Leaf and culm silicification of Pampas grass (*Cortaderia selloana*) developed on different soils from Pampean region, Argentina

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Abstract. Amorphous silica accumulation in grasses is widely described in numerous species; however, work done in relation to the factors affecting this accumulation in the tissues and different type of cells, is still incipient. Here, we analysed a perennial tussock grass, *Cortaderia selloana* (Schult. & Schult.f.) Asch. & Graebn. (Pampas grass), a native and widely spread species from South America, which is also considered an invasive plant in many regions of the world. We analysed the relation between silicification process, soil type and environment, and anatomical features. Silicophytolith content and distribution in plants was analysed through calcination and staining techniques and SEM-EDAX analyses. Silicophytolith content was higher in leaves (2.48–5.54% DW) than in culms (0.29–0.43% DW) and values were similar to other tussock grasses. A relationship between the habit of a grass species and the potential maximum content of amorphous silica is suggested. Plants grown in soils from modified environments with high silica content (Anthrosol and Tecnosol) produced a higher content of amorphous silica. In leaves, the distribution of silicified cells was conditioned by leaf xeromorphic features. Soil Si content and environmental conditions (radiation, disturbance) are more relevant in silicification process than phenological factors. Within leaves, the accumulation of amorphous silica is conditioned by anatomy and transpiration.

Introduction

Grasses, along with other families such as Equisetaceae, Cyperaceae and Arecaceae, biomineralise abundant quantities of amorphous silica in their tissues, in forms known as silicophytoliths (Hodson *et al*. 2005; Katz 2014; Exley 2015). These deposits can be found in inter- or intracellular spaces, as well as beneath the cuticle forming a cuticle-Si double layer (Sangster *et al*. 2001; Prychid *et al*. 2003). They have a taxonomic importance, especially in grasses, since some specific morphologies can be associated to specific families and subfamilies (e.g. Metcalfe 1960; Twiss 1992; Prychid *et al*. 2003). The accumulation of amorphous silica contribute to the anti-herbivore defence of plants (Massey and Hartley 2006; Massey *et al*. 2007; Reynolds *et al*. 2012; Cooke and Leishman 2012; Soininen *et al*. 2013), prevents fungal attacks (e.g. Ma 2004), affects leaf optical properties and UV-absorbance

(Agarie *et al*. 1996; Schaller *et al*. 2013; Klančnik *et al*. 2014), reinforces the organ structure under specific stresses (Lux *et al*. 2002; Schoelynck *et al*. 2010; Fernández Honaine *et al*. 2013) and ameliorates Al toxicity by the induction of the Si-Al co-deposition (Hodson and Evans 1995; Hodson 2016).

Many studies have dealt with the factors involved in the ability of a species to take up silica from soils, and the relation between uptake and total plant SiO_2 content (e.g. Ma and Takahashi 2002; Ma and Yamaji 2006). Although genetic factors mainly determine silica accumulation in plant species, other environmental factors also influence the silicophytolith production such as water balance, fire, grazing pressure and soil silicon availability, among others (Jones and Handreck 1967; Handreck and Jones 1968; de Melo *et al*. 2010; Cooke and Leishman 2012; Soininen *et al*. 2013; Quigley and Anderson 2014; Katz *et al*. 2015). Particularly, work completed in relation

to the factors that affect the accumulation of amorphous silica in the tissues (i.e. the formation of silicophytoliths) and/or the silicification of the different type of cells, is still incipient (e.g. Motomura *et al*. 2000, 2002, 2004; Sangster *et al*. 2001; de Melo *et al*. 2010; Fernández Honaine and Osterrieth 2012; Fernández Honaine *et al*. 2013). The main results of such studies showed that the deposition of silica in tissues (mainly leaf tissue) depends on leaf side (adaxial or abaxial), organ age and position in the plant and type of cell. The deposition of silica is the result of water loss through transpiration, so those tissue sites that have higher transpiration rates (for example, those more exposed to solar radiation or to higher temperatures) may have higher amounts of silica biomineralisations (Sangster *et al*. 2001; Ma and Takahashi 2002). Also, since silica deposition is not a reversible process, older organs or tissues may be more densely silicified than younger (Motomura *et al*. 2000; Sangster *et al*. 2001; Fernández Honaine *et al*. 2013). However, the effects of these factors can be overlapped and/or be more relevant at different times (Fernández Honaine and Osterrieth 2012).

