


RE-AL THEMATIC SERIES

RESEARCH ARTICLE

Inoculating native microorganisms improved soil function and altered the microbial composition of a degraded soil

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Restoration managers inoculate microorganisms to enhance soil function and improve restoration success, but the efficacy of these inoculations in real-world conditions is still unclear. We conducted a field experiment to test whether applying extruded seed pellets inoculated with native microbes affected soil properties related to ecosystem function in severely degraded mine soil. We found that inoculating with bacteria did not affect soil carbon, metabolic quotient (a measure of microbial stress), or basal respiration, but increased soil nitrogen by 75%, substrate-induced respiration by 147% and reduced carbon-to-nitrogen ratio by 44% compared to the control. This suggests that the bacteria inoculant contained free-living N fixers that increased the soil N content. Thus, inoculating with bacteria could supplement nitrogen fertilizers in degraded soils during soil restoration. However, we found that inoculating with a mix of bacteria and cyanobacteria did not affect any of the soil properties. This finding is counter to results in laboratory studies, suggesting that field tests are critical for understanding real-world outcomes of microbial inoculation. Finally, we found that soil microbial composition was changed by the inoculation with a mix of bacteria and cyanobacteria. None of the treatments significantly changed the diversity of soil microbial communities. Our data suggest that microbial inoculation could improve some aspects of ecosystem function and thus provide beneficial effects that might facilitate restoration of degraded sites.

Key words: biocrust, dryland restoration, microorganisms, pellets, soil function, soil nutrient

Implications for Practice

- (1) Inoculating bacteria into extruded pellets increases the nitrogen content of degraded soil. This may facilitate restoration of degraded ecosystems and could decrease the need for nitrogen fertilization.
- (2) Mixing bacteria and cyanobacteria had no effect on soil functions. This may suggest that increasing microbial diversity may not always lead to improved soil functions.
- (3) Microbial inoculation via extruded pellets onto degraded soils changes the structure of the soil community composition suggesting that resident soil communities can be changed over time.

Introduction

Bacteria and cyanobacteria are key components of the soil microbial community that affect many aspects of ecosystem function, including infiltration and retention of moisture (Colica et al. 2014), control of soil erosion (Chamizo et al. 2017), and nutrient cycling (Chamizo et al. 2018; Muñoz-Rojas et al. 2018). There has been some interest in using native microbial inoculation to restore degraded soils (Rossi 2020). However, evidence for the

effect of microbial inoculation on the function of degraded soil has been mixed, and evidence is mostly from laboratory and

Author contributions: FAD, MMR conceived the project; FAD, MMR, TEE executed the experiment. FAD performed all the data analysis and the writing of the manuscript; ATM assisted in formulation of hypothesis and results interpretation; NML assisted with all bioinformatic data analyses and interpretation; all authors contributed to the revision of the manuscript.

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doi: 10.1111/rec.14025

Supporting information at:

<http://onlinelibrary.wiley.com/doi/10.1111/rec.14025/supinfo>

greenhouse studies (Chamizo et al. 2018; Muñoz-Rojas 2018; Roncero-Ramos et al. 2022). We have limited information about the effect of inoculation on soil function under field conditions in drylands. We address this knowledge gap by quantitatively assessing whether adding extruded seed pellets inoculated with native bacteria and cyanobacteria communities affect soil properties in degraded dryland soil.

The soil properties we have selected for the study are soil carbon, nitrogen, carbon:nitrogen ratio, soil respiration, and metabolic quotient. These variables provide a strong overview of the microbial impact on soil function. Soil carbon, nitrogen content, and carbon:nitrogen ratio are strongly correlated with soil fertility (Harris 2009). Microbial respiration is associated with nutrient cycling (decomposition and mineralization (Schimel & Schaeffer 2012) and soil carbon storage (Reichstein & Beer 2008). We also measured microbial metabolic quotient, which is used as an indicator of stress within soil communities (West & Sparling 1986; Wardle & Ghani 1995).

First, we tested the hypothesis that the addition of extruded seed pellets inoculated with either native bacteria communities or a mix of bacteria and cyanobacteria communities would increase soil carbon, nitrogen, carbon:nitrogen ratio, soil respiration, and decrease metabolic quotient relative to the uninoculated counterpart. Bacteria and cyanobacteria drive biogeochemical processes in dryland soils (van der Heijden et al. 2008; Delgado-Baquerizo et al. 2020). It has been suggested that returning these microorganisms to degraded soils would improve soil biogeochemical processes without the need to apply chemical fertilizers (which has been the traditional practice) (Koziol et al. 2018). However, most studies on the use of microorganisms to facilitate soil function have been done with live soil inocula and conducted under glasshouse conditions. Thus, it is not clear whether the dried inocula commonly used in field situations (e.g. Khan et al. 2007; Schoebitz et al. 2013) are effective, especially if abiotic conditions are unfavorable, as is often the case in arid lands (Schimel & Schaeffer 2012). Our study addresses this knowledge gap.

