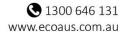
Crinolyn and Windella Ramsar Sites – Soil Seedbank Assessment 2023

NSW Department of Planning and Environment – Environment and Heritage Group





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Template 2.8.1

Executive summary

This report documents a soil seedbank assessment of the Crinolyn and Windella Ramsar sites, to complement field vegetation surveys and mapping undertaken from 12 April to 16 April 2023 and detailed in the report *Vegetation survey and mapping of Crinolyn and Windella Ramsar sites 2023* by Eco Logical Australia (ELA 2023).

Soil samples were collected during field vegetation surveys from six plots within the Crinolyn Ramsar site and six plots within the Windella Ramsar site and transported to a glasshouse facility in south-east Queensland for germination.

The germination trial was conducted in the glasshouse over 18 weeks from June until October 2023. Soil samples from each plot were distributed between two experimental trays, with one tray subjected to a submerged treatment whilst the other tray subjected to a damp (i.e. regularly watered) treatment for the duration of the experiment. Emerging seedlings were harvested and recorded once identification was possible. Where necessary, some seedlings were re-potted into a deeper substrate to further develop until such time that they could be identified.

Total seedling abundance and species richness obtained from each taxa were calculated for each experimental container and each plot. Data was visually inspected to describe patterns in soil seedbank composition and structure between plots and between the Crinolyn and Windella Ramsar sites. Species recorded from the soil seedbank were also compared to those observed during field vegetation surveys to investigate likely contributions of the soil seedbank to vegetation dynamics.

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Abbreviations

Abbreviation	Description
ADS40	Airborne Digital Sensor 40
CRIN	Crinolyn
DPE-EHG	NSW Department of Planning and Environment – Environment and Heritage Group
ELA	Eco Logical Australia
ha	Hectare
NSW	New South Wales
РСТ	Plant Community Type
RBGS	Royal Botanic Gardens Sydney
SSB	Soil Seedbank
WIND	Windella

Introduction

Persistent soil seedbanks typically represent a significant component of the floristic diversity in temporary wetlands and floodplains, harbouring propagules of many plant species through periods of unfavourable conditions (e.g., floods and droughts). In dryland wetlands, a high diversity of plant species often establishes from soil seedbanks in the damp conditions which follow the drawdown of floodwaters (Capon et al. 2016). It is generally expected that fewer species tend to germinate from soil seedbanks during submerged conditions although some aquatic species can require full inundation to trigger germination. Investigating soil seedbanks in such wetlands enables a more complete floristic assessment of the vegetation, since many species may be absent from the extant flora recorded during single-event field surveys. Soil seedbank assessments thus provide an indication of past extant vegetation communities, as well as their potential responses to future conditions.

This report accompanies a more detailed report (ELA 2023) of the vegetation and floristics of the Crinolyn and Windella Ramsar sites of the lower Gingham Watercourse in the Gwydir Wetlands west of Moree, New South Wales (NSW). Soil samples were collected from six field survey plots (Figure 1-2) to enable an assessment of the covert, in addition to the overt, flora present at each Ramsar site. The key objectives of this assessment were to:

- Determine the composition and structure of the soil seedbank in each plot and Ramsar site
- Describe spatial variability in soil seedbank composition between plots and Ramsar sites
- Compare soil seedbank assemblages to extant vegetation assemblages recorded during field surveys.

Results are intended to help inform environmental water management for both the Crinolyn and Windella Ramsar sites by providing an indication of flora species within the soil seedbank and their response to watering (both damp and submerged) treatments.

