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1 **Impacts on freshwater macrophytes produced by small invertebrate herbivores:**
2 **Afrotropical and Neotropical wetlands compared**

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18

19 **Abstract**

20 We compare invertebrate herbivory upon 13 macrophyte species in freshwater wetland systems
21 located in two global ecozones, the Afrotropics and Neotropics, in the context of biotic and
22 environmental factors influencing these wetlands. The two ecozones are climatically-similar
23 regions, with similar water chemistry, but experience contrasting grazing and disturbance
24 pressures from large mammalian herbivores. Our results for macrophytes show that small
25 invertebrates removed significantly more lamina biomass per leaf in Neotropical macrophytes
26 (6.55%) than Afrotropical ones (4.99%). Overall, the results indicate that under-estimation of up
27 to 15.6% of leaf biomass may occur if plant tissue removal by invertebrate herbivores is not
28 included in estimates of plant biomass. Regarding the contrasting grazing and disturbance
29 pressures from large herbivores influencing these wetlands, seven mammal species (especially the
30 Black Lechwe antelope, *Kobus leche*) were observed impacting macrophytes in the Afrotropical
31 wetlands, while in the Neotropics, only much smaller rodents, capybara (*Hydrochoerus*
32 *hydrochaeris*) were sporadically observed. We discuss the relevance of results for invertebrate
33 herbivory in the context of both the methodological approach, and the importance of large
34 mammalian herbivores as biotic factors additionally impacting macrophyte populations in these
35 subtropical to tropical wetlands.

36

37 **Key words:** Herbivorous mammals; freshwater ecosystems; grazing damage; tropics

38

40 **Introduction**

41 Historically, both the abundance of herbivores and the influence of herbivory (produced by
42 invertebrates and larger grazing animals) have been little considered as a biotic process
43 influencing macrophyte communities within freshwater ecosystems (e.g., Newman, 1991; Cyr
44 and Pace, 1993; Lodge et al., 1998). Macrophytes (“aquatic photosynthetic organisms, large
45 enough to see with the naked eye, that actively grow permanently or periodically submerged
46 below, floating on, or up through the water surface” of inland freshwater or brackish waterbodies:
47 Chambers et al., 2008) were considered mainly as providers of physical substrate for periphyton,
48 habitat for invertebrates and fish, and a source of detritus for invertebrate detritivores (e.g.,
49 Selford 1918; Newman, 1991; Wetzel, 2001; Thomaz and da Cunha, 2010). However, evidence is
50 now mounting that herbivores can substantially affect both macrophyte abundance, and the
51 structure and functioning of freshwater ecosystems that support macrophyte communities
52 (Coetzee et al., 2011; Bakker et al., 2016a, b; Grutters et al., 2016; Wood et al., 2016).
53 Furthermore, these studies have shown that herbivory may substantially impact macrophyte
54 biomass, with median values for percentage removal of 44 - 48 % (Bakker et al., 2016a), which
55 are generally higher than those recorded for the impacts of herbivory on terrestrial vegetation
56 (Cyr and Pace, 1993; Bakker et al., 2016a). Most work on macrophyte herbivory has concentrated
57 on temperate ecosystems and has generally neglected tropical or sub-tropical ecosystems, with
58 studies of invertebrate herbivory impacts in warm-water systems hitherto primarily focused on
59 insects used or proposed as biological control agents of invasive macrophytes (e.g., Coetzee et al.,
60 2011; Sacco et al, 2013; Cabrera Walsh et al., 2017; Bownes, 2018; Strange et al. 2018).

61

62 Previous studies suggest that macrophyte biomass and productivity can be high in tropical and
63 sub-tropical freshwater ecosystems (e.g., Boar et al., 1999; Morison et al., 2000; Silva et al.,
64 2009; Bottino et al., 2014). Such productivity is likely to support invertebrate herbivory, and there
65 is evidence from the Neotropics that biomass directly removed by invertebrate grazing can be up
66 to 27% of the leaf lamina biomass, and up to 26% of the lamina biomass per m² of vegetation
67 (Franceschini et al., 2010). There has also been some work, mainly in the Neotropics, on the
68 effects of invertebrate herbivory on naturally-occurring macrophyte populations which suggests
69 that invertebrate damage influences the seasonal decay of macrophyte populations and that
70 herbivores may strongly affect detrital inputs from macrophyte sources (Medeiros dos Santos and
71 Esteves, 2002; Poi de Neiff and Casco, 2003). Whether determined by destructive (Soti and
72 Volin, 2010) or non-destructive methods (Gonçalves et al., 2010), it is highly likely that

73 measurements of macrophyte biomass and production which do not take into account the biomass
74 removed by invertebrate herbivores will underestimate true plant biomass and production values
75 (Esteves, 2011). Also, the number of studies of biodiversity and ecosystem-functioning involving
76 macrophytes in subtropical and tropical freshwater systems, in the context of the relevant biotic
77 and environmental factors that influence their functioning, has been increasing in recent years
78 (e.g., Murphy et al., 2003; Padial et al., 2008; Varandas Martins et al., 2013; Bottino et al., 2014;
79 Tapia Grimaldo et al., 2016, 2017; Kennedy et al., 2015, 2017; Trindade et al., 2018). However
80 knowledge of the effect of invertebrate herbivores on warm-water macrophyte populations, and in
81 particular their biomass and production values remains very limited.

82 Both the Afrotropics and Neotropics are global ecozones with substantial areas of freshwater
83 ecosystems supporting rich macrophyte γ -diversity and productivity, with plants playing an
84 important role in the functioning of such ecosystems (e.g., Morison et al., 2000; Wetzel, 2001;
85 Chambers et al., 2008; Silva et al., 2009; Murphy et al., 2019; Murphy et al., 2020). However,
86 there are a number of ecological dissimilarities between these two warm-climate regions of the
87 Earth. Amongst these is the distinct difference between the two ecozones, in the abundance of
88 large herbivores impacting wetland systems. This is of particular interest here because these large
89 grazing animals may act both as competitors with, and sources of direct and indirect damage to
90 invertebrate herbivores associated with aquatic macrophytes.

91 In the Afrotropical ecozone, grazing by wild mammalian herbivores is known to be an important
92 biotic factor influencing ecosystem processes, though studied mainly in terrestrial rather than
93 freshwater ecosystems (Cristoffer and Peres, 2003; Asner et al. 2009; Hamandawana, 2012;
94 Hrabar and Du Toit, 2014), despite the fact that many of the large African mammalian herbivores
95 feed in wetlands, especially during the dry season (Chabwela and Ellenbrook, 1990; Redfern et
96 al., 2003). In Afrotropical wetlands macrophytes and their associated invertebrate herbivore
97 assemblages hence frequently coexist with a high diversity of large mammal herbivores, with
98 individual body weights in the range 40 – 6300 kg (Stuart and Stuart, 2006). Often such animals
99 are present at high abundance. For example, in one of the target areas of this study, the
100 Bangweulu Swamp of Northern Zambia, a recent survey (Viljoen, 2011) showed the presence of
101 large numbers (c. 75,000 animals across an area of 243 km²) of the semi-aquatic antelope Black
102 Lechwe [*Kobus leche* subsp. *smithemani* (Lydekker, 1900)], primarily feeding on floodplain and
103 aquatic vegetation. Large herbivores may also act as a biotic factor modifying nutrient cycling in
104 warm-water wetland systems, as well as potentially causing substantial direct disturbance impacts

105 on invertebrate assemblages and their host plant communities (e.g., via trampling), in addition to
106 direct feeding damage (Zamora and Gómez, 1993; Bakker and Nolet, 2014; Bakker et al., 2016a).
107 The Neotropical ecozone is very different in this regard (Cristoffer and Peres, 2003). In wetlands
108 of this ecozone, and certainly in northeastern Argentina, large mammal herbivore species of more
109 than 80 kg body weight are almost absent [with the exception of very small numbers of Swamp
110 Deer: *Blastocerus dichotomus* (Illiger, 1815)], and invertebrate herbivore assemblages only
111 coexist with a low abundance and diversity of mainly smaller mammalian herbivorous species
112 [especially the large rodent, Capybara: *Hydrochoerus hydrochaeris* (Linnaeus, 1766)], which
113 feeds on floodplain and aquatic vegetation, though grazing impacts may not always be severe
114 (Milne et al., 2008; Ramos et al., 2018). Despite the evidence for substantial ecological
115 differences between these two warm-climate regions, there has been no previous attempt to
116 characterize invertebrate herbivore assemblages on naturally-occurring macrophyte populations in
117 the Afrotropics and Neotropics, with the exception of a concurrent study (Franceschini et al.,
118 2020 accepted) that shows substantial differences in abundance and composition of such
119 invertebrates from the two ecozones.

120 In the light of the differences in environmental and biotic pressures, such as large animal
121 herbivory, acting upon wetland systems in the two ecozones, a question which arises is whether
122 the impact of invertebrate herbivores on freshwater macrophytes in Afrotropical and Neotropical
123 wetlands might also differ. The primary hypothesis assessed here was that damage caused by
124 small invertebrate herbivores is an extensive process affecting freshwater tropical and subtropical
125 macrophytes, but quantitatively differs between the two ecozones. Secondly, we examined the
126 possibility that the presence of large herbivores may be one relevant biotic factor influencing any
127 such observed differences in macrophyte-invertebrate herbivory interactions in warm-freshwater
128 wetlands of the two ecozones.

129

130 **Materials and Methods**

131

132 **Study sites, herbivores and macrophyte species**

133 In the Afrotropics, sampling was conducted at seven sites in the Northern and Eastern Provinces
134 of Zambia, within three well-protected conservation areas: Kasanka National Park and the
135 Bangweulu Game Management Area (Northern Province), and South Luangwa National Park
136 (Eastern Province). In the Neotropics, study areas were located in northeastern Argentina,

137 comprising ten sites within the Riachuelo and Paraguay Basin, and Paraná River floodplains,
138 including the international Ramsar protected area of the Chaco Wetlands. (Fig. 1).

