

1 **ON THE RELATIONSHIP BETWEEN HOLOCENE ENVIRONMENTAL**  
2 **VARIABILITY AND DIATOM COMPOSITION IN THE PEÑA LAGOON, SE**  
3 **URUGUAY**

4 CUÑA-RODRÍGUEZ CAROLINA<sup>1</sup>, PIOVANO EDUARDO<sup>1</sup>, DEL PUERTO  
5 LAURA<sup>2</sup>, INDA HUGO<sup>2</sup>, GARCÍA-RODRÍGUEZ FELIPE<sup>3</sup>

6 <sup>1</sup> CICTERRA–CONICET and Universidad Nacional de Córdoba, Ciudad Universitaria,  
7 5000 Córdoba, Argentina. ([ccunarodriguez@gmail.com](mailto:ccunarodriguez@gmail.com); [eduardopiovano@unc.edu.ar](mailto:eduardopiovano@unc.edu.ar))

8 <sup>2</sup> Centro Universitario Regional del Este, Universidad de la República, Maldonado and  
9 Rocha, Uruguay. ([lau2phy@yahoo.com](mailto:lau2phy@yahoo.com); [hif@adinet.com.uy](mailto:hif@adinet.com.uy))

10 <sup>3</sup> Departamento de Geociencias, CURE-Rocha, Universidad de la República, Uruguay,  
11 Ruta 9 intersección ruta 15 s/n, Rocha, Uruguay. ([felipegr@fcien.edu.uy](mailto:felipegr@fcien.edu.uy))

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15 Corresponding author: Cuña Rodriguez Carolina ([ccunarodriguez@gmail.com](mailto:ccunarodriguez@gmail.com))

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23 **Abstract**

24 Uruguayan Southern lagoons exhibit high Holocene resolution paleoenvironmental -  
25 paleoclimatic records for inferring long-term regional changes. The multiproxy analysis  
26 of three sediment cores allowed to recognize Holocene climatic variability from the  
27 paleolimnological record of Peña lagoon over the last 2,458 yr BP. Four main stages were  
28 identified throughout the record. The first (2,458 – 1,500 cal yr BP) was characterized as  
29 a shallow meso – eutrophic system with high abundances of aerophilic benthic species  
30 (i.e., *Hantzschia amphioxys*, *Nitzschia brevissima*, *Frustulia* sp., *Luticola goeppertiana*),  
31 epiphytic taxa (i.e. *Epithemia adnata*, *Eunotia* spp., *Rophalodia gibba*) and planktonic  
32 taxa (i.e. *Aulacoseira ambigua* and *A. granulata*). The second stage showed a noticeable  
33 change in the diatom assemblage dominated by fresh-brackish benthic species *Staurosira*  
34 *construens*, but also fluctuations in the abundance of *Aulacoseira ambigua* and *A.*  
35 *granulata*, which indicates the occurrence of temperate to cold and semiarid climatic  
36 conditions, including intervals of high rainfall. The core chronology allowed us to ascribe  
37 this stage to the Little Ice Age (LIA). The third stage, post 390 cal yr BP, showed the  
38 highest proportion of freshwater planktonic species throughout the entire core, thus  
39 indicating the development of a eutrophic system under relatively warm and wet  
40 conditions, which were assigned to the Current Warm Period. After *ca.* 1962 AD, a sharp  
41 increase in the abundance of epiphytic species (i.e., *Cocconeis placentula*, *Eunotia* spp,  
42 *Epithemia adnata* and *Encyonema minutum*) highlights the onset of the fourth stage,  
43 which was characterized by littoral expansion and consequently, the proliferation of  
44 associated macrophytes due to anthropogenic impacts.

45 **Key words:** Diatoms. Holocene. Southeastern Uruguay. Paleoecology

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47 **RELACIÓN ENTRE LA VARIABILIDAD AMBIENTAL HOLOCENA Y**  
48 **COMPOSICIÓN DIATOMOLÓGICA EN LA LAGUNA PEÑA, SE URUGUAY**

49 **Resumen**

50 Las lagunas costeras del sudeste uruguayo son sistemas naturales que exhiben registros  
51 paleoclimáticos y paleoambientales de alta resolución temporal para analizar la  
52 variabilidad ambiental holocena. El análisis “*multi-proxy*” de tres testigos sedimentarios  
53 permitió identificar la variabilidad climática Holocena en la laguna Peña durante los  
54 últimos 2458 años. Se identificaron cuatro estadios, el más antiguo (2458 a 1500 años cal  
55 AP), caracterizando un sistema somero meso – eutrófico, con abundancias relativas altas  
56 de especies bentónicas aerófilas (*Hantzschia amphioxys*, *Nitzschia brevissima*, *Frustulia*  
57 *sp.*, *Luticola goeppertiana*), especies epifíticas (*Epithemia adnata*, *Eunotia spp.*,  
58 *Rophalodia gibba*) y especies planctónicas (*Aulacoseira ambigua* y *A. granulata*). La  
59 segunda fase (1415 - 390 años cal. AP) se identifica por alto contenido de la especie  
60 bentónica dulce acuícola – salobre *Staurosira construens* y fluctuaciones de la  
61 abundancia de *Aulacoseira ambigua* y *A. granulata*, infiriendo condiciones climáticas  
62 templadas - frías y semiáridas con intervalos de precipitación. La cronología sedimentaria  
63 permite relacionar esta fase con la Pequeña Edad de Hielo. La tercera fase (posterior a  
64 390 años cal AP) presenta la mayor proporción de especies dulceacuícolas planctónicas,  
65 indicando el desarrollo de un sistema eutrófico bajo condiciones cálidas y húmedas,  
66 asociadas al Periodo Cálido Actual (PCA). Posterior a los *ca.* 1962 AD, el aumento de  
67 especies epifíticas (*Cocconeis placentula*, *Eunotia spp.*, *Epithemia adnata* y *Encyonema*  
68 *minutum*) infiere un sistema léntico con proliferación de macrófitas y una zona litoral  
69 ampliamente desarrollada, debido al impacto antrópico.

70 Palabras clave: Diatomeas. Holoceno Tardío. Sudeste Uruguayo. Paleoecología

71 URUGUAYAN coastal lagoon systems provide a multiple set of  
72 geomorphological elements as well as sedimentological, geochemical and biological  
73 indicators useful to reconstruct past environmental changes as well as the most recent  
74 anthropogenic impacts (Iriarte, 2006; del Puerto et al., 2011, Inda, 2016). Most of these  
75 lentic systems, developed after the Holocene marine transgression at around 5,500 cal. yr  
76 BP (García-Rodríguez et al., 2001; Bracco et al., 2005; Inda et al., 2006), offer valuable  
77 paleolimnological records for reconstructing climatic and environmental changes  
78 occurred in the region during the Holocene (Bracco et al., 2005; del Puerto et al., 2006,  
79 2011, 2013; Garcia-Rodriguez et al., 2001, 2002a,2002b, 2004a, 2004b, 2004c; Garcia-  
80 Rodriguez & Witkowski, 2003; Inda et al., 2006, 2016). A general overview of the  
81 Holocene climate variability and associated geological processes along the Uruguayan  
82 coastal setting can be found in García-Rodríguez et al., (2011).

