Enhancing native seedling establishment on degraded agricultural land with topsoil pellets

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Abstract

Background and aims

Agricultural activities can degrade soils and promote weeds, posing challenges in restoring native plant species. Removing contaminated topsoil, a restoration method used in some agricultural systems, reduces soil nutrients, and eliminates weeds both aboveground and in soil seed bank before direct seeding. However, it also diminishes native soil seed banks and beneficial soil microbes. We investigated the potential of encasing seeds in pellets containing fresh topsoil to improve seedling performance and establishment on 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. We explored the effect of these pellet varieties on seedling emergence and growth of six native species common in this community, as well microbial activity in the soil.

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 *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

Overall, our results demonstrate that using seed enhancement technologies in combination with direct seeding can improve the emergence of one native species on degraded agricultural soil, potentially facilitating the recovery of degraded ecosystems.

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). 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 ecosystems encompass a continuum of disturbance and degradation, from low-

intensity grazing with modest ecological impacts to high-intensity grazing or cropping that can result in complete shifts in species composition. Given that agriculture affects approximately 32% of the Earth's terrestrial surface (Ritchie and Roser 2013), restoring degraded agricultural land provides an opportunity to address climate change and improve biodiversity, and enhance ecosystem function (Etter et al. 2020; Strassburg et al. 2020; Yang et al. 2020).

Restoring plant diversity in degraded agricultural sites requires addressing both altered abiotic and biotic conditions (Brown et al. 2017; Prober et al. 2002). The primary barriers to the recovery of native species in post-agricultural sites are twofold. Firstly, the elevated soil nutrients resulting from fertilizer application promotes the growth of non-native invasive species, leading to a proliferation of the weed seed bank. Secondly, seed limitation of native species hinders natural recovery which is further exacerbated by landscape fragmentation reducing dispersal opportunities (Gibson-Roy et al. 2010; Lunt 1997; Yates and Hobbs 1997). To tackle these challenges, topsoil scalping is often carried out to reduce soil nutrients and non-native invasive species seed banks through the removal of the upper soil layer (Brown et al. 2017; Gibson-Roy et al. 2010). In addition, restoration of native species requires further measures, including direct seeding and ongoing non-native invasive species control (Brown et al. 2017; Gibson-Roy et al. 2010).

A major downside 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 has a beneficial relationship with over 90% of plant families, making them essential for re-establishing plant diversity (Emam 2016; Koch et al. 2006; Neuenkamp et al. 2019).

Typically, seeds are used to return native species to degraded ecosystems to overcome seed limitation. At large scales, direct and broadcast seeding are preferred as they are less expensive than topsoil relocation and tubestock planting (Palma and Laurance 2015; Rokich et al. 2000; Souza and Engel 2018). However, establishing plants from seeds can be challenging, often resulting in less than 10% establishment (Beveridge et al. 2019; Leger et al. 2019). 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 (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 products 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. 2019a; 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 involved the use of stockpiled topsoil as well as the isolation and incorporation of its components, such as AMF and cyanobacteria, 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, it is important to note that 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 demonstrated increased emergence of *Acacia inaequilatera* Domin by 48% and 55% respectively, cyanobacteria also increased the 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).

The potential benefits of using fresh topsoil, which naturally contains AMF and soil microbes without requiring culturing has been underexplored in pellet technologies. Notably, Alfonzetti et al. (2022) found that incorporating fresh topsoil into pellets resulted in increased seedling biomass for both 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). Using pellets to deliver the beneficial components of fresh topsoil to the embedded native seeds after topsoil scalping could potentially improve the success of seed-based restoration and soil microbial health on degraded soils simultaneously.

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. 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 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 the Environment 2018). 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 the Environment 2018; 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%) of it falling during the winter months (June - August) (Bureau of Meteorology 2023). The average summer temperatures of 23.3^{o}C are more than double that of the winter months (8.7^{o}C), while only receiving 20% of the annual rainfall (Bureau of Meteorology 2023).