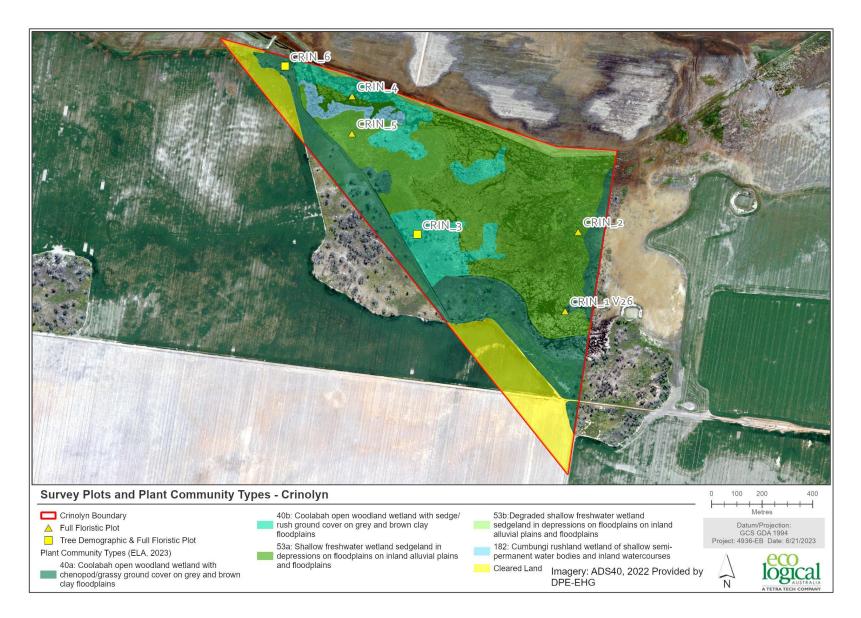


Figure 1: Crinolyn full floristic and tree demographic / full floristic plot locations. Imagery captured August 2022.

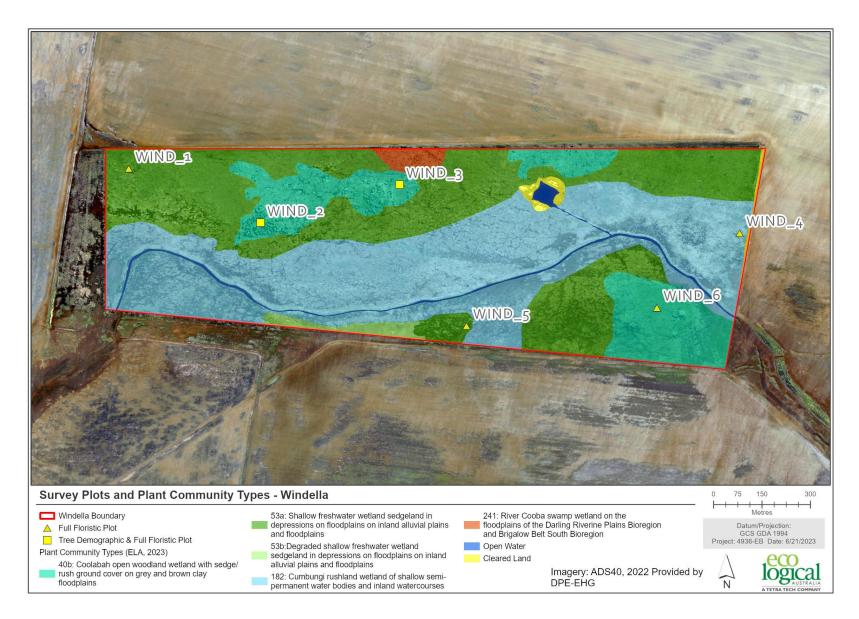


Figure 2: Windella full floristic and tree demographic / full floristic plot locations. Imagery captured August 2022.

Methods

Sample collection

Soil seed bank samples were collected during field surveys undertaken in April 2023 from six plots in Crinolyn Ramsar site and six plots in Windella Ramsar site (Table 1; Eco Logical Australia 2023). Plots were distributed across several Plant Community Types (PCTs) present within both Ramsar sites. Within each plot, five soil sub-samples were collected from each of the four corners and the centre of each plot and aggregated together in a single sample bag for transport to the glasshouse. Soil samples collected at all sites were dry at the time of sampling.