83 Previous paleolimnological reconstructions from the Peña Lagoon (Figure 1)  
84 based on the analysis of opal phytolith and isotopic records, highlighted the development  
85 of three climatic stages during the last 2,458 cal yr BP (del Puerto et al., 2013) The first,  
86 spanning from 2,458 cal years BP until 700 AD, was characterized by prevailing  
87 temperate and humid conditions. The second, lasted from 700 AD until 1,200 AD, was  
88 comparatively warmer and wetter and was assigned to the Medieval Warm Period. This  
89 stage was not uniform and included a colder and drier pulse. The third climatic stage  
90 extended from 1,200 AD until the present, and it showed high variability, with three  
91 dry/cold phases reaching their maximums at 1,300, 1,600 and 1,900 AD matching with  
92 the Little Ice Age (González-Rouco et al., 2003; Bracco et al., 2005; Piovano et al., 2009,  
93 Córdoba et al., 2014, Villalba, 1994).

94 In this paper, we explored biological and physical indicators of Holocene climatic  
95 variability from the paleolimnological record of Peña lagoon. Since, multiproxy studies

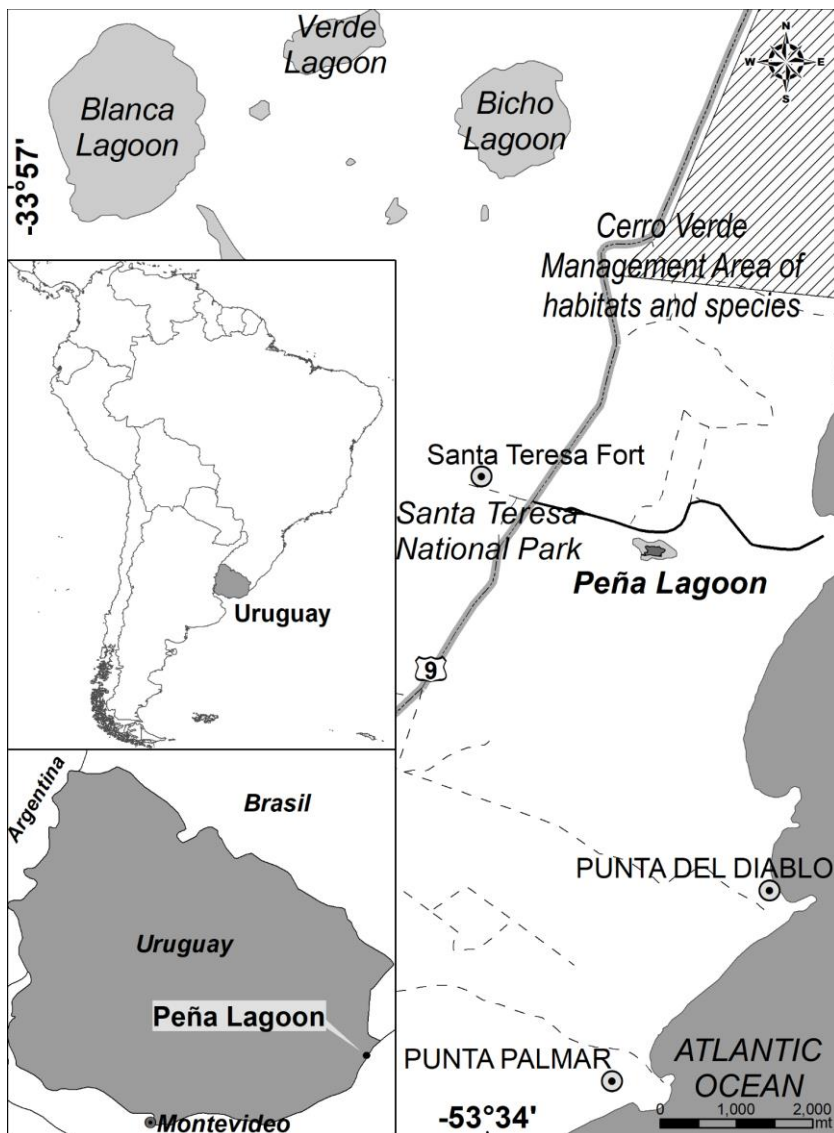
96 provide the means to identify sensitivities, strengths, and weaknesses of different proxies  
97 related to the environmental forcing (Birks & Birks, 2006), the analysis of both diatom  
98 assemblages and facies analysis are included to strengthen previous environmental  
99 reconstructions mostly based on isotopes and the opal phytolith record (del Puerto et al.,  
100 2013). Diatoms are microscopic algae, abundant in almost all aquatic habitats. They are  
101 sensitive organisms, that respond to environmental factors, influencing some water  
102 variables (i.e., pH, salinity, water level fluctuations, and trophic status), representing one  
103 of the biological indicator that have been widely used to paleoenvironmental  
104 reconstructions (Battarbee, 2000; Battarbee et al., 2002; Lamper & Sommer, 2007; Smol,  
105 2008). Similarly, sedimentological features, such as grain size and magnetic  
106 susceptibility, may help to delineate depositional dynamics, clastic, biological, and/or  
107 authigenic sediment sources (Sandgren & Snowball 2002; Ver Straeten et al., 2011).  
108 Moreover, the study of phytoliths assemblages allow to infer the paleovegetation in the  
109 local area reflecting climatic and environmental characteristics (Fredlund & Tieszen,  
110 1994; Lu & Liu, 2003a, b). Although this proxy present taphonomic problems it does not  
111 always reflect the original plant communities precisely (Lu et al., 2006).

112 Our results showed that the combined analyses of biological indicators with data  
113 derived from stable isotopic composition of organic matter, geochemistry, and physical  
114 proxies (i.e., magnetic susceptibility, sedimentary facies, among others) allow to infer  
115 past lake-catchment changes related to climate change during the 2,458 yr BP. Finally,  
116 this paper provides new results to be included in the general framework of the MATES  
117 Program (Multiproxy Approach for Tracking Environmental changes in Southern South  
118 America) which aims to integrate paleoclimate research across Argentina and Uruguay.

