

1 **Seasonal composition and activity of the intertidal macrobenthic**

2 **community of Caleta Valdés (Patagonia, Argentina) applying *in situ* and *ex***

3 ***situ* experimental protocols**

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28 **Abstract**

29 Caleta Valdés (CV) is a coastal lagoon of the Patagonian Atlantic coast located  
30 in the Península de Valdés declared as Humanity Mundial Patrimony due to its  
31 remarkable biodiversity, ecosystemic richness, and pristine state. Marine  
32 mammal populations are well documented in this area but few studies have been  
33 carried out on the local macrobenthic communities. The goals of this study were  
34 (a) to evaluate for the first time the seasonal variation of the structure and activity  
35 (i.e., sediment reworking) of the intertidal macrobenthic communities of CV, and  
36 (b) to validate an *ex situ* experimental protocol for future ecotoxicological studies.

37 To do so, sedimentary (granulometry, water content, and organic matter) and  
38 biological (macrobenthic assemblages, biodiffusive-like and advective sediment  
39 reworking components) parameters were analyzed using a combination of *in situ*  
40 and *ex situ* measurements. Overall, polychaete and crustacean dominated the  
41 macrobenthic community. The highest abundances were found in Spring-  
42 Summer along with a predominance of biodiffusors versus conveyors. *Ex situ* and  
43 *in situ* measurements demonstrated similar results, thus allowing validation of an  
44 *ex situ* experimental procedure for macrobenthic community and functioning  
45 studies. In addition, these results provide a first baseline of benthic information  
46 on CV that will be helpful to monitor the effects of potential pollution in Patagonian  
47 coastal systems.

48

#### 49 **Keywords**

50 Biodiversity, bioturbation, sedimentary matrix, soft-bottom macrofauna, protected  
51 areas.

52

#### 53 **1. Introduction**

54 The Patagonian coast is about 3000 kilometers long with a high value in terms of  
55 biodiversity (Esteves and Arhex, 2009). This coast is used by migratory birds and  
56 marine mammals to rest, feeding and mating (Yorio, 2009). Moreover, it includes  
57 zones of reproduction and breeding of fish, crustaceans, and mollusks sustaining  
58 one of the most productive temperate marine ecosystems in the world (Vázquez,  
59 2004). As in other marine coastal environments, the food webs are mainly  
60 sustained by phytoplankton (Smetacek, 1999). Particularly, in these coasts,  
61 upwelling phenomena increase primary production (Acha et al., 2004). Due to its

62 remarkable biodiversity and ecosystemic richness, big areas of this coast have a  
63 special protection status (e.g. Península de Valdés was established as Humanity  
64 Mundial Patrimony in 1999, North Zone San Jorge gulf was defined as  
65 Patagonian Austral Inter-jurisdictional Marine Coastal Park in 2007 and Punta  
66 Tombo as Natural Protected Area in 1979, among others). Despite these  
67 protection measures, there are diverse and growing anthropogenic activities  
68 constituting a direct threat to the ecosystem. Therefore, studies about the  
69 seasonal dynamics of the macrobenthic communities constituting a particular  
70 interest at ecological level but also for integrated coastal zone management  
71 purposes.

72

73 Indeed, the soft-bottom benthic organisms play a key role in nutrient cycles,  
74 pollutant metabolism, dispersion, and burial of the organic matter and secondary  
75 production processes (Hopkinson et al., 1999; Snelgrove, 1998). These  
76 organisms have reduced mobility (Pearson and Rosenberg, 1978; Teixeira et al.,  
77 2012) and therefore they are highly sensitive to the physical and chemical  
78 changes of the sediment matrix (Dauvin et al., 2010; Muniz et al., 2005). For this  
79 reason, this group of organisms is useful as an indicator of ecological state in  
80 impacted sites and/or in monitoring programs (e.g. Teixeira et al., 2012). Through  
81 the excavation, burrow ventilation, and/or mud and organic matter ingestion  
82 (Taghon and Greene, 1992), they induce the transport of particles (sediment  
83 reworking) and fluids also known as bioturbation (Kristensen et al., 2012).  
84 Community bioturbation is a good integrator of macrofaunal functioning as it  
85 incorporates various aspects of behavior (e.g. feeding, locomotion, burrow  
86 building) and may vary depending on the community structure and local

87 heterogeneity (Solan et al., 2019). Differences in bioturbation over time may also  
88 reflect the abiotic and/or biotic changes of not only the benthic but also the pelagic  
89 ecosystem. Particularly, bioturbation affects processes such denitrification  
90 (Gilbert et al., 2003), nitrification (Aller et al., 1998), sulfate reduction (Canavan  
91 et al., 2006), benthic fluxes (Aller and Aller, 1998; Mermillod-Blondin et al., 2005;  
92 Michaud et al., 2005; Mortimer et al., 1999) and microbe dynamics (Goñi-Urriza  
93 et al., 1999; Papaspyrou et al., 2006).

94

95 Reworking organisms can be classified into functional groups based on the  
96 different ways in which the organisms behave and on the resulting transport of  
97 particles within the sedimentary column. According to Kristensen et al. (2012),  
98 these organisms can be differentiated into biodiffusors, upwards and downward  
99 conveyors, and regenerators. Biodiffusors are species whose activities produce  
100 a local, constant, and random mixture of sediment over short distances. Upwards  
101 and downward conveyors are species vertically distributed that respectively feed  
102 head down and deposit its dregs in the surface of the sediment or inversely, and  
103 finally the regenerators, excavator species that transfer the sediment from the  
104 bottom to the surface. In ecosystems, the complex communities are then able to  
105 generate various patterns and intensities of sediment reworking depending on  
106 their functional composition (e.g. Duport et al., 2007). Moreover, it can be  
107 modulated by the environmental conditions via changes in organisms behavior.  
108 For example, the burrowing polychaetes *Hediste diversicolor* can switch from  
109 deposit-feeding to filter-feeding if the phytoplankton concentration is high enough  
110 (Riisgård, 1994) and *Alitta virens* has been shown to have a temperature  
111 dependence of sediment reworking activity (Ouellette et al., 2004).

112

113 The goals of this study were (a) to evaluate for the first time the seasonal variation  
114 of the structure and the activity (i.e., sediment reworking) of the intertidal  
115 macrobenthic communities of Caleta Valdés and, (b) to validate an *ex situ*  
116 experimental protocol for future ecotoxicological studies.

117

## 118 **2. Materials and methods**

### 119 *2.1. Study area*

120 Caleta Valdés (CV) is a north-south-oriented coastal lagoon located at the  
121 eastern side of Península Valdés. This is 30 km long and has its mouth at the  
122 southern end where the water exchange is produced at two and four nudes of  
123 velocity. A gravel bank limits the lagoon, generating a channel of 200 m width  
124 and a mean depth of 5 m. The enclosed north zone presents a marsh that cover  
125 a 25% of the total surficial of the system (Esteves et al., 1993). The tidal regimen  
126 is semidiurnal with a mean high of 5 m. The sampling site for the experimentation  
127 was chosen in the muddy north continental zone of CV (42°15'53" S, 63°40'50"  
128 W; Figure 1) due to its macrofauna richness and the absence of anthropogenic  
129 pollution. Currently, this natural reserve is protected of human activities so that  
130 strict control is carried out on touristic and smaller productive activities, which are  
131 developed in the area.

132

### 133 *2.2. Sampling*

134 Sediment cores were sampled by hand in autumn (April 2011), winter (July 2011),  
135 spring (November 2011), and summer (February 2012) using 10 cm diameter  
136 and 25 cm length PVC cores, in agreement with previous bioturbation studies

137 (Ferrando et al., 2015; Hedman et al., 2011; Quintana et al., 2007; Timmermann  
138 et al., 2002). At each season, at low tide, eight cores were vertically and randomly  
139 pushed down to 20 cm sediment depth approximately. Then, for *ex situ*  
140 experiments, four of them were immediately collected by hand and transported  
141 to the laboratory. The other four cores were embedded in the sediments for *in*  
142 *situ* assessments. In addition to sediment sampling, 60 L of seawater were also  
143 collected in plastic containers to be used for the *ex situ* experiments. This  
144 procedure was applied for each studied season.

145

### 146 2.3. Incubation conditions

#### 147 2.3.1. *In situ* experiments

148 In order to assess biological reworking activity, in each sediment core, 4 g of  
149 luminophores (inert fluorescent particles, Duport et al., 2007) of two sizes and  
150 colors (pink: 65-125  $\mu\text{m}$ ; green: 125-355  $\mu\text{m}$ ) were homogeneously spread at the  
151 sediment surface at initial time (T=0 day). Luminophores sizes were selected  
152 according to the two major size distribution modes of sedimentary particles in the  
153 studied site (Sturla Lompré et al., 2018). After 13 days of *in situ* incubation, the  
154 cores were withdrawn and carried out to the laboratory. Then, the sediment cores  
155 were sliced to provide 0.5 cm thick sediment layers from the surface to 2 cm  
156 depth and 1 cm thick sediment layers from 2 to 17 cm depth. Each sediment slice  
157 was separated in four equal parts that were randomly distributed to perform the  
158 different analyses.

159

#### 160 2.3.2. *Ex situ* experiments

161 Similarly, for each season, the four cores containing the sediments sampled at  
162 CV were distributed in a 56 L tank that was then filled until the cores were totally  
163 submerged with the seawater collected the same day in the same sampling  
164 station. To assess biological reworking activity in a no water flow system where  
165 no hydrodynamic loss of tracers could occur, in each sediment column, 2 g of the  
166 two types of luminophores (pink: 65-125  $\mu\text{m}$  and green: 125-355  $\mu\text{m}$ ) were  
167 homogeneously spread at the sediment surface at the initial time (T=0 day). The  
168 system was kept continuously aerated (air bubbling). Sediment cores were  
169 incubated for 13 days at a similar ambient temperature to the *in situ* assessment.  
170 Then, they were manually collected and processed identically as for the *in situ*  
171 experiments.