Site	Plot No.	PCT No.	Extant vegetation survey type
Crinolyn	CRIN_1 V26	53a	Full floristic 20 m x 20 m (0.04 ha)
	CRIN_2	53a	Full floristic 20 m x 20 m (0.04 ha)
	CRIN_3	40b	Tree demographic 50 m x 50 m (0.25 ha) / Full floristic 20 m x 20 m (0.04 ha)
	CRIN_4	40b / 53a	Full floristic 20 m x 20 m (0.04 ha)
	CRIN_5	53a	Full floristic 20 m x 20 m (0.04 ha)
	CRIN_6	40a	Tree demographic 25 m x 100 m (0.25 ha) / Full floristic 20 m x 20 m (0.04 ha)
Windella	WIND_1	53a	Full floristic 20 m x 20 m (0.04 ha)
	WIND_2	40b	Tree demographic 50 m x 50 m (0.25 ha) / Full floristic 20 m x 20 m (0.04 ha)
	WIND_3	40b	Tree demographic 50 m x 50 m (0.25 ha) / Full floristic 20 m x 20 m (0.04 ha)
	WIND_4	182	Full floristic 20 m x 20 m (0.04 ha)
	WIND_5	53a / 182	Full floristic 20 m x 20 m (0.04 ha)
	WIND_6	40b	Full floristic 20 m x 20 m (0.04 ha)

Table 1: Crinolyn and Windella survey plots

Germination trial

A germination trial was conducted in a glasshouse facility in south-east Queensland over 18 weeks from June until September 2023. Aggregated soil samples from each plot were distributed between two clear plastic trays (17cm x 12cm x 3.5cm), each with four drainage holes in the bottom, to a depth of 3 cm. For each plot, one of these experimental trays was placed inside a larger foil container to enable flooding of the soil. Each of the two experimental trays were then subjected to one of two watering regimes for the duration of the experiment: 1.) damp (i.e. regularly watered - once per week) or 2.) submerged to a depth of 2 cm above the soil line using the larger foil container. A total of 24 trays were prepared, including 12 trays for damp treatment and 12 trays for submerged treatment. Water delivery to samples was automated to ensure soils did not dry out for damp treatments and to maintain 2 cm inundation depth for submerged treatments, with samples visited by staff at least once per week throughout the duration of the trail.

During the experiment, emerging seedlings were harvested and recorded once identification was possible. Where necessary, seedlings were re-potted into a deeper substrate to further develop until identification was possible.

Data analysis

Species of emerging seedlings were initially assigned as either native or exotic according to information provided by <u>NSW Flora Online (RBGS, 2023)</u>. Total seedling abundance and the species richness (native, exotic and overall) based on each taxa were calculated for each experimental container and each plot. Data were visually inspected to describe patterns in soil seed bank composition and structure between plots and Ramsar sites. A comparison of species recorded from the soil seed bank and during field vegetation surveys was also conducted to investigate the likely contribution of the soil seed bank to vegetation dynamics.

Limitations

The species identifications were limited by the growth of the seedlings and in some cases relied on growth of flowers and/or seed and fruiting material. For this reason, the trial duration was extended from 12 to 15 and then finally 18 weeks. Where identification to the species level was not possible, genus or family was recorded. Where seedlings died or did not grow to a stage where formal identification was possible, these individuals were included in the total abundance calculations but excluded from the species richness data analysis.

Results

Soil seed bank species richness

At least 989 seedlings representing 25 species from 17 families emerged from soil seedbanks (SSBs) during the glasshouse experiment (Table 2). Of these, 84% were native species. The most frequently occurring native species was *Juncus aridicola*, which emerged from 20 experimental trays (twelve damp conditions, eight submerged conditions), with the native grass *Diplachne fusca*, emerging from thirteen experimental trays (eight damp conditions, five submerged conditions). The exotic species *Rorippa palustris* was the most frequently occuring exotic species, emerging from three experimental trays (two damp conditions, one submerged conditions).

Nine (36%) species only emerged from a single plot (i.e., damp and submerged combined; Table 2) and were not recorded in any other sample plot. The number of species emerging overall from samples was higher for the Crinolyn Ramsar site (22 species) compared to the Windella Ramsar site (17 species).

The highest species richness emerged from experimental trays which were under submerged conditions, however, both treatments had relatively similar species richness overall (with differences of one or two additional species between the treatments) (Table 2). Mean species richness was also similar for both treatments which for damp treatment was 4.7 species and for submerged treatment was 4.8 species.



Plate 1: Sample CRIN_CS2 (submerged treatment) showing high species richness for the SSB experiment (a total of six native species germinating).

Total species richness of seedlings germinating from each plot (i.e., damp and submerged combined) ranged from two to seven species (Figure 3). WIND_1 and WIND_5 had the highest species richness of emerging seedlings overall, while the lowest species richness was recorded from CRIN_1 and WIND_3. Mean species richness per plot was generally similar for the two Ramsar sites, where the Crinolyn Ramsar site mean was 4.6 and Windella Ramsar site mean was slightly higher at 4.9.

