



28 **Keywords**

29 Nutrient enrichment, carbohydrate, oligotrophic soft-water lakes, sucrose, starch, aquatic  
30 macrophytes.

## 31 Introduction

32 Local and regional eutrophication are at the origin of major biodiversity loss for submerged  
33 aquatic vegetation due to both, organic matter (OM) load to sediments and light environment  
34 deterioration (Hautier et al., 2009; Moss et al., 2013, among others). Soft-water oligotrophic  
35 lakes from Northern and Central Europe (e.g. Denmark and the Netherlands) have experienced  
36 eutrophication at a regional scale derived by intensive agriculture, which peaked during the late  
37 twentieth century (Portielje & Van der Molen, 1997; Baastrup-Spohr et al., 2013), with  
38 consequent major impacts for aquatic macrophytes (e.g. Arts, 2002; Sand-Jensen et al., 2000;  
39 Pulido et al., 2012). Further appropriate management has resulted in a significant improvement  
40 of water quality. Also, some projects have focused on restoration actions devoted to improve  
41 and, whenever possible, recover some of these ecosystems and its endangered flora (i.e.  
42 isoetids *Lobelia dortmanna*, *Littorella uniflora* and *Isoetes* spp.; Pulido et al., 2011a, 2011b;  
43 Baastrup-Spohr et al., unpublished data). However, isoetid plant recovery has been found to be  
44 low and requires further research.

45 The Pyrenean high mountain range (Southern Europe) holds hundreds of soft-water oligotrophic  
46 lakes and ponds that shelter an important representation of European isoetids in its southern  
47 geographical limit of distribution (Murphy, 2002; Gacia et al., 1994). Signs of regional increase  
48 in N deposition (Catalan et al., 1994) and shifts towards lake P eutrophication have been  
49 detected within the last decades in the Pyrenees (Camarero & Catalan, 2012), while livestock  
50 farming and damn construction have locally threatened few of those systems (Gacia &  
51 Ballesteros, 1996, 1998; Chappuis et al., 2011).

52 *Isoetes lacustris* is a rather common aquatic perennial quillwort present in soft-water  
53 oligotrophic lakes throughout Europe. This species plays a key role in the systems it colonizes  
54 by increasing redox potential and subsequently oxidizing the soluble nitrogen forms found in  
55 sediment pore-water due to high root oxygen leaking (Smolders et al., 2002; Gacia et al., 2009).  
56 This increase in the redox potential leads to an enhancement of denitrification (Risgaard-  
57 Petersen & Jensen, 1997) and phosphorous immobilization that fosters the recovery to  
58 oligotrophic conditions (Jones et al., 1994). Moreover, *I. lacustris* has a CAM photosynthetic

59 pathway (Gacia & Ballesteros, 1993a), which is a carbon dioxide concentrating mechanism that  
60 diminishes the threat of carbon starvation in soft-water lakes (Keeley, 1998).

61 Light is an essential resource for plant photosynthesis that can often limit growth and  
62 reproduction. Variation of irradiance results in changes from the physiological up to the  
63 community level. In aquatic systems, light may be strongly reduced by water column  
64 characteristics (i.e. water colour, suspended solids concentration and plankton) and by  
65 epiphytes presence (Bornette & Puijalon, 2001). A way to bypass a reduced irradiance income  
66 is to increase the photosynthetic surface area, a response already described for *I. lacustris*  
67 (Gacia & Ballesteros, 1993b). However, when light irradiance is below the compensation point,  
68 *I. lacustris* declines rapidly and many plants die after two months (Rorslett & Johansen, 1995).

69 Sediment organic matter is also an important factor that influences the growth and distribution of  
70 aquatic plants (e.g. Barko & Smart, 1983, 1986; Sousa et al., 2009). Specifically, *I. lacustris*  
71 thrives in oligotrophic lakes, where growth can be nutrient limited (Gacia & Ballesteros, 1994).  
72 Previous *in situ* experiments showed that fertilized plots produced more leaves and lost fewer  
73 leaves than unfertilized plots (Gacia, 1992). However, *I. lacustris* populations decline and  
74 disappear if nutrient increase persists and reaches eutrophic conditions (Arts, 2002; Salgado et  
75 al., 2010; Kowalewski et al., 2013).

76 Carbohydrates synthesis and allocation in the plant depend on light and nutrients, among other  
77 factors. Patterns of allocation are critically important for plant growth (Levin, 2009). Most of the  
78 carbon dioxide assimilated by plants during photosynthesis is channeled into the synthesis of  
79 soluble carbohydrates to be exported to sink organs, where they are stored as non-soluble  
80 carbohydrates (Rosa et al., 2009). Under abiotic stress plant soluble carbohydrates fluctuate,  
81 carbon dioxide assimilation changes and source-sink partitioning also changes (Rosa et al.,  
82 2009). Carbohydrate production and allocation has been studied for some aquatic plants (e.g.  
83 Keeley et al., 1983; Vermaat & Verhagen, 1995), but not for *I. lacustris*.

84 Light reduction and organic matter enrichment are two major environmental problems in aquatic  
85 systems. Although efforts to reduce eutrophication have been successful in some countries, the  
86 situation is getting worse worldwide. In addition, reduction in nutrient load has not been  
87 sufficient in many systems to restore eutrophic water bodies (Scheffer et al., 1993; Brock &

88 Starrett, 2003) mainly due to the high organic matter accumulated in lake sediments. Actually,  
89 both factors co-occur when lakes are dammed, under cattle pressure or even with the operation  
90 of aquaculture facilities (see Kraska et al., 2013). Damming results in a prompt reduction of light  
91 because of water level increase and re-suspension of sediments and particles coming  
92 especially from the newly flooded area (Gacia & Ballesteros, 1998). In parallel, water column  
93 nutrients and suspended organic matter increases because of the decomposition of flooded  
94 terrestrial vegetation and the eventual die-off of aquatic vegetation (Gacia & Ballesteros, 1998;  
95 Camargo et al., 2005). Cattle waste may be deposited on adjacent land and enter the lake  
96 through runoff or enter straight into the lake when cattle are standing on the shallow littoral  
97 areas (Croel & Kneitel, 2011; Chappuis et al., personal observations). Dung and urine input  
98 results in an increase of nutrients and turbidity related to algal proliferation (Croel & Kneitel,  
99 2011; Bond et al., 2014). Cattle waste may have global effects on ponds and small shallow  
100 lakes but only shows local effects on big lakes (Riera et al., 2014). When it comes to  
101 submerged aquatic plants, the slow growing isoetids are the less competitive in front of  
102 eutrophication. Therefore, it is of particular interest the study of the impact of the interaction  
103 between reduced light and increased organic matter in sediments, in *Isoetes lacustris*, a widely  
104 extended species highly threatened by eutrophication.

