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RESEARCH ARTICLE

Plant recruitment from the soil seed bank depends on topsoil stockpile age, height, and storage history in an arid environment

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The topsoil native seed bank is a valuable resource in post-mining rehabilitation capable of providing a cost-effective means for restoring plant diversity. However, the value of the native soil seed bank is affected by topsoil management practices especially long-term stockpiling. Salvaged topsoil is usually stockpiled until mining operations are completed and landforming has been performed. This study compared the germinable soil seed bank of freshly harvested topsoil against the effects of stockpile age (stored for 1 and 3 years) and depth (top and bottom 50 cm of 2-m-high stockpiles). Total native seedling emergence was greater from the 1-year-old stockpile but was dominated by annuals including species commonly found on disturbed sites. Seedling emergence of perennial species was more than 4-fold greater in fresh topsoil than in the 1-year-old stockpiles, and 2-fold greater than the bottom 50 cm of 3-year-old stockpiles, with no difference in the top 50 cm of the 3-year-old stockpiles. Although there was no difference in total emergence between the top and bottom of stockpiled topsoil, there was greater emergence of the ecologically important *Triodia* grass genus from the top than bottom of 3-year-old stockpiles. Species composition of the seed bank in the 1-year-old stockpile was different to fresh topsoil and the 3-year-old stockpile possibly reflecting differences in species composition of vegetation present when topsoil was initially salvaged for stockpiling, confounding comparisons between years. This study demonstrates the importance of conducting soil seed bank analysis of stockpiled topsoil to be used in restoration programs.

Key words: dryland rehabilitation, mine site, Pilbara, restoration ecology, *Triodia*

Implications for Practice

- Assessment of the native seed bank is required to determine the capacity of topsoil to contribute to the regeneration of a rehabilitated plant community.
- Once the soil seed bank has been assessed, the species present and seed quantities required for sowing can be calculated, allowing for the accurate assessment of seeding success.
- The germinable seed bank of *Triodia* spp. is greater in the top layer of 3-year-old stockpiled topsoil. The likely reason for this difference is the replenishment of the soil seed bank from reestablished plants on top of topsoil stockpiles.
- Removal of gravel/rock from salvaged topsoil can reduce the volume of topsoil stockpiles by half. However, the benefit of gravel/rock in providing recruitment niches needs to be considered.

Introduction

Ecological restoration of ecosystems impacted by human activity is of major global importance (Perring et al. 2015) with recent global targets aimed at restoring 15% of degraded ecosystems by 2020 (CBD 2012). With increasing demand for ecosystem restoration, there will be a corresponding increase in global demand for seeds to restore vegetation communities impacted by human activity (Merritt & Dixon 2011). Therefore, it is imperative that the most efficient use of native seeds is made to reduce the financial cost of collecting, storing, and sowing seeds, and to minimize the environmental impact of wild seed collecting through optimizing the use of all sources of regenerative materials including the soil seed bank in salvaged topsoil (Merritt & Dixon 2011; Erickson et al. 2016).

The native seed bank contained in topsoil provides an important source of seed for the restoration of disturbed sites (Carrick & Kruger 2007; Koch 2007; Rokich & Dixon 2007; Scoles-Sciulla & DeFalco 2009; Hall et al. 2010; Golos 2013; Merritt et al. 2016) and in some cases the only practical method of restoring some species to site (Rokich & Dixon 2007). As well as providing seeds, topsoil also contains the appropriate

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mycorrhizal and bacterial symbionts for promoting the establishment and persistence of plant species indigenous to the local environment (Bell et al. 2003; Jasper 2007; Muñoz-Rojas et al. 2016*a*, 2016*b*). However, the value of salvaging the native soil seed bank can be affected by topsoil management practices, especially topsoil stockpiling (Rokich et al. 2000; Koch 2007; Golos & Dixon 2014). Hence, it is important to assess the size and species present in the soil seed bank to determine the capacity of topsoil for vegetation reestablishment (Merritt et al. 2016). Assessment of the soil seed bank will also inform the species and quantities of seeds required for collecting and sowing. Optimizing seed usage and preventing over-collecting of seeds reduce their associated environmental impact on wild sources; cost of collecting, storage, and direct seeding, combined with the respreading of a stockpiled topsoil soil seed bank, may provide a more balanced restored plant community (Grant et al. 2007).

Mine site restoration is undertaken in a planned process as disturbed areas become available, with direct return of topsoil only rarely achieved in the mining sector (Koch 2007). As a consequence, it is common for many mine sites to stockpile topsoil in preparation for future mine site restoration. Topsoil stockpiles can cover large areas thus increasing the mine site footprint and hence the area and costs associated with larger areas of restoration. As a consequence, mining companies are interested in developing salvaged topsoil stockpiles with a higher profile but reduced land area; however, little is known of the consequences of height on the soil seed bank viability in the salvaged material. This is despite recommendations indicating maximum heights of 1–3 m (Commonwealth of Australia 2006).

Some studies have examined the effect of topsoil stockpiling and to a lesser extent the depth of topsoil stockpiles on the soil seed bank. In southwest Western Australia, studies examining soil seed bank changes over time of stockpiled sandy *Banksia* woodland topsoil (Rokich et al. 2000) and stockpiled gravelly sandy Jarrah forest topsoil (Tacey & Glossop 1980; Koch et al. 1996) found a decrease in seedling recruitment with increasing age of stockpiled topsoil. Also, Rokich et al. (2000) found no difference in seed viability of seeds buried at 1 and 3 m storage depths. In the United Kingdom, Dickie et al. (1988) reported, for topsoil stockpiled for differing periods, significantly lower viable seed count with older topsoil stockpiles and increasing stockpile depth in clay topsoil. In contrast, in the United States, Johnson and West (1989) found an increase in viable seed count with older stockpiles with no difference in seed viability with increasing stockpile depths. The contradictory results between Dickie et al. (1988) and Johnson and West (1989) are probably due to confounding effects such as differences in topsoil stripping depths, season of stripping, and vegetation cover leading to differences in the initial size of the topsoil seed bank at the time of topsoil stripping and storage.