In Pampean region, where grasslands constitute the main and dominant plant community, some research has been conducted in relation to grass silicification, mainly from a paleobotany and anatomical viewpoint (Zucol 1999; Gallego and Distel 2004; Fernández Honaine *et al*. 2006, 2008). The silicification process and its relation with some environmental and phenological factors have been studied in *Bothriochloa laguroides* (DC.) Herter (Fernández Honaine and Osterrieth 2012). This species is a C_4 panicoid grass, native to America and widely distributed in Pampean plains (Cabrera 1976). Soil silica availability conditions silica accumulation in mature leaves of *B. laguroides*, and silicification of some cell types is related to ageing and type of habitat (Fernández Honaine and Osterrieth 2012). In order to advance the comparative study of silicification processes in pampean grasses, we analysed a perennial tussock grass. *Cortaderia selloana* (Schult. & Schult.f.) Asch. & Graebn. (Pampas grass) is a native grass from South America, but in many regions of the world this grass is considered an invasive plant (Lambrinos 2002; Domènech *et al*. 2005, 2006; Domènech and Vila 2008). Although it is a cosmopolitan species, in Argentina it is usually developed on sandy and wet soils and it is extended on coastal sectors of the Pampean Plains, where it conforms monoespecific communities (Cabrera 1976; Monserrat 2010). Like the rest of the grasses, *C. selloana* produces silicophytoliths in its tissues and organs, and some authors have also described the main morphologies in mature plants (Lanning and Eleuterius 1989; Zucol 1999).

In this work, we analysed if the silicification process in leaves and culms of *C. selloana*, a grass with tussock habit and high plasticity in colonising different environments in pampean soils, is influenced by the same factors that affect the silicification of leaves of *B. laguroides* (a typical and smaller pampean grass). Particularly, we hypothesised that: (i) the accumulation of amorphous silica in *C. selloana* is higher than other pampean grass species due to its tussock habit; (ii) the silica content in the plant varies depending on soil type and/or soil Si content; (iii) the amount and distribution of silicified cells along a single plant vary with the age and position of the organ, and anatomy. In order to test these hypotheses, the following aims were raised: to (1) quantify the silica content (measured as silicophytoliths)

in culms and leaves of *C. selloana* collected from different environments; (2) analyse the relation between soil type and/or soil Si content and plant Si content; and (3) analyse the distribution of silicified cells in relation to anatomy, organ age and position. Finally, we compared the results with previous research.

Materials and methods

Sampling sites and environmental characteristics

The south-east sector of Pampean Plains, Argentina, has a subhumid/humid mesothermal climate with little to no water deficiency, according to the Thornthwaite classification of climates (Burgos and Vidal 1951). The climate is temperate, with mean annual precipitation of 940.6 mm, mean annual temperature of 13.8°C, with a mean of 20°C during the hottest month (January) and a mean of 7.3°C during the coolest month (July) (Servicio Meteorológico Nacional 2010). This region includes geomorphological units such as: the Ranges, the Perirange hills, the Fluvio-eolian Plain and the Coastal Plain (Schnack *et al*. 1982; Osterrieth 1998; Martínez 2001; del Rio *et al*. 2012). The Ranges unit belongs to the Tandilia system and is constituted by a group of table-like hills with a flat top. The Perirange hills are a relief of morphologically complex hills, which were originated from processes of primary eolian accumulation, later modified by superficial wash (Osterrieth and Martínez 1993).The Fluvio-eolian Plain presents a very gentle slope relief. The Coastal Plain comprises a system of beaches and dunes and is characterised by active morphodynamics and different soil types buried by diverse cycles of coastal dunes and estuarine sediments (Osterrieth 1998). The main soils developed in this study area are Argiudolls, Hapludolls, Alfisols and Entisols (Osterrieth *et al*. 2014*b*). The dominant vegetation type are grasslands, and some introduced trees, but in many sectors the agricultural activities have replaced the native communities (Cabrera 1976).

Cortaderia selloana (Schult. & Schult.f.) Asch. & Graebn. plants were collected from different natural and anthropic environments located on SE Pampean Plain: (1) coastal dunes and interdunes (two sites: Querandí and Mar de Cobo sites); (2) quartzite quarry (Batán city); and (3) fluvieolian plain (roadside) (Fig. 1; Table 1).

