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RESEARCH

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Contrasting plant growth performance of invasive polyploid and native diploid *Prosopis* is mediated by the soil bacterial community

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Abstract

Background Soil microbial communities affect above-ground plant diversity and community composition by influencing plant growth performance. Several studies have tested the effect of soil bacterial microbiome on growth performance of native and invasive plants, but the influence of specific bacterial isolates has not been investigated. Here, we investigated the effects of soil bacterial exclusion by soil sterilization and by inoculation of *Streptomyces* rhizobacterial isolates on the growth performance of native and invasive *Prosopis* congeners.

Results Plant growth performance of invasive *P. juliflora* was significantly reduced when grown in sterilized soils, whereas native *P. cineraria* showed enhanced growth performance in the sterilized soils. When grown in the soil inoculated with the specific *Streptomyces* isolate from *P. juliflora* (PJ1), the growth performance of invasive *P. juliflora* was significantly enhanced while that of native *P. cineraria* seedlings was significantly reduced. However, inoculation of *P. cineraria* and *P. juliflora* seedlings with *Streptomyces* isolate from the rhizosphere of native *P. cineraria* (PC1) had no significant effect on the growth performances either of *P. juliflora* or *P. cineraria*.

Conclusion Our study reveals that invasive *P. juliflora* experiences positive feedback from the non-native soil bacterial community, while the native *P. cineraria* experiences negative feedback from its soil bacterial community. Our results provide fresh experimental evidence for the enemy release hypothesis, and further our understanding of the contrasting growth-promoting effects of differentially recruited microbial species belonging to the same genus (*Streptomyces*) in the rhizospheres of alien invasive and native plants.

Keywords Soil bacteria, *Streptomyces*, Invasive, *Prosopis juliflora*, *Prosopis cineraria*, Soil microbiota

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Background

Rapid spread of invasive plants and the resulting threat to native ecosystems, ecosystem services and their biodiversity is a global concern. Global trade and human cultural exchanges have resulted in numerous plant species navigating across geographical barriers and extending to new habitats (Kleunen et al. 2015). Many of these plants have got established as invasive species in the introduced ranges and have significantly affected the ecosystem services and regional economies (Bai et al. 2013). Invasive alien species experience several ecological interactions in their introduced ranges and the relative strength of such associations can impact their invasiveness (Catford et al. 2009; Traveset and Richardson 2020). Among various theories and models of plant invasion, enemy release hypothesis (ERH) offers the most plausible explanation for the rapid growth and establishment of exotic plant species (Keane and Crawley 2002). The core notion of this hypothesis is that exotic species are less negatively impacted by enemies in newly introduced surroundings than native species because they are freed from enemies like herbivores, parasites, or pathogens that may have constrained their growth in native ecosystems. The core tenet of this hypothesis is that natural enemies play a significant role in population regulation, and that the diminished regulation of exotic species by natural enemies is the primary driver of their increased abundance and spread outside of their native areas.

Soil microbes plays an important role in mediating the interaction between native and invasive plants as well as spread of the latter (Fahey and Florey 2022). For instance, the escape of invasive plant species from their co-evolved pathogens in non-native ranges provides them a competitive advantage over native plants (Mitchell and Power 2003; Allen et al. 2017), while some invasive plants are further endowed with microbial communities which directly inhibit growth of native plant species in their vicinity (Eppinga et al. 2006; Mangla et al. 2008; Flory and Clay 2013) or suppress the growth of microbes beneficial for the native plant communities (Bozzolo and Lipson 2013), suggesting that the microbial communities associated with exotic invasive plants play a significant role in shaping community structure of invaded ranges. Furthermore, soil mutualists such as rhizobia and mycorrhizal fungi can indirectly influence competitive interactions between native and invasive plants through differential effects on nutrient acquisition (Abbott et al. 2015; Marler et al. 1999), signifying that soil microbes can have substantial effects on plant community composition (Ehrenfeld et al. 2005; Mordecai 2011; Callaway and Rout 2010). Several studies have reported that invasive plants amend the endemic soil microbiome to facilitate their establishment in novel ranges (Si et al. 2013) and that plant–microbial interactions play an important role in mediating their establishment success in non-native range (Klironomos 2002; Van der Putten et al. 2007). However, there are limited studies on understanding the effects of specific microbial isolates associated with native and invasive plant species that impact their respective growth performances. Therefore, this study fills that gap and provides empirical evidence of the contrasting effects of specific bacterial isolates of the rhizospheres of native and invasive *Prosopis* congeners.

Prosopis genus comprises 44 species including some of the most aggressive invasive species in the world, like *P. glandulosa, P. velutina, P. juliflora*, and *P. pallida* (Burkart 1976; Pasiecznik et al. 2001). These invasive species have altered the global distribution patterns of other native plants, including its own non-invasive congeners like *P. cineraria*. Furthermore, *P. juliflora* is a polyploid across its invaded range ($2n = 4x = 56$), while its native congener, *P. cineraria* is a diploid ($2n = 28$) (Trenchard et al. 2008). Notably, the plant species with higher ploidy are shown to experience higher invasion success (Pandit et al. 2011; Te Beest et al. 2012) and often show better adaptability to fluctuating environments (Leitch and Leitch 2008; Richardson et al. 2000). Invasion success of exotic polyploids have been attributed to certain physiological characters such as enhanced ecological tolerance, higher growth and reproductive potential, efficient dispersal strategies, self-compatibility, and higher phenotypic plasticity (Baker 1965; Rejmanek and Richardson 1996; Pyšek and Richardson 2008). Furthermore, Thébault et al. (2010) compared the rhizosphere microbial activity between native and introduced cytotypes of *Senecio inaequidens* and found that a higher amount of microbial biomass carbon was associated with native and invasive tetraploids as compared to the native diploids. Similarly, in case of *Centaurea stoebe*, the soil bacterial diversity was higher in native tetraploids than native diploids and even higher in introduced tetraploids (Thébault et al. 2010), suggesting that ploidy impacts the microbial communities associated with a plant species which ultimately influences their establishment success in the introduced environment.