139 The study sites were chosen to provide conditions strongly contrasting in terms of abundance,
140 richness and body weight of mammalian herbivores present (Stuart and Stuart 2006; Quintana et
141 al., 2012; Schivo et al., 2010; Marques 1988), but similar in terms of water chemistry (e.g., see data
142 on pH and conductivity presented below for the study sites). Although northeastern Argentina is
143 geographically subtropical and the Northern and Eastern Provinces of Zambia are tropical, the
144 latter areas are located at high altitude (500 - 1200 m above sea level, a.s.l.) whilst the former is
145 low-lying (60 - 80 m a.s.l.), so that in fact the two study areas are climatically quite closely
146 comparable, with similar rainfall and temperature ranges across the year. The Köppen climate
147 scheme designates the study area in Zambia as "humid subtropical climate", whilst that in
148 Argentina is classified as "warm oceanic climate/ humid subtropical climate".

149 In this paper, we define "large herbivores" to include terrestrial, semiaquatic or aquatic vertebrates
150 that obtain some or all of their food from freshwater macrophytes, whereas phytophagous insects
151 and gastropods, are referred to as "small invertebrate herbivores".

152 In Afrotropical wetlands, small invertebrate herbivores coexist with a substantial range and
153 abundance of large herbivores, mainly mammals (Fig. 1). These include several antelope species,
154 Plains Zebra [*Equus quagga* subsp. *burchellii* (Gray 1824)], Hippopotamus [*Hippopotamus*
155 *amphibius* (Linnaeus, 1758)], African Savannah Elephant [*Loxodonta africana* (Blumenbach,
156 1797)], and Buffalo [*Syncerus caffer* (Sparrman, 1779)], as well as omnivorous species, such as
157 Yellow Baboon [*Papio cynocephalus* subsp. *cynocephalus* (Linnaeus, 1766)], also known to
158 include macrophytes in their diet. In Neotropical wetland systems, small invertebrate herbivores
159 coexist with only a low abundance of mammalian semiaquatic herbivores, mainly Capybara (*H.*
160 *hydrochaeris*), Swamp Deer *B. dichotomus*, Coypu [*Myocastor coypus* (Molina, 1782)] and the
161 Red Marsh Rat [*Holochilus brasiliensis* (Desmarest, 1819)].

162 In each ecozone, sites and macrophyte species were selected to include representatives of each of
163 the four usually-distinguished functional groups ("life-forms") of aquatic plants (Chambers et al,
164 2008): free-floating (FF), floating leaf-rooted (FR), emergent (E), and submersed (S) species.
165 Species were identified using specific guides for each ecozone (Arbo and Tressens, 2002;
166 Kennedy and Murphy 2012) and nomenclature was confirmed following The Plant List
167 (www.theplantlist.org). In the Afrotropical wetlands, the macrophytes studied were *Pistia*
168 *stratiotes* L. (FF: Araceae), *Nymphaoides indica* (L.) Kuntze (FR: Menyanthaceae), *Nymphaea*

169 *nouchali* var. *caerulea* (Savigny) Verdc. (FR: Nymphaeaceae), *Trapa natans* L. (FR: Trapaceae),
170 *Potamogeton nodosus* Poir. (FR: Potamogetonaceae), *Cyperus papyrus* L. (E: Cyperaceae) and
171 *Potamogeton octandrus* Poir. (S: Potamogetonaceae). Although *P. octandrus* sometimes has
172 floating leaves present, only assessments of damage to its more abundant submersed leaves were
173 included in this study. In the Neotropical wetlands the macrophytes studied were *P. stratiotes* and
174 *N. indica* (also present in the sites in Zambia), as well as *Nymphaea prolifera* Wiersema (FR:
175 Nymphaeaceae), *Hydrocleys nymphoides* (Humb. and Bonpl. ex Willd.) Buchenau (FR:
176 Lymnocharitaceae), *Eichhornia azurea* (Sw.) Kunth (FR: Pontederiaceae), *Cyperus giganteus*
177 Vahl (E: Cyperaceae), *Thalia multiflora* Horkel ex Körn. (E: Marantaceae) and *Potamogeton*
178 *illinoensis* Morong (S: Potamogetonaceae).

179

180 **Assessment of damage by small invertebrate herbivores on Afrotropical and Neotropical** 181 **macrophytes**

182 We sampled mature leaves and stems of freshwater macrophyte populations in a range of
183 freshwater habitats, during the decline (winter dry season) period of the plant growth cycle (June
184 to September in 2012 and 2013, respectively in Argentina and Zambia). Sampling dates were
185 chosen in the dry season in both countries primarily because wetland macrophyte populations are
186 more accessible for sampling purposes. Leaf damage was evaluated in free-floating, floating-leaf
187 rooted, and submersed macrophytes, and stem damage was evaluated in emergent species (both
188 *Cyperus* species are leafless plants). Invertebrate taxa producing leaf and stem damage were
189 identified as a part of a concurrent study on invertebrate herbivore assemblages in both ecozones
190 (Franceschini et al., 2020 accepted).

191 For all macrophyte species, we collected three samples of 10 leaves or stems at random from
192 different individuals of each plant species per site (N=30 leaves or stems per macrophyte species
193 and site). Two plant species, the FF *P. stratiotes* and the FR *N. indica*, were each sampled in both
194 ecozones and the S *P. octandrus* was sampled in two sites from the Afrotropics (N=60 leaves per
195 macrophyte species). In total 390 leaves were assessed in the ten FF, FR and S macrophyte
196 species considered, whereas a total of 90 stems were assessed in the three E macrophyte species
197 included in this study. In each macrophyte population, samples included the edge and the centre
198 of the vegetation stand (one and two samples, respectively, collected at random from each part of
199 the stand, and separated as much as was possible from each other to maximise independence of
200 the data).

201 Total number of leaves and stems damaged and non-damaged by small invertebrate herbivore
202 grazing were counted and compared in each macrophyte species. The following categories of
203 invertebrate damage were distinguished and separately measured (Labandeira, 1998):

- 204 i. Surface abrasions: caused when epidermis and mesophyll were not completely removed,
205 and most basal tissue persists in the affected areas of the leaf lamina. This type of damage
206 was not found when processing petioles and stems.
- 207 ii. Holes: involved complete removal of tissues of the leaf lamina.
- 208 iii. Galleries: parenchyma and vascular tissues inside stems and petioles were removed or
209 affected as a consequences of necrosis. Total numbers of leaf petioles per plant species
210 affected by galleries were also counted. Galleries produced by miners were not found when
211 processing leaf laminas.

212 Biomass removed by herbivores (surface abrasions and holes) per leaf was calculated indirectly
213 using the data for damaged lamina area. Area damaged by invertebrate herbivores (surface
214 abrasions and holes) was measured by a photographic procedure, using ImageJ 1.44 (Rasband
215 1997-2016), for each sampled leaf. Due to the small size of submersed leaves of *P. octandrus*,
216 damage was quantified for this species with ImageJ using a stereoscopic microscope, analyzing
217 leaves previously preserved in 70% ethanol.

218 Damaged area data were converted to biomass removed following different procedures and
219 equations for surface abrasions and holes. Surface abrasion was assessed by the difference in
220 weight between the area with this type of damage and the same size area without damage. We cut
221 leaf circles of 6-7 mm diameter, depending on macrophytes species, using a perforating punch.
222 The surface abrasion biomass was calculated on the basis of the mean weight of 30 circles with
223 surface abrasion and the same number of circles of the same size from undamaged areas, using
224 equation (1), below (Franceschini et al., 2010):

$$(1) \quad b_s = \frac{\sum \frac{ad_s \cdot (Wn - Wd)}{a_s}}{N}$$

225
226 Where b_s is surface abrasion biomass (g), ad_s is the damaged area by surface abrasion (cm²), a_s is
227 area of the circle (cm²), Wn is the mean weight of undamaged circles (g), Wd is the mean weight
228 of damaged circles with surface abrasion (g), and N is the total number of leaves.

229 The average weight of undamaged circles was used to calculate the biomass removed in holes
230 produced by small invertebrate herbivore grazing. Because tissues are removed completely in the
231 affected areas, equation (2) was used to calculate the biomass removed by this damage
232 (Franceschini et al., 2010):

$$(2) \quad b_h = \frac{\sum \frac{ad_h \cdot Wn}{a_s}}{N}$$

234 Where b_h is hole biomass, ad_h is the area damaged by holes (cm^2), a_s is the area of the circle
235 (cm^2), Wn is the mean weight of undamaged circles (g), and N is the total number of leaves. For
236 each lamina, total biomass removed was calculated as the sum of the damage produced by surface
237 abrasion plus damage by holes. Removed biomass (holes, surface abrasions and total) and lamina
238 biomass were used to calculate the percentage of lamina biomass removed by invertebrate
239 herbivores per leaf. Leaf circles and leaf lamina were previously dried for 72 hours at 60 °C to
240 obtain constant dry weight values.

241 To compare invertebrate herbivory on macrophytes from Afrotropical and Neotropical wetlands,
242 we quantified, and compared between ecozones, the percentage of total biomass removed (by
243 both surface abrasions and holes) per leaf produced by small invertebrate herbivores. The two sets
244 of data percentages (i.e., % removed by surface abrasion and % removed by hole), were also
245 assessed separately and compared between ecozones. We used values of percentage of biomass
246 removed by invertebrates per leaf instead of absolute values due to the high variability of leaf
247 biomass and size observed in the different species and functional groups of aquatic macrophytes
248 included in this study.

249

250 **Assessing biotic and environmental factors influencing study sites**

251 The presence of different species of large herbivores in the study areas was noted during
252 fieldwork, by direct observation or from the presence of fresh footprints, either by walking
253 through the wetland survey areas, or from a game-viewing vehicle (in areas where large
254 carnivores were present).

