



The Potential of Biocrust-Forming Cyanobacteria to Enhance Seedling Growth of Native Semi-arid Plants Through Seed Biopriming

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Received: 21 December 2023 / Accepted: 4 October 2024 / Published online: 17 November 2024
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Abstract

In drylands, extreme environmental conditions pose a challenge for restoration, especially on a large scale. Direct seeding is the most cost-effective approach to restore large areas, but it requires improvements to enhance seedling survival and establishment. For this purpose, biopriming seeds with cyanobacteria is promising due to their plant growth-promoting properties. We evaluated the effect of seed biopriming with native biocrust-forming cyanobacteria on seed germination and radicle length of four native plant species, two perennials (*Macrochloa tenacissima* and *Thymus hyemalis*) and two annuals (*Plantago ovata* and *Stipa capensis*), chosen for their ubiquity in Mediterranean drylands. Treatments included seed biopriming with cyanobacteria inoculants (biomass + exudate), seed priming only with the cyanobacterial exudate, and controls (BG11 culture medium and distilled water). Biopriming effect was assessed individually for four native biocrust-forming cyanobacteria species: *Nostoc commune*, *Tolypothrix distorta*, *Trichocoleus desertorum*, and *Leptolyngbya frigida*. Seed biopriming showed no effect on germination with similar rates among treatments (on average, $45 \pm 12.6\%$ for *M. tenacissima*, $38.8 \pm 12.4\%$ for *T. hyemalis*, $91.7 \pm 9.8\%$ for *P. ovata* and $77.6 \pm 10.8\%$ for *S. capensis*). However, biopriming significantly affected radicle length of annual plants. Radicles were significantly increased in *P. ovata* when treated with cyanobacterial exudates (15–30% increase over control), and in *S. capensis* when treated with cyanobacterial biomass + exudate (26–42% increase over control). These results indicate that the effect of seed biopriming is species specific, but selecting the appropriate plant species and biopriming treatment can enhance plant survival and establishment in drylands, making restoration efforts more successful.

Keywords Cyanobacteria exudate · Cyanobacteria biomass · Annual plants · Germination · Radicle length · Restoration

Handling Editor: Abdul Latif Khan.

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Introduction

Drylands, representing almost half of the Earth's land surface and sustaining one-third of the global population, are among the most susceptible ecosystems to land degradation (Právělie 2016). Estimates point to 10–20% of global drylands being already degraded and around 12 million hectares being newly degraded every year due to climate change and land use intensification (Burrell et al. 2020). In this context, restoration activities aimed at preserving and restoring ecosystem services and societal goods provided by drylands are critical to the well-being of society (Yirdaw et al. 2017; Maestre et al. 2021). However, ecosystem restoration is particularly challenging for drylands, where low and unpredictable precipitation, extreme temperatures, low soil fertility, and seed quality hinder restoration efforts (Hoover et al. 2020). In fact, passive restoration of degraded drylands is

slow and, in many cases, hardly achievable depending on the scale and severity of degradation. On the other hand, active restoration practices often fail because of the mentioned environmental stresses (Shackelford et al. 2021), being the permanent or seasonal water scarcity one of the main factors limiting restoration success (Duncan et al. 2019). This problem will be exacerbated in the next decades given the predicted increase in aridity and drought frequency and magnitude (Mirzabaev et al. 2022), requiring the development of more efficient restoration techniques that also cope with future climatic scenarios (Zvinavashe et al. 2021). Besides, to achieve the global ecological restoration objectives, economically and logistically viable strategies should be developed to face large-scale restoration projects as well as to act on hard-to-reach areas. In this context, seeding arises as an easier and spatially manageable strategy, considered as the most cost-effective strategy for most species (Pérez et al. 2019; Shackelford et al. 2021), regardless of high seed mortality rates in drylands (James et al. 2011).

The transition between germination and seedling emergence is the main bottleneck for native plant recruitment in drylands, where typically only 5–10% of seeds reach the establishment phase (Kildisheva et al. 2016). Usually, seedling mortality is extremely high during summer drought (James et al. 2011) and seedlings that overcome the critical phase of establishment increase considerably their survival probability (Chambers 2000). Seed and seedling stage are critical to enhance ecological restoration success in drylands (Brown et al. 2021) and to face the high demand of native seed within the U.N. Therefore, improvements are needed at the Decade of Ecosystem Restoration aiming to restore 350 million hectares at global scale. To advance on this line, seed enhancement technologies have emerged in the last decades. These techniques are based on physical manipulation, such as mechanical abrasion, cold and warm stratification, dry or moist heat, and dry post-ripening, or on the application of phytohormones (Kildisheva et al. 2020) and other materials to the seed to favor germination and seedling growth (Madsen et al. 2016). In particular, methods to harden and stimulate seeds in which biological components like bacteria, fungi, algae, or cyanobacteria act as boosters are recently attracting special attention (Chakraborti et al. 2022). These are commonly known as biopriming techniques.

Biopriming has been used since the 1990s (Callan et al. 1990), with most studies focused on crop plants (e.g., wheat, rice, and corn) (Singh et al. 2023; Srivastava et al. 2023), especially to prevent fungal infections (Singh et al. 2020) and to combat abiotic stresses, such as salinity (Biswas et al. 2023). In contrast, research efforts on the effect of biopriming on wild plants have been marginal, mainly because the investment return does not occur in the short term. This situation is currently changing due to the benefits that biopriming can have on the germination and establishment of plants

in natural ecosystems (Zulueta-Rodríguez et al. 2015; Chitra and Jijeesh 2021). This technique can be especially relevant in drylands, where biopriming using bacteria and fungi with PGP (plant growth-promoting organisms) properties can increase seed resistance to environmental stress and pre-adapt plants to upcoming changes in temperature and precipitation regimes due to ongoing climate change (Fazeli-Nasab et al. 2021). Among the different organisms that can be used for biopriming, cyanobacteria are emerging as one of the most interesting candidates. These organisms have been successfully used as bio-inoculants to promote seed germination and plant growth in agriculture (Essa et al. 2015; Moorthy and Delfe 2019) because of their ability to synthesize a great number of substances that act as biofertilizers and antipathogenic agents (Hashtroudi et al. 2013). These substances include growth-promoting regulators, such as cytokinins, gibberellins, or auxins (Machado-de-Lima et al. 2023), as well as vitamins, amino acids, polypeptides, biotin, metabolites that act as allelochemical agents, proteins, antibacterial and antifungal substances, and polymers, especially exopolysaccharides that improve exoenzyme activity (Kumar et al. 2019). Most results have been reported for cyanobacteria isolated from aquatic systems, used individually or as a consortium, and applied on agricultural plants (Prabha et al. 2019; El-Habet and Elsadany 2020), finding that the effects were greatly variable among different cyanobacterial species (Yadav et al. 2022). Since biopriming effects strongly vary depending on the cyanobacteria species used as inoculant, as well as the plant species, it is essential to consider both when evaluating the effectiveness of this technique on the performance of either agricultural or wild plants for which very few data exist (Muñoz-Rojas et al. 2018; Chua et al. 2020; Machado-de-Lima et al. 2023).

In drylands, biopriming with cyanobacteria to promote seed germination can be especially advantageous as these organisms are ubiquitous components of soil communities that colonize the soil surface of open areas between plants, also known as biological soil crusts or biocrusts (Weber et al. 2022). Biocrust-forming cyanobacteria play a primary role as pioneer colonizers of barren soils and drivers of biocrust succession (Chilton et al. 2018) thanks to their ability to stabilize the soil surface and fix the atmospheric carbon and nitrogen, contributing to soil fertility (Xiao et al. 2022). Moreover, they exert multiple effects on plants, such as enhancing seed germination and plant growth, providing tolerance to abiotic (salinity, drought, and extreme temperatures) and biotic (pathogens that are destroyed by the defense enzymes released by cyanobacteria) stress (Kollmen and Strieth 2022). As a result, cyanobacteria species have been isolated from biocrusts and used as inoculum to restore dryland degraded soils (Román et al. 2018, 2021; Roncero-Ramos et al. 2019b). However, biocrust-forming cyanobacteria have been hardly used for seed biopriming to

promote germination as well as plant growth in restoration assays. To our knowledge, very few studies have evaluated the impact of biopriming with biocrust-forming cyanobacteria on the germination rate and seedling development of dryland plants and all of them assessed the effects of cyanobacterial consortia on seeds from a few Australian perennial plant species (Muñoz-Rojas et al. 2018; Chua et al. 2020; Machado-de-Lima et al. 2023), but no attempt with plants from other different continent has been conducted. Furthermore, the isolated effect of each of the cyanobacteria species forming the inoculum was not evaluated in these studies. This aspect is crucial to screen the most viable ones in terms of seed germination and plant growth promotion.