In an earlier study, involving native diploid and invasive polyploid *Prosopis* congeners, we established that selective enrichment of plant growth promoting communities from diverse phyla like *Actinomycetota, Acidobacteria, Chloroflexi*, etc., including higher abundance of pathways involved in antimicrobial synthesis and degradation, contributes to the invasion success of exotic polyploid *Prosopis juliflora* over its native diploid *Prosopis cineraria* (Kaushik et al. 2021). Higher abundance of antimicrobial pathways in *P. juliflora* rhizobiome was further corroborated by the presence of relatively higher proportion of

Actinomycetota belonging to the genus *Streptomyces* using culture-based methods.

In this study, we investigated the role of soil microbes in mediating plant invasion by testing the effect of soil bacterial community on growth of native and invasive *Prosopis* congeners. We used microbial exclusion (soil sterilization) and reciprocal inoculation (inoculating rhizosphere bacteria of native species in the soils of invasive species and vice versa) experiments to assay the comparative growth performances of invasive and native *Prosopis* species. We hypothesized that invasive *Prosopis juliflora* benefits more from the mutualistic endemic soil microbes compared to its native congener, *P. cineraria* and that the microbial communities associated with the invasive *P. juliflora* limit the growth of native *P. cineraria.*

Methods

Rhizosphere isolate cultures

Rhizosphere soil samples of *P. juliflora* and *P. cineraria* were collected from Jodhpur, Rajasthan, India, as described in Kaushik et al. (2021). Soil sample (100 mg) was dissolved in 1 ml of double distilled water, serially diluted, and plated on mineral media containing pyruvate, malate, and succinate as the carbon sources (Biebl and Pfennig 1981; Lakshmi et al. 2011). The mineral medium contained: KH_2PO_4 (0.5 g l^{-1}), $MgSO_4{\cdot}7H2O$ (0.2 g l^{-1}), NaCl (0.4 g l^{-1}), NH_4Cl (0.6 g l^{-1}), $CaCl_2{\cdot}2H_2O$ (0.05 g l^{-1}), sodium pyruvate (1.0 g l^{-1}), malic acid (1.0 g l^{-1}), succinic acid (1.0 g l^{-1}), yeast extract (0.3 g l^{-1}), ferric citrate (5 ml l^{-1} from a 0.1% w/v, stock) and trace element solution SL 7 (1 ml l^{-1}). SL 7 contained: HCl (25% v/v; 1 ml), $ZnCl_2$ (70 mg l^{-1}), $MnCl_2{\cdot}4H_2O$ (100 mg l^{-1}), H_3BO_3 (60 mg l^{-1}), $CoCl_2{\cdot}6H_2O$ (200 mg l^{-1}), $CuCl_2{\cdot}H_2O$ (20 mg l^{-1}), $NiCl_2{\cdot}6H_2O$ (20 mg l^{-1}), $NaMoO_4{\cdot}2H_2O$ (40 mg l^{-1}) (Biebl and Pfennig 1981; Lakshmi et al. 2011). Plates were incubated at 30 °C for 3 days after which several bacterial colonies resembling Actinomycetota were sub-cultured and purified as single colonies. The purified colonies were then picked and put in 10 µl of double-distilled autoclaved water in a polymerase chain reaction (PCR) tube and centrifuged at 8000 rpm for 2 min. The dissolved colonies were amplified using colony polymerase chain reaction (PCR) and were sent for 16S sequencing for the identification of bacterial isolates. The sequences thus obtained were identified using EzBioCloud BLAST (Kim et al. 2012) to determine the closest bacterial species. Among the identified rhizobacterial isolates (Table 1), members belonging to the genus *Streptomyces* were the most common. Due to their preponderance in the rhizosphere of *Prosopis* congeners, *Streptomyces* species were chosen for investigating and understanding the effects of specific isolates on the plant growth performance of the alien invasive and native species of *Prosopis*. More specifically, members belonging to the genus *Streptomyces* are known to be efficient colonizers of the rhizosphere which generally act as plant growth promoters due to pathogen suppression and phytohormone production (Olanrewaju and Babalola 2019), but may rarely act as plant growth inhibitors due to antibiotic production against other plant growth promoting microbes (Murao and Hayashi 1983). Notably, the response of *Streptomyces* isolates associated with phylogenetically related native, diploid, and invasive, polyploid plants on their respective growth performances was hitherto unknown. For these garden experiments, we randomly selected one bacterial isolate each from the rhizospheres of invasive *P. juliflora* and native *P. cineraria* and are referred to as PJ1 and PC1, respectively. These isolates from *P. juliflora* and *P. cineraria* were identified as *Streptomyces pratensis* and *S. coelescens*, respectively (Table 1).

Table 1 16S rRNA gene sequence identity of the axenic bacteria isolated from the rhizosphere of *P. juliflora* (PJ) and *P. cineraria* (PC)

Culture id	Phylum	Closest hit taxon	Percentage identity (%)
PJ1	Actinomycetota	*Streptomyces pratensis*	97.6
PJ2		*Streptomyces diastaticus*	98.9
PJ3		*Streptomyces drozdowiczii*	98.3
PJ5		*Isoptericola nanjingensis*	99.6
PJ6		*Brevibacterium frigoritolerans*	99.5
PC1		*Streptomyces coelescens*	99.2
PJ4	Bacillota	*Bacillus muralis*	100
PC2		*Bacillus muralis*	99.6