Second, we tested the hypothesis that adding extruded seed pellets inoculated with a mix of bacteria and cyanobacteria would show a stronger effect than inoculating with only bacteria. In drylands, bacteria and cyanobacteria often associate for mutual benefits in soils (Paerl et al. 2000). Cyanobacteria provide carbon and nitrogen resources to bacteria and protect bacteria by providing exopolysaccharide that ameliorates soil conditions to enhance bacterial growth (Mugnai et al. 2020). Bacteria can provide growth stimulation to cyanobacteria (Salomon et al. 2003). Furthermore, many glasshouse studies show that bacteria and cyanobacteria show complementary effects that enhance growth and nutrient cycling beyond what a single species or population can achieve alone (Paerl et al. 2000). It has been suggested that bacteria and cyanobacteria together might be effective under a wider range of conditions than bacteria in isolation (Nelson et al. 2021; Rossi et al. 2022). However, the effect of inoculating extruded seed pellets with a mix of bacteria and cyanobacteria on soil function under field conditions in degraded drylands has never been studied.

Third, we tested the hypothesis that adding extruded seed pellets inoculated with native bacteria and mixed bacteria and cyanobacteria to degraded soil would change the composition and increase the diversity of the soil microbial community. It has been suggested that inoculating microorganisms can shift the composition of soil microbial communities to populations that facilitate ecosystem restoration (Koziol et al. 2018). However, whether the inoculated microbial species would complement or reduce the existing microbial population to effect the change is still unclear. Some studies suggest that exogenous inoculation of microorganisms into the soil will not have any effect on the soil community due to the soil community's inherent resilience to temporary disturbance (Trabelsi & Mhamdi 2013). Other studies suggest that inoculation of microorganisms will change the soil community even in subtle ways (Mawarda et al. 2020). In this study, we inoculated with microbes cultured from local soil, so the existing microorganisms at our field sites are expected to have been pre-exposed to similar native microorganisms. Therefore, we predicted that the addition of the inoculant will increase the relative abundance of the inoculated taxa in the soil communities without adversely impacting the microbial diversity of the soil communities. However, several responses are possible. An increase in community diversity following microbial inoculation could suggest that inoculated microbial taxa are coexisting with and complementing the resident microbial community (Albertsen et al. 2006). However, if the inoculated microbial taxa die out, are already present in the degraded soils, or replace resident microbial taxa, inoculation might not affect overall diversity (Antunes et al. 2009). Finally, if inoculated microbes outcompete and displace resident microbial communities, we could see a substantial change in community composition and a decrease in diversity (Koch et al. 2011; Islam et al. 2021).

Overall, our study aimed to advance our understanding of how inoculated native microorganisms affect soil properties and interact with native microbial communities in degraded soils under field conditions. We hope that this research will help scientists to develop more effective inoculants for the future.

Methods

Study Area, Experimental Design, and Inoculant Preparation

We conducted our field study in a purpose-built rain exclusion shelter that closely mimics environmental conditions of mine rehabilitation in a decommissioned area nearby an active mine site in the Pilbara region in Western Australia (23°21'55.30"S, 119°40'31.4"E) (see Erickson et al. 2016, 2023 for more details of the facility). The distance between the near-natural research facility and the active mining activities is about 2 km and the mining operations do not influence any conditions of the facility. The climate in this region is classified as arid to semi-arid with annual rainfall ranging from 250 to 400 mm per year (Sudmeyer 2016). Evaporative demand exceeds 3000 mm per annum. Soils are red shallow stony loam with very low fertility, having spinifex (*Triodia* spp.) as the dominant vegetation (McKenzie et al. 2009). Due to massive earthworks and excavations on the site, the vegetation has been

removed and the topsoil (surface layer of soil which is the primary source of seeds, nutrients, and microbial communities) stockpiled for subsequent use in soil reconstruction for land rehabilitation.

We used a nested plot design to undertake the experiment. First, we used three experimental blocks (400 cm × 200 cm × 25 cm) filled with the iron-rich bedrock soil (mine substrate). The experimental blocks were about 1 m apart from each other. Within each block, we created three separate subplots. Within each subplot we nested 27 rectangular subunits (rectangular grids 30 cm × 30 cm) that held an individual treatment. The subplots were 0.5 m apart. Each subplot was randomly assigned to one of three treatments.

The treatments comprised extruded seed pellets (as defined below) containing *Triodia epactia* and *Acacia inaequilatera* seeds, commonly used for restoration in the study area (Erickson et al. 2016). Extruded seed pellets are a seed enhancement technology that embeds seeds within a soil-based slurry which is either molded or extruded into a pellet to enhance the delivery of plant growth enhancers to the soil (Erickson et al. 2021). We used extruded seed pellets (hereafter referred to as “pellets”) because they have shown potential to be used to encapsulate and deliver seeds and microorganisms into arid soils (Dadzie et al. 2022). Pellets were subjected to three different treatments:

