Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

# Ectomycorrhizal compatibility of *Lactarius quieticolor* and *Tuber floridanum* with *Pinus elliottii* and *Pinus taeda*

## Compatibilidade ectomicorrízica de *Lactarius quieticolor* e *Tuber floridanum* com *Pinus eliotti* e *Pinus taeda*

L. C. Dias[a], R. F. Ramos[a], J. B. Magalhães[a]*, V. G. Handte[a], G. V. Ferreira[a], P. H. Santiago[b], D. L. Scheid[a], L. A. Tabaldi[b], E. Gasparin[c], T. Grebenc[d] and Z. I. Antoniolli[a]

[a]Universidade Federal de Santa Maria – UFSM, Departamento de Solos, Santa Maria, RS, Brasil
[b]Universidade Federal de Santa Maria – UFSM, Departamento de Biologia, Santa Maria, RS, Brasil
[c]Universidade Federal de Santa Maria – UFSM, Departamento de Ciências Florestais, Santa Maria, RS, Brasil
[d]Slovenian Forest Institute, Department of Forest Physiology and Genetics, Ljubljana, Slovenia

**Abstract**

This study evaluated ectomycorrhizal compatibility, functional effects, and the inoculum-dose response of *Lactarius quieticolor* and *Tuber floridanum* in *Pinus elliottii* and *Pinus taeda*, aiming to understand the role of fungus-host specificity in the expression of growth and physiological responses. Basidiomes of *Lactarius* spp. used as the inoculum source were identified through morphological and molecular analyses, confirming the identity as *L. quieticolor*. The symbiont *L. quieticolor* formed ectomycorrhizae on both *Pinus* species, whereas *T. floridanum* established an association only with *P. elliottii*, representing the first experimental record of this interaction. In *P. elliottii*, both fungi elicited integrated responses regardless of dose, with increases in growth, root biomass and electron transport rate, while *L. quieticolor* also increased root length, phosphorus content and modulated initial chlorophyll fluorescence. In *P. taeda*, effects were more limited and dose-dependent: increases in shoot nitrogen content, electron transport rate and root colonization were observed only at the highest inoculum concentrations. Multivariate analyses revealed a clear separation between control and inoculated plants even as the fungal treatments, indicating specific functional signatures. Altogether, the results demonstrate that ectomycorrhizal compatibility is not universal among congeneric *Pinus* species and highlight the potential of *L. quieticolor* and *T. floridanum* as functional ectomycorrhizal symbionts, with implications for seedling production and the ecology of forest systems.

**Keywords:** ectomycorrhizae, host-fungus compatibility, Pinaceae, plant growth, edible fungi.

**Resumo**

Este estudo avaliou a compatibilidade ectomicorrízica, os efeitos funcionais e a resposta à dose de inóculo *de Lactarius quieticolor* e *Tuber floridanum* em *Pinus elliottii* e *Pinus taeda*, visando compreender o papel da especificidade fungo-hospedeiro na expressão do crescimento e do desempenho fisiológico. Basidiomas de *Lactarius* spp. utilizados como fonte de inóculo foram identificados por análises morfológicas e moleculares, confirmando a identidade de *L. quieticolor*. O simbionte *L. quieticolor* formou ectomicorrizas em ambas as espécies de Pinus, enquanto *T. floridanum* estabeleceu associação apenas com *P. elliottii*, constituindo o primeiro registro experimental dessa interação. Em *P. elliottii*, ambos os fungos promoveram respostas integradas, independentemente da dose, com incrementos no crescimento, na biomassa radicular e na taxa de transporte de elétrons, enquanto *L. quieticolor* também aumentou o comprimento radicular, o teor de fósforo e modulou a fluorescência inicial da clorofila. Em *P. taeda*, os efeitos foram mais restritos e dependentes da dose, sendo observados aumentos no teor de nitrogênio da parte aérea, na taxa de transporte de elétrons e na colonização radicular apenas nas maiores concentrações de inóculo. As análises multivariadas evidenciaram clara separação entre plantas controle e inoculadas e discriminaram os tratamentos fúngicos, indicando assinaturas funcionais específicas. Em conjunto, os resultados demonstram que a compatibilidade ectomicorrízica não é universal entre espécies congêneres de *Pinus* e destacam o potencial de *L. quieticolor* e *T. floridanum* como simbiontes ectomicorrízicos funcionais, com implicações para a produção de mudas e para a ecologia de sistemas florestais.

**Palavras-chave:** ectomicorriza, compatibilidade fungo-hospedeiro, Pinaceae, crescimento de plantas, fungos comestíveis.

---

*e-mail: juliano.borela@outlook.com

Received: February 1, 2026 – Accepted: March 26, 2026
Editor: Takako Matsumura Tundisi

## 1. Introduction

In forest ecosystems, primary productivity and functional stability are strongly conditioned by soil nutrient availability and genetic material, especially in environments characterized by imbalances in the relationships between carbon and other essential elements such as nitrogen (N) and phosphorus (P) (Simon et al., 2022; Sardans et al., 2023). Under these conditions, ectomycorrhizal (ECM) associations constitute a key mechanism for nutrient acquisition and allocation, enhancing the adaptive capacity of tree species in nutritionally restrictive environments. However, the establishment and efficiency of these associations are not random, being modulated by biochemical, physiological and evolutionary filters imposed by the host. Thus, ectomycorrhizal compatibility emerges as a selective process in which only certain fungi become functionally integrated into the plant's metabolism.

For *Pinus* cultivation, recent evidence shows that ectomycorrhizal association promotes increased plant biomass and greater carbon allocation and fixation (Zhou et al., 2022; Delard et al., 2024). Subsequent studies have shown that ectomycorrhizae contribute to plant protection against pathogens through physical barriers, resource competition and induction of host defense responses (Wang et al., 2022; Ramos et al., 2023). Under water deficit conditions, the symbiosis has been associated with improved water-use efficiency, enhanced plant water status and maintenance of physiological activity (Sebastiana et al., 2019). Additionally, evidence indicates that ectomycorrhizae directly modulate the photosynthetic apparatus, promoting higher photochemical efficiency (Zhou et al., 2022). Together, these effects can translate into higher survival rates, improved early growth and better seedling quality following field planting. The utilization of specific mycorrhizal inoculants as a strategy to optimize the initial development and morphological parameters of tree species is a growing trend in sustainable forestry practices, particularly in southern Brazil (Trovato et al., 2024; Santos et al., 2024).

Alongside advances in the eco-physiological understanding of mycorrhizae, contemporary forestry has incorporated approaches focused on valuing ecosystem services and the circular bioeconomy (Fuentealba et al., 2025; Ramos, 2026). In this context, mycosilviculture, defined as the integration of commercial tree species with edible ectomycorrhizal fungi, emerges as a promising alternative for diversifying forest production. Fungal genera such as *Lactarius* and *Tuber* stand out not only for their ecological relevance but also for their high gastronomic value, contributing to the transformation of monocultures into multifunctional productive systems (Xu et al., 2023).

