Effects of Ectomycorrhizal Fungi on the Physiological and Ecological Characteristics of Larix gmelinii under Salt Stress

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Research Article

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Abstract

Soil salinization has become a major constraint on afforestation and forest regeneration in Northeast China, limiting the growth and development of *Larix gmelinii*. Enhancing the salt tolerance of *L. gmelinii* is therefore of considerable importance, and ectomycorrhizal fungi (ECMF) inoculation has been shown to improve plant stress resistance. However, the mechanisms by which ECMF enhance salt tolerance in *L. gmelinii* remain poorly understood. In this study, *L. gmelinii* seedlings inoculated with an ECMF consortium consisting of *Boletus edulis* and *Gomphidius rutulus* were subjected to 120 mM NaCl stress. Photosynthetic characteristics, antioxidant enzyme activities, osmotic adjustment substances, ion contents, and metabolite profiles were analyzed to elucidate the physiological mechanisms underlying ECMF-mediated salt tolerance. The results showed that ECMF inoculation promoted the growth of *L. gmelinii* under salt stress. ECMF alleviated the decline in net photosynthetic rate caused by stomatal limitation and maintained photosynthetic performance. Under salt stress, *L. gmelinii* primarily scavenged reactive oxygen species through increased peroxidase activity, activation of ascorbate and aldarate metabolism, and enhanced flavonoid biosynthesis. Furthermore, ECMF inoculation enhanced galactose metabolism and amino acid biosynthesis, resulting in increased soluble sugar and soluble protein contents and improved osmotic adjustment capacity. In addition, ECMF improved ion homeostasis by promoting K^+ translocation to shoots while restricting Na^+ transport and enhancing Na^+ retention in roots. These findings demonstrate that ECMF enhance salt tolerance in *L. gmelinii* through coordinated regulation of photosynthesis, antioxidant defense, osmotic adjustment, and ion homeostasis, providing a theoretical basis for the application of ECMF in saline-alkali land afforestation.

1. Introduction

Soil salinization is one of the major global land degradation issues(Hassani et al. 2021), and northeastern China represents a key salinized region characterized by extensive distribution(Dai et al. 2025) (accounting for 19.7% of the total land area of the Songnen Plain)(Ma et al. 2024) and rapid expansion(Ding et al. 2025). Soil salinization exerts multiple inhibitory effects on plant growth and development, thereby posing substantial challenges to afforestation projects(Acharya et al. 2024). When salt concentrations exceed the tolerance threshold of plants, excessive Na^+ accumulation disrupts ionic homeostasis and induces ion toxicity(Liang et al. 2024), thereby interfering with the synthesis of proteins and carbohydrates(Miller et al. 2010). Simultaneously, osmotic stress leads to cellular dehydration and reduced photosynthetic capacity(Anaya et al. 2025). More importantly, salt stress triggers excessive accumulation of reactive oxygen species (ROS)(Acharya et al. 2024), resulting in antioxidant system imbalance(Miller et al. 2010) and membrane lipid peroxidation damage(Gao et al. 2024).

Larix gmelinii, a coniferous species in the Pinaceae family, is widely used for afforestation in Northeast China. It plays an important role in timber production, protective forests, ecological conservation in cold northern regions, landscape greening, and wood resource development(Liu et al. 2025a; Gao et al. 2023). However, among major afforestation species, *L. gmelinii* exhibits relatively low salt tolerance under NaCl stress(Liu Guifeng 1998). With the increasing severity of soil salinization, the growth and biomass

accumulation of salt-sensitive afforestation species are restricted, leading to difficulties in forest establishment and reduced effectiveness in ecological protection, landscape function, and timber production(Li et al. 2025).

Ectomycorrhizal fungi (ECMF) commonly establish symbiotic associations with the roots of coniferous species, including members of the Pinaceae family(Ramírez-Mendoza et al. 2026), and play an important role in enhancing plant tolerance to environmental stresses(Zwiazek et al. 2019). Under salt stress conditions, ECMF have been shown to promote the accumulation of osmotic adjustment substances and enhance antioxidant enzyme activities, thereby alleviating salt-induced damage in host plants(Xu et al. 2024; Shi et al. 2025; Ding et al. 2026). Furthermore, previous studies have demonstrated that co-inoculation with *Boletus edulis* (Be) and *Gomphidius rutulus* (Gr) can significantly improve the stress resistance of *Larix gmelinii*(Yuanyuan 2020). However, despite the recognized benefits of ECMF symbiosis, the mechanisms by which ECMF regulate growth and enhance salt tolerance in *L. gmelinii* under saline conditions remain largely unclear.

Therefore, this study employed pot experiments combined with untargeted metabolomics to systematically compare growth performance, photosynthetic physiology, antioxidant mechanisms, osmotic adjustment, ion homeostasis, and metabolic characteristics between non-inoculated and ECMF-inoculated (*Be* + *Gr*) *L. gmelinii* seedlings under different salt concentration gradients. The objectives of this study were to: (1) evaluate the growth-promoting effects of ECMF inoculation on *L. gmelinii*; (2) investigate the adaptive mechanisms of *L. gmelinii* under salt stress; and (3) elucidate the physiological mechanisms by which ECMF enhance salt tolerance in *L. gmelinii*. These findings will provide a theoretical basis for improving the survival rate of *L. gmelinii* plantations in saline-alkaline soils and for future related studies.

2. Materials and Methods

2.1 Plant materials and growth conditions

The experiment was conducted in a controlled growth chamber at Northeast Forestry University, Heilongjiang Province, China (45°43' N, 126°38' E). *Larix gmelinii* seeds were used as plant materials and were provided by the Greater Khingan Mountains State-owned Forest Administration, Inner Mongolia, China.

2.2 Ectomycorrhizal fungi inoculation

Boletus edulis (Be) was provided by the Chinese Academy of Forestry, while *Gomphidius rutulus* (Gr) was obtained from the China General Microbiological Culture Collection Center (CGMCC).

The inoculum suspensions of *B. edulis* and *G. rutulus* were prepared separately by mixing fungal cultures with sterile water at a ratio of 1:1 (v/v). After transplantation into pots, 5 mL of each inoculum

suspension was evenly injected into the rhizosphere soil surrounding each seedling from four directions using a syringe. The seedlings were then maintained in the growth chamber for an additional 14 weeks.

2.3 Experimental design

The treatment without salt stress or ECMF inoculation was used as the control (CK). The treatment groups included: (1) salt stress without ECMF inoculation (S); (2) ECMF inoculation without salt stress (M); and (3) combined salt stress and ECMF inoculation (S + M).

After 14 weeks of ECMF inoculation, salt stress was imposed using NaCl at a concentration of 120 mM based on preliminary experimental results. Each pot received a total of 500 mL of NaCl solution, applied at 100 mL per irrigation every 2 days. Plants in the non-salt treatments received an equal volume of distilled water. Seedlings were harvested on day 32 after salt treatment and stored at − 80°C for subsequent analyses.

