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Preliminary study of prairies forested with *Eucalyptus sp*. at the northwestern Uruguayan soils

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"Capsule": *The forestation of Uruguayan natural prairie soil does not always ensure an increase of soil carbon sink.*

Abstract

The land cover change of Uruguayan Forestal Plan provoked biogeochemical changes on horizon Au_1 of Argiudols; in native prairies which were replaced by monoculture *Eucalyptus sp*. plantation with 20 year rotations as trees. Five fields forested and six natural prairies were compared. The results not only show a statistical significant soil acidification, diminution of soil organic carbon, increase of aliphaticity degree of humic substances, and increase of affinity and capacity of hydrolytic activity from soil microbial communities for forested sites with *Eucalyptus sp*. but also, a tendency of podzolization and/or mineralization by this kind of land cover changes, with a net soil organic lost of 16.6 tons ha^{-1} in the horizon Au_1 of soil under *Eucalyptus sp*. plantation compared with prairie. Besides, these results point out the necessity of correction of the methodology used by assigned Uruguayan commission to assess the national net emission of greenhouse gases, since the mineralization and/or podzolization process detected in forested soil imply a overestimation of soil organic carbon. The biochemical parameters show a statistical significant correlation between the soil organic carbon status and these parameters which were presented as essential for the correct evaluation of Uruguayan soil carbon sink.
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Keywords: Carbon dioxide; Land cover change; Soil organic carbon; Soil carbon sink; *Eucalyptus sp*

1. Introduction

Changes in land-use management and increased burning of fossil fuels have elevated atmospheric CO_2 concentrations (Vitousek et al., 1997). For this reason, one of the most important environmental topics is a strategy to reduce the atmospheric CO_2 levels to mitigate global climatic change. Since the upper meter of the soil profile is one of the compartments with the highest carbon content, the management of land cover changes have been identified as a key process that can help to reduce the atmospheric carbon levels (Eswaran et al., 1995; Jobbagy and Jackson, 2000). Based on this fact, the Kyoto Protocol proposed the promotion of forestation to increase soil carbon storage as one tool to mitigate the global climate change (Eve et al., 2002; Health et al., 2002; Johnson et al., 2002; Lal, 2002; Palmer et al., 2002; Schumann et al., 2002). That international environmental Protocol incentivized the forestation and reforestation of aforested land (e.g., agricultural lands, prairie lands) in developing countries under the hypothesis that always this kind of land use conversion to perennial forest implies a positive effect on the soil carbon sink (Honty, 2000; IGBP, 1998; Ver et al., 1999).

In Uruguay, 400 thousand hectares have been forested with *Pinus* (*taeda* and *eliotti*) and *Eucalyptus* (*grandis and globulus*). Eighty percent of this forestation have been with *Eucalyptus sp*., having an expected rate of plantation establishment of about 50,000–65,000 hectares per year (MGAP, 1999; Morales and Ribeiro, 1999). The private commercial forestation using exotic

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fast growing tree species has been promoted since 1989 when the new National Forestal Law declared that 20% of the national territory were established as "soils of forestal priority". As the main landscape of Uruguay is grassland (140,000 km^2, 87% of the national territory), the most part of the forest plantations would currently occur in regions with prairie land cover (MGAP, 1999; Morales and Ribeiro, 1999; Pérez-Arrarte, 1993). The planting of *Eucalyptus sp*. has been criticized because of concerns that soil would be degraded on account of nutrient depletion and biological changes (Caffera et al., 1991; Morales and Ribeiro, 1999; Pérez-Arrarte, 1993).

In the last ten years, the northwestern regions of Uruguay the *Eucalyptus spp*. plantations have reached annual forestation rates of 300%. That zone has a potential forest soil area of 822,054 hectares, approximately 23% of the national soil declared as forestal priority, of which approximately 57,069 hectares are currently forested by monoculture *Eucalyptus spp*. (approximately an 80%) or *Pinus*.; 20,000 hectares correspond to a 20 year old *Eucalyptus* monoculture (Dirección Forestal, 2001; MGAP, 1999). Then it should be expected that the radical and durable substitution of vegetation cover brought about changes in the quantity and quality of soil organic matter by itself. Such changes may also influence soil fertility, and soil physical and chemical properties. The soil quality effects associated with *Eucalyptus sp*. plantations could promote negative soil biochemical changes (Aggangan et al., 1999; Caffera et al., 1991; Grierson and Adams, 2000); for example: soil acidification (Bandzouzi, 1993; Bernhard-Reversat, 1999; Loubelo, 1990), iron leaching, allelopathic activities (Bernhard-Reversat, 1999) and a high C:N ratio of litter (Aggangan et al., 1999; Khanna, 1994; Madeira et al., 1989). Additionally, as most part of scientific experience on land cover change is related to ecosystems where forests were replaced by grasslands or crops; or grassland replaced by crops, the environmental effects of the currently Uruguayan land cover change are yet unknown (Altesor et al., 1998).

