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Comparison of seedling emergence and seed extraction techniques for estimating the composition of soil seed banks

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Summary

1. Characterization of the seed bank is one of the most important demographic assessments that can be undertaken for a plant community. Overlapping generations, evidence of past above-ground vegetation and histories of invasion and disturbance are recorded in the seed bank.

2. Two broad approaches have been used to elucidate seed bank components – sifting-sorting techniques and germinability assays. The utility of these approaches varies with community type and habitat although a common theme among studies has been the quest for an efficacious method. Here, we compare the two approaches for semi-arid ephemeral wetlands: seed extraction through flotation and seedling emergence.

3. Species composition of the soil seed bank differed dramatically depending on the technique, with only 19 species common to both methods and a total of 66 species detected using both procedures.

4. Both techniques provided similar estimates of seed density and species richness of the seed bank in the top 5 cm of soil. However, samples collected from 5 cm to 20 cm had lower seed densities using the flotation technique than with the seedling emergence technique.

5. Differences in seed detectability between the two approaches may be related to seed size, seed dormancy and specific germination requirements.

6. The community composition of soil seed banks for ephemeral wetlands depends on the choice of technique.

Key-words: functional groups, *Phyla canescens*, seed size, species composition

Introduction

Several methods have been used to estimate the density and composition of soil seed banks, and there has been much debate on the utility of the different approaches (e.g. Bernhardt *et al.* 2008; Brown 1992; Gross 1990). The 'seedling emergence method', which involves spreading a thin layer of soil over a sterilized medium in a glasshouse, and identifying and counting emerging seedlings, is commonly used (Ter Heerdt *et al.* 1996; Ter Heerdt, Schutter, & Bakker 1999). This method gives a good indication of the readily germinable seed bank but does not provide a complete assessment of the seed bank flora unless the soil sample is maintained for extended periods of time at simulated habitat conditions (Thompson & Grime 1979). Indeed, the seedling emergence method can dramatically underestimate the density of the seed bank due to errors associated with seed dormancy and specific environmental requirements for germination (Brown 1992; Bernhardt *et al.* 2008; Wright & Clarke 2009). This may be particularly so for wetland species, in which emergence from the soil seed bank is largely determined by the water regime (Casanova & Brock 2000; Crossle & Brock 2002).

A second method, regarded as providing a better estimate of total seed bank densities, is seed extraction from the soil (Malone 1967; Brown 1992). This can be achieved through 'rinsing', flotation in a salt solution or other high specific gravity liquid, or a combination of both (Malone 1967; Gross 1990; Devictor, Moret, & Machon 2007; Bernhardt *et al.* 2008). Seeds are then hand sorted under a microscope and identified. Seed extraction is not commonly used as it is time consuming, ineffective at finding small seeded species and may overestimate the viable seed bank by including non-viable seeds (Thompson, Bakker, & Bekker 1997; Baskin & Baskin 1998). Also, depending on when samples are collected, seeds may lose viability before the next germination season, i.e. if you

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determine the seed content several months before the next germination season, you do not know if these seeds would have survived until the germination season.

Studies comparing these two methods have found contrasting results (Johnson & Anderson 1986; Brown 1992). Seed extraction methods detected greater seed densities and species richness of a forest soil seed bank compared with seedling emergence techniques (Brown 1992). Johnson & Anderson (1986) reported similar estimates for seed density but greater species richness of the soil seed bank with the emergence method. For wetland studies, Poiani & Johnson (1988) detected higher seed densities with the extraction method but similar species richness, and Bernhardt *et al.* (2008) found that the seedling emergence method failed to detect nearly 90% of all germinable seeds found via the 'rinsing method'. Despite large differences between the two approaches, seedling emergence is the most commonly used method and to our knowledge the only one that has been used for ephemeral wetlands in Australia (Brock, Theodore, & O'Donnell 1994; Capon & Brock 2006; Robertson & James 2006; Porter, Kingsford, & Brock 2007).