- (1) *Control treatment*: No microbial inoculant added to the pellets.
- (2) *Bacteria treatment*: Free-living culturable whole soil bacteria communities inoculated into the pellets. We collected soil samples from natural (undisturbed) areas adjacent to the study site and cultured them to obtain native bacteria communities and bacteria enriched with cyanobacteria consortia for the inoculation. A detailed description of the inoculant preparation can be found in Dadzie et al. (2022). Briefly, 1 g of soil was inoculated in 25 mL of sterile 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 cultures were incubated for 7 days at 30°C, and then a subsample of 1 mL was transferred to a fresh sterile media and incubated for an extra 7 days. We pipetted 1 mL of the bacteria growing in the fresh media as culturable whole soil bacteria inoculant.
- (3) *Mixed bacteria and cyanobacteria treatment*: Two species of cyanobacteria (*Leptolyngbya* spp. and *Microcoleus* spp.) were inoculated into the pellets along with their associated bacteria. We did not use axenic cultures of cyanobacteria as pure cyanobacteria have a very short lifespan compared to cyanobacteria with its associated bacteria (Rossi et al. 2022). The bacteria used in this treatment were not free-living but those associated with cyanobacteria and living in the cyanosphere (Fig. 2A; Rossi et al. 2022). *Leptolyngbya* spp. and *Microcoleus* spp. were selected because they are representative of the study area and have previously shown the ability to survive desiccation (Jiménez-González et al. 2022; Muñoz-Rojas et al. 2018). The cyanobacteria consortium and the associated bacteria were growing together in a BG11 media at 28°C under dark: light cycles at 16:8 h. The irradiance was set to 70 $\mu\text{mol m}^{-2} \text{s}^{-1}$ as this

has been found to be optimum for cyanobacteria growth (Muñoz-Rojas et al. 2018). We constantly aerated the culture with sterile air. When enough cyanobacteria biomass was obtained, we filtered the biomass and resuspended cyanobacteria cells in autoclaved distilled water. The cyanobacteria were centrifuged at ×5,000 rpm for 15 minutes. Subsamples of the wet weight of cyanobacteria consortia were used as inoculants.

Each soil × treatment combination was replicated 36 times. There were 9 subplots with 12 pellets per subplot. Pellets were placed in the rectangular grid of each subplot with 20 cm buffer spaces around each pellet.

Pellet Preparation and Microbial Inoculation

We used soils that had been stockpiled on the site for >15 years as a substrate to prepare the pellets. A detailed description of the pellet preparation and microbial inoculation can be found in Dadzie et al. (2022). Briefly, we screened stockpiled soil through a 5-mm sieve to remove stones and plant debris. We weighed 6 g of the soil and, mixed it with 1.5 mL of distilled autoclaved water, and molded the resultant mixture in a silicone tray (2.7 cm diameter × 1.5 cm depth). Each pellet was subjected to one of the two microbial treatments. Following restoration practices in the study area, we added 25 viable seeds (20 seeds of *T. epactia* and 5 seeds of *A. inaequilatera*) to the pellet and allowed them to dry for 24 hours. For bacteria, we inoculated pellets with one milliliter of the bacteria culture and supplemented it with 0.5 mL distilled autoclaved water before molding the pellets.

To compose the pellets with a mix of bacteria and cyanobacteria, and because the bacteria are already associated with cyanobacteria, we inoculated the soil pellets with 0.3 g wet-weight cyanobacteria per gram soil (Román et al. 2020). We added distilled autoclaved water to the pellets to maintain a moisture content of 1.5 mL before the drying process.

The pellets were placed in the center of each subunit and subjected to four events of 30 mm $\text{hour}^{-1} \text{day}^{-1}$ irrigation. The four events were applied every 2nd day within a 7-day period followed by 10 mm $\text{hour}^{-1} \text{day}^{-1}$ irrigation applied once every 4 weeks following the study by Stock et al. (2020). The total irrigation for the experiment was 200 mm of water spread over 28 weeks.

After 28 weeks, we collected soil samples from the center of the rectangular grid where the pellet was placed. We used a hand shovel to collect 5 cm × 5 cm to 1 cm depth of the topsoil (about 5 g soil). There were a total of 108 samples across the three treatments. Within each plot, we pooled soil samples of the same treatments together. We had three main soil samples per treatment across the plots. For the pooled treatment within the plot, the soil was sieved through 2-mm wire mesh to remove stones and plant roots, thoroughly homogenized, and divided into three subsamples. One subsample was separated for DNA analysis and stored at −20°C, another subsample was stored at 4°C for two weeks and used for microbial respiration analyses. A third subsample was air-dried and used for C and N analyses.

Analyses of Soil C, N, Basal Respiration, Substrate-Induced Respiration, and Metabolic Quotient

We measured the following soil variables:

Total soil carbon and total soil nitrogen: We ground air-dried soil samples into a fine powder using mortar and pestle and weighed 0.5 g soil into Eppendorf tubes and submitted them to Mark Wainwright Analytical Centre (UNSW Sydney, Australia) for analysis. Total carbon and nitrogen were determined by the complete combustion of soils using an Elemental Analyser (Elementar Analysensysteme GmbH [EA] 2017).

Carbon-to-nitrogen ratio: Carbon and nitrogen content were used to calculate the C:N ratio of the soil.

Basal and substrate-induced respiration: Basal and substrate-induced respiration were determined using the MicroResp method (Campbell et al. 2003). We weighed 0.7 g of soil into wells and added 0.3 mL of deionized water. Each well was covered with a parafilm to reduce evaporation and incubated at 20°C. After 4 days the well incubation was interrupted and 25 µL of autoclaved water or dissolved glucose was added to the well to determine basal respiration and substrate-induced respiration, respectively. The concentration of the glucose solution was 30 mg/g of soil. We sealed the well with a creosol gel to prevent the escape of gases and incubated it again. The incubation lasted for 6 hours and the released CO₂ from the soil was trapped in the creosol gel, resulting in a color change. The color change was analyzed spectrophotometrically to determine the amount of evolved CO₂. Basal respiration (a measure of microbial activity in soil; ISO 2002) was measured as the mean CO₂ that evolved from the soil after water was added to air-dried soils in a chamber. Substrate-induced respiration was determined as the efflux of CO₂ after glucose was added to the soil (Cesarz et al. 2022).

