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ORIGINAL PAPER

Particulate organic carbon is a sensitive indicator of soil degradation related to overgrazing in Patagonian wet and mesic meadows

Andrea Soledad Enriquez · María Victoria Cremona

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Abstract Patagonian meadows are key for the development and sustainability of rural environments. However, they have been degraded due to a combination of weather conditions and overgrazing. Total soil organic carbon (TOC) has been found as a moderate indicator of meadow soil degradation by long-term heavy grazing. The aim of this study was to evaluate the particulate organic carbon (POC) (a more labile fraction of the soil organic matter) as an ecological indicator for assessing soil changes in natural Patagonian meadows, related to different long-term grazing managements. We worked in three locations along a precipitation gradient in Northern Patagonia. Each location has wet and mesic meadows with good and poor grassland condition, attributed to two long-term grazing pressures (light and heavy). We sampled soil throughout the plant-growing season following the natural soil moisture fluctuations, and quantified the POC content, the POC:TOC ratio, and the $F_{>53}$ proportion. Along the different sampling dates, significant differences between good versus poor grassland condition were found in many cases in POC, a few in POC:TOC ratio, and none in $F_{>53}$ proportion. The POC varies over time, in association with changes in the soil moisture (adjusted R^2 vary between 0.76 and 0.99); therefore, the sampling date must be standardized (we recommend dry periods). The POC appears to be a sensitive indicator when assessing the effect of different soil management practices on soil quality, an important step for meadow conservation, restoration and sustainable use, but further research is needed to validate this exploratory study.

Keywords Patagonian wetlands · Long-term use · Indicator · Soil organic matter fractionation · Sustainable management

A. S. Enriquez (✉)
Consejo Nacional de Investigaciones Científicas y Técnicas, Bariloche, Argentina
e-mail: enriquez.andrea@inta.gob.ar

A. S. Enriquez · M. V. Cremona
Instituto Nacional de Tecnología Agropecuaria – EEA Bariloche, Modesta Victoria 4450, 8400 Bariloche, Río Negro, Argentina

Introduction

Patagonian meadows are semiarid wetlands, which stay as key ecosystems for the development and sustainability of rural environments. These meadows develop in bottom valleys along a strong West–East precipitation gradient (Jobbágy et al. 1995). Here, the scarce water from precipitation, runoff and groundwater accumulates and generates seasonally saturated soils. Temporary flooding in meadows promotes a primary production of plants (mostly herbs, grasses and wetland sedges) higher than the typical spatially heterogeneous steppe vegetation around them, and

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concentrates cattle (López et al. 2005; Buono et al. 2010; Irisarri et al. 2012). The overgrazing pressure over the last 100 years (Willis 1914), coupled with hostile weather conditions and a lack of sustainable management practices, have led to the degradation of about 30% of meadows in Patagonia (Del Valle et al. 1998; Chimner et al. 2011).

Ecological indicators can be employed as the base of monitoring programs, which are essential for developing sustainable management plans for any ecosystem. These indicators would not only be able to show early signs of environmental change, but might also be used to diagnose the causes of the change (Dale and Beyeler 2001). Total soil organic carbon (TOC) is often used as an indicator of soil quality (Manley et al. 1995) and to assess disturbances such as tillage (Liu et al. 2016), fire (Gaitán et al. 2007) or grazing (Eldridge et al. 2016). Several studies have demonstrated that soil organic matter (SOM) dynamics in wet areas (under hydromorphic conditions) can be influenced by environmental changes caused by land use and management (de Sousa et al. 2015). Other works specifically have found that overgrazing reduce total SOM (García Martínez 2005). In Patagonian meadows undergoing long-term overgrazing, we have recently found a moderate signal of change in SOM, with a tendency to the loss of total SOM on heavy grazed areas, and with no change in short periods of time (Enriquez et al. 2015).

The TOC cannot always express land use changes (de Figueiredo et al. 2010). The SOM fractionation techniques have been developed to separate the more labile and lighter fractions (particulate organic matter—POM) from the more stable and heavier fractions (Cambardella et al. 2001; Bastida et al. 2008). The POM is the transitory fraction of the SOM in an intermediate state between recent litter and humidified and stable organic matter (Cambardella et al. 2001). It was found that POM can be very sensitive to different practices of land use and soil management (Janzen et al. 1992; Cambardella et al. 2001; Motta et al. 2007). After a SOM fractionation, the POM or the particulate organic carbon (POC) may be used as more sensitive indicators than SOM or TOC, respectively.

The proper management of meadows in Patagonia is fundamental for the long-term sustainability of the grazing system in the region ("green economy", Programme des Nations Unies pour l'environnement 2011). The aim of this study was to evaluate the POC as an ecological indicator for assessing soil changes in natural Patagonian meadows, related to the long-term overgrazing. Soil quality indicators are site-specific and thus must be selected for each soil and management system individually. As Patagonian meadows have highly fluctuating hydroperiods, we first studied POC behavior over time, analyzing whether POC varied among successive sampling dates with different soil moisture conditions. Then, we evaluated the sensitivity of the SOM fractionation technique (POC content, the $F_{>53}$ proportion, and the POC:TOC ratio) to indicate differences between sites with contrasting grassland condition (good vs. poor), attributed to two long-term grazing pressures (light and heavy). As an indicator should be generalizable, we conducted exploratory samplings to analyze whether all above patterns replicated in wet and mesic meadows of three study locations with different mean annual precipitation, where these environments can be found. With above information, we discuss if POC could potentially be used as an overall good indicator of soil quality of Patagonian meadows.

