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PRIMARY RESEARCH PAPER

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

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

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additionally impacting macrophyte populations in these subtropical to tropical wetlands.

Keywords Herbivorous mammals · Freshwater ecosystems · Grazing damage · Tropics

Introduction

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

Previous studies suggest that macrophyte biomass and productivity can be high in tropical and subtropical freshwater ecosystems (e.g., Boar et al., 1999; Morison et al., 2000; Silva et al., 2009; Bottino et al., 2014). Such productivity is likely to support invertebrate herbivory, and there is evidence from the Neotropics that biomass directly removed by invertebrate grazing can be up to 27% of the leaf lamina biomass and up to 26% of the lamina biomass per m^2 of vegetation (Franceschini et al., 2010). There has also been some work, mainly in the Neotropics, on the effects of invertebrate herbivory on naturally occurring macrophyte populations which suggests that invertebrate damage influences the seasonal decay of macrophyte populations and that herbivores may strongly affect detrital inputs from macrophyte sources (Medeiros dos Santos & Esteves, 2002; Poi de Neiff & Casco, 2003). Whether determined by destructive (Soti & Volin, 2010) or non-destructive methods (Gonçalves et al., 2010), it is highly likely that measurements of macrophyte biomass and production which do not take into account the biomass removed by invertebrate herbivores will underestimate true plant biomass and production values (Esteves, 2011). Also, the number of studies of biodiversity and ecosystem functioning involving macrophytes in subtropical and tropical freshwater systems, in the context of the relevant biotic and environmental factors that influence their functioning, has been increasing in recent years (e.g., Murphy et al., 2003; Padial et al., 2008; Varandas Martins et al., 2013; Bottino et al., 2014; Tapia Grimaldo et al., 2016, 2017; Kennedy et al., 2015, 2017; Trindade et al., 2018). However, knowledge of the effect of invertebrate herbivores on warm-water macrophyte populations and in particular their biomass and production values remains very limited.

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

In the Afrotropical ecozone, grazing by wild mammalian herbivores is known to be an important biotic factor influencing ecosystem processes, though studied mainly in terrestrial rather than freshwater ecosystems (Cristoffer & Peres, 2003; Asner et al. 2009; Hamandawana, 2012; Hrabar & Du Toit, 2014), despite the fact that many of the large African mammalian herbivores feed in wetlands, especially during the dry season (Chabwela & Ellenbrook, 1990; Redfern et al., 2003). In Afrotropical wetlands, macrophytes and their associated invertebrate herbivore assemblages, hence, frequently coexist with a high diversity of large-mammal herbivores, with individual body weights in the range 40–6300 kg (Stuart & Stuart, 2006). Often, such animals are present at high abundance. For example, in one of the target areas of this study, the Bangweulu Swamp of Northern Zambia, a recent survey (Viljoen, 2011) showed the presence of large numbers (c. 75,000 animals across an area of 243 km^2) of the semiaquatic antelope Black Lechwe [*Kobus leche* susbsp. *smithemani* (Lydekker, 1900)], primarily feeding on floodplain and aquatic vegetation. Large herbivores may also act as a biotic factor modifying nutrient cycling in warm-water wetland systems, as well as potentially causing substantial direct disturbance impacts on invertebrate assemblages and their host plant communities (e.g., via trampling), in addition to direct feeding damage (Zamora & Gómez, 1993; Bakker & Nolet, 2014; Bakker et al., 2016a). The Neotropical ecozone is very different in this regard (Cristoffer & Peres, 2003). In wetlands of this ecozone, and certainly in northeastern Argentina, large-mammal herbivore species of more than 80 kg body weight are almost absent [with the exception of very small numbers of Swamp Deer: *Blastocerus dichotomus* (Illiger, 1815)], and invertebrate herbivore assemblages only coexist with a low abundance and diversity of mainly smaller mammalian herbivorous species [especially the large rodent, Capybara: *Hydrochoerus hydrochaeris* (Linnaeus, 1766)], which feed on floodplain and aquatic vegetation, though grazing impacts may not always be severe (Milne et al., 2008; Ramos et al., 2018). Despite the evidence for substantial ecological differences between these two warm climate regions, there has been no previous attempt to characterize invertebrate herbivore assemblages on naturally occurring macrophyte populations in the Afrotropics and Neotropics, with the exception of a concurrent study (Franceschini et al., 2020) that shows substantial differences in abundance and composition of such invertebrates from the two ecozones.