## 119 **METHODS**

### 120 *Study site and climate setting*

121 Peña lagoon is a freshwater lagoon (34°00'13'' S - 53° 33'10'' W), located in a  
122 narrow sedimentary fringe called “La Angostura”, situated between the Atlantic Ocean  
123 and the Negra Lagoon (Fig. 1). The catchment and lagoon area are 0.5 Km<sup>2</sup> and *ca.* 0.05  
124 Km<sup>2</sup> respectively. The maximum water depth is 1.8 m (del Puerto et al., 2013). The lagoon  
125 is located in Santa Teresa National Park, which has been drastically modified during the  
126 20<sup>th</sup> Century. More information about the site setting and vegetation can be found in del  
127 Puerto et al., (2013).



128  
129 **Figure 1.** Geographical location of Peña Lagoon. Gray point represents coring stations.

130           Peña Lagoon is part of a group of small marginal water bodies located on the 10  
131 – 20 m a.s.l. contour lines of the Uruguayan coast (Kruk et al., 2006). The topography  
132 indicates that, in contrast to the major coastal lagoons, this aquatic system was originated  
133 as the result of fluvial damming by movement of the sand dunes (Bracco et al., 2011).

134           The study region is located along the boundary between subtropical and temperate  
135 regions of Southeastern South America (Cervený, 1998). South America regional climate  
136 distribution is defined by continental north-to-south variations, east-west asymmetries  
137 (given by the presence of the Andes), land mass shape and the boundary conditions  
138 imposed by a cold southeastern Pacific and a warm southwestern Atlantic (Garreaud et  
139 al., 2009). Extending Eastward the Andes and covering a vast lowland area from  
140 Colombia and Venezuela up to the Argentinean Pampas in the south, it is the most  
141 outstanding geographical feature that provides a unique environment for the development  
142 of a Monsoon-like circulation (Zhou and Lau, 1998; Vera et al., 2006). Summertime  
143 climate in Southeastern South America is linked to the South Atlantic Convergence Zone  
144 (SACZ) in the form of a rainfall seesaw: increased rains in the SACZ are correlated with  
145 decreased rainfall in SESA (Doyle and Barros 2002). The SACZ, in turn, may be forced  
146 by South Atlantic SST anomalies (Barreiro et al. 2002, 2005). The wind and water mass  
147 regime are controlled by the interaction between the tropical anticyclone of the South  
148 Atlantic and the migratory polar anticyclone (Fonzar, 1994).

149           In the study area, the Atlantic influence causes moderate daily and annual thermal  
150 amplitude with high levels of relative humidity. Mean temperature is 17 °C and mean  
151 historical total annual precipitation is 1200 mm (PROBIDES, 1999; IBERSIS, 2001).  
152 Interannual climate variability is influenced by El Niño Southern Oscillation (ENSO). El  
153 Niño episodes are mostly associated with anomalously wet conditions while drought  
154 anomalies are observed during La Niña events. However, ENSO at a regional scale

155 exhibits significant seasonal fluctuations, such as impacts on rainfall, which show  
156 considerable variability during the 20th Century. Decadal and interdecadal variability are  
157 possibly forced by the Pacific Decadal Oscillation (PDO) and the Antarctic Oscillation  
158 (AAO) over South America (Barreiro and Tippman, 2008; Garreaud et al., 2009).

### 159 *Core collection, sampling and previous analysis*

160 Cores LP1 and LP2 (95 and 156 cm long respectively) were taken in 2010 using  
161 a piston corer. Both cores were retrieved very closely, thus, a composite core LP1-LP2  
162 can be considered. A third core LP3 (106 cm long) was collected in 2014 with the same  
163 methodology used in 2010. The sampling sites, of the cores are shown in Fig 1. The  
164 opening procedure, dating, geochemistry, organic matter and isotopes analysis for the  
165 core LP1 - LP2 are described in detail by del Puerto et al. (2013). Samples for diatoms  
166 and grain size analysis were taken every 2 cm in the LP1 core (0 -95 cm) and in the basal  
167 part of the LP2 core (95 – 156 cm), both cores represent the entire record of Peña Lagoon  
168 of 156 cm long. The sedimentological description was performed on core LP3. Core  
169 correlation between LP3 and both LP1 - LP2 was established through the inspection of  
170 sedimentological features such as sedimentary structures, magnetic susceptibility core  
171 profiles, grain size values and content of sedimentary organic matter.

### 172 *Sedimentological analysis: grain size, magnetic susceptibility measurement*

173 The sediment grain-size was measured in samples from the LP1 and the LP2 cores  
174 using a laser diffraction grain size analyzer (HORIBA LA-950; Centro de Investigaciones  
175 en Ciencias de la Tierra (CICTERRA). Samples were pretreated with 20 mL of 30% H<sub>2</sub>O<sub>2</sub>  
176 to eliminate the organic matter, and with 20 mL HCl (10%) to remove carbonates. Finally,  
177 samples were rinsed with deionized water and dispersed in 10 mL of (NaPO<sub>3</sub>)<sub>6</sub> solution  
178 to prevent particles from aggregating. Grain size data were analyzed using the statistical  
179 program GRADISTAT 8.0. Sediment description was performed according to



180 Schnurrenberger et al. (2003). The Munsell chart was utilized to characterize sediment  
181 color. The volume specific magnetic susceptibility ( $\kappa$ ) of sediments was measured on the  
182 surface of the split half core at 1 cm intervals with a Bartington F-sensor. Values are given  
183 in  $10^{-6}$  SI (dimensionless). Sedimentary core LP3 was inspected through XR radiograph  
184 in the Department of Image at the Universidad Nacional de Cordoba Argentina (UNC) to  
185 further identify sedimentary structures.

### 186 *Diatom analysis*

187 Samples for diatom analyses ( $n = 39$ ) were pre-treated with  $H_2O_2$  for organic  
188 matter removal and with HCl for carbonate removal as indicated in Metzeltin and Garcia-  
189 Rodriguez (2003). Permanent microscope slides were mounted using Entellan resin  
190 (Refractive Index: 1.54).

191 Slides were inspected at 1000x magnification with oil immersion using an  
192 Olympus BX53 light microscope. A minimum of 400 diatom valves was counted in each  
193 slide along randomly selected transects according to Battarbee *et al.* (2002). The relative  
194 abundances of individual species were calculated by dividing the number of valves from  
195 each species by the total number of valves counted on each slide. Diatoms were identified  
196 to species level using the appropriate keys (Metzeltin et al., 2005, Metzeltin and García-  
197 Rodríguez 2003, Krammer and Lange-Bertalot 1986, 1988, 1991a, 1991b; Frenguelli,  
198 1941, 1945; Round, 1990; ANSP Algae Image Database). Ecological information of  
199 diatom taxa preferences (i.e trophic status, moisture and salinity) was extracted from  
200 Round et al. (1990), Denys (1991), Van Dam et al. (1994), Rühland et al. (2003), Hassan  
201 (2010) and Solak et al. (2012).

