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Soil carbon isotope ratios in forest-grassland toposequences to identify vegetation changes in southern Brazilian grasslands

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ABSTRACT

The Southern Grasslands or Pampa biome covers 176,496 km^2 (2.07%) of the Brazilian territory, concentrated in the southernmost state with 63% covered by this biome. When adding Uruguay and Argentina, the biome area reaches 750,000 km^2. Rangelands are being substituted by crops and commercial eucalyptus, with potential impacts on ecological and hydrological response of watersheds and river basins. Studies of isotopic natural abundance may be used to understand vegetation dynamics in the past. We evaluated the natural abundance of ^{13}C in soil profiles (ten soil layers down to 1.40 m) at different positions of the landscape, in order to associate it with the vegetation dynamics in the studied environment. Two grassland areas and a forest area in the Pampa region of southern Brazil were sampled. Isotopic signature δ^{13}C in the soil profile of the two grassland areas was dissimilar: one area is in the range of C4 plant formation, and the other followed the same trend up to the first 1.00 m but from this depth on δ^{13}C is in an intermediate range, which could have occurred due to the influence of C3 and C4 plants. Forest vegetation presents a clear change of predominant vegetation in a more-recent past period. Characteristic values of soil formed by C3 photosynthetic cycle plants are predominant in surface layers, whereas those of C4 plants predominate in deeper soil layers. Our hypothesis that soil classes, texture, and position in landscape are related to the presence/absence of riparian forest, as evaluated by the ^{13}C isotopic abundance in soils from these sites, is accepted. Quantification of isotopic natural abundance allowed verifying changes in vegetation based on photosynthetic types predominant in the environments of grassland and native forests of the Pampa biome, and may provide information to manage this ecosystem.

1. Introduction

Rich in biodiversity, the Pampa biome or southern grasslands is a mix of ecological formations in a single eco-landscape with intense flow of matter, energy and wildlife (IBGE, 2004). This biome covers 750,000 km^2 in South America, with 176,496 km^2 of this area in the southernmost Brazilian state where it occupies 63% of the territory and represents 90% of rangelands of the state (MMA, 2016).

A mosaic of grassland and forest, the Pampa biome has been affected by climate change in past geological times and by the occurrence of natural and anthropic disturbances, such as fire and grazing (Behling, 2002; Overbeck et al., 2007). Geomorphology, hydromorphism, edaphic factors, species origin time on site, wind, and plant dispersal syndromes also contribute to plant distribution patterns in the environment (Rizzini, 1997; Quadros and Pillar, 2002).

Grassland is the predecessor to forest in the Pampa biome (Lindman, 1906; Rambo, 1956; Behling et al., 2005). Once dominant in extensive areas from past periods of cold and dry climates, grasslands have been naturally losing ground to forests due to current climate (Behling, 2002). In this context, the geological and climatic events associated to the floristic centers and flows constitute the foundations on which the proposed model to classify the southern Brazilian primary vegetation is built (Leite, 2002).

Currently, there is great discussion among scientists and society about the effect of the conversion of grasslands to agriculture and forestry, in terms of biodiversity loss (Overbeck et al., 2007, 2013; Roesch et al., 2009; Bond and Parr, 2010; Bolzan et al., 2016), water consumption and soil degradation (Rodrigues et al., 2014; Reichert et al., 2017). Lack of knowledge on the dynamics between grassland and forest contributes to improper conservation and management of grasslands and forests.

Studies of isotopic natural abundance may be used to fill gaps in the

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understanding of vegetation dynamics, to trace patterns and identify physiological mechanisms in organisms; to trace energy flows in food chains; to understand paleodiet; and to establish nutrient cycling paths in terrestrial and aquatic ecosystems (Pereira and Benedito, 2007). In recent years, studies on isotopic natural abundance have often been used to describe the dynamics of soil organic carbon (Balesdent et al., 1987; Desjardins et al., 1994; Ehleringer et al., 2000) or even to reconstruct vegetation changes over time (Boutton et al., 1998; Biedenbender et al., 2004; Pessenda et al., 1998, 2010).

Carbon naturally has two stable isotopes (^{13}C and ^{12}C) and approximately 98.89% of all the carbon present in nature is in the form of ^{12}C and only 1.11% in the form of ^{13}C. The use of ^{13}C as a vegetation change tracker is possible since C3 cycle (or Calvin cycle) plants fix atmospheric CO_2 by means of the Rubisco (ribulosebisphosphate carboxylase/oxygenase) enzyme, while C4 plants utilize the PEP carboxylase (phosphoenolpyruvate carboxylase) enzyme. As the Rubisco enzyme has a lower affinity for CO_2, this enzyme discriminates the heavy isotope of carbon (^{13}C) compared to the light isotope (^{12}C) much more than PEP carboxylase. Thus, the $^{13}C/^{12}C$ ratio (isotope ratio) is not identical in all natural materials, mostly due to isotopic fractionation that takes place during biological, physical and chemical processes (Taiz and Zeiger, 2006).

Thus, C3 cycle plants accumulate less ^{13}C (Farquhar et al., 1989; Taiz and Zeiger, 2006). C3 photosynthetic cycle plants exhibit isotopic values ($\delta^{13}C$) ranging from − 20.0‰ to − 32.0‰, averaging − 27.0‰, while $\delta^{13}C$ values of C4 plant species range from − 9.0‰ to − 17.0‰, averaging − 13.0‰ (Farquhar et al., 1989; Boutton, 1991; Silva et al., 2013).