105 In this study, we aim at experimentally testing the relative importance of light availability, organic  
106 matter enrichment, and its interaction in the collapse of healthy populations of *I. lacustris*. To do  
107 so we conducted an *in situ* shading and sediment organic matter enrichment experiment in a  
108 small Pyrenean pond vegetated with a dense *I. lacustris* population. The experiment was  
109 conducted in the course of the growing period (July to September). We followed plant  
110 performance by using growth indicators (number of leaves, leaf length, and leaf production),  
111 individual survival (mortality) and carbon balance indicators (levels of soluble and non-soluble  
112 carbohydrates in leaves and corms).

## 113 **Methods**

114 The experiment was conducted in summer 2006 in Lake Baciver Petit (42° 41' 49"N, 0° 59'  
115 52"E), a remote pond located in the Central Pyrenees (Val d'Aran, Spain) at 2307 m a.s.l. and  
116 only accessible by foot. Lake Baciver Petit is surrounded by lakes and ponds in a plateau

117 covered by alpine grasslands where livestock roams (o remains) for a couple months in  
118 summer. The pond is small (1 ha), shallow (2 m of maximum depth) and has oligotrophic soft-  
119 waters (see Gacia et al., 2009 for detailed water chemistry composition). The pond had two  
120 differentiated communities: a monospecific population of *I. lacustris* and a mixed assemblage  
121 with *Isoetes echinospora*, *Sparganium angustifolium* and *Subularia aquatica* (Gacia et al.,  
122 2009). The *I. lacustris* population completely covered the bottom of the lake (100 % cover), with  
123 an average leaf biomass of 244.2 g/m<sup>2</sup> (Gacia et al., 2009) and an average belowground  
124 biomass of 195.1 g/m<sup>2</sup> (unpublished data of the authors).

### 125 The experiment

126 To test for the impact of light reduction and organic matter enrichment in *I. lacustris*, we  
127 conducted an *in situ* experiment where plots of the population were manipulated to get different  
128 levels of light attenuation and fertilization. A total of 18 plots of 30 x 30 cm were randomly  
129 chosen within the dense *I. lacustris* population situated at 1.6 m depth. Each plot, containing  
130 both, plants and sediment, was detached from the bottom and brought to pots of the same area  
131 for easier manipulation. The plots were moved close to water surface for labeling and leaf  
132 marking and further returned on site to follow leaf growth, plant performance and plant mortality  
133 over the course of the experiment. Previous essays showed that this procedure did not affect *I.*  
134 *lacustris* performance or survival (Gacia & Ballesteros, 1994).

135 The experimental design consisted of a two-factorial orthogonal model where light and organic  
136 matter enrichment were fixed factors. Light treatment had three levels (control, 65% and 35% of  
137 incident light) and organic matter enrichment had two levels (control and 10% organic matter  
138 enrichment). The light treatments at 65% and 35% irradiance will be referred to as L65 and L35,  
139 respectively. Levels of light attenuation correspond to the average light levels found at the lower  
140 bathymetric distribution of *Isoetes lacustris* in the lakes present in the area of study (L65) and at  
141 5.5 meters depth (L35), where no *Isoetes lacustris* are found in non-disturbed lakes, although  
142 they can survive there if transplanted or after damming (authors pers. obs.). Light attenuation  
143 was obtained with neutral shading screens set at 20 cm above the plant canopy and covering  
144 an area wider than the plots to avoid light coming from the sides. The percentage of light  
145 attenuation was measured below the water surface and below the screen at canopy level at

146 noon on a clear day using a SPQA spherical quantum sensor LiCor coupled to a LiCor Li-1000  
147 data logger. Organic matter enrichment was achieved by covering the plots with a 1 cm layer of  
148 lake sediment mixed with fresh horse faeces collected from nearby grasslands. The mixture was  
149 prepared *in situ*, spread evenly on top of the plots forming a layer at the base of the plants that  
150 covered only the first centimetre of the leaves of the 9.8 cm canopy height. Samples of the lake  
151 surface sediment and of the homogenised mixture with horse faeces were taken to the  
152 laboratory and their organic matter content was calculated by weight loss-on-ignition following  
153 the protocol described in Chappuis et al. (2014). Results showed that the mixture had reached  
154 an organic content enrichment of the 10 % of the dry weight (DW), which would correspond to  
155 levels found in a pond highly frequented by livestock. Dung input leads to an increase of  
156 nutrients in the sediment and also increases the adjacent water orthophosphate, conductivity  
157 and turbidity, while dissolved oxygen decreases (Croel & Kneitel, 2011), which are water quality  
158 changes related to a shift toward a eutrophic state. Plots with organic matter enrichment were  
159 located at least 3 m away from the non-enriched plots to avoid contamination.

160 *Isoetes lacustris* performance and plant survival was followed from early summer (July, 14th) to  
161 early fall (September, 26th), the period of maximum leaf growth for this species (Gacia &  
162 Ballesteros, 1994). Twelve plants from each plot were randomly selected and the leaves  
163 marked using the method described in Gacia & Ballesteros (1994). Plant performance was  
164 assessed by measuring the number of leaves produced and lost over the course of the  
165 experiment, total leaf length and leaf biomass production at the end of the experiment. To  
166 reproduce the leaf growth model under each treatment, 32 non marked plants were collected  
167 and the leaves of each individual sorted and ordered from the inner (1) to the most external (n)  
168 position. The length of each leaf was measured and mean biomass (dry weight) of each leaf  
169 was calculated according to its position in the corm. A leaf growth function was fitted for each  
170 treatment and leaf biomass production was estimated (Gacia & Ballesteros, 1994). From each  
171 experimental treatment, six individuals were frozen to further analysis of total and soluble  
172 carbohydrate content.

173 Sediment redox potentials were measured in the field at the end of the experiment. Two  
174 replicates were done per treatment and measures were taken from 0 to -8 cm as described in  
175 Gacia et al. (2009).

176 Carbohydrate analysis

177 Since there was no previous information on the presence of carbohydrates in *I. lacustris* we first  
178 verified and determined the carbohydrate concentration in all organs (i.e. leaves of different  
179 ages, corms and roots) of control plants. We sorted and analysed independently leaves of  
180 different ages (i.e. different position in the rosette) based on the fact that young growing leaves  
181 of aquatic plants usually have higher soluble carbohydrate content compared to older ones  
182 (Alcoverro et al., 2001 and others).

183 Consequently, we sorted leaves in age classes: a) position 1 and 2 from the centre of the  
184 rosette, corresponding to the youngest leaves that were grown during the course of the  
185 experiment (latest two months); b) positions 3 to 5 corresponding to leaves that may have  
186 partially grown over the course of the experiment and that were born in the same summer; and  
187 finally c) positions 6 to 9 which had to be born in the previous growing season (see Gacia &  
188 Ballesteros, 1994). Corms and roots were analysed separately. We did not further proceed to  
189 analyse the reserves in roots because levels were below detection in control plants. Samples of  
190 the five control individuals were analysed together in a composite for the different tissues and  
191 leaf ages and so were 2-3 individuals of the same plot resulting in nine replicates per treatment.