Although recent studies have shown that the decline in soil seed bank viability is slower in arid environments (Scoles-Sciulla & DeFalco 2009; Golos & Dixon 2014), no study has investigated what effect topsoil stockpile age and depth has on the topsoil seed bank in a warm/hot, xeric environment or for topsoil stripped from skeletal/rocky soils. The aim of this study is to investigate the effect of topsoil stockpile age and stockpile depth on seedling recruitment from the topsoil seed bank and hence the potential of salvaged and stored topsoil for restoring native vegetation to post-mine sites in an arid environment.

Methods

Study Site

The study took place at an iron ore mine site located approximately 180 km east of Port Hedland in Western Australia's Pilbara region (lat 20°37′S, long 120°18′E). The climate is semiarid, with a mean annual precipitation of 372 mm (75% falling in the summer wet season from December to March), and mean maximum/minimum temperature of 40.9/26.6°C in January and 27.1/11.9°C in July (nearest weather station Marble Bar 84 km away; Australian Bureau of Meteorology 2015). The vegetation is a spinifex (*Triodia* R.Br.) hummock grassland with scattered mixed shrubs and small trees (e.g. *Acacia* Mill. and *Eucalyptus* L'Her. spp.), growing on shallow stony/gravelly soils on the plateau of mesa outcrops (Smith et al. 2012; Erickson & Merritt 2016; Muñoz-Rojas et al. 2016*b*).

One- and three-year-old topsoil stockpiles selected for this study were created by stripping topsoil to 20 cm and "paddock dumping" (haul trucks dumping successive loads side by side) to approximately 2 m high. The stockpile surface profile was undulating with approximately 50 cm variation in height. Vegetation cover and structure differed between topsoil stockpiles and undisturbed vegetation. On 1-year-old stockpile vegetation cover was less than 10% and less than 0.7 m tall. Vegetation cover on the 3-year-old topsoil stockpile was composed of an upper layer (>2 m) dominated by *Acacia* species with circa 50% cover, and a lower stratum (<0.7 m) dominated by *Triodia* species with circa 25% cover, and a small percentage of short-lived herbs and perennial shrubs. There was evidence that *Acacia*, empty seed pods on plants and ground, and *Triodia*, remnant inflorescences and dehisced florets on the ground, species had seeded. Undisturbed vegetation was dominated by a 40% cover of *Triodia epactia* S.W.L. Jacobs with scattered larger shrubs (>2 m) of *Acacia* species and *Grevillea wickhamii* Meisn.

Ex Situ Analysis for the Effects of Topsoil Age and Storage Depth

In October 2009, before the commencement of the wet season (December–March), topsoil was stripped with a front-end loader, from an area approximately 200 m^2, from the top and bottom 50 cm of a 1-year-old (30 × 30 m) and a 3-year-old (75 × 40 m) stockpile. Topsoil was also freshly stripped from 0.5 ha to 20 cm depth in an undisturbed site. This gave 100 m^3 of topsoil for each topsoil storage treatment (top 1-year-old, bottom 1-year-old, top 3-year-old, bottom 3-year-old, and freshly stripped). Immediately after stripping of topsoil and temporary stockpiling, awaiting set-up of the in situ analysis (see below), 1 L of soil was taken at random, passed through a 6.7 mm sieve to remove gravel/rock and organic matter (except seed), until a total of 12 L of sifted topsoil had been collected for each of the five topsoil storage age/depth treatments. The total quantity of topsoil sifted to make each 12 L replicate was recorded to determine gravel/rock content of topsoil and to normalize volumes before undertaking statistical analysis. This process was replicated three times for each topsoil age/depth treatment and each sifted 12 L sample was placed into a calico bag and transported to Kings Park and Botanic Garden in sealed drums. Samples were stored in drums for five weeks outdoors in dry, shaded conditions at ambient temperature before processing. The effectiveness of estimating the soil seed bank by seedling emergence method has been enhanced with the development of smoke application as a tool to stimulate germination of Australian species (Dixon et al. 1995; Lloyd et al. 2000; Rokich & Dixon 2007; Golos 2013; Erickson et al. 2016). To allow pair-wise comparisons for the effect of aerosol smoke on seedling emergence, 2 L of topsoil was passed through a soil sample splitter (riffle). Each 1 L sample was then spread, approximately 1 cm deep, which is unlikely to inhibit germination of viable seeds in the sample (Golos 2013), onto a seedling punnet tray, lined with a perforated fabric cloth covered with 2 cm of washed white sand. Each tray was labeled with topsoil treatment, smoke treatment, replicate number, and tray number. This resulted in a combined 180 tray experiment (five topsoil age/depth treatments × two smoke treatments × three replicates, each replicate consisting of six 1-L/tray). Each tray for smoking was then placed in a smoke shed and smoked applied for 1 hour according to the method developed by Dixon et al. (1995). All trays were maintained in a greenhouse, irrigated, and scored for number of emergent seedlings and species. Irrigation commenced at the beginning of January 2010, with seedling counts beginning two days later. Final seedling counts were completed in March 2010, when no seedlings had emerged in the previous 28 days. Some seedlings were transferred into pots for growing on to aid identification.

In Situ Analysis for the Effects of Topsoil Age and Storage Depth

The site chosen for in situ analysis was an area cleared more than two years previously during mining operations and it consisted of a red shallow stony/gravelly substrate that is typical of the lateritic hills and mesa outcrops of the area (Muñoz-Rojas et al. 2016*b*). Any regrowth was cleared with a bulldozer before plots were installed. Immediately after topsoil samples were taken for ex situ analysis from temporary stockpiles, remaining topsoil was then spread, using a front-end loader, to approximately 20 cm depth into six replicates of 10 × 9 m randomly placed plots. Each plot was separated by at least 1 m. Within each treatment plot a 9 × 8 m monitoring plot was pegged out and seedling emergence counts (alive and dead) were made in June, October 2010, and January 2012. Size differences and presence/absence of cotyledons were used to discern new seedlings from old seedlings between counts.

