

Germinative behaviour of ten tree species in white-water floodplain forests in central Amazonia

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Abstract Amazonian floodplain forests (known as várzea) are classified into high or low várzea depending on the spatial position on the plains. This topographic feature exposes the terrain over different time periods of inundation, causing a major limiting factor for tree seedling establishment. We hypothesize that, strategically, most of the seeds produced by trees in low várzea forests

germinate faster and in synchrony (temporally concentrated germination), and that their seedlings tend to have cotyledons without reserve or foliaceous cotyledons (PEF). By contrast, seeds produced by high-várzea specialist trees exhibit slower and temporally scattered germination, and their seedlings tend to have reserve storage cotyledons (CHR). Generalist species may show no clear pattern or may be related to high-várzea species. To test this hypothesis, diaspores of 10 tree species were collected: five of low-várzea specialist trees, three of high-várzea specialist trees and two of generalist species. Seedling emergence and morphology were monitored daily in a nursery for a period of 150 days of being subjected to non-flooded (sown directly in várzea soil) and flooded conditions (15 days in water before sowing in the same soil). The seedling emergence of low-várzea species showed an increase of 37% in germinability whereas high-várzea and generalist species exhibited a decrease of 38% and 35% of germinability, respectively. Foliaceous cotyledons were preferentially found in seedlings of low-várzea species, and storage cotyledons were more common in those of high-várzea species, indicating how cotyledon morphology may determine the amount and use of resources available to a seedling during the first stages of establishment and growth. Conservation plans aiming for the maintenance of ecosystem services must consider these strategies.

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Introduction

Every tree species will always demonstrate an intrinsic and more suitable germination period over its lifespan. More precisely, the factors that influence germination and how species adjust to this stage in situ are the central questions to be answered by studies of germination ecology, as the time that one species takes to germinate can influence reproductive success and may develop standards of success that will perpetuate a species throughout time. These investigations can be extremely useful to better understand forest systems and consequently their conservation, rehabilitation and management (Bawa et al. 1990; Baskin and Baskin 1998; Lucas et al. 2012). This is especially true in regions with high biodiversity and obviously complex natural systems such as the Amazon (ter Steege et al. 2013).

About 14% of the Amazon basin is covered by floodplains forests (Melack and Hess 2010), and, according to the fertility of soil stands, Amazonian floodplains may be divided into poor and acidic igapó soils and nutrient-rich várzea soils (Furch 1997; 2000). The reason for this simple dichotomy is related to the amount of nutrients present in and carried by waters that annually inundate the forests along rivers. Igapó forest receive low amounts of nutrients from very acidic black water whereas várzeas are associated with white-water rivers with high concentrations of dissolved solids rich in nutrients that are annually deposited in their alluvial plains, which cover a very extensive area of about 400,000 km² of the Amazon region (Junk et al. 2011). Still related to várzea forests and based on seasonal flooding intensity and duration, várzeas can be divided into two main zones: the high-elevation (high-várzea) zone and the low-elevation (low-várzea) zone (Wittmann et al. 2002; Wittmann et al. 2004). In each of these zones, there are naturally different environmental conditions for colonization by new plant individuals.

In central Amazonia, the amplitude of water levels can annually oscillate by approximately 10 m, defining periodically a terrestrial and an aquatic phase in floodplains (Junk et al. 1989). Spatially, such water pulses may have an extremely different effect due to the duration of the inundation, promoting for a certain zone a short, long or extremely long aquatic phase, for instance. Trees inhabiting areas under this flooding regime must develop traits and strategies that enable them to survive in both phases (Scarano et al. 1997; Parolin et al. 2003; Scarano et al. 2003). The varying degree of flood

tolerance among flooded forest species is reflected in the distribution of plant communities: They follow gradients along river channels that vary according to topography, duration of inundation and water depth (Junk et al. 1989; Wittmann et al. 2004; Wittmann et al. 2006).

Diaspores (fruits and seeds) and seedlings of Amazonian floodplain trees interacts with river water at different times, and the recurrent inundation cycle in floodplains is considered the most restraining factor affecting seedling establishment. Thus, traits favouring seed flotation, seed survival and seed germination even after prolonged periods of being submersed in water are important in promoting successful seedling establishment (Kubitzki and Ziburski 1994; Lopez 2001; de Oliveira Wittmann et al. 2010; Lucas et al. 2012; Maurenza et al. 2012; Conserva et al. 2013). This is particularly significant because diaspores can be subjected to variable periods of water saturation and hypoxia in waterlogged conditions, which may threaten seed viability (Baskin and Baskin 1998).

In addition of affecting germination, plant growth under varying environmental conditions may cause changes in seed characteristics, such as size, structure, and amounts and types of stored compounds. These changes can be related to seed germination and may help explain how certain environmental factors cause variations in germination responses (Baskin and Baskin 1998).

For several várzea tree species, seedling formation, including all its essential and functional structures, occurs during the aquatic phase (Parolin 2001a), when seedlings already show morphological adaptations for survival during intensive inundation (Junk 1989), such as adventitious roots, lenticels and stem hypertrophy (Parolin 2001a; Parolin 2001b). However, when completely submerged, the diaspores of some species do not germinate, and in some situations, although they may form radicles, proper seedling formation does not occur (Parolin et al. 2003).