Metabolic quotient: The results obtained from the basal and substrate-induced respiration were used to calculate the metabolic quotient which is the ratio of soil basal respiration to substrate-induced respiration (Wardle & Ghani 1995).

Analysis of Soil Microbial Community

Relative microbial abundance: All samples were handled aseptically. To determine the microbial community composition of the composite soil samples ($n = 9$), we extracted DNA from 0.25 g from each of the collected soil samples using DNeasy PowerSoil Kit (Qiagen) and following the manufacturer's instructions. Extracted products were submitted to Ramaciotti Centre for Genomics (UNSW Sydney, Australia) where 16S rRNA amplicon sequencing was performed. V1–V3 were the targeted region, and the primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 519R (5'-GWATTACCGCGGCKGCTG-3') were used for that purpose. The library pool was sequenced using a MiSeq Reagent Kit v3 on a Miseq System using 2 × 300 bp pair-end chemistry. After reads were obtained, the data was processed following Machado et al. (2021). The forward and reverse end reads were checked for quality using FASTQC (Andrews 2010). Sequences with Phred quality score <30 were trimmed using Trimomatic (Bolger et al. 2014). After trimming and rechecking for sequence quality, we paired and assembled the forward and reverse reads using Pear with statistical testing to automatically discard low-

probability pairs (Zhang et al. 2014). Sequences were then checked for chimeras using USEARCH (Edgar 2010). We processed the obtained 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 into single reads and classified using the SILVA database v132 (Gurevich et al. 2013). Sequences were then clustered into operational taxonomic units (OTUs) based on 97% sequence similarity using the OptiClust algorithm (Westcott & Schloss 2017). We selected a representative OTU with the *get.oturep* command from Mothur and set an assignment method using the NCBI database (BLAST+v2.9.0) (Altschul et al. 1997). The assigned OTU was used to construct an abundance plot using “ggplot2” (Wickham 2016) (Fig. S1).

Microbial diversity: We measured microbial diversity using data obtained from microbial sequencing. We first rarefied the data to a minimum of 10,000 reads to obtain an even depth of the sequences and prepared them for diversity analyses using the *phylo-seq* package (McMurdie & Holmes 2013). We used the *Microbiome* package (Lahti et al. 2012–2019) to estimate Shannon and inverse-Simpson diversity indices which are indicators of microbial species diversity and evenness respectively (Grice et al. 2009).

Data Analysis

All statistical analyses were performed in R and R studio (R Core Team 2022, version 4.0.0). To test the hypothesis of bacteria and mixed bacteria and cyanobacteria inoculation on carbon, nitrogen, carbon to nitrogen ratio, basal respiration, and substrate-induced respiration, we fitted a generalized linear model with a Gaussian distribution of residuals using LME4 package. Because we combined all the soil at the block level, we set block as a fixed effect variable rather than a random variable. Most variables satisfied assumptions such as normality and homogeneity of variance for each model. However, substrate-induced respiration did not meet the model assumptions criteria despite undertaking different transformation tests. We therefore used non-parametric Kruskal–Wallis tests for this variable. We used model summary to estimate differences between treatments.

To test for effects of bacteria, and mixed bacteria and cyanobacteria inoculation on soil microbial taxa, we rarefied the sequenced microbial data. We fixed separate models for each taxa. We used a *glm* with gaussian distribution model and set microorganisms as the main effects. Significant differences were obtained from the summary of the model. We further used non-metric multidimensional scaling (NMDS) from the ‘vegan’ package (Dixon 2003) to determine the microbial community dissimilarity and visualized groupings among the microorganisms (following Machado et al. 2021).

Results

Effect of Microbial Inoculation on Soil Function

Adding pellets inoculated with native bacteria increased soil nitrogen content by 75% ($p = 0.01$; Fig. 1B), increased

