Oecologia Australis
25(1):22-33, 2021
https://doi.org/10.4257/oeco.2021.2501.03

APPLICABILITY OF SEED BANK ASSESSMENT METHODS IN WETLANDS: ADVANTAGES AND DISADVANTAGES

Francielli Bao[1,], Marco Antonio de Assis[1] and Arnildo Pott[2]*

[1] Universidade Estadual Paulista, Instituto de Biociências, Avenida 24 A, 1515 - Jardim Bela Vista, CEP:13506-900, Rio Claro, SP, Brazil.

[2] Universidade Federal de Mato Grosso do Sul, Programa de Pós-Graduação em Biotecnologia e Biodiversidade, Cidade Universitária, Av. Costa e Silva - Pioneiros, MS, 79070-900, Campo Grande, MS, Brazil.

E-mail: franbao@yahoo.com.br (*corresponding author); massis@rc.unesp.br; arnildo.pott@gmail.com.

Abstract: The soil seed bank is the primary source of regeneration in wetlands and has different assessment methods that vary according to the objective of the study. We evaluated the seed bank composition using three methods: seedlings emergence (EME), seedlings emergence with submersion trays in water (SUB) and screening and counting seeds (COU), and finally, we evaluated the applicability of COU to assess seed predation. The abundance and species richness were evaluated for two years at the end of the flood and dry seasons, in the Brazilian Pantanal. The abundance and species richness differed significantly between methods and seasons. The COU method showed the highest richness (84) and abundance (95.023) followed by EME and SUB. The SUB method reflected only the aquatic community. In the flood season, EME and COU methods showed similar species composition. There were no differences between COU and EME + SUB. The main advantage of COU method was the possibility to assess the seed predation, and we detected that *Croton trinitatis* had 32% of predated seeds. We consider that wetland ecosystems can be sampled by both methods COU and EME + SUB; however, the complete method that can be used for different purposes is COU, also, must be considered the infrastructure and objective of each study.

Keywords: diaspore; floodplain; predation seeds; regeneration mechanism; seedlings.

INTRODUCTION

The seed bank is considered the primary source of regeneration for different ecosystems in wetlands, due the high seed turnover (Bell & Clark 2004, Brock 2011). Wetlands that are characterized by extreme seasonal events, such as the Brazilian Pantanal, have many species dependent on the seed bank to remain in the soil in long periods of flood or drought (Souza *et al.* 2016, Bao *et al.* 2017). In this context, the regeneration mechanisms and seasonal floods are essential in the role of structuring the spatial and temporal species distribution (Daniel *et al.* 2019). Therefore, the presence and absence of many species are conditioned to the ability to tolerate the environmental conditions through the replenishment of the seed bank (Greulich *et al.* 2019).

In wetlands ecosystems, different life forms interact over the year, through the input and output of aquatic and amphibious plants between the seed bank and vegetation during the flood season, and terrestrial plants during the dry season (Bao *et al.* 2017). The presence of different life forms over the year can lead to numerous difficulties to evaluate seed banks in wetlands (Leck & Simpson

1987), mainly due to the presence of many seeds of annual plants in the persistent seed banks (Brock *et al.* 2003). Monitoring and controlling annual plants require more sampling effort (Baskin *et al.* 2019), especially, because they are small and disperse readily, germinating throughout the year (Sanou *et al.* 2019). In terms of soil sampling and collection, as well as species identification, there is a clear need to address different assessment methods of the plant community (Jarman *et al.* 1991).

The two conventional seed bank assessment methods are seedlings emergence and seeds screening (by washing and sieving the soil and seeds counting) (Bonis *et al.* 1995). Both have advantages and disadvantages that we will describe here in detail. Some general points have already been described by other authors, such as, the seedlings emergence method has the main advantage allowing species identification, but it does not reveal all species present in the soil, due to seed dormancy (Thompson *et al.* 1997, Boedeltje *et al.* 2002, Bonis *et al.* 1995, Capon 2007). Also, for wetlands it is required to flood part of the soil samples to evaluate the aquatic community (Brock 1994, Hölzel & Otte 2001, Bao *et al.* 2017). The biggest disadvantage of the seeds screening method is that it does not show seeds viability (Bonis *et al.* 1995). However, no study has made specific approaches and suggestions about how and why to apply each method.

Another critical point is the lack of information about seed banks assessment methods regarding the number of predated seeds, not allowing to understand the dynamics of native plant populations and predator control (Solbreck & Knape 2017). Consistent seed loss by predation has a potential impact on plant abundance, distribution, competition, life cycle traits and or other adaptations (*e.g.* Harper 1977, Crawley 2000). One option would be through the seed bank assessment methods, as in studies of arboreal plants, where the topsoil seeds are separated and counted (Harper 1977). However, for herbaceous plants, that exhibit small seeds it is not easy, an alternative would be the seeds screening method.

The seed bank is the primary regeneration mechanism in wetlands, therefore, understanding the functionality of each assessment method is important to choose the best way to sample an aquatic environment. We compared three seed bank assessment methods: seedlings emergence in greenhouse (EME), seedlings emergence under submersion (SUB), and seeds screening and counting (COU), in a seasonal grassland in the Brazilian Pantanal. Our main objective was to evaluate the capacity of each method in quantifying the seed banks abundance and species richness, as well as to discuss the applicability of each method in a seasonal wetland, and evaluate the possibility to estimate herbaceous seed predation using the seed screening method (COU).