Some studies demonstrate that, in nutrient-rich várzea forests, seedlings tend to form foliar cotyledons due to reduced nutrient requirements from the mother plant (Parolin 2001b; Lucas et al. 2012). Furthermore, phanerocotylar seedlings are assumed to be more resistant to prolonged inundation, while cryptocotylar seedlings, due to their reserves, quickly develop vegetative structures to avoid inundation (Coops and van der Velde 1995; Scarano and Franco 1998; Parolin 2002). Thus, the functional morphology of seedlings could be related to

germination time or to other biotic and abiotic factors that affect seedling establishment success (Ng 1978; Garwood 1996; Khurana and Singh 2001; Zanne et al. 2005).

Studies on várzea trees usually compare species germination in environments with different hydrological regimes and varying nutrient status (Scarano 1998; Lopez 2001; Parolin et al. 2003; de Oliveira Wittmann et al. 2010), or even they consider the buoyancy of diaspores when in contact with water (Scarano et al. 2003; de Oliveira et al. 2007). Only a few tree species with non-floating seeds are known to germinate while submerged, as observed by Conserva (2007) in *Calycophyllum spruceanum*, *Hura crepitans* and *Piranhea trifoliata*. However, these studies used water columns with a depth of only 10 cm in the experimental setting, thus allowing full light and oxygen to reach the submerged seeds. Some authors have tested the effects of different immersion or submersion periods (Ferreira et al. 2009; Parolin and Junk 2002; Lucas et al. 2012; Maurenza et al. 2012) and monitor, not only germination and seedling development in water, but also the ability of seedlings to survive in these conditions and to establish after the aquatic period (de Melo et al. 2015). Nevertheless, waterlogged diaspores were observed for periods ranging from 15 to 150 days, thus making it difficult to compare germination success. The wide range of seed viability and intra-specific germination variability indicates that more studies are required to better understand the germinative behaviour of seeds submersed in water under distinct periods for floodplain tree species. In addition, in no previous study is germination discussed in terms of synchronicity, concentration or scattering germination over a given period, nor is seedling functional morphology considered a feature associated with seedling emergence rates and speed of germination. In dynamic environments, such as várzea forests, these characteristics can be relevant to seedling establishment.

When the differential colonization of tree species occurs as a function of selective filter related to flooding conditions, germinative behaviour is expected to reflect the responses to these conditions. If this holds, our hypothesis is that more diaspores of species from low várzea forests germinate faster and in synchrony in flood conditions, and their seedlings tend to have foliaceous cotyledons that are resistant to prolonged inundation. On the other hand, diaspores of species from high várzea forests under the same conditions present lower and slower germination, without a fine synchrony, and tend

to have reserve storage or absorption cotyledons to avoid the effects of inundation. Generalist várzea species may show no clear pattern or may be related to high-várzea specialist species, because in the high-várzea zone environmental selective pressure is less intense.