Application of seed biopriming with biocrust cyanobacteria for dryland restoration requires first to evaluate the contribution of this strategy to native plant growth promotion, including a wide range of target plant species and different life forms (i.e., annuals and perennials) used in dryland restoration. Typically, assays have been conducted with perennial species (Muñoz-Rojas et al. 2018; Chua et al. 2020; Machado-de-Lima et al. 2023), but the use of annual plants is certainly relevant for three reasons: (i) grow rapidly, (ii) improve soil properties and prevent erosion during first stages of restoration, and (iii) are closely associated with cyanobacteria-dominated biocrusts in drylands ecosystems, potentially involving facilitation relationships (Boeken et al. 2004; Rodríguez-Caballero et al. 2019). Furthermore, most previous studies on seed biopriming with cyanobacteria were conducted using commercial strains from aquatic environments (Prabha et al. 2019). Therefore, the viability of native soil biocrust-forming cyanobacteria as bio-inoculants for native plant recruitment for ecosystem restoration should be evaluated. Finally, it is interesting to explore whether germination and seedling of native plants is influenced by either the cyanobacterial culture or the cyanobacterial exudates since the latter would facilitate the production and manipulation of the bioprimer and, ultimately, biopriming application.

To fill in these gaps, in this study, we examined the individual effect of seed biopriming with four native biocrust-forming cyanobacteria species (including N fixing and non-N fixing) on germination and radicle elongation of four indigenous plants of Mediterranean drylands. Plant selection included two annual (*Plantago ovata*, *Stipa capensis*) and two perennial (*Macrochloa tenacissima*, *Thymus hyemalis*) plant species that exhibit potential for restoration due to the ecosystem services they provide such as increased fertility, stability and infiltration (Pueyo et al. 2013), increased biodiversity and forage, reduced fire risk (Cortina et al. 2009), and reduced erosion and attraction of pollinators (Barbir et al. 2016).

Additionally, we also evaluated whether the effect of seed biopriming on germination and radicle elongation was

attributed to the effect of the whole cyanobacterial culture (biomass together with the cyanobacterial exudates) or exclusively to the cyanobacterial exudates. We hypothesized that seed biopriming with cyanobacteria would have a positive effect on both germination and radicle length in the four plant species, and induced effects would be greater when seeds are bioprimed with N-fixing cyanobacteria compared to non-N-fixing cyanobacteria.

Materials and Methods

Vegetation Species Selection and Seed Sampling and Preparation

We examined the effect of biopriming with biocrust-forming cyanobacteria on seeds from four plant species representative of Mediterranean drylands, having different life strategies and that usually coexist with biocrusts: (i) two annual plants, i.e., *Plantago ovata* Forssk. and *Stipa capensis* Thunb. and (ii) two perennials, i.e., *Thymus hyemalis* Lange and *Macrochloa tenacissima* (L.) Kunth (Table 1). Seeds from annuals were collected in the field from wild populations in Huércal de Almería (near Almería city, 36° 52' 52.6" N 2° 27' 19.3" W) in April 2020, while seeds from perennials were obtained from the germplasm bank of CECOUAL (Scientific Collection Center of University of Almería), which includes seeds from dryland ecosystems within the province of Almería. Seeds were stored in darkness at room temperature for 4 months. Before the biopriming experiment, we assessed seed viability by carrying out germination tests on a range of seeds with different morphologies and colors (representing different degrees of maturity) for each of the plant species. These tests were conducted in a culture chamber at 25 °C with a photoperiod of 16 h of light and 8 h of darkness (hereafter 16/8) and lasted 15 days for annual species and 30 days for perennial ones. Based on these results, we chose suitable seeds using a stereomicroscope (Leica MZ 12, Wetzlar, Germany). The seed selection varied depending on the species. For *S. capensis*, seeds of dark brown color and with more hairiness were chosen. In *P. ovata*, selection was based on the presence of a whitish coating externally covering the seeds. In *T. hyemalis*, seeds with a spherical shape and black color were chosen, and finally, for *M. tenacissima*, seeds without any cracks or missing parts at the ends, and with a dark green color, were selected. No pre-treatment of the seeds was necessary to break dormancy, but seed disinfection was conducted. To sterilize the seeds, they were shaken for 1 min in 50-mL Falcon tubes containing a 5% (w/v) sodium hypochlorite solution. After this exposure, they were washed twice with distilled water to remove the residual sodium hypochlorite.

Table 1 List of the four native plant species from Mediterranean drylands used in this study, specifying the type of life form and the restoration interest

Sp. (Family)	Life form (photosynthetic pathway)	Restoration interest	Reference
<i>Thymus hyemalis</i> (Lamiaceae)	Perennial shrub (C3)	Attraction of pollinators Soil fertility and stability Increased infiltration Nurse plant	Barbir et al. (2016)
<i>Plantago ovata</i> (Plantaginaceae)	Annual grass (C3)	Soil fertility and stability Increased infiltration	Pueyo et al. (2013)
<i>Macrochloa tenacissima</i> (Poaceae)	Perennial grass (C3)	Fire risk reduction Source of food for grazers Soil fertility and stability Increased infiltration Nurse plant Wildlife refuge	Cortina et al. (2009)
<i>Stipa capensis</i> (Poaceae)	Annual grass (C3)	Soil fertility and stability Increased infiltration	Pueyo et al. (2013)

Cyanobacteria Species Selection and Culture

We used indigenous cyanobacteria species isolated from biocrusts located in semi-arid ecosystems of southeastern Spain, specifically in Almeria province (Roncero-Ramos et al. 2019a). From our stock cultures, we chose two non-heterocystous cyanobacteria (*Leptolyngbya frigida* CAU10 UAM 837 and *Trichocoleus desertorum* CAU7 UAM832) and two heterocystous cyanobacteria (*Nostoc commune* CANT2 UAM 817 and *Tolypothrix distorta* CANT7 UAM 825). Selection of these species was based on their different functional traits (nitrogen fixation, filamentous growth form, stress tolerance, desiccation, and UV radiation resistance), their wide distribution in arid and semi-arid ecosystems in Almeria (Roncero-Ramos et al. 2019a), and their optimized cultivation in liquid media (Roncero-Ramos et al. 2022). Each cyanobacteria species was cultured individually in sterilized flasks using a BG11 culture medium and constant temperature and light conditions (Román et al. 2018; Roncero-Ramos et al. 2019b). We monitored the growth curve of each cyanobacterial culture until they reached steady-state conditions. At that point, the biomass of each species was concentrated or diluted to obtain a concentration of 2 g L^{-1} , required for the biopriming experiments.

Seed Biopriming and Germination Monitoring

Seeds were bioprimed separately with each of the selected cyanobacteria species according to the following treatments: (i) biopriming with cyanobacteria biomass and the culture medium, including cyanobacterial exudates and the growth medium (hereafter “biomass + exudate”); (ii) priming with the cyanobacteria exudates, which includes both the exudates released by cyanobacteria as well as the

growth medium (hereafter “exudate”); (iii) seeds immersed in distilled water; and (iv) in the culture medium BG11 as controls treatments. For biomass + exudate priming, seeds were immersed in the different inocula already prepared consisting of isolated cultures of each cyanobacterium (biomass and the medium where they were grown) at a concentration of 2 g L^{-1} . For the exudate priming, the inoculum cultures were centrifuged for 10 min at 9000 rpm (Wongchawalit and Prem Suriya 2015) and the supernatants containing the culture medium and the substances released by each cyanobacteria species were collected and used to immerse the seeds.

A total of 4000 seeds were bioprimed, 1000 of each plant species. They were separated into groups of 100 seeds, corresponding to each treatment, in small beakers containing 20 mL of the different solutions. The beakers were then shaken on an orbital shaker for 24 h (Prabha et al. 2019) at 180 rpm (to ensure adequate mixing) at room conditions. After shaking, each group of 100 seeds was subdivided into 4 subsets of 25 seeds. These seeds were placed on filter papers in 90 mm Petri dishes (4 Petri dishes per treatment) which were incubated in a growth chamber with a photoperiod of 16/8 and a temperature of 25°C . Seeds were watered with 4 mL of distilled water every day. Germination was monitored daily, for 15 days in the case of annuals plants, and for 30 days in the case of the perennials. We defined germination as the development of a conspicuous radicle protrusion measuring at least 2 mm through the seed coat (Dessi et al. 2021).

