Effects of soil treatments on the establishment of *Acacia* and *Eucalyptus* following gravel extraction

K.C. Madhu and Sean M. Bellairs

Research Institute for the Environment and Livelihoods, Charles Darwin University, Darwin, NT 0909, Australia
Email: sean.bellairs@cdu.edu.au

Abstract

Sources of construction materials such as gravel are sought near urban areas, including near Darwin, and these sites are required to be rehabilitated. However, few studies have investigated techniques to improve seedling establishment on gravel rehabilitation sites in northern Australia. This study investigated seedling establishment of the locally dominant tree species, Darwin Stringybark (*Eucalyptus tetrodonta*), Darwin Woollybutt (*E. miniata*) and the shrub, Western Salwood (*Acacia lamprocarpa*), on a gravel rehabilitation area near Howard Springs and in a shade house at Charles Darwin University, Darwin. The aim was to determine the effects of six treatments, including scarification, topsoil and application of leaf litter, and combinations of these treatments, on the emergence and survival of *Acacia* and *Eucalyptus* seedlings. Seedling establishment on the compacted gravel substrate was minimal, whereas application of litter or scarification of the gravel surface was found to increase seedling emergence and establishment. Seedling emergence was much greater in the shade house for all species (41%) than under field conditions (8%) suggesting that water stress was likely the major factor affecting survival in the field. Greater wind disturbance, seedling herbivory and erosion could have been additional factors. However, the application of topsoil had relatively little benefit for emergence, although later survival of *E. miniata* seedlings in topsoil was high. We suggest that altering or modifying the microsites of the gravel extraction areas by relatively easy and cost-effective methods such as scarification and application of litter can improve the seedling establishment of native woody species and help in rehabilitation of gravel extraction sites. Application of litter and scarification of the substrate are likely to enhance water availability for seedlings, possibly by reducing heating and evaporation, and by increasing water infiltration.

Introduction

Demand for construction materials such as gravel is increasing globally. Worldwide, the annual demand for construction aggregates in 2019 is estimated at over 51 billion tonnes (Freedonia 2016). Local demand for construction materials is substantial and has resulted in clearing of approximately 17 ha of native vegetation per year in the Darwin region for gravel extraction (Price *et al.* 2005). In the Darwin region, gravel is extracted from shallow borrow pits, after initial clearing of vegetation and stockpiling of the upper soil layer (Setterfield *et al.* 1993). In comparison to unmined sites, these processes can reduce the moisture-holding capacity, reduce nutrient (especially available nitrogen) availability and increase penetration resistance to root growth due to surface compaction (Johnson 1987; Schwenke *et al.* 1999). Even when fertiliser is added, nutrients are easily leached from the sites (Johnson 1987) and lateritic soils can have great capacity for immobilisation of phosphorus making it unavailable for plant growth (Bell 1985). Alterations to the hydrology following gravel extraction also have substantial impacts on the vegetation (Johnson 1987; Setterfield *et al.* 1993). A study in the Czech Republic by Rehounkova & Prach (2006) found that hydrology was the most important local site factor influencing the course of vegetation succession, but other significant influences were surface soil texture, soil pH and the presence of nearby communities containing desired species.

Unaided natural revegetation of gravel borrow pits is typically slow and generally unsuccessful. Setterfield *et al.* (1993) found that gravel borrow pits for road construction in Kakadu National Park recovered very little compared to their original vegetation. Similarly, Price *et al.* (2005) found that recovery of vegetation in the Darwin region was poorer in gravel mines than in sand mines. They found that only 41% of native vegetation species recovered in gravel mine sites compared to the species richness in control sites, with only 2% recovery of tree density, although the sites were up to 27 years old. It is very rare that the locally dominant *Eucalyptus* species re-establish in gravel extraction sites (Setterfield *et al.* 1993), although more recent observations suggest some *Eucalyptus* have established (Chris Brady pers. comm.). The extent to which the disturbed sites eventually recover their ecological character after gravel extraction is unknown. Poor natural establishment can be due to physical and chemical changes to the substrates, lack of nutrients, and limited or excessive water (Johnson 1987; Corbett 1999). Even in undisturbed soils of the wet-dry tropics, low levels of nitrogen and phosphorus limit the growth of native species (Bell 1985) and this is exacerbated by loss, disturbance or stockpiling of topsoil (Schwenke *et al.* 1999). In such circumstances, research on rehabilitation is essential for better outcomes, yet most research has focused on rehabilitation of larger mining sites; very little research has focused on the rehabilitation of gravel mine sites.

