

RESEARCH ARTICLE

Extreme environments as sources of fungal endophytes mitigating climate change impacts on crops in Mediterranean-type ecosystems

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Societal Impact Statement

Climate change is predicted to increase drought and soil salinity in Mediterranean-type ecosystems (MTEs), posing a significant threat to global food security. Genetic modification of crops to counteract this threat is expensive and has not met with universal support, and alternatives are hence needed to enhance crop production in MTEs. Here, fungal endophytes from the Atacama Desert, High Andes and Antarctica inoculated onto three crops were found to alleviate the negative effects of drought and salinity on plant performance. The study concludes that extremophile endophytes might be used to enhance crop performance as the climate of MTEs changes over future decades.

Summary

- Climate change will curtail the ability to provide sufficient food for our rapidly expanding population. Improvements to crop production in changing environments, particularly Mediterranean-type ecosystems (MTEs), which are increasingly subjected to drought and salinisation, are hence urgently needed. Here, we explored the possibility that fungal endophytes from extreme environments can be used to enhance crop yield, survival and tolerance to environmental stresses.
- Plants of lettuce, tomato and bell pepper were inoculated with up to six species of endophytic fungi isolated from the Atacama Desert, the High Andes and Antarctica. They were then exposed in the field for up to 120 days in each of three summers to current climatic conditions or to a future climate scenario simulating increased drought and soil salinisation.
- Compared with uninoculated plants, the yield and survival of inoculated crops were increased by up to two-fold under the future climate scenario. These effects were in part attributable to the improved water balance of inoculated crops exposed to drought and salinisation. The inocula also increased the concentrations of total phenols and proline in leaves and decreased lipid peroxidation when plants

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were subjected to increased aridity and salinity. A mixed inoculum of six endophytes from the extreme environments conferred the most beneficial effects on crop performance, with a commercially available inoculum having fewer positive effects on crops.

- We conclude that the inoculation of crops with endophytes from extreme environments may be a viable solution to sustaining crop production in MTEs exposed to rapid climate change.

KEYWORDS

climate change, crop production, drought, extremophiles, fungal endophytes, global food security, Mediterranean-type ecosystems, salinity

1 | INTRODUCTION

Food demand is currently rising owing to sustained human population growth (Tilman et al., 2011), with the world's population being predicted to reach 9.7 billion by 2050 (United Nations, 2019). By then, global crop production will need to have risen by at least 50% compared with current output in order to meet the increasing demand (van Dijk et al., 2021). However, climate change is likely to exacerbate food security problems by reducing crop yield, survival and quality (Hochman et al., 2017). Crop production in Mediterranean-type ecosystems (MTEs), which experience hot and dry summers and supply much of the world's food (Malek & Verburg, 2018; del Pozo et al., 2019), is under particular threat. Over the last century, climate change in MTEs has caused air temperatures to rise and rainfall to decline (Garreaud et al., 2017), with climate models forecasting further warming and reduced precipitation in MTEs by the end of the 21st century (Williams, 2017). Importantly for MTEs, warming and drought, coupled with high evapotranspiration during spring and summer, strongly reduce water availability and lead to the salinisation of soils (Queiros et al., 2022; Zeng et al., 2021).

It is hence important to boost the yields of crops grown in MTEs and their resilience to climate change in order to meet future food demand (Rashid et al., 2021). Yet, most crops are grown in high-yield monocultures under suboptimal conditions that are altering more quickly than the ability of plants to adapt to change, likely owing to the narrowed genetic variability of modern crops (Bailey-Serres et al., 2019). Although genetically modified (GM) crops can mitigate the negative effects of climate change while maintaining productivity (Gamalero & Glick, 2022), their use is a long-term, cost-intensive strategy that has produced limited stress tolerance benefits (Arif et al., 2020; Chourasia et al., 2022). Moreover, despite clear benefits to farmers and end-users, GM crops have not been widely adopted because of opposition from both producers and consumers (Turnbull et al., 2021). Acceptance of GM crops has also been stifled by debate arising from concerns over their potential adverse effects on biodiversity, food safety and allergenicity (Evanega et al., 2022; Talas-Oğraş, 2011). These factors have led to the establishment of regulatory barriers, moratoriums and prohibitive policies towards the

cultivation of GM crops for human consumption (Gamalero & Glick, 2022; Turnbull et al., 2021).

Alternatives to GM crops are hence needed to rapidly enhance crop production and resilience to environmental stresses. One such alternative is to use beneficial micro-organisms, such as fungal endophytes, to improve crop performance (Acuña-Rodríguez et al., 2022; Bailey-Serres et al., 2019). Endophytes colonise almost all plants in natural terrestrial ecosystems, with their benefits varying between the environments from which they originate (Garnica et al., 2022; Rodríguez & Redman, 2008). For example, those from coastal and geothermal settings confer tolerance to salt and heat, respectively, a phenomenon termed 'habitat-adapted symbiosis' (*sensu*; Rodríguez et al., 2008), which is thought to arise from the activation of distinct metabolic pathways under specific selective pressures (Ballesteros et al., 2020; Rodríguez et al., 2008). Crop performance in MTEs might therefore be enhanced by endophytes from arid and saline soils, such as those in desert, montane and Antarctic environments (Bertini et al., 2022; Poveda et al., 2022). Although these underexplored environments are largely untapped because of their geographic isolation and inhospitable conditions, they are considered to be rich sources of endophytes (Saikkonen et al., 2004) that might synthesise a plethora of natural products capable of enhancing host stress tolerance (Tiwari & Bae, 2022).

Plants growing in extreme environments may thus harbour novel endophytes that can confer beneficial effects on crops in a sustainable and environmentally friendly manner (García-Latorre et al., 2021; Verma et al., 2021). However, the majority of previous studies that have examined the effects of endophytes on crops have inoculated single endophytes, rather than multiple strains, onto plants (Poveda et al., 2022). Thus, there is scant evidence that inoculation with multiple endophytes improves crop production. Therefore, the main objective of the present study was to investigate how inoculation with up to six extremophile fungal endophytes affects the performance of three crops grown in the field under current climatic conditions and under increased aridity and salinity, simulating a future climate change scenario for MTEs. This allowed us to test the hypothesis that combinations of endophytes mitigate the negative effects of aridity and salinity on crops.