We measured four variables; (i) germination percentage, which is the number of germinated seeds with respect to the number of seeds used for each subset (25 seeds) multiplied by 100; (ii) germination time, which is the average number of days it has taken for the seeds of a subset to germinate; (iii) T_{50} , which is the number of days required for the half of

the seeds in each subset to reach germination; and (iv) slope around the inflection point of the cumulative germination curves, which was calculated by solving the b term of a log-logistic function fitted by non-linear least squares regression for each replicate of each treatment, using the *drm* function included in *drc* package (Ritz et al. 2015). The following equation was used:

$$G(t) = \frac{d}{1 + \exp\{-b[\log(t) - \log(e)]\}}$$

where G : Fraction of germinated seeds at time t ; d : Germinable fraction; e : Median germination time for the germinable fraction; and b : Slope around the inflection point.

Radicle Length Measurement

Seven days after germination, each seedling was placed over a black cardboard with a reference scale bar to increase contrast and then photographed. Individual radicles were digitized as individual objects using BeePen tool from QGIS 3.10.7 (QGIS Development Team 2019), and from these, the total length of each radicle [mm] was obtained. Before digitization, images were rescaled to real dimensions using the reference scale added in each picture.

From germination and radicle length data, we also determined the Germination Index (GI), which considers biopriming effects on both germination and radicle elongation. It was calculated according to the following equation proposed by Zucconi et al. (1981):

$$GI = \frac{(Ge(\%) \times REe)}{(Gdw(\%) \times REdw)}$$

where GI : Germination index; $Ge(\%)$: Seed germination percentage in presence of extract (exudate or biomass + exudate); $Gdw(\%)$: Seed germination percentage in distilled water; REe : Mean of radicle length (mm) in presence of extract (exudate or biomass + exudate); and $REdw$: Mean of radicle length (mm) in distilled water.

GI equal to 1 indicate no effect of the treatment compared to the controls. Values lower than 1 indicate inhibition by the treatment, while values higher than 1 indicate promotion on germination and radicle length by the treatment.

Cyanobacteria Cultures and Exudate Characterization

The content of exopolysaccharides was determined in both exudates and cyanobacterial cultures (biomass + exudate). To do this, 1 mL of the solutions (exudates or biomass + exudates) was placed on glass vials and the phenol–sulfuric assay was applied (DuBois et al. 1956). The absorbance

was measured at 488 nm with a UV–vis spectrophotometer Thermo Helios Gamma 9423 UVG 1002 E (Thermo Electron Corporation, Waltham, MA, USA) (DuBois et al. 1956), and the carbohydrate content was calculated according to a calibration curve built using glucose as standard (Chamizo et al. 2020).

To detect and quantify phytohormones in the exudates and cultures (biomass + exudate) of each cyanobacterial species, samples were sent to CEBAS-CSIC (Center for Applied Soil Science and Biology of the Segura) to perform ultra-performance liquid chromatography–quadrupole/time of flight mass spectrometry (UPLC–QToF–MS/MS). Specifically, the following pool composed of salicylic acid (SA), indole-3-acetic acid (AIA), indole-3-butyric acid (IBA), gibberellic acid (GA3), 6-benzylaminopurine (BAP), abscisic acid (ABA), and jasmonic acid (JA) was determined. Phytohormone concentration was quantified in the samples, each one replicated three times, after filtering through a 0.22-μm filter. 5 μL of the filtered sample extracts were injected into a high-resolution UPLC–QToF–MS/MS equipment (UPLC equipped with a Waters Acquity I-Class Plus (Waters Corporation, Milford, MA, USA) coupled to a QToF–MS maXis Impact Series (Bruker Daltonics, Billerica, MA, USA) with a resolution of $\geq 55\,000$ FWHM). The chromatographic conditions tested were based on Müller and Munné-Bosch (2011). Ionization by electrospray (ESI) was used as the source MS with positive polarity. Broadband collision induced dissociation (+bbCID) was employed for the MS/MS analysis with collision energy applied of 24 eV. Additional parameters included a voltage source of 4.5 kV, drying temperature of 200 °C, a nebulizer pressure of 2.0 bar, a N_2 flow rate of 9 L/min, and a mass range of 50–1200 Da. Sodium formate (HCOONa) served as the reference standard.

Data Analysis

We analyzed the effect of our treatments (water, culture medium, biomass + exudate, and exudate of the different cyanobacterial species) on germination percentage, germination time, median germination time (hereafter T_{50}), slope around the inflection point (b parameter in the equation), radicle length, secondary roots and GI for each plant species, using a nested ANOVA and the Tukey's post hoc comparison test ($p < 0.05$). The factors nested from most generalist to most specific were as follows: (i) priming type (no priming including water and culture medium and priming including the rest of treatments), (ii) inoculum type (water, culture medium for the no priming treatment and priming with the four cyanobacterial species, *N. commune*, *T. distorta*, *T. desertorum*, and *L. frigida* (either biomass + exudates or exudates)), and (iii) treatment type (water, culture medium for the non-priming treatment, and treatment with exudate or

biomass + exudate for the different cyanobacteria species). Finally, a one-way ANOVA was performed to analyze the differences in EPS concentration in both exudates and biomass + exudates of the four cyanobacterial species for each plant. Post hoc comparisons were performed using Tukey's test with a significance level set at $p < 0.05$. Before data analysis, variables were tested for normality and homogeneity of variance using the Shapiro–Wilk and Levene's test. All the analyses were conducted in R version 4.2.3 (R Core Team 2023) using the *stats* (R Core Team 2023) and *rsq* (Zhang 2022) packages and the *ggplot2* package (Wickham 2011) for boxplots.

Results

Seed Germination

In absence of any priming treatment (water control), the four plant species differed in germination percentage, germination time, and slope around the inflection point of cumulative germination curves (Table 2). *S. capensis* and *P. ovata* showed the highest germination percentage, the shortest germination time and the steepest slope (lowest b-value). Comparatively, *M. tenacissima* and *T. hyemalis* showed lower germination percentages. Differences were found in the time for germination between the two perennial plants, where *T. hyemalis* showed much longer time than *M. tenacissima*, which showed values closer to those shown by the annual plants (Table 2). The slopes in perennial species were smoother, with *T. hyemalis* having the lowest slope (higher b-value) (Table 2). Notably, the pattern in germination curves differed between plants (Fig. 2). *S. capensis*, *P. ovata*, and *M. tenacissima* showed an exponential cumulative curve, while *T. hyemalis* showed a linear trend. The germination rate of *S. capensis* and *P. ovata* increased exponentially during the first three days, after which they reached a plateau with values between 64–86% and 82–100%, respectively. A similar response was observed in *M. tenacissima* (Fig. 2), but in this case, stabilization occurred at day 7 with a germination rate between 35 and 54% depending on the bioprimer treatment and cyanobacteria species. In the case of *T. hyemalis*, no plateau was reached since seeds germinated daily. With

regards to the T_{50} , we also detected differences among plant species mainly associated with their life strategy. T_{50} was just 1 day for the annual plants, while it increased to 6 days for *M. tenacissima* and was the highest for woody shrub *T. hyemalis*, which reached more than 50% of the seeds germinated after 28 days (Fig. 2).

The nested ANOVA showed that priming, inoculum type, treatment type, as well as their interactions, had no significant effect on seed germination percentage of the four plant species tested (Fig. 1; Table S1). Regardless of this lack of significance in most cases, we observed that seed priming with the exudate of the four cyanobacterial species generally performed better than priming with the biomass + exudate for the four plant species (Fig. 1). Overall, exudates increased germination with respect to biomass + exudate by $11.5 \pm 14.6\%$ in *S. capensis* (p -value = 0.0421, η^2_{sq} = 0.1306), $8.6 \pm 9.4\%$ in *P. ovata* (p -value = 0.0524, η^2_{sq} = 0.1197), $44.2 \pm 16.2\%$ in *T. hyemalis* (p -value = 0.0011, η^2_{sq} = 0.3014), and $3 \pm 14.2\%$ in *M. tenacissima* (p -value = 0.7969, η^2_{sq} = 0.0022). It is worth noting that in the case of *P. ovata*, seed priming with *T. desertorum* exudates led to 100% seed germination (Fig. 1).

In contrast to the results of germination percentage, the nested ANOVA revealed a significant effect of the interaction between priming and inoculum on germination time in *S. capensis*, as well as a significant effect of priming and the interaction between priming, inoculum and treatment in *M. tenacissima*. In *S. capensis*, the water treatment resulted in the shortest germination time (1.19 ± 0.11 days), while all other treatments extended it. For *M. tenacissima*, all treatments shortened germination time compared to water (3.31 ± 0.61 days), with the biomass + exudates treatment achieving a 23% reduction.