Pellet production

A pilot study (Appendix A) was conducted to determine the optimal composition of fresh topsoil in pellets. Pellets with varying proportions of fresh topsoil were subject to wetting and drying cycles to assess their structural integrity and their ability to 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 if the amount of topsoil affected our response variables. Topsoil samples were collected from a reference site with 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. Pellets were produced by combining the ingredients (detailed in Table 1) with water to create a slurry/paste. This slurry was then poured into moulds 11 cm in circumference and 1 cm deep, resulting in a total volume ca. 9.6 cm^3. 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.

Table 1
Pellet recipes show the percentage of dry ingredients for the base pellet, and the 30%, 50% and 70% topsoil pellets used during the field experiment

Ingredients %	Pellet treatment			
	Base pellet	Topsoil 30	Topsoil 50	Topsoil 70
Calcium bentonite	15	10	10	10
Diatomaceous earth	25	20	20	20
Sand	60	40	20	0
Topsoil	0	30	50	70

Experimental design

The study compared four pellet recipes, which consisted of a base pellet made from commercially available ingredients 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 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. Details about the six species used and the exact 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 watered with 9 L of water per block (to simulate a ~ 1 mm rain event) weekly until the arrival of autumn rains at the end of May.

Table 2
Details of species used during the field experiment showing family, species, Monocot/Dicot, 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/ Dicot	1000 seed weight (g)	Seed treatment	Seeds per unit
Asparagaceae	*Arthropodium milleflorum*	Monocot	0.96	No treatment	5
Asteraceae	*Chrysocephalum apiculatum*	Dicot	0.05	Cleaned	5
Fabaceae	*Glycine tabacina*	Dicot	6.40	Scarified	3
Linaceae	*Linum marginale*	Dicot	1.14	No treatment	4
Poaceae	*Bothriochloa macra*	Monocot	1.30	Remove	4
Poaceae	*Rytidosperma caespitosum*	Monocot	0.63	Remove	4

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 dicot species) or singular cotyledon or coleoptile (for monocot species) broke through the surface of the pellets or soil and became visible above-ground. 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, four soil samples were 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 insufficient levels of emergence. The soil samples were air-dried at 15^{o}C for 7 days, sieved through a 2mm mesh, and stored at 4 ^{o}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 ^{0}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 25ml 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 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 package (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 (Dean et al. 2004).

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 treatment 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 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.

Results

Seedling emergence

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). Seedling emergence of *C. apiculatum* 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 30% topsoil pellets *G. tabacina* seeds were 92% less likely to emerge and 97% less likely when treated with base pellets and 50% topsoil pellets (Fig. 2d, Table 3). There were no significant effects of SET application on the emergence of *R. caespitosum* and *L. marginale* (Fig. 2e and c, Table 3).

Table 3

Summary of GLMM analysis for the proportion of seedling emergence in each species across all SET treatments and control (non-enhanced seed). Significant results are denoted with asterisks: * indicates p-value < 0.05, ** indicates p-value < 0.01, and *** indicates p-value < 0.001

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** **
	30% Topsoil pellet	0.66	0.32–1.37	0.269
	50% Topsoil pellet	0.68	0.31–1.53	0.355
	70% Topsoil 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** **
	30% Topsoil pellet	5.90	2.82–12.38	**< 0.001*** **
	50% Topsoil pellet	6.81	3.12–14.88	**< 0.001*** **
	70% Topsoil 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
	30% Topsoil pellet	1.42	0.83–2.42	0.201
	50% Topsoil pellet	0.97	0.57–1.68	0.922
	70% Topsoil 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** **
	30% Topsoil pellet	0.08	0.02–0.34	**0.001** **
	50% Topsoil pellet	0.03	0.00–0.26	**0.002** **

Species	*Predictors*	*Odds Ratio*	*Confidence intervals*	*p-value*
	70% Topsoil 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
	30% Topsoil pellet	0.79	0.38–1.64	0.532
	50% Topsoil pellet	0.58	0.28–1.20	0.143
	70% Topsoil pellet	0.61	0.30–1.25	0.178

Seedling height

The topsoil 30% pellet had a significant negative effect on seedling height of *C. apiculatum* (Fig. 3a, Table 4). Similarly, for *R. caespitosum*, all topsoil 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.

