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MULTIFUNCTIONALITY OF LARGE RIVERS

Combined effect of elevated CO_2 level and temperature on germination and initial growth of *Montrichardia arborescens* (L.) Schott (Araceae): a microcosm experiment

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Abstract IPCC predictions indicate an increase in temperatures by 1.5–7°C in some Amazonian regions during the twenty-first century. These changes could disrupt the present distribution patterns of organisms, including wetland plant species. In this work, we determined in microcosms the effects of scenarios combining elevated temperature and atmospheric CO_2 concentration on the germination and initial growth of the arborescent Amazonian aquatic macrophyte *Montrichardia arborescens*. Seeds were germinated, and seedlings produced were monitored over a 5-month period in four microcosms: Control: ambient temperature and CO_2 level; Mild: Control + 1.5°C and + 200 ppm CO_2; intermediate: control + 2.5°C and + 400 ppm CO_2; Extreme: Control + 4.5°C and + 850 ppm of CO_2. Rapid light response curves and Fv/Fm values taken in seedlings showed a decrease in electron transportation rate with CO_2 and temperature elevation. Mild and Intermediate treatments stimulated biomass production; Extreme treatment and Control produced similar results. The severe climatic changes expected in the future may negatively influence carbon accumulation in *M. arborescens*. Since aquatic macrophytes in Amazonian wetlands and wetlands worldwide are key plant

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We dedicate this manuscript to the memory of Dra. Mônica Elisa Bleich a great and beloved colleague from the MAUA Group who left us early and suddenly.

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species, further studies are needed to predict their fate in a global change perspective.

Keywords Amazonian floodplains · Nutrient poor black-water · Igapó · Aquatic macrophytes · Physiology · Climate change

Introduction

Average global surface temperatures are projected to increase by 1.5–5.8°C over the twenty-first century. In the Amazon Basin, an increase in temperatures by ca. 1.5–7.0°C is expected in some areas (IPCC, 2013). Over the decade 1984–1993, the rate of CO_2 increase was on average 1.5 μmol/mol/year and that trend will probably continue over the current century, resulting in concentrations approaching 560 μmol/mol, twice the pre-industrial value by the middle or second half of the twenty-first century (Schimel et al., 2000). The steadily increasing human population and economic activities are likely to lead to even higher CO_2 concentrations (approximately 700 μmol/mol) by the end of the XXI century. Projected increases in mean temperature are expected to greatly disrupt present patterns of plant and animal distributions. These climatic changes would reduce plant water availability and thereby increase drought stress for many Amazonian species (Piedade et al., 2013). Moreover, large changes in the patterns of warming, wind, and rainfall, which will entail extreme weather events, will directly affect the duration and intensity of flooding and the dry season and fire events in Amazonian wetlands (Cochrane & Laurance, 2002; IPCC, 2007, 2013; Nobre et al., 2007).

The Amazonian rainforest is one of the main storage compartments of terrestrial carbon (Turcq et al., 2002), with estimated values of 74–127 Mg C/ha/year in soil stock and 148–180 Mg C/ha/year in above ground biomass (Malhi et al., 2009a). The region is also responsible for the annual variation in carbon sequestration rates (Turcq et al., 2002), since it is influenced by climate variations, especially during the rainy season when the occurrence of El Niño may reduce the radiation quantity during dry years, due to the elevation of the temperature of the tropical North Atlantic Ocean, leading to the occurrence of more fire events and contributing to the increase in carbon emissions (Tian et al., 1998; Lewis et al., 2011; Vasconcelos et al., 2013). Extrapolated over the whole basin, CO_2 flux from flooded areas was found to be of comparable magnitude to some estimates of carbon sequestration in upland forests (Hess et al., 2003). Net primary production (NPP) of aquatic herbaceous plants in Amazonian floodplains varies between 30 and 50 t/ha/year (Junk & Piedade, 1993), contributing substantially to the carbon budget of the region (Piedade et al., 2001). The decomposition of herbaceous plants promotes the enrichment of the aquatic system and associated floodplains, during both the aquatic and terrestrial phases of the hydrologic cycle (Piedade et al., 2001, 2010).