Querandí site is a mining area located on the Partido of Gral. Madariaga (37°23′20.06″S, 57°5′37.13″W), where sand extraction activity is conducted. Mar de Cobo site is a coastal town located in the Partido of Mar Chiquita (37°45′58.22″S, 57°27′25.04″W). Both Querandí and Mar de Cobo sites are included in the Coastal Plain geomorphological unit (Martínez 2001) (Table 1). Quartzite quarries, Batán city (38°30′S, 57°45′W), are located in the Perirange hills. They constitute anthropogenic diggings 20 m depth on average, made for quartzite extraction. Once this activity finished, incipient soil horizons are developed, named anthropic Regosols (*sensu* IUSS Grupo de Trabajo WRB 2007), characterised by a depth that ranges between some millimetres to more than 10 cm (Osterrieth *et al*. 2012). As a consequence of the mining activities, triturated materials are accumulated into batteries of ~5 m high, where *C. selloana* individuals usually grow. The Fluvieolian Plain is one of the four geomorphological units of the SE sector of Buenos Aires
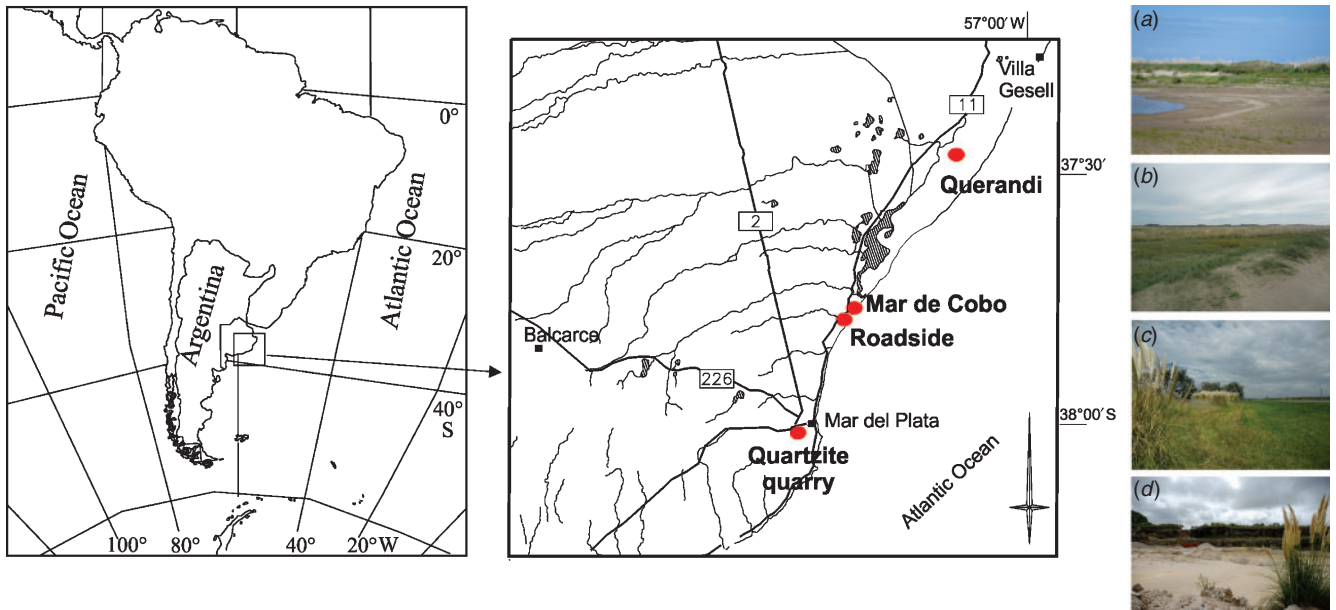
Fig. 1. Location of collection sites (dots) and panoramic views of the environments (*a–d*). (*a*) Querandi site, (*b*) Mar de Cobo dunes and interdunes, (*c*) Roadside, and (*d*) Quartzite quarry. Photographs were provided by the authors (MFH and NB).

province. The plant specimens were collected from roadside (37°47′46.3″S, 57°29′13.1″W), where some *C. selloana* populations are developed, since the rest of the landscape is dominated by crops and pastures (Table 1).

Soil Si content

In each study site, at least three soil samples of the first 0–15 cm were collected. Soil samples (700 g) were air-dried, saturated with distilled water and left to stand for 24 h. Soil saturated paste was obtained by centrifugation at 3000 rpm, filtered and the silica (SiO_2) concentration was determined through UV-VIS spectrophotometry by means of silicomolybdate method (APHA–AWWA–WPFC 1998).

Silicophytolith content in plants

Plants of *C. selloana* were selected from the four study sites on December 2009 (quartzite quarry and Querandi site) and March 2012 (Mar de Cobo site and roadside). In each site, the culms and leaves of 5–8 mature plants in floristic stage were sampled. Silicophytoliths from the different organs were extracted through a calcination technique, which destroys the organic matter, dissolves calcium crystals and releases silicophytoliths (Labouriau 1983). After this treatment, ashes mainly compound by these amorphous silica biomineralisations produced along the plant life were obtained. The content of silicophytoliths (SC) was calculated as the percentage of dry weight (Fernández Honaine *et al.* 2005):

$$SC = \text{silicophytolith content} (\%)$$
$$= (\text{weight of ash obtained after calcination technique/}$$
$$\text{initial weight}) \times 100. \quad (1)$$

In order to analyse the variation in silicophytolith content along a whole plant, a more detailed study was carried out. The

leaf flag and between seven or eight consecutive leaves (below the leaf flag) from the floral culm, and leaves from basal region were sampled in plants from two sites with different soil types (Mar de Cobo and roadside sites). Counting the leaves from the shoot apex downwards, the superior leaf is the first one (younger leaf) and the inferior is the 6th–7th leaf (older leaf). These group of leaves were subjected to calcination technique (see above), and the total content of silicophytoliths of each leaf along the plant and between site collection was obtained.