In Brazil, the predominance of highly weathered and chemically restrictive soils increases *Pinus* dependence on ectomycorrhizal associations (Simon et al., 2022). Although the functionality of these symbioses is widely supported by the presence of specialized structures for active nutrient exchange between plants and fungus (Gross et al., 2004), taxonomic identification of mycobionts remains challenging. In commercial plantations, the high occurrence of morphologically similar species and infraspecific varieties of fungus difficult precise distinction based solely on morphological characters (Wartchow et al., 2013). In this context, molecular characterization becomes indispensable to overcome the limitations of classical taxonomy and ensure correct identification of isolates. Accurate identification is crucial for mapping the expansion of exotic mycobiota, since the ectomycorrhizal niche in the Southern Hemisphere has been progressively occupied by true truffles, such as *Tuber borchii* Vittad., a species that shows high ecological plasticity and success in establishing functional ectomycorrhizae (Loewe-Muñoz et al., 2024).

The record of *Tuber floridanum* Grupe, Sulzbacher and M. E. Smith in pecan orchards in southern Brazil (Grupe et al., 2018) represents a unique opportunity to understand its ecological plasticity. Unlike assisted introductions, this fungus is already acclimated to the subtropical environment. The ability of this truffle to colonize exotic gymnosperms such as *Pinus elliottii* Engelm. and *P. taeda* L., thereby crossing the barrier of its original host niche associated with angiosperms, constitutes a relevant gap in our understanding of ectomycorrhizal community dynamics in novel environments (Rudawska et al., 2025). Accordingly, based on the hypothesis that, although ectomycorrhizal associations are common in *Pinus*, the degree of compatibility and the resulting functional and morphophysiological effects may vary among congeneric *Pinus* species depending on the ectomycorrhizal fungal partner, this study aimed to evaluate the symbiotic association and morphophysiological effects of inoculating *Tuber floridanum* and *Lactarius quieticolor* Romagn. in seedlings of *P. taeda* and *P. elliottii.*

## 2. Materials and Methods

The experiment was conducted in a greenhouse at the Soil Department of the Universidade Federal de Santa Maria (UFSM), located in Santa Maria, Rio Grande do Sul, Brazil, under mean temperature of 25 °C and a relative humidity of 70%. Seeds of *Pinus elliottii* and *P. taeda* were donated by the Departamento de Diagnóstico e Pesquisa Agropecuária (DDPA), linked to the Secretaria da Agricultura, Pecuária, Produção Sustentável e Irrigação (SEAPI), Rio Grande do Sul, Brazil, and by company Smurfit Westrock Brazil. To overcome dormancy, seeds were surface-sterilized with 70% ethanol for 1 min, rinsed and soaked in distilled water for 24 h. Afterwards, seeds were sown in boxes of sterilized sand (three autoclave cycles at 121 °C for 30 min) and stratified at 5 °C for 30 days (Delard et al., 2024).

### *2.1. Fungal material*

Fungal inocula were obtained from fresh sporocarps collected in the central region of Rio Grande do Sul (RS), Brazil (Figure 1). Basidiomes of *Lactarius* spp. were collected in commercial *Pinus* spp. Plantations located in the municipality of São Pedro do Sul, RS (Figures 2A-C). Ascomata of *Tuber floridanum* Grupe, Sulzbacher and M.E. Sm. were collected in January of 2025 from a 15-year-old pecan (*Carya illinoinensis*) orchard, located in Cachoeira do Sul/RS (Figures 2E and 2F). The orchard is managed under

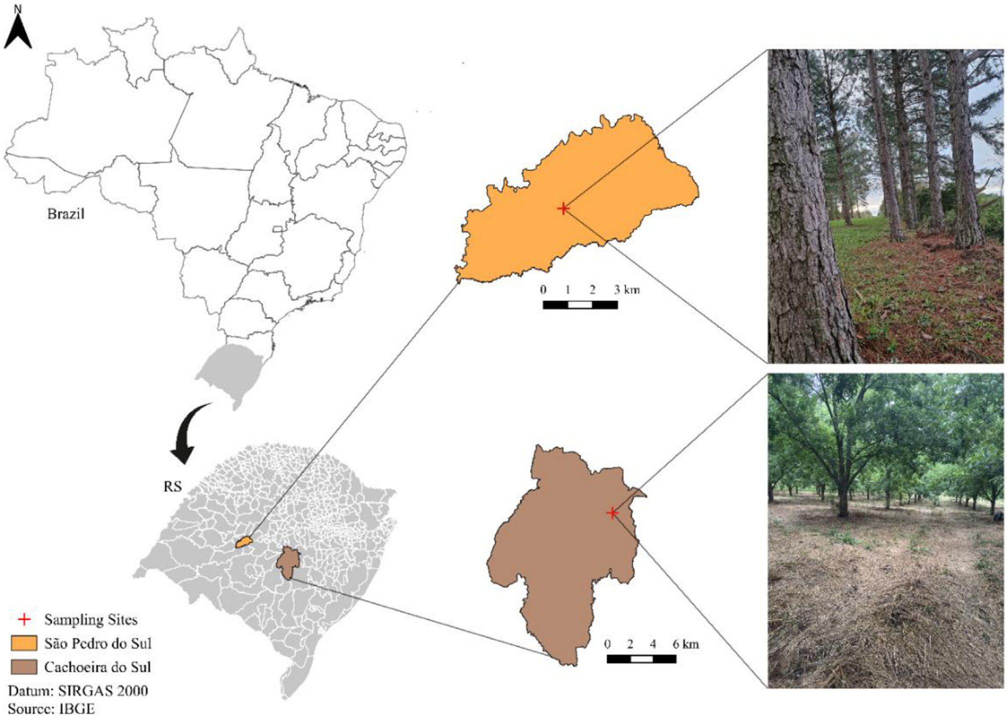

**Figure 1.** Graphic representation of the collection sites used to obtain the inocula.

sprinkler irrigation and receives regular applications of organic manure and dolomitic limestone. Ground cover plants are maintained using forage turnip (*Raphanus sativus*) and white oat (*Avena sativa*). The inocula were kept at −20 °C until use.

### *2.2. Morphological, molecular and phylogenetic characterization of Lactarius*

Taxonomic identification of *Lactarius* was initially performed based on macroscopic characters of fresh specimens, recording pileus and stipe coloration, concentric zonation and the presence/color of latex. Identification followed the taxonomic key and morphological descriptions proposed by Nuytinck and Verbeken (2005). The color standardization and nomenclature used the catalog of Kornerup and Wanscher (1978). Exsiccates of *L. quieticolor* were deposited in the Herbarium of the Department of Biology, Federal University of Santa Maria (SMDB), under accession SMDB 25541.

For taxonomic confirmation, genomic DNA was extracted from tissue of the pileus of the *Lactarius* basidiomes used as the inoculum source. Pileus fragments were previously macerated in liquid nitrogen until a fine powder was obtained. DNA extraction followed the protocol of the commercial Quick-DNA Fungal/Bacterial Miniprep kit (Zymo Research), according to the manufacturer's instructions. DNA integrity and quality were assessed by electrophoresis on 0.8% (w/v) agarose gel prepared in 0.5× TBE buffer. Electrophoresis was run under a constant voltage of 80-120 V using a 1 kb molecular weight marker (Ludwig Biotecnologia, Alvorada, Rio Grande do Sul, Brazil)

The internal transcribed spacer (ITS) region of the rDNA, including the 5.8S gene, was amplified by polymerase chain reaction (PCR) using the universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al., 1990). Reactions were prepared in a final volume of 25 µL, containing: 2.5 µL 10× PCR buffer, 2.5 µL dNTPs, 1.5 µL $MgCl_2$, 0.5 µL Taq DNA polymerase, 1 µL of each primer and 13 µL Milli-Q water, totaling 22 µL of reaction mix, to which 3 µL of template DNA were added. Reactions were performed in triplicate, including a negative control without template DNA.