For most plant species, recruitment is limited primarily by microsite availability rather than seed availability (Crawley 1990). Microsite conditions at a small scale affect seed germination and seedling establishment and this is particularly important for small-seeded species such as *Eucalyptus* (Battaglia & Reid 1993). Important characteristics of microsites which can affect seed germination and seedling establishment include topographical features, soil texture, amount of litter, and presence of rocks or woody debris (Eldridge *et al.* 1991; Oswald & Neuenschwander 1993; Titus & del Moral 1998). *Eucalyptus* seedling establishment has been found to be affected by various factors including soil moisture, drought, temperature, light, overstorey shading, canopy gap, litter accumulation, grazing, plant competition, and insect and fungal attack (Setterfield *et al.* 1993; Li *et al.* 2003). Litter cover effects on *Acacia*, *Corymbia* and *Eucalyptus* can vary with species and soil type (Saragih & Bellairs 2015). In addition to that, disturbances like fire, grazing, cultivation, addition of fertiliser and soil compaction (or their interactive/ combined effects) also affect the establishment of eucalypt seedlings (Li *et al.* 2003). Small scale variation in soil conditions, even at the scale of tens of centimetres, can affect germination and establishment of *Eucalyptus* (Battaglia & Reid 1993). At older mine sites that had similar grass cover to that of the unmined savanna sites and where litter cover was substantial, eucalypt establishment can be successful (Saragih *et al.* 2015). Demonstrating this, across 14 gold mine rehabilitation sites south of Darwin, the average density of *Eucalyptus*/*Corymbia* seedlings in 12 year old mine rehabilitation sites was 528 ± 131 seedlings ha^{-1} (Saragih *et al.* 2015).

The aim of this study was to investigate whether seedling establishment of common Northern Territory savanna tree and shrub species can be improved by treatments to alter and create establishment microsites in gravel extraction areas. Specifically, the effects of scarification of the gravel surface, application of litter and application of soil and their combined effects on seedling emergence and growth of Darwin Stringybark (*Eucalyptus tetrodonta*), Darwin Woollybutt (*E. miniata*) and Western Salwood (*Acacia lamprocarpa*) were investigated in a gravel extraction site in the field and also in a shade house at Charles Darwin University.

Methods

The field trial was carried out at Scrubby Creek in the Howard Springs region (12.794 ºS, 131.224 ºE), 31 km east of Darwin. The site was formerly *Eucalyptus tetrodonta* dominated savanna woodland that had been cleared for gravel extraction. Trees and shrubs were cleared and burnt, then the top 0.2 m of topsoil and remaining vegetation were stockpiled (Keith Joy pers. comm.). After extraction of the gravel, the topsoil was spread out in 0.3 m high heaps over the site, enabling unaided vegetation recruitment to occur.

Three common local woody savanna species were chosen for the study: *Eucalyptus miniata, E. tetrodonta* and *Acacia lamprocarpa*. The two *Eucalyptus* are dominant overstorey trees while *Acacia lamprocarpa* is a large shrub to small tree growing from 4–12 m high. Seeds of all three species were obtained from Greening Australia.

Seed germination testing using four replicates of 25 seeds per species was carried out. Each replicate was placed in a plastic 9 cm diameter Petri dish lined with two filter papers soaked with 70 ml of 0.08 gm L^{-1} of Banrot 400 WP Fungicide. For *Acacia lamprocarpa,* four replicates of 25 seeds were placed in boiling water for 1 minute before being placed in Petri dishes like the the other seeds. The Petri dishes were incubated at 30 ºC in a controlled temperature incubator with a 12 hour light/dark regime and monitored for germination three times a week for four weeks. Germination (presence of an emergent radical) was recorded. Germinated and dead (soft seeds showing discoloration or decomposition of tissue) seeds were removed and percent germination was calculated after four weeks.

