

1 Main carbon sources supporting primary and secondary production in a
2 disturbed semiarid wetland from central Spain

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18 **Abstract**

19 Approximately 95% of the total carbon (C) in wetlands is typically found as particulate
20 organic matter (POM). In this study, we evaluated the main C sources of a semiarid
21 floodplain wetland in central Spain under disturbance. For this, we used stable isotopes (^{13}C
22 and ^{15}N) and the Bayesian mixing model SISUS. We show that the allochthonous C derived
23 from wastewater discharges have distinctive $\delta^{13}\text{C}$ values compared to the C of sites that did
24 not receive wastewater discharges. Wastewater discharges affected the isotopic signatures of
25 dissolved organic carbon as well as $\delta^{15}\text{N}$ values of primary producers (epiphyton, filamentous
26 algae and *Scirpus maritimus*). $\delta^{15}\text{N}$ values showed a greater response to the effects of
27 environmental degradation. The salt-tolerant marginal tree *Tamarix canariensis* was the main
28 autochthonous contributor to POM (69 to 84%), while the main source of energy for the
29 secondary production was the submerged macrophyte *Ceratophyllum submersum*. *Tamarix*
30 *canariensis* was also the main component of the diet for macroinvertebrates, followed by
31 POM. Other species such as *Cladium mariscus*, an emblematic aquatic plant in the region,
32 showed a very low contribution to POM (less than 20%), although it represented a relatively
33 important energy source for zooplankton (up to 47%). This relatively low contribution is
34 probably due to the drastic reduction in the abundance of this species during the last 50 years.
35 In contrast, *Phragmites australis*, which has multiplied its abundance by twenty-two since
36 1950, does not seem to contribute significantly to POM or to the diet of invertebrates (less
37 than 22%), although it can represent up to 54% zooplankton's diet. Isotopic results confirm
38 that in this eutrophied and disturbed wetland, the contribution of macrophytes to the food
39 web as autochthonous organic sources is lower than expected based on their biomass.

40

41 **Keywords:** carbon sources; SISUS; Daimiel; primary producers; secondary producers;
42 trophic interactions; wetland

43 1. Introduction

44 Approximately 95% of the total carbon (C) found in wetlands is in the form of particulate
45 organic matter (POM), which is generated through the decomposition of litterfall and detrital
46 production (Reddy & DeLaune, 2008). Wetlands can produce their own organic matter (i.e.,
47 autochthonous sources, including remains of vegetation like leaves, roots, rhizomes and
48 detrital material from algae, microorganisms, invertebrates, vertebrates, etc.), but they can
49 also receive organic C from allochthonous sources. Allochthonous sources include dissolved
50 and particulate organic C from diffuse sources, including those derived from agriculture and
51 wastewater effluents of different types (Reddy & DeLaune, 2008). Allochthonous
52 contributions are generally very important to support the metabolism of aquatic ecosystems
53 and they are, in most river systems, the largest contributor to the organic C budget (Carpenter
54 *et al.*, 2005), affecting significantly the configuration and regulation of food webs (Polis &
55 Strong, 1996; Huxel & McCann, 1998)

56 Wetland POM consists of a mixture of terrestrial, lacustrine and riverine organic
57 remains, all subject to additional physical, chemical and biological transformations (Hedges,
58 Clark & Come, 1988), with the intensity and direction of water exchange between the river
59 and the floodplain conditioning the balance of organic matter (Albuquerque & Mozeto, 1997;
60 Valett *et al.*, 2005) The role of wetlands in the retention and transformation of organic matter
61 has been shown in numerous studies (Newbold *et al.*, 1982; Puckett *et al.*, 1993; Sánchez
62 Carrillo, Álvarez Cobelas & Angeler, 2001; Valett *et al.*, 2005). However, the balances of
63 organic matter of wetlands are hard to establish, mainly due to the difficulty of identifying the
64 main sources of organic C in these systems. This difficulty resides in the presence of multiple
65 molecular forms and organic matter sources, as well as the high spatiotemporal variability in
66 the activity of these ecosystems (Hedges *et al.*, 1988; Albuquerque & Mozeto, 1997).
67 Different complex markers, such as organic matter fatty acid composition (Mortillaro *et al.*,