Materials and methods

Study area and experimental design

Three locations were selected along a West–East precipitation gradient in North Patagonia, Argentina, located approximated 100 km away from each other and in different ecological regions (Fig. 1a): (a) Pre-Andes Range (Bariloche, with a mean annual precipitation of 638 mm), (b) Occidental Hills and Plateaus (Pilcaniyeu, with a mean annual precipitation of 269 mm), and (c) Oriental Hills and Plateaus (Jacobacci, with a mean annual precipitation of 170 mm). The mean annual temperature for the three locations is around 8 °C (Bariloche: 8.8 °C, Pilcaniyeu 7.7 °C, and Jacobacci 8.2 °C). Precipitation (rainfall and snow) is concentrated in the autumn–winter months (from May to August), whereas the growing season is dry (spring–summer months, from December to March). Meadow soils are often saturated during winter months and normally dried out throughout the growing season (Chimner et al. 2011).

Meadows in Patagonia show an internal gradient of soil moisture determined by their topography, and can form two different types of meadows: (1) wet

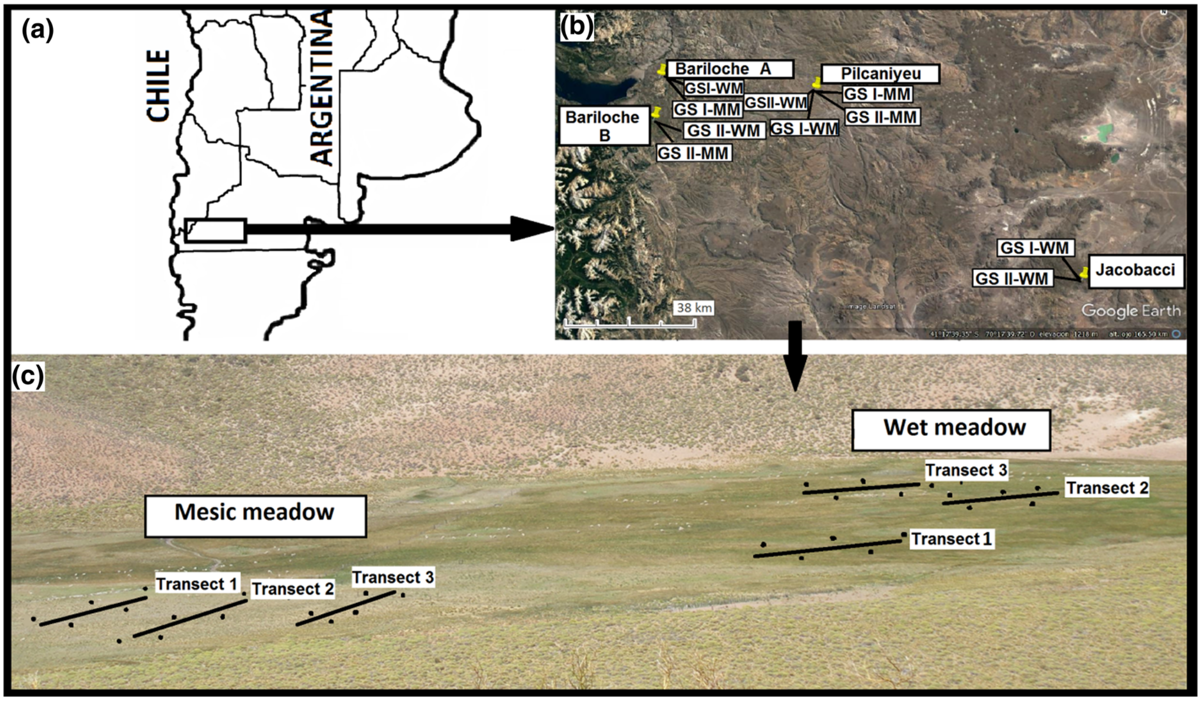

Fig. 1 **a** Selected locations in Southern Argentina, Río Negro province. **b** Google Earth image (2016) showing meadow types (*WM* wet meadow, *MM* mesic meadow) and grazing site levels (*GSI* Grazing Site I-good, *GSII* Grazing Site II-poor). **c** Example of the sampling design for each grazing site level: a soil composite sample consisting of five subsamples randomly collected along three transects. This photograph shows a meadow with good grassland condition at Pilcaniyeu

meadows dominated by *Juncus balticus* in the lower, central and wetter areas, with the presence of saturated soils in the rain season (hydromorphic soils) classified as Histosols (López et al. 2005); and (2) mesic meadows dominated by *Festuca pallescens* in relatively higher, peripheral and dryer areas, classified as Aquic Mollisols (Bran et al. 1998). In Bariloche and Pilcaniyeu locations, both wet and mesic meadows were found and selected for this work. In Jacobacci, the mesic meadow was absent, and its wet meadow soil was classified as Aquic Mollisol (Bran et al. 1998). In this work, we defined 5 study zones, as a result of the combination of each type of meadow by each location (Fig. 1b).

Within each study zone, we characterized grassland condition in different grazing sites, using local Range Plant Community Type Guides (Bonvissuto et al. 2008). These guides are based on quantitative variables such as aerial net primary production, plant cover, and floristic composition (Table 1), as well as on other qualitative variables specific for this project, such as superficial salinization, soil cover or plant vigor. Within each study zone, we contrasted all measured parameters and defined two grazing site levels: Grazing Site I, related to areas with good grassland condition, and Grazing Site II, related to areas with poor grassland condition (Fig. 1).

