Plant Soil
https://doi.org/10.1007/s11104-024-06554-5

RESEARCH ARTICLE

Exploring the potential of topsoil pellets to improve native seedling establishment on degraded agricultural land

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Received: 1 August 2023 / Accepted: 11 February 2024

Abstract
Background and aims Agricultural activities can degrade soils and promote weeds, posing challenges to native species restoration. In agricultural restoration, removing contaminated topsoil is a method designed to reduce elevated soil nutrients caused by fertilisation. This strategy targets weed control by eliminating both aboveground weeds and their soil seed bank before direct seeding. However, it also diminishes native soil seed banks and beneficial soil microbes. We investigated the potential of fresh topsoil pellets containing seeds to improve seedling performance in a degraded grassy woodland where topsoil had been removed.
Methods We tested various pellet recipes, including one using commercial ingredients and three with different topsoil proportions (30%, 50%, and 70%). The study was conducted in a degraded grassy woodland in southeastern Australia, where topsoil was removed for restoration. We explored the effect of these pellet varieties on seedling emergence and growth of six native species common in this community, as well as microbial activity in the soil surrounding the seedlings.
Results Pellets significantly improved the emergence of *Chrysocephalum apiculatum*, providing evidence of their effectiveness. However, pellets significantly reduced *Arthropodium milleflorum* and *Glycine tabacina* emergence. *Linum marginale* and

Responsible Editor: Rafael S. Oliveira.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-024-06554-5.

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Rytidosperma caespitosum emergence remained unaffected by pellets. One species, *Bothriochloa macra,* had insufficient emergence for analysis. The microbial activity of the soil surrounding *Rytidosperma caespitosum* seedlings was significantly improved by pellets, with no significant effects observed for other species.

Conclusion Our results demonstrate that topsoil pellets improved the emergence of one native species, but reduced emergence for two others, indicating species-specific responses to pelleting.

Keywords Grassy woodlands · Fresh topsoil · Seed enhancement technologies · Soil microbes

Introduction

Restoration of degraded ecosystems has highly variable outcomes, with actions often requiring decades to achieve a shift from degraded ecosystem states (Atkinson et al. 2022; Isbell et al. 2019). Complex restoration is required to address a wide array of barriers or thresholds that can delay or prevent ecosystem recovery (Jones et al. 2018). Agricultural sites span a continuum of disturbance and degradation, ranging from low-intensity grazing with modest ecological impacts to high-intensity grazing or cropping that can result in complete shifts in species composition (Dorrough and Scroggie 2008; Li et al. 2017). While agriculture impacts approximately 32% of the Earth's terrestrial surface (Ritchie and Roser 2013), the global increase in abandoned agricultural land (Cramer et al. 2008; Isbell et al. 2019) presents an opportunity to restore extensive areas, potentially addressing climate change, improving biodiversity, and enhancing ecosystem function (Etter et al. 2020; Strassburg et al. 2020; Yang et al. 2020).

The main barriers to native species recovery in highly degraded agricultural sites are twofold. First, elevated soil nutrients resulting from fertiliser application create unfavourable soil conditions for many native Australian grassland species (Standish et al. 2006). These elevated soil nutrients such as nitrogen (N), and phosphorus (P) foster the growth of non-native weed species, leading to a proliferation of the weed seed bank, restricting the emergence and growth of native species (Cole and Lunt 2005; Gibson-Roy et al. 2010a). Second, seed limitation of native species hinders natural recovery, which is further exacerbated by landscape fragmentation, reducing dispersal opportunities (Gibson-Roy et al. 2010b; Standish et al. 2007; Svejcar et al. 2017; Yates and Hobbs 1997).

Typically, highly degraded grassland and grassy woodlands in Australia are challenging to restore, as most restoration methods are ineffective (Brown et al. 2017; Gibson-Roy et al. 2010a; Gibson-Roy et al. 2010b). However, topsoil removal has been successful in the restoration of highly degraded post-agricultural sites in grasslands and grassy woodlands in southeastern Australia (Brown et al. 2017; Gibson-Roy et al. 2010a). Topsoil removal aids recovery by reducing soil nutrients and non-native species seed banks through the extraction of the upper soil layer (Brown et al. 2017; Gibson-Roy et al. 2010a). This approach is typically carried out in sites where recovery using less intensive methods is unlikely, making it essential for enabling the restoration of sites that would otherwise remain unsuitable (Gibson-Roy et al. 2010a; Gibson-Roy et al. 2010b). The main cost associated with this method is the cost of seed (Gibson-Roy 2023). Therefore, any techniques that can improve seedling success rate (which is commonly low) effectively reduces the cost of this approach.

A significant drawback of topsoil scalping is that it removes topsoil resources (i.e., microbial communities, vital nutrients) that are ecologically important and often in short supply during restoration (Ferreira and Vieira 2017). Topsoil contains microbes like cyanobacteria and arbuscular mycorrhizal fungi (hereafter AMF) that improve seedling performance (Pitaktamrong et al. 2018; Román et al. 2020). AMF and cyanobacteria enhance soil fertility, increase water and nutrient acquisition by plants, and promote plant growth and health (Begum et al. 2019; Singh et al. 2016). AMF also have a beneficial relationship with over 90% of plant families (Van Der Heijden et al. 2008; Wang and Qiu 2006). This symbiotic relationship and their ability to enhance soil fertility, makes them crucial for the success of seed-based restoration, especially in low-fertility degraded soils (Coban et al. 2022; Rivera et al. 2014).

Seeding is a common approach to reintroduce native species in degraded ecosystems, aiming to overcome seed scarcity. Direct and broadcast seeding are especially important at larger scales due to their cost-effectiveness in comparison to topsoil relocation and tubestock planting (Palma and Laurance 2015; Rokich et al. 2000; Souza and Engel 2018). However, establishing native species for restoration from seeds can be challenging, often resulting in less than 10% seedling establishment (Ceccon et al. 2016; James et al. 2011). Additionally, the scarcity of native Australian seeds adds complexity to seed-based restoration, making them unsuitable for numerous large-scale restoration projects (Gibson-Roy et al. 2021; Merritt and Dixon 2011; Pedrini et al. 2023).

