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Explanatory variables associated with diversity and composition of aquatic macrophytes in a large subtropical river floodplain

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ABSTRACT

The structure of aquatic macrophyte assemblages can be affected by myriad factors, including physical, chemical and morphometric characteristics. We describe the patterns of plant species diversity and composition and vegetation height in aquatic ecosystems and their potential determinants at a regional scale in the Middle Paraná River floodplain. Sampling was conducted in twenty-three water bodies, including secondary channels, connected lakes and disconnected lakes. We compared the macrophyte species richness, composition and vegetation height in water bodies with different degrees of connectivity with the river and assessed the most important abiotic explanatory variables (morphometric, physical and chemical) associated with these assemblage attributes. Species accumulation curves showed that species richness did not differ between water bodies with different degrees of connectivity, although it tended to be lowest in secondary channels. Species richness was specifically associated with depth, conductivity, percent of solar radiation reaching the bottom and nitrate. Macrophyte assemblage composition was related primarily to the degree of connectivity, as rare species mostly occurred in disconnected lakes. Composition was also related to the degree of wind exposure (fetch), distance to shoreline and depth. Finally, vegetation height (a surrogate for biomass) was associated with the ammonium and sediment organic matter. Thus, a combination of morphometric and abiotic factors explained species richness, while degree of connectivity and morphometry explained assemblage composition. Vegetation height was explained by nutrients. These results suggest that a single set of variables is not sufficient to explain different aspects of macrophyte assemblages.

1. Introduction

Aquatic macrophytes comprise a variety of species belonging to different life forms that colonize almost all types of shallow waters (Sculthorpe, 1967). The richness and composition of macrophyte assemblages are affected by a myriad of factors, which include physical and chemical characteristics and morphometry of the water body (Murphy et al., 2003; Johnston and Brown, 2013; Kisson et al., 2013; Steffen et al., 2014; Azzella et al., 2014). At fine spatial scales, macrophytes are likely to be sensitive to the physical and chemical characteristics of the water and sediment, generating patterns which are also mirrored at broader scales (Bornette and Puijalon, 2011; Neiff et al., 2014). For example, while free-floating species usually thrive in water with high nutrient concentrations, submerged plants are mainly associated with light availability (Chambers and Kalff, 1985). Alternativelly, morphometric characteristics especialy affect macrophytes on broader spatial scales. Wave disturbance, measured indirectly by degree of wind exposure (fetch), for instance, can influence macrophytes by changing the rates of sedimentation and the resuspension process or by causing direct damage to these plants (Madsen et al., 2001; Zhang et al., 2014). The littoral slope is another important morphometric variable that influences the establishment of rooted macrophytes, determining the biomass of submerged plants (Duarte and Kalff, 1986) and affecting the dissipation pattern of wave energy and sediment dynamics (Azza et al., 2007).

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Particularly in river floodplain systems, patterns of richness and composition of macrophytes species are also affected by the degree of connectivity between the water body and river (Neiff, 1979; Junk et al., 1989; Monção et al., 2012; Rooney et al., 2013). In more connected water bodies floating species are usually removed by flushing and submerged species are commonly limited by increase in water column turbidity, both caused by disturbance events of strong floods (e.g., Madsen et al., 2001; Zhang et al., 2014). In contrast, macrophyte assemblages colonizing disconnected lakes, which are more stable, may be structured by biotic interactions, such as interspecific competition (Grime, 1974; Thomaz et al., 2007). As the degree of connectivity with the river regulates the magnitude of disturbance caused by floods (Monção et al., 2012; Rooney et al., 2013), intermediate disturbance hypothesis (Grime, 1974; Ward and Tockner, 2001) is quite probable to be supported by macrophytes assemblages colonizing these ecosystems.

Many studies using morphometric and limnological variables to explain macrophyte richness and composition have been conducted in rivers and lakes in temperate regions (e.g., Rooney et al., 2013). In large subtropical river-floodplain systems, several studies explored the effects of flood pulse on macrophytes (Thomaz et al., 2007; Neiff et al., 2011), while others tested the correlation between macrophyte assemblage attributes and the degree of connectivity with the river, lake morphometry, and physical and chemical factors (e.g., Murphy et al., 2003; Neiff and Poi de Neiff, 2003). However, morphometric and abiotic variables are usually not studied in combination. Therefore, it is necessary to test the combined importance of the degree of connectivity with the river, lake morphometry and water physic-chemistry for macrophyte assemblage attributes in large river-floodplain ecosystems, especially in Neotropical regions where these ecosystems are highly representative.

Along the Paraná River system, the middle course is the richest stretch in macrophyte diversity (Neiff et al., 2014). The middle Paraná course has been studied from different perspectives, and investigations related to the composition, the structure and successional dynamics have been conducted in this area (e.g., Neiff and Orellana, 1972; Neiff, 1975; Franceschi and Lewis, 1979; Lewis and Franceschi, 1979; Neiff, 1979; Sabattini et al., 1983). Nevertheless, despite having a high diversity of macrophytes, the morphometric and abiotic factors associated with macrophyte richness and composition are unknown in this part of the Paraná, as well as in other large subtropical rivers. Strong influences of physical and chemical factors on macrophyte assemblages are expected, especially during the dry season, when lakes and channels differ from one another with regards to morphometry and other physical and chemical features (Thomaz et al., 2007). Thus, the high environmental heterogeneity (in terms of abiotic factors) provided by the isolation of lakes during low water levels may be a key factor for the maintenance of high macrophyte diversity in river-floodplain systems.

Considering the assumptions of the intermediate disturbance hypothesis, we first tested the hypothesis that along a gradient of increasing connectivity, connected lakes (with intermediate disturbance) contain the highest species richness, followed by disconnected lakes and secondary channels (with lower and higher levels of disturbance, respectively). Second, we tested whether macrophyte composition differed between different degrees of connectivity. Finally, we used selected morphometric and abiotic environmental data to explore the likely determinants of macrophyte richness, composition and vegetation height. To reach our targets, we studied a wide variety of habitats within a large spatial scale (approximately 184 km^2) in the floodplain.

2. Methods

2.1. Study area

The Middle Paraná River (Fig. 1A–C) extends from its confluence with the Paraguay River (27°29′ S; 58°50′ W) to the city of Diamante (Argentina) (32°4′ S; 60°32′3″ W) and covers an area of 2,600,000–2,800,000 km^2 (Iriondo and Paira, 2007). Its main channel has a braided pattern with variable width (0.4–8 km) and several anabranches. Sediment deposition has almost filled the river valley, forming a complex floodplain with islands, bars, levees, secondary channels and shallow lakes (Paira and Drago, 2007). The dry season, when the water level is low and some lakes are isolated from one another (<10.5 m a.s.l.), usually occurs from July to December, while the flooding season (>13.2 m a.s.l) occurs between January and June (Drago, 2007). Due to its middle position along the river course, the Middle Paraná River serves as habitat for species common to both the Upper and the Lower Paraná River, establishing a continuum or biological corridor between them (Sabattini and Lallana, 2007).

2.2. Methods

Twenty-three water bodies (nine disconnected lakes, eight connected lakes and six secondary channels) distributed along the Middle Paraná River floodplain were sampled twice (from March to June 2012, and October 2012 to January 2013) during the dry seasons (Fig. 1C). We sampled during the dry season because, in general, individual habitats (see Thomaz et al., 2007) and macrophyte assemblages (Padial et al., 2009; Neiff et al., 2014) within a floodplain differ the most during this period (however, see Mayora et al. (2013)). Thus, a gradient of habitats with distinct connectivity to the main river could be used to assess the association of environmental variables and macrophyte assemblage attributes.