Since very minor changes occur in the $\delta^{13}C$ signal between plant material and soil organic matter (Martinelli et al., 2009), it is possible to evaluate the change in vegetation through the natural abundance of $\delta^{13}C$ based on the photosynthetic types predominant in the environment. Isotope analyses of soil organic carbon have been used to improve the understanding of vegetation-climate interactions (Guillet et al., 1988; Pessenda et al., 1996; Gouveia et al., 2002).

From the above it is clear C4 species occur naturally in riparian areas of the Pampa Biome and form the native vegetation predominant in these areas, even if climatic changes from the Holocene have favored the development of forest vegetation (C3 species), as previously demonstrated (Pillar et al., 2002; Pillar, 2003; Boldrini, 2009; Behling et al., 2009; Setubal, 2010). Furthermore, analyses of $\delta^{13}C$ in the soil profile can be used to evaluate the contribution of current and past vegetation of riparian areas.

We hypothesized that soil classes, texture, and position in landscape are related to the presence/absence of riparian forest, based on the ^{13}C isotopic abundance in soils from these sites. The objective of this study was to evaluate the natural abundance of ^{13}C in soils profiles at different positions in landscapes under areas of native grassland and forest, in order to associate it with the vegetation dynamics in the Pampa biome (Southern Grasslands) environment.

2. Material and methods

2.1. Study sites

The study was conducted in two forest farms: Cerro do Batovi and Santa Olga, both located in a transition between the Depressão Central and Escudo Rio-grandense regions in the Rio Grande do Sul state, Brazil (Fig. 1). The average altitude of the area is 150 m, and the climate of the region according to the Köppen classification is humid subtropical (Cfa), with average annual temperature of 18 °C and average annual rainfall of 1800 mm (Alvares et al., 2013).

Native vegetation of the Pampa biome is herein named grassland, which refers to a vegetation type predominantly composed of grasses and other herbaceous plants, classified as a steppe in the international phytogeographic system (Bilenca and Miñarro, 2004). The occurrence of forest vegetation in areas of permanent preservation was classified as Broadleaved Forest Seasonal Riverine, of Subtropical Upper plain (Oliveira-Filho, 2009).

The study areas are located in legal reserve (LR) and permanent preservation areas (PPA), as they border a perennial watercourse (Brasil, 2012). Current predominant vegetation is native grassland, but there are also native forest areas in Santa Olga. Therefore, there are three study areas defined by location and land use: Batovi Grassland (BG), Santa Olga Grassland (SOG), and Santa Olga Middle Forest (SOF). Both forest fragments are composed of native vegetation, at a distance of approximately 2830 m from the oldest forest fragment (a likely source of propagules).

The site was composed of typical Pampa biome landscape characterized by mosaics containing grassland and forest fragments bordering watercourses or isolated patches. Therefore, the study area properly represents the characteristics of the biome, except for the southeastern mountainous region and the coastal region, which have distinct geological formation and vegetation formation from the other regions.

From the natural resources survey of the Radam Brazil Project (IBGE, 1975), it is believed that in the last 30 to 40 years the land has been used for livestock. The campestral vegetation present in most of the relief served as natural pasture, while the forests provided shelter and shade to the animals. On the other hand, the campestral and forest vegetation, which were not isolated, suffered disturbances, such as trampling and selective grazing by the animals. Typically, these grasslands support less than one beef-cattle animal unit per hectare. The BG has had no influence of cattle grazing for over a year and six months, SOG and SOF for more than six years (Fig. 2).

2.2. Sampling and soil and vegetation analyses

Soil was sampled in soil profiles (13 in BG, 12 in SOG, and 1 in SOF) in summit, backslope and footslope landscape positions in nine transects (Figs. 3 and 4). The soil profiles were described and soil classified according to the Brazilian System of Soil Classification (EMBRAPA, 2013) and IUSS Working Group (WRB, 2014) (Table 1). The sites for the sampling of the profiles were chosen in toposequences due to the heterogeneity of soils and vegetation, since preliminary observations indicated there is a vegetation-landscape position association in the Pampa biome.

Disturbed soil samples were taken to analyze ^{13}C isotopic abundance in the soil layers of 0–0.05, 0.05–0.10, 0.10–0.20, 0.20–0.30, 0.30–0.40, 0.40–0.60, 0.60–0.80, 0.80–1.00, 1.00–1.20, and 1.20–1.40 m. After collection, soil samples were prepared by manually removing residues and visible roots. Subsequently, samples were dried at 40 °C to constant weight, passed through a 2-mm sieve, and macerated in a ball mill.

Samples of plant material were also prepared from the main tree and herbaceous species occurring respectively in forest and grassland, and plant material from the three landscape positions of each toposequence evaluated in SOG and BG. The samples were collected, stored, washed with distilled water, dried at 60 °C, and finally macerated in a ball mill.

Isotopic $^{13}C/^{12}C$ concentration was determined in a Flash 1112 Advantage autoanalyzer coupled to a Delta V Advantage Isotope Ratio Mass Spectrometer, both from Thermo Scientific. The results of the isotopic analysis were expressed in units of δ (‰), determined in accordance with the international Pee Dee Belemnite (PDB) standard (Faure and Mensing, 2005) as in Eq. (1):

$$\delta^{13}C = \left(\frac{R_{sample} - R_{PDB}}{R_{PDB}}\right) \times 10^3 ‰ \tag{1}$$

where: $\delta^{13}C$ is the isotopic composition; R_{sample} is the isotopic ratio of $^{13}C/^{12}C$; R_{PDB} is the ratio of the international PDB standard.