Seed enhancement technologies (SETs) are used in restoration to overcome seedling emergence barriers associated with seed-based restoration (Brown et al. 2021; Madsen et al. 2016b). SETs involve the application of additives or physiological alteration of seeds to improve seed delivery, protection, germination, or seedling performance making greater use of scare seed resources (Brown et al. 2021; Erickson et al. 2021; Madsen et al. 2016b). One type of SETs, extruded pellets (hereafter referred to as pellets), incorporates seeds into a soil slurry containing a range of additives such as organic material, water holding crystals, soil, and mineral products. This mixture is then extruded through a die or shaped using moulds (Brown et al. 2021; Dadzie et al. 2022; Erickson et al. 2019; Madsen et al. 2016b). The pellets and their various components are often designed to improve the conditions in which the seeds germinate and establish, therefore improving the success of seed-based restoration (Brown et al. 2021; Madsen et al. 2016a; Madsen et al. 2016b).

Recent advancements in restoration techniques have explored the use of stockpiled topsoil and the isolation and incorporation of its components, such soil microbes, in conjunction with SETs. One study found that pellets containing stockpiled topsoil (stored for over 10 years) did not significantly affect seedling emergence, microbial activity, or the soil bacterial community (Stock et al. 2020). However, prolonged storage of topsoil exceeding 6 months can lead to a decline in AMF diversity and microbial activity (Amir et al. 2022). The isolation of microbes and incorporation into pellets has been more successful, for example, isolated cyanobacteria in pellets have been effective in restoring soil biota and establishing biocrusts on degraded soils (Román et al. 2020). Additionally, incorporating isolated native bacteria and cyanobacteria into pellets has led to a 48% and 55% increase in the emergence of *Acacia inaequilatera* Domin respectively, while cyanobacteria increased emergence of *Triodia epactia* S.W.L.Jacobs (Dadzie et al. 2022). Although only a few studies have examined the effects of AMF in pellet technologies, both seed coating and pellets with AMF have shown increased root colonisation and plant growth (Colla et al. 2015; Pitaktamrong et al. 2018).

Topsoil relocation is a common practice used to restore soil function and native seed banks in severely degraded sites, including post-agricultural and mining environments (Brown et al. 2017; Bulot et al. 2017; Koch 2007). Incorporating fresh topsoil in pellet SETs offers potential benefits in terms of logistics, costs, and outcomes by delivering concentrated nutrients and microbes to the microsite of enclosed plant species. This targeted delivery method, as opposed to spreading topsoil out across the entire site, efficiently uses a limited resource and ensures the desired species are contained within the topsoil, minimising the encouragement of weeds across the site. The potential benefits of using fresh topsoil, which naturally contains AMF and soil microbes without requiring culturing has been underexplored in pellet technologies. Indeed, to our knowledge only one other study has tested this. Here, Alfonzetti et al. (2022) found that incorporating fresh topsoil into pellets increased seedling biomass for two study species. However, it reduced the emergence of one species while showing no significant effect on the other. Furthermore, these outcomes were contingent upon the soil conditions at the planting site (Alfonzetti et al. 2022). Hence, the outcomes of using fresh topsoil are understudied with variable results, and there has been no prior investigation into using varying quantities of topsoil within pellets.

Using pellets to deliver the beneficial components of fresh topsoil to the embedded native seeds after topsoil scalping could potentially improve both the success of seed-based restoration and soil microbial health on degraded soils. We used six model species to examine the effect of topsoil pellets on emergence. These species encompassed various life-forms (grasses, forbs, and a legume) commonly found in the target community, considering that most SET studies highlight species-specific outcomes. Considering the chosen species, we expect that all species should benefit from the addition of fresh topsoil containing

AMF and specifically, the legume species should benefit from soil bacteria.

We address the following questions:

1. How does the use of pellets containing fresh local topsoil affect seedling emergence and early growth of native plant species on degraded agricultural sites where topsoil has been removed? Further, does the proportion of topsoil included in the pellet affect seedling emergence and growth?
2. Does the incorporation of fresh local topsoil in pellets contribute to an increase in soil microbial activity on degraded agricultural sites where topsoil has been removed?

Method

Study site

This study was conducted at the Burrumbuttock Woodland restoration site (−35.835, 146.794), located in southern New South Wales, Australia. The restoration site was established through the collaborative efforts of the Corowa District Landcare and Wirraminna Environmental Education Centre. The site is a post-agricultural land-use area where topsoil scalping was carried out in 2019 to remove weeds, including aboveground vegetation and the soil seed bank (Fig. 1). Prior to scalping, the site was a degraded Box-Gum grassy woodland (formally referred to as White Box - Yellow Box - Blakely's Red Gum Grassy Woodland and Derived Native Grasslands), which is nationally listed as a critically endangered ecological community (Department of Climate Change, Energy, the Environment and Water 2023). This community is characterised by three main species of *Eucalyptus*, they are yellow box (*E. melliodora* A.Cunn. ex Schauer), white box (*E. albens* Benth.), and Blakely's red gum (*E. blakelyi* Maiden.) (Department of Climate Change, Energy, the Environment and Water 2023; Keith 2004). Common understorey species are native grasses e.g., Wallaby-grass (*Rytidosperma caespitosum* [Gaudich.] Connor & Edgar.) and Red grass (*Bothriochloa macra* [Steud.] S. T. Blake.), and forbs (e.g., *Arthropodium* spp. and *Chrysocephalum* spp.). Non native grasses such as common couch *(Elytrigia repens* [L.] Desv. ex Nevski.) and serrated tussock (*Nassella trichotoma* [Nees.] hack.ex arechav.) are also common. The land was primarily used for sheep grazing until 2012, and prior to grazing, it was subject to broad acre cropping and cultivation for 30 years. The restoration aim is to return some of the species that were lost due to decades of agricultural practices. The average annual precipitation over a 29-year period is 582.4 mm, with the majority (30%) falling during the winter months (June - August) (Bureau of Meteorology 2023). The average summer temperatures of 23.3 °C are more than double that of the winter months (8.7 °C), while only receiving 20% of the annual rainfall (Bureau of Meteorology 2023).