192 All plant tissues were analysed following the method described in Alcoverro et al. (1999). Starch  
193 and sucrose were expressed as percentage of dry weight (DW). Sucrose and starch contents  
194 were added to obtain the total non-structural carbohydrates (TNC) of each organ. The total  
195 amount of carbohydrate per plant was calculated considering the relative weight and  
196 carbohydrate concentration of the corm and each leaf.

197 Statistical analysis

198 Data were analysed with a 2-Factorial ANOVA. When the interaction was significant (i.e. leaf  
199 length) we run one-way ANOVA per each factor independently. When the analysis of variance  
200 rejected the null hypothesis of no difference in light treatments, we used a post-hoc test  
201 (Tukey's HSD test) to determine which groups differed. We run a one-way ANOVA to test for  
202 carbohydrate differences among corm and leaf age classes of reference plants. Occasionally,  
203 ANOVA assumptions of normality (Chi-square test) and heteroscedasticity (Cochran test) were

204 not met but provided that the working sampling size was large enough, we considered that the  
205 ANOVA F statistic should be robust enough (Underwood, 1981). When such assumptions were  
206 not met, we set the significance level to  $p > 0.01$  to minimize the risk of making a type I error. All  
207 statistical analyses and graphs were performed with the software R (version 2.12.2) and p-value  
208 was set at 0.05.

209 It would be interesting to make comparisons among treatments of the total, leaf and  
210 belowground biomass of the plants, as well as the total amount of carbohydrates in corms,  
211 leaves or plants. However, we did not make these comparisons, as data was biased and not  
212 representative of the average individuals for some treatments. The surviving individuals of the  
213 fertilization treatment (especially when combined with light reduction: L65+OM and L35+OM)  
214 had few leaves and small corms in average. We had to select the biggest plants within the  
215 fertilized treatments in order to have enough biomass to analyse the carbohydrate content. In  
216 contrast, average plants were selected for the non-fertilized treatments. However, we tested  
217 carbohydrate concentration as it is a relative measure and thus we assumed that the bias was  
218 much reduced.

## 219 **Results**

### 220 Growth indicators and mortality

221 The growth response of *I. lacustris* in the different plots after 72 days of the beginning of the  
222 experiment is compiled in Table 1 and Fig. 1. The reduction of light in non-fertilized plots  
223 resulted in a general lengthening of the new leaves, at both L65 and L35 treatments. No other  
224 significant effects were detected in *Isoetes* morphology, growth and mortality because of light  
225 reduction. On the contrary, multiple effects were detected on plants undergoing organic matter  
226 enrichment (Table 1 and Figure 1). Specifically, organic matter enrichment led to a decrease in  
227 the number of new leaves and an increase in leaf mortality that consequently resulted in a  
228 decrease of leaf production. The general leaf lengthening associated with low light irradiances  
229 negatively interacted with the increase in organic matter in the sediment, resulting in lower  
230 lengthening than expected for the plots fertilized and under a 35% of incident light (L35+OM;  
231 Table 1 and Fig. 1). Leaves of plants from the organic enriched plots showed necrotic spots and  
232 a lower turgidity than plants from reference plots.

233 No plant mortality was detected at reference and at L65 light conditions. Low mortalities (8.3%  
234 on average) were measured in plots at L35 (Fig. 1), although this reduction was not significant  
235 (see Table 1). In contrast, organic matter enrichment not only reduced leaf production but also  
236 increased significantly plant mortality, especially when combined with light reduction to an  
237 average of 34% of dead plants (see Fig. 1).

238 Redox potential displayed similar values along the first 8 cm of sediment under reference  
239 conditions as previously reported for *I. lacustris* populations (Gacia et al., 2009). Although  
240 ANOVA did not detect changes in redox potential, it ranged between an average value of 413  
241 mV under reference conditions and an average value of 274 mV under organic matter  
242 enrichment and at L35 light conditions.

243 Carbon balance indicators

244 *Isoetes lacustris* at reference conditions showed presence of both sucrose and starch in leaves  
245 and corms (Fig. 2). The amount of sucrose varied for leaves occupying different position in the  
246 plant rosette (i.e. having different ages). The youngest leaf, occupying the first and central  
247 position, had the highest sucrose content (18% DW), followed by the leaf occupying the second  
248 position (12% DW) and then the remaining leaves that showed similar lower values (< 8.5%  
249 DW; Fig. 2). The starch content of leaves showed low variation among different leaf ages (Fig.  
250 2). The corm had a very large amount of starch (up to 20% DW) while the amount of sucrose  
251 was lower (3.6% DW) than for the leaf tissue. We did not detect either sucrose or starch in the  
252 roots. Leaves 1 and 2 had significantly more percentage of sucrose than the remaining leaves  
253 and the corm (Table 2). Corm had a significant higher concentration of starch than the leaves  
254 (Table 2).

255 The patterns of sucrose and starch accumulation in the plants were consistent through the  
256 treatments (Fig. 3). Hence, sucrose (as percentage of dry weight) was always higher in the  
257 leaves compared to the corm regardless of the experimental treatments, while the opposite  
258 occurred for the starch. Sucrose increased in corm and all leaves especially under L35 light  
259 conditions and organic matter enrichment (Table 3 and Fig. 3). Corm starch was significantly  
260 reduced at L35 light treatment (Table 3 and Fig. 3).

261 Similar trends were observed when analyzing the total carbohydrate percentage per plant  
262 (Table 3 and Fig. 4): light reduction at L35 increased sucrose and decreased starch, and  
263 organic matter enrichment also increased sucrose. Despite these changes, the total non-  
264 structural carbohydrates (TNC, total sucrose and starch) did not significantly change though the  
265 different treatments (Table 3 and Fig. 4). However, the proportion between sucrose and starch  
266 did change as observed in the ratio sucrose:TNC (Table 3 and Fig. 4). Specifically, both light  
267 reduction at L35 and organic matter enrichment had significant effects in increasing sucrose  
268 proportion (and decreasing starch proportion). The more extreme values were obtained always  
269 at L35 light reduction combined with organic matter enrichment (Fig. 4).

## 270 **Discussion**

271 Both, strong light attenuation (L35) and organic matter enrichment had a stress effect on  
272 *Isoetes lacustris* from Lake Baciver Petit. Reduction of light availability at L65 had almost no  
273 effect on *I. lacustris* other than the lengthening of new leaves. However, a reduction of light  
274 availability at L35 increased the number of dead leaves and major effects were observed on the  
275 proportion of carbohydrate reserves (sucrose percentage increased and starch percentage  
276 decreased) as expected from light limitation. Organic matter enrichment had major effects on *I.*  
277 *lacustris* growth (more dead leaves and less new leaves, which led to a decrease in the number  
278 of leaves and in leaf production) but little effect on carbohydrates (only sucrose percentage  
279 increased). The combination of L35 light irradiance and organic matter enrichment had the  
280 strongest negative effects on *Isoetes* growth, survival and carbohydrate reserves.

