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NOTA TÉCNICA

Actividad microbiana y enzimática en suelos forestales y agrícolas

Microbial and enzymatic activities in forest plantations and agricultural field soils

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RESUMEN

El objetivo de este estudio fue caracterizar la actividad microbiana y enzimática en horizontes minerales superficiales bajo plantaciones forestales y suelos agrícolas en la región de Ventania, Provincia de Buenos Aires, Argentina. El pH del suelo en las plantaciones forestales fue en promedio 5.0 comparado con 6.2 en los suelos agrícolas adyacentes. En las plantaciones forestales, también hubo aumentos estadísticamente significativos en el carbono orgánico total y en el carbono orgánico particulado grueso (COPg), en el contenido de N total y en la relación C:N en comparación con los suelos agrícolas. La tasa de respiración microbiana fue mayor en las plantaciones forestales (17,8 ± 1,5 mg CO_2 100 g^{-1} $día^{-1}$) en comparación con los suelos agrícolas (10,2 ± 1,4 mg CO_2 100 g^{-1} $día^{-1}$). Se observó una estrecha relación entre la respiración y el aumento de COPg. En ambos sistemas, la actividad de la enzima fosfatasa fue muy elevada debido a la alta tasa de sorción de fósforo (58 %) que presentan los suelos de Ventania. El uso de la tierra forestal y agrícola afectó la dinámica del carbono y nitrógeno del suelo de diferentes maneras. Los resultados sugieren que la movilización de N y S fue mayor en las plantaciones forestales.

Palabras clave: actividad biológica, respiración microbiana, sulfatasa, quitinasa.

ABSTRACT

We characterized the microbial and enzymatic activity in upper mineral horizons under forest plantations and agricultural fields in the Ventania region, Province of Buenos Aires, Argentina. The pH in the soil of forest plantations was, on average, 1.2 units lower than in the adjacent agricultural fields. In the forest plantations, there were also significant increases in total organic carbon, and coarse particulate organic carbon (POCc), total N content, and the C:N ratio. The microbial respiration rate was higher in the forest plantations (17.8 ± 1.5 mg CO_2 100 g^{-1} day^{-1}) in comparison to the agricultural fields (10.2 ± 1.4 mg CO_2 100 g^{-1} day^{-1}). A close relationship between respiration and the POCc increase was observed. The enzymatic activity of sulphatase and chitinase was also greater in forest plantations, indicating greater mobilization of N and S. In both systems, the phosphatase enzyme activity was high as expected given the high phosphorus sorption rate (58 %) of the soils in Ventania. Forest and agriculture land use affected soil carbon and nitrogen dynamics in different ways. Microbial respiration is positively associated with coarse particulate organic carbon level. The results suggest that N and S mobilization was greater in forest plantations.

Key words: biological activity, microbial respiration, sulphatase, chitinase.

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1. INTRODUCTION

The biological activity of soils plays an important role in the edaphic structure and functioning since microorganisms (e.g., bacteria, fungi, yeasts), together with earthworms and insects participate in the decomposition of organic matter (OM). At the same time, root exudates, fungal hyphae, and bacterial secretions unite soil mineral particles with organic materials. This improves the soil habitat by attracting many organisms and accelerating nutrient cycling. Decay products from invertebrate increase soil aggregation, resulting in greater water infiltration, aeration, and rooting (Clapperton, 2003).

Decomposition is a biological process that occurs normally in all production systems, but the speed of this process varies widely (Ramos *et al.* 2021). In agricultural soils, biological activity is affected by cultivation (Saggar *et al.*, 2001). Time is needed after tilling and other practices to restore the soil biologically and mechanically. Biological activity can be assessed by measuring microbial respiration or enzyme activity.

Enzyme activities provide information on the soil qualitative state as microorganisms, plants, algae, and insects are capable of secreting enzymes. The activity of the chitinase enzyme is related to the breakdown of chitin and is important in both the C and N cycles. Chitin is mainly a constituent of fungal cell walls and is also present in arthropod exoskeletons. It is a polysaccharide made up of N-acetylglucosamine units (N-acetyl-D-glucos-2-amine) linked together by β-1,4 bonds, which is the most abundant natural polymer in soils after cellulose.