Pellet production

A pilot study was conducted testing various proportions of ingredients to determine the optimal composition of pellets. Pellets with varying proportions of fresh topsoil were subject to wetting and drying to assess their structural integrity and their ability to

Fig. 1 Left: an aerial photo of the topsoil scalped site at Wirraminna Environmental Education Centre at Burrumbuttock, southern New South Wales, Australia. The red outline indicates the portion of scalped site where the field trial was conducted. Right: a photo of the site at the start of the field trial showing the bare scalped soil

break down in response to hydration. The results from the pilot study guided the formulation of the pellet recipes used in the subsequent field study (Table 1). Due to the high sand content of the topsoil, it was necessary to decrease the percentage of sand as the proportion of topsoil was increased to maintain a consistent clay content in the pellets across all treatments (Table 1). Hence, the topsoil treatments differed mainly in the proportion of topsoil to enable us to determine how various amounts affected our response variables.

Topsoil samples were collected using a shovel from the top 5 cm of a roadside reference site containing remnant native vegetation, located approximately 500 m from the study site. The topsoil was sieved (2 mm) to remove larger seeds and coarse organic material. Once sieved the topsoil was used to produce pellets within 14 days. These pellets were created by combining the sieved topsoil with the commercially available ingredients (detailed in Table 1) and water to create a slurry/paste. The slurry was then poured into moulds 11 cm in circumference and 1 cm deep, resulting in a total volume ca. 9.6 cm^3. Each pellet encapsulated seeds of a single species, seeds were inserted into the wet slurry, positioning them in the centre of the mould, and then covered with additional slurry to completely enclose the seeds. The pellets were left to air dry at room temperature until they hardened sufficiently to be removed from the moulds (ca. 2-3 days).

Experimental design

The study compared four pellet recipes, which consisted of a base pellet made from commercially available ingredients (Table 1) and three topsoil pellets with varying quantities of fresh untreated topsoil (30%, 50% and 70%), compared with a control of non-enhanced seeds, precision sown 3-5 mm into the soil profile. The base pellet was included to determine seedling responses to SET application in the absence of topsoil.

We used a randomised block design with eight replicate blocks, each divided into 30 nested sub-plots (Fig. S1). Each sub-plot contained 20 units of a single treatment comprised of either pellets or non-enhanced control seeds, arranged in clusters to simulate pellet seed distribution. This proof-of-concept experiment investigated the effect of four pellet treatments on the emergence responses of six model native species, two grasses; *Rytidosperma caespitosum* (Gaudich.) Connor & Edgar. and *Bothriochloa macra* (Steud.) S. T. Blake., three non-leguminous forbs; *Arthropodium milleflorum* (DC.) J. F. Macbr., *Chrysocephalum apiculatum* (Labill.) Steetz., and *Linum marginale* (A. Cunn.) ex Planch and one leguminous forb; *Glycine tabacina* (Labill.) Benth. All study genera form symbiotic relationships with AMF, while *Glycine* species' also establish symbiotic associations with nitrogen-fixing rhizobacteria, leading to the formation of root nodules (Frew 2021; Gibson-Roy et al. 2014; Raza et al. 2020). The seeds used in this study were exclusively sourced from the Wirraminna Environmental Education Centre and cultivated within their seed production area. All seeds used in this study were collected in the year 2020, except for *R. caespitosum*, which included seeds from both 2019 and 2020. Before pellet production, all seeds were stored in a cool, dry environment in a refrigerator at 4 °C. Details about the six species used and the exact

Table 1 Recipe details of pellets, displaying percentages (%) and weights (g) of commercially available ingredients (Calcium bentonite, diatomaceous earth, and sand) alongside locally sourced topsoil, used to produce 1000 pellets

Ingredients	Base Pellet		Topsoil 30% Pellet		Topsoil 50% Pellet		Topsoil 70% Pellet	
	%	Weight (g)	%	Weight (g)	%	Weight (g)	%	Weight (g)
Ca Bentonite	10	1008	10	672	10	672	10	672
Diatomaceous earth	20	1680	20	1344	10	672	20	1344
Sand	70	4032	40	2688	30	2016	0	0
Topsoil	0	0	30	2016	50	3360	70	4032
Average pellet weight (g)		3.87		4.21		4.33		4.56

Also included is the average pellet weight (g) for all pellet types used in the field study at Wirraminna Environmental Education Centre, New South Wales number of seeds within each unit for individual species are outlined in Table 2. The field experiment was installed on 30th of April 2021, to coincide with autumn rains. However, due to a lack of rain following installation, the site was hand watered using a watering can with 9 L of water per block (to simulate a ~ 1 mm rain event) weekly for three weeks until the arrival of autumn rains at the end of May.

Data collection

Data were collected regularly over a 12-month period to capture critical emergence and early life-stages during the first growing season. Seedling emergence was recorded when cotyledons (for eudicot species) or singular cotyledon or coleoptile (for monocot species) broke through the surface of the pellets or soil and became visible aboveground. Seedling emergence data were collected tri-weekly in May, June, and July. After seedlings emerged, they were marked with a coloured pin to indicate the emergence date. By August, the rate of seedling emergence had begun to plateau for four of the six species (*R. caespitosum, C. apiculatum, L. marginale* and *A. milleflorum*) and data collection for emergence ceased. Due to the high rate of *R. caespitosum* emergence, units containing more than three seedlings were thinned down to three individuals on the 6th of August to reduce intraspecific competition among seedlings. For all remaining individuals, data were collected on survival and plant height every two weeks until early December 2021, after which all further sampling was conducted monthly for the remainder of the trial. Due to low seedling emergence(<1%), *B. macra* was excluded from all analyses. *A. milleflorum, L. marginale,* and *G. tabacina* were not monitored past the emergence phase due to low levels of emergence and high mortality (<1% seedlings were alive at the conclusion of the experiment).