281 The lack of negative responses of the plants at L65 of light compared to the control plants  
282 indicates that growth was largely saturated by light in the studied pool for the time course of the  
283 experiment. Actually, *I. lacustris* performed slightly better at L65 of incident light, which strongly  
284 suggests photo-inhibition and/or UV stress at reference conditions (Hussner et al., 2010; Pulido  
285 et al., *in prep.*).

## 286 Growth indicators

287 Light reduction resulted in a general lengthening of new leaves towards increasing their  
288 photosynthetic surface, proving the high plasticity of the photosynthetic tissues of this species

289 (Hickey, 1986). Differences in the leaf lengthening among the two shading levels were not  
290 significant, suggesting limitation in leaf length as described in other isoetids (Madsen et al.,  
291 2002) to maintain lacunae CO<sub>2</sub> transport towards the leaf tips. Light reduction, at least to these  
292 levels, seems to play a secondary role for *Isoetes* growth and survival as also seen by Abeli et  
293 al. (2012).

294 The load of up to 10 % of organic matter in the upper sediment layer had a clear negative  
295 impact on *I. lacustris* resulting in a general deterioration of the leaves throughout the  
296 appearance of necrosis spots (i.e. dead photosynthetic tissue) and loss of turgidity.  
297 Consequently, leaf production (number) and survival (increase of leaf mortality) decreased  
298 Death of photosynthetic tissue could be related to a large number of causes, but heavy organic  
299 sediment enrichment causes accumulation of intracellular ammonia in aquatic flowering plants  
300 (Pulich, 1986), which is at the origin of cellular death due to ammonia toxicity (Smolders et al.,  
301 2003). This stress was particularly strong when associated with severe light limitation (L35+OM)  
302 since plants could not provide the extra-carbohydrate demand to bind ammonia through  
303 synthesis of soluble amino acids (Smolders et al. 2000), which is the mechanism to prevent the  
304 accumulation of highly toxic free ammonium in the cells (Burkholder et al., 1992). This may help  
305 to explain the shortening of new leaves observed under this treatment. The presence of other  
306 phytotoxics, such as sulphides and methane, can be mostly ruled out since we did not detect  
307 reduced sediment conditions. In addition, there is low sulphates and sufficient iron in the lake  
308 catchment (Gacia et al., 1994) to precipitate sulphites from the interstitial water (Smolders et al.,  
309 2003; Holmer et al., 1998).

310 *Isoetes* sporangia are attached at the base of the leaves. Consequently, a reduction in the total  
311 number of leaves and in the new leaf formation is probably reflected into a lower reproductive  
312 performance (Vöge, 1997). Therefore, two mechanisms spring from organic matter enrichment  
313 in reducing *I. lacustris* population: an immediate reduction because of phytotoxics that cause  
314 plant death and a future reduced spore bank that will difficult population recovery.

### 315 Mortality

316 Mortality did not increase under light reduction and moderate mortality resulted under organic  
317 matter enrichment at 100% irradiance. Nevertheless, the combination of light reduction and

318 organic matter enrichment highly enhanced mortality and helps explaining *Isoetes* die-offs  
319 observed after damming.

320 A real example is Lake Baciver (a lake close to the pond where the experiments were  
321 performed). This lake had extensive meadows of *Isoetes lacustris* between 0.5 and 2.3 meters  
322 depth (Ballesteros et al., 1989) before a dam was built in 1990. After the impact, the lake  
323 showed a decrease in light availability for *I. lacustris* populations, a chlorophyll peak in the water  
324 column and a high deposition of organic matter on the upper sediment layer (i.e. eutrophication;  
325 Gacia & Ballesteros, 1996, 1998). Two years after the damming, *Isoetes* specimens had all  
326 leaves severely damaged and no new leaves were produced in summer, leading to an almost  
327 complete die-off of the population (Gacia & Ballesteros, 1996, 1998). The results of the present  
328 experiment help explaining the collapse of *I. lacustris* population as a longer experiment would  
329 probably result in 100% mortality in the plots with severe light reduction and labile organic  
330 matter enrichment. Indeed, *Isoetes* species are generally considered to be absent from highly  
331 eutrophic sites (Pearsall, 1920; Hutchinson, 1975) and they decline and even disappear when  
332 lakes undergo eutrophication (e.g. Almestrand & Lundh, 1951; Wallsten, 1981; Brouwer &  
333 Roelofs, 2001).

#### 334 Carbon balance indicators

335 Severe light reduction (L35) resulted in a general increase of the sucrose concentration in both  
336 old leaves and corms. This response was coupled to a reduction in the corm starch content, as  
337 expected from mobilization of soluble carbohydrates derived from starch in order to keep  
338 growing in situations of light limitation (e.g. Lavery et al., 2009; Gacia et al., 2012).  
339 Consequently, the ratio between sucrose and TNC increased.

340 The presence of high organic matter concentration in the upper sediment layer resulted in  
341 significant differences only in sucrose percentage in leaves and plants that also resulted in an  
342 increase of the sucrose:TNC ratio. However, the plants undergoing organic enrichment visually  
343 had smaller corms or were dead and, consequently, their total amount of carbohydrate reserves  
344 was probably lower than plants under reference conditions.

345 Starch concentration was a good indicator of stress only for light reduction while sucrose  
346 percentage was a good stress indicator for light reduction and organic matter enrichment. Both  
347 light decrease and organic matter enrichment, alone and combined, increased soluble  
348 carbohydrate concentrations as observed for other abiotic stresses such as drought, salinity,  
349 low temperatures, flooding and low nutrients (Kovach et al., 1992; Rosa et al., 2009). Actually,  
350 the regulation of carbon partitioning between source and sink tissues is important for plant  
351 growth and development (Koch, 1996; Roitsch, 1999). Indeed, sucrose, as well as other sugars,  
352 has been recognized as important signal molecules in source-sink regulation (Roitsch, 1999).

### 353 Natural carbohydrate reserves comparison within aquatic macrophytes

354 We found significant levels of both, sucrose and starch in *I. lacustris* leaves and corms. The  
355 corm appears to be a reservoir of carbohydrates (high starch levels) that may be converted to  
356 soluble, mobile forms to be derived to other plant compartments during winter (Klimes et al.,  
357 1999) or used in response to any stress situation such as the ones described here (i.e. light  
358 impairment or organic matter enrichment). Indeed, high carbohydrate content has been  
359 described as an adaptation for perennial, long living species at extreme (cold and very short  
360 growing period) environmental conditions (Klimes et al., 1999). The levels of sucrose (i.e. a  
361 transport carbohydrate) were higher in the youngest non-mature leaves (positions 1 and 2)  
362 compared to the rest. This pattern fits with the model that indicates that young leaves generally  
363 function as C sinks, whereas older leaves act as a C source (Zimmerman & Alberte, 1995;  
364 Marbà et al., 2002) and it has been already reported for leaves occupying different position in  
365 the isoetid *Lobelia dortmanna* (Farmer & Spence, 1987).