Regarding T_{50} , nested ANOVA showed significant effects for *S. capensis* and *M. tenacissima*. In *S. capensis*, the significant effects were due to the interaction between priming and inoculum, as well as to the combined effect of the three factors. The treatments *T. distorta* biomass + exudate and culture medium significantly doubled the median germination time. For *M. tenacissima*, the interaction between priming and inoculum was statistically significant (Table S2). Although no effect was detected between treatments in the Tukey analysis, some trends were identified. Control

Table 2 Germination percentage, germination time and slope around the inflection point of the cumulative germination curves (average [b (slope)], minimum [b min], and maximum [b max]) of each plant species for the control water

	Germination (%)	Germination time (days)	b (slope)	b min	b max
<i>S. capensis</i>	82 ± 12	1.2 ± 0.1	0.46 ± 0.07	0.38	0.52
<i>P. ovata</i>	92 ± 6.5	1.2 ± 0.2	0.40 ± 0.04	0.33	0.43
<i>T. hyemalis</i>	31 ± 8.9	15.7 ± 3.7	3.70 ± 0.38	3.21	4.12
<i>M. tenacissima</i>	50 ± 4	3.4 ± 0.7	2.05 ± 0.47	1.68	2.71

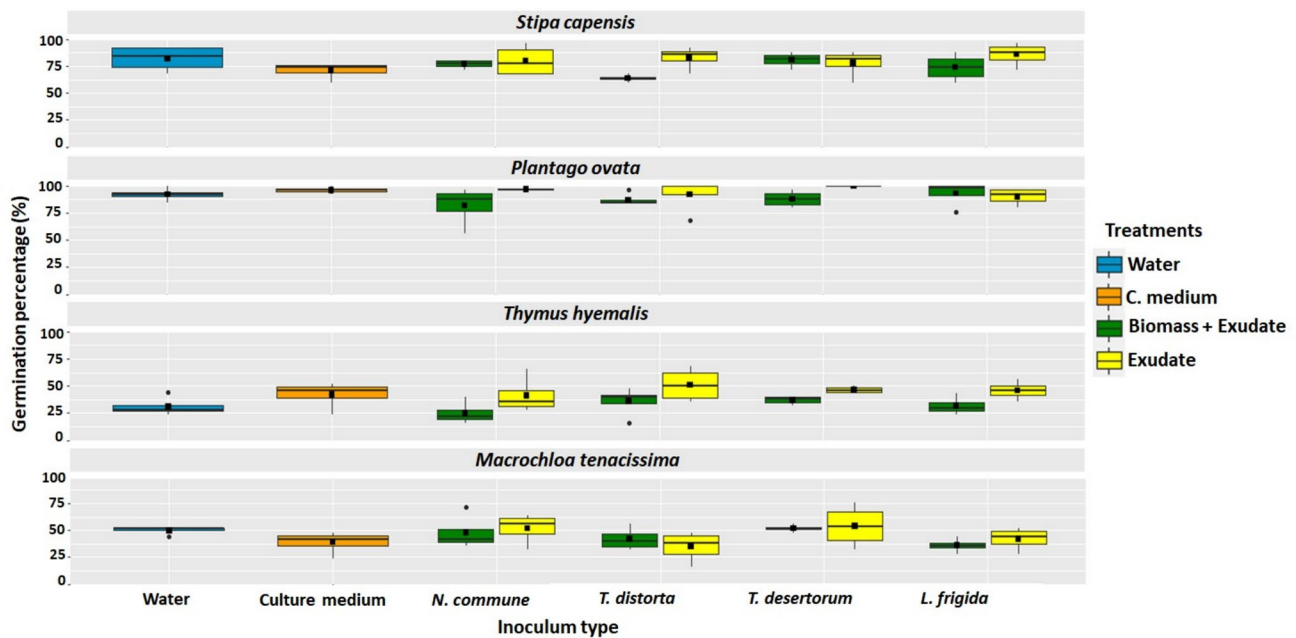


Fig. 1 Biopriming effect on seed germination percentage in *S. capensis*, *P. ovata*, *T. hyemalis*, and *M. tenacissima*. Boxes represent 50% of the central data (starting at the first percentile (Q1) and ending at the third percentile (Q3)), with the black line inside representing the median and the black square representing the mean. Vertical segments arising from the boxes represent the minimum and maximum values omitting the outliers and the black dots correspond to the out-

liers. Blue and orange bars indicate the water and growth medium control treatments, respectively, while the green and yellow bars indicate the cyanobacteria biomass + exudate and exudate treatments, respectively. No significant differences between the treatments and the controls (water and culture medium) were found for any of the plant species. Nested ANOVA results are shown in Table S1 (Color figure online)

water, *N. commune* exudate, *T. desertorum* exudate and biomass + exudate reduced T_{50} by more than half compared to the other treatments.

Finally, no differences were found in the slope around the inflection point between the different cyanobacteria treatments (both exudates and biomass + exudate) compared to controls for the different plant species (Fig. 2; Tables S3, S4), meaning that the priming treatments did not induce differences in the germination rate over time compared to water and medium controls.

Radicle Elongation

The results of the nested ANOVA showed a significant effect of priming, inoculum type and treatment type on radicle elongation. These effects varied depending on the plant and cyanobacteria species and were only detected in annual plants (Fig. 3; Table 3, S1). In the case of *S. capensis*, all biomass + exudate treatments caused a significant increase in radicle length compared to water, ranging from 26 to 42% with no significant differences between cyanobacterial species (Fig. 3). Priming (p -value = 0.0058, η^2_{sq} = 0.0139), inoculum (p -value = 0.0002, η^2_{sq} = 0.0396), and treatment (p -value = 2.63×10^{-8} , η^2_{sq} = 0.0727) also had a significant effect on the number of secondary or lateral roots in

S. capensis. The biomass + exudates treatments produced an increase of 43% to 300% in the number of secondary roots compared to the exudates alone. Despite these differences, a significant effect was only found in the case of the biomass + exudate treatment of *T. desertorum* which led to a significantly higher number of secondary roots (7.5 ± 4) compared to the water (3 ± 2.5) and culture medium (2.5 ± 0.6) (Fig. S1). In *P. ovata*, *N. commune* exudate and both *T. distorta* exudate and *T. distorta* biomass + exudate had a significant positive effect on radicle elongation, showing increases of 31, 31, and 29%, respectively, with respect to water and culture medium (Fig. 3). Although the exudates of the non-N-fixing species did not induce significant changes compared to the controls in *P. ovata*, we detected a tendency of this treatment to increase radicle length, e.g., by 16% in *T. desertorum* and 15% in *L. frigida*. Similarly, the exudate treatment showed a positive effect on radicle length compared to the biomass + exudate treatment; however, differences were not significant due to the large variability within treatments (Table 3; Fig. 3). *N. commune* exudate increased by 27%, *T. distorta* exudate by 1%, *T. desertorum* exudate by 8%, and *L. frigida* exudate by 83%, with respect to their corresponding biomass + exudate treatment. Finally, it is worth mentioning that *L. frigida* biomass + exudate showed a negative effect on radicle length, causing a