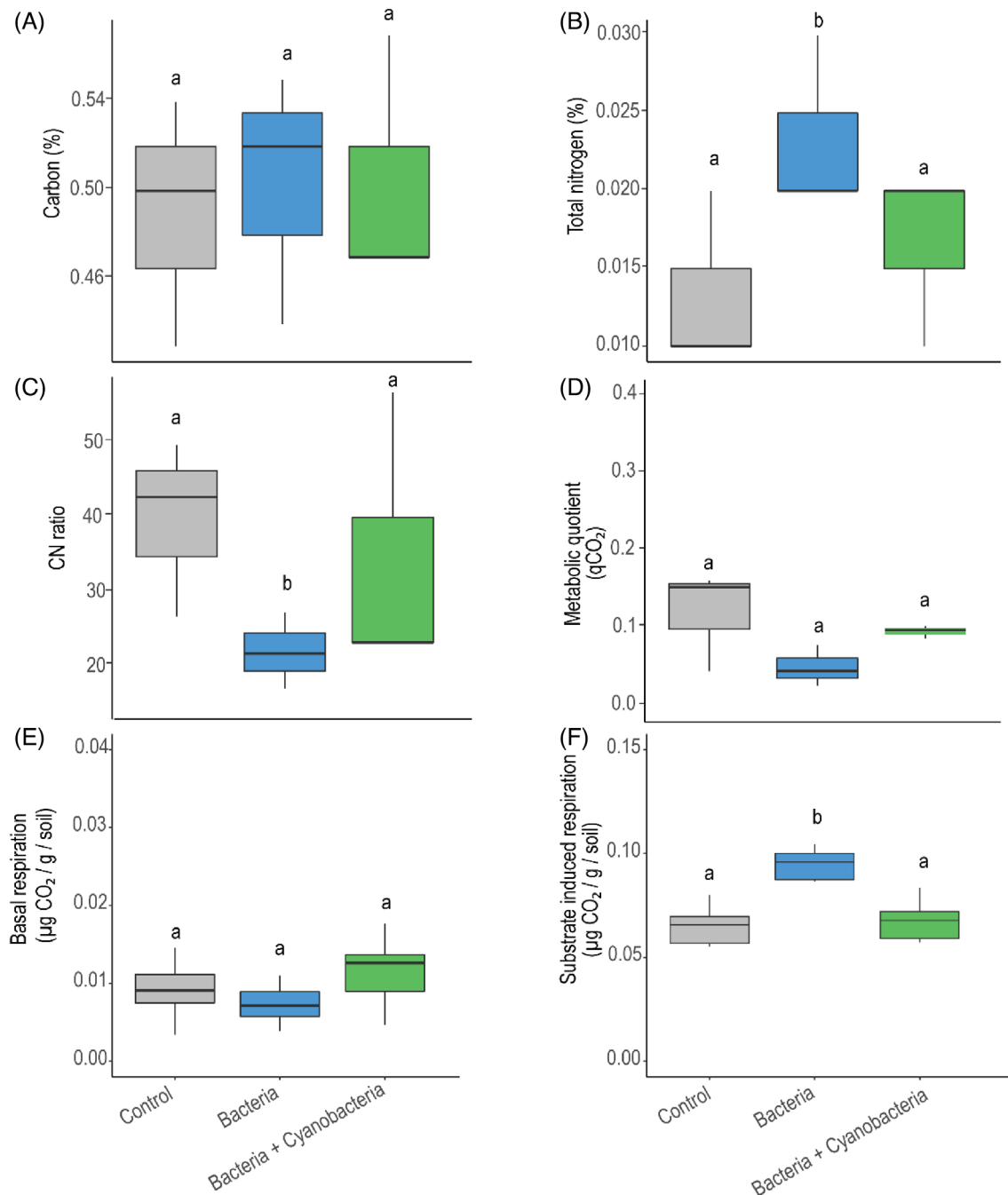


Figure 1. Effect of inoculating native microorganisms on selected soil properties ((A) total soil carbon, (B) total soil nitrogen (C) CN ratio (D) metabolic quotient (E) basal respiration, and (F) substrate-induced respiration) in a degraded land. Soil samples were analyzed after 28 weeks of inoculation in the field. “Control” represents treatments that did not receive any microbial inoculation. “Bacteria” represents treatments inoculated with culturable whole soil native bacteria communities. “Bacteria + Cyanobacteria” represents treatments inoculated with a mix of native bacteria and cyanobacteria. Different letters above graphs indicate treatments that were significantly different at 95% confidence interval.

substrate-induced respiration by 147% ($p = 0.007$; Table S2; Fig. 1F) and decreased carbon to nitrogen ratio by 44% ($p = 0.04$; Fig. 1C) compared to the control, but did not significantly change soil carbon, basal respiration, or metabolic quotient (all $p > 0.10$; Table S2; Fig. 1A, 1D, & 1E).

Adding pellets inoculated with a mix of bacteria and cyanobacteria did not have a significant effect on any of the soil properties measured (all $p > 0.06$; Table S2; Fig. 1).

Contrary to our predictions that inoculating a mix of bacteria and cyanobacteria would show stronger effect than inoculating