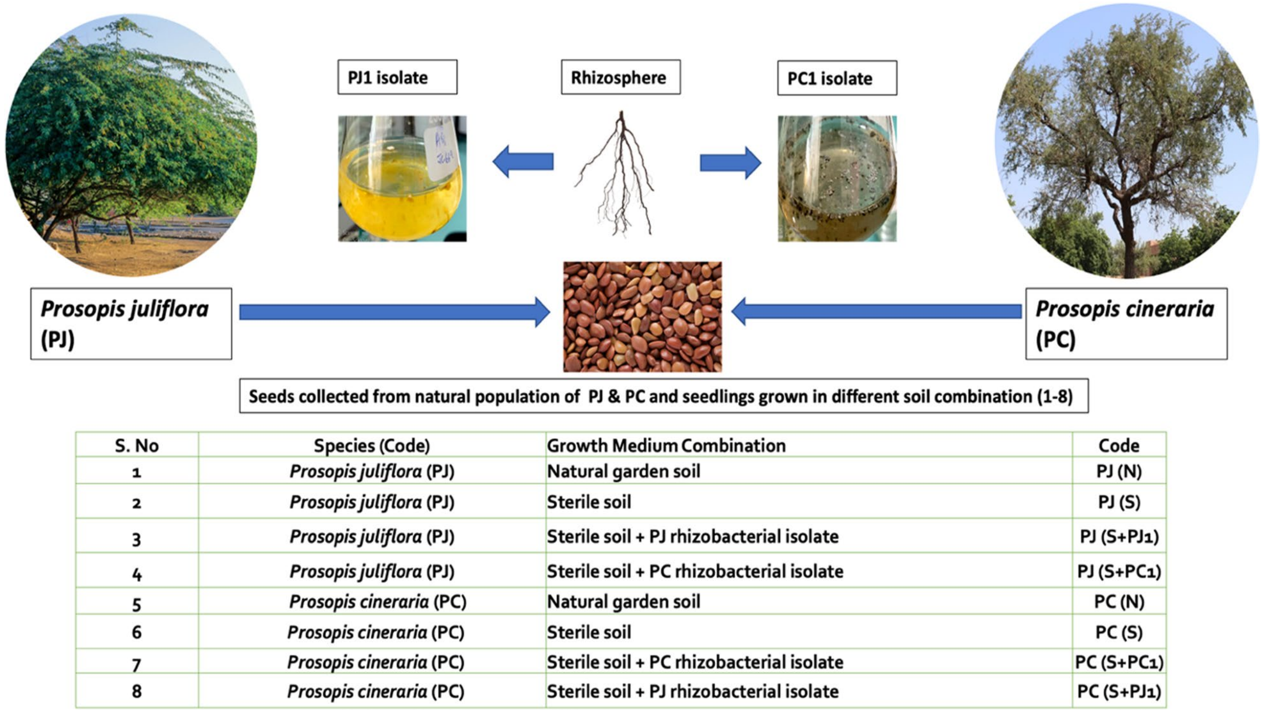
Rhizobacterial isolates PJ1 and PC1 were cultured using modified Biebl and Pfennig medium broth and allowed to grow for a week at 28–30 °C. The antibiotics streptomycin (0.4 g l^{-1}), ampicillin (0.2 g l^{-1}) and cycloheximide (0.025 g l^{-1}) were added to the media to avoid contamination. The cultures were maintained until the optical density of the medium broth was 0.5 @O.D.$_{600}$ (10^7–10^8 CFU/ml). After the optimum growth was achieved, the culture suspension was centrifuged at 6000 rpm for 10 min, washed and recentrifuged in double-distilled autoclaved water to get rid of the media salts. The cultures were washed twice using double-distilled water before being dissolved in 15–20 ml of double-distilled autoclaved water, to prepare the inoculum for garden experiments.

Experimental design

The garden experiments were conducted at the School of Life Sciences, University of Hyderabad, India, between

January 2020 and April 2020. Seeds of *Prosopis juliflora* and *P. cineraria* were collected from the natural populations in Jodhpur, Rajasthan, India. Natural garden soil collected from the University of Hyderabad greenhouse facility was sterilized three times by autoclaving at 121 °C at 15 psi for 1 h for 3 consecutive days. The experiments were conducted in the greenhouse facility at the University of Hyderabad, India, with ambient temperature of 26 ± 2 °C under natural conditions. Before sowing, seeds of *P. juliflora* and *P. cineraria* were surface sterilized using 4% sodium hypochlorite (NaOCl) inside a laminar hood. The seeds were then rinsed with double-distilled autoclaved water and kept on moist filter paper inside the laminar hood until their radicles emerged. Four or more germinated seedlings of each species were transferred to a pre-sterilized pot. Four treatments (experiments) were carried out comprising seedlings of native and invasive *Prosopis* individuals. The following experimental combinations were investigated (Fig. 1): (i) seedlings of *P. juliflora* and *P. cineraria* grown in the natural garden soil; (ii) seedlings of *P. juliflora* and *P. cineraria* grown in the sterile garden soil; (iii) seedlings of *P. juliflora* grown in sterile soil and inoculated with its own rhizobacterial isolate (PJ1); (iv) seedlings of *P. juliflora* grown in sterile soil and inoculated with rhizobacterial isolate from *P. cineraria* (PC1), and (v) seedlings of *P. cineraria* grown in sterile soil inoculated with its own isolate (PC1); (vi) seedlings of *P. cineraria* grown in sterile soil inoculated with rhizobacterial isolate of *P. juliflora* (PJ1). Five or more replicate pots per treatment with four or more seedlings in each were raised and assayed for different plant growth parameters. However, in one or few replicates in some cases (e.g., *P. cineraria* seedlings grown in sterile soil inoculated with PJ1 isolate) though four or more seedlings were transferred but only one was established. Finally, a total of 15 seedlings per treatment were assessed for plant growth performance.

The seedlings from each treatment were grown for a 12-week duration and harvested for measurements related to plant growth performance. Plant growth parameters like root length, shoot length, root biomass (dry weight), shoot biomass (dry weight) and number of thorns were measured for each treatment. For dry weight estimation, the harvested seedlings were dried in an oven maintained at 65 °C for 24 h. All downstream statistical analysis was carried out using GraphPad Prism, wherein normal distribution and the homogeneity of variance were tested using the Shapiro–Wilk and D'Agostino and Pearson normality test. For statistical evaluation of differences between treatments, a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test ($\alpha = 0.05$) was applied to understand the impact of sterilization and microbial inoculation on plant growth performance.



