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RESEARCH ARTICLE

Native bacteria and cyanobacteria can influence seedling emergence and growth of native plants used in dryland restoration

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Abstract

1. Seed-based ecosystem restoration has huge potential to restore degraded drylands. However, fewer than 10% of directly sown seeds transition to established seedlings. One of the potential factors restricting plant establishment in degraded soils is the low abundance and diversity of native soil micro-organisms. In this study, we investigated whether returning indigenous bacteria and cyanobacteria consortia to degraded dryland soils improved seedling emergence, survival and growth of native plants.
2. We inoculated 'culturable whole soil' native heterotrophic bacteria and biocrust cyanobacteria individually and as a mixed inoculant into extruded pellets containing *Acacia inaequilatera* (Fabaceae) and *Triodia epactia* (Poaceae) seeds. The pellets were planted in an active minefield for 28 weeks and seedling emergence and total biomass of plants were determined.
3. Cyanobacteria and bacteria inoculants increased the emergence of *A. inaequilatera* by 55% and 48%, respectively. Seedling emergence in *T. epactia* was increased by 20% by cyanobacteria but was not increased by bacteria. The only effect of inoculation on seedling survival or mass per surviving seedling in either species was an 11% reduction of the growth of *T. epactia* seedlings that were inoculated with cyanobacteria.
4. *Synthesis and applications*: Our results suggest that the benefit of micro-organisms on plant establishment is both species specific and life stage specific, with particularly strong benefits in the early stages of recruitment. Our experiment was conducted under shade and with additional water, so a worthwhile future direction would be to quantify the effect of inoculation under unmodified

field conditions. It would also be worthwhile monitoring the outcomes for longer than 28 weeks. Since seedling emergence is one of the critical challenges in dryland restoration, our study provides direct evidence in the use of native micro-organisms to potentially improve seedling emergence in seed-based dryland restoration.

KEYWORDS
bacteria, cyanobacteria, dryland, extruded pellets, micro-organisms, native, restoration, seed enhancement, soil biocrust

1 | INTRODUCTION

Plant recruitment from seed is a critical challenge in seed-based restoration in arid and semi-arid ecosystems. Commonly, less than 10% of seeds establish in these ecosystems (James et al., 2011). One factor that contributes to the low success rate of directly seeded plants in disturbed sites is the low abundance and diversity of native soil microbial communities (Wang et al., 2019). Micro-organisms can affect plant growth (Thakur et al., 2021), and in turn, plants can affect the composition of soil microbial communities (Jaunatre et al., 2014, but see Yang et al., 2022), potentially leading to positive plant–soil feedback cycles that enhance both plant growth and survival, and microbial abundance and diversity (van der Putten et al., 2016). In sites with disturbed soils, microbial communities may shift to a less mutualistic relationship with plants, which can have substantial consequences for plant establishment (Koziol et al., 2018). There is mounting evidence that translocating native microbes from undisturbed ecosystems to disturbed ecosystems improve the establishment of native plants (Wubs et al., 2016). However, translocating soil at a landscape scale would require the extraction of large quantities of topsoil from healthy sites, which would likely degrade these donor sites. Here, we used an innovative non-destructive approach to transfer whole soil bacteria and cyanobacteria consortia from a healthy site to a disturbed site to assist in seed-based restoration.

Our primary objective was to test whether seeds inoculated with whole soil native heterotrophic bacteria (hereafter bacteria) and biocrust cyanobacteria (hereafter cyanobacteria) from an undisturbed site could improve seedling emergence, survival and growth in degraded land. There are mixed responses to the use of micro-organisms for restoration in literature, with studies showing positive (Muñoz-Rojas et al., 2018), neutral (Chua et al., 2020) or negative effects (Koziol et al., 2018). The source and microbial strains used for inoculation studies may partially account for the differences in microbial effect on plant growth (Moreira-Grez et al., 2019). For instance, laboratory cultured exogenous strains can show poor performance compared to locally sourced strains (Middleton et al., 2015). Similarly, studies that have inoculated multiple microbial strains have benefited from the synergistic effect of inter-microbial relationships (Vahter et al., 2020). However, it is unknown how inoculating culturable whole soil native bacteria and cyanobacteria consortia influence the seed recruitment process in arid ecosystems. We address this knowledge gap by testing the hypothesis that inoculating seeds with culturable whole soil native micro-organisms from undisturbed ecosystems would increase seedling emergence, survival and growth potential in disturbed arid lands.