MATERIAL AND METHODS

Study area

The study was carried out in the subregion of Abobral, Pantanal, Mato Grosso do Sul (Central-west, Brazil; 19º29'27.3" S; 57º01'55.9" W, Figure S1a). The grasslands are flooded annually during the summer (between April and August) (Silva & Abdon 1998), with pluvial and fluvial fluctuations, with maximum (7.34 m) and minimum level (2.37 m) of the Miranda river (data collected at *Base de Estudos do Pantanal - BEP*, between 2005 and 2015). Due to long periods of flooding, part of the native grasslands was partially replaced by cultivated species, as the exotic *Urochloa humidicola* (Rendle) Morrone & Zuloaga (Poaceae). This grass is tolerant to flooding but has high invasion value (Bao *et al.* 2015) and it is being object of study for native species control and maintenance (Bao *et al.* 2014, 2018, 2020).

We conducted samplings on the flood and dry hydrophases of the Pantanal: two at the end of the dry period (September) and two at the end of the flood period (July; Figure S1b). We took the samples in eight seasonal ponds, with 1 km between them. The Brazilian Pantanal is composed by several seasonal ponds (Pott & Silva 2015), which present variation in the vegetation structure (Bao *et al.* 2015). To achieve higher amplitude of seeds capture, we collected soil samples along three contour lines (*i.e.* transects), representing relative elevation differences of low, mid, and high (Figure S1c). We established three transects that were marked during the flood season, following the water level, one located in the lowest zone, in the middle of the pond = low (ca. 60 cm deep), one at the pond edge = mid (ca. 30 cm deep) and one in the higher zone, on the external part = high (ca. 1 cm deep) (*e.g.* Bao *et al.* 2018). In each area (seasonal pond), we collected soil samples randomly (using a table of random numbers from 1 to 50 m) (*e.g.* Bao *et al.* 2014, 2015, 2017).

Seed bank assessment methods

In each transect, we sampled five random replicates, and we took three soil samples, with 20x20cm (Figure S2a-b) and 3cm deep (Figure S2c) each. We chose this size to increase precision in estimating species richness in the seed bank (Bao *et al.* 2014, Butler & Chazdon 1998). The samples were stored in plastic bags (Figure S2d) and transported to the greenhouse. We used three methods to evaluate the seed bank: Method I – Seedlings emergence from drained soil in a greenhouse (EME); Method II – Seedlings emergence with soil submersion in water (SUB); Method III – Seeds screening and counting (COU). For each method, we collected the same amount of soil, 120 samples each, totalling 360 soil samples.

Method I : Seedlings emergence in the greenhouse (EME) - the soil samples were spread in plastic trays (30cm x 30cm x 10cm; Figure S3), over 2 cm layer of sterilized washed sand under ambient temperature, the seedlings emerged were counted, identified, and removed to avoid competition to new seedlings (Thompson *et al.* 1997).

Method II: Seedlings emergence under submersion (SUB) - was determined by spreading soil samples in plastic trays (30 cm x 30 cm x 10 cm) and then submerged in tanks under 90 cm of water (simulating flood) for three months (filamentous algae were removed when necessary) (Figure S4).

Method III: Seeds screening and counting (COU) - was applied to determinate the number of viable seeds in the soil by manual seeds counting. For this, soil samples were washed through a set of sieves with three mash sizes (0.25 mm, 0.35 mm and 0.50 mm) to trap seeds of distinct sizes (Bonis *et al.* 1995, Mcfarland & Shafer 2011), and determined the total number of seeds in the sediment (Simpson *et al.* 1989). The retained seeds were preserved in alcohol 50 %. In the laboratory, we counted and identified the seeds under stereoscopic microscope (Figure S5). In this method, it was evaluated whether it is possible to find predated seeds through marks or damage on the tegument.

Species identification was made by comparison with specimens in the Herbarium CGMS, of Universidade Federal de Mato Grosso do Sul (UFMS), consulting specialized books (*e.g.* Pott & Pott 1994, Pott & Pott 2000, Kissmann & Groth 1992, 1995, 1997), identification manuals (*e.g.* Kaul 1978, Groth 1983, Kaul 1985, Gil & Bove 2006, Souza & Giulietti 2014), and consulting specialists on plants of the Pantanal. Species were presented according to APG IV- Angiosperm Phylogeny Group (2016).

Statistical analysis

First, we built species accumulation curves to assess the potential of three seed bank sampling methods: seedlings emergence (EME), seedlings emergence under submersion (SUB) and seeds screening and counting (COU), as well as evaluated richness and abundance between the flood and dry seasons. Then, to evaluate the efficiency of the methods regarding seed bank species richness and abundance during the flood and dry seasons, we constructed species accumulation curves for each season. The species richness and abundance data fit into a Poisson distribution. Analysis of variance (Two-Way ANOVA) was used to test the effect of seasons (flood and dry) and methods (EME, SUB and COU) on species richness and abundance and, between methods and seasons interaction. One-Way ANOVA was used to test the efficiency of the EME+SUB methods together, disregarding the effect of seasonality. All analyses were performed in R environment (R Core Team 2020), using vegan (Oksanen *et al.* 2017), permute (Simpson 2016), lattice (Deepayan 2008) and BiodiversityR (Kindt & Coe 2005) packages.