At the conclusion of the experiment, the aboveground biomass of all *R. caespitosum and C. apiculatum* plants were harvested, the biomass was oven dried for three days at 80 °C and weighed. Four soil samples were also taken from the top 5 cm of each SET treatment in all eight blocks. Samples were collected from the soil beneath *R. caespitosum* and *C. apiculatum* seedlings, as well as from areas where seedlings of *L. marginale* and *A. milleflorum* had emerged but subsequently died. No samples were collected for *G. tabacina* and *B. macra*, due to insufficient levels of emergence. The soil samples were air-dried at 15 °C for 7 days, sieved through a 2 mm mesh, and stored at 4 °C to preserve the microbial community. The samples were then transported to UNSW Centre for Ecosystem Science & Evolution & Ecology Research, where the soil 'microresp' method (Campbell et al. 2003) was used to determine the soil microbial activity in the presence of glucose (substrate-induced respiration). For this, 0.5 g of air-dried soil was weighed into 96-well plates, and 100 µl of distilled water was used to activate the microorganisms. After four days of incubation at a constant temperature of 25 °C, the set-up was removed, and 25 µl of glucose solution was added to the soil. The glucose solution was prepared by dissolving 4 g glucose in 25 ml of distilled water. The efflux of CO_2 was trapped with a creosol red gel for 6 hours. A change in colour of the creosol red due to CO_2 evolution was determined calorimetrically using a spectrophotometer. The amount of CO_2

Table 2 Details of species used during the field experiment showing family, species, monocot/eudicot, 1000 seeds weight (g), seed pre-treatment (remove = seeds removed from florets, clean = seeds cleaned from chaff, and scarified = exterior of the seeds broken with sandpaper) and number of seed in each experimental unit (pellet or cluster of non-enhanced seeds)

Family	Species	Monocot/ Eudicot	1000 Seed Weight (g)	Seed Pre-treatment	Seeds Per Unit
Asparagaceae	*Arthropodium milleflorum*	Monocot	0.96	No treatment	5
Asteraceae	*Chrysocephalum apiculatum*	Eudicot	0.05	Cleaned	5
Fabaceae	*Glycine tabacina*	Eudicot	6.40	Scarified	3
Linaceae	*Linum marginale*	Eudicot	1.14	No treatment	4
Poaceae	*Bothriochloa macra*	Monocot	1.30	Remove	4
Poaceae	*Rytidosperma caespitosum*	Monocot	0.63	Remove	4

evolved from the soil (expressed as ugCO_2-C/g) was used as a proxy for microbial activity.

Data analysis

All statistical analyses were conducted using the R statistical software version 4.3.2 (R Development Core Team 2019). Generalised Linear Mixed Models (GLMMs) were used to examine the effect of pellets containing fresh topsoil on seedling emergence. A binomial distribution of errors was used to account for the binary nature of the response variable, considering the number of successes (seedlings that emerged) and failures (seedlings that did not emerge) within a fixed number of Bernoulli trails (total number of seeds in each nested sub-plot) (Zuur et al. 2009). Due to the blocking design of the experiment, 'block' was designated as a random effect in all models to account for non-independence (Harrison 2015; Zuur et al. 2009). The response variable (seedling emergence) was modelled as a function of SET treatment which was specified as a categorical variable with five levels: Non-enhanced, Base, Topsoil 30%, Topsoil 50%, and Topsoil 70%. The control non-enhanced seed was set as the reference category. GLMMs were fitted using the 'glmer' function within the lme4 package (Bates et al. 2015; Dean et al. 2004). Significant results indicate SET treatments differ from that of the control (non-enhanced seeds).

Linear Mixed Models (LMMs) were used to analyse the effects of SET treatments on seedling growth (height and biomass) and microbial activity. LMM was chosen as the appropriate method due to the continuous nature of the response variables, which follow a Gaussian distribution. LMMs were fit using the lmer function within the lme4 package (Bates et al. 2015). Separate analyses were conducted to investigate the impact of SET treatment on seedling height, aboveground biomass, and microbial activity. In each analysis, the response variable was modelled as a function of SET treatment, which was specified as a categorical variable with five levels: Non-enhanced, Base, Topsoil 30%, Topsoil 50%, and Topsoil 70%. The control non-enhanced seed was used as the reference category in all analyses. Due to low levels of seedling emergence and high mortality in other species, only seedling height and aboveground biomass data for *C. apiculatum* and *R. caespitosum* (after thinning) were analysed. Microbial activity was assessed for all species except *G. tabacina* and *B. macra*, which exhibited low levels of emergence. Significant results indicate SET treatments differ from that of the control (non-enhanced seeds).

Results

Seedling emergence

Pellet SETs had limited benefits on seedling emergence for most of the study species. For *A. milleflorum,* seedling emergence was significantly lower in the base pellet and the topsoil 70% pellet treatments, with seedlings being 0.34 and 0.47 times less likely to emerge, respectively, compared to the non-enhanced seed (Fig. 2A, Table 3). Conversely, for *C. apiculatum*, seedling emergence was significantly greater in the base pellet, topsoil 30%, and topsoil 50% pellet treatments (Fig. 2B, Table 3), resulting in 2.69, 5.90-, and 6.81-times higher likelihood of emergence, respectively, compared to non-enhanced seeds. For *Glycine tabacina*, seedling emergence was significantly reduced by the base pellet, topsoil 30% and topsoil 70% pellet treatments (Fig. 2D, Table 3). As a result, when treated with topsoil 30% pellets *G. tabacina* seeds were 92% less likely to emerge and this likelihood further decreased to 97% when treated with base pellets and topsoil 50% pellets, compared with non-enhanced seeds (Fig. 2D, Table 3). There were no significant effects of SET application on the emergence of *L. marginale* and *R. caespitosum* (Fig. 2C and E, Table 3).

Seedling height and aboveground biomass

Pellet SETs had an overall negative effect on seedling height. The topsoil 30% pellets significantly reduced seedling height of *C. apiculatum* compared to non-enhanced seeds (Fig. 3A, Table 4). Similarly, for *R. caespitosum*, all pellet treatments had a significantly lower height than the non-enhanced controls (Fig. 3B, Table 4). However, the estimates for some treatments have wide confidence intervals, indicating high levels of variability within these results. There were no significant differences in the aboveground biomass for *R. caespitosum* and *C. apiculatum* across all pellet treatments (Fig. S2, Table S1).