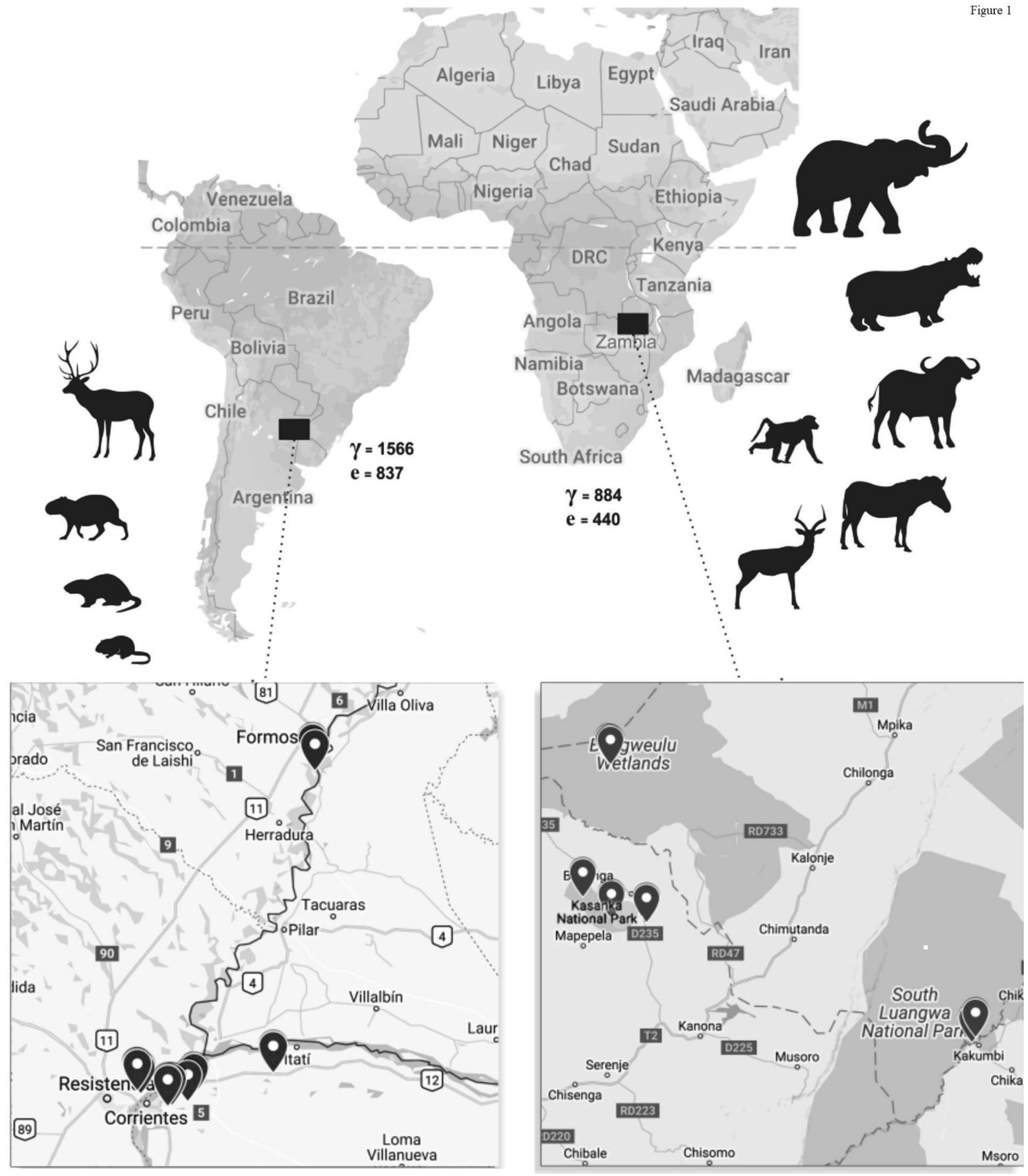
In the light of the differences in environmental and biotic pressures, such as large-animal herbivory, acting upon wetland systems in the two ecozones, a question which arises is whether the impact of invertebrate herbivores on freshwater macrophytes in Afrotropical and Neotropical wetlands might also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

Materials and methods

Study sites, herbivores and macrophyte species

In the Afrotropics, sampling was conducted at seven sites in the Northern and Eastern Provinces of Zambia, within three well-protected conservation areas: Kasanka National Park and the Bangweulu Game Management Area (Northern Province), and South Luangwa National Park (Eastern Province). In the Neotropics, study areas were located in northeastern Argentina, comprising ten sites within the Riachuelo and Paraguay Basin, and Paraná River floodplains, including the international Ramsar protected area of the Chaco Wetlands. (Figure 1).