366 *I. lacustris* carbohydrates concentrations detected at natural conditions are comparable to those  
367 of other freshwater macrophytes given that natural concentration of soluble and non-soluble  
368 carbohydrates is highly variable depending on species, organ and season (e.g. Muztar et al.,  
369 1979; Keeley et al., 1983; Owens & Madsen, 1998; Gao et al., 2014). Overall, helophytes and  
370 hydrophytes show similar carbohydrate concentration values, but the highest values correspond  
371 to hydrophytes (i.e. *Trapa* sp. and *Hygrophyla polysperma*; Kovach et al., 1992; Gao et al.,  
372 2014). Belowground organs (rhizomes, corms, tubers and roots), resistance structures (turions)  
373 and reproductive organs (kernels) tend to have higher non-soluble carbohydrate concentrations

374 than green organs (leaves and stems) but no clear patterns are observed with percentage data.  
375 The levels encountered here for *Isoetes lacustris* are in the upper range when compared to  
376 other hydrophytes (e.g. *Ceratophyllum*, *Myriophyllum*, *Potamogeton*, *Isoetes bolanderii*, among  
377 others; Muztar et al., 1979; Keeley et al., 1983; Vermaat & Verhagen, 1995; Xie et al., 2014).

#### 378 Conservation and management considerations

379 High numbers of livestock, especially in areas with small lakes and ponds or highly frequented  
380 lakes, can have strong negative effects on *Isoetes* populations and other aquatic plants (Croel &  
381 Kneitel, 2011). Reducing the number of animals and building a fence around the whole lake  
382 perimeter to prevent animal access into the water may be effective strategies to avoid plant  
383 mortality and system deterioration. We suggest fencing ponds and small lakes as well as  
384 building drinking troughs nearby the water bodies as a way to preserve aquatic biodiversity in  
385 areas with livestock. Damming should be avoided as *Isoetes lacustris* populations may not  
386 recover for decades, as seen in Lake Baciver.

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391 **References**

- 392 Abeli, T., E. Barni, C. Siniscalco, C. Amosso, & G. Rossi, 2012. A cost-effective model for  
393 preliminary site evaluation for the reintroduction of a threatened quillwort. *Aquatic Conservation-*  
394 *Marine and Freshwater Ecosystems* 22:66-73.
- 395 Alcoverro, T., R.C. Zimmerman, D.G. Kohrs & R.S. Alberte, 1999. Resource allocation and  
396 sucrose mobilization in light-limited eelgrass *Zostera marina*. *Marine Ecology Progress Series*  
397 187:121-131.
- 398 Alcoverro, T., M. Manzanera & J. Romero, 2001. Annual metabolic carbon balance of the  
399 seagrass *Posidonia oceanica*: the importance of carbohydrate reserves. *Marine Ecology*  
400 *Progress Series* 211:105-116.
- 401 Almestrand, A. & A. Lundh, 1951. Studies on the vegetation and hydrochemistry of Scanian  
402 Lakes I-III. C.W.K. Gleerups Forlag, Lund.
- 403 Arts, G.H.P., 2002. Deterioration of Atlantic soft water macrophyte communities by acidification,  
404 eutrophication and alkalinisation. *Aquatic Botany* 73:373-393.
- 405 Baastrup-Spohr, L., L.L. Iversen, J. Dahl-Nielsen & K. Sand-Jensen, 2013. Seventy years of  
406 changes in the abundance of Danish charophytes. *Freshwater Biology* 58:1682-1693.
- 407 Ballesteros, E., E. Gacia & L. Camarero, 1989. Composition, distribution and biomass of benthic  
408 macrophyte communities from an alpine lake in Central Pyrenees: the lake Baciver. *Annals*  
409 *Limnologie* 25:177-184.
- 410 Barko, J.W., & R.M. Smart, 1983. Effects of organic matter additions to sediment on the growth  
411 of aquatic plants. *Journal of Ecology* 71:161-175.
- 412 Barko, J.W., & R.M. Smart. 1986. Sediment related mechanisms of growth limitation in  
413 submersed macrophytes. *Ecology* 67:1328-1340.
- 414 Bond, T., D. Sear & T. Sykes, 2014. Estimating the contribution of in-stream cattle faeces  
415 deposits to nutrient loading in an English Chalk stream. *Agricultural Water Management*  
416 131:156-162.

417 Bornette, G. & S. Puijalon, 2011. Response of aquatic plants to abiotic factors: a review.  
418 Aquatic Sciences 73:1-14

419 Brock, W.A., & D. Starrett, 2003. Managing systems with non-convex positive feedback.  
420 Environmental & Resource Economics 26:575-602.

421 Brouwer, E. & J.G.M. Roelofs, 2001. Degraded soft-water lakes: possibilities for restauration.  
422 Restoration Ecology 9:155-166.

423 Burkholder, J.M., K.M. Mason & H.B. Glasgow, 1992. Water-column nitrate enrichment  
424 promotes decline of eelgrass *Zostera marina*; evidence from a seasonal mesocosm experiment.  
425 Marine Ecology Progress Series 81(2):163-178.

426 Camarero, L. & J. Catalan, 2012. Atmospheric phosphorus deposition may cause lakes to revert  
427 from phosphorous limitation back to nitrogen limitation. Nature Communications 3:118.

428 Camargo, J.A., A. Alonso & M. de la Puente, 2005. Eutrophication downstream from small  
429 reservoirs in mountain rivers of Central Spain. Water Research 39:3376-3384.

430 Catalan, J., L. Camarero, E. Gacia, E. Ballesteros & M. Felip, 1994. Nitrogen in the Pyrenean  
431 lakes (Spain). Hydrobiologia 274(1):17-27.

432 Chappuis, E., E. Gacia & E. Ballesteros, 2011. Changes in aquatic macrophyte flora over the  
433 last century in Catalan water bodies (NE Spain). Aquatic Botany 95:268-277.

434 Chappuis, E., E. Gacia & E. Ballesteros, 2014. Environmental factors explaining the distribution  
435 and diversity of vascular aquatic macrophytes in a highly heterogeneous Mediterranean region.  
436 Aquatic Botany 113:72-82.

437 Croel, R.C. & J.M. Kneitel, 2011. Cattle waste reduces plant diversity in vernal pool  
438 mesocosms. Aquatic Botany 95:140-145.

439 Farmer, A.M. & D.H.N. Spence, 1987. Environmental control of the seasonal growth of the  
440 submersed aquatic macrophyte *Lobelia dortmanna* L. New Phytologist 106:289-299.

