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Research paper

Effects of *Trichoderma* Pers. Application on the Growth, Physiological Responses, and Rhizosphere Soil Enzyme Activities of Three Coastal Tree Species Under Different Soil Salinity Levels

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ABSTRACT

Soil salinization is a major cause of land degradation along the western coast of Taiwan. Afforestation has the potential to improve soil quality, enhance ecological functions, and increase land resilience. In this study, a planting experiment was conducted in the salt-affected degraded land of Qigu, using three native coastal tree species. The experiment aimed to investigate the effects of microbial inoculants under different soil salinity conditions by applying *Trichoderma harzianum* Rifai (strain T2). Results showed that *Trichoderma* Pers. significantly increased the activity of most rhizosphere soil enzymes in low-salinity areas and enhanced invertase and alkaline phosphatase activity in *Hibiscus tiliaceus* L. and *Calophyllum inophyllum* L., respectively, under high-salinity conditions. Additionally, *Trichoderma* promoted arylsulfatase activity across all tree species and salinity conditions, demonstrating its potential to enhance sulfate transformation in soils. In terms of seedling growth, *Trichoderma* improved the Fv/Fm and root collar diameter of *Melia azedarach* L. under high salinity, indicating enhanced salt tolerance. It also promoted the ΦPSII of all species across different salinity levels, suggesting widespread benefits to the photosynthetic system. Furthermore, microbial biomass carbon was highly positively correlated with enzyme activity, particularly alkaline phosphatase and urease, which were closely related to plant height growth. These findings indicated that soil microorganisms play a crucial role in improving soil quality and promoting plant growth. In conclusion, although excessive salinity inhibits tree growth, the application of *Trichoderma* improves rhizosphere enzyme activity and promotes seedling growth and physiological health, making it a promising soil amendment for enhancing afforestation success in coastal salt-affected areas.

Keywords: afforestation, plant growth-promoting fungi (PGPF), microbial biomass carbon (MBC), soil amendment, salt tolerance

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INTRODUCTION

Taiwan contains approximately 25,000 ha of salt-affected soils, primarily distributed along the western coastal region. The main causes of soil salinization include excessive fertilization, improper irrigation practices (Chen et al. 2015), and the over-extraction of groundwater, which leads to seawater intrusion. As saline groundwater infiltrates inland, soil salinity gradually increases (Nicholls et al. 2011, Negm et al. 2017). Afforestation and reforestation of salt-affected degraded lands have received increasing attention due to their critical roles in ecological restoration, biodiversity conservation, carbon sequestration, and climate change mitigation (Gupta et al. 2016, Zhang 2020). Afforestation also serves as a natural strategy to mitigate soil salinity by facilitating salt uptake by plants, lowering groundwater levels through evapotranspiration, and improving soil physical and chemical properties through organic matter input (Barrett-Lennard 2002, Shaygan and Baumgartl 2022). However, elevated soil salinity increases osmotic stress, which hinders water uptake by plants, and can also result in ion toxicity and nutrient imbalances—factors that adversely affect plant growth and development (Osman 2018). Therefore, using growth-promoting agents to enhance seedling growth during the early stages of afforestation is one of the key factors for successful establishment in salt-affected soils. One promising approach is the application of plant growth-promoting microorganisms (PGPM), including specific fungi, bacteria, and actinomycetes. These microorganisms can release bioactive compounds, such as vitamins, phytohormones, and enzymes, that stimulate plant growth, enhance stress tolerance, and induce immune responses that help suppress pathogens. In addition, PGPM can secrete exopolysaccharides that promote the formation of stable soil aggregates, thereby improving soil water retention and nutrient availability. As such, PGPM are increasingly regarded as key agents for restoring degraded land and achieving sustainable agriculture and forestry (Ramachandran and Radhapriya 2016, Naik et al. 2019, El-Saadony et al. 2022).

Trichoderma spp. are filamentous, saprophytic fungi widely distributed in the environment and are now commonly used to suppress crop diseases and enhance plant growth and yield (Zin and Badaluddin 2020). Numerous studies have demonstrated that their application can effectively improve soil microbial community structure, increase microbial abundance, and positively influence soil quality (Mao and Jiang 2021, Wu et al. 2022). *Trichoderma* Pers. also promotes root system development in plants (Contreras-Cornejo et al. 2009) and engages in both rhizospheric and endophytic symbioses. When present in the rhizosphere as free-living microorganisms, they can detect host plant location and initiate colonization toward the roots, enhancing nutrient uptake by decomposing organic matter and providing nutrients. Some strains can also enter plant tissues and establish endophytic symbiosis, further promoting plant growth (Contreras-Cornejo et al. 2024). The effects of *Trichoderma* inoculation are not limited to the colonized root zone but can influence the entire plant system (Abdullah et al. 2021). Research has shown that *Trichoderma* can modulate rhizosphere soil properties, such as nutrient availability and soil enzyme activities, while enhancing plant tolerance to both biotic and abiotic stresses (Mastouri et al. 2010, Asghar and Kataoka 2021). The interaction between *Trichoderma* and

plants involves complex physiological, biochemical, and molecular mechanisms that enhance disease resistance, promote nutrient acquisition, strengthen carbohydrate metabolism, and stimulate phytohormone synthesis (Stewart and Hill 2014, Sharma et al. 2017). Previous studies have also shown that *Trichoderma* application can increase concentrations of plant osmolytes, improve the efficiency of the glutathione cycle, upregulate antioxidant enzyme activity, and enhance the expression of stress-related genes (Ahmad et al. 2015, Zhang et al. 2016, Fu et al. 2017). Among the various species, *Trichoderma harzianum* Rifai is particularly notable for its high environmental adaptability and strong antagonistic activity against pathogens. Studies have demonstrated that the application of *T. harzianum* can significantly enhance plant growth and development, promote root elongation, improve nutrient uptake, and alleviate oxidative stress in plant tissues under salt stress conditions (Zhang et al. 2019, Sabzi-Nojadeh et al. 2024). Furthermore, *T. harzianum* has been reported to improve photosynthetic performance by increasing chlorophyll content and net photosynthetic rate (Zhao et al. 2021).

Soil enzymes are secreted by plant roots and soil microorganisms or released into the soil following cell lysis and are therefore commonly referred to as extracellular enzymes. These enzymes are involved in a wide range of biochemical processes and play a critical role in soil nutrient transformation. They convert nutrients into bioavailable forms that can be readily utilized by plants and are considered important indicators of nutrient cycling in soils (Song et al. 2012, Li et al. 2014). Moreover, soil enzyme activities respond to environmental changes more rapidly and sensitively than physical or chemical soil properties, making them effective indicators of soil ecosystem status (Araújo et al. 2013, Zhang et al. 2015). The relationship between soil salinity and enzyme activity has emerged as an important area of study. For instance, in long-term restored coastal saline soils, reductions in salinity have been associated with increased activities of dehydrogenase, urease, amylase, and alkaline phosphatase (Xie et al. 2017). Similarly, in highly salinized paddy fields, soil amendments have been shown to enhance enzyme activities and simultaneously improve rice yields (Che et al. 2023). Most studies have found a negative correlation between soil salinity and enzyme activity (Guan et al. 2014). Among these, urease activity is particularly sensitive to salinity and alkalinity, making it a reliable indicator of soil quality (Zhang et al. 2014). Different enzymes contribute uniquely to nutrient cycling. For example, urease facilitates the hydrolysis of nitrogen-containing organic compounds, thereby enhancing nitrogen transformation and its bioavailability (Liang et al. 2003). Alkaline phosphatase, on the other hand, plays a key role in the phosphorus cycle by converting organic phosphorus into inorganic forms that can be absorbed by plants (Dick and Burns 2011).

As a biological fertilizer, *Trichoderma* has been shown to enhance the activity of soil enzymes, such as urease, phosphatase, and catalase, thereby improving nutrient availability and stimulating microbial activity in the soil (Ji et al. 2020). These enzymes play distinct roles in nutrient cycling, and previous studies have demonstrated the benefits of *Trichoderma* application in enhancing enzyme activities in saline soils and promoting plant salt tolerance (Fu et al. 2019, Cheng et al. 2023). However, most existing research has focused on agricultural systems and crop yield improvement, while studies investigating the relationship between soil enzyme activity

and tree growth in afforestation contexts remain limited. Moreover, the symbiotic interaction between *Trichoderma* and plants is inherently complex, and its effectiveness in field applications often varies depending on environmental conditions, microbial strains, and plant species. Many studies have also lacked field-based validation (Santoyo et al. 2024). In light of this, the present study aimed to evaluate whether *Trichoderma* application can improve plant growth and soil quality under salt stress across different tree species. Specifically, this experiment investigated the effects of *Trichoderma* on rhizosphere soil enzyme activity within planting holes, as well as on the growth and physiological responses of seedlings in afforestation sites located in salt-affected coastal areas. The goal was to clarify the specific benefits of *Trichoderma* application for afforestation efforts on degraded saline lands.

MATERIALS AND METHODS

Experimental site

The experimental site was located at the exit of the Qigu Yancheng Interchange on Provincial Highway 61 in Qigu District, Tainan City, Taiwan (23°09'14.5"N, 120°05'16.8"E). The site, approximately 2.5 ha in area, was formed by the accumulation of construction spoil, stones, and pre-existing salinized soil. Due to its proximity to a lagoon, the groundwater level is high and easily influenced by seawater intrusion, resulting in soil salinity that poses challenges to afforestation and greening (Figure 1).

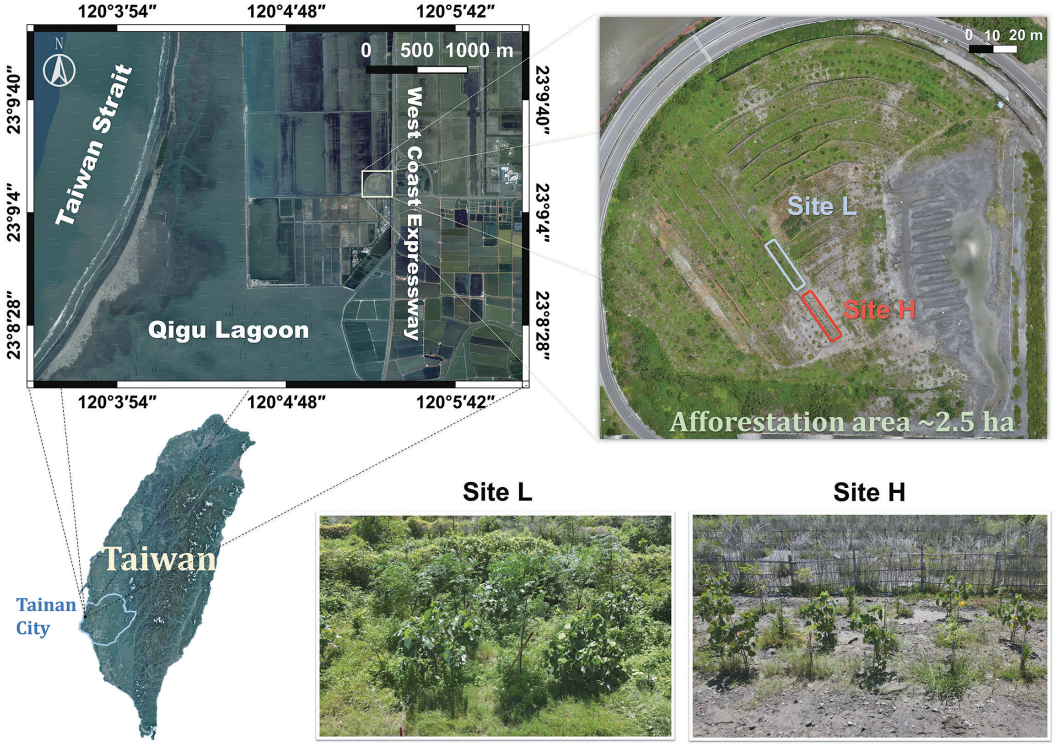

Figure 1. Locations of the study area and soil salinity sites. The sites are denoted as Site L (low soil salinity) and Site H (high soil salinity); Qigu Lagoon (七股潟湖).

In 2023, the highest monthly average temperature occurred in July (29.5℃, with a maximum of 34.3℃, while the lowest monthly average temperature occurred in January (17.0℃), with a minimum of 8.8℃. Annual precipitation totaled 1,134.5 mm, of which approximately 94% fell between June and September. The low-salinity area of the site is approximately 50 cm higher in elevation than the high-salinity area, and this microtopographic variation influences the redistribution of salts through rainfall-induced leaching and accumulation, resulting in distinct salinity zones. Surface soil (0-30 cm) was sampled in February 2023. In the high-salinity zone (H), the mean electrical conductivity of saturated soil paste (EC_e) was 67.01 dS m^{-1}, and the sodium adsorption ratio (SAR) was 34.83. By contrast, the low-salinity zone (L) exhibited a mean EC_e of 0.98 dS m^{-1} and a SAR of 11.80.

Experimental design and soil treatment

The experiment was conducted in March 2023 in a field site characterized by distinct natural variation in soil salinity. Two salinity sites (high and low) were designated as the main-plot treatments, each comprising three replicate plots (main plots), resulting in a total of six main plots. Within each replicate plot, 18 planting holes (50 cm × 50 cm × 50 cm) were established at a spacing of 1.3 m, totaling 108 planting holes across all plots. Three tree species with potential for carbon sequestration or salt tolerance were used in the trial: *Melia azedarach* L., *Hibiscus tiliaceus* L., and *Calophyllum inophyllum* L. *Trichoderma* treatment included two levels: inoculation with *T. harzianum* (T) and a non-inoculated control (C). The inoculated group received a granular formulation of the commercial T2 strain (Long-Ying Biotechnology Co., Ltd.), diluted at 1:200 (v/v) and applied directly to the center of each planting hole (30 cm × 30 cm in area and 10-30 cm deep). The control group received no fungal inoculant. To reduce initial salt stress, the soil in each planting hole was amended with a 1:2 (v/v) mixture of unfertilized peat and native soil. Within each main plot, six treatment combinations (three species × two *Trichoderma* levels) were randomly assigned, with three biological replicates per combination. The overall experiment followed a split-plot design, with salinity as the main-plot factor and species and *Trichoderma* treatment as sub-plot factors. In total, the experiment included 12 treatment combinations (3 × 2 × 2), each with 9 seedlings (three plots × three replicates), resulting in 108 seedlings. Because of the highly uneven temporal distribution of rainfall at the site, supplemental drip irrigation was implemented. Each planting hole was irrigated for 20 minutes at 6 a.m. and 6 p.m. on days without rainfall, based on a rain sensor, using a drip system with a flow rate of 7.8 L h^{-1}.

The experimental treatment lasted until August 2023, for a total duration of approximately 150 days. Soil respiration measurements were conducted in the planting holes in August, followed by soil sampling. At the canopy edge (10-30 cm from the trunk, depending on the species), the surface soil was carefully removed, and soil was carefully scraped toward the plant center using a steel brush until the roots were exposed. Rhizosphere soil was then collected at a depth of 15-20 cm. The rhizosphere soil is defined as the zone influenced by root exudates, with its extent determined by factors, such as soil moisture, the residence time of exudates, and the rate of exudation. This zone typically extends approximately 0.2 to 61 mm from the root surface (Raynaud 2010). In this study,

to obtain sufficient material for analysis, soil was collected within 3 cm of the root surface. The rhizosphere soil was promptly sieved (2 mm) upon return to the laboratory and stored at -20℃ for subsequent microbial biomass carbon (MBC) and enzyme activity assays. To determine soil moisture content, 5 g of rhizosphere soil from each planting hole was oven-dried at 105℃ to constant weight. Based on these data, MBC and enzyme activity results from fresh soil were converted to dry weight (DW) units. Additionally, at the conclusion of the experiment in August, bulk soil properties from the 0-30 cm layer of each planting hole were analyzed. In the high-salinity area, the mean electrical conductivity of saturated paste (EC_e) was 11.44 dS m^{-1}, and the SAR was 8.46. By contrast, the low-salinity area had an average EC_e of 0.65 dS m^{-1} and a SAR of 1.44.

Physiological parameters and seedling growth

Physiological measurements were conducted in August 2023 using a LI-600 porometer/fluorometer (LI-COR Inc., Lincoln, Nebraska, USA). Measurements were taken at 10 p.m. (dark-adapted) and 9 a.m. (light-adapted), with each session lasting approximately 1 hr. After dark adaptation, the maximum quantum efficiency of photosystem II (Fv/Fm) was measured. Following light adaptation, the stomatal conductance (g_{sw}), electron transport rate (ETR), and effective quantum yield of photosystem II (ΦPSII) were recorded. For each tree, three fully expanded young leaves were selected from the third leaf position below the apical bud. The average of the three measurements was used as the representative value for that individual. Seedling growth was assessed in both May and August 2023 by measuring tree height and root collar diameter (RCD). RCD was measured in millimeters using a vernier caliper, and tree height was measured in centimeters from the RCD mark to the apical bud using a tree height ruler. The relative growth rate (RGR) was calculated using the formula proposed by Hunt (1982):

$$\mathrm{RGR} = \frac{(\ln S_2 - \ln S_1)}{(t_2 - t_1)} \quad \text{.....(1)}$$

where S_1 and S_2 represent the tree height or RCD at the initial and final measurements, and t_1 and t_2 represent the corresponding time points. The RGR of the tree height is expressed as RGR_{height} (cm cm^{-1} day^{-1}), and that of RCD as RGRRCD (mm mm^{-1} day^{-1}).

Soil respiration

Soil respiration was measured using the alkali absorption method, which estimates the intensity of respiration based on the amount of carbon dioxide (CO_2) released from the topsoil and absorbed by an alkali solution (Kirita 1971). A plastic tripod was placed 10-20 cm from the base of the seedling, supporting a 6 cm diameter Petri dish containing 10 mL of 2 M NaOH. The dish was covered with an inverted 300 mL transparent polystyrene cup (covering an area of approximately 44 cm^2). The rim of the cup was gently pressed into the surrounding soil to secure the setup, which was left undisturbed in the field for 24 hrs. A nearby patch of soil was covered with polyethylene film, and a blank control was prepared using the same apparatus. After the incubation period, the NaOH solution was rinsed into a centrifuge tube using deionized water and adjusted to a final volume of 50 mL. In the laboratory, a 5 mL aliquot was taken from each sample, mixed with 20 mL of deionized water, and one drop of phenolphthalein indicator was added. The mixture was titrated with 0.01 M HCl until it became colorless. Then, one drop

of bromophenol blue indicator was added, and titration continued with 0.01 M HCl until the solution turned yellow. The volume of HCl used was recorded as V_1. The same procedure was applied to the blank control, and the volume used was recorded as V_0. Soil respiration was calculated using Formula 2:

$$\text{Soil respiration (mg } CO_2 \text{ m}^{-2} \text{ h}^{-1}) = \frac{(V_0 - V_1)}{A \times T} \times N \times E \quad \text{.....(2)}$$

where A is the soil surface area covered (m^2), T is the incubation time (h), N is the concentration of titrated HCl (mol L^{-1}), and E is the conversion factor that accounts for unit conversions, the molar mass of CO_2, and the final solution volume (in this study, E = 440).

Microbial biomass carbon (MBC)

MBC in rhizosphere soil was determined using the chloroform-fumigation extraction method proposed by Vance et al. (1987). Two sets of 10 g soil samples were prepared. For the control group, 0.5 M K_2SO_4 was added and the mixture was shaken for 1 hr. For the fumigation group, the 10 g soil was fumigated with alcohol-free chloroform in a glass vacuum desiccator for 24 hrs (in the dark at 22℃), followed by adding 0.5 M K_2SO_4, and was also shaken for 1 hr.

Both samples were filtered through glass fiber filter paper (Whatman GF/C). The resulting filtrates were analyzed for dissolved organic carbon (DOC) using a total organic carbon analyzer (Vario TOC, Elementar, Germany). The difference in DOC between the fumigated and control samples was used to calculate MBC according to Fang et al. (2020), using Formula 3:

$$\text{MBC (mg kg}^{-1} \text{ DW soil)} = \frac{(DOC_{fumigated} - DOC_{control})}{k_{ec} \times W} \quad \text{.....(3)}$$

where $DOC_{fumigated}$ and $DOC_{control}$ are the DOC concentrations (mg) of the fumigated and control samples, respectively; k_{ec} is the conversion coefficient (0.45); and W is the dry weight of the fresh soil sample after conversion (kg).

Soil enzyme activity

The activity of seven soil enzymes in rhizosphere soil was measured: protease, urease, alkaline phosphatase, dehydrogenase, arylsulfatase, β-glucosidase, and invertase. For each enzyme, background correction was conducted using both soil-free and substrate-free blanks. The assay methods for each enzyme are described below:

1. Protease (EC 3.4.21-24)

One gram of soil was mixed with 2.5 mL of sodium caseinate solution and incubated at 50℃ for 1 hr. Then, 1 mL of 17.5% trichloroacetic acid was added, followed by centrifugation at 4,000 rpm for 10 minutes. A 0.5 mL aliquot of the supernatant was mixed with 3 mL of 2.8 N sodium carbonate and 1 mL of Folin phenol reagent (diluted 1:3). After standing for 15 minutes, the absorbance of the supernatant was measured at 700 nm. Protease activity was expressed as the amount of tyrosine released (μg tyrosine h^{-1} g^{-1} DW soil) (Ladd and Butler 1972).