172

## 173 2.4. Analytical procedures

### 174 2.4.1. Sediment characterization

175 A quarter of each sediment slice was used to determine sediment granulometry  
176 (only in autumn 2011) by laser beam diffraction (Partica LA-950; HORIBA  
177 Instruments, Inc.), and for the four seasons, water content and organic matter by  
178 oven drying at 105 °C during 24 h and muffle furnace ignition at 450 °C during 4  
179 h, respectively. Eight granulometry fractions were obtained: fine silt < 6  $\mu\text{m}$ ,  
180 medium silt 6-20  $\mu\text{m}$ , coarse silt 20-60  $\mu\text{m}$ , very fine sand 60-125  $\mu\text{m}$ , fine sand  
181 125-200  $\mu\text{m}$ , medium sand 200-600  $\mu\text{m}$ , coarse sand 600-1000  $\mu\text{m}$ , and very  
182 coarse sand 1000-2000  $\mu\text{m}$ .

183

### 184 2.4.2. Macrobenthic communities

185 The remaining three quarters of each sediment slice were fixed and dyed with a  
186 neutralized 4% formaldehyde solution and Rose Bengal colorant, respectively.  
187 Then, the samples were sieved, first with a 500  $\mu\text{m}$  mesh and then with a 44  $\mu\text{m}$   
188 mesh. The sediment retained in the first mesh was preserved in ethanol 70% for  
189 the identification and counting of the macrobenthic organisms. The sorting of  
190 organisms (for the four seasons) was done to the lowest possible taxonomic level  
191 using stereoscopic and optic microscopes in the laboratory with reference  
192 material and dichotomous keys (Banse and Hobson, 1974; Blake and Ruff, 2007;  
193 Hartman, 1968, 1969; Orensanz et al., unpublished data, among others). Only  
194 the whole organisms or anterior fragments of each taxon were recorded.

195

#### 196 2.4.3. Bioturbation

197 The sediments retained in the 44  $\mu\text{m}$  mesh were used (see above) to quantify  
198 sediment reworking. The sediments were homogenized and subsampled to  
199 quantify the luminophores percentage using a microplate reader (Biotek, Synergy  
200 Mx) at  $\lambda_{\text{ex}}/\lambda_{\text{em}}$ : 460/500 and 565/602 nm for the pink and green luminophores,  
201 respectively (Majdi et al., 2014). For each sediment column at the four seasons,  
202 we obtained the vertical luminophores profile at the experimental final time (13  
203 days) from the percentage of luminophores found in each layer concerning the  
204 total amount in the core. The quantification of sediment reworking was then  
205 evaluated from the distributions of luminophores by the gallery-diffusor model  
206 (François et al., 2002). This model allows describing both the biodiffusion-like  
207 transport ( $D_b$  coefficient) due to the continuous displacement of the tracers and  
208 the nonlocal advective displacement of the tracers ( $r$  coefficient). The best fit  
209 between observed and modeled tracer distribution with depth (i.e., producing the



210 best  $D_b$  and  $r$  coefficients couple) was estimated by the least-squares method  
211 (Gilbert et al., 2007).

212

#### 213 2.4.4. Statistical data analysis

214 Variation between seasons (autumn, winter, spring and summer) and  
215 experimental conditions (I: *in situ* and E: *ex situ*) of water content and organic  
216 matter (OM) were evaluated by core through two-way ANOVA considering  
217 seasons and conditions as fixed factors (n=32) (Statistica, version 7). A square  
218 root transformation was applied to satisfy the homogeneity of variances  
219 assumption. The differences between pairs of seasons and conditions were  
220 tested through Tukey test for multiple mean comparisons (Statistica, version 7).

221

222 A comparison of the mean abundance by core of dominant taxa between seasons  
223 and I and E experimental conditions were evaluated through two-way ANOVA  
224 considering seasons and conditions as fixed factors (n=32) (Statistica, version 7).  
225 A square root transformation was applied to satisfy the homogeneity of variances  
226 assumption. The differences between pairs of seasons and conditions were  
227 tested through Tukey test for multiple mean comparisons (Statistica, version 7).

228

229 Biological data analyses were performed using the software package PRIMER  
230 7.0 (Plymouth Marine Laboratory, UK). The sorting using the total abundance of  
231 each taxa by core recorded in the I and E experiments at the four seasons was  
232 carried out with a nonmetric multidimensional scaling (nMDS) analysis (Bray-  
233 Curtis index; group average link; square root). In addition, a Permutational  
234 Multivariate Analysis of Variance (PERMANOVA) was applied to assess the

235 effect of the seasons, I and E experimental conditions, and the interaction  
236 between both factors (Bray-Curtis index; fourth root and 9999 permutations)  
237 (PRIMER v7). A Pair-wise PERMANOVA was applied to analyze the significant  
238 differences between seasons and conditions (Bray-Curtis index; fourth root and  
239 9999 permutations) (Anderson et al. 2008). Moreover, Specific richness (S), total  
240 abundance (N), and the Shannon diversity index (H') were calculated by layer  
241 and core (I and E) at the four seasons. Variation of each biological parameter  
242 between seasons and conditions was evaluated through two-way ANOVA  
243 considering seasons and conditions as fixed factors (n=32) (Statistica, version 7).  
244 The differences between pairs of seasons and conditions were tested through  
245 Tukey test for multiple mean comparisons.

246

247 A comparison of the bio-diffusion ( $D_b$ ) and bio-advection ( $r$ ) coefficients calculated  
248 for each experimental condition (I and E) was performed through the non-  
249 parametric Kruskal-Wallis one-way analysis of variance by ranks (Kruskal and  
250 Wallis, 1952) (Statistica, version 7). Moreover, a one-way ANOVA was applied to  
251 test the differences between seasons (n=16). The differences between pairs of  
252 seasons were tested through Fisher LSD test for multiple mean comparisons  
253 (Statistica, version 7). A Kruskal-Wallis and Pair-wise Multiple Comparisons  
254 analysis were carried out to test the differences as a non-parametric alternative  
255 due to lack of homogeneity of variances of data in some cases.

256

### 257 **3. Results**

#### 258 *3.1. Characterization of the sediment*

259 The granulometry profiles were similar for the *in situ* and the *ex situ* sediments  
260 showing a predominance of medium sand (200-600  $\mu\text{m}$ ) (Figure 2). Moreover,  
261 the fine fractions ( $< 200 \mu\text{m}$ ) were highest at the first two centimeters of the  
262 sedimentary columns. Regards *in situ* sediments, the maximum percentages of  
263 water content ( $58.5 \pm 8.2\%$ ) and organic matter (OM) ( $1.7 \pm 0.1\%$ ) were recorded  
264 in the first layer (0-0.5 cm) in winter and autumn, respectively (Online Resource  
265 1). In spring, the maximum percentages of water content ( $48.5 \pm 19.6\%$ ) and OM  
266 ( $1.3 \pm 0.2\%$ ) were found in the fourth layer (1.5-2 cm) and the first layer (0-0.5  
267 cm), respectively. In summer, the maximum percentages of water content ( $33.3$   
268  $\pm 0.8\%$ ) and OM ( $0.9 \pm 0.0\%$ ) were recorded in the second (0.5-1 cm) and second  
269 and third layers (0.5-1.5 cm), respectively. These patterns were similar to those  
270 recorded in *ex situ* conditions (Online Resource 1). Comparing seasons and  
271 experimental conditions (I and E), percentages of water content did not show  
272 significant effect of the interaction of these factors (Two-way ANOVA,  $p = 0.15$ ,  $n$   
273  $= 32$ ) but highly significant effect of each factor ( $p < 0.01$ ,  $n = 16$ ). Percentages  
274 of water content recorded in I and E conditions for winter and summer were  
275 similar (Tukey test  $p = 0.23$  and  $p = 0.06$ ;  $n = 8$ , respectively). Contrary, in autumn  
276 and spring, this parameter was significantly higher I than E (Tukey test  $p < 0.01$ ;  
277  $n = 8$ ) (Figure 3). Moreover, in I conditions, water content showed not significant  
278 differences (Tukey test  $p > 0.05$ ;  $n = 16$ ) among autumn, winter and spring, but  
279 did (Tukey test  $p < 0.01$ ;  $n = 16$ ) in summer compared to other seasons. By  
280 contrast, in E conditions, in winter water content was significantly higher (Tukey  
281 test  $p < 0.01$ ;  $n = 16$ ) than in other seasons (Figure 3). Percentages of OM  
282 showed significant effect of the interaction season and experimental condition  
283 (Two-way ANOVA,  $p = 0.01$ ,  $n = 32$ ) and just significant effect of season ( $p <$

284 0.01, n = 16). Only significant differences between I and E conditions were  
285 recorded in spring ( $p = 0.026$ , n = 8). Comparing seasons, at both conditions, the  
286 highest values were recorded in autumn with significant differences (Tukey test  
287  $p < 0.01$ ; n = 16) from the rest seasons. At I conditions, OM significant differences  
288 ( $p < 0.01$ ; n = 16) were between winter and spring from summer; conversely, E  
289 conditions presented significant differences ( $p < 0.01$ , n = 16) between winter  
290 from spring and summer (Figure 3).