Thermocycling conditions consisted of an initial denaturation at 95 °C for 3 min, followed by 35 cycles of: denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. PCR products were visualized by electrophoresis on 0.8% agarose gel prepared in 0.5× TBE buffer. For visualization, 10 µL of each sample were mixed with 1 µL of Loading Dye® (Sinapse Biotecnologia) and 3 µL of the fluorescent dye GelRed® Nucleic Acid Gel Stain (Biotium). Remaining amplified products were enzymatically purified using ExoSAP-IT (Applied Biosystems). Bidirectional Sanger sequencing was performed by ACTGene (Alvorada, Rio Grande do Sul, Brazil) using an ABI 3500 Genetic Analyzer (Applied Biosystems).

Obtained sequences (forward and reverse) were inspected and manually edited in UGENE v.53 to generate consensus sequences. Taxonomic identity was verified via BLASTn (Basic Local Alignment Search Tool) against the NCBI GenBank database. Sequences showing >99%

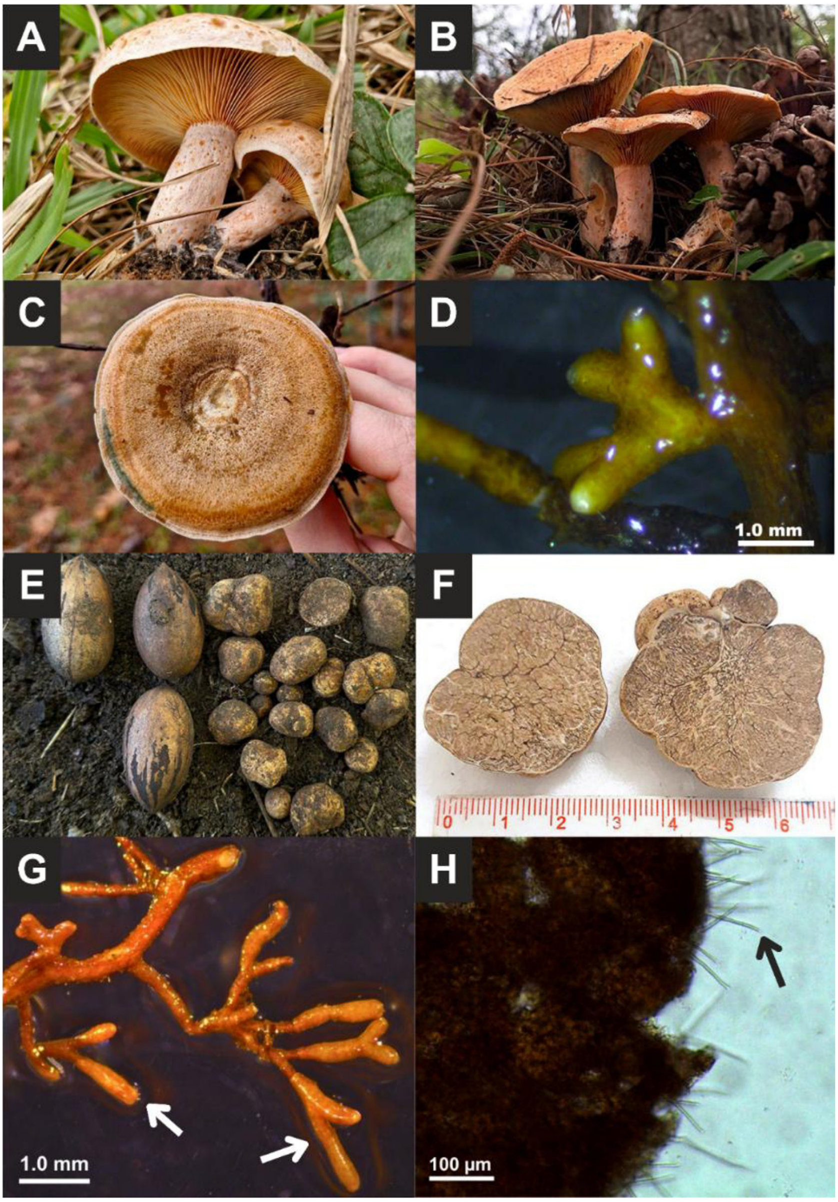

**Figure 2.** Basidiomes and ectomycorrhizal structures of *Lactarius quieticolor* and *Tuber floridanum* collected in forest systems. (A-D) *Lactarius quieticolor* collected in a commercial *Pinus* plantation. (A) Young basidiome showing a convex pileus and well developed lamellae; (B) More mature basidiomes with pileus plano-to slightly depressed; (C) Top view of the pileus highlighting orange coloration and concentric zonation; (D) Ectomycorrhiza formed on roots of *Pinus elliottii* and *P. taeda*; (E-H) *Tuber floridanum* collected in a pecan orchard (*Carya illinoinensis*); (E) Recently collected ascocarps showing size variation, shown alongside pecan nuts for scale; (F) Transverse section of a *T. floridanum* ascocarp showing the gleba; ruler in centimeters; (G) Typical *Tuber* ectomycorrhizal structure formed on *P. elliottii*, with arrows indicating ectomycorrhizae; (H) Microscopic detail showing the presence of cystidia in the ectomycorrhiza, a feature typical of ascomycetes. Scale bars as indicated in the images.

similarity and E-value close to zero relative to reference (voucher) specimens were considered for identification. The generated sequences were deposited in GenBank under accession numbers GenBank PX901894 to PX901897.

For phylogenetic analysis, the sequences were aligned with reference sequences of the genus *Lactarius* (Section *Deliciosi*) retrieved from GenBank using the MUSCLE algorithm implemented in MEGA 11 (Tamura et al., 2021). *Lactarius indigo* was used as the outgroup. The phylogenetic tree was constructed by the Maximum Likelihood method based on the Kimura 2-parameter substitution model with Gamma distribution (K2P+G), determined as the best fit for the data. Clade robustness was assessed by bootstrap analysis with 1,000 replicates. Treatment of gaps and missing data followed partial deletion with a 70% site coverage cutoff.

### *2.3. Preparation of the inoculum and experimental design*

The spore aqueous solution was prepared according to the methodology described by Marozzi et al. (2017). Two independent trials were carried out, one for each tree species (*P. taeda* and *P. elliottii*), under a completely randomized design (CRD). Treatments were arranged in a factorial scheme (2 × 3) + 1, consisting of two fungal species (*Lactarius* spp. and *Tuber floridanum*) and three inoculum doses (2, 4 and 6 g $L^{-1}$ of spore aqueous solution per unit of substrate), plus an additional uninoculated control for each *Pinus* species. The experiment was conducted with five replicates per treatment (single seedling plot), using flexible polyethylene (PE) pots with a capacity of 1.7 L (Nutriplan®).

The substrate used (Carolina Soil® internal class LXXXVI) consisted primarily of sphagnum peat, expanded vermiculite, and charred rice husk, supplemented with mineral fertilizer (NPK), with pH adjusted to 6.5 by the addition of dolomitic limestone and electrical conductivity of 0.7 mS $cm^{-1}$. Prior to use, the substrate was sterilized by autoclaving at 121 °C for 2 h. Irrigation was applied manually once daily, as needed to maintain adequate moisture.