2. Urease (EC 3.5.1.5)

Five grams of soil were treated with 1 mL of toluene for 15 minutes, followed by the sequential addition of 10 mL of 10% urea solution and 20 mL of citrate buffer (pH 6.7). After thorough mixing, the sample was incubated at 37℃ for 24 hrs. The resulting solution was filtered using a 0.45 μm

polytetrafluoroethylene (PTFE) syringe filter. A 3 mL aliquot of the filtrate was mixed with 4 mL of sodium phenoxide (1.35 M) and 3 mL of sodium hypochlorite (0.9% active chlorine). After standing for 20 minutes, the mixture was brought to a final volume of 50 mL, and absorbance was measured at 578 nm. Urease activity was expressed as the amount of ammonium nitrogen produced (μg NH_4^+-N h^{-1} g^{-1} DW soil) (Guo et al. 2012).

3. Alkaline phosphatase (EC 3.1.3.1)

Ten grams of soil were sequentially mixed with 2 mL of toluene, 10 mL of 0.05 M disodium phenyl phosphate, and 10 mL of boric acid buffer (pH 9.4). After incubation at 37℃ for 3 hrs, the sample was centrifuged at 4,000 rpm for 10 minutes. The supernatant was mixed with 0.5 mL of 2% 4-aminoantipyrine and 0.5 mL of 8% potassium ferricyanide. The mixture was then filtered using a 0.45 μm PTFE syringe filter, and absorbance was measured at 510 nm. Alkaline phosphatase activity was expressed as the amount of phenol released (μg phenol h^{-1} g^{-1} DW soil) (An et al. 2009).

4. Dehydrogenase (EC 1.1.1.1)

Five grams of soil were sequentially mixed with 0.05 g of calcium carbonate, 1 mL of 3% triphenyl tetrazolium chloride (TTC), and 2 mL of deionized water. The mixture was incubated at 37℃ for 24 hrs. After incubation, the soil was transferred to a Büchner funnel lined with Whatman No. 1 filter paper and washed with methanol until the filtrate became colorless. The filtrate was then collected and brought to a final volume of 50 mL. Absorbance was measured at 485 nm. Dehydrogenase activity was expressed as the amount of 1,3,5-triphenylformazan (TPF) produced (μg TPF h^{-1} g^{-1} DW soil) (Jyot et al. 2015).

5. Arylsulfatase (EC 3.1.6.1)

One gram of soil was mixed with 4 mL of acetate buffer, 0.25 mL of toluene, and 1 mL of p-nitrophenyl sulfate solution. The mixture was incubated at 37℃ for 1 hr, followed by the addition of 1 mL of 0.5 M calcium chloride and 4 mL of 0.5 M sodium hydroxide. After centrifugation at 4,000 rpm for 10 minutes, the absorbance of the supernatant was measured at 400 nm. Arylsulfatase activity was expressed as the amount of p-nitrophenol (PNP) released (μg PNP h^{-1} g^{-1} DW soil) (Tabatabai and Bremner, 1970).

6. β-Glucosidase (EC 3.2.1.21)

One gram of soil was mixed with 0.25 mL of toluene, 4 mL of modified universal buffer (MUB), and 1 mL of p-nitrophenyl-β-D-glucoside (PNG) solution. The mixture was incubated at 37℃ for 1 hr, then centrifuged at 4,000 rpm for 10 minutes and filtered through a 0.45 μm PTFE syringe filter. Absorbance of the filtrate was measured at 400 nm. β-Glucosidase activity was expressed as the amount of 4-nitrophenol (PNP) released (μg PNP h^{-1} g^{-1} DW soil) (Eivazi and Tabatabai 1988).

7. Invertase (EC 3.2.1.26)

Two grams of soil were sequentially mixed with 15 mL of 8% sucrose solution, 5 mL of phosphate buffer, and 0.25 mL of toluene. The mixture was incubated at 37℃ for 24 hrs and then filtered using a 0.45 μm PTFE syringe filter. One mL of the filtrate was mixed with 3 mL of 3,5-dinitrosalicylic acid (DNS) and heated in a boiling water bath for 5 minutes. After cooling for 3 minutes under running water, absorbance was measured at 508 nm. Invertase activity was expressed as the amount of glucose produced (μg glucose h^{-1} g^{-1} DW soil) (Zheng et al. 2017).

Statistical analysis

Statistical analyses were conducted using a general linear model (GLM) to

perform a split-plot three-way analysis of variance (ANOVA), with salinity as the main-plot factor and tree species and *Trichoderma* treatment as sub-plot factors. The significance level was set at $\alpha = 0.05$. When significant effects were detected, Tukey's honestly significant difference (HSD) test was applied for post hoc comparisons among treatment means. To examine the relationships among plant growth, physiological, and soil-related parameters, Spearman's rank correlation analysis was performed, and correlation coefficients were reported as ρ. All statistical analyses and visualizations were carried out using R software (v. 4.3.2; R Development Core Team 2023), SPSS 28 (IBM Corporation, Armonk, NY, USA), and SigmaPlot 14.0 (Systat Software Inc., San Jose, CA, USA).

RESULTS

Three-factor analysis of soil properties

Three-way ANOVA of soil properties indicated that both soil respiration and MBC were significantly influenced only by the main effect of salinity, with no significant effects from *Trichoderma* treatment or tree species. Among soil enzymes, alkaline phosphatase exhibited a significant *Trichoderma* × tree species interaction, while invertase showed a significant three-way interaction among salinity, *Trichoderma*, and tree species. The remaining enzymes generally displayed significant salinity × *Trichoderma* interactions (Table 1). Effect plots indicated that both soil respiration and MBC were significantly higher under

Table 1. Three-way ANOVA results of soil properties

	Soil respiration		**MBC**		**Protease**	
Variables	*F*	*p* value	*F*	*p* value	*F*	*p* value
Sal	50.177	**< 0.001**	64.295	**< 0.001**	105.778	**< 0.001**
Tri	0.922	0.342	0.645	0.430	5.386	**0.025**
Spe	0.949	0.394	1.345	0.279	0.492	0.614
Sal × Tri	1.788	0.188	0.031	0.863	4.943	**0.031**
Sal × Spe	0.876	0.423	0.734	0.490	0.067	0.935
Tri × Spe	0.665	0.519	1.849	0.179	0.164	0.849
Sal × Tri × Spe	1.182	0.315	0.307	0.738	0.308	0.736
	Urease		**Alkaline phosphatase**		**Dehydrogenase**	
Variables	*F*	*p* value	*F*	*p* value	*F*	*p* value
Sal	123.301	**< 0.001**	280.297	**< 0.001**	189.336	**< 0.001**
Tri	30.947	**< 0.001**	12.296	**< 0.001**	31.014	**< 0.001**
Spe	2.777	0.072	8.231	**< 0.001**	3.570	**0.036**
Sal × Tri	15.189	**< 0.001**	3.421	0.071	37.089	**< 0.001**
Sal × Spe	2.710	0.077	0.529	0.593	1.093	0.343
Tri × Spe	0.008	0.992	3.711	**0.032**	2.397	0.102
Sal × Tri × Spe	0.461	0.633	0.732	0.486	0.701	0.501
	Arylsulfatase		**β-glucosidase**		**Invertase**	
Variables	*F*	*p* value	*F*	*p* value	*F*	*p* value
Sal	122.055	**< 0.001**	8.981	**0.004**	54.386	**< 0.001**
Tri	42.403	**< 0.001**	8.012	**0.007**	26.890	**< 0.001**
Spe	8.460	**< 0.001**	8.949	**< 0.001**	4.164	**0.022**
Sal × Tri	8.630	**0.005**	8.978	**0.004**	0.513	0.477
Sal × Spe	2.027	0.143	1.772	0.181	7.766	**0.001**
Tri × Spe	0.477	0.623	0.594	0.556	0.662	0.521
Sal × Tri × Spe	0.242	0.786	2.711	0.077	10.746	**< 0.001**

The *p*-values less than 0.05 are indicated in bold. Abbreviations: Sal (Salinity), Tri (*Trichoderma*), Spe (Species), and MBC (microbial biomass carbon).

low-salinity conditions, with no significant effects observed from tree species or *Trichoderma* treatment (Figure 2a, b). For rhizosphere soil enzyme activities, protease and urease showed similar patterns—both were significantly elevated under low salinity, and *Trichoderma* application further enhanced their activity under these conditions (Figure 2c, d). Alkaline phosphatase activity was generally higher under low-salinity conditions. Moreover, *Trichoderma* application eliminated species-specific differences in enzyme activity, significantly enhancing activity in *C. inophyllum* across both salinity levels, indicating consistent benefits in the absence of a three-way interaction (Figure 2e, f). Dehydrogenase, arylsulfatase, and β-glucosidase showed similar trends. Enzyme activities were generally higher under low salinity, and *Trichoderma* application further enhanced activity under these conditions, reflecting a salinity × *Trichoderma* interaction. Notably, arylsulfatase was the only enzyme to show a significant increase in activity under high salinity following *Trichoderma* treatment. By contrast, β-glucosidase activity in the control group (without *Trichoderma*) was not significantly affected by salinity (Figure 2g-i). A significant main effect of tree species was found for all three enzymes, with *M. azedarach* consistently exhibiting the highest rhizosphere enzyme activity (Figure 2j-l). Finally, invertase activity was significantly affected by a three-way interaction. *Trichoderma* application significantly increased invertase activity in the rhizosphere of *M. azedarach* under low salinity (Figure 2m) and in *H. tiliaceus* L. under high salinity (Figure 2n). In *C. inophyllum*, invertase activity was not significantly influenced by the salinity × *Trichoderma* interaction, but remained significantly higher under both low salinity and *Trichoderma* treatment, indicating a generally positive effect across salinity levels (Figure 2o).

Three-factor analysis of seedling growth and physiological parameters

Three-way ANOVA of seedling growth and physiological parameters showed that all measured variables were significantly affected by salinity, *Trichoderma* treatment, and tree species, except for the ETR, which showed no significant main effect from *Trichoderma*. A significant salinity × *Trichoderma* interaction was observed only for stomatal conductance (g_{sw}), while ETR was not affected by any interactions. By contrast, ΦPSII, Fv/Fm, and RGR_{RCD} exhibited significant interactions between tree species and either salinity or *Trichoderma*, and RGR_{RCD} additionally showed a significant three-way interaction among salinity, *Trichoderma*, and tree species (Table 2). Effect plots indicated that g_{sw} was generally higher under low salinity, with *Trichoderma* significantly enhancing g_{sw} only under the low-salinity conditions (Figure 3a). ETR exhibited a significant main effect of salinity, with higher values in the low-salinity group, but was not significantly affected by *Trichoderma* or any interactions (Figure 3b). Both g_{sw} and ETR showed significant main effects of tree species, with *H. tiliaceus* exhibiting the highest values (Figure 3c, d). For ΦPSII, Fv/Fm, and RGRRCD, significant tree species × salinity interactions were observed. *Melia azedarach* and *H. tiliaceus* generally had significantly higher values under low salinity, whereas *C. inophyllum* showed no significant salinity response. Under high salinity, ΦPSII in *M. azedarach* was strongly suppressed (0.57), while *H. tiliaceus* maintained a consistently high Fv/Fm (0.82) (Figure 3e-g). *Trichoderma* application significantly enhanced ΦPSII,

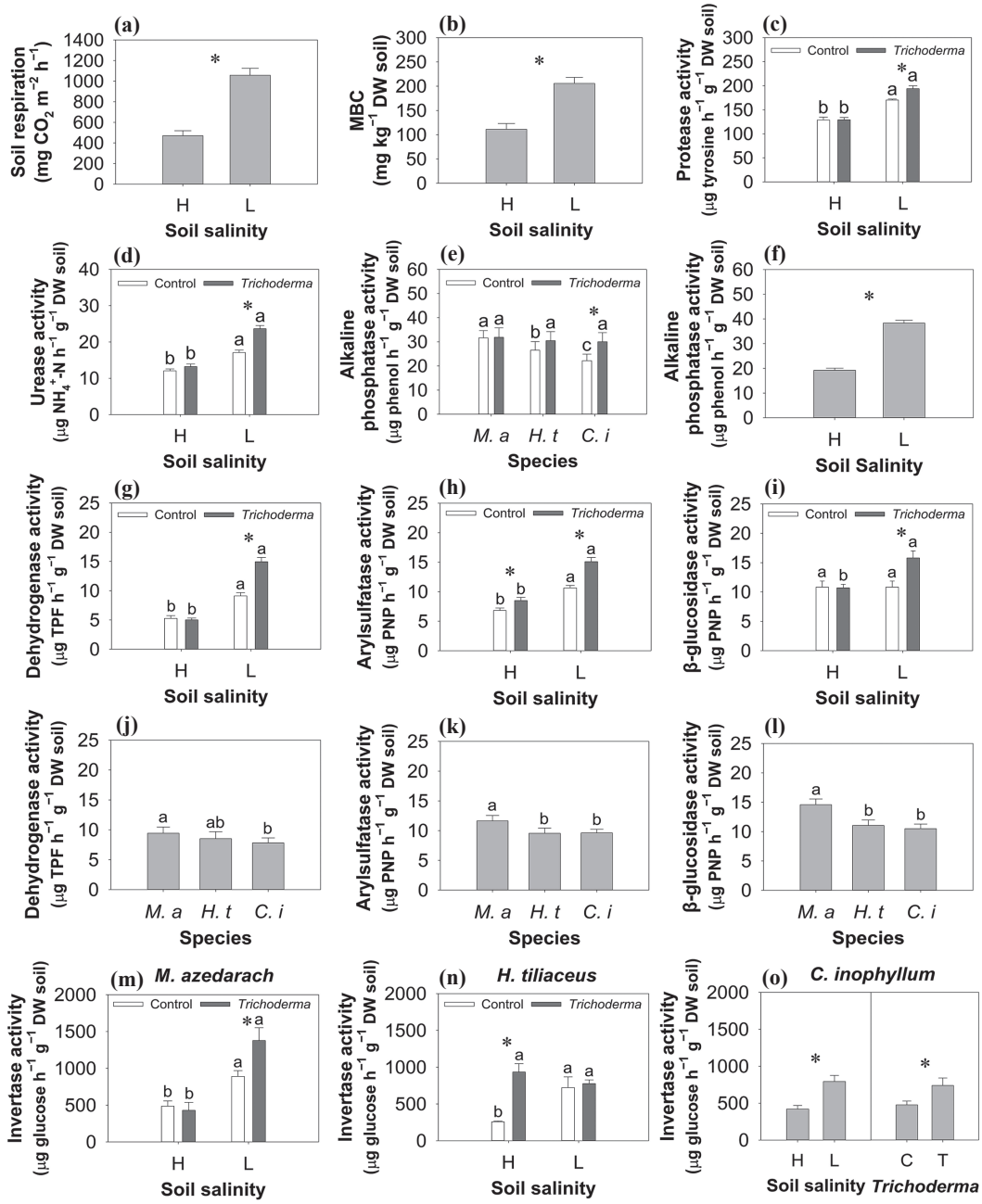

Figure 2. Effects of soil salinity, *Trichoderma* treatment, and tree species on (a) soil respiration, (b) microbial biomass carbon (MBC), and (c-o) soil enzyme activities. Panels show the main effects and interaction effects of soil salinity levels (H: high salinity, L: low salinity), *Trichoderma* treatments (Control [C], *Trichoderma* [T]), and tree species (*M. a*: *Melia azedarach*; *H. t*: *Hibiscus tiliaceus*; *C. i*: *Calophyllum inophyllum*) on different soil parameters. Bars represent mean values and standard errors. Different letters indicate significant differences between groups, and an asterisk (*) indicates significant differences between adjacent bars (α = 0.05).

Table 2 Three-way ANOVA results of the growth and physiological responses

	g_{sw}		ETR		ΦPSII	
Variables	*F*	*p* value	*F*	*p* value	*F*	*p* value
Sal	50.061	**< 0.001**	4.416	**0.041**	57.403	**< 0.001**
Tri	9.840	**0.003**	2.025	0.161	13.785	**< 0.001**
Spe	144.444	**< 0.001**	31.815	**< 0.001**	24.319	**< 0.001**
Sal × Tri	4.336	**0.043**	1.453	0.234	0.906	0.346
Sal × Spe	0.742	0.482	1.051	0.358	17.017	**< 0.001**
Tri × Spe	1.350	0.269	0.208	0.813	0.697	0.503
Sal × Tri × Spe	0.006	0.994	2.176	0.125	0.686	0.509
	Fv/Fm		**RGR_{RCD}**		**RGR_{height}**	
Variables	*F*	*p* value	*F*	*p* value	*F*	*p* value
Sal	26.936	**< 0.001**	10.286	**0.002**	96.734	**< 0.001**
Tri	11.787	**0.001**	38.324	**< 0.001**	20.962	**< 0.001**
Spe	30.339	**< 0.001**	192.646	**< 0.001**	42.344	**< 0.001**
Sal × Tri	0.733	0.396	0.042	0.839	1.466	0.232
Sal × Spe	7.627	**0.001**	8.511	**< 0.001**	18.531	**< 0.001**
Tri × Spe	3.526	**0.037**	16.833	**< 0.001**	2.915	0.064
Sal × Tri × Spe	1.448	0.245	2.387	0.103	8.618	**< 0.001**

The *p*-values less than 0.05 are indicated in bold. Abbreviations: Sal (Salinity), Tri (*Trichoderma*), Spe (Species), g_{sw} (stomatal conductance), ETR (electron transport rate), ΦPSII (quantum yield of photosystem II), Fv/Fm (maximum quantum efficiency of PSII), RGR (relative growth rate), and RCD (root collar diameter).

with no significant interactions with salinity or tree species, indicating a consistent promotive effect across salinity levels and species (Figure 3h). For Fv/Fm and RGR_{RCD}, *Trichoderma* showed a significant positive effect only in *M. azedarach* (Figure 3i, j). Regarding the RGR of seedling height (RGR_{height}), *Trichoderma* significantly promoted growth only in *C. inophyllum* under low-salinity conditions. In *M. azedarach* and *H. tiliaceus*, only a significant main effect of salinity was observed, with higher values in the low-salinity group (Figure 3k-m).