The study sites were chosen to provide conditions strongly contrasting in terms of abundance, richness and body weight of mammalian herbivores present (Marques 1988; Stuart & Stuart 2006; Quintana et al., 2012; Schivo et al., 2010), but similar in terms of water chemistry (e.g., see data on pH and conductivity presented below for the study sites). Although northeastern Argentina is geographically subtropical and the Northern and Eastern Provinces of Zambia are tropical, the latter areas are located at high altitude (500–1200 m above sea level, a.s.l.) while the former



is low lying (60–80 m a.s.l), so that in fact the two study areas are climatically quite closely comparable, with similar rainfall and temperature ranges across the year. The Köppen climate scheme designates the study area in Zambia as “humid subtropical climate”, while the study area in Argentina is classified as “warm oceanic climate/humid subtropical climate”.

In this paper, we define “large herbivores” to include terrestrial, semiaquatic, or aquatic vertebrates that obtain some or all of their food from freshwater

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

macrophytes, whereas phytophagous insects and gastropods are referred to as “small invertebrate herbivores”.

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

In each ecozone, sites and macrophyte species were selected to include representatives of each of the four usually distinguished functional groups (“life forms”) of aquatic plants (Chambers et al., 2008): free-floating (FF), floating-leaf-rooted (FR), emergent (E), and submersed (S) species. Species were identified using specific guides for each ecozone (Arbo & Tressens, 2002; Kennedy & Murphy 2012) and nomenclature was confirmed following The Plant List (www.theplantlist.org). In the Afrotropical wetlands, the macrophytes studied were *Pistia stratiotes* L. (FF: Araceae), *Nymphoides indica* (L.) Kuntze (FR: Menyanthaceae), *Nymphaea nouchali* var. *caerulea* (Savigny) Verdc. (FR: Nymphaeaceae), *Trapa natans* L. (FR: Trapaceae), *Potamogeton nodosus* Poir. (FR: Potamogetonaceae), *Cyperus papyrus* L. (E: Cyperaceae) and *Potamogeton octandrus* Poir. (S: Potamogetonaceae). Although *P. octandrus* sometimes has floating leaves present, only assessments of damage to its more abundant submersed leaves were included in this study. In the Neotropical wetlands the macrophytes studied were *P. stratiotes* and *N. indica* (also present in the sites in Zambia), as well as *Nymphaea prolifera* Wiersema (FR: Nymphaeaceae), *Hydrocleys nymphoides* (Humb. and Bonpl. ex Willd.) Buchenau (FR: Lymnocharitaceae), *Eichhornia azurea* (Sw.) Kunth (FR: Pontederiaceae), *Cyperus giganteus* Vahl (E: Cyperaceae), *Thalia multiflora* Horkel ex Körn. (E: Marantaceae) and *Potamogeton illinoensis* Morong (S: Potamogetonaceae).

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

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

For all macrophyte species, we collected three samples of 10 leaves or stems at random from different individuals of each plant species per site ($N = 30$

leaves or stems per macrophyte species and site). Two plant species, the FF *P. stratiotes* and the FR *N. indica,* were each sampled in both ecozones and the S *P. octandrus* was sampled in two sites from the Afrotropics ($N = 60$ leaves per macrophyte species). In total 390 leaves were assessed in the ten FF, FR, and S macrophyte species considered, whereas a total of 90 stems were assessed in the three E macrophyte species included in this study. In each macrophyte population, samples included the edge and the center of the vegetation stand (one and two samples, respectively, collected at random from each part of the stand, and separated as much as was possible from each other to maximize independence of the data).

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

(i) Surface abrasions: caused when epidermis and mesophyll were not completely removed, and most basal tissue persists in the affected areas of the leaf lamina. This type of damage was not found when processing petioles and stems.

(ii) Holes: involved complete removal of tissues of the leaf lamina.

(iii) Galleries: parenchyma and vascular tissues inside stems and petioles were removed or affected as a consequences of necrosis. Total numbers of leaf petioles per plant species affected by galleries were also counted. Galleries produced by miners were not found when processing leaf laminas.

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

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

$$b_s = \frac{\sum \frac{ad_s.(Wn-Wd)}{a_s}}{N}, \qquad (1)$$

where b_s is surface abrasion biomass (g), ad_s is the damaged area by surface abrasion (cm^2), a_s is area of the circle (cm^2), Wn is the mean weight of undamaged circles (g), Wd is the mean weight of damaged circles with surface abrasion (g), and N is the total number of leaves.