2 | METHODS

2.1 | Fungal endophytes

The endophytes selected for study were *Penicillium fuscoglaucum* and *Penicillium glabrum* from *Distichlis spicata* in the Atacama Desert, *Penicillium thomii* and *Penicillium crustosum* from *Hordeum comosum* in the High Andes and *Penicillium rubens* and *Penicillium bialowiezense* from *Deschampsia antarctica* in maritime Antarctica (Figure 1). The endophytes, which had been isolated from surface-sterilised root fragments of each plant species, were identified through sequencing of the partial internal transcribed spacer region and large subunit genes (Torres-Díaz et al., 2021). Previous studies have shown that each of the six isolates colonises the roots of lettuce (*Lactuca sativa*), tomato (*Solanum lycopersicum*) and bell pepper (*Capsicum annuum*) grown in monoculture (Acuña-Rodríguez et al., 2019; Molina-Montenegro et al., 2016, 2020), with hyphae growing on root surfaces and apoplastically between epidermal cells (Acuña-Rodríguez et al., 2020).

2.2 | Field experiments

Six hundred seedlings each of lettuce, tomato and bell pepper were obtained from seeds germinated in a glasshouse under

semi-controlled temperature and light conditions ($22 \pm 5^\circ\text{C}$ and $760 \pm 96 \mu\text{mol m}^{-2} \text{s}^{-1}$, with a 14/10 h day/night photoperiod). Four hundred seedlings of each species, grown in sterilised soil in transparent plastic containers, were randomly selected and inoculated when they had grown at least four expanded leaves and 3-cm-long roots. Inocula of each *Penicillium* isolate, obtained from single conidia cultured on potato dextrose agar (PDA) medium with chloramphenicol ($100 \mu\text{g mL}^{-1}$), were incubated in the glasshouse for 2 weeks. Conidia were then harvested from the medium by adding sterile water to its surface, followed by gentle agitation with a sterile spreader. Viability, as determined by the method of Greenfield et al. (2016), was found to be >95%. Four inocula were then prepared. Three consisted of dual inocula of the two *Penicillium* species from each extreme environment (i.e., the Atacama Desert, the High Andes and Antarctica) in 1:1 proportions, while the fourth consisted of a mixed inoculum of all six species. Each inoculum, consisting of approximately 2×10^7 conidia in 2 mL of sterile water, was applied directly to the rhizospheres of 100 seedlings of each crop species. This procedure was repeated twice, at 0 and 15 days, to ensure the establishment of endophytes. At 30 days after inoculation, the roots of two plants per treatment were sampled, cleared in 10% KOH (w/v) and stained with trypan blue (0.4%) in an acid glycerol solution. Colonisation of roots by isolates was verified by light microscopy at 400 \times magnification (Motic BA310). Plant response to a commercial product was also assessed by

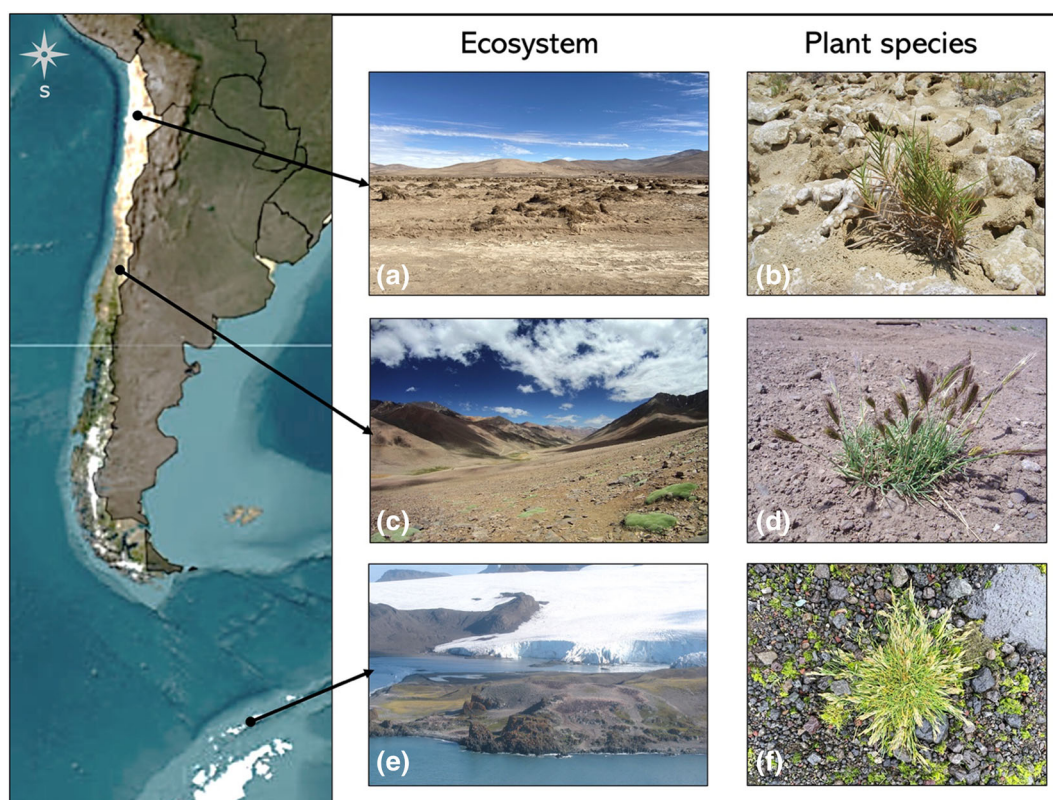


FIGURE 1 Extreme environments and plant species from which fungal endophytes were isolated. (a) The Atacama Desert (photo: M.A. Molina-Montenegro) and (b) *Distichlis spicata* (photo: Royal Botanic Gardens, Kew), (c) the High Andes of central Chile (photo: I.S. Acuña-Rodríguez) and (d) *Hordeum comosum* (photo: M. Mihoc) and (e) Maritime Antarctica (photo: M.A. Molina-Montenegro) and (f) *Deschampsia antarctica* (photo: F. Carrasco-Urra).

inoculating 100 seedlings of each crop species with Sáfer Mycorrizas M.A. (Agrobiological Sáfer, Medellín, Colombia), a mixture of the arbuscular mycorrhizal fungi (AMF) *Glomus*, *Scutellospora*, *Acaulospora* and *Entrophospora* spp., which has been shown to improve crop growth and stress tolerance (Torres-Díaz et al., 2021). Finally, 100 control plants of each crop species were mock inoculated with approximately 2 mL of distilled, filtered (0.45 µm) water.