255 Trampling damage to macrophyte vegetation, produced by mammalian herbivores, was scored on
256 a semi-quantitative scale of 1 = no disturbance due to trampling by animals, to 4 = major
257 trampling damage. Water turbidity, which is affected by resuspension of sediments caused by

258 large-animal trampling disturbance of waterbody substrates, was recorded in the Afrotropical sites
259 as photosynthetically-active radiation (PAR) absorbance coefficient: $k \text{ m}^{-1}$, calculated from PAR
260 measurements taken using an underwater PAR sensor at two points in the water column: just
261 below surface and at a standard depth, usually 0.22 m (Moore and Murphy, 2015). In the
262 Neotropics, water turbidity was recorded as Secchi depth (m), considering also maximum water
263 depth (m) as a complement of this variable. Other environmental parameters measured at the
264 sampling sites were pH, conductivity ($\mu\text{S cm}^{-1}$), water temperature ($^{\circ}\text{C}$), and visually-assessed
265 flow class (class 1= static or very slow flow; 2 = slow flow: “pool”; 3 = moderate flow: “glide”; 4
266 = fast flow: “white water showing”: Lang and Murphy, 2011). Field meters used in Zambia were
267 a Handylab pH/temperature LF12 meter, HI98311 conductivity meter, and a SKYE SKP210
268 underwater PAR sensor. Field meter used in Argentina were a handylab
269 pH/temperature/conductivity Hanna meter.

270 **Statistical analysis**

271 To assess the extent of invertebrate herbivory damage on macrophytes from Neotropical and
272 Afrotropical wetlands, we used a Chi-square Test (χ^2) with Yates correction for continuity to
273 compare number of damaged leaves and stems quantified in the field (observed values) with
274 respect to expected values (H_0 : number of damaged leaves = number of non-damaged ones) for
275 samples from the Afrotropics and Neotropics. Two mensurative analyses (Hurlbert, 1984) were
276 conducted to compare invertebrate herbivory in Afrotropical and Neotropical macrophytes, using
277 as variables percentage biomass removed per leaf (total damage), as well as abrasion and hole
278 damage, assessed separately. First, we made a general comparison between ecozones using the
279 percentage of biomass removed by invertebrates on leaves of S, FF and FR macrophyte species.
280 Data for herbivory damage were normalized by $\log_{10}(x+1)$ transformation, then assessed for
281 significance using General Linear Models (GLM) with LSD Fisher post hoc mean separation
282 tests. We incorporated an *a priori* function to model the heterogeneous variances. Second, the
283 same approach was used to compare invertebrate herbivory per leaf on plants of *P. stratiotes* and
284 *N. indica*, occurring both in the Afrotropics and Neotropics. Differences were considered to be
285 statistically significant at $p < 0.05$. Statistical analyses were performed using Infostat Software,
286 with R interface (Di Rienzo et al., 2017)

287

288 **Results**

289 **Damage by small invertebrate herbivores on Afrotropical versus Neotropical freshwater**
290 **macrophytes**

291 Number of leaves with occurrence of invertebrate damage was significantly higher than number
292 of non-damaged leaves in both Afrotropical and Neotropical macrophytes ($\chi^2 > 3.84$, $df = 1$). More
293 than 70% of sampled leaves had damage produced by small invertebrate herbivores. Leaves with
294 invertebrate damage reached up to 72% of the sampled leaves in Afrotropics and 95% in
295 Neotropics (Fig. 2a). Almost all FR macrophytes studied had petioles containing galleries made
296 by endophagous invertebrate herbivores. Total number of petioles damaged by endophagous
297 larvae reached 83.3% in the Neotropical *E. azurea*, but was only 26.7% in the Afrotropical *N.*
298 *nouchali* var. *caerulea* and 13.3% in Neotropical *N. prolifera*, while the value was less than 7% in
299 the other Afrotropical and Neotropical macrophyte species.

300 Non-significant differences were found between total numbers of damaged and non-damaged
301 stems of emergent macrophytes ($\chi^2 < 3.84$, $df = 1$) in both ecozones (Fig. 2b). Galleries made by
302 endophagous herbivores were found in stems of the emergent macrophytes from both ecozones.
303 In Afrotropical stems, galleries were mainly produced by Lepidoptera larvae whereas galleries
304 from Neotropical stems were produced by adults and larvae of Curculionidae. The percentage
305 length of stems damaged by these herbivores was high in the Afrotropics (42.6%) and
306 intermediate to low in the Neotropics (25.9 to 7.1%).

307

308 A more detailed analysis of leaf herbivory by small invertebrates, quantifying percentage of total
309 biomass removed per leaf lamina (abrasion + holes) on floating and submersed macrophyte
310 species included in this study (Fig. 3a), indicated that invertebrates removed significantly more
311 lamina biomass in plants from the Neotropics than the Afrotropics (GLM, $p = 0.0084$; $N = 390$
312 leaves). In fact, percentage of total biomass removed by small invertebrate herbivores per leaf
313 was 1.31 times greater in Neotropical macrophytes ($6.55 \pm 0.66\%$) than Afrotropical ones ($4.99 \pm$
314 0.66%). In the Afrotropics, the highest percentage of biomass removed by invertebrates was
315 recorded on *Trapa natans* ($8.38 \pm 0.61\%$) and damage was produced exclusively by larvae and
316 adults of the semiaquatic chrysomelid *Donacia* sp. In Neotropical macrophytes, the percentage of
317 total biomass removed by small invertebrate herbivores per leaf reached up to $15.63 \pm 2.56\%$ on
318 *Nymphoides indica* and damage was produced mainly by semiaquatic weevils, grasshoppers and
319 caterpillars.

320 When type of damage, abrasion and hole, were assessed separately, non-significant differences on
321 biomass removed as abrasion damage were obtained comparing Afrotropical and Neotropical
322 macrophytes (GLM, $p= 0.2140$; $N= 390$ leaves). Abrasion damage was absent in submersed
323 leaves of the Afrotropical *P. octandrus* and the Neotropical *P. illinoensis*. In contrast to abrasion
324 damage, hole damage occurred in all leaf laminas sampled in Afrotropical as well in Neotropical
325 macrophytes. When biomass removed as holes was compared in leaves from both ecozones,
326 invertebrate herbivores produced significantly more hole damage on Neotropical than
327 Afrotropical macrophytes (GLM, $p< 0.0001$; $N= 390$ leaves). Leaves from Neotropical
328 macrophytes had 1.73 times more hole damage than those from Afrotropical ones (Fig. 3b-c). On
329 almost all macrophytes from Afrotropics and Neotropics, secondary infection by fungi and
330 bacteria was noted at grazing scars, which increased the affected tissues on leaves, especially in
331 the FR macrophytes included in this study.

332 When invertebrate herbivory was compared only in the two macrophyte species which occurred
333 in both ecozones, *Pistia stratiotes* and *Nymphoides indica*, the results show differences between
334 plants from Afrotropics and Neotropics. Total biomass removed (abrasion + holes) by
335 invertebrate herbivores on the FF *P. stratiotes* was significantly higher for Neotropical compared
336 to Afrotropical plants (GLM, $p< 0.0001$, $N= 60$), with mean total biomass removed by
337 invertebrates per leaf 3.95 times greater in Neotropics than in Afrotropics ($8.38 \pm 1.36\%$ versus
338 $2.12 \pm 0.52\%$). The same trend was obtained comparing abrasion damage (GLM, $p= 0.0023$, $N=$
339 60) and hole damage (GLM, $p< 0.0001$, $N= 60$) between ecozones. Values for mean biomass
340 removed as abrasion and hole were 17.4 and 3.64 times greater in plants from the Neotropics than
341 those from the Afrotropics, respectively (Fig. 4a-c).

342 A significant difference was also observed regarding invertebrate herbivory of *N. indica* between
343 Afrotropics and Neotropics for total biomass removed per leaf (GLM, $p= 0.0090$, $N= 60$), with a
344 mean total biomass removed per leaf 1.7 times greater for Neotropics plants ($15.6 \pm 2.56\%$) than
345 for Afrotropical ones ($8.93 \pm 2.64\%$). When types of damage were compared, biomass removed
346 by holes was 2.1 times greater for Neotropical plants than for Afrotropical plants (Fig. 4c), with
347 significant differences between plants from both ecozones (GLM, $p= 0.0004$, $N= 60$). On the
348 other hand, biomass removed by abrasion was 3.8 times greater in Afrotropics than in Neotropics
349 (Fig. 3b), with significant differences between plants from both ecozones (GLM, $p= 0.0004$, $N=$
350 60).

351 Despite the fact that the general trend showed that biomass removed by invertebrate herbivores
352 per leaf was higher in Neotropical macrophytes than those from the Afrotropics, almost all *P.*
353 *stratiotes* and *N. indica* plants, from both ecozones, showed herbivory damage to their leaves. The
354 total number of leaves with invertebrate damage was significantly higher than the number of non-
355 damaged leaves for both *N. indica* ($\chi^2= 14.01$ for both ecozones, $df= 1$) and *P. stratiotes* ($\chi^2= 7.35$
356 for Afrotropics; $\chi^2= 12.15$ for Neotropics, $df= 1$).