Since no studies have compared seed extraction with seedling emergence for semi-arid ephemeral wetlands in Australia, it is unknown whether the commonly used seedling emergence technique is sufficient for detecting the composition and density of soil seed banks. What we do know however, is that only a fraction of viable seeds germinated in response to one flood event in soil samples from an ephemeral wetland in South Africa (Brock & Rogers 1998) that emergence is largely determined by the water regime used (Casanova & Brock 2000; Crossle & Brock 2002; Nicol, Ganf, & Pelton 2003; Robertson & James 2006), and is strongly seasonal (Britton & Brock 1994). Hence, to gain an accurate estimate of the complete soil seed bank for wetlands, seedling emergence trials should incorporate a range of watering treatments and seasonal conditions, i.e. requiring much glasshouse space and time.

The density of viable seeds usually decreases with depth in non-tilled soils (Thompson *et al.* 1997) and consequently in most seed bank studies, samples are taken from the soil surface to around 10 cm depth. Recent studies have reported that for vertic soils seasonal drying and cracking can influence the vertical distribution of seeds in the soil seed bank (Espinar, Thompson, & Garcia 2005; Espinar & Clemente 2007). Species with small diaspores and those whose seed is shed during dry periods (when cracks are open) were found to have increased seed densities at 12–16 cm depth in the soil (Espinar *et al.* 2005; Espinar & Clemente 2007). The current study was conducted in semi-arid ephemeral wetlands on cracking clay soils, and hence we were also interested in the vertical distribution of the soil seed bank.

The aim of the current study was to compare seed extraction with seedling emergence for assessing the composition, richness and density of the soil seed bank at different soil depths of some semi-arid ephemeral wetlands in Australia. We were also particularly interested in finding the best method for estimating the seed bank of an important wetland weed (*Phyla canescens*, Verbenaceae) in these communities.

Materials and methods

STUDY AREA

The study was conducted in the Lower Gwydir Wetlands (29°19′S, 149°17′E) and Macquarie Marshes (30°30′S, 147°28′E) in western NSW, Australia. The climate is semi-arid with an average annual rainfall of 600 mm in the Gwydir Wetlands and 445 mm in the Macquarie Marshes, with most of this falling in summer (Bureau of Meteorology, unpublished data). Samples were collected from two properties – 'Old Dromana' in the Gwydir Wetlands and 'Wilgara' in the Macquarie Marshes, in winter (June) 2007. Both wetlands are temporary and subject to substantial water level fluctuations and an extended dry period had occurred prior to sampling. Vegetation was dominated by *Paspalum distichum*, *Phyla canescens*, *Eleocharis plana* and *Eleocharis sphacelata*. Soils are clays that crack deeply on drying.

SOIL SEED BANK SAMPLING

Sampling was stratified into two different hydrological areas ('wet' and 'dry') within each property, which was based on landholder knowledge. The 'wet' sites were generally inundated annually either from natural flooding or environmental flow releases and the 'dry' sites less frequently (*c.* every 5 years). Within each of these four sites (i.e. Old Dromana 'wet', Old Dromana 'dry', Wilgara 'wet' and Wilgara 'dry') 20, 2 × 2 m quadrats were sampled. In this study, we were interested only in the comparison of the two techniques; differences in soil seed banks in relation to hydrology and flooding is the subject of another paper. In each quadrat, five soil cores (5 cm diameter) were collected randomly and carefully fractionated (to avoid contamination) into depth categories of 0–5, 5–10 and 10–20 cm. Soil samples were then transported to the laboratory, air dried and stored at room temperature until use. The five cores from each quadrat were bulked within depth categories, mixed well and divided into two subsamples (using a soil cutter) of 100 g each, with 100 g allocated to each trial.

SEED EXTRACTION TRIAL

Seeds were extracted from the soil via flotation in a salt solution using a modified method of Malone (1967) in September 2007. Each 100 g soil sample was initially immersed in 1 L of potassium carbonate solution (500 g L^{-1} water). This solution was thoroughly agitated for 30 s and left for 1 h to allow the organic matter to become suspended. Initially, we tried a centrifugal separation technique (Buhler & Maxwell 1993), whereby small samples were centrifuged in K_2CO_3 supernatant at 4000 *g* for 5 min. However, we found this method to be inadequate since organic matter did not separate well from the heavy clay soils. After the solutions had settled in beakers for 1 h, organic matter was decanted and rinsed gently through a series of sieves (400 and 50 μm) to remove soil particles. This also removed residual potassium carbonate solution and facilitated counting and sorting of seeds since large and small seeds became separated.