It is important to mention that there are neither ungrazed areas in the region due to the long-term widespread grazing, nor controlled long-term exclosures or unused fields. Long-term grazing was further confirmed by interviews with land owners; grazing by livestock (mostly sheep) in both grazing levels occurred continuously and season-long (early October to mid-April) for at least the last 20 years. Also, there was no evidence of other disturbances such as fire or widespread erosion at the selected meadows, and all contrasted grazing sites levels shared similar type of soil and similar topographical characteristics (slope < 5–10%), so the experimental units were treated similarly within each study zone.

Inference of this work is intended to apply independently to each study zone. We expected that different combinations of locations and meadow type

Table 1 Classification of meadow-grassland condition based on Bonvissuto et al. (2008): meadow type (wet and mesic meadow); annual aerial net primary production ± standard error (ANPP, g m^{-2} $year^{-1}$); plant cover-abundance scale as (a) 95–100%, and (b) 75–95%, following Daubenmire (1959); main plant species at each grazing site

Grazing site levels(1)	Locations	Meadow type	ANPP	Plant cover (%)	*Agrostis pyrogea*	*Agrostis* sp.	*Alopecurus* sp.	*Azorella trifurcata*	*Boopis gracilis*	*Carex* spp.
I	Bar	Wet	714(103)	a						X
		Mesic	261(18)	a						X
	Pil	Wet	607(44)	a			X			X
		Mesic	222(40)	a	X			X	X	X
	Jac	Wet	300(26)	a		X				X
II	Bar	Wet	169(49)	a						X
		Mesic	37(6)	b						X
	Pil	Wet	93(15)	a						X
		Mesic	74(18)	b	X			X	X	X
	Jac	Wet	118(14)	a			X			

Grazing site levels(1)	Locations	Meadow type	*Deschampsia caespitosa*	*Distichlis* spp.	*Festuca pallescens*	*Hordeum halophyllum*	*Holcus lanatus*	*Hordeum pubiflorum*	*Juncus balticus*	*Nitrophila australis*
I	Bar	Wet	X				X	X	D	
		Mesic			D					
	Pil	Wet					X	X	D	
		Mesic			D	X			X	X
	Jac	Wet		X				X	D	
II	Bar	Wet					X	X	D	
		Mesic			D					
	Pil	Wet					X	X	D	
		Mesic		X	D	X			X	X
	Jac	Wet	X	D			X		D	

Grazing site levels(1)	Locations	Meadow type	*Perennial herbs and shrubs*	*Poa lanuginosa*	*Poa pratensis*	*Pratia repens*	*Puccinelia pusilla*	*Rumex acetosella*	*Taraxacum officinale*	*Trifolium repens*
I	Bar	Wet			X					X
		Mesic			X				X	X
	Pil	Wet			X				X	X
		Mesic				X	X		X	X
	Jac	Wet	X	X			X			

Table 1 continued

Grazing site levels[(1)]	Locations	Meadow type	*Perennial herbs and shrubs*	*Poa lanuginosa*	*Poa pratensis*	*Pratia repens*	*Puccinelia pusilla*	*Rumex acetosella*	*Taraxacum officinale*	*Trifolium repens*
II	Bar	Wet			X					X
		Mesic			X			X	X	X
	Pil	Wet			X					X
		Mesic				X	X		X	X
	Jac	Wet	X	X	X		X		X	X

X present, *S* dominant

[(1)]Grazing Site I, related to areas with good grassland condition, and Grazing Site II, related to areas with poor grassland condition

may have different relationships between the SOM fractions and soil moisture and/or grassland conditions for a variety of reasons (e.g., mean annual precipitation, plant diversity, edaphic conditions, etc.). However, as an ecological indicator should be generalizable, we replicated our exploratory study in the three different locations to evaluate the pattern of response in a variety of situations, where meadows can be found.

Soil sampling

Three composite samples were collected in each grazing site level. Each composite sample consisted of five soil subsamples collected in a zigzag manner along a 20 m line transect, arranged in the direction of meadow drainage (Fig. 1c). Each line transect is considered one experimental unit (n = 3). Subsamples were taken at two soil depth (0–15 cm, and 30–40 cm), where the root density is concentrated (Enriquez et al. 2015). This sampling effort was repeated at four dates throughout the plant-growing season (November 2011 and January, March, and April 2012), selecting the transects randomly in each sampling date. The composite samples from 0 to 15 cm and 30 to 40 cm were separately air-dried and stored until analyzed. The groundwater level was manually recorded from monitoring wells with previously installed PVC pipes.

Laboratory analysis

Immediately after field work, gravimetric soil water content was determined for each subsample, at 105 °C, for 48 h (Sparks et al. 1996), and then they were mixed. Each composite sample (from five subsamples) was sieved through a 2 mm mesh and then a fraction was ground in a porcelain mortar and sieved again (< 0.05 mm). Each 0.05 mm-composite sample was analyzed to determine TOC following the Walkley and Black (1934) method. We performed a physical fractionation of the soil organic matter (SOM) by the particle size technique (soil texture), following Galantini and Suñer (2008) adapted from Cambardella et al. (2001), who proposed to start the fractionation procedure from soil samples sieved through 2 mm. The 2 mm-composite samples were dispersed with hexametaphosphate (0.5%: 5 g $(NaPO_3)n$ L^{-1}) in a 1:3 proportion, stirred for 18 h on a horizontal shaker at 150 rpm, and finally wet sieved with demineralized water on a № 270 sieve (53 µm). The material retained on the sieve with particles between 53 µm and 2 mm was defined as $F_{>53}$, and consisted of organic waste (as well as a sand fraction) associated with the most labile fraction of the SOM. The proportion of $F_{>53}$ (%) was determined with respect to the total soil sample used for fractionation (100%). E.g., initial total soil sample 20 g; Amount of $F_{>53}$: 10 g; Proportion of $F_{>53} = 50\%$. The collected material ($F_{>53}$) was oven-dried at 60 °C for 24–48 h, and subsequently weighed and ground in a porcelain mortar and sieved (< 0.05 mm), to be analyzed for their organic carbon levels as for TOC (Walkley and Black 1934), thereby determining the POC. Dispersed soil fraction with particles smaller than 53 µm was washed and discarded, but it is considered that stable OC to be equal to TOC minus POC. Thereby, $\%\ F_{<53} = 100\% - \%\ F_{>53}$.