441 Gacia, E., 1992. Ecologia dels macròfits submergits dels estanys del Pirineu: estructura i  
442 dinàmica de les poblacions de l'Estany Baciver (Vall d'Aran). University of Barcelona,  
443 Barcelona. Doctoral Thesis.

444 Gacia, E. & E. Ballesteros, 1993a. Diel acid fluctuations in Pyrenean *Isoetes* species - The  
445 effects of seasonality and emersion. *Archiv für Hydrobiologie* 128:187-196.

446 Gacia, E. & E. Ballesteros, 1993b. Population and individual variability of *Isoetes lacustris* L.  
447 with depth in a Pyrenean lake. *Aquatic Botany* 46:35-47.

448 Gacia, E. & E. Ballesteros, 1994. Production of *Isoetes lacustris* in a Pyrenean lake: seasonality  
449 and ecological factors involved in the growing period. *Aquatic Botany* 48:77-89.

450 Gacia, E. & E. Ballesteros, 1996. The effect of increased water level on *Isoetes lacustris* L in  
451 Lake Baciver, Spain. *Journal of Aquatic Plant Management* 34:57-59.

452 Gacia, E. & E. Ballesteros, 1998. Effects of building up a dam in a shallow high mountain lake  
453 (Baciver, Central Pyrenees). *Oecologia Aquatica* 11:55-66.

454 Gacia, E., E. Ballesteros, L. Camarero, O. Delgado, A. Palau, J.L. Riera & J. Catalan, 1994.  
455 Macrophytes from lakes in the eastern Pyrenees: community composition and ordination in  
456 relation to environmental factors. *Freshwater Biology* 32:73-81.

457 Gacia, E., E. Chappuis, A. Lumbreras, J.L. Riera & E. Ballesteros, 2009. Functional diversity of  
458 macrophyte communities within and between Pyrenean lakes. *Journal of Limnology* 68:25-36.

459 Gacia, E., N. Marbà, J. Cebrián, R. Vaquer-Sunyer, N. Garcias-Bonet & C.M. Duarte, 2012.  
460 Thresholds of Irradiance for seagrass (*Posidonia oceanica*) meadow metabolism: An  
461 experimental approach. *Marine Ecology Progress Series* 466:69-79.

462 Gao, H., J. Cai, W. Han, H. Huai, Y. Chen & C. Wei, 2014. Comparison of starches isolated  
463 from three different *Trapa* species. *Food Hydrocolloids* 37:174-181.

464 Hautier, Y., P.A. Niklaus & A. Hector, 2009. Competition for light causes plant biodiversity loss  
465 after eutrophication. *Science* 324:636-638.

466 Hickey, R.J., 1986. *Isoetes* megaspore surface morphology: nomenclature, variation and  
467 systematic importance. *American Fern Journal* 76:1-16.

468 Holmer, M., H.S.Jensen, K.K. Christensen, C. Wigand, F.O. Andersen, 1998. Sulfate reduction  
469 in lake sediments inhabited by the isoetid macrophytes *Littorella uniflora* and *Isoetes lacustris*.  
470 *Aquatic Botany* 60:307-324.

471 Hussner, A., H.P. Hoelken & P. Jahns, 2010. Low light acclimated submerged  
472 freshwater plants show a pronounced sensitivity to increasing irradiances. *Aquatic Botany*  
473 93:17-24.

474 Hutchinson, G.E., 1975, *A Treatise on Limnology*. Limnological Botany. Wiley, New York.

475 Jones, C.G., J.H. Lawron & M. Shachak, 1994. Organisms as ecosystem engineers. *Oikos*  
476 69:373-386.

477 Keeley, J.E., 1998. CAM photosynthesis in submerged aquatic plants. *Botanical Review*  
478 64:121-175.

479 Keeley, J.E., C.M. Walker & R.P. Mathews, 1983. Crassulacean acid metabolism in *Isoetes*  
480 *bolanderi* in high elevation oligotrophic lakes. *Oecologia* 58:63-69.

481 Klimes, L., J. Klimesova & H. Cizkova, 1999. Carbohydrate storage in rhizomes of *Phragmites*  
482 *australis*: the effects of altitude and rhizome age. *Aquatic Botany* 64:105-110.

483 Koch, K.E., 1996. Carbohydrate-modulated gene expression in plants. *Annual Review of Plant*  
484 *Physiology and Plant Molecular Biology* 47:509-540.

485 Kovach, C.W., J.P. Kurdziel, R. Bowman, J. Wagner & J.M. Lawrence, 1992. The effects of  
486 stress and disturbance on proximate composition, allocation of production, photosynthesis,  
487 respiration, and chlorophyll levels in *Hygrophila polysperma* (Roxb) Anders (Acanthaceae).  
488 *Environmental and Experimental Botany* 32:479-486.

489 Kowalewski, G.A., R. Kornijów, S. McGowan, M. Woszczyk, M. Suchora, K. Bałaga, A.  
490 Kaczorowska, M. Gąsiorowski, K. Szeroczyńska & A. Wasilowska, 2013. Persistence of  
491 protected, vulnerable macrophyte species in a small, shallow eutrophic lake (eastern Poland)  
492 over the past two centuries: Implications for lake management and conservation. *Aquatic*  
493 *Botany* 106:1-13.

494 Kraska, M., P. Klimaszuk, & R. Piotrowicz, 2013. Anthropogenic changes in properties of the  
495 water and spatial structure of the vegetation of the Lobelia lake Lake Modre in the Bytw  
496 Lakeland. *Oceanological and Hydrobiological Studies* 42:302-313.

497 Lavery, P.S., K. McMahon, M. Mulligan & A. Tennison, 2009. Interactive effects of timing,  
498 duration of experimental shading on *Amphibolis griffithii*. Marine Ecology Progress Series  
499 394:21-33.

500 Levin, S.A., 2009, The Princeton Guide to Ecology. Princeton University Press, Princeton.

501 Madsen, T.V., B. Olesen & J. Bagger, 2002. Carbon acquisition and carbon dynamics by  
502 aquatic isoetids. Aquatic Botany 73:351-371.

503 Marbà, N., M.A. Hemminga, M.A. Mateo, C.M. Duarte, Y.E.M. Mass, J. Terrados & E. Gacia,  
504 2002. Carbon and nitrogen translocation between seagrass ramets. Marine Ecology Progress  
505 Series 226:287-300.

506 Moss, B., E. Jeppesen, M. Sondergaard, T. Lauridsen & Z.W. Liu, 2013. Nitrogen, macrophytes,  
507 shallow lakes and nutrient limitation: resolution of a current controversy? Hydrobiologia 710:3-  
508 21.

509 Murphy, K.J., 2002. Plant communities and plant diversity in soft-water lakes of northern  
510 Europe. Aquatic Botany 73:28-324.

511 Muztar, A.J., S.J. Slinger & J.H. Burton, 1979. Chemical composition of aquatic macrophytes.  
512 IV. Carotenoids, soluble sugars and starch in relation to their pigmentation, and ensiling potential.  
513 Canadian Journal of Plant Science 59:1093-1098.