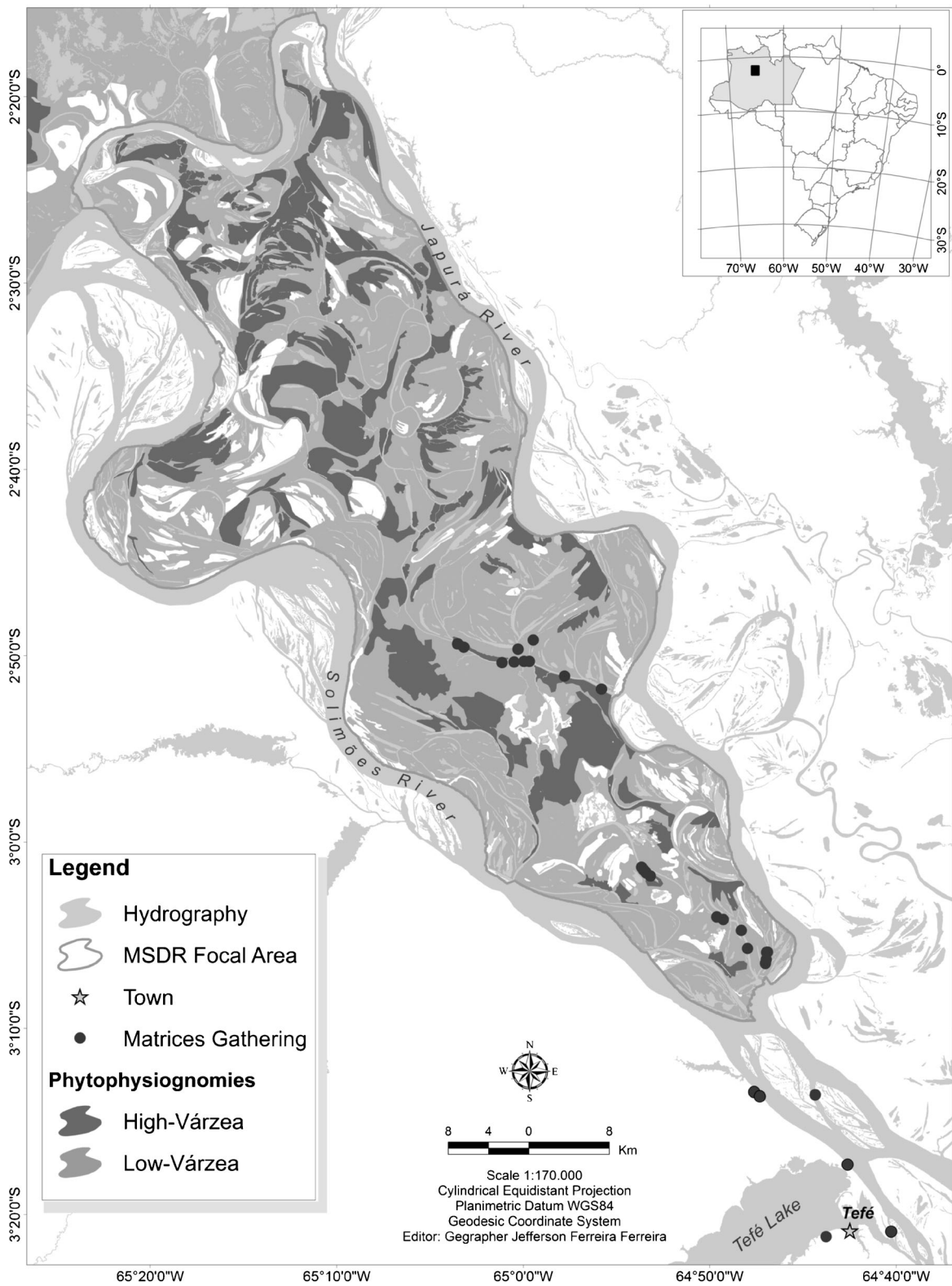
Material and methods

Study site

Mamirauá Sustainable Development Reserve (MSDR; 2°51' S, 64°55' W) is situated about 70 km NW of the city of Tefé, Amazonas, and covers an area of 1,124,000 ha, including parts of the rivers Japurá, Solimões and Auati-Paraná (Fig. 1). The area is characteristically composed of white-water flooded várzea forests, where from 2009 to 2012, the mean flooding amplitude was 13 m (Conserva et al. 2013). Mean monthly temperature ranges between 23°C and 32°C; and rainfall average is 1,730 mm per year (Mamirauá Institute for Sustainable Development – IDSMD 2010). The floodplain in the region of the study site is comprised of a mosaic of lake systems (Fig. 1).

Species selection

In central Amazonia, the low várzea forest is characterized by flood heights above 3 m in average and flood duration may last up to 250 days per year, while in high várzea forests, flood levels have heights below 3 m in average, with maximum flood duration of 50 days per year (Junk 1989; Wittmann et al. 2002; Wittmann et al. 2004). Tree species are distributed along the plains according to their tolerance and traits for coping with flooding in typical zones along the flooding gradient and may be divided in three specific categories: low-várzea zone specialists or trees more tolerant to longer period of inundation, high-várzea zone specialists or trees less tolerant to inundation, and generalist species or tree naturally distributes along the entire gradient of inundation. We selected 10 common tree species widely distributed in white-water floodplains in the Amazon basin (Wittmann et al. 2013), representing the three categories: (1) five typical tree species of low-várzea zones: *Duroia duckei* Huber (Rubiaceae), *Handroanthus barbatus* (E. Mey.) Mattos (Bignoniaceae), *Ilex inundata* Poepp. ex Reissek (Aquifoliaceae), *Piranhea trifoliata* Baill. (Picodendraceae) and the várzea



◀ **Fig. 1** Map of the Mamirauá Sustainable Development Reserve (MSDR), situated about 70 km NW of the city of Tefé, Amazonas, highlighting the different phytophysionomies of the várzea forests (low várzea and high várzea). Black point are mother plants (Matrices Gathering) marked for the collection of diaspores

endemic species *Laetia corymbulosa* Spruce ex Benth. (Salicaceae); (2) three typical tree species of high-várzea zones: *Cedrela odorata* L. (Meliaceae), *Guarea guidonia* (L.) Sleumer (Meliaceae) and *Ocotea cymbarum* Kunth (Lauraceae); and finally (3) two generalists tree species: *Calycophyllum spruceanum* (Benth.) K. Schum. (Rubiaceae) and *Pseudobombax munguba* (Mart. & Zucc.) Dugand (Malvaceae). For each of the selected species we evaluated germination behaviour, and compared seedling emergence and their morphology.

Naturally mature diaspores from each of the tree species were collected from at least five individual mother trees, that were separated from each other by a minimum distance of 100 m. Collecting were at the beginning and at the peak of the flooding seasons (between April and June of 2009, 2010 and 2012), which are periods of maximum fructification for most of the várzea tree species (Kubitzki 1985; Schöngart et al. 2002). We have collected diaspores in different years because some species did not produce fruits yearly, or their production was very low in a given year. Therefore, seeds of *D. duckei*, *H. barbathus*, *I. inundata*, *L. corymbulosa* and *P. munguba* were collected in 2009; while seeds of *C. spruceanum* and *P. trifoliata* were collected in 2010 and seeds of *C. odorata*, *G. guidonia* and *O. cymbarum* were collected in 2012.

Diaspores were stored in plastic bags and labelled with the date and sampling site, then placed in a styro-foam box and transported to the Forest Ecology Laboratory at Mamirauá Institute. In the laboratory, dehiscent fruits of *C. spruceanum*, *C. odorata*, *G. guidonia*, *H. barbathus*, *L. corymbulosa*, *P. trifoliata* and *P. munguba* were placed on plastic trays and left at room temperature until spontaneous opening and seed liberation occurred. For the indehiscent fruits, the seeds used in no-flooded experiments were manually extracted; for the flood tests (as described below), simulating a natural condition, the whole fruits were immersed, as occurred with *D. duckei*, *I. inundata* and *O. cymbarum*. After the waterlogging period, the fruits were washed and the seeds were manually extracted before sowing.