The macrophytes were sampled using quadrats (1 m × 1 m) that were placed along transects perpendicular to the shoreline. The number of transects in each lake ranged from one to five, according to the total area of the water body. Within each transect, the distance between successive quadrats was constant, and the number of quadrats per transect varied from one to sixteen according to the length of the macrophyte stand (3–50 m from the shoreline toward the limnetic zone). This procedure takes into account habitat area and the size of the macrophyte stands, ensuring a representative inventory of aquatic flora. In addition, this procedure accounts for the zonation along depth gradients and the variation of spatial distribution in the margins, allowing the investigation of associations between macrophyte assemblage attributes and environmental variables.

Inside each 1 m × 1 m quadrat, macrophyte species were visually scored for percent cover to provide an estimate of abundance. Rakes were used to sample the submersed macrophytes. We also measured the total height of the vegetation (a proxy for biomass) in each quadrat. The collected material was transported to the Instituto Nacional de Limnología (INALI-UNL-CONICET), and the plants were identified according to Cabrera (1968), Pott and Pott (2000) and others (complete list in Appendix S1). Despite some taxa were not identified to species level, for the sake of simplicity, we used the term species to represent taxonomic units.

Environmental parameters were characterized at different scales encompassing physical and chemical variables and morphometric features. In each transect, we measured the conductivity and pH with a portable water checker (Hanna®). The Secchi depth was determined in each transect and was used to estimate the light extinction coefficient (k) according to Padial and Thomaz (2008). We used k and the depth of the water column to calculate the mean

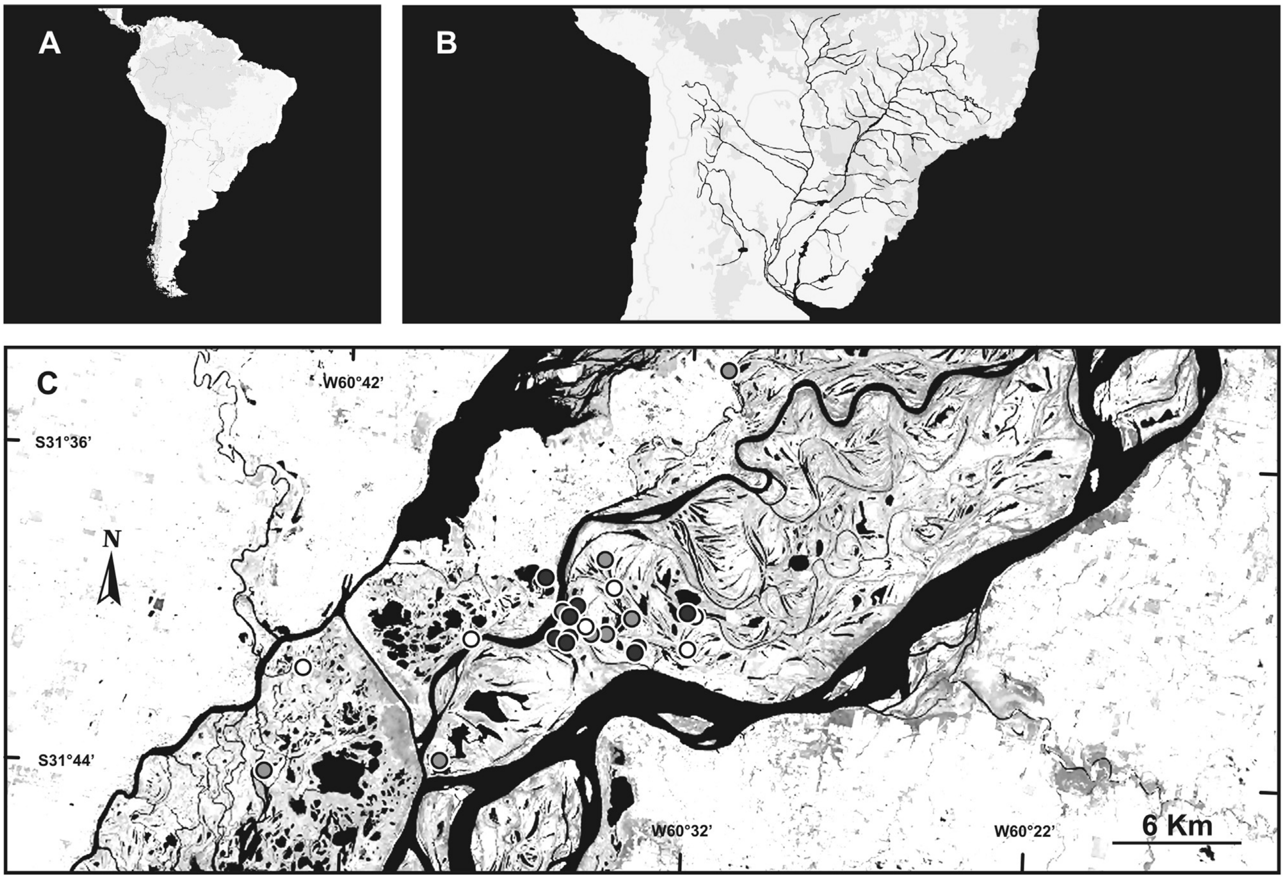

Fig. 1. The map of South America (A) highlighting the drainage basin and the main tributaries of the Paraná River (B), in addition to the sites where surveys were conducted (C). The locations of the disconnected lakes (dark gray), connected lakes (light gray) and secondary channels (white) are indicated by circles.

percent (%) light reaching the bottom, which represents the amount of light available for plants growing from the sediment. Mean distance to shoreline was measured in each quadrat as an indication of stand length. The littoral slope was calculated using depth measurements and distances between quadrats along each transect. The fetch, a surrogate for wave disturbance, was also calculated for each transect. To obtain the fetch we measured the maximum distance of open water in a straight line from the macrophyte stand to the furthest point to the shoreline or an island. In each water body, we determined the total phosphorus, nitrate and ammonium (using HACH, Greenberg et al., 1985). For this purpose, we sampled water superficially from a point within the macrophyte stand, but near the open water or the limnetic region of the water body. A grab sample of sediment was collected from within the macrophyte stand in an area close to the shoreline, and the organic matter content of the sediment was determined using ignition in a muffle furnace at 500 °C for 3 h.

2.3. Data analysis

To compare species richness between the disconnected lakes, connected lakes and channels, we used species accumulation curves and rarefaction procedure to standardize sampling effort between the ecosystem types. We used the presence and absence of macrophyte species from each quadrat to generate the curves for each ecosystem type. Comparisons between the curves were conducted on the basis of a standardized sampling effort, and we used 95% confidence intervals to evaluate differences in species richness. We used these same data to estimate the true species richness through nonparametric estimators (Chao 2, ICE, Jackknife 1 and Jackknife 2). Smooth curves of species richness estimators accumulated over samples were obtained after 999 randomisations without replacement, and the performance of richness estimators was evaluated after analysing the precision of estimation (95% confidence intervals) and stabilization of curves (Magurran, 2005). The rarefaction curves and estimators of species richness were calculated using EstimateS 9.10 (Colwell, 2013) and SPADE (Chao and Shen, 2009).

