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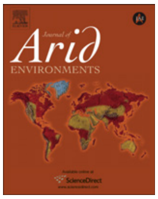

Realistic soil C sink estimate in dry forests of western Argentina based on humic substance content

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ABSTRACT

Due to high temporal variation of soil organic matter in arid regions, estimates of annual sequestered C might be overestimate. We assessed the soil stable organic matter (humic substances) in the transitional area between Dry Chaco and Monte eco-regions in western Argentina, as an approach to estimate realistic soil C sink. Soil samples were taken during wet and dry seasons in four sites along precipitation gradient. In each site three soil cover situations (under tree, under shrubs and on bare soils) were sampled ($n = 5$) and the quantity and type of residues (tree and shrub leaves, woody material, grasses and forbs) were recorded. Soil organic matter and humic substances (humic and fulvic acids) content were analyzed and non-humic substances were calculated by the differences between organic matter and humic substances. Soil humic substance proportion respect to SOM was low (20%) in all sites and it did not correspond with the precipitation gradient. Non-humic substances were lower in wet season indicating high C lability. The most important factors that affected soil humic substance content were the type and quantity of organic residues and soil cover type. Our results suggest that previous C sink estimations in Argentina dry forest probably are overestimated.

1. Introduction

The C in soil organic matter (SOM) is 3.3 times greater than the atmospheric pool and 4.5 times greater than that of the biotic pool (Simpson et al., 2007). The soil C sink results from the balance between organic residues input and CO_2 emission by microbial activity (Abril and Bucher, 2001; Abril and Noé, 2007; Reeder et al., 2001).

Although SOM in arid regions is very scarce, drylands occupy about 43% of the world's land. Accordingly, estimates suggest that the annual C sequestration in the world's drylands would be in the range 1.0–1.3 Gt. (Squires, 1998). However, C dynamics in drylands is complicated by their marked climatic variability, which determines well defined pulses of biological activity (Abril and Bucher, 1999; Abril et al., 2005; Austin et al., 2004). For example, in the western Chaco, Abril and Bucher (2001) found 50% of seasonal SOM variations.

In tropical regions, Leng et al. (2009), reported that due to the high microbial activity during rainfall events, soils could become a source instead a sink of C. In consequence, in arid land it would expected that C sink may be overestimated if SOM data were obtained during dry periods (Almendros et al., 2005; Aranda and Oyonarte, 2006; Satrio et al., 2009). Moreover, SOM in arid regions has high spatial heterogeneity attributed to a differential plant distribution, which generates areas of high deposition of organic residues and high decomposition rate due to the improved environmental conditions (moderate temperatures, higher infiltration, greater water holding capacity, etc.), present beneath the plant canopy (Bisigato et al., 2009; Rietkerk et al., 2004; Rotundo and Aguiar, 2005).

It is widely known that SOM is a heterogeneous substance, which includes a low molecular weight fraction (non-humic compounds) and a high molecular weight fraction (humic substances) (Prentice and Webb, 2010). The non-humic fraction is easier decomposable by microorganisms and it can undergo leaching (Marinari et al., 2010). Contrarily, the humic substances are highly resistant to biodegradation and they are strongly associated to soil mineral phase (Ivanov et al., 2009). Accordingly, the organic C in humic substances realistically reflects C sequestration in the soils (Leng et al., 2009).

Many factors affect the proportion of both SOM fractions, non-humic and humic, particularly the climatic conditions and the quantity and chemical characteristics of organic residues (Abril et al., 2009; Almendros et al., 2005; Kovaleva and Kovalev, 2009). For example, in warm–wet regions, the very fast litter decomposition prevent humus formation, while organic residues with high proportion of lignin and cellulose result in high soil

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humus content (Bahri et al., 2008; Kovaleva and Kovalev, 2009; Lopez et al., 2006).

Moreover, humic substances can be separated into fulvic and humic acids on the basis of their solubility. Fulvic acids are soluble in both alkali and acid, and humic acids are soluble in alkali but precipitate in acid. These fractions differ in molecular size and functional group content, particularly in aromatic group proportion (D'Orazio and Senesi, 2008; Prentice and Webb, 2010). Accordingly, humic acids are more polymerized and aromatized than fulvic acids, and in consequence, the humic/fulvic acids proportion indicate the SOM maturity degree (Aranda and Oyonarte, 2006).