Silicophytolith distribution in epidermal tissues

Selected leaves were analysed for silica distribution along plant individuals: flag leaf, culm leaves number 1, 3 and 5 (numbered from apex to base of the culm), and basal leaf. The blades were separated from the rest of the leaves and they were divided in three equidistant sections: basal, median and apical. Fragments were cleared with acetic acid and hydrogen peroxide (1 : 1) for 48 h at 60°C, according to Motomura *et al.* (2000), dehydrated in an ethanol series and then stained with phenol crystals (Fernández Honaine and Osterrieth 2012). Phenol crystals were diluted with a minimum quantity of ethyl alcohol before staining the fragments. Phenol crystals stained silica cells with a rose colour (Johansen 1940; Fernández Honaine and Osterrieth 2012). The stained fragments were mounted in immersion oil and observed under Leitz Wetzlar D35780 microscope (Ernest Leitz GMBH Wetzlar) at ×400 magnification. From each slide (which includes 2–3 fragments of a section of a blade) silicified and not silicified epidermal cells in 1.9–2.09 mm^2 were counted and the relative frequencies of each category were calculated.

Some leaves were observed under a scanning electron microscope (SEM) (JEOL JSM-6460 LV) at Universidad Nacional de Mar del Plata, Argentina. Leaf samples were first fixed for 12 h with glutaraldehyde 3% in pH 7.2–7.4 phosphate buffer solution, followed by dehydration through an alcohol

Table 1.   Geomorphological, pedological and botanical characteristics of the sites studied

Study site	Geomor-phological unit (Martínez 2001)	Soil type (IUSS Grupo de Trabajo WRB 2007)	Soils Texture	Organic matter (%)	Phytogeographic area (Soriano *et al.* 1992)	Vegetation Dominant species in the site
Querandi	Coastal plain	Arenosol	Sandy loam (fine to medium sands)	0.6	Flooding Pampa	Poaceae (*Cynodon dactylon* var. *dactylon*, *Cortaderia selloana*, *Digitaria aequiglumis* var. *aequiglumis*, *Panicum gouinii*, among others), Asteraceae (*Baccharis spicata*, *Achyrocline saturoides*) and Cyperaceae (*Androtrichum trigynum*, *Eleocharis bonariensis*, *Schoenoplectus* spp.) (Fernández Montoni *et al.* 2014)
Mar de Cobo	Coastal plain	Arenosol	Sandy (fine sands)	4.97	Flooding Pampa	Poaceae (*Lagurus ovatus*, *Eleusine tristachya*, *Polypogon elongatus*, *Sporobolus indicus*, *Eragrostis* sp.), Asteraceae (*Pluchea sagittalis*, *Ambrosia tenuifolia*) and Apiaceae (*Hydrocotyle bonariensis*) among others
Quarzite quarry	Perirange hills	Technosol: batteries of triturated material (clasts of quartz) of 5 m high	Psefites (61–73%), Psammites (22–33%)	0.38	Southern Pampa	Poaceae (*Dactylis glomerata*, *Jarava plumosa*), Asteraceae (*Senecio pampeanus*, *Baccharis* spp., *Achyrocline satureioides*, *Cynara cardunculus*), Cyperaceae (*Schoenoplectus californicus*) and Juncaceae (*Juncus imbricatus*) (Álvarez *et al.* 2012)
Roadside	Fluvio-eolian Plain	Anthrosol: mix of actual soil, quaternary sediments and material of construction	Silt-clay	4.03	Southern Pampa	*Stipa* and *Piptochaetium* species, mostly replaced by crops

series (50, 70, 80, 90, 95 and 100%). Finally, they were dried in HMDS (hexamethyldisilazane), mounted in aluminium discs and coated with gold. The identification of silicified cells was carried out by X-ray energy dispersive spectroscopy (EDS). The system used was an EDAX Genesis XM4-Sys 60, equipped with multichannel analyser EDAX mod EDAM IV, Sapphire Si (Li) detector and super ultra-thin window of Be, and EDAX Genesis ver. 5.11 software, (Tokyo, Japan).

Data analyses

Differences between silica content in different soil types were tested by Unpaired *t*-test for each pair of sites separately (Zar 1984).

The total mean values of silicophytolith content from leaves and culms of plants collected from different sites were subjected to ANOVA tests. In order to test if there were differences among the leaves of different age and position along an individual plant, and collected in different sites, a two-way ANOVA test

for unbalanced data was applied, where type of leaf and site collection were the factors.

Finally, we analysed if there were differences between the relative frequencies of silicified and not silicified cells through different tests (parametric and non-parametric), depending on the hypothesis evaluated and the assumptions achieved (Zar 1984). Differences between epidermis were evaluated through *t*-tests for paired samples; and between leaf type or leaf section in adaxial and abaxial epidermis through Kruskal–Wallis and ANOVA tests. Lastly, we tested if there were differences in the type of cell that became silicified between type of leaf and leaf section, through two-way ANOVA tests (two factors: type of leaf and leaf section).

