



Leaf consumption by invertebrate aquatic shredders in the Amazon: effects of climate change and microbial conditioning

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Abstract

We grew leaves of *Montrichardia arborescens* in four microcosm chambers with different temperatures and CO₂ concentrations simulating the scenarios of expected climate change. These leaves were used to feed shredders (*Phylloicus*) and to assess the effects of changes in leaf quality on their consumption. We also evaluated the effect of detritus conditioning by microorganisms on leaf consumption. We hypothesized that leaves of plants grown under different environmental conditions could offer substrata of different qualities to microorganisms colonizing them, and, consequently the shredder consumption rate would differ according to leaf conditioning. The microcosm chambers for plant growth simulated three different combined air temperature and CO₂ scenarios, relative to the real-time (control) current conditions in Manaus-Brazil. The leaf consumption experiment was performed only in the control chamber. Specific leaf area was positively affected by predicted climate change, while tannins were detected only in leaves of plants grown in chambers simulating a changed climate. Other leaf detritus parameters were similar in all chambers. Shredders showed higher consumption rates in leaves developed under mild and intermediate conditions in relation to control. Shredder consumption was similar in conditioned and unconditioned treatments. Thus, shredder consumption was influenced more by the intrinsic quality of leaves than by microorganism conditioning, but we were not able to show effects of climate change on leaf quality that could explain differences in shredder consumption.

Keywords Aquatic insects · Conditioned leaves · *Montrichardia arborescens* · *Phylloicus* · Temperature and CO₂ increase

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Introduction

Macrophytes colonize different aquatic environments and can alter ecosystems by influencing nutrient cycling (Esteves and Camargo 1986; Levi et al. 2015) and oxygen dynamics (Desmet et al. 2011), as well as water velocity and transparency (Wang et al. 2015). They also affect the composition of aquatic communities by increasing the availability and complexity of habitats offering protection from predators (Junk 1973; Grutters et al. 2015). Invertebrates can use aquatic macrophytes as a food source by consuming their living or decaying leaves (Watson and Barmuta 2011; Grutters et al. 2016) or material that is adhered to the plant surface (e.g., fine particulate organic matter and periphyton; Martins et al. 2011; Paice et al. 2016; Silveira et al. 2016).

Invertebrates can consume leaves of different plant species (Watson and Barmuta 2011). In general, detritus quality positively influences the rate of leaf consumption (Carreira et al. 2014; Grutters et al. 2015). Thus, invertebrates

consume larger amounts of debris with higher concentrations of nutrients and lower concentrations of secondary and structural compounds (Navarro et al. 2013; Rezende et al. 2014; Navarro and Gonçalves Jr. 2017). Microbial conditioning positively affects leaf consumption by invertebrates (Biasi et al. 2019). Microbial colonization increases detritus palatability due to increased protein content and due to the decomposition of refractory compounds by enzymatic action (Findlay 2010).

In the Amazon region predicted climate change by 2100 is expected to increase temperature by up to 7 °C, and atmospheric CO₂ concentration would reach up to 870 ppmv (IPCC 2007). Alterations in rainfall are likely to result in changes in the region's hydrological regime (Guimberteau et al. 2017). These climatic changes can result in alterations in the physiology, morphology, and species composition of aquatic macrophyte communities (Cao and Ruan 2015; Dhir 2015). Lopes et al. (2018) recorded an increase in leaf and root biomass in mild and intermediate climate change scenarios for the same individual plants (*Montrichardia arborescens* (L.) Schott) used in the present study. In general, little information exists on the combined effect of increased temperature and CO₂ on the chemical constitution of leaves of aquatic macrophytes. However, for terrestrial plants in general, increases in temperature and CO₂ are expected to decrease nitrogen content and increase the concentration of polyphenols and tannins (e.g., Wang et al. 2012; Gherlenda et al. 2015; Zhang et al. 2017; Jeong et al. 2018). The causes of alterations to leaf quality under scenarios of increased temperature and CO₂ due to climate change are complex (Rothman et al. 2015). They may be related to the increase of secondary metabolite production due to growth inhibition under stress conditions (Gairola et al. 2010), or to the control of the concentrations of secondary metabolite in leaf tissues in response to the availability of carbon (C) and nitrogen (N) in the environment (Carbon–Nutrient Balance Hypothesis; Bryant et al. 1983). Regardless of the regulatory mechanisms involved, climatic changes may result in lower-quality leaves, thus negatively affecting their consumption and rate of decomposition in aquatic environments (Tuchman et al. 2003; Dray et al. 2014).