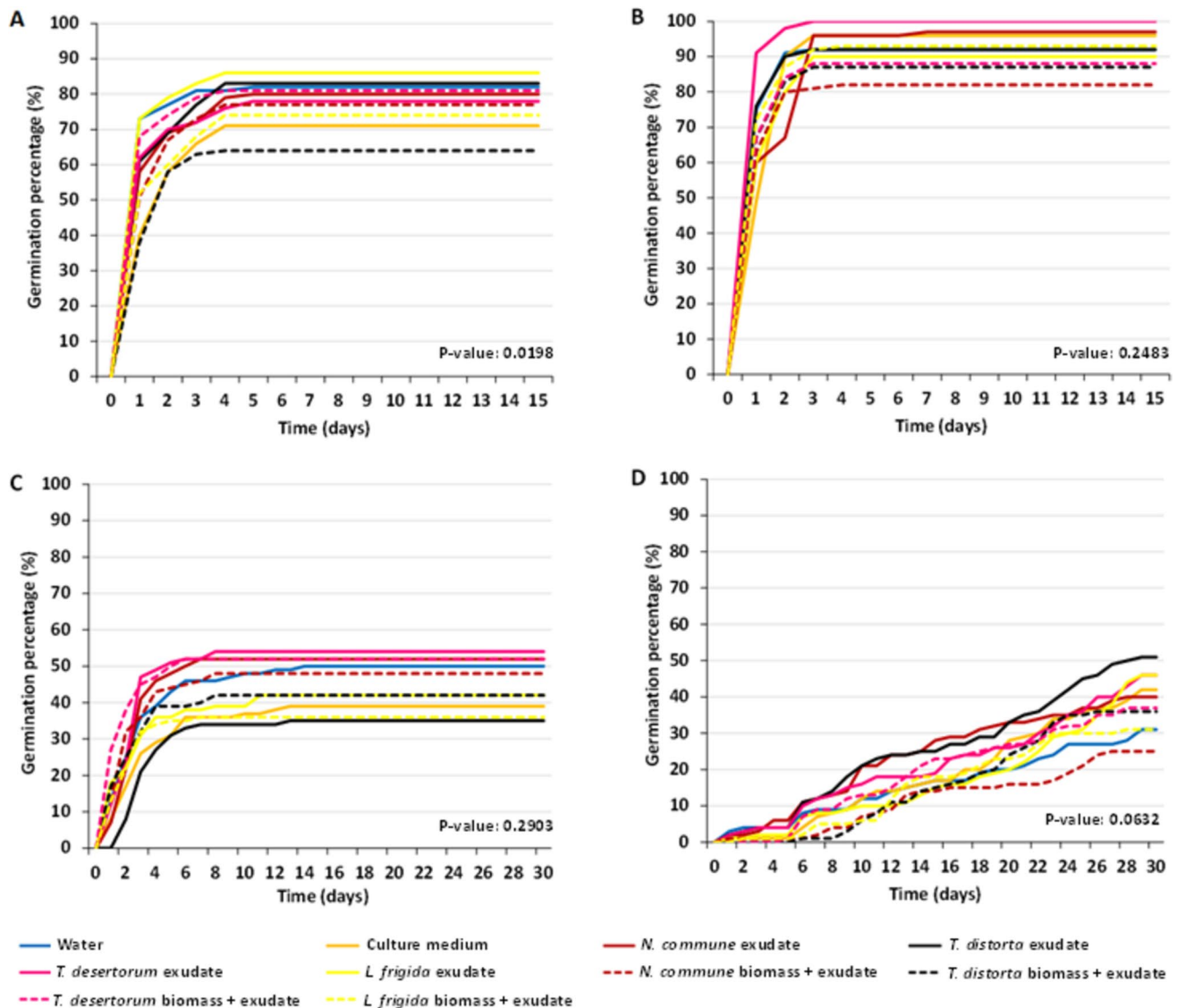


Fig. 2 Germination accumulation curves over time for each treatment and plant species: *S. capensis* (A), *P. ovata* (B), *M. tenacissima* (C), and *T. hyemalis* (D). The blue line corresponds to the water control, while the orange line corresponds to the culture medium. For the four cyanobacteria species, exudates are represented by continuous lines,

while biomass + exudates are represented by dashed lines: *N. commune* (dark red), *T. distorta* (black), *T. desertorum* (fuchsia), and *L. frigida* (yellow). The differences in the slopes of the curves are shown in Table S3 and Nested ANOVA results are shown in Table S4 (Color figure online)

decrease of 37% and 28% compared to water and culture medium, respectively (Fig. 3).

For the perennial species, none of the two types of inocula (exudate or biomass + exudate) promoted a significant effect on radicle elongation compared to the water treatment (Fig. 3; Table S1). For *T. hyemalis*, the exudates from *T. desertorum* and *L. frigida* showed a positive but non-significant trend on radicle growth, increasing by 10 and 6%, respectively, compared to water and culture medium. In contrast, biomass + exudate of this non-heterocystous cyanobacteria species displayed an opposite effect compared to the exudates, decreasing radicle length by 39 and 48%,

respectively, relative to controls (Fig. 3). For *M. tenacissima*, none of the cyanobacteria treatments differed significantly from the water treatment, but it is noteworthy the potential inhibitory effect of the culture medium that resulted in a 33% decrease in radicle length compared to the water treatment. The summarized effects of the priming treatments on radicle elongation for the four plant species can be seen in Table 3.

The GI (Fig. S2; Table S5) showed a similar pattern to radicle length but no significant effect of priming, inoculum and treatment was found for any of the plant species due to large variability between replicates. Similar to the effect on radicle length, a positive trend of *N. commune*

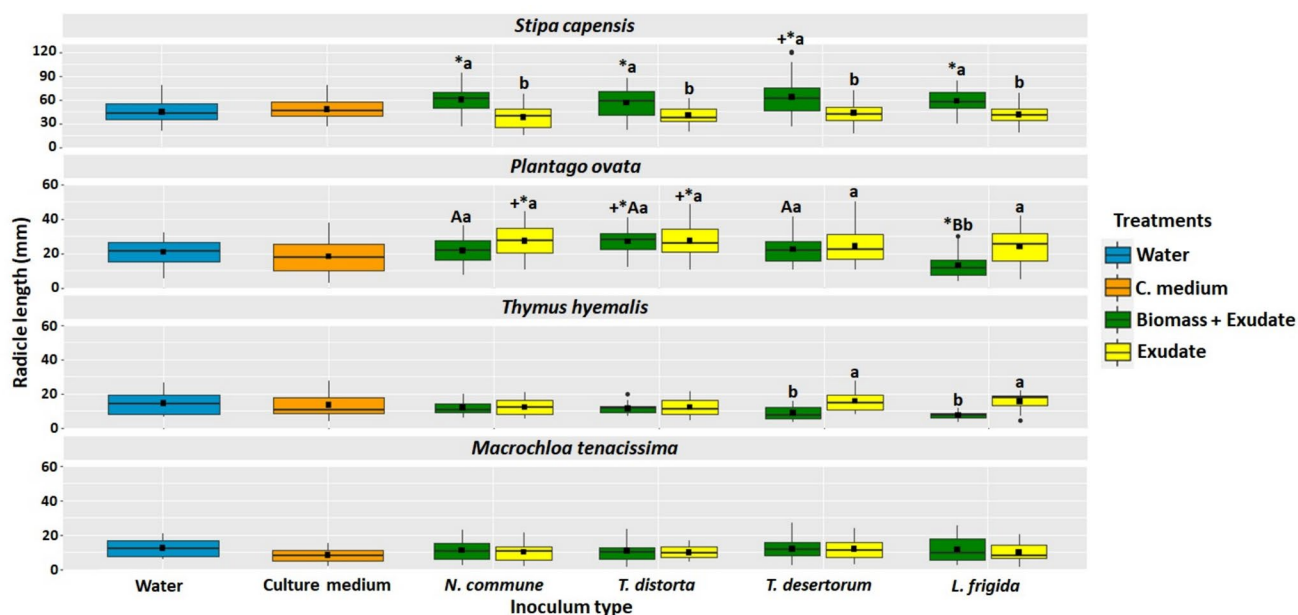


Fig. 3 Biopriming effect on radicle elongation in *S. capensis*, *P. ovata*, *T. hyemalis*, and *M. tenacissima*. Boxes represent 50% of the central data (starting at the first percentile (Q1) and ending at the third percentile (Q3)), with the black line inside representing the median and the black square representing the mean. Vertical segments arising from the boxes represent the minimum and maximum values omitting the outliers and the black dots correspond to the outliers. Blue and orange bars indicate the water and growth medium control treatments, respectively, while the green and yellow bars indicate the cyanobacteria biomass + exudate and exudate treat-

ments, respectively. Lower case letters indicate significant differences between exudate and biomass + exudate for each cyanobacteria (p -value < 0.05). Capital letters located above the green boxes indicate significant differences among the four biomass + exudate treatments for the same plant species, while capital letters located above the yellow boxes indicate significant differences between the four exudate treatments. Significant differences of the treatment versus water are indicated with an asterisk, while differences versus culture medium are indicated with a cross (Color figure online)

Table 3 Categorical summary of the effects of different priming treatments on radicle elongation in *S. capensis*, *P. ovata*, *T. hyemalis* and *M. tenacissima*. B + Ex Cyanobacteria biomass + exudate, Ex Cyanobacteria exudate

	Water	Culture medium	<i>N. commune</i>		<i>T. distorta</i>		<i>T. desertorum</i>		<i>L. frigida</i>	
			B+Ex	Ex	B+Ex	Ex	B+Ex	Ex	B+Ex	Ex
<i>S. capensis</i>										
<i>P. ovata</i>										
<i>T. hyemalis</i>										
<i>M. tenacissima</i>										

Green cells denote positive effects vs water, yellow ones denote no effect vs water, and orange ones denote negative effects vs water

(1.3 ± 0.1) and *T. desertorum* (1.2 ± 0.3) biomass + exudate treatments for *S. capensis* and the exudate treatments for *P. ovata* were observed for GI. The GI of *T. hyemalis* was positively influenced by *T. desertorum* (1.8 ± 0.2) and *L. frigida* (1.8 ± 0.2) exudates, whereas a negative trend of *L. frigida* biomass + exudate was found on the GI of *P. ovata* and *T. hyemalis*.