Samples were then dried at 60 °C overnight, and the following day a fan-forced articulator was used to separate viable seeds from non-viable seeds and organic chaff. Seeds were then isolated from the remaining organic matter using a dissecting microscope and forceps. Viability of seeds was inferred by applying light pressure to seeds with forceps. When seeds resisted this pressure, they were deemed viable and recorded. Although this testing procedure was less rigorous than other viability tests, such as tetrazolium and germination-based tests, it was quicker and cheaper and is commonly used in seed bank

research (Ball & Miller 1989; Wright & Clarke 2009). Exposure to K_2CO_3 can reduce germination of some species depending on length of exposure time and hence, if viability of seeds is to be determined, then the effects of exposure must be tested for the species of interest (Buhler & Maxwell 1993). The extracted seeds were then identified to species, where possible, using specimens from the University of New England herbarium. Fourteen species remained unidentified, all with a mean abundance of $< 0{\cdot}5$.

SEEDLING EMERGENCE TRIAL

The seedling emergence trial was commenced in July 2008 after soils had dried for *c*. 12 months. Dry storage has been shown to enhance germination of many species from the soil seed bank (Thompson & Grime 1983) and in similar wetland types (Casanova & Brock 1996, 2000) although it may mean the loss of species with transient seed banks. Each 100 g soil sample was spread on seedling trays ($13{\cdot}9 \times 8{\cdot}5 \times 5$ cm) over sterilized potting mix. This provided a soil depth of *c*. 2 cm which we gauged would provide adequate surface area for germination. Trays were then placed in an unheated glasshouse. Trays were disturbed regularly to mix the soil after rate of seedling emergence had slowed. Five control trays containing only sterilized potting mix were also prepared to check for contamination from the glasshouse environment. Trays were kept continually saturated by an automatic irrigation system. As seedlings emerged, they were identified, counted and removed on a weekly basis. Species that could not be identified were transplanted and grown until they could be identified. The trial was finished when emergence had stopped (May 2009), hence the trial ran for 11 months. Six species remained unidentified at the end of the trial due to lack of reproductive material. The temperature in the glasshouse ranged from a minimum of 2 °C to a maximum of 30 °C and light was from natural light that penetrated the glass.

DATA ANALYSIS

We used paired *t*-tests to compare the total seed density, species richness and density of *P. canescens* between the seedling emergence and seed extraction methods. The nonparametric Mann–Whitney test was used where data did not meet the assumptions of normality. Statistical analyses were conducted using SYSTAT version 12 (Systat Software Inc., San Jose, CA, USA). Species composition of the soil seed bank in the top 5 cm was analysed by non-metric multidimensional scaling ordination (NMDS) using PC-ORD version 4·25 (MjM Software, Gleneden Beach, OR, USA). Dissimilarities between all pairs of samples were calculated using the Bray–Curtis dissimilarity coefficient. The differences in each study site between the two methods in NMDS configuration were tested by multi-response permutation procedures using PC-ORD (Biondini, Bonham, & Redente 1985). Species were allocated to functional groups based on categories developed by Brock & Casanova (1997) and Leck & Brock (2000). These groups have been determined on the basis of their germination, establishment and reproduction in the presence or absence of water.

Results

SEED DENSITIES

We found no significant difference between the two methods in the density of seeds found in the top 5 cm of the soil seed bank ($P = 0{\cdot}7$, Table 1). The extraction method detected a mean of 97 ± 9 seeds per 100 g soil ($N = 80$) and the emergence method of a mean of 94 ± 10 seeds per 100 g soil ($N = 80$). Significant differences were found in total seed density between the two methods from samples collected at depths of 5–10 cm ($P < 0{\cdot}001$), with significantly lower seed densities detected in the extraction trial (extraction mean $3{\cdot}6 \pm 0{\cdot}5$ compared to emergence mean $21 \pm 2{\cdot}7$, Table 1). This pattern was consistent in samples collected from a soil depth of 10–20 cm, with a mean of 0·5 seeds per 100 g ($N = 80$) detected in the extraction technique compared to a mean of 4·9 seeds per 100 g ($N = 80$) detected in the emergence technique ($P < 0{\cdot}01$, Table 1). The extraction method detected significantly greater densities of *P. canescens* seed than the emergence method in the top 5 cm of soil and at depths of 5–10 cm, but not at 10–20 cm (Table 1, $P < 0{\cdot}001$).