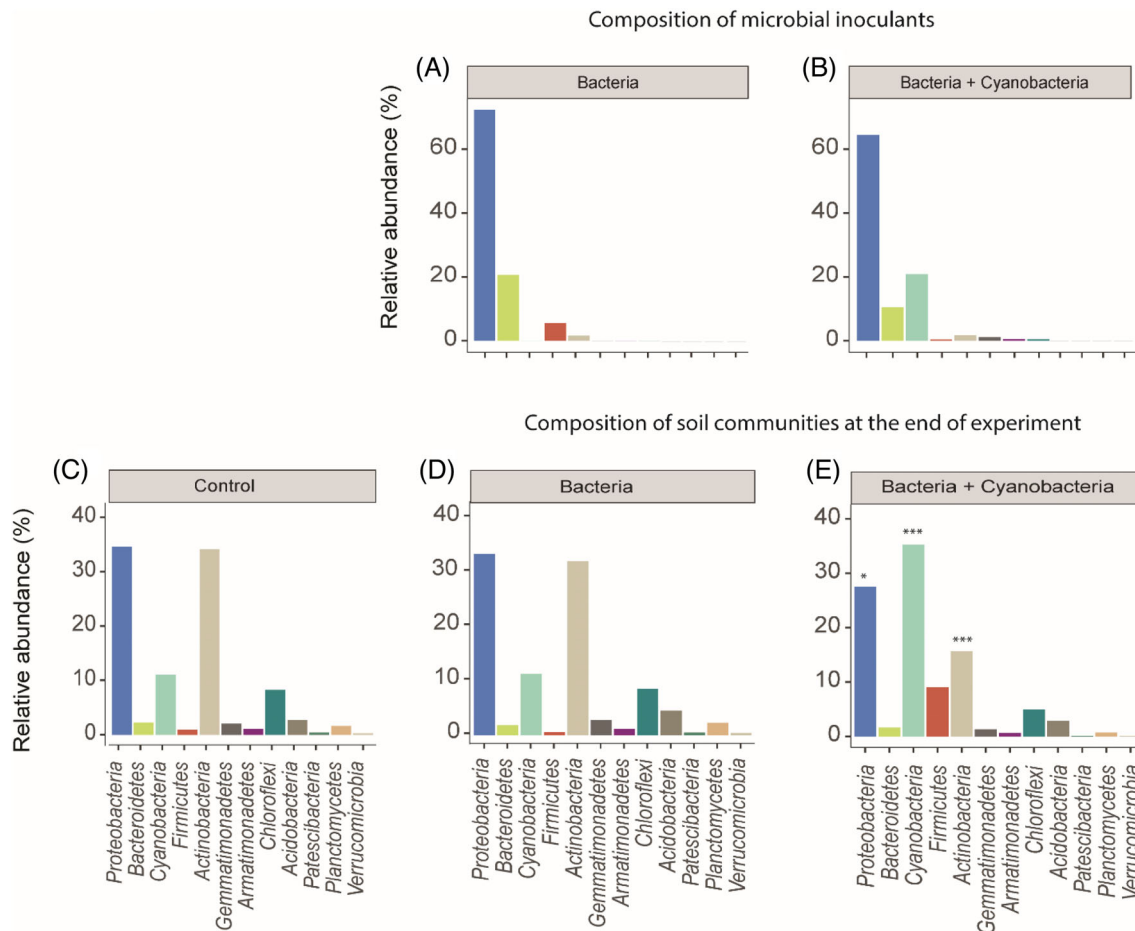


Figure 2. Effect of microbial inoculation on relative abundance and microbial inoculants and resident microbial taxa 28 weeks after microbial inoculation. (A, B) The microbial composition and relative abundance at the phylum level of the microbial inoculants. (C–E) The relative abundance of resident microbial taxa after 28 weeks of inoculation with culturable whole soil bacteria and a mix of bacteria and cyanobacteria. The figure shows the 12 dominant microbial taxa after sequencing. Each treatment was replicated three times.

with only bacteria, none of the six soil properties were significantly higher after addition of pellets inoculated with a mix of bacteria and cyanobacteria compared to addition of pellets with bacteria as a single inoculant (Fig. 1). In fact, the substrate-induced respiration was 136% higher in the bacteria treatment compared to the mix of bacteria and cyanobacteria treatment ($p = 0.005$; Table S2; Fig. 1F).

Effect of Microbial Inoculation on Soil Community Composition and Diversity

Addition of pellets inoculated with bacteria did not have a significant effect on the relative abundance of any of the soil taxa ($p > 0.23$ for all taxa; Fig. 2D). In contrast, addition of pellets inoculated with mixed bacteria and cyanobacteria more than tripled the relative abundance of cyanobacteria in the community compared to the control ($p < 0.001$; Fig. 2E; Table S3) but decreased the relative abundance of Actinobacteria ($p < 0.001$; Table S3) by 50% and Proteobacteria by 7% ($p = 0.01$; Table S3) compared to the control.

Addition of pellets inoculated with native microorganisms did not impact the overall diversity of the microbial community (Fig. 3). Both Shannon diversity and inverse Simpson analysis showed similar microbial diversity among all treatments ($p > 0.1$ for all treatments; Table S2), while NMDS analysis also showed no clear distinction in the microbial community composition among treatments (Fig. 4).

Discussion

Addition of pellets inoculated with bacteria increased soil nitrogen content, substrate-induced respiration and decreased carbon-to-nitrogen ratio, but did not affect soil carbon content, metabolic quotient, or basal respiration. These results partially support our hypothesis that addition of inoculated pellets would increase metrics related to soil function. Our findings are also consistent with previous evidence that inoculation of bacteria to degraded soil can improve some soil properties (Delgado-Baquerizo et al. 2016; Philippot et al. 2013).

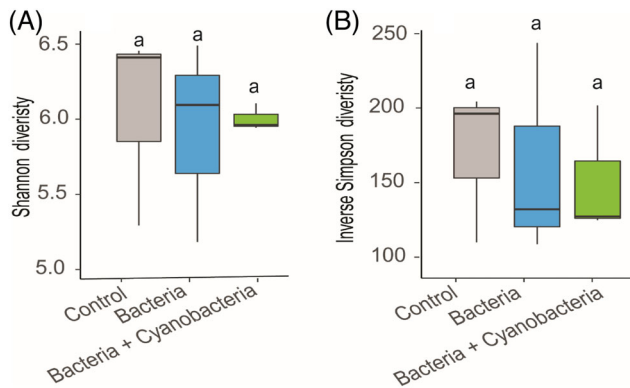


Figure 3. Effect of bacteria and a mix of bacteria and cyanobacteria inoculation on resident soil microbial diversity 28 weeks after inoculation.