Statistical Analysis

For in situ analysis, seedling counts for each tray were aggregated for each replicate 12 L sample/treatment. Also, before statistical analysis was undertaken, the volume of un-sieved topsoil sampled to make up each replicate 12 L sample (range 17–24 L for each replicate/treatment) was used to normalize seedling number to 20 L/sample (10 L smoked and 10 L unsmoked). One-way analysis of variance (ANOVA) was made to compare means between topsoil treatments (1-year-old top 50 cm, 1-year-old bottom 50 cm, 3-year-old top 50 cm, 3-year-old bottom 50 cm, and freshly stripped). A two-way ANOVA, excluding fresh topsoil, was made to detect differences between topsoil depth and age treatments, and interactions. In addition, for ex situ data, a two-way ANOVA was made to analyze the effects of smoke and topsoil treatment. Multiple pairwise comparisons, adjusted using Fisher's protected least significant difference (LSD) after ANOVA, were used for comparison of the means. The ANOVA assumptions of normality were tested using the Shapiro–Wilk test and homogeneity tested using Bartlett's test. To conform data with ANOVA assumptions of normality and homogeneity of variances, all seedling emergence counts were square root transformed prior to statistical analysis (Zar 2010). The differences were assumed to be significant when the probability was 5% or less. To examine the similarity of species composition of soil seed banks among fresh and stockpiled topsoil, hierarchical cluster analysis was performed with the Bray–Curtis index of similarity and the species by topsoil age/depth matrix using abundance data. GenStat Release 12.1 (VSN International Ltd., Hemel Hempstead, U.K.) was used for all statistical analysis.

Results

Gravel/rock Content of Harvested Topsoil

There was no difference in mean percentage gravel content (>6.7 mm fraction) between topsoil samples ($F_{[4,10]} = 0.43$, $p = 0.784$). Mean percentage gravel content of topsoil samples ranged from 37.7 to 43.5% with a mean of 40.4%. However, the mean percentage content of greater than 6.7 mm fraction in topsoil is underestimated as some rocks were too large to be included in samples and is estimated to be an additional 10%.

Ex Situ Analysis for the Effects of Topsoil Age and Storage Depth

Mean total seedling emergence was one order of magnitude greater ($F_{[4,20]} = 60.41$, $p < 0.001$) from freshly stripped topsoil than stockpiled topsoil, with no difference between 1-year-old and 3-year-old topsoil (Fig. 1). Only nine species emerged from all topsoil samples, with the highest species richness ($F_{[4,20]} = 9.36$, $p < 0.001$) from freshly stripped topsoil and the top 50 cm of 3-year-old topsoil stockpile and the lowest species richness from 1-year-old topsoil stockpile and bottom 50 cm of 3-year-old topsoil stockpile (Fig. 2). Over three quarters of seedlings emerging from topsoil samples consisted of only two species, *Goodenia stobbsiana* F. Muell. (59% of all seedlings) and *Bulbostylis barbata* (Rottb.) C.B. Clarke (17%), with less than 3% of seedlings represented by the two most important genera (by vegetation cover) (Burbidge 1943; Van Etten 1998;

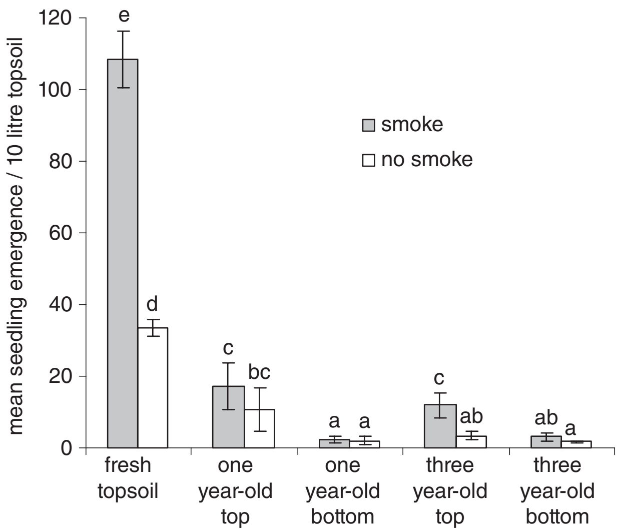

Figure 1. Ex situ mean seedling emergence (normalized to 10 L unsieved topsoil) from smoked and unsmoked topsoil sampled from freshly stripped topsoil, and 1-year-old and 3-year-old stockpiled topsoil sampled at 0–50 cm (top) and 150–200 cm (bottom) depth ($n=3$). Error bars represent ± SE. Different letters indicate significant differences according to Fisher's protected LSD ($p<0.05$).

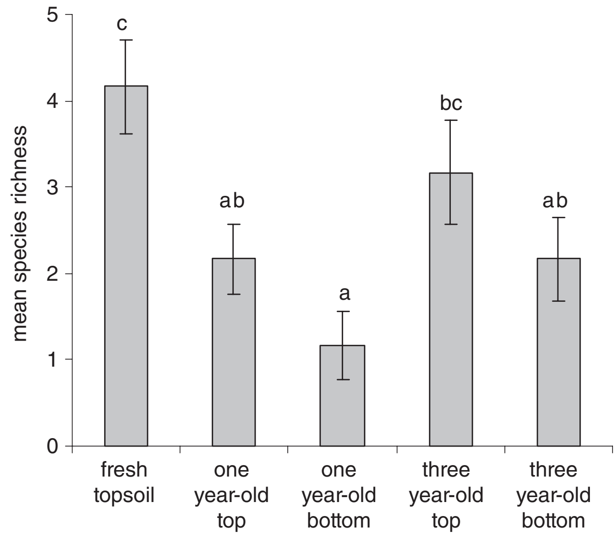

Figure 2. Ex situ mean species richness in 20 L topsoil (normalized unsieved topsoil samples; 10 L smoked, 10 L unsmoked) collected from freshly stripped topsoil and 1-year-old and 3-year-old topsoil stockpiles stripped at 0–50 cm (top) and 150–200 cm (bottom) depth ($n=6$; three smoked, three unsmoked). Error bars represent ± SE. Different letters indicate significant differences according to Fisher's protected LSD ($p<0.05$).