Correlation analysis of measured variables

The correlations among soil respiration, MBC, rhizosphere enzyme activities, seedling growth, and physiological parameters are shown in Figure 4. Except for β-glucosidase, all enzyme activities exhibited strong and significant positive correlations with both soil respiration and MBC (ρ = 0.52-0.80, $p < 0.001$). By contrast, β-glucosidase showed generally weaker correlations with other enzymes (ρ = 0.45-0.58), while the correlations among the remaining enzymes were consistently strong (ρ = 0.64-0.86). Photosynthetic parameters ΦPSII and Fv/Fm were significantly and positively correlated with most enzyme activities other than β-glucosidase, although the strength of these correlations varied (ρ = 0.33-0.64, $p < 0.01/0.001$). Notably, both parameters were most strongly correlated with urease. Stomatal conductance (g_{sw}) and ETR were significantly and strongly correlated with each other (ρ = 0.68, $p < 0.001$), but showed relatively weak correlations with soil-related variables. Regarding growth metrics, the RGR of tree height (RGR_{height}) showed moderate to strong positive correlations with rhizosphere soil properties and physiological traits (ρ = 0.46-0.76, $p < 0.01/0.001$), with the strongest correlation observed with Fv/Fm (ρ = 0.76). Among the enzyme activities, RGR_{height} was most strongly correlated with alkaline phosphatase (ρ = 0.69) and urease (ρ = 0.71). By contrast, the RGR of RCD (RGR_{RCD}) showed generally weak correlations with other variables, with a relatively stronger

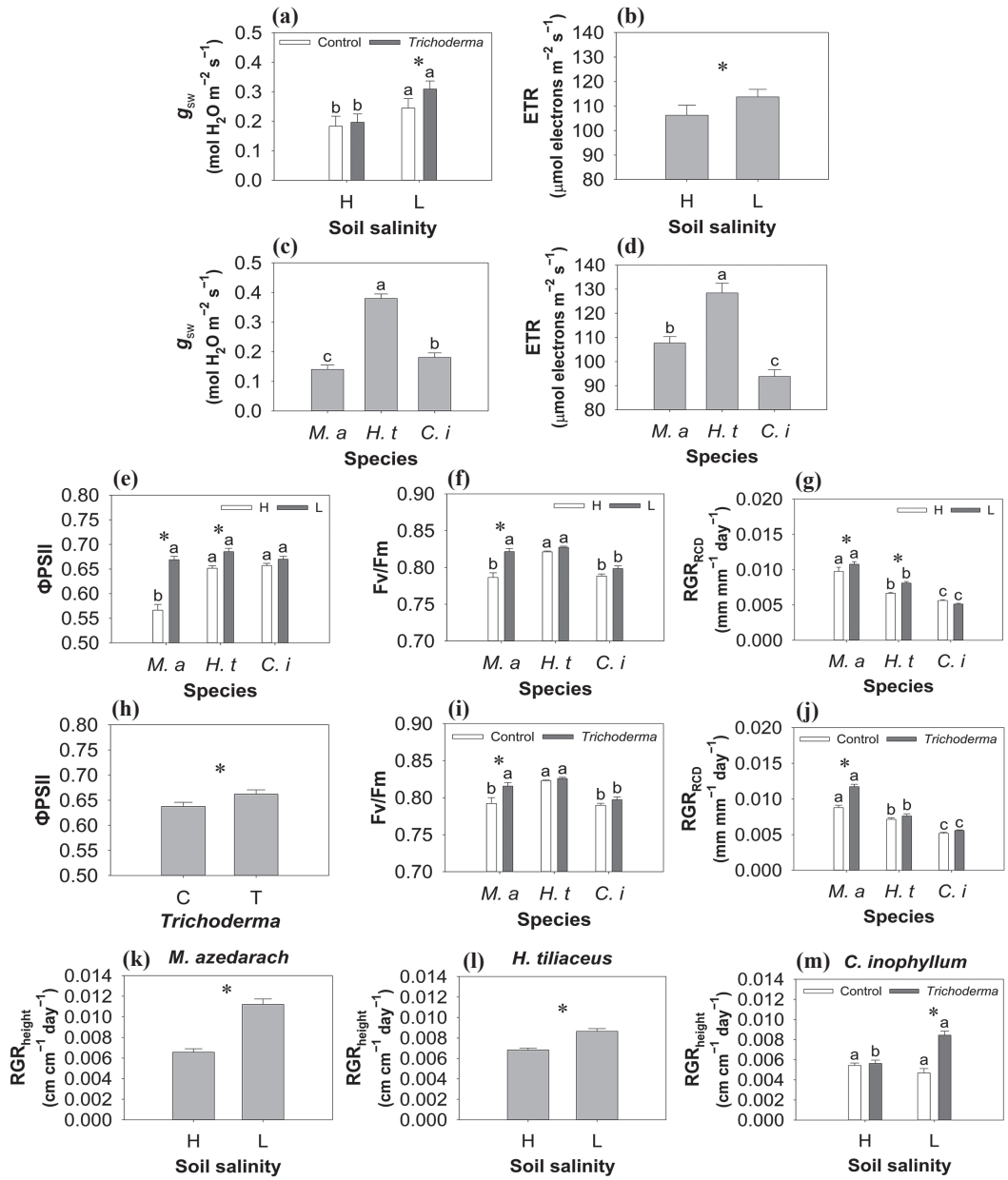

Figure 3. Effects of soil salinity, *Trichoderma* treatment, and tree species on growth and physiological responses. Panels show the main effects and interaction effects of soil salinity levels (H: high salinity, L: low salinity), *Trichoderma* treatments (Control [C], *Trichoderma* [T]), and tree species (*M. a*: *Melia azedarach*; *H. t*: *Hibiscus tiliaceus*; *C. i*: *Calophyllum inophyllum*) on different soil parameters. Bars represent mean values and standard errors. Different letters indicate significant differences between groups, and an asterisk (*) indicates significant differences between adjacent bars (α = 0.05). Abbreviations: g_{sw} (stomatal conductance), ETR (electron transport rate), ΦPSII (quantum yield of photosystem II), Fv/Fm (maximum quantum efficiency of PSII), RGR (relative growth rate), and RCD (root collar diameter).

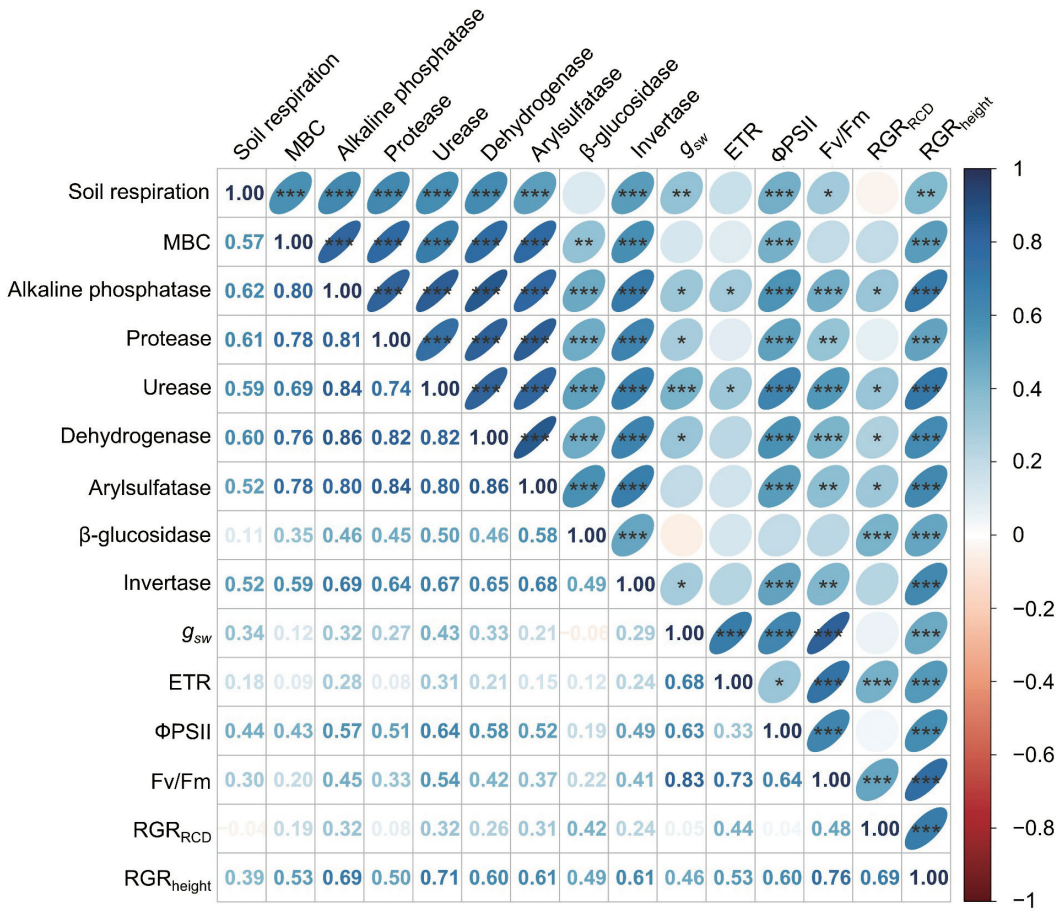

Figure 4. Spearman correlation coefficients (ρ) between parameters from soil properties and growth and physiological responses (n = 60). Color intensity represents the strength of relationships between parameters, with blue indicating positive correlations and red indicating negative correlations. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Abbreviations: MBC (microbial biomass carbon), g_{sw} (stomatal conductance), ETR (electron transport rate), ΦPSII (quantum yield of photosystem II), Fv/Fm (maximum quantum efficiency of PSII), RGR (relative growth rate), and RCD (root collar diameter).

association observed only with β-glucosidase (ρ = 0.42, $p < 0.001$).

DISCUSSION

Under coastal saline conditions, plant roots tend to accumulate large amounts of sodium (Na^+) and chloride (Cl^-) ions, leading to nutrient imbalances and reduced water uptake due to a lower osmotic potential outside the root system. This results in physiological drought (Mahajan and Tuteja 2005). Salt stress also induces the accumulation of reactive oxygen species (ROS), which impair the performance of photosystem I (PSI), photosystem II (PSII), and ETR (Oukarroum et al. 2015). This study revealed significant interspecific differences in the responses of native coastal tree species to salt stress. Among the three

species, *M. azedarach* was the most salt-sensitive, showing significant reductions in ΦPSII and Fv/Fm—two key indicators of photosynthetic efficiency—as well as a substantial suppression of height growth. By contrast, although *H. tiliaceus* exhibited salt-induced inhibition in both height and radial growth, its Fv/Fm remained unaffected, suggesting that PSII function remained stable and chloroplasts were not significantly damaged. Likewise, in *C. inophyllum*, both ΦPSII and Fv/Fm were unaffected by salinity, indicating that photosynthetic efficiency was maintained. The lack of apparent growth inhibition in *C. inophyllum* may be related to its characteristically slower growth during the first year after transplantation. Slow growth under environmental stress, such as salinity, is considered an adaptive trait that has evolved over time, enabling plants to conserve essential cellular materials and energy, maintain physiological stability, and enhance tolerance to stress (Zhu 2001). The salt tolerance characteristics observed in this study generally correspond to the species' natural coastal distributions. *Hibiscus tiliaceus* and *C. inophyllum* are typical front-shore coastal tree species (Kuo et al. 2014, Hsu et al. 2023) and likely possess greater inherent salt tolerance. Previous studies have reported that *H. tiliaceus* has effective Na^+/K^+ regulatory mechanisms, allowing it to adapt well to high-salinity environments and avoid ion toxicity (Yang et al. 2011). Meanwhile, *C. inophyllum* possesses thick, leathery leaves, and its well-developed cuticle and mesophyll structure effectively reduce water loss and protect against salt spray damage (Arora and Dagar 2019).

The main sources of soil enzymes are secretions from plant roots and soil microorganisms (Nannipieri et al. 2012). However, elevated soil salinity imposes osmotic stress on soil biota, resulting in reduced microbial biomass (Yan et al. 2015), decreased microbial community activity (Azadi et al. 2021), and suppressed microbial respiration (Sritongon et al. 2022). High salinity can also impair root growth, limiting the synthesis of extracellular enzymes (Hernández et al. 2006) and ultimately leading to reduced soil enzyme activity. In this study, the activities of all tested soil enzymes—except β-glucosidase—were reduced under high-salinity conditions, a trend consistent with findings from previous research (Pan et al. 2013, Singh 2016, Kumar et al. 2021). Boyrahmadi and Raiesi (2018) similarly observed that increasing soil salinity suppressed enzyme activity regardless of vegetation presence or type, indicating that salinity plays a more dominant role than plant species in influencing soil enzyme dynamics. This pattern aligns with the results of this study, in which most enzymes exhibited no significant interaction between salinity and species and generally showed lower activity in the high-salinity conditions. On the other hand, β-glucosidase, a key enzyme involved in cellulose degradation and the conversion of cellulose into sugars required for microbial metabolism, is widely recognized as an important indicator of soil quality (Martinez and Tabatabai 1997). In this study, its correlations with other enzymes were relatively weak, likely because its activity was not significantly suppressed by salinity. This may be attributed to the addition of peat to the planting holes. Peat is rich in cellulose, which can be decomposed into cellobiose—the catalytic substrate for β-glucosidase—thereby enhancing its activity and potentially offsetting the inhibitory effects of salinity (Vepsäläinen et al. 2004).

Bioremediation is considered an environmentally friendly approach for restoring salt-

affected areas. By leveraging plant–microbe interactions to overcome the adverse effects of salinity on plant growth, this method offers significant application potential (Arora et al. 2017). Results from this study showed that most soil enzyme activities were influenced by the interaction between *Trichoderma* inoculation and salinity levels. In low-salinity areas, *Trichoderma* significantly enhanced soil enzyme activities, whereas its effects were relatively reduced under high-salinity conditions, suggesting that salt stress inhibited the functional efficacy of *Trichoderma*. As noted by Rath and Rousk (2015), increased salinity elevates osmotic pressure in the environment, leading to microbial dehydration. To adapt, microbes expend substantial energy on synthesizing intracellular osmolytes (e.g., amino acids and carbohydrates), shifting resource allocation from growth to survival and reducing the production of proteins, including enzymes. Furthermore, high salinity may denature proteins, further decreasing enzyme solubility and activity. Nevertheless, this study found that *Trichoderma* inoculation still enhanced arylsulfatase activity under high-salinity conditions across all tested tree species. Arylsulfatase is a key enzyme in the soil sulfur cycle, responsible for mineralizing organic sulfur compounds into sulfate (SO_4^{2-}), which can be absorbed by plants (Klose et al. 2011). A study by Baum and Hrynkiewicz (2006) on *Salix* L. species demonstrated that arylsulfatase activity is primarily governed by the composition of rhizospheric saprophytic fungal communities (including *Trichoderma*), with a relatively minor influence from tree species differences. Similarly, our findings suggest that *Trichoderma* inoculation has cross-species potential for enhancing arylsulfatase activity in the rhizosphere of coastal tree species. Maintaining stable sulfur levels in plant tissues is crucial for salt tolerance, as sulfur is required for the synthesis of proteins and amino acids, including key compounds, such as membrane transport proteins (which help regulate ionic balance), osmoprotectants like proline, and antioxidants, such as glutathione (Nazar et al. 2011). Under high-salinity conditions, plants may release nutrients or signaling molecules via root exudates to interact with *Trichoderma* and other soil microorganisms, thereby enhancing arylsulfatase activity in the rhizosphere. This process helps stabilize sulfate availability and promotes sulfur uptake, ultimately improving plant tolerance to salinity stress (Zenda et al. 2021, Neemisha et al. 2022).

Notably, a significant three-way interaction for invertase activity was observed only in *H. tiliaceus*, where *Trichoderma* treatment significantly enhanced enzyme activity under high-salinity conditions. Compared to the other species, *H. tiliaceus* exhibits both rapid growth and high salt tolerance, along with a relatively well-developed root system capable of producing greater quantities of root exudates. Salt-tolerant species can also modulate the composition of their root exudates to recruit beneficial microbes that help mitigate salt stress (Kumar et al. 2023). Soluble sugar content in plant tissues is recognized as an important physiological indicator of salt tolerance (Liang et al. 2018). Salt-tolerant species often increase soluble sugar concentrations in their roots to regulate osmotic pressure and counteract salt stress (Rahneshan et al. 2018). Higher sugar concentrations in roots can elevate sucrose levels in root exudates, which are subsequently released into the soil (Hennion et al. 2019). Indeed, previous research has shown that salt-tolerant plant genotypes tend

to release higher levels of soluble sugars in their root exudates (Wu et al. 2012). Once released into the soil, sucrose can induce *Trichoderma* to produce invertase, which hydrolyzes sucrose into smaller sugars (Nakkeeran et al. 2020). Therefore, the observed enhancement of invertase activity by *Trichoderma* in *H. tiliaceus* may be attributed to the specific composition of its root exudates. By contrast, *C. inophyllum*, although highly salt-tolerant, exhibits slower early growth and may release fewer root exudates. Previous research has found no significant differences in root soluble sugar and starch contents in *C. inophyllum* across different salinity gradients (Su et al. 2024), suggesting that carbohydrate accumulation in the roots is not a primary strategy for salt tolerance and may explain the weak association with rhizospheric invertase activity. Similarly, the effects of *Trichoderma* on promoting alkaline phosphatase activity varied among tree species, with relatively more pronounced responses observed in *H. tiliaceus* and *C. inophyllum*, both characterized by high salt tolerance. Collectively, these results indicate that the ability of *Trichoderma* to enhance soil enzyme activity depends on complex, enzyme-specific interactions with plant traits, which in turn influence soil nutrient availability and plant growth performance.

From the growth and physiological monitoring results of different tree species, *Trichoderma* demonstrated a notable promotive effect on height growth in the slow-growing *C. inophyllum*, particularly under low-salinity conditions. In *M. azedarach*, *Trichoderma* treatment significantly increased both Fv/Fm and the RGR of RCD across salinity levels, suggesting its potential to enhance growth and physiological responses in salt-sensitive species. Under salt stress, a plant's capacity to scavenge ROS is widely recognized as a key determinant of salt tolerance (Parida and Das 2005). The field survival of various species in coastal environments is also closely linked to the degree of oxidative stress experienced by the plant (Su et al. 2024). Previous studies have shown that *Trichoderma* forms non-pathogenic symbiotic associations with plant roots, reprogramming the host immune system, inducing the expression of defense-related genes, and boosting antioxidant enzyme activity. It can also secrete antibiotics and other secondary metabolites that protect plants from both biotic and abiotic stressors (Hidangmayum and Dwivedi 2018, Khan et al. 2021). In this study, the salt-sensitive *M. azedarach* likely accumulated substantial ROS under high-salinity conditions. However, the *Trichoderma*-induced increase in antioxidant enzyme activity likely contributed to ROS detoxification, reducing damage to the photosynthetic system and thereby supporting improved plant growth (Zhang et al. 2016). This was further supported by increased ΦPSII values, indicating that *Trichoderma* enhanced photosynthetic efficiency across species and salinity conditions.

Increased soil salinity is widely recognized as a major environmental constraint on the growth and proliferation of soil microorganisms (Sardinha et al. 2003). Due to their high sensitivity to environmental disturbances, soil microbial activities—such as respiration, microbial biomass, and enzyme activity—are considered early indicators of changes in soil ecosystems. Singh (2016) noted that these microbial parameters serve as potential indicators for assessing soil degradation and the effectiveness of land management practices, and are generally suppressed under increasing salinity. In this study, soil respiration, MBC, and various enzyme

activities were generally positively correlated, indicating that salinity broadly inhibits microbial-associated indicators. The relatively weak correlation between soil respiration and other soil parameters may be attributed to methodological limitations: CO_2 release from soil respiration was measured in situ using surface soil with a restricted masking area. By contrast, MBC and enzyme activities were assessed using rhizosphere soil, resulting in stronger inter-parameter correlations. Because planting was in its early stage, height growth was more responsive to soil condition differences than radial growth, and it showed stronger associations with alkaline phosphatase and urease activity. Nitrogen and phosphorus—key macronutrients essential for synthesizing proteins, chlorophyll, and nucleic acids—are especially important in early plant development. Urease catalyzes the hydrolysis of urea into ammonia, which is subsequently transformed into plant-available forms, such as nitrate and ammonium. Alkaline phosphatase catalyzes the hydrolysis of ester-phosphate bonds in organic phosphorus compounds, releasing phosphate ions that can be absorbed by plants and microorganisms (Shi et al. 2019). Therefore, the activity of these two enzymes likely played a critical role in nitrogen and phosphorus release during early afforestation, contributing to observed differences in seedling growth. Moreover, Fv/Fm—a key indicator of PSII efficiency—was strongly correlated with both plant height and the two enzyme activities. This underscores the importance of a stable foliar photosynthetic system for seedling performance in saline-alkaline soils. As such, Fv/Fm can serve as a reliable physiological indicator for evaluating plant salt tolerance (Percival 2005).

CONCLUSIONS

Salt stress constrains seedling growth in afforestation areas, and the effectiveness of *Trichoderma* in improving soil quality and seedling performance varies by tree species, enzyme type, and salinity level. In this study, *Trichoderma* generally enhanced rhizosphere soil enzyme activity more effectively under low-salinity conditions. Under high salinity, its application promoted the activity of certain rhizosphere soil enzymes—such as invertase in *H. tiliaceus* and alkaline phosphatase in *C. inophyllum*—possibly due to species-specific differences in the composition and quantity of root exudates. Regardless of salinity levels, *Trichoderma* significantly increased arylsulfatase activity across all tree species, indicating its potential to promote sulfate transformation in soil. With respect to plant performance, *Trichoderma* improved the height growth of *C. inophyllum* under low-salinity conditions and enhanced both leaf Fv/Fm and the RGR of RCD in *M. azedarach* under both salinity levels, suggesting increased salt tolerance. It also consistently improved leaf ΦPSII values across tree species and salinity conditions, indicating broad benefits for photosynthetic efficiency. Overall, plant growth was significantly associated with rhizosphere enzyme activity. Among the enzymes, urease and alkaline phosphatase—key mediators of nitrogen and phosphorus conversion—emerged as critical factors in promoting plant growth in saline-alkali soils. MBC, which reflects the microbial biomass in soil, showed strong correlations with soil enzyme activities, indicating that the size of the rhizosphere microbial community significantly affects enzyme secretion and activity, thereby influencing soil nutrient availability. Overall, these findings demonstrate that applying

Trichoderma during coastal afforestation provides multiple benefits across different tree species and salinity levels, including enhanced seedling growth, physiological responses, and rhizosphere enzyme activities. This highlights its strong potential for improving afforestation outcomes and promoting soil restoration in salt-degraded environments.

LITERATURE CITED

Abdullah NS, Doni F, Mispan MS, et al. 2021. Harnessing *Trichoderma* in agriculture for productivity and sustainability. Agronomy 11(12):2559. https://doi.org/10.3390/agronomy11122559

Ahmad P, Hashem A, Abd-Allah EF, et al. 2015. Role of *Trichoderma* harzianum in mitigating NaCl stress in Indian mustard (*Brassica juncea* L.) through antioxidative defense system. Front Plant Sci 6:868. https://doi.org/10.3389/fpls.2015.00868

An SS, Huang YM, Zheng FL. 2009. Evaluation of soil microbial indices along a revegetation chronosequence in grassland soils on the Loess Plateau, Northwest China. Appl Soil Ecol 41(3):286-92. https://doi.org/10.1016/j.apsoil.2008.12.001

Araújo ASF, Cesarz S, Leite LFC, Borges CD, Tsai SM, Eisenhauer N. 2013. Soil microbial properties and temporal stability in degraded and restored lands of Northeast Brazil. Soil Bio and Biochem 66:175-81. https://doi.org/10.1016/j.soilbio.2013.07.013

Arora S, Dagar JC. 2019. Salinity tolerance indicators. In: Dagar J, Yadav R, Sharma P, editors. Research Developments in Saline Agriculture. Singapore: Springer. p 155-201.