68 2011), the comparative composition of carbohydrates, proteins, lignin, lipids, char and
69 "carbonyl" groups (Nelson & Baldock, 2005; Rodríguez-Murillo, Almendros & Knicker,
70 2011) have been widely used to elucidate the source and destination of the organic matter in
71 aquatic environments and to characterize nutrient utilization by heterotrophic organisms
72 (Teranes & Bernasconi, 2000; Mortillaro *et al.*, 2011). Stable isotopes have also been used to
73 determine the contribution of POM to food webs (Sullivan & Moncreiff, 1990; Fry, 1991;
74 Bunn & Boon, 1993; Keough, Sierszen & Hagley, 1996; Grey, Jones & Sleep, 2001; Bunn,
75 Davies & Winning, 2003; Pace *et al.*, 2004). For example, Bunn & Boon (1993) measured
76 ^{13}C and ^{15}N in Australian floodplain wetlands (Billabongs) to evaluate the contribution of
77 POM to three food webs and found that none of the conspicuous organic matter sources of
78 the site (terrestrial, submerged, floating and emergent aquatic macrophytes) were the source
79 of C for most consumers. In contrast, Keough *et al.* (1996) showed that two food webs in a
80 coastal wetland of Lake Superior depended on the C fixed by phytoplankton, although they
81 also found no evidence of a direct connection between the most abundant macrophytes and
82 the fish fauna analysed. Although $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ have proved useful to distinguish between
83 aquatic and terrestrial primary producers and their influence on the metabolism of wetlands,
84 their signals are often masked by the different contributions of organic matter from different
85 photosynthetic sources such as phytoplankton or terrestrial and aquatic C_3/C_4 plants
86 (Townsend-Small, McClain & Brandes, 2005).

87 In recent years, there has been greater use of mixing models using stable isotopes to
88 quantify the diet or the path of organic matter in food webs (Phillips, 2001; Phillips & Gregg,
89 2003; Benstead *et al.*, 2006; Zeug & Winemiller, 2008). These models are based on mixture
90 sets of two or more stable isotopes (typically $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and are a powerful tool to
91 determine the relative contribution of each of the sources in those organisms that have more
92 than one potential source and may themselves be isotopically distinct (Phillips, 2001). In a

93 previous study, (Rodríguez-Murillo *et al.*, 2011) characterized the organic matter in sediments
94 of “Las Tablas de Daimiel” to determine its origin and transformations using $\delta^{13}\text{C}$ nuclear
95 magnetic resonance and elemental analysis and applying a molecular mixture model. Their
96 results indicate that macrophytes (emergent or submerged) are the main source of organic
97 matter in wetland soils and that their quality depend more on drastic changes, such as the
98 effect of fires, than on external inputs. However, the contribution of different
99 autochthons/allochthonous carbon sources in sustaining the food web of this wetland is
100 unknown.

101 The aim of this study was to determine the main sources of C in “Las Tablas de
102 Daimiel”, a semiarid floodplain wetland from central Spain, through the isotopic signatures
103 ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of plants, phytoplankton, algae and detrital bacteria. We assessed how
104 different forms of biomass (i.e., C sources) are incorporated into both organic matter and
105 primary producers. We also evaluated the effect that organic material from urban wastewater
106 discharge has on the food web of this wetland. Moreover, we also collected information about
107 potential organic sources that support communities in different habitats of the wetland
108 (coastal areas, shallow areas free of emergent vegetation, deep areas and river channels). This
109 information is essential to define the functions that support the food web and may be useful
110 and necessary for mitigation projects and wetland restoration.

111

112 **2. Material and methods**

113 *1.1 Study site*

114 This study was conducted in the "National Park Las Tablas de Daimiel" (hereafter referred to
115 as NPTD), a unique wetland in the Euro-Mediterranean region (39° 08' N, 3° 43' W, Fig. 1).
116 Currently, the ecosystem encompasses the heart of the Biosphere Reserve "La Mancha
117 Húmeda" ($\approx 8.000 \text{ km}^2$), a major wetland region of southern Europe (Sánchez-Carrillo &

118 Angeler, 2010). NPTD potentially floods an area of 15.9 km² and the maximum average
119 depth is 0.9 m. The flooded area has great annual seasonal fluctuations that lead to drastic
120 changes in the habitats during the dry cycles (Angeler *et al.*, 2001).