Results

Soil Si content

Silica content (SiO_2) in soil solution ranged between 473 and 2015 $\mu mol\,L^{-1}$, with a mean content of 1486 $\mu mol\,L^{-1}$. Entisolls (Querandí and Mar de Cobo sites) were the soils with lower

silica content ($1378 \pm 212 \, \mu mol \, L^{-1}$) compared with Tecnosol (quartzite quarry, $1496 \pm 563 \, \mu mol \, L^{-1}$) and Anthrosol (roadside, $1795 \pm 200 \, \mu mol \, L^{-1}$). Statistically significant differences were observed between Querandí site and Roadside ($F = -3.154$, $P = 0.0195$) (Fig. 2).

Silicophytolith content in plants and distribution in epidermal tissues

Silicophytolith content in culms ranged between 0.29 and 0.43% DW, with a mean of 0.36%, and differences among sites were insignificant (ANOVA $F = 2.25$, $P = 0.11$) (Fig. 2). Comparing the same type of leaf (the two below the leaf flag), the leaf values ranged between 2.48 and 5.54%, with a mean of 4.04%. There were statistically significant differences between leaf values (ANOVA $F = 15.01$; $P = 0.012$), and they were higher at quartzite quarry and roadside sites (Fig. 2).

The silicophytolith content of leaves of different age and position in the plant ranged between 2.3 and 4.17% DW in plants from Mar de Cobo site, and between 4.23 and 5.93% in plants from Roadside site (Table 2). A two way ANOVA test showed that there were differences between sites but not between leaves (Table 3).

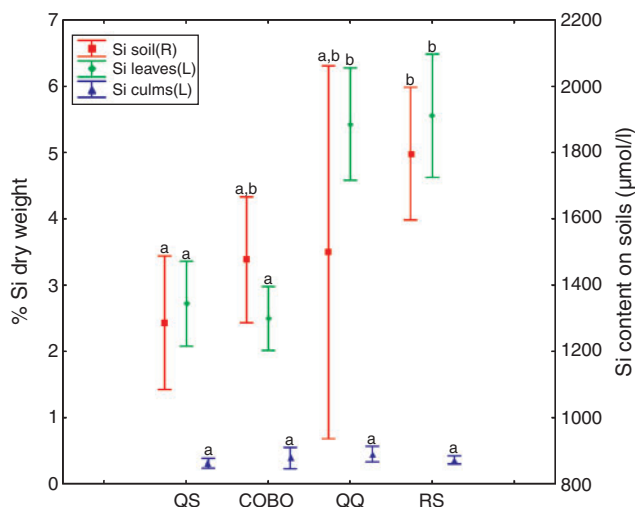Considering all the plants and types of leaf, the total percentage of silicified cells (silicophytoliths) ranged between 20.31 and 30.81% in adaxial epidermis and 23.73 and 31.26% in abaxial epidermis (Table 4). Statistical tests carried out on the data showed that there are no differences in the values between epidermis ($t = 1.71$, $P = 0.1$, *t*-test for paired samples); between leaves, considering a single epidermis ($F = 0.47$, $P = 0.97$ for abaxial; $F = 3.95$, $P = 0.47$ for adaxial, Kruskal–Wallis test); or between different sections along a same leaf ($F = 0.39$, $P = 0.68$ for abaxial, $F = 0.02$, $P = 0.97$ for adaxial, ANOVA tests) (Table 4).

In abaxial epidermis, the main silicified cells are costal and intercostal short cells, and they represent the 62.5 and 23.5% of the total abaxial silicified cells respectively. Two-way ANOVA tests showed that intercostal long cells are silicified in higher proportion in basal leaves ($F = 3.54$, $P = 0.013$, for leaf factor), whereas the rest of the cell types did not show differences between leaf position or section (Fig. 3).

Costal short cells (bilobate morphology) represent the 82.9% of the total adaxial silicified cells. Costal prickles (5.32%), intercostal prickles (4.95%) and costal rectangular short cells (4.73%) complete the type of cells that were silicified. Statistically significant differences (two way ANOVA tests, $P < 0.05$) were observed in the percentages of costal prickles between leaf position (higher in basal leaves), and in the intercostal prickles between leaf sections (higher in apical sectors) (Fig. 3).

Discussion

Silicophytolith content in relation to the habit of the grass

The values of silicophytolith content in leaves (mean value 4.04% DW) and culms (0.36%) of *Cortaderia selloana* range within values commonly described for plant species (0.1–10%) (Jones and Handreck 1967; Epstein 1994; Hodson *et al.* 2005). Silicophytolith contents in *C. selloana* represent the lower limit

Fig. 2. Soil Si content and silicophytolith content in leaves and culms in plants collected in Querandí site (QS), Mar de Cobo site (COBO), quartzite quarry (QQ) and roadside (RS). Values are the mean + s.d. Within each variable, different letters indicate a significant difference among the values ($P < 0.05$).