Phytohormone and EPS Characterization of Cyanobacterial Inoculants

The two types of cyanobacterial inoculants (biomass + exudates and exudates) showed differences in phytohormone composition and concentration depending on the nitrogen-fixing capacity of the cyanobacteria (Table 4). In the exudates of non-N-fixing cyanobacteria (*T. desertorum* and *L. frigida*), none of the analyzed phytohormones were detected (Table 4). In contrast, in the exudates of N-fixing cyanobacteria species (*N. commune* and *T. distorta*), 3 out of 7 phytohormones were present, i.e., salicylic acid (SA), jasmonic acid (JA), and abscisic acid (ABA) (Table 4). In particular, JA showed low concentrations in the two cyanobacteria species, while SA and ABA were found in higher proportions. The latter was produced in the highest concentration by the two heterocystous cyanobacteria and especially by *T. distorta*. Opposite to the exudates, phytohormones were detected in the biomass + exudate of the non-N-fixing cyanobacteria, but in very low concentrations. ABA was detected in both species, while JA was only detected in *T. desertorum* and a new phytohormone, 6-benzylaminopurine (BAP), was detected in *L. frigida* (Table 4). Concerning the N-fixing cyanobacteria species, some of the phytohormones present in the exudates were no longer present in the biomass + exudate. In *N. commune*, there was a significant reduction in the concentration of ABA and JA compared to its exudate and SA was no longer detected. In *T. distorta*, SA and ABA were not detected and the concentration of JA increased compared to its exudate (Table 4).

Exopolysaccharide content also differed between the exudates and the biomass + exudates and greatly depended on cyanobacterial species in both (exudates, p -value = 2.20×10^{-16} , $\eta^2_{sq} = 0.9929$; biomass + exudates, p -value = 0.0103, $\eta^2_{sq} = 0.8283$). In the exudates, *L. frigida* was the cyanobacterial species with the highest EPS concentration, followed by *T. distorta* and *T. desertorum*. Conversely, *N. commune* showed the lowest EPS concentration, with values 3 times lower than the rest of the cyanobacteria species (Table 4). The exopolysaccharide content of the biomass + exudate was much higher than that present in the exudates. Among species, *T. distorta* and *L. frigida* showed the highest EPS concentration, while *N. commune* and *T. desertorum* showed the lowest EPS concentration. The EPS content of the biomass + exudates of the four cyanobacteria

species was 21, 17.1, 40, and 26.4 times higher, respectively, than that detected in the exudates (Table 4). The EPS content of the biomass + exudates showed a similar pattern among cyanobacteria species. *T. distorta* (1099 mg/L) and *L. frigida* (961 mg/L) were the cyanobacteria that showed the highest EPS concentration. However, in contrast with that observed in the exudates, *T. desertorum* showed the lowest values (575 mg/L), showing *N. commune* (760 mg/L) intermediate values.

Discussion

Our study revealed that biopriming with native biocrust-forming cyanobacteria had no effect on seed germination. However, substantial effects on radicle length were detected. The enhancing effects in radicle length were mainly observed for the annual species, which indeed, in their natural environment usually co-inhabit with cyanobacteria-dominated biocrusts in the open spaces among perennial plants (Rodríguez-Caballero et al. 2019). On the whole, biopriming effects depended on the plant species, species of cyanobacteria used, and the type of inoculum (exudate or biomass + exudate), which evidences that biopriming effect was species specific.

Biopriming Effect on Germination

Seed biopriming has been reported to improve germination and growth parameters of different wild and agricultural plant species (Ghorbanpour and Hatami 2014; Mahmood et al. 2016; Houida et al. 2022). Thus, we hypothesized that seed biopriming with different inoculum types based on biocrust-forming filamentous cyanobacteria species would exert a positive effect on the germination of various perennial and annual plants that usually cohabit with biocrusts in Mediterranean semi-arid environments. Contrary to what was expected, neither priming with cyanobacteria exudates nor with cyanobacteria biomass + exudate induced a significant positive effect on the germination of the selected plant species (Fig. 1). In general, biopriming treatments exhibited no effect on germination but also importantly, none of them showed an inhibitory impact on seed germination. Our results agree with the scarce previous studies which found no effect of biopriming with cyanobacteria on seed germination of 7 out of 8 evaluated wild plant species (Muñoz-Rojas et al. 2018; Chua et al. 2020). Contrasting results are reported for agricultural species, for which cyanobacterial biopriming is commonly reported to enhance germination of crops, such as rice (Yadav et al. 2022), tomato (Righini et al. 2021), cumin, coriander, or fennel (Kumar et al. 2013). The positive effects found in these studies were always species specific (Kumar et al. 2013; Yadav et al. 2022). Moreover,

Table 4 Phytohormone (ng/mL) and EPS (mg/L) concentration of cyanobacterial exudates and biomass + exudates (cyanobacteria culture concentration 2 g L⁻¹). [M + H] is the sum of the molar mass of the phytohormone and the atomic mass of a proton (H⁺). n.d. means not detected

	Heterocystous cyanobacteria			Non-heterocystous cyanobacteria					
	<i>N. commune</i>			<i>T. distorta</i>			<i>T. desertorum</i>		
	Ex	B + Ex		Ex	B + Ex		Ex	B + Ex	<i>L. frigida</i>
Salicylic acid (SA) [ng/mL] [M + H] + = 139.0309	196.10	n.d		72.70	n.d		n.d	n.d	n.d
Indole-3-butyric acid (IBA) [ng/mL] [M + H] + = 204.1019	n.d	n.d		n.d	n.d		n.d	n.d	n.d
Indole-3-acetic acid (AIA) [ng/mL] [M + H] + = 176.0706	n.d	n.d		n.d	n.d		n.d	n.d	n.d
Gibberellic acid (GA3) [ng/mL] [M + H] + = 347.1489	n.d	n.d		n.d	n.d		n.d	n.d	n.d
6-benzylaminopurine (BAP) [ng/mL] [M + H] + = 226.1087	n.d	n.d		n.d	n.d		n.d	n.d	5.39
Abscisic acid (ABA) [ng/mL] [M + H] + = 265.1434	651.96	8.45		1822.34	n.d		n.d	4.98	7.69
Jasmonic acid (JA) [ng/mL] [M + H] + = 211.1328	21.81	4.52		8.97	19.13		n.d	8.67	n.d
Exopolysaccharides (EPS) [mg/L]	18.99 ± 1.20 D	759.63 ± 2.62 ab		52.43 ± 1.11 B	1099.45 ± 152.23 a		21.82 ± 1.27 C	575.07 ± 125.57 b	56.38 ± 2.79 A
									961.11 ± 127.56 ab

Letters indicate statistically significant differences in EPS concentration. Capital letters indicate differences among exudates and lowercase letters indicate differences among biomass + exudates
B + Ex cyanobacteria biomass + exudate, *Ex* cyanobacteria exudate