The enzyme β-glucosidase acts upon the β1,4 bonds that join two molecules of glucose or glucose substitutes, e.g., disaccharide cellobiose. It is an exocellulase with specificity for various β-D-glucoside substrates that catalyzes the hydrolysis of these substrates to produce glucose. The cellobiohydrolase hydrolyzes cellulose whereas β-glucosidase hydrolyzes cellobiose. The xylosidase that hydrolyzes xylose is also included in this group of enzymes, being a pentose that is important in the carbon cycle. Both the phosphatase and the sulphatase are also hydrolytic enzymes closely related to the P and S cycles, respectively. The natural substrates upon which they act are diverse molecules of organic origin present in soils.

In Ventania, there have been no characterizations of soil biological activity. If the soil biological activity has not been affected by land use, it should be close to the level considered optimal. The production of 20 to 25 mg CO_2 100 g soil^{-1} day^{-1} is considered adequate according to the analysis by one of the authors (P. Zalba, personal communication). Enzyme activities also provide information for characterizing different environments. The study of hydrolytic enzyme activities is very useful as they are related to the breakdown of polymers, which would likely differ under differences land uses.

2. MATERIALS AND METHODS

Study area

The study area is the Ventania mountain range, in the Province of Buenos Aires, Argentina. The region belongs to the morpho-structural domain "Positivo de Ventania" where two main units have been defined: the "Sistema Serrano" and the "Nivel de Planación General" (González Uriarte, 1984).

The mean annual precipitation averaged 700 mm from 1900 to 2009. The potential evapotranspiration for the same periods was 1100 mm. The rainiest seasons are summer and autumn with 32 % and 28 % of the total precipitation, respectively. Rainfall is highly variable in the region (Scian, 2010).

The mean annual temperature is 15.0 ºC. The mean temperature in the warmest month (January) is 23.2 ºC and that of the coldest month (July) is 7.5 ºC. The average frost-free period extends from October to April. Predominant winds come from the northwest. The windiest season is summer with average speeds of 24 $km.h^{-1}$. According to the Thornthwaite water balance, there is a marked water deficiency during the summer months. The water replacement period begins in March-April and lasts until October when consumption of the water accumulated in the soil begins (Scian, 2010).

The soils in the study area belong to the Mollisols order, suborder Udol (Soil Survey Staff, 2014). The fertile soils that characterize the natural ecosystems in Ventania are from the Holocene period (Amiotti *et al.*, 2000). Their formation is linked to a combination of factors that include calcareous parental material (loess or loessoid sediments), and edaphoclimatic conditions that resulted in the development of herbaceous strata rich in plants in the *Poaceae* family. The mineralogy of the clay fraction is mixed, with a predominance of illite and illite-smectite interlayer (Blanco *et al.*, 2003).

The land is apt for agriculture, forestry, and livestock production. Both winter cereals such as wheat (*Triticum aestivum* L.), oats *(Avena sativa* L.), barley (*Avena sativa* L.) and rye (*Secale cereale* L.), and summer crops such as corn (*Zea mays* L.), sorghum (*Sorghum* spp.), and sunflower (*Helianthus annuus* L.) are grown. Forest plantations are mainly composited of coniferous species.

Treatments and soil sampling

We sampled soils of similar texture from seven forest plantations consisting of species of *Pinus halepensis* Mill., *Pinus radiata* D. Don, and *Pinus pinaster* Aiton. The soil texture of the sampled sites was similar and, therefore, confounding effects of granulometry on changes in the content of humic substances and associated nutrients were avoided (Galantini *et al.*, 2004). The forest plantations were planted between 1949 and 1958.