RESULTS

Species accumulation curves generated from the plot data suggested that most species in the community were sampled. There was a significant interaction between season for predicting species richness (Figure 1); it was meanly in EME (Figure 1a) and SUB (Figure 1b), especially in COU method (Figure 1c). Season interacted with methods and influenced species richness. Such result is relevant since it gives support to the importance of the assessment seed banks

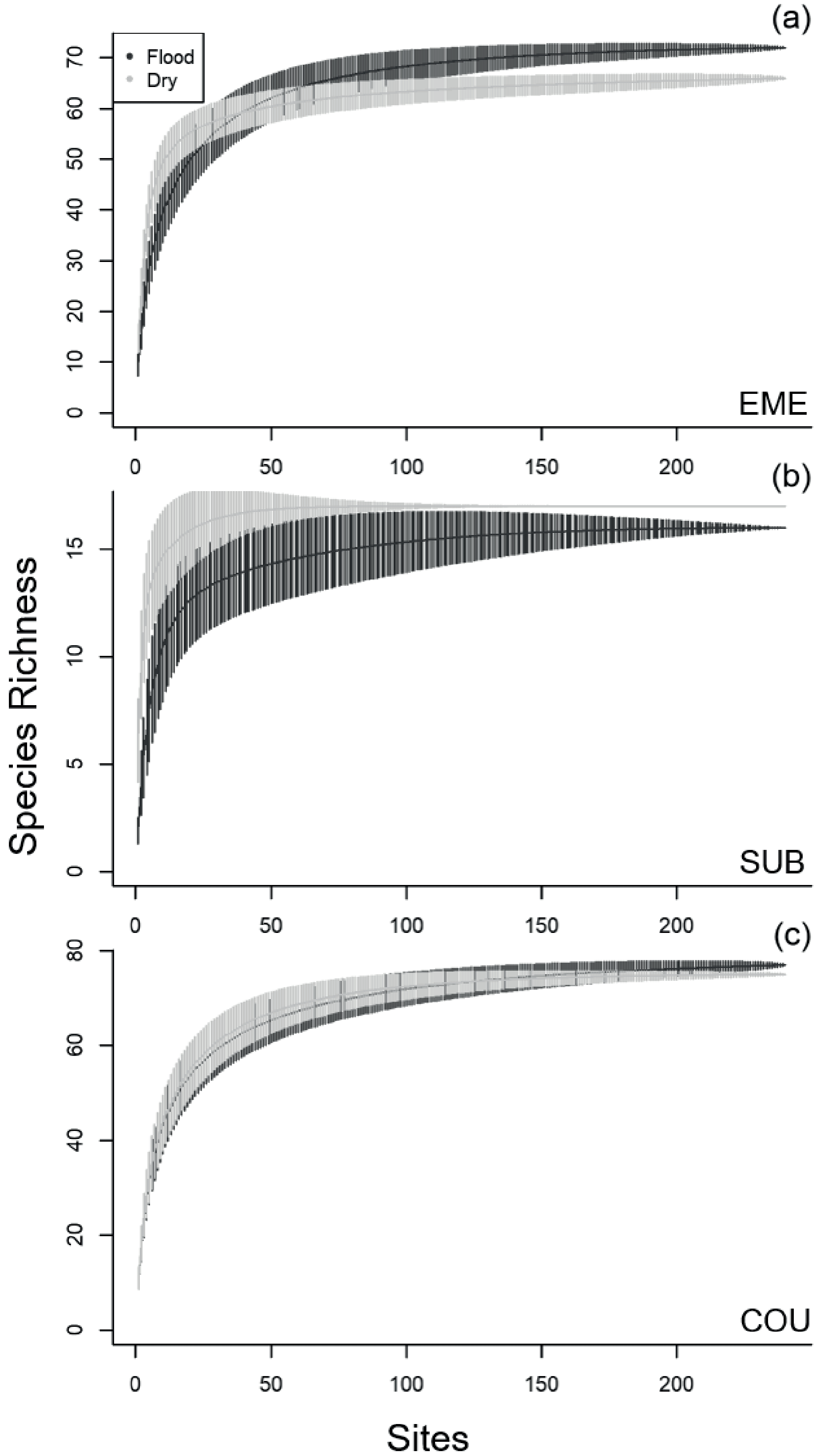

Figure 1. Sample-based species accumulation curves in flood and dry season in a seasonally flooded grassland area in the Pantanal wetland (Central-West Brazil) for (**a**) emergence method (EME), (**b**) submerged (SUB) and (**c**) direct seed counting (COU).

methods in both seasons: dry and flood, showing a curve well-adjusted to the sampling effort.

Species richness and abundance in different methods of seed bank evaluation

We recorded 117 species in the seed bank methods (60 % annuals and 40 % perennials). Individually, 80 species were sampled in the seedlings emergence method (EME) and, 19 species were exclusive in EME; 19 species were sampled in submerged (SUB) and 84 species in seeds counting (COU), where 28 were exclusive in COU. However, 16 species remained unidentified in the latter. Overall, most species were Poaceae (18 species), followed by Cyperaceae (15), Asteraceae (8), Plantaginaceae (7), Alismataceae, Euphorbiaceae and Fabaceae (6 each), and Rubiaceae and Malvaceae (5). Together, these represented over 63 % of the species found in all methods (Table S1). In general, The COU method showed the highest abundance (95.023) followed by EME (64.285) and SUB (19.705).

The abundance and richness between methods and seasons differed significantly (Table 1). The dry season showed less species abundance (± 106.07) and richness (± 11.61) in EME than COU, that showed higher abundance (± 162.19) and richness (± 19.24), differing significantly in both variables, of abundance ($F_{4.25}$ = 234.32, $p < 0.001$, Figure 2a) and richness ($F_{4.25}$ = 153.27, $p < 0.001$, Figure 2b). Regarding the data from the flood season, EME and COU did not differ in terms of abundance ($F_{4.25}$ = 237.33, p = 0.891, Figure 2a) and richness ($F_{4.25}$ = 123.34, p = 0.128, Figure 2b).

The SUB method, as expected, reflected only the aquatic community, and differed significantly from the other methods in abundance (Figure 2a) and richness (Figure 2b). Thirteen species that are essentially aquatic were not sampled in the EME method. The SUB and EME methods shared the amphibious species *Bacopa australis, B. myriophylloides, B. salzmannii, Eleocharis minima, Helanthium tenellum* and *Rotala ramosior*. The COU method, on the other hand, presented all species that were found in SUB.