291

### 292 3.2. *Macrobenthic communities*

293 A total of 27 taxa (I and E conditions) were found in CV including sixteen taxa of  
294 crustaceans, nine taxa of polychaetes, and one taxon of oligochaetes and  
295 nemerteans (Table 1). Mean abundance of the six most dominant taxa ( $> 4.8\%$ )  
296 are shown in Figure 4. All taxa except *Axiiothella* sp. were more abundant in the  
297 spring-summer period. This was pronounced in summer for the polychaete  
298 *Exogone molesta* (Syllidae), the most abundant species (37.4% of total  
299 macroinfauna). In addition, the two-way ANOVA showed only for *Scoloplos* sp.  
300 and *E. molesta* highly significant ( $p = 0.00$ , n = 32) and significant ( $p = 0.02$ , n =  
301 32) effect of the interaction (season and experimental condition) and I and E  
302 factor, respectively. On the contrary, all the dominant taxa showed a highly  
303 significant effect ( $p = 0.00$ , n = 32) of the season factor. Particularly, the  
304 differences between pairs of seasons showed not significant differences (Tukey  
305 test  $p > 0.05$ ; n = 32) for *Axiiothella* sp. and between autumn and winter all the  
306 cases (Tukey test  $p > 0.05$ ; n = 32). Moreover, abundance recorded in these  
307 seasons showed highly significant differences (Tukey test  $p < 0.01$ ; n = 32) with  
308 spring and summer, which were similar in most cases (Figure 4). Finally, only for

309 *E. molesta* in spring were detected significant differences (Tukey test  $p < 0.05$ ;  $n$   
310 = 32) between experimental conditions.

311

312 The nMDS showed a good representation (stress = 0.15) and grouped spring and  
313 summer samples, which were located on the opposite side of winter samples.  
314 Autumn samples were in an intermediate position close to spring-summer it. In  
315 addition, I and E faunistic composition were similar all the seasons (Figure 5).  
316 Moreover, the interaction between the factors season and experimental condition  
317 (I and E) were not significant (PERMANOVA,  $p > 0.01$ ) meanwhile the effect of  
318 each factor was it ( $p < 0.01$ ) (Table 2). The Pair-wise PERMANOVA showed  
319 highly significant differences ( $p < 0.01$ ) between all the seasons in both  
320 experimental conditions (I and E) meanwhile the differences between I and E  
321 were highly significant ( $p < 0.01$ ) only in spring.

322

323 Vertical profiles of the community parameters (S, N and  $H'$ ) showed a similar  
324 trend for the four seasons with maximum values in the first five centimeters of the  
325 sedimentary columns and a decrease with depth (Figure 6). The two-way ANOVA  
326 for each community parameter showed only significant effect of the interaction  
327 (season and experimental condition) for  $H'$ , meanwhile the effect of both factors  
328 were significant for S and  $H'$  ( $p < 0.01$ ,  $n = 32$ ) and just factor season for N ( $p <$   
329  $0.11$ ,  $n = 32$ ). Regarding the differences between experimental conditions (I and  
330 E), significantly higher values I than E were recorded in spring  $H'$  (Tukey test  $p =$   
331  $0.023$ ,  $n = 8$ , respectively). Particularly, at I condition, the highest S and N values  
332 were recorded in spring and summer while  $H'$  values were relatively constant  
333 between seasons (Figure 7). Moreover, S and N showed strong differences

334 between autumn and winter versus spring and summer (Tukey test  $p = 0.00$  n  
335 =16), and were no differences between autumn versus winter (Tukey test  $p =$   
336 0.00; n = 18). Similarly, N showed strong differences between all the seasons  
337 (Tukey test  $p = 0.00$ ; n = 16) except between autumn versus winter (Tukey test  $p$   
338 = 0.15; n = 16). Regard to E condition, S had no significant differences among  
339 autumn, winter and spring (Tukey test  $p > 0.05$ , n = 16) but did between winter  
340 and summer (Tukey test  $p = 0.029$ , n = 16). Total abundance were similar  
341 between autumn versus winter (Tukey test  $p = 0.17$ , n = 16) and spring (Tukey  
342 test  $p = 0.06$ , n = 16), and in summer was significantly higher than the other  
343 season (Tukey test  $p < 0.01$ , n = 16). Likewise, H' in autumn, spring and summer  
344 were similar (Tukey test  $p = 0.99$ , n = 16), but in winter showed differences  
345 compared to spring and autumn (Tukey test  $p = 0.006$  and  $p = 0.016$ ; n = 16,  
346 respectively).

347

348 Overall, the macrobenthic community was functionally composed of biodiffusors  
349 (surficial biodiffusors, epifaunal biodiffusors and gallery-biodiffusors),  
350 regenerators, and upward conveyors (Table 1). Upward conveyors predominated  
351 in autumn (61%) and winter (76%). Spring showed more balanced repartition  
352 between upward conveyors and biodiffusors, and this latter finally represented  
353 81% of the functional groups in summer (Figure 8).

354

### 355 3.3. Bioturbation

356 The depth distributions of the luminophores for the two particles range sizes (pink  
357 and green) were similar (Kruskal test  $p > 0.05$ ). Although the main bioturbation  
358 activity was recorded until 2 cm depth, both tracers were found buried down to

359 13 to 17 cm depth within the different sediment cores and seasons (Figure 9).  
360 The shapes of the tracer's distribution were also the same whatever the tracers  
361 and seasons showing a combination between a biodiffusive-like distribution from  
362 the surface completed by the presence of tracers deeper down. Thus, all tracers  
363 (pink and green) data were pooled together and the gallery-diffusor model  
364 (François et al., 2002) was used to calculate the apparent bio-diffusion ( $D_b$ ) and  
365 bio-advection ( $r$ ) coefficients within each core. Like previously observed for the  
366 majority of physical, chemical, and biological parameters, whatever the season  
367 the calculated coefficients did not show differences between the sediments  
368 incubated *in situ* and *ex situ* conditions (Kruskal-Wallis test  $p > 0.05$ ;  $n = 16$ ). In I  
369 conditions, nor  $D_b$  nor  $r$  showed season effect (Kruskal-Wallis test  $p = 0.5$  and  
370 one-way ANOVA  $p = 0.05$ ;  $n = 16$ , respectively). Contrary, in E conditions, highly  
371 significant differences were found for the  $D_b$  coefficient between autumn versus  
372 spring and summer versus autumn and winter (Fisher LSD test  $p < 0.01$ ;  $n = 16$ ).  
373 Moreover, the  $r$  coefficient showed significant differences between spring versus  
374 winter (Multiple comparisons test  $p < 0.01$ ;  $n = 16$ ) (Figure 10). Particularly, the  
375  $D_b$  highest average values were found in autumn and winter ( $1.71 \pm 0.14$  and  
376  $1.49 \pm 0.31$   $\text{cm}^2 \text{ year}^{-1}$ , respectively). Nevertheless, the results were highly  
377 variable in spring and summer. Finally, maximum values of  $r$  were recorded in  
378 spring and summer (Figure 10).

379

#### 380 **4. Discussion**

381 The seasonal composition and functioning (sediment reworking activity) of the  
382 intertidal macrobenthic community of Caleta Valdés (CV) applying an *in situ* and  
383 *ex situ* experimental protocol were evaluated. The macrofaunal invertebrates (>

384 0.5 mm) occupy almost every trophic level in marine ecosystems and influence  
385 the physical, chemical, and biological structure surroundings (Lenihan and  
386 Micheli, 2001). Moreover, it is recognized that soft-sediment ecosystems are  
387 driven by complex interactions between water column processes, organic matter  
388 inputs and their utilization by benthic populations, and the hydrodynamic and  
389 sedimentary conditions (Barry and Dayton, 1991; Snelgrove and Butman, 1994).  
390 The coastal sediments of shallow environments play a key role in nutrient  
391 recycling because they can provide even greater fluxes to the water column than  
392 those from the continent (Clavero et al., 2000; Niencheski and Jahnke, 2002).  
393 Moreover, numerous studies highlight the importance of physical processes such  
394 as the velocity and intensity currents as the main environmental factors that  
395 influence the benthic systems (Pastor de Ward, 2000). These factors determine  
396 the granulometry of the sediment (Brown and McLachlan, 2010; Dauvin et al.,  
397 2004; Muniz and Pires, 2000, among others) and food availability (Brown and  
398 McLachlan, 2010; McLachlan and Dorvlo, 2005), modifying the composition of  
399 the communities. In the present study, the superficial layers (0-2 cm) were  
400 enriched in fine-grained particles with a relatively high percentage of water  
401 content and organic matter, meanwhile in the sandy deeper sediment, these  
402 parameters decreased to lower and almost uniform values. Similar results were  
403 recorded in sediments from Caleta Sara (San Jorge gulf, Patagonia Argentina),  
404 applying a similar methodology (Romanut, 2019). Particularly, the benthic  
405 organisms are able to change the local geochemical conditions through the  
406 reworking of sediment particles and irrigation during feeding, excretion, and  
407 locomotion within and through different sediment layers (Flach and Heip, 1996;  
408 Kristensen and Holmer, 2001; Venturini et al., 2011). Therefore, it is not surprising



409 that in our study the bioturbation activity was concentrated mainly in the first  
410 centimeters of the sedimentary columns, i.e., 50% of the luminophores were  
411 finally retained (Gambi et al., 1998; Gambi and Bussotti, 1999; Jorein, 1999;  
412 Venturini et al., 2011) where the higher densities of macroorganisms were  
413 observed. In fact, the water-sediment interface, considered as a large sink of  
414 organic matter and oxygen, is an area of intense biological activity and  
415 remineralization (Nixon, 1981). Particularly, the combined effect of particulate  
416 and fluid transport on sediment biogeochemical processes is reflected in the  
417 vertical color transition (from brown to olive green/black) of the sediment profile  
418 (Lyle, 1983). This color transition is dictated by the change from iron  
419 (oxyhydr)oxides at the surface to black sulphidic phases at depth (Statham et al.,  
420 2017) correlating with a variety of environmental drivers (Solan et al., 2019).  
421 Moreover, depending on the biogeochemical transformations developed in this  
422 zone by the benthic communities, the sediment may be source of some nutrients  
423 and sink for others (Cabrita and Brotas, 2000).