Our second objective was to compare the effect of bacteria versus cyanobacteria on seedling emergence. The feeding strategy of micro-organisms affects their metabolic functions and enzymatic activities which, in turn, affect the plants they support (Ge et al., 2018). Heterotrophic micro-organisms such as bacteria rely on plant exudates and plant detritus as a carbon source to sustain their metabolic activities. Conversely, autotrophs such as cyanobacteria transform atmospheric CO_2 into organic carbon for metabolic function. Having the capability to thrive independently from the support of plants while improving other bacteria growth makes cyanobacteria a favourable option that could likely show a higher effect size than bacteria (Chaudhary et al., 2019). Therefore, we hypothesise that cyanobacteria will be more beneficial to plants than bacteria during early plant establishment.

Our third objective was to determine whether a combination of bacteria and cyanobacteria results in higher seedling emergence and survival than treatments of either bacteria or cyanobacteria in isolation. Interactions between different types of microbes can be positive, neutral or negative (Porter et al., 2020), yet within a similar niche, micro-organisms can also exhibit resource use exclusivity such that the use of a resource for the growth and activity of one microbe may not be impacted by the presence or absence of the other (Ronda & Wang, 2022). Also, cyanobacteria can associate with and promote the growth of other heterotrophic bacteria (Bowker et al., 2018). Since cyanobacteria use atmospheric carbon while bacteria use soil carbon, we predict that these groups of micro-organisms would tend to have synergistic effects on plant establishment and growth. That is, we hypothesise that combined inoculation of bacteria and cyanobacteria will result in higher seedling emergence and growth than with each taxon in isolation.

2 | MATERIALS AND METHODS

This study was conducted in an active iron ore mine site in Newman, in the Pilbara region of Western Australia (23°21′55.30″S, 119°40′31.4″E), and did not require any ethical approval.

The climate is semi-arid with mean annual rainfall ranging from 250 to 400 mm per year (Sudmeyer, 2016). Precipitation is highest (72%) during the summer periods (December to March) and is often associated with tropical cyclones and large summer thunderstorms. Mean maximum temperatures range from 31 to 39°C from November to April and 28 to 29°C from May to October (BOM, 2020). The soils are classified as stony loam with very low fertility and are predominantly vegetated by spinifex (*Triodia* spp.) grassland interspersed with irregularly scattered shrubs and trees (McKenzie et al., 2009).

We set up the experiment on reconstructed post-mining soil materials (that originated from iron-rich bedrock and were used in rehabilitation as the growing medium), separated into three blocks (400 cm x 200 cm x 25 cm) under a purpose-built rain-out shelter (100% rain exclusion; see Erickson et al., 2017). The soils had a high silt content (12%–18%) with a bulk density of 1.5 g/cm^3 (Table S1). Within each block, we set up three plots (2 m × 1 m). We programmed an automated irrigation system that dispensed a total amount of 200 mm of water over the entire experimental period. The total amount of irrigation was based on estimates from long-term weather data and the pattern of irrigation was based on field evidence that has demonstrated that 4 events of ca. 30 mm per (400 cm × 200 cm) over 7 days, maximise the likelihood of germination and emergence in Pilbara species (Lewandrowski et al., 2017). Hence, in the first 7 days, 10 mm of water per (400 cm × 200 cm) was dispensed for 3 h every second day. Follow-up irrigation events of 10 mm per (400 cm × 200 cm) were applied once every 4 weeks.

We used seeds of *Triodia epactia* (i.e. seeds removed from the floret structure following Erickson et al., 2016) and *Acacia inaequilatera*. We selected these two native plant species because they were common to the Australian arid zone vegetation and are often targeted in restoration projects in the Pilbara (Erickson et al., 2017). The seeds were pre-treated in 1% calcium hypochlorite ($Ca[OCl]_2$) for 30 min and washed three times in distilled autoclaved water. Prior to use, seeds of *Acacia inaequilatera* were heat treated in 90°C hot water for 1 min to break physical dormancy (Erickson et al., 2016).