2.4 Measurements

2.4.1 Growth parameters

Plant height was determined by measuring the vertical distance from the plant base to the apex. Root morphological parameters, including total root length, root surface area, root volume, root tip number, and root branching number, were measured using a root scanner. Plant samples were dried at 80°C to constant weight for determination of belowground and aboveground biomass.The root to shoot ratio (RSR) was calculated as:

RSR=Shoot biomass / Root biomass(Dietrich et al. 2025)

2.4.2 Photosynthetic parameters

Photosynthetic parameters, including net photosynthetic rate (Pn), intercellular CO_2 concentration (Ci), stomatal conductance (Gs), and transpiration rate (Tr), were measured using an LI-6400XT portable photosynthesis system according to the method described by Jia et al(Jia et al. 2025).

2.4.3 Physiological and biochemical parameters

Superoxide dismutase (SOD) activity was determined using the nitroblue tetrazolium (NBT) method(Constantine N. Giannopolitis 1977), while peroxidase (POD) activity was measured using the guaiacol method(Xu et al. 2026). Malondialdehyde (MDA) content was determined using the thiobarbituric acid method(D. Mark Hodges 1999), and relative electrolyte conductivity (REC) was measured using a conductivity meter(Fan et al. 2025). Proline (Pro) content was determined using the acid ninhydrin method(Troll and Lindsley 1955), soluble sugar (SS) content using the anthrone colorimetric method(Liu et al. 2025b), and soluble protein (SP) content using the Coomassie Brilliant Blue method(Liu et al. 2025b).

2.4.4 Ion content analysis

Na^+ and K^+ concentrations were determined using microwave plasma–atomic emission spectrometry(Chen et al. 2020).

The transport ratio (TR)(Liu et al. 2019) was calculated as:

TR(X)= Shoot (X) / Root (X).

The transport selection ratio (TS)(Wang et al. 2002) was calculated as:

TS(K^+/ Na^+)=Shoot (K^+/ Na^+) / Root (K^+/ Na^+)

2.4.5 Untargeted metabolomics analysis

Untargeted metabolomics analysis was performed by Shanghai Lu-Ming Biotech Co., Ltd. (Shanghai, China). Samples were extracted using a methanol–water solution. The supernatant was collected, filtered through a 0.22 μm membrane, and transferred into LC vials for liquid chromatography–mass spectrometry (LC–MS) analysis.

Metabolic profiling was performed using an ACQUITY UPLC I-Class system coupled with a VION IMS QTOF mass spectrometer (Waters Corporation, Milford, MA, USA) under both positive and negative electrospray ionization (ESI) modes.

Raw LC–MS data were processed using Progenesis QI V2.3 software (Nonlinear Dynamics, Newcastle, UK)(Halime et al. 2025). Compound identification was performed based on accurate mass-to-charge ratio (*m/z*), MS/MS fragmentation patterns, and isotope distribution using the PMDB database(Udayakumar et al. 2010). Differential metabolites from four comparison groups, including A (S/CK), B (M/CK), C (S + M/S), and D (S + M/M), were subjected to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis(Hiroyuki Ogata 1999).

2.5 Statistical analysis

Data are presented as mean ± standard error (SE). One-way analyses of variance (ANOVA) were performed using SPSS 27.0 software (SPSS Inc., Chicago, IL, USA). Significant differences among treatment means were determined using Duncan's multiple range test at $P < 0.05$, and different letters were used to indicate significant differences among treatments. Figures were generated using Origin 2022 software (OriginLab Corporation, Northampton, MA, USA).

3. Results

3.1 Effects of salt stress and ECMF inoculation on the growth of *Larix gmelinii*

As shown in Fig. 1, no significant differences were observed between the S and CK groups in plant height, root biomass, shoot biomass, or root shoot ratio. Compared with CK, the M treatment

significantly increased plant height, root biomass, and shoot biomass, reaching 24.70 cm, 0.3687 g, and 0.96 g, respectively. Moreover, both root and shoot biomass in the M group were significantly higher than those in the other treatment groups. The S + M treatment showed a significantly higher root biomass than CK, with a value of 0.4957 g, whereas no significant differences were observed for the other growth parameters.

3.2 Effects of salt stress and ECMF inoculation on root morphology of *Larix gmelinii*

As shown in Table 1, no significant differences were observed between the S and CK groups in root morphological parameters, including total root length, root surface area, root volume, root tip number, and root branching number. The M treatment exhibited significantly higher values for total root length, root surface area, root volume, root tip number, and root branching number than all other treatments, reaching 1115.82 cm, 128.58 cm^2, 1.19 cm^3, 4227.33, and 4730.67, respectively. Compared with the CK and S groups, the S + M treatment also significantly increased total root length, root surface area, root volume, and root branching number, with corresponding values of 590.23 cm, 59.47 cm^2, 0.48 cm^3, and 2107.00, respectively.

3.2 Effects of salt stress and ECMF inoculation on root morphology of *Larix gmelinii*

Table 1
Effect of Salt stress and ECMF on the root of *Larix gmelinii*.

Treatment	Length (cm)	SurfArea (cm^2)	RootVolume (cm^3)	Tips	Forks
CK	182.98 ± 8.99c	17.03 ± 1.37c	0.13 ± 0.01c	452.67 ± 134.12c	491.67 ± 120.47c
S	218.92 ± 7.03c	20.48 ± 1.17c	0.15 ± 0.02c	636.67 ± 46.84bc	664.00 ± 67.55c
M	1115.82 ± 70.20a	128.58 ± 0.98a	1.19 ± 0.10a	4227.33 ± 772.68a	4730.67 ± 617.50a
S + M	590.23 ± 73.43b	59.47 ± 6.18b	0.48 ± 0.07b	1852.00 ± 131.01b	2107.00 ± 208.71b

Note: CK, control without salt stress or ECMF inoculation; S, 120 mM NaCl treatment; M, ectomycorrhizal fungi (ECMF) inoculation (*Boletus edulis* + *Gomphidius rutulus*); S + M, combined 120 mM NaCl treatment and ECMF inoculation.

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3.3 Effects of salt stress and ECMF inoculation on photosynthetic characteristics and chlorophyll content of Larix gmelinii

Table 2
Effect of Salt stress and ECMF on the photosynthsis and chlorophyll of *Larix gmelinii*.

Treatment	Pn ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	Ci ($\mu mol\ CO_2\ mol^{-1}$)	Gs ($mol\ H_2O\ m^{-2}\ s^{-1}$)	Tr ($mmol\ H_2O\ m^{-2}\ s^{-1}$)	Chlorophyll content ($mg\ g^{-1}$)
CK	4.16 ± 0.09b	413.45 ± 26.01ab	0.04 ± 0.01b	1.05 ± 0.19b	1.68 ± 0.35a
S	3.27 ± 0.03c	331.95 ± 2.32b	0.02 ± 0.00c	0.61 ± 0.00c	1.62 ± 0.06a
M	5.44 ± 0.40a	451.73 ± 9.74a	0.06 ± 0.00a	1.57 ± 0.03a	2.17 ± 0.27a
S + M	5.45 ± 0.33a	335.11 ± 47.72b	0.04 ± 0.01bc	0.97 ± 0.08b	1.75 ± 0.05a

Note: CK, control without salt stress or ECMF inoculation; S, 120 mM NaCl treatment; M, ectomycorrhizal fungi (ECMF) inoculation (*Boletus edulis* + *Gomphidius rutulus*); S + M, combined 120 mM NaCl treatment and ECMF inoculation.