Data analysis

To characterize the natural variation of POC along the plant-growing season, we performed an ANOVA for every grazing site in good grassland condition (Grazing Sites I), comparing the POC content as the response variable of the model among sampling dates.

To study the POC as an indicator of meadow-grassland condition throughout the plant-growing season, we performed an ANOVA with a completely randomized factorial model for each study zone (location × type of meadow) and sampling soil depth. In the model, POC was the response variable and we considered two factors: "sampling date" with four levels (November, January, March, and April) and "grazing site" with two levels (Grazing Site I-good, and Grazing Site II-poor). When the interaction was significant, we made the analysis for each sampling date to study the comparison between grazing site levels, as the main factor of interest.

We evaluated the differences of $F_{>53}$ proportion, and of POC:TOC ratio between the two grazing site levels, at each sampling date by using a non-parametric analyses (Kruskal–Wallis) for each study zone, to compare with POC responses.

All above statistical analyses were performed using *SAS V.9* (SAS 2002–2003 SAS Institute Inc., Cary NC, USA).

In order to evaluate in each study zone the relationship between POC content and soil moisture, and the rate of change of these variables throughout the 2011–2012 plant-growing season, we fitted the best but simplest model for each variable (exponential regressions or polynomial regressions of 1st degree), for each soil sample depth, and each meadow type. We used the AIC criteria to select the best model for each case. After this, we compare both models (soil moisture vs. POC) using a global adjustment (Motulsky and Christopoulos 2004) to compare the parameters of each equation and using the adjusted R^2 as a statistical indicator of goodness of fit and the significance of the common selected model (Archontoulis and Miguez 2015), by using Prism4 software (GraphPad, San Diego, CA, USA). When exponential or polynomial I models could not be possible to use, we declare "no fit". All above steps were made for both grazing site levels.

Results

Around 30% of the soil sampled belong to a fraction with particles between 2 mm and 0.053 mm ($F_{>53}$) (Table 2). The POC content was high until 40 cm deep, but decreased with soil depth (Fig. 2). Total organic carbon content (TOC) was always higher than the POC content (Table 3). The same patterns were observed in all study zones, for both grazing site levels and both soil depth, and at every sampling date (Tables 2, 3; Fig. 2).

POC relationship to soil moisture throughout the plant-growing season

The water table level and the soil moisture varied between sampling dates in all study zones and in both grazing site levels (Fig. 3). Soil moisture varied differently in time for each type of meadow (Fig. 3). All patterns of water table and soil moisture variation registered in Grazing Sites I were similar in Grazing Sites II, but lower amplitude of change between November and April was observed in the first ones (Fig. 3).

The ANOVA performed for Grazing Sites I did not show a general pattern of significant increase or decrease in the POC content throughout the 2011–2012 plant-growing season (Fig. 2). Significant

Table 2 Soil fractions proportions (%) of particles larger than 53 µm and less than 2 mm ($F_{>53}$) (± standard error) in the three different locations, meadow types (wet and mesic meadow), and grazing site levels (Grazing Site I and Grazing Site II), by soil depths (cm) and sampling dates (along the 2011–2012 plant growing season)

Location	Soil depth	Grazing site levels	November-2011	January-2012	March-2012	April-2012
Wet meadow						
Bariloche	0–15	Grazing Site I	52(1)	44(3)	42(1)	41(3)
		Grazing Site II	48(1)	42(2)	54(14)	43(1)
	30–40	Grazing Site I	42(1)	39(0)	36(1)	38(1)
		Grazing Site II	42(2)	38(1)	37(3)	35(3)
Pilcaniyeu	0–15	Grazing Site I	42(3)	37(1)	32(4)	43(3)
		Grazing Site II	28(1)	31(3)	21(2)	26(1)
	30–40	Grazing Site I	33(1)	25(2)	25(2)	28(1)
		Grazing Site II	25(1)	28(1)	23(1)	20(4)
Jacobacci	0–15	Grazing Site I	13(2)	13(3)	12(1)	12(1)
		Grazing Site II	11(1)	17(1)	18(6)	13(1)
	30–40	Grazing Site I	28(3)	13(2)	25(7)	11(1)
		Grazing Site II	24(4)	30(2)	31(4)	27(3)
Mesic meadow						
Bariloche	0–15	Grazing Site I	48(1)	39(2)	42(5)	37(2)
		Grazing Site II	46(10)	51(1)	46(0)	46(1)
	30–40	Grazing Site I	40(1)	39(0)	40(1)	37(1)
		Grazing Site II	51(2)	51(1)	47(1)	47(1)
Pilcaniyeu	0–15	Grazing Site I	27(1)	26(3)	18(1)	24(1)
		Grazing Site II	28(1)	32(3)	22(2)	27(2)
	30–40	Grazing Site I	24(2)	21(1)	18(1)	24(1)
		Grazing Site II	26(1)	32(3)	25(0)	29(1)

Different letters show significant differences between the grazing site levels ($p < 0.05$)

differences appeared mostly between extreme dates of the sampled season, and we only found a few significant differences in the POC content between consecutive sampling dates (Fig. 2).