Laboratory studies have been conducted to assess the direct effects (e.g., survival rate and growth) and indirect effects (decreased detritus quality) of climate change on invertebrate shredders (e.g., Tuchman et al. 2003; Navarro et al. 2013; Martins et al. 2017a). In Amazonian streams, species of the trichopteran genus *Phylloicus* are important shredders of coarse organic matter, because they use leaves for food and case-building (Landeiro et al. 2008; Martins et al. 2015, 2017a). Leaves of plants growing under different temperatures and CO₂ concentrations were collected to evaluate the effects of expected climate change on leaf quality and on their consumption by shredders (*Phylloicus*

elektoros Prather 2003). We also evaluated the effect on leaf consumption due to detritus conditioning. We tested the hypothesis that leaves of plants grown under different environmental conditions predicted by the IPCC (2007) models could offer different qualities of substrata to microorganisms colonizing them, and, as a consequence, the shredder consumption rate will differ according to leaf conditioning.

Methods

The experiment was carried out in microcosm chambers in which changes in CO₂ concentration and air temperature were adjusted in real time to simulate the forecasts in the Amazon for the year 2100 (IPCC 2007). The control chamber maintained real time current conditions of CO₂ and air temperature at Manaus, state of Amazonas, Brazil. An additional three chambers simulated conditions of temperature and CO₂ increase in relation to the control chamber (mild condition: temperature = +1.5 °C; CO₂ = +220 ppmv; intermediate condition: temperature = +3 °C; CO₂ = +420 ppmv; and extreme condition: temperature = +4.5 °C; CO₂ = +870 ppmv; Martins et al. 2017a). All chambers maintained similar humidity levels (~70%) and the same photoperiod (12:12 h; Martins et al. 2017a). Plants were grown in all chambers; however, experiments on leaf consumption by shredders were performed in the control chamber, where temperature was 26.4 ± 0.4 °C and the CO₂ concentration was 392.2 ± 11.9 ppmv.

Leaves of *M. arborescens* were used in the experiment, a species popularly known as “Aninga” that is common in flooded areas in the Amazon region (Lopes et al. 2016, 2017). The plant material was obtained from a research study conducted by the MAUA/INPA group (Ecology, Monitoring and Sustainable use of Wetlands; for details see Lopes et al. 2018), in which infructescences of *M. arborescens* were collected along a 100-m stretch of the edge of the Negro River in the municipality (county) of Iraduba in the state of Amazonas, Brazil. Seeds were removed from fruit pulp and planted in pots (Lopes et al. 2018) that were placed in the four chambers ($n = 50$) with expected conditions of the IPCC scenarios (see Table 1), and their germination was monitored. The plants remained in the same conditions for 162 days from April to September 2012 and were subsequently dried in an oven until reaching a constant mass (60 °C, ~72 h). Most of the leaves used in our experiments were green and not senescent, since previous studies in the Amazon have recorded *Phylloicus* feeding on green leaves of *Mabea speciosa* Müll. Arg. (Euphorbiaceae) (Landeiro et al. 2008; Martins et al. 2015).

Table 1 Climatic conditions of the microcosm chambers in which the leaves of *Montrichardia arborescens* grew between April and September 2012 (data from Lopes et al. 2018)

	CO ₂ (ppmv)	Temperature (°C)	Humidity (%)
Control	427.00 ± 66.44	27.45 ± 0.65	73.52 ± 2.19
Mild	600.81 ± 47.54	29.10 ± 0.60	74.40 ± 3.87
Intermediate	814.41 ± 39.48	30.04 ± 0.72	73.58 ± 3.24
Extreme	1253.37 ± 45.22	31.78 ± 0.69	73.41 ± 2.86