Arora S, Singh AK, Sahni D. 2017. Bioremediation of salt-affected soils: Challenges and opportunities. In: Arora S, Singh A, Singh Y, editors. Bioremediation of Salt Affected Soils: An Indian Perspective. Cham, Switzerland: Springer. p 275-301.

Asghar W, Kataoka R. 2021. Effect of co-application of *Trichoderma* spp. with organic composts on plant growth enhancement, soil enzymes and fungal community in soil. Arch of Microbiol 203(7):4281-91. https://doi.org/10.1007/s00203-021-02413-4

Azadi N, Raiesi F. 2021. Salinization depresses soil enzyme activity in metal-polluted soils through increases in metal mobilization and decreases in microbial biomass. Ecotoxicology 30(6):1071-83. https://doi.org/10.1007/s10646-021-02433-2

Barrett-Lennard EG. 2002. Restoration of saline land through revegetation. Agric Water Manag 53(1-3):213-26. https://doi.org/10.1016/S0378-3774(01)00166-4

Baum C, Hrynkiewicz K. 2006. Clonal and seasonal shifts in communities of saprotrophic microfungi and soil enzyme activities in the mycorrhizosphere of *Salix* spp. J Plant Nutr Soil Sci 169(4):481-7. https://doi.org/10.1002/jpln.200521922

Boyrahmadi M, Raiesi F. 2018. Plant roots and species moderate the salinity effect on microbial respiration, biomass, and enzyme activities in a sandy clay soil. Biol Fertil Soils 54:509-21.

Che W, Piao J, Gao Q, Li X, Li X, Jin F. 2023. Response of soil physicochemical properties, soil nutrients, enzyme activity and rice yield to rice straw returning in highly saline-alkali paddy soils. J Soil Sci Plant Nutri 23(3):4396-411. https://doi.org/10.1007/s42729-023-01358-5

Chen ZS, Hseu ZY, Tsai CC. 2015. Soil degradation. In: The soils of Taiwan, editors. World Soils Book Series. Dordrecht, Netherlands: Springer. p 111-24.

Cheng L, Xu Z, Zhou X. 2023. Application of *Trichoderma* species increases plant salinity resistance: A bibliometric analysis and a meta-analysis. J Soil Sediment 23(7):2641-

53. https://doi.org/10.1007/s11368-023-03557-0

Contreras-Cornejo HA, Macías-Rodríguez L, Cortés-Penagos C, López-Bucio J. 2009. *Trichoderma virens,* a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in *Arabidopsis*. Plant Physiol 149(3):1579-92. https://doi.org/10.1104/pp.108.130369

Contreras-Cornejo HA, Schmoll M, Esquivel-Ayala BA, González-Esquivel CE, Rocha-Ramírez V, Larsen J. 2024. Mechanisms for plant growth promotion activated by *Trichoderma* in natural and managed terrestrial ecosystem. Microbiol Res 281:127621. https://doi.org/10.1016/j.micres.2024.127621

Dick RP, Burns RG. 2011. A brief history of soil enzymology research. Methods Soil Enzymol 9:1-34. https://doi.org/10.2136/sssabookser9.c1

Eivazi F, Tabatabai MA. 1988. Glucosidases and galactosidases in soils. Soil Bio Biochem 20(5):601-6. https://doi.org/10.1016/0038-0717(88)90141-1

El-Saadony MT, Saad AM, Soliman SM, et al. 2022. Plant growth-promoting microorganisms as biocontrol agents of plant diseases: Mechanisms, challenges and future perspectives. Front Plant Sci 13:923880. https://doi.org/10.3389/fpls.2022.923880

Fang Y, Singh BP, Collins D, Armstrong R, Van Zwieten L, Tavakkoli E. 2020. Nutrient stoichiometry and labile carbon content of organic amendments control microbial biomass and carbon-use efficiency in a poorly structured sodic-subsoil. Bio Fertil Soils 56(2):219-33. https://doi.org/10.1007/s00374-019-01413-3

Fu J, Liu Z, Li Z, Wang Y, Yang K. 2017. Alleviation of the effects of saline-alkaline stress on maize seedlings by regulation of active oxygen metabolism by *Trichoderma asperellum*. PLoS One 12(6):e0179617. https://doi.org/10.1371/journal.pone.0179617

Fu J, Xiao Y, Wang YF, Liu ZH, Yang KJ. 2019. *Trichoderma* affects the physiochemical characteristics and bacterial community composition of saline-alkaline maize rhizosphere soils in the cold-region of Heilongjiang Province. Plant Soil 436:211-27. https://doi.org/10.1007/s11104-018-03916-8

Guan Z, Luo Q, Chen X, et al. 2014. Saline soil enzyme activities of four plant communities in Sangong River basin of Xinjiang, China. J Arid Land 6:164-73. https://doi.org/10.1007/s40333-013-0223-6

Guo H, Yao J, Cai M, et al. 2012. Effects of petroleum contamination on soil microbial numbers, metabolic activity and urease activity. Chemosphere 87(11):1273-80. https://doi.org/10.1016/j.chemosphere.2012.01.034

Gupta SR, Dagar JC, Kumar M. 2016. Tree plantations in saline environments: Ecosystem services, carbon sequestration and climate change mitigation. In: Dagar J, Minhas P, editors. Agroforestry for the Management of Waterlogged Saline Soils and Poor-Quality Waters. Advances in Agroforestry, Vol. 13. New Delhi, India: Springer. p 181-95. https://doi.org/10.1007/978-81-322-2659-8_11

Hennion N, Durand M, Vriet C, et al. 2019. Sugars en route to the roots. Transport, metabolism and storage within plant roots and towards microorganisms of the rhizosphere. Physiol Plant 165(1):44-57. https://doi.org/10.1111/ppl.12751

Hernández-Allica J, Becerril JM, Zárate O, Garbisu C. 2006. Assessment of the efficiency of a metal phytoextraction process with biological indicators of soil health. Plant Soil 281:147-58.

Hidangmayum A, Dwivedi P. 2018. Plant responses to *Trichoderma* spp. and their tolerance to abiotic stresses: a review. J Phar-

macogn Phytochem 7(1):758-66.

Hsu CH, Fang WT, Chiu HK, Kao WC, Huang TS. 2023. Coastal forest structure survey and associated land crab population in Suao Dakenggu community, Yilan, Taiwan. Diversity 15(4):515. https://doi.org/10.3390/d15040515

Hunt R. 1982. Plant growth curves: The functional approach to plant growth analysis. London, UK: Edward Arnold Ltd. 260 p.

Ji S, Liu Z, Liu B, Wang Y, Wang J. 2020. The effect of *Trichoderma* biofertilizer on the quality of flowering Chinese cabbage and the soil environment. Sci Hortic 262:109069. https://doi.org/10.1016/j.scienta.2019.109069

Jyot G, Mandal K, Singh B. 2015. Effect of dehydrogenase, phosphatase and urease activity in cotton soil after applying thiamethoxam as seed treatment. Environ Monit Assess 187:298.

Khan MR, Parveen G, Zaid A, Wani SH, Jogaiah S. 2021. Potential of *Trichoderma* species in alleviating the adverse effects of biotic and abiotic stresses in plants. In: Jogaiah S, editor. Biocontrol Agents and Secondary Metabolites. Cambridge, UK: Woodhead Publishing. p 85-112. https://doi.org/10.1016/B978-0-12-822919-4.00005-3

Kirita H. 1971. Re-examination of the absorption method of measuring soil respiration under field conditions. IV. An improved absorption method using a disc of plastic sponge as absorbent holder. Jap J Ecol 21:119-27.

Klose S, Bilen S, Ali Tabatabai M, Dick WA. 2011. Sulfur cycle enzymes. Methods Soil Enzymol 9:125-59. https://doi.org/10.2136/sssabookser9.c7

Kumar A, Singh S, Mukherjee A, Rastogi RP, Verma JP. 2021. Salt-tolerant plant growth-promoting *Bacillus pumil*us strain JPVS11 to enhance plant growth attributes of rice and improve soil health under salinity stress. Microbiol Res 242:126616. https://doi.org/10.1016/j.micres.2020.126616

Kumar N, Haldar S, Saikia R. 2023. Root exudation as a strategy for plants to deal with salt stress: an updated review. Environ Exp Bot 216:105518. https://doi.org/10.1016/j.envexpbot.2023.105518

Kuo YL, Wang HH, Peng SH, Yang YP. 2014. Composition, structure, and preliminary restoration efforts of a tropical coastal forest at Siangjiaowan, Southern Taiwan. Taiwan J For Sci 29(4):267-84. https://doi.org/10.7075/TJFS.201412_29(4).0003

Ladd JN, Butler JHA. 1972. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. Soil Bio Biochem 4(1):19-30. https://doi.org/10.1016/0038-0717(72)90038-7

Li J, Pu L, Han M, Zhu M, Zhang R, Xiang Y. 2014. Soil salinization research in China: Advances and prospects. J Geogr Sci 24:943-60. https://doi.org/10.1007/s11442-014-1130-2

Liang W, Ma X, Wan P, Liu L. 2018. Plant salt-tolerance mechanism: a review. Biochem Biophys Res Commun 495(1):286-91. https://doi.org/10.1016/j.bbrc.2017.11.043

Liang Y, Yang Y, Yang C, Shen Q, Zhou J, Yang L. 2003. Soil enzymatic activity and growth of rice and barley as influenced by organic manure in an anthropogenic soil. Geoderma 115(1-2):149-60. https://doi.org/10.1016/S0016-7061(03)00084-3

Mahajan S, Tuteja N. 2005. Cold, salinity and drought stresses: an overview. Arch Biochem Biophys 444(2):139-58.

Mao T, Jiang X. 2021. Changes in microbial community and enzyme activity in soil under continuous pepper cropping in response to *Trichoderma hamatum* MHT1134 application. Sci Rep 11(1):21585. https://doi.org/10.1038/s41598-021-00951-x

Martinez CE, Tabatabai MA. 1997. Decomposition of biotechnology by-products in soils. J Environ Qual 26(3):625-32. https://doi.org/10.2134/jeq1997.00472425002600030006x

Mastouri F, Björkman T, Harman GE. 2010. Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings. Phytopathology 100(11):1213-21. https://doi.org/10.1094/PHYTO-03-10-0091

Naik K, Mishra S, Srichandan H, Singh PK, Sarangi PK. 2019. Plant growth promoting microbes: Potential link to sustainable agriculture and environment. Biocatal Agric Biotechnol 21:101326. https://doi.org/10.1016/j.bcab.2019.101326

Nakkeeran S, Rajamanickam S, Vanthana M, Renukadevi P, Muthamilan M. 2020. Harnessing the perception of *Trichoderma* signal molecules in rhizosphere to improve soil health and plant health. In: Sharma A, Sharma P, editors. *Trichoderma*. Rhizosphere Biology. Singapore: Springer. p 61-79.

Nannipieri P, Giagnoni L, Renella G, et al. 2012. Soil enzymology: Classical and molecular approaches. Bio Fertil Soils 48:743-62. https://doi.org/10.1007/s00374-012-0723-0

Nazar R, Iqbal N, Masood A, Syeed S, Khan NA. 2011. Understanding the significance of sulfur in improving salinity tolerance in plants. Environ Exp Bot 70(2-3):80-7. https://doi.org/10.1016/j.envexpbot.2010.09.011

Neemisha, Kumar A, Sharma P, Kaur A, Sharma S, Jain R. 2022. Harnessing rhizobacteria to fulfil inter-linked nutrient dependency on soil and alleviate stresses in plants. J Appl Microbiol 133(5):2694-716. https://doi.org/10.1111/jam.15649

Negm AM, Saavedra O, El-Adawy A. 2016. Nile Delta biography: Challenges and opportunities. In: Negm A, editor. The Nile Delta. The Handbook of Environmental Chemistry, vol 55. Cham, Switzerland: Springer. p 3-18.

Nicholls RJ, Marinova N, Lowe JA, et al. 2011. Sea-level rise and its possible impacts given a 'beyond 4℃ world' in the twenty-first century. Phil Trans R Soc A. 369(1934):161-81. http://doi.org/10.1098/rsta.2010.0291

Osman KT. 2018. Saline and sodic soils. In: Osman KT, editor. Management of Soil Problems. Cham, Switzerland: Springer. p 255-98.

Oukarroum A, Bussotti F, Goltsev V, Kalaji HM. 2015. Correlation between reactive oxygen species production and photochemistry of photosystems I and II in *Lemna gibba* L. plants under salt stress. Environ Exp Bot 109:80-8. https://doi.org/10.1016/j.envexpbot.2014.08.005

Pan C, Liu C, Zhao H, Wang Y. 2013. Changes of soil physico-chemical properties and enzyme activities in relation to grassland salinization. Eur J Soil Biol 55:13-9. https://doi.org/10.1016/j.ejsobi.2012.09.009

Parida AK, Das AB. 2005. Salt tolerance and salinity effects on plants: a review. Ecotox Environ Safe 60(3):324-49. https://doi.org/10.1016/j.ecoenv.2004.06.010

Percival GC. 2005. Identification of foliar salt tolerance of woody perennials using chlorophyll fluorescence. HortScience 40(6):1892-7.

Rahneshan Z, Nasibi F, Moghadam AA. 2018. Effects of salinity stress on some growth, physiological, biochemical parameters and nutrients in two pistachio (*Pistacia vera* L.) rootstocks. J Plant Interact 13(1):73-82. https://doi.org/10.1080/17429145.2018.1424355

Ramachandran A, Radhapriya P. 2016. Restoration of degraded soil in the Nanmangalam Reserve Forest with native tree species: Effect of indigenous plant growth-promoting bacteria. Sci World J 2016(1):5465841. https://doi.org/10.1155/2016/5465841

Rath KM, Rousk J. 2015. Salt effects on

the soil microbial decomposer community and their role in organic carbon cycling: a review. Soil Bio Biochem 81:108-23. https://doi.org/10.1016/j.soilbio.2014.11.001

Raynaud X. 2010. Soil properties are key determinants for the development of exudate gradients in a rhizosphere simulation model. Soil Bio Biochem 42(2):210-19. https://doi.org/10.1016/j.soilbio.2009.10.019

Sabzi-Nojadeh M, Pouresmaeil M, Amani M, Younessi-Hamzekhanlu M, Maggi F. 2024. Colonization of *Satureja hortensis* L. (Summer savory) with *Trichoderma harzianum* alleviates salinity stress via improving physio-biochemical traits and biosynthesis of secondary metabolites. Ind Crops Prod 208:117831. https://doi.org/10.1016/j.indcrop.2023.117831

Santoyo G, Orozco-Mosqueda MDC, Afridi MS, Mitra D, Valencia-Cantero E, Macías-Rodríguez L. 2024. *Trichoderma* and *Bacillus* multifunctional allies for plant growth and health in saline soils: Recent advances and future challenges. Front Microbiol 15:1423980. https://doi.org/10.3389/fmicb.2024.1423980

Sardinha M, Müller T, Schmeisky H, Joergensen RG. 2003. Microbial performance in soils along a salinity gradient under acidic conditions. Appl Soil Ecol 23(3):237-44. https://doi.org/10.1016/S0929-1393(03)00027-1

Sharma V, Salwan R, Sharma PN. 2017. The comparative mechanistic aspects of *Trichoderma* and probiotics: Scope for future research. Physiol Mo Plant Pathol 100:84-96. https://doi.org/10.1016/j.pmpp.2017.07.005

Shaygan M, Baumgartl T. 2022. Reclamation of salt-affected land: a review. Soil Syst 6(3):61. https://doi.org/10.3390/soilsystems6030061

Shi S, Tian L, Nasir F, et al. 2019. Response of microbial communities and enzyme activities to amendments in saline-alkaline soils. Appl Soil Ecol 135:16-24. https://doi.org/10.1016/j.apsoil.2018.11.003

Singh K. 2016. Microbial and enzyme activities of saline and sodic soils. Land Degrad Dev 27(3):706-18. https://doi.org/10.1002/ldr.2385

Song Y, Song C, Yang G, Miao Y, Wang J, Guo Y. 2012. Changes in labile organic carbon fractions and soil enzyme activities after marshland reclamation and restoration in the Sanjiang Plain in Northeast China. Environ Manag 50:418-26. https://doi.org/10.1007/s00267-012-9890-x

Sritongon N, Sarin P, Theerakulpisut P, Riddech N. 2022. The effect of salinity on soil chemical characteristics, enzyme activity and bacterial community composition in rice rhizospheres in Northeastern Thailand. Sci Rep 12(1):20360. https://doi.org/10.1038/s41598-022-24902-2

Stewart A, Hill R. 2014. Applications of *Trichoderma* in plant growth promotion. In: Gupta VK, Schmoll M, Herrera-Estrella A, Upadhyay RS, Druzhinina I, Tuohy MG, editors. Biotechnology and Biology of *Trichoderma*. Amsterdam, Netherlands: Elsevier. p 415-28.

Su TH, Shen Y, Chiang YY, et al. 2024. Species selection as a key factor in the afforestation of coastal salt-affected lands: Insights from pot and field experiments. J Environ Manag 360:121126. https://doi.org/10.1016/j.jenvman.2024.121126

Tabatabai MA, Bremner JM. 1970. Arylsulfatase activity of soils. Soil Sci Soc Am J 34(2):225-9. https://doi.org/10.2136/sssaj1970.03615995003400020016x

Vance ED, Brookes PC, Jenkinson DS. 1987. An extraction method for measuring soil microbial biomass-C. Soil Biol Biochem 19:703-7. https://doi.org/10.1016/0038-0717(87)90052-6

Vepsäläinen M, Erkomaa K, Kukkonen S,

Vestberg M, Wallenius K, Niemi RM. 2004. The impact of crop plant cultivation and peat amendment on soil microbial activity and structure. Plant Soil 264:273-86. https://doi.org/10.1023/B:PLSO.0000047763.46795.cb

Wu FZ, Zhou XG, Bao J. 2012. Effects of NaCl stress on the main components of root exudates of different salt-tolerant cucumber varieties. Chin J Agric Sci 45:4415-27. [in Chinese].

Wu J, Zhu J, Zhang D, et al. 2022. Beneficial effect on the soil microenvironment of *Trichoderma* applied after fumigation for cucumber production. PLos One 17(8):e0266347. https://doi.org/10.1371/journal.pone.0266347

Xie X, Pu L, Wang Q, Zhu M, Xu Y, Zhang M. 2017. Response of soil physicochemical properties and enzyme activities to long-term reclamation of coastal saline soil, Eastern China. Sci Total Environ 607:1419-27. https://doi.org/10.1016/j.scitotenv.2017.05.185

Yan N, Marschner P, Cao W, Zuo C, Qin W. 2015. Influence of salinity and water content on soil microorganisms. Int Soil Water Conserv Res 3(4):316-23. https://doi.org/10.1016/j.iswcr.2015.11.003

Yang G, Chen X, Tang T, et al. 2011. Comparative genomics of two ecologically differential populations of *Hibiscus tiliaceus* under salt stress. Funct Plant Biol 38(3):199-208. https://doi.org/10.1071/FP10228

Zenda T, Liu S, Dong A, Duan H. 2021. Revisiting sulphur—The once neglected nutrient: It's roles in plant growth, metabolism, stress tolerance and crop production. Agriculture 11(7):626. https://doi.org/10.3390/agriculture11070626

Zhang F, Wang Y, Liu C, et al. 2019. *Trichoderma harzianum* mitigates salt stress in cucumber via multiple responses. Ecotox Environ Safe 170:436-45. https://doi.org/10.1016/j.ecoenv.2018.11.084

Zhang HS, Zai XM, Wu XH, Qin P, Zhang WM. 2014. An ecological technology of coastal saline soil amelioration. Ecol Eng 67:80-8. https://doi.org/10.1016/j.ecoleng.2014.03.022

Zhang J. 2020. Soil environmental deterioration and ecological rehabilitation. In: Zhang J., editor. Study of Ecological Engineering of Human Settlements. Singapore: Springer. p 41-82.