Although a wide literature is available on the transformation of organic residues in humic substances from agricultural perspective (Adani et al., 2006; Brunetti et al., 2007; Poirier et al., 2003; Senesi et al., 2007), scant attention has been devoted on soil humic fraction dynamics from natural ecosystems (Egli et al., 2007; Zancada et al., 2003; Zanelli et al., 2006) and few information is available relative on arid–semiarid regions (Almendros et al., 2005; Aranda and Oyonarte, 2005).

Dry forests cover a large area in Argentina (320 000 km^2). The western boundary of Argentina dry forests has an E-W precipitation gradient, accordingly, vegetation decreases in density and biodiversity (Cabido et al., 1993). Most of the dry forest areas have been cleared or support a high grazing pressure and forest relicts are very scarce (Abril and Bucher, 1999; Abril et al., 2005). In previous studies on soil C balance in this region, high SOM and CO_2 temporal variation was detected (Abril and Noé, 2007; Abril et al., 2005). Therefore, realistic estimate of soil C sink must be reassessed based on stable organic matter. However, data of humic substances are very scarce for the region (Abril et al., 2009).

In consequence, our objectives were to characterize the SOM fractions along a precipitation gradient (500–100 mm) in the transitional area between Dry Chaco and Monte eco-regions in western Argentina, as an approach to estimate realistic soil C sink, and to identify the main factors influencing SOM stability. We assess soil humic substance dynamics in relation to: a) amount of precipitation; b) type and quantity of organic residues; c) soil cover type (tree canopy, shrub canopy and bare soil); and d) seasonal climatic variation.

We hypothesized that due to the litter type and vegetation structure, no humified organic matter proportion is higher in zones with lower precipitations, which lead higher risk of C losses by climatic conditions. In consequence C sink estimates based on total C could be less realistic according to aridity increase.

2. Materials and methods

2.1. Study area

The transitional area between Dry Chaco–Monte eco-regions in western Argentina covers the area between 31°–32° S and 65°–68° W (Fig. 1). The Dry Chaco is a forest whose tree layer is dominated by *Aspidosperma quebracho-blanco* and, to a lesser degree, by *Prosopis* spp., with an abundant shrub layer of *Larrea divaricata*, *Mimozyganthus carinatus* and *Acacia furcatispina,* and presence of grasses (genera: *Trichloris Gouinia, Setaria* and *Pappophorum*) in sites with lowest woody cover. Mean annual rainfall is 500 mm and mean annual temperature is 20 °C. The Monte eco-region is characterized by an extensive shrubland dominated by *Larrea* spp, interspersed with open forest of *Prosopis* spp. The herb layer is composed of grasses, mainly perennial Poaceae C4 species (genera *Pappophorus, Digitaria, Trichloris, Aristida* and *Sporobolus*). Mean annual rainfall and temperature range between 80–350 mm and 13–15.5 °C, respectively (Cabrera, 1976).

Four forest relict sites with similar land use (grazing) were selected from satellite images, based on the difference of reflectance that shows the green vegetation in the visible band (20%) and in the near infrared (60%) (Paruelo and Lauenroth, 1998), on a 500-km transect from Pocho Department (Córdoba province) to Lavalle department (Mendoza province). The selected sites were named according to precipitation annual average as follow: a) 500 mm, El Cadillo (31° 19′ S and 65° 30′ W) **EC**; b) 350 mm, La Ripiera (32° 19′ S and 66° 02′ W) **LR**; c) 200 mm, Quebracho de la Legua (32° 21′ S and 66° 55′ W) **QL**; and d) 100 mm, Telteca (32° 19′ S and 68° 00′ W) **TE** (Fig. 1).