121 The National Park is what remains of an extensive floodplain that covered an area of
122 150-250 km² in the early twentieth century (Sánchez-Carrillo & Angeler, 2010). Since the
123 1950s, the extent of the wetland has been drastically reduced and its ecological integrity is
124 seriously threatened (Cirujano, Álvarez-Cobelas & Sánchez-Andrés, 2010; Sánchez-Carrillo
125 & Angeler, 2010). The uniqueness and richness of the landscape used to be characterized by
126 the seasonal mixture of surface, sulphate-rich waters with more fresh and stable groundwater
127 (Coronado Castillo *et al.*, 1974); however, currently this system works very differently from
128 the biogeochemical point of view, and many external inputs of organic matter from the
129 watershed (a large fraction of this wastewater is untreated or insufficiently treated) has
130 boosted internal production and turned the wetland into turbid waters (Alvarez-Cobelas *et al.*
131 2001; Sánchez-Carrillo *et al.* 2001). Thus, NPTD has become a hypertrophic wetland,
132 receiving both high organic loads from wastewater with insufficient treatment as well as
133 diffused agricultural pollution that reaches the basin through the Gigüela River (Sánchez-
134 Carrillo & Angeler, 2010). More information about Las Tablas de Daimiel can be found in
135 (Sánchez-Carrillo & Angeler, 2010). Physical-chemical water conditions during the study
136 period in different parts of the wetland are shown in Table 1.

137

138 2.2 Collection of potential wetland C sources

139 The living C reservoirs collected were: seven macrophytes (four emerging: *Cladium mariscus*,
140 *Phragmites australis*, *Typha dominguensis* and *Scirpus maritimus*, and three submerged:
141 *Potamogeton pectinatus*, *Ceratophyllum submersum* and *Chara hispida* var. *major*, the latter
142 being the most abundant charophyte in the Park), *Tamarix canariensis* (forming large clusters

143 in coastal wetland areas and flourishing in marginal areas), epiphytic algae, filamentous algae,
144 phytoplankton and microbial mats. Complete charophytes were collected and cleaned of
145 sediments. For the rest of plants, we collected green leaves and, when present, rhizomes.
146 Periphyton was collected by scraping submerged wood substrate, macrophytes and rocks.
147 Filamentous algae were collected in sites where they were present. The roots from the three
148 most abundant species of wetland were also analysed (*C. mariscus*, *P. australis* and *T.*
149 *dominguensis*).

150 We separated phytoplankton from the zooplankton by filtration with Nyltal filters
151 (45µm) and then filtering a volume of 10 L with GF/F filters (0.2 µm). Microbial mats
152 including cyanobacteria such as *Microcoleus chthonoplastes* (Barón, 2011) were collected by
153 carefully removing the protruding layer on the substrate with a spatula and then passing the
154 remaining material through a 1-mm mesh.

155 As the secondary producers from this study, we collected zooplankton and benthic
156 macroinvertebrates. Invertebrates were collected using dredged sediments, storing live
157 specimens without preservatives. Invertebrate species were identified as *Crocothemis*
158 *erytrhaea* (Odonata), *Naucoris maculatus* (Heteroptera) and *Chironomus* sp. (Diptera).
159 Zooplankton was filtered from 40 L of water through a Nyltal filter, and this material was
160 subsequently slurred and filtered with 0.45 µm filters before being oven-dried at 80 °C.
161 Invertebrates were analysed as a composite samples because it was not possible to obtain
162 sufficient mass of each species at each site.

163 We also collected sediment samples, from which visible vegetation remains were
164 eliminated. The water C pool was estimated as the sum of particulate organic C (POC),
165 dissolved organic C (DOC) and dissolved inorganic C (DIC), which were collected at each
166 site. Samples for $\delta^{13}\text{C}$ -DIC were taken into 60 ml vials and were acidified to pH <2 with 1 ml
167 of H_2SO_4 10 N (Carpenter et al., 2005). For analysis of $\delta^{13}\text{C}$ -POC and DOC, 250 ml of water

168 were filtered through a mesh GF/F (0.7 μm). For $\delta^{13}\text{C}$ -POC, we scraped the filter residue,
169 which was then oven-dried at 40 °C. $\delta^{13}\text{C}$ -DOC was obtained after evaporating the water
170 obtained from the filtrate at 40 °C (Pace *et al.*, 2007).

171 During the summer of 2011 (June-July) samples were taken along a longitudinal
172 gradient to cover the environmental spatial heterogeneity of the wetland (Fig. 1), including
173 inland areas (n = 7 sites; A1: A7), coastal areas (n = 5 sites, B1: B5), channels (n = 3 sites, C1:
174 C3), and deep zones (n = 2 sites, D1: D2; Fig. 1). M1 is the channel where the wastewater
175 from Villarrubia de los Ojos discharged after treatment. The interior areas are shallow (about
176 50-70 cm deep) and emerging macrophyte patches with vegetation-free zones can be found.
177 The coastal areas were usually covered with emergent macrophytes, with reeds and tamarisk
178 predominantly present. These areas were drying up over the dry season. The deepest areas
179 correspond to the terminal part of the wetland, with depths of 3-4 m, with emergent
180 vegetation restricted to the shoreline and without submerged macrophytes.