424

425 Macrobenthic fauna is a key element of many marine and estuarine monitoring  
426 programs but those usually do not (or cannot) take into account distribution  
427 patterns at different spatial-temporal scales (Ysebaert and Herman, 2002). In the  
428 present study, the abundance of the dominant taxa and the bioturbation activity  
429 (especially in depth) were globally higher in spring-summer ( $D_b = 1.04 \pm 0.59 \text{ cm}^2 \text{ y}^{-1}$  and  $r = 12.60 \pm 3.22 \text{ y}^{-1}$ ) than in the rest of the seasons ( $D_b = 1.59 \pm 0.26 \text{ cm}^2 \text{ y}^{-1}$  and  $r = 4.81 \pm 5.85 \text{ y}^{-1}$ ). The  $D_b$  and  $r$  values recorded at CV were in the  
431 range of those measured in Mediterranean Sea lagoons, the Thau lagoon, and  
432 the Carteau Cove by Duport et al. (2007) and Gilbert et al. (2015), respectively.

434 Nevertheless, we can go no further in the comparison between sites because the  
435 sediment mixing intensity is mainly driven by population biomass (Matisoff, 1982;  
436 Reible et al., 1996), density (Duport et al., 2006; Ingalls et al., 2000; Mermillod-  
437 Blondin et al., 2001; Sun et al., 1999), and interspecific variability within functional  
438 groups (François et al., 1999). Moreover, it can be modulated by environmental  
439 conditions (e.g. Gilbert et al., 2021; Hollertz and Duchêne, 2001; Maire et al.,  
440 2007; Nogaro et al., 2008; Orvain and Sauriau, 2002; Ouellette et al., 2004).

441

442 The maximums of macrofauna abundance recorded in spring-summer in this  
443 study were similar to those found at Cerro Avanzado (Golfo Nuevo, Patagonia  
444 Argentina) which was associated with higher juvenile occurrence and primary  
445 productivity (Lizarralde et al., unpublished data). This increase could be also  
446 influenced by the closeness to the Península Valdés frontal system, which is one  
447 of the best-known tidal fronts on the northern Patagonian continental shelf  
448 (Derisio et al., 2014). These fronts are generated usually within the same water  
449 mass where the boundary between stratified (offshore) and coastal vertically  
450 mixed water is found (Sabatini and Martos, 2002). In temperate climates,  
451 seasonal thermoclines are established near the surface during late spring and  
452 summer. This structure is maintained until autumn when stratification breaks  
453 down (Acha et al., 2004; Carreto et al., 1986; Glorioso, 1987). The fronts are  
454 zones where the lateral and vertical mixes are increased producing an increase  
455 of the primary and secondary production (Mann and Lazier, 2005; Olson and  
456 Backus, 1985; Yamamoto et al., 2000). The vertical circulation not only promotes  
457 the fertilization by nutrients in the surface (Mann and Lazier, 2005) but also the  
458 sinking water with the exportation of particles and organisms to the deepest strata

459 facilitating the persistence of big invertebrates and vertebrates benthic  
460 populations (Sournia, 1994; Turley et al., 2000). The highest concentrations of  
461 *Zygochlamys patagonica* found in the region have also been related to the  
462 formation of this front (Bogazzi et al., 2005). Viñas and Ramírez (1996) have  
463 reported previously eggs and nauplii of copepods peaking in frontal waters off  
464 Península Valdés at highest chlorophyll “a” concentration zones. The importance  
465 of the fronts to the benthic communities lies not only on the high primary  
466 production but also by vertical fluxes that transport food particles to the bottom  
467 and by the weakening of the vertical stratification that allowing increasing the  
468 particulate material sink rate. This material rich in labile OM is consumed and  
469 incorporated into the benthic biomass. Particularly, all dominant taxa recorded in  
470 CV except *Axiothella* sp. were more abundant in the spring-summer period. The  
471 feeding modes are not defined specifically for *Exogone molesta* (Syllidae),  
472 *Cerathocephale* sp. (Nereididae), *Scoloplos* sp. (Orbiniidae), Oligochaeta sp.  
473 (Oligochaeta), and *Leuroleberis poulsoni* (Crustacea). Nevertheless, the  
474 members of the studied taxa generally are identifying as carnivores, filters,  
475 selective or no selective sediment feeders and scavengers, respectively.  
476 Therefore, these taxa could tend to move from the surface to the most depth zone  
477 of the sedimentary column, producing an increase of the bioturbation activity in  
478 the whole column at this period.

479

480 From a functional point of view, the overall high activity of the spring-summer  
481 community (i.e., increased  $D_b$  and  $r$  coefficients) produced a higher deep  
482 repartition of particles than those recorded in autumn and winter, even when in  
483 summer the community was dominated by biodiffusors meanwhile in autumn and

484 winter predominated upwards conveyors. This clearly demonstrates that the  
485 repartition of particles within sediments is strongly dependent of the interactions  
486 between the different functional groups rather than the presence of a dominant  
487 group and that we cannot simply assume that the dominance of one functional  
488 group automatically produces a dominant sediment reworking process within a  
489 community. In spring-summer period in CV by example, the increase of  
490 subsurface bioturbative transport may have feed the deeper advective process  
491 resulting in a global deeper burying of surface particles by the community.

492

493 Regarding *in situ* and *ex situ* comparing, in general terms, the community  
494 parameters profiles did not differ between the sediment incubated in field  
495 conditions versus those incubated in the laboratory. Moreover, there was a high  
496 similarity in the faunistic composition in both experiments. Consistently, there  
497 were no differences in the bioturbation activity of macrobenthic organisms for  
498 both experimental conditions. Thus allowing validation of an *ex situ* experimental  
499 protocol for macrobenthic community and functioning studies. To the best of our  
500 knowledge, there are no previous studies allowing such protocol to be validated.  
501 These results are particularly useful as a reliable alternative for ecotoxicological  
502 studies in protected environments where *in situ* addition of pollutants to the  
503 sedimentary matrix won't be feasible (see Ferrando et al., 2015, 2019; Sturla  
504 Lompré et al., 2018; Romanut, 2019; among others).

505

## 506 **5. Conclusions**

507 The seasonal composition and functioning of the intertidal macrobenthic  
508 community of CV were reported for the first time, contributing to the knowledge

509 of this vulnerable ecosystem threatened by anthropogenic activities. In addition,  
510 *ex situ* and *in situ* measurements demonstrated similar results, thus allowing  
511 validation of an *ex situ* experimental protocol for macrobenthic community and  
512 functioning studies. The information generated will allow assessing pollutant  
513 effects on macrobenthic communities inhabiting sediments from a natural  
514 environment without disturbing the ecosystem. In addition, these results provide  
515 a first baseline of benthic information on CV that will be helpful to monitor the  
516 effects of potential pollution in Patagonian coastal systems.

517

## 518 **6. Author contributions**

519 **Agustina Ferrando:** Conceptualization, Methodology, Investigation, Writing -  
520 Original Draft, Writing - Review & Editing, **Julieta Sturla Lompré:** Investigation,  
521 Writing - Review & Editing, **Emilia Gonzalez:** Investigation, **Marcos Franco:**  
522 Investigation, **Marta Commendatore:** Methodology, Investigation, Writing -  
523 Original Draft, Funding acquisition, **Marina Nievas:** Investigation, Writing -  
524 Review & Editing, Funding acquisition, **Cécile Militon:** Investigation, **Georges**  
525 **Stora:** Supervision, Funding acquisition, **José Luis Esteves:** Conceptualization,  
526 Methodology, Investigation, Writing - Original Draft, Project administration,  
527 Funding acquisition, **Philippe Cuny:** Investigation, Writing - Review & Editing,  
528 Project administration, Funding acquisition, **Franck Gilbert:** Conceptualization,  
529 Methodology, Investigation, Writing - Review & Editing, Project administration,  
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531

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541

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887 **Tables**888 **Table 1** Macrobenthic species mean abundance (n=4) in *in situ* and *ex situ*889 sediments for the four seasons. I: *In situ*; E: *Ex situ*