Table 4
Summary of LMM analysis for seedling height for *R. caespitosum* and *C. apiculatum across* all SET treatments and control (non-enhanced seed). Significant results are denoted with asterisks: * indicates p-value < 0.05, ** indicates p-value < 0.01, and *** indicates p-value < 0.001

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
	30% Topsoil pellet	-80.68	-132.67 – -28.68	**0.003** **
	50% Topsoil pellet	-12.79	-67.90–42.32	0.645
	70% Topsoil 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*** **
	30% Topsoil pellet	-104.82	-133.35 – -76.28	**< 0.001*** **
	50% Topsoil pellet	-58.34	-88.47 – -28.22	**< 0.001*** **
	70% Topsoil pellet	-52.00	-81.62 – -22.38	**0.001** **

Microbial activity

The topsoil treatments showed mixed results across the four species, with only *R. caespitosum* showing a significant increase in microbial activity in response to the topsoil 50% treatment. The other three species did not show any significant differences in microbial activity among the treatments (Fig. 4a, Table 5).

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 seed), *G. tabacina* was not included due to low levels of emergence. Significant results are denoted with asterisks: * indicates p-value < 0.05, ** indicates p-value < 0.01, and *** indicates p-value < 0.001

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

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. 2019b; Gornish et al. 2019). However, the potential of incorporating fresh topsoil in pellets for the restoration of sites with degraded soils, such as mine sites, remains largely unexplored in the literature. To address this knowledge gap, we investigated the effect of pellets containing varying amounts of fresh topsoil on seedling emergence, seedling height, 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 was generally low for most of the species investigated, with levels below 15%, except for *R. caespitosum*. This low level of field emergence is not uncommon in direct seeding practices (Grossnickle and Ivetić 2017). For one species, *Chrysocephalum apiculatum*, we found increased seedling emergence in the pellet treatments, this was 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 regardless of the inclusion of topsoil, but the highest emergence occurred in the topsoil 50% pellets, with a 6.8 times greater likelihood of emergence compared to non-enhanced seeds. However, for *A. milleflorum* and *G. tabacina*, seedling emergence was significantly reduced by pelleting in most of the treatments. The field emergence for these species, even in non-enhanced seed, was very low, suggesting that they may not be suitable candidates for restoration, at least with the seed batch we used. Future studies should investigate methods to enhance their field emergence. *Linum marginale* and *R. caespitosum* seedling emergence was not affected by any of the treatments, with *L. marginale* having overall low emergence, and *R. caespitosum* having reasonable emergence of around 40%.

Many studies exploring seedling emergence in SETs have found species-specific results (Alfonzetti et al. 2022; Dadzie et al. 2022; Stock et al. 2020), and it is unclear what attributes affect performance in pellets. Indeed, the other study exploring fresh topsoil in pellets found adverse effects on the emergence of one species but null effects on another (Alfonzetti et al. 2022). Here, our primary focus was to establish a proof of concept regarding the potential of topsoil pellets to improve emergence. Species from the community were selected based on availability, and in the hope that at least one species would emerge successfully enabling us to examine treatment effects. Hence, the improved emergence in *C. apiculatum* supports the notion that pellets containing topsoil of up to 50% can improve seedling emergence. The two species that pellets negatively affected emergence had very low emergence across all treatments and are currently not suitable for restoration.

Mechanical or direct seeding is the most common approach to reintroduce species to degraded sites where topsoil has been removed (Gibson-Roy et al. 2010; Gibson-Roy et al. 2010). *Rytidosperma caespitosum* is typically not used in restoration due 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 explored seedling height as a measure of plant growth and health in the treatments. Here, we only had enough individuals for two species at the end of the field trial to explore this. For both species, we found significant reductions in height in the pellet treatments, for *Chrysocephalum apiculatum* seedlings the reduction was found in the topsoil 30% treatment, and for *R. caespitosum* seedlings reductions were found in all pellet treatments. 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. Plant height can be related to plant survival as larger plants are more likely to survive the summer drought period (Gardiner et al. 2019). Here, we finished our trial in the second autumn after commencement and the seedlings of the species investigated in height survived through the first summer drought period, a critical time when mortality typically occurs (Morgan 2001). Furthermore, although pellet treatments had a negative effect on seedling height, there was no significant difference in aboveground biomass for both species across all pellet treatments (Fig S2, Table S1). This suggests a shift in growth allocation, where shorter seedlings exhibited a higher leaf production. Further research is needed to determine the optimal topsoil pellet composition for different species 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. 2010; 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. However, the results in this study suggest that when fresh topsoil is contained within the microsite of seeds, the effects are more species-specific or less effective, possibly due to the reduced topsoil quantities compared to topsoil relocation studies (or quality). 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 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 much more involved and costly for restoration practitioners and may not be feasible 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 native species recovery on degraded sites. 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.