In each plantation, three samples were taken randomly at 50 cm from the tree trunks and at 0 - to 20 - cm depth. Three samples were taken simultaneously at random at 0 - to - 20 cm depth in adjacent fields with similar number of years of agricultural use. Information about the agricultural history of the fields was provided by farmers. In all cases, wheat, corn, and sunflower were sown after a fallow period to accumulate soil water. Occasionally, fescue (*Festuca* spp.) and alfalfa (*Medicago sativa* L.) pastures were grown for periods of at least three years as part of rotations to maintain soil fertility. The land uses included in the study were 1) Forest plantations of more than 60 years of age surrounded by a perimeter fence that prevented cattle access, and 2) agricultural fields with more than 6a years of agriculture use mostly under winter and summer cereals and pastures.

Laboratory measurements and statistical analysis

Chemical analyses were made on all soil samples and the three granulometric fractions to study the effect of land use on the SOC distribution.

In soil samples, it were determined: pH in soil-water suspension, 1:2.5 ratio; total organic carbon (TOC, g kg^{-1}) by the dry combustion method using a Leco-Dry Combustion Analyzer; total nitrogen (TN, g kg^{-1}), by the semi-micro-Kjeldahl method (Bremner, 1996); organic phosphorus by the Saunders and Williams method obtained from the difference between the phosphorus extracted with 0.5 M sulphuric acid from a sample calcinated at 550ºC and from a sample without calcination (Kuo, 1996); single point phosphorus sorption index (PSI) using a 30 mg solution of P L^{-1} prepared from 0.112 g of $NH_4PO_4H_2$ L^{-1} (Ron *et al.*, 1995); as well as sand, silt, and clay percentage obtained by the Robinson pipette method (Gee and Orr, 2002).

The physical fractionation of the soil previously sieved with a 2-mm mesh was carried out by wet sieving to separate three granulometric fractions (Galantini, 2005): coarse of 100-2000 µm, intermediate of 50-100 µm, and fine less than 50 µm.

The SOC (g kg^{-1}) of the coarse, medium, and fine fractions was determined by the dry combustion method with a Leco-Dry Combustion Analyzer, obtaining the carbon associated with the mineral fraction (MOC, 0-50 µm), the fine particulate (POCf, 50-100 µm) and the coarse particulate (POCc, 100-2000 µm), respectively.

The soil microbial activity was characterized using the respiration technique. The activity of enzymes involved in different soil metabolic processes such as phosphatase, sulphatase, chitinase, β-glucosidase, cellobiohydrolase and laxilosidase, was evaluated. The phosphatase and sulphatase activities were determined to assess their effect on P and S metabolisms, respectively. To characterize the activity related to N metabolism, the sum of chitinase plus leusin amino peptidase (LEU or LAP) activities was used. The latter enzyme did not show activity, so only the chitinase activity is mentioned. Regarding C metabolism, the activities of cellobiohydrolase, xylosidase, and of β-glucosidase (BGLU or BG), were evaluated. Even though there are several enzymes that hydrolyze polymers, the latter enzyme is supposed to be the most important one in the carbon pathway.

The enzymatic activities were measured directly in the soil suspension in a relatively short time to ensure the occurrence of extracellular activities as the microorganisms did not have time to colonize the substrate. Hydrolytic enzymes were analyzed using fluorine-methylumbelliferyl-linked substrates and the oxidative enzymes were determined colorimetrically using L-3,4-dihydroxyphenylalanine. The enzymatic activities were calculated and expressed in nmol h^{-1} g^{-1} of dry soil. The samples were incubated at 20 ºC. To approximate the environmental pH of the soil, the samples were analyzed at pH 5 obtained by suspending 1 g of soil in 100 ml of 50 mM sodium acetate buffer solution (Sinsabaugh, 2008).

The land use effects (forest and agriculture) on the studied variables were tested in ANOVA analyses with land uses as fixed factors. Tests of least significant differences were performed for comparing means. We used the INFOSTAT software (Di Rienzo *et al.*, 2013) for the statistical analyses.

