Growth of a native versus an invasive submerged aquatic macrophyte differs in relation to mud and organic matter concentrations in sediment

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ABSTRACT

Eutrophication has reduced the colonization of submerged plants in some freshwater ecosystems, and organic matter (OM) together with grain size in sediment may mediate such decreases. We tested the isolated and combined effects of sediment mud and OM concentrations on the early growth of two species of submerged macrophytes, Egeria najas (native) and Hydrilla verticillata (invasive). We hypothesized that the sediment OM concentration has more important effects than the mud concentration on plant growth and that E. najas is more successful than H. verticillata in highly organic sediment. We tested these hypotheses using mesocosms with several combinations of mud and OM concentrations in the sediment. We used plant length, the number of lateral branches, root dry mass and total plant dry mass as response variables. Both OM and mud were found to be important determinants of the growth of both species, but the former had a stronger influence than the latter. However, the responses of all plant attributes differed between the species. For example, the growth of E. najas increased linearly with increasing OM concentration, but H. verticillata responded to this variable with a quadratic tendency. The decrease in the growth of the invasive species at high OM concentrations may be associated with its lower tolerance to phytotoxic compounds released by the decomposition that occurs in more organic sediment. In conclusion, our experimental data support the importance of the sediment OM in the growth of the native E. najas and invasive H. verticillata. However, future studies should investigate the adaptation mechanisms that allow both plants to colonise such distinct sediment types.

1. Introduction

Eutrophication has reduced the presence of submerged macrophytes in some freshwater ecosystems over the last 100 years (Sand-Jensen et al., 2000; Seddon et al., 2000). Sediment organic matter (OM), which usually increases with eutrophication, is one of the factors used to explain this tendency (Irfanullah and Moss, 2004; Liu et al., 2004). Nutrients typically increase with the OM concentration in the sediment (Barko and Smart, 1986; Newbolt et al., 2008), which could account for higher plant growth in more organic sediment (Pulido et al., 2011). However, after a threshold, OM may hinder plant growth through the release of phytotoxins (Barko and Smart, 1986; Wu et al., 2009). Because of its importance for macrophytes growth, sediment OM, along with other environmental factors, can influence entire macrophyte communities by determining the patterns of species distribution (Chappuis et al., 2014). Several studies have indicated that an increase in sediment OM may inhibit the growth and survival of some submerged plant species (Barko and Smart, 1986; Terrados et al., 1999). Thus, sediment OM could be a filter preventing invasion by non-native macrophytes, such as the extremely invasive submerged macrophyte Hydrilla verticillata (L.f.) Royle, which is highly productive in its introduced ranges (Bianchini Jr. et al., 2010) but is sensitive to phytotoxins released by organic sediment (Barko and Smart, 1986; Wu et al., 2009). This species has successfully invaded ecosystems and caused ecological and economic impacts worldwide (Cook and Lüönd, 1982; Sousa, 2011); thus, studies of its ecology are of great interest. It has recently appeared in and colonised the Upper Paraná River (Brazil), but it grows poorly in sites with highly organic sediment (Sousa et al., 2009, 2010). The decline of H. verticillata in its native range (Asia) is also attributed to eutrophication and the consequent increase in sediment OM (Yan et al., 1997; Qiu and Wu, 1998). However, the OM concentration in sediment does not seem...
to compromise the success of several Neotropical macrophytes, such as Egeria najas (Planck), Cabomba furcata Schult. & Schult. f. and Myriophyllum aquaticum (Vell.) Verdcourt, which are found in habitats with high OM concentrations, such as floodplain lakes (Sousa et al., 2009; Sousa et al., 2009).

In addition to OM, other sediment features, such as grain size, are also determinants of submerged macrophyte success. Increased plant growth in fine sediment appears to be a response to nutrient availability (Boeger, 1992). Most aquatic macrophytes grow poorly in coarse sediment compared with fine sediment because the latter increases nutrient diffusion and exchange between the roots and the sediment (Barko and Smart, 1986).