Seeds of the three species were sown into 0.5 x 0.4 m plots, with various substrate conditions, at the Scrubby Creek field site on 7 March 2018. Rainfall included 56 mm on 5 and 6 March on the days prior to planting, 143 mm from 7–31 March, 78 mm in May, and 0 mm in June (Howard Springs rainfall data from www.bom.gov.au). The control was the bare gravel base, 'Litter' had 122 gm of local *Eucalyptus* woodland litter applied on the gravel surface, 'Topsoil' had 0.02 m depth of topsoil placed on the gravel, and 'Scarified' had the surface broken up to 0.02 m depth with a mattock. Other treatments were combinations, with 'Scarified plus litter' and 'Topsoil plus litter'. Fifty seeds were sown for each treatment within a 0.4 m x 0.3 m area in the middle of the plot (Figures 1, 2). *Eucalyptus miniata* and *Acacia lamprocarpa* had six replicates per treatment. *Eucalyptus tetrodonta* had only sufficient seeds for the control, litter, scarified and topsoil treatments and for 25 seeds per plot.

Similar treatments were applied in a shade house at Charles Darwin University. Gravel from the Boral Quarry to the north of Gunn Point Road was used. Plastic trays (0.42 x 0.32 x 0.20 m depth) had six holes drilled to allow drainage. Gravel was hardened by compressing and moistening simultaneously to make a compact hardpan substrate similar to that of the field. After making the hardpan, the same treatments were applied as for the field site; that is, litter was applied at an equivalent rate of 70 gm per tray, the top 0.02 m was scarified using a hand cultivator, and 0.02 m of topsoil from the field site was overlaid. The same combinations of treatments were also applied as with the

Figure 1. Overview of one of the treatment areas on the gravel rehabilitation field site, with small mounds of topsoil surrounding the treatment area applied as part of general rehabilitation of the site. (Sean Bellairs)

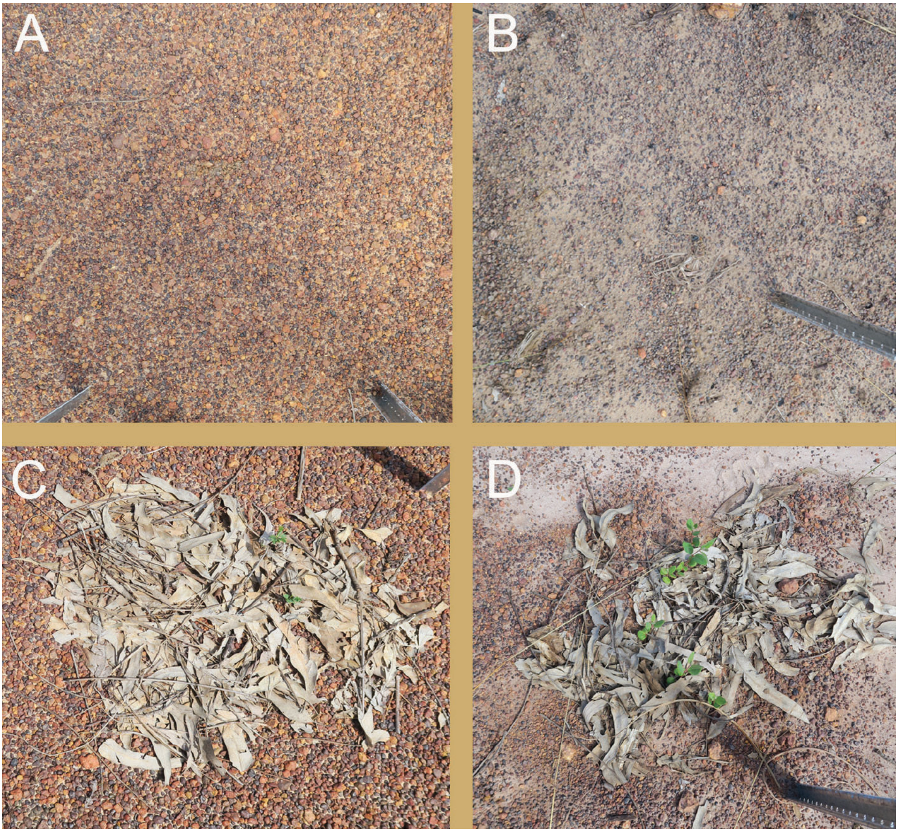

Figure 2. Photos of the treatments in the field site one month after application, including: (**A**) the bare gravel base, (**B**) topsoil, (**C**) leaf litter and (**D**) scarification plus leaf litter. (Sean Bellairs)