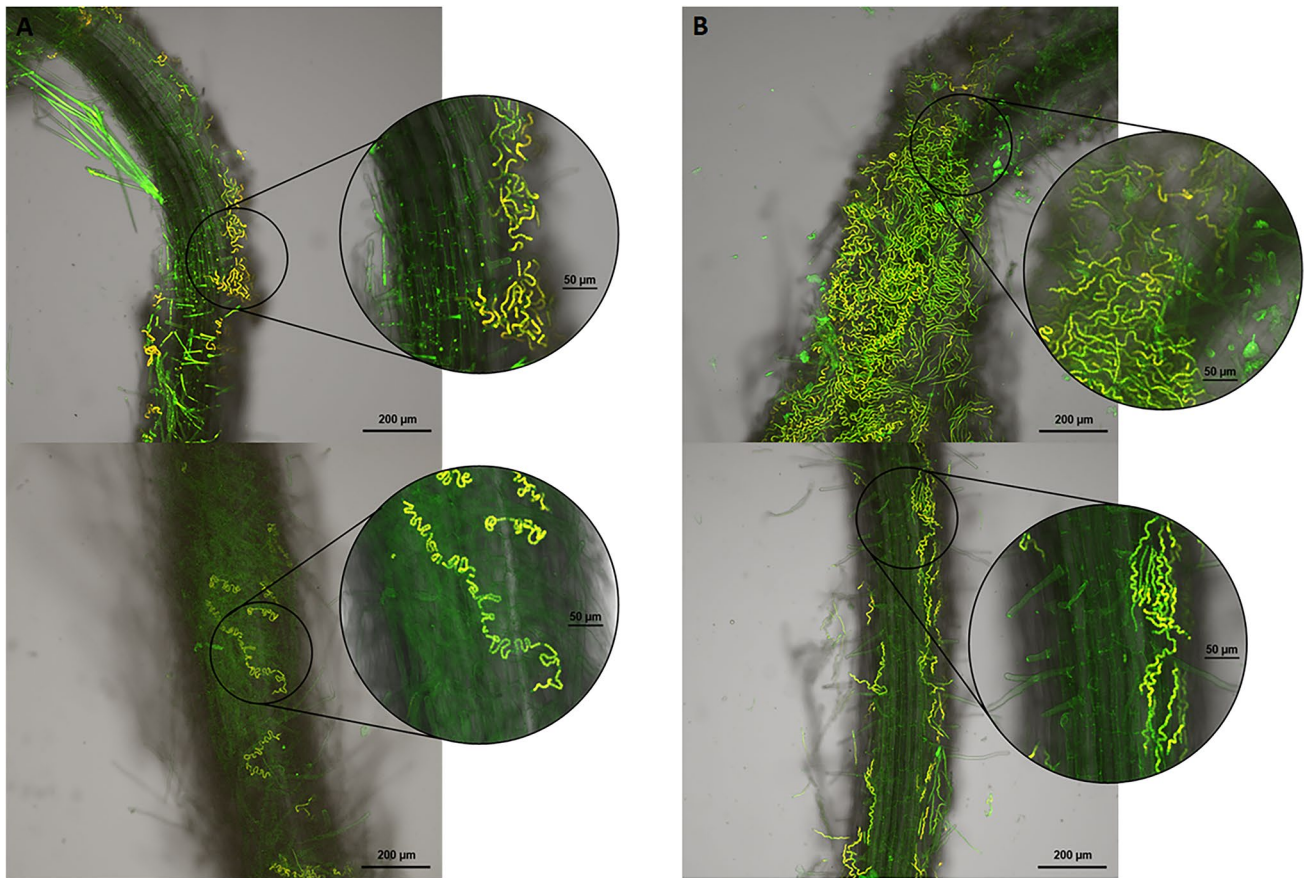


Fig. 4 **A** Radicle colonization by *N. commune* in *S. capensis* (upper image) and *P. ovata* (lower image). **B** Radicle colonization by *T. desertorum* in *S. capensis* (upper image) and *P. ovata* (lower image). Green emission corresponds to suberin and lignin of the radicle, and yellow color represents the mixture of the union of the red and green emission which corresponds to chlorophyll *a* of the cyanobacteria filaments.

For each plant species, photos were taken at 10× magnification (left image) and 30× magnification (right image). These images were taken with a motorized inverted microscope Nikon Eclipse Ti-E property of Research Central Services of University of Almería (Color figure online)

certain plant species can exhibit germination inhibition in the presence of cyanobacteria (Saqrane et al. 2008) or undergo both inhibition and potentiation depending on the cyanobacteria used as primer. For example, an inhibitory effect was found for *Zea mays* L. seeds when primed with *Microcystis* (Saqrane et al. 2008), but an improved effect was found when primed with a consortium of N-fixing cyanobacteria (Sharma et al. 2020).

Biopriming Effect on Radicle Elongation

Biopriming promoted a significant increase in radicle length mainly in the annual plant species. In the case of *S. capensis*, radicle elongation was favored by the biopriming with the cyanobacteria biomass + exudate, which promoted an average increase of 31–42% over the control and exhibiting the biomass + exudate of *T. desertorum*, the highest effect. In contrast, radicle elongation in *P. ovata* was promoted by exudate biopriming, with an increase in radicle

elongation of 15–31% compared to the controls, reaching the highest values with the two N-fixing species (*N. commune* and *T. distorta*) (Fig. 3). These effects of cyanobacteria biomass + exudate and exudate treatments on radicle elongation can be attributed to two factors: (i) the production and release of biologically active compounds like amino acids, vitamins, siderophores, polysaccharides, or phytohormones (gibberellins, cytokinins, and auxins), which can improve plant growth and increase seed and fruit production (Nowruzi et al. 2021) and (ii) the colonization of the external and internal tissues of the radicle by cyanobacterial filaments (Álvarez et al. 2020). When colonization occurs, an exchange pathway is established where the cyanobacteria are supplemented with sugars provided by the host plant and the roots are supplied with nitrogen and secondary metabolites present in the cyanobacterial exudates (Kollmen and Strieth 2022). Using fluorescence and scanning electron microscope images, we verified the action of cyanobacteria filaments surrounding *S. capensis* radicles, likely creating

a more favorable microenvironment and providing an extra input of nutrients for root growth (Fig. 4A, B). Cyanobacterial filaments that were closely anchored to the radicle also appeared in *P. ovata*, but their density and distribution along the radicle were lower (Fig. 4A, B). This can explain the positive effect of the cyanobacterial biomass + exudate promoting radicle elongation in *S. capensis* but absent in *P. ovata*. The root elongation-stimulating effect induced by cyanobacteria in the annual species can have huge relevance for their establishment in drylands where water is scarce, and a well-developed root system is essential for survival (Scholes 2020). Importantly, for *S. capensis* the treatments with biomass + exudate and especially, the biomass + exudate of *T. desertorum* caused an increase in the development of secondary or lateral roots (Fig. S1), which is likely to benefit the survival and growth of this species under field conditions. The bundle-forming cyanobacteria *T. desertorum* has been described as a pioneering biocrust colonizer that strongly stabilizes soils through EPS secretion, allowing the subsequent colonization of other biocrust components and vegetation (Roncero-Ramos et al. 2019a). This important stabilizing effect can be strengthened by the promotion of longer radicles and a greater number of secondary roots in annuals, which in turn will contribute to soil stabilization and the reduction of soil erosion. Similarly, Salamah et al. (2020) found that on rice plant seeds inoculated with *Nostoc* strains, the filaments were able to colonize the root, protecting the meristematic zones with their exopolysaccharides and promoting lateral root development.

This positive effect of biopriming on radicle elongation in annual plants can be explained by the process of coevolution between biocrusts and annuals. In Mediterranean drylands, perennial vegetation usually appears in isolated patches, known as fertility islands, while both biocrusts and small annual species dominate open areas (Rodríguez-Caballero et al. 2019), where they do not compete for water, nutrients, or light with vascular plants. As biopriming has been carried out with one of the more ubiquitous components of biocrusts, i.e., cyanobacteria, we hypothesized that this co-inhabitation for a long time may have led to synergies between both components, leading to beneficial effects for both. Many of the studies aimed at restoring degraded drylands have used shrubs, but the results have often been unsuccessful (Rinella et al. 2015), and more promising outcomes have been found for herbaceous plants (Shackelford et al. 2021). So, annual plants could play a relevant role in restoration as they can stabilize the soil with their root systems during the first stages after restoration (De Baets et al. 2007), as well as provide soil organic matter as litter and leave a significant seed bank for the following year (Shackelford et al. 2021). Thus, the action of the cyanobacterial inoculum promoting longer radicles will facilitate the establishment of annual plants that will contribute to

make these habitats more fertile and erosion resistant. These effects will also favor the colonization of perennial plants, increasing biodiversity (Oliver et al. 2015). In addition, the positive effect of biopriming on the radicle elongation of annual plants compared to perennials can be explained by their short life cycle and rapid growth rates, so that the possible effects of biopriming become evident more quickly. The absence of observed effects in perennial species could be related to the slower development and longer life expectancy of these life forms (Friedman 2020), making early effects more difficult to distinguish. Thus, more time is likely required to detect biopriming effects (if existing) and it would be necessary to wait for higher plant developmental stages to detect differences in growth or at flowering and fruiting level. An improvement in any of the plant development stages will increase the chance for the plant to survive and grow in harsh environments as those from drylands.

Nonetheless, the inhibitory effect of *L. frigida* biomass + exudate on radicle length in *P. ovata* (compared to water and its exudate) and *T. hyemalis* (compared to its exudate) was noteworthy. Both *P. ovata* and *T. hyemalis* have small spherical seeds and both plant species release hemicellulose-rich mucilage (Pan et al. 2022) that naturally envelops the seed. We hypothesize that this mucilaginous layer together with the dense biofilm formed by the tight junction of the narrow filaments of *Leptolyngbya* (Roncero-Ramos et al. 2019a) could constitute a physical barrier in the early stages of germination, limiting the exchange of water and nutrients between the radicle and the cyanobacteria, causing a decrease in radicle growth. This dense biofilm is detected in both plant species, being more evident in *T. hyemalis*, where there is a clear colonization of both seeds and mucilage (Fig. S3). In *M. tenacissima*, although results are not significant due to large variability within treatments, we found a potential inhibitory effect on radicle elongation caused by the culture medium. This effect can be explained by the composition of the BG11 medium which is mainly composed of salts (Roncero-Ramos et al. 2019b). These salts act as a powerful inhibitor of germination and radicle growth for alpha grass (Moulessehouli et al. 2022). The exudate and biomass + exudate treatments did not show this inhibitory effect most probably because cyanobacteria have served as a buffer, biosorbing these compounds and retaining the salt in the exopolysaccharides (Maggioli et al. 2022).