Leaf parameters

The chemical composition of leaves grown in microcosms with all climatic conditions was determined using air-dried leaves, following the methods of Graça et al. (2005). In each chamber, we used leaves of 16 plants that had been grown under these conditions to obtain their chemical composition. Leaves of other plants ($n = 34$) were used in the consumption experiment. Thus, in each chamber we obtained only one value for each leaf chemical variable. Phosphorus (%) contents were determined by nitric-perchloric digestion and subsequent spectrophotometry. Polyphenol (%) contents were obtained using the Folin-Ciocalteu method and subsequent spectrophotometry. Tannin (%) contents were measured using the radial diffusion method, while cellulose (%) and lignin (%) contents were obtained by the gravimetric method using acetone and sulfuric acid. The specific leaf area (SLA) is an inverse proxy of leaf toughness and was obtained from the ratio of disc area (mm²) to leaf dry mass (mg; Huang et al. 2007). SLA was measured using conditioned and unconditioned disks.

Treatment of leaves prior to consumption

Disks of *M. arborescens* (8 mm in diameter) were cut from each leaf, avoiding the central rib. The disks were then divided into two groups, one to be conditioned and one to remain unconditioned. Conditioned disks were incubated in fine-mesh litter bags for 7 days in the Barro Branco stream (Reserva Ducke—02°55' and 03°01'S; 59°53' and 59°59'W; Manaus) to leach out water-soluble compounds and to condition the leaves with fungi and other microorganisms (Martins et al. 2017a). The leaf disks could not be left in the field for an extended period of time due to their low toughness; a preliminary study to determine the incubation time found the disks reached an advanced state of decomposition after 7 days. The stream is bordered by dense riparian forest and has acidic water (pH = 4.6 ± 0.1), high dissolved oxygen concentration (6.6 ± 0.1 mg l⁻¹), low electrical conductivity (10.7 ± 0.4 μS cm⁻¹) and an average water temperature of 24.5 ± 0.5 °C (Martins et al. 2014).

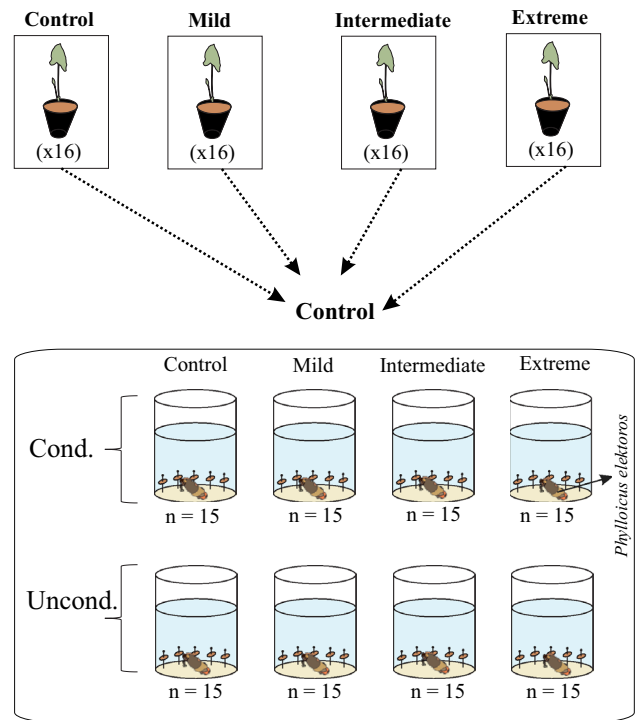


Fig. 1 Sample design of the experiment evaluating consumption by shredders using leaf disks of *Montrichardia arborescens* growing under four simulated climatic conditions. Leaves were collected from 16 pots in each microcosm. *Cond.* Conditioned leaf. *Uncond.* Unconditioned leaf

Unconditioned disks were immersed in distilled water (48 h) to permit leaching of water-soluble compounds to avoid overestimating shredder consumption. We did not perform direct measurement of leaf conditioning. The disks (conditioned and unconditioned) were subsequently lyophilized (24 h) and weighed on a precision balance. Freeze-drying is effective for revival of various Hyphomycetes species (Aiyer 2018).