Seeds were placed in one of four treatments:

2.1 | Control pellets

We used extruded pellets (hereafter referred to as 'soil pellets' or 'pellets') made from stockpiled topsoil (see Appendix Table S1 for differences between topsoil and overburden physicochemical properties) as the carrier for the seeds. We formulated the soil pellets after Stock et al. (2020) and modified them following Román et al. (2020). The aim was to make a pellet that would retain its structural integrity during transportation without crumbling and at the same time disintegrate upon irrigation to allow seedling emergence. To form the pellets, we collected topsoil stockpiled for >15 years and screened it through a 5-mm mesh to remove large gravel fraction and plant debris. Soils were unsterilised because (1) we aimed to mimic the common practice in the mining sector where unsterilised stockpiled topsoil is respread on degraded soil during the rehabilitation (Muñoz-Rojas et al., 2018), (2) we did not want to suggest methods that may be experimentally feasible but practically impossible to implement. Hence, we mixed 1.5 ml of distilled autoclaved water with 6 g of the soil in a concave silicone-mould tray to form the pellets (2.7 cm diameter × 1.5 cm depth) and air-dried them for 24 h. Before drying, we inserted 25 viable seeds (20 seeds of *T. epactia* and five seeds of *A. inaequilatera*) into each soil pellet.

2.2 | Pellets inoculated with whole soil bacteria communities

Pellets were identical to the control pellets, except that they were made with 1 ml of bacterial culture and 0.5 ml of autoclaved distilled water instead of 1.5 ml of distilled water. To obtain the soil bacteria, we collected soil samples from a pristine undisturbed ecosystem adjacent to the study site with vegetation cover mainly composed of *Triodia* sp. and *Acacia* sp. The soil was mixed thoroughly, and a composite sample was taken for bacterial growth. An enrichment culture was prepared by mixing 1 g soil in a sterile 50 ml nutrient broth (beef extract 1 g/L, yeast extract 2 g/L, peptone 5 g/L, sodium chloride 5 g/L, agar 15 g/L). The culture was incubated for 7 days at 30°C. After 7 days, we transferred 1 ml aliquot from the enrichment culture, into a fresh sterile nutrient broth and incubated for another 7 days. Since the focus of the experiment was to examine the effect of culturable whole soil bacteria communities on seedling emergence, we did not assess the concentration of the individual bacteria cells in the inoculum, instead, bacteria concentration was measured volumetrically.

2.3 | Pellets inoculated with cyanobacteria consortia

Pellets were identical to the control pellets, except that they were made with 1.8 g cyanobacteria suspended in 1.5 ml autoclaved distilled water. We used a native consortium that had already been grown in BG11 media at 25°C under constant 80 µmol m^{-2} s^{-1} in our laboratory (Muñoz-Rojas et al., 2018). Briefly, cyanobacteria were isolated by inoculating field soils on a solid BG11 media and incubated at 30°C. Cyanobacteria growth on media was repeatedly isolated into fresh liquid BG11 media (Martins et al., 2019). We filtered cyanobacteria cultures to separate the cyanobacteria biomass from the BG11 media. The residue from filtration was centrifuged at 10,000 rpm for 15 min to separate extracellular metabolites from the wet biomass. A subsample of the centrifuged cyanobacteria was oven-dried to determine the moisture content in the wet cyanobacteria biomass. We inoculated the pellets with 0.3 g wet weight cyanobacteria per gram of soil following Román et al. (2020).

2.4 | Pellets inoculated with combined culturable whole soil bacteria communities and cyanobacteria consortia

Pellets were identical to the controls except they were inoculated with 0.5 ml of bacteria cultures and 0.9 g of cyanobacteria consortia suspended in 1 ml of autoclaved distilled water.

Each pellet–microbe treatment combination was replicated 36 times across the plots and each treatment received equal amounts of the simulated rainfall. At the end of the experiment, we observed that the pellets had disintegrated and mixed with the soil substrate.

Three weeks after planting, we counted the emerged seedlings. After 28 weeks, we recounted the seedlings that had survived (seedling survival) and harvested the above-ground plant biomass. We dried the plants at 60°C in an oven for 72 h and weighed them on a balance (Mettler AE 200) accurate to 1 mg. We measured mass per surviving seedlings as the aboveground dry biomass of seedlings that had emerged and were still alive after 28 weeks. We also estimated the final biomass produced from 100 seeds sown as the total dry aboveground biomass that would be produced if we sowed 100 seeds for each treatment. This measure differs from the mass per surviving seedling as it includes non-emerged and non-survived seedlings (individuals with zero biomass following Finch et al., 2022).