514 Owens, C.S. & J.D. Madsen, 1998. Phenological studies of carbohydrate allocation in *Hydrilla*.  
515 Journal of Aquatic Plant Management 36:40-44.

516 Pearsall, W.H., 1920. The aquatic vegetation of the English lakes. Journal of Ecology 8:163-  
517 201.

518 Portielje, R. & D.T. Van der Molen, 1998. Trend-analysis of eutrophication variables in lakes in  
519 The Netherlands. Water Science and Technology 37:235-240.

520 Pulich, W.M., 1986. Variation in leaf soluble amino-acids and ammonium content in subtropical  
521 seagrasses related to salinity stress. Plant Physiology 80:283-286.

522 Pulido, C., E.C.H.E.T. Lucassen, O. Pedersen & J.G.M. Roelofs, 2011a. Influence of quantity  
523 and lability of sediment organic matter on the biomass of two isoetids, *Littorella uniflora* and  
524 *Echinodorus repens*. Freshwater Biology 56:939-951.

525 Pulido, C., D.J.H. Keijzers, E.C.H.E.T. Lucassen, O. Pedersen & J.G.M. Roelofs, 2011b.  
526 Elevated alkalinity and sulfate adversely affect the aquatic macrophyte *Lobelia dortmanna*.  
527 *Aquatic Ecology* 46:283-295.

528 Pulido C, Sand-Jensen K, Lucassen E, Roelofs, JGM, Brodersen KP, Pedersen O (2012)  
529 Improved prediction of vegetation composition in NW European softwater lakes by combining  
530 location, water and sediment chemistry. *Aquatic Sciences* 74:351-360.

531 Riera, J.L., M. Felip, E. Chappuis, J. Tresserra, L. Camarero, 2014. Efectes de l'activitat  
532 ramadera sobre les aportacions de nitrogen als estanys del Parc Nacional d'Aigüestortes i  
533 Estany de Sant Maurici. In: La investigació al Parc Nacional d'Aigüestortes i Estany de Sant  
534 Maurici: IX Jornades sobre Recerca al Parc Nacional d'Aigüestortes i Estany de Sant Maurici.  
535 pp 19-29.

536 Risgaard-Petersen, N. & K. Jensen, 1997. Nitrification and denitrification in the rhizosphere of  
537 the aquatic macrophyte *Lobelia dortmanna* L. *Limnology and Oceanography* 42:529-537.

538 Roitsch, T., 1999. Source-sink regulation by sugar and stress. *Current Opinion in Plant Biology*  
539 2:198-206.

540 Rørslett, B. & S.W. Johansen, 1995. Dynamic response of the submerged macrophyte, *Isoetes*  
541 *lacustris*, to alternating light levels under field conditions. *Aquatic Botany* 51:223-242.

542 Rosa, M., C. Prado, G. Podazza, R. Interdonato, J.A. González, M. Hilal & F.E. Prado, 2009.  
543 Soluble sugars: metabolism, sensing and abiotic stress. *Plant Signaling and Behavior* 4:388-  
544 393.

545 Salgado, J., C. Sayer, L. Carvalho, T. Davidson & I. Gunn, 2010. Assessing aquatic macrophyte  
546 community change through the integration of palaeolimnological and historical data at Loch  
547 Leven, Scotland. *Journal of Paleolimnology* 43:191-204.

548 Sand-Jensen, K., T. Riis, O. Vestergaard & S.E. Larsen, 2000. Macrophyte decline in Danish  
549 lakes and streams over the past 100 years. *Journal of Ecology* 88:1030-1040.

550 Scheffer, M., S. H. Hosper, M. L. Meijer, B. Moss, and E. Jeppesen, 1993. Alternative equilibria  
551 in shallow lakes. *Trends in Ecology & Evolution* 8:275-279.

552 Smolders, A.J.P., E. Lucassen & J.G.M. Roelofs, 2002. The isoetid environment:  
553 biogeochemistry and threats. *Aquatic Botany* 73:325-350.

554 Smolders, A.J.P., L.P.M. Lamers, C. den Hartog & J.G.M. Roelofs, 2003. Mechanisms involved  
555 in the decline of *Stratiotes aloides* L. in The Netherlands: sulphate as a key variable.  
556 *Hydrobiologia* 506-509: 603-610.

557 Smolders, A.J.P., M.C. van Riel, & J.G.M. Roelofs, 2000. Accumulation of free amino acids as  
558 an early indication for physiological stress (nitrogen overload) due to elevated ammonium levels  
559 in vital *Stratiotes aloides* L. stands. *Archiv für Hydrobiologie* 150:169-175.

560 Sousa, W.T.Z., S.M. Thomaz, K.J. Murphy, M.J. Silveira, & R.P. Mormul, 2009. Environmental  
561 predictors of the occurrence of exotic *Hydrilla verticillata* (L.f.) Royle and native *Egeria najas*  
562 Planch. in a sub-tropical river floodplain: the Upper River Parana, Brazil. *Hydrobiologia* 632:65-  
563 78.

564 Underwood, A.J., 1981. Techniques of analysis of variance in experimental marine biology and  
565 ecology. *Oceanography and Marine biology: Annual Review* 19:513-605.

566 Vermaat, J.E. & F.C.A. Verhagen, 1995. Freezer-independent preservation of carbohydrate  
567 samples from water plants: a methodological test. *Aquatic Botany* 51:155-161.

568 Vöge, M., 1997. Plant size and fertility of *Isoetes lacustris* L in 20 lakes of Scandinavia: A field  
569 study. *Archiv für Hydrobiologie* 139:171-185.

570 Wallsten, M., 1981. Changes of lakes in Uppland, central Sweden, during 40 years.  
571 *Evolutionary Biology Centre. Uppsala Universitet*, p. 84.

572 Xie, D., D. Yu, C. Xia & W. You, 2014. Stay dormant or escape sprouting? Turion buoyancy and  
573 sprouting abilities of the submerged macrophyte *Potamogeton crispus* L. *Hydrobiologia* 726:43-  
574 51.

575 Zimmerman, R.C., R.S. Alberte, 1995. Light availability, root anoxia and patterns of carbon  
576 allocation in the marine angiosperm *Zostera marina* L. (eelgrass). *Plant Physiology* 108:24-24.