The number of native species emerging ranged from one to six between plots, with four plots recording the maximum native species richness of six (CRIN_4, WIND_1, WIND_2 and WIND_5) (Figure 2). The number of exotic species emerging ranged from zero to two between plots and was highest at plot CRIN_5 (Figure 3). There was no correlation between native and/or exotic species richness per plot and the PCT in which the plot was located.

Table 2: Species present in the SSB at Crinolyn and Windella Ramsar sites. N.B. * indicates exotic species. Symbols indicate if a species emerged from this plot's sample under either regularly watered (damp) conditions (■), submerged conditions (△) or both (●).

			Emerged	from Crino	lyn SSB				Emerged	from Wind	lella SSB			
Species	Family	Common name	CRIN_1 V26	CRIN_2	CRIN_3	CRIN_4	CRIN_ 5	CRIN_6	WIND_ 1	WIND_ 2	WIND_ 3	WIND_ 4	WIND_ 5	WIND_ 6
Alternanthera denticulata	Amaranthaceae	Lesser joyweed		Δ	•	•			•			•		•
Ammannia multiflora	Lythraceae	Jerry-jerry						Δ		Δ			Δ	
Asteraceae sp.	Asteraceae	-												
Bulbine bulbosa	Asphodelaceae	-									Δ			
Cyperus difformis	Cyperaceae	Dirty dora	Δ	Δ	Δ	Δ	Δ	Δ	Δ					
Damasonium minus	Alismataceae	Starfruit								Δ				
Diplachne fusca	Poaceae	Brown beetle grass		•	•	•	Δ		•	•	•	Δ	•	•
Dysphania pumilio	Amaranthaceae	Small crumbweed		•				•						
Echinochloa crus- galli*	Poaceae	Barnyard grass					•							
Einadia nutans	Amaranthaceae	Climbing saltbush						•						
Eleocharis plana	Cyperaceae	-												
Juncus aridicola	Juncaceae	-	•		•	•		•	•			•	•	•
Ludwigia octovalvis	Onagraceae	-	Δ	•		Δ		Δ				Δ	Δ	
Lythrum hyssopifolia	Lythraceae	-				•			•	•			•	•
Medicago polymorpha*	Fabaceae	Burr medic					•							

			Emerged	from Crinolyn SS	5B		Emerge	d from Winde	ella SSB		
Myriophyllum sp.	Haloragaceae	-		•	•				•		
Peplidium sp.	Phrymaceae	-			•						
Phyla canescens*	Verbenaceae	-									
Portulaca oleracea	Portulacaceae	-		Δ							
Pseudognaphalium luteoalbum	Asteraceae	-				•					
Ranunculus undosus	Ranunculaceae	Swamp buttercup		Δ							
Rorippa palustris*	Brassicaceae	Yellow cress		Δ							
Rumex sp.	Polygonaceae	Dock						Δ		Δ	•
Solanum esuriale	Solanaceae	Quena	Δ								
Trianthema sp.	Aizoaceae	-		Δ							
Wahlenbergia sp.	Campanulaceae	-									