Soon after we set up the experiment, a hard lockdown imposed in Australia due to the COVID-19 pandemic precluded travel from New South Wales to Western Australia to collect and transport soil samples from the field. Therefore, even though seedling emergence and survival could be monitored, we were unable to obtain data on changes in the microbial communities in the field as the experiment progressed. This remains a worthwhile direction for future work in this field.

To determine the composition of the cyanobacteria and bacteria inoculants, 1 ml of both cultures was collected for DNA extraction. The DNA extraction was done using DNeasy PowerSoil Kit (100; Qiagen, 2020) following the manufacturer's instructions. The extracted DNA was sent to Ramaciotti Center for Genomics for 16S rRNA sequencing where the V3-V4 regions were amplified in a Miseq Illumina platform. The V3-V4 regions were amplified with a primer set CYA 359 and 781a/b as described in Nübel et al. (1997). After sequence reads were obtained, we processed the raw data with OTUreporter v1.0.0-beta (9b72c8e) pipeline (https://bitbucket.org/xvazquezc/otureporter) based on Mothur, v1.39.5 (http://www.mothur.org/—Schloss et al., 2009). The sequences were aligned and classified using the SILVA database v132 (Quast et al., 2012). Sequences were then clustered into operational taxonomic units (OTUs) based on 97% similarity using OptiClust algorithm (Westcott & Schloss, 2017). Representative sequences for each OTU were selected with the *get.oturep* command from Mothur and searched against the NCBI database using BLAST+v2.9.0 (−max_hsps 1 -evalue 1e-5 -max_target_seqs 20; Altschul et al., 1997). The resulting OTU table was then used to construct an abundance plot using R studio.

2.5 | Data analysis

Analyses were conducted in R studio (R Core Team, 2019; version 4.0.0). To test the hypotheses that bacteria communities and cyanobacteria consortia improved seedling emergence and survival, we ran a generalised mixed-effects model with binomial distribution using the LME4 package (Bates et al., 2015). The response was the number of seedlings that emerged out of the total number of seeds in the pellet. Bacteria and cyanobacteria were considered the main effects and pellet and plot position as random effects. We used the EMMEANS package (Lenth, 2018) to conduct pairwise comparisons of treatments.

Neither mass per surviving seedling nor final biomass per 100 sown seeds was normally distributed, so the data were $\log_{10}$-transformed before analysis. We used a linear mixed-effect model with the GLMMTMB package (Brooks et al., 2017) for the analysis. The differences between treatments for the log-transformed biomass data were calculated using pairwise comparisons from the EMMEANS package. For estimating the overall treatment effect on final biomass produced from 100 seeds sown, we used the Tweedie family within 'glmmTMB', which models continuous data with a proportion of the data equal to zero (Foster & Bravington, 2013), to account for seeds that emerged and those that failed and had zero biomass (Lecomte et al., 2013). We used violin plots to visualise results as they provide density distribution with '*ggplot2*'.

3 | RESULTS

Analysis of the composition and relative abundance of the inoculant revealed that *Enterobacteriales* and *Flavobacteriales* were the most abundant orders in the bacterial inoculant while *Nostocales* and *Synechococales* were the main orders in the cyanobacteria inoculant (Figure 1). At the genus level, *Chryseobacterium* spp. (20.6%) and *Achromobacter* spp. (20.1%) were the co-abundant genera in the bacterial inoculant while *Leptolynbya* spp. (7.4%) and *Microcoleus* spp. (12.1%) were the most abundant genera in the cyanobacteria inoculant.

Compared to the control, cyanobacteria inoculation increased emergence of *A. inaequilatera* by 55% (mean ± standard error in control = 1.39 ± 0.19; with cyanobacteria = 2.14 ± 0.21; $p = 0.007$; Figure 2a) and *T. epactia* by 20% (mean ± standard error in control = 6.86 ± 0.38; with cyanobacteria = 8.33 ± 0.55, $p = 0.045$; Figure 2b). Inoculation with bacteria increased *A. inaequilatera* emergence by 48% (mean ± standard error with bacteria = 2.06 ± 0.2, $p = 0.01$; Figure 2a) but had no significant effect on *T. epactia* (mean ± standard error with bacteria = 7.39 ± 0.52, $p = 0.48$; Figure 2b). The co-inoculation of bacteria and cyanobacteria increased *A. inaequilatera* emergence by 32% (1.86 ± 0.25, $p = 0.01$) compared to the control but was similar to the control in *T. epactia* (6.22 ± 0.63, $p = 0.49$). There was no difference in seedling emergence between bacteria and cyanobacteria treatments ($p > 0.56$, Figure 2a,b).