To investigate the possible effects of environmental variables on species richness and composition, we pooled the data from quadrats after re-scaling our sampling units to water bodies. Explanatory variables were averaged when necessary to adjust for the scale of response variables. This method was used to avoid potential pseudo-replication (e.g., dependency of quadrats within transects) while maintaining valid relationships between the response and explanatory variables.

To identify the most important explanatory variables associated with macrophyte richness and vegetation height, we used the Multimodel Inference of Akaike Information Criterion (AIC_c; corrected for a small number of samples) based on the all-subsets approach (Burnham and Anderson, 2002). This approach consists of constructing all possible linear models using different combinations of explanatory variables. For each model, the AIC_c was obtained and then the Akaike weights were calculated, which indicate the probability of each model to be the best model of the set (Burnham and Anderson, 2002; Johnson and Omland, 2004). Inferences were made using Multimodel Inference (Burnham and Anderson, 2002) by analysing the relative importance of the variables and the standardized regression coefficients of the weight-averaged model, both obtained from the total set of models using the Akaike weights. The 95% confidence intervals were used to assess the statistical effects of the variables. Explanatory variables considered in this analysis were conductivity, nitrate, ammonium, total phosphorus, sediment organic matter, proportion of radiation reaching the bottom, pH (chemical and physical variables), distance to the shoreline, depth, littoral slope, fetch (morphometric variables) and degree of connectivity with the river. The degree of connectivity was expressed as 0, 1 or 2, indicating secondary channels, connected lakes and disconnected lakes, respectively. Proportion of radiation reaching the bottom was not included in the vegetation height model because only plants above the water surface were used for this response variable. Some variables (distance to the shoreline, fetch, total phosphorus, nitrate, slope, depth and degree of connectivity) were log $(x+1)$ transformed to improve the assumption of normality and linearity before analysis. Differences in sampling effort were controlled by including in every model the number of quadrats pooled when re-scaling the sampling units, which might allow estimating the pure effects of explanatory variables (e.g., Mitchell et al., 2010). Multimodel Inferences of AIC_c were performed using Spatial Analysis in Macroecology (SAM v4.0; Rangel et al., 2010).

To assess whether the species composition differed among the channels, connected lakes and disconnected lakes, we employed a permutational multivariate analysis of variance (PERMANOVA). This analysis was performed using the Jaccard dissimilarity based on the presence and absence data of the macrophyte species. The type III sums of squares were used to account for the unbalanced statistical design (Quinn and Keough, 2002), and a total of 9999 permutations were used to assess the significance of the *pseudo*-F statistic derived from the PERMANOVA. Significant differences between particular groups were investigated with a posteriori pairwise comparisons using *pseudo*-t statistic also derived from the PERMANOVA (Anderson, 2001). Effects of sampling effort were removed in these analyses by including the number of pooled quadrats as a covariate when running tests. PERMANOVAs were performed in PRIMER 6.1.13, PERMANOVA + 1.0.3 add on (Anderson et al., 2008; Clarke and Gorley, 2006).

To identify the subset of environmental variables that best correlated with the composition of the macrophyte species, we used the *BioEnv* routine (Clark and Ainsworth, 1993). This routine correlates the dissimilarity matrix of the species composition (Jaccard dissimilarity on the presence and absence data) with each possible dissimilarity matrix combining different environmental variables (based on Euclidean distances). The subset of environmental variables that best explained the composition of the macrophyte species was the one with the highest correlation (denoted by standardized Mantel statistic (r_M)). To assist interpreting the results obtained by the *BioEnv* routine, we reported correlations between the dissimilarity matrix of the macrophyte species and the dissimilarity matrices based on single environmental variables. For the same purpose, cumulative correlations considering the variables indicated by the *BioEnv* routine were also reported. For these analyses, the effect of sampling effort was removed by partialling out a matrix of Euclidean dissimilarity of the number of pooled quadrats. The significance of the standardized Mantel statistics was assessed using 99999 permutations. The same environmental variables were used and log $(x+1)$ transformed as those used in the Multimodel inference of the AIC_C. The *BioEnv* and Mantel tests were performed in R using the vegan package (Oksanen et al., 2013).

Finally, to visually inspect the multivariate patterns of species composition, we used Canonical Analysis of Principal Coordinates (CAP; Anderson and Willis, 2003). For this analysis, an ordination of sampling sites was produced using Jaccard dissimilarity of species presence and absence data, constrained by the environmental variables contained in the best subset indicated by *BioEnv*. The effect of sampling effort was removed by partialling out the effect of the number of pooled quadrats before constraints. Canonical Analysis of Principal Coordinates was performed in R using the vegan package (Oksanen et al., 2013).

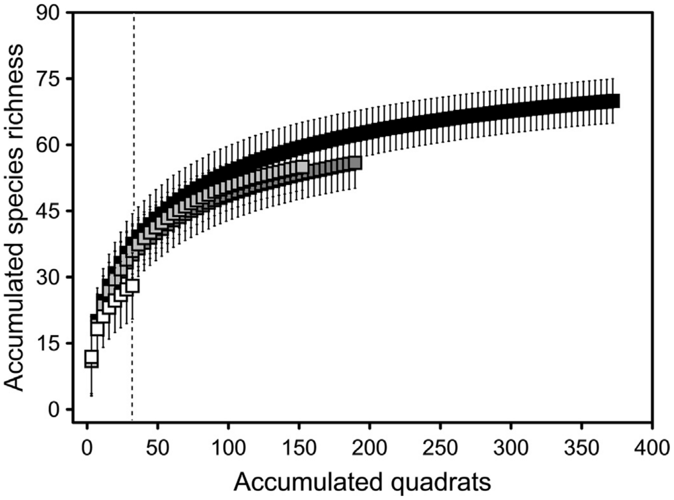

Fig. 2. The macrophyte species accumulation curves with 95% confidence intervals of total water bodies (black), disconnected lakes (dark gray), connected lakes (light gray) and secondary channels (white). Details of the comparisons between total samples, disconnected lakes, connected lakes and secondary channels on the basis of a standardized sampling effort and estimates of true species richness are given in Table 1.

3. Results

The species richness in each water body ranged between 4 in a secondary channel and in a disconnected lake and 23 in another disconnected lake. According to the relative frequency of occurrence, the most frequent species were *Salvinia* spp. (0.710 ± 0.050; mean ± standard error) and *Ludwigia peploides* (Kunth) P. H. Raven (0.668 ± 0.050), while between the rarest were *Cyperus virens* Michx., *Eleocharis* sp., *Polygonum hydropiperoides* Michx., *Polygonum lapathifolium* L. and *Solanum glaucophyllum* Desf. (0.003 ± 0.003 each species) (Fig. S1). Considering the candidate variables used to explain variation in macrophyte assemblages, our results indicated a broad gradient of morphometric, physical and chemical characteristics occurring among disconnected lakes, connected lakes and secondary channels (Fig. S2).

The species accumulation curves tended to reach an asymptote for the total samples in the disconnected and connected lakes, indicating that our sampling effort was sufficient to survey most of the species in the region (Fig. 2). In general, species richness estimators performed well (except Chao2 for secondary channels, which clearly presented problems of precision and stabilization – see Fig. S3) and provided very similar values among different indexes indicating that estimates of number of species were consistent (Table 1; Fig. S3). In accordance with the accumulation curves, all estimators produced values that were similar to the observed species richness (Table 1), indicating that the estimated and observed species richness were close. Rarefied species richness was lowest in secondary channels and highest in connected lakes, followed by disconnected lakes, a pattern which was also consistent among estimators (Table 1). Despite this trend, no significant differences were found when richness was compared across species accumulation curves using rarefaction or across most of species richness estimators, as evidenced by overlapping confidence intervals (except for Jackknife 1; Table 1). Thus, these results conservatively indicate that species richness did not significantly differ between different degrees of connectivity (Fig. 2; Table 1).