In order to understand the potential soil quality changes from the establishment of *Eucalyptus* plantations in Uruguay and to fill the gap of knowledge of this land cover change, soils under a 20 year old *Eucalyptus* plantation were compared with similar soils under natural prairie vegetation. We tested the hypothesis that commercial *Eucalyptus sp*. plantations in Acuic Argiudols soils (Altamirano et al., 1976) can markedly reduce rates of carbon storage in the A horizon, and that negative changes in carbon biogeochemistry is mediated by raising the rate of carbon mineralization by soil microbial communities.

The present paper deals with the change in soil texture, particle size distribution, acidity, organic carbon content, and aliphaticity degree of humic substances of Argiudols soils. The last parameter was determined because the ratio E4/E6 of absorbancies is independent on carbon concentration. The absorbancy parameter allows a good evaluation of changes of aliphaticity degree, humic substances molecules with higher absorbance values imply a major quantity of carbon localized in lateral aliphatic chains instead of aromatic rings (Ghosh and Schnitzer, 1979; Kumada, 1987; Salfeld, 1972; Schnitzer, 1972; Stevenson, 1982). The changes of soil carbon biochemistry were assessed by the evaluation of enzymatic kinetic parameters of fluorescein diacetate (3′,6′-diacetylfluorescein or FDA) hydrolysis. This enzymatic parameter is the result of the expression of lipases, proteases and esterases by soil microbial communities. The biochemical assay assesses the relationship of soil microbiota with the surrounding carbon substrate. This assay allowed us to determine the biochemical changes of the soil carbon substrate breakdown under the *Eucalyptus* cover compared with the prairie cover (Adam and Duncan, 2001; Dick, 1994; Hoitink and Boehm, 1999; Inbar et al., 1991; Perucci, 1992; Schnürer and Rosswall, 1982; Swisher and Carroll, 1980).

2. Material and methods

2.1. Site and soil description

The study area is located in northwestern Uruguay (32° 20,57′ S 58° 02,13′ W), 61 m above sea level. The mean annual rainfall is 1218 mm, without dry period. The mean temperature is 7 °C in winter and 30 °C in summer (DNM, 1995). The studied soils closely fit the description of Acuic Argiudols, sandy loam, with horizon Au_1 of 18 cm depth, and are related with parent material derived from clay sediments and Cretaceous sands (Altamirano et al., 1976). This kind of soil covers an area of 141,969 ha in the Queguay River basin. This basin, which belongs to Paysandú Department, Northwestern of Uruguay, was entirely declared to forestal priority (CONEAT, 1979).

The sampling of soils for this study was performed in the Queguay River basin, and for the selection of sampling sites, all the *Eucalyptus sp*. plantations more than 20 years old were selected from a satellite image (LANDSAT 7^{TM}). Then we chose those sites with the same edaphic characteristics, altitude and slope position on Argiudol soils. Finally, the natural prairies selected to be sampled, were those with same edaphic characteristics, altitude and slope position on Argiudol soils as the *Eucalyptus sp*. plantations. The experimental design was an unpaired design. It was formed by five forested plots and six prairy plots (as control), each one of 100 m^2. Every plot was divided into a sampling matrix with cells of 1 m^2; from which five cells were randomly selected for sampling. A soil corer (5 cm of internal diameter, 20 cm of length) was used for sampling. Finally, the A1 horizons (approximately 18 cm) were separated from the next horizon and the five samples from the A1 horizon taken from each site was pooled. The samples were taken in the middle summer of 2001.

2.2. Laboratory procedures

All samples from the same plot were pooled, sieved by 2-mm sieve, and frozen at −20 °C until analyses. The core samples were oven dried at 105 °C for 24 h for bulk density determinations (Madeira, 1992). Soil acidity was determined in water (1:2 w/v) by the McLean method (1982). Organic carbon content was determined using an aliquot soil sieved with the 2 mm sieve by mass loss on ignition at 500 °C for 6 h (Adam and Duncan, 2001), and the Van Bemmelen factor of 1.724 to the calculations (Nelson and Sommers, 1982). This is a valid methodology for the Uruguayan soils with clay contents lower than 10% (Altamirano et al., 1976). Aliphaticity degree of humic substances was determined with the ratio of absorbances 472 and 665 nm of humic substances, extracted with benzene-ethanol by Waksman's method (Schnitzer, 1972; Stevenson, 1982; Kumada, 1987).