Sub-tropical lakes in the Upper Paraná River floodplain accumulate sediment with a high mud concentration (grain size <0.062 mm) together with high quantities of OM (Rosin et al., 2010; Ragonha et al., 2013). Some of these lakes are colonized by the native submerged species E. najas, but they are apparently less prone to colonization by the non-native H. verticillata (Sousa et al., 2009; Sousa et al., 2009). Although OM and mud may explain the distribution of these and other species of macrophytes, it is difficult to distinguish the effects of both variables on plant success because they are positively correlated in natural ecosystems. Thus, experimental manipulations of these two important sediment features are necessary to determine their individual and interactive roles in plant growth.

In this study, we assessed both isolated and interaction effects between OM and mud on the success of E. najas and H. verticillata in mesocosms. We chose these two species because the former is very common in several natural and man-made ecosystems in the Neotropical region, while the latter has a great invasive potential. We asked the following questions: (i) which sediment feature (OM or mud) is the most important determinant of plant establishment and growth? (ii) How do these species respond to increases in OM and mud in sediment? We hypothesized that (i) sediment OM is the variable that best explains plant performance compared with mud and that (ii) E. najas growth will be less affected than H. verticillata growth in OM and mud-rich sediment. We predicted that dry mass, plant length and the number of lateral branches, which are surrogates of plant success, are not limited by high OM and mud concentrations for E. najas, but that these factors are negatively affected in H. verticillata.

2. Materials and methods

Our experiments were conducted on the campus of the University of Maringá (South Brazil). Apical portions of healthy macrophytes (15 cm long), sediment and riparian forest litter were collected from the Upper Paraná River floodplain (22°45′S, 53°15′W and 22°45′S, 53°30′W). Fragments of both species of macrophytes were collected from a site at the main river channel where the two species co-existed. Litter was dried at 60 °C to a constant weight and ground in a mill to obtain a homogeneous powder, which was used to manipulate the sediment OM concentrations. All natural sediment was incinerated at 560 °C for four hours to eliminate OM, and it was then sieved according to the method described by Wentworth (1922) and Suguio (1973) to remove all mud (silt + clay; grain size <0.062 mm). The two fractions of OM-free sediment (one with particle sizes of <0.062 mm and the other with particle sizes of >0.062 mm) were saved for later use in the experiment. This procedure produced mud-free and OM-free sediment.

To assess the independent effect of OM, litter powder was added to the OM-free sediment >0.062 mm to produce a gradient of 0, 2, 4, 7, 12 and 22% g DW OM. Then, these different sediments were mixed with OM-free mud sediment (<0.062 mm particle size, collected by sieving as described above) to obtain a gradient of 0, 5, 15, 25, 40 and 55% g DW mud. This procedure allowed for all possible mixtures of mud and OM, producing a total of 36 combinations, which are (the procedure and all combinations) shown in Fig. 1. These percentages were chosen to simulate the range found in the field in the Paraná River floodplain (Sousa et al., 2009, 2010 Rosin et al., 2010; Ragonha et al., 2013). The treatment with 2% g DW mud and 5% g DW OM can be considered to be a control because it is similar to the condition found at the site where both macrophyte species co-existed (Sousa et al., 2010).

The apical portions of E. najas and H. verticillata without adventitious roots or branches were planted in pots (8 x 8 x 10 cm) with the sediment treatments shown in Fig. 1. Each pot contained one apical portion. We used three replicates for the treatment pots (a total of 108 pots for each species). Although the number of replicates can be considered to be low, our procedure produced a gradient of sediment OM and mud that permitted the use of
regression analysis to evaluate our data (see below). This analysis circumvented the limited number of replicates. The pots were randomized inside of three tanks (161 × 111 × 73 cm), and each tank received a replicate to ensure replicate independence. The tanks were filled with tap water (approximately 500 L), which was replaced every 10 days to avoid phytoplankton accumulation. All tanks remained outdoors; thus, the incidence of light was similar to that experienced by plants grown in natural ecosystems.

Water temperature, dissolved oxygen and saturation, pH and conductivity were measured at the sub-surface every 10 days. In addition, sediment was collected from each pot to measure total nitrogen (N) (Mackereth et al., 1978) and total phosphorus (P) concentrations (Goltherman et al., 1978). Mud content was determined as previously stated and OM content was measured after incineration at 560 °C for four hours.

The experiment ended when the plants reached the water surface (60 days). Macrophytes were removed from the tanks to measure the following attributes: plant length (cm), the number of lateral branches, root dry mass and total dry mass (obtained by drying in an oven at 60 °C until a constant weight was reached).