Table 1
Number of quadrats, species recorded and rarefied species richness for macrophytes sampled in all water bodies and specifically in disconnected lakes, connected lakes and secondary channels. Upper and lower boundaries of 95% confidence intervals are given within brackets.

Ecosystem type	Number of quadrats	Species richness		Species richness estimators			
		Number of species recorded	Rarefied species richness	ICE	Chao 2	Jackknife 1	Jackknife 2
Total	400	67.0 (62.2–71.9)	36.1 (30.1–42.1)	72.1 (68.5–84.4)	72.1 (68.1–90.4)	76.0 (70.7–88.6)	79.0 (71.0–103.1)
Disconnected lakes	208	54.0 (49.5–58.5)	34.8 (29.4–40.2)	60.1 (55.8–74.6)	58.5 (55.0–74.6)	63.0 (57.7–75.6)	65.0 (57.3–89.9)
Connected lakes	156	54.0 (48.8–59.3)	36.6 (31.1–42.1)	60.7 (56.0–75.8)	60.4 (55.5–81.1)	63.9 (58.3–77.0)	67.9 (59.1–92.2)
Secondary channels	36	28.0 (20.5–35.5)	28.0 (20.5–35.5)	35.9 (30.1–56.8)	59.1 (32.7–233.8)	35.8 (31.1–47.7)	42.4 (34.1–62.2)

Considering the explanatory variables we investigated to explore the likely determinants of macrophyte richness, a total of 4095 possible models were generated (Table S1). Multimodel Inference of the AIC_C showed that the weight-averaged model explained 28% of the total variance ($R^2_{adj} = 0.28$). The most important variables were the depth, conductivity, nitrate, and the proportion of radiation reaching the bottom. All of these variables (except nitrate) had positive associations with richness, according to the weight-averaged model (depth: $\beta = 0.604$ (95% CI = 0.026–1.182); conductivity: $\beta = 0.266$ (0.091–0.441); nitrate: $\beta = -0.237$ (−0.382 to −0.092); proportion of radiation reaching the bottom: $\beta = 0.528$ (0.097–0.960); Fig. 3A and B). The slope, phosphorus and fetch were also associated with species richness; however, these variables were not considered for interpretation due to their low contribution according to the weight-averaged model (absolute values of $\beta \leq 0.147$).

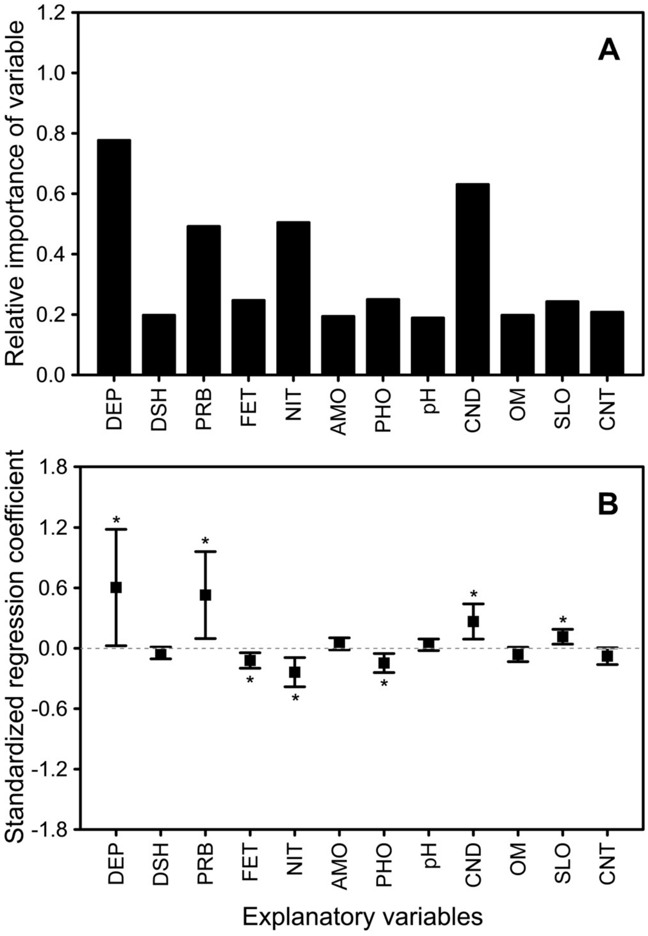

Fig. 3. The Multimodel Inference of Akaike Information Criterion (AIC*c*) used to explain species richness, demonstrating the relative importance of the variables (A) and weight-averaged model (B). Explanatory variables considered were depth (DEP); distance to the shoreline (DSH); proportion of radiation reaching the bottom (PRB); fetch (FET); nitrate (NIT); ammonium (AMO); total phosphorus (PHO); conductivity (CON); organic matter content of the sediment (OM); slope (SLO); degree of connectivity of water bodies (CNT). All models included number of pooled quadrats to remove differences in sampling effort ($\beta = 0.604$ (95% CI = 0.026–1.182)). Asterisks indicate positive or negative effects with 95% confidence intervals not including zero.

In regard to all possible combination of variables used to explore the likely determinants of the vegetation height, a total of 2047 possible models were generated (Table S2). The weight-averaged model obtained by the Multimodel Inference of the AIC_C explained only 5% of the total variance. Despite of the weak explanatory power of the weight-averaged model, the most important variables were ammonium and sediment organic matter; the former had a positive association with the vegetation height ($\beta = 0.391$ (0.121–0.660)), whereas the latter had a negative association with the height ($\beta = -0.473$ (−0.121 to −0.660)); Fig. 4A and B). The fetch, distance to the shoreline, phosphorus and conductivity were also associated with height; however, these variables were not considered for interpretation due to their low contribution according to the weight-averaged model (absolute values of $\beta \leq 0.163$).

We found significant differences in the PERMANOVA analysis (*pseudo-F* = 2.45; $p < 0.001$), indicating that the composition of the macrophyte species differed between the degrees of connectivity. Pairwise comparisons indicated that disconnected lakes were significantly different from connected lakes (*pseudo-t* = 1.86; $p < 0.001$) and from secondary channels (*pseudo-t* = 1.42; $p = 0.010$). However, the composition of the macrophyte species did not differ between secondary channels and connected lakes (*pseudo-t* = 0.97; $p = 0.520$). Exploratory analyses of the data showed that rare species (more frequent in the disconnected lakes) were primarily responsible for the differences between the degrees of connectivity (Fig. S1).

Among the environmental variables explaining the composition of the macrophyte species, the subset including degree of connectivity, fetch, distance from shoreline and depth presented the strongest correlation ($r_M = 0.31$), as evidenced by *BioEnv*. In this subset, the degree of connectivity was the variable with the strongest individual correlation, followed by fetch, distance to the shoreline and depth (Table 2). Patterns of species composition constrained by these explanatory variables are also evidenced by the biplot of the Canonical Analysis of Principal Coordinates (Fig. 5). This finding indicates that the degree of connection, in conjunction with the degree of wind exposure and diversity of microhabitat (in terms of distance to shoreline and depth), are the primary determinants of the structure of the macrophyte assemblage.