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

$$b_h = \frac{\sum \frac{ad_h.Wn}{a_s}}{N}, \qquad (2)$$

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

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

Assessing biotic and environmental factors influencing study sites

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

Trampling damage to macrophyte vegetation, produced by mammalian herbivores, was scored on a semi–quantitative scale of 1 = no disturbance due to trampling by animals, to 4 = major trampling damage. Water turbidity, which is affected by resuspension of sediments caused by large-animal trampling disturbance of waterbody substrates, was recorded in the Afrotropical sites as photosynthetically active radiation (PAR) absorbance coefficient: $k\ \mathrm{m}^{-1}$, calculated from PAR measurements taken using an underwater PAR sensor at two points in the water column: just below surface and at a standard depth, usually 0.22 m (Moore & Murphy, 2015). In the Neotropics, water turbidity was recorded as Secchi depth (m), considering also maximum water depth (m) as a complement of this variable. Other environmental parameters measured at the sampling sites were pH, conductivity ($\mu\mathrm{S\ cm}^{-1}$), water temperature (°C), and visually–assessed flow class (class 1 = static or very slow flow; 2 = slow flow: “pool”; 3 = moderate flow: “glide”; 4 = fast flow: “white water showing”: Lang & Murphy, 2011). Field meters used in Zambia were a Handylab pH/temperature LF12 m, HI98311 conductivity meter, and a SKYE SKP210 underwater PAR sensor. Field meter used in Argentina was a handylab pH/temperature/conductivity Hanna meter.

Statistical analysis

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

Results

Damage by small invertebrate herbivores on Afrotropical versus Neotropical freshwater macrophytes

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

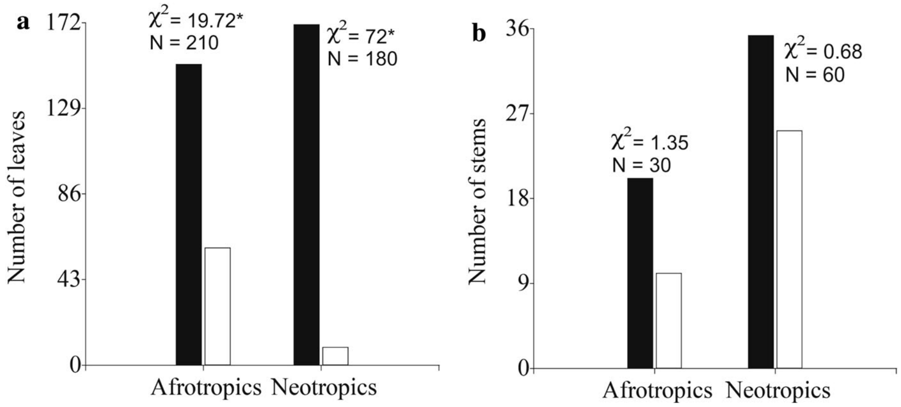
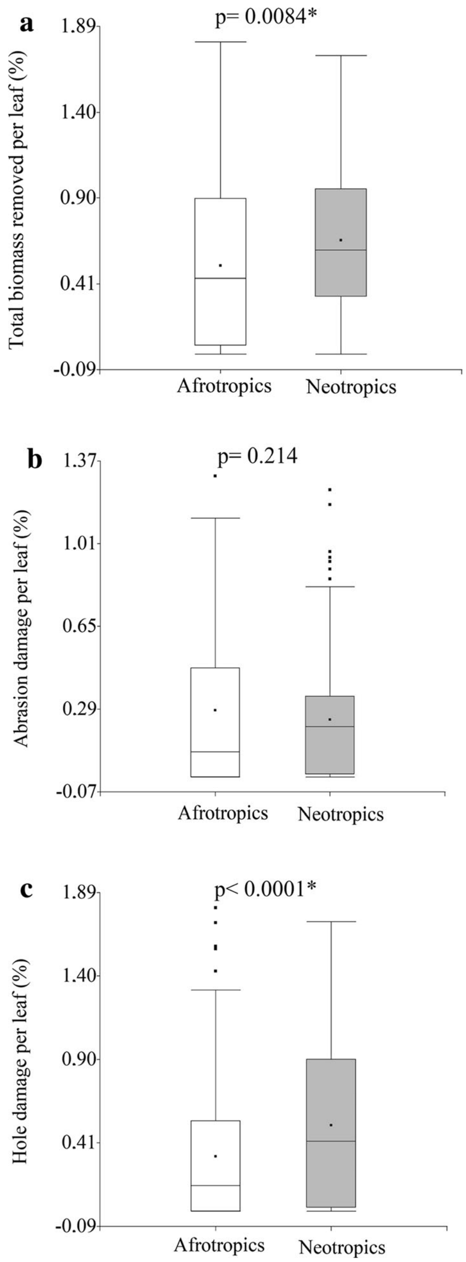