Considering the EME + SUB complementary methods, there was an increase in richness (± 21.14) and abundance (± 179.11). They did not differ from the COU method in richness ($F_{4.25}$ = 13.70, p = 0.039, Table 1) and abundance ($F_{4.25}$ = 19.94, p = 0.045, Table 1) in both seasons.

Evaluation of seed predation in the counting method (COU)

Among 84 species found in seeds counting method (COU), predation was possible to verify in only one specie, *Croton trinitatis*, that presented the largest size in the seed bank (± 4.1 mm length x 3 mm width). *C. trinitatis* was one of the ten species that had the highest abundance (1225 seeds) and represented 2.3 % of the seed bank

Table 1. Analysis of variance of the abundance and average seedling/seed richness between the seasons (flood and dry) and methods (EME, SUB and COU), in seasonally flooded grasslands in Pantanal wetland (Central West Brazil).

	Response variable	Source of variation	Df	F value	p value
Two-Way ANOVA	Seedling/seeds richness	Season	1	169.70	<0.001
		Methods	2	247.45	<0.001
		Season*Methods	2	37.22	<0.001
		Residuals	1434		
	Seedling/seeds abundance	Season	1	17.64	<0.001
		Methods	2	173.57	<0.001
		Season*Methods	2	8.33	<0.001
		Residuals	1434		
One-Way ANOVA	Seedling richness (COU x SUB + EME)	Methods	2	13.70	0.039
		Residuals	717		
	Seedling abundance (COU x SUB + EME)	Methods	2	19.94	0.045
		Residuals	717		

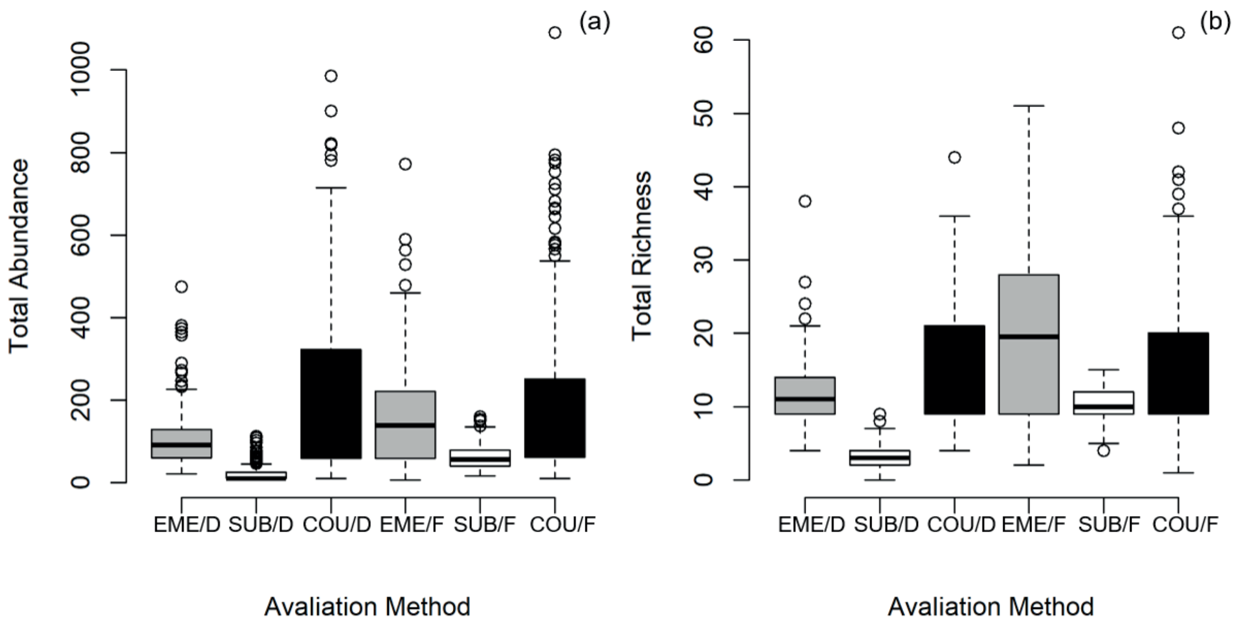

Figure 2. Analysis of variance (ANOVA) of species regarding (**a**) abundance of seeds and (**b**) species richness, between seed bank evaluation methods (EME, SUB, COU) in two seasons (dry (D) and flood (F)), in the Pantanal wetland (Central-West Brazil).

in COU (Table S1), and of these, 32 % presented predation.

DISCUSSION

Species richness and abundance in different methods of seed bank evaluation

The seed bank reflects most of the species that are present in the established vegetation, however many aquatic plants (*e.g. Limnocharis flava, Hydrocleys parviflora*), are not sampled in vegetation studies due to the difficulty of sampling when the field is flooded (Bao *et al.* 2015). This can be seen in our study, where we found high richness (117 species) within the seed bank. In general, the seed banks assessment methods showed high species richness and abundance, the highest percentage of annual species (60 %) is characteristic of seasonal wetlands around the world (Oliveira *et al.* 2015, Souza *et al.* 2016, Anthony 2019, Guan *et al.* 2019). The annual species quickly show germination and reproduction in both seasons, and high seeds amount in their fruits (Farnsworth & Bazzaz 1995), those characteristics increased the abundance in the seed bank.