Temperature is one of the most important climatic factors affecting the distribution range of many species, including aquatic and wetland plants. It affects their physiology, seed germination, periodicity and rate of seasonal growth, and onset of dormancy (Short & Neckles, 1999). On the other hand, rising levels of atmospheric CO_2 are predicted to have significant direct effects on global vegetation (Bowes, 1993), including aquatic plant communities (Wetzel & Grace, 1983; Edwards, 1995). Despite the fact that nature of the physiological adaptations may vary greatly between species according to the genetic load as a function of the availability of environmental resources (Pepper et al., 1998), typical responses to short-term CO_2 enrichment include increases in photosynthesis, growth, total biomass, root/shoot ratio, and tissue carbon/nitrogen ratio (Short & Neckles, 1999). In Amazonian floodplain forest trees, the annual changes in water level owing to the flood pulse (Junk et al., 1989) influence parameters directly responsible for obtaining daily carbon, such as photosynthetic capacity and quantum efficiency and the uptake and use of nutrients in soil, which influences primary metabolism (Parolin, 2001; De Simone et al., 2002). Thus, the increase in CO_2 concentration and temperature associated with changes in the flooding regime and precipitation are expected to affect the ecophysiological responses and carbon uptake of plant species colonizing floodplain ecosystems (Piedade et al., 2013).

Analyzing the combined effects of CO_2 and temperature on plants is important because changes in CO_2 concentrations are global, and different ecosystems in very different temperature regimes will be affected, irrespective of any past or future changes in temperature (Morison & Lawlor, 1999). The effect of the interaction of increased temperature and CO_2 concentration in plants has been discussed by many authors (see Morison & Lawlor, 1999), but its effect on aquatic macrophytes has been poorly investigated. However, a huge variety of aquatic plants colonize the Amazonian floodplains, estimated at 388 species alone for central Amazonia (Junk & Piedade, 1997). Rooted aquatic macrophytes, such as *Montrichardia arborescens* (L.) Schott (Araceae), are subject to typical stresses of the aquatic environment, such as waves, oxygen shortage, instability of the substrate, and also the periodic variation of the water level which causes drastic changes in the environment (Piedade & Junk, 2000). *Montrichardia arborescens* occurs in the fertile white-water várzea and the poor acidic igapó flooded forests (Lopes et al., 2014). Although being an aquatic emergent macrophyte, *M. arborescens* occupies a position along the floodplain more related to the first succession stage of pioneer trees and shrubs with which it may compete due to its arboreal habit (Lopes et al., 2015). The climate change studies on emergent macrophytes are important owing to their crucial role in the ecosystem functioning in aquatic habitats (Ojala et al., 2002).

The aim of this study was to determine the responses of an Amazonian aquatic macrophyte, *M. arborescens*, in scenarios with substantial changes in temperature and atmospheric CO_2 concentrations according to the predictions of the IPCC (2007). Our study was designed to evaluate the responses of this species to four different scenarios of CO_2 concentration and temperature to characterize the potential effect of a CO_2-enriched atmosphere on germination and seedling establishment. We aimed to determine whether climatic changes would interfere with responses of germination and growth parameters and photosynthesis. Our hypothesis was that moderate elevation of temperature and CO_2 should stimulate seed germination and seedling growth, while high concentrations could inhibit germination and growth of seedlings.

Methods

Species investigated

Montrichardia arborescens, known as aninga, has a broad distribution along the river shores in the Amazon Basin, forming monospecific stands at the edges of floodplain forests (Lopes et al., 2015). The species can also be found in high densities in Central America and in isolated spots in the states of Minas Gerais and Bahia (Brazil) (http://splink.cria.org.br). In the Amazon Basin, *M. arborescens* may be found in nutritionally rich environments, such as the white-water rivers (várzeas), but especially in poor environments, such as black-water rivers (igapós) and in the brackish estuarine areas of the Amazon River (Lopes et al., 2015).

Mature infructescences of *M. arborescens* were collected in April 2012 from eight individuals (one infructescence/individual) approximately 100 m apart along the Negro River, the biggest black-water river in Central Amazonia (Sioli, 1984), in the locality of Praia Grande, Iranduba (Amazonas, Brazil). The pulp of the fruits was removed, and 200 similarly healthy seeds were chosen for the experiments in the microcosms. The seeds were placed in plastic pots (8 cm in height and 8 cm in diameter) with a vermiculite substrate, with one seed per pot. Trays with water were placed beneath the pots to maintain a high moisture level in the substrate. Each treatment had 50 replicates. The experiment was conducted from April 4 to September 13, 2012 (162 days).