Fig. 2 Total number of leaves (**a**) and stems (**b**) damaged (black bars) and non-damaged (white bars) by small herbivores (invertebrates) in freshwater macrophytes from Afrotropical (Afro) and Neotropical (Neo) wetlands. Macrophyte "life forms": *(FF)* free-floating, *(FR)* floating rooted, *(S)*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

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

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

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



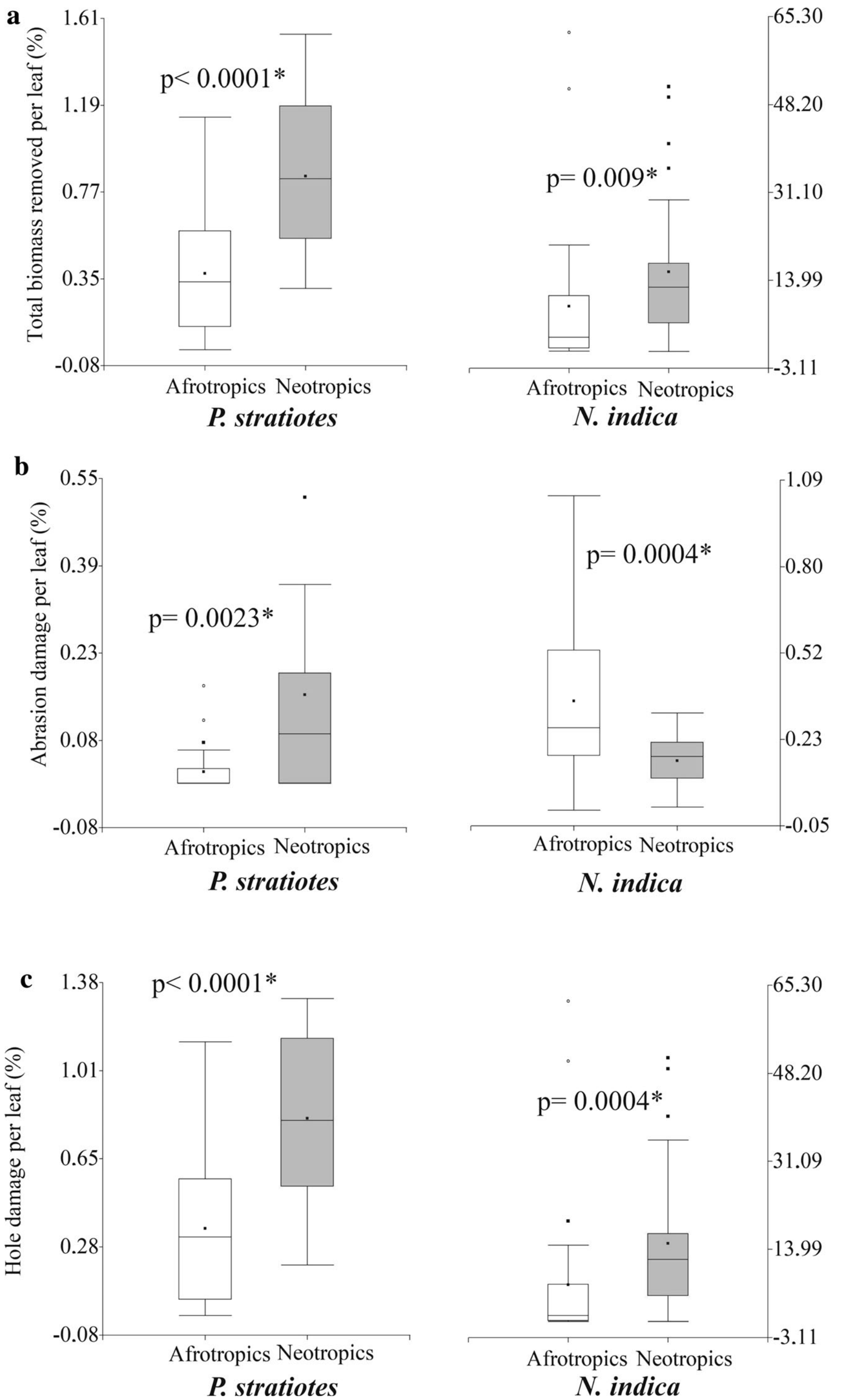
◀**Fig. 3** Biomass removed by small herbivores (invertebrates) per leaf in freshwater macrophytes from Afrotropical (white color) and Neotropical (gray color) wetlands. Values quantifying herbivory impact are expressed as percentage (%) of total biomass removed (surface abrasions + holes) (**a**), abrasion damage (**b**), and hole damage (**c**) per leaf lamina. Total number of leaves quantified on *Pistia stratiotes* (FF: Afro & Neo), *Nymphoides indica* (FR: Afro & Neo), and *Potamogeton octandrus* (S: Afro) were 60 per macrophyte species. In *P. octandrus* only submersed leaves were included. Number of leaves quantified in *Eichhornia azurea* (FR: Neo), *Hydrocleys nymphoides* (FR: Neo), *Nymphaea nouchali* var. *caerulea* (FR: Afro), *Nymphaea prolifera* (FR: Neo), *Potamogeton nodosus* (FR: Afro), *Trapa natans* (FR: Afro), and *Potamogeton illinoensis* (S: Neo) were 30 per macrophyte species. (*) in the *p* values indicates significantly different outcomes for pairwise comparisons between ecozones, using GLM (df = 358, values significant at $P < 0.05$). In box plots, box indicates quartiles Q1 and Q3, and central line and dot indicate median and mean values, respectively; whiskers show quantiles 0.05 and 0.95, and external dots represent outliers. Data were transformed to Log_{10} $(x + 1)$. See caption of Fig. 2 for other abbreviations