field trials. *Eucalyptus miniata* and *Acacia lamprocarpa* had six replicate trays per treatment with 50 seeds per tray. *Eucalyptus tetrodonta* had only the control and gravel plus topsoil treatments, with only four replicates and 25 seeds per tray due to limited seed availability.

Seeds were placed on the surface of gravel, scarified gravel and soil, whereas seeds were placed before the litter was applied in the litter treatments. The field experiment relied on natural rainfall. Irrigation in the shade house was applied three times a day for 10 minutes. The number of seedlings that emerged was observed each fortnight for two months in the field until May 2018. The seedlings were recorded in the field, with a photograph taken of each plot on each monitoring occasion. In the shade house, seedling emergence was recorded three times a week for two months. Seedlings were marked with toothpicks and dead plants were recorded. Up to eight seedlings per tray or plot were randomly selected and had their height (from shoot apical meristem to the surface of the substrate) measured at the end of the experiment.

Data analysis for seedling emergence in the field trial involved each species being separately analysed using GLM with a binomial distribution. The data were binomial, as each of the 50 seeds could either emerge or not by the end of the trial. The treatments were fixed effects, and block was included as a factor. The gravel control was the baseline for the intercept in the models. Multiple comparisons of means were analysed using Tukey contrasts to identify differences between individual pairs of treatments.

Seedling emergence in the shade house was analysed similarly, except there were no blocks and, to account for over-dispersion, a quasibinomial distribution was used. Analyses were performed using the packages 'lme4' and 'MASS' version 7.3-47 in the R environment 3.4.1 (R Core Team 2017), with a significance level of $P \leq 0.05$.

For plant height analysis of field treatments, GLM was used to allow the unbalanced design with different numbers of plants per treatment. For plant height analysis of shade house treatments, ANOVA was used, as the number of plants used for height measurement in each treatment was relatively uniform.

Results

Untreated seeds of *Acacia lamprocarpa* had 32 ± 3% germination, and boiling the seeds reduced germination; therefore the seeds used in the subsequent trials were not boiled. Germination of *Eucalyptus tetrodonta* was 44 ± 20% and *E. miniata* was 38 ± 11%. The *Acacia* had 96% of seeds germinate within 17 days and the *Eucalyptus* species had all seeds germinate within 9 days.

In the shade house, application of topsoil and litter on the gravel resulted in 57 ± 6% emergence, which was significantly higher than the scarified gravel treatment or compacted bare gravel (Figure 3). Application of litter, or litter and topsoil, increased emergence of *A. lamprocarpa* to above 40%, which was significantly greater than the 15 ± 4% emergence of the seeds sown onto compacted bare gravel. Mean emergence from the scarified gravel treatment at 28 ± 5% was not significantly different to the gravel or scarified gravel treatments with applied litter.

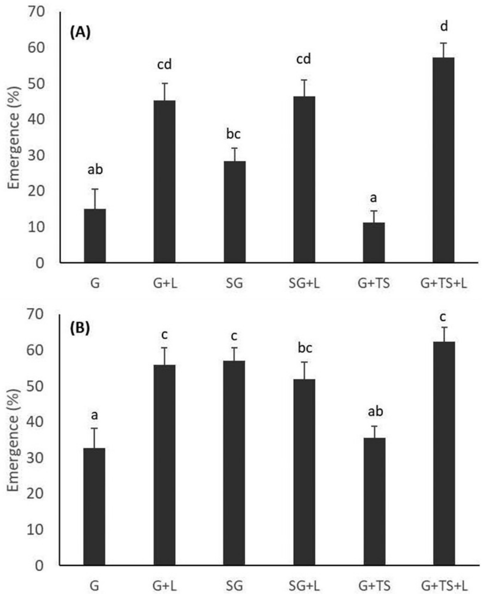

Figure 3. Emergence in the shade house of (**A**) *Acacia lamprocarpa* and (**B**) *Eucalyptus miniata* in gravel base (G), gravel base plus litter (G+L), scarified gravel (SG), scarified gravel plus litter (SG+L), gravel base plus topsoil (G+TS) and gravel base plus topsoil plus litter (G+ST+L). Bars that do not share the same letter above the bar are significantly different (GLM, quasibinomial with post hoc Tukey test, P<0.05).