Our results suggest that addition of pellets inoculated with bacteria might be able to decrease the need for nitrogen fertilizer application in this ecosystem in which undisturbed soils have characteristically low nitrogen content in the range of 0.02–0.1% (Bateman et al. 2019; Kneller et al. 2018). One of the issues when applying chemical fertilizers to degraded soils in

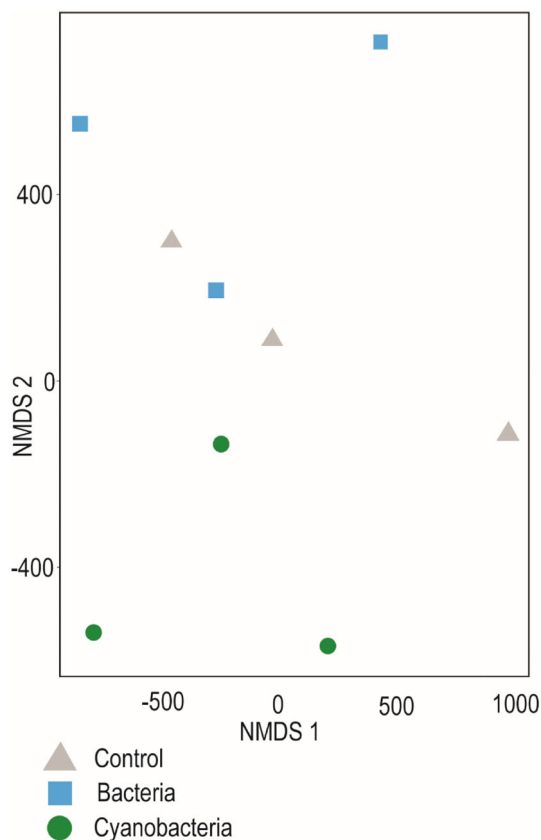


Figure 4. Non-metric Multi-Dimensional Scaling of the microbial community composition at the OTU level, for each treatment, based on Bray–Curtis similarity. 2D stress: 0.07. Each treatment was replicated three times.

drylands is that it sharply increases the soil N content and promotes rapid growth of introduced species that may overshadow the growth of targeted plant species (Liu et al. 2018). Inoculating native bacteria to the soil, increased nitrogen to levels similar to an undisturbed soil in the area (Bateman et al. 2019). This suggests that bacteria inoculation has the potential to increase soil nitrogen content without promoting infestation of introduced species. Also, if bacteria inoculation is restoring nitrogen levels to pre-disturbed states, it can provide an economic advantage to restoration budgets by reducing chemical fertilization in the field.

Adding pellets inoculated with bacteria resulted in an increase in nitrogen content but did not significantly affect soil carbon content. One possible explanation for this result is that both the bacteria inoculant and the soil microbial community, after adding pellets inoculated with bacteria, were dominated by diazotroph bacteria in the Proteobacteria clade which fix nitrogen but do not affect carbon content (Koirala & Brözel 2021). Addition of pellets inoculated with bacteria may have lowered the carbon-to-nitrogen ratio, which has previously been found to facilitate microbial activity (Jilkova et al. 2020).

Surprisingly, addition of pellets inoculated with mixed bacteria and cyanobacteria did not affect any of the soil properties we measured. The observation is contrary to many cyanobacteria-assisted studies where soil C and N were increased (Kumar et al. 2013; Román et al. 2018; Roncero-Ramos et al. 2019; Toribio et al. 2022). We provide three possible explanations for the observed differences found in the literature and from our study. First, most studies on ecological restoration in which cyanobacteria increased soil C and N were either glasshouse or laboratory studies (Chamizo et al. 2018; Muñoz-Rojas et al. 2018; Roncero-Ramos et al. 2019), which necessarily have soil conditions different to those found in the field (Ryan & Graham 2018). Second was the unfertilized nature of our field site. Some of the field inoculation studies that have shown a positive effect of cyanobacteria on soil C and N were conducted in agricultural fields (Maqubela et al. 2009; Manjunath et al. 2016; Alobwede et al. 2019). Agricultural fields are often fertilized to have a higher nutrient status (Zhang et al. 2018; Liu et al. 2020) which could boost microbial activity compared to seldomly fertilized dryland restoration sites (Barbosa et al. 2010). Thus, our results suggest that adding pellets inoculated with a mix of bacteria and cyanobacteria do not promote soil restoration under field conditions, at least under the low-nutrient conditions at our field site.

Third, the analysis of relative abundance revealed an increase in cyanobacteria after the inoculation process. We are confident that the bacteria and cyanobacteria mixture was successfully delivered, leading to the establishment of these microbes in the soil and the formation of an initial biological soil crust. This establishment most likely occurred during the initial phase of the experiment when consistent irrigation was provided. However, as the experiment progressed, the irrigation was reduced, exposing the microbes to the environmental conditions present in the area. It is likely that cyanobacteria became inactive or died due to desiccation (Lan et al. 2014), and thus did not affect soil properties. An alternative approach that might prevent the inactivity of the delivered inoculant would involve pre-acclimating

the inoculum to the stressful conditions found in the target area (Giraldo-Silva et al. 2018; Antoninka et al. 2020). Investigating the efficacy of such an approach would be a worthy direction for future research.