The 1800 seedlings were planted during the austral summer into field plots consisting of ploughed and tilled soil at Quinamavida, Región del Maule, Central Chile (35°47'S, 71°26'W). They were randomly planted in rows 0.5 m apart, with 0.2 m between individuals. Rows were randomly assigned to either a current climate control (131 mg NaCl dissolved in 45 mL of distilled water, equivalent to a 50 mM solution, applied daily to each plant) or a future climate treatment consisting of an aridity-salinity treatment (219 mg NaCl dissolved in 25 mL of distilled water, equivalent to a 150 mM solution, applied daily to each plant) simulating the effects of a climate change scenario on the Mediterranean Central Chile zone (Molina-Montenegro et al., 2020). The control represented 100% of the water normally added to reach marketable size in the crops, while the future climate treatment imposed a 45% water deficit (Molina-Montenegro et al., 2020). Fertiliser (0.2 g L⁻¹ of Phostrogen, Solaris, NPK, 14:10:27) was applied every 30 days. Experiments lasted for 60 days (*C. annuum*), 90 days (*L. sativa*) or 120 days (*S. lycopersicum*) and were repeated in three consecutive austral summers. Summertime rainfall in Central Chile is sparse (typically one low-intensity event per month; <https://www.cr2.cl>), and carry-over of NaCl between treated and control rows is hence likely to have been negligible. In a similar study applying the same future climate treatment, concentrations of NaCl measured weekly in soil did not alter during the course of a 3-month experiment ($F_{1,48} = 1.09$; $p = .13$) (Molina-Montenegro et al., 2020).

2.2.1 | Crop survival and yield

Survival was recorded weekly. Yield was measured at the end of each experiment after oven-drying the aboveground biomass of lettuce and the fruits of tomato and bell pepper at 62°C for 96 h (Molina-Montenegro et al., 2020).

2.2.2 | Biochemical mechanisms of environmental tolerance

Leaf phenol and proline concentrations and lipid peroxidation were measured in three fully expanded leaves from 25 randomly selected plants in each experimental group at the end of each experiment in 2021. Total leaf phenolic compound concentrations were determined spectrophotometrically using the Folin-Ciocalteu method and expressed as µmol phenols g⁻¹ fresh weight, as described by Ballesteros et al. (2022). Phenolic compounds in plant tissues provide antioxidant capacity by scavenging free radicals and reactive oxygen

species (ROS) associated with abiotic stresses and are thus an important indicator of plant health (Shi et al., 2022). Foliar proline concentration, which was used as a biochemical indicator of stress tolerance, was measured using a spectrophotometric procedure (Molina-Montenegro et al., 2016) and expressed as µmol proline g⁻¹ fresh weight tissue. Lipid oxidative degradation was estimated using the thiobarbituric acid reaction method, which measures the concentration of cellular malondialdehyde (MDA), an index of general lipid peroxidation and a proxy for cell damage arising from ROS. TBA reactive substances (TBARS) were measured spectrophotometrically, and lipoperoxidation was expressed as mmol TBARS g⁻¹ fresh weight, as described by Acuña-Rodríguez et al. (2022). Leaf carbon isotope discrimination, a proxy for water use efficiency, was measured in three expanded leaves from 10 randomly selected plants in each experimental group at the end of the experiments in 2021. Leaf δ¹³C (‰), determined by isotope ratio mass spectrometry at the Laboratory of Biogeochemistry and Applied Stable Isotopes at the Pontificia Universidad Católica de Chile, was calculated following Farquhar et al. (1989) using Vienna Pee Dee Belemnite calcium carbonate as the standard (Iso-Analytical, Crewe, UK).

2.3 | Statistical analyses

All analyses were performed using R v.4.2.2 (R-CoreTeam, 2022). Two-way ANOVA was used to test for the main and interaction effects of inocula and climate on the survival, yield, foliar phenol and proline concentrations, lipid peroxidation and δ¹³C signature of each crop species. Analyses were conducted on 3-year survival and yield averages and, for other responses, on data measured at the end of each experiment. Tukey's honestly significant difference (HSD) *a posteriori* tests were used to determine between-group differences. Assumptions for parametric statistical testing were verified for each response dataset. To summarise the effects of inocula under the current climate control and the future climate treatment, we calculated the effect sizes (Cohen's d, with Hedge's correction) of each inoculum relative to uninoculated controls for each response, and also combined data to estimate pooled effect sizes. Effect size analyses were conducted with the 'cohen.d' and 'rma' functions in the packages *effect-sizes* and *metafor* (Viechtbauer, 2010).

3 | RESULTS

3.1 | Crop survival and yield

Inocula and climate both had highly significant main effects on the survival and yield of each crop species (Table 1). However, highly significant inocula × climate interactions were recorded, indicating that responses to the inocula depended on aridity and salinity (Table 1). Broadly, the inocula had only moderate effects on plant survival under current climatic conditions (Figure 2). Compared with uninoculated plants exposed to the current climate control, no

TABLE 1 Results from two-way ANOVA models showing the main and interaction effects of inocula (mix of fungal endophytes) and the future climate treatment (simulating aridity and salinity in Mediterranean-type ecosystems) on the survival, yield and foliar $\delta^{13}\text{C}$ signature of three crop species. Survival and yield were averaged over three experiments. Significant probability values ($p < .05$) are shown in red.