We did not observe a common pattern for the POC content throughout the 2011–2012 plant-growing season among study zones or grazing site levels (Figs. 2, 3). However, the POC in many study zones follow their soil moisture variation, with adjusted R^2 from 0.76 to 0.99 for models of wet meadow, and adjusted R^2 between 0.94 and 0.99 for models of mesic meadow (Table 4). Six fitted models between the patterns of variation of soil moisture and POC were found in Grazing Sites I, and three fitted models were found in Grazing Sites II (Table 4).

Evaluation between grazing site levels

Throughout the plant-growing season, and for most study zones, we found POC values that were significantly higher in Grazing Site I than in Grazing Site II (Fig. 2). The factorial analyses with both "sampling date" and "grazing site level" as factors showed many interactions, with the exception of wet meadow of Bariloche (0–15 cm), Pilcaniyeu (30–40 cm), and Jacobacci. The results of the variation of POC along the plant-growing season for cases without interaction are not shown.

The differences in the POC content between Grazing Site I and Grazing Site II also varied along the sampling dates. In wet meadows, at 0–15 cm, the POC reduction ranged between 16 and 35%, 16 and 80%, and 0 and 42% for Bariloche, Pilcaniyeu, and Jacobacci, respectively; at 30–40 cm, the POC

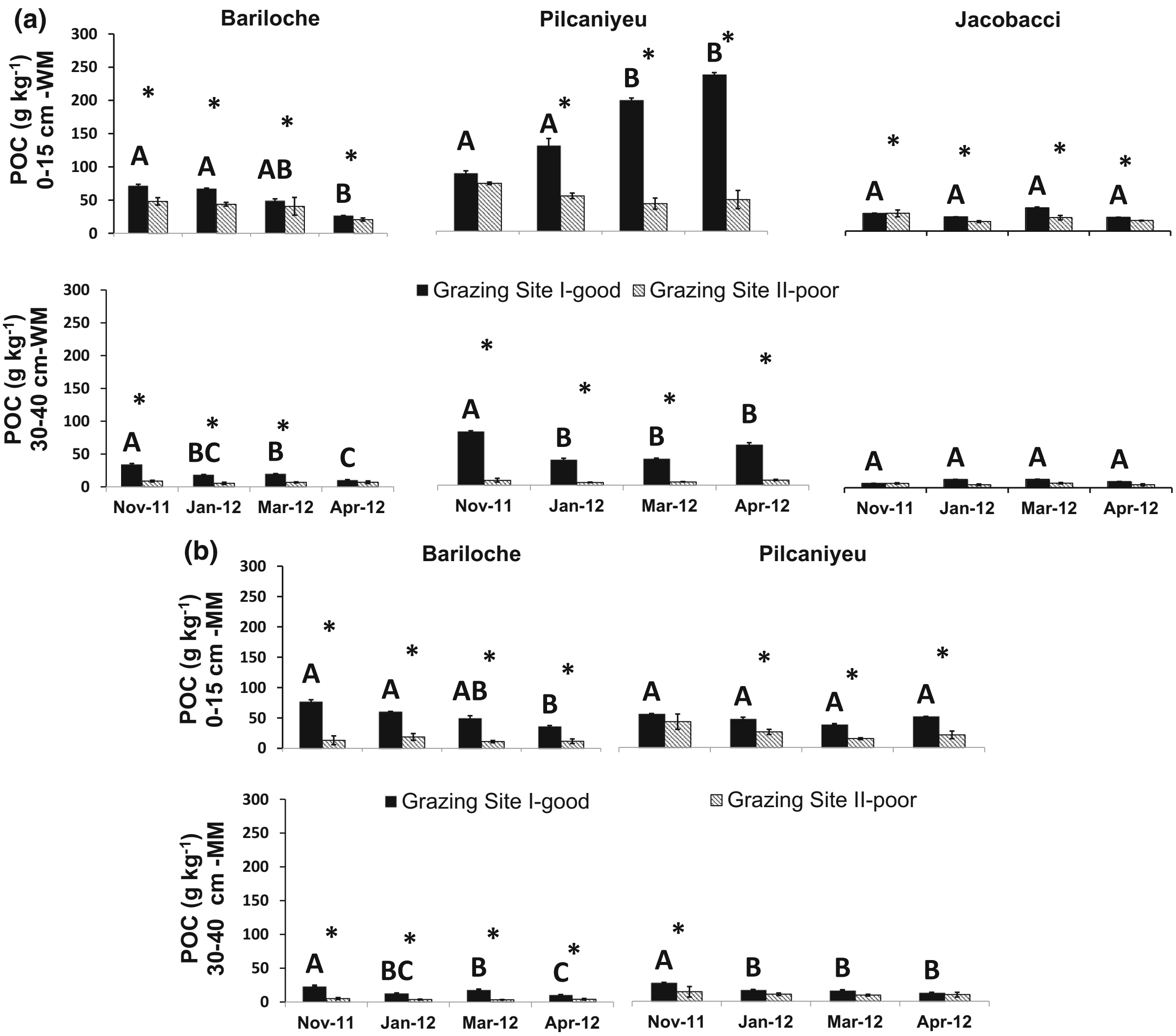

Fig. 2 Variation of the particulate organic carbon (POC) along the 2011–2012 plant-growing season for each location, by soil depths (0–15, and 30–40 cm), and for each meadow type: **a** wet meadows (WM) and **b** mesic meadows (MM). Asterisks show significant differences ($p < 0.05$) in the POC content between Grazing Site I and Grazing Site II. Different letters show significant differences between sampling dates ($p < 0.05$) only for Grazing Site I level (black bars). Bars show standard error

reduction ranged between 32 and 74%, 87 and 91%, and 0 and 53% for Bariloche, Pilcaniyeu, and Jacobacci, respectively. In mesic meadows, at 0–15 cm, the POC reduction ranged between 67 and 83%, and 23 and 62%, for Bariloche, and Pilcaniyeu, respectively; at 30–40 cm, the POC reduction ranged between 62 and 82%, and 26 and 50%, for Bariloche, and Pilcaniyeu, respectively (Fig. 2).