SPECIES RICHNESS

We found marginally greater mean species richness in samples from 0 cm to 5 cm soil depth using the extraction method compared with seedling emergence ($P = 0{\cdot}05$) with a mean of $7{\cdot}3 \pm 0{\cdot}2$ species detected in the extraction trial compared with $6{\cdot}8 \pm 0{\cdot}2$ species in the emergence trial (Table 1). However, in total, fewer species were found via the extraction method compared with the emergence trial (total species richness = 45 in the extraction trial and 48 for emergence). For samples collected from deeper in the soil (5–10 cm and 10–20 cm), mean species richness was significantly lower using the extraction technique than emergence ($P < 0{\cdot}001$, 5–10 cm and 10–20 cm, Table 1).

SPECIES COMPOSITION

Large differences in species composition of the soil seed bank (top 5 cm) between the two methods are evident in the ordination (Fig. 1). The two methods are clearly separated along axis one. There appears to be more variability in the extraction technique as indicated by the spread of samples, with some separation into sites. Within each site, significant differences were found between the extraction and emergence techniques (Table 2).

No clear patterns emerged in relation to functional group classification and the ability of either method to detect particular functional groups (Table 3). Rather, differences in detectability appear species-specific and may be related to other traits and/or dormancy and germination requirements. Specifically, species undetected in the extraction trial were mostly small seeded, e.g. *Cyperus difformis* and *Ammannia multiflora* (< 2 mm, Table 4) and species undetected in the emergence trial, such as *Eleocharis plana* and *E. sphacelata*, were most likely absent because of seed dormancy and/or inappropriate germination conditions.

The species composition of the soil seed bank at greater depths in the soil also differed between the two methods (Table 5). Similar species were driving the differences between the treatments as found in the samples from the soil surface.

Table 1. Comparison of the mean (±1SE) seed density, density of *Phyla canescens* and species richness per 100 g soil sample in the extraction vs. seedling emergence method ($N = 80$)

	Total seed densities		*Phyla canescens*		Species richness	
Soil depth (cm)	Extraction	Emergence	Extraction	Emergence	Extraction	Emergence
0–5	97·1 ± 9	93·9 ± 10	48·6 ± 7	16·3 ± 7	7·3 ± 0·2	6·8 ± 0·2
5–10	3·6 ± 0·5	21 ± 3	1·2 ± 0·2	0·3 ± 0·1	1·7 ± 0·1	3·2 ± 0·1
10–20	0·5 ± 0·1	4·9 ± 0·1	0·2 ± 0·06	0·2 ± 0·05	1·2 ± 0·04	2·5 ± 0·1

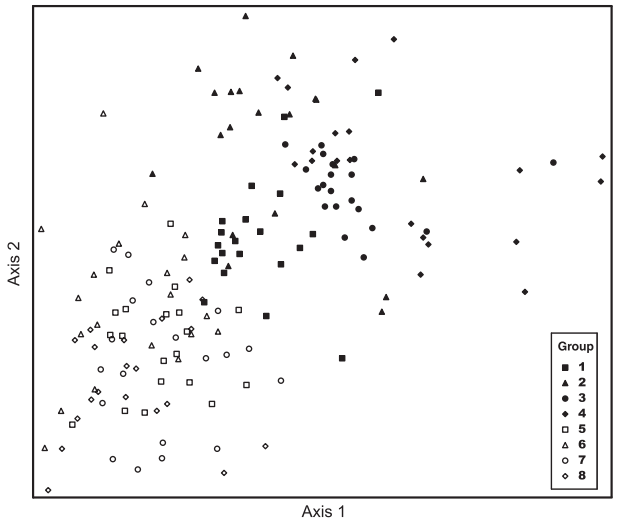

Fig. 1. Non-metric multidimensional scaling ordination of the soil seed bank data from the soil surface (0–5 cm) based on the extraction technique (treatments 1–4) and the seedling emergence technique (5–8). Ordination is based on seed density data. Treatments are: 1 = extraction OD dry, 2 = extraction OD wet, 3 = extraction WL dry, 4 = extraction WL wet, 5 = emergence OD dry, 6 = emergence OD wet, 7 = emergence WL dry, 8 = emergence WL wet. Minimum stress = 20. OD dry, Old Dromana 'dry'; OD wet, Old Dromana wet; WL dry, Wilgara dry; WL wet, Wilgara wet.