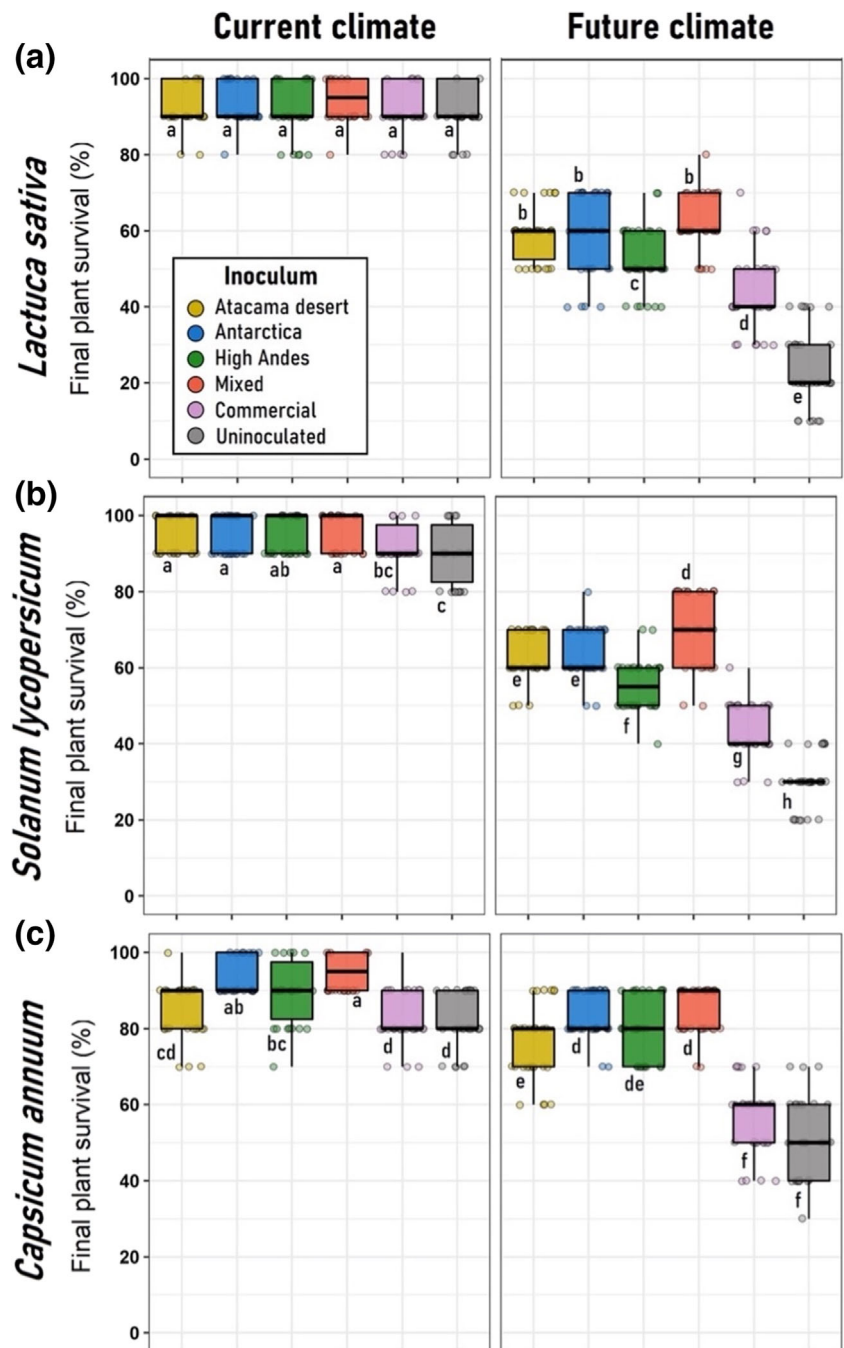
Response	Species	Factor	d.f.	SS	MS	F	p
Survival	<i>Lactuca sativa</i>	Inocula	5	5771.85	1154.37	77.38	<.0001
		Climate	1	56,044.81	56,044.81	3756.94	<.0001
		Inocula \times climate	5	4459.63	891.93	59.79	<.0001
		Residuals	108	1611.11	14.92		
	<i>Solanum lycopersicum</i>	Inocula	5	7622.96	1524.59	74.84	<.0001
		Climate	1	46,413.33	46,413.33	2278.47	<.0001
		Inocula \times climate	5	4034.44	806.89	39.61	<.0001
		Residuals	108	2200	20.37		
	<i>Capsicum annuum</i>	Inocula	5	10,002.96	2000.59	124.49	<.0001
		Climate	1	8112.59	8112.59	504.83	<.0001
		Inocula \times climate	5	2369.63	473.93	29.49	<.0001
		Residuals	108	1735.56	16.07		
Yield	<i>L. sativa</i>	Inocula	5	66,812.27	13,362.45	288.51	<.0001
		Climate	1	92,796.41	92,796.41	2003.59	<.0001
		Inocula \times climate	5	28,986.35	5797.27	125.17	<.0001
		Residuals	108	5002.03	46.32		
	<i>S. lycopersicum</i>	Inocula	5	3955.84	791.17	33.86	<.0001
		Climate	1	290,345.61	290,345.61	12,425.63	<.0001
		Inocula \times climate	5	1903.09	380.62	16.29	<.0001
		Residuals	108	2523.6	23.37		
	<i>C. annuum</i>	Inocula	5	5546.51	1109.3	66.23	<.0001
		Climate	1	8579.57	8579.57	512.24	<.0001
		Inocula \times climate	5	416.77	83.35	4.98	.0003
		Residuals	108	1808.91	16.75		
Foliar $\delta^{13}\text{C}$ signature	<i>L. sativa</i>	Inocula	5	70.71	14.14	69.32	<.0001
		Climate	1	102.97	102.97	504.74	<.0001
		Inocula \times climate	5	27.55	5.51	27.01	<.0001
		Residuals	48	9.79	0.2		
	<i>S. lycopersicum</i>	Inocula	5	43.57	8.71	48.15	<.0001
		Climate	1	159.09	159.09	878.94	<.0001
		Inocula \times climate	5	9.82	1.96	10.85	<.0001
		Residuals	48	8.69	0.18		
	<i>C. annuum</i>	Inocula	5	36.17	7.23	482.9	<.0001
		Climate	1	82.46	82.46	5504.5	<.0001
		Inocula \times climate	5	14.04	2.81	187.46	<.0001
		Residuals	48	0.72	0.01		

Abbreviations: d.f., degrees of freedom; F, F-statistic; MS, mean square; p, probability values; SS, sum of squares.

effects of inocula were recorded on the survival of lettuce (Figure 2a). However, with the exception of the dual inoculum from the Atacama Desert applied to bell pepper, all inocula from extreme environments enhanced the survival of tomato and bell pepper by 11%–19% under the current climate control (Figure 2b,c). In contrast, when subjected to the future climate treatment, the inocula from extreme environments substantially increased crop survival, with the dual inocula from the Atacama Desert, the High Andes and

Antarctica, and the mixed inoculum increasing crop survival by 1–2 fold relative to uninoculated controls (Figure 2a–c). Despite this, the mean survival of lettuce and tomato plants inoculated with extremophile endophytes and exposed to the future climate treatment was consistently lower than that of all plants in the current climate control (Figure 2a,b). Under the future climate treatment, the 3-year average survival of plants treated with the mixed inoculum was highest, with, compared with uninoculated plants, 67%–166% increases

FIGURE 2 Effects of inocula and the future climate treatment (simulating aridity and salinity in Mediterranean-type ecosystems) on the survival of (a) *Lactuca sativa*, (b) *Solanum lycopersicum* and (c) *Capsicum annuum*. For each crop species, distinct letters indicate significant ($p < .05$) differences between means according to Tukey's honestly significant difference (HSD) *a posteriori* tests. Each box shows the mean (solid horizontal line) and interquartile range derived from 300 replicate plants. The experiment was repeated in three consecutive years. Mean \pm SD are shown.



in the survival of each crop species treated with all six endophytes from the Atacama Desert, the High Andes and Antarctica (Figure 2). The commercial inoculum did not affect crop survival under the current climate control but increased the survival of lettuce and tomatoes under the future climate treatment by 46%–83% (Figure 2a,b).