181

182 2.3 Sample preparation and analysis

183 We removed carbonates from *Chara hispida* var. *major* and *Ceratophyllum submersum* with
184 5% HCl (Demopoulos, Fry & Smith, 2007). Carbonates of sediment were removed using 1M
185 HCl (Demopoulos *et al.*, 2007). Subsequently, all samples were washed repeatedly with
186 distilled water and successively oven-dried at 40 °C (invertebrates), 60 °C (vegetation; Boon
187 & Bunn 1994) and 80 °C (detritus, phytoplankton and zooplankton). Subsequently all plant,
188 detrital and invertebrate samples were crushed mechanically and manually, with the help of
189 an agate mortar, and were separated using a 1 mm mesh.

190

191 2.4 Stable isotope analysis

192 The analyses of the ratio of $^{12/13}\text{C}$ and $^{14/15}\text{N}$ stable isotopes were determined by Isotope
193 Ratios Mass Spectrometry (IRMS) in the Stable Isotope Laboratory at the University of
194 Arizona, United States (http://www.geo.arizona.edu/research/iso_lab.html) Two standards
195 were used: Peedee belemnite (PDB) for C and atmospheric N_2 for N. The results are
196 expressed in parts per thousand (‰) with respect to differences with the standards according
197 to the following equation: $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ (‰) = $[(R_{\text{sample}} / R_{\text{standar}}) - 1] \times 10^3$, where $R = ^{13}\text{C}$
198 $/^{12}\text{C}$ and $^{15}\text{N} / ^{14}\text{N}$. The analytical precision of the replicas of the analysis was less than 0.2 ‰.
199

200 2.5. Statistical analysis

201 Differences in $\delta^{13}\text{C}/\delta^{15}\text{N}$ of organic sources were evaluated according to two criteria, for
202 which we used ANOVAs. (1) The position of each sampling point was considered to
203 determine if there were differences between the different habitats studied, i.e. the (i) inner
204 zone subjected to the influence of municipal wastewater discharges, (ii) littoral, (iii) channels
205 and (iii) deep zones. (2) To evaluate the effects of wastewater discharges in $\delta^{13}\text{C}/\delta^{15}\text{N}$ -organic
206 sources we considered the following categories: (i) entrance, (ii) discharge area of Gigüela
207 river, (ii) intermediate zone, and (iii) end zone (Navarro bridge dam). Finally, the contribution
208 of autochthonous sources of C in the secondary production was determined using the
209 Bayesian model SISUS in R (Erhardt, 2010).

210

211 3. Results

212 The results of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the major C sources from NPTD and the relative
213 position of each organism is represented in Fig. 2.

214

215 *$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of nonliving reservoirs*

216 Non-living C reservoirs of the wetland showed wide variation in ^{13}C . $\delta^{13}\text{C}$ values of DOC
217 were highly enriched (average $-8.83\text{‰} \pm 2.78$; Fig. 2) and there were no significant
218 differences in terms of their spatial position ($\text{df}=2$, $F=3.562$, $p=0.096$). Instead, significant
219 differences related to the effect of wastewater discharges were associated with less negative
220 $\delta^{13}\text{C}$ values of DOC near the entry point ($\text{df}=3$, $F=6.053$, $p=0.010$). Meanwhile, the $\delta^{13}\text{C}$ of
221 POC was greatly depleted ($-26.89 \pm 4.36 \text{‰}$), showing very homogeneous signatures around
222 the wetland.

223 DON and PON showed similar isotopic signatures (average: $7.66 \pm 1.98\text{‰}$ and $7.82 \pm$
224 3.02‰ respectively). $\delta^{15}\text{N}$ values of DON values showed no significant differences according
225 to their spatial position ($\text{df}=2$, $F=0.944$, $p=0.440$), but did show significant differences related
226 to the presence of wastewater discharge that were related to more enriched values near the
227 entry point ($\text{df}=3$, $F=17.035$, $p<0.001$).

228 Sedimental detritus had $\delta^{13}\text{C}$ values that were less depleted than $\delta^{13}\text{C}$ of POC
229 (average $-17.96 \pm 3.52 \text{‰}$; Table 2), showing no significant differences according to the
230 criteria studied. However, $\delta^{15}\text{N}$ of sedimental detritus (average: $7.39 \pm 2.44\text{‰}$) did vary
231 depending on the effect of wastewater discharge, being also more positive near the entry
232 point ($\text{df}=3$, $F=3.897$, $p=0.040$). $\delta^{13}\text{C}$ of DIC were on average $-7.74 \pm 0.87 \text{‰}$ and only
233 showed significant differences depending on the spatial location ($\text{df}=2$, $F=4.473$, $p=0.020$).