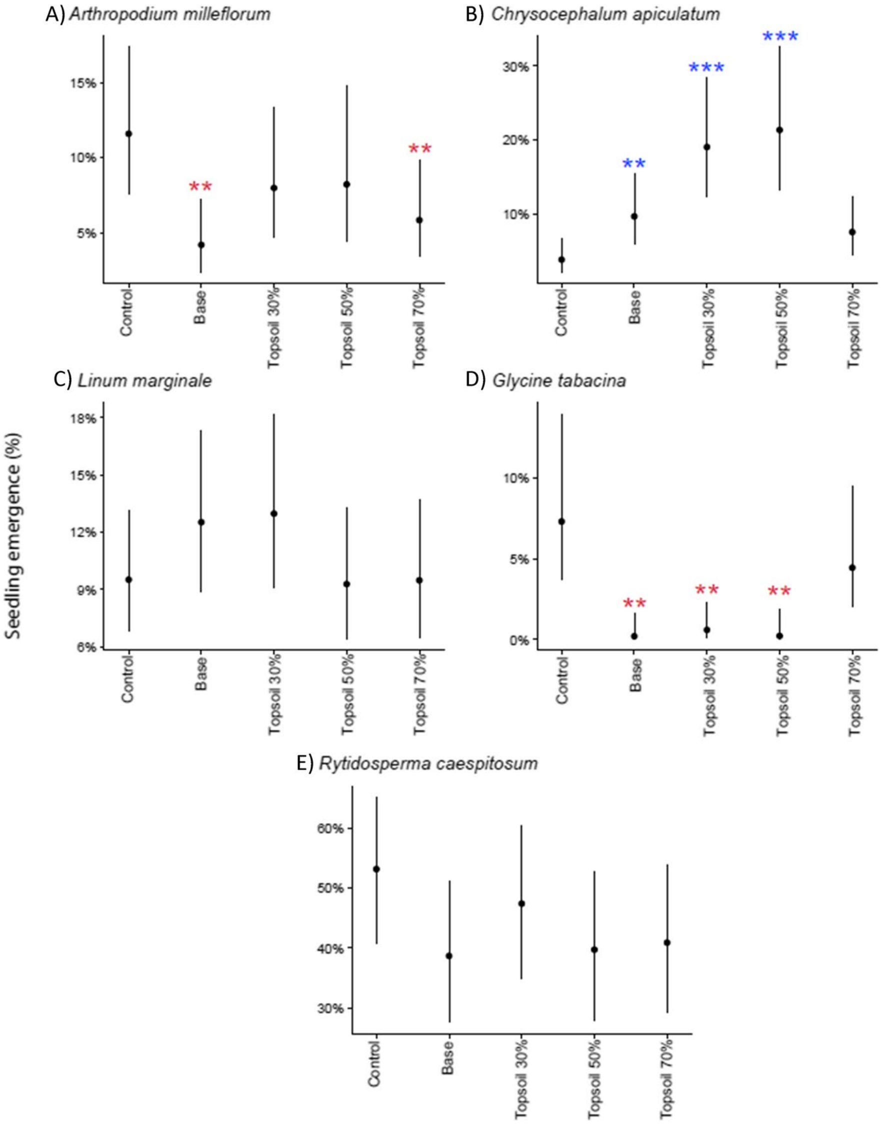


Fig. 2 Percentage of emerged seedlings from all SET treatments and control (non-enhanced seeds) for the five study species. The figure displays means and error bars representing 95% confidence intervals. Significant results indicate differences between the SET treatments in relation to the control (non-enhanced seeds). Significance levels are denoted with asterisks: * indicates p value <0.05, ** <0.01, and *** <0.001. Red asterisks indicate a decrease, while blue asterisks indicate an increase relative to the control

Microbial activity

The topsoil pellet treatments had limited effects on microbial activity. Specifically, the topsoil 50% treatment significantly increased microbial activity in the soil surrounding *R. caespitosum* seedlings (Fig. 4D, Table 5). Additionally, the topsoil 70% pellets demonstrated a trend towards increasing microbial activity around *R. caespitosum* (Fig. 4D, Table 5). However, the other three species did not show any significant differences in microbial activity among the treatments (Fig. 4, Table 5).

Discussion

Recently, there have been significant advancements in seed enhancement technologies designed to overcome specific challenges associated with seed-based restoration (Brown et al. 2021; Davies et al. 2018; Erickson et al. 2019; Gornish et al. 2019). However, the potential of incorporating fresh topsoil in pellets for the restoration of sites with degraded soils, such as scalped agricultural sites and mine sites, remains largely unexplored in the literature. To address this knowledge gap, we investigated the effect of pellets

Table 3 Summary of GLMM analysis for the proportion of seedling emergence in each species across all SET treatments and control (non-enhanced seeds)

Species	Predictors	Odds Ratio	Confidence Intervals	*p* value
Arthropodium milleflorum	(Intercept)	0.13	0.08 – 0.21	**<0.001*** **
	Base pellet	0.34	0.16 – 0.70	**<0.01** **
	Topsoil 30% pellet	0.66	0.32 – 1.37	0.269
	Topsoil 50% pellet	0.68	0.31 – 1.53	0.355
	Topsoil 70% pellet	0.47	0.23 – 0.98	**<0.05***
Chrysocephalum apiculatum	(Intercept)	0.04	0.02 – 0.07	**<0.001*** **
	Base pellet	2.69	1.28 – 5.65	**<0.01** **
	Topsoil 30% pellet	5.90	2.82 – 12.38	**<0.001*** **
	Topsoil 50% pellet	6.81	3.12 – 14.88	**<0.001*** **
	Topsoil 70% pellet	2.05	0.96 – 4.37	0.063
Linum marginale	(Intercept)	0.11	0.07 – 0.15	**<0.001*** **
	Base pellet	1.36	0.81 – 2.30	0.250
	Topsoil 30% pellet	1.42	0.83 – 2.42	0.201
	Topsoil 50% pellet	0.97	0.57 – 1.68	0.922
	Topsoil 70% pellet	1.00	0.57 – 1.73	0.989
Glycine tabacina	(Intercept)	0.08	0.04 – 0.16	**<0.001*** **
	Base pellet	0.03	0.00 – 0.23	**0.001** **
	Topsoil 30% pellet	0.08	0.02 – 0.34	**0.001** **
	Topsoil 50% pellet	0.03	0.00 – 0.26	**0.002** **
	Topsoil 70% pellet	0.59	0.21 – 1.69	0.327
Rytidosperma caespitosum	(Intercept)	1.14	0.69 – 1.88	0.614
	Base pellet	0.55	0.27 – 1.13	0.105
	Topsoil 30% pellet	0.79	0.38 – 1.64	0.532
	Topsoil 50% pellet	0.58	0.28 – 1.20	0.143
	Topsoil 70% pellet	0.61	0.30 – 1.25	0.178

Significant results are denoted with asterisks: * indicates *p* value <0.05, ** <0.01, and *** <0.001

containing varying amounts of fresh topsoil on seedling emergence, seedling height and aboveground biomass, and microbial activity on degraded post-agricultural land where topsoil had been removed. We found variable effects on seedling emergence depending on the species investigated and the proportion of topsoil in the pellet.