Due to asynchrony in seed emergence, sowing of *P. elliottii* occurred 60 days before *P. taeda*. Consequently, transplanting and inoculation were performed simultaneously, but on seedlings of different chronological ages: 90 days for *P. elliottii* and 30 days for *P. taeda*. Thus, the total plant ages at the end of the experiment differed between species (270 and 210 days, respectively). However, the effective experimental period was standardized and synchronized, with both species cultivated for 180 days after inoculation until harvest. Statistical comparisons were performed exclusively within each *Pinus* species, ensuring that possible ontogenetic differences did not affect the interpretation of the ectomycorrhizal inoculation effects.

### *2.4. Morphological assessments and ectomorrhizal association*

At the end of the experimental period (180 days after inoculation), plants were harvested and the root system was carefully washed under running water to remove substrate. Shoot height (H) was measured with a graduated ruler and collar diameter (DC) was measured with a digital caliper. Dry biomass of shoots and roots was determined by gravimetry after drying in a forced-air circulation oven at 65 °C until constant mass. For assessment of ectomycorrhizal colonization, root fragments of 1.5-2.0 cm were randomly selected and examined under a stereomicroscope. Percentage colonization was estimated using the gridline intersect method (Brundrett et al., 1996), counting mycorrhizal and non-mycorrhizal root tips until a total of at least 200 apices per plant was reached. Morphological identification of ectomycorrhizae was based on the characteristics described by Agerer (1991), distinguishing the morphotypes formed by the inoculated isolates from possible contaminants.

### *2.5. Physiological and nutritional analyses*

Fluorescence of chlorophyll *a* was measured on needles from the upper third of the pine needles between 08:00 and 11:00 h using a modulated light fluorometer (Junior-PAM, Walz, Germany). Needles were dark-adapted for 30 min prior to measurement. Minimum fluorescence ($F_0$), maximum fluorescence ($F_m$), the maximum quantum efficiency of photosystem II ($F_v/F_m$), and the relative electron transport rate (ETR) were determined using saturating light pulses of 10,000 µmol photons $m^{-2}$ $s^{-1}$ (Rangel et al., 2023). For nutritional analysis, the dried shoot tissue of each sample was ground in a Wiley-type mill. Determination of nitrogen (N) and phosphorus (P) contents followed Tedesco et al. (1995), consisting of sulfuric acid digestion with hydrogen peroxide ($H_2O_2$), followed by N quantification by Kjeldahl distillation and P quantification by colorimetry (molybdenum-blue method).

### *2.6. Statistical analyzes*

The morphological, physiological and nutritional data were initially evaluated for residual normality and homogeneity of variances using the Shapiro-Wilk and Levene tests, respectively. When necessary, data were transformed using the bestNormalize package. After meeting the assumptions, data were submitted to analysis of variance (ANOVA). First, the effect of fungal inoculation was assessed through simple comparisons between Control vs. *Lactarius quieticolor* and Control vs. *Tuber floridanum* for *P. elliottii* (Table 1S) and *P. taeda* (Table 2S) (Supplementary Material). Subsequently, the effect of fungal dose on the response variables was investigated. When significant effects were detected, means were compared by Tukey's test ($P < 0.05$), using the *ExpDes.pt* package. To evaluate the overall effect of treatments on the set of variables, a permutational multivariate analysis of variance (PERMANOVA) based on Euclidean distance with 9,999 permutations was performed (Table 3S). Additionally, a principal component analysis (PCA) was applied to visualize treatment dispersion. All analyses were conducted in the R statistical environment (R Development Core Team, 2024), using the *ExpDes.pt*, *bestNormalize*, *vegan* and *ggplot2* packages.

## **3. Results**

The identity of the fungus used as the inoculum source for the *Lactarius* treatment was initially inferred from macroscopic characters of basidiomes collected in the field, which indicated affinity with the genus *Lactarius*, showing orange coloration, presence of latex and morphology typical of species of section *Deliciosi* (Figures 2A-C). These characteristics are similar among taxa such as *Lactarius deliciousus* and *L. quieticolor* (Silva-Filho et al., 2020). The collected basidiomes (*n* = 13) had total heights ranging from 40 to 120 mm, pileus diameters from 50 to 80 mm, stipe diameters from 5 to 20 mm, and individual fresh masses between 11.2 and 68.5 g. The pileus was convex to planoconvex, sometimes slightly depressed centrally, orange to orange-brown in color, with a smooth to slightly fibrillose surface and frequently concentric zones (Figures 2A-C). Lamellae were subdecurrent to decurrent and densely arranged, and the stipe was central, cylindrical and similar in color to the pileus. The context was firm and exuded orange latex, features consistent with the *Lactarius* sect. *Deliciosi* complex. Sequencing of the 18S rDNA gene and phylogenetic analysis showed high similarity with *Lactarius quieticolor* sequences deposited in public databases (GenBank: PX901894 to PX901897), with the isolates grouping in the same clade as that species in the dendrogram, thus confirming the taxonomic identification of the material used (Figure 3).

Inoculation of *L. quieticolor* (Figure 2D) and *T. floridanum* (Figure 2G) in *Pinus elliottii* resulted in the formation of ectomycorrhizae, whereas in *Pinus taeda* only *L. quieticolor* (Figure 2D) established a mycorrhizal association. In *P. elliottii*, residual colonization was recorded in the control treatment (~1.0%), attributed to other ectomycorrhizal morphotypes. Nevertheless, colonization levels were higher in the inoculated treatments (11.62% for *Lactarius* and 8.92% for *Tuber*, Figure 4A). In *P. elliottii*, both fungal species significantly influenced plant height, root dry mass and the electron transport rate (ETR) (Table 1S, Figure 4). Root length (Figure 4B), P content (Figure 4I) and initial chlorophyll fluorescence ($F_0$) (Figure 4K) responded exclusively to inoculation with *L. quieticolor*, whereas collar diameter (Figure 4G) and shoot dry mass (Figure 4F) were influenced only by *T. floridanum*. For *P. taeda*, inoculation with *T. floridanum* did not produce a significant effect (Table 2S), while *L. quieticolor* increased shoot nitrogen content (Figure 5H) and ETR (Figure 5J).

For *P. elliottii*, collar diameter and $F_0$ showed only a simple effect of the fungus factor (Table 1S). Collar diameter was greater in plants inoculated with *T. floridanum* (3.73 mm) compared to those inoculated with *L. quieticolor* (3.09 mm) (Figure 4G), whereas $F_0$ values were lower in plants inoculated with *L. quieticolor* (Figure 4K). For *P. elliottii*, some variables showed a significant interaction between fungal species and inoculum dose (Table 1S, Table 1). The greatest heights were observed at doses of 2 and 6 g $L^{-1}$ for *T. floridanum*. Root length was maximized at 4 g $L^{-1}$ for *L. quieticolor* and at 2 g $L^{-1}$ for *T. floridanum*: at 2 g $L^{-1}$ *T. floridanum* promoted greater root length, while at 4 and 6 g $L^{-1}$ *L. quieticolor* produced the highest values (Table 1). Root and shoot dry masses were higher at 2 g $L^{-1}$ for *T. floridanum*. Ectomycorrhizal colonization in *P. elliottii* was not influenced by inoculum dose ($P = 0.4497$, Table 1S). In *P. taeda*, only *L. quieticolor* formed ectomycorrhizae, with higher colonization percentages (Figure 6A) and higher shoot nitrogen contents (Figure 6B) observed at doses of 4 and 6 g $L^{-1}$.