Table 2. Mean and s.d. of silicophytolith content (% DW) of different leaves collected along a plant, developed in Mar de Cobo and roadside sites

Leaf	Mar de Cobo site		Roadside site	
	Mean	s.d.	Mean	s.d.
Leaf flag	3.99	0.75	5.54	2.29
Leaf 1	2.30	0.61	5.93	1.33
Leaf 2	2.66	0.37	5.16	0.55
Leaf 3	2.76	0.17	5.22	1.14
Leaf 4	3.00	0.48	5.40	1.04
Leaf 5	3.18	0.60	5.60	1.31
Leaf 6	4.17	1.13	5.19	1.33
Basal leaf	4.14	2.44	4.23	1.10

Table 3. Two-way ANOVA test for unbalanced data
Factors analysed were site collection and leaf type. Significant differences are indicated: ***, $P < 0.001$; d.f., degrees of freedom

Factor	Sum of squares	d.f.	Mean square	F	P(F)
Site collection	46.9560218	1	46.9560218	22.0439477	5.14E-05***
Leaves	3.88903404	6.96164384	0.55557629	0.26082053	0.96405586
Interaction site × leaves	11.9837928	6.95529197	1.7119704	0.80370066	0.58990594
Error	66.0333936	31	2.13010947	–	–
Total	128.862242	46	–	–	–

Table 4.   Percentages of silicified cells in the three leaf sections (basal, media and tip) of adaxial and abaxial epidermis of the five leaves analysed, and total percentages of the whole leaves
Values are the mean (s.d.) (*n* = 4)

Type of leaf	Epidermis	Total leaf %	Leaf section Basal %	Media %	Tip %
Leaf flag	Adaxial	20.31 (10.17)	17.61 (9.76)	20.63 (7.82)	22.54 (16.31)
	Abaxial	31.26 (7.54)	34.83 (7.84)	30.04 (8.81)	27.76 (9.14)
Leaf 1	Adaxial	23.66 (10.12)	20.89 (6.73)	24.70 (10.77)	25.21 (13.04)
	Abaxial	28.85 (9.30)	31.45 (11.40)	28.23 (8.15)	25.12 (11.87)
Leaf 3	Adaxial	26.18 (2.08)	30.36 (4.02)	30.68 (9.05)	29.25 (10.64)
	Abaxial	23.73 (9.06)	25.49 (10.08)	28.89 (11.77)	27.56 (8.65)
Leaf 5	Adaxial	27.45 (0.62)	29.13 (2.61)	27.58 (1.88)	25.44 (3.17)
	Abaxial	31.04 (5.30)	26.11 (8.07)	32.53 (5.80)	35.52 (7.17)
Basal leaf	Adaxial	30.81 (5.04)	32.82 (6.52)	30.23 (4.68)	28.77 (7.35)
	Abaxial	29.92 (8.24)	22.23 (9.37)	33.91 (11.14)	35.06 (8.18)

of the range known for other grass species in the pampean region (Gallego *et al.* 2004; Fernández Honaine *et al.* 2008). Also, some previous studies in *C. selloana* from other regions corroborate its low content (considered as % DW) (Lanning and Eleuterius 1989). Research carried on species of the same subfamily showed values higher than the ones obtained here (0.72–7.02% in leaves of *Chionochloa* sp, 4.8% for *Phragmites australis*, 5.17% for *Danthonia montevidensis*) (Marx *et al.* 2004; Fernández Honaine *et al.* 2008; Mercader *et al.* 2010). However, silicophytoliths content of *C. selloana* is comparable to other tussock grasses such as *Paspalum quadrifarium* (mean of 3.98%), *Phragmites australis* (4.8%) and *Sorghastrum pellitum* (3.91%) (Fernández Honaine *et al.* 2008; Mercader *et al.* 2010). It may be likely that tussock grasses or high biomass producer species accumulate low quantities of amorphous silica (measured as percentage of dry weight), so as to avoid an extra mass to the organism per unit volume of structural material (Raven 1983). So, it may be possible that the maximum total content of amorphous silica in a plant responds to physical and structural laws of the specific individuals; and so a relation between total biomass and the potential content of amorphous silica in a species may exist and may be regulated (Schoelynck *et al.* 2010).

Silicophytolith content in relation to soil type and Si soil content

As it was previously reported (e.g. Jones and Handreck 1967; Handreck and Jones 1968; Ma and Takahashi 2002), the Si-rich soils (anthropogenic soils from disturbed sites: Anthrosol in roadside site, and Tecnosol in quartzite quarry site) support the plants with higher silicophytolith content in leaves. In addition to soil characteristics, some environmental features may explain the higher accumulation of amorphous silica in those sites. For instance, the Quartzite quarry site represents anthropogenic diggings of 20 m depth in average and since the floor and the vertical walls (10–20 m high) are made of quartzites, the light reflexion and the temperature inside the site are high. These special characteristics led to a higher transpiration rate of the plants developed there. If silica deposition on tissues is related to transpiration process, a higher silica content in plants grown in these areas can be expected (e.g. Ma and Takahashi 2002; Ma and Yamaji 2006).