3. RESULTS AND DISCUSSION

The soil texture was similar in forest plantations and agricultural fields (Table 1) with soils having loamy to sandy loam textures. Clay content varied between 15.5 % and 26.5 %, whereas silt content ranged between 15.4 % and 36 %, and sand content between 41.1 % and 65 %. A sharp decrease in pH, and significant increases in SOC and in the C:N ratio were observed in the forest plantations.

Table 1. Chemical properties in forest plantations and agriculture fields in Ventania, Argentina.

System	pH	SOC %	Nt %	C:N	Texture
Forest plantation	5.0 ± 0.3 a	3.6 ± 0.8 a	0.27 ± 0.04 a	13.2 ± 1.0 a	Loam to loamy sand
Agricultural field	6.2 ± 0.2 b	2.5 ± 0.7 b	0.22 ± 0.03 b	11.7 ± 0.6 b	Loam to loamy sand

SOC: Soil organic carbon; Nt: Total nitrogen; C:N: Carbon: nitrogen ratio. Values are means ± one standard deviation. Different letters within each column indicate highly significant differences ($P \leq 0.01$).

The lower N values observed in agricultural fields could be attributed to the loss of N in this system because plant residue loss does not occur in the forest plantations. Because of the marked increase in SOC, especially in the less transformed organic material, the C:N ratio is also higher in the forest plantations (Amiotti *et al.*, 2012).

In the forest plantations, the superficial organic contributions produce a greater increase of SOC in the coarsest fraction (Table 2). This increase in SOC mainly corresponds to young or particulate organic matter that was considered in this study to be greater than 100 µm. This organic matter closely related to the most recent organic contributions (Galantini and Suñer, 2008) are significantly higher in the forest plantations as shown by the SOC enrichment (Table 2). In this regard, the transformation of organic materials follows the sequence: coarse particulate OC (POCc) > fine particulate OC (POCf) > OC associated with the mineral fraction (MOC). A marked accumulation of material larger than 100 µm was observed in the forest plantations, suggesting that plant residues are more resistant to decomposition, although there is also evidence that under agricultural soil the POCc decreases (because of tilling (Blanco-Moure *et al.*, 2017)

Table 2. Soil organic carbon content in different particulate fractions in forest plantations and agricultural fields in Ventania, Argentina.

System	**POCc > 100 µm (%)**	**POCf 50 – 100 µm (%)**	**MOC < 50 µm (%)**
Forest plantation	5.0 ± 3.1 a	1.30 ± 0.7 a	4.3 ± 1.6 a
Agricultural field	2.5 ± 1.9 b	1.92 ± 1.1 a	3.5 ± 0.9 a

POCc: Particulate organic carbon, coarse; POCf: Particulate organic carbon, fine; MOC: Mineral organic carbon. Values are means ± one standard deviation. Different letters within each column indicate significant differences ($P \leq 0.05$).

No significant differences were observed in MOC between the two systems. However, there is a trend towards a higher MOC content under the trees, indicating that the humification conditions were comparable. There were no significant differences in the POCf content corresponding to the 50 to 100 µm fractions.

Greater biological activity was observed in the forest plantations compared to the agricultural fields ($P \leq 0.01$). The soil respiration in the agricultural fields was classified as moderately low averaging 10.2 ± 1.4 mg CO_2 100 g^{-1} day^{-1}, whereas in the forest plantations was moderate with an average value of 17.8 ± 1.5 mg CO_2 100 g^{-1} day^{-1} (Zarafshar *et al.*, 2020).

Lower biological activity in agricultural fields may be related to the use of agrochemicals that are commonly employed to achieve higher crop yields. The use of herbicides and pesticides can reduce the diversity of soil-dwelling microorganisms, and edaphic mesofauna (Chavez-Bedoya *et al.*, 2013). Comparing soil respiration and the changes produced in the physically separated organic fractions of the two systems, a positive relationship can be observed between respiration and the POCc increase. Several studies indicated that this fraction is the most dynamic and most rapidly used by soil microorganisms (Galantini and Rosell, 1997; Haynes, 2005; Duval *et al.*, 2018).