As shown in Table 2, the S treatment significantly decreased net photosynthetic rate (Pn), stomatal conductance (Gs), and transpiration rate (Tr) compared with CK, reaching 3.27 μmol CO_2 m^{-2} s^{-1}, 0.02 mol H_2O m^{-2} s^{-1}, and 0.61 mmol H_2O m^{-2} s^{-1}, respectively. In contrast, the M treatment significantly increased Pn, Gs, and Tr compared with CK, with values of 5.44 μmol CO_2 m^{-2} s^{-1}, 0.06 mol H_2O m^{-2} s^{-1}, and 1.57 mmol H_2O m^{-2} s^{-1}, respectively. The S + M treatment showed a significantly higher Pn than CK, with a value of 5.45 μmol CO_2 m^{-2} s^{-1}.

Although the M treatment exhibited significantly higher Ci, Gs, and Tr values than the S + M treatment, no significant difference in Pn was observed between these two treatments. In addition, no significant differences in chlorophyll content were observed among the four treatments.

3.3 Effects of salt stress and ECMF inoculation on oxidative stress and antioxidant responses of Larix gmelinii

As shown in Fig. 2, no significant differences in superoxide dismutase (SOD) activity or malondialdehyde (MDA) content were observed among the four treatments. The S treatment significantly increased peroxidase (POD) activity and relative electrolyte conductivity (REC) compared with CK, reaching 52.98 μmol g^{-1} min^{-1} and 72.92%, respectively. No significant differences in POD activity or REC were observed between the M and CK treatments.

The S + M treatment showed a significantly higher POD activity than CK, reaching 72.71 μmol g^{-1} min^{-1}, and this value was also significantly higher than those of the S and M treatments. Although no significant difference in REC was observed between the S + M and CK treatments, the REC value in the S + M treatment was significantly higher than that in the M treatment, reaching 68.00%.

3.4 Effects of salt stress and ECMF inoculation on osmotic regulatory substances in *Larix gmelinii*

As shown in Fig. 3, the S treatment significantly increased soluble sugar (SS), soluble protein (SP), and proline (Pro) contents compared with CK, reaching 643.81 mg g^{-1}, 171.97 mg g^{-1}, and 1.47 mg g^{-1}, respectively. In the M treatment, only SS content was significantly higher than that in CK, with a value of 487.12 mg g^{-1}. The S + M treatment significantly increased SS and SP contents compared with CK, reaching 693.21 mg g^{-1} and 175.91 mg g^{-1}, respectively.

Furthermore, SS and SP contents in the S + M treatment were significantly higher than those in the M treatment but did not differ significantly from those in the S treatment. In contrast, Pro content in the S + M treatment showed no significant difference compared with the M treatment but was significantly lower than that in the S treatment, with a value of 0.55 mg g^{-1}.

3.5 Effects of salt stress and ECMF inoculation on ion homeostasis in aboveground and belowground tissues of Larix gmelinii

Table 3
Effect of Salt stress and ECMF on the Ion homeostasis of *Larix gmelinii*.

Treatment	Root K^+/Na^+ ratio	Shoot K^+/Na^+ ratio	Na^+ transport ratio	K^+ transport ratio	Transport selection ratio
CK	0.29 ± 0.01b	0.30 ± 0.01b	2.08 ± 0.05a	2.18 ± 0.06b	1.05 ± 0.01c
S	0.11 ± 0.00c	0.15 ± 0.00d	1.91 ± 0.05b	2.68 ± 0.05a	1.40 ± 0.05b
M	0.45 ± 0.01a	0.49 ± 0.00a	1.64 ± 0.03c	1.78 ± 0.01c	1.08 ± 0.02c
S + M	0.10 ± 0.00c	0.18 ± 0.00c	1.65 ± 0.02c	2.80 ± 0.00a	1.69 ± 0.02a

Note: CK, control without salt stress or ECMF inoculation; S, 120 mM NaCl treatment; M, ectomycorrhizal fungi (ECMF) inoculation (*Boletus edulis* + *Gomphidius rutulus*); S + M, combined 120 mM NaCl treatment and ECMF inoculation.

As shown in Table 3, both the S and S + M treatments exhibited significantly lower K^+/Na^+ ratios in belowground and aboveground tissues, as well as lower Na^+ transport ratios, compared with CK. In contrast, K^+ transport ratios and transport selection ratios were significantly higher in both treatments than in CK.

The M treatment showed significantly higher K^+/Na^+ ratios in both belowground and aboveground tissues than CK, whereas Na^+ and K^+ transport ratios were significantly lower.

Compared with the M treatment, the S + M treatment showed significant differences in all parameters except Na^+ transport ratio. Specifically, K^+/Na^+ ratios in both belowground and aboveground tissues were significantly lower, whereas K^+ transport ratio and transport selection ratio were significantly higher. Compared with the S treatment, significant differences were observed in all parameters except belowground K^+/Na^+ ratio and K^+ transport ratio in the S + M treatment. The aboveground K^+/Na^+ ratio and transport selection ratio were significantly increased, whereas the Na^+ transport ratio was significantly decreased.

3.6 Effects of salt stress and ECMF inoculation on metabolic responses of *Larix gmelinii*

In the S + M/CK comparison (Fig. 5A), all detected metabolites were classified, and the top nine categories were individually presented, while the remaining categories were grouped as "Others." Among these, lipids and lipid-like molecules accounted for the largest proportion (28.44%). A total of 13,191 differential metabolites were identified, of which 6,425 were significantly upregulated and 6,736 significantly downregulated (Fig. 5B). The heatmap of differential metabolites showed continuous

upregulation of lipid metabolism-related molecules, amino acids and their derivatives, and glycosides (Fig. S1). KEGG enrichment analysis revealed that these metabolites were primarily involved in the "Flavonoid biosynthesis" pathway, followed by the "Ascorbate and aldarate metabolism" pathway (Fig. 5C). Principal component analysis (PCA) demonstrated a clear separation between the S + M and CK groups (PC1 = 83.2%, PC2 = 7.11%; Fig. S2), and orthogonal partial least squares discriminant analysis (OPLS-DA) further confirmed the distinct metabolic profiles of the two groups (Fig. S3).

KEGG pathway enrichment analysis was performed on the identified differential metabolites (Fig. 6). Ascorbate and aldarate metabolism, aminoacyl-tRNA biosynthesis, and ABC transporters were significantly enriched across all comparison groups.Among the S/CK, M/CK, and S + M/M comparison groups, the pathways with the highest enrichment factors were flavonoid biosynthesis, pyruvate metabolism, and aminoacyl-tRNA biosynthesis, respectively. Notably, among the significantly enriched pathways in the S + M/S group (Fig. 6C), arginine biosynthesis exhibited the highest enrichment factor, followed by arginine and proline metabolism.

4. Discussion

Plant height and biomass are important indicators of plant growth status, reflecting the integrated outcome of multiple physiological processes rather than simple biomass accumulation alone(Xu et al. 2024). In the present study, the M treatment inoculated with *Boletus edulis* and *Gomphidius rutulus* showed significantly higher plant height, biomass (both root and shoot), and root morphological parameters (including total root length, root surface area, root volume, root tip number, and root branching number) than the CK treatment, indicating a pronounced growth-promoting effect of this ECMF combination on *Larix gmelinii*. Under salt stress conditions (S + M treatment), this growth-promoting effect was still maintained, as evidenced by significant increases in root biomass and root morphological traits, although no significant changes were observed in shoot biomass. Furthermore, the S + M treatment exhibited significantly higher values for most root morphological parameters than CK, except for root tip number, further suggesting that ECMF inoculation maintained its stimulatory effects on root development under salt stress conditions.