2.1. Data analyses

Correlations between OM, mud, and N and P concentrations were examined to assess the effects of mud and OM on nutrient concentrations. These correlations also allowed us to assess the independence of the OM and mud concentrations in the treatments.

To test the effects of OM and mud on the plant attributes, we used multiple regression analyses. We assessed the assumptions of multicollinearity and homogeneity through the visual inspection of residuals. This exploratory analysis revealed distinct tendencies for both species. A linear tendency was observed for E. najas, whereas a quadratic curve in response to OM was clear for H. verticillata (Figs. 3 and 4). However, the linear and quadratic models were tested for both species, after which we selected the best model (based on residual analyses) that explained their development. Because OM and mud usually co-vari in the field (Burone et al., 2003; Magni et al., 2008), we included a coefficient in the models to test the interaction between OM × mud. The linear models were described as follows:

\[ z = \text{constant} + b(\text{OM}) + c(\text{mud}) + d(\text{OM} \times \text{mud}) \] (1)

where \( z \) = macrophyte attribute; \( b \) = partial coefficient for the relationship with OM; \( c \) = partial coefficient for the relationship with mud; and \( d \) = partial coefficient for the interaction of OM × mud. The model with the quadratic parameter was determined by the following equation:

\[ z = \text{constant} + b(\text{OM}) + c(\text{OM}^2) + d(\text{mud}) + f(\text{OM} \times \text{mud}) \] (2)

where \( z \) = macrophyte attribute; \( b \) = partial coefficient for the relationship with OM; \( c \) = partial coefficient for the relationship with OM \( 2 \) (quadratic term); \( d \) = partial coefficient for the relationship with mud; and \( f \) = partial coefficient for the interaction of OM × mud.

All analyses were conducted using Statistica TM 7.0.a software package.

No statistical comparison of growth between the two species was performed, i.e., no interaction terms were investigated, because the species were fitted by two different models (quadratic versus linear). We believe that these results are sufficient to draw conclusions about interactions, i.e., differing species responses in the presence of varying OM and mud concentrations.

Table 1

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Means and standard deviations (SDs) of the abiotic water variables measured in the tanks during the experiment. The means were obtained from samples collected over time.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abiotic water variables</td>
<td>Tank 1</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>28.3 ± 0.57</td>
</tr>
<tr>
<td>Oxygen (%)</td>
<td>134.5 ± 15.25</td>
</tr>
<tr>
<td>Oxygen (mg L⁻¹)</td>
<td>6.9 ± 0.76</td>
</tr>
<tr>
<td>pH</td>
<td>8.3 ± 0.37</td>
</tr>
<tr>
<td>Conductivity (mS cm⁻¹)</td>
<td>150.7 ± 12.34</td>
</tr>
</tbody>
</table>

3. Results

The water features were very similar among the tanks, indicating that all of the treatments were conducted under similar abiotic conditions (Table 1). High oxygen levels, alkaline pH levels and warm temperatures predominated, which are similar to the conditions found in the natural habitat of the Upper Paraná River floodplain, where plants grow during the spring and summer.

The sediment N concentrations varied between 1.59 and 2.98 mg/g DW, and P concentrations ranged from 0.03 to 2.37 mg/g DW. The treatments with lower OM and mud concentrations also had low levels of N and P (Fig. 2). Indeed, nutrient concentrations were highly correlated with that of OM (N: \( r = 0.94; p < 0.001 \); P: \( r = 0.78; p < 0.001 \)). Correlations between nutrient and mud concentrations were less strong, although they were also significant (N: \( r = 0.18; p = 0.05 \); P: \( r = 0.48; p < 0.001 \)). Mud and OM concentrations were not correlated (r = 0.01; p = 0.901), indicating that these two predictive variables varied independently in our treatments. This independence is an important pre-assumption to test our hypotheses.

The responses of E. najas and H. verticillata to changes in the OM concentration were stronger than those for mud (Figs. 3 and 4). However, the attributes of E. najas exhibited different tendencies than those of H. verticillata in response to the varying OM and mud concentrations (Figs. 3 and 4).