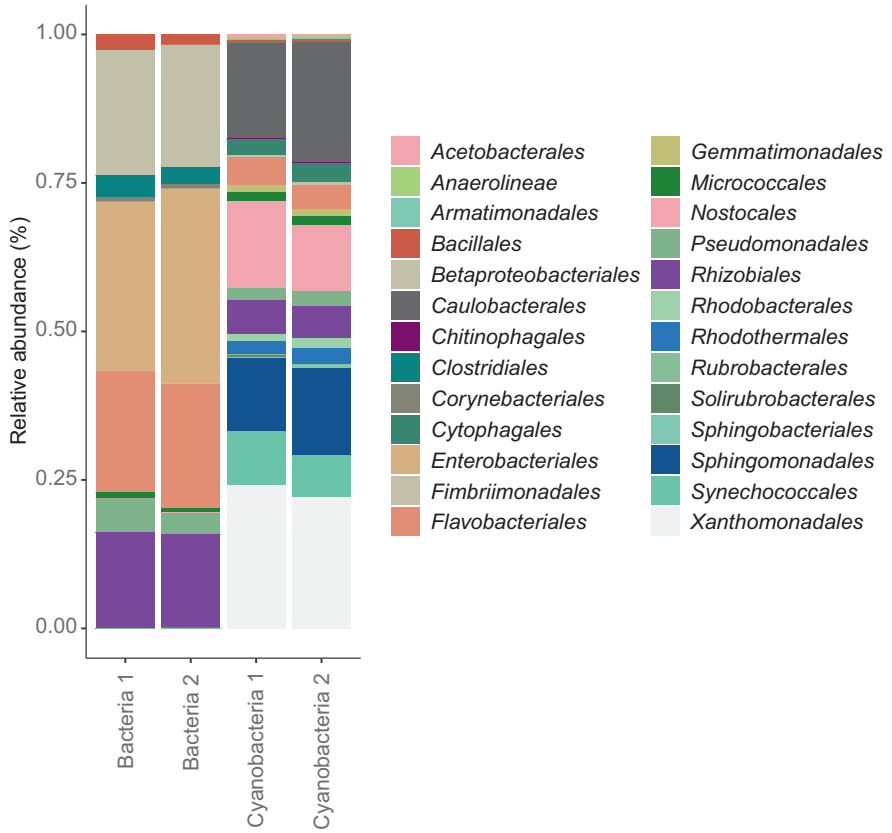

FIGURE 1 Relative abundance (%) at the taxonomic order level for the bacteria and cyanobacteria inoculants after cultures were mixed with soil pellets. Two replicates were extracted for DNA and sequenced. The names on the x-axis show the respective replicate of inoculant compositions.

None of the treatments influenced seedling survival in either species (Figure 2c,d). In *T. epactia*, survival of the emerged seedlings ranged from 67% to 70% of total seeds sown (means ranged from 7.39 ± 0.52 to 8.33 ± 0.55, $p > 0.18$). In *A. inaequilatera*, nearly all (86%) of the emerged seedlings dried up and died by the end of the experiment. This mortality was not related to treatment (means ranged from 0.25 ± 0.07 to 0.42 ± 0.09, and all $p > 0.27$).

In *T. epactia*, cyanobacteria inoculation was associated with a mean mass per surviving seedling 11% lower than in the control (mean ± standard error in control = 1.24 ± 0.35, mean ± standard error with cyanobacteria = 1.14 ± 0.24, $p = 0.03$) while the other treatments showed no significant difference relative to the control (1.22 ± 0.3 for bacteria treatment and 1.24 ± 0.28 for bacteria and cyanobacteria treatment, $p > 0.26$, Figure 2e,f).