Microcosm experiment

The microcosm was set up at the Laboratory of Genetics and Molecular Evolution—LEEM, INPA/Manaus (AM, Brazil). The microcosm itself consisted of four climatic chambers subjected to a daily 12-h photoperiod regime (12 h light and 12 h dark), and independently controlled humidity, temperature, and CO_2 concentrations [Control (environmental), Mild, Intermediate and Extreme] (Table 1). The climatic conditions of the rooms as described above were programmed according to the scenarios forecasted for 2100 by the IPCC (2007). Since in the natural environment, temperature and CO_2 vary throughout the day, the rooms were designed to simulate these fluctuations in the enriched rooms over 24 h. Therefore, the Control room mimicked in real time the current conditions of CO_2 level, temperature, and humidity in the surrounding forested environment (Table 1).

Table 1 Programmed values of CO_2 concentration, temperature, and humidity for the microcosm

Treatment	CO_2 (ppm)	Temperature (°C)	Humidity (%)	IPCC Scenario (2007)
Control	±400	±30	±75	–
Mild	Control +200	Control +1.5	±75	B1
Intermediate	Control +400	Control +2.5	±75	B2
Extreme	Control +850	Control +4.5	±75	A2

Parameters measured

Germination, considered the protrusion of the radicle, was evaluated daily from the beginning of the experiment, and the number of seedlings produced was calculated for each treatment at the end of the experiment. The following parameters were evaluated and calculated: the percentage of germination (G%), initial time of germination (ITG), and final time of germination (FTG). Median time of germination (t) was calculated as $\bar{t} = \sum_{i=1}^{k} n_i t_i / \sum_{i=1}^{k} n_i$, where t_i is time from the start of the experiment to the ith observation (day); n_i is number of seeds germinated in time i (not the accumulated number, but the number corresponding to the ith observation); and k is last time of germination (Labouriau, 1983). Germination speed index (GSI) was determined as GSI = (G1/N1) + (G2/N2) + (G3/N3) + $\cdots$ + (Gn/Nn), where G1, G2, G3,…, Gn = number of germinated seeds in the first, second, third, and last count; and N1, N2, N3,…, Nn = number of days at first, second, third, and last count (Maguire, 1962) of number of seeds germinated. In the seedlings produced in the microcosms at the end of the experiment, morphological and physiological traits were measured as described below.

(1) *Morphological traits*: number of leaves, length of roots and shoot (cm), total biomass (shoot plus root; g); seed biomass.

(2) *Physiological traits*:

- 2.1 The content of chlorophyll a, chlorophyll b, and total chlorophyll of leaves was measured with a portable Clorofilog (Falker, Brazil). Chlorophyll was measured on the abaxial and adaxial surfaces of one leaf per plant. The chlorophyll index was converted to chlorophyll (μmol/mm^2) by the equations: total chlorophyll = 1.64 X Clorofilog index + 17.00, $R^2 = 0.88$; chlorophyll a = y = 0.81 X Clorofilog index—11.65, $R^2 = 0.72$; and chlorophyll b = 0.25 X Clorofilog index +1.38, $R^2 = 0.90$ (Lopes et al., 2015)
- 2.2 The maximal photochemical yield of PSII was measured in ten plants in each room. The 'current photochemical capacity' of PSII (Bolhár-Nordenkampf & Öquist, 1993) or 'intrinsic efficiency' (Maxwell & Johnson, 2000) was assessed with a Mini-PAM (Walz, Effeltrich, Germany). The maximal photochemical yield (Y = Fv/Fm) was measured in dark-adapted leaves (10 min), when a saturation pulse induces maximal fluorescence yield (Fm) and maximal variable fluorescence (Fv), which are considered reliable measures of the potential quantum yield of photosystem II. In dark-adapted leaves (all reaction centers of the electron transport chain oxidized), Fv/Fm is a parameter for measuring the maximum relative electron transport rate of PSII (Krause & Weis, 1991). Healthy leaves usually show Fv/Fm values between 0.66 and 0.80, while lower values indicate intracellular or physiological changes (calculated according to Bolhár-Nordenkampf & Götzl, 1992 and Waldhoff et al., 2000 for several common floodplain species).
- 2.3 Rapid light curves (RLC) in three plants in each treatment were additionally carried out. This measure shows the photosynthetic electron transport rate (ETR). Responses to short-term irradiance were measured using increasing irradiation in

nine steps within 3 min, with each irradiation period lasting 10 s.