S. No	Species (Code)	Growth Medium Combination	Code
1	*Prosopis juliflora* (PJ)	Natural garden soil	PJ (N)
2	*Prosopis juliflora* (PJ)	Sterile soil	PJ (S)
3	*Prosopis juliflora* (PJ)	Sterile soil + PJ rhizobacterial isolate	PJ (S+PJ1)
4	*Prosopis juliflora* (PJ)	Sterile soil + PC rhizobacterial isolate	PJ (S+PC1)
5	*Prosopis cineraria* (PC)	Natural garden soil	PC (N)
6	*Prosopis cineraria* (PC)	Sterile soil	PC (S)
7	*Prosopis cineraria* (PC)	Sterile soil + PC rhizobacterial isolate	PC (S+PC1)
8	*Prosopis cineraria* (PC)	Sterile soil + PJ rhizobacterial isolate	PC (S+PJ1)

Fig. 1 Experimental design. Rhizosphere bacterial isolates obtained from the native *Prosopis cineraria* (PC1) and invasive *Prosopis juliflora* (PJ1) were used for soil inoculation in different combinations (1–8) as indicated above

Results

Root length and root biomass of *Prosopis juliflora*

The seedlings of *P. juliflora* recorded a mean root length of 14.93 ± 0.45 cm when grown in the garden soil, but exhibited a significant reduction (11.33 ± 0.46 cm) when grown in the sterile soil (Figs. 2, 3a). The *P.*

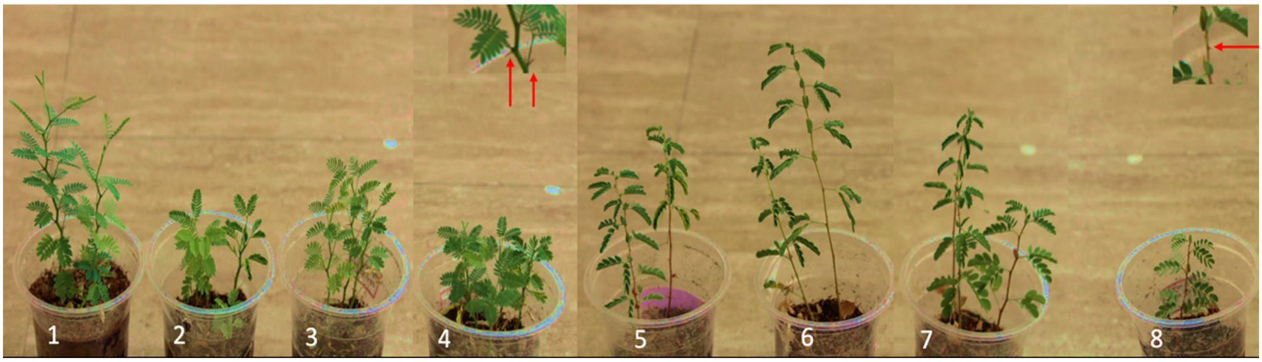


Fig. 2 Effect of soil biota exclusion and inoculation of PC1 and PJ1 isolates on the plant growth performance of *Prosopis juliflora* (PJ) and *Prosopis cineraria* (PC). The numbers (1–8) refer to different growth medium combination, namely PJ in natural garden soil (1); PJ in sterile soil (2); PJ in sterile soil inoculated with PJ1 bacterial isolate (3); PJ in sterile soil inoculated with PC1 bacterial isolate (4); PC in natural garden soil (5); PC in sterile soil (6); PC in sterile soil inoculated with PC1 bacterial isolate (7); PC in sterile soil inoculated with PJ1 bacterial isolate (8). All the seedlings represent 12-week-old seedlings at the end of the experiments and the figures on top right corners indicate the presence of thorns in the *Prosopis* congeners. Thorns in PJ seedlings were more abundant and larger as compared to those of PC seedlings