Declarations

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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.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interest

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

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Figures

Figure 1

Left: an aerial photo of the topsoil scalped site at Wirraminna Environmental Education Centre at Burrumbuttock, New South Wales. 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

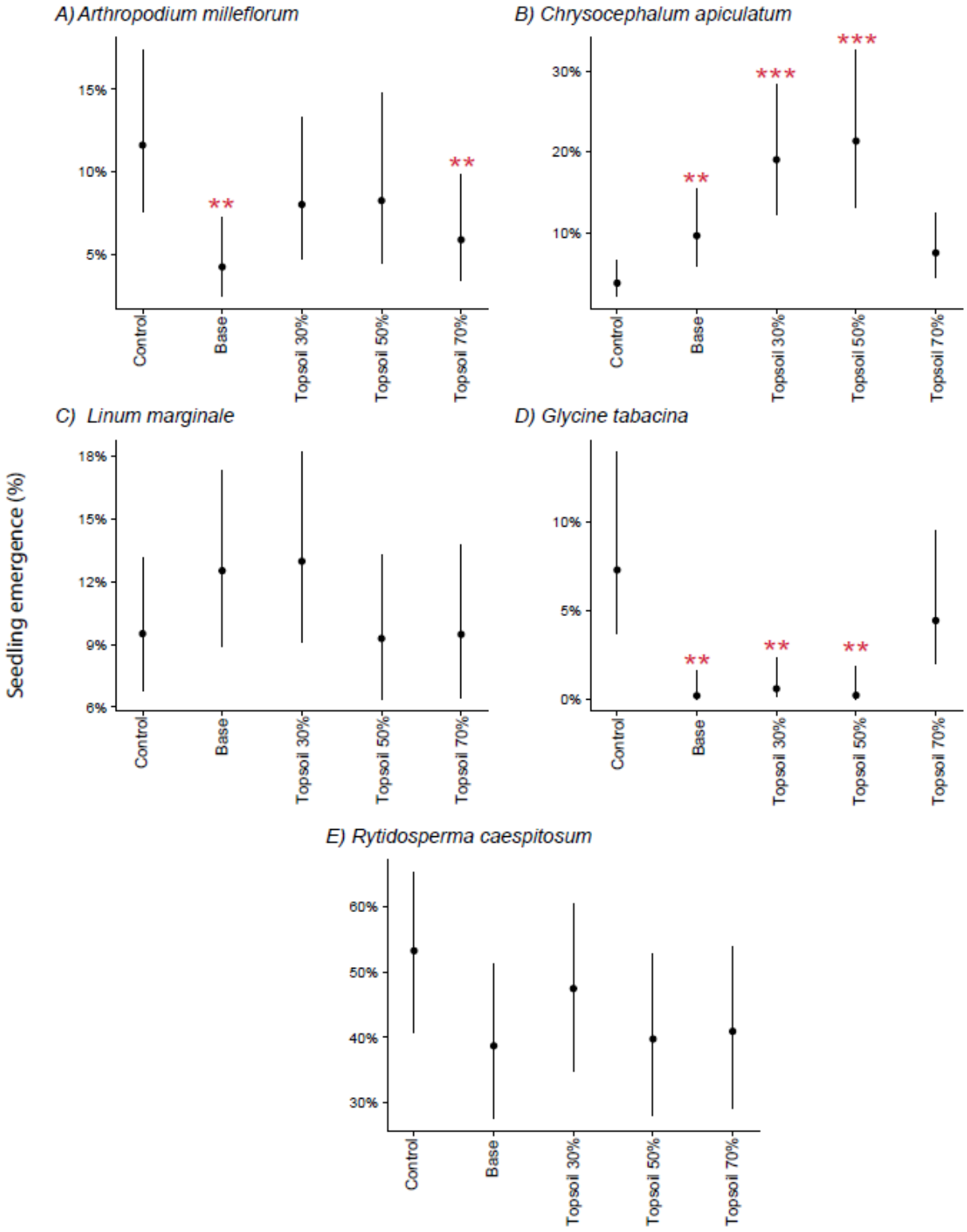

Figure 2