	F.G. <sup>a</sup>	Autumn		Winter		Spring		Summer	
		I	E	I	E	I	E	I	E
<b>Polychaeta</b>									
<i>Exogone molesta</i>	GB	13.8	14.0	3.3	0.3	47.5	1.8	210.5	198.0
<i>Ceratocephale</i> sp.	GB	1.8	1.5			41.5	19.5	34.3	29.3
<i>Axiiothella</i> sp.	UC	16.3	21.8	13.5	17.3	18.8	19.0	6.5	9.0
<i>Scoloplos</i> sp.	GB	0.5	2.3	0.3		31.0	19.8	4.8	5.3
<i>Levinsenia gracilis</i>	SB	1.5	5.8	0.3		0.8			0.3
<i>Pionosyllis</i> sp.	GB					2.3		4.8	1.5
<i>Capitella "capitata"</i>	UC			2.5	5.8				
<i>Chone mollis</i>	SB	0.8	2.5			1.3	0.3	0.8	0.8
<i>Brania</i> sp.	GB	0.3							
<b>Oligochaeta</b>									
Oligochaeta sp.	GB	15.5	16.0	1.0	6.5	28.5	32.5	37.5	58.8
<b>Crustacea</b>									
<i>Leuroleberis poulseni</i>	SB	8.3	10.8	0.8	0.5	31.5	23.0	26.5	34.5
Ostracoda sp. 1	SB	2.8	2.5	17.8	8.0	15.5	8.0	6.0	5.3
<i>Phoxocephalopsis</i> sp.	SB	1.3	2.8	4.8	2.3		1.5	0.8	0.8
Ostracoda sp. 2	SB			5.8	2.5		0.5	0.5	4.0
<i>Anacalliax argentinensis</i>	GB	1.5	2.8	3.0	0.8	0.8	0.3	2.0	0.8
<i>Paranthura</i> sp.	SB	0.5	0.8					2.0	2.5
Gammaridae sp. 1	SB	3.3		0.3			0.3	0.3	
<i>Paranebalia</i> sp.	GB			1.8	0.8				1.3

<i>Caprella scaura</i>	SB	0.5	0.5	1.0			
Pseudocumatidae sp.	SB				0.8	1.0	
<i>Cyrtograpsus angulatus</i>	R	0.8			0.3		
Cirolanidae sp.	SB			0.3		0.3	0.3
Gammaridae sp. 2	SB		0.5		0.3		
Corophioidea sp.	SB						0.5
Leptostraca sp.	EB			0.3			
Anthuroidea sp.	SB					0.3	
<b>Nemertea</b>							
Nemertea sp.	GB				1.5	0.5	

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891 <sup>a</sup> F.G.: Functional group; Biodiffusors species presented in three sub-groups as  
892 EB: Epifaunal biodiffusors; SB: Surficial biodiffusors and GB: Gallery biodiffusors;  
893 R: Regenerators; UC: Upward conveyors. For a complete description of the  
894 different functional groups, see Kristensen et al. (2012).

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896 **Table 2** Summary PERMANOVA. Res: Residuals; df: Degrees of freedom; SS:  
897 Sum of squares; MS: Middle squares; mc: Monte Carlo simulation; \*\*  $p$  (mc) <  
898 0.01

Source	df	SS	MS	Pseudo-F	$p$ (mc)
Experimental condition	1	2013.5	2013.5	5.9166	0.001**
Season	3	15226	5075.4	14.914	0.0001**
Experimental condition x Season	3	1240.5	413.51	1.2151	0.2721
Res	24	8167.5	340.31		
Total	31	26648			

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## 908 **Figures captions**

909 **Figure 1.** Sampling site in Caleta Valdés (CV; Península de Valdés, Patagonia  
910 Argentina). *Ex situ* cores sampled and *in situ* cores embedded in the intertidal  
911 zone.

912 **Figure 2.** Mean contribution percentage of each granulometry fraction with depth  
913 (n = 4) in Caleta Valdés sediments

914 **Figure 3.** Water content and organic matter (OM) by core (mean  $\pm$  SD; n = 4)  
915 measured in Caleta Valdés sediments for the four different seasons. I: *In situ*; E:  
916 *Ex situ*. Significant differences ( $p < 0.05$ ) tested between conditions are  
917 represented with different letters (uppercase for *in situ*, and lowercase for *ex situ*).  
918 For each season, significant differences ( $p < 0.05$ ) between *in situ* and *ex situ* are  
919 represented with an asterisk

920 **Figure 4.** Abundance of the dominant species by core (mean + SD; n = 4) in  
921 Caleta Valdés sediments for the four seasons. I: *In situ*; E: *Ex situ*. Significant  
922 differences ( $p < 0.05$ ) tested between conditions are represented with different  
923 letters (uppercase for *in situ*, and lowercase for *ex situ*). For each season,  
924 significant differences ( $p < 0.05$ ) between *in situ* and *ex situ* are represented with  
925 an asterisk

926 **Figure 5.** Nonmetric multidimensional scaling (nMDS) analysis (Bray-Curtis  
927 index; group average link; square root) of total abundance by core (n = 4) in  
928 Caleta Valdés sediments for the different four seasons. I: *In situ*; E: *Ex situ*

929 **Figure 6.** Macrobenthic community parameters by layer (mean + SD; n = 4) in  
930 the *in situ* and *ex situ* Caleta Valdés sediments for the four seasons. S: Specific  
931 richness; N: Total abundance; H': Shannon diversity I: *In situ*; E: *Ex situ*.

932 **Figure 7.** Macrobenthic community parameters by core (mean + SD; n = 4) in  
933 Caleta Valdés sediments for the four seasons. I: *In situ*; E: *Ex situ*. Significant  
934 differences ( $p < 0.05$ ) tested between conditions are represented with different  
935 letters (uppercase for *in situ*, and lowercase for *ex situ*). For each season,  
936 significant differences ( $p < 0.05$ ) between *in situ* and *ex situ* are represented with  
937 an asterisk

938 **Figure 8.** Functional groups in Caleta Valdés sediments (pooled data between *in*  
939 *situ* and *ex situ* sediments) (n = 8) for the four seasons. Biodiffusors species  
940 presented in three sub-groups as EB: Epifaunal biodiffusors; SB: Surficial  
941 biodiffusors and GB: Gallery biodiffusors; R: Regenerators; UC: Upward  
942 conveyors. For a complete description of the different functional groups, see  
943 Kristensen et al. (2012)

944 **Figure 9.** Luminophores percentage (pooled data between both size of  
945 luminophores particles) in each layer (mean + SD; n = 8) for the *in situ* and *ex*  
946 *situ* Caleta Valdés sediments by season

947 **Figure 10.** Bio-diffusion ( $D_b$ ) and bio-advection ( $r$ ) coefficients in Caleta Valdés  
948 sediments (pooled data between both size of luminophores particles) by season  
949 and experimental condition (mean  $\pm$  SD; n = 8) Significant differences ( $p < 0.05$ )  
950 tested between conditions are represented with different letters (uppercase for *in*  
951 *situ*, and lowercase for *ex situ*). For each season, significant differences ( $p < 0.05$ )  
952 between *in situ* and *ex situ* are represented with an asterisk

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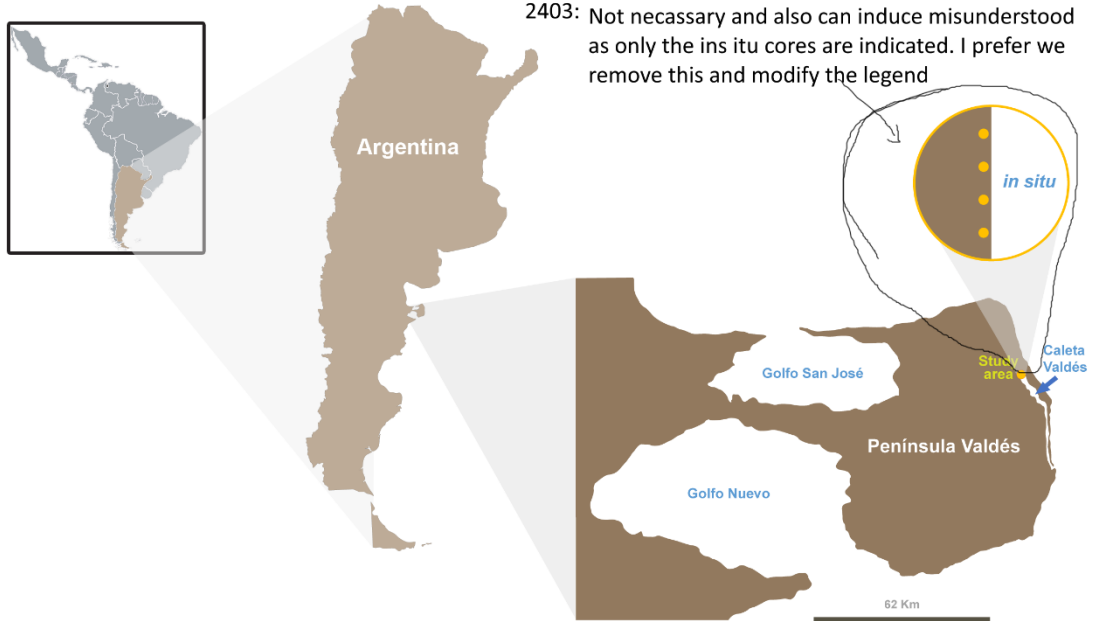




955 Fig.1

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2403: Not necessary and also can induce misunderstanding as only the ins itu cores are indicated. I prefer we remove this and modify the legend