Shredder sampling and consumption experiments

In February 2013, we collected 110 larvae of *P. elektoros* from the Barro Branco stream. The individuals were transported to the laboratory and were acclimatized for 72 h in a plastic box containing mineral water and leaves from the stream (Martins et al. 2017a). During acclimatization, the water was constantly aerated. The photoperiod was 12:12 h.

In all treatments the mean water temperature was 24.9 ± 0.7°C, the pH was 6.2 ± 0.4 and the dissolved oxygen was 5.8 ± 0.3 mg L⁻¹. The experiment lasted 1 week. We set up 110 experimental arenas containing mineral water (500 mL) and incinerated sand (burned at 450 °C for 4 h; Martins et al. 2017a, b), with a single individual of *P. elektoros* per arena. In each arena, we included five leaf

disks. The arenas were divided into eight treatments (see Fig. 1) with two factors: temperature and CO₂ (four levels) and conditioning (two levels). The number of replicates varied from 10 to 15 for each treatment. We observed all arenas daily to record dead or pupated individuals. Any dead individuals were removed.

After the experiment, the disks were removed from the arenas, freeze-dried (~ 24 h) and weighed on a precision balance. Leaf consumption was calculated as the difference between initial and final disk mass divided by the duration time of the experiment. The leaf masses for arenas with dead or pupated individuals were not considered in the calculation of the consumption rate.

To evaluate the combined effects of temperature and CO₂ on leaf parameters, we first used Principal Component Analysis (PCA-Axis 1) to determine climatic conditions, because in our study, these climate parameters are associated, varying together in the chambers. Thus, we used PCA-Axis 1 as a proxy for the gradient of climatic conditions. Second, we used a generalized linear model (% data; quasi-binomial distribution) to evaluate the relationships between the chemical composition of the leaves (polyphenols, tannins, lignin, cellulose, and phosphorus) and climatic conditions (PCA-Axis 1).

The consumption rates were compared among the conditions under which the leaves had grown (control, mild, intermediate, and extreme) and conditioning treatments (conditioned and unconditioned) using a two-way Analysis of Variance (ANOVA) and a Tukey test. We also used two-way ANOVA to compare the SLAs among the conditions under which the leaves had grown and the conditioning treatments. All statistical analyses were performed with R software.

Results

Chemical composition

The combined elevation of temperature and CO₂ simulating the predicted conditions of different climatic scenarios did not significantly alter the chemical composition of leaves of *M. arborescens* ($p > 0.05$; Table 2). Under all climate conditions, the leaves of the species showed similar values contents of polyphenols (~ 1.4%), lignin (~ 30%), cellulose (~ 11%), and phosphorus (~ 0.2%). Although tannins could not be detected in the control, they were present in the three simulated scenarios (mild, intermediate, and extreme) with similar concentrations (~ 2.5%).

The interaction between the climatic conditions under which the plant had grown and the conditioning tended to affect SLA ($F_{2,64} = 1.86$; $p = 0.053$). In conditioned leaves there was a linear increase of the SLA of plants grown under the increasingly altered climate conditions from the control ($67.18 \pm 12.86 \text{ mm}^2 \text{ g}^{-1}$) to the extreme condition ($77.63 \pm 7.41 \text{ mm}^2 \text{ g}^{-1}$). In unconditioned leaves there was lower SLA for plants grown under the intermediate climate condition ($40.11 \pm 3.74 \text{ mm}^2 \text{ g}^{-1}$) and higher SLA under the extreme condition ($43.73 \pm 2.46 \text{ mm}^2 \text{ g}^{-1}$). In addition, we recorded a difference in SLA due to climatic conditions under which the plant had been grown ($F_{3,112} = 3.93$; $p = 0.011$). The SLAs of leaves grown under the control condition ($54.50 \pm 16.13 \text{ mm}^2 \text{ g}^{-1}$) were ~ 11% lower than those under the extreme scenario ($60.68 \pm 18.07 \text{ mm}^2 \text{ g}^{-1}$). On the other hand, the consumption of leaves grown under the mild ($55.75 \pm 13.85 \text{ mm}^2 \text{ g}^{-1}$) and intermediate ($56.26 \pm 18.43 \text{ mm}^2 \text{ g}^{-1}$) scenarios were similar to all other scenarios. Moreover, SLA was lower in unconditioned ($42.14 \pm 4.35 \text{ mm}^2 \text{ g}^{-1}$) than in conditioned leaves ($71.45 \pm 10.23 \text{ mm}^2 \text{ g}^{-1}$; $F_{1,112} = 465.56$; $p < 0.001$).