Statistical analyses

ANCOVA was used to evaluate the effect of the treatments on morphological traits, with the time available for plant's growth as co-variable. ANOVA was used to evaluate the effect of the treatments on physiological traits and germination parameters. The differences between the factors were estimated a posteriori by the Tukey test. The non-parametric Kruskal–Wallis test was applied when at least one of the assumptions of ANOVA was not met, followed by of the Dunnett test. The statistical analyses were performed with Systat 12 (SYSTAT, 2007).

Results

During the experimental period, the climatic conditions in the microcosms varied in response to outside-room ambient changes. The CO_2 concentration varied between 350 and 500 ppm in the control treatment, 550–680 in the Mild treatment, 770–890 in the Intermediate treatment, and 1200–1330 in the Extreme treatment (Table 2). The temperature varied between 26 and 28°C in Control, 28–30°C in Mild, 29–31°C in Intermediate, and 31–33°C in Extreme treatments (Table 2). The humidity varied between 69 and 77% in all treatments during the experiment period (Table 2).

The elevation in CO_2 concentration together with increased temperature did not exert significant effects on any of the germination parameters analyzed (Table 3). The median time of germination tended to be shorter with increasing CO_2 and temperature in treatments, but differences were not significant ($F_{15\ 3} = 2.82$, $P = 0.075$). The maximum time for germination was 163 days in the control treatment, and the minimum was 44 days in the control, intermediate, and extreme treatments.

The seedling morphology did not change with CO_2 and temperature elevation. The number of leaves was similar between the treatments with averages ranging from 1.7 to 2.0 leaves. A higher number of leaves occurred in the control, 4 leaves, and the other treatments showed a maximum of 3 leaves, but this difference occurred in response to the time of growth of each individual. There was an effect on elongation of the roots in response to the elevation in CO_2 and temperature and the time of growth (Table 4; Fig. 1), although this effect was not reflected in root biomass (Table 4). There were no effects of the treatments on shoot length (Table 4), but there was an increase in shoot biomass with increase in CO_2 and temperature (Table 4). The root/shoot ratio did not change with treatments (Table 4). Total biomass was influenced by the treatments and time (Table 4; Fig. 2). The treatments with mild and intermediate conditions showed higher values of total biomass than did the control ($P < 0.05$), whereas the treatment with extreme conditions showed values similar to the control ($P > 0.05$).

The physiological parameters were affected by CO_2 and temperature. Fv/Fm was lower in the Extreme than Intermediate treatment (Table 4; Fig. 3); the time of growth did not influence this parameter. RLC of leaves in the control, Mild, and Intermediate treatments showed a maximum ETR of 6.3, 6.5, and

Table 2 Average values of microcosm climatic conditions per month in the experiment period

Month	CO_2 (ppm)				Temperature (°C)				Humidity (%)			
	1	2	3	4	1	2	3	4	1	2	3	4
Apr	507.26	682.94	888.83	1331.47	26.77	28.31	29.26	31.15	75.30	76.96	76.50	76.05
May	498.86	628.32	821.60	1272.03	26.84	28.76	29.36	31.19	75.47	79.05	76.51	76.73
Jun	437.03	596.12	807.00	1258.43	27.24	28.93	29.81	31.51	74.88	76.38	75.09	74.45
Jul	402.13	571.75	795.52	1227.45	27.42	28.97	30.05	31.55	73.67	74.43	74.28	72.95
Aug	371.14	567.96	798.18	1225.32	28.09	29.67	30.80	32.53	70.03	69.31	69.73	69.79
Sep	345.60	557.76	775.32	1205.49	28.35	29.93	30.97	32.75	71.78	70.27	69.37	70.47

Where 1 = control, 2 = mild; 3 = intermediate; 4: extreme treatments

Table 3 Index of germination (median) of *Montrichardia arborescens:* percentage of germination (G%), initial time of germination (ITG), median germination time (*t*), final time of germination (FTG), the germination speed index (GSI)

Treatment	G%	ITG (days)	*t* (days)	FTG (days)	GSI
Control	44.00	63.00	94.27	135.20	0.05
Mild	38.00	67.60	86.99	114.20	0.05
Intermediate	34.00	61.40	82.69	108.60	0.05
Extreme	42.00	58.40	72.67	105.40	0.06

5.0 µmol, respectively, at approximately 200 PPFD, and the Extreme treatment showed a maximum ETR of 4.5 at approximately 100 PPFD and slightly decreasing ETR at higher light intensities (Fig. 4). ETR was related to treatment (ANCOVA; $F = 6.16$, $P < 0.001$), with the Extreme treatment showing lower values than the other treatments.