234

235 *3.2 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the living reservoirs*

236 Regarding primary producers, charophyte showed enriched $\delta^{13}\text{C}$ values ($-14.94 \pm 0.43 \text{‰}$;
237 Table 2), while emerging macrophytes presented depleted $\delta^{13}\text{C}$ values ($\approx -28 \text{‰}$). Regarding
238 $\delta^{15}\text{N}$ values, the lowest average values were those of *T. canariensis* ($4.85 \pm 1.12 \text{‰}$) and the
239 highest by *S. maritimus* ($11.03 \pm 2.88\text{‰}$). Only phytoplankton showed significant spatial
240 differences in $\delta^{15}\text{N}$ ($p=0.028$, Table 3). The effect of wastewater discharge in $\delta^{15}\text{N}$ of primary

241 producers was not significant, although the $\delta^{15}\text{N}$ of epiphyton was marginally affected by the
242 distance to the water inlet ($R^2=0.5174$; $p=0.057$). The effect of wastewater on $\delta^{13}\text{C}$ of primary
243 producers was not significant. Secondary production was depleted in $\delta^{13}\text{C}$, mainly in the case
244 of invertebrates (-27.80 ‰), with zooplankton having slightly more enriched values (average:
245 $-23.50\pm 5.43\%$). In contrast $\delta^{15}\text{N}$ of secondary producers was very rich, especially that of
246 zooplankton.

247

248 *3.4 Contribution of C sources to wetland organic matter, primary producers and secondary* 249 *production*

250 Using SISUS, we showed that *T. canariensis* was the main source contributing to POM (69-
251 84%), followed by the roots of the most abundant emergent macrophytes ($\approx 19\%$) and, with
252 similar contribution, *P. australis*, *C. mariscus* and *T. dominguensis* ($\approx 15\%$; Table 2).
253 According to SISUS, C sources that contribute to wetland secondary production vary widely.
254 *C. submersum* appears as the main diet of zooplankton, although POC and microbial mats
255 also represent a significant contribution (Table 2. *T. canariensis* appeared to be the main diet
256 of invertebrates (Table 2).

257

258 **4. Discussion**

259 In this study, the $\delta^{13}\text{C}$ of both C living and nonliving reservoirs were more dependent on the
260 physical-chemical characteristics of water and sediments than on other habitat features, while
261 the effects of wastewater discharges mainly affected the isotopic signature of the dissolved
262 fraction of organic matter and sediment detritus. Regarding $\delta^{15}\text{N}$, primary producers such as
263 epiphyton, filamentous algae and *S. maritimus* did show a clear decrease as we moved away
264 from the entry point as seen in other systems influenced by wastewater discharge (Costanzo
265 *et al.*, 2001). These results suggest that while both $\delta^{13}\text{C}$ of both C living and nonliving

266 reservoirs are good markers of environmental conditions, $\delta^{15}\text{N}$ of primary producers are
267 better indicators of disturbance, thus providing complementary information.

268 The isotopic fractionation that occurred in phytoplankton during photosynthesis in the
269 PNTD is outside the normal range of observation (-13.4 ‰ $\delta^{13}\text{C}$ in our case, -20 to 30 ‰;
270 Jones et al., 1998), which can be associated with the composition of phytoplankton species,
271 temperature and even the ability to use HCO_3^- by algae, with greater affinity for $\delta^{13}\text{C}$ and,
272 thus, with less fractionation (Bade *et al.*, 2006). Furthermore, $\delta^{13}\text{C}$ of aquatic plant values
273 reveal a distinctive C fixation pathway according to the species: while macrophytes generally
274 are in the range of C_3 plant values, *C. hispida* var. *major* and *P. pectinatus* have C_4 plant
275 signatures (Keeley, 1990). $\delta^{13}\text{C}$ of *C. submersum* values show a possible contamination
276 because carbonate values should be close to those of C_4 plants (Sensuła *et al.*, 2006).
277 Nevertheless, some authors like (Raz-Guzman, A. & L.A., 1992) do not considered it feasible
278 to categorize aquatic vegetation exclusively as C_3 or C_4 because the relationship between
279 $\delta^{13}\text{C}$ and the photosynthetic mechanism is not as strict as in the case of terrestrial vegetation,
280 and also aquatic vegetation reflects HCO_3^- isotopic composition. And this, in turn, depends on
281 the salinity, which can interfere with the signatures and obscure the carboxylation process.