Eucalyptus miniata showed a similar pattern of emergence in the shade house with over 50% emergence in the treatments where litter was applied over gravel, scarified gravel or topsoil. Scarifying the gravel alone resulted in similar levels of emergence to the litter treatments, and all these treatments had significantly higher emergence than from the compacted gravel base (33 ± 6%). Only topsoil application did not improve emergence over that of the compacted gravel base (the control).

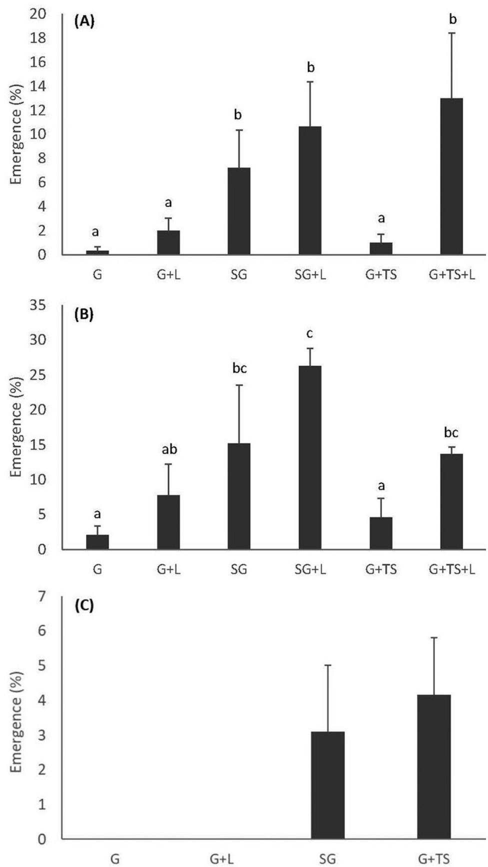

Figure 4. Emergence in the field plots of (**A**) *Acacia lamprocarpa*, (**B**) *Eucalyptus miniata* and (**C**) *E. tetrodonta* in gravel base (G), gravel base plus litter (G+L), scarified gravel (SG), scarified gravel plus litter (SG+L), gravel base plus topsoil (G+TS) and gravel base plus topsoil plus litter (G+ST+L). Bars that do not share the same letter above the bar are significantly different (GLM, binomial, with post hoc Tukey test, $P<0.05$).

Eucalyptus tetrodonta emergence from compact gravel in the shade house was 16 ± 8%.

In the field trial, mean emergence of all three species was much lower than that which occurred in the shade house. Emergence of *A. lamprocarpa* on compacted bare gravel was only 0.3 ± 0.3% (Figure 4). Emergence was significantly greater if the gravel base was scarified or if litter was applied. Application of 0.02 m depth of topsoil did not significantly increase emergence, unless litter was applied. One scarified gravel plot had 98% emergence of *Acacia* in comparison to the treatment mean of 7.3 ± 2.1%. This was excluded from statistical analysis as it was clearly an outlier.

Eucalyptus miniata emergence on bare gravel was only 2.0 ± 1.3%, whereas for *E. tetrodonta* no emergence on bare gravel occurred in the field (Figure 4). For *E. miniata,* the highest mean emergence of 17.3% resulted from the application of litter to scarified gravel, but scarifying the gravel or applying litter and topsoil also significantly increased emergence. Topsoil alone or litter applied to the gravel base did not significantly improve emergence. No treatments significantly improved emergence of

E. tetrodonta, although some emergence occurred in the scarified gravel plots and in the plots where soil was applied, and no emergence occurred in the untreated gravel.