Erickson et al. 2016), *Triodia* (11 seedlings in total) and *Acacia* (six seedlings in total) (Table 1). Mean seedling emergence of grasses was more than 2-fold higher ($F_{[4,20]}=52.61$, $p<0.001$) from freshly stripped topsoil than 3-year-old topsoil stockpile. Mean seedling emergence of grasses was lowest from the bottom 50 cm of 3-year-old topsoil stockpile samples and 1-year-old topsoil stockpiles samples where no grass seedlings emerge. There was no difference ($p>0.05$) in seedling emergence of *Acacia* spp. between topsoil samples, with overall seedling emergence counts extremely low to nonexistent (e.g. 1-year-old topsoil stockpile samples). *Goodenia stobbsiana* seedling emergence was largely limited to freshly stripped topsoil samples (97% of all seedlings) and *Dysphania rhadinostachya* (F. Muell.) A.J.Scott seedling emergence was largely limited to 1-year-old topsoil stockpile samples (94% of all seedlings) (Table 1). A separate analysis, excluding fresh topsoil, found mean seedling emergence was 4-fold greater ($F_{[1,20]}=16.42$, $p<0.001$) from the top 50 cm than the bottom 50 cm of topsoil stockpiles.

There was an interaction between topsoil treatment and smoke with mean seedling emergence more than 3-fold greater ($F_{[4,20]}=6.22$, $p=0.002$) (Table S1, Supporting Information) in freshly stripped topsoil treated with smoke than without smoke (Fig. 1). In contrast, there was no difference in seedling emergence when smoke was applied to top or bottom 50 cm of 1-year-old topsoil and no difference with the bottom 50 cm of 3-year-old topsoil, but there was a difference in seedling emergence when smoke was applied to the top 50 cm of 3-year-old topsoil.

Species richness of emergent seedlings was also greater with smoke ($F_{[1,20]}=21.16$, $p<0.001$) (Table S2). Only six species/genera were identified from topsoil samples of which three species, *Triodia* spp., *B. barbata*, and *G. stobbsiana*, showed a positive response to smoke while the other three species, *Acacia ptychophylla* F. Muell., *D. rhadinostachya*, and *Salsola australis* R.Br., plus three unidentified dicot species showed no response.

In Situ Analysis for the Effects of Topsoil Age and Storage Depth

Total seedling emergence in 1-year-old topsoil was 3-fold greater than fresh topsoil and 4-fold greater than 3-year-old topsoil ($F_{[4,25]}=6.41$, $p=0.001$) (Fig. 3). However, 84% of seedlings in 1-year-old topsoil were *D. rhadinostachya* compared to only 12% in 3-year-old and 2% in fresh topsoil. Most seedlings from 1-year-old topsoil emerged after the first year (Fig. 4). When *D. rhadinostachya* is excluded from the analysis, total seedling emergence was greater in fresh topsoil ($F_{[4,25]}=3.9$, $p=0.013$) than in 1-year-old and the bottom 50 cm of 3-year-old topsoil, but no different to the top 50 cm of the 3-year-old topsoil. There was no difference in mean species richness between topsoil treatments (fresh 13.7 ± 0.8 species/plot, 1-year-old 11.0 ± 0.8 species/plot, 3-year-old 14.2 ± 0.8 species/plot), although there was a trend for lower species richness from 1-year-old topsoil ($F_{[4,25]}=2.28$, $p=0.089$).

A total of 30 species were found emerging from all topsoil treatments but only 20 species had mean seedling emergence of greater than one seedling per plot (Fig. 5). Species composition of emergent seedlings differed between fresh and stockpiled topsoil. For species emerging at greater than one seedling per plot, *G. stobbsiana* and *Grevillea wickhamii* were one order

Table 1. Ex situ mean seedling emergence (± SE) from 20 L (normalized unsieved topsoil samples; 10 L smoked, 10 L unsmoked) collected from freshly stripped topsoil and 1-year-old and 3-year-old topsoil stockpiles stripped at 0–50 cm (top) and 150–200 cm (bottom) depth ($n = 6$; three smoked, three unsmoked). Different letters indicate significant differences according to Fisher's protected LSD ($p < 0.05$).

		One-year-old Topsoil		*Three-year-old Topsoil*	
	Fresh Topsoil	*Top*	*Bottom*	*Top*	*Bottom*
Acacia ptychophylla	0.5(0.2)	0.0(0.0)	0.0(0.0)	0.1(0.1)	0.3(0.2)
Dysphania rhadinostachya	0.7(0.3)[a]	12.2(4.3)[b]	1.4(0.7)[a]	0.2(0.2)[a]	0.0(0.0)[a]
Salsola australis	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.2(0.2)	0.4(0.2)
Goodenia stobbsiana	55.3(11.9)[b]	0.5(0.3)[a]	0.5(0.4)[a]	0.8(0.7)[a]	0.0(0.0)[a]
Triodia spp.	1.0(0.5)[b]	0.0(0.0)[a]	0.0(0.0)[a]	0.6(0.5)[ab]	0.2(0.2)[a]
Bulbostylis barbata	11.7(4.8)[d]	0.0(0.0)[a]	0.0(0.0)[a]	4.4(1.6)[b]	0.8(0.3)[c]

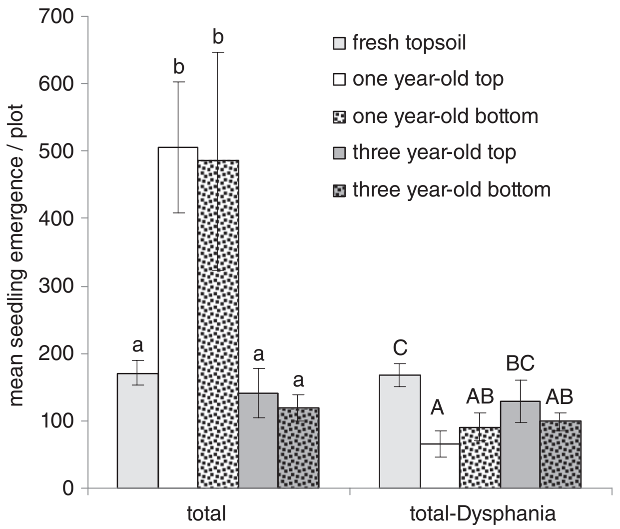

Figure 3. In situ mean total seedling emergence and total less *Dysphania rhadinostachya* emergence from freshly stripped topsoil and 1-year-old and 3-year-old topsoil stockpiles stripped at 0–50 cm (top) and 150–200 cm (bottom) depth and spread at 20 cm depth in 9 × 8 m plots ($n = 6$). Error bars represent ± SE. Different letters indicate significant differences according to Fisher's protected LSD ($p < 0.05$).