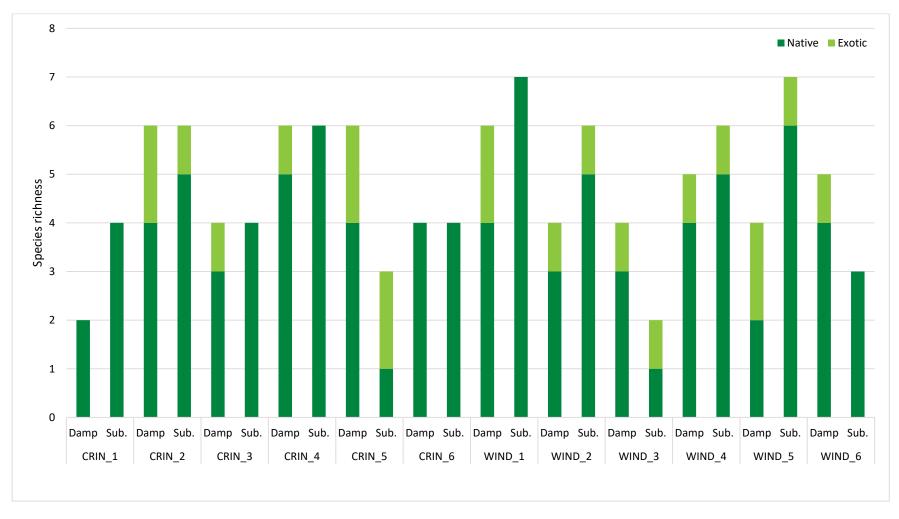


Figure 3: Total species richness (native and exotic) for each plot. NB. Sub. = Submerged.

Soil seedling abundance

The abundance of seedlings emerging overall from samples was slightly higher for the Crinolyn Ramsar site (total seedlings 530) than the Windella Ramsar site (total seedlings 459).

The experimental trays which showed the highest abundance of seedling germination were from samples exposed to the submerged conditions (Table 2). This was generally shown by three samples with the highest abundance of seedlings under submerged conditions (with >90 seedlings), which was related to the presence of *Cyperus difformis* and/or *Juncus aridicola* in these samples.

Total abundance of seedlings germinating during the experiment at the time of harvest ranged from 6 to 103 and varied between plot samples (Figure 4 and Plate 2).





Plate 2: Variation in seedling abundance from highest total seedlings in WIND_1 (submerged treatment) (left) and low total seedling abundance in CRIN_1 (damp treatment)

The highest number of seedlings (>50 seedlings) overall, emerged from samples collected from plots CRIN_2 and WIND_1, with the lowest number emerging from plot WIND_3 (6 seedlings) and CRIN_1 (8 seedlings). Both CRIN_2 and WIND_1 plots were located in the same PCT (PCT 53: Shallow freshwater wetland sedgeland in depressions on floodplains on inland alluvial plains and floodplains), however, there was no other correlation between PCT and seedling abundance amongst plots. Mean seedling abundance per plot was similar for the two Ramsar sites, where Crinolyn recorded a mean of 44 seedlings per site and Windella recorded a mean of 38 seedlings per site.

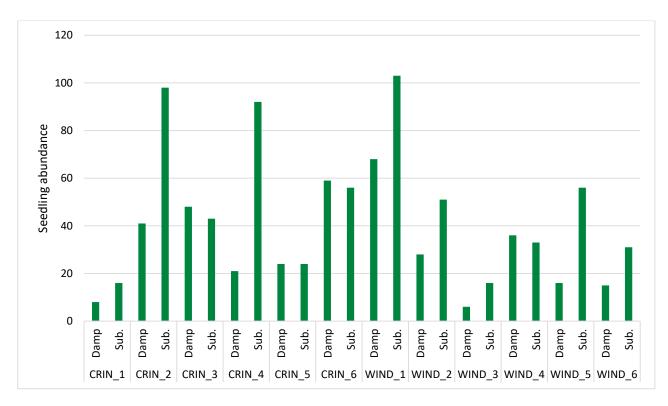


Figure 4: Total seedling abundance for each plot. NB. Sub. = Submerged.

Soil seedbank composition and structure

A total of 83 species were recorded from both the extant vegetation survey and the soil seedbank samples. Of these, 58 species were only observed from the extant vegetation survey, and not observed throughout the duration of the soil seedbank germination trial. Whilst this could indicate that the soil seedbank does not contain reproductive material for these species, it is more likely that seed is present and simply did not germinate during the trial. This may be a result of samples not experiencing the right conditions for these seeds to grow or being unable to grow sufficiently during the trial to allow for identification.

Seven species were only present in the soil seedbank and not previously recorded during field vegetation surveys. These species were all native and mostly comprised herbs/forbs. The unidentified Asteraceae sp. did not contain any reproductive material to aid in identification to the species level (nor to determine whether it was native or exotic) and this species, therefore, has the potential to have been recorded within the existing extant vegetation list. Species that were recorded within the soil seedbank during the glasshouse experiment, that had not previously been recorded in the extant vegetation are listed below:

- Asteraceae sp. (Crinolyn and Windella)
- Bulbine bulbosa (Crinolyn and Windella)
- Lythrum hyssopifolia (Crinolyn and Windella)
- Myriophyllum sp. (Crinolyn and Windella)
- Peplidium sp. (Crinolyn and Windella)
- Pseudognaphalium luteoalbum (Crinolyn and Windella)
- Wahlenbergia sp. (Crinolyn only).