Cyanobacteria inoculation increased the final biomass per 100 seeds sown by seven times more than the control in *A. inaequilatera* (mean ± standard error in control = 1.64 ± 0.34; mean ± standard error with cyanobacteria = 11.14 ± 1.31; Figure 2g,h). Bacteria inoculation did not influence the final biomass per 100 seeds sown in either plant species (2.45 ± 0.44 for *A. inaequilatera* and 45.6 ± 2.85 for *T. epactia*, $p > 0.12$ in both species). For the mixed microbial treatment, the final biomass per 100 seeds sown in *A. inaequilatera* was 2.5 times higher than the control (4.33 ± 0.68, $p = 0.0001$) but similar to the control in *T. epactia* (51.4 ± 3.16, $p = 0.64$; Table S3). The combined bacteria and cyanobacteria treatment did not lead to higher emergence, survival or mass per surviving seedling than did inoculation with either cyanobacteria or bacteria individually, in any treatment in either plant species ($p > 0.7$ in all cases, Figure 2; Table S3).

4 | DISCUSSION

We found that the effect of inoculating native seeds with native bacteria and/or cyanobacteria consortia on plant establishment under simulated rainfall was species dependent and life stage dependent. Overall, there were only beneficial effects on seedling emergence, no effect on survival in either species, and either neutral or negative effects on the mass per surviving seedling. The beneficial effect on seedling emergence could be influenced by the inoculated micro-organisms since both bacteria and cyanobacteria inoculants had abundant micro-organisms that show plant growth-promoting properties (Chhetri et al., 2022; Román et al., 2018). Also, *Chromobacter* spp. and *Chryseobacterium* spp. show multi-metal tolerance which makes them suitable inoculants for the iron-ore rehabilitation field (Benmalek et al., 2014; Ma et al., 2009). This observation suggests that using native microbial consortia adapted to the local environment might offer indirect benefits. However, it must be noted that our result is based on plants grown under shaded and irrigated field conditions; thus, a worthwhile future direction would be to quantify the effect of inoculation under unmodified field conditions.

The declining effect of microbes on plant growth through our experiment (Figure 2) might be caused by either the inoculated micro-organisms declining in abundance and diversity through time (Martínez-Viveros et al., 2010) or because later life stages