357

358 **Biotic and environmental factors influencing study sites**

359 The presence of herbivorous mammals was clearly a biotic factor more likely to influence the
360 Afrotropical sites rather than those located in the Neotropics. In total seven species of large
361 herbivores, mostly large mammals, were observed damaging macrophytes in the Afrotropical
362 wetland sites, while in the Neotropical wetlands, only grazing damage caused by Capybara (*H.*
363 *hydrochoerus*) was sporadically observed in the study sites. Other Neotropical small mammalian
364 herbivores, such as Red Marsh Rat (*H. brasiliensis*) and Coypu (*M. coypus*), and the larger
365 Swamp Deer (*B. dichotomus*) were not personally observed during sampling at the study sites in
366 Argentina, but are known to be present (Table 1). In the Afrotropics, Black Lechwe (*K. leche*),
367 was the most important wetland antelope species in Bangweulu, Puku [*Kobus vardonii*
368 (Livingstone, 1857)] in both South Luangwa and Kasanka [together with lower use of wetland
369 habitat by Impala: *Aepyceros melampus* (Sundevall, 1847); and Sitatunga: *Tragelaphus spekii*
370 (Speke, 1863) in Kasanka]. Other large grazing mammals, like Hippopotamus (*H. amphibius*)
371 were also observed in substantial numbers in waterbodies located in all three areas, as well as
372 African Savannah Elephant (*L. africana*) in Kasanka and South Luangwa. The smaller Yellow
373 Baboon (*P. cynocephalus* subsp. *cynocephalus*), which is an omnivorous animal, was also
374 observed feeding on macrophytes (especially *P. stratiotes*) and for this reason is considered here
375 as another large herbivore (Table 1). Trampling and grazing were particularly intense in the
376 Bangwuelu Swamps, produced by the high population density of the Black Lechwe, but severe
377 damage to macrophyte populations was also observed in South Luangwa, produced by the
378 activities of Hippopotamus and elephants. However, some waterbodies within these wetlands
379 were not used by large herbivores, usually either because the water is too deep for them to gain
380 access, or due to a high presence of aquatic predators, especially Nile Crocodile: *Crocodylus*
381 *niloticus* (Laurenti, 1768).

382 Contrasting influences of biotic pressures associated with the presence or near-absence of large
383 herbivores were recorded as being highly likely to impact populations of the two macrophyte
384 species, *Pistia stratiotes* and *Nymphoides indica* that occurred in both ecozones. The Afrotropical
385 *P. stratiotes* (in Mushroom Lagoon, South Luangwa) was observed to be heavily influenced by
386 trampling and/or herbivory of elephants, Hippopotamus, Puku and Impala antelope, and baboons.
387 In the same ecozone, *N. indica* in Shoebill A Lagoon (Bangweulu) was observed to be damaged
388 by both trampling and herbivory, mainly by Black Lechwe antelopes.

389 In contrast, both *P. stratiotes* and *N. indica* in their Neotropical sites (Antequera 1 and La Antena
390 Lake, respectively) experienced, at most, only low impact from mammalian herbivores (score 1),
391 and then only from sporadic capybara grazing and trampling in the study sites. During sampling
392 in Zambia, we also verified by personal observation that plants of a third species, *Potamogeton*
393 *octandrus*, were severely damaged by large herbivore activity in lagoon areas used by Black
394 Lechwe antelopes (Shoebill A), with many stems and leaves broken off the plants (Table 1,
395 Online Resource 1).

396 With regard to the full set of environmental factors measured, there were non-significant
397 differences between Afrotropical and Neotropical wetlands for pH (GLM, $p= 0.404$; $N= 14$),
398 temperature (GLM, $p= 0.201$; $N= 14$) and conductivity (GLM, $p= 0.550$; $N= 14$). However,
399 Afrotropical wetlands had significantly greater intensity of disturbance by large herbivores
400 (GLM, $p= 0.003$; $N= 14$) and faster flowing water (GLM, $p= 0.022$; $N= 14$) compared to those in
401 the Neotropics.

402 The intensity of environmental disturbance due to the presence of antelopes using the lagoon
403 habitat was also observed to differ considerably at sites sampled within the Afrotropical wetlands
404 (see Online Resource 1). The lagoon sites Shoebill A (in Bangweulu: a very slow-flowing riverine
405 lagoon, forming part of the Lukulu River) and Mushroom Lagoon (an enclosed lagoon in South
406 Luangwa) both had substantial trampling damage by mammals (scored at 3), while the other
407 African sites only had low to intermediate disturbance from large herbivore usage (scored 1 or 2).
408 In the two most-disturbed sites (Shoebill A Lagoon; Mushroom Lagoon) underwater PAR
409 absorbance coefficients (k) were $>20.0\text{ m}^{-1}$, indicating very high turbidity (black or dark brown
410 muddy water) due to the constant re-suspension of sediment caused by regular mammal
411 trampling, whereas in the other less-disturbed sites values calculated for k were all $<5.0\text{ m}^{-1}$,
412 indicating clear water. In contrast, sites in the Neotropical wetlands all had little or no visible
413 evidence of disturbance due to trampling by large herbivores (all sites scored at 1), and the

414 lagoons studied here all had high to intermediate levels of transparency, measured as Secchi
415 depth, due to the absence of resuspension of sediment by mammals, or from other causes. In
416 Argentina, Antequera 1, Antequera 2 and Herradura Lake 2 had slightly lower water transparency
417 and silty-clay sediments, while the other Neotropical sites, had higher water transparency and
418 sandy sediments (Online Resource 1).

419

420 **Discussion**

421 The data that we present suggest that invertebrate herbivory is an extensive process impacting
422 subtropical to tropical freshwater macrophyte populations in the study areas. A higher number of
423 invertebrate-damaged than undamaged leaves was observed in almost all the macrophyte species
424 examined, while half of the stems sampled showed damage caused by invertebrate herbivore
425 grazing in Afrotropical as well in Neotropical wetlands. These results support the findings of
426 Bakker et al. (2016a), and Newman and Rotjan (2013) regarding the role of invertebrate
427 herbivores in freshwater ecosystem functioning. Our study includes macrophyte species
428 representing all four of the usually-distinguished functional groups (“life-forms”) of aquatic
429 plants (Chambers et al., 2008), extending the findings of previous work, which focused on
430 emergent and submersed macrophyte species (Bakker et al., 2016a). Our results indicate that
431 invertebrate herbivory can also be an important ecological process affecting free-floating and
432 floating-leaf rooted plants in both ecozones. This study and a concurrent one (Franceschini et al.,
433 2020 accepted) are the first to compare herbivore-macrophyte interactions for small (invertebrate)
434 herbivores in Neotropical and Afrotropical freshwater ecosystems, in the context of the presence,
435 or near-absence, of large (mammalian) herbivores. Though emphasising the impacts of
436 invertebrate herbivory on aquatic plants, our results also provide an initial insight into the
437 importance of large-mammal herbivores as a biotic factor that may influence invertebrate-
438 macrophyte relationships in tropical and subtropical freshwater systems.

439 The high number of damaged leaves and biomass removed by invertebrates in FF and FR
440 macrophytes, and the fact that half of sampled stems had galleries in E macrophytes, suggest that
441 abundance and number of species of semiaquatic external feeders (e.g., grasshoppers,
442 planthoppers, weevils, moth caterpillars) and endophagous invertebrate herbivores (e.g., larvae
443 and adults of weevils and lepidopteran) could be higher than previously reported on invertebrate
444 assessments of Afrotropical and Neotropical wetlands (e.g. Poi de Neiff and Neiff, 2006; Poi de
445 Neiff, 2003; Albertoni et al., 2007; Wantzen et al., 2016; Ferreira et al., 2012). Also, a high
446 number of semiaquatic invertebrate herbivores was recorded on these plants by an associated

447 study of invertebrate herbivore assemblages conducted during our fieldwork program in
448 Afrotropical and Neotropical wetlands (Franceschini et al., 2020 accepted). Our results also agree
449 with those of Wissinger (1999), who pointed out that many works on wetland invertebrates are
450 biased toward collecting and studying invertebrates of purely aquatic taxa, and suggested that
451 herbivory by insects on above-waterline parts of macrophytes might be higher than previously
452 expected.

453

454 Plant tissue loss due to invertebrate herbivory could influence estimates of macrophyte biomass
455 from the Neotropics (Franceschini et al., 2010) as well as other wetland ecosystems (e.g. Jacobsen
456 and Sand-Jensen 1994; Nachtrieb et al., 2011), but this can vary in importance between plant
457 species. Despite the fact that mean amount of biomass removed per leaf reached up to 4.99% and
458 6.55% of the leaf lamina in Afrotropical and Neotropical macrophytes, respectively, our results
459 suggest that in the particular cases of the Neotropical macrophytes *N. indica* and *P. stratiotes*,
460 neglecting the effect of small invertebrate herbivores would result in even bigger underestimation
461 of leaf lamina biomass, up to 15.63% and 8.38% of leaf lamina.

462

463 It should be noted that the sampling work for this study was conducted during the dry (winter)
464 season, in both Zambia and Argentina, when plants generally show lower rates of growth than
465 during the summer period. There is evidence that invertebrate herbivores are much more active,
466 and consequently cause much more damage to plants (up to five times as much as during the
467 winter period), during the summer plant growth season in Neotropical as well as Palearctic
468 temperate aquatic systems (Jacobsen and Sand-Jensen, 1994; Franceschini et al., 2010). It is
469 hence reasonable to assume that invertebrate damage in spring and summer could be higher than
470 the values reported here for macrophytes in Afrotropical and Neotropical wetlands.

471 Our findings agree with previous results (Franceschini et al., 2010) which suggest that quantifying
472 the type of invertebrate damage (surface abrasion and holes) to assess biomass removed is
473 important for methodological reasons, especially in ecosystems where enclosure or exclosure
474 field experimentation is difficult or even impossible (e.g., due to the risk of damage to plots by
475 large animals, extreme water level fluctuations, high abundance of aquatic predator like Nile
476 crocodile or other problematic fieldwork issues common in tropical and subtropical wetlands). In
477 addition, type of damage is also ecologically important because it reflects the predominance of
478 different guilds and taxa in the invertebrate herbivore assemblages associated with a particular

479 freshwater macrophytes species. Thus, for example, a different trend was seen for abrasion
480 damage on *P. stratiotes* and *N. indica* comparing Afrotropical versus Neotropical plants, which
481 indicate differing abundances of scrapers (mainly snails) in the invertebrate herbivore
482 assemblages associated with these plants (Franceschini et al., 2020 accepted).