increased the affected tissues on leaves, especially in the FR macrophytes included in this study.

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

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

Despite the fact that the general trend showed that biomass removed by invertebrate herbivores per leaf


a
Total biomass removed per leaf (%)
1.61
1.19
0.77
0.35
-0.08
p< 0.0001*
Afrotropics Neotropics
P. stratiotes
65.30
48.20
31.10
13.99
-3.11
p= 0.009*
Afrotropics Neotropics
N. indica
b
Abrasion damage per leaf (%)
0.55
0.39
0.23
0.08
-0.08
p= 0.0023*
Afrotropics Neotropics
P. stratiotes
1.09
0.80
0.52
0.23
-0.05
p= 0.0004*
Afrotropics Neotropics
N. indica
c
Hole damage per leaf (%)
1.38
1.01
0.65
0.28
-0.08
p< 0.0001*
Afrotropics Neotropics
P. stratiotes
65.30
48.20
31.09
13.99
-3.11
p= 0.0004*
Afrotropics Neotropics
N. indica

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

was higher in Neotropical macrophytes than those from the Afrotropics, almost all *P. stratiotes* and *N. indica* plants, from both ecozones, showed herbivory damage to their leaves. The total number of leaves with invertebrate damage was significantly higher than the number of non-damaged leaves for both *N. indica* ($\chi^2 = 14.01$ for both ecozones, df = 1) and *P. stratiotes* ($\chi^2 = 7.35$ for Afrotropics; $\chi^2 = 12.15$ for Neotropics, df = 1).

Biotic and environmental factors influencing study sites

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

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

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

With regard to the full set of environmental factors measured, there were non-significant differences between Afrotropical and Neotropical wetlands for pH (GLM, $P = 0.404$; $N = 14$), temperature (GLM, $P = 0.201$; $N = 14$) and conductivity (GLM,

Table 1 Large herbivore (mammal) species and damage impacts (sensu Grime 1979) on freshwater macrophytes from Afrotropical (Afro) and Neotropical (Neo) wetlands, hosting invertebrate herbivores

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 *Loxodonta africana* LM: 2800–6300 kg (2)	Trampling grazing	*Pistia stratiotes, Azolla nilotica, A. pinnata* (1)	Afrotropics: South Luangwa, Kasanka, Bangweulu	Kasanka: 30–50 (5)
Hippopotamus *Hippopotamus amphibius* LM: 1000–> 2000 kg (2)	Trampling grazing	*Pistia stratiotes, Azolla nilotica, A. pinnata* (1)	Afrotropics: South Luangwa, Kasanka, Bangweulu	Kasanka: 100–200 (5); Luangwa: 20,000 (2)
Sitatunga antelope *Tragelaphus spekei* LM: 55–115 kg (2)	Trampling grazing	*Cyperus papyrus* (2), *Nymphaea nouchali* var. *caerulea* (1)	Afrotropics: Kasanka	Kasanka 500–1000 (5)
Puku antelope *Kobus vardonii* LM: 62–74 kg (2)	Trampling	*Nymphaea nouchali* var. *caerulea, Pistia stratiotes, Azolla nilotica, A. pinnata* (1)	Afrotropics: Kasanka, South Luangwa	Kasanka: 5000-7000 (5)
Impala antelope *Aepyceros melampus* LM: 40–50 kg (2)	Trampling	*Pistia stratiotes, Azolla nilotica, A. pinnata* (1)	Afrotropics: South Luangwa	
Black lechwe antelope *Kobus leche* LM: 80–100 kg (2)	Trampling grazing	*Nymphaea nouchali* var. *caerulea, Nymphoides indica, Potamogeton octandrus, Cyperus* sp. (1)	Afrotropics: Bangweulu Swamps	Bangweulu: 75,000 Kasanka: 0–2 (5)
Baboon *Papio cynocephalus cynocephalus* LM: 12–45 kg (2)	Grazing	*Pistia stratiotes* (1)	Afrotropics: South Luangwa National Park, Kasanka National Park	
Capybara *Hydrochoerus hydrochaeris* LM: 35–75 kg (4)	Trampling grazing	*Eichhornia azurea, Pistia stratiotes, Eichhornia crassipes; Hydrochleys nymphoides; Nymphoides indica* (1); E, FF and FR macrophytes (3) (4)	Neotropics: Riachuelo River Basin, Paraná Floodplain, Paraguay River Basin	
Red marsh rat *Holochilus brasiliensis* SM: 0.9–3.7 kg (6)	Grazing	Terrestrial and semiaquatic vegetation (4)	Neotropics: not seen in this study	
Swamp deer *Blastocerus dichotomus* LM: 80–125 kg (4)	Trampling grazing	Terrestrial and semiaquatic grasslands (4)	Neotropics: not seen in this study	