Once the seedlings had emerged there were no significant effects of treatments on growth rates.

Mortality of emerged seedlings began within one month of emergence. Data on individual treatments for *E. tetrodonta* are not shown, as a total of only eight seedlings emerged. For *A. lamprocarpa* and *E. miniata,* mortality tended to be highest in the bare gravel and gravel plus litter plots (Table 1). Overall mortality tended to be lowest in the plots with scarified gravel or soil and litter. Scarified gravel had similarly low mortality for *A. lamprocarpa* but high mortality for *E. miniata.* Conversely, soil applied to gravel resulted in low mortality for *E. miniata* and high mortality for *A. lamprocarpa,* but the number of emerging seedlings was relatively low.

Table 1. Mortality of *Acacia lamprocarpa* and *Eucalyptus miniata* seedlings from March until May 2018 in the field trial. The number of emerging and surviving seedlings is the total for all plots.

	Acacia lamprocarpa			*Eucalyptus miniata*		
	Emerge #	Died #	Mortality %	Emerge #	Died #	Mortality %
Gravel	1	1	100%	5	2	40%
Gravel + Litter	6	3	50%	18	9	50%
Scarified Gravel	69	9	13%	33	12	36%
Scarified Gravel + Litter	33	5	15%	52	9	17%
Gravel + soil	3	2	67%	11	0	0%
Gravel + soil + litter	39	8	21%	45	7	16%

Discussion

For successful land restoration, research focused on the factors affecting establishment and finding cost-effective solutions is essential. As expected, surface treatments applied to the gravel base that remained after the gravel had been extracted affected emergence of *Acacia* and *Eucalyptus* seedlings. There was a dramatic increase in emergence with scarification of the gravel and the litter application treatments.

In this study of 700 seeds sown into the untreated gravel base, only six emerged and only three survived until May. It is quite likely that none of those 700 seeds would have produced seedlings that continued to survive throughout the first dry season. Recovery of vegetation in gravel extraction sites is very poor in the Darwin region (Price *et al.* 2005) and this study found very poor performance of seedlings of all studied species when sown on the untreated gravel base. Similarly, Setterfield *et al.* (1993) found that gravel borrow pits for road construction in Kakadu National Park recovered very little compared to their original vegetation. This may change with sufficient time, but is yet to be demonstrated.

There are several potential reasons for poor performance on the gravel substrate. There might be a physical hindrance to root growth (Rokich *et al.* 2001) or an inability to store sufficient water due to very low porosity (Ceacero *et al.* 2012). By hindering root growth, the compacted gravel substrate might also cause nutrient deficiencies in the seedlings. Hence, due to the physical properties of the gravel substrate, as well as prolonged lack of water and lack of nutrients in the abandoned gravel pit, young seedlings in the field might be more vulnerable to drought and have impaired anchorage, thus limiting their growth and survival.

We found that scarification of the gravel surface improved seedling emergence. Producing heterogeneity in the surface media, and creating microsites with more favourable conditions for seed germination and seedling establishment, can greatly assist tree establishment (Battaglia & Reid 1993). Setterfield (2002) found that seedling regeneration was limited by both seed supply and microsite availability in the Australian tropical savanna around Darwin. Scarification of the gravel surface affects water infiltration by creating hollows to trap water, which increases soil moisture. It also promotes litter accumulation in the hollows and the depressions may be shaded, which reduces temperature. Research done on seed germination and establishment of *Eucalyptus delegatensis* in different microsites (hillock, depression and flat site) in Tasmania showed that small scale variation in soil conditions even at the scale of tens of centimetres affected its germination and establishment (Battaglia & Reid 1993). Setterfield (2002) also found that shallow scarification of the soil surface in a tropical savanna increased seedling establishment of *Eucalyptus miniata* two to four times more than without disturbance. Likewise, seedling emergence of *Eucalyptus blakelyi* and *Acacia dealbata* was lower for seeds sown on untreated surfaces compared to seeds sown on scarified soil as seeds become shallowly buried and soil-to-seed contact is improved (Clarke & Davison 2001).