4. Discussion

Our study builds on earlier works assessing the relationships between aquatic macrophytes and environmental variables (Kisson et al., 2013; Murphy et al., 2003; Neiff et al., 2014; Rooney et al., 2013; Steffen et al., 2014), and it indicates the extent to which macrophyte richness and composition are related to

Table 2
Standardized Mantel statistics (r_M) for the correlation between the dissimilarity matrix of macrophyte species composition and dissimilarity matrices based on individual environmental variables. Cumulative r_M for variables composing the best subset of environmental variables indicated by *BioEnv* is also shown. Sampling effort was controlled by partialling out the dissimilarity matrices based on number of pooled quadrats. Numbers in brackets indicate the order which variables were accumulated. Abbreviations refer to depth (DEP), distance to the shoreline (DSH), proportion of radiation reaching the bottom (PRB), fetch (FET), nitrate (NIT), ammonium (AMO), total phosphorus (PHO), conductivity (CND), organic matter content of the sediment (OM), slope (SLO), and degrees of connectivity (CNT). Bold values indicate significant correlations ($\alpha = 0.05$).

Explanatory variable	Standardized Mantel statistics for individual variables		Standardized Mantel statistics accumulated for variables indicated by *BioEnv*	
	r_M	p	r_M	p
DEP	0.09	0.189	**(4) 0.31**	**<0.001**
DSH	0.10	0.157	**(3) 0.31**	**<0.001**
PRB	0.02	0.404		
FET	**0.23**	**0.007**	**(2) 0.30**	**<0.001**
NIT	−0.02	0.556		
AMO	0.04	0.309		
PHO	0.07	0.228		
pH	0.08	0.208		
CND	0.03	0.346		
OM	0.00	0.467		
SLO	−0.10	0. 813		
CNT	**0.23**	**<0.001**	**(1) 0.23**	**<0.001**

morphometric, physical and chemical characteristics of water bodies and their degree of connectivity with the river. Our study differs from these earlier works in two main ways. Firstly, although the Middle Paraná is considered the richest stretch in macrophyte diversity (Neiff et al., 2014), the correlation of macrophyte assemblage attributes and a combination of the degree of connectivity with the river, morphometric and abiotic factors was previously unknown in the water bodies of this stretch of the Paraná River. Indeed, we showed that different combinations of variables are necessary to explain different assemblage attributes. Morphometry and abiotic variables explained macrophyte species richness and morphometry and degree of connectivity explained macrophyte composition, but nutrients alone explained plant height. Secondly, our investigation showed the importance of the degree of wind exposure, which has been considered in a few earlier works in large tropical ecosystems (Barbosa et al., 2014) but has been previously overlooked in studies of smaller water bodies. We highlight that these two conclusions were the result

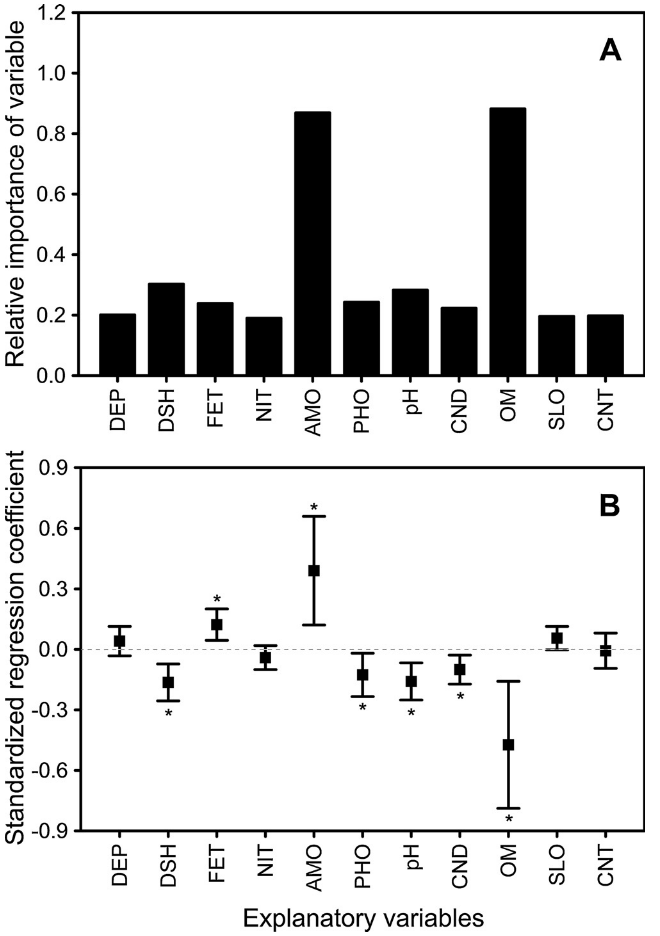

Fig. 4. The Multimodel Inference of Akaike Information Criterion (AIC_c) used to explain the macrophyte height, demonstrating the relative importance of the variables (A) and the weight-averaged model (B). Explanatory variables considered were depth (DEP); distance to the shoreline (DSH); fetch (FET); nitrate (NIT); ammonium (AMO); total phosphorus (PHO); conductivity (CON); organic matter content of the sediment (OM); slope (SLO); degree of connectivity of water bodies (CNT). All models included number of pooled quadrats to remove differences in sampling effort ($\beta = 0.187$ (95% CI = −0.191 to 0.565)). Asterisks indicate positive or negative effects with 95% confidence intervals not including zero.

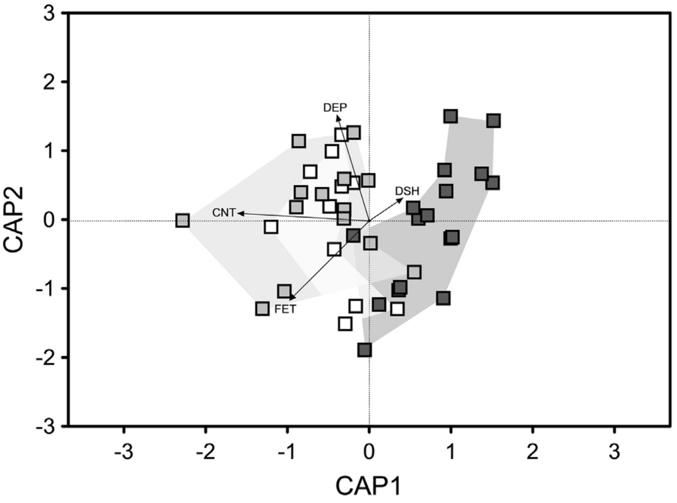

Fig. 5. Canonical Analysis of Principal Coordinates (CAPSCALE) plot of the macrophyte assemblages sampled in disconnected lakes (dark gray), connected lakes (light gray) and secondary channels (white). CAP1 and CAP2 accounted for 50.09% and 23.31% of the constrained variance, respectively. Arrows indicate the direction and relative importance of environmental variables. Abbreviations refer to degrees of connectivity (CNT), depth (DEP), fetch (FET) and distance to the shoreline (DSH).

of an investigation of a multitude of different aquatic ecosystems distributed over a large spatial scale.