202 The vertical distribution of the most abundant diatoms (*i.e.* those species with  
203 relative abundance higher than 3% in at least three intervals) was plotted against core

204 depth using C2 software (Juggins, 2005). Diatom zones were determined using  
 205 constrained cluster analysis (CONISS) using the software Tilia v. 2.0.38.

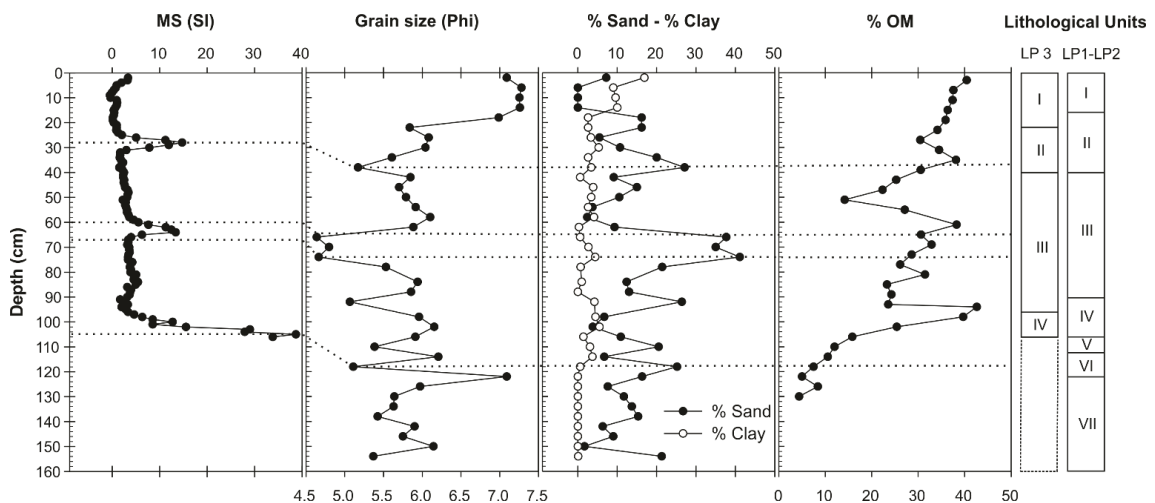
206 **Geochemistry**

207 Isotopic composition of organic matter ( $\delta^{13}\text{C}$ ) as well as C/N ratios were used to  
 208 infer the sedimentary organic matter source/composition in Peña Lagoon. Data were  
 209 taken from del Puerto et al. (2013). The stable carbon isotope composition ( $\delta^{13}\text{C}$ ) and the  
 210 ratio Carbon-Nitrogen (C/N) can be employed to assess the origin and composition of  
 211 sedimentary organic matter (Lamb et al., 2006).

212 **RESULTS**

213 **Sedimentology and geochemistry**

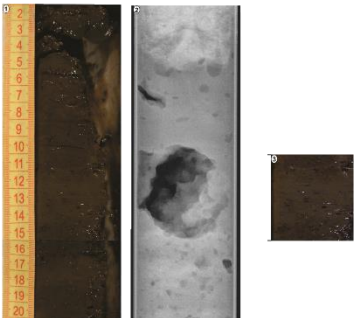
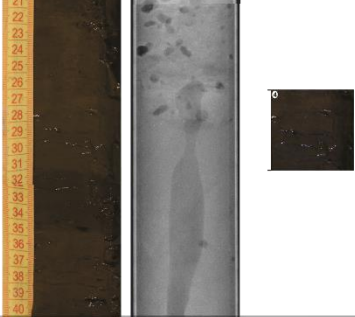

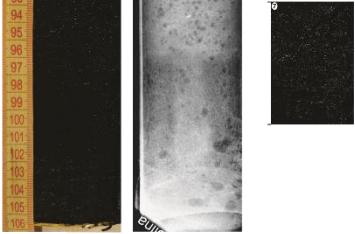
214 With the aim of establishing a stratigraphic correlation between cores LP3 and the  
 215 composite core LP1 - LP2, we compared magnetic susceptibility values throughout core  
 216 LP3 to grain size variations of core LP1 - LP2. Since high MS values observed in core  
 217 LP3 match with coarser sediments in LP1 and LP2 (Fig.2) both variables were  
 218 simultaneously used as stratigraphic markers for core correlation. Core LP3 showed a  
 219 uniform pattern of magnetic susceptibility (average 4.7 SI) that was interrupted by distinct  
 220 shifts to higher peak values (maximum 38.7 SI) at 19, 62 and 104 cm depth.



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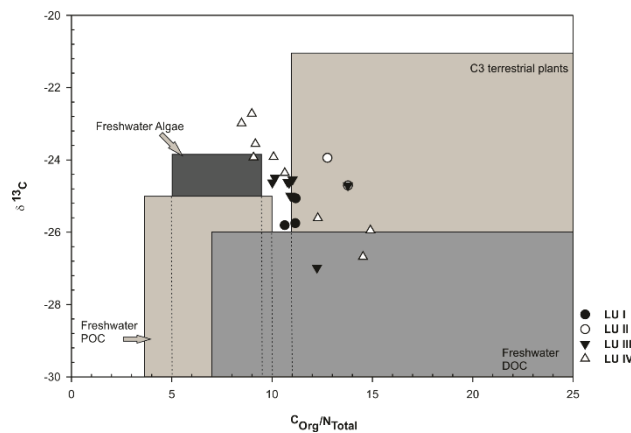
222 **Figure 2.** Correlation of the core LP3 with the LP1 - LP2 composite core. Dotted lines  
223 indicates the stratigraphic correlation between magnetic susceptibility (MS) of the LP3  
224 core with grain size, percentage of sand - clay and percentage of sedimentary organic  
225 matter measured in the LP1 - LP2 composite core (Published data by del Puerto et al.,  
226 2013 and provided by the authors). The right side plot shows the correspondence of  
227 lithological units of the LP3 core with those identified by del Puerto et al (2013). Based  
228 on physical data, LU V, VI and VII from core LP1 - LP2 were considered within LU IV  
229 identified in LP3.

230           The core LP3 consisted of massive to banded and laminated, dark-gray – black,  
231 sandy-silty muds, with abundant fibrous plant remains. Based on the sedimentological  
232 features, magnetic susceptibility, grain size, OM content and sediment color (Fig. 2 and  
233 3), the sedimentary record was subdivided into four lithological units (LU): LU IV (106  
234 –93 cm): Massive Sandy Muds; LU III (93 – 41 cm): Banded organic-rich Sandy Muds;  
235 LU II (41 – 20 cm): Massive Sandy Muds and LU I (20 – 0 cm): Banded Medium Silt  
236 Muds. A summary of lithological units characteristics, photographs and XR radiographs  
237 is presented in Figure 3.