Litter improved the establishment success of the *Acacia* and *Eucalyptus* seedlings in our trials irrespective of topsoil application. Similarly, Facelli *et al.* (1999) observed higher densities of *Eucalyptus obliqua* seedlings in microsites with litter compared to those sites without it, due to enhancement of the humidity in the microenvironment surrounding the seeds. Litter favours seedling establishment in water-limited areas by reducing evaporative water loss from the soil, thus enhancing seed germination, growth and seedling survival (Enright & Lamont 1989). Removal of vegetation and topsoil from the gravel extraction site reduces the nutrient resources as most of the nutrient content is in the living biomass and the upper organic soil. Also, the moisture-holding capacity of gravel pits is reduced by loss of organic material and by surface compaction (Johnson 1987). Therefore, additional benefits for water and nutrition resources for plant growth can be expected as the litter breaks down. However, litter can be detrimental in some circumstances. If it is applied too thickly on top of the seeds, it may shade the seedlings, thus preventing photosynthesis, and the seedlings may die without emerging from the litter (Facelli *et al.* 1999). Seedling mortality in the shade house in our study was very low

(0–4%), but some mortality appeared to be due to litter promoting rotting of seedlings under moist conditions.

Due to weather conditions at the site, particularly occasional storms, loose leaf litter applied on the top of the gravel substrate was moved by wind and rain, and some plots were left with little or no litter at the end of the trial. For better results from litter application, the rehabilitation sites might be treated with leaf litter cut as foliage and small branches so that it cannot be easily moved by wind and rain.

An unexpected result was that a thin layer of topsoil was less favourable for seedling emergence than the application of a thin layer of leaf litter. During emergence, the topsoil is easily eroded by storms and the tiny root of the emerging seedling can be easily exposed and killed. An interesting observation was that although a 0.02 m depth of topsoil did not promote emergence, the 14 seedlings that emerged in topsoil over gravel had better overall survival than those growing on gravel. Once the seedling is able to establish in soil, the soil likely provides a better source of nutrients and water. However, in the Northern Territory, topsoil can also promote vigorous growth of grasses and numerous weed species, and sometimes competition can inhibit successful tree establishment and survival (Fawcett 1995). At our study site, clumps of grasses were only present in the piles of topsoil (although not in our treatment plots).

The regular supply of water in the shade house strongly influenced the germination, growth and survival of the seedlings. Seedling emergence was much greater in the shade house for all species (41%) than in the field (8%), suggesting that water stress was likely the major factor affecting survival in the field, however greater wind disturbance, seedling herbivory and erosion could have been additional factors. Similarly, Wilson & Bowman (1994) recorded 60–70% seed germination of *Eucalyptus miniata* and *E. tetrodonta* in nursery conditions compared to 3–15% in their field trial. In our trial, mortality of emerged seedlings was negligible in the shade house (0–4%) compared to the overall mortality rate of seedlings in field of 21%, indicating that mortality due to water stress and other factors is continuing to occur.

Seed availability is a major issue for restoration of gravel extraction sites. We did not observe any natural emergence of *Acacia* or *Eucalyptus* seedlings in our trial area outside our plots. When gravel extraction occurs, trees are cleared over substantial areas and seed dispersal of *Eucalyptus* species in particular is limited. They have no particular dispersal mechanisms and the seeds typically fall within a distance equivalent to the canopy height of the tree (Booth 2017). Over time, some *Eucalyptus* species of the savanna can also produce shoots from root buds, where lateral surface roots grow into the rehabilitation area. *Acacia lamprocarpa* does have a small yellow aril on its seed and so its seeds are adapted for ant dispersal (Dunlop *et al.* 1995). However, ants tend to remove seeds from disturbed areas and carry them into woodland rather than from the woodland into the open disturbed sites (Andersen & Morrison 1998). We also found that limited commercial seed supplies were a limiting factor for the trial.

For successful restoration of gravel extraction sites, research focused on finding solutions to factors affecting the revegetation success on those sites is necessary. The results of field experiments investigating different treatments and ways of improving soil conditions for plant growth can be used to substantially improve restoration outcomes. For example, scarification and application of litter can improve *Eucalyptus* establishment. Supplementary watering during the first dry season may also be an option at some sites but the effects of this on the developing seedlings would need to be investigated further.

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