The field emergence of non-enhanced seeds for all species was approximately 8%, with the exception of *R. caespitosum* (~40%). While this rate aligns with other direct seeding studies (Gibson-Roy et al. 2007), our aim was to enhance seedling establishment, reducing costs associated with seed purchases and making more efficient use of limited seed resources. Notably, for *Chrysocephalum apiculatum*, we found increased seedling emergence in the pellet treatments—specifically in the topsoil 30% and 50% treatments, as well as the base pellet, which did not contain topsoil. Hence, for *C. apiculatum*, pelleting improved emergence irrespective of the presence of topsoil. The highest emergence occurred in the topsoil 50% pellets, with *C. apiculatum* showing an increase to 20% from 4% in the non-enhanced seeds. From a practitioner perspective, achieving a significant improvement in direct seeding to 20% is noteworthy and expands the range of species suitable for restoration via direct seeding. Although we did not observe a corresponding increase in microbial activity for this species, indicating that the topsoil may have improved soil nutrient conditions (though this does not explain the improvements in the base pellet). While some species showed no significant effect of topsoil pellets on emergence (*Linum marginale* and *R. caespitosum*,) the single positive result provides proof of concept, which requires further development.

Mechanical or direct seeding is the most common approach to reintroduce species to degraded sites where topsoil has been removed (Gibson-Roy et al. 2010a; Gibson-Roy et al. 2010b). *Rytidosperma caespitosum* is typically not used in restoration due

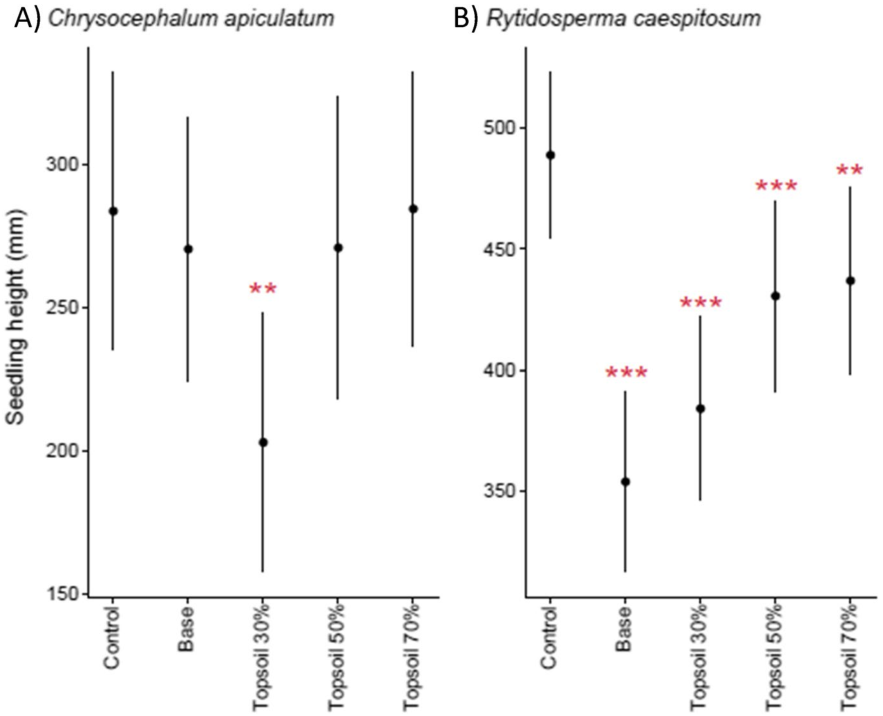


Fig. 3 Seedling height at the conclusion of the experiment for *C. apiculatum* and *R. caespitosum* in all SET treatments and controls (non-enhanced seeds). The figure displays means and error bars representing 95% confidence intervals. Significant results indicate differences between the SET treatments in relation to the control (non-enhanced seeds). Significance levels are denoted with asterisks: * indicates *p* value <0.05, ** <0.01, and *** <0.001. Red asterisks indicate a decrease relative to the control

Table 4 Summary of LMM analysis for seedling height for *R. caespitosum* and *C. apiculatum* across all SET treatments and control (non-enhanced seeds)