The total chlorophyll measured on the abaxial and adaxial sides of the leaf did not show significant differences between the treatments ($P > 0.05$), as well as the ratio Chl *a*/Chl *P* ($P > 0.05$). The minimum value of total chlorophyll on the adaxial side was 19.9 µmol/mm^2 in the intermediate treatment, and the maximum was 42.7 µmol/mm^2 in the control (Table 5).

Discussion

Higher CO$_2$ concentrations and increased temperature influenced the initial growth of *M. arborescens* in our experiments. Plants exposed to the highest CO$_2$ concentrations and temperatures (extreme treatment) showed a lower accumulation of biomass and lesser electron transportation in the leaves compared to the Mild and Intermediate treatments.

The faster development of plants at warmer temperatures may result in substantial shortening of the growth period in some annual species. This results in less time for carbon fixation and biomass accumulation (Rawson, 1992; Wheeler et al., 1996; Bowes, 1996). Most studies of terrestrial plants have revealed that an increase in CO$_2$ level generally causes greater increases in growth at higher temperatures, beyond the optimal temperatures (Chen et al., 1994). In the present study, the exposure of *M. arborescens* to the Extreme treatment with the highest conditions of temperature and CO$_2$ showed no differences in germination parameters and total biomass when compared with the Control treatment. However, plants in the Mild and Intermediate treatments showed higher values of biomass compared to the control, indicating that the temperature and CO$_2$ concentration in the Extreme treatment were higher than optimal for *M. arborescens*.

Table 4 Results of the ANCOVA to effect of temperature and CO$_2$ elevation (variable) and growth time (co-variable) of *Montrichardia arborescens* seedlings

Source	*df*	*F*-ratio	*P* value
Roots elongation			
Treatment	3	5.081	0.003
Growth time	1	27.270	0.000
Error	63		
Roots biomass			
Treatment	3	2.005	0.122
Growth time	1	25.037	0.000
Error	68		
Shoot length			
Treatment	3	0.431	0.731
Growth time	1	20.956	0.000
Error	66		
Shoot biomass			
Treatment	3	3.390	0.023
Growth time	1	35.922	0.000
Error	65		
Root/shoot biomass			
Treatment	3	0.807	0.495
Growth time	1	6.259	0.015
Error	65		
Total biomass			
Treatment	3	3.496	0.020
Growth time	1	40.960	0.000
Error	66		
Fv/Fm			
Treatment	3	3.555	0.025
Growth time	1	0.025	0.876
Error	32	3.555	

Studies with aquatic macrophytes analyzing the effects of the interaction of elevated CO$_2$ and temperature are still scant. Idso et al. (1987) demonstrated that the relative increase in net photosynthesis of *Azolla pinnata* var. *pinnata* and *Eichhornia crassipes*

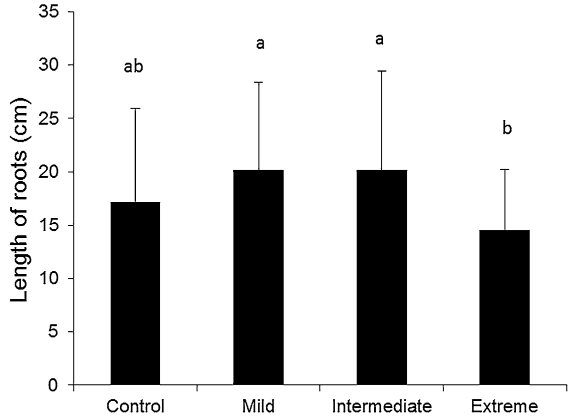

Fig. 1 Length of roots of *Montrichardia arborescens* in each treatment. Medians with standard deviation