282 In the PNTD, previous studies determined that the emergent vegetation can contribute
283 up to 65% organic matter deposited on the wetland (autochthonous organic matter; Sánchez-
284 Carrillo *et al.* 2001). Our results indicate that the main contribution to POM was from *T.*
285 *canariensis*, while emerging and submerged macrophytes represented less than 20%. Since
286 most of the carbon containing macrophytes is recalcitrant (Mann, 1988), it is expected that
287 most of these plant remains entering the detrital pool will eventually be degraded by fungi
288 and bacteria (Kuehn *et al.*, 2000). However, the conditions prevailing in the years prior to
289 sampling (2006-2010) as well as the biomass removed might be masking the actual
290 contribution of emergent macrophytes to the detritus in the system.

291 Some studies show that *Tamarix* sp. has a low litter quality, which tends to reduce the
292 biodiversity of macroinvertebrates and increases its accumulation in sediments (Bailey,
293 Schweitzer & Whitham, 2001). The strong spread of this species in the park during the dry
294 years prior to sampling could be masking their actual effects during wet cycles when it is
295 confined to the margins of the wetland (Bailey *et al.*, 2001). POM showed isotopic signatures
296 consistent with macrophytes, which may indicate that their importance probably varies
297 temporarily and needs to be re-evaluated in a long dry-wet cycle. In any case, the importance
298 of *T. canariensis* in secondary production is surprising, despite its low nutritional value.

299 The Chironomidae family, the most abundant of the benthic macroinvertebrates
300 during our study, was found to be related to *Tamarix* sp. seeds (Campobasso, 2017) and with
301 some algae species (Maasri *et al.*, 2008). The proliferation of *T. canariensis* during the dry
302 years has resulted in that, for many areas, it has been the dominant riparian plant species.
303 This could have increased its importance for secondary production, which should have
304 become dominated by macroinvertebrates that tolerate its low litter quality (Bailey *et al.*,
305 2001). Other species such as *C. mariscus*, emblematic of Daimiel, show a very low
306 contribution to POM (less than 20%), although they represent a relatively important energy
307 source for zooplankton (up to 47.17%), probably as a result of a drastic reduction in coverage
308 suffered during the last 50 years (Cirujano *et al.*, 2010). Instead, *P. australis*, which has
309 increased by twenty-two times since 1950 (Alvarez-Cobelas *et al.*, 2001), but does not
310 contribute significantly to the POM, or invertebrates (less than 22%) can represent up to 54%
311 zooplankton's diet. Gratton & Denno (2006) demonstrated in a coastal wetland that the large
312 biomass of *P. australis* was not functionally integrated in the local food web compared with
313 other plants such as *Spartina*. The small contribution of *P. australis* in Las Tablas is
314 paradoxical considering the great amount of biomass generated annually (M.D. Ribeiro,

315 2005). We speculate that this matter is quickly assimilated by microorganisms, but that it does
316 not represent a significant of macroinvertebrates's diet.

317 The direct contribution of phytoplankton to zooplankton was high (up to 68%). POM
318 was the main zooplankton's diet, confirming what has been observed in other aquatic
319 ecosystems, even from other latitudes (Jones *et al.*, 1999; Grey, Jones & Sleep, 2000).
320 However, in the NPTD, $\delta^{13}\text{C}$ of zooplankton were more enriched than those of POM,
321 contradictory to most freshwater ecosystems (depleted in comparison with the POM; Grey &
322 Jones 1999; Jones *et al.* 1999). Isotopic relations between zooplankton and food sources can
323 be masked by the dominance of a particular species, as well as the season and the habitat
324 where this is collected (Grey & Jones, 1999; Grey *et al.*, 2000)

325 In this study, we showed that invertebrates trophically depend on the vegetation but
326 not on macroalgae, contrary to the results of Finlay, Khandwala & Power (2002) but similar
327 to Bunn *et al.* (2003) in an Australian stream. Finlay *et al.* (2002) found that most consumer
328 invertebrates (e.g., mayflies, caddisflies, blackflies, Hydropsychidae, stoneflies, Hemiptera
329 and Odonata) depended on algal production, mainly composed by *Cladophora glomerata*. In
330 NPTD, it is possible that dry cycles modify macroinvertebrate trophic patterns or that in this
331 category of semiarid lentic systems the macroalgae's contribution to secondary production is
332 lower than expected. In any case, our results should be strengthened with more isotopic
333 studies in different hydrological and environmental conditions to capture all the possible
334 variability of sources and trophic processes.

335

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472 **Table 1.** Physicochemical characteristics of the five sampling zones in June 2011.