Photosynthesis is the primary source of energy for plant growth, and changes in photosynthetic parameters reflect the carbon assimilation capacity of plants, which is often among the earliest physiological processes affected by environmental stress(Muhammad et al. 2020). In the present study, the S treatment showed significantly lower net photosynthetic rate (Pn), stomatal conductance (Gs), and transpiration rate (Tr) than CK, whereas intercellular CO_2 concentration (Ci) showed no significant difference, indicating that salt stress inhibited photosynthesis mainly through stomatal limitation. Moreover, KEGG enrichment analysis (Fig. 6A) showed that carbon fixation in photosynthetic organisms was significantly enriched under salt stress, which reflect an adaptive response to stomatal closure and limited CO_2 diffusion, enhancing carbon assimilation efficiency and improving water use efficiency(Wang et al. 2024). In contrast, the M treatment exhibited opposite trends, with significantly higher Pn, Gs, and Tr than CK, suggesting that ECMF inoculation enhanced stomatal conductance and transpiration pull,

thereby promoting CO_2 uptake and photosynthetic capacity. However, this promotive effect on photosynthesis was partially altered under salt stress. In the S + M treatment, Gs and Tr were significantly lower than those in the M treatment but showed no significant difference compared with CK, whereas Pn remained comparable to the M treatment and significantly higher than both CK and S treatments, indicating that ECMF inoculation maintained a relatively high photosynthetic capacity under salt stress while reducing dependence on stomatal conductance. In addition, the lack of significant differences in chlorophyll content among treatments suggests that the reduction in photosynthetic rate under salt stress was primarily attributed to stomatal limitation rather than chlorophyll degradation.

Salt stress is known to exacerbate oxidative stress in plants and generally induces antioxidant defense responses. Malondialdehyde (MDA) content and relative electrical conductivity (REC) are widely used indicators of lipid peroxidation and membrane integrity, reflecting the extent of oxidative damage in plant tissues(Liu et al. 2024). In the present study, no significant differences in superoxide dismutase (SOD) activity were observed among the four treatments. This result may be attributed to the constitutive role of SOD as a primary antioxidant defense enzyme that provides continuous protection against reactive oxygen species (ROS) accumulation(Jomova et al. 2024), Maintaining relatively stable SOD activity may also help reduce the metabolic costs associated with stress responses(Cannea and Padiglia 2025). In contrast, POD activity was significantly increased in both the S and S + M treatments compared with the CK treatment. This finding suggests that POD-mediated ROS scavenging represents an important antioxidant strategy of *Larix gmelinii* under salt stress. Consistent with this result, the ascorbate and aldarate metabolism pathway contained a large number of differential metabolites (Fig. 5C). As a key component of the AsA–GSH cycle, ascorbate as an essential electron donor in POD-catalyzed reactions and plays a critical role in H_2O_2 detoxification(Rao et al. 2025; Anjum et al. 2016a). Therefore, the enhanced POD activity together with the activation of ascorbate and aldarate metabolism can contribute to more efficient ROS scavenging under 120 mM NaCl stress. In addition, flavonoid biosynthesis was the most enriched metabolic pathway, exhibiting the highest number of differential metabolites in Fig. 5C and significant enrichment in Fig. 6C. Flavonoids are well recognized as important non-enzymatic antioxidants that directly scavenge ROS and maintain cellular redox homeostasis. The activation of flavonoid biosynthesis therefore suggests that both enzymatic and non-enzymatic antioxidant systems were involved in the response of *L. gmelinii* to salt stress. Notably, neither MDA content nor REC differed significantly between the S + M and CK treatments. The maintenance of membrane damage indicators at control levels suggests that ECMF inoculation effectively alleviated oxidative damage and contributed to the preservation of membrane stability under salt stress(Wang et al. 2025). In conclusion *L. gmelinii* primarily relies on POD-dependent ROS detoxification and flavonoid-mediated antioxidant defense rather than substantial changes in SOD activity when exposed to moderate salt stress. ECMF inoculation further strengthened these antioxidant pathways, thereby maintaining membrane integrity and enhancing salt tolerance (Fig. 7).

Salt and alkaline stress generally intensifies oxidative stress in plants and induces changes in antioxidant enzyme activities. Malondialdehyde (MDA) content and relative electrolyte conductivity (REC) are widely used indicators of membrane lipid peroxidation, reflecting membrane damage and cellular

redox homeostasis(Liu et al. 2024). In the present study, no significant differences in superoxide dismutase (SOD) activity were observed among the four treatments, which may be attributed to the role of SOD as a baseline defense enzyme that provides constitutive protection(Jomova et al. 2024). The relatively stable SOD activity may help reduce metabolic costs associated with stress adaptation(Cannea and Padiglia 2025). However, peroxidase (POD) activity in both the S and S + M treatments was significantly higher than that in CK, suggesting that under salt stress, *Larix gmelinii* mainly relies on POD-mediated reactive oxygen species (ROS) scavenging pathways to alleviate oxidative damage, regardless of ECMF inoculation. KEGG enrichment analysis (Fig. 5A–D) showed that ascorbate and aldarate metabolism was significantly enriched across all treatments. This pathway is closely associated with the ascorbate–glutathione (AsA–GSH) cycle, which works in coordination with POD to detoxify H_2O_2(Rao et al. 2025). Ascorbic acid (AsA) serves as an important electron donor in antioxidant reactions and can indirectly influence POD activity(Anjum et al. 2016b). These results further suggest that under 120 mM NaCl stress, *Larix gmelinii* primarily detoxifies H_2O_2 through the POD/APX pathway by converting it into H_2O, thereby mitigating oxidative damage (Fig. 6). The S + M treatment showed significantly higher POD activity than the S treatment, indicating that ECMF inoculation enhanced the ability of plants to scavenge reactive oxygen species under stress conditions. In addition, the significant enrichment of ascorbate and aldarate metabolism in the S + M/S and M/CK comparisons (Fig. 5B, D) further supports that both ECMF inoculation and its interaction with salt stress can improve antioxidant capacity. Moreover, the S + M treatment showed no significant differences in MDA content and REC compared with CK, indicating that ECMF inoculation effectively maintained membrane stability under salt stress(Wang et al. 2025).