We observed that some amphibious species, e.g., *Bacopa australis*, *Eleocharis minima*, *Ludwigia octovalvis* and *Rotala ramosior* were found in all methods and both seasons. Some perennial species from the families Poaceae, Fabaceae and Cyperaceae can remain in the environment owing to the sprout banks and vegetative propagation (Combroux *et al.* 2002), some species may lose their aerial parts during the flood season but regrow during the dry season (Bao *et al.* 2020). However, in temporary wetlands, most aquatic plants such as Alismataceae remain in the environment due to the deposit of seeds in the soil (Tuckett *et al.* 2010), within the three months that the flood occurs these aquatic species lose seeds (germination) and store seeds in the seed bank again (reproduction and dispersion) (Souza *et al.* 2016, Bao *et al.* 2018). In this regard, it is noticeable that the maintenance of several species of temporary wetlands depends on the efficiency of the seed banks to germinate.

Individually, the COU method revealed greater abundance and richness, mainly due to the genuinely aquatic species, which we did not record in EME in the dry season. COU is the only method that shows all the floristic composition and, can be used in temporary and permanent wetlands (*e.g.* Greulich *et al.* 2019, Yang 2020). Perhaps the worst difficulty and disadvantage of this method is the lack of comprehensive database with seed images for species comparison and identification, so the researchers spend time with screening and identification. In contrast, the EME method has the advantage of identification which is easier through comparisons with specimens from botanical collections or with plants collected in the field, and specialists, or wait for doubtful seedlings to grow. It is the most used method by researchers in different wetlands (*e.g.* Bao *et al.* 2014, Hazelton *et al.* 2018, Kohagura *et al.* 2020).

We detected an interaction between seasonality and, methods COU and EME. The flood season increased similarity between COU and EME in abundance and richness, possibly due to the seed rains of many aquatic plants (Bornette & Puijalon 2009), as well as the highest transport of seeds by hydrochory from neighbouring areas (Baldwin *et al.* 2001). Furthermore, the intrinsic characteristics of species adaptation to the aquatic environment can promote the germination of some macrophytes even at low water levels in the soil (Brewer & Parker 1990), this is another advantage of EME method. However, when the richness and abundance of the EME and SUB methods was estimated together, we found potential to reflect the same species composition as COU. Although EME and SUB are considered to illustrate only alive plants that germinate under ideal environmental conditions, the specific issues of the total seed bank composition can be elucidated only with the COU method (Brock 1994). Our results emphasize that EME + SUB methods together reflected the same composition as COU, and these findings can assist researchers when there is little knowledge about seeds and species present in the environment.

The SUB method individually requires more infrastructure for the storage of tanks and daily care with the accumulation of algae that limit the entry of light. However, the best advantage is to reveal the aquatic community without having to be in the field during flood periods (van der Valk & Davis 1978). The SUB method can be considered complementary, because does not represent the entire plant composition of temporary wetlands. However, many studies have already applied the method for different purposes in Brazilian wetlands (e.g. Oliveira *et al.* 2015, Souza *et al.* 2016, Bao *et al.* 2018) and around the world (e.g., Brock 1994, Bernhardt *et al.* 2008, Boedeltje *et al.* 2002). Thus, we considered that the advantages and disadvantages of the methods are more attributed to the goal of the study and the evaluation work of each method than to the results that reflect species richness and abundance in temporary wetlands (Table 2).

Evaluation of seed predation in the counting method (COU)

It is not easy to assess predation within the seed bank, as it is difficult to detect what can be a mark of predation or what can be a tegument injury. However, in seeds of *Croton trinitatis*, the predation was evident due to the pattern found in all seeds that presented the mark. In this case, the seed size may have been a determining factor in seed bank predation, as already reported (*e.g.* Thompson *et al.* 1977), *C. trinitatis* showed the largest and the most abundant seed found, which may lead to increased predation (Boutin *et al.* 2006). The COU method not only served to count the number of predated seeds but also brought the total abundance of *C. trinitatis* in the seed bank, which can assist in population studies. Also, many insects that were present in the soil could be sieved and separated; thus, a possible list of predators could be obtained in future studies.

The mechanisms underlying species diversity maintenance are complex to understand due to the variety of the species characteristics (*e.g.* life cycle (perennial or annual) and life forms (aquatic, amphibious and terrestrials). The seed bank assessment methods aid in communities and population ecology studies, especially in wetlands that have difficult access during the flood season. The SUB method is effective for revealing the macrophytes that exist in the field. The richness and abundance of the community and, seasonality promote the reproductive success of a large species group (well distributed in aquatic, amphibious and terrestrial species). Notably, this wetland is species-rich community, the composition of the EME and COU methods was close, especially in flood season. Thus, all combination methods: COU and EME and/or EME + SUB showed enough results to reflect the species composition of this wetland. However, we consider that COU is the most complete of the three methods, besides reflecting the total species composition in the vegetation, covering all life cycles and life forms it also aids the predation assessment within the seed bank.

Table 2. Advantages, disadvantages, problems and, suggestions between methods (EME, SUB and COU).

Methods	**Advantages**	**Disadvantages**	**Problems**	**Suggestions**
Emergence of seedlings (EME)	It shows the community of terrestrial and amphibious plants; Better to identify; It allows the creation of a database of images of plants in different stages of life.	It does not show all seedlings, some late or dormant seeds remain in the soil without germinating.	The self-contamination of annual plants that produce fruits quickly;	It is important to transplant seedlings that are not identified, draw and create identification patterns; Make daily counts and remove plants to avoid self-contamination; Make seedling exsiccates to help herbariums in future studies that require identification;
Submerged (SUB)	It shows of the aquatic community; It allows the creation of a database of images of aquatic plants in different stages of life.	It is a complementary method, it does not work alone in temporary ponds, it does not show terrestrial plants.	Many algae appear that must be removed to avoid blocking the entrance of light and take nutrients; Macrophytes show quickly vegetative propagation, e.g., *Helanthium tenellus*.	The water in tanks must be removed weekly to count seedlings and remove plants that have flowers and fruits, to avoid self-contamination; Keep the water level low in the tanks at the beginning to increase the initial germination; Use screens in the tanks to avoid mosquitoes and prevent pollinators.
Seeds Counting (COU)	It reflects the entire plant community (aquatic, terrestrial and amphibious plants); It allows the creation of a database of images of seeds; It makes possible to evaluate seed predation in the seed bank.	It spends so much time and is detailed; Many seeds remain unidentified; The seeds are too small for tetrazolium testing.	Difficult to identify seeds, there are few identification guides and specialists; Some seeds that are the same genus present high similarity in their morphological characteristics, which makes it difficult to separate in species, such as those of the genus *Echinodorus* sp., *Eleocharis* sp. and *Euphorbia* sp.; Insect eggs can confuse seed separation.	It needs a working team to assist in screening and counting seeds; Illustrate or photograph and print the images to find identification patterns; In the beginning, separate all similar seeds and once you are familiar with, you can start counting and identification, it accelerates the process; Do not use 70 % alcohol to store seeds because it can damage the color of the seeds (I suggest 40 % or 50 %).