Similar responses were observed for yield, with moderate effects of the inocula on this parameter under current climate conditions but substantial positive effects under the future climate change scenario (Figure 3). When exposed to the current climate control, all inocula from extreme environments increased the yield of lettuce by 5%–7%, the mixed inoculum increased that of tomato by 6%, and the High

Andes, Antarctic and mixed inocula increased that of bell pepper by 2%–11%, relative to uninoculated plants (Figure 3a–c). The future climate treatment reduced the yields of uninoculated plants by 18%–60%, but, under this treatment, all dual and mixed inocula from the Atacama Desert, High Andes and Antarctica counteracted these reductions by significantly increasing lettuce, tomato and bell pepper yields by 31%–35%, 22%–28% and 7%–15%, respectively, relative to uninoculated controls (Figure 3a–c). As for survival, and notably for tomato, the mean yields of plants inoculated with extremophile endophytes and exposed to the future climate scenario were lower than those of plants in the current climate control (Figure 3a–c). Plants treated with the mixed inoculum typically exhibited the highest yields,

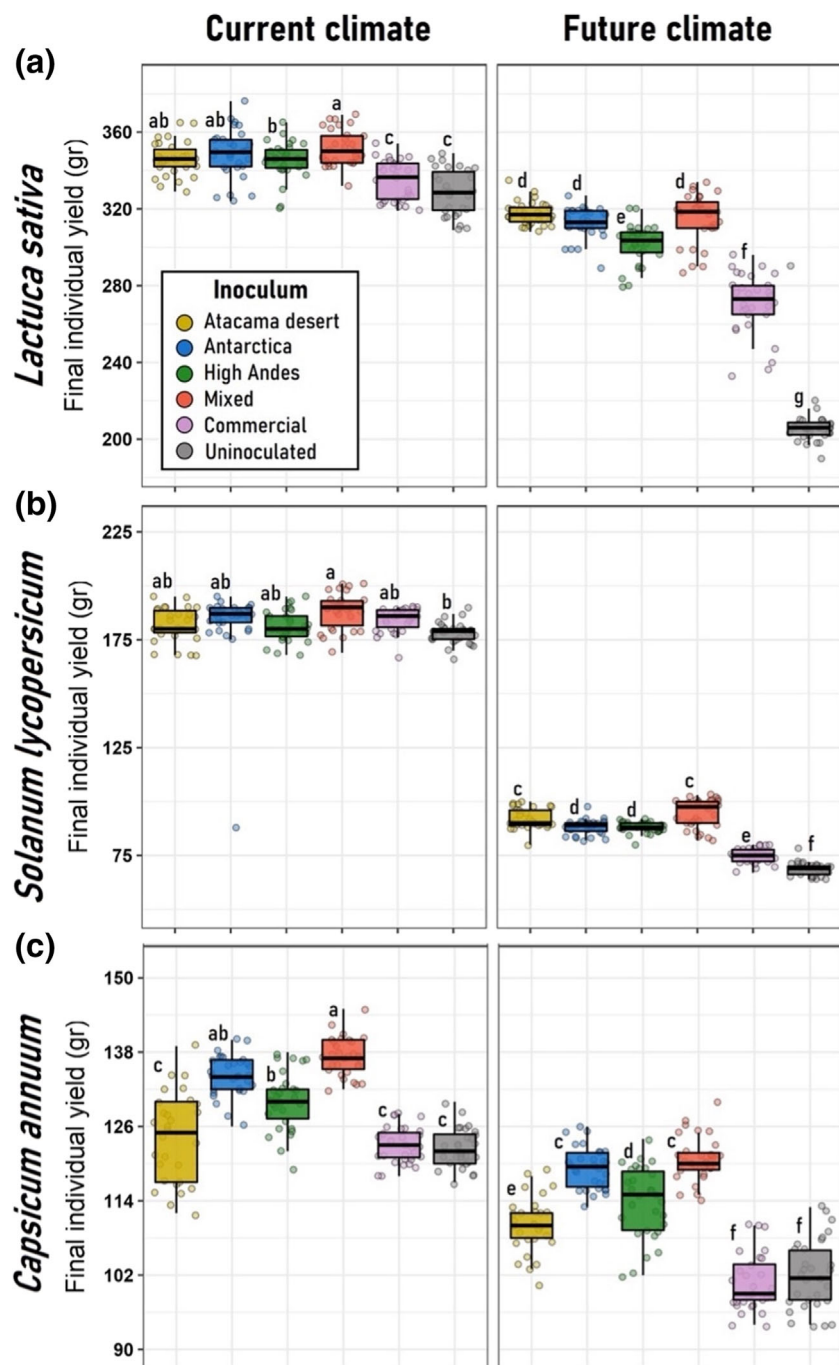


FIGURE 3 Effects of inocula and the future climate treatment (simulating aridity and salinity in Mediterranean-type ecosystems) on the final yield of (a) *Lactuca sativa*, (b) *Solanum lycopersicum* and (c) *Capsicum annuum*. For each crop species, distinct letters indicate significant ($p < .05$) differences between means according to Tukey's honestly significant difference (HSD) *a posteriori* tests. Each box shows the mean (solid horizontal line) and interquartile range derived from 300 replicate plants. The experiment was repeated in three consecutive years. Mean \pm SD are shown.

both under the current climate control and the future climate treatment (Figure 3a–c). The commercial inoculum only enhanced the yields of lettuce and tomatoes by up to 24% under the future climate treatment (Figure 3a–c). There were few differences between years in crop survival or yield (Figures S1–S2).

3.2 | Biochemical mechanisms of environmental tolerance

As for survival and yield, highly significant main and interaction effects of inocula and climate were recorded on foliar $\delta^{13}\text{C}$ signature

(Table 1), phenol and proline concentrations and lipid peroxidation (Table S1). With the exception of the High Andes dual inoculum applied to lettuce and tomato, and the dual inoculum from the Atacama Desert applied to tomato, the inocula from extreme environments depleted the leaf $\delta^{13}\text{C}$ of plants exposed to the current climate control by 0.5–1.0‰ (Figure 4). The future climate treatment substantially (3–5‰) enriched ^{13}C in the leaves of uninoculated plants (Figure 4). However, all dual and mixed inocula from extreme environments counteracted the enrichment effect of drought and salinity by strongly depleting foliar ^{13}C in each crop species by 3–5‰, compared with uninoculated controls (Figure 4a–c). The largest depletions in leaf ^{13}C , under both the current climate control and the future climate

treatment, were induced by the mixed inoculum (Figure 4a–c). Other than depleting leaf ^{13}C of tomatoes exposed to the future climate treatment by 1.8‰, the commercial inoculum did not affect $\delta^{13}\text{C}$ signatures (Figure 4).