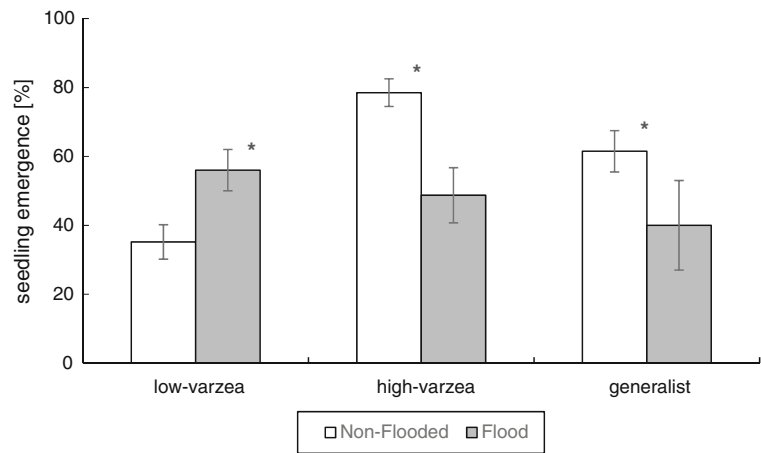
Single samples of soil, about 20 cm deep, were collected before the flooding period close to the mother trees and homogenized to form the soil mixture that was used as substrate in all germination trays. The average soil pH ranged from 4.98 to 5.3 and total organic matter was between 34 and 44 g·kg⁻¹. The silty clay texture comprised 477 g·kg⁻¹ of silt (0.05–0.002 mm) and 514 g·kg⁻¹ of clay (< 0.002 mm).

Germination tests

Diaspores from different mother plants of each species were mixed, producing one homogenized sample to increase representativeness and genetic variability, and eliminating dependence between diaspores of the same individual. Thus, experiments were setting up without any undergoing seed storage period. From the combined samples of each species, four random replicates of 25 seeds were sown in plastic trays (45 cm × 28 cm × 10 cm) using várzea soil as the substrate (non-flooded condition). Another set of four random replicates of 25 diaspores was placed on trays with water 10 cm deep, remaining submerged, floating or both for 15 days before sowing on trays with várzea soil (flood condition). We chose specifically the 15-days interval because this period of immersion is likely to be a sufficient period to have an impact on the germination behaviour. Also, this period might be considered the minimum time necessary for seed water absorption and metabolic activation (Lopez 2001; de Oliveira Wittmann et al. 2007; de Oliveira Wittmann et al. 2010; Lucas et al. 2012). Additionally, this was the interval in which we obtained responses for seeds of every species in all replications and supports our field observations of the germination process in both low and high-várzea zones.

During the three-year study, the same greenhouse was used as a nursery where all germination tests were performed, with screens made of 50% shadow mesh, relative solar radiation levels of $483 \pm 33 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-2}$ and temperature varying between 26°C and 36°C. Radiation averages, which were recorded in a natural environment under canopy and in clearings, were 33 and 528 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-2}$, respectively, indicating that the plants received between 4% and 59% of the total incident radiation (902 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-2}$) recorded in the field (Wittmann and Junk 2003; Conserva 2007). Therefore, we can assume that the greenhouse simulated light conditions of the latter successional stages in várzea forests (ca 50% shaded).

Fig. 2 Seedling emergence (%) of low-várzea specialist, high-várzea specialist and generalists tree species in non-flooded (white) and flood conditions (grey). The columns represent seedling emergence for all species grouped by zone preference, and the bars represent the standard error. Asterisks (*) indicate that the mean difference is significant at the 0.05 level



Seedling characteristics

Seedling emergence was observed daily since the first day of sowing until 150 days later. Seedlings were also classified into categories based on three cotyledon characteristics: position, texture and exposure. Following these criteria, the following three seedling types were identified: (1) cryptocotylar hypogeal (CHR), with cryptic reserve storage or absorption cotyledons; (2) phanerocotylar hypogeal (PHR), with exposed reserve

storage or absorption cotyledons; and (3) phanerocotylar epigeal (PEF), with foliaceous cotyledons (sensu Garwood 1996; Ferraz and Calvi 2011).

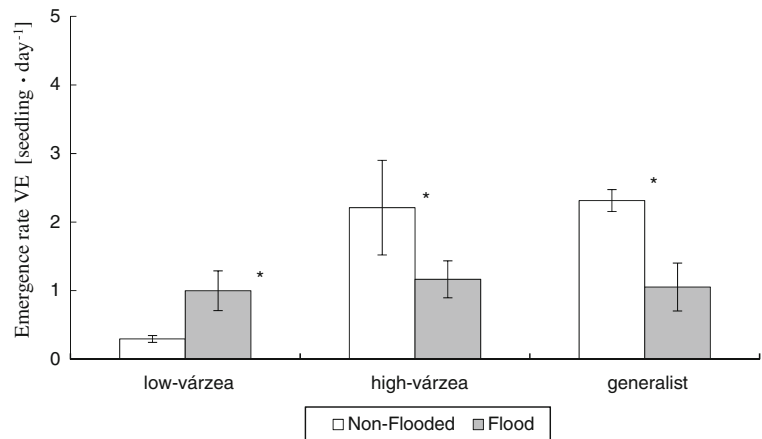
Measurements and statistical analysis

Using daily counts for up to 150 days, we calculated measurements for germinability or seedling emergence (%), mean emergence time (\bar{t}), mean emergence rate (\bar{v}) and emergence rate (VE), coefficient of variation of

Table 1 Summary of ANOVA results for seed germination measurements of tree species that preferably colonize different zones of flooded várzea forests in central Amazonia