ACKNOWLEDGEMENTS

The author thanks to the Brazilian governmental agencies CNPq for scholarship and INAU (Instituto Nacional de Ciência e Tecnologia de Áreas Úmidas), for financial support to the project. To Vali Joana Pott and Gisele Catian for assistance on seeds identification.

REFERENCES

Anthony, R. S. 2019. Seed bank and Invertebrate Potential of Moist-Soil Managed Wetland Units in New Mexico and West Texas. Master thesis. College of Agricultural and Natural Resource Sciences of Sul Ross State University.

APG IV. 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Botanical Journal of the Linnean Society, 181, 1–20. DOI: 10.1111/boj.12385

Baldwin, A. H., Egnotovich, M. S., & Clarke, E. 2001. Hydrologic change and vegetation of tidal freshwater marshes: field, greenhouse, and seed-bank experiments. Wetlands, 21, 519–531. DOI: doi.org/10.1672/0277-5212(2001)021[0519:HCAVOT]2.0.CO;2

Bao, F., Pott, A., Ferreira, F. A., & Arruda, R. 2014. Soil seed bank of floodable native and cultivated grassland in the Pantanal wetland: effects of flood gradient, season and species invasion. Brazilian Journal of Botany, 37, 239–250. DOI: doi.org/10.1007/s40415-014-0076-z

Bao, F., Assis, M. A., Arruda, R., & Pott, A. 2015. Effects of *Urochloa humidicola* on plant diversity in native grasslands in a Neotropical wetland. Wetlands, 35, 841–850. DOI: 10.1007/s13157-015-0673-z

Bao, F., Elsey-Quirk, T., Assis, M. A., & Pott, A. 2017. Seed bank of seasonally flooded grassland: experimental simulation of flood and post-flood. Aquatic Ecology, 50, 1–13. DOI: doi.org/10.1007/s10452-017-9647-y

Bao, F., Elsey-Quirk, T., Assis, M.A., Arruda, R., & Pott, A. 2018. Seasonal flooding, topography, and organic debris in interact to influence the emergence and distribution of seedlings in a tropical grassland. Biotropica 1, 1–9. DOI: 10.1111/btp.12550

Bao, F., Elsey-Quirk, T., Assis, M.A., Souza, E.B., & Pott, A. 2020. Do aquatic macrophytes limit the invasion potential of exotic species in Pantanal grassland? Wetlands, 40, 135–142. DOI: doi.org/10.1007/s13157-019-01168-5

Baskin, C. C., Baskin, J. M., & Chester, E. W. 2019. Long-term persistence of summer annuals in soil seed banks of seasonally dewatered mudflats. Plant Ecology, 220, 731–740. DOI: doi.org/10.1007/s11258-019-00948-7

Bell, D. M., & Clarke, P. J. 2004. Seed-bank dynamics of Eleocharis: can spatial and temporal variability explain habitat segregation? Australian Journal of Botany, 52, 119–131. DOI: 10.1071/BT03024

Bernhardt, K. G., Koch, M., Kropf, M., Ulbel, E., & Webhofer, J. 2008. Comparison of two methods characterising the seed bank of amphibious plants in submerged sediments. Aquatic Botany, 88, 171–177. DOI: doi.org/10.1016/j.aquabot.2007.10.004

Boedeltje, G., Heerdt, G. N. J., & Bakker, J. P. 2002. Applying the seedling-emergence method under waterlogged conditions to detect the seed bank of aquatic plants in submerged sediments. Aquatic Botany, 72, 121–128. DOI: doi.org/10.1016/S0304-3770(01)00224-8

Bonis, A., Lepart, J., & Grillas, P. 1995. Seed bank dynamics and coexistence of annual macrophytes in temporary and variable habitat. Oikos 74, 81–92. DOI: 10.2307/3545677

Bornette, G., & Puijalon, S. 2009. Macrophytes: Ecology of Aquatic Plants. In: Encyclopedia of Life Sciences. pp. 1–9 Chichester: John Wiley & Sons, ltd. DOI: 10.1002/9780470015902.a0020475

Boutin, S., Wauters, L. A., McAdam, A. G., Humphries, M. M., Tosi, G., & Dhondt, A. A. 2006. Anticipatory reproduction and population growth in seed predators. Science 314, 1928–1930. DOI: 10.1126/science.1135520

Brewer, C. A., & Parker, M. 1990. Adaptations of macrophytes to life in moving water: upslope limits and mechanical properties of stems. Hydrobiologia 194, 133–142. DOI: doi.org/10.1007/BF00028414

Brock, M. A. 1994. Aquatic vegetation of inland wetlands. In: Groves, R. H. (Ed.). Australian Vegetation. pp. 437-466. Cambridge University Press.