Zhang S, Gan Y, Xu B. 2016. Application of plant-growth-promoting fungi *Trichoderma longibrachiatum* T6 enhances tolerance of wheat to salt stress through improvement of antioxidative defense system and gene expression. Front Plant Sci 7:1405. https://doi.org/10.3389/fpls.2016.01405

Zhang X, Dong W, Dai X, et al. 2015. Responses of absolute and specific soil enzyme activities to long term additions of organic and mineral fertilizer. Sci Total Environ 536:59-67. https://doi.org/10.1016/j.scitotenv.2015.07.043

Zhao ZJ, Hu JD, Chen K, Wei YL, Li JS. 2021. Effect of salt-tolerant *Trichoderma* ST02 on salt tolerance of tomato seed and seedling. Sci Technol Eng 21(7):2632-9. [In Chinese] https://doi.org/10.3969/j.issn.1671-1815.2021.07.010

Zheng L, Zhang M, Xiao R, Chen J, Yu F. 2017. Impact of salinity and Pb on enzyme activities of a saline soil from the Yellow River delta: A microcosm study. Phys Chem Earth Parts A/B/C 97:77-87. https://doi.org/10.1016/j.pce.2016.11.001

Zhu JK. 2001. Plant salt tolerance. Trends Plant Sci 6(2):66-71. https://doi.org/10.1016/S1360-1385(00)01838-0

Zin NA, Badaluddin NA. 2020. Biological functions of *Trichoderma* spp. for agriculture applications. Ann Agric Sci 65(2):168-78. https://doi.org/10.1016/j.aoas.2020.09.003

研究報告

施用木黴菌在不同土壤鹽度下對三種濱海樹種生長、生理反應及根際土壤酵素活性之影響

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摘 要

土壤鹽化是臺灣西部沿海土地退化的重要原因，透過植樹造林可改善土壤品質、強化生態功能並提升土地韌性。本研究於七股鹽害劣化地選擇三種濱海常見的原生樹種進行栽植試驗，透過添加哈茨木黴菌(*Trichoderma harzianum* Rifai, strain T2)確立微生物製劑在不同土壤鹽分條件下，對植物生長及土壤酵素活性的影響。結果顯示，木黴菌顯著提升了低鹽區多數根際土壤酵素活性，並在高鹽條件下提升黃槿(*Hibiscus tiliaceus* L.)的蔗糖酶及瓊崖海棠(*Calophyllum inophyllum* L.)的鹼性磷酸酶活性。此外，無論鹽分條件如何，木黴菌均能提高不同樹種的根際硫酸脂酶活性，顯示其促進土壤硫酸鹽轉化的潛力。在苗木生長方面，木黴菌在高鹽條件下提升了耐鹽性較低的楝樹(*Melia azedarach* L.)的Fv/Fm及根領直徑生長，改善其耐鹽性；木黴菌也促進了各樹種在不同鹽度條件下的ΦPSII，顯示其對光系統效能的廣泛改善效果。此外，根際土壤微生物生物量碳與各酵素活性呈高度正相關，其中鹼性磷酸酶和尿素酶的活性與植株高度生長密切相關，表明土壤微生物對土壤品質的改善和植株生長具有重要影響。綜上所述，雖然過高的鹽分會抑制樹木生長，但透過施用木黴菌能改善根際土壤酵素活性及促進苗木生長與生理健全，可做為造林初期良好的土壤改良劑，提升濱海造林的成效。

關鍵詞：植樹造林、植物促生真菌、微生物生物量碳、土壤改良劑、耐鹽性

劉宥廷、江曜宇、賴志銘、林鴻志、羅方岑、蘇子豪。2025。施用木黴菌在不同土壤鹽度下對三種濱海樹種生長、生理反應及根際土壤酵素活性之影響。台灣林業科學 40(2): 89-132。

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緒言

臺灣鹽害土壤面積約25,000 ha，主要分布在西部沿岸，成因為過度施肥或不當的灌溉(Chen et al. 2015)，以及超用地下水導致沿海地區受到海水入侵，土壤隨著含鹽地下水的入滲逐漸鹽化(Nicholls et al. 2011, Negm et al. 2017)。鹽害退化地的新植或復育造林(afforestation/reforestation)是一個重要且日益受到重視的領域，主要因其在生態保護、生物多樣性的維護、碳吸存及對抗氣候變化方面具關鍵作用(Gupta et al. 2016, Zhang 2020)。植樹造林也是減緩土壤鹽分累積的方法之一，可透過植體鹽分累積、蒸散作用降低地下水位、及有機質的輸入來改善土壤的物化性質(Barrett-Lennard 2002, Shaygan and Baumgartl 2022)。然而土壤鹽分增加會提高土壤的滲透勢，導致植物水分吸收受阻，同時也會造成離子毒害或養分供給失衡等，不利植物的生長與發育(Osman 2018)。因此，在造林前期透過生長促進劑來提升苗木的生長，是鹽害土壤成功造林的關鍵之一。其中，應用植物促生微生物(plant growth-promoting microorganisms, PGPM)如特定的真菌、細菌及放線菌等，能夠釋放維生素、激素和酵素等生物活性化合物(bioactive compounds)，有效刺激植物生長同時提升逆境耐受性，並誘導植物提升對病原體的免疫反應以抑制病害；還能分泌胞外多醣(exopolysaccharides)促進土壤團粒結構的形成，改善土壤水分及養分特性，被認為是恢復退化土地及達成永續農林業的重要關鍵(Ramachandran and Radhapriya 2016, Naik et al. 2019, El-Saadony et al. 2022)。

木黴菌屬(*Trichoderma* spp.)為絲狀真菌類，是廣泛存在於環境中的腐生性真菌，目前已普遍被運用於減緩作物的病害及提高生長與產量(Zin and Badaluddin 2020)。許多研究顯示，其施用能有效改善土壤的微生物群落結構，增進微生物豐度，對土壤品質具有正面影響(Mao and Jiang 2021, Wu et al. 2022)，同時也能促進植物的根系發展(Contreras-Cornejo et al. 2009)。木黴菌同時具有根際(rhizosphere)共生和內生(endophytic)特性，當其存在於根際作為自由生活的微生物時，能夠偵測宿主植物位置，並開始向根系生長進行定殖，透過分解有機物質和提供營養來促進植物的養分吸收；部分菌種也能進入植物組織共生，增強植物生長表現(Contreras-Cornejo et al. 2024)。此外，木黴菌接種的影響並不局限於定殖部位，而是能影響整個植物系統(Abdullah et al. 2021)。研究結果表明，木黴菌能夠影響植物根際的土壤特性，如養分利用性及土壤酵素活性等，同時可協助植物抵抗生物及非生物逆境 (Mastouri et al. 2010, Asghar and Kataoka 2021)。木黴菌與植物交互關係相當複雜，涵蓋生理、生化及分子層面的多重機制，能夠增強植物的抗病能力、促進養分吸收、強化碳水化合物代謝以及植物激素的合成(Stewart and Hill 2014, Sharma et al. 2017)。過去研究指出，施用木黴菌可增加植體滲透調節物質濃度，提升穀胱甘肽(glutathione)循環的效率，增加抗氧化酵素活性以及相關基因表達量等(Ahmad et al. 2015, Zhang et al. 2016, Fu et al. 2017)。其中，哈茨木黴菌(*T. harzianum* Rifai)是具有高環境適應性及病原菌拮抗能力的菌種，在過去針對鹽分逆境的研究中顯示，施用哈茨木黴菌能顯著提升植物的生長發育、促進根系發展增進養分吸收、及減緩植體氧化壓力等效益(Zhang et al. 2019, Sabzi-Nojadeh et al. 2024)。此外，哈茨木黴菌還能促進植物光合系統的效能，增加葉綠素含量並提升淨光合作用速率(Zhao et al. 2021)。

土壤酵素(soil enzyme)係指植物根系及微生物分泌，或是細胞凋亡後進入土壤的酵素，因此又稱為胞外酵素(extracellular enzyme)。土壤酵素參與土壤中多樣化的生化反應，在土壤養分轉換的過程中發揮關鍵作用，能將養分轉化為植物可使用的有效態，是土壤養分循環的重要指標(Song et al. 2012, Li et al. 2014)。此外，土壤酵素活性對環境變化的反應更為敏感和快速，比物化性質更能即時反映土壤生態系的狀況(Araújo et al. 2013, Zhang et al. 2015)。其中，關於土壤鹽分與土壤酵素的關係更是重要的研究焦點，如濱海鹽土經長期復育下，脫氫酶(dehydrogenase)、尿素酶(urease)、澱粉酶(amylase)和鹼性磷酸酶(alkaline

phosphatase)都因為土壤鹽分下降而提高了活性(Xie et al. 2017)。而在高鹽鹼化的水稻田中則發現，透過介質改良不僅提升土壤酵素的活性，同時也提高了水稻的產量(Che et al. 2023)。多數的實驗顯示土壤酵素活性與土壤的鹽分呈現負相關(Guan et al. 2014)，其中尿素酶的活性對鹽分和鹼化程度較為敏感，可作為土壤品質的指標(Zhang et al. 2014)。各類酵素對於土壤養分具有不同的影響，如尿素酶能促進含氮有機物水解，從而增進了氮的轉化和生物利用(Liang et al. 2003)；鹼性磷酸酶則參與磷循環，能將有機磷轉化為無機態，供植物利用(Dick and Burns 2011)。

木黴菌作為生物性肥料，能有效提升土壤酵素的活性，例如尿素酶、磷酸酶和過氧化氫酶(catalase)等，從而改善土壤中養分的有效性並增強微生物活性(Ji et al. 2020)。各類酵素在養分循環的過程中提供了不同的幫助，在過去的研究中已經揭示許多應用木黴菌對於鹽化土壤的酵素活性改善效益，及植物耐鹽性的促進效果(Fu et al. 2019, Cheng et al. 2023)。然而，多數前人研究普遍聚焦於農地及對作物產量的提升效益，有關於造林地土壤酵素的活性變化與樹木生長的關聯性研究則相對稀少。此外，木黴菌與植物間的共生關係較為複雜，實際應用時的成效常因不同環境、菌種或植物物種等而存在差異，同時許多研究也缺乏了野外驗證(Santoyo et al. 2024)。據此，為了更加了解不同樹種於鹽分逆境下，施用木黴菌是否具有植物生長或土壤品質的改善效益，本試驗調查了鹽害地區的造林地中，施用木黴菌對植穴根際的土壤酵素活性，以及對苗木的生長和生理反應等影響，期可釐清木黴菌應用於濱海鹽害劣化地造林中的具體效益。

材料與方法

實驗地點

本研究試驗地點位於臺南市七股區台61線七股鹽埕交流道出口(23°09’14.5”N, 120°05’16.8”E)，為工程棄置土方、石塊及既存鹽化土壤堆置而成，基地面積約2.5 ha，因鄰近潟湖，地下水位高且易受海水影響，導致基地土壤受到鹽害，造林綠化相當困難(Figure 1)。試驗地於2023年之月高溫發生於7月，平均29.5℃，最高溫34.3℃；月低溫發生於1月，平均17.0℃，最低溫8.8℃。2023年降雨量1,134.5 mm，集中分布於6-9月(約占全年雨量94%)。試驗基地低鹽區地勢比高鹽區高約50 cm，微地形差異導致降雨對土壤鹽分的淋洗與匯聚，形成明顯的鹽分分區。經2023年2月檢測表層0-30 cm土壤後顯示，高鹽區(H)平均土壤飽和土糊導電度(EC_e)為67.01 dS m^{-1}，鈉吸附比(sodium adsorption ratio, SAR)為34.83；低鹽區(L)平均EC_e為0.98 dS m^{-1}，SAR為11.80。

試驗設計與土壤採樣處理

本試驗於2023年3月進行，選定具自然土壤鹽分差異的高鹽與低鹽區作為主因子處理區，於各區設置三條重複樣帶(主區 replicates)，總計六個主區。每條樣帶挖設18個植穴 (50 cm × 50 cm × 50 cm)，行株距為1.3 m，合計配置108個植穴。供試樹種為具碳匯效益或耐鹽潛力之楝樹(*Melia azedarach* L.)、黃槿(*Hibiscus tiliaceus* L.)及瓊崖海棠(*Calophyllum inophyllum* L.)。木黴處理則分為接種哈茨木黴菌(T)與未接種對照組(C)，接種組使用市售T2菌株粒劑(龍瑩生物科技股份有限公司)，以1:200 (v/v)稀釋後施用於植穴中心30 × 30 cm、深10-30 cm處；對照組則未添加菌劑。為降低植穴初期鹽分壓力，植穴內土壤以無肥泥炭土與現地原土按1:2 (v/v)混合改良。各主區內共配置三種樹種 × 兩種菌處理組合(共6組)，並對每組設置三株生物重複，於主區內隨機配置。試驗共包含12種處理組合(3樹種 × 2木黴處理 × 2鹽分)，每組合計9株樣本(3主區 × 每區3株)，總計108株苗木。由於試驗地降雨時間分布高度不均，本研究另採用植穴滴灌供水方式，連結雨水感測器於未降雨日之6 a.m.及6 p.m.各灌溉20 min (7.8 L h^{-1})。

試驗處理持續至同年8月，總計約150天。於8月時進行植穴土壤呼吸(soil respiration)測定，並於測定完畢後進行土壤採樣。於樹冠邊際(依不

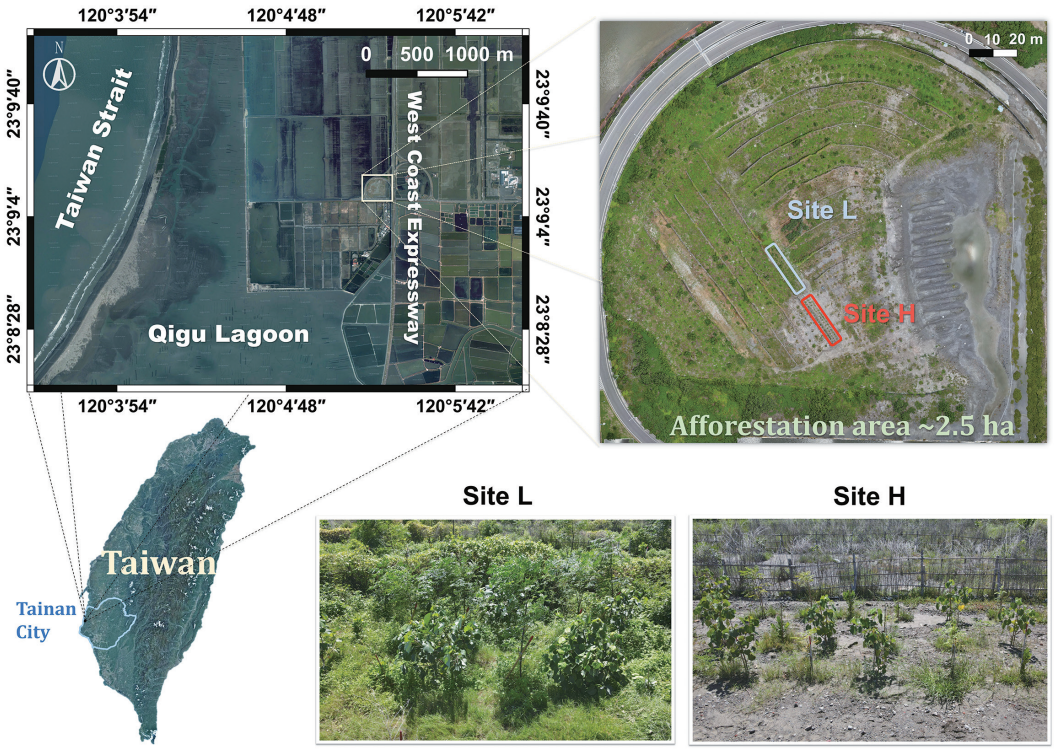

Figure 1. Locations of the study area and soil salinity sites. The sites are denoted as Site L (low soil salinity) and Site H (high soil salinity); Qigu Lagoon (七股潟湖).

同物種約距植株10-30 cm處)刨開表土並往植株中心以鋼刷小心刮除土壤，直至根部裸露後，收集深度15-20 cm處的根際土壤。根際土壤被定義為植物根分泌物能夠移動的範圍，受土壤含水率、根分泌物壽命及分泌速率等因素影響，約距離根系0.2-61 mm不等(Raynaud 2010)。本研究為獲取足量土壤進行分析，採樣範圍設定於根系周圍3 cm內。根際土壤攜回實驗室後盡速過篩(2 mm)，保存於-20℃環境，待後續進行微生物生物量碳(microbial biomass carbon, MBC)及酵素活性檢測。各植穴根際土壤取5 g以105℃烘至恆重測定含水率，依結果將新鮮土壤的MBC及酵素活性轉換為單位乾土重(dry weight, DW)之結果。此外，8月試驗結束時另針對植穴0-30 cm土壤性質進行測定，高鹽區平均土壤EC_e為11.44 dS m^{-1}，SAR為8.46；低鹽區平均土壤EC_e為0.65 dS m^{-1}，SAR為1.44。

生理參數與苗木生長

生理參數於2023年8月使用LI-600 porometer/fluorometer (LI-COR Inc., Lincoln, Nebraska, USA)分別於10 p.m.和9 a.m.測量暗適應及光適應相關參數(測定時間皆約1 h)。於夜間暗適應後量測葉片的光系統II最大光化學潛能(Fv/Fm)，並於上午光適應後則量測氣孔導度(g_{sw})、電子傳遞速率(electron transport rate, ETR)和光系統II實際光量子產量(ΦPSII)。各樹種每株量測頂芽向下第3葉位起，充分展開的年輕葉片共3片，後取平均值作為此株測值。苗木生長量測則於2023年5月及8月測定樹高(height)和根領直徑(root collar diameter, RCD)。RCD使用游標卡尺量測(mm)；樹高則使用樹高尺量測根領直徑標記處到頂芽讀數(cm)。針對二時間測定結果以公式1計算相對生長率(relative growth rate, RGR)(Hunt 1982)：

$$RGR = \frac{(\ln S_2 - \ln S_1)}{(t_2 - t_1)} \quad \text{.....(1)}$$

式中S_1及S_2為前及後測樹高或根領直徑、t_1及t_2為前及後測時間，樹高之RGR表示為RGR_{height} (cm cm^{-1} day^{-1})、RCD之RGR表示為RGR_{RCD} (mm mm^{-1} day^{-1})。

土壤呼吸

土壤呼吸採用鹼吸收法(alkali absorption method)進行測定，以鹼液吸附表土釋出的二氧化碳(CO_2)量來評估土壤呼吸的強度(Kirita 1971)。將塑膠三腳架立於距植株10-20 cm處的表土上方，上置盛裝10 mL 2M NaOH之直徑6 cm培養皿，並以300 mL的聚苯乙烯透明塑膠杯倒蓋(遮罩面積約44 cm^2)，杯緣輕撥外側土壤固定，並於現地放置24 h。另以聚乙烯塑膠膜覆蓋鄰近處土壤，以相同裝置製作空白組。於處理結束後，以去離子水將培養皿液體洗入離心管中，並定量至50 mL。攜回實驗室後取5 mL加入20 mL去離子水及酚酞指示劑1滴，以0.01 M HCl滴定至無色。再加入溴酚藍指示劑1滴，以0.01 M HCl滴定至黃色(滴定量mL計為V_1)；並以相同方法測定空白組(滴定量mL計為V_0)，依公式2計算土壤呼吸：

$$\text{Soil respiration (mg } CO_2 \text{ m}^{-2} \text{ h}^{-1}) = \frac{(V_0 - V_1)}{A \times T} \times N \times E \quad \text{.....(2)}$$

式中A為遮罩土壤面積(m^2)、T為處理時間(h)、N為滴定HCl濃度(M)、E為轉換係數(綜合單位轉換、CO_2莫耳質量及檢測體積等，此處為440)。

微生物生物量碳(MBC)

針對根際土壤的MBC，參考Vance et al. (1987)提出之氯仿熏蒸浸提法(chloroform-fumigation extraction method)進行分析。取10 g土壤做為對照組，加入0.5 M K_2SO_4後震盪1 h。另取10 g土壤做為熏蒸組，於玻璃真空乾燥器以無酒精氯仿熏蒸24 h (黑暗環境、22℃)，再加入0.5 M K_2SO_4後震盪1 h。二組土壤皆以玻璃纖維濾紙(Whatman GF/C)過濾，收集濾液後以總有機碳分析儀(Vario TOC, Elementar, Germany)測定溶解性有機碳(dissolved organic carbon, DOC)含量差異以評估土壤MBC (Fang et al. 2020)。MBC的計算方式如公式3：

$$\text{MBC (mg kg}^{-1} \text{ DW soil)} = \frac{(DOC_{fumigated} - DOC_{control})}{k_{ec} \times W} \quad \text{.....(3)}$$

式中$DOC_{fumigated}$及$DOC_{control}$分別為熏蒸組及控制組的DOC含量(mg)，k_{ec}為轉換係數(0.45)、W為新鮮土壤轉換後乾重(kg)。

土壤酵素活性

本實驗測量了根際土壤之蛋白酶(protease)、尿素酶(urease)、鹼性磷酸酶(alkaline phosphatase)、脫氫酶(dehydrogenase)、硫酸酯酶(arylsulfatase)、β-葡萄糖苷酶(β-glucosidase)及蔗糖酶(invertase)，共7個土壤酵素的活性。各酵素皆進行無土壤空白組及無基質空白組以扣除背景值，酵素活性檢測方法分述如下：

(1) 蛋白酶 (EC 3.4.21–24)

取1 g土壤加入2.5 mL酪蛋白酸鈉溶液，混合均勻後於50℃環境孵育1 h，再加入 1 mL 17.5%的三氯乙酸，以4,000 rpm離心10 min後，取0.5 mL上清液加入3 mL 2.8 N 碳酸鈉和1 mL福林酚試劑(Folin phenol reagent；稀釋3倍)，靜置15 min後取上清液量測700 nm吸光值，測定結果以酪胺酸(tyrosine)生成量表示蛋白酶活性(μg tyrosine h^{-1} g^{-1} DW soil)(Ladd and Butler 1972)。

(2) 尿素酶 (EC 3.5.1.5)