C. selloana is considered as an important invasive species in diverse regions (Lambrinos 2002; Domènech *et al.* 2005). In general, the invasion to a specific area is determined by the presence of soil disturbance, which would favour the development of this species (Domènech *et al.* 2006; Domènech and Vila 2008). The invasion of species to a specific region not only modifies the composition and structure of the natural communities, but also has an impact on the dynamics of different element cycles. For instance, the introduction of certain plant species, whose silicophytolith production is high, will affect the influx/efflux of Si/silica in invaded environments, since they will stimulate the transport of Si from deeper horizons and will promote Si efflux (Struyf *et al.* 2007; Schoelynck *et al.* 2014). Accordingly, the introduction and expansion of *C. selloana* in new environments, affects not only to the specific composition of the community (communities become monoespecific), but also may affect the dynamics and rates of influx and efflux of Si/silica on soils. In a direct way, *C. selloana* may increase the silica pool in soils as its silicophytolith production is higher in disturbed sites; whereas in an indirect way, it may affect natural communities attributes (specific composition), modifying the natural silicophytolith input in these environments (Borrelli *et al.* 2010; Osterrieth *et al.* 2014*a*, 2014*b*, 2015).

Distribution of silicified cells and plant anatomy relation

Since silica deposition is continuous along plant life, and until now there has been no evidence of any type of dissolution processes in tissues, it follows that older tissues/organs should have more amorphous silica accumulated than younger ones (Blackman 1968; Ma and Takahashi 2002; Fernández Honaine *et al.* 2013). However, the results obtained here do not match these general rule, since in a same plant of *C. selloana*, the total content of silicophytoliths (calculated as % DW) or silicified cells (calculated as percentage of silicified cells) varied more between sampling sites than between leaf position or age. These findings may indicate that environmental conditions and/or soil type represent a stronger or more influential factor on *C. selloana* silicification process, than the phenological stage or ageing.

Further, considering that silica accumulation occurs as a consequence of transpiration, it is expected that more exposed (to solar radiation) tissues or organs will have a higher content
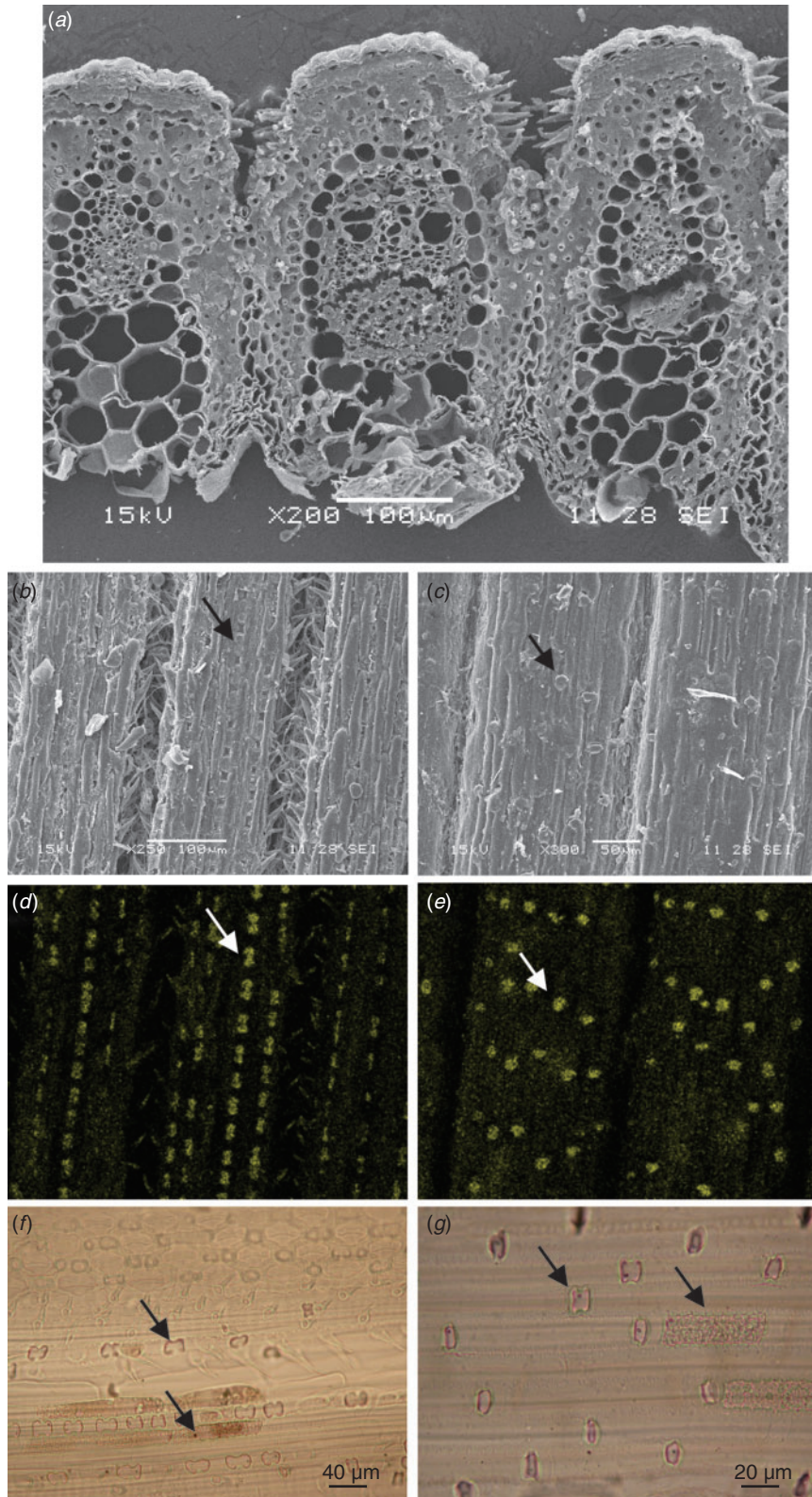
Fig. 3. Photographs taken under scanning electron (*a–e*) and optical microscopes (*f–g*). (*a*) Transversal view of a leaf of *Cortaderia selloana*, (*b*) superficial view of adaxial epidermis of the leaf, (*c*) superficial view of abaxial epidermis of the leaf, (*d*) mapping of Si of the photograph in (*b*), (*e*) mapping of Si of the photograph in (*c*), (*f*) superficial view of silicified cells (stained with phenol) in adaxial epidermis, (*g*) superficial view of silicified cells (stained with phenol) in abaxial epidermis. Arrows indicate silicified cells.