In regard to species richness, our findings demonstrate no difference between the degrees of connectivity, in spite of the tendency of secondary channels to have a smaller number of species. In addition, it is highly likely that the number of species recorded in this study provides a reasonable representation of the real richness of both the floodplain (gamma diversity) and the local richness of the lakes (alpha diversity). This is suggested by the overlap of the confidence intervals of observed species richness and those of estimated species richness. Nevertheless, the upper limits of the confidence intervals suggest that additional samples could allow for new species to be recorded.

In contrast to the results from other wetlands in which the number of species was affected by the degree of connectivity (Monção et al., 2012; Thomaz et al., 2007), our findings indicate that there was no difference in the species richness with respect to the connectivity. Our results therefore do not support our hypothesis related to the intermediate disturbance. However, the number of species in the secondary channels showed a tendency to be lower than that of the lakes, and this finding has been associated with a higher water speed (Sabattini and Lallana, 2007). In channels, current represents a stress factor that impedes the establishment of macrophytes and allows the development only of those plants adapted to live in flowing habitats. Indeed, mechanical factors, such as current velocity, are the dominant factors structuring macrophyte composition (Steffen et al., 2014). Thus, plants that are highly flexible and have more resistant tissues generally occur in flowing environments (Monção et al., 2012). Therefore, the high species richness in lakes may be due to the potential of plant establishment and the possibility of the development of macrophyte patches due to the absence of flow in these water bodies.

In this study, we employed an observational approach and therefore can only make inferences about the influence of the selected variables on macrophyte richness, height and composition. However, we are able to speculate about the potential explanatory mechanisms for our main results by using the findings of other investigations. In particular, with regard to variables that are likely to affect species richness, we found the strongest correlations for depth, percent radiation reaching the bottom, conductivity and nitrate. Thus, it seems that the morphometry, along with some physical and chemical factors, are important explanatory variables of macrophyte richness and that combinations of different processes may generate patters of macrophyte richness in floodplain habitats.

For example, depth may have been important to explain the macrophyte richness because macrophytes are commonly organized along depth gradients in the littoral zone of water bodies (Santos and Thomaz, 2007). In general, shallower areas are dominated by rooted emergent species; however, as the depth of the littoral zone increases, other life forms such as floating species, epiphytes or submerged species appear among the emergent plants. Given the sloping nature of the littoral zones, an increasing depth should enable a greater variety of life forms as species are replaced along depth gradients (Santos and Thomaz, 2007), resulting in higher species richness. Indeed, depth has been considered an important variable explaining the macrophyte assemblage structure in other habitats in the Middle Paraná River floodplain (Sabattini and Lallana, 2007) and in other freshwater ecosystems (Azzella et al., 2014).

The percent light reaching the bottom can also drive the structure of macrophyte assemblages, especially by its effects on submerged species. Percent of light reaching the bottom can restrict macrophyte richness by affecting the germination of seeds from the sediment (Kettenring et al., 2006) and by impeding the establishment of propagules through a reduction in photosynthetic activity. Nevertheless, different species of macrophytes may have specific light requirements (e.g., Schwarza et al., 2002), and the positive association of the percent light reaching the bottom and macrophyte species richness may be the result of improving the likelihood for different species to establish and coexist. The same positive effect of light availability at the surface of the sediment on species richness has also been recorded in the Upper Paraná River (e.g., Sousa et al., 2011), suggesting the importance of this variable.

Among chemical factors, conductivity is importantly related to the richness of aquatic plants (Murphy et al., 2003). Nevertheless, and in agreement with other studies (e.g., Lacoul and Freedman, 2006), the influence of conductivity on richness may instead be related to its indirect relationship with nutrient availability and alkalinity, making it difficult to determine a direct influence of this variable on the richness. However, with specific regard to other nutrients, nitrate commonly affects macrophyte growth (e.g., Wersal and Madsen, 2011), which may suggest at least two emerging mechanisms to explain the negative association with species richness. The first one considers that some species may be more efficient than others with regard to biomass accumulation. These fast-growing macrophytes may develop densely, increasing nitrate uptake, and in turns, may outcompete and exclude other coexisting species (James et al., 2005; Weisner and Thiere, 2010). The second considers an increase in nitrate uptake with species richness, suggesting that some processes of ecosystem function could be driven by biodiversity, as predicted by niche complementarily. Indeed, as diversity of plant species increases, productivity is also expected to increase due to complementary interactions among species and their resource use (Tilman et al., 2001).

Vegetation height, which was used as a proxy for macrophyte biomass, was associated with the chemical factors (i.e., ammonium and organic matter content of the sediment) that represented resource availability. For example, ammonium was the most important variable explaining the vegetation height, and its positive association with vegetation height may indicate that N is the limiting nutrient for plant growth. Indeed, experiments carried out in lentic habitats of the Middle and Lower Paraná basin showed that nitrogen is more limiting than phosphorus for plant growth (Carignan and Neiff, 1992; Bonetto et al., 1994). Thus, habitats with higher concentrations of nitrogen should promote growth and contain taller plants. In contrast to ammonium, we found a negative association between vegetation height and organic matter. The organic matter of the sediment has been shown to have a positive effect on plant growth, most likely due to liberation of nitrogen and/or phosphorus during mineralization (Sand-Jensen and Sondergaard, 1979). However, high quantities of organic matter consume a large amount of oxygen from sediment and release phytotoxins, which have negative effects on the growth of both emergent and submerged macrophytes (Barko and Smart, 1983; Wu et al., 2009). Therefore, this process is more likely to support our results.

With regard to composition of macrophytes species, the assemblages we sampled were, in general, quite similar to the ones recorded in other lakes of the Middle Paraná (Sabattini and Lallana, 2007). Particularly with regard to differences in the degree of connectivity with the river, we found that the occurrence of some rare species was enough to distinguish disconnected lakes from connected lakes and secondary channels. In general, disconnected lakes tend to differ from water bodies with greater degrees of connectivity because they are less exposed to the disturbance and homogenization effects of floods (Thomaz et al., 2007). Consequently, this type of water body is most likely subjected to local driving forces that act with different intensities, creating more stable habitats with distinct characteristics (Thomaz et al., 2007). Given that some species need specific types of habitats to succeed and that many rare species are specialists and require rare resources (Spitale, 2012), disconnected lakes could contribute favorably to the establishment of these species. This idea is in accordance with our finding that rare species were more likely to be found in disconnected lakes and may explain the importance of the degree of connectivity influencing composition of macrophyte species (Table S2; Fig. 3).

Other than the degree of connectivity with the river, the most important abiotic variables associated with macrophyte assemblage composition were the distance to the shoreline, depth and degree of wind exposure. The distance to the shoreline is an indicator of stand length, which represents the degree of development of the macrophytes patch. Therefore, the farther the distance from the shoreline, the more probable it is that the macrophyte stand is in an advanced successional stage and, consequently, presents a different composition of macrophyte species (e.g., Santos and Thomaz, 2008). With regard to depth, changes in species composition are quite likely explained by the same processes affecting species richness. Therefore, the influences of distance to the shoreline and depth in macrophyte species composition may result from species replacement due to successional process and zonation along depth gradients.

Finally, the importance of wind exposure for plant communities in tropical regions has been demonstrated in large man-made lakes where the wave exposure negatively affects macrophytes (Barbosa et al., 2014). However, our results highlight that even in small lakes, similar to the ones we investigated, this morphometric variable may be an important determinant of the macrophyte assemblage composition. Habitats where the degree of wind exposure is larger should favor macrophyte species that are more robust against mechanical injury and are more strongly rooted, given that free-floating and weakly anchored species may be carried away. Thus, as the amount of wind exposure disturbance increases, rooted emergent species and rooted species with floating leaves should dominate. Indeed, we found that the rooted *Nymphoides indica* (L.) Kuntze and *Paspalum repens* Bergius dominated habitats with greater amounts of wind exposure.