483

484 Our results comparing herbivory on macrophytes species from two climatically-similar ecozones,
485 show that plants from Neotropical wetlands, lacking large herbivores as a source of biotic
486 pressure, showed higher biomass removed per leaf, but similar numbers of damaged leaves when
487 compared with those from Afrotropical wetlands where large herbivores are an important biotic
488 factor. This was also seen in the results obtained for the comparison of the two macrophyte
489 species which occur in both ecozones, *P. stratiotes* and *N. indica*. Our finding of higher biomass
490 removed per leaf in Neotropical macrophytes, in comparison with those from Afrotropical
491 wetlands is in agreement with our results for assessment of invertebrate assemblages
492 (Franceschini et al., 2020 accepted), which showed a higher abundance of herbivorous taxa in
493 Neotropical macrophyte populations compared with Afrotropical ones.

494 The presence of large mammalian herbivores is known to be a biotic factor which modifies many
495 “top down” and “bottom up” processes (such as nutrient cycling) that influence macrophytes in
496 aquatic ecosystems (Bakker and Nolet, 2014; Bakker et al., 2016a), including modification of
497 nutrient concentrations in water. Our data do not permit an assessment of the relevance of plant
498 nutrient content as a factor potentially influencing invertebrate herbivory. However, it is entirely
499 possible that this might differ between ecozones as an indirect result of the differences in nutrient
500 conditions potentially produced by the presence or absence of large mammals. In addition, plant
501 stoichiometry, including both chemical defenses and nutrient content, as well as plant
502 productivity are usually considered to be important factors determining food quality and quantity
503 for herbivores feeding on macrophyte communities (Dorn et al., 2001; Bakker et al., 2016a), and
504 all may be differentially impacted by the intensity of usage of wetland waterbodies by large
505 animals. Abundance, body size, and taxonomical and functional composition of the invertebrate
506 herbivore assemblages, as well as feeding selectivity (generalists versus specialists: *sensu* Barone,
507 1998), competition and predation are also important factors affecting macrophyte-invertebrate
508 herbivore interactions (Newman, 1991; Cronin et al., 1998; Bakker et al., 2016a; Franceschini et
509 al., 2020 accepted). Thus, to achieve better understanding of the factors that control invertebrate
510 herbivory on freshwater macrophytes in Afrotropical and Neotropical wetlands, future research
511 should consider both such “bottom up” and “top down” factors.

512 Considering the relevant biotic and environmental factors that could influence damage by
513 invertebrate herbivores on freshwater macrophytes, our findings suggest likely impacts from large
514 herbivores affecting (with stronger impact), some 78% of macrophyte species examined at the
515 Afrotropical sites, and (to a limited extent only), about 50% of the macrophyte species examined
516 in the Neotropical wetlands, which is broadly in line with outcomes reported elsewhere (Stuart
517 and Stuart, 2006; Quintana et al., 2012; Madnes et al., 2010; Schivo et al., 2010). We have
518 provided evidence here that grazing by small invertebrate herbivores may substantially affect leaf
519 lamina biomass, but the damage done by trampling and grazing produced by large herbivores also
520 appears likely to be important in influencing macrophyte populations, especially in wetlands that
521 support high densities of these animals. We did not quantify such effects, and further research is
522 needed to determine the importance of large-animal herbivory and trampling compared with
523 invertebrate grazing for warm-water macrophyte populations. This is particularly important
524 because although high estimates of macrophyte biomass and productivity are usually given in
525 studies of tropical and subtropical ecosystems (e.g., Boar et al., 1999; Morison et al., 2000; Silva
526 et al., 2009), it is likely that they underestimate true values incorporating the effects of herbivore
527 damage. Furthermore, damage by large herbivores is quite likely to be higher in the dry season,
528 rather than during the main plant growth periods of the year because extreme drying (of
529 Afrotropical wetlands in particular) tends to concentrate animals around remaining water sources,
530 thus increasing disturbance to the plants living in such waterbodies (Chabwela and Ellenbrook,
531 1990; Redfern et al., 2003). If true this would represent an opposite trend to that observed for
532 damage by small invertebrate herbivores on subtropical macrophytes, which is usually greater
533 during the main plant growth period of the year (Franceschini et al., 2010).

534 Although the effects on macrophytes of mammalian herbivores like capybara, as seen in
535 Neotropical wetland systems such as the Iberá Swamps in Argentina, appeared to be less
536 substantial (e.g., Borges and Gonçalves Colares, 2007; Corriale and Herrera, 2014) than the
537 impacts of (bigger and more abundant) large herbivores in Africa, it should not be forgotten that
538 other herbivorous organisms also occur, in Afrotropical and Neotropical freshwater wetland
539 systems which were not included in our study. Important amongst these are waterfowl for
540 example, large flocks of White-faced Whistling Duck [*Dendrocygna viduata* (Linnaeus, 1766)]
541 were observed feeding on macrophytes in the Bangweulu Swamp (Franceschini et al., 2020
542 accepted), and also large non-obligate herbivorous fish such as piraputanga [several species in the
543 genus *Brycon* (J.P. Müller and Troschel, 1844)], occurring, for example, in the southern Pantanal
544 wetlands of Brazil (Reys et al., 2009).

545 In systems such as the Zambian floodplain lagoons studied here, where trampling by large
546 animals occurs at sometimes high intensity, it is likely that the additional damage produced by
547 this disturbance will exacerbate any damage caused by large-herbivore grazing. In addition,
548 where regular trampling within the waterbodies by large herbivores results in resuspension of
549 sediments there is likely to be an increase in water turbidity. In this murky water the resulting
550 reduction in available light for submersed macrophyte photosynthesis may decrease plant
551 productivity for this group of macrophytes. There are known quantitative relationships between
552 the amount of sediment resuspension produced by environmental disturbance in shallow-water
553 systems (Murphy and Eaton, 1983) and the intensity of such disturbance required to produce
554 sufficient turbidity to adversely affect submersed macrophyte production. Although these
555 relationships were derived from studies of propeller-disturbance of sediments produced by boat
556 movements in shallow temperate canals it is highly likely that similar effects on water turbidity
557 could occur due to the daily impacts of thousands of antelope hooves on the sediment of shallow
558 tropical lagoons and rivers. Furthermore, the associated invertebrate populations of damaged
559 plants will also, as a result, likely be exposed to serious and potentially fatal damage by large
560 herbivore activities (including incidental predation), as has been observed in terrestrial
561 ecosystems (Zamora and Gómez, 1993).

562 In mensurative ecological field studies (Hurlbert, 1984) of the type we report here it is rarely
563 possible to distinguish the relative importance of location of the study sites from actions occurring
564 at those locations (such as differential intensities of large-animal grazing and trampling
565 disturbance impacting the invertebrate and macrophyte populations studied). We are fully aware
566 of the issues of pseudoreplication in producing unsupportable claims in field ecological studies
567 (Hurlbert, 1984), and consequently we make no claims for cause and effect of the differences in
568 environmental factors impacting the study sites in the two ecozones, in influencing invertebrate
569 effects on their macrophyte populations. However, we do provide statistical evidence for the
570 existence of observed differences in invertebrate grazing impacts on macrophyte populations
571 between sites with and effectively without large mammalian herbivores, providing a starting point
572 for future work to examine these issues in more detail. Such work is clearly needed to disentangle
573 the sets of factors which determine the interplay of large- and small invertebrate-herbivore
574 interactions with macrophytes, and with each other, in these warm-water systems.

575 Our results indicate that invertebrate herbivory is an important ecosystem process damaging
576 macrophyte species that occur in both Afrotropical and Neotropical wetlands and in some cases
577 this damage can be great enough to substantially influence estimates of leaf biomass. In general,

578 the observed impacts of invertebrate grazing on macrophytes were greater in the Neotropics than
579 Afrotropics. The findings support our primary hypothesis that damage caused to tropical and
580 subtropical freshwater macrophytes by small invertebrate herbivores is an extensive process
581 affecting freshwater tropical and subtropical macrophytes, but that the intensity of grazing
582 impacts differs quite substantially between the two ecozones, even in the case of two macrophyte
583 species that occur in both ecozones. More generally, we provide new evidence to support the view
584 that herbivory is an important process influencing freshwater ecosystem functioning (Bakker et
585 al., 2016a, b; Grutters et al., 2016; Wood et al., 2016). Regarding our secondary hypothesis, that
586 large herbivores, when present, may be a relevant biotic factor influencing macrophyte-
587 invertebrate herbivory interactions in warm-freshwater wetlands, the findings are inconclusive.
588 We found observational evidence that the disturbance produced by large herbivore activity in
589 warm-water wetland systems both damages macrophyte populations directly, and interacts with
590 the grazing damage produced by small invertebrate herbivores in affecting the macrophyte
591 populations of such systems. Although our results cannot be used to ascribe cause and effect here,
592 they can provide a starting point for further work aimed at understanding the interactions of
593 macrophytes with both small invertebrate and large herbivores in warm-water wetland systems.
594 Finally, more field research (for example the use of simulated damage or enclosure experiments
595 to assess the relative importance of small invertebrate and large mammalian herbivores on
596 macrophyte production, e.g., Milne et al., 2008; Soti and Volin, 2010; Ramos et al., 2018) is
597 clearly needed to understand and predict the role and impacts of small and large herbivores in
598 tropical and subtropical ecosystems, in which anthropogenic disturbances may generate dramatic
599 declines in biodiversity and habitat complexity.