The SSB samples from plots within the Crinolyn Ramsar site expressed 14 species that have been previously recorded in the extant vegetation species list for the Crinolyn site and one species (*Echinochloa crus-galli*) that had been recorded in the Windella Ramsar site extant vegetation, but not at Crinolyn. The soil seedbank samples from plots within the Windella Ramsar site expressed 10 species that have been previously recorded in the extant vegetation species list for the Windella site and two species (*Damasonium minus* and *Diplachne fusca*) that had been recorded in the Crinolyn Ramsar site extant vegetation, but not at Windella.

Table 3: Species observed in the extant vegetation survey and the SSB samples at Crinolyn and Windella Ramsar sites. N.B. * indicates exotic species. Symbols indicate if a species was observed for this Ramsar site only in the extant vegetation during field surveys (\blacksquare), only from the SSB during the glasshouse experiment (\triangle) or was recorded from both (\bullet).

Species	Crinolyn	Windella
Alternanthera denticulata	•	•
Alternanthera nodiflora	-	•
Amaranthus sp.		-
Ammannia multiflora	•	•
Asteraceae sp.	Δ	Δ
Aster subulatus*	•	•
Boerhavia dominii		-
Bulbine bulbosa	Δ	Δ
Centipeda cunninghamii	-	
Convolvulus sp.		-
Conyza bonariensis*		-
Cucumis myriocarpus subsp. leptodermis*	•	-
Cynodon dactylon		-
Cyperus bifax	•	-
Cyperus difformis	•	•
Cyperus sp.	•	•
Damasonium minus		Δ
Digitaria sp.	•	-
Diplachne fusca	•	Δ
Duma florulenta	•	•
Dysphania pumilio	•	•
Echinochloa crus-galli*	Δ	•
Einadia nutans	•	-
Eleocharis plana	•	•
Eleocharis pusilla		
Enchylaena tomentosa	•	-
Enteropogon acicularis	•	-
Eucalyptus coolabah	•	•

Euphorbio drummondiiIHalioragis glouca-Heliotropium europaeum*ILachnagrostis filformisILachnagrostis filformisILepidium pseudohyssopifolumILepidium sp.ILepidium sp.ILudwigia pepioides subsp. montevidensisIMolva parvilfora*IMolva parvilfora*IMolva parvilfora*IMolva parvilfora*IMolva parvilfora*IPapidium sp.IMolva parvilfora*IPapidium sp.IMolva parvilfora*IPapidium sp.IMolva parvilfora*IPapidium sp.IPapidium sp.IPapidium jubiforumIPapidium jubiforumIPapidium jubiforumIPapidium sp.IPapidium sp.IPapi	Species	Crinolyn	Windella
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Heliotropium europaeum*ILucus aridicolo9Lachagrostis filiformis9Lepidium pseudohyssopifolum9Lepidium sp.9Ludwigia peploides subsp. montevidensis9Ludwigia peploides subsp. montevidensis9Malva parviffora*9Malva parviffora*9Malva parviffora*9Marsileo drummondii9Mediago polymorpha*9Oenothera stricta*9Panicum decompositum9Paspalum distichum9Paspalum distichum9Persicaria sp.9Phyla canescens*9Phyla canescens*9Portulace acea9Portulace acea9Ranunculus pumilio-Ranunculus pumilio-Rumex brownii-Rumex brownii-Rumex spSolsol tragus-Solsol tragus-Solsol tragus-Solsol tragus-Solsol tragus-Solsol tragus-Solsol tragus-Solsol tragus	Euphorbia drummondii	•	•
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Ranunculus undosus•Rapistrum rugosum*•Rorippa palustris*•Rumex brownii•Rumex sp.•Salsola tragus•	Pseudognaphalium luteoalbum	Δ	Δ
Rapistrum rugosum*Image: Compage: Compage	Ranunculus pumilio	-	•
Rorippa palustris*••Rumex brownii••Rumex sp.••Salsola tragus••	Ranunculus undosus	•	•
Rumex browniiImage: Comparison of the second se	Rapistrum rugosum*	•	-
Rumex sp.•Salsola tragus-	Rorippa palustris*	•	•
Salsola tragus -	Rumex brownii	•	
	Rumex sp.	•	•
	Salsola tragus	•	-
Sclerolaena bicornis var. horrida -	Sclerolaena bicornis var. horrida	•	-
Sclerolaena birchii -	Sclerolaena birchii	•	-