Under stress conditions, plants typically accumulate osmolytes such as soluble sugars, soluble proteins, and proline to maintain cellular osmotic balance(Liu et al. 2024). In the present study, the S treatment showed significantly higher soluble sugar, soluble protein, and proline contents than CK, indicating that non-inoculated *Larix gmelinii* enhanced the synthesis of osmolytes to maintain cellular homeostasis under salt stress, consistent with the general response pattern to salinity stress. In the M treatment, only soluble sugar content was significantly higher than that in CK. Although the S + M treatment showed significantly higher soluble sugar and soluble protein contents than CK and no significant differences compared with the S treatment, proline content was significantly lower than in the S treatment and comparable to CK, which differs from the commonly observed pattern that increased proline accumulation is associated with enhanced salt tolerance. This suggests that, under salt stress, ECMF-inoculated *L. gmelinii* mainly relies on soluble sugars and soluble proteins for osmotic regulation. KEGG analysis (Fig. 5C) showed that, compared with the S treatment, the S + M treatment (S + M/S) significantly enriched arginine and proline metabolism as well as arginine biosynthesis, with arginine biosynthesis showing the highest enrichment factor. Proline and arginine share functional similarities in stress responses. Proline acts as an osmolyte and contributes to ROS scavenging through osmotic adjustment(Renzetti et al. 2024), whereas arginine serves as a precursor for polyamine biosynthesis(Stecker et al. 2022) (e.g., spermidine and spermine) (Hu et al. 2025), which are involved in antioxidant defense. Both amino acids are associated with glutamate metabolism. Glutamate can be

converted into proline via P5CS-mediated NADPH-dependent reduction, or enter arginine biosynthesis through the N-acetylglutamate synthase (NAGS) pathway(Winter et al. 2015). These results suggest that ECMF inoculation may enhance the arginine biosynthetic branch under salt stress, potentially partially substituting arginine-related metabolic functions for proline, thereby contributing to reduced proline accumulation. In addition, recent studies suggest that proline may function more importantly as a signaling molecule than as a compatible solute in higher plants(Renzetti et al. 2024; Spormann et al. 2023; Koc et al. 2024). Inoculated plants also showed enrichment in galactose metabolism (Fig. 5C), producing soluble sugars such as galactose, raffinose, and inositol-galactosides, which represent important soluble sugars in plants(Nishizawa et al. 2008). Additionally, amino acids and their derivatives were abundant in the metabolome (Fig. 5C, Fig. S1), serving as precursors for soluble protein synthesis(Amira et al. 2005). The increased abundance and enrichment of these metabolites likely contributed to the observed increases in soluble sugar and protein contents in the S + M group. Collectively, ECMF inoculation enables *L. gmelinii* to regulate osmotic balance primarily through soluble sugars and proteins, reducing reliance on proline for osmotic adjustment (Fig. 7).

Fine regulation of ion homeostasis is a key mechanism by which ectomycorrhizal fungi (ECMF) enhance host salt tolerance(Wang et al. 2026). In this study, both the S and S + M treatments exhibited significantly lower K^+/Na^+ ratios in roots and shoots compared with CK, indicating that 120 mM NaCl disrupted ion homeostasis in *Larix gmelinii* and promoted Na^+ accumulation in roots. In the M treatment, the K^+ transport ratio was significantly lower than that in CK, suggesting that ECMF inoculation reduced K^+ transport from roots to shoots, likely due to preferential retention of nutrients within the root system rather than translocation to shoots. However, the S + M treatment showed significantly higher K^+ transport ratio and transport selectivity than both CK and M treatments, indicating that ECMF-mediated regulation of K^+ allocation is strongly dependent on the presence of salt stress. The S + M treatment exhibited a significantly higher root K^+/Na^+ ratio than the S treatment, characterized by increased K^+ retention and reduced Na^+ accumulation in roots. This may be associated with the significant enrichment of ascorbate and aldarate metabolism and arginine biosynthesis, which could alleviate ROS-induced activation of K^+ efflux channels(Pottosin et al. 2020; Hanin et al. 2016; Assaha et al. 2017), thereby reducing K^+ loss. Alternatively, enrichment of the ABC transporter pathway may contribute to improved ion homeostasis by regulating the expression of ion transport proteins(Assaha et al. 2017), thereby reducing K^+ efflux and Na^+ uptake. Under salt stress, ECMF may preferentially promote K^+ transport to shoots to support aboveground growth (Fig. 7). In addition, the S + M treatment showed significantly lower Na^+ transport ratios than CK and S treatments, suggesting restricted Na^+ translocation to shoots and enhanced sequestration in roots, potentially through vacuolar compartmentalization mediated by ECMF symbiosis(Mansour 2022).

5. Conclusion

Inoculation with *Boletus edulis* and *Gomphidius rutulus* (Be + Gr) significantly promoted the growth of *Larix gmelinii*, and this effect was maintained under salt stress. ECMF inoculation alleviated the negative effects of salt-induced stomatal limitation on photosynthesis, thereby maintaining a relatively high net

photosynthetic rate. Under salt stress, *L. gmelinii* primarily relied on increased POD activity, activation of ascorbate and aldarate metabolism, and enhanced flavonoid biosynthesis to scavenge reactive oxygen species, while ECMF inoculation further strengthened these antioxidant pathways and improved salt tolerance. In addition, ECMF enhanced galactose metabolism and amino acid biosynthesis, leading to increased soluble sugar and soluble protein contents and consequently improved osmotic adjustment capacity. Although ECMF promoted K^+ retention in roots under non-stress conditions, salt stress induced preferential K^+ translocation to shoots and restricted Na^+ transport, thereby improving ion homeostasis and supporting plant growth under saline conditions. Overall, ECMF enhanced salt tolerance in *Larix gmelinii* through the coordinated regulation of photosynthetic performance, antioxidant defense, osmotic adjustment, and ion homeostasis.

Declarations

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Author Contribution

Yuxuan Wang: Writing-review & editing and Formal analysis; Yan Zhang: Writing - original draft preparation; Wei Yang: Investigation; Fei Wang: Resources and supervision; Laiye Qu: Funding acquisition and supervision.

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Data Availability

All data are available upon reasonable request to the corresponding author.