Percentage of seedlings emerged from all SET treatments and control (non-enhanced seeds) for the five study species. The figure displays medians with upper and lower quartiles (± SE). Significant results are denoted with asterisks: * indicates p-value < 0.05, ** indicates p-value <0.01, and *** indicates p-value <0.001

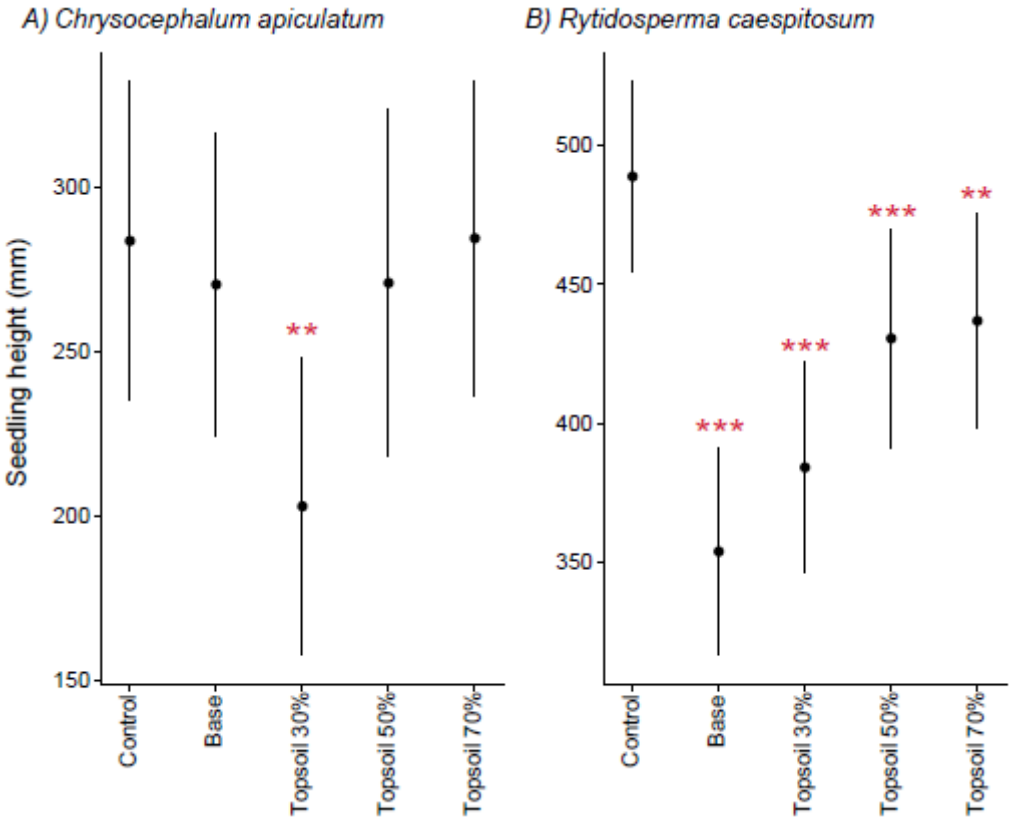

Figure 3

Seedling height for *C. apiculatum* and *R. caespitosum* in all SET treatments and controls (non-enhanced seed). The figure displays medians with upper and lower quartiles (± SE). Significant results are denoted with asterisks: * indicates p-value < 0.05, ** indicates p-value <0.01, and *** indicates p-value <0.001