Species	Crinolyn	Windella
Sclerolaena muricata	•	•
Sesbania cannabina	•	
Sida corrugata	•	
Sida trichopoda	•	-
Solanum esuriale	•	-
Solanum nigrum*	•	•
Sonchus oleraceus*		
Sporobolus caroli	•	-
Stellaria angustifolia		-
Tetragonia tetragonioides	•	-
Tribulus terrestris*	•	-
Typha domingensis	•	
Urochloa panicoides*	-	
Vachellia farnesiana	•	
Verbena gaudichaudii		
Verbena supina*		
Wahlenbergia sp.	Δ	-
Xanthium occidentale*		
Xanthium spinosum*	•	-

Conclusion

Soil seedbank species richness and abundance

At least 989 seedlings representing 25 species from 17 families emerged from SSBs during the germination trial, the majority of which were native species (84%).

Slightly higher species richness emerged from experimental trays which were under submerged conditions, however, both treatments had relatively similar species richness overall, with mean species richness of 4.7 and 4.8 species for damp vs submerged treatments, respectively. Mean species richness per plot was also generally similar for the two Ramsar sites, where the Crinolyn Ramsar site mean was 4.6 species and the Windella Ramsar site mean was 4.9 species.

The abundance of seedlings emerging overall from samples was slightly higher for the Crinolyn Ramsar site (total seedlings 530) than the Windella Ramsar site (total seedlings 459). Total abundance of seedlings germinating during the experiment at the time of harvest ranged from 6 to 103 and varied considerably between plot samples. The two highest abundance of seedlings emerged from plots WIND_1 (171 seedlings) and CRIN_2 (139 seedlings) which were both located within PCT 53, however, no other PCT-related correlations were evident. Mean seedling numbers per plot was similar for the two Ramsar sites, where the Crinolyn sites recorded a mean of 44 seedings per site, whilst Windella sites had a mean of 38 seedlings per site.

Mean seedling abundance was considerably higher for submerged treatment samples (52 seedlings per plot), compared to damp treatment samples (31 seedlings per plot). When compared to mean species richness results from both sets of treatments, these results indicate that submerging the seedbank increases relative seedling abundance without an associated increase in species diversity. This result correlates with data from field surveys which indicated relatively high vegetated ground cover and relatively low floristic diversity.

Soil seedbank contribution to vegetation dynamics

83 species were recorded from both the extant vegetation field survey and the soil seedbank germination trial. Of these, 58 species were only observed from the extant vegetation field survey and not observed in the soil seedbank germination trial. Seven species (including six identifiable native species) were recorded from the SSB samples, whilst not being recorded in the extant vegetation field surveys. The recording of these species in this study highlights the importance of sampling the SSB and the value that SSB germination trials can provide in enhancing understanding of the floristic composition and vegetation dynamics of dryland wetland vegetation communities, such as those present at the Crinolyn and Windella Ramsar sites.

Implications for vegetation resilience

The soil seedbank for the Crinolyn and Windella Ramsar sites provides sources of propagules for regeneration of vegetation following disturbances including drought and flood. The results of the soil seedbank experiment identified 25 species from 17 families within the SSBs across the two Ramsar sites, the majority of which were native species (84%). This does, however, represent only a proportion (30%) of the total cumulative species lists for the two sites recorded from both extant vegetation field surveys and species identified from the seedbank trials.

Within the limitations of the experimental design, the two soil treatments (damp and submerged) had relatively similar species richness overall, however, submerged treatments recorded considerably higher overall and mean seedling abundance. This indicates that the species that germinated were responsive to some level of water treatment and resilient to both fully submerged and frequently watered conditions. Given this, the results of the seedbank trial indicate the presence of a predominantly native floristic seedbank within the Crinolyn and Windella Ramsar sites, which is responsive to water treatment, such as the delivery of environmental water to these sites.

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