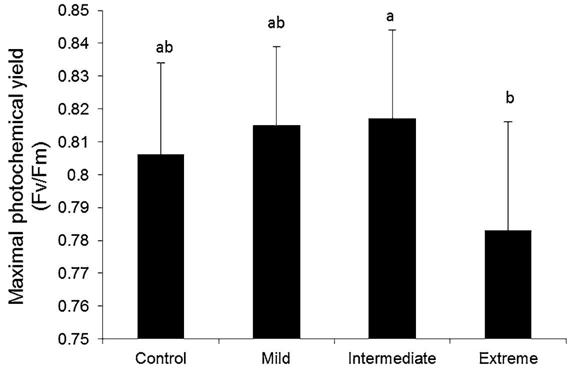

Fig. 3 The maximal photochemical yield (Fv/Fm) values of *Montrichardia arborescens* from each CO_2/temperature conditions. Medians with standard deviation

due to CO_2 enrichment was higher during the spring and fall than during winter. Allen et al. (1990) reported that there were significant interactive effects of CO_2 and temperature on photosynthesis in *Nymphaea marlic*. Chen et al. (1994) showed that the relative increase in *Hydrilla verticillata* growth by 700 ppm of CO_2 was less at 32°C than 15 and 25°C. In our study, *M. arborescens* showed similar results with a decline in total biomass at 31–33°C and around 1200 ppm of CO_2 compared to treatments with lower temperature and CO_2 concentration, indicating that the analyses of the scenario considering both CO_2 and temperature are more appropriate, since these effects in nature are combined. Indeed, Idso et al. (1987) and Chen et al. (1994) demonstrated that the effect of CO_2 enrichment is strongly temperature-dependent.

Working with seedlings of *Hymenaea courbaril*, L. Aidar et al. (2002) noted that when reserves were mobilized and growth occurred primarily at the expense of an internal source of carbon (cotyledons), the effect of elevated CO_2 in the external environment was significantly less. In *M. arborescens*, like in other monocotyledons, cotyledons are highly modified leaves with a scutellum specialized to absorb stored reserves from the endosperm, and a coleoptile, which serves as a protective cap that covers the part that will become the stem and leaves of the plant. This could

Fig. 2 Dry weight (shoot plus root) of *Montrichardia arborescens* in each treatment. Medians with standard error

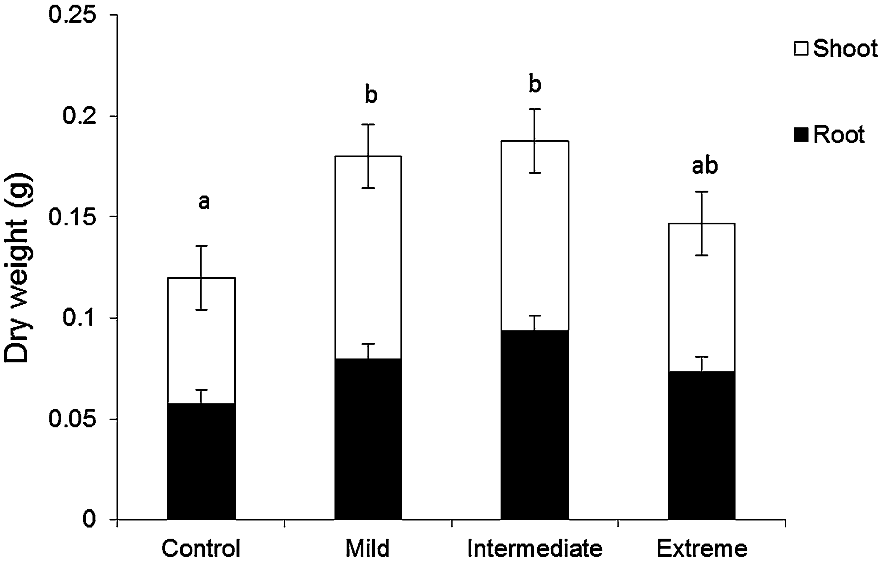

Fig. 4 Quadratic functions fitted to the data of rapid light curves measured in the leaves of *Montrichardia arborescens,* submitted to different temperatures and CO_2 concentrations. *ETR* electric transportation rate, *PPFD* photosynthetic photon flux density (μmol photons/m^2/second)