Source of variation	<i>D.f.</i>	Mean square	<i>F</i> -value	<i>P</i> -values	Mean square	<i>F</i> -value	<i>P</i> -values
		Emergence (<i>E</i>)			Mean germination time (\bar{t})		
Treatment	1	195.397	0.594	0.4442	306.433	0.279	0.5994
Treatment × Species (zone preference)	7	1,174.735	3.571	0.0031	280.555	0.256	0.9682
Species × Treatment	9	2,095.104	6.369	< 0.001	300.126	0.273	0.9793
zone preference × Treatment	2	5,316.396	16.160	< 0.001	368.627	0.336	0.7162
Residual	55	328.976			1,097.691		
		Mean emergence rate (\bar{v})			Emergence rate (VE)		
Treatment	1	0.156	29.650	< 0.001	1.070	0.909	0.3446
Treatment × Species (zone preference)	7	0.143	27.077	< 0.001	3.859	3.276	0.0056
Species × Treatment	9	0.136	25.837	< 0.001	4.748	4.030	0.0005
zone preference × Treatment	2	0.113	21.497	< 0.001	7.858	6.670	0.0025
Residual	55	0.005			1.178		
		Coefficient of variation of germination time (CV_t)			Synchrony (<i>Z</i>)		
Treatment	1	555.693	5.464	0.0234	< 0.001	0.003	0.9569
Treatment × Species (zone preference)	7	221.376	2.177	0.0518	0.030	3.105	0.0078
Species × Treatment	9	229.542	2.257	0.0327	0.025	2.566	0.0153
zone preference × Treatment	2	258.123	2.538	0.0889	0.007	0.679	0.5113
Residual	55	101.694			0.010		

Fig. 3 Emergence rate (VE) of species comparing the non-flooded (*white*) and flood (*grey*) conditions. The *columns* represent the emergence rate of all species grouped by zone preference, and the bars represent the standard error. Asterisks (*) indicate that the mean difference is significant at the 0.05 level



germination time (CV_i) and synchrony (Z), according to Ranal and Santana (2006). The mean germination rate (\bar{v}) is defined as reciprocal of the mean germination time ($1 / \bar{t}$). The emergence rate (VE) or Maguire's index is a time-weighted cumulative germination that measures the speed of germination and quantifies the seedling vigour. If the germination occurs soon after sowing, the index value will be greater than if this occurs later. This is the great advantage of the index, that in fact, measures germination rate. Synchrony or the Z index was used to evaluate the degree of overlapping in the seed germination or seedling emergence processes. Then, $Z = 1$ when the germination of all seeds occurs at the same time and $Z = 0$ when at least two seeds could germinate, one at a time (Ranal and Santana 2006).

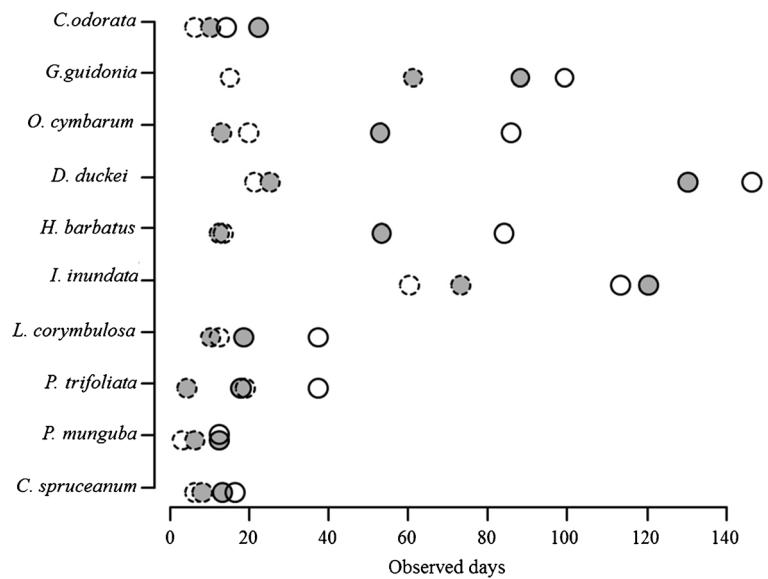
To test the differences among the three species categories (low-várzea specialists, high-várzea specialists and generalists), a nested ANOVA was used, with the species nested within zone preference, together with each treatment and their respective interactions both between and within each group [treatment \times species (zone preference)]. After checking the differences among the three categories, we tested the effect of species (*C. spruceanum*, *C. odorata*, *D. duckei*, *G. guidonia*, *H. barbathus*, *I. inundata*, *L. corymbulosa*, *O. cymbarum*, *P. trifoliata* and *P. munguba*) and inundation condition (non-flooded and flood group) on seed germination measurements. The nested ANOVA and the comparisons among the three categories and between non-flooded and flooded conditions were made using the Tukey test at 0.05 significance, and processed with SISVAR software (Ferreira 2014).

We also used different species classifications based on the functional morphology of the seedlings (CHR, PHR and PEF as fixed factor), together with each treatment and their respective interactions both between and within each category (treatment \times morphology) to test for the association with seedling emergence (%), mean emergence rate (\bar{v}) and synchrony (Z), using the same nested ANOVA model. Comparisons among the seedling functional morphology groups and between the non-flooded and flood conditions within the same categories (CHR, PHR and PEF) were made using the Tukey pair wise post hoc test at 0.05 significance (SISVAR software). Although the functional morphology of seedlings does not overlap with the different types of zone preference (low-várzea specialists, high-várzea specialists and generalists), we use this classification to test what other characteristic could explain species' responses to flooding, since seedling functional morphology could be related to germination time or to other biotic factors that affect seedling establishment success. All germination measurements were tested, but only seedling emergence (%), mean emergence rate (\bar{v}) and synchrony (Z) showed significant differences.