Photographs, XR radiographs (negative) and LU of LP3	Lithological Unit Description
	<p><b>LU_I Banded Medium Silt Muds</b></p> <p>Dark grey medium silt mud (10YR 4/1)  Organic matter 35.9 - 40%  Magnetic susceptibility (SI) -0.5 - 3.3  Grain size (Phi) 7.0 - 7.3</p>
	<p><b>LU_II Massive sandy Muds</b></p> <p>Dark grey sandy mud (10YR 4/1)  Organic matter 25.3 - 38%  Magnetic susceptibility (SI) 0.9 - 14.7  Grain size (Phi) 5.2 - 6.1</p>
	<p><b>LU_III Banded organic rich Sandy Muds</b></p> <p>Dark grey sandy mud (10YR 4/1) fining upward with plant remains  Organic matter 14 - 38%  Magnetic susceptibility (SI) 1.7 - 13.4  Grain size (Phi) 4.7 - 6.1</p>
	<p><b>LU_IV Massive Sandy Muds</b></p> <p>Black coarse to fine sandy medium silt (10YR 2/1)  Organic matter 15.8 - 42%  Magnetic susceptibility (SI) 1.9 - 38.7  Grain size (Phi) 5.1 - 7.1</p>

239 **Figure 3.** Description of LP3 lithological units: (1) Photograph of the sedimentary record  
 240 LP3 (106 cm) and (2) corresponding X ray image, (3) banded zone of LU I (10 – 15 cm),  
 241 (4) massive sandy mud of LU II (26 – 31 cm), (5) fibrous plants remains zone of LU III  
 242 (48 – 57 cm), (6) sandy sediments present in LU III (84 – 90 cm) and (g) black sandy  
 243 mud zone of LU IV (93 – 100 cm).

244 The relationship between  $\delta^{13}\text{C}$  and C/N ratio values for each lithological unit is  
 245 shown in Fig. 4. In the LU IV,  $\delta^{13}\text{C}$  values ranged between  $-26.7\text{‰}$  and  $-23.0\text{‰}$ , while  
 246 C/N values ranged between 8.5 and 14.9. The LU III, exhibited  $\delta^{13}\text{C}$  values between  $-$   
 247  $27.0\text{‰}$  and  $-24.5\text{‰}$  and C/N values between 10.0 and 13.7. In the LU II,  $\delta^{13}\text{C}$  ranged  
 248 between  $-24.7\text{‰}$  and  $-23.9\text{‰}$  and C/N values ranged between 12.7 and 13.7. The LU I  
 249 showed  $\delta^{13}\text{C}$  values ranging between  $-25.8\text{‰}$  and  $-23.9\text{‰}$ , and C/N ratios ranging  
 250 between 10.6 and 12.7.



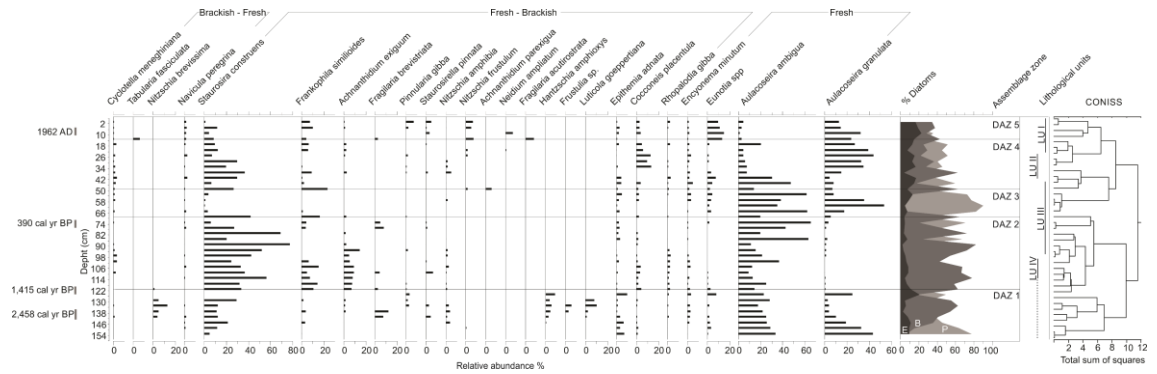
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252 **Figure 4.** The relationship between  $\delta^{13}\text{C}$  values and C/N ratios sediment cores (LP1 –  
 253 LP2) (Published data by del Puerto et al., 2013 and provided by the authors), including  
 254 typical ranges of sources according to data presented by Meyers (1994) and Lamb et al.  
 255 (2006).

256 *Diatoms*

257 A total of 109 species were identified in 39 samples selected from cores LP1 (0 –  
 258 95 cm) and LP2 (95 – 156 cm). The vertical distribution of the most abundant diatom  
 259 species, the percentage of diatom groups (i.e., Planktonic, Benthic and Epiphytic) and the  
 260 Diatom Association Zones (DAZ), inferred from the stratigraphic constrained cluster  
 261 analysis are presented in Figure 5.

262



263 **Figure 5.** Relative abundance of diatom species of cores LP1 (0 – 95 cm) and LP2 (95 –  
 264 106 cm). Percentage of groups of diatoms; planktonic (P), benthic (B) and epiphytic (E).  
 265 Clustering groups, lithological units (LU) and Diatom Assemblage Zones (DAZ) are  
 266 shown to the right of the plot.

267 Those species with a relative abundance lower than 3% were excluded from the  
 268 statistical analysis as they are considered as rare species (Whiting and Mc Intire 1985, in  
 269 Hassan et al., 2006). Therefore, a set of 26 representative co-dominant species with a  
 270 relative abundance  $\geq 3\%$  in at least two intervals are presented in Figure 5.

271 Cluster analysis allowed us to identify five diatom zones (DAZ) (Fig. 5). DAZ 1,  
 272 encompassed the basal section of the sedimentary record (156 – 122 cm), and was  
 273 dominated by *Staurosira construens* Ehrenberg, *Aulacoseira ambigua* (Grunow)  
 274 Simonsen, *Aulacoseira granulata* (Ehrenberg) Simonsen, *Fragilaria brevistriata*  
 275 (Grunow) Van Heurck, *Encyonema minutum* (Hilse) D.G.Mann in Round, *Nitzschia*

276 *amphibia* Grunow, *Hantzschia amphioxys* (Ehrenberg) Grunow, *Eunotia* spp, *Epithemia*  
277 *adnata* (Kützing) Brébisson, *Nitzschia brevissima* Grunow, *Frustulia* sp., *Luticola*  
278 *goeppertiana* (Bleisch ex Rabenhorst) D.G.Mann in Round and *Rhopalodia gibba*  
279 (Ehrenberg) O. Müller. The most abundant diatom species in this zone consisted of  
280 freshwater planktonic species *Aulacoseira ambigua* (26%), *Aulacoseira granulata* (18%)  
281 from 156 cm to 148 cm, and 148 to 122 cm the benthic – brackish/freshwater species of  
282 *Staurosira construens* (13%). DAZ 1 exhibited a mean value of 55.5% of benthic  
283 aerophilic taxa (i.e., *Hantzschia amphioxys*, *Nitzschia brevissima*, *Frustulia* sp., *Luticola*  
284 *goeppertiana*) from which 19.5% were epiphytic (i.e., *Epithemia adnata*, *Eunotia* spp.,  
285 *Rhopalodia gibba*) and 25% planktonic. In the interval 134 - 122 cm there was an increase  
286 in epiphytic species (Fig. 5).