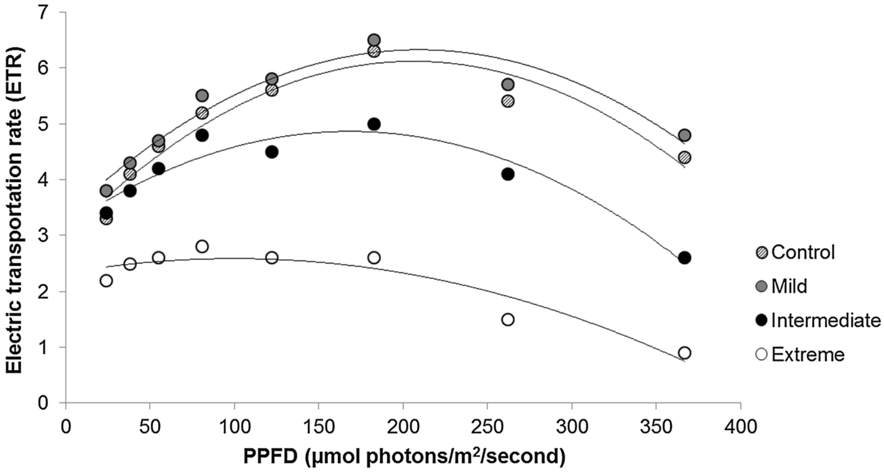

Table 5 Average values of chlorophyll (μmol/mm^2) at adaxial side of the leaf of *Montrichardia arborescens*

Treatment	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll
Control	24.7 ± 4.9	4.2 ± 1.3	28.9 ± 5.3
Mild	26.7 ± 5.0	4.7 ± 2.4	31.5 ± 7.2
Intermediate	23.7 ± 4.6	3.4 ± 1.0	27.2 ± 4.6
Extreme	24.5 ± 4.0	4.1 ± 1.2	28.6 ± 5.0

influence the effect of CO_2 on the growth of the species. Since the seed remained fixed to the seedlings in all plants germinated in the microcosms, this may eventually mitigate the effect of the external elevation of CO_2 level. Another explanation for the absence of effect of treatment on germination parameters could be the combined effect of CO_2 and temperature. The rise in temperature leads to an increase in respiration rate, affecting the germination capacity, since temperature accelerates biochemical reactions and metabolism of seeds (Perez-Garcia & Gonzalez-Benito, 2006). This accelerates the breakdown of reserves stored in the supporting tissues, which are more rapidly transported and assimilated in the embryonic axis. As a result, the increase in CO_2 concentration reduces metabolic activity and favors the conservation of seeds of tropical forest species and crops for longer periods (Khurana & Singh, 2001). This occurs because increasing CO_2 levels reduce metabolic activity and sharply lower the deterioration and aging of seeds, favoring the maintenance of quality during storage as stated for soybean seeds by several authors (Ainsworth et al., 2002; Schmidt, 2007). On the other hand, higher temperatures increase metabolic rates in early germination (Carvalho & Nakagawa, 2000).

In contrast to reports in the literature of higher CO_2 concentrations causing an increase in plant height in comparison to the control (Poorter, 1993), in the present study, *M. arborescens* did not show differences in this parameter between treatments with different CO_2 levels. Ojala et al. (2002) found a reduction in shoot length of the emergent macrophyte *Equisetum fluviatile* in response to CO_2 elevation, and an increase in response to temperature elevation, but a non-significant effect when evaluating the interaction between temperature and CO_2 concentration. Therefore, it is important to consider the joint effect of factors such as increased temperature and CO_2 enrichment on plant growth in future climate scenarios.

Effects of elevated CO_2 on carbon allocation between roots and shoots are variable (Bazzaz, 1990; Rogers et al., 1983), but the general conclusion of studies on root growth under elevated CO_2 concentration is that the root mass increases (Norby, 1994). As described for *E. fluviatile* during 5 months of observation (Ojala et al., 2002), in *M. arborescens*, only a discrete increase in root growth took place when CO_2 concentration was 600–800 ppm, coupled with elevated temperature.