Table 2 Percentages (%) of polyphenols, tannins, lignin, cellulose, and phosphorus in leaves of *Montrichardia arborescens* grown under four simulated climatic conditions

	Polyphenols (%)	Tannins (%)	Lignin (%)	Cellulose (%)	Phosphorus (%)	SLA (mm ² g ⁻¹)
Control	1.44	B.D.L	30.16	10.56	0.21	54.50 ± 16.13
Mild	1.45	2.43	31.27	10.36	0.20	55.75 ± 13.85
Intermediate	1.45	2.50	30.36	11.74	0.23	56.26 ± 18.43
Extreme	1.45	2.49	29.77	10.67	0.25	60.68 ± 18.07
R ²	0.28	0.12	0.18	0.42	0.69	0.93
P	0.278	0.537	0.535	0.767	0.115	0.023

B.D.L Below detection limit (0.025 mg tannin; Hagerman 1987). SLA Specific leaf area

R² and P values from generalized linear model to evaluate the relationship between the chemical composition of the leaves and climatic conditions (PCA-Axis 1)

Phylloicus elektoros survival

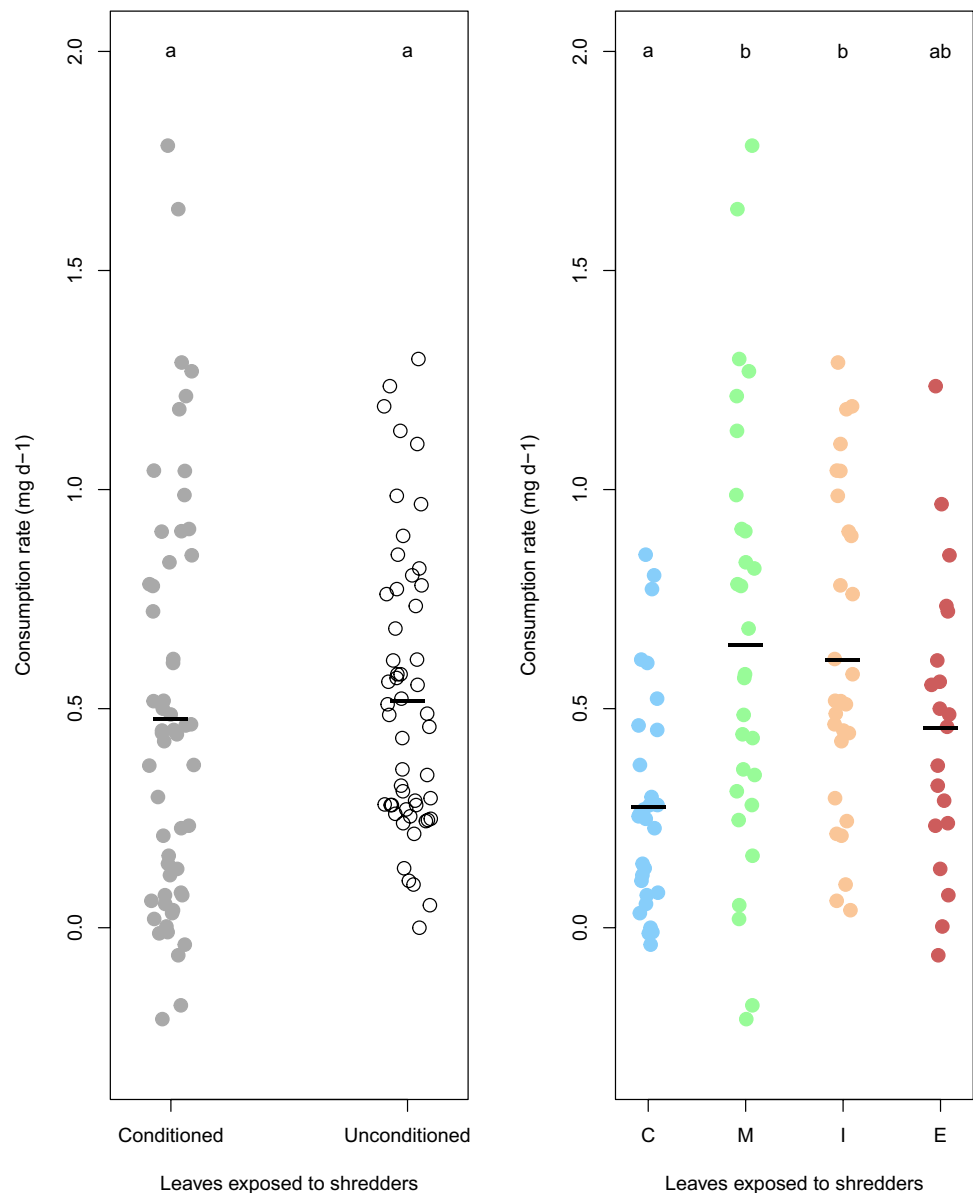
During the experiment two individuals died (1.8%), both in the treatment with unconditioned leaves grown under the extreme conditions. We recorded only one pupa in the treatment with extreme condition and unconditioned leaves.