取5 g的土壤加入1 mL甲苯混合15 min後，依序加入10 mL 10%尿素及20 mL檸檬酸鹽緩衝液(pH6.7)，混合均勻置於37℃環境孵育24 h，以0.45 μm polytetrafluoroethylene (PTFE) syringe filter過濾後，取3 mL濾液依序加入4 mL苯酚鈉(1.35 M)、3 mL次氯酸鈉(active chlorine 0.9%)，靜置20 min後

定量至50 mL，量測578 nm吸光值，測定結果以銨態氮(NH_4^+-N)生成量表示尿素酶活性(μg NH_4^+-N h^{-1} g^{-1} DW soil)(Guo et al. 2012)。

(3) 鹼性磷酸酶 (EC 3.1.3.1)

取10 g土壤依序加入2 mL 甲苯、10 mL 苯基磷酸二鈉(0.05 M)以及10 mL 硼酸緩衝液(pH 9.4)，混合均勻後於37℃環境孵育3 h，以4,000 rpm離心10 min後，加入0.5 mL 2% 4-氨基安替比林和0.5 mL 8% 鐵氰化鉀，再以0.45 μm PTFE syringe filter過濾，取濾液量測510 nm吸光值，測定結果以苯酚(phenol)生成量表示鹼性磷酸酶活性(μg phenol h^{-1} g^{-1} DW soil)(An et al. 2009)。

(4) 脫氫酶 (EC 1.1.1.1)

取5 g土壤依序加入0.05 g 碳酸鈣、1 mL 3% triphenyl tetrazolium chloride (TTC)和2 mL DI water，混合均勻後置於37℃環境孵育24 h，將土壤轉移至裝有濾紙(Whatman No. 1)的布氏漏斗中，以甲醇清洗土壤至濾液無色後，收集濾液並定量至50 mL，取濾液量測485 nm吸光值，測定結果以1,3,5-triphenylformazan (TPF)生成量表示脫氫酶活性(μg TPF h^{-1} g^{-1} DW soil)(Jyot et al. 2015)。

(5) 硫酸酯酶 (EC 3.1.6.1)

取1 g土壤加入4 mL的醋酸鹽緩衝液、0.25 mL的甲苯及1 mL的對硝基苯硫酸溶液，並於37℃環境孵育1 h後，加入1 mL的氯化鈣(0.5 M)和4 mL氫氧化鈉(0.5 M)，以4,000 rpm離心10 min後，取上清液量測400 nm吸光值，測定結果以PNP生成量表示硫酸酯酶活性(μg PNP h^{-1} g^{-1} DW soil)(Tabatabai and Bremner 1970)。

(6) β-葡萄糖苷酶 (EC 3.2.1.21)

取1 g土壤加入0.25 mL的甲苯、4 mL的modified universal buffer (MUB)以及1 mL的p-nitrophenyl-β-D-glucoside (PNG)溶液，並於37℃環境孵育1 h，以4,000 rpm離心10 min後，再以0.45 μm PTFE syringe filter過濾，取濾液量測400 nm吸光值，測定結果以4-nitrophenol (PNP)生成量表示β-葡萄糖苷酶活性(μg PNP h^{-1} g^{-1} DW soil)(Eivazi and Tabatabai 1988)。

(7) 蔗糖酶 (EC 3.2.1.26)

取2 g土壤依序加入15 mL 8% 蔗糖溶液、5 mL 磷酸緩衝液及0.25 mL 甲苯，混合均勻後置於37℃環境孵育24 h，以0.45 μm PTFE syringe filter過濾後，取1 mL濾液與3 mL 3,5-Dinitrosalicylic acid (DNS)混合均勻，並以沸水浴加熱5 min，流水冷卻3 min後量測508 nm吸光值，測定結果以葡萄糖(glucose)生成量表示蔗糖酶活性(μg glucose h^{-1} g^{-1} DW soil)(Zheng et al. 2017)。

統計分析

統計分析採用一般線性模型(general linear model, GLM)進行三因子裂區變異數分析(split-plot three-way analysis of variance, ANOVA)，其中鹽度為主因子(whole plot)，樹種與木黴菌處理為子因子(sub-plot)。顯著水準設定為 $\alpha = 0.05$，並針對具顯著差異之固定因子或交互作用，進行Tukey 事後檢定(Tukey's HSD test)。此外，為探討植物生長、生理與土壤參數間的關聯性，採用斯皮爾曼等級相關分析(Spearman's rank correlation analysis)，並以 ρ 表示相關係數。所有統計分析與繪圖作業使用 R 軟體(v. 4.3.2; R Development Core Team, 2023)、SPSS 28 (IBM Corporation, Armonk, NY, USA)及 SigmaPlot 14.0 (Systat Software Inc., San Jose, CA, USA)執行。

結果

土壤性質之三因子分析

由土壤性質之三因子變異數分析結果顯示，土壤呼吸及MBC僅呈現顯著的鹽度主效應。而在各酵素活性中，鹼性磷酸酶具有顯著的木黴菌及樹種交互作用；蔗糖酶出現顯著的鹽度、木黴菌及樹種之三因子交互作用；其餘酵素則普遍呈現顯著的鹽度及木黴菌交互作用(Table 1)。從效應結果圖中可發現，土壤呼吸及MBC呈現低鹽度下顯著較高的趨勢，而樹種及木黴菌處理則未有顯著影響(Figure 2a, b)。在根際土壤酵素活性方面，蛋白酶與尿素酶趨勢相近，皆於低鹽度下顯著較高，其中木黴菌可於低鹽度條件下進一步提升二

Table 1. Three-way ANOVA results of soil properties

	Soil respiration		MBC		Protease	
Variables	*F*	*p* value	*F*	*p* value	*F*	*p* value
Sal	50.177	**< 0.001**	64.295	**< 0.001**	105.778	**< 0.001**
Tri	0.922	0.342	0.645	0.430	5.386	**0.025**
Spe	0.949	0.394	1.345	0.279	0.492	0.614
Sal × Tri	1.788	0.188	0.031	0.863	4.943	**0.031**
Sal × Spe	0.876	0.423	0.734	0.490	0.067	0.935
Tri × Spe	0.665	0.519	1.849	0.179	0.164	0.849
Sal × Tri × Spe	1.182	0.315	0.307	0.738	0.308	0.736
	Urease		**Alkaline phosphatase**		**Dehydrogenase**	
Variables	*F*	*p* value	*F*	*p* value	*F*	*p* value
Sal	123.301	**< 0.001**	280.297	**< 0.001**	189.336	**< 0.001**
Tri	30.947	**< 0.001**	12.296	**< 0.001**	31.014	**< 0.001**
Spe	2.777	0.072	8.231	**< 0.001**	3.570	**0.036**
Sal × Tri	15.189	**< 0.001**	3.421	0.071	37.089	**< 0.001**
Sal × Spe	2.710	0.077	0.529	0.593	1.093	0.343
Tri × Spe	0.008	0.992	3.711	**0.032**	2.397	0.102
Sal × Tri × Spe	0.461	0.633	0.732	0.486	0.701	0.501
	Arylsulfatase		**β-glucosidase**		**Invertase**	
Variables	*F*	*p* value	*F*	*p* value	*F*	*p* value
Sal	122.055	**< 0.001**	8.981	**0.004**	54.386	**< 0.001**
Tri	42.403	**< 0.001**	8.012	**0.007**	26.890	**< 0.001**
Spe	8.460	**< 0.001**	8.949	**< 0.001**	4.164	**0.022**
Sal × Tri	8.630	**0.005**	8.978	**0.004**	0.513	0.477
Sal × Spe	2.027	0.143	1.772	0.181	7.766	**0.001**
Tri × Spe	0.477	0.623	0.594	0.556	0.662	0.521
Sal × Tri × Spe	0.242	0.786	2.711	0.077	10.746	**< 0.001**

The *p*-values less than 0.05 are indicated in bold. Abbreviations: Sal (Salinity), Tri (*Trichoderma*), Spe (Species), and MBC (microbial biomass carbon).

酵素活性(Figure 2c, d)。鹼性磷酸酶除了在低鹽度條件下整體活性較高外，施用木黴菌還能消除原樹種之間的鹼性磷酸酶活性差異，顯著促進瓊崖海棠的酵素活性，並在不同鹽分下皆具效益(無三因子交互作用)(Figure 2e, f)。脫氫酶、硫酸酯酶及β-葡萄糖苷酶等整體趨勢接近，在鹽度及木黴菌的交互作用中，普遍出現低鹽度組活性相對較高、及低鹽度處理下施加木黴菌具活性促進效果；其中僅硫酸酯酶觀察到在高鹽度條件下施加木黴菌可顯著提升酵素活性，而β-葡萄糖苷酶則在未添加木黴菌的控制組中，鹽分差異未造成顯著影響(Figure 2g-i)；此外，三酵素皆存在顯著的樹種主效應，普遍以棟樹的根系酵素活性顯著最高(Figure 2j-l)。最後，蔗糖酶受三因子交互作用影響，添加木黴菌能在低鹽組顯著提升棟樹根際的蔗糖酶活性(Figure 2m)；黃槿則在高鹽組添加木黴菌後有較高的蔗糖酶活性(Figure 2n)；而瓊崖海棠根際的蔗糖酶活性不受鹽度與木黴菌之間交互作用的影響，但在低鹽組和添加木黴菌處理下均顯著較高，表明木黴菌的添加在不同鹽度條件下均具有促進效益(Figure 2o)。

苗木生長及生理參數之三因子分析

由苗木生長及生理參數之三因子變異數分析結果顯示，除了ETR在木黴菌處理下未呈現顯著主效應外，其餘各測定參數皆具有鹽度、木黴菌及樹種等顯著的主效應。而在交互作用檢定中，顯著的鹽度與木黴菌交互作用僅出現於g_{sw}；而ETR未觀察到任何顯著的交互作用；ΦPSII、Fv/Fm及RGR_{RCD}等在樹種與鹽度或木黴菌之間存在顯著交互作用；RGR_{RCD}則出現顯著的鹽度、木黴菌及樹種之三因子交互作用(Table 2)。從效應結果圖中可發現，g_{sw}除了在低鹽度條件下整體皆較高外，施用木黴菌也僅於低鹽組具有促進效

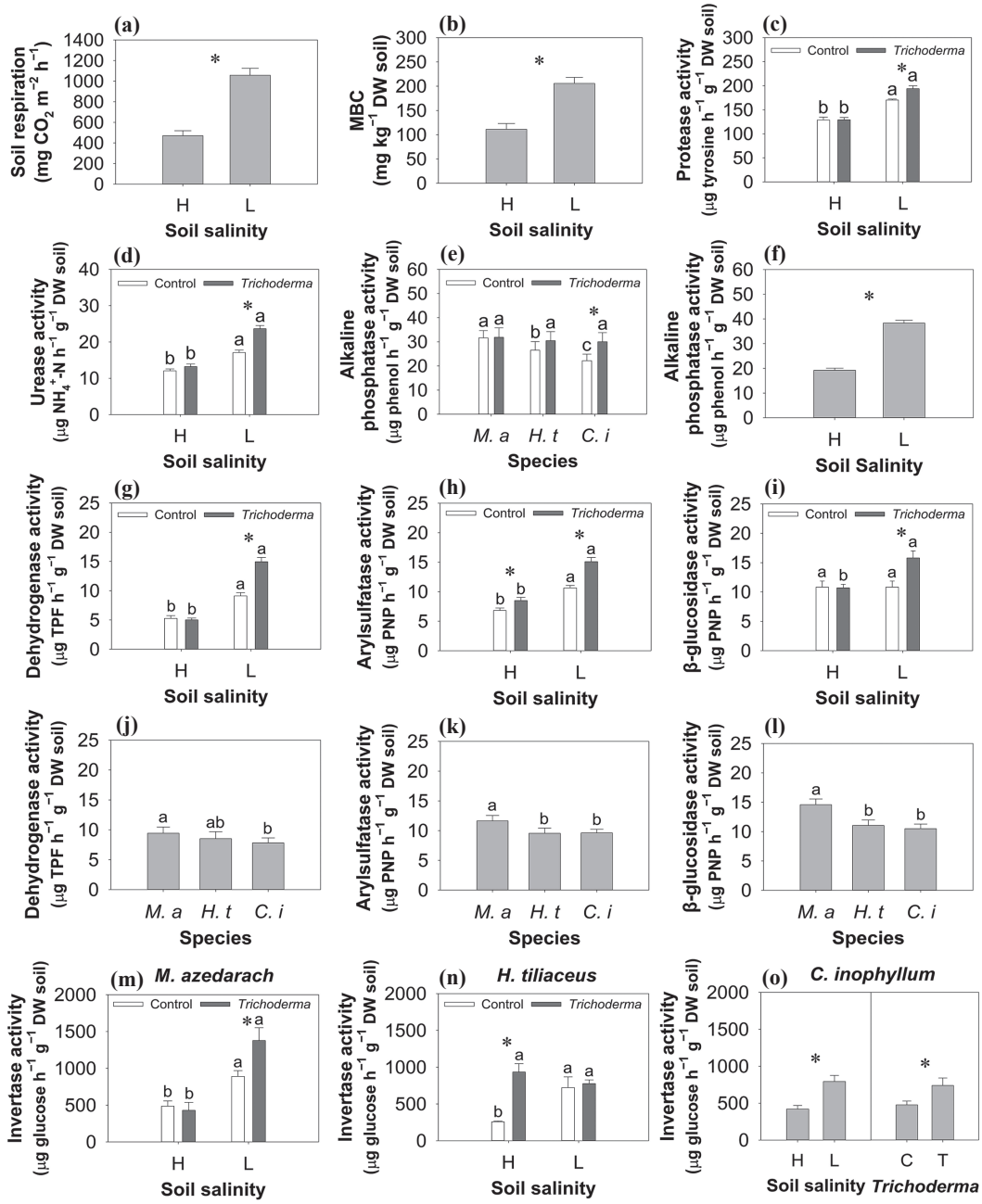

Figure 2. Effects of soil salinity, *Trichoderma* treatment, and tree species on (a) soil respiration, (b) microbial biomass carbon (MBC), and (c-o) soil enzyme activities. Panels show the main effects and interaction effects of soil salinity levels (H: high salinity, L: low salinity), *Trichoderma* treatments (Control [C], *Trichoderma* [T]), and tree species (*M. a*: *Melia azedarach*; *H. t*: *Hibiscus tiliaceus*; *C. i*: *Calophyllum inophyllum*) on different soil parameters. Bars represent mean values and standard errors. Different letters indicate significant differences between groups, and an asterisk (*) indicates significant differences between adjacent bars (α = 0.05).

Table 2 Three-way ANOVA results of the growth and physiological responses

	g_{sw}		ETR		ΦPSII	
Variables	*F*	*p* value	*F*	*p* value	*F*	*p* value
Sal	50.061	**< 0.001**	4.416	**0.041**	57.403	**< 0.001**
Tri	9.840	**0.003**	2.025	0.161	13.785	**< 0.001**
Spe	144.444	**< 0.001**	31.815	**< 0.001**	24.319	**< 0.001**
Sal × Tri	4.336	**0.043**	1.453	0.234	0.906	0.346
Sal × Spe	0.742	0.482	1.051	0.358	17.017	**< 0.001**
Tri × Spe	1.350	0.269	0.208	0.813	0.697	0.503
Sal × Tri × Spe	0.006	0.994	2.176	0.125	0.686	0.509
	Fv/Fm		**RGR_{RCD}**		**RGR_{height}**	
Variables	*F*	*p* value	*F*	*p* value	*F*	*p* value
Sal	26.936	**< 0.001**	10.286	**0.002**	96.734	**< 0.001**
Tri	11.787	**0.001**	38.324	**< 0.001**	20.962	**< 0.001**
Spe	30.339	**< 0.001**	192.646	**< 0.001**	42.344	**< 0.001**
Sal × Tri	0.733	0.396	0.042	0.839	1.466	0.232
Sal × Spe	7.627	**0.001**	8.511	**< 0.001**	18.531	**< 0.001**
Tri × Spe	3.526	**0.037**	16.833	**< 0.001**	2.915	0.064
Sal × Tri × Spe	1.448	0.245	2.387	0.103	8.618	**< 0.001**

The *p*-values less than 0.05 are indicated in bold. Abbreviations: Sal (Salinity), Tri (*Trichoderma*), Spe (Species), g_{sw} (stomatal conductance), ETR (electron transport rate), ΦPSII (quantum yield of photosystem II), Fv/Fm (maximum quantum efficiency of PSII), RGR (relative growth rate), and RCD (root collar diameter).

果(Figure 3a)；ETR則僅於鹽度主效應中顯示低鹽組整體較高(Figure 3b)。此外，g_{sw}及ETR皆存在顯著的樹種主效應，以黃槿的測值顯著最高(Figure 3c, d)。在ΦPSII、Fv/Fm及RGR_{RCD}的樹種與鹽度交互作用中，楝樹與黃槿普遍於低鹽顯著高於高鹽組，而瓊崖海棠則皆未因不同鹽度條件而觀察到顯著差異；其中在高鹽度條件下，楝樹的ΦPSII受到大幅抑制(0.57)，黃槿則維持穩定較高的Fv/Fm (0.82)(Figure 3e-g)。在木黴菌處理方面，於ΦPSII顯示添加木黴菌後具有顯著提升的主效應，且木黴菌與樹種或鹽度之間不存在顯著的交互作用，這表明木黴菌對ΦPSII的促進效益在不同鹽度和樹種間均一致存在(Figure 3h)。Fv/Fm和RGR_{RCD}的趨勢相似，施用木黴菌後僅在楝樹中顯示出顯著的促進效果(Figure 3i, j)。最後在RGR_{height}方面，木黴菌僅於低鹽環境下，對瓊崖海棠的樹高相對生長率具提升成效，而在楝樹及黃槿中皆只存在顯著的鹽分主效應，並以低鹽組相對較高(Figure 3k-m)。

各測值之相關性分析

土壤呼吸、MBC、根際土壤各酵素活性及苗木生長與生理測值間的相關性分析結果如Figure 4。其中除β-葡萄糖苷酶外，其餘各酵素活性皆與土壤呼吸及MBC間呈現顯著高度正相關(ρ = 0.52-0.80, $p < 0.001$)；同時β-葡萄糖苷酶與其他酵素的相關性多數較弱(ρ = 0.45-0.58)，其他酵素彼此間的相關性則普遍較強(ρ = 0.64-0.86)。光合系統相關參數ΦPSII及Fv/Fm亦與β-葡萄糖苷酶外的多數酵素呈顯著正相關，惟相關程度不一(ρ = 0.33-0.64, $p < 0.01/0.001$)，其中二光合參數主要與尿素酶的相關性較高。而g_{sw}及ETR則僅於彼此間呈顯著高度正相關(ρ = 0.68, $p < 0.001$)，與土壤性質的相關性則相對較弱。最後在生長參數方面，樹高相對生長率與根際土壤性質及各生理參數普遍呈現顯著中高度正相關(ρ = 0.46-0.76, $p < 0.01/0.001$)，其中在生理測值方面與Fv/Fm高度相關(ρ = 0.76)；在酵素活性方面則與鹼性磷酸酶(ρ = 0.69)及尿素酶(ρ = 0.71)的相關性較高。相較之下，根領直徑相對生長率與各測值間的相關性較弱，於各酵素中僅與β-葡萄糖苷酶的相關性相對明顯(ρ = 0.42, $p < 0.001$)。

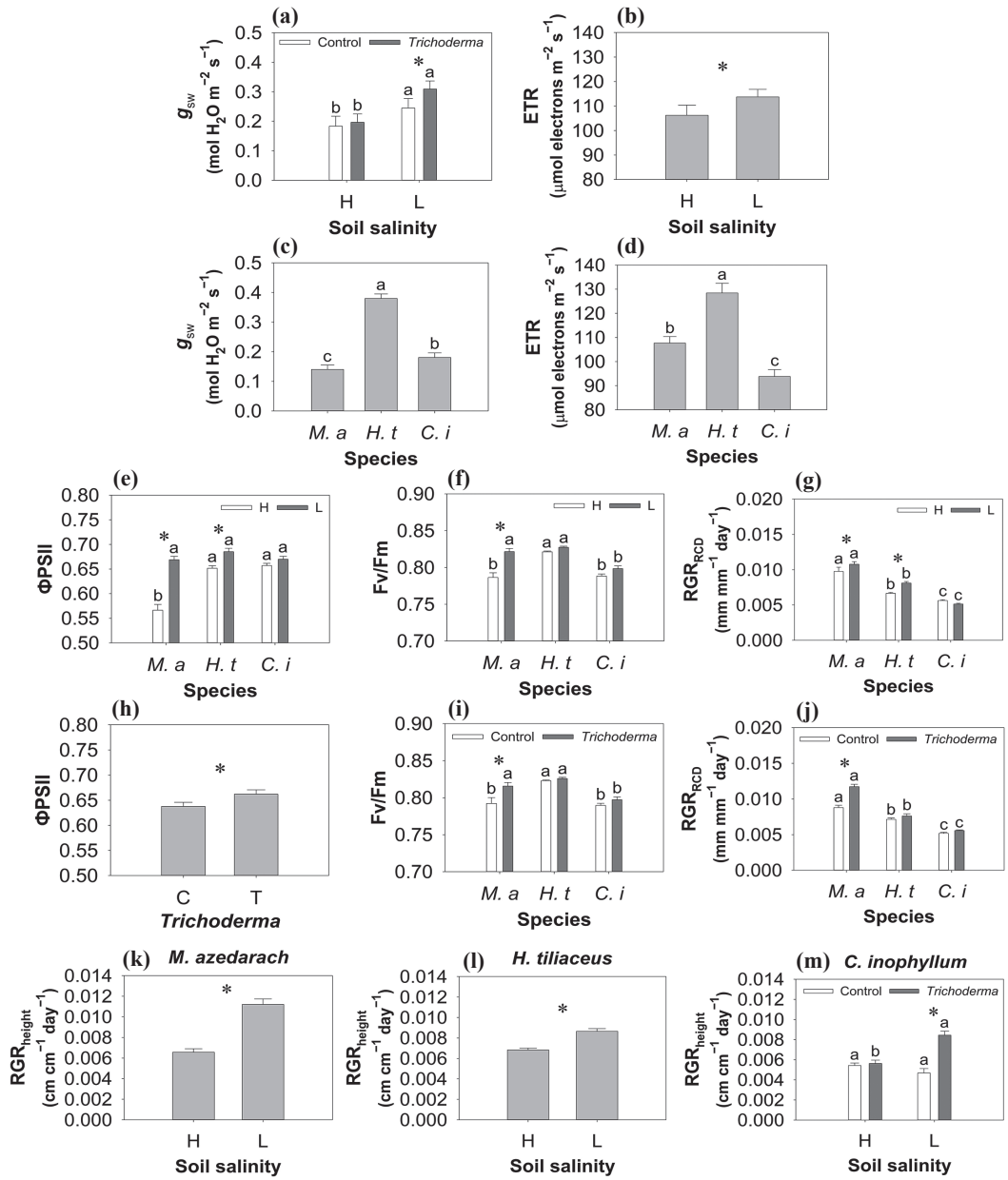

Figure 3. Effects of soil salinity, *Trichoderma* treatment, and tree species on growth and physiological responses. Panels show the main effects and interaction effects of soil salinity levels (H: high salinity, L: low salinity), *Trichoderma* treatments (Control [C], *Trichoderma* [T]), and tree species (*M. a*: *Melia azedarach*; *H. t*: *Hibiscus tiliaceus*; *C. i*: *Calophyllum inophyllum*) on different soil parameters. Bars represent mean values and standard errors. Different letters indicate significant differences between groups, and an asterisk (*) indicates significant differences between adjacent bars (α = 0.05). Abbreviations: g_{sw} (stomatal conductance), ETR (electron transport rate), ΦPSII (quantum yield of photosystem II), Fv/Fm (maximum quantum efficiency of PSII), RGR (relative growth rate), and RCD (root collar diameter).