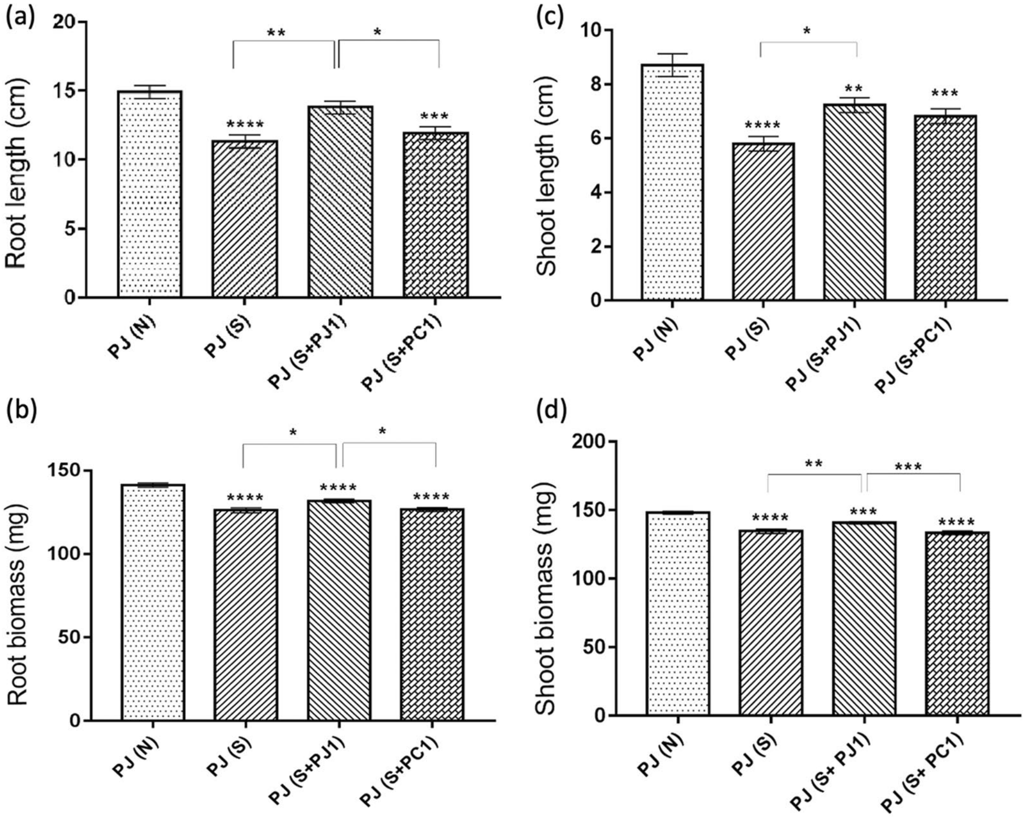


Fig. 3 Effect of soil inoculum on root length (**a**), root biomass (**b**), shoot length (**c**) and shoot biomass (**d**) of *Prosopis juliflora*. PJ: *Prosopis juliflora*; PJ1: *Streptomyces* isolated from *P. juliflora* rhizosphere; PC1: *Streptomyces* isolated from *Prosopis cineraria* rhizosphere; (N), Natural garden soil; (S), Sterilized soil. All statistical analyses were carried out using one-way ANOVA followed by Tukey's post hoc test. $n = 15$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. Error bars represent standard error

juliflora seedlings grown in the soil inoculated with the *Streptomyces* isolate from its own rhizosphere (PJ1) exhibited significantly enhanced mean root length (13.80 ± 0.48 cm) compared to the seedlings grown in the sterile soil (11.33 ± 0.46 cm; Figs. 2, 3a). However, there was no significant change in their mean root length when seedlings were grown in the soil inoculated with the *Streptomyces* rhizosphere isolate from the native *P. cineraria* (PC1) (11.91 ± 0.45; Figs. 2, 3a).

The mean root biomass of *P. juliflora* seedlings was the highest when grown in natural garden soil (141.3 ± 1.2 mg; Figs. 2, 3b). The seedlings exhibited significant reduction in mean root biomass when grown in the sterile soil (126.2 ± 1.5 mg). The seedlings of *P. juliflora* when grown in the soil inoculated with its own rhizosphere isolate (PJ1) showed enhanced mean root biomass (131.8 ± 1.1 mg) compared to the seedlings grown in the sterile soil (126.2 ± 1.5 mg; Figs. 2, 3b). However, no significant difference was observed in the mean root biomass of *P. juliflora* seedlings grown in the sterile soil (126.2 ± 1.5 mg) and those grown in the soil inoculated with rhizosphere isolate of the native *P. cineraria*, PC1 (126.7 ± 1.3 mg; Figs. 2, 3b).

Shoot length, shoot biomass and number of thorns of *Prosopis juliflora*

The mean shoot length of *P. juliflora* seedlings grown in the natural garden soil was significantly higher (8.71 ± 0.42 cm) compared to the seedlings grown in sterile soil (5.80 ± 0.27 cm; Figs. 2, 3c). However, *P. juliflora* seedlings grown in the soil inoculated with rhizobacterial isolate from *P. juliflora* (PJ1) recorded a higher shoot length (7.23 ± 0.26 cm) while the seedlings grown in the soil inoculated with the rhizobacterial isolate from native *P. cineraria* (PC1) recorded a reduced shoot length (6.82 ± 0.26 cm; Figs. 2, 3c). There was a significant increase in the shoot length of the *P. juliflora* seedlings when grown in soil inoculated with its own rhizobacterial isolate, PJ1 (7.23 ± 0.26 cm; Figs. 2, 3c) compared to the seedlings grown in sterile soil (5.80 ± 0.27 cm; Figs. 2, 3c).

The seedlings of *P. juliflora* grown in the natural garden soil yielded significantly higher mean shoot biomass (148.2 ± 1.1 mg) compared to the seedlings grown in the sterile soil (134.7 ± 1.6 mg; Figs. 2, 3d). *P. juliflora* seedlings yielded significantly higher mean shoot biomass when grown in the soil inoculated with its own rhizosphere bacterial isolate, PJ1 (140.9 ± 0.9 mg; Fig. 3d) compared to the mean shoot biomass of *P. juliflora* seedlings grown in the sterile soil (134.7 ± 1.6 mg; Figs. 2, 3d). However, there was no significant change in the mean shoot biomass of *P. juliflora* seedlings when grown in the soil inoculated with the rhizosphere bacterial isolates from native *P. cineraria*, PC1 (133.6 ± 1.3 mg; Figs. 2, 3d) compared to the seedlings grown in the sterile soil (134.7 ± 1.6 mg; Figs. 2, 3d).

Mean number of thorns was the highest in the seedlings of invasive *P. juliflora* grown in natural garden soil (15 ± 0.9) compared to significantly reduced mean number of thorns in *P. juliflora* seedlings grown in the sterile soil (11 ± 0.5; Figs. 2, 4). However, there was no significant difference in the mean number of thorns in the invasive *P. juliflora* seedlings when grown either in the sterilized soil (11 ± 0.5) or when grown in the soil inoculated with its own rhizosphere bacterial isolate PJ1 (11 ± 0.8) or when grown in the soil inoculated