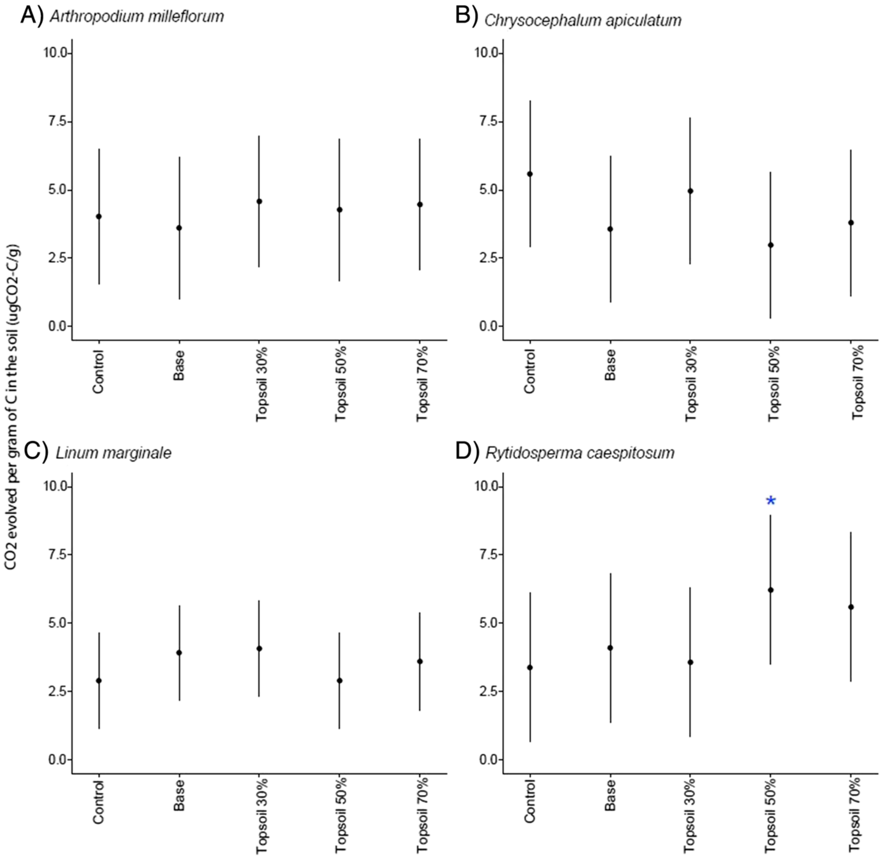
Species	Predictors	Estimate	Confidence Intervals	*p* value
Chrysocephalum apiculatum	(Intercept)	283.74	235.18 – 332.30	**<0.001*** **
	Base pellet	−13.26	−69.72 – 43.20	0.641
	Topsoil 30% pellet	−80.68	−132.67 – −28.68	**0.003** **
	Topsoil 50% pellet	−12.79	−67.90 – 42.32	0.645
	Topsoil 70% pellet	0.79	−56.06 – 57.64	0.978
Rytidosperma caespitosum	(Intercept)	488.89	454.54 – 523.25	**<0.001*** **
	Base pellet	−135.08	−162.31 – −107.86	**<0.001*** **
	Topsoil 30% pellet	−104.82	−133.35 – −76.28	**<0.001*** **
	Topsoil 50% pellet	−58.34	−88.47 – −28.22	**<0.001*** **
	Topsoil 70% pellet	−52.00	−81.62 – −22.38	**0.001** **

Significant results are denoted with asterisks: * indicates p value <0.05, ** <0.01, and *** <0.001

to its non-deep physiological dormancy and a floret structure that makes it difficult to disperse effectively via mechanical seeders (Berto et al. 2021; Grice et al. 1995). These findings suggest that pellets could be an effective method for dispersing *R. caespitosum*, as no negative effects of pelleting were found.

We also found negative effects of pelleting on seedling emergence of two species, indicating the presence of emergence barriers. Specifically, *A. milleflorum* and *G. tabacina*, seedling emergence was significantly reduced by pelleting in most treatments. Their field emergence, even in non-enhanced seed, was very low, suggesting that they may not be suitable candidates for seed-based restoration in degraded post-agricultural sites, at least with the particular seed batch used. Comparable low emergence has been reported for other *Arthropodium* and *Glycine* species (Gibson-Roy et al. 2007). Here, it is unclear why species respond differently to the pelleting treatments. The reduced emergence could be attributed to 'hardsetting' of pellets, which creates an additional physical barrier for enclosed seeds (Daniells 2012; Davies et al. 2018). Hardsetting is the formation of a crust-like surface on the pellets

Fig. 4 Microbial activity measured as the amount of CO_2 evolved per g of C in the soil for four species from all SET treatments and control (non-enhanced seeds). *G. tabacina* was not included due to low levels of emergence. The figure displays means and error bars representing 95% confidence intervals. Significant results indicate differences between the SET treatments in relation to the control (non-enhanced seeds). Significance levels are denoted with asterisks: * indicates p value <0.05, ** <0.01, and *** <0.001. Blue asterisks indicate an increase relative to the control



during dry periods (Daniells 2012; Davies et al. 2018). Further refinement is necessary to address any potential hardsetting resulting from the pellet formulation and to optimise pellet SETs, ensuring no unintended costs to seedling emergence. One potential avenue for improvement is the exploration of different size pellet SETs to accommodate species characteristics such as seed size and shape (Baughman et al. 2023).

The results from this study and others suggest that the proportion of fresh topsoil in pellet SETs may a be crucial factor influencing their ability to enhance seed-based restoration. Another study exploring fresh topsoil in pellets found adverse effects on the emergence of one species but null effects on another (Alfonzetti et al. 2022). This study only examined a small quantity (2.3% of all ingredients) of fresh topsoil in pellets, potentially lacking adequate concentrations of healthy soil microbial communities, diminishing their effectiveness in pellet SETs. In our study, the improved emergence in *C. apiculatum* supports the notion that pellets containing topsoil of up to 50% (and probably 70%) can improve seedling emergence.

For both species, which had sufficient abundances to analyse height and biomass at the conclusion of the study, we observed a reduction in height in the pellet treatments, while biomass was not affected. Specifically, *Chrysocephalum apiculatum* seedlings had reduced height in the topsoil 30% treatment, and *R. caespitosum* seedlings had reductions in all pellet treatments. Despite the negative effect of pellet treatments on seedling height, there was no significant difference in aboveground biomass for both species across all pellet treatments (Fig. S2, Table S1). Previous studies have found delays in seedling emergence in pellets which reduces time aboveground for plant growth compared to non-enhanced seeds (Brown et al. 2019; Ritchie et al. 2020), which could explain the reduced height in our study. However, the results suggest a shift in growth allocation, where shorter seedlings exhibited a higher leaf production. While plant height is often linked with survival, as larger

Table 5 Summary of LMM analysis for microbial activity measured as the amount of CO_2 evolved per gram of C in the soil for four species in all SET treatments and control (non-enhanced seeds), *G. tabacina* was not included due to low levels of emergence