For most of the study zones and for both grazing site levels, the POC:TOC ratios were very similar, with a slight tendency towards higher ratios in Grazing Sites I (0.63 ± 0.16) compared to Grazing Sites II (0.53 ± 0.16) (Table 3). Along the plant-growing season, the POC:TOC ratio did not show as many significant differences between Grazing Site I and Grazing Site II as the POC (13 of 40 against 30 of 40, respectively) (Fig. 2; Table 3). The $F_{>53}$ proportion did not show significant differences between Grazing Site I and Grazing Site II, at any sampling date (Table 2).

Despite the different locations in North Patagonia, the different amount of rain water they receive, and the different amount of TOC in their soils, the POC

Table 3 Average POC:TOC (± standard error) in the three different locations, meadow types (wet and mesic meadow), and grazing site levels (Grazing Site I and Grazing Site II), by soil depths (cm) and sampling dates (along the 2011–2012 plant growing season)

Location	Soil depth	Grazing site level	November-2011	January-2012	March-2012	April-2012
Wet meadow						
Bariloche	0–15	Grazing Site I	0.8(0.2)	0.6(0.1)	0.7(0.2)	0.5(0.1)
		Grazing Site II	0.6(0.1)	0.6(0.1)	0.5(0.1)	0.4(0.1)
	30–40	Grazing Site I	0.6(0.1)a	0.3(0.1)a	0.4(0.0)a	0.3(0.1)
		Grazing Site II	0.2(0.0)b	0.2(0.1)b	0.2(0.0)b	0.2(0.1)
Pilcaniyeu	0–15	Grazing Site I	0.6(0.2)	0.4(0.3)a	1.3(0.1)a	0.9(0.1)
		Grazing Site II	0.8(0.0)	0.8(0.1)b	0.8(0.1)b	0.8(0.2)
	30–40	Grazing Site I	0.7(0.0)	0.6(0.3)	0.9(0.1)a	0.9(0.1)
		Grazing Site II	0.5(0.1)	0.3(0.0)	0.4(0.0)b	0.6(0.1)
Jacobacci	0–15	Grazing Site I	0.5(0.1)	0.8(0.1)	0.6(0.1)	0.8(0.1)
		Grazing Site II	0.3(0.1)	0.9(0.0)	0.7(0.1)	0.8(0.2)
	30–40	Grazing Site I	0.6(0.1)	0.9(0.1)a	0.8(0.1)	0.8(0.1)a
		Grazing Site II	0.7(0.2)	0.5(0.0)b	0.6(0.0)	0.5(0.1)b
Mesic meadow						
Bariloche	0–15	Grazing Site I	0.9(0.1)a	0.7(0.0)a	0.6(0.2)a	0.6(0.1)a
		Grazing Site II	0.4(0.1)b	0.3(0.1)b	0.3(0.0)b	0.3(0.1)b
	30–40	Grazing Site I	0.4(0.1)	0.2(0.1)	0.4(0.1)a	0.3(0.1)
		Grazing Site II	0.3(0.1)	0.2(0.1)	0.2(0.0)b	0.2(0.1)
Pilcaniyeu	0–15	Grazing Site I	0.9(0.0)	0.8(0.2)	0.7(0.1)	0.9(0.1)
		Grazing Site II	0.8(0.1)	0.8(0.1)	0.4(0.1)	0.7(0.2)
	30–40	Grazing Site I	0.6(0.1)	0.5(0.1)	0.5(0.1)	0.5(0.1)
		Grazing Site II	0.4(0.1)	0.6(0.1)	0.5(0.1)	0.5(0.2)

Different letters show significant differences between the grazing site levels ($p < 0.05$)

patterns and trends found were similar in all study zones and in both grazing site levels (Fig. 2; Table 4).

Discussion

This first general evaluation of the partially decomposed organic matter in meadows of three locations of Patagonia showed high contents of POC in relationship to their total organic carbon (TOC) accumulation. A similar pattern was found in "veredas" wetlands from the State of Goias, Brazil (de Sousa et al. 2015). This high POC content might be attributed to the extensive root system and aerial biomass of foraging plants that characterize these meadows (Enriquez et al. 2015), which are also found in other grasslands (e.g., Franzluebbers and Stuedemann 2002; Loss et al. 2013). Labile soil organic matter (SOM) represents a primary source of energy for heterotrophic organisms and a reserve of nutrients (Galantini and Suñer 2008); therefore, these high levels of POC are meaningful for the natural functioning of this soil ecosystem.

In this work, the differences found between the two grazing site levels were mainly related to the long-term grazing activity. Using an ecological perspective, we attributed current good meadow-grassland conditions to the past "light grazing pressure" (long-term grazing below the natural carrying capacity), and current poor meadow-grassland conditions to a "heavy grazing pressure" (long-term grazing above the natural carrying capacity). As we describe above in the methodology section, the attribution of the grazing site levels to the long-term grazing pressures is based on information related to our previous experience, observational descriptions, and historical land use information.