Zone	pH	Conductivity mS cm-1	TOM mg/L	TOC mg C/L	DOC mg C/L	TN mg/L de N	NH ₄ ⁺ mg NH ₄ ⁺ /L	NO ₃ ⁻ mg NO ₃ ⁻ /L	TP mg P/L	PO ₄ ³⁻ mg PO ₄ ³⁻ /L	Chl- <i>a</i> μg/L
Inner	8.17±0.05	2.46±0.23	368.00±145.96	8.69±0.87	7.24±0.33	0.90±0.15	0.04±0.02	0.15±0.02	0.11±0.12	0.02±0.01	8.72±4.46
Littorals	7.88±0.38	2.56±0.12	327.00±45.77	12.00±2.85	8.53±2.48	1.11±0.29	0.04±0.04	0.12±0.02	0.06±0.06	0.02±0.01	6.00±4.36
Channels	8.09±0.06	2.20±0.26	314.67±46.40	7.27±2.10	7.08±2.09	2.30±2.1	0.55±0.73	3.91±6.0	0.15±0.17	0.06±0.01	22.69±17.24
Depth zones	8.29±0.24	2.19±0.04	262.00±31.11	9.50±0.37	6.48±0.01	0.84±0.05	0.03±0.00	0.17±0.04	0.02±0.02	0.02±0.00	2.84±2.87
Wastewares discharges	8.06±0.03	2.35±0.99	296±196.61	5.97±1.15	5.46±0.92	5.89±6.44	3.93±6.45	3.75±4.20	0.52±0.73	1.09±1.83	80.62± 96.33

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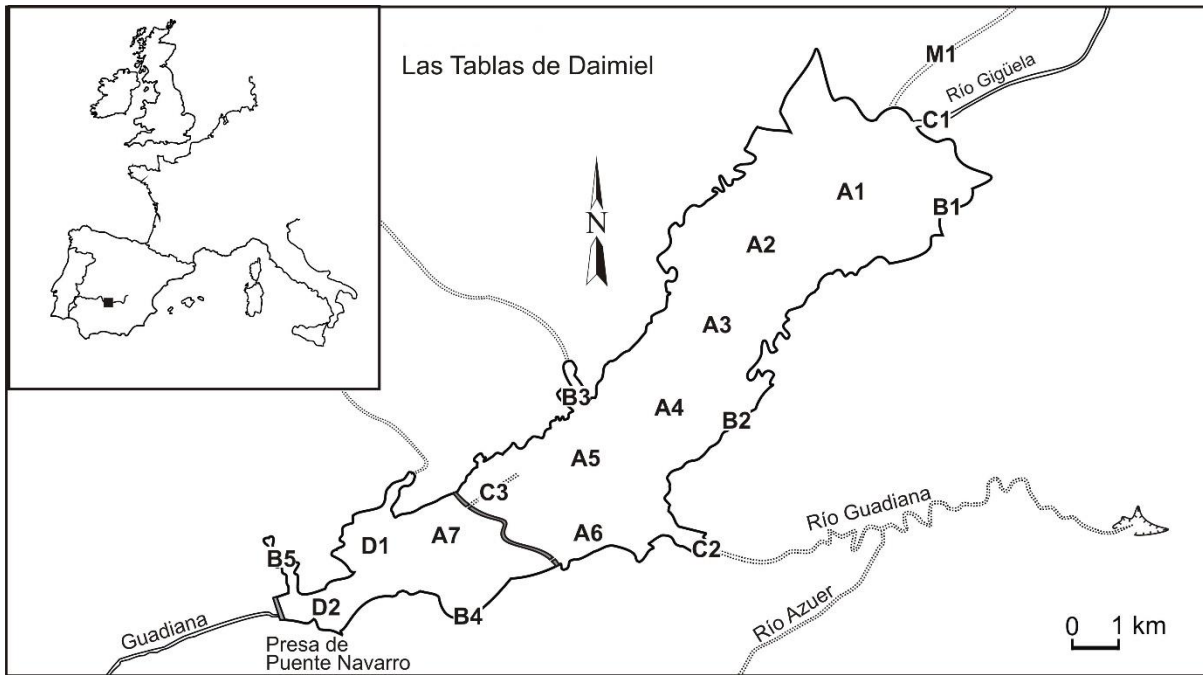
479 **Table 2.** Range of contribution (%) of the main C sources (primary producers and sediment
 480 detritus) to particulate organic matter (POM) and secondary production in summer 2011,
 481 based on SISUS.
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	POM		Invertebrates		Zooplankton	
	Min	Max	Min	Max	Min	Max
Sedimen detritus	0	2.89	0.00	3.87	0.00	46.28
DOC (intermediate zone)			0.00	2.02	0.00	24.29
DOC (end zone)			0.00	2.08	0.00	24.92
DOC (entrance zone)			0.00	1.74	0.00	20.83
POC			0.00	29.08	0.00	80.64
Phytoplankton	0.00	4.23	0.00	5.66	0.00	67.90
Periphyton	0.00	4.97	0.00	6.64	0.00	63.54
filamentous algae	0.00	5.49	0.00	7.35	0.00	63.83
Microbial mats	0.00	6.40	0.00	8.55	0.00	77.87
<i>C. hispida</i> var. <i>major</i>	0.00	2.23	0.00	2.98	0.00	35.71
<i>C. submersum</i>	0.00	8.60	0.00	11.50	0.00	92.35
<i>P. pectinatus</i>	0.00	3.14	0.00	4.20	0.00	50.37
<i>S. maritimus</i>	0.00	14.58	0.00	20.41	0.00	48.06
<i>T. dominguensis</i>	0.00	15.52	0.00	21.75	0.00	51.21
<i>C. mariscus</i>	0.00	15.67	0.00	20.95	0.00	47.17
<i>P. australis</i>	0.00	16.21	0.00	21.66	0.00	54.00
<i>T. canariensis</i>	69.46	83.74	59.16	78.71	0.00	41.22
Roots	0.00	19.02				