Multivariate analysis indicated a significant effect of inoculation on the set of evaluated variables (Table 3S). For *P. elliottii*, PERMANOVA revealed a significant global model ($P < 0.001$), with differences between control and *L. quieticolor* ($P < 0.001$), control and *T. floridanum* ($P = 0.002$), and between the fungi ($P = 0.0109$). These results were corroborated by principal component analysis, in which the first two axes explained 48.6% of the total variation (PC1 = 33.9% and PC2 = 14.7%), showing separation between control and inoculated plants and discrimination among fungal treatments (Figure 7A). For *P. taeda*, the global model (Table 3S) was also significant ($P = 0.0025$), with a difference between control and *L. quieticolor* ($P = 0.0303$). No difference

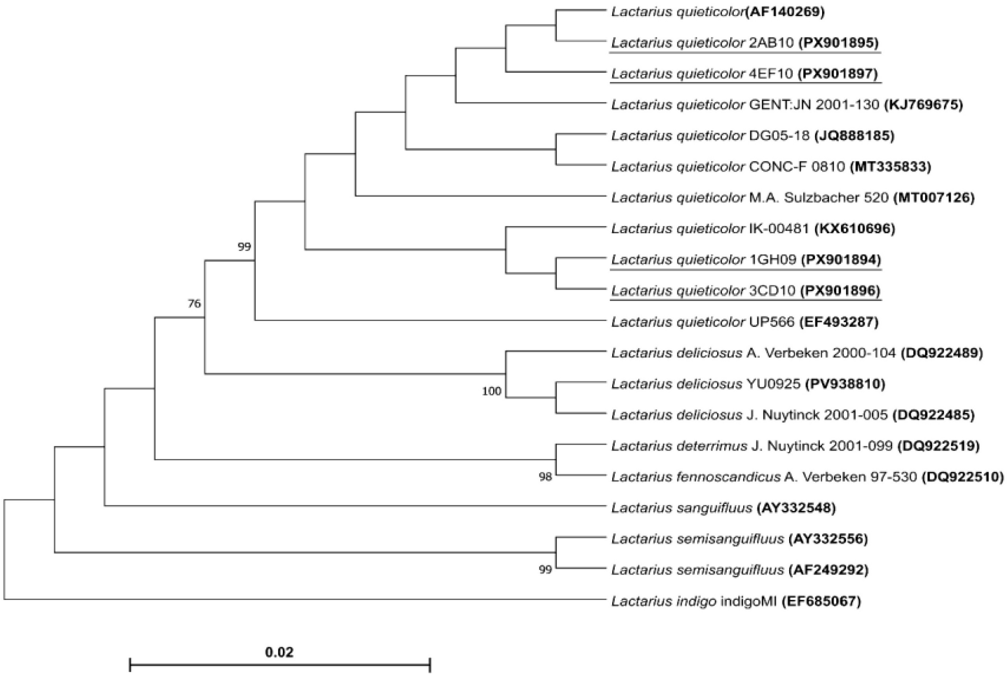

**Figure 3.** Phylogenetic tree inferred by the Maximum Likelihood method using the Kimura 2-parameter model with Gamma distribution (K2P+G). Isolates obtained in this study clustered with *Lactarius quieticolor* (bootstrap support 99%). Values at nodes indicate bootstrap support (%) above 50% (1,000 replicates). The scale bar (0.02) represents the number of nucleotide substitutions per site. The tree was rooted with *Lactarius indigo* as the outgroup. Codes in parentheses refer to GenBank accession numbers.

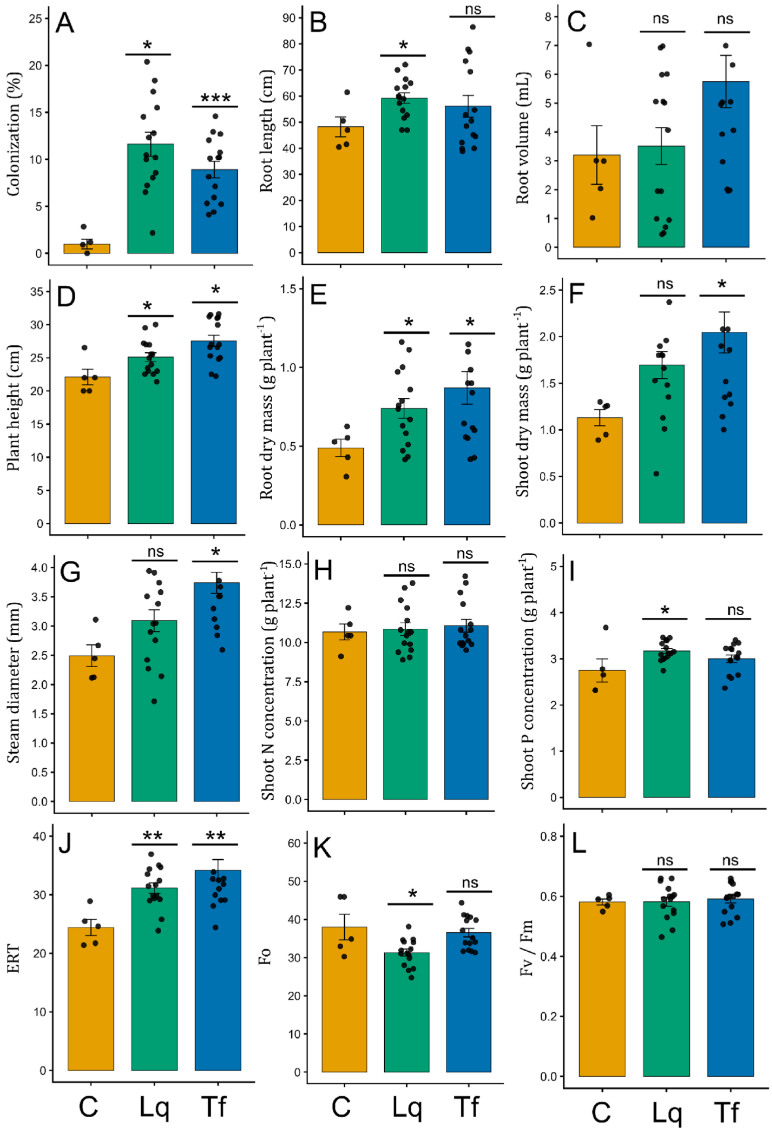

**Figure 4.** Effect of ectomycorrhizal inoculation on morphological, nutritional and physiological variables of *Pinus elliottii*. (A) Colonization; (B) root length; (C) root volume; (D) plant height; (E) root dry mass; (F) shoot dry mass; (G) stem diameter; (H) shoot nitrogen content; (I) shoot phosphorus content; (J) electron transport rate (ETR); (K) minimum fluorescence ($F_0$); (L) maximum quantum efficiency of photosystem II ($F_v/F_m$). Treatments correspond to control plants (C) and plants inoculated with *Lactarius quieticolor* (Lq) and *Tuber floridanum* (Tf). Bars represent means (*n* = 5 for the control and *n* = 15 for each fungal species) ± standard error, and black dots show individual values. Comparisons were made between the control and each fungus. ns = not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

between control and *T. floridanum* ($P = 0.9277$) was found, and a distinction between the fungi ($P < 0.001$), in agreement with the PCA (Figure 7B), whose first two axes explained 40.8% of the total variation (PC1 = 21.3% and PC2 = 19.5%).