Leaf consumption by *P. elektoros* larvae

The individuals of *P. elektoros* consumed conditioned and unconditioned leaves from plants grown under all microcosm conditions (Fig. 2). The interaction between the climatic conditions under which the plant had grown

and the conditioning had no significant effect on leaf consumption ($F_{3,99} = 2.13$; $p = 0.101$). However, we recorded a difference in consumption of leaves due to the climatic conditions under which the plant had grown ($F_{3,99} = 5.99$; $p < 0.001$; Fig. 2). The consumption of leaves grown in the mild (0.65 ± 0.50 mg d⁻¹) and intermediate (0.62 ± 0.37 mg d⁻¹) scenarios was up to 2.3 times higher than for leaves grown in the control scenario (0.28 ± 0.25 mg d⁻¹). On the other hand, the consumption of leaves grown in the extreme scenario (0.46 ± 0.33 mg d⁻¹) was similar to those from all other scenarios. However, no difference in consumption was observed between conditioned (0.49 ± 0.47 mg d⁻¹) and unconditioned leaves (0.53 ± 0.33 mg d⁻¹; $F_{1,99} = 0.44$; $p = 0.507$).

Fig. 2 Rate of leaf consumption rate by *Phylloicus elektoros* in treatments with leaves of *Montrichardia arborescens* grown under four simulated climatic conditions. Leaves were conditioned by microorganisms (gray circles) or unconditioned (black circles). C control chamber, M mild chamber, I intermediate chamber, E extreme chamber. Black bar = mean values. Different letters indicate significant differences between treatments



Discussion

We recorded similar values for polyphenols, lignin, cellulose and phosphorus in the leaves of *M. arborescens* that were grown under the four tested climatic conditions indicating that the combined elevation of temperature and CO₂ did not affect leaf tissues in this study. Tannin and SLA were affected positively by predicted climate change. Shredders showed higher consumption rates in leaves grown under mild and intermediate conditions, but their consumption did not differ due to leaf conditioning. This indicates that leaf consumption could be stimulated by the different temperatures and concentrations of CO₂ under which these leaves had grown.

Chemical composition

We did not find effects of the tested climatic scenarios on leaf polyphenols, lignin, cellulose or phosphorus. However, field studies and meta-analyses with leaves of terrestrial plants have found decreases in nitrogen and phosphorus concentrations and an increase in C:N ratio, leaf thickness, specific mass, and concentrations of polyphenols, among other factors, in response to a joint increase of atmospheric temperature and CO₂ (Murray et al. 2013; Gherlenda et al. 2015). For example, Dray et al. (2014) recorded a decrease in N concentration and increases in C:N ratio and lignin in *Betula pendula* leaves exposed to increased atmospheric CO₂, while *Alnus glutinosa* leaves were not affected.