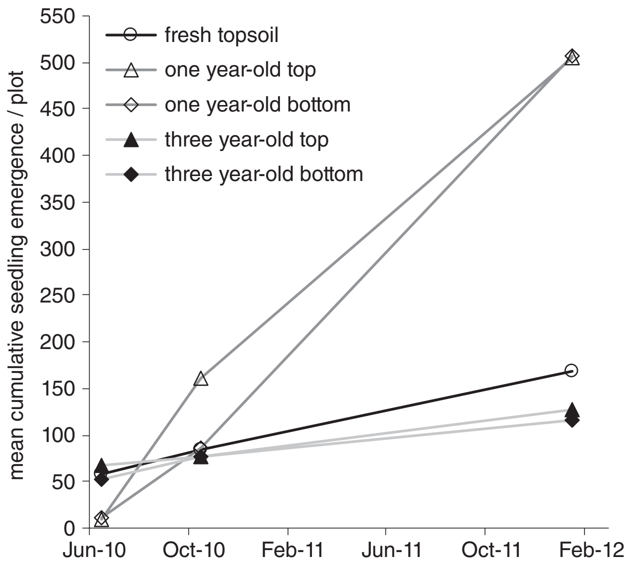

Figure 4. In situ mean cumulative seedling emergence from freshly stripped topsoil and 1-year-old and 3-year-old topsoil stockpiles stripped at 0–50 cm (top) and 150–200 cm (bottom) depth and spread at 20 cm depth in 9 × 8 m plots ($n = 6$).

of magnitude greater from freshly stripped topsoil, whereas *Aristida contorta* F.Muell. only emerged from 1-year-old topsoil and seedling emergence of *Dysphania rhadinostachya*, *Ptilotus calostachyus* F.Muell., and *Aristida inaequiglumis* Domin were one to two orders of magnitude greater in 1-year-old topsoil. *Indigofera monophylla* DC. was only found emerging from 3-year-old topsoil and seedling emergence of *Tephrosia* Pers. spp. was one order of magnitude greater from 3-year-old topsoil. Mean seedling emergence of *Acacia* species was 6-fold greater from fresh topsoil and 8-fold greater from 3-year-old topsoil than from 1-year-old topsoil. *Senna notabilis* (F.Muell.) Randell seedling emergence was more than 5-fold greater from freshly stripped and 3-year-old topsoil than from 1-year-old topsoil. For *Triodia* species, ecologically the most important component of the vegetation community, mean seedling emergence was 3-fold greater ($F_{[4,25]} = 8.4$, $p < 0.001$) from fresh and the top 50 cm of 3-year-old topsoil than 1-year-old topsoil and the bottom 50 cm of 3-year-old topsoil.

With the exception of one sample, cluster analysis of species composition showed soil seed banks segregating into distinct clusters within topsoil of different ages (Fig. 6). One-year-old topsoil formed a distinct cluster which had a higher degree of similarity among soil seed banks than with fresh and 3-year-old topsoil. Although there was segregation between fresh and 3-year-old topsoil, freshly stripped and 3-year-old topsoil formed a distinct cluster with a higher similarity between them than with 1-year-old topsoil. The top and bottom 50 cm of topsoil stockpiles did not form any distinct clusters.

Discussion

Total seedling emergence from topsoil stored for one year was greater than fresh topsoil and topsoil stored for three years. This is largely due to emergence of the annual *Dysphania rhadinostachya* (85% of seedlings) with most seedling emergence occurring in the second year after topsoil spreading and potentially from seeds produced from seedlings emerging during the