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485 **Fig. 1.** Location of "National Park Las Tablas de Daimiel" and sampling sites. Inland areas (n
486 = 7 sites; A1: A7), littorals (n = 5 sites, B1: B5), channels (n = 3 sites, C1: C3), and deep
487 zones (n = 2 sites, D1: D2; Fig. 1). M1 is the channel where the wastewater from "Villarrubia
488 de los Ojos" discharges after treatment.
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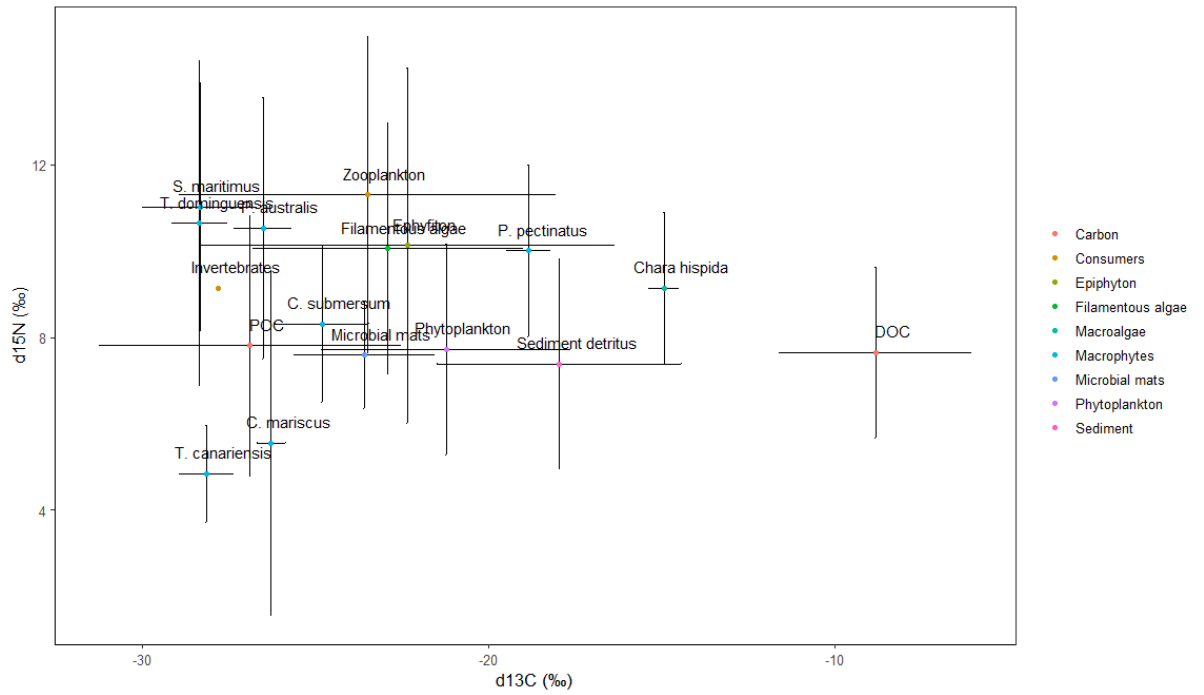
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500 **Fig. 2.** $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the main C sources and secondary production (invertebrates
 501 and zooplankton) at NPTD. Errors bars are SE for the same group across habitats.
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