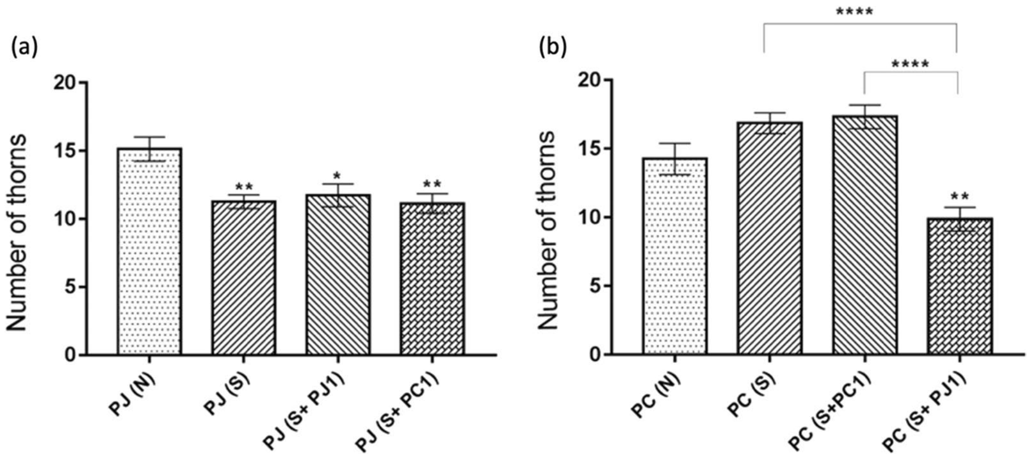


Fig. 4 The effect of soil inoculum on number of thorns in *Prosopis juliflora* seedlings (**a**) and *Prosopis cineraria* seedlings (**b**). PJ: *Prosopis juliflora*; PC: *Prosopis* cineraria; PJ1: *Streptomyces* isolated from *P. juliflora* rhizosphere; PC1: *Streptomyces* isolated from *Prosopis cineraria* rhizosphere; (N), natural garden soil; (S), sterilized soil. All statistical analyses were carried out using one-way ANOVA followed by Tukey's post hoc test. *n* = 15, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Error bars represent standard error

with rhizosphere bacterial isolates of *P. cineraria* PC1 (11 ± 0.7; Figs. 2, 4).

Root length and root biomass of native *Prosopis cineraria*

Seedlings of the native *P. cineraria* when grown in natural garden soil exhibited significantly lower mean root length (11.93 ± 0.35 cm) compared to the seedlings grown in the sterile soil (14.83 ± 0.62 cm; Figs. 2, 5a), but a marginal increase in root length was observed in the seedlings when grown in the soil inoculated with its own *Streptomyces* rhizosphere isolate, PC1 (14.95 ± 0.87 cm; Figs. 2, 5a). However, the root length of native *P. cineraria* seedlings grown in the sterile soil inoculated with the rhizobacterial isolate of invasive *P. juliflora*, PJ1 was significantly lower (9.68 ± 0.30 cm) compared to the seedlings grown in the natural garden soil (11.93 ± 0.35 cm), sterile soil (14.83 ± 0.62 cm) or the sterile soil inoculated with *Streptomyces* isolate, PC1 from native *P. cineraria* (14.95 ± 0.87 cm) (Figs. 2, 5a).

The mean root biomass of *Prosopis cineraria* seedlings grown in the garden soil (136.4 ± 1.0 mg, Fig. 5b) was significantly lower than those of its seedlings grown in sterile soil (141.5 ± 1.5 mg; Figs. 2, 5b) and a marginal increase was observed in the root biomass of *P. cineraria* seedlings grown in the soil inoculated with PC1 isolate (137.4 ± 1.2 mg; Figs. 2, 5b) compared to those grown in garden soil. The mean root biomass of native *P. cineraria* seedlings grown in sterile soil inoculated with rhizobacterial isolates of invasive *P. juliflora* was significantly lower (124.4 ± 1.2 mg; Figs. 2, 5b) compared to its seedlings grown in natural garden soil (136.4 ± 1.0 mg; Figs. 2, 5b), sterile soil (141.5 ± 1.5 mg; Figs. 2, 5b) or sterile soil inoculated with its own rhizobacterial isolate PC1 (137.4 ± 1.2 mg; Figs. 2, 5b).

Shoot length, shoot biomass and number of thorns of native *Prosopis cineraria*

The seedlings of native *P. cineraria* recorded a mean shoot length of 7.11 ± 0.22 cm when grown in the garden soil and there was a significant increase in their shoot length when these seedlings were grown in the sterilized soil (8.90 ± 0.47 cm; Figs. 2, 5c). However, a marginal increase was observed in the shoot length of seedlings grown in sterile soil inoculated with its own rhizobacterial isolate