For E. najas, all attributes (except for root DW) increased linearly and significantly with increasing OM (Table 2 and Fig. 3). By contrast, mud affected only the length of E. najas, and this effect was less noticeable than the effects caused by OM (Table 2 and Fig. 3). Indeed, the OM × mud interaction term was significant, indicating that all of the response variables increased much faster with increasing OM when there was an also increase in the mud concentration (Table 2 and Fig. 3).

Similar to the observations in E. najas, OM significantly influenced all attributes of H. verticillata (Table 2 and Fig. 4). However, all attributes of the latter species increased with changes in OM until a threshold was reached, after which a decrease was observed (Fig. 4). Indeed, the quadratic term was significant for all attributes measured in H. verticillata (Table 2). The mud concentration had a significant effect only on plant length and total dry mass, and its effects on these attributes were also weaker than those caused by OM (Table 2 and Fig. 4).

4. Discussion

Our results showed that both OM and mud are determinants of macrophyte success but that the former has a greater importance than the latter. The results also showed that both macrophyte species responded differently to increase in OM. Thus, our hypotheses that (i) OM is more influential than mud on macrophyte growth and that (ii) the native macrophyte E. najas is more resistant to OM accumulation in sediment were supported. Our results are relevant because our experimental design allowed us to distinguish the
effects of OM and mud on macrophyte growth, which is not possible in the field, where both components usually co-vary (Burone et al., 2003; Magni et al., 2008), making it difficult to assess their independent effects.

In contrast with *E. najas*, the invasive *H. verticillata* showed decreases in all attributes after an OM threshold was reached, as demonstrated by the significance of the quadratic term. It appears that approximately 7% OM is the optimum level for *H. verticillata* development. These results are in accordance with others obtained in situ, showing that this species is still able to grow in sites with <10% OM in the sediment but does not colonize sites with >13% OM (Sousa et al., 2009; Sousa et al., 2009).

Although there was a decrease in *H. verticillata* growth in highly organic sediment in our experiment, this species was still able to grow in the high OM treatments. Thus, sediment OM is not the only factor that explains the low success of *H. verticillata* in the lakes of the Upper Paraná, and other factors are certainly involved (see discussion below).

The increases in plant length, the number of lateral branches, and plant biomass in sediments with <10% OM support the view that these attributes may be involved in the dominance of *H. verticillata* at sites with low to medium concentrations of organic matter in the sediment in situ. First, the growth of this macrophyte allows it to reach the water surface and to reduce underwater light.
is a typical characteristic of canopy-forming species that concentrate the photosynthetic tissues close to the water surface. Second, the development of a greater number of lateral branches, whose fragmentation contributes to plant dispersion (Madsen and Smith, 1999; Martin and Valentine, 2014), further enhance the dominance of this species in the Paraná River. On the other hand, a decrease in \textit{H. verticillata} growth in highly organic sediment is most likely related to its sensitivity to phytotoxins released during the decomposition of OM, such as methane and sulfur dioxide (Barko and Smart, 1986; Wu et al., 2009; Marín-Muñiz et al., 2014). Thus, after a threshold point, the negative effects of OM outperform the positive effect of nutrients, which increases continuously with increasing OM. There is also evidence that biomechanical properties of submerged macrophytes (e.g., tensile force, bending force and structural stiffness) are negatively affected by nutrient-rich sediment (Zhu et al., 2013).

Although data from previous studies in the field in sites of the Upper Paraná River (Sousa et al., 2009; Sousa, 2011) and the results of the present study suggest that \textit{H. verticillata} is affected negatively by high sediment OM concentrations, other investigations have demonstrated that this species is able to grow in a wide range of OM concentrations (0–60%; Barko and Smart, 1986; Sousa, 2011). The unsuccessful colonization of sites with high levels of OM in the sediment in the Upper Paraná floodplain may have occurred because the plants that invaded this area originated from populations that were highly sensitive to sediment OM, as observed in its native range (Wu et al., 2009). Alternatively, other environmental conditions, such as the elevated temperatures found in the Upper Paraná (up to 30 °C during summer; Sousa et al., 2010) can contribute to the acceleration of the release of phytotoxins, even at low OM concentrations, causing toxic effects on \textit{H. verticillata}.