The three most dominant species associated with the seed extraction technique were *E. plana*, *E. sphacelata* and *Einadia nutans*. Common species detected in the seedling emergence trial were *C. differmis*, *Juncus aridicola* and *Myriophyllum variifolium*.

Discussion

The most striking result of our comparison of soil seed bank techniques was the large disparity in the species composition of the soil seed bank. Indeed, only 19 species were common to both methods of a total of 66 species. The choice of technique will be determined by the types of questions being asked but most commonly an accurate description of the species composition of the soil seed bank is sought. Using both techniques is often not practical and hence we recommend conducting a pilot study to determine species that occur in the seed bank and their relative detectability in each method (see also Ter Heerdt *et al.* 1996, 1999). In the current study, the most frequently recorded species in the emergence trial were *J. aridicola* and *C. differmis*, together accounting for 70% of the total seed densities. These species were either undetected, or rarely detected, in the extraction trial and if removed from the total densities, stark differences in seed density emerge, with *c.* 7300 seeds in the extraction trial compared with *c.* 2300 in the emergence trial. Hence, unless one is particularly interested in these two species the extraction trial will give a better estimation of seed densities for the range of species found.

Table 2. Summary of the multi-response permutation procedures analysis comparing within sites extraction with emergence

Extraction vs. emergence	*P*-value	*A*
OD dry	< 0·0001	0·27
OD wet	< 0·0001	0·32
WL dry	< 0·0001	0·41
WL wet	< 0·0001	0·34

The A statistic is a descriptor of within-group homogeneity compared with random expectation. A maximum value of $A = 1$ indicates that all species within the group are identical. OD dry, old Dromana 'dry'; OD wet, old Dromana wet; WL dry, Wilgara dry; WL wet, Wilgara wet.

Similarity in species composition may be improved by using a smaller mesh size when sieving to ensure small-seeded species are not lost. Most of the species undetected in the extraction trial were small seeded (< 2 mm) and may have been lost through sieving. In subsequent work, we have modified our technique by decanting the organic matter through filter paper rather than sieving, hence no seed loss is expected. However, finding the small seeds amongst the organic matter may still be difficult. Alternatively, soil used in the seedling emergence trial could be subject to flotation after germination to detect species that failed to germinate. This may provide an alternative to using many glasshouse treatments to break dormancy for many species and give an estimate of readily germinable and non-germinable fractions of the seed bank.

Previous studies have found the composition of wetland soil seed banks to be determined largely by the water regime used (e.g. Casanova & Brock 2000; Crossle & Brock 2002). Despite this, we found no differences in detectability between the two techniques in relation to the functional group classification. Hence, the saturated soil used in our seedling emergence trial had similar detectability for all functional groups, including species that respond to inundation (amphibious responders) and terrestrial species. A number of species however, were

Table 3. Composition of the soil seed bank in the top 5 cm of soil and functional group classification as determined by the seed extraction and seedling emergence methods