Tannins, on the other hand, were detected in leaves of plants grown in chambers simulating climate change but not in the control. These secondary metabolites have high structural and chemical variability (Salminen and Karonen, 2011), differing between individuals of the same species and even between parts or developmental stages of a given individual (Häring et al. 2007; Salminen and Karonen, 2011). Their quantity and composition can be influenced by seasonal variation (Feeny 1970; Salminen et al. 2004) related to changes in temperature and CO₂, among other factors. Higher tannin values have been found to be positively associated with increased temperature (Top et al. 2017) and increased CO₂ (Koricheva et al. 1998), which improve carbon acquisition and, in turn, stimulate the production of phenolic compounds such as tannins (Bryant et al. 1987). Tannins are important inhibitors of herbivory and, in combination with certain proteins, enhance resistance against the decomposition of tissues (Volz et al. 2001).

Since leaves in all chambers had the same age and they were subjected to the combined effects of increased

temperature and CO₂ (which would increase carbon acquisition and defenses), the detection of tannins in CO₂-enriched chambers was not surprising. However, the unexpected higher consumption of leaves, especially those grown in the mild and intermediate scenarios (up to 2.3 times higher than the control), was intriguing. One possible explanation may relate to SLA. Leaves grown in the control had SLA ~ 11% lower than those grown under the extreme scenario, resulting in softer leaves in the extreme chamber. On the other hand, biomass increase in *M. arborescens* (see Lopes et al. 2018) together with increased SLA, indicate that plants in the mild and intermediate chambers probably also produced softer leaves owing to faster growth and development of aerenchyma tissues in the intracellular spaces of which tannins accumulate (Grundhöfer and Gross 2001; Grundhöfer et al. 2001). Therefore, a higher consumption of leaf material may be the result of softer leaves.

Exposure time may have an influence on chemical modifications in plants, and this effect may be more pronounced in adult plants. Young plants can invest an abundance of CO₂ resources primarily into growth, and only subsequently invest in the production of refractory compounds. This may be the case for the leaves of the young *M. arborescens* used in this study.

Leaf consumption

Greater consumption was recorded for leaves that developed under mild and intermediate conditions. However, we still cannot identify the main variables that can affect leaf consumption in our study. Tannins were recorded only in leaves from the mild, intermediate and extreme chambers, and the concentration of this chemical compound is known to be negatively associated with leaf consumption by invertebrates (Adams et al. 2005). Tannin is highly hydrophilic and leaches rapidly when in contact with water (Gonçalves et al. 2012). Thus, immersion of disks in streams or water before the experiment may have reduced or eliminated possible differences between the chambers in the effects of tannin on leaf consumption by *P. elektoros*. Peñuelas and Estiarte (1998) recorded increases of soluble phenolics and condensed tannins with increased CO₂, but they did not record effects on leaf decomposition.

Higher SLA values were recorded under high temperature and CO₂. However, due to high values of SLA in all scenarios (> 54.5 mm² g⁻¹), it is possible that this variable does not explain the higher consumption of leaves that were grown in the mild and the intermediate scenarios. For example, the SLA value of *Hevea brasiliensis* is 3.08–3.44 times lower than that of *M. arborescens*. *Hevea brasiliensis* has the softest leaf of 51 riparian species from Manaus and

Table 3 Minimum, maximum, mean and standard deviation (SD) of chemical and structural compounds of 51 plant species of riparian forest in Central Amazon (unpublished data) and *Montrichardia arborescens* (Mean \pm SD)

	Riparian species			<i>Montrichardia arborescens</i>
	Min	Max	Mean \pm SD	
Specific leaf area (mm ² mg ⁻¹ mg)	3.60	17.66	8.70 \pm 3.42	56.80 \pm 16.67*
Toughness (mg)	111.14	1181.48	438.99 \pm 250.41	87.93 \pm 27.96**
Carbon (%)	36.78	45.92	43.78 \pm 1.81	41.51 \pm 3.96**
Nitrogen (%)	0.57	2.18	1.21 \pm 0.36	3.30 \pm 0.14**
Phosphorus (%)	0.01	0.09	0.03 \pm 0.02	0.21 \pm 0.07**
Potassium (%)	0.10	1.35	0.21 \pm 0.18	1.91 \pm 0.40**
Calcium (%)	0.06	3.91	1.05 \pm 0.94	0.91 \pm 0.45**
Magnesium (%)	0.04	0.42	0.18 \pm 0.10	0.32 \pm 0.07**
Polyphenols (%)	1.08	17.76	6.25 \pm 4.09	1.45 \pm 0.01*
Tannin (%)	0.00	7.82	2.72 \pm 1.84	2.47 \pm 0.04*
Cellulose (%)	14.89	42.80	27.95 \pm 6.44	10.83 \pm 0.62*
Lignin (%)	31.98	73.70	47.70 \pm 9.10	30.39 \pm 0.64*