Although practitioners sometimes aim to harness the synergistic potential between bacteria and cyanobacteria (Kumar et al. 2013), we found no evidence that addition of pellets inoculated with mixed bacteria and cyanobacteria is more effective than addition of pellets inoculated only with bacteria. One possibility is that resource flow between bacteria and cyanobacteria association only benefited the cyanobacteria because the relative abundance of the cyanobacteria more than tripled. If this was the case, the increased growth of cyanobacteria could have overshadowed the growth and activities of the associated bacteria to have any effect on the soil (Nelson et al. 2021).

Adding pellets inoculated with mixed bacteria and cyanobacteria tripled the relative abundance of cyanobacteria and decreased the relative abundance of Actinobacteria in the soil by 50%. The massive reduction in the relative abundance of Actinobacteria suggests that the cyanobacteria component of the inoculum may have outcompeted the Actinobacteria. Alternatively, the cyanobacteria might have released secondary metabolites that may have affected the Actinobacteria community (Machado et al. 2017). Actinobacteria has been suggested to play an important role in the nitrogen cycle in dryland soils (Zhang et al. 2018). Perhaps the reduction of Actinobacteria populations under mixed bacteria and cyanobacteria treatment contributed to the nonsignificant effect on nitrogen fixation in the soil. However, the fact that the relative abundance of cyanobacteria increased as a result of addition of pellets inoculated with a mix of bacteria and cyanobacteria is consistent with the idea that cyanobacteria have a strong ability to establish themselves even in extreme soil environments (Muñoz-Rojas et al. 2018; Adessi et al. 2021).

A remarkable but unexpected observation is that the relative abundance of different microbial clades in the soil community was minimally modified after addition of pellets inoculated with bacteria, yet the bacteria inoculants increased the soil nitrogen content. One could query whether the increased nitrogen under bacteria treatment could have been caused by the composition of the pellets. However, if that were the case, then all treatments (including the control) would have shown increased nitrogen content since all the treatments were made from similar pellets. Since we did not observe a general increase in nitrogen content across treatments, we attribute changes in the soil nitrogen to the microbial inoculants. It is possible that the inoculated bacteria communities were able to fix N in the soil at the early stages of inoculation but died over time leaving a lasting change on the soil N and no impact on the soil communities (Martínez-Viveros et al. 2010). There is evidence that the microbial effect on the ecosystem is more effective at an early stage of inoculation than later (Dadzie et al. 2022) possibly because the density of active cells in inoculants reduces over time in the field (Martínez-Viveros et al. 2010). Thus, re-inoculation might be required to re-establish viable bacteria cells to prolong the effect on ecosystems.

Addition of inoculated pellets did not significantly change the microbial diversity of the soil. The result is unexpected since the inoculation was predicted to increase the low diversity of degraded soil. One possibility is that many of the inoculated taxa may have died either at the pellet drying stage or after interacting with the soil communities (Deaker et al. 2012; Berninger et al. 2018). The inoculants failing to establish viable populations might partly explain some of the failures of microbial inoculation to improve soil function in dryland (Berruti et al. 2017; Hart et al. 2018). Another possibility is that all the inoculated taxa (which were sourced from local native microbial communities) were already present in the degraded soil, so inoculation did not contribute new taxa to the soil community. The latter situation is possible if the degraded soil had remnant microbial taxa akin to the pre-disturbed state of the soil or had been recolonized by microbial taxa through wind dispersal from surrounding sites since disturbance (eg. dust storms). Our results do suggest that using native microorganisms as inoculants will not alter the soil microbial diversity with unknown taxa that could be deleterious to ecosystem function (Mawarda et al. 2020).

Understanding the effect of native microbes on ecosystem function is crucial for dryland restoration. Our work provides strong evidence that the inoculation of native bacteria into degraded soil can enhance some aspects of soil function such as N fixation and soil respiration, while still maintaining the diversity of the microbial communities.

Acknowledgments

This research received funding from the Australian Research Council Discovery Early Career Research Award DE180100570, the Hermon Slade Foundation (HSF 1808), and in collaboration with the Department of Industry, Innovation and Science, Australian Government's Global Innovation Linkages program (GIL 53873) project "Eco-engineering solutions to improve mine-site rehabilitation outcomes." The project was also supported by the Holsworth Wildlife Research Endowment and The Ecological Society of Australia and M.M.R. is supported by the by the Spanish Ministry of Science and Innovation (RYC2020-029255-I, TED2021-132332A-C22, and PID2021-123097OA-I00). Data and R scripts can be accessed at https://osf.io/ndhvr/?view_only=b8eed925c997400aa7ff96d888e58007. Open access publishing facilitated by University of New South Wales, as part of the Wiley - University of New South Wales agreement via the Council of Australian University Librarians.

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Supporting Information

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

Table S1. Physicochemical properties of inoculated pellets and soil substrate in the block.

Table S2. Effect of microbial inoculations on selected soil parameters.

Table S3. Effect of microbial inoculant on the relative abundance of individual soil taxon.

Coordinating Editor: Elise Gornish

Received: 3 May, 2023; First decision: 30 May, 2023; Revised: 12 September, 2023; Accepted: 13 September, 2023