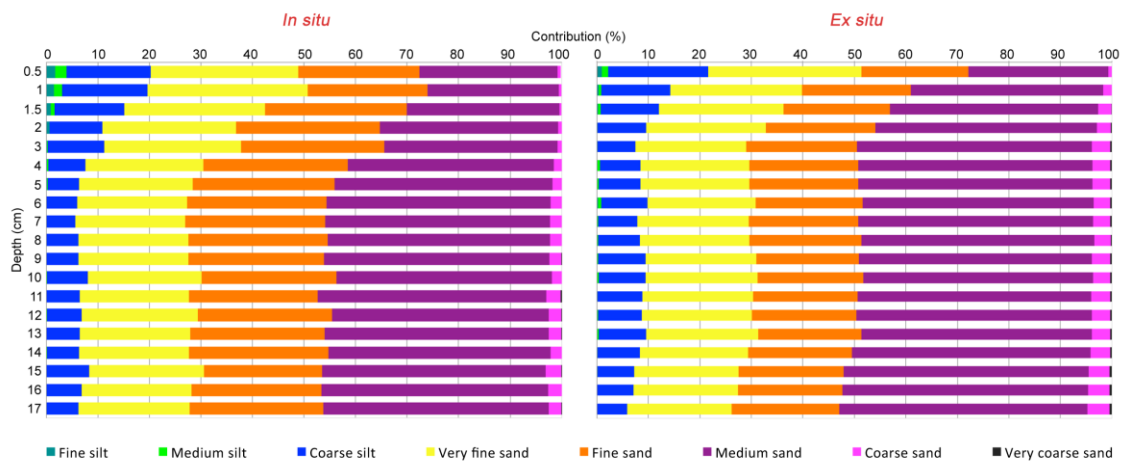
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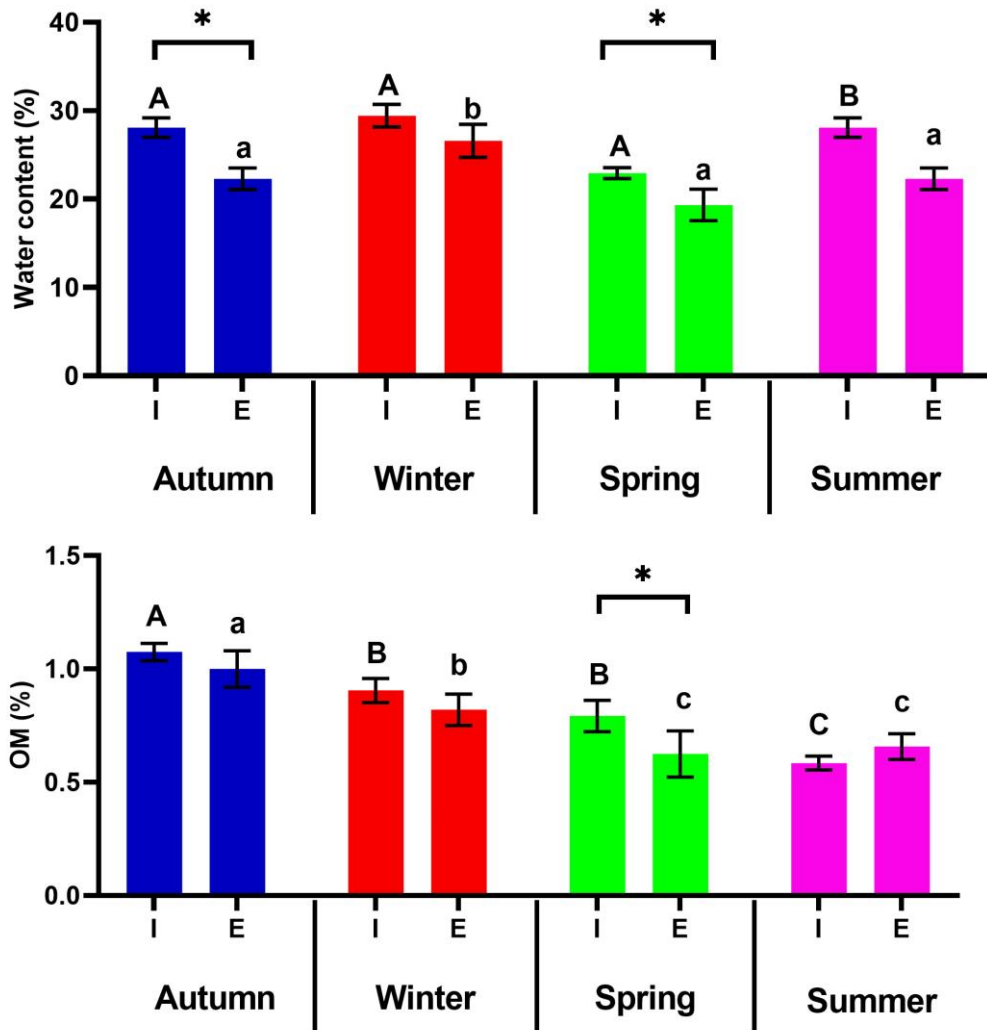
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982 Fig. 3