287 In DAZ 2 (122 – 70 cm) the benthic brackish/freshwater species *Staurosira*  
288 *construens* showed a relative abundance of 40%, while *Aulacoseira ambigua* exhibited a  
289 relative abundance of 27%. In addition, lower frequencies of *Cyclotella meneghiniana*  
290 Kützing, *Frankophila similioides* Lange-Bertalot and U. Rumrich, *Achnantheidium*  
291 *exiguum* (Grunow) Czarnecki, *Fragilaria brevistriata*, *Epithemia adnata*, *Cocconeis*  
292 *placentula* Ehrenberg and *Rhopalodia gibba*, were observed. The relative abundance of  
293 benthic taxa increased to a mean value of 66.5%, while the planktonic species *Aulacoseira*  
294 *granulata* decreased sharply (Fig. 5).

295 In DAZ 3 (70 – 50 cm) the relative abundance of planktonic taxa increased,  
296 reaching here the highest value (62%) of the entire core, dominated by *Aulacoseira*  
297 *ambigua* (43%) and *Aulacoseira granulata* (25%). The benthic taxa decreased to 26%,  
298 and low proportions of *Staurosira construens*; *Epithemia adnata*, *Rhopalodia gibba*,  
299 *Eunotia* spp. and *Encyonema minutum* were observed throughout this zone (Fig. 5).

300 In DAZ 4 (50 – 14 cm) planktonic and benthic taxa reached the 43% and 33%  
301 respectively. DAZ 4 was dominated by *Staurosira construens*, and *Aulacoseira*  
302 *granulata*, with lower proportions of *Aulacoseira ambigua* and *Cocconeis placentula*,  
303 showing higher abundances towards the upper section of the zone. Conversely, lower  
304 percentages of *Frankophila similioides*, *Encyonema minutum*, *Nitzschia amphibia*,  
305 *Epithemia adnata*, *Eunotia* spp and *Cyclotella meneghiniana*, were registered in DAZ 4.  
306 The percentage of *Aulacoseira ambigua* was higher than that of the upper section of the  
307 zone, while *Aulacoseira granulata* displayed lower values in the basal section of the zone  
308 (Fig. 5).

309 In DAZ 5 (14 – 2 cm), diatom assemblages were dominated by *Eunotia* spp and  
310 *Aulacoseira granulata*, but *Staurosira construens*, displayed a decreasing upward trend.  
311 *Aulacoseira ambigua* showed the lowest abundances of the core. In the subsurface  
312 sediments of this zone, the occurrence of *Nitzschia frustulum*, *Nitzschia ampliatum*,  
313 *Pinnularia gibba*, *Staurosirella pinnata* and *Tabularia fasciculata* was observed (Fig. 5).

## 314 **DISCUSSION**

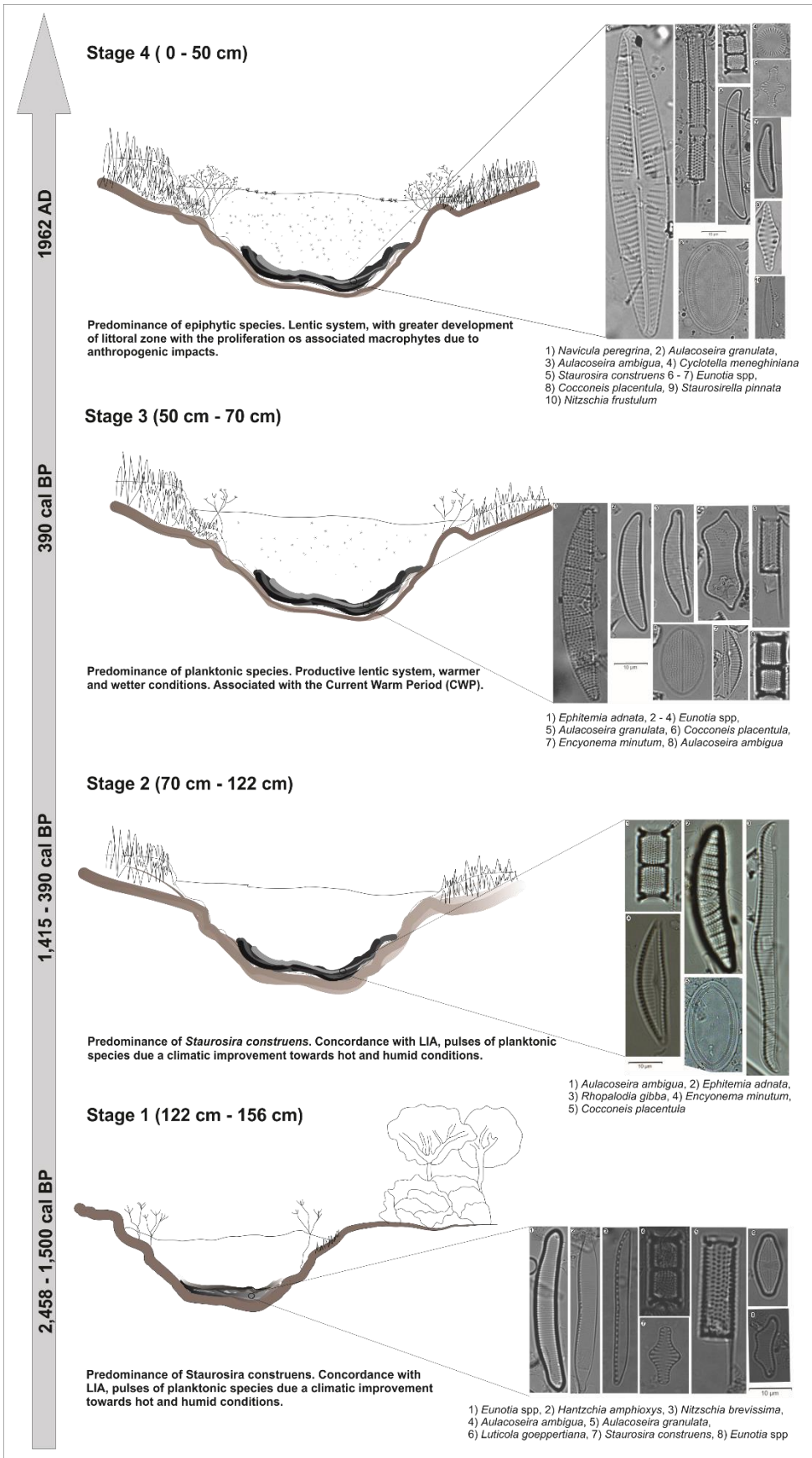
315 The combined analysis of geochemical, sedimentological proxy-data (Fig. 2 and  
316 4) and diatom assemblages (Fig. 5) allowed to infer distinct changes in the environmental  
317 conditions of Peña Lagoon throughout the past 2,458 yr BP.