Table 1 continued

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
Coypu *Myocastor coypus* SM: 4–10 kg (4)	Trampling grazing	Terrestrial and aquatic plants (4)	Neotropics: not seen in this study	

Pistia stratiotes (FR: Neo & Afro), *Azolla nilotica* (FF: Afro), *Azolla pinnata* (FF: Afro), *Eichhornia crassipes* (FF: Neo), *Eichhornia azurea* (FR: Neo), *Hydrochleys nymphoides* (FR: Neo), *Nymphaea nouchali* var. *caerulea* (FR: Afro), *Nymphaea prolifera* (FR: Neo), *Nymphoides indica* (FR: Neo & Afro), *Potamogeton nodosus* (FR: Afro), *Trapa natans* (FR: Afro), *Potamogeton octandrus* (S: Afro), *Potamogeton illinoensis* (S: Neo), *Cyperus papyrus* (E: Afro). See text for life form abbreviations. Abundance of large mammalian herbivores is expressed as number of individuals recorded (where data available) in the study sites by different authors. Large mammalian herbivores (LM) include animals with more than 10 kg body mass and small mammalian herbivores (SM) those with less than 10 kg body mass (Bakker et al. 2016b). Source of the information: *(1)* this study, *(2)* Stuart & Stuart, 2006, *(3)* Schivo et al., 2010, *(4)* Quintana et al., 2012, *(5)* F. Willems (pers. com.), *(6)* Marques (1988)

$P = 0.550$; $N = 14$). However, Afrotropical wetlands had significantly greater intensity of disturbance by large herbivores (GLM, $P = 0.003$; $N = 14$) and faster flowing water (GLM, $P = 0.022$; $N = 14$) compared to those in the Neotropics.

The intensity of environmental disturbance due to the presence of antelopes using the lagoon habitat was also observed to differ considerably at sites sampled within the Afrotropical wetlands (see Online Resource 1). The lagoon sites Shoebill A (in Bangweulu: a very slow-flowing riverine lagoon, forming part of the Lukulu River) and Mushroom Lagoon (an enclosed lagoon in South Luangwa) both had substantial trampling damage by mammals (scored at 3), while the other African sites only had low to intermediate disturbance from large herbivore usage (scored 1 or 2). In the two most disturbed sites (Shoebill A Lagoon; Mushroom Lagoon) underwater PAR absorbance coefficients (k) were $> 20.0\ \mathrm{m}^{-1}$, indicating very high turbidity (black or dark brown muddy water) due to the constant resuspension of sediment caused by regular mammal trampling, whereas in the other less disturbed sites values calculated for k were all $< 5.0\ \mathrm{m}^{-1}$, indicating clear water. In contrast, sites in the Neotropical wetlands all had little or no visible evidence of disturbance due to trampling by large herbivores (all sites scored at 1), and the lagoons studied here all had high to intermediate levels of transparency, measured as Secchi depth, due to the absence of resuspension of sediment by mammals, or from other causes. In Argentina, Antequera 1, Antequera 2 and Herradura Lake 2 had slightly lower water transparency and silty clay sediments, while the other Neotropical sites had higher water transparency and sandy sediments (Online Resource 1).