2.2. Sampling design

Our sampling design took into consideration that the analyzed situations were unique in terms of their management history. Therefore, no true replicates were available (Hurlbert, 1984). In each site, two sets of samples were taken: the first during the wet

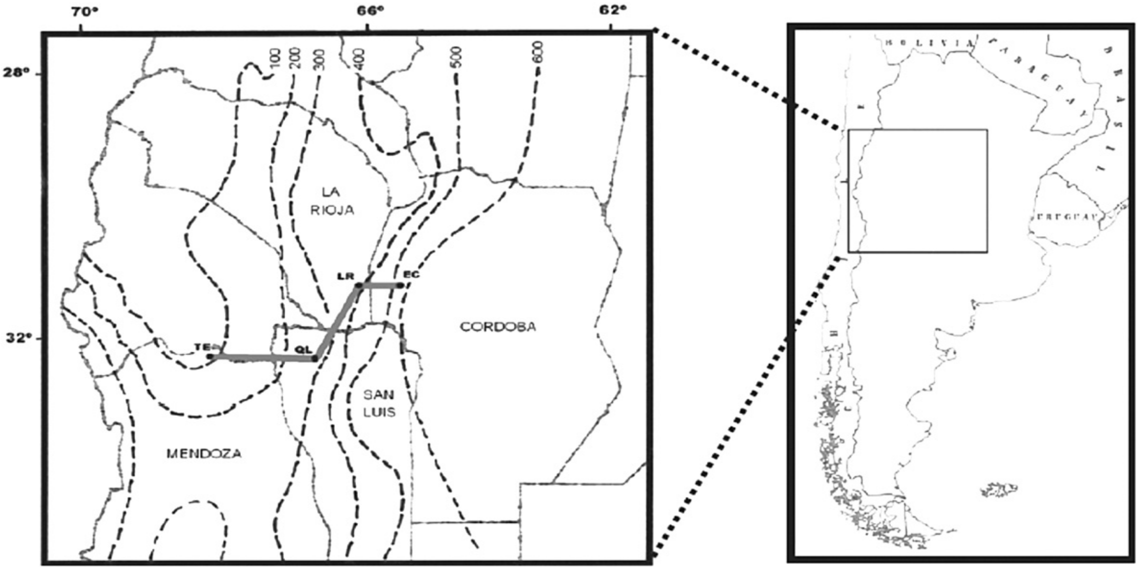

Fig. 1. Location of study sites along precipitation gradient (500 mm–100 mm) between Dry Chaco–Monte eco-regions in western Argentina. EL: El Cadillo, LR: La Ripiera, QL: Quebracho de la Legua, and TE: Telteca.

season (summer, February 2005) and the second during the dry season (winter, September 2005). Special care was taken to sampling the four sites within the same week of each season. Each set consisted of 15 surface litter (0.16 m^2) and soil samples (0–20 cm deep) selected under 5 trees and 5 shrubs and 5 in bare soils, along 200 m linear transect. In each transect (1 m on each side of the transect line) the vegetation composition was recorded and soil cover was estimated as the area of plant vertical projection (trees, shrubs, grasses and forbs), on the soil (Feral et al., 2003).

2.3. Laboratory analysis

Soil samples were air-dried for 24 h, sieved through a 2 mm mesh, and stored at 4 °C until processing. On the first sampling date, soils were characterized according to the following physical variables: soil moisture, texture, conductivity, and pH, using the standard methods recommended by Soil Science Society of America (Klute, 1986). For each soil sample, we measured SOM content by the wet method of Walkley and Black (Nelson and Sommers, 1982) and the humic substances content (HS) by alkali extraction (NaOH). From alkaline extract, the humic (HA) and fulvic acids (FA) were separated by acid precipitation (H_2SO_4) following Jouraiphy et al. (2005). In surface-litter samples, total biomass and litter plant components (woody plant leaves; woody material; grasses and forbs) were measured by dry weight (80 °C). Climate data for each site were obtained from the records of the nearest meteorological stations (range 5–10 km).

2.4. Calculations and statistical analyses

The following calculations were made with soil data: HI: humification index, (HA/SOM); PI: polymerization index (HA/FA), and non-humic substances (NHS) by the difference between SOM and HS (Abril et al., 2009; Marinari et al., 2010). Differences in organic matter fractions among sites and vegetation covers were analyzed using ANOVA. Means were compared using the least significant difference test (LSD) ($P \leq 0.05$). Differences between seasons for each parameter were tested using a paired t test ($P \leq 0.05$).