318 Four lithological units (LU) were defined according to changes in grain size  
319 composition as well as in the magnetic susceptibility ratio, which are considered as  
320 indicators of different sediment sources. Coarser sediments in lithological unit II - III with  
321 high MS ratios are attributed to an increased input of sandy sediments from the lake  
322 watershed.



323           According to Meyers (1994) the  $\delta^{13}\text{C}$  and C/N ratio values allowed us to infer a  
324 mixed source of sedimentary organic matter with signals of freshwater microalgae and  
325 C3 terrestrial plants in lithological unit IV (Fig. 4), where an environment with important  
326 proliferation of grasses and phytoplankton/microphytobenthos was inferred by del Puerto  
327 et al., (2013). Similar environmental conditions in the source of sedimentary organic  
328 matter were observed in LU III and LU I, based on  $\delta^{13}\text{C}$  and C/N ratio values (Fig. 4).  
329 However, in LU II, both isotopic and C/N values indicate that the organic matter  
330 completely derived from the C3 terrestrial plants (Fig. 4). Therefore, the changes in the  
331 isotopic composition of OM and C/N ratios allowed us to reliably infer past changes in  
332 organic matter composition where signals of freshwater microalgae and continental C3  
333 plants were the most important sources.

334           The variability in diatom assemblages (DAZ 1-5) combined with physical and  
335 chemical proxies indicate four main stages during the past 2,458 yr BP in Peña Lagoon,  
336 as depicted in Fig. 6.



338 **Figure 6.** Holocene main climatic variability stages in Peña Lagoon over the last 2,458  
339 cal yr BP.

340 *Stage 1*

341 This stage is recorded from 122 to 156 cm (i.e., DAZ 1). The age of basal  
342 sediments is unknown, but the interval of 127.5 cm was dated at 2,458 cal yr BP and the  
343 top at 1,415 cal yr BP. Sediments are dominantly sandy-muds (Fig. 2 - 3) with a  
344 coarsening trend at the basal part of the core (ca.  $\phi$  5.3) thus suggesting sandy sediment  
345 inputs from the surrounding lagoon area. Allochthonous inputs of organic matter derived  
346 from the watershed can be considered according to  $\delta^{13}\text{C}$  values (-23.6‰), which indicate  
347 a terrestrial plant inputs (Meyers, 1994; Wei et al., 2010) (Fig. 4). Previous results also  
348 inferred such external inputs using the OP/OBP index (opal phytolith:other biosiliceous  
349 particles ratio) as a proxy, which showed both the highest values of the record and  
350 dominance of C4 phytoliths (del Puerto et al., 2013). The uppermost section of stage 1  
351 (dated at 1,415 yr BP) could be ascribed to warm temperate and humid conditions,  
352 corresponding to the Medieval Warm Period (MWP; ca. 1500 years BP), inferred for the  
353 Pampean region by Piovano et al. (2009) and in Uruguay by Perez et al. (2016) as warmer  
354 and more humid pulses with variations in rainfall and wind patterns for 1,200 cal yr BP.

355 Benthic taxa characteristic of moist or temporarily dry sediments (Denys, 1991;  
356 Van Dam et al., 1994) accounted for 55% of the diatom abundance from which 19.5%  
357 consisted of epiphytic taxa, thus indicating the presence of aquatic plants associated to a  
358 shallow system with a well-developed littoral zone. Li et al. (2015) inferred similar  
359 conditions based on high abundances of epiphytic taxa (i.e., *Epithemia adnata*, *Cocconeis*  
360 *placentula*) in south-western China. In addition, a meso eutrophic brackish system (e.g.,  
361 Denys, 1991; Van Dam et al., 1994), with significant water turbulence and associated  
362 turbidity can be inferred from the occurrence of the planktonic species *Aulacoseira*

363 *granulata* and *Aulacoseira ambigua* in the basal section of the core (150 - 156 cm).  
364 Moreover, *A. granulata* is considered a thermophilic diatom linked to water temperatures  
365 higher than 15°C (Rioual et al., 2007), this taxa have been reported in modern Pampean  
366 lakes sediments from Argentina in temperatures ranging from 7 up to 25 °C (Hassan,  
367 2015) The decreasing upward trend in the abundance of both planktonic species, together  
368 with the occurrence of *Hantzschia amphioxys*, *Nitzschia brevissima*, *Frustulia* sp. and  
369 *Luticola goeppertiana* in DAZ 1, suggest a reduction in the water column productivity of  
370 the system, higher salinity (~ 0.9 – 1.8‰, Denys, 1991; Van Dam et al., 1994), cooler  
371 conditions, and a decrease in water turbidity, possibly as a result of a reduction in windy  
372 conditions. The fine grain size fraction of sediments above 140 cm depth, indicate lower  
373 runoff from the catchment.

374 Results are consistent with previous reconstructions which analyzed the phytolith  
375 record of Negra lagoon, where a warm/wet period was also identified between 1,980 ± 40  
376 yr BP and 930 ± 45 yr BP, although an intermediate drier/colder episode has been  
377 proposed (Bracco et al., 2005a; 2005b; 2010; del Puerto, 2009) Furthermore,  
378 paleolimnological studies in the southern Pampa plains of Argentina suggested that it is  
379 fairly acceptable to assume that during the middle–late Holocene, the ratio of evaporation  
380 to precipitation was higher, thus leading to salinization, low water levels and possible  
381 desiccation of lakes (Stutz et al., 2012). In the northern region of the Pampa plain, a  
382 paleolimnological record indicates brackish to saline conditions with pulses of short-  
383 periodic freshwater conditions for 4,840 – 1,200 cal. yr BP (Stutz et al., 2012), as well as  
384 dry conditions during most of the Holocene (Piovano et al., 2009).