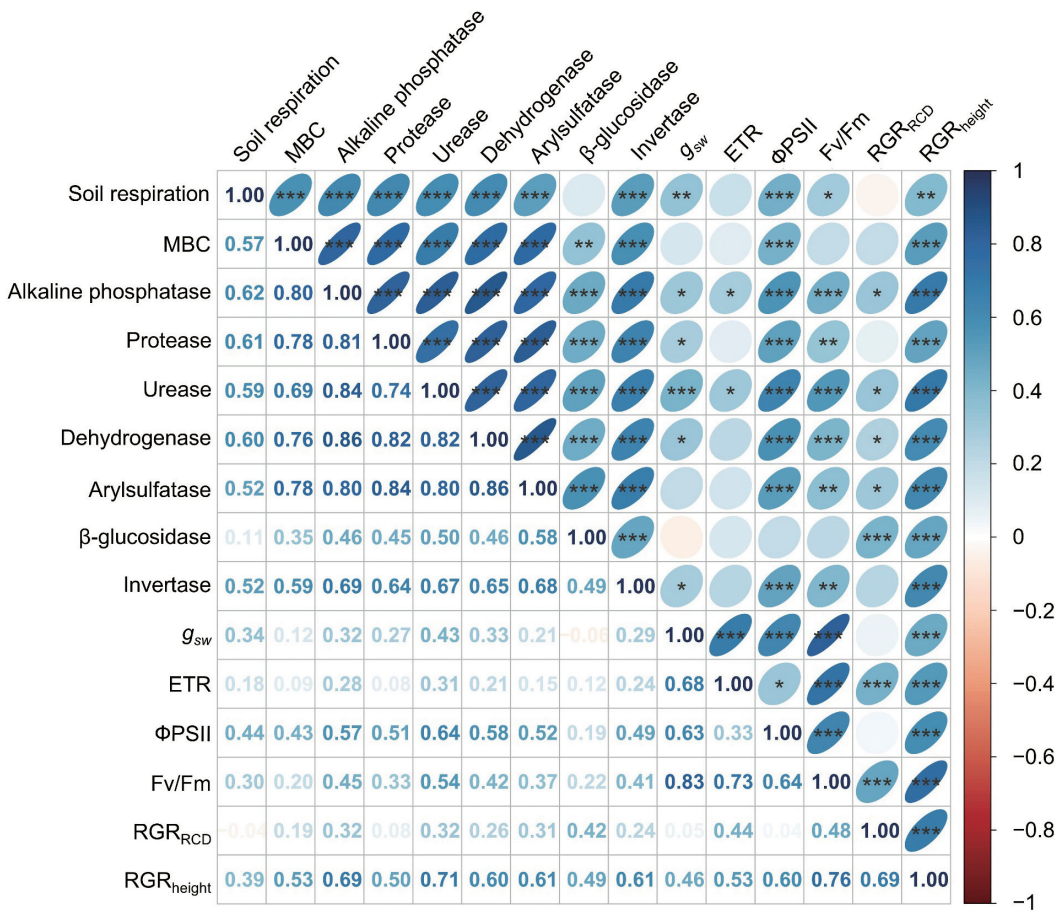

Figure 4. Spearman correlation coefficients (ρ) between parameters from soil properties and growth and physiological responses (n = 60). Color intensity represents the strength of relationships between parameters, with blue indicating positive correlations and red indicating negative correlations. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Abbreviations: MBC (microbial biomass carbon), g_{sw} (stomatal conductance), ETR (electron transport rate), ΦPSII (quantum yield of photosystem II), Fv/Fm (maximum quantum efficiency of PSII), RGR (relative growth rate), and RCD (root collar diameter).

討論

在濱海鹽分逆境下，植物根系容易累積大量的鈉及氯離子，除了導致養分失調外，還會因外界高鹽濃度導致根系周圍滲透勢降低，進而引發生理乾旱(physiological drought)，阻礙水分吸收(Mahajan and Tuteja 2005)。鹽分逆境同時會導致細胞活性氧物質(reactive oxygen species, ROS)的累積，並抑制PSI、PSII及ETR等效能(Oukarroum et al. 2015)。本研究結果發現，不同濱海原生樹種對於鹽分逆境的反應存在顯著差異，其中棟樹對於鹽分較敏感，反映光合系統狀態的ΦPSII及Fv/Fm皆受鹽分影響而顯著降低，高度生長也受到大幅度的抑制。相對地，黃槿在高度生長及徑向生長上雖受到鹽分抑制，但其Fv/Fm不受鹽分處理影響，顯示PSII保持穩定，葉綠體未受明顯損傷。此外，瓊崖海棠的ΦPSII及Fv/Fm也不受鹽分影響，維持良好的光合效能，其生長則未觀察到

明顯的鹽分抑制，可能與出栽後第一年生長相對其他樹種緩慢有關。當植物面對環境壓力如鹽分逆境時，成長緩慢被認為是一種長期演化下的適應性特徵，有助於節省構建細胞的基礎物質及能量，維持穩定的生理狀態和增加耐受性以應對逆境壓力(Zhu 2001)。試驗樹種的耐鹽特性整體符合植群的天然分布，其中黃槿及瓊崖海棠屬前岸喬木植群(Kuo et al. 2014, Hsu et al. 2023)，對於鹽分的耐受性可能較高。前人研究亦指出，黃槿具備良好的鈉鉀調節能力，在高鹽分環境適應良好，可避免細胞毒害(Yang et al. 2011)；瓊崖海棠則具有厚革質葉片特徵，其厚實的葉肉及發達的角質層能有效防止鹽霧損傷及避免水分散失(Arora and Dagar 2019)。

土壤酵素的主要來源是植物根系和土壤微生物的分泌(Nannipieri et al. 2012)。然而，較高的土壤鹽度會導致土壤生物面臨滲透調節逆境，從而減少土壤微生物的生物量(Yan et al. 2015)、降低微生物群落的活性(Azadi et al. 2021)、以及抑制土壤微生物的呼吸作用(Sritongon et al. 2022)。同時，高鹽度還會影響植物根系的生長，減少胞外酵素的合成量(Hernández et al. 2006)，最終導致土壤酵素活性受到抑制。本次實驗結果顯示，除了β-葡萄糖苷酶外，其餘所有土壤酵素的活性皆因較高的鹽度而降低，此趨勢與多數前人研究結果一致(Pan et al. 2013, Singh 2016, Kumar et al. 2021)。Boyrahmadi and Raiesi (2018)的研究顯示，隨著土壤鹽度增加，無論植被有無或種類區別，土壤酵素活性均受到抑制，這表明相比樹種差異，土壤鹽分對酵素活性的影響更為顯著。此現象與本試驗結果相似，即在鹽分逆境下，多數土壤酵素未顯示出顯著的鹽分與樹種交互作用，且普遍呈現高鹽組活性低於低鹽組的趨勢。另一方面，β-葡萄糖苷酶作為纖維素水解的重要分解酶之一，負責轉化土壤微生物所需的糖類養分，是土壤品質的重要指標(Martinez and Tabatabai 1997)。在本研究中，β-葡萄糖苷酶與其他酵素的相關性較低，主要是因為其於高鹽環境下並未受到顯著抑制，此結果可能與植穴中添加的泥炭土有關。由於泥炭土含有大量的纖維素，能分解為β-葡萄糖苷酶的催化底物(纖維二糖)，從而促進了該酵素的活性(Vepsäläinen et al. 2004)，使得鹽分對其抑制作用可能受到減弱。

在鹽害地區採用生物修復方式，被認為是更具環境友善的復育方法，利用植物與微生物交互作用(plant-microbe interaction)克服鹽害逆境對植物生長的影響，具有巨大的應用潛力(Arora et al. 2017)。本研究結果顯示，大部分土壤酵素活性受木黴菌與鹽度交互作用影響，其中木黴菌處理在低鹽區顯著提升了土壤酵素活性，而在高鹽條件下效果相對較差，顯示鹽分逆境對木黴菌的作用產生抑制。Rath and Rousk (2015)指出，鹽度提高會增加環境中的滲透壓，導致微生物細胞失水，為了適應這一逆境，微生物需耗費大量能量在胞內累積滲透調節物質(如胺基酸和碳水化合物)，這使得它們將資源從生長轉向生存，同時減少了蛋白質(包括酵素)的生產。此外，高鹽濃度也會使蛋白質變性，進一步降低酶的溶解度和活性。然而，本研究結果顯示，施用木黴菌仍能促進各樹種在高鹽條件下的硫酸脂酶活性。由於硫酸脂酶能夠將有機硫化物礦化為硫酸鹽(SO_4^{2-})供植物吸收，是土壤硫循環的重要關鍵(Klose et al. 2011)。從Baum and Hrynkiewicz (2006) 針對柳屬(*Salix* L.)的研究表明，硫酸脂酶活性受到根際腐生真菌(包含木黴菌)的群落組成所主導，樹種差異的效應則不明顯。本研究結果亦表明，木黴菌的施用對於提升濱海樹種根際土壤硫酸脂酶活性，具有跨物種的應用潛力。而植體硫含量的穩定對於耐鹽性也非常重要，硫是合成許多關鍵化合物的必要營養元素，涉及蛋白質及胺基酸的合成，如細胞膜轉運蛋白可調控離子平衡、滲透調節物質如脯胺酸的合成、及抗氧化物質如穀胱甘肽的合成等(Nazar et al. 2011)。因此在高鹽條件下，植物可能通過根分泌物提供養分或進行信號傳遞，協同木黴菌及其他微生物來增強根際硫酸脂酶的活性，從而穩定硫酸鹽的供應，促進植物對硫元素的吸收以增強其耐鹽性(Zenda et al. 2021, Neemisha et al. 2022)。

值得注意的是，在蔗糖酶的三因子交互作用中，僅黃槿在高鹽度下經木黴菌處理後蔗糖酶活

性顯著提高。與其他樹種相比，黃槿同時具備速生和耐鹽的特性，其相對發達的根系可以產生更多的根分泌物。同時耐鹽性高的樹種可透過調整根分泌物成分，吸引更多有益微生物來緩解鹽分壓力(Kumar et al. 2023)。過去研究指出，植體可溶糖含量被視為耐鹽性的重要生理指標(Liang et al. 2018)，而耐鹽性較高的植物能透過提高根部的可溶糖含量，調整植體滲透壓以抵抗鹽分逆境(Rahneshan et al. 2018)。根部較高濃度的糖類會增加根分泌物中的蔗糖含量，並將其釋放至土壤中(Hennion et al. 2019)。如過去研究即發現，耐鹽性較高的植物品系其根分泌物中可溶糖含量也顯著較高(Wu et al. 2012)。而蔗糖釋放至土壤後，可誘導木黴菌產生蔗糖酶，將蔗糖轉化為小分子糖類(Nakkeeran et al. 2020)。因此木黴菌僅對於黃槿根際具有蔗糖酶的促進效益，可能與該樹種根分泌物的特性有關。相較之下，瓊崖海棠作為高耐鹽性樹種，其初期生長較為緩慢，根分泌物釋出量可能有限。前人研究指出，瓊崖海棠根部的可溶糖與澱粉含量在不同梯度鹽分條件下均無顯著差異(Su et al. 2024)，顯示碳水化合物的根部累積並非其耐鹽策略，與根際蔗糖酶的關聯性可能不顯著。相似地，木黴菌對於鹼性磷酸酶的促進效益，在各樹種中成效不一，對於耐鹽性較高的黃槿及瓊崖海棠亦相對明顯。綜上顯示，木黴菌對於土壤酵素活性的提升效果，會因不同酵素種類存在與植物間複雜的交互作用，進而改變土壤不同養分的有效性，影響植物的生長表現。

從各樹種生長及生理監測結果可發現，木黴菌對於生長緩慢的瓊崖海棠具有良好的樹高生長促進效果，惟主要於低鹽區成效較為顯著。然而從楝樹的結果中表明，木黴菌處理可以提升不同鹽度下的Fv/Fm及根領直徑相對生長率，顯示對於耐鹽性較差的樹種，木黴菌可以改善其生長及生理反應。在鹽分逆境下，植物對於有害自由基的清除能力，被認為是影響耐鹽性的重要關鍵(Parida and Das 2005)。不同物種在濱海地區的野外存活表現，也與植體的氧化壓力高度相關(Su et al. 2024)。過去研究指出木黴菌在植物根系的共生特性，能夠以非致病性方式重塑植物的免疫系統，誘導防禦相關基因，提升植物抗氧化酵素活性，也能分泌抗生素及其他次級代謝物來保護植物，對於植物抵抗生物性及非生物性逆境皆具成效(Hidangmayum and Dwivedi 2018, Khan et al. 2021)。本試驗中由於鹽分敏感的楝樹在高鹽環境下可能產生大量ROS，而木黴菌誘導的抗氧化酵素活性增加，有助於清除植體累積的ROS以減緩光系統損傷，進而改善植物生長狀況(Zhang et al. 2016)。從ΦPSII的結果也能證實，木黴菌對於光合系統效能的提升，具有跨樹種及鹽度的促進效益。

土壤中的鹽分增加，被認為是影響土壤微生物生長及增殖最主要的環境限制因子(Sardinha et al. 2003)。土壤微生物活性對於環境擾動的敏感性高，能夠即時反映土壤生態系的微小變化。Singh (2016)指出土壤微生物活動(如土壤呼吸)、微生物生物量及土壤酵素等，是評估土壤退化程度和土地使用管理有效性的潛在指標，而當土壤鹽分增加時各類指標普遍會受到影響。在本研究中，土壤呼吸、MBC及各項土壤酵素普遍呈現正相關性，顯示各類土壤微生物相關指標多受鹽分抑制。其中，土壤呼吸與其他土壤參數的相關性程度相對較弱，主要由於本研究測定方式以現地表土進行CO_2釋放量測定，且遮罩面積有限；相對地，MBC與土壤酵素皆採根際土壤進行測定，二者的關聯性較高。另一方面，由於出栽時間尚短，植物的樹高生長對於土壤性質差異的反應，相對徑向生長更為明顯，並與鹼性磷酸酶及尿素酶的相關性程度較高。氮與磷是植物生長不可或缺的巨量元素，參與蛋白質、葉綠素及核酸等重要生物分子的合成。其中，尿素酶可將尿素分解為氨，進一步轉化為植物可吸收的硝酸鹽及銨鹽，提供植物所需的氮素。鹼性磷酸酶則催化有機磷化合物中的磷酸酯鍵(ester-phosphate bonds)的水解，釋放磷酸鹽供植物與微生物吸收(Shi et al. 2019)。因此，在造林初期，這兩種酵素的活性對土壤中氮磷養分的釋放具有重要影響，並可能導致植物生長出現顯著差異。此外，反映PSII健康狀態的Fv/Fm與樹高生長高度相關，且與尿素酶和鹼性磷酸酶的相關性也較高。這顯示葉部光合

系統的穩定性對於鹽鹼地植株的生長至關重要。而Fv/Fm可作為評估植物對鹽分逆境耐受性的良好生理指標(Percival 2005)。

結論

鹽分逆境造成造林地苗木生長受限，而木黴菌的應用對於改善土壤品質及苗木生長的效應，因不同樹種、土壤酵素種類或鹽分條件而異。本試驗結果顯示，木黴菌對植物根際土壤酵素的促進效益，普遍在低鹽度條件下較為明顯。而在高鹽度環境中，木黴菌的施用有助於提升高耐鹽性樹種的根際土壤酵素活性，如黃槿的蔗糖酶及瓊崖海棠的鹼性磷酸酶等，並可能與根分泌物的成分與釋出量有關。此外，無論鹽分條件差異，木黴菌均可顯著提高各樹種的硫酸脂酶活性，顯示其促進土壤硫酸鹽轉化的潛力。在生長表現方面，木黴菌在低鹽度條件下能促進瓊崖海棠的樹高增長。而在不同鹽度條件下，木黴菌對楝樹的葉部Fv/Fm值和根領直徑均有提升效果，顯示其耐鹽性得以增強。同時，木黴菌在不同樹種及鹽度條件下均能顯著提升葉部ΦPSII，展現其對光合系統改善的廣泛效益。綜合結果表明，植物生長與根際土壤酵素活性具有顯著相關性。其中，尿素酶和鹼性磷酸酶在氮磷養分的轉化中扮演重要角色，是促進鹽鹼地植株生長的關鍵因素。此外，反映土壤微生物生物量的MBC與各土壤酵素活性高度相關，顯示根際微生物群落的規模顯著影響酵素的分泌與活性，並進一步影響土壤養分的有效性。綜上顯示，於濱海地區造林過程中施用木黴菌製劑，對於不同樹種的生長、生理反應或土壤酵素活性多具正面效益，能夠增進鹽害劣化地的造林及土壤復育成效，具有良好的應用潛力。

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引用文獻

Abdullah NS, Doni F, Mispan MS, et al. 2021. Harnessing *Trichoderma* in agriculture for productivity and sustainability. Agronomy 11(12):2559. https://doi.org/10.3390/agronomy11122559

Ahmad P, Hashem A, Abd-Allah EF, et al. 2015. Role of *Trichoderma* harzianum in mitigating NaCl stress in Indian mustard (*Brassica juncea* L.) through antioxidative defense system. Front Plant Sci 6:868. https://doi.org/10.3389/fpls.2015.00868

An SS, Huang YM, Zheng FL. 2009. Evaluation of soil microbial indices along a revegetation chronosequence in grassland soils on the Loess Plateau, Northwest China. Appl Soil Ecol 41(3):286-92. https://doi.org/10.1016/j.apsoil.2008.12.001

Araújo ASF, Cesarz S, Leite LFC, Borges CD, Tsai SM, Eisenhauer N. 2013. Soil microbial properties and temporal stability in degraded and restored lands of Northeast Brazil. Soil Bio and Biochem 66:175-81. https://doi.org/10.1016/j.soilbio.2013.07.013

Arora S, Dagar JC. 2019. Salinity tolerance indicators. In: Dagar J, Yadav R, Sharma P, editors. Research Developments in Saline Agriculture. Singapore: Springer. p 155-201.

Arora S, Singh AK, Sahni D. 2017. Bioremediation of salt-affected soils: Challenges and opportunities. In: Arora S, Singh A, Singh Y, editors. Bioremediation of Salt Affected Soils: An Indian perspective. Cham, Switzerland: Springer. p 275-301.