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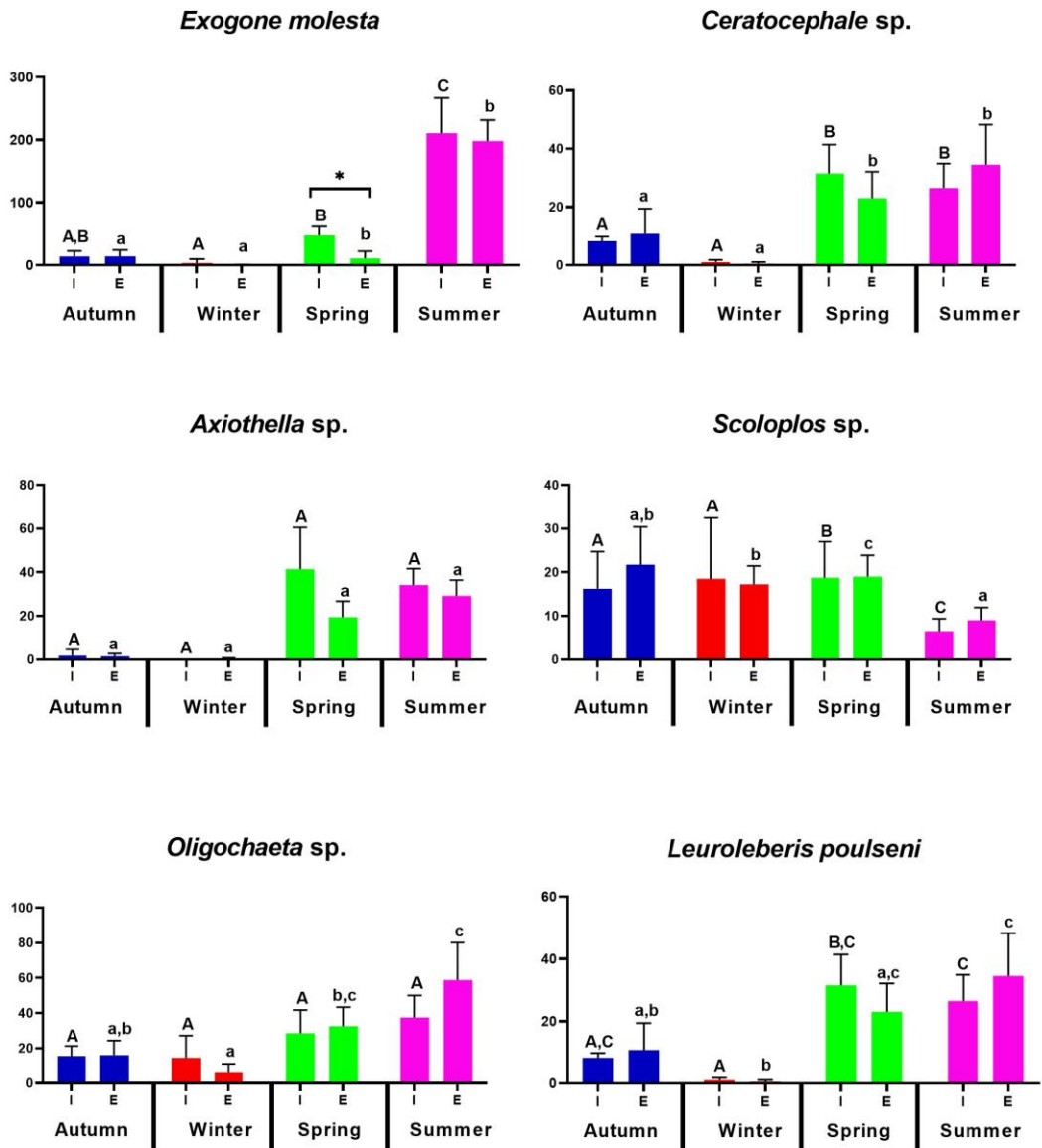
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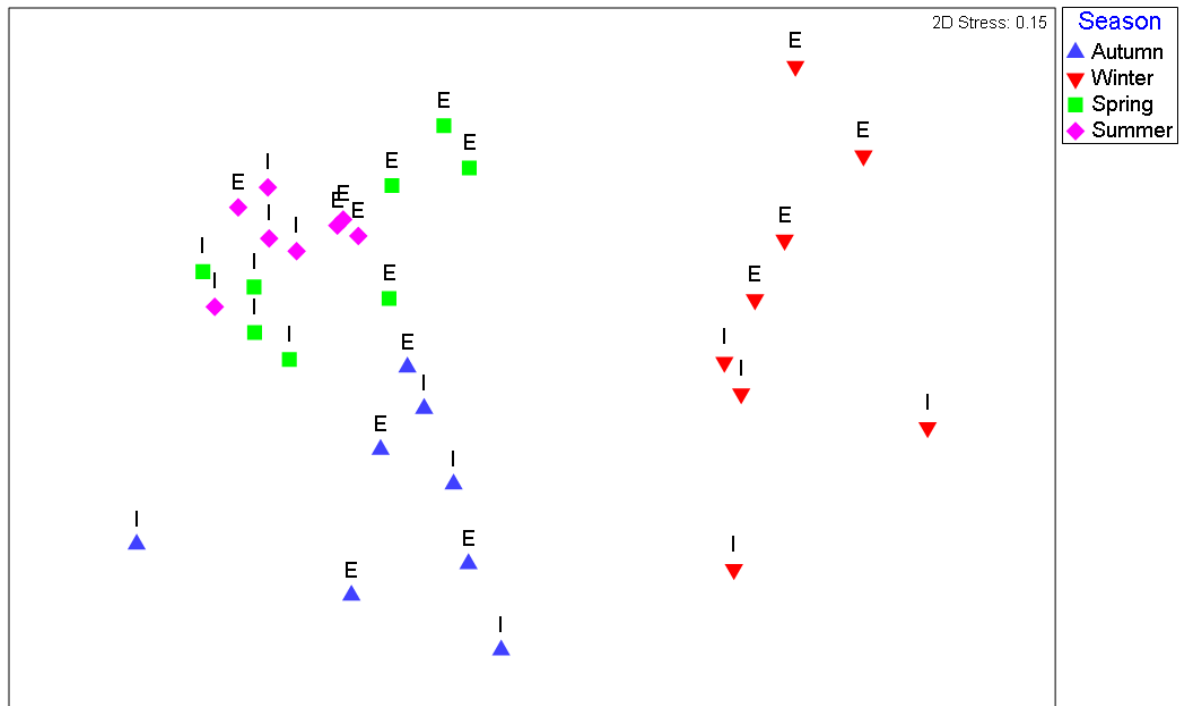
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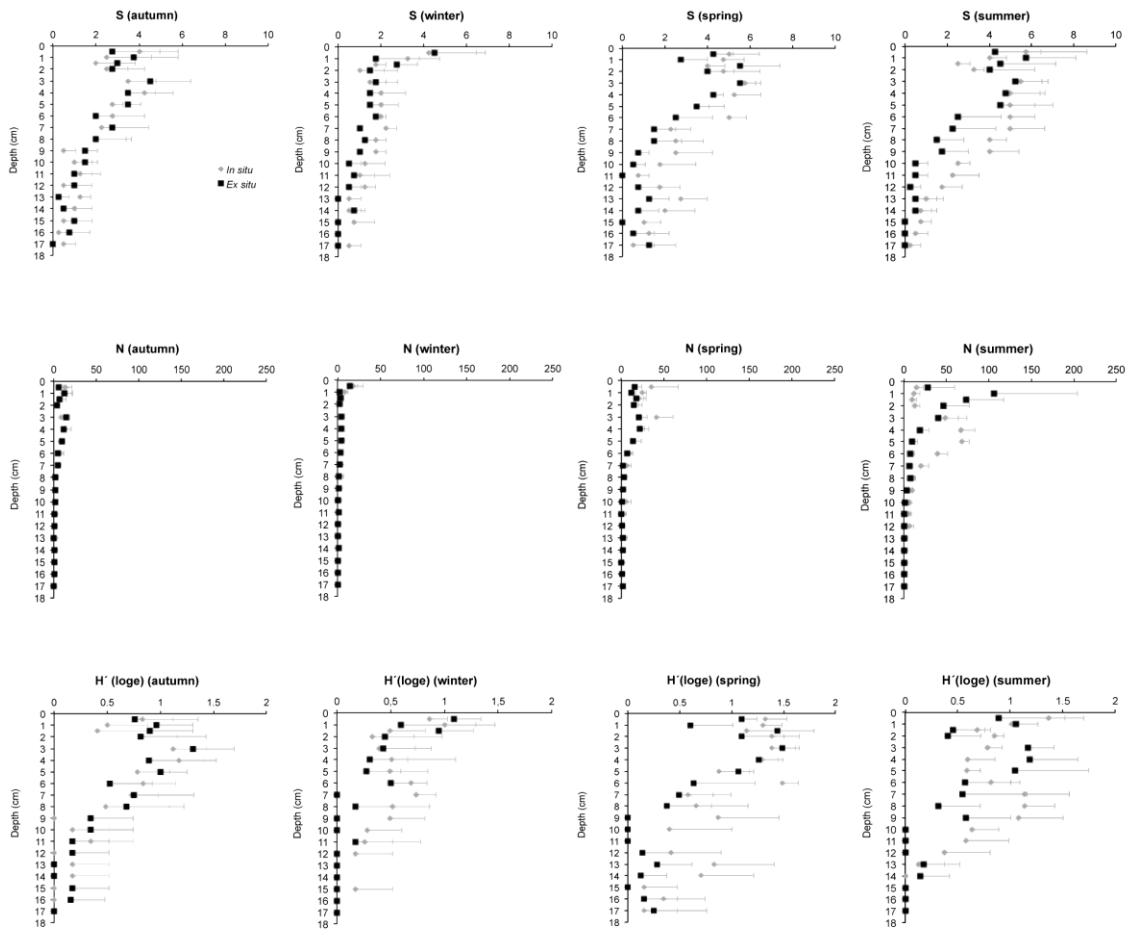
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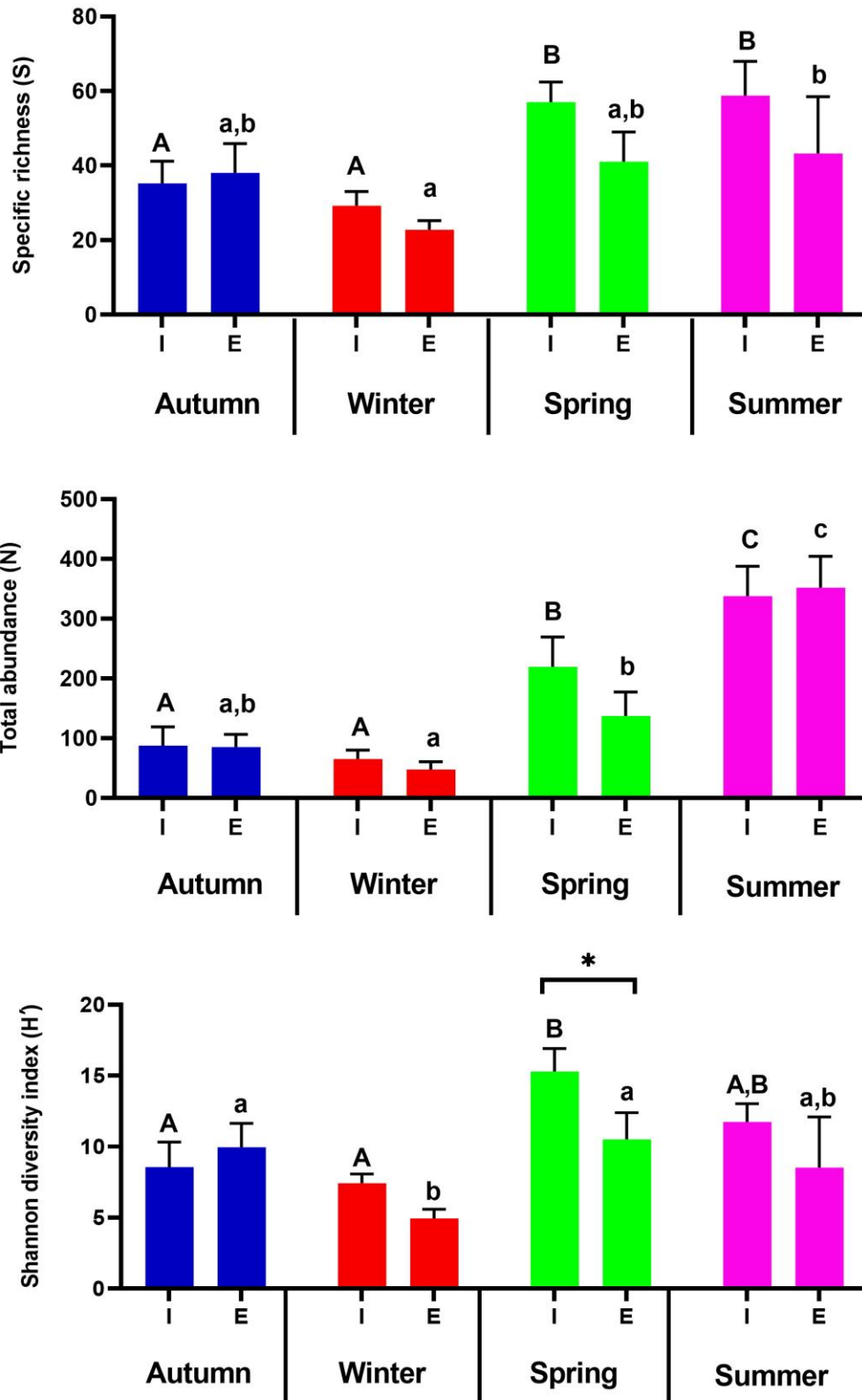
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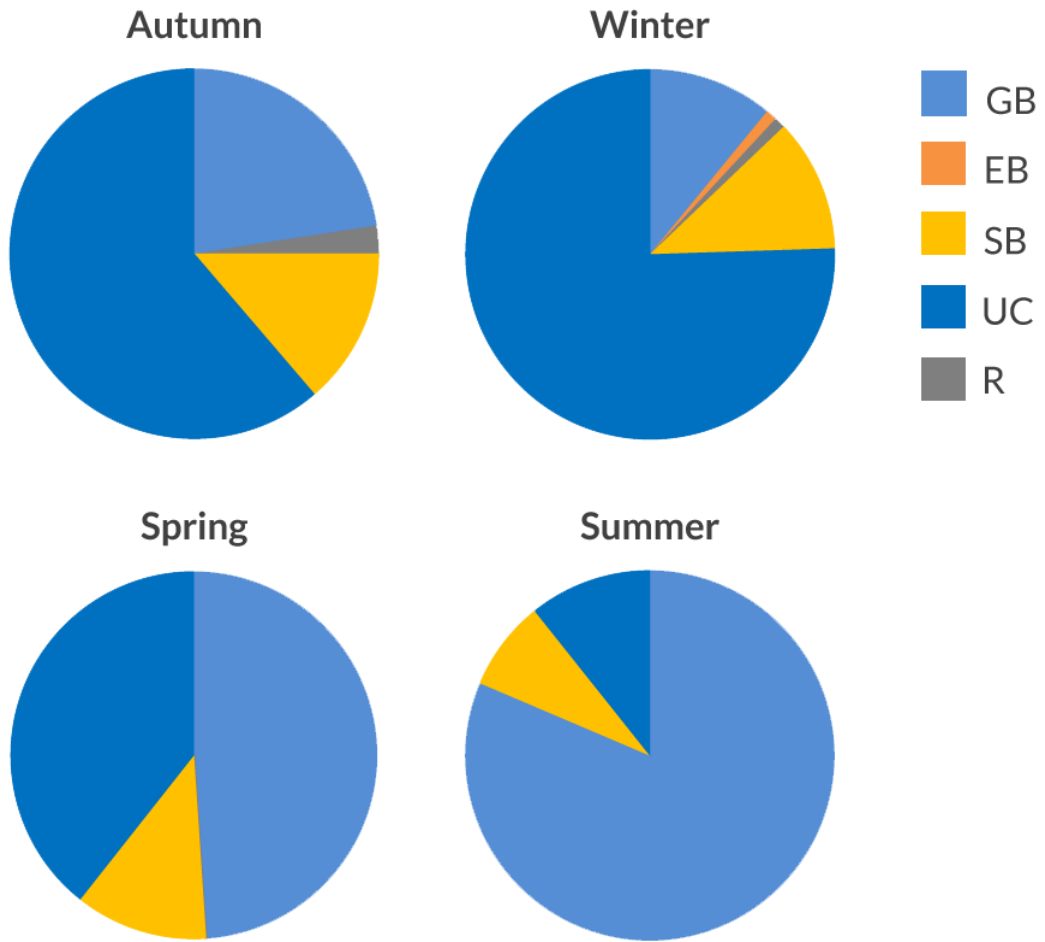