The measurements of RLC in leaves exposed to different levels of CO_2 and temperature showed that the potential for photosynthetic activity of PSII decreased when the CO_2 concentration and temperature were very high, as occurred in the Extreme treatment. Relative ETR is an approximation of the rate of electrons pumped through the photosynthetic chain (Beer et al., 2001), and can provide a reliable assessment of photosynthetic activity, by integrating the leaf's ability to tolerate light fluctuation, as well as reflecting its immediate short-term light history (Schreiber et al., 1997; White & Critchley, 1999). Therefore, it can be assumed that the rates of *M. arborescens* photosynthesis were also reduced in the higher CO_2 and temperature treatments. Wang et al. (2012) concluded from a meta-analysis study that the increase in Fv/Fm of herbaceous and crop species with elevated CO_2 concentration only occurs at an elevated temperature, but no effect on woody and non-crop species was reported. The Fv/Fm values of *M. arborescens* were between 0.69 and 0.86, indicating that the leaves were healthy, using the standard values established for aquatic macrophytes (Koch et al., 2007). However, the Extreme treatment showed a significant reduction of approximately 3.0% in Fv/Fm values together with a reduction in ETR of about 60%, indicating that the photosynthetic apparatus may be damaged under such conditions. This tendency probably could be confirmed with increasing time of exposure of plants to Extreme treatment.

Although seedlings of *M. arborescens* responded by increasing the parameters evaluated when subjected to the Mild and Intermediate treatments (600–800 ppm CO_2), when in the Extreme treatment (1200 ppm), there was a decline in carbon incorporation. These results are consistent with observations by Hartz-Rubin & DeLucia (2001) who recorded an increase in biomass in 14 species of herbaceous plants enriched with CO_2 between 370 and 800 ppm. However, as found for wheat by Grotenhuis & Bugbee (1997) and also in *M. arborescens* in this study, when CO_2 enrichment was above 900 ppm, a decrease in plant biomass takes place. At higher CO_2 concentrations, the photosynthesis rate also decreases as demonstrated in cotton (Reddy et al., 1995) and rice (Baker et al., 1990). However, this response is in contrast to that of the emergent macrophyte *E. fluviatile*, in which maximum biomass was influenced by increase in temperature and not by higher CO_2 levels (600–700 ppm) or by increase in temperature together with CO_2. The different responses of *M. arborescens* and *E. fluviatile* may be explained by the morphology of these species. While *M. arborescens* is an arborescent plant, tall and with big leaves, *E. fluviatile* has slender side branches with scale leaves.

In the Amazon floodplain, with low nutrient levels, the quantity of floating plants and herbaceous shoreline vegetation is low (Piedade et al., 2010). In extremely nutrient-poor and acidic rivers, aquatic macrophytes may only be represented by small patchy stands of *M. arborescens* along the margins of the floodplain forest (Junk & Howard-Williams, 1984). Climate models predict that from the middle of this century on, the Amazon rainforest may give way to a *cerrado-type* vegetation (Oyama & Nobre, 1999; Jenkins et al., 2005) or a semi-deciduous forest (Malhi et al., 2009b). Under such conditions, the Amazonian wetlands may become a refuge for species of rain forests, minimizing the effects of decreased precipitations (Piedade et al., 2013). Our results with *M. arborescens* indicate that when CO_2 level and temperature increased in the range of 600–900 ppm and 28–30°C, plant biomass was stimulated; however, in the Extreme treatment (ca. 1200 ppm and 33°C), biomass values declined due to physiological limitations. Over time, this could lead to smaller populations of the species, which is one of the most abundant aquatic macrophytes in Amazonian floodplains, especially in the igapó system (Lopes et al., 2014). The composition of aquatic macrophytes in the black-water igapó is much lower than that in the white-water várzea counterpart (Piedade et al., 2010). Therefore, if other aquatic plants in the igapó would also decline in growth and biomass under climate change, severe reductions in the number of species and floristic composition of aquatic macrophytes may be expected in these areas. This may lead to an impoverishment of food chains and the carbon budget of this already fragile ecosystem.

Conclusions

No germination parameters of *M. arborescens* were affected by temperature and CO_2 concentration in a microcosm study. However, the initial growth of *M. arborescens* was influenced, resulting in a higher accumulation of biomass in the seedling subjected to CO_2 concentrations in the range of 600–900 ppm and temperatures between 28 and 30°C, but in the Extreme treatment, biomass accumulation was in the same range as that of the control, owing to physiological stress confirmed by Fv/Fm and ETR rates. Therefore, long-term investigations of interactions of CO_2 and temperature effects, like the proposed IPCC scenarios, are needed to clarify their effects on Amazonian aquatic macrophytes and the whole ecosystem.

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