Results

The seedling emergence of low-várzea specialist tree species showed an increase of 37%, when comparing to the non-flooded and flood conditions (Fig. 2). While for high-várzea specialist and generalist tree species, there was a decrease of 38% and 35%, respectively (Fig. 2). For all germination measurements, with the

Fig. 4 Time (in days) for the first germination (dotted circle) and the last germination (plain circle) in both flood (filled circle) and non-flooded (open circle) conditions for the tree species of high-várzea zones (*C. odorata*, *G. guidonia* and *O. cymbarum*), low-várzea zones (*D. duckei*, *H. barbatus*, *I. inundata*, *L. corymbulosa*, *P. trifoliata*) and generalist species (*C. spruceanum*, *P. unguba*)



exception of \bar{t} and CV_t the interaction between habitat preference and treatment was significant (Table 1).

For the mean emergence rate (\bar{v}), only low-várzea tree specialists showed significant difference, with a faster seedling emergence in flood than in the non-flooded condition (supplementary material Table 1). The emergence rate (VE) of low-várzea specialists was also higher in the flood condition (Fig. 3), while the high-várzea specialists and generalists tree species showed higher emergence rates (VE) in the non-flooded condition than in the flood condition (Fig. 3).

Regarding the other germination measurements evaluated, there were no significant differences between the treatments within the same zone preference (lower case letters in Table S1 in the Electronic Supplementary Material).

The seedling emergence of *H. barbatus* and *L. corymbulosa*, specialists from the low-várzea zone, increased by 59% and 28%, respectively, with the immersion of diaspores in water (Table S2 in the Electronic Supplementary Material). The seedling emergence of *C. odorata* and *G. guidonia*, both specialists from the

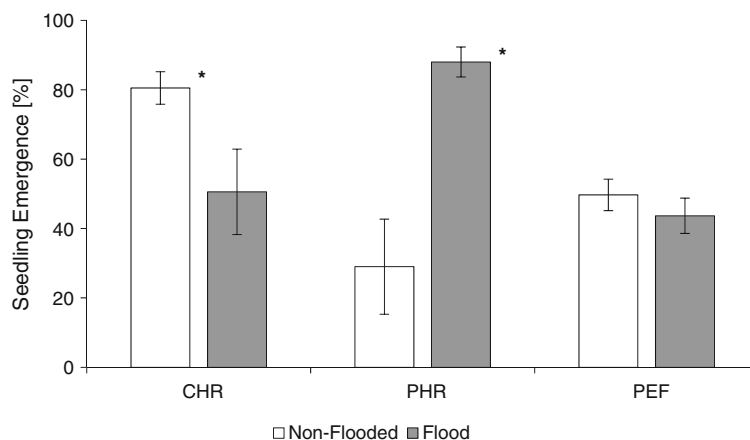


Fig. 5 Seedling emergence (%) of species grouped into three distinct seedling functional morphology categories: cryptocotylar hypogeal with reserve storage or absorption cotyledons (CHR); phanerocotylar hypogeal with reserve storage or absorption cotyledons (PHR) and phanerocotylar epigeal with foliaceous

cotyledons (PEF). The three categories were compared using non-flooded (white) and flood (grey) conditions. Asterisks (*) indicate that the mean difference is significant at the 0.05 level. The line bars represent the standard error

Table 2 Summary of ANOVAs for seed germination measurements of tree species grouped according to their functional seedling morphology (CHR, PHR and PEF)

Source of variation	<i>D.f.</i>	Mean square	<i>F</i> -value	<i>P</i> -values	Mean square	<i>F</i> -value	<i>P</i> -values
		Emergence (<i>E</i>)			Mean germination time (\bar{t})		
Morphology	2	2,425.681	4.117	0.0205	1,593.699	1.773	0.1776
Treatment	1	264.002	0.448	0.5055	212.303	0.236	0.6285
Morphology × Treatment	2	5,252.546	8.915	0.0004	462.730	0.515	0.5999
Residual	68	589.186			898.890		
		Mean emergence rate (\bar{v})			Emergence rate (<i>VE</i>)		
Morphology	2	0.1078	2.969	0.0580	5.995	3.012	0.0558
Treatment	1	0.1583	4.362	0.0405	1.215	0.611	0.4373
Morphology × Treatment	2	0.0303	0.836	0.4380	1.250	0.628	0.5367
Residual	68	0.0363			1.990		
		Synchrony (<i>Z</i>)					
Morphology	2	0.0830	6.894	0.0019			
Treatment	1	0.0408	0.000	0.9941			
Morphology × Treatment	2	0.0043	0.361	0.6985			
Residual	68	0.0120					

high-várzea zone, was reduced by 29% and 59.5%, respectively, in flood condition (Table S2 in the Electronic Supplementary Material). Also, seedling emergence for the generalist species *P. munguba* was reduced by 38% in the flood condition (Table 2 in the Electronic Supplementary Material). The five remaining species did not present statistically significant differences in their germination between treatments (Table 2 in the Electronic Supplementary Material).

The effect of non-flooded and flood condition on seed germination measurements varied greatly among species, mainly when we consider the beginning and the end of the process of germination during the observed 150 days (Fig. 4). The mean emergence rate (\bar{v}) of *L. corymbulosa* was higher in the flood condition; the emergence rate (*VE*) of *P. trifoliata* was higher in the flood condition, while in *C. odorata* and *P. munguba*, it was higher in the non-flooded condition; the synchrony (*Z*) of *I. inundata*, *C. spruceanum* and *P. munguba* had significant differences when comparing the non-flooded and flood conditions (grey lowercase letters in Table S2 in the Electronic Supplementary Material).