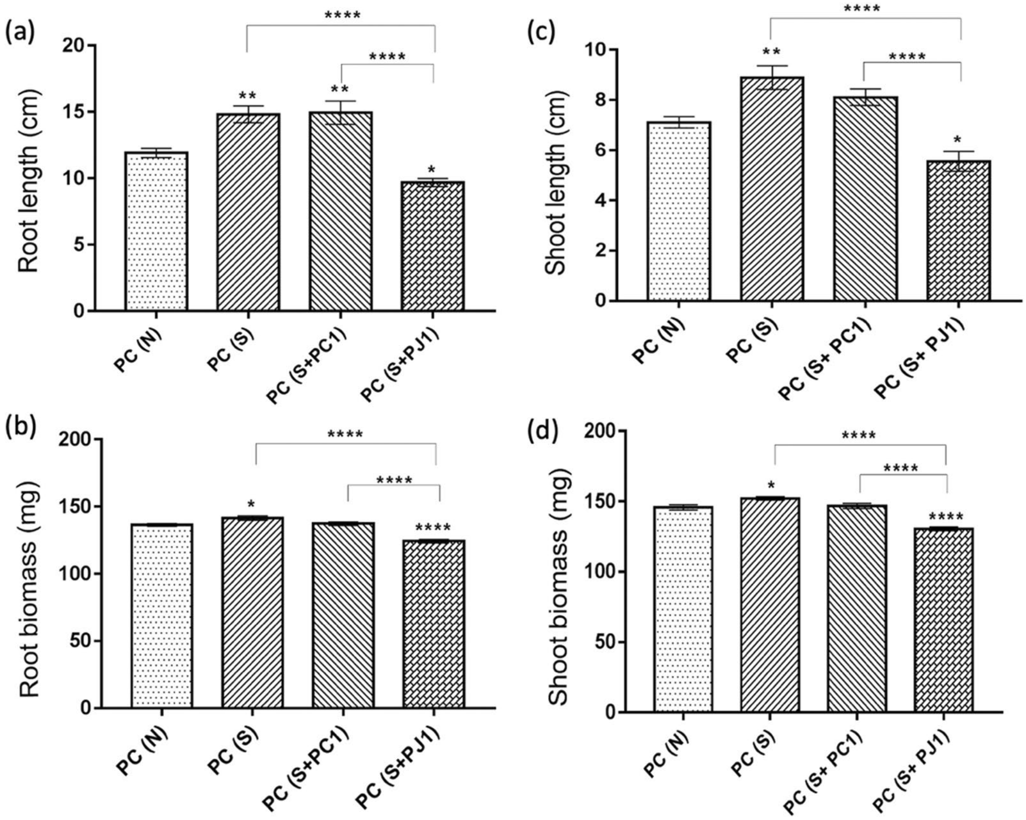


Fig. 5 Effect of soil inoculum on root length (**a**), root biomass (**b**), shoot length (**c**) and shoot biomass (**d**) of *Prosopis cineraria*. PC: *Prosopis cineraria*; PJ1: *Streptomyces* isolated from *Prosopis juliflora* rhizosphere; PC1: *Streptomyces* isolated from *P. cineraria* rhizosphere; (N), natural garden soil; (S), sterilized soil. All statistical analyses were carried out using one-way ANOVA followed by Tukey's post hoc test. $n = 15$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Error bars represent standard error

PC1 (8.10 ± 0.33; Figs. 2, 5c) compared to that of the seedlings grown in sterile soil (8.90 ± 0.47 cm). The mean shoot length of *P. cineraria* seedlings grown in sterile soil inoculated with rhizobacterial isolates of *P. juliflora* PJ1 was significantly lower (5.56 ± 0.40 cm) compared to the shoot length of the seedlings grown in garden soil (7.11 ± 0.22 cm), sterilized soil (8.90 ± 0.47 cm) or sterile soil inoculated with its own rhizobacterial isolate PC1 (8.10 ± 0.33 cm) (Figs. 2, 5c).

The mean shoot biomass of the *P. cineraria* seedlings grown in natural garden soil was significantly lower (145.8 ± 1.7 mg) than the seedlings grown in sterile soil (152.2 ± 1.2 mg; Figs. 2, 5d). However, there was no significant difference in the mean shoot biomass of the native *P. cineraria* seedlings grown in the sterile soil (152.2 ± 1.2 mg) and those grown in the soil inoculated with its own rhizobacterial isolate PC1 (146.8 ± 1.7 mg; Figs. 2, 5d). The mean shoot biomass of *P. cineraria* seedlings grown in the sterile soil inoculated with rhizosphere isolate of *P. juliflora*, PJ1 was significantly lower (130.6 ± 1.3 mg) compared to the seedlings grown in the garden soil (145.8 ± 1.7 mg), sterile soil (152.2 ± 1.2 mg) or sterile soil inoculated with PC1 isolate from native *P. cineraria* (146.8 ± 1.7 mg) (Figs. 2, 5d).

There was no significant difference in the mean number of thorns between the seedlings of *P. cineraria* grown either in sterilized soil (16 ± 0.7; Figs. 2, 4) or the soil inoculated with its own rhizosphere bacterial isolate PC1 (17 ± 0.9; Figs. 2, 4). However, the least number of thorns was observed in the *P. cineraria* seedlings grown in the soil inoculated with rhizosphere bacterial isolate of *P. juliflora* PJ1 (9 ± 0.8; Figs. 2, 4).

Discussion

Invasive plants are known to alter soil communities to gain competitive advantage over native species (Si et al. 2013). In this study, we tested the hypothesis that invasive *Prosopis juliflora*, a globally widespread tropical invasive, may benefit more from the associated mutualistic soil microbial community in its invaded range compared to its native congener, *P. cineraria*. In an earlier study, we had shown that *P. juliflora* recruits a unique set of rhizosphere bacterial communities that proffer it an adaptive advantage mediated by various metabolic pathways (Kaushik et al. 2021). In this study, we further demonstrate the competitive advantage of exotic *P. juliflora* over native *P. cineraria* by providing empirical evidence of the differential impacts of soil bacterial isolates on growth performance of exotic and native *Prosopis* congeners. Our results show that exclusion of soil microbes by soil sterilization produces contrasting effects on the two congeners, evaluated in terms of growth parameters such as root length and biomass, shoot length and biomass, and number of thorns (Figs. 2, 3, 4, 5).

Soil microbial exclusion reduced growth performance of invasive *P. juliflora* as indicated by reduced above and below-ground growth parameters (Figs. 2 and 3), suggesting positive feedback from the endemic soil communities on the growth performance of the exotic species. In contrast, exclusion of the soil biota resulted in enhanced growth performance (Figs. 2 and 5) of the native congener, indicating negative feedback from the native soil biota on growth parameters of the native *P. cineraria*. These results are consistent with earlier studies involving invasive cogongrass *Imperata cylindrica* and native *Pinus palustris* and *Aristida stricta*, in which the invader species were reported to show higher biomass in live soil than sterile soil, while the native species exhibited significantly enhanced biomass upon soil sterilization (Fahey and Florey 2022). Our results, therefore, provide further conclusive evidence of positive soil feedback in case of the exotic invasive species and negative feedback in case of the native plant species.

Our study also points out that the number of thorns in alien *P. juliflora* seedlings reduced significantly (Fig. 4) when grown in sterilized soil, indicating the role and importance of soil microbes in development of thorns, which comprise above-ground defense mechanism such as against herbivory in the invasive *P. juliflora*. However, there was no effect of soil sterilization on the number of thorns in case of native *P. cineraria*. Our results signify the importance of complete soil microbiome in mediating plant defense especially in case of the alien invasive plants such as *P. juliflora* further corroborating the role of soil microbes as mediators of plant defensive phenotype and above-ground interactions with herbivores (Schädler and Ballhorn 2016).