The soil was oven dried at 80 °C for 48 h, then 20 g of each sample was attacked with HCl 0.1 N and oxidizing with H_2O_2 (150% v/v). Soil texture and particle size distribution were then determined by Survey Staff (1979) and Folk and Ward (1957) methods, respectively.

The rate of fluorescein diacetate (3′,6′-diacetylfluorescein)(FDA) hydrolysis was estimated according to the Adam and Duncan method (2001). Two grams of soil (fresh weight, sieved <2 mm) were placed in 20 ml tubes and 10 ml of 60 mM potassium phosphate buffer pH 7.6 were added. To start the reaction, 0.1 ml of stock solution (2 mg FDA ml-1 stock solution) was added to the reaction tubes. Blanks were prepared without the addition of FDA substrate along with a suitable number of sample replicates. The flasks were stoppered and the contents shaken by hand. The flasks were then placed in a shaker at 25 °C for 20 minutes. Once removed from incubator, 15 ml of chloroform/methanol (2:1 v/v) were added immediately to terminate the reaction, and the samples shaken by hand. The supernatant from each sample was filtered (Whatman, N° 2) into 20-ml tubes, and the filtrate measured at 490 nm on a Shimadzu UV-1603 UV-visible spectrophotometer. The fluorescein produced by reaction was quantified using a calibration curve built with adequately fluorescein concentrations.

The Michaelis–Menten constant (Km) and maximum enzyme velocities (Vmax) were determined using fluorescein diacetate dissolved in acetone in 10 ml phosphate buffer (60 mM; pH 7.6), with seven final concentrations between 4–40 μg FDA ml-1 and computed from Lineweaver–Burk linear transformation of Michaelis–Menten:

$$\frac{1}{v_i} = \frac{1}{V_{\max}} + \frac{1}{[S]}\frac{K_m}{V_{\max}}$$

In order to test the reproducibility of this method, all chemical and biochemical assays were replicated at least three times for each treatment. Normal distribution and variance homogeneity were performed by Shapiro-Wilks' W and Bartlett's tests, respectively; and statistical analysis used Student's t test for unbalanced independent samples. All data were analyzed using statistical software STATISTICA 4.0.

3. Results and discussion

3.1. Soil texture and particle size distribution

The soil texture of the samples was mainly sandy loam or loam sandy, with a mean size particle that belongs to medium sands with poor selection processes. These results fit the properties of these soils described by the Uruguayan soil survey (Altamirano et al., 1976). The particle size distribution was positive with a tendency to fine size particle. For soils under 20 year old *Eucalyptus sp.* plantations and natural prairie, these results do not allow us to detect differences of erosion dynamics between both land covers; because, kurtosis results did not show a decrease of fine particles (Fig. 1).

3.2. Soil acidity

In general the soil analyzed was acid, both in prairie (range 5.19–5.51) and *Eucalyptus sp.* plantations (range 5.38–5.76). The t Student test results showed a highly significant statistical soil acidification, 0.27 units in

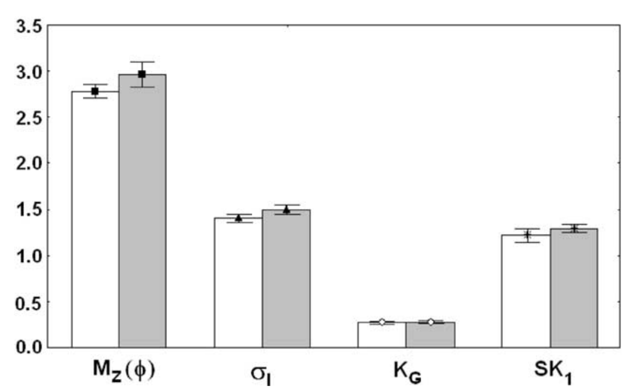

Fig. 1. Mean particle size (M_z), selection degree (σ_I), asymmetry degree (SK_I) and kurtosis (K_G) results of particle distribution analysis from soils under 20 years old commercial *Eucalyptus sp.* plantations (white bars) and natural prairies (grey bars). Bar graphs represent the mean, and whiskers the range mean minus standard error to mean add to standard error.

forest sites (Fig. 2). This decrease of pH is not edaphologically important; it shows a change of soil chemical characteristics that agrees with the results of Loubelo (1990), Bandzouzi (1993) and Bernhard-Reversat (1999). However, this soil acidification does not reach 0.5 to 1 unit, reported by Loubelo (1990) and Bandzouzi (1993) for the top layer of 7–11 years old *Eucalyptus* plantation, by the natural acid characteristics of the prairie soil studied by this research.