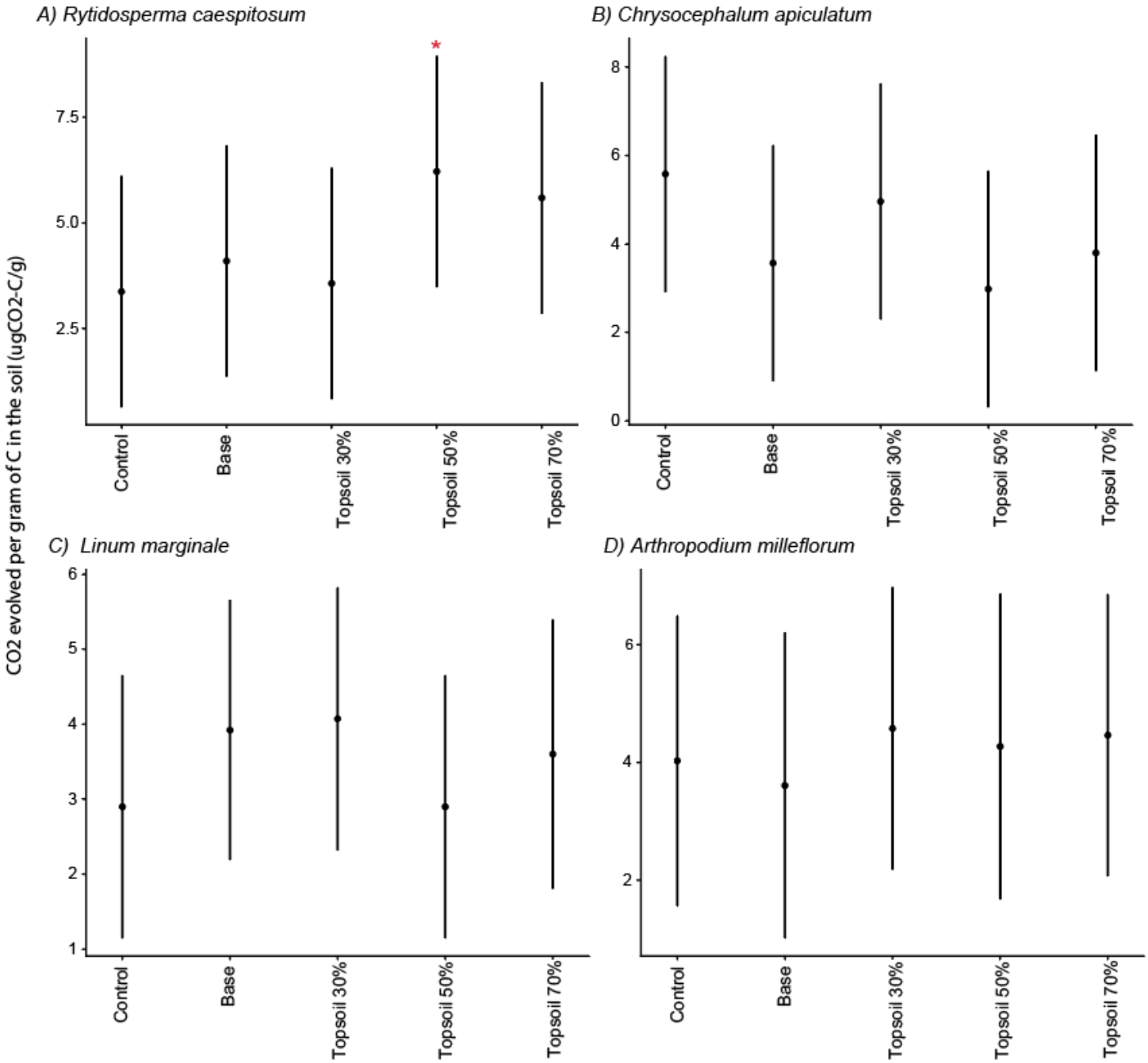

Figure 4

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 seed), *G. tabacina* was not included due to low levels of emergence. The figure displays medians with upper and lower quartiles (± SE). Significant results are denoted with asterisks: * indicates p-value < 0.05, ** indicates p-value <0.01, and *** indicates p-value <0.001

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