The classification of seedlings according to the three distinct groups of cotyledon characteristics (CHR, PHR and PEF; Table 3, Fig. S1 and Fig. S2 in the Electronic Supplementary Material) showed that two specialist species of high várzea forests (*G. guidonia* and *O. cymbarum*)

presented CHR cotyledons and had greater germinability under the non-flooded conditions (Fig. 5 and summary of ANOVA in the Table 2). Only one specialist of low várzea forests presented PHR cotyledons (*H. barbathus*) and germinated more in flood condition (Fig. 5). All the other species fit into the PEF group (*D. duckei*, *I. inundata*, *L. corymbulosa*, *P. trifoliata*, *C. odorata*, *C. spruceanum* and *P. munguba*); the seedling emergence of this last group did not show any difference between the non-flood and flood condition (Fig. 5 and summary of ANOVA in the Table 2). However, species with PEF had at least one seedling emerge at a minimum interval of 20 days in both conditions. On the other hand, for the three species with hypogeal reserve cotyledons (*H. barbathus*, *G. guidonia* and *O. cymbarum*), emergence took more than 40 days (*VE* [seeds·day⁻¹] section of Table S2 in the Electronic Supplementary Material).

Discussion

As a general rule in Amazon várzea forests, the differentiated colonization of tree species is well defined along the flooding gradient and reflects the degree of trees' adaptation to hypoxic and anoxic conditions

(Piedade et al. 2010; Assis and Wittmann 2011). The results of this study support the initial hypothesis when diaspores are grouped according to their zone affinity, since diaspores of species from the low-várzea zone germinate more and faster under flood condition, while diaspores of high-várzea zone species germinate more and faster under non-flood condition. However, the germination behaviour of the diaspores of the 10 species evaluated here did not follow the same pattern since species occupying different flooding positions (high-várzea and low-várzea specialists and generalists) responded differently to waterlogging conditions. Furthermore, the synchronized fruit production of these species (Kubitzki and Ziburski 1994; de Oliveira Wittmann et al. 2007) was not associated with synchronization and uniformity of seedling emergence over time, namely *D. duckei* and *G. guidonia*, which had a low daily frequency and stable emergence after 100 days of sowing. Thus, the degree of germination success of these species was based on the capacity of their diaspores to spread germination over time, permitting recruitment in the environment of at least part of the formed seedlings.

In their study of 31 species of Amazonian wetland trees, Parolin et al. (2003) found higher germination rates for diaspores of specialists from the low-várzea zone. In fact, high germination rates in species of the low-várzea zone may favour establishment and reduce the impact of mortality caused by subsequent floodings (Parolin 2002; de Oliveira Wittmann et al. 2007). On the other hand, the small number of representatives of species from the high-várzea zone in this study does not allow for generalizations on seed capacity to emerge in this environment. The three high-várzea specialists studied, namely *C. odorata*, *G. guidonia* and *O. cymbarum*, regardless of the fruit or seed, presented diaspores that sink at first contact with water. In this case, the delayed onset of germination could be advantageous (Scarano et al. 2003) and may explain the high percentage of seedling emergence in the non-flooded condition and corresponding reduction of emergence in the treatment with previous water contact for 15 days.

The submersion of the diaspores in water for 15 days increased seedling emergence or was not significant for low-várzea specialists. Previous studies have also confirmed that contact with water favoured higher rates of germination for floodplain species such as *Garcinia brasiliensis*, *Crateva bentamii*, *Tabernaemontana* sp., *Casearia aculeata* (Lucas et al. 2012), *Salix martiana*,

Laetia corymbulosa and *Vitex cymosa* (de Oliveira Wittmann et al. 2007; de Melo et al. 2015).

In the case of the generalist *P. munguba*, contact with water reduced seedling emergence, corroborating the findings of Lucas et al. (2012), who reported the complete loss of diaspore viability after 28 days in water. However, increased germination rates when diaspores had contact with water have also been reported for *P. munguba* (de Oliveira Wittmann et al. 2007). We observed in diaspores of *P. munguba* that the imbibition for one week led to radicle protrusion, but not to seedling formation, when the diaspores began to rot. Thus, seed germination and plant establishment relate to the diaspores' survival capacity, both during and after the flooding period. In fact, a strong ability to respond to post-flooding events, represented by oxygen toxicity and soil drying, for example, may play a major role in determining the competitive success of a given species in seasonally flooded environments (de Melo et al. 2015).

For low-várzea specialists, the degree of success in seed germination depends on greater floatability and efficient avoidance of decomposition, which may be related to amounts of stored compounds in the endosperm (de Melo et al. 2015). The species *H. barbathus* and *P. trifoliata* are certainly good examples of species that exhibit tolerance; with water contact for more than 100 days, they form seedlings that survive after transplantation in soil. In the field, *D. duckei*, *I. inundata*, *L. corymbulosa*, *P. trifoliata* and *H. barbathus* have buoyant fruits and, at the time of dispersion, have either a fleshy or hard (*P. trifoliata*) pericarp. *H. barbathus* is the exception with its impermeable seed coat. A fleshy pericarp or an impermeable seed coat could inhibit the effects of submergence on seed germination, as they may serve as a mechanical barrier that prevents rapid seed imbibition (Mora et al. 2013). In our study, after the waterlogging period, the fruits were washed and seeds were manually extracted before sowing. In the cases of *P. trifoliata* and *H. barbathus*, the fruits had previously opened, and the seeds were used in the flood treatment. Furthermore, all low-várzea specialist species studied here presented diaspores with abundant endosperm (Barroso et al. 1999) and, under the hypoxic and anoxic conditions, the endosperm may have been used to maintain physiological activities (Baskin and Baskin 1998). Thus, the loss of morphological dormancy through endosperm consumption would explain why germination increased with time underwater (Mora et al. 2013).