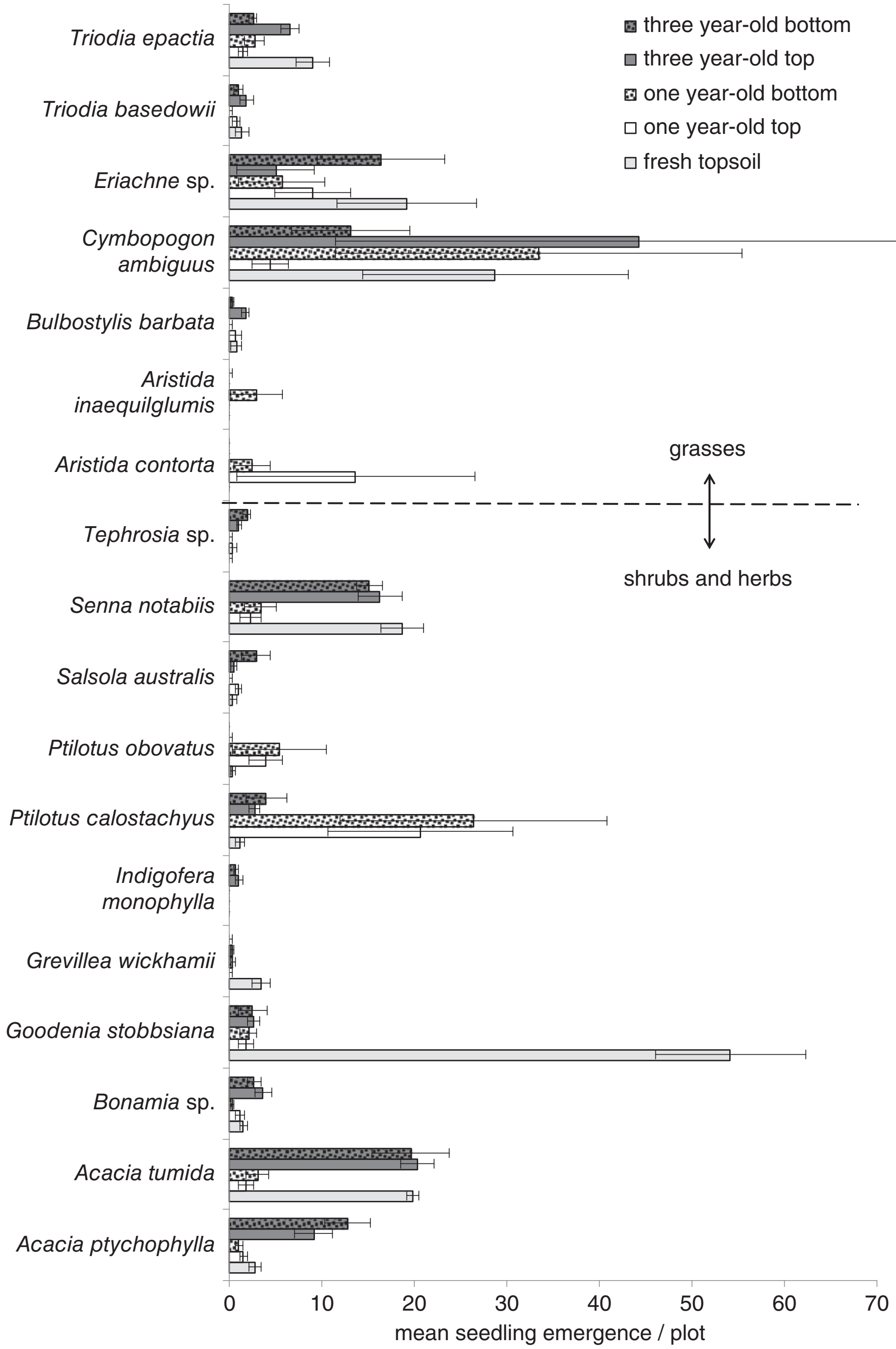

Figure 5. In situ mean seedling emergence of species with greater than one seedling/plot emerging from freshly stripped topsoil and 1-year-old and 3-year-old topsoil stockpiles stripped at 0–50 cm (top) and 150–200 cm (bottom) depth and spread at 20 cm depth in 9 × 8 m plots ($n = 6$). Error bars represent ± SE. NB seedling emergence/plot for *Dysphania rhadinostachya* not shown 3.3 ± 1.6 in fresh topsoil, 440.7 ± 93.6 in 1-year-old top, 394.8 ± 156.5 in 1-year-old bottom, 12.0 ± 4.5 in 3-year-old top, and 19.4 ± 7.1 in 3-year-old bottom.

first year. However, *D. rhadinostachya* is physiologically dormant (see Erickson et al. 2016, p 77) so seedling emergence in the second year could have been from seeds already present in the seed bank of topsoil stockpiles. Excluding *D. rhadinostachya*, total seedling emergence was greater from fresh topsoil and the top 50 cm of 3-year-old topsoil. Differences or lack of differences in seedling emergence between freshly stripped and stored topsoil could be confounded by real differences in the topsoil seed bank at the time of initial topsoil stripping and storage operations. Indeed cluster analysis showed the species composition of the soil seed bank of 1-year-old topsoil was markedly different to freshly stripped and 3-year-old topsoil.

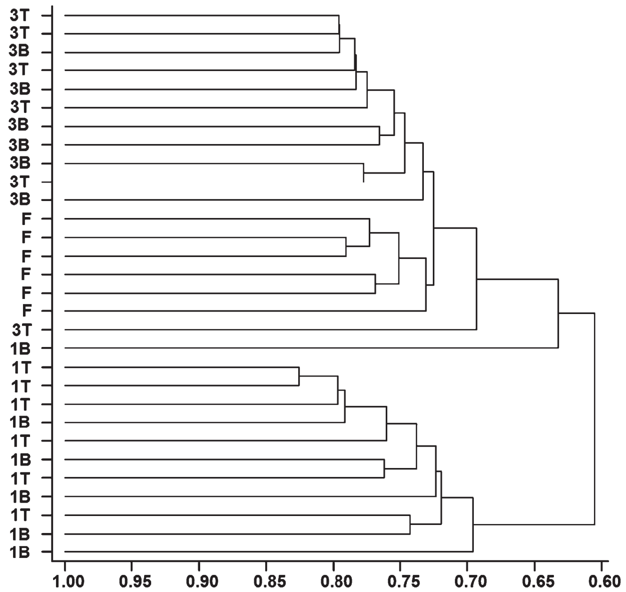

Figure 6. Cluster analysis showing the degree of similarity in species composition of seedlings emerging in situ from soil seed banks in freshly harvested (F), top 50 cm and bottom 50 cm of 3-year-old topsoil (3T and 3B) and 1-year-old topsoil (1T and 1B).

Species composition of 1-year-old topsoil is typical of disturbed mine sites dominated by weedy annuals or short-lived perennials such as *Dysphania*, *Ptilotus*, *Salsola*, and *Aristida* spp. that are associated with mining operations in the greater Pilbara region (Golos 2013; Golos & Dixon 2014). Also, very few longer lived perennials such as *Triodia* and *Acacia* species, which are numerically dominant perennial species in *Triodia* hummock grasslands of the Pilbara region, were present (Burbidge 1943; van Etten 1998; Erickson et al. 2016). This highlights the importance of assessing the topsoil seed bank before undertaking vegetation restoration so as to identify species that are not present or in low numbers that will require supplemental seeding (Merritt et al. 2016). In this study, 3-year-old topsoil will likely restore similar vegetation community to undisturbed sites whereas 1-year-old topsoil is likely to be different and will require supplemental seeding to achieve a similar vegetation community.