3.3. Soil organic carbon

The soil organic carbon level of soils under natural prairies agrees with the characteristics attributed to Argiudols by Uruguayan soil survey (Altamirano et al., 1976). However, the soil organic carbon level of soil under the *Eucalyptus sp.* plantations showed a statistically significant decrease of 0.68% (Fig. 2). That result implies a negative disruption of soil organic carbon stock under *Eucalyptus sp.* plantations. As the soil bulk density mean was 1.35 both in soil under *Eucalyptus sp.* plantations and soil under natural prairie (data not showed). This result agrees with García's studies about soil under natural prairie to this kind of Uruguayan soils (García, 1980). Therefore, we can calculate a net lost of soil organic carbon of 16.6 tons of carbon per hectare with *Eucalyptus sp.* plantations.

Table 1
Correlation results of parameters measured in soils under 20-years old *Eucalyptus sp.* plantation and natural prairie. *, **, ***, $P<0.10$, $P<0.05$, $P<0.01$, respectively

	Acidity	Soil organic carbon	Ratio E4/E6	Km
Acidity				
Soil organic carbon	0.27			
Ratio E4/E6	−0.23	−0.56		
Km	0.41	0.60*	−0.64*	
Vmax	−0.73*	−0.53	0.12	−0.64*

3.4. Aliphaticity degree of humic substances

A statistically significant increase of absorbances ratio 472 and 665 nm (ratio E4/E6) of humic substance UV-spectra showed the soil under *Eucalyptus sp.* plantations. Therefore, for forested soil there was a diminution of humic substances polymerization in forest soil (Fig. 2); which it is associated with a podzolization process (Bravard and Righi, 1992).

3.5. Km and Vmax

For the statistical analysis of maximal initial velocity (Vmax) of FDAse activity, this result must be transformed by natural logarithms to get its normal distribution and variance homogeneity. Both the diminution of Michaelis–Menten constant (Km) (30.16 points) and the increase of Vmax (31.14 points) of activity under *Eucalyptus sp.* plantation soils showed highly significant statistical differences (Fig. 2). As the fluorescein diacetate hydrolytic activity follows a Michaelis–Menten kinetic (Adam and Duncan, 2001), the results above imply an increase of affinity by substrate (or reaction rate) and the enzyme concentration. The biochemical changes show an increase of the capacity and affinity of the soil microbial communities to break-down the soil organic carbon.

3.6. Correlation

The correlation analysis results showed a significant statistical correlation between Km and soil organic carbon, Km and E4/E6, Vmax and soil acidity, Km and Vmax (Table 1). The positive correlation between Km and soil organic carbon implies that any increase of FDAse substrate affinity will produce a diminution of soil organic carbon concentration. Complementary, the negative correlation between Km and E4/E6 expresses that any diminution of Km (an increment of its affinity by carbon substrate) implies an increase of fulvic acids generation, which is strongly linked with podzolization processes. The negative correlation, between Vmax and

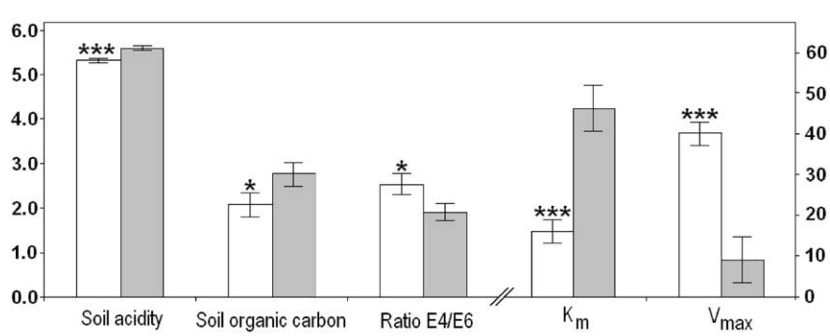

Fig. 2. Soil acidity, soil organic carbon, ratio E4/E6, K_m and V_{max} results from soils under 20 years old *Eucalyptus sp.* plantation (white bars) and natural prairie (grey bars). Bar graphs represent the mean; and whiskers are the range mean minus standard error to mean add to standard error. *, **, ***, $P<0.10$, $P<0.05$, $P<0.01$, respectively.

soil acidity, shows that lower soil pH improves the conditions for enzyme production involved in FDAse activity. Moreover, the negative correlation between Km and Vmax expresses that the induced activity by lower soil pH produces an hydrolytic activity with better affinity and capacity.