## 385 ***Stage 2***

386 This stage is recorded by sandy mud sediments entirely matching DAZ 2 (122 cm  
387 – 70 cm), which was dominated by the benthic species *Staurosira construens* with pulses

388 of increased abundance of *Aulacoseira ambigua* (Fig. 5). The age of the section is ca.  
389 1,415 cal. yr BP (117 cm) while the top corresponds to 390 cal yr BP (73 cm).

390 High abundances of the fragilarioid species *Staurosira construens* (Stoermer,  
391 1993 in Fey et al., 2009), which are also in agreement with the disappearance of a  
392 thermophilic species *A. granulata* allowed us to infer cold conditions during this stage.  
393 Likewise, del Puerto et al. (2013) reported an increase of pooid and chloridoid phytolith  
394 morphotypes as well as an increase in the temperature:humidity (T:H) index, thus  
395 suggesting lower average temperature values, and either more arid or highly seasonal  
396 conditions. Above 90 cm depth, an allochthonous input from runoff processes due to  
397 increased rainfall was inferred based on changes in the relative abundance of *S.*  
398 *construens*, and *A. ambigua*. In addition, changes in the relative abundance of *S.*  
399 *construens*, and the increase in *A. ambigua* indicate a reduction in salinity to < 0.9 (Van  
400 Dam et al., 1994; Alcántara et al., 2002). At the same level, the coarser sediments, high  
401 content of sedimentary OM, high values of C/N ratio and a  $\delta^{13}\text{C}$  can be attributed to  
402 higher external inputs. In agreement with this, high terrestrial inputs and lower mean  
403 annual temperatures were inferred by del Puerto et al. (2013) based on an increase in  
404 phytoliths of winter grasses. Considering the age of the uppermost section of this stage,  
405 it can be assigned to the Little Ice Age (LIA). Moreover, other paleolimnological records  
406 from Southern Uruguay (Bracco et al., 2011a, 2011b) indicate a climatic deterioration  
407 linked to the Little Ice Age (LIA) with estimated chronologies between 800 - 200 yr BP,  
408 thus suggesting semiarid climatic conditions with intervals of rainfall increase. In the  
409 central plain of Argentina, high salinities and low lake levels for the LIA were identified.  
410 Such conditions persisted until the early 1970s, after which extreme pulses of positive  
411 water balances were inferred (Villalba, 1994; Piovano et al., 2004; 2009; Córdoba et al.,  
412 2014)

413 **Stage 3** (after 390 cal yr BP)

414 This stage matches entirely with DAZ 3 (50 cm – 70 cm) where a clear increase  
415 in the abundance of planktonic freshwater species *Aulacoseira granulata* and *A. ambigua*,  
416 together with higher C/N ratios, suggest an autochthonous contribution to the bulk  
417 organic matter (Fig. 4) with a diminished external input which is also supported by the  
418 finer grain sediment size. Both planktonic species are considered eutrophic freshwater  
419 taxa. High abundances of these taxa were observed during conditions of increasing  
420 eutrophic conditions in the Baltic Sea (Andrén, 1999) and in the Bothnian sea (Andrén et  
421 al., 2016). In the Southern argentinean pampas, *A. granulata* was the dominant species  
422 under high nutrient loading and turbid conditions in the lake Lonkoy, associated with  
423 higher water levels and low salinities (Hassan, 2013). Comparatively higher abundances  
424 of planktonic diatoms in the Peña Lagoon can be attributed to the onset of warmer  
425 conditions, as previously reported by del Puerto et al. (2013) based on the increase in  
426 small grass cells. The presence of *A. ambigua* and *A. granulata* species suggest higher  
427 water column trophic state conditions (Bicudo et al., 2016) during stage 3 compared to  
428 stage 2.

429 The presence of sandy muds (part of LU II) and C3 terrestrial plant sources of  
430 sedimentary organic matter (Fig. 4), in addition to the presence of genus *Aulacoseira*,  
431 indicate fairly windy conditions during this stage. *Aulacoseira* has been used in many  
432 geographical regions as a proxy for strong wind stress, turbulent water, and nutrient  
433 upwelling conditions (Wang et al., 2008). Furthermore, del Puerto et al. (2013) observed  
434 variability in phytolith composition and inferred colder and drier conditions by 300 yr  
435 BP, which is in agreement with the aeolian sand input into the water body.

436 **Stage 4**

437           The uppermost 50 cm of the sedimentary record that include DAZ 4 and DAZ 5,  
438 consisted of finer grain size and higher content of OM, thus reflecting higher primary  
439 productivity since 1962 AD to the present. High proportions of epiphytic species such as  
440 *Cocconeis placentula*, *Eunotia* spp, *Epithemia adnata* and *Encyonema minutum* suggest  
441 a eutrophic lentic system with a well-developed littoral zone associated with macrophyte  
442 proliferation. Based on the increase in phytoliths of the morphotype Oryzoide a similar  
443 paleoenvironment was reported by del Puerto et al. (2013), where an increase in  
444 hydrophilic vegetation might have been triggered by warm and humid conditions. The  
445 higher trophic state can be inferred from the increasing upward trend in sedimentary OM  
446 and acidic waters, as suggested by the increase of *Eunotia* spp, which are characteristic  
447 of humic waters, where macrophyte degradation is commonly observed (Eloranta y  
448 Soinninen, 2002). Similar changes were reported in the top 10 cm of the  
449 paleolimnological record (attributed to the last century) of Lake Lonkoy in Argentina,  
450 which were ascribed to the agricultural impact (Hassan, 2013). Even though, in the  
451 surrounding area of Peña Lagoon there are no significant agricultural practices, there is a  
452 water treatment plant which throws the residuary sediment waste into the Peña lagoon  
453 thus leading the proliferation of macrophytes.

#### 454 **FINAL REMARKS**

455           The diatom assemblages, organic matter composition and sedimentological  
456 proxies allowed to recognize four main environmental stages for the last 2,458 cal yr BP:  
457 (i) a shallow meso – eutrophic system with high abundances of aerophilic benthic species,  
458 with high inputs from the watershed, and organic matter signals of C3 plants. This stage  
459 could be ascribed to the Medieval Warm Period (ii) a system dominated by  
460 brackish/freshwater species, with high terrestrial inputs and low temperatures  
461 synchronous with the Little Ice Age, (iii) a system dominated by planktonic freshwater

462 species, high proportion of autochthonous sedimentary organic matter during the Current  
463 Warm Period, and (iv) a eutrophic system with high proportions of epiphytic taxa, and  
464 the proliferation of macrophytes in the littoral zone due to recent human impacts.

465 The results remarks, the importance of developing paleolimnological research at  
466 a regional scale in South East of South America in order to evaluate the timing and  
467 magnitude of climatic changes during the Holocene as well the most recent response of  
468 aquatic systems to human activities.

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