Species	Predictors	Estimates	Confidence interval	*p* value
Arthropodium milleflorum	(Intercept)	4.03	1.56 – 6.50	**0.002****
	Base pellet	−0.42	−3.26 – 2.42	0.766
	Topsoil 30% pellet	0.55	−2.11 – 3.21	0.676
	Topsoil 50% pellet	0.24	−2.60 – 3.09	0.862
	Topsoil 70% pellet	0.44	−2.22 – 3.10	0.74
Chrysocephalum apiculatum	(Intercept)	5.58	2.91 – 8.25	**<0.001*****
	Base pellet	−2.02	−4.82 – 0.78	0.152
	Topsoil 30% pellet	−0.62	−3.42 – 2.18	0.653
	Topsoil 50% pellet	−2.60	−5.40 – 0.20	0.067
	Topsoil 70% pellet	−1.79	−4.59 – 1.02	0.204
Linum marginale	(Intercept)	2.90	1.14 – 4.65	**0.002****
	Base pellet	1.02	−0.21 – 2.25	0.1
	Topsoil 30% pellet	1.17	−0.09 – 2.44	0.067
	Topsoil 50% pellet	0	−1.26 – 1.26	0.999
	Topsoil 70% pellet	0.7	−0.61 – 2.02	0.285
Rytidosperma caespitosum	(Intercept)	3.38	0.65 – 6.11	**0.017***
	Base pellet	0.72	−1.91 – 3.36	0.581
	Topsoil 30% pellet	0.19	−2.44 – 2.83	0.883
	Topsoil 50% pellet	2.84	0.20 – 5.47	**0.036***
	Topsoil 70% pellet	2.22	−0.42 – 4.85	0.096

Significant results are denoted with asterisks: * indicates *p* value <0.05, ** <0.01, and *** <0.001

plants are more likely to survive the summer drought period (Gardiner et al. 2019), reduced seedling height in our study was not associated with decreased seedling survival. Instead, it is likely more closely tied to belowground growth (Grossnickle 2012). Seedlings of both species survived through the critical first summer drought period, a time when mortality typically occurs (Morgan 2001). As such, topsoil pellets are a viable option for these two species due to increased emergence in *C. apiculatum* and neutral effects in *R. caespitosum*. Further research is needed to determine the optimal topsoil pellet composition to maximise their growth and establishment on degraded sites.

Soil microbes play a crucial role in maintaining soil function, fertility, and plant growth and survival (Begum et al. 2019; Singh et al. 2016). Yet topsoil scalping can negatively impact soil microbial diversity and health by removing the upper layers where microbes are often found (Eilers et al. 2012; Gibson-Roy et al. 2010a; Seuradge et al. 2017). Although the SET treatments did not have a significant effect on the emergence of *R. caespitosum* seedlings, we did observe an increase in microbial activity for this species in the topsoil 50% treatment, as indicated by elevated measurements of CO_2 evolved per g of C in the soil. While we were unable to identify the specific microbial taxa responsible for this increase in activity, the findings suggest that pelleted topsoil could potentially improve microbial activity and support plant growth on degraded sites. However, we did not find increased activity for any other species or treatments.

The transfer of soil from an intact reference site after the removal of degraded topsoil has been identified as one of the best methods for restoring soil microbial activity, enhancing physicochemical properties of soil, and for facilitating native species recovery (Bulot et al. 2017; Wubs et al. 2016). Based on our findings, it can be inferred that including 50% of topsoil in pellets is necessary to benefit microbial activity, and this also had the greatest improvements in seedling emergence (although this may vary depending on topsoil source). Our results suggest that when fresh topsoil is contained within the microsite of seeds, the effects are more species-specific or less effective. This is possibly due to the reduced topsoil quantities (or quality) compared to topsoil relocation studies. The effects of topsoil may be compromised by the presence of plant and soil pathogens from the topsoil source or the scalped soil, which could have a negative effect on seedling emergence and plant health (Alfonzetti et al. 2022; Emam 2016). Furthermore, any beneficial bacterial or fungal communities contained in the fresh topsoil may be depleted or damaged as a result of the wetting and drying process during pellet production (John et al. 2010; McIntyre et al. 2007). It is unclear from this study and others whether it is the handling of topsoil during the pellet production procedure, the quality of topsoil used, or the presence of plant and soil pathogens that drive these variable responses. We know that when topsoil components such as fungi, bacterial communities and cyanobacteria are isolated and then incorporated into SETs, they can improve seedling emergence, growth, and properties of degraded soils (Colla et al. 2015; Dadzie et al. 2022; Román et al. 2020). However, this isolation process is more complex and costly for restoration practitioners, making it currently impractical for large-scale restoration.

Further investigations are needed to fully explore the potential of fresh topsoil in pellets for seed-based restoration. It is crucial to consider the impact of the wetting and drying process on microbial and fungal communities during pellet production (John et al. 2010; McIntyre et al. 2007). Our study and other SET research highlight the need for species-specific responses to be considered, which may require a large-scale study with a diverse range of species. This study should consider species factors such as dormancy, life form and seed size to establish patterns and drivers for species-specific responses. Despite these uncertainties, our findings indicate that fresh topsoil pellets can be a valuable method for facilitating the recovery of some native species recovery on degraded sites. However, further research is required to optimise the use of fresh topsoil in pellets to promote seedling emergence of many species and long-term plant health with fewer costs to seedling emergence.

Acknowledgements We would like to acknowledge Sue Schilg and Judy Frankenberg from the Wirraminna Environmental Education Centre for providing the research site and supplying seeds to use in both the pilot study and the field trial. We would also like to acknowledge Emily Flint for assisting with the installation of the field trial.

Authors' contributions TPM, JNP, TEE, DGN conceived the idea and designed the experiment; TPM, DGN analysed the data; FAD conducted the soil analysis; TPM and JNP led the writing of the manuscript. All authors contributed to the drafts and gave final approval.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions TEE also acknowledges financial support for his position from the Australian Seed Scaling Initiative, Project 3.13, as part of the Cooperative Research Centre for Transformations in Mining Economies (CRC TiME) program. Miriam Muñoz-Rojas is supported by the Spanish Ministry of Science and Innovation (RYC2020-029255-I, TED2021-132332A-C22 and PID2021-123097OA-I00).

Data availability The datasets generated during and analysed during the current study are available on the Figshare repository under the https://doi.org/10.6084/m9.figshare.25052837.v1

Declarations

Competing interests The authors have no relevant financial or non-financial interest to disclose.

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