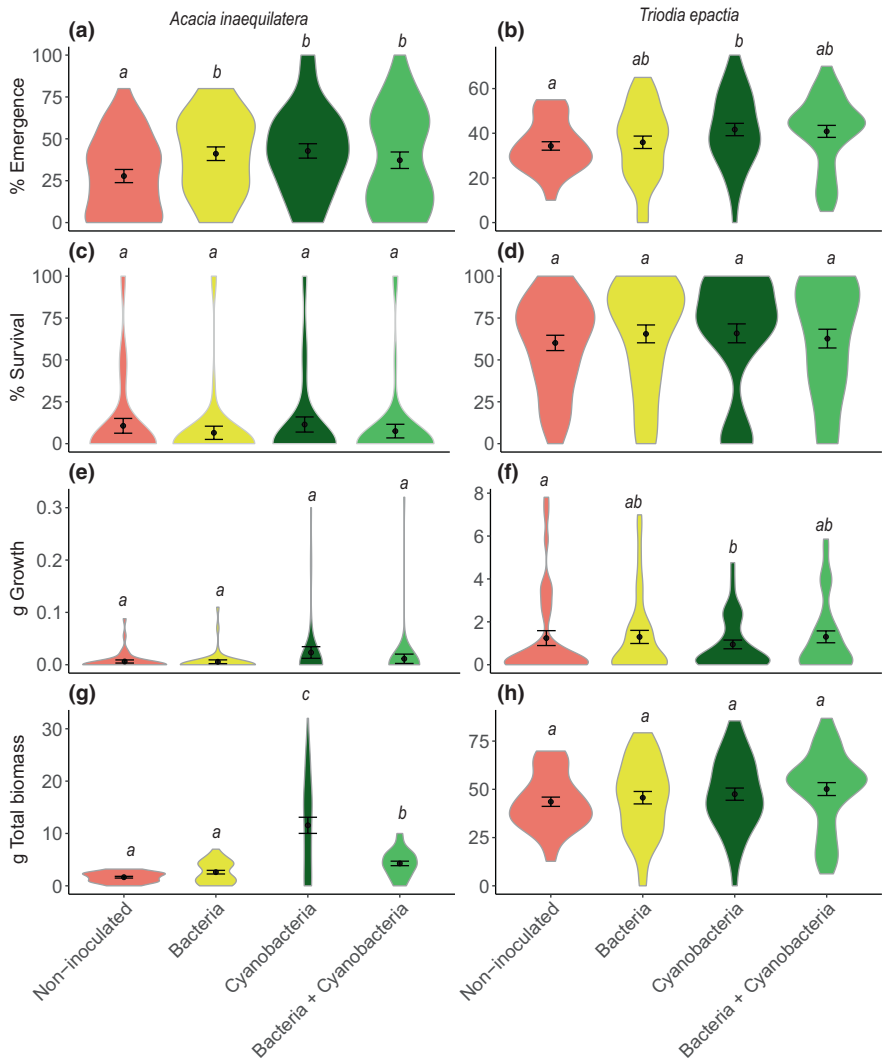

FIGURE 2 Seedling emergence (a, b), survival of emerged seedlings after 28 weeks (c, d), and growth of surviving seedlings after 28 weeks (e, f) and total biomass (g, h) in *a. inaequilatera* and *T. epactia*. 'Control' refers to the treatment with seeds in pellets that did not receive any microbial inoculation. 'Bacteria' refers to the treatment in which pellets received bacteria inoculation and 'cyanobacteria' the treatment in which pellets received cyanobacteria inoculation. 'Mix' refers to the treatment in which pellets received both bacteria and cyanobacteria inoculants. The same letters above graphs indicate no significant differences between treatments.

of the plant do not respond to the inoculated micro-organisms (Torres-Cortés et al., 2018). Future studies could distinguish between these two possibilities by (1) tracking the microbial community composition in the soil through time (we were unable to do this because COVID-19 regulations in Australia prevented us from reaching our field sites), and (2) giving booster doses of inoculant during seedling establishment, to test whether inoculant increases growth and survival of established seedlings. The latter idea was tested by Wang et al. (2021) who observed a beneficial effect of microbial inoculation on plant growth at the early stages but found that repeated inoculations of the same micro-organisms were ineffective in the later growth stages. Another study found that microbial community composition changes at different growth stages (Torres-Cortés et al., 2018). Therefore, one possibility of achieving a positive microbial effect throughout the plant growth stages may require inoculations of different micro-organisms at each developmental stage.

Microbial inoculation substantially increased seedling emergence in *A. inaequilatera*, but did not increase seedling survival in either species. One possibility is that seedling survival is more strongly affected by factors such as drought and nutrient limitation than by micro-organisms (Nuske et al., 2021; Moles & Westoby, 2004). Our experiment was irrigated, but we did not include any source of C or N in the pelletised soils. Thus, another possibility is that a shortage of C or N could have limited microbial growth and removed any positive effect on plant survival. A worthwhile direction for future research would be to run inoculation experiments in sites with varying fertility levels, to test whether the effects of microbial inoculation are greater at more fertile sites.

Many *A. inaequilatera* plants dried up and died while adjacent *T. epactia* stayed healthy. The fact that dried plants were visible suggests that herbivory was not responsible for this mortality (Turcotte et al., 2014), but it was not possible to tell whether the deaths were related to drought (perhaps exacerbated by insufficient below-ground mutualists) or to pathogen attack. Although inoculation increased survival in *T. epactia* more than it did in *A. inaequilatera* (Figure 2); we only have one woody and one herbaceous species, and thus cannot reach any conclusions about factors that affect species' responses to inoculation. However, it would be interesting for future studies to extend our work by asking whether the effect of inoculant differs according to species' growth forms. We predict that since grasses are non-woody

species, their extensive fine root morphology could enhance their resource uptake to induce a higher survival rate compared to woody species during early plant establishment in dryland ecosystem restoration (Peltzer & Köchy, 2001).

The different effect of microbial inoculation on our two study species (Figure 2) aligns with previous evidence for substantial variation in the effect of microbial inoculation on different plant species and in different situations (Pringle & Bever, 2008). A worthwhile direction for future research would be to obtain data for a range of different species, and different sites, to test hypotheses about the types of situations and taxa that are most likely to benefit from microbial inoculation. For instance, it would be valuable for practitioners to know whether microbial inoculation has a stronger effect in arid lands than in more mesic sites, in forests than in grasslands, or in more fertile sites, and to determine whether inoculation was more effective on plant species that have N-fixing symbionts, or species with different growth forms such as grasses, shrubs or trees.