Plant group	Family	Species name	Extraction Freq. %	Mean	Emergence Freq. %	Mean
Amphibious responders	Alismataceae	*Damasonium minus*	10	0·2		
	Cyperaceae	*Eleocharis sphacelata*	40	7·9		
	Elatinaceae	*Elatine gratioloides*			2·5	0·02
	Haloragaceae	*Myriophyllum variifolium*			51·2	3·2
	Marsilaceae	*Marsilea drummondii*	23·7	0·8	3·7	0·06
	Onagraceae	*Ludwigia peploides*	46·2	3·5	20	0·4
Amphibious tolerators	Asteraceae	*Centipeda minima*			13·7	0·9
	Cyperaceae	*Cyperus difformis*			83·7	16·6
		Eleocharis plana	71·5	7·5		
	Juncaceae	*Juncus aridicola*	28·7	5·3	100	48·5
	Lythraceae	*Ammannia multiflora*			28·7	0·98
	Poaceae	*Alopecurus geniculatus*			1·25	0·01
		Lachnagrostis filiformis			8·7	0·2
		Paspalum distichum	25	0·4	31·2	0·5
	Ranunculaceae	*Ranunculus pumilio*	2·5	0·05	18·7	0·2
		Ranunculus undosus	28·7	2·5	10	0·1
	Verbenaceae	*Phyla canescens*	92·5	48·3	85	16·3
Terrestrial damp	Amaranthaceae	*Alternanthera denticulata*			8·75	0·1
	Asteraceae	*Aster subulatus*			1·25	0·01
		Conyza bonariensis	1·25	0·01		
		Soliva anthemifolia	1·25	0·01	2·5	0·04
	Brassicaceae	*Rorippa lacinata*	6·2	0·15	53·7	1·3
	Caryophyllaceae	*Stellaria angustifolia*			8·7	0·1
	Fabaceae	*Aeschynomene indica*			1·25	0·01
	Poaceae	*Amphibromus neesii*			7·5	0·3
	Polygonaceae	*Rumex tenax*	1·25	0·02	3·7	0·05
	Scrophulariaceae	*Gratiola pedunculata*			2·5	0·02
		Mimulus gracilis			6·5	0·1
Terrestrial dry	Aizoceae	*Trianthema triquetra*	5	0·1		
	Amaranthaceae	*Chenopodium pumilio*	5	0·09	42·5	1·5
		Einadia nutans	55	4	3·7	0·05
		Salsola kali	13·7	0·3		
	Asteraceae	*Calotis hispidula*	1·25	0·01		
		Cirsium vulgare	3·7	0·04	2·5	0·02
		Euchiton sphaericus			2·5	0·02
		Xanthium spinosum	5	0·07	2·5	0·02
	Brassicaceae	*Capsella bursa-pastoris*	7·5	0·09	2·5	0·02
		Sisymbrium irio			10	0·15
	Euphorbiaceae	*Chamaesyce drummondii*	3·2	0·1	7·5	0·1
	Fabaceae	*Medicago polymorpha*	50	7·2	18·7	0·3
		Medicago laciniata			1·25	0·02
	Malvaceae	*Malvastrum americanum*	1·25	0·01		
	Poaceae	*Hordeum leporinum*			2·5	0·02
	Polygonaceae	*Polygonum aviculare*	32·5	3·2	21·2	1·3
	Portulaceae	*Portulaca oleracea*	7·5	0·2	2·5	0·02
	Zygophyllaceae	*Tribulus terrestris*	2·5	0·07		

Unidentified species have not been included in this table.
Freq. % = percent frequency of occurrence in 80 samples. Functional groups are classified according to Brock & Casanova (1997) on the basis of germination, establishment and reproduction in the presence or absence of water.

undetected by using one watering regime in the glasshouse, and this may be due to lack of viability or to dormancy and germination requirements. For example, large numbers of *E. sphacelata* seed were detected in the extraction trial, mostly at Old Dromana 'wet', but no seedlings germinated in the glasshouse. Seed of this species exhibits seasonal dormancy and will only germinate after days to weeks of inundation (Bell & Clarke 2004). Since the collection of the soil samples for this trial, above-ground vegetation at the Old Dromana 'wet' site has switched from dominance by the weed *P. canescens* to dominance by the native *E. sphacelata* (with almost 100% cover of this species; Price, Gross, & Whalley in press). Hence, determining the seed bank dynamics of these two species is important.

We were particularly interested in the best method for detecting seed of the invasive forb *P. canescens* for further

studies in these wetlands. *Phyla canescens* is a serious weed of floodplains in Australia, impacting on conservation values of significant wetlands and the productivity of grazing enterprises (Earl 2003). This species dominated the seed bank in the extraction trial, making up *c*. 50% of total seed densities. The extraction method provided greater estimates of *P. canescens* seeds, with an average difference between the two methods of *c*. 30 seeds per 100 g soil. The extraction method may overestimate seed densities by including non-viable seed (Thompson *et al*. 1997; Baskin & Baskin 1998), and although we did not examine viability in the current study, previous estimates suggest *c*. 50% seed viability from samples taken from the soil seed bank (Macdonald 2008). Hence, the seedling emergence trial likely gives an underestimate of the *P. canescens* seed bank even if viability is taken into account. The specific germination requirements of *P. canescens* are abundant moisture, alternating temperatures and light (Macdonald 2008). These conditions were met in the glasshouse but some seeds may not have reached the soil surface where they would be exposed to and received adequate light, despite samples being mixed regularly.