Brock, M. A., Nielsen, D. L., Shiel, R. J., Green, J. D., & Langley, J. D. 2003. Drought and aquatic community resilience: the role of eggs and seeds in sediments of temporary wetlands. Freshwater Biology 48, 1207–1218. DOI: 10.1046/j.1365-2427.2003.01083.x

Brock, M. A. 2011. Persistence of seed banks in Australian temporary wetlands. Freshwater Biology 56, 1312–1327. DOI:10.1111/j.1365-2427.2010.02570.x

Butler, B. J., & Chazdon, R. L. 1998. Species Richness, spatial variation, and abundance of the soil seed bank of a secondary tropical rain forest. Biotropica 30, 214–222. DOI: doi.org/10.1111/j.1744-7429. 1998.tb00056.x

Capon, S. J. 2007. Effects of flooding on seedling emergence from the soil seed bank of a large desert floodplain. Wetlands 27, 904–914. DOI: https://doi.org/ftdmh4

Combroux, I. C., Bornette, G., & Amoros, C. 2002. Plant regenerative strategies after a major disturbance: the case of a riverine wetland restoration. Wetlands 22, 234–246. DOI: doi.org/10.1672/0277-5212(2002)022[0234:PRSAAM]2.0.CO;2

Crawley, M. J. 2000. Seed predators and plant population dynamics. In: Fenner, M. (Ed.). Seeds: the ecology of regeneration in plant communities. pp. 167-182. CABI. DOI: 10.1079/9780851994321.0167

Deepayan S. 2008. Lattice: Multivariate Data Visualization with R. Springer, New York. ISBN 978-0-387-75968-5

Farnsworth, E. J., & Bazzaz, F. A. 1995. Inter- and intra-generic differences in growth, reproduction, and fitness of nine herbaceous annual species grown in elevated CO_2 environments. Oecologia 104, 454–466. DOI: 10.1007/BF00341343.

Gil, A. S. B., & Bove, C. P. 2006. *Eleocharis* R. Br. (Cyperaceae) no estado do Rio de Janeiro, Brasil. Biota Neotropica 7, 163–193. DOI: doi.org/10.1590/S1676-06032007000100020.

Greulich, S., Richard, C., & Marc, V. 2019. Soil seed banks in the floodplain of a large river: A test of hypotheses on seed bank composition in relation to flooding and established vegetation. Journal of Vegetation Science 30, 732–745. DOI: doi.org/10.1111/jvs.12762

Growth, D. 1983. Estudo morfológico das unidades de dispersão e respectivas plantas de seis espécies invasoras da família Cyperaceae. Planta Daninha 5, 25–38. DOI: doi.org/10.1590/S0100-83581983000100005

Guan, B., Chen, M., Elsey-Quirk, T., Yang, S., Shang, W., Li, Y., Tian, X & Han, G. 2019. Soil seed bank and vegetation differences following channel diversion in the Yellow River Delta. Science of the Total Environment 693, 133600. DOI: doi.org/10.1016/j.scitotenv.2019.133600

Harper, J. L. 1977. Population biology of plants. Population biology of plants. London: Academic Press: p. 857.

Hölzel, N., & Otte, A. 2001. The impact of flooding regime on the soil seed bank of flood-meadows. Journal Vegetation of Science 12, 209–218. DOI: 10.2307/3236605

Jarman, N. M., Dobberteen, R. A., Windmiller, B., & Lelito, P. R. 1991. Authenticity: evaluation of created freshwater wetlands in Massachusetts. Ecological Restoration 9, 26–29.

Kaul, R. B. 1978. Morphology of germination and establishment of aquatic seedlings in Alismataceae and Hydrocharitaceae. Aquatic Botany 5, 139–147. DOI: doi.org/10.1016/0304-3770(78)90057-8

Kaul, R. B. 1985. Reproductive phenology and biology in annual and perennial Alismataceae. Aquatic Botany 22, 153–164. DOI: doi.org/10.1016/0304-3770(85)90044-0

Kindt, R., & Coe, R. 2005. Tree diversity analysis. A manual and software for common statistical methods for ecological and biodiversity studies. World Agroforestry Centre (ICRAF), Nairobi, p. 203.

Kissmann, K. G., & Growth, D. 1997. Plantas infestantes e nocivas. Tomo I, 2ªed. São Paulo: BASF, p. 824.

Kissmann, K. G., & Groth D. 1992. Plantas infestantes e nocivas. Tomo II, 2ªed. São Paulo: BASF, p. 798.

Kissmann, K. G., & Groth, D. 1995. Plantas infestantes e nocivas. Tomo III, 2ªed. São Paulo: BASF, p. 683.

Kohagura, T. D. C., Souza, E. B. D., Bao, F., Ferreira, F. A., & Pott, A. 2020. Flood and fire affect the soil seed bank of riparian forest in the Pantanal wetland. Rodriguésia 71, e00052018. DOI: doi.org/10.1590/2175-7860202071013

Leck, M. A., & Simpson, R. L., 1987. Seed bank of a freshwater tidal wetland: turnover and relationship to vegetation change. American Journal of Botany 74, 360–370. DOI: 10.2307/2443812

Mcfarland, D. G., & Shafer, D. J. 2011. Protocol considerations for aquatic plant seed bank assessment. Journal of Aquatic Plant Management 49, 9–19.

Oksanen, J. F., Blanchet, G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E. & Wagner, Z. H. 2017. Vegan: Community Ecology Package. R package version 2, 4–3. https://CRAN.R-project.org/package=vegan

Oliveira, P. C., Torezan, J. M., & Nunes da Cunha, C. 2015. Effects of flooding on the spatial distribution of soil seed and spore banks of native grasslands of the Pantanal wetland. Acta Botanica Brasilica 29, 400–407. DOI: dx.doi.org/10.1590/0102-33062015abb0027

Pott, A., & Pott, V. J. 1994. Plantas do Pantanal. Brasília. EMBRAPA, Corumbá. p. 320.