Discussion

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

The high number of damaged leaves and biomass removed by invertebrates in FF and FR macrophytes, and the fact that half of sampled stems had galleries in E macrophytes, suggest that abundance and number of species of semiaquatic external feeders (e.g., grasshoppers, planthoppers, weevils, moth caterpillars) and endophagous invertebrate herbivores (e.g., larvae and adults of weevils and lepidopteran) could be higher than previously reported on invertebrate assessments of Afrotropical and Neotropical wetlands (e.g., Poi de Neiff, 2003; Poi de Neiff & Neiff, 2006; Albertoni et al., 2007; Ferreira et al., 2012; Wantzen et al., 2016). Also, a high number of semiaquatic invertebrate herbivores was recorded on these plants by an associated study of invertebrate herbivore assemblages conducted during our fieldwork program in Afrotropical and Neotropical wetlands (Franceschini et al., 2020). Our results also agree with those of Wissinger (1999), who pointed out that many works on wetland invertebrates are biased toward collecting and studying invertebrates of purely aquatic taxa, and suggested that herbivory by insects on above-waterline parts of macrophytes might be higher than previously expected.

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

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

Our findings agree with previous results (Franceschini et al., 2010) which suggest that quantifying the type of invertebrate damage (surface abrasion and holes) to assess biomass removed is important for methodological reasons, especially in ecosystems where enclosure or exclosure field experimentation is difficult or even impossible (e.g., due to the risk of damage to plots by large animals, extreme water level fluctuations, high abundance of aquatic predator like Nile crocodile or other problematic fieldwork issues common in tropical and subtropical wetlands). In addition, type of damage is also ecologically important because it reflects the predominance of different guilds and taxa in the invertebrate herbivore assemblages associated with a particular freshwater macrophyte species. Thus, for example, a different trend was seen for abrasion damage on *P. stratiotes* and *N. indica* comparing Afrotropical versus Neotropical plants, which indicate differing abundances of scrapers (mainly snails) in the invertebrate herbivore assemblages associated with these plants (Franceschini et al., 2020).

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

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

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

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

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

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

Our results indicate that invertebrate herbivory is an important ecosystem process damaging macrophyte species that occur in both Afrotropical and Neotropical wetlands and in some cases this damage can be great enough to substantially influence estimates of leaf biomass. In general, the observed impacts of invertebrate grazing on macrophytes were greater in the Neotropics than Afrotropics. The findings support our primary hypothesis that damage caused to tropical and subtropical freshwater macrophytes by small invertebrate herbivores is an extensive process affecting freshwater tropical and subtropical macrophytes, but that the intensity of grazing impacts differs quite substantially between the two ecozones, even in the case of two macrophyte species that occur in both ecozones. More generally, we provide new evidence to support the view that herbivory is an important process influencing freshwater ecosystem functioning (Bakker et al., 2016a, b; Grutters et al., 2016; Wood et al., 2016). Regarding our secondary hypothesis, that large herbivores, when present, may be a relevant biotic factor influencing macrophyte-invertebrate herbivory interactions in warm freshwater wetlands, the findings are inconclusive. We found observational evidence that the disturbance produced by large herbivore activity in warm-water wetland systems both damages macrophyte populations directly, and interacts with the grazing damage produced by small invertebrate herbivores in affecting the macrophyte populations of such systems. Although our results cannot be used to ascribe cause and effect here, they can provide a starting point for further work aimed at understanding the interactions of macrophytes with both small invertebrate and large herbivores in warm-water wetland systems. Finally, more field research (for example the use of simulated damage or exclosure experiments to assess the relative importance of small invertebrate and large mammalian herbivores on macrophyte production, e.g., Milne et al., 2008; Soti & Volin, 2010; Ramos et al., 2018) is clearly needed to understand and predict the role and impacts of small and large herbivores in tropical and subtropical ecosystems, in which anthropogenic disturbances may generate dramatic declines in biodiversity and habitat complexity.

Conclusions

We conclude here that damage by small invertebrate herbivores is an extensive process impacting subtropical to tropical macrophyte populations in the study wetlands, with invertebrate assemblages causing more damage per leaf in Neotropical macrophytes than Afrotropical ones. This damage may be modified by other biotic factors. We observed substantial differences in the incidence of damage from mammalian herbivores, and associated damage due to trampling and resuspension of sediments, between populations of almost all Afrotropical macrophyte species examined, and those from the Neotropical sites. Thus, the presence of large mammalian herbivores may be a relevant biotic factor influencing invertebrate herbivory in warm freshwater wetlands. Future research should be carried out in order to understand better the interaction between macrophyte populations and their associated invertebrate herbivore assemblages in these warm freshwater wetlands, comparing sites with and effectively without large mammalian herbivore pressure. Our work provides a starting point to examine these issues in more detail.

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