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1034 Fig. 8

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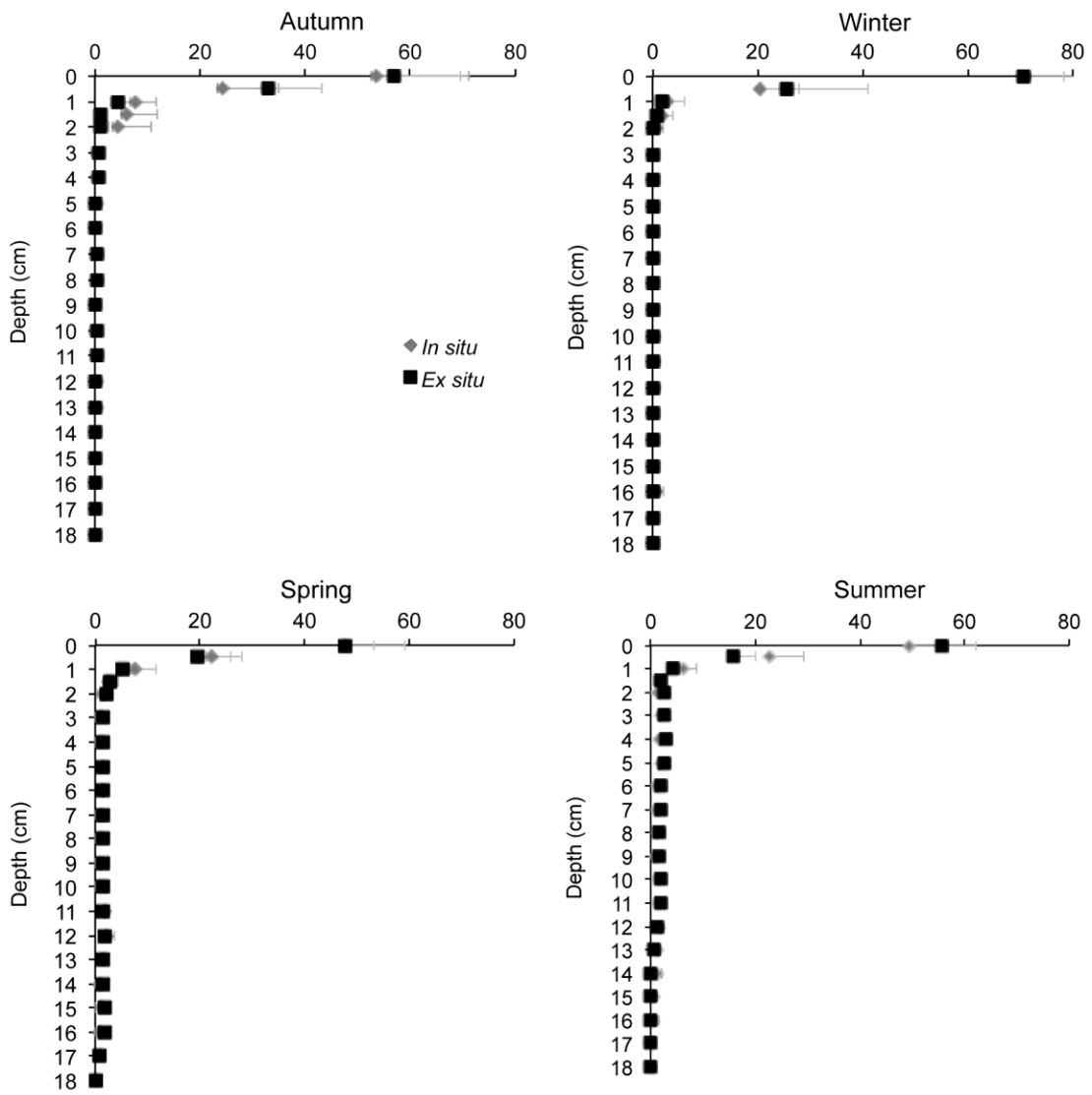
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1046 Fig. 9



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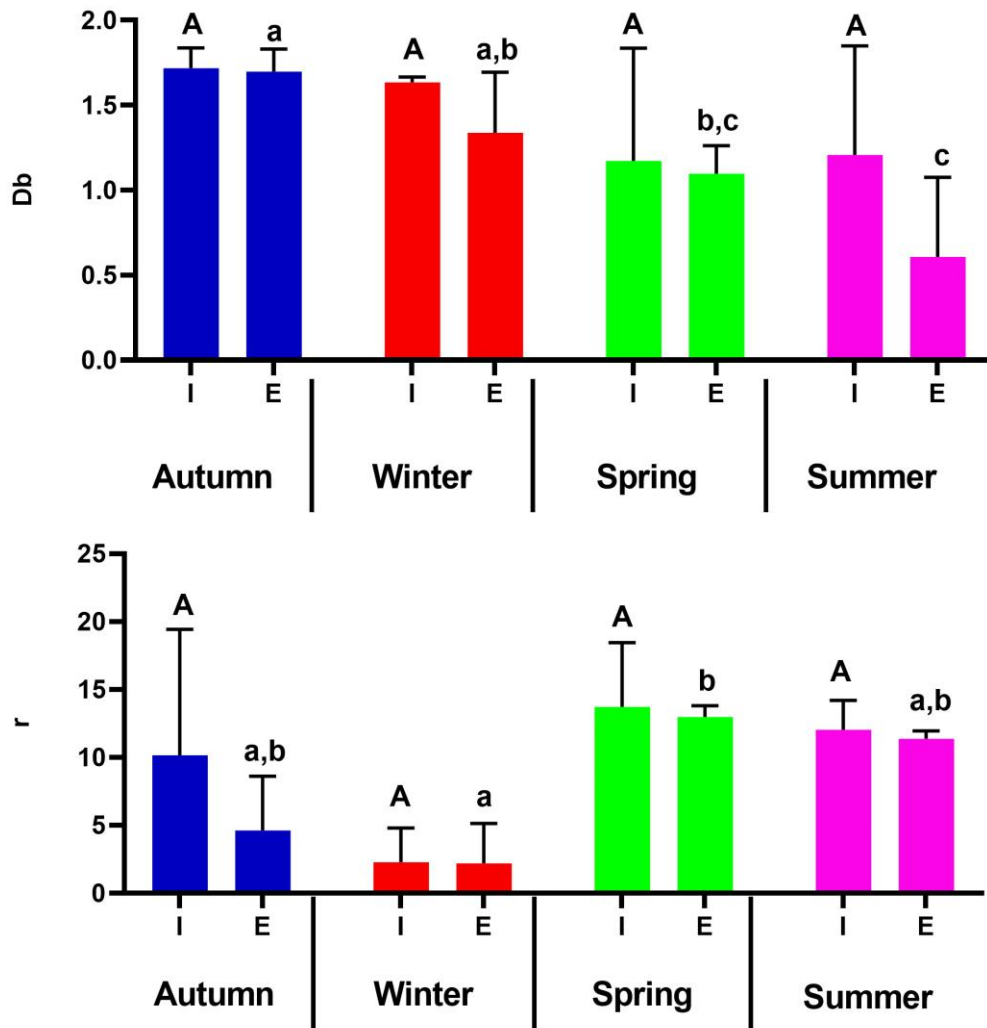
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1056 Fig. 10



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