## 4. Discussion

### 4.1. Morphology and molecular identification of Lactarius

Identification based exclusively on morphological characters has proven insufficient to discriminate species within the *Lactarius* sect. *Deliciosi* complex, where taxonomic errors are frequently reported (Lee et al., 2019; Kaewgrajang et al., 2020). In this context, molecular confirmation of *Lactarius quieticolor* through phylogenetic analyses using DNA markers (Figure 3) was essential to ensure the correct identity of the fungus used as the inoculum source in this study, distinguishing it from cryptic and morphologically similar taxa. In southern Brazil, Silva-Filho et al. (2020) first confirmed the occurrence of *L. quieticolor* in 12 sites distributed across four municipalities in the state of Rio Grande do Sul (Itaara, São Francisco de Paula, Santa Maria and São Gabriel) and in

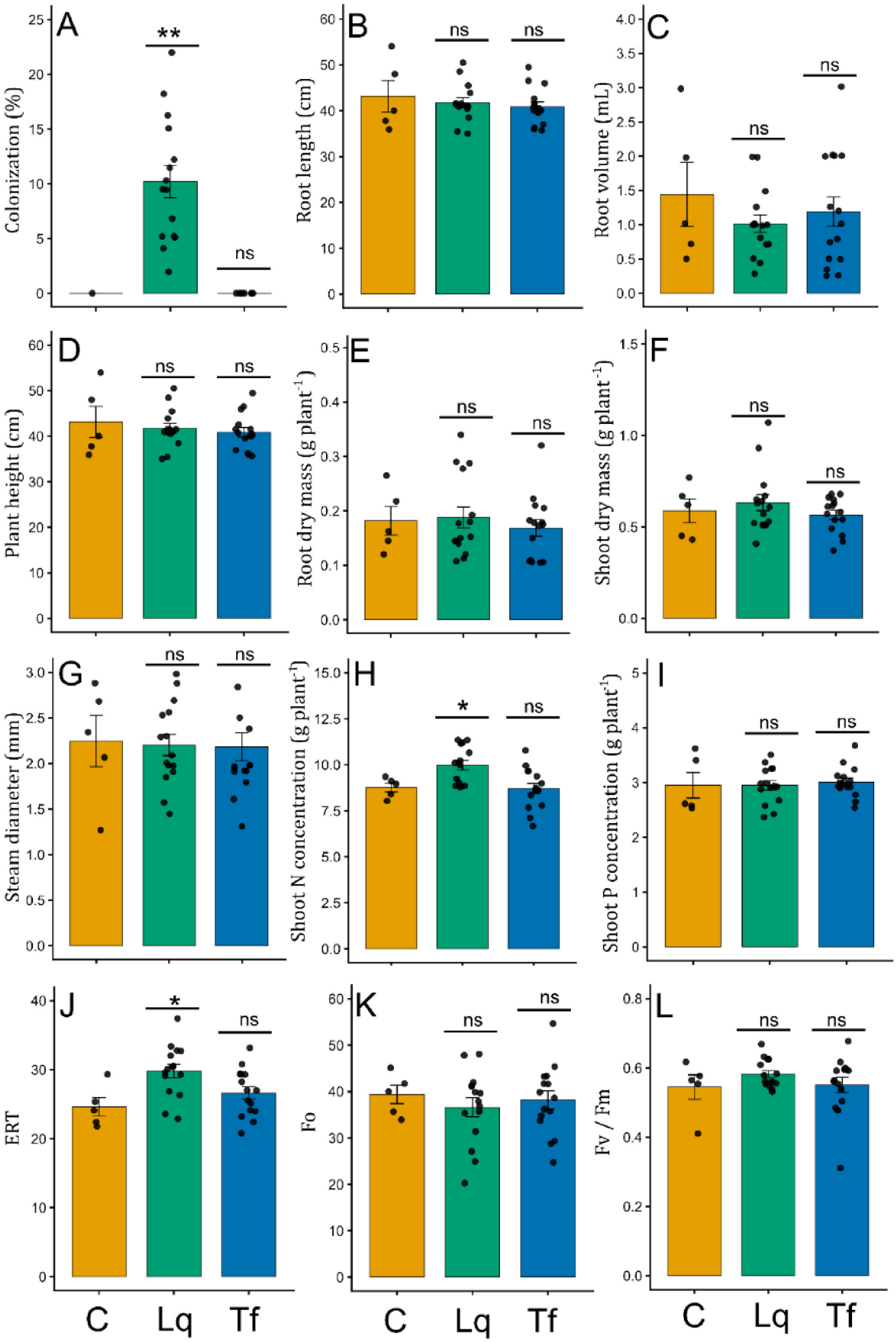

**Figure 5.** Effect of ectomycorrhizal inoculation on morphological, nutritional and physiological variables of *Pinus taeda*. (A) colonization; (B) root length; (C) root volume; (D) plant height; (E) root dry mass; (F) shoot dry mass; (G) stem diameter; (H) shoot nitrogen content; (I) shoot phosphorus content; (J) electron transport rate (ETR); (K) minimum fluorescence ($F_0$); (L) maximum quantum efficiency of photosystem II ($F_v/F_m$). Treatments correspond to control plants (C) and plants inoculated with *Lactarius quieticolor* (Lq) and *Tuber floridanum* (Tf). Bars represent means (*n* = 5 for the control and *n* = 15 for each fungal species) ± standard error, and black dots show individual values. Comparisons were made between the control and each fungus. ns = not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

three sites within a single municipality in Santa Catarina (Urupema), all associated with *Pinus* stands. The collection site used in the present study corresponds to an additional locality (São Pedro do Sul, RS), distinct from those previously reported, thus extending the known distribution of the species in the southern region of the country. This result reinforces the broad adaptation of *L. quieticolor* to pine plantations in Brazil and the importance of using molecular

**Table 1.** Mean values of plant height (cm), root length (cm), root dry mass (g), and shoot dry mass (g) in *Pinus elliottii* as a function of the interaction between fungal species (*Lactarius quieticolor* and *Tuber floridanum*) and inoculant dose (2, 4, and 6 g $L^{-1}$ of basidioma).

| | | **Plant height (cm)** | |
|---|---|---|---|
| | 2 g $L^{-1}$ | 4 g $L^{-1}$ | 6 g $L^{-1}$ |
| *Lactarius* | 25.04 B | 25.50 | 24.78 B |
| *Tuber* | 29.38 Aa | 23.96 b | 29.34 Aa |
| | | **Root length (cm)** | |
| *Lactarius* | 52.70 Bb | 67.30 Aa | 47.72 Ab |
| *Tuber* | 76.82 Aa | 47.2 Bb | 44.21 Bb |
| | | **Root Dry Mass (g)** | |
| *Lactarius* | 0.77 B | 0.87 | 0.57 |
| *Tuber* | 1.28 Aa | 0.56 b | 0.77 b |
| | | **Shoot Dry Mass (g)** | |
| *Lactarius* | 1.55 B | 1.93 | 1.59 |
| *Tuber* | 2.92 Aa | 1.31 b | 1.91 b |

Means followed by different letters differ from each other by the Tukey test ($P \leq 0.05$). Capital letters compare fungal species within each dose, and lowercase letters compare doses within each fungal species.