To avoid confounding factors in assessing changes in soil seed bank during storage, it is ideal to investigate changes in the topsoil seed bank of a single stockpile over time rather than investigating differences in topsoil seed bank of different aged stockpiles at one point in time. This avoids any differences in the initial soil seed bank due to temporal differences in seed production and seed rain (Facelli et al. 2005; Williams et al. 2005; Csontos 2007); differences between sites such as landforms (Yan et al. 2005; Golos 2013); management practices such as time since fire (Williams et al. 2005; Koch et al. 2009); the method and timing of topsoil harvesting operations which can all affect the size and diversity of the soil seed bank (Koch 2007; Rokich & Dixon 2007; Golos 2013); and method and type of machinery used and operator experience (Golos 2013). However, comparing seedling emergence from topsoil stockpiles over time can be affected by variation in rainfall between years (that directly influences seed production rates) confounding differences between years. This can be avoided by use of controlled ex situ environments that provide standard and ideal germination conditions but also allow the application of aerosol smoke and other germination enhancing products (e.g. GA_3 in Hoyle et al. 2013) to more accurately assess the viable seed bank. However, ex situ analysis is limited by area available for spreading topsoil that can miss species that have small or very patchy soil seed banks. Indeed 5-fold greater number of species was identified in situ than ex situ. Irrigation of topsoil spread into large plots in situ can reduce the effect of rainfall differences between years and also in situ analysis has the advantage of providing representative light and temperature conditions.

Despite the likelihood of real differences in the initial size and composition of the topsoil seed bank confounding comparisons between topsoil stockpiles and fresh topsoil, there is less likelihood of the same confounding factors affecting comparisons between the top and bottom 50 cm within topsoil stockpiles. There was no difference in total seedling emergence between the top and bottom 50 cm of stored topsoil; however, for the ecologically important *Triodia* species, seedling emergence was greater in the top 50 cm than bottom 50 cm of 3-year-old topsoil. The difference in *Triodia* seedling emergence between the top and bottom 50 cm of topsoil stockpiles is most likely due to fresh seed being input into the topsoil seed bank from vegetation growing on the stockpile compounded by seed decay from wetter, anaerobic conditions found at the bottom 50 cm of topsoil stockpiles (Pakeman et al. 2012; Golos & Dixon 2014). The sampling range of 0–50 cm probably masks a larger difference in the germinable seed bank between the top and bottom layer of 3-year-old topsoil stockpiles as most of the new seed input would be concentrated in the top 10 cm (Golos 2013).

In contrast to in situ analysis, ex situ analysis found total seedling emergence was higher from freshly stripped topsoil than from both 1-year-old and 3-year-old topsoil. The reason for differences in findings between ex situ and in situ analysis could be due to ex situ application of smoke stimulating germination of seeds (Dixon et al. 1995; Erickson et al. 2016). Smoke did enhance seedling emergence from fresh topsoil and the top 50 cm of 3-year-old topsoil which is where fresh seed inputs would have been most prominent. Another reason for the lack of differences detected in situ is the effect of plants emerging and flowering in the first year and setting seeds which consequently germinated in the second year. Also, there could have been seed migration from surrounding vegetation.

Increasing the height of topsoil stockpiles can reduce the area covered by topsoil stockpiles and hence the area of the mine foot print required to store topsoil for multiple years. However, this will decrease the ratio of seed replenishment to volume of topsoil stockpile and will dilute the effect of seed bank replenishment, as found here for the ecologically important *Triodia* species. Alternatively, the volume, and therefore costs of building and maintaining of stockpiles, can be reduced by removing gravel and rock (by screening) that made up over 40% (>6.7 mm fraction) of the salvaged topsoil volume. The benefits of removing gravel/rocks include reduced handling costs, more concentrated soil seed bank, and possibly higher seedling emergence with shallower spread resulting in lower loss of seedlings due to deep burial as found for salvaged topsoil in biodiverse forest restoration in southwest Australia (Koch 2007). However, rock may provide benefits such as microsites for seeds and reduction of soil loss to wind and water erosion, particularly on slopes (Golos 2013).

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LITERATURE CITED

Bell J, Wells S, Jasper DA, Abbott LK (2003) Field inoculation with arbuscular mycorrhizal fungi in rehabilitation of mine sites with native vegetation, including *Acacia* spp. Australian Systematic Botany 16:131–138

Burbidge NT (1943) Ecological succession observed during regeneration of *Triodia pungens* R.Br. after burning. Journal of the Royal Society of Western Australia 28:149–156

Carrick PJ, Kruger R (2007) Restoring degraded landscapes in lowland Namaqualand: Lessons from the mining experience and from regional ecological dynamics. Journal of Arid Environments 70:767–781

CBD (2012) Aichi biodiversity targets, convention of biological diversity. http://www.cbd.int/sp/targets/ (accessed 12 May 2015)

Commonwealth of Australia (2006) Leading practice sustainable development program for the mining industry—mine rehabilitation. Department of Industry, Tourism and Resources, Canberra, Australia

Csontos P (2007) Seed banks: ecological definitions and sampling considerations. Community Ecology 8:75–85

Dickie JB, Gajjar KH, Birch P, Harris JA (1988) The survival of viable seeds in stored topsoil from opencast coal workings and its implications for site restoration. Biological Conservation 43:257–265

Dixon KW, Roche S, Pate JS (1995) The promotive effect of smoke derived from burnt native vegetation on seed germination of Western Australian plants. Oecologia 101:185–192

Erickson TE, Merritt DJ (2016) Introduction to plant diversity of the Pilbara. Pages 1–6. In: Erickson TE, Barrett RL, Merritt DJ, Dixon KW (eds) Pilbara seed atlas and field guide: plant restoration in Australia's arid northwest. CSIRO Publishing, Dickson, Australia

Erickson TE, Barrett RL, Merritt DJ, Dixon KW (2016) Pilbara seed atlas and field guide: plant restoration in Australia's arid northwest. CSIRO Publishing, Dickson, Australia