600 **Conclusions**

601 We conclude here that damage by small invertebrate herbivores is an extensive process impacting
602 subtropical to tropical macrophyte populations in the study wetlands, with invertebrate
603 assemblages causing more damage per leaf in Neotropical macrophytes than Afrotropical ones.
604 This damage may be modified by other biotic factors. We observed substantial differences in the
605 incidence of damage from mammalian herbivores, and associated damage due to trampling and
606 resuspension of sediments, between populations of almost all Afrotropical macrophyte species
607 examined, and those from the Neotropical sites. Thus, the presence of large mammalian
608 herbivores may be a relevant biotic factor influencing invertebrate herbivory in warm freshwater
609 wetlands. Future research should be carried out in order to understand better the interaction
610 between macrophyte populations and their associated invertebrate herbivore assemblages in these

611 warm freshwater wetlands, comparing sites with and effectively without large mammalian
612 herbivore pressure. Our work provides a starting point to examine these issues in more detail.

613

614

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849 **Table 1:** Large herbivore (mammal) species and damage impacts (*sensu* Grime 1979) on freshwater macrophytes from Afrotropical (Afro) and Neotropical
850 (Neo) wetlands, hosting invertebrate herbivores. *Pistia stratiotes* (FR: Neo & Afro), *Azolla nilotica* (FF: Afro), *Azolla pinnata* (FF: Afro), *Eichhornia*
851 *crassipes* (FF: Neo), *Eichhornia azurea* (FR: Neo), *Hydrochleys nymphoides* (FR: Neo), *Nymphaea nouchali* var. *caerulea* (FR: Afro), *Nymphaea prolifera*
852 (FR: Neo), *Nymphoides indica* (FR: Neo & Afro), *Potamogeton nodosus* (FR: Afro), *Trapa natans* (FR: Afro), *Potamogeton octandrus* (S: Afro),
853 *Potamogeton illinoensis* (S: Neo), *Cyperus papyrus* (E: Afro). See text for life-form abbreviations. Abundance of large mammalian herbivores is expressed as
854 number of individuals recorded (where data available) in the study sites by different authors. Large mammalian herbivores (LM) include animals with more
855 than 10 kg body mass, small mammalian herbivores (SM) those with less than 10 kg body mass (Bakker et al. 2016b). Source of the information: (1): this
856 study; (2): Stuart and Stuart, 2006; (3): Quintana et al., 2012; (4): Schivo et al., 2010; (5): F. Willems (*pers. com.*); (6): Marques (1988)

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Large herbivore species/ Type of mammal and body mass	Impact	Macrophyte species hosting small invertebrate herbivores	Bioregion and wetlands systems	Large herbivore abundance at the study sites
African Savanna Elephant <i>Loxodonta africana</i> LM: 2800-6300 kg (2)	trampling grazing	<i>Pistia stratiotes</i> , <i>Azolla nilotica</i> , <i>A. pinnata</i> (1)	Afrotropics: South Luangwa, Kasanka, Bangweulu	Kasanka: 30-50 (5)
Hippopotamus <i>Hippopotamus amphibius</i> LM: 1000->2000 kg (2)	trampling grazing	<i>Pistia stratiotes</i> , <i>Azolla nilotica</i> , <i>A. pinnata</i> (1)	Afrotropics: South Luangwa, Kasanka, Bangweulu	Kasanka: 100-200 (5); Luangwa: 20.000 (2)
Sitatunga antelope <i>Tragelaphus spekei</i> LM: 55-115 kg (2)	trampling grazing	<i>Cyperus papyrus</i> (2), <i>Nymphaea nouchali</i> var. <i>caerulea</i> (1)	Afrotropics: Kasanka	Kasanka 500-1,000 (5)
Puku antelope <i>Kobus vardonii</i> LM: 62-74 kg (2)	trampling	<i>Nymphaea nouchali</i> var. <i>caerulea</i> , <i>Pistia stratiotes</i> , <i>Azolla nilotica</i> , <i>A. pinnata</i> (1)	Afrotropics: Kasanka, South Luangwa	Kasanka: 5,000-7,000 (5)
Impala antelope <i>Aepyceros melampus</i> LM: 40-50 kg (2)	trampling	<i>Pistia stratiotes</i> , <i>Azolla nilotica</i> , <i>A. pinnata</i> (1)	Afrotropics: South Luangwa	
Black lechwe antelope <i>Kobus leche</i> LM: 80-100 kg (2)	trampling grazing	<i>Nymphaea nouchali</i> var. <i>caerulea</i> , <i>Nymphoides indica</i> , <i>Potamogeton octandrus</i> , <i>Cyperus</i> sp. (1)	Afrotropics: Bangweulu Swamps	Bangweulu: 75,000 Kasanka: 0-2 (5)
Baboon <i>Papio cynocephalus cynocephalus</i> LM: 12-45 kg (2)	grazing	<i>Pistia stratiotes</i> (1)	Afrotropics: South Luangwa National Park, Kasanka National Park	
Capybara <i>Hydrochoerus hydrochaeris</i> LM: 35-75 kg (4)	trampling grazing	<i>Eichhornia azurea</i> , <i>Pistia stratiotes</i> , <i>Eichhornia</i> <i>crassipes</i> ; <i>Hydrochleys nymphoides</i> ; <i>Nymphoides</i> <i>indica</i> (1); E, FF and FR macrophytes (3) (4) (5)	Neotropics: Riachuelo River Basin, Paraná Floodplain, Paraguay River Basin	
Red marsh rat <i>Holochilus brasiliensis</i> SM: 0.9-3.7 kg (6)	grazing	Terrestrial and semiaquatic vegetation (3)	Neotropics : not seen in this study	
Swamp deer <i>Blastocercus dichotomus</i> LM: 80-125 kg (4)	trampling grazing	Terrestrial and semiaquatic grasslands (3)	Neotropics: not seen in this study	

Coypu
Myocastor coypus
SM: 4-10kg (4)

trampling
grazing

Terrestrial and aquatic plants (3)

Neotropics: not seen in this
study

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861 **Figure captions**

862 **Fig. 1** Wetland study areas in Afrotropics (right) and Neotropics (left), supporting small
863 invertebrate herbivores on macrophytes and large mammalian herbivores (named in text)
864 acting as a biotic factor impacting macrophyte habitats. Total ecozone macrophyte γ -
865 diversity and number of ecozone-endemic (e) macrophyte species recorded are also
866 indicated. Sites are shown with latitude and longitude coordinates. Afrotropical sites:
867 Kasanka, Njelele Stream (12°36'31.1"S, 30°23'59.6"E); Kasanka, Fibwe Stream
868 (12°35'30.1"S, 30°15'07.0"E); Kasanka, Luwombwa River (12°30'08.9"S, 30°07'52.1"E);
869 Bangweulu, Shoebill A Lukulu River* (11°57'04.0"S, 30°14'22.7"E); Bangweulu, Shoebill
870 C Lukulu River (11°57'16.0"S, 30°14'52.3"E); South Luangwa, Hippo Lagoon
871 (13°06'09.4"S, 31°46'41.0"E); South Luangwa, Mushroom Lagoon* (13°04'48.8"S,
872 31°47'36.7"E). Neotropical sites: Paiva Lake (27°29'02.7"S, 58°44'51.3"W); Aeroclub Lake
873 (27°28'48.8"S, 58°43'55.0"W); La Antena Lake (27°22'03.0"S, 58°20'01.0"W); Antequera 1
874 (27°26'08.6"S, 58°51'26.1"W); Antequera 2 (27°25'41.7"S, 58°52'12.8"W); Medina Lake
875 (27°26'36.2"S, 58°38'43.8"W); Municipal Lake (27°28'01.5"S, 58°40'12.6"W); El Puente
876 Lake (27°26'23.7"S, 58°51'14.1"W)*; Herradura Lake 1 (26°17'28.3"S, 58°10'53.1"W)*;
877 Herradura Lake 2 (26°18'37.5"S, 58°10'18.7"W)*. (*) indicates effects of mammal
878 herbivores on macrophytes discussed in text for these sites

879 **Fig. 2** Total number of leaves (a) and stems (b) damaged (black bars) and non-damaged
880 (white bars) by small herbivores (invertebrates) in freshwater macrophytes from
881 Afrotropical (Afro) and Neotropical (Neo) wetlands. Macrophyte "life forms": (FF): free
882 floating, (FR): floating rooted, (S): submersed, (E) emergent. Number of leaves were
883 quantified in *Pistia stratiotes* (FF: Afro & Neo; N=60), *Eichhornia azurea* (FR: Neo;
884 N=30), *Hydrocleys nymphoides* (FR: Neo; N=30), *Nymphaea nouchali* var. *caerulea* (FR:
885 Afro; N=30), *Nymphaea prolifera* (FR: Neo; N=30), *Nymphoides indica* (FR: Afro & Neo;
886 N=60), *Potamogeton nodosus* (FR: Afro; N=30), *Trapa natans* (FR: Afro; N=30),
887 *Potamogeton octandrus* (S: Afro; N=60) and *Potamogeton illinoensis* (S: Neo; N=30). In *P.*
888 *octandrus* only submersed leaves were included. Number of stems were quantified in *Thalia*
889 *multiflora* (E: Neo; N=30), *Cyperus giganteus* (E: Neo; N=30) and *Cyperus papyrus* (E:
890 Afro; N=30). (*) indicates significantly different outcomes with χ^2 (df=1, with Yates
891 correction for continuity) between number of damaged leaves or stems quantified (observed
892 values) compared to expected values under null hypothesis (H_0 : number of damaged leaves
893 = non-damaged leaves)

894 **Fig. 3** Biomass removed by small herbivores (invertebrates) per leaf in freshwater
895 macrophytes from Afrotropical (white colour) and Neotropical (grey colour) wetlands.
896 Values quantifying herbivory impact are expressed as percentage (%) of total biomass
897 removed (surface abrasions + holes) (a), abrasion damage (b) and hole damage (c) per leaf
898 lamina. Total number of leaves quantified on *Pistia stratiotes* (FF: Afro & Neo),
899 *Nymphoides indica* (FR: Afro & Neo) and *Potamogeton octandrus* (S: Afro) were 60 per
900 macrophyte species. In *P. octandrus* only submersed leaves were included. Number of
901 leaves quantified in *Eichhornia azurea* (FR: Neo), *Hydrocleys nymphoides* (FR: Neo),
902 *Nymphaea nouchali* var. *caerulea* (FR: Afro), *Nymphaea prolifera* (FR: Neo),
903 *Potamogeton nodosus* (FR: Afro), *Trapa natans* (FR: Afro), and *Potamogeton illinoensis*

904 (S: Neo) were 30 per macrophyte species. (*) in the p values indicates significantly
905 different outcomes for pairwise comparisons between ecozones, using GLM (df=358,
906 values significant at $p<0.05$). In box-plots, box indicates quartiles Q1 and Q3, central line
907 and dot indicate median and mean values, respectively; whiskers show quantiles 0.05 and
908 0.95, external dots represent outliers. Data were transformed to $\text{Log}_{10}(X+1)$. See caption
909 of Fig 2 for other abbreviations.