5. Conclusion

Our results showed that a simple set of variables was not sufficient to explain macrophyte assemblages in a large (relatively) pristine subtropical floodplain. Indeed, a combination of factors including morphometry, physical and chemical variables, and the degree of connectivity with the river, are important explanatory variables of richness and community composition. We also show the importance of each set of factors varies according to the response variable: a combination of morphometry, nutrients and light best explained macrophyte richness; morphometry and degree of connectivity explained macrophyte composition; and macrophyte height was explained mainly by nutrients. We also found that species richness does not differ between the degrees of connectivity, and thus, our hypothesis about intermediate disturbance was not supported. However, the main difference in the macrophyte composition between environments was the presence of rare species mainly in disconnected lakes. The possibility that such species depend exclusively on certain conditions in these lakes (e.g., the distance to the shoreline, wind disturbance and ammonium in the water column) suggests that the disconnected lakes of the Middle Paraná River floodplain should be considered important areas for assessing biodiversity conservation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aquabot.2014.11.003.

References

Anderson, M.J., Willis, T.J., 2003. Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. Ecology 84, 511–525.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46.

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. Permanova+ for PRIMER. Primer-E, Plymouth, UK.

Azza, N., van de Koopel, J., Denny, P., Kansiime, F., 2007. Shoreline vegetation distribution in relation to wave exposure and bay characteristics in a tropical great lake, Lake Victoria. J. Trop. Ecol. 23, 353–360.

Azzella, M.M., Bolpagni, R., Oggioni, A., 2014. A preliminary evaluation of lake morphometric traits influence on the maximum colonization depth of aquatic plants. J. Limnol. 73, 400–406.

Barbosa, M.V.M., Kobayashi, J.T., Pelicice, F.M., 2014. Morphometric and biotic variables as potential predictors of *Ludwigia sedoides* (Humb. and Bonpl.) Hara in a large Amazonian reservoir. Ann. Limnol. Int. J. Lim. 50, 163–171.

Barko, J.W., Smart, R.M., 1983. Effects of organic matter additions to sediment on the growth of aquatic plants. J. Ecol. 71, 161–175.

Bonetto, C., Cabo, L., Gabellone, N., Vinocur, A., Donadelli, J., Unrein, F., 1994. Nutrients dynamics in the deltaic floodplain of the Lower Paraná River. Arch. Hydrobiol. 131, 277–295.

Bornette, G., Puijalon, S., 2011. Response of aquatic plants to abiotic factors: a review. Aquat. Sci. 73, 1–14.

Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multimodel Inference: A Practical Information – Theoretic Approach, 2nd ed. Springer, pp. 515.

Cabrera, A.L., 1968. Flora de la Provincia de Buenos Aires. Colección Científica INTA, Buenos Aires.

Carignan, R., Neiff, J.J., 1992. Nutrient dynamics in the floodplain ponds of the Paraná River (Argentina) dominated by the water hyacinth *Eichhornia crassipes*. Biogeochemistry 17, 85–121.

Chambers, P.A., Kalff, J., 1985. Depth distribution and biomass of submersed aquatic macrophyte communities in relation to Secchi depth. Can. J. Fish. Aquat. Sci. 42, 701–709.

Chao, A. and Shen, T., 2009. SPADE - Species Prediction And Diversity Estimation. Version 2009/Feb. Persistent URL: chao.stat.nthu.edu.tw/.

Clark, K.R., Ainsworth, M., 1993. A method of linking multivariate community structure to environmental variables. Mar. Ecol. Prog. Ser. 92, 205–219.

Clarke, K.R. and Gorley, R.N., 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E. Plymouth; UK.

Colwell, R.K., 2013. EstimateS: Statistical estimation of species richness and shared species from samples. Version 9.

Drago, E.C., 2007. The physical dynamics of the river-lake floodplain system. In: Iriondo, M., Paggi, J.C., Parma, M.J. (Eds.), The Middle Paraná River. Limnology of a Subtropical Wetland. Springer, Berlin, pp. 83–212.

Duarte, C.M., Kalff, J., 1986. Littoral slope as a predictor of the maximum biomass of submerged macrophyte communities. Limnol. Oceanogr. 31, 1072–1080.

Franceschi, E.A., Lewis, J.P., 1979. Notas sobre la Vegetación del Valle santafecino del Río Paraná (República Argentina). ECOSUR. 6 (11), 55–82.

Greenberg, A.E., Trussell, R.R., Clesceri, L.S. (Eds.), 1985. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, New York.

Grime, J.P., 1974. Vegetation Classification by Reference to Strategies. Nature 250, 26–31.

Iriondo, M.H., Paira, A.R., 2007. Physical geography of the basin. In: Iriondo, M., Paggi, J.C., Parma, M.J. (Eds.), The Middle Paraná River: Limnology of a Subtropical Wetland. Springer, Berlin, pp. 7–31.

James, C., Fisher, J., Russel, V., Collings, S., Moss, B., 2005. Nitrate availability and hydrophyte species richness in shallow lakes. Freshw. Biol. 50, 1049–1063.

Johnson, J.B., Omland, K.S., 2004. Model selection in ecology and evolution. Trends Ecol. Evol. 19, 101–108.

Johnston, C.A., Brown, T.N., 2013. Water chemistry distinguishes wetland plant communities of the Great Lakes coast. Aquat. Bot. 104, 111–120.

Junk, W.J., Bayley, P.B., Sparks, R.E., 1989. The flood pulse concept in river floodplain systems. Can. Spec. Publ. Fish. Aquat. Sci. 106, 110–127.

Kettenring, M.K., Gardner, G., Galatowitsch, S.M., 2006. Effect of light on seed germination of eight wetland *Carex* species. Ann. Bot. 98, 869–874.

Kisson, L.T.T., Jacob, D.L., Hanson, M.A., Herwig, B.R., Bow, S.E., Otte, M.L., 2013. Macrophytes in shallow lakes: relationships with water, sediment and watershed characteristics. Aquat. Bot. 109, 39–48.

Lacoul, P., Freedman, B., 2006. Relationships between aquatic plants and environmental factors along a steep Himalayan altitudinal gradient. Aquat. Bot. 84, 3–16.

Lewis, J.P., Franceschi, E.A., 1979. Notas sobre la dinámica de la vegetación del valle del río Paraná. Ecosur. 6, 145–163.

Madsen, J.D., Chambers, P.A., James, W.F., Koch, E.W., Westlake, D.F., 2001. The interaction between water movement, sediment dynamics and submersed macrophytes. Hydrobiologia 444, 71–84.

Magurran, A.E., 2005. Species abundance distributions: pattern or process? Funct. Ecol. 19, 177–181.

Mayora, G., Devercelli, M., Giri, F., 2013. Spatial variability of chlorophyll-a and abiotic variables in a river–floodplain system during different hydrological phases. Hydrobiologia 717, 51–63.

Mitchell, C.E., Blumenthal, D., Jarosík, V., Puchett, E.E., Pysek, P., 2010. Controls on pathogen species richness in plants introduced and native ranges: roles of residence time, range size and host traits. Ecol. Lett. 13, 1525–1535.