3. Results

Soils of the four sampling sites were similar in pH, salinity and texture, except for 100 mm site (TE), which exhibited higher alkalinity and lower salinity and 200 mm site (QL), which presented more sandy soils (Table 1). Soil moisture was very low in all sites, although 350 mm (LR) and 100 mm (TE) presented the highest values. Litter mass also differed among sites. Sites with greater tree canopy cover (LR: 350 mm and QL: 200 mm) had the highest litter mass values (Table 1). The 350 mm site (LR) had the greatest woody cover (trees and shrubs) and the lowest proportion of bare soil, whereas the 100 mm site (TE) had the greatest grass cover and bare soil (Table 1). Litter in all sites was mostly composed by leaves and twigs from trees and shrubs, whereas grass material was a scarce litter component in all sites. In 200 mm site (QL) forbs were a significant component, mostly due to the presence of terrestrial bromeliaceae (Fig. 2).

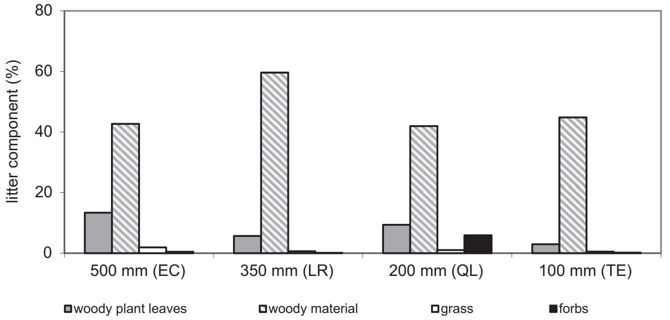

Fig. 2. Litter plant component (%) along precipitation gradient between Dry Chaco–Monte eco-regions in western Argentina. EL: El Cadillo, LR: La Ripiera, QL: Quebracho de la Legua, and TE: Telteca.

The HS proportion respect to SOM content was approximately of the 20%, and it was similar in all sites except in 100 mm site (TE) that presented less proportion (15%). The SOM fractions did not follow a pattern according to the precipitation gradient. All fractions were significantly higher in 350 mm site (LR) and they did not vary in the other sites except in 100 mm site (TE) that presented the lowest values of FA. The humification (HI) and polymerization index (PI) did not significantly vary among sites (Fig. 3). This general pattern among sites was similar in each season, except for NHS and HA which did not show significant differences during wet season ($P = 0.172$ and $P = 0.072$ respectively).

All SOM fractions were higher in soils under tree canopy and lower in bare soils, while the soil under shrub canopy showed intermediate values. However, the HI was higher in bare soils and the PI did not differ among soil cover, although PI tended to higher values in bare soils (Fig. 4). The interaction analysis was significant for site × soil cover ($P = 0.0143$): a) in 200 mm (QL) and 100 mm (TE) sites all the analyzed parameters presented the same general pattern; b) in 350 mm site (LR) only PI showed the general pattern among soil cover; and c) in 500 mm site (EC) only NHS showed the general pattern (Table 2).

Under tree canopy, the SOM fraction values were similar among sites, except the FA content which was higher in 350 mm (LR) and 200 mm (QL) than other sites. Contrarily, all the analyzed parameters presented differences among sites under shrub canopy and bare soil ($P < 0.0001$), except for the HI and PI. Under shrub canopy the highest values in all fractions were detected in 350 mm site

Table 1
Soil characteristics along precipitation gradient between Dry Chaco–Monte eco-regions in western Argentina. Letters indicate significant differences among sites (LSD test, $P \leq 0.05$). EC: El Cadillo; LR: La Ripiera; QL: Quebracho de la Legua; and TE: Telteca.