577 **Table 1** Summary of the factorial two-way ANOVAs of *Isoetes lacustris* growth variables and  
 578 mortality under light reduction and organic matter enrichment

Variable	Effect	df	MS	F value	p-value	Comparison
No. of dead leaves	Light	2	30.19	2.75	0.066	
	F	1	632.52	57.67	0.000	r < F
	Light x F	2	2.34	0.21	0.808	
	Within	180	10.97			
No. of new leaves	Light	2	8.23	7.65	0.000	r, 65% > 35%
	F	1	80.02	74.37	0.000	r > F
	Light x F	2	0.23	0.21	0.807	
	Within	180				
Total no. of leaves	Light	2	10.49	0.74	0.478	
	F	1	1249.05	88.42	0.000	r > F
	Light x F	2	20.81	1.47	0.232	
	Within	180	14.13			
New leaves length (cm)	Light x F	2	0.565	4.693	0.031	
	Light (without F)	2	2.401	10.751	0.010	r < 65%, 35%
	Light (with F)	2	1.921	2.119	0.201	
	F (100% Light)	1	0.807	0.644	0.467	
	F (65% Light)	1	0.042	0.137	0.730	
	F (35% Light)	1	4.86	35.133	0.0041	r > F
Production (mg DW·ind <sup>-1</sup> ·day <sup>-1</sup> )	Light	2	0.002	0.314	0.731	
	F	1	0.396	57.98	0.000	r > F
	Light x F	2	0.007	0.98	0.376	
	Within	210	0.007			
Mortality (% of ind)	Light	2	130.6	1.54	0.254	
	F	1	3378.4	39.85	0.000	r < F
	Light x F	2	120.9	1.43	0.278	
	Within	12	84.4			

579 Levels of light availability: reference (r), 65% of incident light (65%) and 35% of incident light  
 580 (35%). Levels of organic matter enrichment: reference (r) and 10% organic matter enrichment  
 581 (F). When the interaction was significant (i.e. new leaf length) we have run one-way ANOVA  
 582 per each factor independently. Tukey HSD post-hoc comparison tests were run when significant  
 583 differences were detected for light in order to know which levels differed. Abbreviations: df =  
 584 degrees of freedom, MS = Mean square, F value = F test statistic

585 **Table 2** Summary of the factorial one-way ANOVAs of *Isoetes lacustris* carbohydrate storage at  
 586 reference conditions  
 587

Variable	Effect	df	MS	F value	p-value	Comparison
Sucrose (% DW)	Organ	3	71.72	18.30	0.000	corm, L345, L678 < L12
	Within	8	3.92			
Starch (% DW)	Organ	3	144.82	315.7	0.000	corm > L12, L345, L678
	Within	8	0.46			
TNC (% DW)	Organ	3	52.35	1.50	0.286	
	Within	8	34.85			

588 Different *Isoetes* organs were analysed: corm; leaves 1 and 2 (L12); leaves 3, 4 and 5 (L345);  
 589 and leaves 6, 7 and 8 (L678). Tukey HSD post-hoc comparison tests were run when significant  
 590 differences were detected among organs in order to know which ones differed. Abbreviations: df  
 591 = degrees of freedom, MS = Mean square, F value = F test statistic

592 **Table 3** Summary of the factorial two-way ANOVAs of *Isoetes lacustris* carbohydrate storage  
 593 response under light reduction and organic nutrient enrichment  
 594

Variable	Effect	df	MS	F value	p-value	Comparison
Corm sucrose (% DW)	Light	2	55.95	6.92	0.010	r < 35%
	F	1	42.26	5.23	0.041	
	Light x F	2	1.17	0.15	0.866	
	Within	12	8.08			
Corm starch (% DW)	Light	2	163.35	5.96	0.016	65% >35%
	F	1	18.16	0.66	0.431	
	Light x F	2	4.68	0.17	0.845	
	Within	12	27.39			
Leaves sucrose (% DW)	Light	2	54.96	14.99	0.00054	r < 35%
	F	1	34.89	9.52	0.0094	r < F
	Light x F	2	9.20	2.51	0.123	
	Within	12	3.67			
Leaves starch (% DW)	Light	2	0.70	0.69	0.522	
	F	1	1.60	1.56	0.246	
	Light x F	2	0.25	0.25	0.784	
	Within	12	1.02			
Plant sucrose (% DW)	Light	2	60.90	15.67	0.0004	r < 35%
	F	1	39.07	10.05	0.008	r < F
	Light x F	2	7.96	2.05	0.172	
	Within	12	3.88			
Plant starch (% DW)	Light	2	32.06	4.02	0.046	65% > 35%
	F	1	12.72	1.59	0.230	
	Light x F	2	0.36	0.04	0.956	
	Within	12	7.97			
TNC (% DW)	Light	2	22.68	3.14	0.080	
	F	1	7.20	1.00	0.337	
	Light x F	2	9.66	1.34	0.298	
	Within	12	7.21			
Sucrose : TNC	Light	2	0.072	12.66	0.0011	r,65% < 35%
	F	1	0.046	7.72	0.0167	r < F
	Light x F	2	0.002	0.42	0.665	
	Within	12	0.006			

595 Levels of light availability: reference (r), 65% of incident light (65%) and 35% of incident light  
 596 (35%). Levels of organic matter enrichment: reference (r) and 10% organic matter enrichment  
 597 (F). Tukey HSD post-hoc comparison tests were run when significant differences were detected  
 598 for light in order to know which levels differed. Abbreviations: df = degrees of freedom, MS =  
 599 Mean square, F value = F test statistic

600 **Fig. 1** *Isoetes lacustris* growth indicators per plant (a-e) and plant survival (f) under different  
601 treatments (light irradiance of 100%, 65% and 35%; and organic matter enrichment). Error bars  
602 represent the 95% confidence interval. Different letters above bars indicate a significant  
603 difference between treatments; same letters indicate no difference. Abbreviations: NF = no  
604 fertilization, F = fertilization

605 **Fig. 2** Percentage (% DW) of sucrose, starch and total non-structural carbohydrates (TNC) in  
606 *Isoetes lacustris* leaves (according to its position in the leaf rosette), corms and roots under  
607 reference conditions. Different letters (for sucrose) or numbers (for starch) on bars indicate a  
608 significant difference between treatments; same letters/numbers indicate no difference. Leaf  
609 position number 9 had not enough replicates to be included in the ANOVA test and no group  
610 association can be shown

611 **Fig. 3** Percentage (% DW) of sucrose (a) and starch (b) in *Isoetes lacustris* leaves (according to  
612 its position in the leaf rosette), corms and roots under different treatments of light reduction and  
613 organic matter enrichment. Error bars represent the 95% confidence interval. Abbreviations:  
614 100% = 100% of light without fertilization, 65% = 65% of light without fertilization, 35% = 35% of  
615 light without fertilization, 100% F = 100% of light with fertilization, 65% F = 65% of light with  
616 fertilization, 35% F = 35% of light with fertilization

617 **Fig. 4** *Isoetes lacustris* carbon storage indicators per plant under different treatments (light  
618 irradiance of 100%, 65% and 35%; and with/without organic matter enrichment). Error bars  
619 represent the 95% confidence interval. Different letters above bars indicate a significant  
620 difference between treatments; same letters indicate no difference. Abbreviations: NF = no  
621 fertilization, F = fertilization, TNC = total non-structural carbohydrates