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Figures

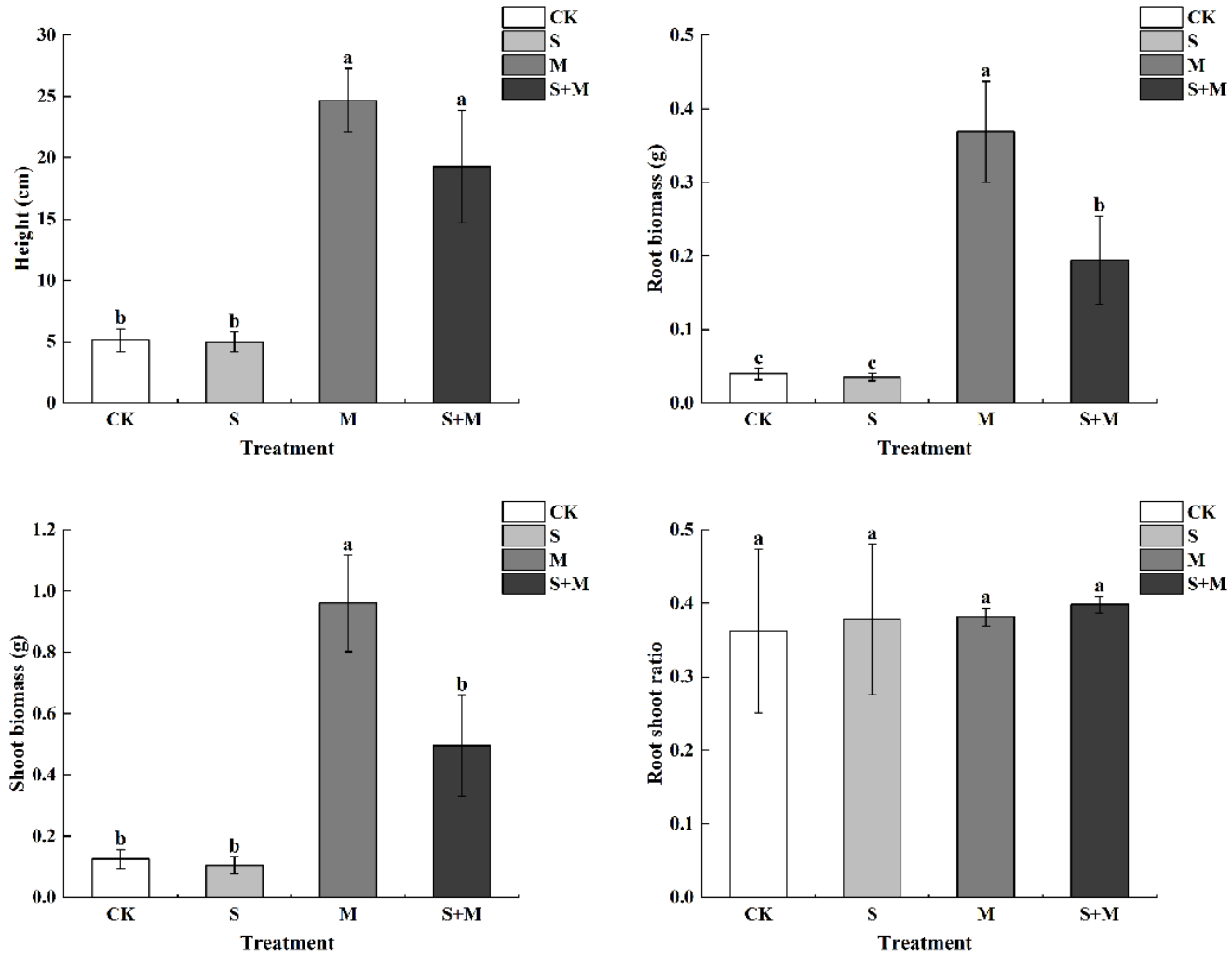

Figure 1

Effect of Salt stress and ECMF stress on grow of *Larix gmelinii*

Note: CK, control without salt stress or ECMF inoculation; S, 120 mM NaCl treatment; M, ectomycorrhizal fungi (ECMF) inoculation (*Boletus edulis* + *Gomphidius rutulus*); S+M, combined 120 mM NaCl treatment and ECMF inoculation.

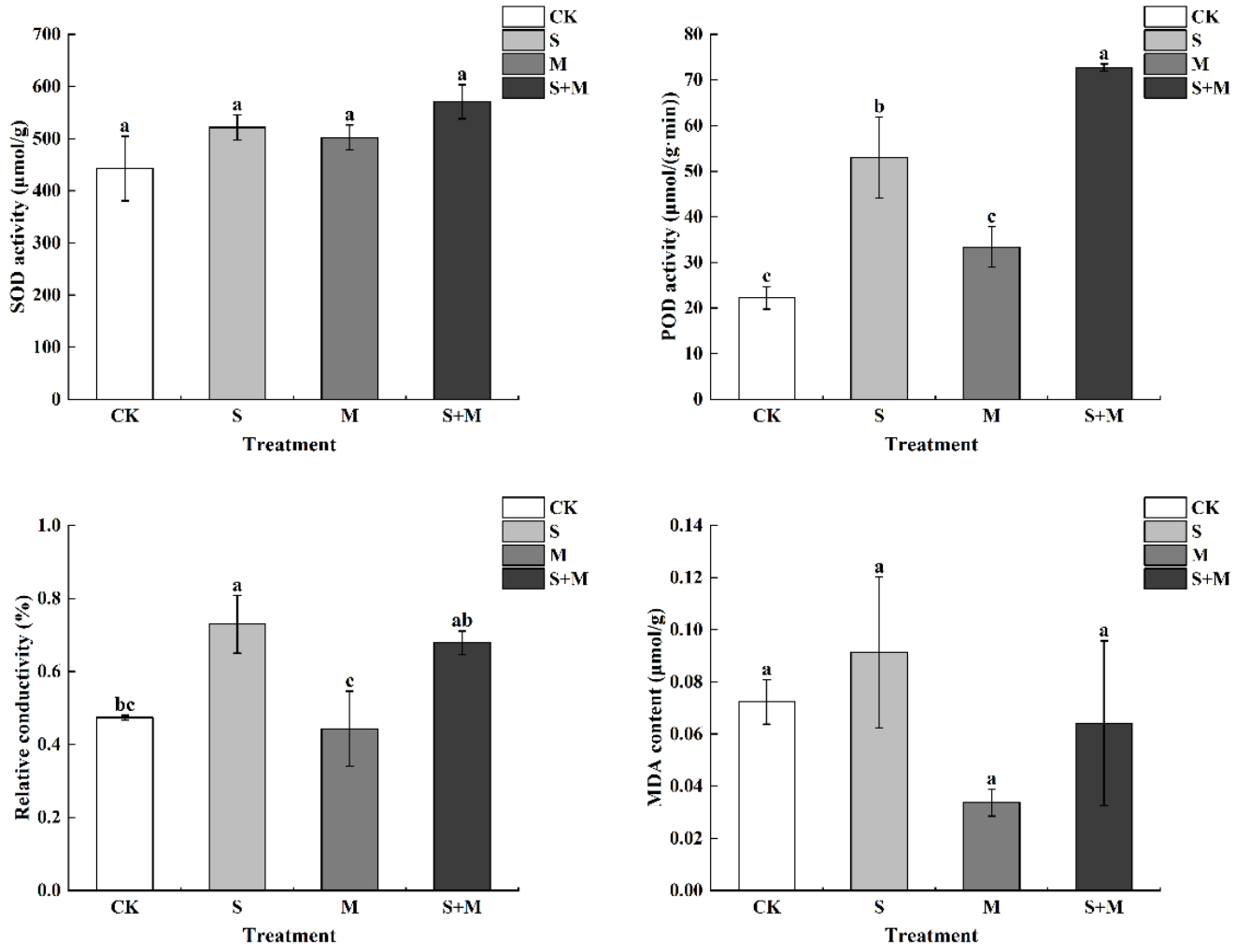

Figure 2

Effect of Salt stress and ECMF on activities of antioxidant enzymes and membrane damage of *Larix gmelinii*

Note: CK, control without salt stress or ECMF inoculation; S, 120 mM NaCl treatment; M, ectomycorrhizal fungi (ECMF) inoculation (*Boletus edulis* + *Gomphidius rutulus*); S+M, combined 120 mM NaCl treatment and ECMF inoculation.