	500 mm (EC)	350 mm (LR)	200 mm (QL)	100 mm (TE)
Soil moisture	3.80 ± 1.83 b	5.38 ± 2.57 a	2.39 ± 1.22 b	6.94 ± 2.36 a
Conductivity (μS cm^{-1})	534.00 ± 8.74 a	405.67 ± 24.01 a	425.00 ± 213.50 a	187.77 ± 46.71 b
pH	7.54 ± 0.33 b	7.54 ± 0.32 b	7.48 ± 0.31 b	8.73 ± 0.51 a
Texture	Sandy-loam	Sandy-loam	Sandy	Sandy-loam
Organic matter (mg g^{-1})	18.5 ± 2.1 b	24.7 ± 2.0 a	15.3 ± 2.1 bc	13.1 ± 2.9 c
Soil cover (%)				
Canopy trees	8	20	15	11
Canopy shrubs	38	72	51	31
Grass and forbs	12	13	30	45
Bare soil	40	12	15	53
Litter mass (g m^{-2})	385.6 ± 74.9 b	769.1 ± 226.5 a	531.8 ± 116.5 ab	472.1 ± 317.4 b

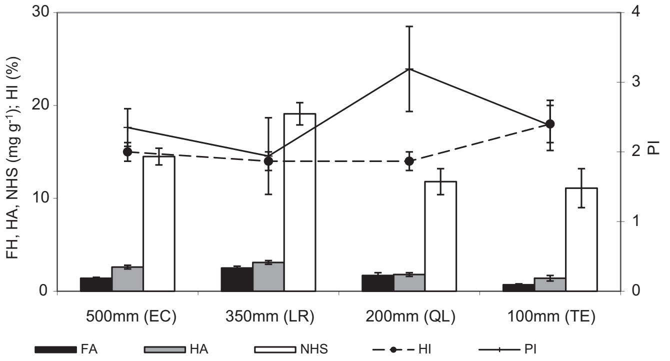

Fig. 3. Soil organic matter fractions (mg g^{-1}), humification index (%) and polymerization index (mean, $n = 30$), along precipitation gradient between Dry Chaco–Monte eco-regions in western Argentina. EL: El Cadillo, LR: La Ripiera, QL: Quebracho de la Legua, and TE: Telteca. FA: fulvic acids; HA: humic acids; NHS: non humic substances; HI: humification index (HA/SOM); PI: polymerization index (HA/FA). Bars indicate SE.

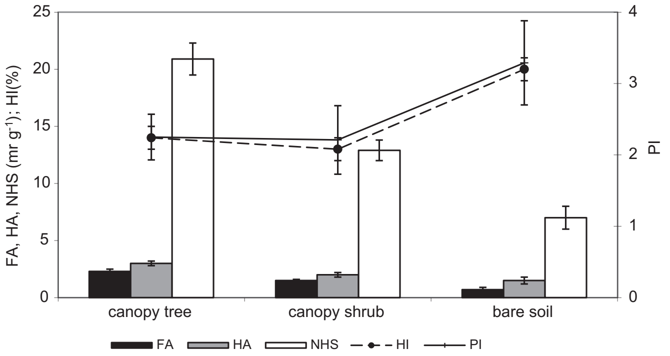

Fig. 4. Soil organic matter fractions (mg g^{-1}), humification index (%) and polymerization index under different soil cover situations (tree canopy, shrub canopy and bare soil) at Dry Chaco–Monte eco-region in western Argentina. FA: fulvic acids; HA: humic acids; NHS: non humic substances; HI: humification index (HA/SOM); PI: polymerization index (HA/FA). Bars indicate SE.

(LR), although NHS were also elevated in 500 mm site (EC). In bare soils the highest values in all analyzed parameters were detected in the two sites with the highest precipitations (EC: 500 mm and LR: 350 mm) (Table 2).

During wet season the NHS content significantly decreased, in consequence the HI increased (Fig. 5). However, the analyses for each site showed that in wet season the NHS decreasing was significant only in 350 mm site (LR) (13.9 vs. 24.1% $P = 0.0001$), while the HI increasing was significant in all sites except in 200 mm (QL).

4. Discussion

SOM stable (HS) values detected in our results (20% of total SOM) are lower than mentioned for other arid–semiarid zones. For example, Aranda and Oyonarte (2005) find 34% of humic substances in a xeric shrubland in Spain (200 mm) and Almendros et al. (2005) find 40% in South African savannas. However, in both places, the polymerization indexes are lower than those for dry forest of the arid western region of Argentina (0.9 and 1.05 respectively vs. 2.46), which indicate that humic substances in dry forests of Argentina have high stability and in consequence low fragility in terms of conservation of the ecosystem (Aranda and Oyonarte, 2005).