We found an 11% reduction in the mass per surviving seedling of *T. epactia* under cyanobacteria inoculation but neutral effects from the other inoculants (Figure 2f). This result suggests species-specific responses to different taxa of micro-organisms, and it is contrary to the findings of Bao et al. (2021) and Sharma et al. (2020) that beneficial microbial inoculation increases plant biomass production. One possibility for the observed difference is that the higher emergence of seedlings in the presence of beneficial micro-organisms may lead to increased intraspecies competition and reduced growth of individual plants, as observed in cyanobacteria inoculation on *T. epactia* (Figure S1). This is in line with the study of Bhattacharjee et al. (2009) and Knochel et al. (2010) who found intraspecies plant competition from increased plant density, thereby reducing biomass production. This observation raises the question of quantity of plant emergence over quality in plant establishment during restoration. Such a dilemma is managed in forest restoration programmes by allowing many seedlings to emerge and grow and later subjecting them to selective thinning (Cameron, 2002). However, in dryland restoration with limited funds, such additional cost and labour should be tailored to the purpose and interest of the restoration project and the stakeholders (Castillo et al., 2021).

Our prediction that combined bacteria and cyanobacteria would improve seedling emergence, survival and growth was not supported (Figure 2). In contrast to previous studies that have reported synergistic effects from the inoculation of microbial consortia (Vahter et al., 2020; Xie et al., 2018), in our study, the combined cyanobacteria and bacteria treatment was not the best performer in any metric for any species except in the final biomass produced after sowing 100 seeds in *A. inaequilatera* (Figure 2). Given that similar or sometimes higher results are obtained for inoculating a single rather than a mixed taxon, we suggest that focusing on a single inoculant such as cyanobacteria might save time and effort.

Inoculation with cyanobacteria increased the final biomass produced from 100 seeds sown seven-fold for *A. inaequilatera*, but neither inoculant had a significant effect on the final biomass from 100 seeds sown in *T. epactia*. Despite the increase observed in *A. inaequilatera*, the final biomass produced from 100 seeds sown was several orders lower than what has been reported in other studies in dryland ecosystems (Chaudhary et al., 2016; del Mar Alguacil et al., 2011). The difference in literature and our study could be related to the use of microbial consortium in our study which includes both beneficial and antagonistic micro-organisms as opposed to the use of single beneficial micro-organisms reported in most studies (Morris et al., 2007). Considering that we bulk cultured soils, it is possible that the inoculants contain other micro-organisms that might be detrimental to plant growth. Thus, future studies can test the effect of single microbial inoculants against the use of whole soil microbial consortium to determine the effect size of the different inocula on plant growth. Also, our result on the effect of microbial inoculation on final biomass is short term (lasting only 28 weeks); thus, it will be worthwhile for future studies to conduct microbial inoculation studies in the long term to monitor the outcome on plant growth.

5 | CONCLUSION

We show that returning native micro-organisms to the soil can improve native seedling emergence, with trade-offs in seedling growth in some plant species but no effect on the survival of the emerged plants. Our study also shows a taxa-specific microbial effect on seedling emergence with no synergistic effect of inter-taxon inoculation on plant growth. Since inoculation with cyanobacteria significantly improved seedling emergence of both *Triodia* and *Acacia* while bacteria significantly improved emergence of *Acacia* seedlings only, we predict that the use of cyanobacteria as a bio-inoculant will yield beneficial results in dryland ecosystems. Overall, our findings highlight the potential benefits of indigenous micro-organisms during early plant life stages in seed-based dryland restoration programmes.

AUTHOR CONTRIBUTIONS

Frederick Asankom Dadzie, Miriam Muñoz-Rojas and Todd E. Erickson designed and carried out the experiment. Frederick Asankom Dadzie conducted the data analysis and the writing of the manuscript. Angela T. Moles helped in formulating the hypothesis, data analyses and manuscript write-up. Miriam Muñoz-Rojas and Todd E. Erickson helped in the experimental layout and planning and field data collection. Eve Slavich helped with all the statistical analysis. All authors contributed to the revision of the manuscript.

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CONFLICT OF INTEREST

Authors had no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data and codes are available via Open Science Framework repositiory: Dadzie et al. (2022). Data. https://doi.org/10.17605/OSF.IO/9WB8S.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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