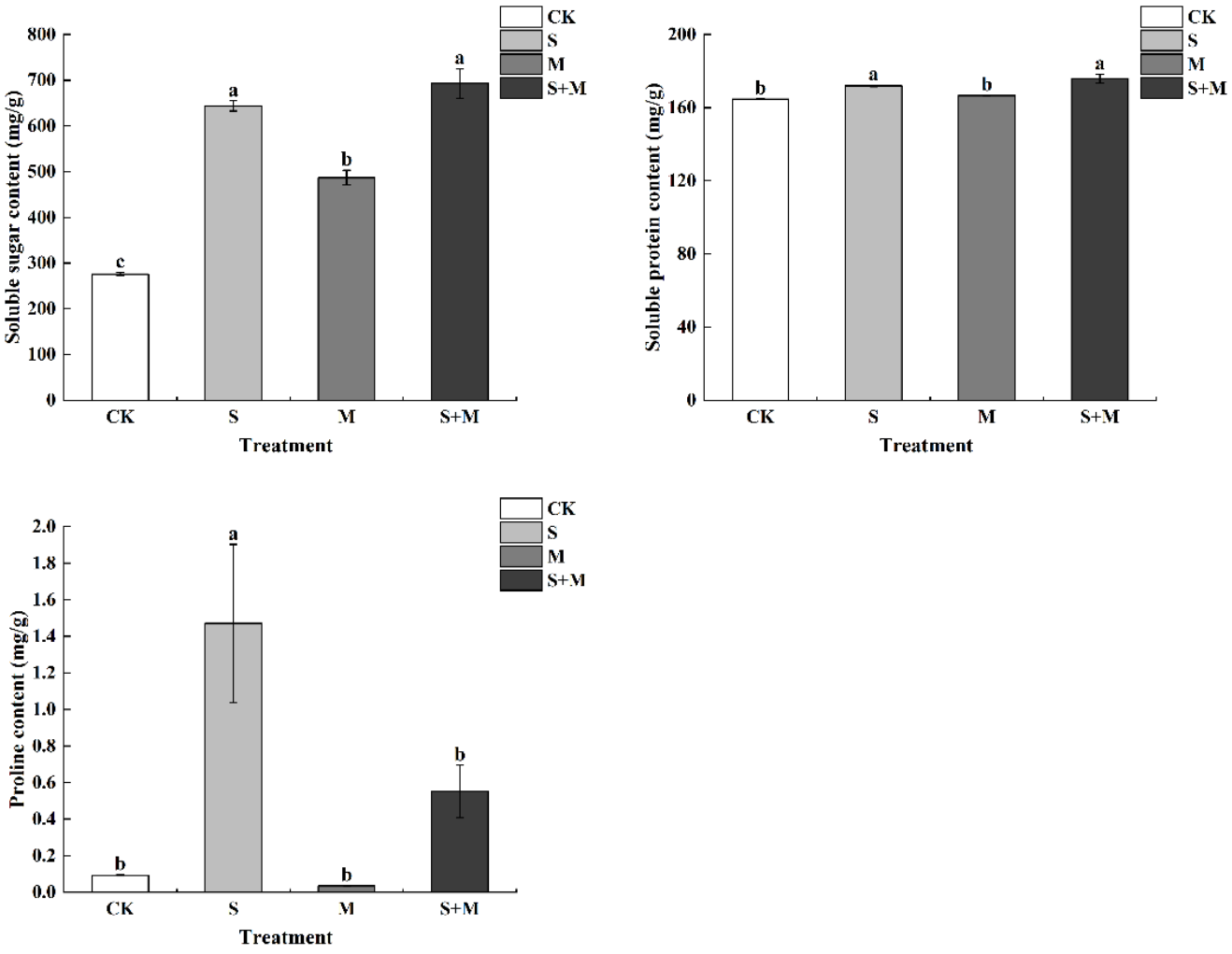

Figure 3

Effect of Salt stress and ECMF on Osmotic adjustment substances of *Larix gmelinii*

Note: CK, control without salt stress or ECMF inoculation; S, 120 mM NaCl treatment; M, ectomycorrhizal fungi (ECMF) inoculation (*Boletus edulis* + *Gomphidius rutulus*); S+M, combined 120 mM NaCl treatment and ECMF inoculation.

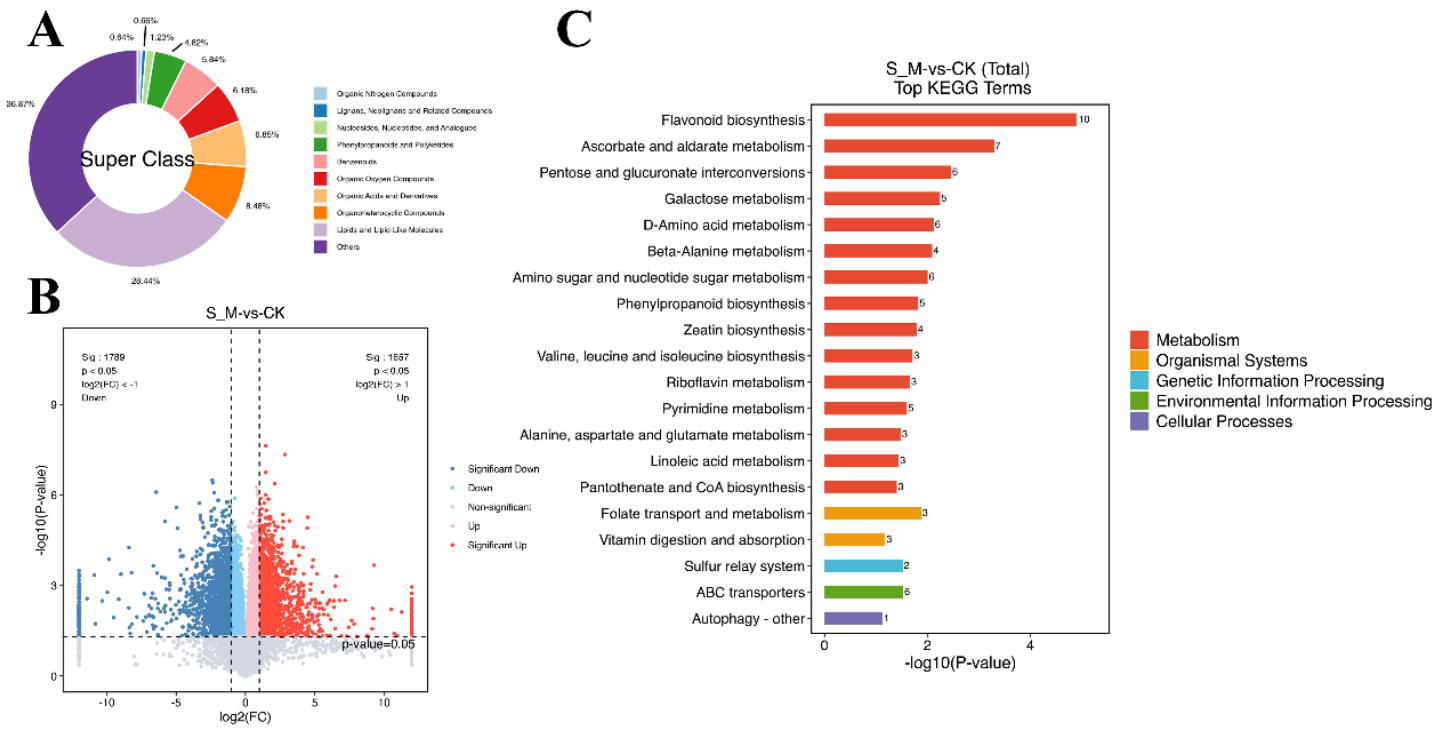

Figure 4

Figure 5. Metabolomic responses of *Larix gmelinii* under salt stress and ECMF inoculation(S+M/CK).