These studies also clearly demonstrate that the presence of native soil biota facilitated the growth performance of alien invasive *P. juliflora* in its non-native range, but negatively impacted the growth of native *P. cineraria*. These results point out that the native species tend to be restricted by the endemic soil microbes which have co-evolved alongside the host flora during evolutionary time (Lankau 2012). Conversely, the absence of restrictive microbial community in alien *P. juliflora* and facilitation of its growth performance provides further evidence to the enemy release hypothesis, which posits that the reduced loads of the natural enemies like pathogens, predators, or parasites in the non-native ranges aid plant invasion (Keane and Crawley 2002). Notably, the absence of inhibitory soil microbes in the non-native range results in an overall positive soil feedback for exotic alien plant species, while the presence of natural pathogens specific to the native plants co-evolved during evolutionary history results in a negative soil feedbacks for the native plants. These results further substantiate our earlier observations on the rhizosphere microbiome of the two congeners in which we showed that the alien invasive *P. juliflora* exhibited selective enrichment of growth-promoting phyla like *Actinomycetota, Chloroflexi, Firmicutes* and *Acidobacteria* and a lower abundance of phytopathogenic genera like *Serratia* compared to its native congener, *P. cineraria* (Kaushik et al. 2021).

The soil inoculation experiments involving seedlings of *P. juliflora* and *P. cineraria* and their respective *Streptomyces* isolates, PJ1 and PC1, respectively, also yielded contrasting results. The root length of *P. juliflora* seedlings grown in sterile soil was significantly lower compared to those grown in soils inoculated with PJ1 isolate, while there was no change in root length when seedlings were grown in the soil inoculated with PC1 isolate (Fig. 3). This result suggests that in absence of any soil microbes, invasive *P. juliflora* showed decreased growth performance than in the presence of its rhizobacterial isolate PJ1. However, in case of native *Prosopis cineraria*, only a marginal increase in root length was observed upon inoculation with its own PC1 isolate compared to the seedlings grown in sterile soil (Fig. 5). However, there was a significant decline in root length of the *P. cineraria* seedlings when grown in soils inoculated with PJ1 isolate (Fig. 5). This result indicates restrictive influence of PJ1 microbial isolate from *P. juliflora* on the growth performance of *P. cineraria*. In other words, the rhizobacteria recruited by *P. juliflora* are not only different from those of native *P. cineraria* but that the microbial community of *P. juliflora* negatively affects growth of native *P. cineraria*, thereby hampering its spread and growth performance.

Our results also show that there was a significant increase in root biomass of *P. juliflora* when its seedlings were grown in soil inoculated with its bacterial isolate PJ1, while no change was observed when its seedlings were grown in soil inoculated with PC1 bacterial isolate (Fig. 3). In contrast, only an insignificant increase in root biomass of native *P. cineraria* was observed when its seedlings were grown in soils inoculated with its own bacterial isolate PC1 (Fig. 5) compared to the seedlings grown in natural garden soil, while a significant decline in root biomass was observed when the native *P. cineraria* seedlings were grown in soils inoculated with PJ1 isolate of *P. juliflora* (Fig. 5). These results clearly point out to uniqueness and differential soil microbiome recruitment strategy of exotic invasive *P. juliflora* which offers it an adaptive advantage over *P. cineraria*. As such, our results are consistent with results of many earlier studies pointing to the role of invasive species in changing native soil microbial community in their non-native ranges to gain invasion success (Batten et al. 2006; Kourtev et al. 2002; Kaushik et al. 2021).

Our results on the comparative growth performance measured in terms of shoot length showed that *P. juliflora* seedlings exhibited a significant increase when grown in soils inoculated with its own rhizobacterial isolate PJ1 compared to when grown in sterile soil (Figs. 2 and 3). However, the seedlings of exotic *P. juliflora* exhibited no significant change in growth performance when grown in soils inoculated with rhizobacterial isolate of *P. cineraria* (PC1). The native *P. cineraria* seedlings exhibited a significant decline in their mean shoot length when the seedlings were grown in soils inoculated with PJ1 isolate, but only a marginal increase was observed when grown in soils with PC1 isolate (Fig. 5). Similarly, we found an increase in shoot biomass of *P. juliflora* seedlings when grown in soil inoculated with its own rhizobacterial isolate (PJ1) while no change was observed when its seedlings were grown in the soils inoculated with *P. cineraria* (PC1) isolate (Figs. 2 and 3). Likewise, for shoot biomass of the native *P. cineraria* seedlings, an insignificant increase was observed when its seedlings were grown in soils inoculated with PC1 isolate compared to the seedlings grown in natural garden soil, but a significant decline was observed in the seedlings when grown in soils inoculated with PJ1 isolate (Figs. 2 and 5). These results signify that exotic invasive *P. juliflora* experienced: (i) reduced growth performance in absence of soil microbes; (ii) better growth performance in presence of its unique soil bacterial community which it recruits differentially and does not share with its native congener, *P. cineraria*; and (iii) lack of increase in growth performance in the presence of non-specific soil microbes unique to its native congener *P. cineraria*. Similarly, the native *P. cineraria* experienced: (i) enhanced growth performance in absence of native soil microbes; (ii) enhanced growth performance in presence of its unique soil bacterial community which it does not share with invasive congener, *P. juliflora*; and (iii) reduced growth performance in the presence of non-specific soil microbes unique to its invasive congener *P. juliflora*. Such results corroborate our earlier findings of differential recruitment of soil microbial communities by native and invasive *Prosopis* congeners (Kaushik et al. 2021) and further our understanding of the divergent legacy effects of microbial species even from the same genus on the respective growth performance of alien invasive and native congeners, which was hitherto unknown.

Conclusion

Our research shows that native *P. cineraria* receives negative feedback from its soil microbial population, but invasive *P. juliflora* receives positive input from the non-native range soil microbial community. Our findings advance our knowledge of the divergent growth-promoting effects of differently recruited microbial species belonging to the same genus (*Streptomyces*) in the rhizospheres of foreign invasive and native plants, and they offer new experimental support for the enemy release theory. Based on these results, we conclude that soil microbes may indirectly affect competition among native and invasive plants through differential impacts on plant growth performance parameters which influence direct competitive interactions. These findings raise new questions about the possible genomic attributes of bacterial genera like *Streptomyces* associated with the invasive plants which could potentially have negative impacts on the growth of native plants. Further experiments are required to decipher the factors which determine the growth-promoting or inhibiting nature of microbial community associated with native and invasive plants.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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