Relative to uninoculated plants exposed to the current climate control, the dual and mixed inocula from extreme environments increased leaf phenol concentrations of lettuce, tomato and bell pepper by 10%–21% (Figure S3). In contrast, under the future climate scenario and compared with uninoculated plants, the dual inocula from extreme environments caused 46%–83% increases in foliar phenol concentration, with the mixed and commercial inocula

eliciting increases in concentrations of 55%–108% and 12%–51%, respectively (Figure S3). Under the current climate, inocula from extreme environments had no effect on foliar proline concentrations in tomatoes but increased concentrations of the amino acid in lettuce and bell pepper leaves by 12%–15% and 31%–63%, respectively (Figure S4). The future climate treatment increased leaf proline concentrations in uninoculated plants, with the dual and mixed inocula from extreme environments further increasing concentrations of the amino acid in lettuce, tomato and bell pepper leaves by 54%–65%, 30%–39% and 44%–57%, compared with uninoculated controls. The largest increases in foliar proline concentrations were

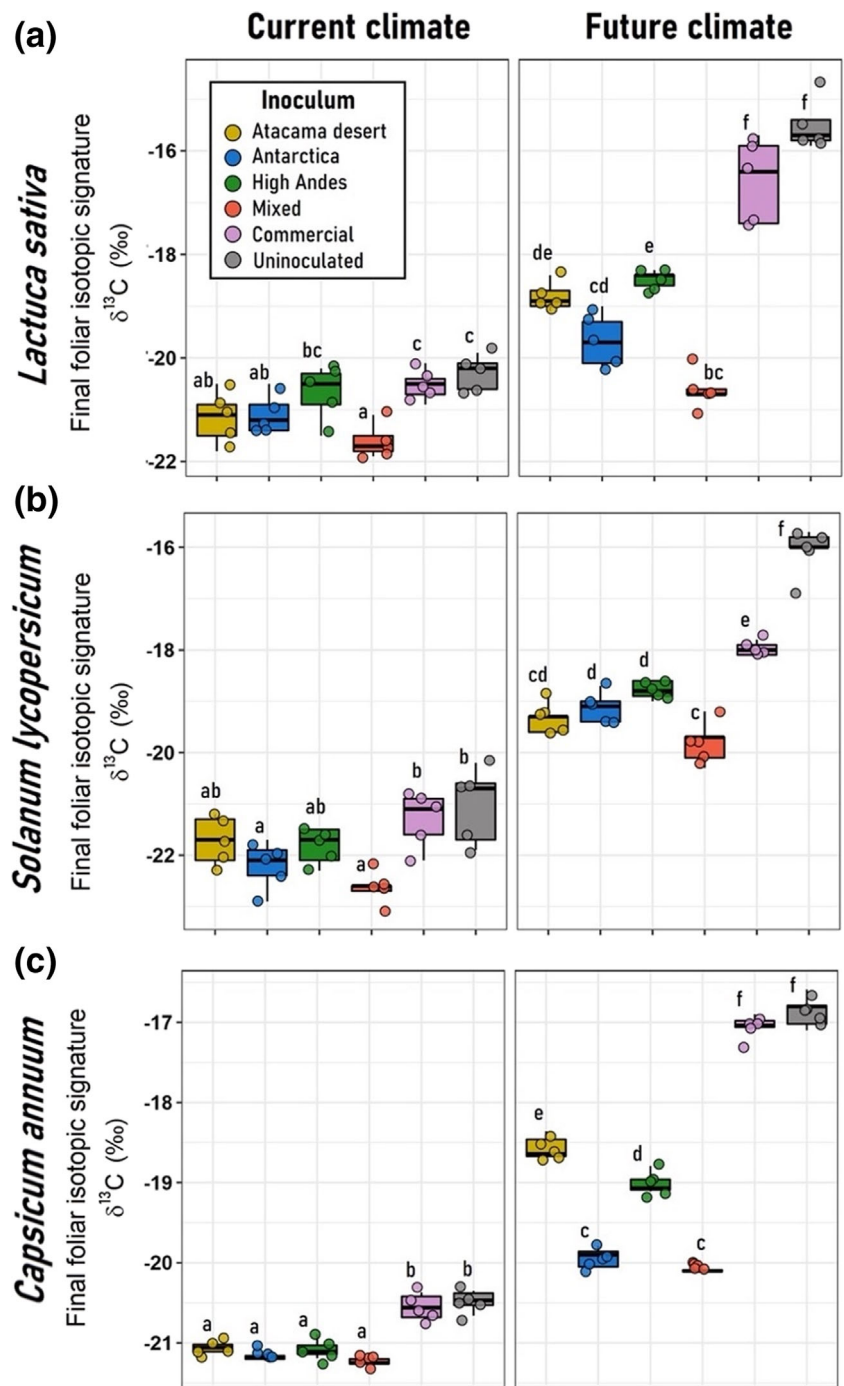


FIGURE 4 Effects of inocula and the future climate treatment (simulating aridity and salinity in Mediterranean-type ecosystems) on the foliar carbon isotopic signature ($\delta^{13}\text{C}$) of (a) *Lactuca sativa*, (b) *Solanum lycopersicum* and (c) *Capsicum annuum*. For each crop species, distinct letters indicate significant ($p < .05$) differences between means according to Tukey's honestly significant difference (HSD) *a posteriori* tests. Each point shows the mean $\delta^{13}\text{C}$ signature of 10 replicate plants. Mean, interquartile range and SD are shown in the boxes.

caused by the mixed inoculum (Figure S4). Lipid peroxidation was typically reduced by the inocula from extreme environments by 8%–35% under current climate conditions and by 68%–107% in all crop species under the future climate scenario, compared with uninoculated controls (Figure S5).

3.3 | Effect size analyses

These analyses illustrated the clear impacts of inocula from extreme environments on crop performance under the future climate scenario. With the exception of lipid peroxidation, which was diminished by inocula under the future climate treatment, positive effects of extremophile endophytes were recorded on all parameters, including pooled effect size (Figure 5). The magnitude of the effect of inocula from extreme environments was markedly superior to that of the commercial product, with Cohen's *d* values for pooled effect sizes on each crop under the future climate treatment being consistently lower for

the commercial inoculum than they were for the dual and mixed inocula from the Atacama Desert, Andes and Antarctica (Figure 5; Table S2). Under current climate conditions, of the 18 parameters that were measured (six in each crop species), 10 were unaffected by the commercial product, seven, six and three parameters were unresponsive to the dual inocula from the Atacama Desert, Andes and Antarctica, and just two did not respond to the mixed inoculum, respectively (Table S3). Under the future climate treatment, the commercial product had no effect on three responses in *C. annuum* (yield, foliar proline concentration and $\delta^{13}\text{C}$ signature), whereas the inocula from extreme environments exerted significant effects on all of the measured responses in the three crop species (Table S3).