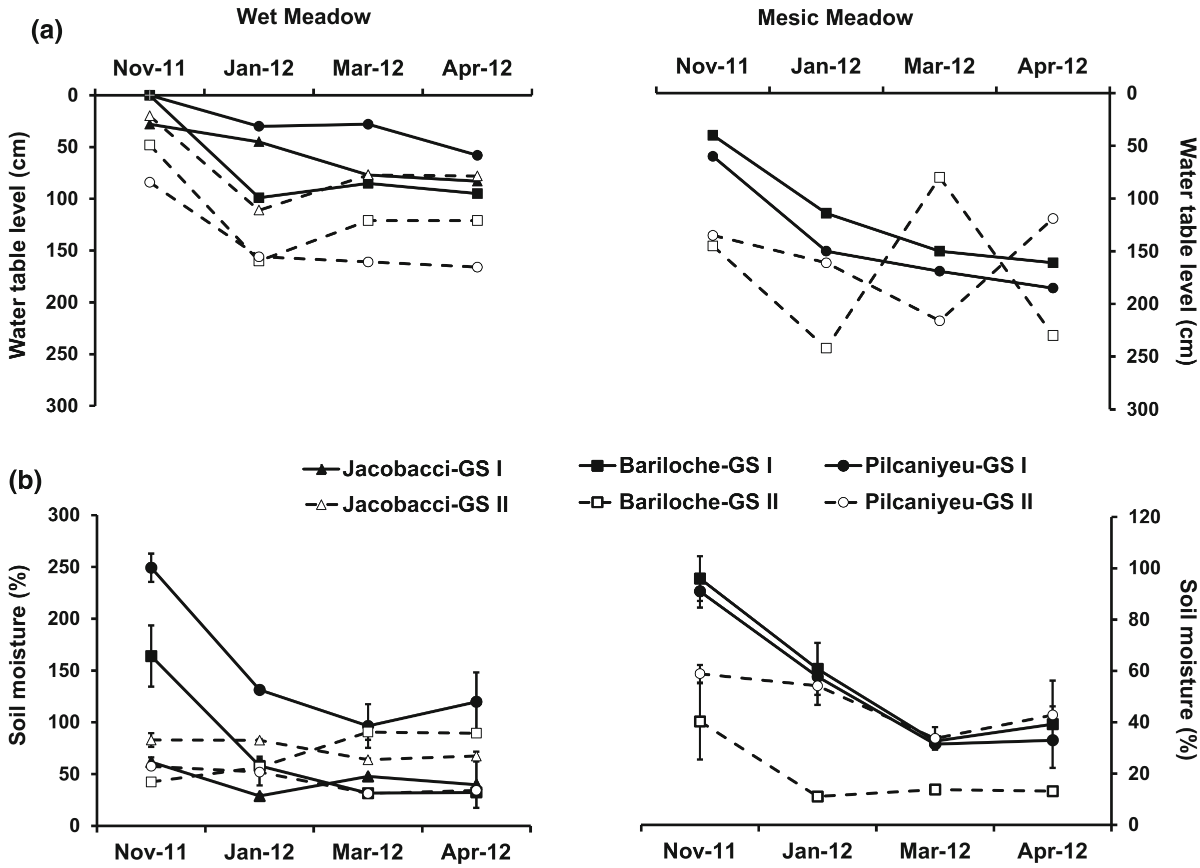

Fig. 3 **a** Water table level and **b** soil moisture at 0–15 cm along the 2011–2012 plant-growing season. Data are from wet meadows and mesic meadows of Bariloche, Pilcaniyeu, and Jacobacci locations, showing the two grazing site levels: Grazing Site I (GS I, solid marker) and Grazing Site II (GS II, empty marker). Bars show standard error for soil moisture (water table values are punctual)

To analyze the stability of POC in our meadows, we studied in the relationship between POC and soil moisture over time. The variations found along the plant-growing season shows that POC is able to vary in short periods of time. The observed trend was a variation of the POC throughout the 2011–2012 plant-growing season, following the soil moisture variation. The POC content varied in time but no common pattern was found for all situations here analyzed. Nevertheless, the variation of POC was reflected in the particular drying-rewetting dynamics of each meadow, as expected. Moreover, as the factors “sampling date” and “grazing site level” interact, POC could not be used as an indicator of changes due to overgrazing at any point of the plant-growing season. Based on these two results, we suggest performing soil samplings during the dry period of the year at standardized dates to help to avoid natural variations that may affect comparison analyses when using POC as an ecological indicator in Patagonian meadows.

The results of this work showed that historical grazing activity seems to have impacted on all meadow types, regardless their location in the West–East precipitation gradient. These results differ from our previous study where TOC was tested (Enriquez et al. 2015) and where not all study zones showed differences between the long-term grazing intensities (grazing site levels). This implies that in our study zones POC highlights degradation processes otherwise masked, and suggests the use of POC as a more sensitive indicator of changes promoted by long-term overgrazing in meadows than TOC. It is important to note that the significant differences found in the POC content between grazing sites levels along the plant-

Table 4 Fitted models for particulate organic carbon (POC) and soil moisture, in the three different locations, meadow types (wet and mesic meadow), and grazing site levels (Grazing Site I and Grazing Site II), by soil depths (cm)