Results from this study are highly relevant as they allow us to identify which type of treatment (cyanobacteria exudate or cyanobacteria biomass + exudate) can stimulate the growth of plant species potentially used for ecological restoration. This knowledge enables the fine-tuning and optimization of specific biopriming techniques that enhance seedling establishment and growth and can be applied in large-scale restoration projects. Last, it is worth mentioning that the plant species evaluated in this study represent

suitable candidates for their use in restoration. The two annual species, due to their rapid germination, contribute to soil stabilization at an early stage, improving soil conditions for later organisms. Besides, *P. ovata* and *T. hyemalis*, due to the presence of a mucilaginous coating on their seeds, adhere better to the substrate and are less susceptible to their detachment by erosive agents. These anatomic and physiologic characteristics together with the right choice of inoculum type and cyanobacterial strain for biopriming can increase the success rate of restoration assays when using seeds.

Influence of Phytohormones and Exopolysaccharides Synthesized by Cyanobacteria

Many effects of seed biopriming on germination and plant growth are related to the supply of bioactive compounds, such as phytohormones (Singh et al. 2018). Freshwater-dwelling filamentous N-fixing cyanobacteria (*Anabaena* sp.) and endophytic cyanobacteria have been reported to produce indole-3-acetic acid, abscisic acid, cytokinins, gibberellins, ethylene, and salicylic acid (Singh et al. 2017). In our study, we found that non-N-fixing cyanobacteria synthesize only small amounts of phytohormones, which are retained inside the cells, making them unavailable to surrounding organisms as they are not released into the environment (not detected in exudates). Other studies partially support this finding by detecting endogenous phytohormones in non-N-fixing species, although they also report the presence of exogenous phytohormones in smaller proportions (Singh 2014; Tan et al. 2021). In contrast, N-fixing cyanobacteria produce higher concentrations of phytohormones, which are released into the surrounding environment, as evidenced by their presence in the exudates. As these molecules are bioavailable, they exert their functions on other organisms and may affect different periods of the life cycle. This finding is consistent with previous studies that also found most cyanobacteria species producing phytohormone belong to the order Nostocales, consisting of species with heterocysts capable of fixing atmospheric nitrogen (Kumar et al. 2019). Nitrogen, besides being a scarce element in the soils, is one of the basic elements for the synthesis of some phytohormones, like cytokinins or auxins (Hnatuszko-Konka et al. 2021; Tan et al. 2021). So, the species with heterocysts can potentially synthesize these phytohormones to a greater extent because they have access to a source of nitrogen unavailable to non-N-fixing cyanobacteria.

The abscisic acid (ABA) was the phytohormone that showed the highest concentration in the cyanobacterial exudates, and especially in *T. distorta* exudates (Table 4). Exopolysaccharides and phytohormones are often secreted by cyanobacteria as a protection against environmental

stresses (Singh et al. 2018). As the selected cyanobacterial strains were isolated from dryland soils, synthesis of these compounds likely responds to a protection mechanism against harsh environmental conditions. In addition, the behavior of cyanobacterial strains concerning phytohormone production varies depending on their morphology: unicellular strains release more phytohormones into the culture medium (they show a marked exogenous production), while filamentous strains accumulate these phytohormones inside (they show endogenous production) (Mazhar et al. 2013). This can also explain the presence of some of the phytohormones analyzed in the biomass + exudates, but not in the exudates. The general absence of cytokinins (BAP), auxins (IBA and AIA) and gibberellins (GA3) in the exudates and biomass + exudates of the tested cyanobacteria species may be explained by the lack of precursors of these molecules in the medium in which they grow. For example, previous studies have found that, if tryptophan is added to the culture medium, the production of indole-3-acetic acid is stimulated (Mazhar et al. 2013). Synthesis of phytohormones by cyanobacteria in response to the addition of precursors should be evaluated in future assays.

Regarding EPS, they have important functions, such as the adhesion of soil particles and the increase of soil stability (Chamizo et al. 2020), entrapment of carbon and nutrients, and protection against extreme temperatures, salinity, and drought (Costa et al. 2018). Through these effects, EPS can also influence seed germination and plant growth, although these effects seem to be species specific (Jian et al. 2015). Exopolysaccharide amount, molecular weight, and chemical composition greatly depend on cyanobacteria species and cultivation conditions (Laroche 2022). Previous works have found cyanobacterial EPS increased germination percentage and plant vigor (Jian et al. 2015) and also alleviated salt stress in saline soils (Arora et al. 2010). Although we did not find a direct relationship between the concentration of released EPS and the response in germination or radicle length, some of the cyanobacteria species showed a high amount of total and released EPS, (i.e., *T. distorta* and *L. frigida*, see Table 4), which could partially explain the positive observed effects of the biopriming treatment with these two cyanobacteria species on the radicle length of the annual plants (Fig. 3). In addition, a positive trend was observed in the radicle length of *T. hyemalis* when primed with cyanobacterial exudates. Thus, in the plant species having a mucilaginous coating (i.e., *P. ovata* and *T. hyemalis*), EPS provided by the biopriming treatment together with the mucilage helped retain phytohormones and other substances in the plant seedlings, likely promoting radicle length.

Conclusion

The effect of biopriming was species specific and strongly depended on both the plant and cyanobacteria species. Biopriming generally showed no effect on seed germination but enhanced radicle elongation in annual plants. Cyanobacterial biomass + exudate and exudates induced differences in radicle length, with some plants performing better with biomass + exudate (e.g., *S. capensis*), others with exudate (e.g., *P. ovata* and *T. hyemalis*) or having no effect (*M. tenacissima*). This positive effect on radicle length will increase the chance for the seedling to survive which, when focused on the ecological restoration of drylands, is of extraordinary importance to ensure a greater probability of success. As an advantage, seed biopriming can reduce the cost of restoration efforts since less volume of inoculum per plant is needed compared to direct soil inoculation or hydroseeding with cyanobacteria. More importantly, with this technique, there is close contact between the cyanobacteria and the seed or seedling tissues, which can favor the transfer of compounds synthesized by these organisms leading to an increase in plant growth. Future research should investigate the biochemical and genetic mechanisms behind the varying effects of different cyanobacteria on plant species.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00344-024-11521-6>.

Acknowledgements Author would like to express our gratitude to Raúl Román, Borja Rodríguez-Lozano, Francisco Martínez Oller, and Adrián Aguilar López for their invaluable help in the realization of the photograph measurements for radicle determination.

Author Contributions Conceptualization, A.A-M., S.C., M.M-R., and Y.C.; methodology and investigation, A.A-M.; formal analysis, A.A-M. and E.R-C.; writing and original draft preparation, A.A-M., S.C., and Y.C.; writing, reviewing, and editing of the manuscript, A.A-M., S.C., E.R-C., M.M-R., and Y.C.; funding acquisition, Y.C. All authors have read and agreed to the published version of the manuscript.

Funding Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature. This work has been part of the projects TED2021-132332B-C21 and BIOPANTFLUX (CNS2023-144749), co-funded by the Spanish Ministry of Science and Innovation- State Research Agency MCIN/ AEI/10.13039/501100011033 and European Union funds "NextGenerationEU/ PRTR", the project REBIOARID (RTI2018101921 B I00) funded by FEDER/Spanish Ministry of Science and Innovation- State Research Agency, the project P_FORT_GRUPOS_2023/20 PPIT-UAL, Junta de Andalucía-ERDF 2021-2027. Objective RSO1.1. Programme: 54.A, and the project R-Forze (PID2021-127631NA-I00) funded by FEDER/Spanish Ministry of Science and Innovation- State Research Agency. A.A-M. was funded by the FPI predoctoral fellowship from the Spanish Ministry of Science and Innovation (PRE2019-090869). S.C. was supported by the EMERGIA program (EMC21_00424), funded by the Council of Economic Transformation, Industry, Knowledge and Universities of the Andalusian regional government. E.R-C. was supported by the Ramon y Cajal fellowship (RYC2020-030762-I). M.M-R was funded from the Spanish Ministry of Science and Innovation (RYC2020-029255-I, TED2021-132332A-C22 and PID2021- 123097OA-I00).

Declarations

Conflict of interest All the authors have no competing interest to declare that is relevant to the content of this article.

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