Diaspores sharing the same total germination percentage do not always spread germination over time in a similar way. Surveys of germination rates of Amazonian floodplain tree species describe differences as not being remarkable; however, they are relevant considering the mean emergence time and rates (Lucas et al. 2012). Distinct patterns of relative frequency observed for the diaspores of the 10 species studied also enlarge the range of strategies for specific seedling establishment. This is critical to maintaining their populations in areas with seasonal flooding, where high rates of sedimentation and soil erosion lead to formation of environments with spatial and temporal heterogeneity (Wittmann et al. 2004). The increase of the mean emergence rate is characteristic of species whose strategies are to establish as soon as possible (Borghetti and Ferreira 2004), as observed for the low-várzea specialists and generalist species *C. spruceanum* and *P. munguba*, in which at least one seedling emerged every day, and for *L. corymbulosa* and *P. trifoliata*, which exhibited emergence every 5 days and 10 days, respectively. The ability to produce a greater number of seedlings in less time favours rapid development in low-várzea zones with a shorter terrestrial phase and high instability in physical conditions (Wittmann et al. 2006).

Almost all várzea forest species under study start germination very soon after dispersion, between seven and 60 days, perhaps because the suitable conditions for seedling growth in this environment last for a short time (Parolin et al. 2003). Thus, earlier germination may be the benefit of having a long initial growing season for some species, favouring establishment during a short terrestrial phase (de Oliveira Wittmann et al. 2007; de Oliveira Wittmann et al. 2010). On the other hand, delaying germination or emergence with a lower number of seedlings per day may prevent mortality of seedlings of less tolerant species that colonize high-várzea zones, such as *G. guidonia* and *O. cymbarum*. The patterns observed for the relative frequency distribution of seedling emergence indicate how species colonize and share physical space for effective establishment. These species exploit opportunities by allocating germination over different time periods, which may lead to optimal use of resources. For example, while the low-várzea specialist species *L. corymbulosa* and *P. trifoliata* exhibit fast seedling emergence, in *D. duckei* and *I. inundata*, this process is much slower.

Another feature that may be associated with tolerance to flooding is the functional morphology of cotyledons.

Parolin et al. (2003) recorded that nine out of 13 studied várzea species had epigeal cotyledons. For these species, seedling emergence and the longevity of the cotyledons were lower in comparison to species with hypogeal cotyledons. Seven species studied, *C. spruceanum*, *P. munguba*, *D. duckei*, *I. inundata*, *L. corymbulosa*, *P. trifoliata* and *C. odorata*, presented epigeal foliaceous cotyledons and germinated less than *O. cymbarum* and *G. guidonia* with hypogeal fleshy cotyledons. In these species, rapid germination and cotyledon emergence probably help to maximize the short period between flooding events (Parolin et al. 2003). Since foliaceous cotyledons possess limited energy reserves, their rapid formation in epigeal germination is responsible for the first photosynthetic activity, which facilitates rapid growth (Hladik and Miquel 1990; Primack 1990).

Nevertheless, it is common to find species with hypogeal fleshy or cryptocotylar seedlings in floodplain forests. The main advantage of this type of seedling is avoidance of submergence: After a few days of germination, they utilize their seed reserves at once to quickly extend their shoots (Scarano 1998; Parolin et al. 2003). This strategy would be more effective in geographic regions with lower flooding depth, and in fact it was related to the species colonizing the high-várzea zones, such as *G. guidonia* and *O. cymbarum*.

With the exception of two species, the classification of low-várzea seedlings as preferentially PEF and those from high-várzea as CHR supports the initial hypothesis, as it indicates how cotyledon functional morphology determines the amount and use of resources available to a seedling during the first stages of growth in floodplain forests, as reported for other tropical forests (Garwood 1996; Kitajima 1996; Ibarra-Manríquez et al. 2001; Khurana and Singh 2001; Zanne et al. 2005).

The species investigated showed a combination of different features associated with seed dispersion, germination and investment in the initial growth of seedlings. This combination of different strategies was consistent with species distribution along the flooding gradient and seems to promote effective population maintenance. Thus, *C. spruceanum*, *C. odorata*, *D. duckei*, *G. guidonia*, *H. barbathus*, *I. inundata*, *L. corymbulosa*, *O. cymbarum*, *P. trifoliata* and *P. munguba* are cited among the 60 most important species based on the Overall Importance Value Index (OIV) of floodplain forests in the Amazon Basin (Wittmann et al. 2006; Wittmann et al. 2013). The fact that these species produce a greater OIV may have a meaning that, in

evolutionary terms, they have developed more effective mechanisms to tolerate flooding (Wittman et al. 2002), such as seed size, germination rates and functional seedling morphology. The ecological relevance of these species to várzea environments must acknowledge the series of intrinsic strategies strongly related to the riverine flood pulse. Effective forest management, rehabilitation and conservation plans that call for the utilization of resources from floodplain forests, as well as maintenance of ecosystem services, must take these strategies into consideration. Prior knowledge about the establishment features of these species is also relevant to understand the threats of climate change and other external stressors, since these threats may exceed the adaptive capacity of species and the resilience of their ecosystems (Enright 2014).

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