Meadow type	Location	Soil depth	Grazing Site I			Grazing Site II		
			Model	Adjusted R^2	Probability ratio	Model	Adjusted R^2	Probability ratio
Wet meadow	Bariloche	0–15	No fit	–	–	No fit	–	–
		30–40	Exponential (–)	0.99	7.05	No fit	–	–
	Pilcaniyeu	0–15	No fit	–	–	Exponential (–)	0.98	6.69
		30–40	Polynomial I	0.76	3.75	No fit	–	–
	Jacobacci	0–15	Polynomial I	0.81	1.82	No fit	–	–
		30–40	No fit	–	–	No fit	–	–
Mesic meadow	Bariloche	0–15	Exponential (–)	0.94	7.01	No fit	–	–
		30–40	No fit	–	–	No fit	–	–
	Pilcaniyeu	0–15	Exponential (–)	0.99	6.23	Exponential (–)	0.99	6.53
		30–40	Exponential (–)	0.99	6.55	Exponential (–)	0.99	6.68

Models were performed using information of all sampling dates (along the 2011–2012 plant-growing season)

growing season were similar for both 0–15 cm and 15–30 cm soil depth. Sampling just in the first depth will give adequate information if POC is used as a monitoring tool on these meadows, even when overall differences in POC between grassland conditions at 30–40 cm depth were greater than at 0–15 cm.

In general, the study of the SOM fractionation technique was mostly focused on agricultural systems (Cambardella and Elliott 1992; Galantini and Suñer 2008; Aguiar et al. 2013), particularly on tillage (Six et al. 2002), crop rotation (Janzen et al. 1992) or crop regrowth (Koutika et al. 2001). Therefore, it has not been extensively applied in natural ecosystems (e.g., Videla et al. 2008; de Sousa et al. 2015). Videla et al. (2008) examined the use of particulate organic matter (POM) as a potential indicator of changes in soils of the Patagonia steppe under grazing. They have found that POM was more affected by grazing intensity than the total organic matter. In agreement with these findings, we found that long-term heavy grazing in meadows of the three studied locations in January, led to an overall reduction of around 42 ± 14% and 58 ± 16% in POC for wet meadows and mesic meadows, respectively. This pattern matches with the average reduction of 40% of soil TOC and of ~ 30% of the total ecosystem C reservoir (soil + below and aboveground biomass) estimated in the same meadows by Enriquez et al. (2015). The carbon in the lighter fraction of the soil ($F_{>53}$) appears to be sensitive enough to show general trends for the whole ecosystem. However, we did not found differences in the $F_{>53}$ proportion, between Grazing Site I and Grazing Site II. This implies for our studied meadows that long-term overgrazing has led to a reduction in the quality of the soil aggregates between 2 mm and 53 μm (reduction of POM) but not in their structure (no reduction of $F_{>53}$ proportion).

In this work we have found a null signal for $F_{>53}$ proportions, and a light signal for POC:TOC ratios as indicators of differences between Grazing Site I and Grazing Site II. For the studied meadows, the $F_{>53}$ proportions and the POC:TOC ratio were not found as sensitive indicators of changes caused by long-term overgrazing as POC. Particularly, the POC:TOC ratio was most related to the TOC signal than to the POC signal. This is showing a high influence of the stable organic carbon on the TOC behavior, which has indirectly estimated in around 70% of each sampled soil (particles smaller than 53 μm; stable OC = TOC − POC).

If we consider the "Model of states and transitions frames" (Bestelmeyer et al. 2010), the moderate signal found in this work for POC:TOC ratio, and for TOC in Enriquez et al. (2015), indicates that these two parameters can be proposed to be used for assessment of structural features of meadow states. For the assessment of functional features related to transitions (Lopez et al. 2011), the POC seems to be better. The three of them could be used to define a framework for sustainable land use or restoration programs of these grasslands, and provide tools for monitoring.

The pattern of variation of the POC along the plant-growing season (soil moisture-POC) was different in the studied meadows with different grazing site levels. Models for meadow with good grassland conditions due to light grazing intensity (Grazing Sites I) were fitted for almost all cases, while only a few models were fitted for the data coming from poor conditions due to heavy grazing intensity (Grazing Sites II). As POC follows the soil moisture content, which is related with water table levels, this result shows that the POC content might be an indicator of structural land degradation, but also supports our suggestion that it might show functional changes of these ecosystems (variations in wetland hydrology).

The results of this work indicate that POC is a suitable ecological indicator for the Patagonian meadows here studied: it is sensitive to the stress caused by long-term grazing on the meadow systems, and the response to the stress can be modeled and predicted. Also, in these meadows POC changed over time during the plant-growing season with a predictable variability, depending of the grassland condition. Although this is an exploratory study with a design that contemplates the variability of environments where these wetlands can be found and their internal structure, we need to increase the number of sampled meadows in the future to validate this work. Nevertheless, the number and variation of sampling areas with common patterns for the parameters here analyzed, allow us to suggest that POC can be a good indicator of status, thresholds and changes in grazed areas of Patagonian meadows. The implementation of the SOM fractionation technique, specifically the POC content as an ecological indicator in the study of meadows in Patagonia, might improve the way these ecosystems are monitored to maintain their environmental sustainability.

Conclusions

We propose to use the POC as a simple and sensitive indicator to examine the ecological structure and functioning of complex ecological systems as Patagonian meadows. The POC is result of a simple methodological analysis, and has shown to be more sensitive to management practices than TOC, making it a potential ecological indicator of disturbances effects in meadows. Because POC has also responded to small environmental changes as soil moisture variations, we suggest that it may be used as a monitoring parameter of short-term changes in these ecosystems. The POC seems to be a potential indicator to monitor and model effectively and efficiently the effect of different soil management practices on meadow conservation, restoration or sustainable use, although further research is needed to validate this exploratory study.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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