The different responses of \textit{H. verticillata} and \textit{E. najas} to the varying OM concentrations may be related to physiological adaptations and root morphology (Sorrell, 1999; Vretare and Weisner, 2000). The acclimatisation of macrophytes to anoxic sediment can be attributed to their ability to translocate oxygen to roots (Pedersen et al., 1998), and this ability may be more developed in \textit{E. najas} considering its evolutionary history in warm Neotropical regions, where unsaturated oxygen concentrations are very common (Sousa et al., 2010).

Despite the great importance of OM determined in our experiment, the mud concentration also had a positive, although lesser, role on macrophyte development, affecting only the lengths of both species and the biomass of \textit{H. verticillata}. The influence of grain size on plant growth is well known (Barko and Smart, 1986; Xiao et al., 2007) and occurs because this factor affects several physical and chemical sediment properties (Volkenborn et al., 2007). For example, fine sediment grains increase nutrient diffusion and exchange, which enhance plant growth (Barko and Smart, 1986). In addition, grain size affects the rates of nutrient infiltration and nutrient loss.
in sediment (Sculthorpe, 1967). As a result, coarse sediment supports lower macrophyte growth rates (Wheeler and Giller, 1982; Barko and Smart, 1986).

Our results also demonstrated that in addition to having isolated effects of OM and mud, these factors interacted to determine E. najas growth. Studies conducted in situ in the Upper Paraná floodplain have shown that E. najas is able to greatly accumulate biomass and attain dominance in floodplain lakes, where sediments with high organic matter and mud concentrations predominate (Sousa et al., 2009, 2010; Rosin et al., 2010; Ragonha et al., 2013). This finding was most likely caused by increased nutrient concentrations with increasing OM concentrations. In addition, nutrient retention increases and nutrient loss decreases with increasing mud concentrations due to its fine particle size. This fact may explain the observation of the highest growth of E. najas at the greatest concentrations of OM and mud and the highest growth of H. verticillata at intermediate concentrations of OM and high concentrations of mud. The interactions between sediment mud and OM also indicated that data on macrophyte growth with sediment characteristics obtained in the field should be interpreted with caution because it is difficult to make conclusions on interactions between factors in the absence of controlled experiments.

Extrapolation of experimental results to the field are not always easy because mesocosms represent a simplification of reality. Although our results add confidence that the OM concentration in sediment may play a role in the patterns of distribution of E. najas and H. verticillata recorded in the Paraná River floodplain, interactions with other factors that were not manipulated in our experiment may also occur, influencing the field results in the Upper Paraná floodplain (Sousa et al., 2009; Sousa, 2011). At least two potential, and not mutually exclusive, factors may interact with sediment, including underwater light and herbivory. Indeed, the sites colonized by H. verticillata in the Upper Paraná are locations at which Secchi depth values are greater, whereas the opposite is true for E. najas (Sousa et al., 2009; Sousa et al., 2009). Regarding herbivory, it has been shown that native macrophytes are preferred by native herbivores over introduced macrophytes (Xiong et al., 2008). Non-choice experiments have indicated that there is at least one species of snail that prefers E. najas to H. verticillata in the Paraná River floodplain (T. Meurer, unpublished). Thus, a com-

**Fig. 4.** The effects of OM and mud concentrations on the measured attributes of Hydrilla verticillata, including plant length (a), the number of lateral branches (b), root dry mass (c) and plant dry mass (d).
bination of sediment type, underwater light and herbivory (enemy release hypothesis) might explain the differential distribution of native and non-native species in the Paraná River floodplain.

In conclusion, our experimental data support the importance of sediment OM in the growth of the native E. najas and invasive H. verticillata, at least in their early stages (two months) of development. In addition, our findings show that these two species respond differently to OM in sediment and that granulometry interacts with OM to determine plant growth. Finally, our results indicate that sediment type alone is not sufficient to explain the absence of H. verticillata in floodplain lakes, where the sediment is rich in OM. Future studies integrating sediment type with other potential factors that affect H. verticillata success, such as biotic resistance and underwater light, are necessary to better explain the distribution of this species in the lakes of the Upper Paraná floodplain. In addition, the assessment of the morphologies and physiologies of these two species, combined with experiments that manipulate sediment OM and oxygen concentrations, will help to explain the mechanisms underlying our results.

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