4 | DISCUSSION

In confirmation of our hypothesis, we found that co-inoculation with endophytic fungi from the Atacama Desert, the High Andes and

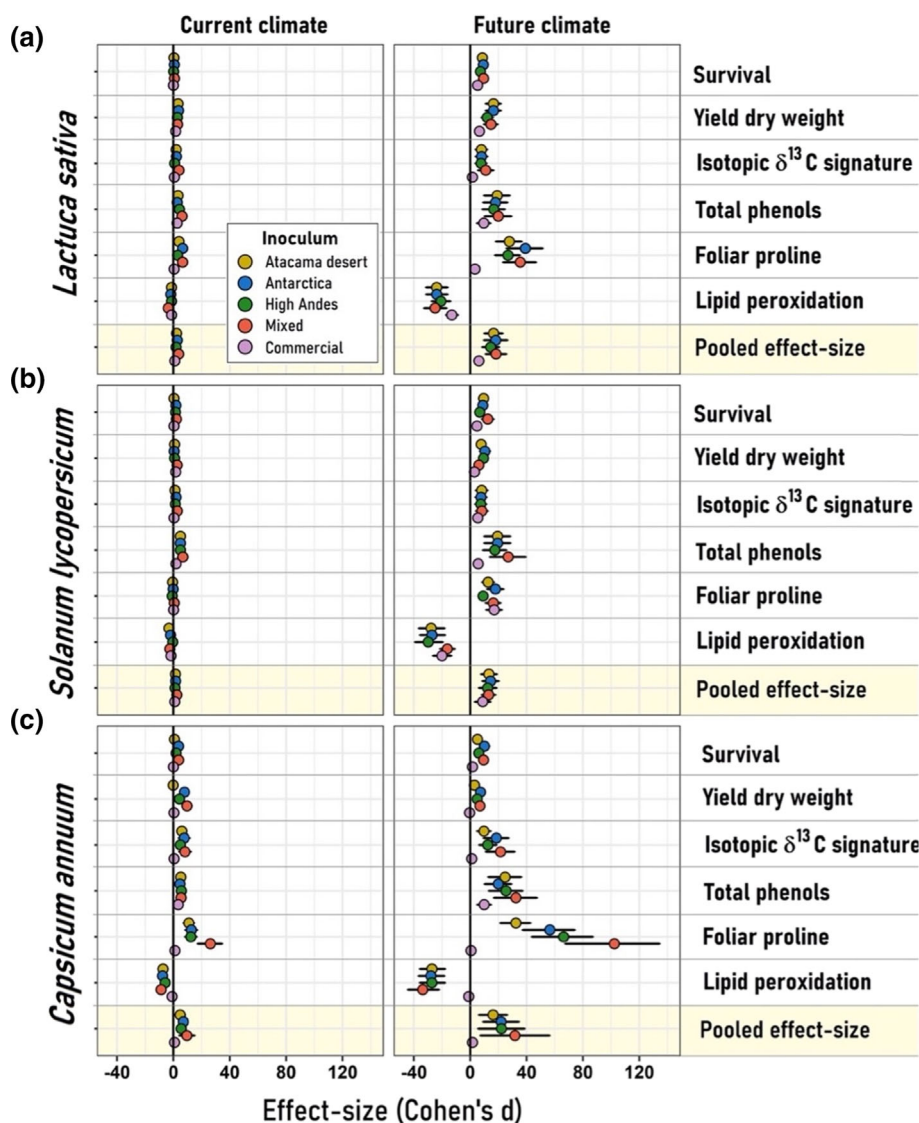


FIGURE 5 Effect sizes (Cohen's *d*, with Hedge's correction) of inocula (fungal endophytes from different ecosystems, a mix of these fungal endophytes, commercial inocula with microorganisms and treatment without microorganisms as control) on survival, yield and physiological variables of *Lactuca sativa*, *Solanum lycopersicum* and *Capsicum annuum* compared with uninoculated controls under the current climate control and the future climate treatment. Points represent the mean effect size of each of five inocula on response variables, with positive and negative means indicating increases and decreases in response variables caused by inocula, respectively. Significant ($p < .05$) effects of inocula are denoted by the 95% confidence intervals (horizontal bars) not overlapping with zero (vertical black line). Pooled effect sizes (highlighted in yellow) were also calculated for each crop species. Note that survival and yield data were those measured at the end of each experiment.

Antarctica partially mitigated the negative effects of climate change on three crops, with amplified positive effects of endophytes on survival and yield being observed when lettuce, tomato and bell pepper plants were grown under increased aridity and salinity, conditions that are anticipated over future decades in MTEs (Williams, 2017). The greatest and most frequent benefits were typically derived from a mixed inoculum of six extremophile endophytes applied to crops, with the positive effects of this inoculum and the dual inocula apparently arising from changes in biochemical tolerance and improved water balance of plants exposed to the future climate scenario (Baron & Rigobelo, 2022). Furthermore, the inocula from extreme environments conferred greater drought and salinity tolerance on crops than a commercially available inoculum of AMF previously reported to enhance the growth and performance of several crop species (Torres-Díaz et al., 2021).

The endophytes studied here consistently increased the concentrations of phenols and proline in the leaves of crops exposed to drought and salinity. Phenols act as signalling molecules in plant-microbe symbioses (Mandal et al., 2010), whereas proline is a stress-associated amino acid synthesised by many fungi, including *Penicillium* (Wang et al., 2017), which is involved in antioxidant responses and improved drought tolerance. The amino acid has important stress-protective functions as a compatible osmolyte and regulates the stabilisation of macromolecules, proteins, antioxidant enzymes and the balance of intracellular redox homeostasis (Fu et al., 2018). Increased membrane stability induced by proline, hence most probably explains the reduced lipid peroxidation in the leaves of inoculated plants exposed to the climate change scenario, and may in part explain the improved survival and yield of crops exposed to drought and salinisation in the present study. The enhanced performance of endophyte-inoculated lettuce, tomato and bell pepper, crop species that are prone to drought stress, is also most probably attributable to improved plant water relations. Consistent with the enrichment of ^{13}C in drought-exposed plant tissues (Farquhar et al., 1989), when uninoculated plants of these crops were exposed to aridity and salinity, foliar $\delta^{13}\text{C}$ was enriched by 3–5% compared with uninoculated plants grown under current climate conditions. However, under the aridity-salinity treatment and salinity treatments, the inocula from extreme environments counteracted these effects by strongly (up to 5%) depleting leaf ^{13}C , suggesting improved plant water balance (Farquhar et al., 1989). The mechanisms by which the endophytes effected these changes to crop water relations remain to be determined. They may be associated with the widely-exploited capacity of *Penicillium* species for secondary metabolite synthesis (Toghueo & Boyom, 2020), their production of phytohormones, such as gibberellin or indole-3-acetic acid, which enable plants to tolerate salinity stress (Leitão & Enguita, 2016), or altered leaf and root morphology of inoculated plants (Aghaei-Dargiri et al., 2021; González-Teuber et al., 2018). Alternatively, it is possible that, as previously posited for AMF (Augé, 2001), dense wefts of hyphae formed by *Penicillium* around roots may bind together primary soil particles, leading to the formation of micro-aggregates that improve soil water retention.