Pott, V. J., & Pott, A. 2000. Plantas aquáticas do Pantanal. EMBRAPA, Corumbá. p. 353.

Pott, A., & Silva, J. S. V. 2015. Terrestrial and aquatic vegetation diversity of the Pantanal Wetland. In: Bergier, I. & Assine M. L. (Eds.). Dynamics of the Pantanal Wetland in South America. pp. 111-151. Springer International Publishing Switzerland.

R core team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.r-project.org/about.html

Sanou, L., Savadogo, P., Zida, D., & Thiombiano, A. 2019. Contrasting land use systems influence soil seed bank composition and density in a rural landscape mosaic in West Africa. Flora, 250, 79–90. DOI: doi.org/10.1016/j.flora.2018.11.013

Silva, J. S. V. & Abdon, M, M. 1998. Delimitação do Pantanal Brasileiro e suas sub regiões. Pesquisa Agropecuária Brasileira 33, 1703–1711.

Simpson, R. L., Leck, M. A. & Parker, V. T. 1989. Seed banks: general concepts and methodological issues. In: Leck, M. A., Parker, V. T. & Simpson, R. L. (Eds.). Ecology of Soil Seed Banks. pp. 3-9. San Diego: Academic Press.

Simpson, G. L. 2016. Permute: Functions for Generating Restricted Permutations of Data. R package version 0.9-4. URL CRAN.R-project.org/package=permute

Solbreck, C., & Knape, J. 2017. Seed production and predation in a changing climate: new roles for resource and seed predator feedback? Ecology 98, 2301–2311. DOI: doi.org/10.1002/ecy.1941

Souza, D. K. L., & Giulietti, A. M. 2014. Flora da Bahia: Pontederiaceae. Sitientibus-série Ciências Biológicas 14, 1–10.

Souza, E. B., Ferreira, F. A., & Pott, A. 2016. Effects of flooding and its temporal variation on seedling recruitment from the soil seed bank of a Neotropical floodplain. Acta Botanica Brasilica 31, 64–75. DOI: doi.org/10.1590/0102-33062016abb0202

Thompson, K., Grime, J. P., & Mason, G. 1977. Seed germination in response to diurnal fluctuations of temperature. Nature 267, 147–149. DOI: doi.org/10.1038/267147a0

Thompson, K., Bakker, J. P. & Bekker, R. M. 1997. The soil seed banks of North West Europe: methodology, density, and longevity. Cambridge University Press, Cambridge, UK. p. 267.

Tuckett, R. E., Merritt, D. J., Hay, F. R., Hopper, S. D., & Dixon, K. W. 2010. Dormancy, germination, and seed bank storage: a study in support of ex situ conservation of macrophytes of southwest Australian temporary pools. Freshwater Biology 55, 1118–1129. DOI: doi.org/10.1111/j.1365-2427.2010. 02386.x

Yang, W. 2020. Assessment of Health Status of Lake Wetland by Vegetation-Based Index of Biotic Integrity (V-IBI). In: Wang, Y. (Ed.). Wetlands and Habitats. pp. 213–223. Boca Raton: CRC Press.

van der Valk, A. G., & Davis, C. B. 1978. The role of seed banks in the vegetation dynamics of prairie glacial marshes. Ecology 59, 322–335.

Supplementary Material:

Table S1. List of species sampled in the soil seed bank (values of total abundance per specie), classified regarding life cycle (annual - A and perennial plants - P), growth forms (aquatic - Aq, amphibious – Am and terrestrial - T) and, methods (EME, SUB and COU), in the sub-region Abobral in the Pantanal wetland, MS, Brazil.

Figure S1. Seasonally flooded grassland in the Pantanal wetland (Central-West Brazil). (**a**) Sampling points including eight seasonal ponds (P1, P2, P3 and P4 in grassland dominated by *Urochloa humidicola*, and P5, P6, P7 and P8 in native grassland), (**b**) representation of the transects following the topographic levels (low, mid and high) in each sampled pond, with five random samples, (**c**) mean monthly level of the Miranda River, arrows showing the sampled seasonal periods (post-dry and post-flood), between the years 2005-2015.

Figure S2. Collection process at Fazenda São Bento in the Abobral subregion, Pantanal, MS, Brazil. (**a**) Sample delimited by 20 x 20 cm, (**b**) Soil collected with the aid of a shovel, (**c**) Seed bank soil 3cm deep, (**d**) Soil stored in a plastic bag to be transported.

Figure S3. Method I: seedling emergence in a greenhouse (EME) (**a**-**g**) trays arranged, (**h**) seedling emergence, (**i**) Leguminosae seedling and (**j**) *Pontederia subovata* seedling.

Figure S4. Method II: seedling emergence with submersion of 30 cm of water (SUB) (**a**) Tanks containing trays with soil, (**b**) trays with seedling emergence, (**c**) *Nymphaea gardneriana* Planch. (**d**) *Sagittaria guayanensis* Kunth (**e**) *Heteranthera limosa* (Sw.) Willd. (**f**) *Limnocharis flava* (L.) Buchenau.

Figure S5. Method III: Seed counting (COU) (**a**), (**b**) Sieves used for soil washing in mesh sizes 0.50, 0.35 and 0.25 mm. (**c**), (**d**) Seeds in pots with 70 % alcohol. (**e**), (**f**) Plate used for screening, counting and identification with the aid of a stereoscopic microscope.

Submitted: 15 May 2020
Accepted: 12 August 2020
Published on line: 01 September 2020
Associate Editor: Bianca Andrade