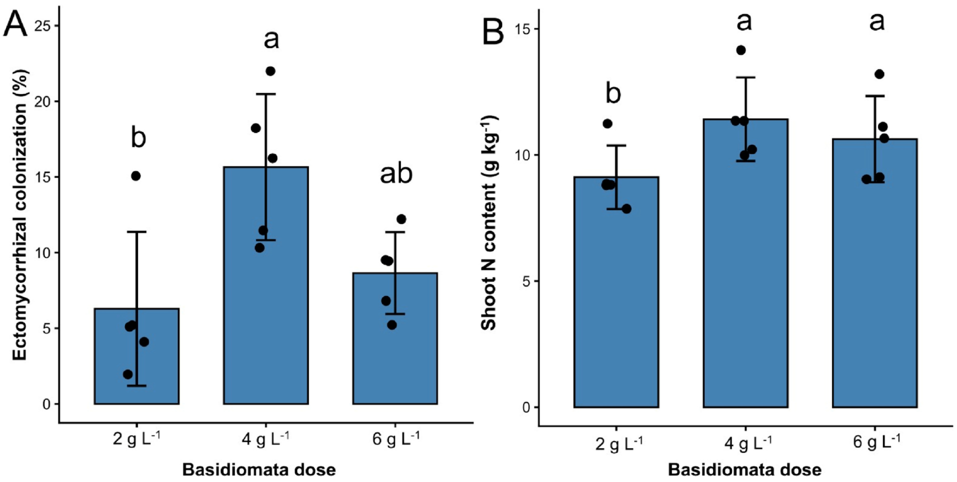

**Figure 6.** Effect of different *Lactarius quieticolor* doses on ectomycorrhizal colonization and nutritional status of *Pinus taeda*. (A) Percentage of ectomycorrhizal root colonization as a function of basidiomata doses (2, 4, and 6 g $L^{-1}$); (B) Shoot nitrogen content (g $kg^{-1}$) in response to the same doses. Bars represent means ± standard deviation, and dots indicate individual replicates.

tools for the accurate characterization of ectomycorrhizal fungi in introduced forest systems (Drewinski et al., 2024).

### *4.2. Ectomycorrhizal formation and host compatibility between Tuber, Lactarius and Pinus*

The formation of ectomycorrhizae in *P. elliottii* after inoculation with *L. quieticolor* and *T. floridanum* confirms the compatibility of these symbionts with this host under controlled conditions, whereas in *P. taeda* only *L. quieticolor* established a mycorrhizal association, suggesting specificity or differential preferences between the fungi and pine species. *Lactarius* is widely reported as an ectomycorrhizal partner of *Pinus* species, including in plantations in the Southern Hemisphere, and is commonly observed in roots of *P. elliottii* and *P. taeda* across diverse localities and edaphoclimatic conditions, which underscores its adaptability and compatibility with these tree hosts (Daniel Chávez et al., 2015; Silva-Filho et al., 2020). In contrast, the evidence of *T. floridanum* forming ectomycorrhizae in *P. elliottii* in the present study represents the first experimental documentation of this species as a pine symbiont, expanding knowledge of the ecology of *T. floridanum*.

### *4.3. Host response to inoculum concentration*

Increasing the inoculation dose of *Lactarius quieticolor* and *Tuber floridanum* in *P. elliottii* did not result in an increase in associative capacity, indicating that the

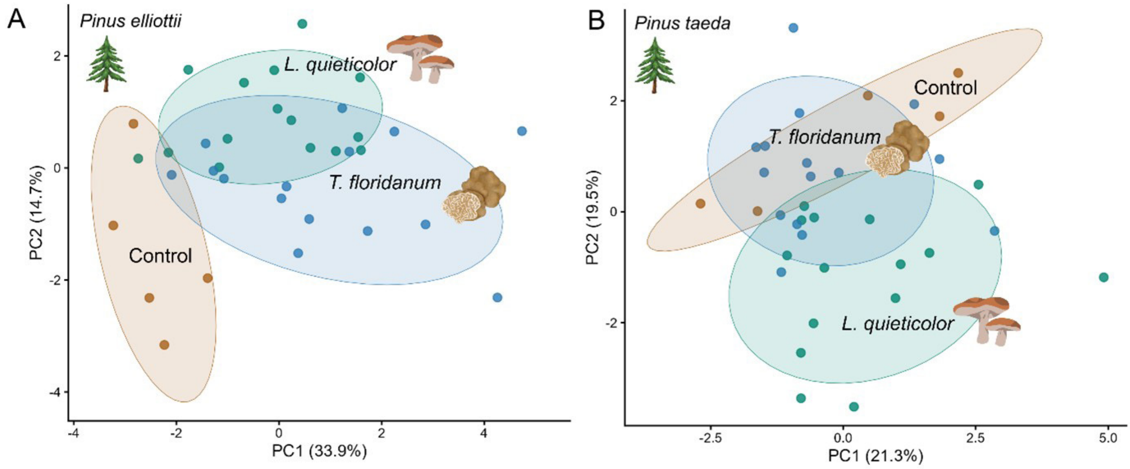

**Figure 7.** Principal component analysis (PCA) based on the set of morphological, nutritional and physiological variables of control and inoculated plants. (A) *Pinus elliottii*; (B) *Pinus taeda*. Points represent experimental units, colored by treatment: control, *Lactarius quieticolor* and *Tuber floridanum*. Ellipses indicate the distribution of treatments around the centroid (95% confidence interval). Values in parentheses on the axes correspond to the percentage of variance explained by each principal component.

lowest tested dose (2 g L$^{-1}$) was sufficient to promote ectomycorrhiza formation in this species. On the other hand, in *P. taeda* an increase in root colonization by *L. quieticolor* was observed with higher doses (4 and 6 g L$^{-1}$), suggesting a positive response to greater inoculum presence (Figure 6A). These results may reflect differences in symbiotic receptivity between the two pine species, with *P. taeda* possibly requiring a higher initial inoculum amount to reach equivalent colonization levels, as previously reported in studies highlighting dose-dependent responses in mycorrhizal interactions (Smith and Read, 2010).

In the present study, relatively high inoculum doses were used, corresponding to 2, 4 and 6 g of fresh mushrooms or truffles per liter of substrate, totaling 4, 8 and 12 g of fresh material per pot (2 L). The relatively high inoculum doses used are knowingly higher than those that would be economically viable for operational application, especially in the case of *Tuber* truffles, which have high commercial value. This strategy was deliberately adopted to maximize the presence of viable propagules and ensure establishment of the ectomycorrhizal symbiosis in the effective combinations observed in this study. A similar approach was discussed by Geng et al. (2009), who used approximately 3 g of fresh ascocarps of *T. indicum* per seedling (in 1 L pots) and emphasized that this amount does not represent an economically viable rate, highlighting the need to define minimum effective doses.