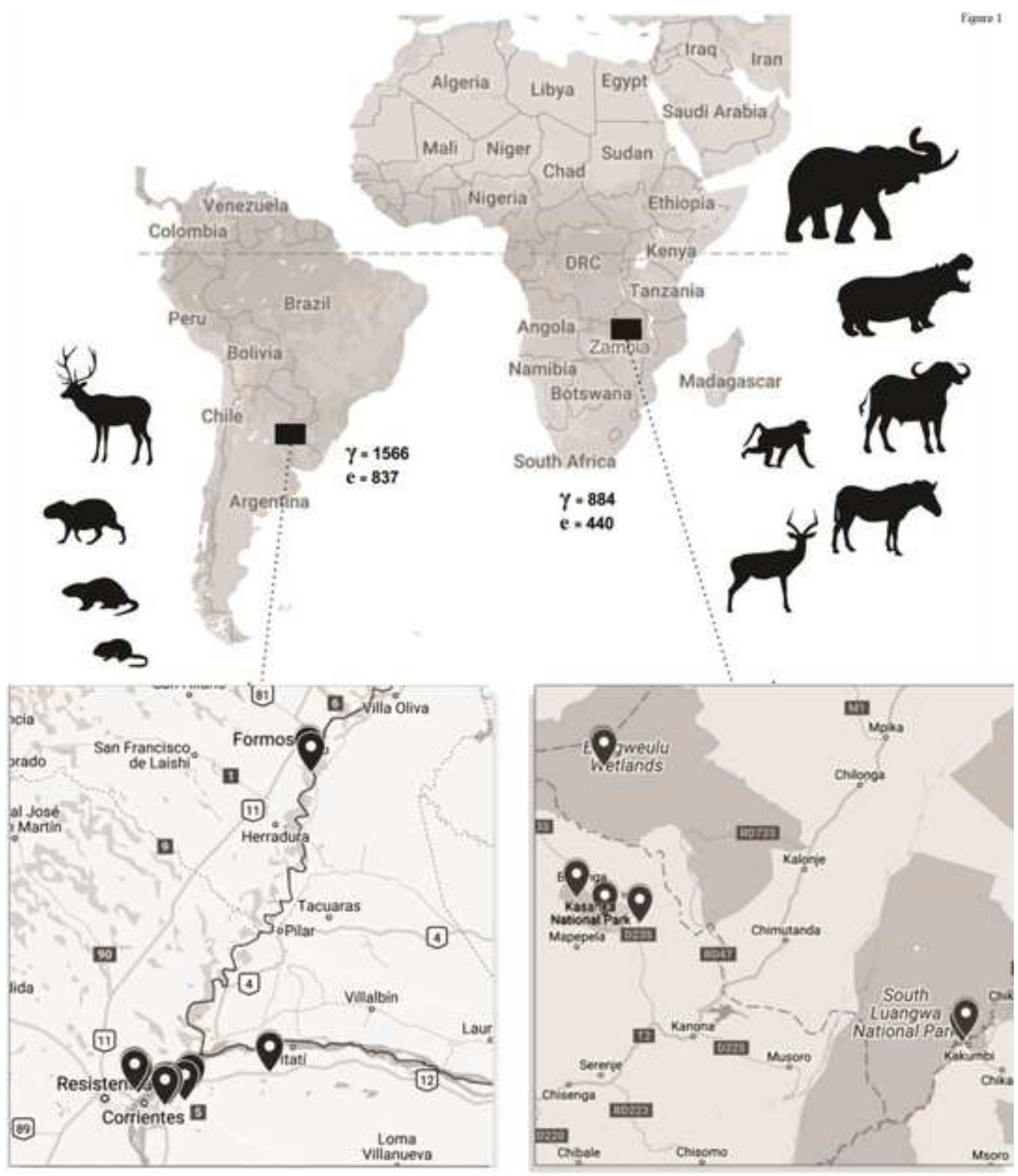
910 **Fig. 4** Herbivory produced by small herbivores (invertebrates) on *Pistia stratiotes* (FF:
911 N=60 leaves) and *Nymphoides indica* (FR: N=60 leaves) in Afrotropics (white colour) and
912 Neotropics (grey colour). Herbivory is expressed as percentage of total (surface abrasions +
913 holes) biomass removed (**a**), abrasion damage (**b**) and holes damage (**c**) per leaf lamina. (*)
914 in the p values indicate significant differences for comparisons, of invertebrate herbivory
915 damage between Afrotropics and Neotropics, using GLM (df=358, values significant at
916 $p<0.05$). In box-plots, box indicates quartiles Q1 and Q3, central line and dot indicate
917 median and mean values, respectively; whiskers show quantiles 0.05 and 0.95, external dots
918 represent outliers. Data were transformed to $\text{Log}_{10}(X+1)$. See caption to Figure 2 for other
919 abbreviations

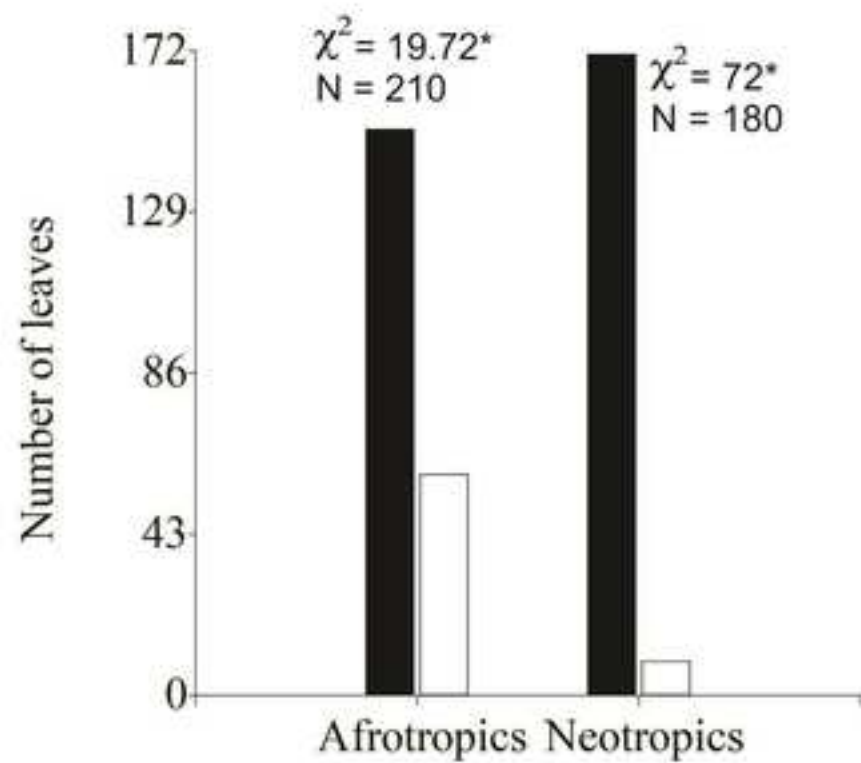
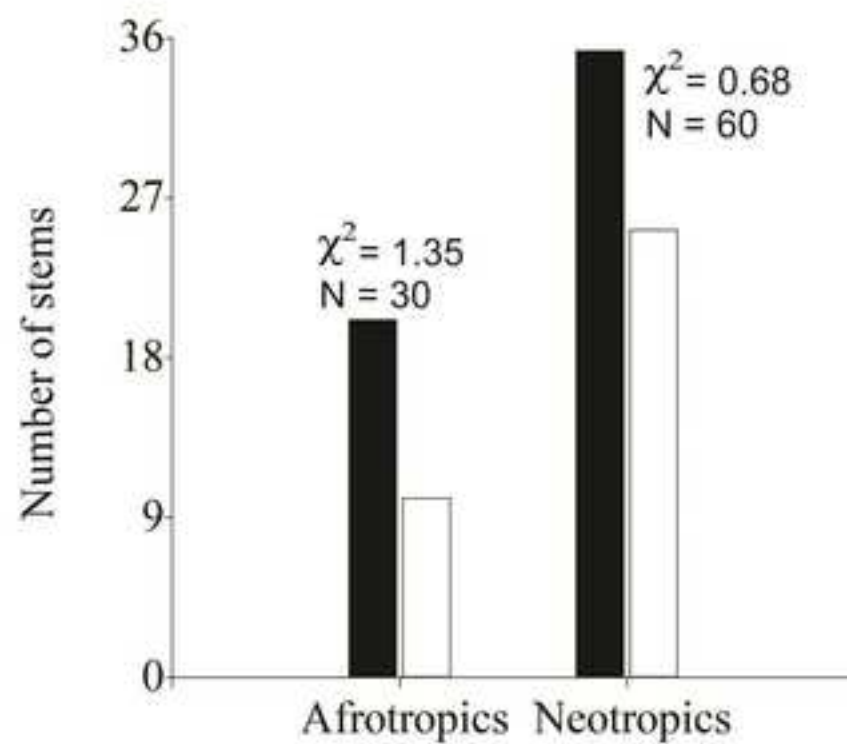
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Figure 1