Facelli JM, Chesson P, Barnes N (2005) Differences in seed biology of annual plants in arid lands: a key ingredient of the storage effect. Ecology 86:2998–3006

Golos PJ (2013) Restoring vegetation on waste rock dumps at the Telfer mine site in Australia's Great Sandy Desert: topsoil management and plant establishment. PhD dissertation. The University of Western Australia, Crawley, Australia

Golos PJ, Dixon KW (2014) Waterproofing topsoil stockpiles minimises viability decline in the soil seed bank in an arid environment. Restoration Ecology 22:495–501

Grant CD, Ward SC, Morley SC (2007) Return of ecosystem function to restored bauxite mines in Western Australia. Restoration Ecology 15(Supplement):S94–S103

Hall SL, Barton CD, Baskin CC (2010) Topsoil seed bank of an Oak-Hickory forest in Eastern Kentucky as a restoration tool on surface mines. Restoration Ecology 18:834–842

Hoyle GL, Venn SE, Steadman KJ, Good RB, Mcauliffe EJ, Williams ER, Nicotra AB (2013) Soil warming increases plant species richness but decreases germination from the alpine soil seed bank. Global Change Biology 19:1549–1561

Jasper DA (2007) Beneficial soil microorganisms of the Jarrah Forest and their recovery in bauxite mine restoration in Southwestern Australia. Restoration Ecology 15(Supplement):S74–S84

Johnson CK, West NE (1989) Seed reserves in stockpiled topsoil on a coal stripmine near Kemmerer, Wyoming. Landscape and Urban Planning 17:169–173

Koch JM (2007) Restoring a Jarrah Forest understorey vegetation after bauxite mining in Western Australia. Restoration Ecology 15(Supplement):S26–S39

Koch JM, Ward SC, Grant CD, Ainsworth GL (1996) Effects of bauxite mine restoration operations on topsoil seed reserves in the jarrah forest of Western Australia. Restoration Ecology 4:368–376

Koch JM, Ruschmann AM, Morald TK (2009) Effect of time since burn on soil seedbanks in the jarrah forest of Western Australia. Australian Journal of Botany 57:647–660

Lloyd MV, Dixon KW, Sivasithamparam K (2000) Comparative effects of different smoke treatments on germination of Australian native plants. Austral Ecology 25:610–615

Merritt DJ, Dixon KW (2011) Restoration seed banks—a matter of scale. Science 332:424–425

Merritt DJ, Golos PJ, Erickson TE (2016) A systematic approach to seed management for restoration. Pages 35–42. In: Erickson TE, Barrett RL, Merritt DJ, Dixon KW (eds) Pilbara seed atlas and field guide: plant restoration in Australia's arid northwest. CSIRO Publishing, Dickson, Australia

Muñoz-Rojas M, Erickson TE, Martini D, Dixon KW, Merritt DJ (2016*a*) Soil physicochemical and microbiological indicators of short, medium, and long term post-fire recovery in semi-arid ecosystems. Ecological Indicators 63:14–22

Muñoz-Rojas M, Erickson TE, Dixon KW, Merritt DJ (2016*b*) Soil quality indicators to assess functionality of restored soils in degraded semiarid ecosystems. Restoration Ecology: this issue

Pakeman RJ, Small JL, Torvell L (2012) Edaphic factors influence the longevity of seeds in the soil. Plant Ecology 213:57–65

Perring MP, Standish RJ, Price JN, Craig MD, Erickson TE, Ruthrof KX, Whiteley AS, Valentine LE, Hobbs RJ (2015) Advances in restoration ecology: rising to the challenges of the coming decades. Ecosphere 6: 1–25, http://dx.doi.org/10.1890/ES1815-00121.00121

Rokich DP, Dixon KW (2007) Recent advances in restoration ecology, with a focus on the Banksia woodland and the smoke germination tool. Australian Journal of Botany 55:375–389

Rokich DP, Dixon KW, Sivasithamparam K, Meney KA (2000) Topsoil handling and storage effects on woodland restoration in Western Australia. Restoration Ecology 8:196–208

Scoles-Sciulla SJ, DeFalco LA (2009) Seed reserves diluted during surface soil reclamation in eastern Mojave Desert. Arid Land Research and Management 23:1–13

Smith GB, Eberhard SM, Perina G, Finston T (2012) New species of short range endemic troglobitic silverfish (Zygentoma: Nicoletiidae) from subterranean habitats in Western Australia's semi-arid Pilbara region. Records of the Western Australian Museum 27:101–116

Tacey WH, Glossop BL (1980) Assessment of topsoil handling techniques for rehabilitation of sites mined for bauxite within the jarrah forest of Western Australia. Journal of Applied Ecology 17:195–201

Van Etten EJB (1998) Mapping vegetation in an arid, mountainous region of Western Australia. Applied Vegetation Science 1:189–200

Williams PR, Congdon RA, Rice AC, Clarke PJ (2005) Germinable soil seed banks in a tropical savanna: seasonal dynamics and effects of fire. Austral Ecology 30:79–90

Yan Q, Liu Z, Zhu J, Luo Y, Wang H, Jiang D (2005) Structure, pattern and mechanisms of formation of seed banks in sand dune systems in northeastern Inner Mongolia, China. Plant and Soil 277:175–184

Zar JH (2010) Biostatistical analysis. 5th edition. Pearson Prentice-Hall, Upper Saddle River, New Jersey

Supporting Information

The following information may be found in the online version of this article:

Table S1. ANOVA of square root transformed mean seedling emergence ex situ. Analysis of topsoil treatment (TS; 1-year-old top 50 cm, 1-year-old bottom 50 cm, 3-year-old top 50 cm, 3-year-old bottom 50 cm and freshly stripped) and smoke (smoked and unsmoked).

Table S2. ANOVA of mean species richness of emergent seedlings ex situ. Analysis of topsoil treatment (TS; 1-year-old top 50 cm, 1-year-old bottom 50 cm, 3-year-old top 50 cm, 3-year-old bottom 50 cm, and freshly stripped) and smoke (smoked and unsmoked).

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