4. Discussion

The physical and textural results of soils under native prairie and 20 year old *Eucalyptus sp*. plantations did not show evidences of differences that could be attributed to changes of erosion dynamics. Therefore, we conclude that commercial forestation with *Eucalyptus sp*. promoted and provoked all the biogeochemical differences detected in our study.

The diminution of soil organic carbon, the increase of affinity and capacity of microbial hydrolytic activity (FDAse), and the diminution of aromatic rings of humic substances make us conclude that the *Eucalyptus sp*. plantations produce a negative disruption on biogeochemical reactions that control the levels of soil organic carbon. This land cover change promoted a diminution of quantity and quality of soil organic carbon, which imply podzolization and/or mineralization in soil horizon Au_1 (Bravard and Righi, 1992).

The soil acidification found agrees with the results of other studies of *Eucalyptus sp*. plantations (Bandzouzi, 1993; Bernhard-Reversat, 1999; Loubelo, 1990). This result points out that, at least, the mechanisms proposed by Bernhard-Reversat (1999) concerning to acidification by organic acid from the litter and iron leaching, can exist in the studied Argiudols. These changes were enough to modify the genetic expression of soil microbial communities, a situation manifested by the change of Km and Vmax parameters. Additionally, the correlation of enzymatic kinetic parameters with the lost of quality and quantity of soil organic carbon makes us conclude that the changes mentioned above are responsible for the lost soil organic carbon.

The loss of 16.6 tons of soil organic carbon per hectare, in soil under 20 year old *Eucalyptus sp*. plantations, points out the necessity of recalculating the net emission of greenhouse gases associated with Uruguayan commercial forestation and claims of soil carbon storage in these forests. Thus, if we consider an equivalent loss of soil organic carbon for the 59,060 ha with *Eucalyptus sp*. plantations established, on the same kind of soils, the soil organic carbon lost only from the Au_1 horizon will be 980 kT of C. This potential loss represents 83% of the net carbon sink calculated for national commercial *Eucalyptus sp*. plantations (approximately 1167.48 kT of C), and 74% of net carbon sink calculated for the national commercial exotic tree plantations (approximately 1319.12 kT of C) (UCG, 1998). This situation could be worst if in the future the entire soil unit (141,000 ha), which belongs to the soil studied, followed the same land cover change.

Our results show a potential serious problem of reports made by the Unit of Global Change, a Uruguayan commission assigned to estimate the national net greenhouse gases emissions (UCG, 1998). This commission followed the IPCC assumptions for its estimations; therefore, it assumed that soil carbon stock did not change with the forestation of prairies, and only considered carbon sink gained through the growth of above-ground biomass of plantations (UCG, 1997; UCG, 1998). However, the soil podzolization and net lost of soil carbon sink found (at horizon Au_1) by our study points out that soil organic carbon was affected negatively by the commercial forestation. These results agree with the evaluations done by IGBP (1998), if the IPCC assumptions was utilized (IPCC, 1996).

5. Conclusion

At the environmental dimension of this land cover change, the commercial *Eucalyptus sp*. plantations in Uruguay should consider mitigation strategies to improve the soil carbon sink, if the podzolization process was confirmed for the most part of the soil forested. Moreover, the evaluations of UCG (1998) should incorporate and define real soil carbon change to find the current carbon sink of the Uruguayan forestation activity. This would be an unavoidable prerequisite for any application for Uruguay to Kyoto Protocol requirements. Evaluations of Vmax and Km parameters appear a good biomarker for fast evaluation of the biogeochemical status of soil organic carbon under *Eucalyptus sp*. plantations.

Finally, our study concludes that to understand the real biogeochemical effects of land cover change, it is necessary to conduct an integrated assessment. This assessment must include physical, chemical and biochemical soil parameters for complete evaluation of changes in the soil profile. This assessment can then be used to elaborate mitigation strategies to minimize negative environmental impacts. This kind of assessment is necessary to get an accurate estimate of the changes in soil carbon sinks attributed to Uruguayan commercial forestation.

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