This POCc fraction, together with the soil organisms related to it, constitutes a potential reserve of nutrients that can be vital for the maintenance of soil quality. In addition, when the edaphic environment is altered, the microorganisms are ideal indicators of contamination by the xenobiotic substances present in agrochemicals (Atlas and Bartha, 2002).

The activity of enzymes involved in different soil metabolic processes such as phosphatase, sulphatase, chitinase, β-glucosidase, cellobiohydrolase and laxilosidase, was evaluated. The phosphatase and sulphatase activities were determined to assess their effect on P and S metabolisms, respectively. To characterize the activity related to N metabolism, the sum of chitinase plus leusin amino peptidase (LEU or LAP) activities was used. The latter enzyme did not show activity so only the chitinase activity is mentioned. Regarding C metabolism, the activities of cellobiohydrolase, xylosidase, and of β-glucosidase (BGLU or BG), were evaluated.

Even though there are several enzymes that hydrolyze polymers, the latter enzyme is supposed to be the most important one in the carbon pathway.

There were significant differences in the enzymatic activity of sulphatase and chitinase that participate in the metabolism of S and N, respectively (Table 3). Both enzymes showed greater activity in the forestry system. It can be inferred, then, that both S and N are more intensely metabolized in forest plantations. Regarding S, pines and birches in southern Poland absorbed and incorporate high levels of S into their woody tissues, at a proportional rate to the degree of contamination with this element (Likus-Cieślik and Pietrzykowski, 2017).

Table 3. Enzyme activity in forest plantations and agriculture field in Ventania, Argentina.

	Enzymes related to the carbon metabolism		
System	**Cellobiohydrolase**	**β-Glucosidase**	**Xylosidase**
Forest plantation	36.6 ± 11.6 a	465.8 ± 151.4 a	39.9 ± 17.9 a
Agricultural field	28.1 ± 16.3 a	414.8 ± 120.0 a	30.1 ± 18.9 a
	Enzymes related to N, P and S metabolism		
	Chitinase	**Phosphatase**	**Sulphatase**
Forest plantation	209.6 ± 61.7 a	2126.7 ± 560.7 a	49.9 ± 14.5 a
Agricultural field	85.4 ± 25.5 b	1845.0 ± 427.2 a	32.5 ± 5.6 b

Values are expressed in mmol of substrate per h^{-1} per g^{-1} of dry soil. Values are means ± one standard deviation. Different letters within each column indicate significant differences ($P \leq 0.05$).

There were no significant differences between land uses in the enzymes involved in C metabolism. Neither were differences in the enzymatic activity of phosphatase, which was much higher than that of the other enzymes studied (Table 3), even though it had been shown, that the availability of this nutrient significantly increases under pine trees (Garay *et al.*, 2015). The availability of P in the soil depends on complex physicochemical and biological balances (Galantini *et al.*, 2007). On one hand, there is a large amount of P in the more labile organic and inorganic fractions (Perrot, 2003) and, on the other hand, the soils in this region have a high PSI (Ron *et al.*, 1995). Both factors, at least in part, could explain the great enzymatic activity of phosphatase observed in the two systems. Traditionally, the soil P retention capacity is evaluated by the PSI. The ability of a soil to release the P retained in the colloids into the environment depends on the soil P sorption capacity as well as the amount of P absorbed (Pose and Zamuner, 2016). The hypothesis that the enzymatic activity is related to the presence of a high content of organic phosphorus is not supported. Organic P values of 236 ± 39.2 mg OP kg^{-1} were determined in the forest plantations and 254 ± 53.9 mg OP kg^{-1} in the agricultural fields. The PSI values in both systems were very high (57.9 ± 3.9 % and vs. 57.8 ± 3.0 % in forest plantations and agricultural fields, respectively) and they did not differ significantly.