Several terrestrial species were also rarely detected in the glasshouse trial compared with seed extraction e.g. *Medicago polymorpha* and *E. nutans*. Seeds of the winter annual *M. polymorpha* are dormant due to a water-impermeable seed coat, which can extend dormancy over several years (Wagner & Spira 1994). *Einadia nutans* however, exhibits no seed dormancy (Leishman & Westoby 1998), thus it is unclear why detectability was low in the emergence trial.

Several species that were commonly and abundantly detected in the soil seed bank of the emergence trial were undetected or rarely detected in the extraction trial. Differences in detectability appear to be related largely to seed size with species with small seeds rarely detected in the extraction. For example, *c*. 1300 seeds of *Cyperus difformis* germinated in the glasshouse, but none were found in the extraction; seed size of this species is 0·6 mm. Further, seeds of *Centipeda minima* (1·5–2 mm) also were not detected in the extraction method.

Density of seeds in the soil seed bank declined monotonically with depth as is usually reported in the literature (Thompson *et al*. 1997). We found no species that had a bimodal depth distribution as has been reported recently in soils that develop cracks (Espinar *et al*. 2005). The seed extraction and seedling emergence techniques gave similar estimates of mean seed density and species richness from the soil surface (top 5 cm). As our soil samples were collected in winter, it is likely that the samples also contain species that have a transient seed bank (Thompson & Grime 1979) and this would account for the large numbers of seed in the 0–5 cm soil depth. The seedling emergence technique had greater seed detectability from samples collected deeper in the soil (5–20 cm). Much of this difference is attributed to high densities of *C. difformis* and *J. aridicola* consistent with samples from the soil surface. The low seed detectability of the extraction method from samples deeper in the soil is likely due to the dominance of small-seeded species at these depths. Seed bank composition deeper in the

Table 4. Seed size of species detected in the emergence trial that were undetected in the extraction trial

Species	Seed size (mm)	Frequency %
Juncus aridicola	0·3	100
Cyperus difformis	0·5–0·7	83
Myriophyllum variifolium	1·5	51
Ammannia multiflora	1·5–2	29
Sisymbrium irio	1	10
Centipeda minima	1·5–2	14
Alternanthera denticulata	1·4	9
Stellaria angustifolia	1	9
Lachnagrostis filiformis	1·6	9
Amphibromus neesii	4	7
Mimulus gracilis	0·25	6·5

Species included are those with > 5% frequency in the emergence trial. Data on seed size is either from Harden (2002) or measured from samples contained in the N.C.W. Beadle Herbarium.

Table 5. Species composition and mean (± 1SE) density of the soil seed bank at 5–10 cm and 10–20 cm soil depth as determined by the seed extraction and seedling emergence method

	Extraction		Emergence	
Species	5–10 cm	10–20 cm	5–10 cm	10–20 cm
Ammannia multiflora			0·6 ± 0·2	
Amphibromus neesi			0·1 ± 0·08	
Centipeda minima			0·1 ± 0·07	
Chenopodium pumilio			0·25 ± 0·07	
Cyperus difformis			3·3 ± 0·5	0·9 ± 0·3
Einadia nutans	0·4 ± 0·1		0·01 ± 0·01	
Eleocharis plana	0·5 ± 0·2			
Eleocharis sphacelata	0·3 ± 0·1			
Juncus aridicola	0·02 ± 0·02		14 ± 2·6	3·2 ± 0·5
Ludwigia peploides		0·04 ± 0·02	0·1 ± 0·04	
Mimulus gracilis			0·35 ± 0·2	0·07 ± 0·07
Myriophyllum variifolium			1·0 ± 0·3	0·3 ± 0·09
Phyla canescens	1·2 ± 0·2	0·2 ± 0·6	0·3 ± 0·1	0·2 ± 0·05
Polygonum aviculare	0·3 ± 0·06			

Species with a total density of < 6 in total from the 80 samples and those not identified have not been included.

soil profile of cracking soils is related to diaspore size, with smaller diaspores more likely to be carried by wind and fall into soil cracks (Espinar & Clemente 2007).

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