4.1. Factors influencing SOM stability

Our results clearly show that the soil humic substances do not correspond with the rainfall gradient. The amount of precipitation (ranges: 100–500 mm) does not seem to be the main factor to regulate the humification processes. Contrarily, the detected differences among sites are defined by vegetation distribution and litter type: the highest HS content (5.7 mg g^{-1}) were detected in 350 mm site, with the highest values of woody cover (92%) and woody litter (62%). Our results agree with Egli et al. (2007) finding that the amount of HA and FA in the soil was dependent on the type and amount of litter. Humification is defined as the transformation of macromorphological and less stable organic compounds into more stable and less biodegradable organic complexes. In consequence, the composition of plant litter greatly influences SOM stability (Kovaleva and Kovalev, 2009; Poirier et al., 2003).

The site with the highest values of all SOM fractions (LR: 350 mm) correspond with the greatest amount of litter (4700 kg ha^{-1}) and the highest tree density and cover (20%), particularly the dominant tree *A. quebracho-blanco* (15%). This type of tree cover contributes with a higher proportion of *A. quebracho-blanco* woody litter (87%) and leaf litter (5.7%). It is well known that

Table 2
Soil organic matter fraction content (mean ± SD) under different soil cover situations (tree canopy, shrub canopy and bare soil) along precipitation gradient between Dry Chaco–Monte eco-regions in western Argentina. Small letters indicate significant differences among sites and capital letters indicate significant differences among soil covers (LSD test, $P \leq 0.05$). EC: El Cadillo; LR: La Ripiera; QL: Quebracho de la Legua; and TE: Telteca. FA: fulvic acids; HA: humic acids; NHS: non humic substances; HI: humification index (HA/SOM); PI: polymerization index (HA/FA).

	500 mm (EC)	350 mm (LR)	200 mm (QL)	100 mm (TE)	*P*
Under trees					
HA (mg g^{-1})	3.0 ± 1.7	3.2 ± 1.8	3.6 ± 2.0 A	2.6 ± 2.5 A	0.5413
FA (mg g^{-1})	1.6 ± 0.7 b	3.3 ± 2.2 a	3.8 ± 2.8 aA	0.9 ± 0.8 bA	<0.0001
NHS (mg g^{-1})	17.0 ± 7.4 A	23.3 ± 7.6	23.1 ± 12.7 A	21.7 ± 18.0 A	0.3041
HI (%)	15 ± 9.8	11 ± 6.6	14 ± 7.4	17 ± 13.6	0.3552
PI	2.11 ± 1.51	1.20 ± 0.76 B	2.73 ± 4.86	2.99 ± 2.36	0.2474
Under shrubs					
HA (mg g^{-1})	2.2 ± 1.4 b	2.9 ± 1.5 a	1.3 ± 0.9 cB	0.8 ± 0.9 cB	<0.0001
FA (mg g^{-1})	1.2 b ± 0.7	2.2 ± 1.2 a	1.1 ± 1.0 bB	0.8 ± 0.8 bA	<0.0001
NHS (mg g^{-1})	13.7 ± 4.3 aAB	17.6 ± 8.8 a	8.9 ± 6.2 bB	7.9 ± 10.3 bB	<0.0001
HI (%)	12 ± 5.1	14 ± 6.8	13 ± 9.4	13 ± 12.6	0.9364
PI	2.64 ± 2.78	1.86 ± 1.93 B	2.78 ± 4.01	1.54 ± 1.59	0.3857
Bare soils					
HA (mg g^{-1})	2.6 ± 1.3 a	3.7 ± 3.01 a	0.8 ± 0.8 bB	0.64 ± 0.7 bB	<0.0001
FA (mg g^{-1})	1.3 ± 0.9 a	2.0 ± 2.2 a	0.3 ± 0.3 bB	0.2 ± 0.2 bB	<0.0001
NHS (mg g^{-1})	11.1 ± 5.1 aB	16.3 ± 12.0 a	4.4 ± 2.4 bB	2.6 ± 4.0 bB	<0.0001
HI (%)	19 ± 13.0	21 ± 19.8	15 ± 16.2	24 ± 19.5	0.5672
PI	2.32 ± 1.40	4.36 ± 6.52 A	4.57 ± 5.09	2.57 ± 3.16	0.4689