The microorganisms present in the soil must develop great enzymatic activity to mobilize and obtain the phosphorus necessary for growth and development. In natural edaphic ecosystems, a 1:1:1 stoichiometric relationship has been found for the enzymes related to C, N, and P acquisition (Sinsabaugh *et al.*, 2008). This finding suggests that the microbiome regulates the production of enzymes based on the need for a nutrient. These authors evaluated 40 ecosystems and concluded that a condition of equilibrium is reached when the existing stoichiometric relationship between the enzymatic activities of C, N and P tends to be equal to one, and therefore the enzymatic relationships between C: N: P would be like each other.

The natural logarithm of the quotient of enzymatic activities is represented in Figure 1. The C: N ratio relates to enzymes that hydrolyze C over those that degrade N. in forest plantations the C: N, C: P and N: P ratios are equal to 1.2; 0.8, and 0.7, respectively, whereas in the agricultural fields these ratios are 1.4; 0.8, and 0.6, respectively. Therefore, as suggested by Sinsabaugh *et al.* (2008), the effect on the microbiome activity of more than 60 years of agriculture and/or afforestation with pine trees produced similar consequences. These similar values can show that the maintenance of element homeostasis by soil microorganisms induces a strong co-regulation of enzymes involved in covering their C, N and P nutrient supply during litter degradation (Bai *et al.*, 2021)

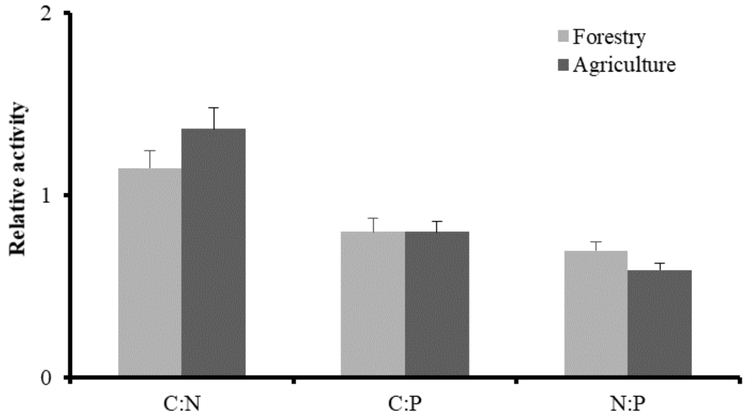

Figure 1. C: N, C: P and N: P enzymatic relationships in forest plantations and agricultural fields in Ventania. Argentina

As the enzymatic activity of phosphatase in the forest plantations and agricultural fields was very high, the C: P and N: P ratios were always less than one. This suggests that microorganisms must have high enzymatic activity to obtain the phosphorus necessary for their development. This is probably related to the high PSI in the loessic soils in the study area. In the natural forests of *Nothofagus pumilio* (Poepp. & Endl.) Krasser in the Patagonia Argentina, the communities of ectomycorrhizal fungi played a predominant role as regulators of N (Truong *et al.* 2019). These are shallow soils with high content of SOC and available P, with N likely as the limiting nutrient, especially at high altitude sites (Moretto and Martınez Pastur, 2014). The enzyme with the highest activity in this soil, and under those environmental conditions, was N-acetyl-glucosaminidase, which is related to the N metabolism (Truong *et al.*, 2019).

4. CONCLUSIONS

In this study, forest plantations showed greater soil C, N concentration, and biological activity in the upper soil compared to agriculture fields. Differences in enzyme activity varied for the studied enzymes with higher activity in forests, for enzymes related to the S and N cycles with no differences for C and P related enzymes. The C:N:P stoichiometry agreed with this result. The phosphatase activity was high in both systems. From a land use perspective, soil under forest would constitute a more conservative systems for major pools (e.g., C and N) compared to losses or lower build-up in soils under agriculture than could be attributed to slower plant residue decomposition and lower soil disturbance because of absence of tiling and agrochemical applications. However, soil acidification in plantations of introduced trees species has been of concern in many regions of the world.

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