Figure 1



a**b**

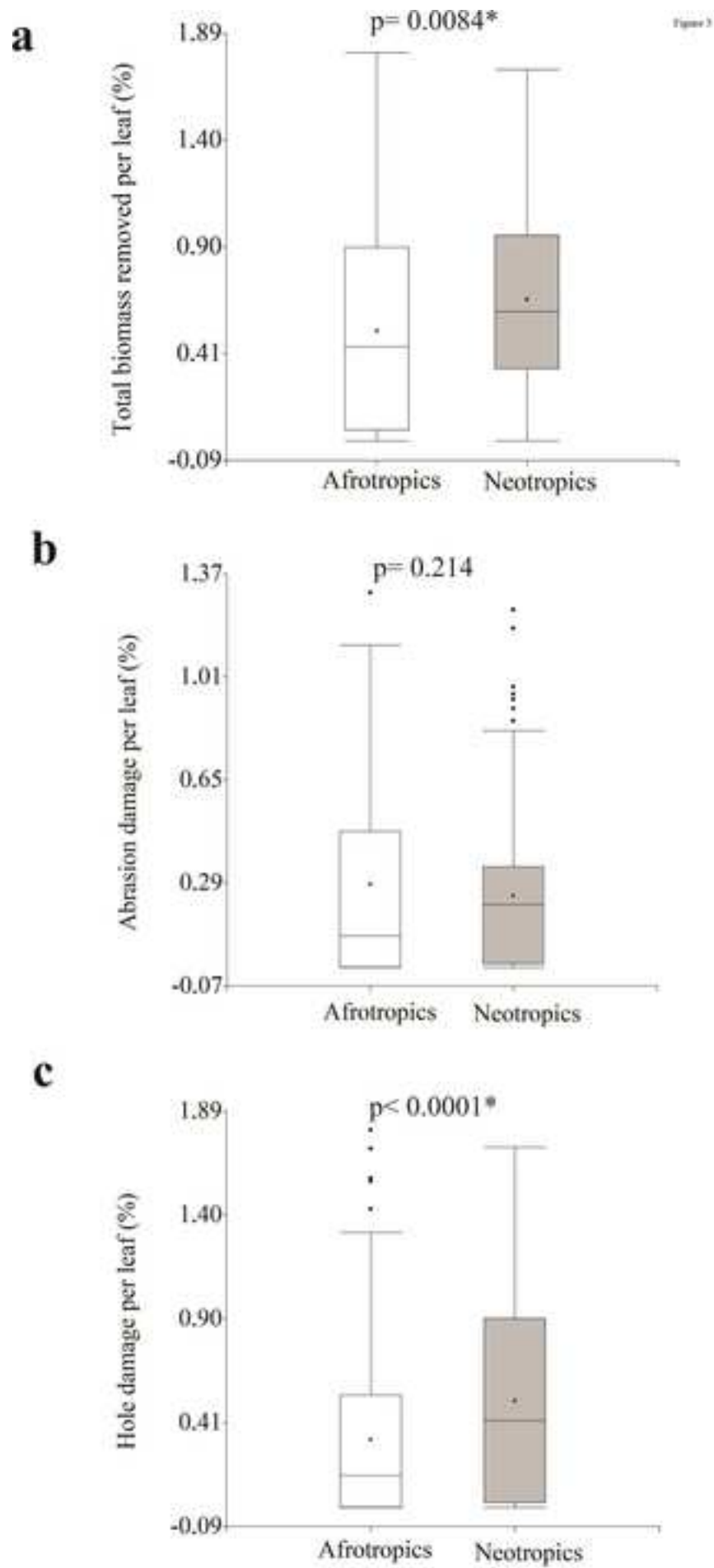
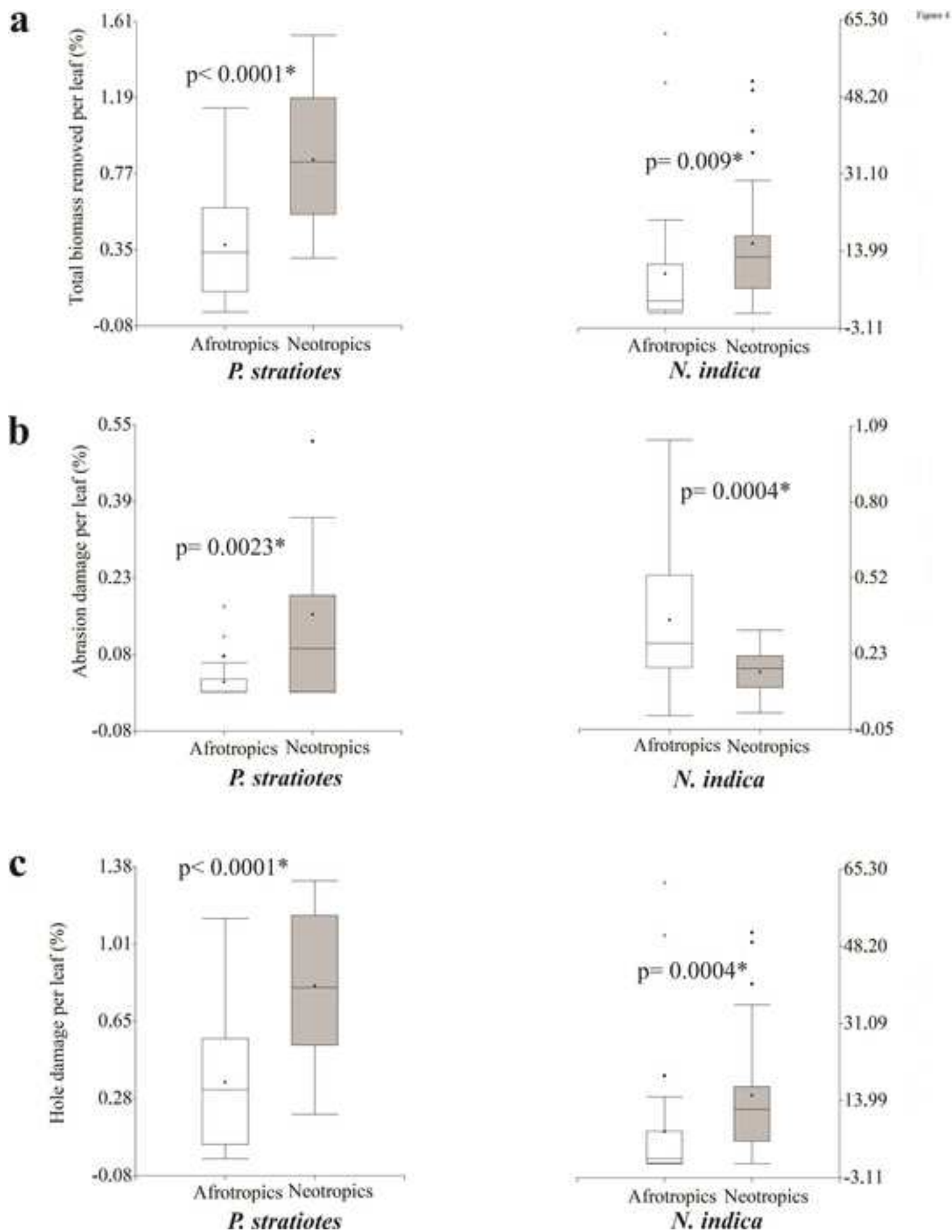


Figure 4



Online Resource 1: Environmental data and effect of large mammalian herbivores (as a biotic factor producing trampling disturbance effects: *sensu* Grime 1979) potentially impacting macrophyte-invertebrate herbivore interactions in Neotropical and Afrotropical wetlands. Flow: assessed on a scale of 1 = static; to 4 = fast-flowing (Lang and Murphy, 2011). Disturbance: expressed on a semi quantitative scale of 1 = no disturbance due to trampling by animals, to 4 = major trampling damage. In Afrotropical sites, turbidity is shown as underwater photosynthetically-active radiation (PAR) absorbance coefficient: $k\ m^{-1}$ with depth (m) at which the deeper of the two underwater PAR measurements was taken (not the maximum depth of the waterbody: Moore and Murphy, 2015). In Neotropical sites turbidity was measured as Secchi depth (m), with maximum water depth (m) also given.

NEOTROPICAL WETLANDS								
	Paiva Lake	Aeroclub Lake	La Antena Lake	Medina Lake	Municipal Lake	Antequera 1 Lake	Antequera 2	Herradura Lake 2
pH	6.44	7.41	7.4	7.30	7.53	6.76	6.6	6.8
Temperature (°C)	19.5	17.0	15.3	16.5	15	20.5	18	16.9
Flow	1	1	1	1	1	1	1	1
Conductivity ($\mu\text{S cm}^{-1}$)	50	65	30	32	30	145	100	67
Secchi depth (m)	>1.24	1.1	>0.3	>1.5	>0.90	0.27	0.25	0.11
Water depth (m)	1.24	4.5	0.3	1.5	0.90	0.53	0.40	2.7
Disturbance	1	1	1	1	1	1	1	1
AFROTROPICAL WETLANDS								
	Kasanka. Njelele Stream	Kasanka. Fibwe Stream	Kasanka. Luwombwa River	Bangweulu. Shoebill Lagoon A, Lukulu River	Bangweulu. Shoebill Lagoon C, Lukulu River	South Luangwa. Mushroom Lagoon		
pH	6.44	7	8	7.4	6.83	7.97		
Temperature (°C)	17.2	14.8	25	16.5	25	19.5		
Flow	2	4	2	1	2	1		
Conductivity ($\mu\text{S cm}^{-1}$)	17	16	20	16	20	625		
$k\ (m^{-1})$	4.76	4.664	1.447	32.19	1.943	21.638		
Depth at which deeper measurement taken (m)	0.22	0.22	0.22	0.15	0.22	0.04		
Disturbance	2	1	1	3	2	3		

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