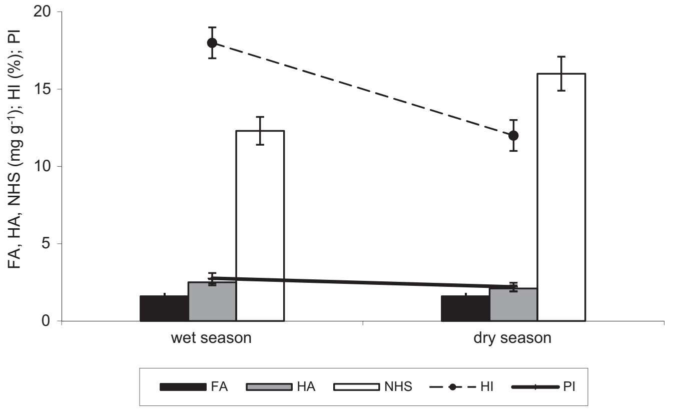

Fig. 5. Seasonal variation of soil organic matter fractions (mg g^{-1}), humification index (%) and polymerization index at Dry Chaco–Monte eco-region in western Argentina. FA: fulvic acids; HA: humic acids; NHS: non humic substances; HI: humification index (HA/SOM); PI: polymerization index (HA/FA). Bars indicate SE.

leaves of *A. quebracho-blanco* are resistant to degradation which results in a more humifiable litter (Torres et al., 2005).

Accordingly, the only site with no presence of *A. quebracho blanco* and with larger bare soil areas (TE: 100 mm) has the lowest values of all SOM fractions. Although in this site, the tree cover (11%) is not the lowest one, the tree cover includes *Prosopis flexuosa* and *Geophroea decorticans* only. It is well known that these leguminous species have small, easily degradable folioles, and in consequence the leaf litter remaining on the floor for short periods.

The site with the highest precipitation records (EC: 500 mm) has lower amount of all SOM fractions than 350 mm site (LR), which corresponds with a lower amount of litter (3856 kg ha^{-1}), tree cover (8%), and *A. quebracho-blanco* density (3%). These data agree with information about the different use of the forest in both sites: there are no records of tree logging in 350 mm site (LR), whereas 500 mm site (EC) has been frequently logged in the last 50 years (Abril et al., 2005).

The trend to highest HI and PI values in bare soils agree with intense sunlight radiation that favors photodegradation process (Austin and Vivanco, 2006), and the warm temperature and dry–wet cycles that improves microbial activity (Huxman et al., 2004), lead to a rapid mineralization of labile fractions with the concomitant survival of the humic fractions most steadily associated to the mineral fraction (Almendros et al., 2005).

The NHS decrease during wet season clearly indicates lability of NHS fraction, due to leaching and/or microbial degradation. It is mentioned that temperate ecosystems have high proportion of recalcitrant compounds within NHS, but in dry forest soils of Argentina, the high NHS lability would result from to scarce litter deposition (due to low primary productivity and high presence of perennials species) and climatic conditions (warm temperature and contrasted dry–wet cycles) which favors the decomposition processes (Abril and Noé, 2007).

The high lability of NHS is more evident in the 350 mm site (LR), because the wet season sampling date was coincident with a 25 mm rainfall, which is well known promotes high leaching of low weight organic molecules and increase of microbial activity (Huxman et al., 2004). The high proportion and lability of NHS fraction agree with the Almendros et al. (2005) who detected an important soluble fraction in soils of South African savannas.

4.2. Realistic C sink

Our results indicate high non-humic substances lability during rainfall periods in agreement with reports in tropical soils (Leng et al., 2009). In consequence, during rainfall periods soil become a source instead a sink of C. Therefore C sequestration information in Argentina dry forest probably is overestimated (Abril and Noé, 2007; Abril et al., 2005). Our prediction was supported by our data because the 100 mm site showed higher no humified organic matter proportion than the other sites (85% vs. 80%), which lead higher risk of C losses by climatic conditions. Therefore, in dry regions C sink estimates could be less realistic according to aridity increase.

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