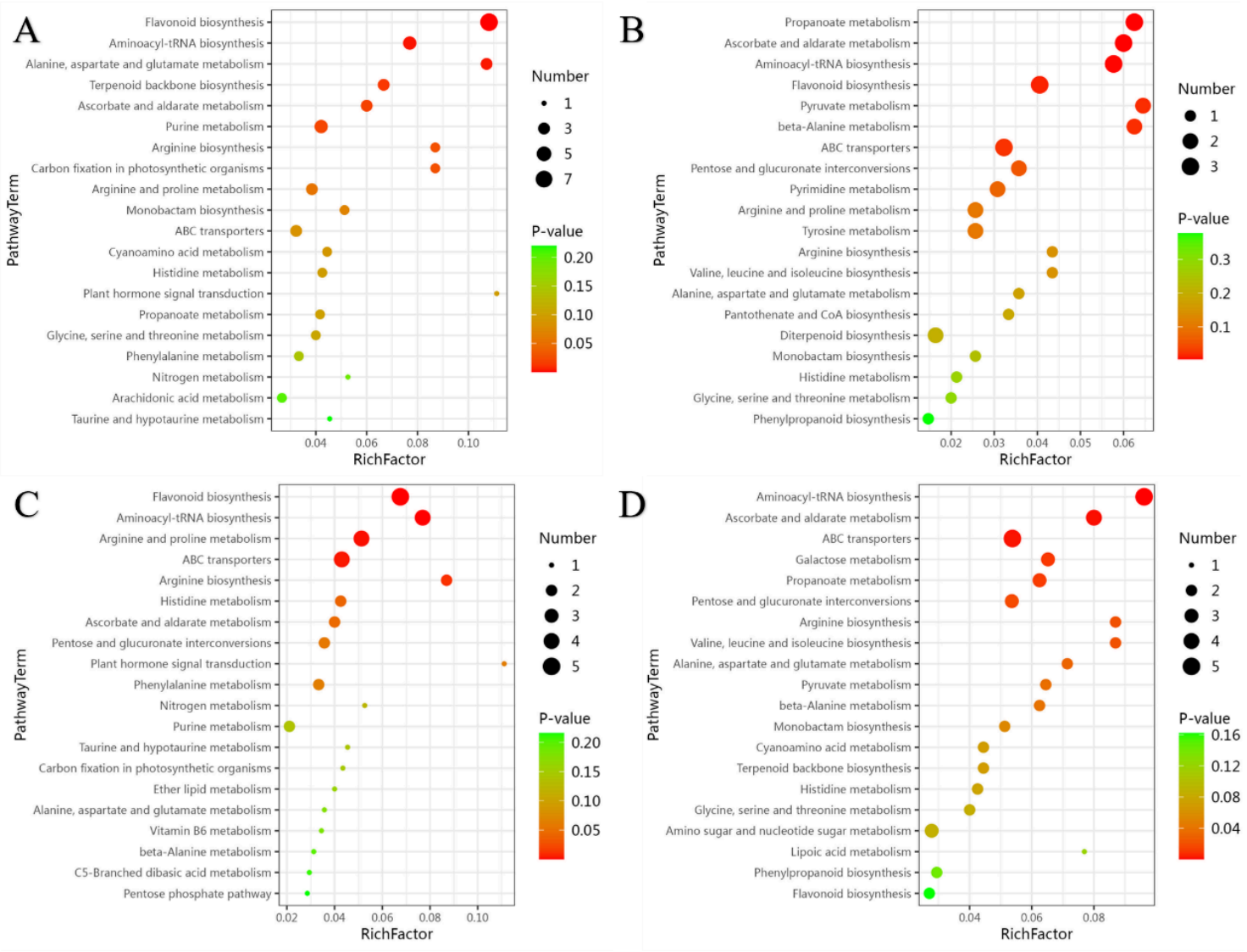

Figure 5

Figure 6 Top 20 signaling KEGG pathways enriched by the differential metabolites

Note: A: S/CK, B: M/CK, C: S+M/S, D: S+M/M.

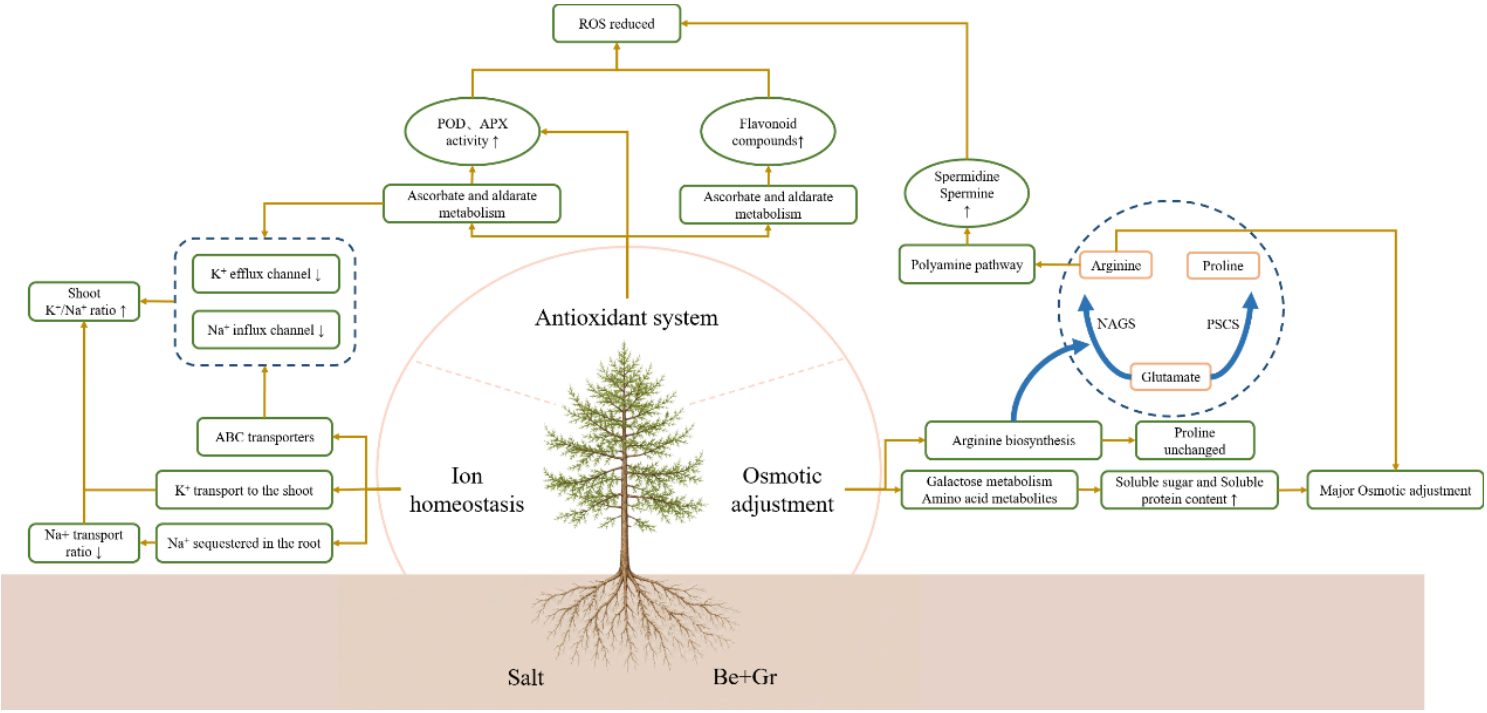

Figure 6

Figure 7 Physiological mechanisms of ectomycorrhizal fungi enhancing salinity tolerance in *Larix gmelinii*

Supplementary Files

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