In agreement with reports that co-inoculation with micro-organisms promotes the growth of plants exposed to stressful abiotic conditions (González-Teuber et al., 2022; Pozo et al., 2021), a mixed inoculum of six endophytes from Antarctica, the Atacama Desert and the High Andes typically confers greater and more frequent benefits on crops than dual inocula or a commercial bioformulation. Our findings thus suggest that microbiome engineering, that is, the application to plants of consortia of micro-organisms, may be used to modulate and extend crop yield, survival and metabolic capability (Hyde et al., 2019; Pozo et al., 2021). This approach, which more closely mimics the colonisation of roots in the natural environment by highly diverse fungal communities (e.g., Vandenkoornhuyse et al., 2002), is deemed to be more efficient than single- or dual-endophyte inoculation because a range of genotypes are more effective in helping plants to tolerate different abiotic stresses (González-Teuber et al., 2022) or shifting abiotic conditions, as occur in environments exposed to climate change (IPCC, 2022). Although the six endophytes in the mixed inoculum are each able to colonise the root cells of lettuce, tomato and bell pepper in monoculture (Acuña-Rodríguez et al., 2019; Molina-Montenegro et al., 2016, 2020), at present, it is unclear which, if any, of these endophytes preferentially colonised crop roots in the present study. Further research, using DNA- or RNA-based techniques, is hence needed to determine whether specific endophytes more efficiently colonise host root tissues than others, and whether individual taxa benefit the growth of specific plant hosts exposed to increased salinity or aridity. We recommend that future bioformulations conferring tolerance to these environmental stresses, possibly arising from the modulation of host plant physiology at the molecular level (Alves et al., 2021; Pozo et al., 2021), should consist of mixtures of endophytes identified in this way.

Species of *Penicillium* have been found to grow endophytically in the tissues of at least 117 plant species in 63 families, including crops such as coffee, quinoa, rapeseed, cotton and common grape vine (Toghueo & Boyom, 2020). The widespread occurrence of members of the genus in plant tissues suggests that they may be used to improve the stress tolerance of a range of economically important crops. Owing to their ease of cultivation on artificial media, sufficient biomass of these fungi for inocula can be rapidly generated at a relatively low cost. They are hence, use probably more suitable for inclusion in bioformulations than some other micro-organisms, notably the AMF. These fungi, which belong to the Glomeromycotina and enhance crop performance in moderately dry soils, have proven beneficial effects on crops through their impacts on water relations, phosphorus acquisition and pathogen resistance (Augé, 2001; Bell et al., 2022; Gosling et al., 2006). However, AMF cannot be grown in the absence of their plant hosts, presenting a significant obstacle to generating sufficient material with which to inoculate crops at the scales necessary to improve yield (Rosikiewicz et al., 2017). Furthermore, AMF are infrequent in extreme environments, where the aridity and/or salinity of desert, Antarctic and montane soils suppress their occurrence in roots (Madouh & Quoreshi, 2023; Nepote et al., 2023; Schmidt et al., 2008). In contrast, ascomycetes in the Aspergillaceae, such as *Penicillium* species,

occur widely in cold and saline habitats (Cantrell et al., 2011), supporting the view that extreme environments may be rich sources of beneficial endophytes (Saikkonen et al., 2004).

Despite the promising use of Antarctic endophytes in agriculture (Acuña-Rodríguez et al., 2022; Molina-Montenegro et al., 2016, 2020), questions remain over the deployment of non-native microorganisms to enhance crop growth. For example, it has been suggested that the introduction of soil microorganisms from Antarctica to Chile may pose biological invasion risks (Núñez et al., 2015). However, *Penicillium* species found in Antarctica may already be present in South America, since this cosmopolitan genus, which forms tiny (typically $<6 \mu\text{m} \times <4 \mu\text{m}$) dry spores, is apparently capable of intercontinental dispersal, with all three operational taxonomic units of the genus recorded in Antarctic soils also being found in soils on other continents (Cox et al., 2019). Nevertheless, in order to avoid inadvertent introductions of pathogens into agricultural soils, we advocate the thorough identification of Antarctic endophytic fungi based on DNA sequence data (Torres-Díaz et al., 2021) and stringent tests of the effects of these fungi on crop performance. It has also been suggested that the introduction of endophytes from Antarctica to South America could alter native soil communities (Núñez et al., 2015). However, the inoculation of *Nothofagus* with Antarctic isolates of *P. rubens* and *P. bialowiezense* has no apparent effects on the diversity or abundance of native rhizosphere bacteria or fungi (Torres-Díaz et al., 2021), suggesting that these endophytes pose negligible risks to soil microbiome composition. Further research is needed to confirm whether or not the four other endophytes studied here affect native microbiomes and to determine potential shifts in the efficacy of extremophile endophytes in the less extreme habitats of MTEs, which could potentially lead to their decreased effectiveness over time or even switching to negative impacts on host plants under altered environmental conditions (Giauque et al., 2019).

The application to crops of microorganisms from extreme environments is a rapid, cost-effective and environmentally-friendly approach that could provide a better alternative to transgenic plants (Ray et al., 2019) and might augment the sustainable agriculture practices required by the new green revolution (Gopal & Gupta, 2016; Pozo et al., 2021). Despite these potential benefits, the use of microbial inoculants is not widespread in farming, mostly probably because of the strict regulatory standards for the registration and commercialization of new bioformulations, the difficulties of mass production, the efficacy of inoculants when applied under field conditions, and resistance from consumers and farmers who prefer traditional food production methods (Poveda et al., 2022). Moreover, there are still few reports of the mechanisms by which endophytes manipulate host plant physiology and exert their effects (Chaudhary et al., 2022; Sarkar et al., 2021), generating further questions about their use in crop production. If extremophile fungi are to be used to enhance global food security by either maintaining or increasing food production levels (Tiwari & Bae, 2022), then further studies are needed on the interplay between microbial inoculants, crop plants and the soil and plant microbiome and how they determine plant ecophysiological and biochemical responses. Nevertheless, the observations here indicate that the use of

endophytic fungi from extreme environments is a promising approach for enhancing crop production in MTEs as the soils in these ecosystems become more arid and saline in future decades.

AUTHOR CONTRIBUTIONS

Cristian Torres-Díaz, Cristian Atala and Marco A. Molina-Montenegro designed the research and, along with Gabriel I. Ballesteros, conducted the experiments. Gabriel I. Ballesteros and Ian S. Acuña-Rodríguez analysed the data, and Gabriel I. Ballesteros, Kevin K. Newsham and Marco A. Molina-Montenegro wrote the article. All authors read and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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