Monção, F.S., Santos, A.M., Bini, L.M., 2012. Aquatic macrophyte traits and habitat utilization in the Upper Paraná River floodplain, Brazil. Aquat. Bot. 102, 50–55.

Murphy, K.J., Dickinson, G., Thomaz, S.M., Bini, L.M., Dick, K., Greaves, K., Kennedy, M.P., Livingstone, S., McFerran, H., Milne, J.M., Oldroyd, J., Wingfield, R.A., 2003. Aquatic plant communities and predictors of diversity in a sub-tropical river floodplain: the upper Rio Paraná, Brazil. Aquat. Bot. 77, 257–276.

Neiff, J.J., 1975. Fluctuaciones anuales en la composición fitocenótica y biomasa de la hidrofitia en lagunas isleñas el Paraná Medio. Ecosur. 2, 153–183.

Neiff, J.J., 1979. Fluctuaciones de la vegetación acuática en ambientes del valle de inundación del Paraná medio. Physis. 38, 43–53.

Neiff, J.J., Orellana, J., 1972. Diferenciación de ambientes en una cuenca isleña del Paraná Medio, sobre la base de las unidades de vegetación y suelos asociados. Asoc. Cien. Nat. Lit. 3, 3–17.

Neiff, J.J., Poi de Neiff, A., 2003. Connectivity processes as a basis for management of aquatic plants. In: Thomaz, S.M., Bini, M. (Eds.), Ecología e Manejo de Macrófitas Aquáticas. Cap. II. Ed. Universidad Estadual de Maringá, Maringá, PR – Brasil, pp. 39–58, 341 pp.

Neiff, J.J., Casco, S.L., Cozar, A., Poi de Neiff, A.S.G., 2011. Vegetation diversity in a Neotropical wetland during two different climatic scenarios. Biodivers. Conserv. 20, 2007–2025.

Neiff, J.J., Casco, S.L., Mari, E.C.K., Di Rienzo, J.A., Poi, A.S.G., 2014. Do aquatic plant assemblages in the Paraná River change along the river's length? Aquat. Bot. 114, 50–57.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, R.P., O'Hara, B.R., Simpson, G.L., Solumos, P., Stevens, M.H.H., Wagner, H., 2013. Vegan: Community Ecology Package. R package version 2.0-10. http://CRAN.R-project.org/package-vegan

Padial, A.A., Thomaz, S.M., 2008. Prediction of the light attenuation coefficient through the Secchi disk depth-empirical modeling in two large Neotropical ecosystems. Limnology 9, 143–151.

Padial, A.A., Thomaz, S.M., Agostinho, A.A., 2009. Effects of structural heterogeneity provided by the floating macrophytes *Eichhornia azurea* on the predation efficiency and habitat use of the small Neotropical fish *Moenkhausia sanctaefilomenae*. Hydrobiologia 624, 161–170.

Paira, A.R., Drago, E.C., 2007. Origin, evolution and types of floodplain waterbodies. In: Iriondo, M.H., Paggi, J.C., Parma, M.J. (Eds.), The Middle Paraná River: Limnology of a Subtropical Wetland. Springer, Berlin, pp. 51–81.

Pott, V.J., Pott, A., 2000. Plantas aquáticas do Pantanal. Centro de Pesquisa Agropecuárica do Pantanal, Corumbá, Embrapa; Brazil.

Quinn, G., Keough, M., 2002. Experimental Design and Data Analysis for Biologists. Cambridge University Press, Cambridge.

Rangel, T.F., Diniz-Filho, J.A.F., Bini, L.M., 2010. SAM: a comprehensive application for spatial analysis in macroecology. Ecography 33, 46–50.

Rooney, R.C., Carli, C., Bayley, S.E., 2013. River connectivity affects submerged and floating aquatic vegetation in floodplain wetlands. Wetlands 33, 1165–1177.

Sabattini, R.A., Lallana, V.H., Marta, M.C., 1983. Inventario y biomasa de plantas acuáticas en un tramo del valle aluvial del Río Paraná medio. Revista de La Asoc. Cienc. Nat. Litoral. 14, 179–191.

Sabattini, R.A., Lallana, V.H., 2007. Aquatic macrophytes. In: Iriondo, M.H., Paggi, J.C., Parma, M.J. (Eds.), The Middle Paraná River: Limnology of a Subtropical Wetland. Springer, New York, pp. 205–226.

Sand-Jensen, K., Sondergaard, M., 1979. Distribution and quantitative development of aquatic macrophytes in relation to sediment characteristics in oligotrophic Lake Kalgaard, Denmark. Freshw. Biol. 9, 1–11.

Santos, A.M., Thomaz, S.M., 2007. Aquatic macrophytes diversity in lagoons of a tropical floodplain: the role of connectivity and water level. Austral Ecol. 32, 177–190.

Santos, A.M., Thomaz, S.M., 2008. Short term fluctuations and succession trends in tropical floodplain vegetation measured by correspondence analysis. Braz. Arch. Biol. Technol. 51, 781–791.

Schwarza, A.M., de Wintonb, M., Hawesa, I., 2002. Species-specific depth zonation in New Zealand charophytes as a function of light availability. Aquat. Bot. 72, 209–217.

Sculthorpe, C.D., 1967. The Biology of Aquatic Vascular Plants. Edward Arnold Publishers, London.

Sousa, W.T.Z., Thomaz, S.M., Murphy, K.J., 2011. Drivers of aquatic macrophyte community structure in a Neotropical riverine lake. Acta Oecol. 37, 462–475.

Spitale, D., 2012. A comparative study of common and rare species in spring habitats. Ecoscience 19, 80–88.

Steffen, K., Leuschner, C., Müller, U., Wiegleb, G., Becker, T., 2014. Relationships between macrophyte vegetation and physical and chemical conditions in northwest German running waters. Aquat. Bot. 113, 46–55.

Thomaz, S.M., Bini, L.M., Bozelli, R.L., 2007. Floods increase similarity among aquatic habitats in river floodplain systems. Hydrobiologia 579, 1–13.

Tilman, D., Reich, P.B., Knops, J., Wedin, D., Mielke, T., Lehman, C., 2001. Diversity and productivity in a long-term grassland experiment. Science 294, 483–485.

Ward, J.V., Tockner, K., 2001. Biodiversity: towards a unifiyng theme for river ecology. Freshw. Biol. 46, 807–819.

Weisner, S.E.B., Thiere, G., 2010. Effects of vegetation state on biodiversity and nitrogen retention in created wetlands: a test of the biodiversity–ecosystem functioning hypothesis. Freshw. Biol. 55, 387–396.

Wersal, R.M., Madsen, J.D., 2011. Influences of water column nutrient loading on growth characteristics of the invasive aquatic macrophyte *Myriophyllum aquaticum* (Vell.) Verdc. Hydrobiologia 665, 93–105.

Wu, J., Cheng, S., Liang, W., Wu, Z., 2009. Effect of organic-rich sediment and belowground sulfide exposure on submerged macrophytes *Hydrilla verticillata*. Bull. Environ. Contam. Toxicol. 83, 497–501.

Zhang, Q., Xu, Y., Huang, L., Xue, W., Sun, G., Zhang, M., Yu, F., 2014. Does mechanical disturbance affect the performance and species composition of submerged macrophyte communities? Nature, 4888, http://dx.doi.org/10.1038/srep04888.