of silicified cells (Ma and Takahashi 2002). However, since the adaxial epidermis (most exposed tissue) of *C. selloana* leaves is characterised by xeromorphic features such as sunken stomata, crypts and hairs, no differences were observed in silicophytolith contents between adaxial (20–30%) and abaxial (27–31%) epidermis (Turner 1994; Dickison 2000; Hassiotou *et al*. 2009). These anatomic characteristics, condition and reduce the water loss by transpiration processes, and therefore silica accumulation is not favoured. As a consequence, leaf anatomy controls silicification process, which is mediated by the water loss of tissues, as it was previously reported (e.g. Ma and Takahashi). Finally, the content of silicified cells obtained in the present study almost doubles the values obtained for other grasses (Motomura *et al*. 2000; Fernández Honaine and Osterrieth 2012). Between 13% and 19% of total cells of the adaxial epidermis of the leaf blades *B. laguroides* was silicified; instead the mean of silicified cells in adaxial epidermis of *Pleioblastus chino* was 11% (Motomura *et al*. 2000; Fernández Honaine and Osterrieth 2012). Differences in abaxial epidermis are also greater: only 8.4% of total cells were silicified in *P. chino* individuals (Motomura *et al*. 2000), compared with 23.73 and 31.26% in *C. selloana*. These results highlight the relevance of silicification process in this species, and the role that this process may have for it; and also shows the important source of silicophytoliths and silicon that these plants represent for the ecosystems.

In grasses , there are two types of cells that became silicified: the short cells that silicify early in the plant development and in a regular way; and other cell types, such as bulliforms, long cells, stomatal complexes and trichomes, whose silicification occurs according to different factors such as phenology, transpiration rate and solar radiation (Blackman 1968; Takeoka *et al*. 1983; Motomura *et al*. 2000, 2002; Fernández Honaine and Osterrieth 2012; Fernández Honaine *et al*. 2013; Fernández Honaine *et al*. 2016). In this study, the silicification of the prickles in adaxial epidermis showed some relation with senescence or solar radiation. Silicified prickles were more abundant in both apical sectors of the leaves (older and more exposed sites) and in basal leaves (more exposed to solar radiation).

In summary, the accumulation of amorphous silica in tissues of *C. selloana*, measured as percentage of DW, is slightly lower than pampean grasses in general, but is comparable to other tussock grasses. The present study suggests some relation between the habit of a grass species and the percentage of silicophytolith content per gram of leaf. In tussock grasses, in order to avoid an extra mass to the organism per unit volume of structural material, the percentage of silicophytolith in leaves is lower than in smaller grasses. Silicification is higher in those plants grown on soils with high silica content and, in this case, developed as a consequence of human activities and/ or disturbance events (Anthrosol and Tecnosol). Also, specific environmental conditions such as a higher radiation (in quartzite quarry) may promote the silicification process. These factors (soil Si content and site conditions) may be more relevant to silicification than organ age or position. However, within each leaf, the anatomy defines the distribution of silicified cells, especially in adaxial epidermis, where xeromorphic features of leaf decrease water loss and prevent amorphous silica accumulation in the cells. Finally, considering that the studied species is a common invasive species in many regions and usually conforms monospecific communities, and that the invasion is favoured by disturbance events and silica accumulation in plants grown in disturbed soils seems to be higher, the introduction and advance of this species in new environments could modify not only community attributes but also the dynamics of certain element cycles, such as Si cycle.

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