Asghar W, Kataoka R. 2021. Effect of co-application of *Trichoderma* spp. with organic composts on plant growth enhance-

ment, soil enzymes and fungal community in soil. Arch of Microbiol 203(7):4281-91. https://doi.org/10.1007/s00203-021-02413-4

Azadi N, Raiesi F. 2021. Salinization depresses soil enzyme activity in metal-polluted soils through increases in metal mobilization and decreases in microbial biomass. Ecotoxicology 30(6):1071-83. https://doi.org/10.1007/s10646-021-02433-2

Barrett-Lennard EG. 2002. Restoration of saline land through revegetation. Agric Water Manag 53(1-3):213-26. https://doi.org/10.1016/S0378-3774(01)00166-4

Baum C, Hrynkiewicz K. 2006. Clonal and seasonal shifts in communities of saprotrophic microfungi and soil enzyme activities in the mycorrhizosphere of *Salix* spp. J Plant Nutr Soil Sci 169(4):481-7. https://doi.org/10.1002/jpln.200521922

Boyrahmadi M, Raiesi F. 2018. Plant roots and species moderate the salinity effect on microbial respiration, biomass, and enzyme activities in a sandy clay soil. Biol Fertil Soils 54:509-21.

Che W, Piao J, Gao Q, Li X, Li X, Jin F. 2023. Response of soil physicochemical properties, soil nutrients, enzyme activity and rice yield to rice straw returning in highly saline-alkali paddy soils. J Soil Sci Plant Nutri 23(3):4396-411. https://doi.org/10.1007/s42729-023-01358-5

Chen ZS, Hseu ZY, Tsai CC. 2015. Soil degradation. In: The soils of Taiwan, editors. World Soils Book Series. Dordrecht, Netherlands: Springer. p 111-24.

Cheng L, Xu Z, Zhou X. 2023. Application of *Trichoderma* species increases plant salinity resistance: A bibliometric analysis and a meta-analysis. J Soil Sediment 23(7):2641-53. https://doi.org/10.1007/s11368-023-03557-0

Contreras-Cornejo HA, Macías-Rodríguez L, Cortés-Penagos C, López-Bucio J. 2009. *Trichoderma virens,* a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in Arabidopsis. Plant Physiol 149(3):1579-92. https://doi.org/10.1104/pp.108.130369

Contreras-Cornejo HA, Schmoll M, Esquivel-Ayala BA, González-Esquivel CE, Rocha-Ramírez V, Larsen J. 2024. Mechanisms for plant growth promotion activated by *Trichoderma* in natural and managed terrestrial ecosystem. Microbiol Res 281:127621. https://doi.org/10.1016/j.micres.2024.127621

Dick RP, Burns RG. 2011. A brief history of soil enzymology research. Methods Soil Enzymol 9:1-34. https://doi.org/10.2136/sssabookser9.c1

Eivazi F, Tabatabai MA. 1988. Glucosidases and galactosidases in soils. Soil Bio Biochem 20(5):601-6. https://doi.org/10.1016/0038-0717(88)90141-1

El-Saadony MT, Saad AM, Soliman SM, et al. 2022. Plant growth-promoting microorganisms as biocontrol agents of plant diseases: Mechanisms, challenges and future perspectives. Front Plant Sci 13:923880. https://doi.org/10.3389/fpls.2022.923880

Fang Y, Singh BP, Collins D, Armstrong R, Van Zwieten L, Tavakkoli E. 2020. Nutrient stoichiometry and labile carbon content of organic amendments control microbial biomass and carbon-use efficiency in a poorly structured sodic-subsoil. Bio Fertil Soils 56(2):219-33. https://doi.org/10.1007/s00374-019-01413-3

Fu J, Liu Z, Li Z, Wang Y, Yang K. 2017. Alleviation of the effects of saline-alkaline stress on maize seedlings by regulation of active oxygen metabolism by *Trichoderma asperellum*. PLoS One 12(6):e0179617. https://doi.org/10.1371/journal.pone.0179617

Fu J, Xiao Y, Wang YF, Liu ZH, Yang KJ. 2019. *Trichoderma* affects the physiochem-

ical characteristics and bacterial community composition of saline-alkaline maize rhizosphere soils in the cold-region of Heilongjiang Province. Plant Soil 436:211-27. https://doi.org/10.1007/s11104-018-03916-8

Guan Z, Luo Q, Chen X, et al. 2014. Saline soil enzyme activities of four plant communities in Sangong River basin of Xinjiang, China. J Arid Land 6:164-73. https://doi.org/10.1007/s40333-013-0223-6

Guo H, Yao J, Cai M, et al. 2012. Effects of petroleum contamination on soil microbial numbers, metabolic activity and urease activity. Chemosphere 87(11):1273-80. https://doi.org/10.1016/j.chemosphere.2012.01.034

Gupta SR, Dagar JC, Kumar M. 2016. Tree plantations in saline environments: Ecosystem services, carbon sequestration and climate change mitigation. In: Dagar J, Minhas P, editors. Agroforestry for the Management of Waterlogged Saline Soils and Poor-Quality Waters. Advances in Agroforestry, Vol. 13. New Delhi, India: Springer. p 181-95. https://doi.org/10.1007/978-81-322-2659-8_11

Hennion N, Durand M, Vriet C, et al. 2019. Sugars en route to the roots. Transport, metabolism and storage within plant roots and towards microorganisms of the rhizosphere. Physiol Plant 165(1):44-57. https://doi.org/10.1111/ppl.12751

Hernández-Allica J, Becerril JM, Zárate O, Garbisu C. 2006. Assessment of the efficiency of a metal phytoextraction process with biological indicators of soil health. Plant Soil 281:147-58.

Hidangmayum A, Dwivedi P. 2018. Plant responses to *Trichoderma* spp. and their tolerance to abiotic stresses: a review. J Pharmacogn Phytochem 7(1):758-66.

Hsu CH, Fang WT, Chiu HK, Kao WC, Huang TS. 2023. Coastal forest structure survey and associated land crab population in Suao Dakenggu community, Yilan, Taiwan. Diversity 15(4):515. https://doi.org/10.3390/d15040515

Hunt R. 1982. Plant growth curves: The functional approach to plant growth analysis. London, UK: Edward Arnold Ltd. 260 p.

Ji S, Liu Z, Liu B, Wang Y, Wang J. 2020. The effect of *Trichoderma* biofertilizer on the quality of flowering Chinese cabbage and the soil environment. Sci Hortic 262:109069. https://doi.org/10.1016/j.scienta.2019.109069

Jyot G, Mandal K, Singh B. 2015. Effect of dehydrogenase, phosphatase and urease activity in cotton soil after applying thiamethoxam as seed treatment. Environ Monit Assess 187:298.

Khan MR, Parveen G, Zaid A, Wani SH, Jogaiah S. 2021. Potential of *Trichoderma* species in alleviating the adverse effects of biotic and abiotic stresses in plants. In: Jogaiah S, editor. Biocontrol Agents and Secondary Metabolites. Cambridge, UK: Woodhead Publishing. p 85-112. https://doi.org/10.1016/B978-0-12-822919-4.00005-3

Kirita H. 1971. Re-examination of the absorption method of measuring soil respiration under field conditions. IV. An improved absorption method using a disc of plastic sponge as absorbent holder. Jap J Ecol 21:119-27.

Klose S, Bilen S, Ali Tabatabai M, Dick WA. 2011. Sulfur cycle enzymes. Methods Soil Enzymol 9:125-59. https://doi.org/10.2136/sssabookser9.c7

Kumar A, Singh S, Mukherjee A, Rastogi RP, Verma JP. 2021. Salt-tolerant plant growth-promoting *Bacillus pumil*us strain JPVS11 to enhance plant growth attributes of rice and improve soil health under salinity stress. Microbiol Res 242:126616. https://doi.org/10.1016/j.micres.2020.126616

Kumar N, Haldar S, Saikia R. 2023. Root exudation as a strategy for plants to deal with

salt stress: an updated review. Environ Exp Bot 216:105518. https://doi.org/10.1016/j.envexpbot.2023.105518

Kuo YL, Wang HH, Peng SH, Yang YP. 2014. Composition, structure, and preliminary restoration efforts of a tropical coastal forest at Siangjiaowan, Southern Taiwan. Taiwan J For Sci 29(4):267-84. https://doi.org/10.7075/TJFS.201412_29(4).0003

Ladd JN, Butler JHA. 1972. Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. Soil Bio Biochem 4(1):19-30. https://doi.org/10.1016/0038-0717(72)90038-7

Li J, Pu L, Han M, Zhu M, Zhang R, Xiang Y. 2014. Soil salinization research in China: Advances and prospects. J Geogr Sci 24:943-60. https://doi.org/10.1007/s11442-014-1130-2

Liang W, Ma X, Wan P, Liu L. 2018. Plant salt-tolerance mechanism: a review. Biochem Biophys Res Commun 495(1):286-91. https://doi.org/10.1016/j.bbrc.2017.11.043

Liang Y, Yang Y, Yang C, Shen Q, Zhou J, Yang L. 2003. Soil enzymatic activity and growth of rice and barley as influenced by organic manure in an anthropogenic soil. Geoderma 115(1-2):149-60. https://doi.org/10.1016/S0016-7061(03)00084-3

Mahajan S, Tuteja N. 2005. Cold, salinity and drought stresses: an overview. Arch Biochem Biophys 444(2):139-58.

Mao T, Jiang X. 2021. Changes in microbial community and enzyme activity in soil under continuous pepper cropping in response to *Trichoderma hamatum* MHT1134 application. Sci Rep 11(1):21585. https://doi.org/10.1038/s41598-021-00951-x

Martinez CE, Tabatabai MA. 1997. Decomposition of biotechnology by-products in soils. J Environ Qual 26(3):625-32. https://doi.org/10.2134/jeq1997.00472425002600030006x

Mastouri F, Björkman T, Harman GE. 2010. Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings. Phytopathology 100(11):1213-21. https://doi.org/10.1094/PHYTO-03-10-0091

Naik K, Mishra S, Srichandan H, Singh PK, Sarangi PK. 2019. Plant growth promoting microbes: Potential link to sustainable agriculture and environment. Biocatal Agric Biotechnol 21:101326. https://doi.org/10.1016/j.bcab.2019.101326

Nakkeeran S, Rajamanickam S, Vanthana M, Renukadevi P, Muthamilan M. 2020. Harnessing the perception of *Trichoderma* signal molecules in rhizosphere to improve soil health and plant health. In: Sharma A, Sharma P, editors. *Trichoderma*. Rhizosphere Biology. Singapore: Springer. p 61-79.

Nannipieri P, Giagnoni L, Renella G, et al. 2012. Soil enzymology: Classical and molecular approaches. Bio Fertil Soils 48:743-62. https://doi.org/10.1007/s00374-012-0723-0

Nazar R, Iqbal N, Masood A, Syeed S, Khan NA. 2011. Understanding the significance of sulfur in improving salinity tolerance in plants. Environ Exp Bot 70(2-3):80-7. https://doi.org/10.1016/j.envexpbot.2010.09.011

Neemisha, Kumar A, Sharma P, Kaur A, Sharma S, Jain R. 2022. Harnessing rhizobacteria to fulfil inter-linked nutrient dependency on soil and alleviate stresses in plants. J Appl Microbiol 133(5):2694-716. https://doi.org/10.1111/jam.15649

Negm AM, Saavedra O, El-Adawy A. 2016. Nile Delta biography: Challenges and opportunities. In: Negm A, editor. The Nile Delta. The Handbook of Environmental Chemistry, vol 55. Cham, Switzerland: Springer. p 3-18.

Nicholls RJ, Marinova N, Lowe JA, et al. 2011. Sea-level rise and its possible impacts given a 'beyond 4℃ world' in the twenty-first

century. Phil Trans R Soc A. 369(1934):161-81. http://doi.org/10.1098/rsta.2010.0291

Osman KT. 2018. Saline and sodic soils. In: Osman KT, editor. Management of Soil Problems. Cham, Switzerland: Springer. p 255-98.

Oukarroum A, Bussotti F, Goltsev V, Kalaji HM. 2015. Correlation between reactive oxygen species production and photochemistry of photosystems I and II in *Lemna gibba* L. plants under salt stress. Environ Exp Bot 109:80-8. https://doi.org/10.1016/j.envexpbot.2014.08.005

Pan C, Liu C, Zhao H, Wang Y. 2013. Changes of soil physico-chemical properties and enzyme activities in relation to grassland salinization. Eur J Soil Biol 55:13-9. https://doi.org/10.1016/j.ejsobi.2012.09.009

Parida AK, Das AB. 2005. Salt tolerance and salinity effects on plants: a review. Ecotox Environ Safe 60(3):324-49. https://doi.org/10.1016/j.ecoenv.2004.06.010

Percival GC. 2005. Identification of foliar salt tolerance of woody perennials using chlorophyll fluorescence. HortScience 40(6):1892-7.

Rahneshan Z, Nasibi F, Moghadam AA. 2018. Effects of salinity stress on some growth, physiological, biochemical parameters and nutrients in two pistachio (*Pistacia vera* L.) rootstocks. J Plant Interact 13(1):73-82. https://doi.org/10.1080/17429145.2018.1424355

Ramachandran A, Radhapriya P. 2016. Restoration of degraded soil in the Nanmangalam Reserve Forest with native tree species: Effect of indigenous plant growth-promoting bacteria. Sci World J 2016(1):5465841. https://doi.org/10.1155/2016/5465841

Rath KM, Rousk J. 2015. Salt effects on the soil microbial decomposer community and their role in organic carbon cycling: a review. Soil Bio Biochem 81:108-23. https://doi.org/10.1016/j.soilbio.2014.11.001

Raynaud X. 2010. Soil properties are key determinants for the development of exudate gradients in a rhizosphere simulation model. Soil Bio Biochem 42(2):210-19. https://doi.org/10.1016/j.soilbio.2009.10.019

Sabzi-Nojadeh M, Pouresmaeil M, Amani M, Younessi-Hamzekhanlu M, Maggi F. 2024. Colonization of *Satureja hortensis* L. (Summer savory) with *Trichoderma harzianum* alleviates salinity stress via improving physio-biochemical traits and biosynthesis of secondary metabolites. Ind Crops Prod 208:117831. https://doi.org/10.1016/j.indcrop.2023.117831

Santoyo G, Orozco-Mosqueda MDC, Afridi MS, Mitra D, Valencia-Cantero E, Macías-Rodríguez L. 2024. *Trichoderma* and *Bacillus* multifunctional allies for plant growth and health in saline soils: Recent advances and future challenges. Front Microbiol 15:1423980. https://doi.org/10.3389/fmicb.2024.1423980

Sardinha M, Müller T, Schmeisky H, Joergensen RG. 2003. Microbial performance in soils along a salinity gradient under acidic conditions. Appl Soil Ecol 23(3):237-44. https://doi.org/10.1016/S0929-1393(03)00027-1

Sharma V, Salwan R, Sharma PN. 2017. The comparative mechanistic aspects of *Trichoderma* and probiotics: Scope for future research. Physiol Mo Plant Pathol 100:84-96. https://doi.org/10.1016/j.pmpp.2017.07.005

Shaygan M, Baumgartl T. 2022. Reclamation of salt-affected land: a review. Soil Syst 6(3):61. https://doi.org/10.3390/soilsystems6030061

Shi S, Tian L, Nasir F, et al. 2019. Response of microbial communities and enzyme activities to amendments in saline-alkaline soils. Appl Soil Ecol 135:16-24. https://doi.org/10.1016/j.apsoil.2018.11.003

Singh K. 2016. Microbial and enzyme activities of saline and sodic soils. Land Degrad

Dev 27(3):706-18. https://doi.org/10.1002/ldr.2385

Song Y, Song C, Yang G, Miao Y, Wang J, Guo Y. 2012. Changes in labile organic carbon fractions and soil enzyme activities after marshland reclamation and restoration in the Sanjiang Plain in Northeast China. Environ Manag 50:418-26. https://doi.org/10.1007/s00267-012-9890-x

Sritongon N, Sarin P, Theerakulpisut P, Riddech N. 2022. The effect of salinity on soil chemical characteristics, enzyme activity and bacterial community composition in rice rhizospheres in Northeastern Thailand. Sci Rep 12(1):20360. https://doi.org/10.1038/s41598-022-24902-2

Stewart A, Hill R. 2014. Applications of *Trichoderma* in plant growth promotion. In: Gupta VK, Schmoll M, Herrera-Estrella A, Upadhyay RS, Druzhinina I, Tuohy MG, editors. Biotechnology and Biology of *Trichoderma*. Amsterdam, Netherlands: Elsevier. p 415-28.

Su TH, Shen Y, Chiang YY, et al. 2024. Species selection as a key factor in the afforestation of coastal salt-affected lands: Insights from pot and field experiments. J Environ Manag 360:121126. https://doi.org/10.1016/j.jenvman.2024.121126

Tabatabai MA, Bremner JM. 1970. Arylsulfatase activity of soils. Soil Sci Soc Am J 34(2):225-9. https://doi.org/10.2136/sssaj1970.03615995003400020016x

Vance ED, Brookes PC, Jenkinson DS. 1987. An extraction method for measuring soil microbial biomass-C. Soil Biol Biochem 19:703-7. https://doi.org/10.1016/0038-0717(87)90052-6

Vepsäläinen M, Erkomaa K, Kukkonen S, Vestberg M, Wallenius K, Niemi RM. 2004. The impact of crop plant cultivation and peat amendment on soil microbial activity and structure. Plant Soil 264:273-86. https://doi.org/10.1023/B:PLSO.0000047763.46795.cb

Wu FZ, Zhou XG, Bao J. 2012. Effects of NaCl stress on the main components of root exudates of different salt-tolerant cucumber varieties. Chin J Agric Sci 45:4415-27. [in Chinese].

Wu J, Zhu J, Zhang D, et al. 2022. Beneficial effect on the soil microenvironment of *Trichoderma* applied after fumigation for cucumber production. PLos One 17(8):e0266347. https://doi.org/10.1371/journal.pone.0266347

Xie X, Pu L, Wang Q, Zhu M, Xu Y, Zhang M. 2017. Response of soil physicochemical properties and enzyme activities to long-term reclamation of coastal saline soil, Eastern China. Sci Total Environ 607:1419-27. https://doi.org/10.1016/j.scitotenv.2017.05.185

Yan N, Marschner P, Cao W, Zuo C, Qin W. 2015. Influence of salinity and water content on soil microorganisms. Int Soil Water Conserv Res 3(4):316-23. https://doi.org/10.1016/j.iswcr.2015.11.003

Yang G, Chen X, Tang T, et al. 2011. Comparative genomics of two ecologically differential populations of *Hibiscus tiliaceus* under salt stress. Funct Plant Biol 38(3):199-208. https://doi.org/10.1071/FP10228

Zenda T, Liu S, Dong A, Duan H. 2021. Revisiting sulphur—The once neglected nutrient: It's roles in plant growth, metabolism, stress tolerance and crop production. Agriculture 11(7):626. https://doi.org/10.3390/agriculture11070626

Zhang F, Wang Y, Liu C, et al. 2019. *Trichoderma harzianum* mitigates salt stress in cucumber via multiple responses. Ecotox Environ Safe 170:436-45. https://doi.org/10.1016/j.ecoenv.2018.11.084

Zhang HS, Zai XM, Wu XH, Qin P, Zhang WM. 2014. An ecological technology of coastal saline soil amelioration. Ecol Eng 67:80-8. https://doi.org/10.1016/j.ecoleng.2014.03.022

Zhang J. 2020. Soil environmental deteriora-

tion and ecological rehabilitation. In: Zhang J., editor. Study of Ecological Engineering of Human Settlements. Singapore: Springer. p 41-82.

Zhang S, Gan Y, Xu B. 2016. Application of plant-growth-promoting fungi *Trichoderma longibrachiatum* T6 enhances tolerance of wheat to salt stress through improvement of antioxidative defense system and gene expression. Front Plant Sci 7:1405. https://doi.org/10.3389/fpls.2016.01405

Zhang X, Dong W, Dai X, et al. 2015. Responses of absolute and specific soil enzyme activities to long term additions of organic and mineral fertilizer. Sci Total Environ 536:59-67. https://doi.org/10.1016/j.scitotenv.2015.07.043

Zhao ZJ, Hu JD, Chen K, Wei YL, Li JS. 2021. Effect of salt-tolerant *Trichoderma* ST02 on salt tolerance of tomato seed and seedling. Sci Technol Eng 21(7):2632-9. [In Chinese] https://doi.org/10.3969/j.issn.1671-1815.2021.07.010

Zheng L, Zhang M, Xiao R, Chen J, Yu F. 2017. Impact of salinity and Pb on enzyme activities of a saline soil from the Yellow River delta: A microcosm study. Phys Chem Earth Parts A/B/C 97:77-87. https://doi.org/10.1016/j.pce.2016.11.001

Zhu JK. 2001. Plant salt tolerance. Trends Plant Sci 6(2):66-71. https://doi.org/10.1016/S1360-1385(00)01838-0

Zin NA, Badaluddin NA. 2020. Biological functions of *Trichoderma* spp. for agriculture applications. Ann Agric Sci 65(2):168-78. https://doi.org/10.1016/j.aoas.2020.09.003