Methods to determine these variables are presented in the supplementary material

*Data obtained from plants grown in microcosms

**Data obtained from plants collected in the field (in floodplain and igapó areas)

had rapid decomposition ($>0.017d^{-1}$) in Amazon streams (unpublished data; see Table 3).

No differences in the other chemical parameters of the leaves were observed among the different climatic conditions, although it remains possible that climatic change causes variation in leaf chemical compounds that were not evaluated in our study, especially carbon. In fact, corroborating this assumption, for the same individual plants used in the present study higher biomass values were measured in the mild and intermediate scenarios, which certainly results from higher rates of carbon fixation as compared to the control and the extreme conditions (Lopes et al. 2018). Studies in temperate regions have recorded a preference of ectotherms for carbohydrate-rich food at higher temperatures, which may result in increased consumption of leaves with higher C:N and C:P ratios due to increased rates of metabolism at these temperatures (Malzahn et al. 2106; Carreira et al. 2017). We suggest investigating N and micronutrients in the leaf tissue in future studies.

Leaf consumption was similar between conditioned and unconditioned treatments. In general, higher consumption of conditioned leaves has been recorded due to increased palatability and increased microbial biomass in detritus (Canhoto and Graça 1995; Tant et al. 2015) and may even compensate for the lower quality of certain plant species (Ferreira et al. 2010). However, leaves with high nutritional quality can be consumed independently of microbial conditioning. For instance, in Amazonian streams it has been observed that the colonization and consumption of soft leaves of *Mabea speciosa* by trichopteran shredders (*Phylloicus* and *Triplectides*) occurred independently of microorganisms (Landeiro et al. 2008; Martins et al. 2015). Moreover, data for leaves

of 51 riparian species from Manaus (unpublished data; see Table 3) indicate that leaves of *M. arborescens* have high palatability, with higher N-proportion and lower toughness than any of these species. This would reduce the influence of conditioning on leaf consumption by *P. elektoros*. *Montrichardia arborescens* leaves from plants collected in floodplain and igapó areas have N- proportion ($3.30 \pm 0.14\%$; unpublished data) similar to the value reported for the N-rich species *A. glutionsa* ($\sim 2.6\text{--}2.9\%$; Gessner and Chauvet 1994; Graça and Cressa 2010; Tonin et al. 2017). On the other hand, the relatively short period of conditioning (7 days) may have made it difficult to determine differences due to conditioning (Bärlocher and Sridhar 2014). However, the fungal biomass either remained constant or decreased during decomposition in studies using leaves of aquatic macrophytes (Kuehn et al. 2000; Carvalho et al. 2015). Thus, it is possible that for the leaves of *M. arborescens* used in the present study, the optimal conditioning time is shorter than that recorded for terrestrial riparian plant leaves (2–8 weeks; Golladay et al. 1983; Arsuffi and Suberkropp 1984). Moreover, in contrast to woody plants, the conditioning phase could be less important in macrophytes detritus.

In conclusion, the intrinsic quality (high SLA) of *M. arborescens* leaves was more important for consumption by shredders than was microorganism conditioning. Variables that differ between scenarios (SLA and tannin) probably were not the main factors affecting the consumption of leaves. Thus, since we did not record a potential negative effect of climate change on leaf quality, it was also not possible to assess the associated direct effects of climate change on shredders. Possibly the effects on leaf quality and palatability will be only evident after a longer period

of exposure of the plants to the microcosm scenarios. Such chronic effects could include genetic mutations, which would require testing over multiple generations. Finally, in our exploratory study several important questions remain open, requiring further investigation into the fate of food chains in Amazonian water bodies under scenarios of global environmental change.

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