### *4.4. Growth, nutrition and ectomycorriza-mediated physiological responses*

The beneficial effects of ectomycorrhizae on growth, mineral nutrition and physiology of conifers are widely documented, from classical studies that demonstrated greater biomass accumulation, root morphological changes and improved nutritional status (Melin and Nilsson, 1957; Marx, 1969; Rwizi et al., 2025), to more recent work that integrates physiological and molecular responses (Heller et al., 2008; Liao et al., 2016; Martin and van der Heijden, 2024). In *Pinus* species, inoculation with ectomycorrhizal fungi has consistently been associated with increased N and P uptake, greater root development, increases in height and stem diameter, as well as changes in photosynthetic efficiency and antioxidant enzyme activity (Rwizi et al., 2025; Zhang et al., 2025). In broader terms, the functional responsiveness of roots to mycorrhizal inoculation, reflecting increases in root volume, dry mass, and physiological status, is a key determinant for successful plant establishment even under diverse environmental pressures (Santos et al., 2023; Barros et al., 2024).

Within this context, *Lactarius* species are frequently reported as effective conifer symbionts, with records of improved early growth, higher biomass production and modulation of physiological parameters in *Pinus* seedlings (Guerin-Laguette et al., 2003; Wei et al., 2024). Such effects are commonly attributed to enhanced soil exploration by the extraradical mycelium, expansion of the absorption zone for low-mobility nutrients, and regulation of host physiological activity, mechanisms consistent with the growth, nutritional, and physiological responses observed in *P. elliottii* in the present study. Consistent with this framework, our results show that inoculation with *L. quieticolor* elicited broad responses in *P. elliottii*, reflected in consistent growth gains (greater root length, height and root dry mass), mineral nutrition (greater P accumulation in shoots) and physiological performance (higher electron transport rate and lower initial chlorophyll fluorescence). In contrast, in *P. taeda* the effects were more restricted, limited to increases in shoot N content and ETR, indicating a lower functional responsiveness to the symbiosis. These results are supported by the fact that, in *P. taeda*, only the highest inoculum doses (4 and 6 g) led to increases in shoot N content, coinciding with higher levels of root colonization. This pattern reinforces the hypothesis that the nutritional benefits observed depend on effective establishment of the symbiosis and suggests that *P. taeda* requires a higher initial inoculum pressure to express measurable physiological and nutritional responses. Together, these findings show that, although both species are susceptible to ectomycorrhizal association, *P. elliottii* exhibits greater responsiveness to inoculation with *L. quieticolor*, highlighting the importance of host-symbiont specificity and initial inoculum pressure for the functional success of the symbiosis.

Although *Tuber* species are more frequently associated with angiosperms (Bonito et al., 2010; Grupe et al., 2018), there is consistent evidence that different taxa of this genus can form ectomycorrhizae with conifers, including *Pinus* species. Natural fruiting of *T. aestivum* has been documented in association with *P. nigra* in Spain, indicating that pines can act as effective hosts under field conditions (Garcia-Montero et al., 2014). Under controlled conditions, *T. indicum* formed ectomycorrhizae with *P. armandii* (Geng et al., 2009), and mycorrhizal synthesis of *T. melanosporum* with *P. halepensis* has also been reported (Domínguez-Núñez et al., 2012). In other controlled experiments, *T. indicum* and *T. panzhihuanense* were able to form ectomycorrhizae in *P. armandii* (Zhang et al., 2025), while *T. borchii* formed mycorrhizae in *P. sylvestris* seedlings (Mrak et al., 2024). Additionally, surveys in *Pinus* forests in North America have recorded the natural occurrence of *T. eburneum* and *T. mujicii* associated with pines (Lemmond et al., 2022). Collectively, these studies reinforce that associations between *Tuber* species and *Pinus* occur both in natural and experimental settings.

Nevertheless, our results demonstrate the existence of specificity in the host-symbiont interaction. Although *T. floridanum* was able to form ectomycorrhizae with *P. elliottii*, resulting in direct benefits to plants such as greater root growth and plant height, higher shoot P concentration, increased root and shoot dry mass, larger collar diameter and higher electron transport rate, the same was not observed in *P. taeda*, despite both belonging to the same genus. These findings indicate that mycorrhizal compatibility is not universal among *Pinus* and *Tuber* species, underscoring the need to investigate the host physiological, biochemical and molecular mechanisms that regulate symbiont recognition and establishment.

### *4.5. Multivariate integration of responses to inoculation*

The significance observed in the PERMANOVA (Table 3S) and the consistent separation of treatments in the PCA (Figure 7) indicate that ectomycorrhizal inoculation triggered integrated changes across the set of morphological, nutritional and physiological variables evaluated, rather than isolated responses. In *P. elliottii*, the distinction among control plants, inoculated plants and between the two fungal species shows that *L. quieticolor* and *T. floridanum* induced distinct functional patterns, reinforcing the notion of fungus-host specificity at the multivariate level. In *P. taeda*, the lack of differentiation between the control and *T. floridanum* and the clear separation of the *Lactarius* treatment (Figure 7) corroborate the univariate results and indicate that only association was functionally relevant for this species. Multivariate approaches have been widely used in mycorrhizal studies to demonstrate that inoculation simultaneously reorganizes plant growth, nutrition and physiological apparatus, allowing identification of global response patterns not fully captured by isolated analyses (Corrêa et al., 2008; van der Heijden et al., 2015; Lamit et al., 2016). In conifers, PCA and PERMANOVA analyses have been employed to show that different ectomycorrhizal fungi generate specific “functional signatures” in their hosts by integrating biomass, nutritional status and physiological parameters (Jones et al., 2012; Li et al., 2021; Valdés et al., 2025). In this context, the multivariate separation observed in the present study reinforces that the established ectomycorrhizae coordinately modified plant performance, supporting the interpretation that inoculation effects reflect systemic changes in host functioning.

## 5. Conclusions

This study records, for the first time, the occurrence of Lactarius quieticolor in new sites in the extreme southern region of Brazil, with confirmation based on morphological and molecular characterization, thereby expanding current knowledge of the distribution of this species in Pinus plantations. The results demonstrate that ectomycorrhizal compatibility and its functional effects are not automatically transferable among congeneric Pinus species and are strongly modulated by host identity.

*Lactarius quieticolor* established functional associations with both evaluated species, but with contrasting responsiveness. In *P. elliottii*, the lowest tested dose (2 g $L^{-1}$) was sufficient to promote broad growth, nutritional, and physiological responses, whereas in *P. taeda* functional benefits were more limited and only evident at higher inoculum doses (4-6 g $L^{-1}$). In contrast, *Tuber floridanum* showed compatibility only with *P. elliottii*, with effective ectomycorrhiza formation and associated plant benefits observed at the tested inoculum doses, constituting the first experimental evidence of ectomycorrhiza formation between this truffle species and a member of the genus *Pinus*.

The dose-dependent responses observed, particularly in *P. taeda*, and the effectiveness of the lowest inoculum dose in *P. elliottii* underscore the relevance of inoculum pressure for the expression of ectomycorrhizal benefits. Together, these results advance the understanding of the functional ecology of ectomycorrhizae and provide a solid conceptual basis for future field-based and applied studies.

## Acknowledgments

The authors thank the Coordination for the Improvement of Higher Education Personnel (CAPES) and the National Council for Scientific and Technological Development (CNPq) (Process No. 303679/2022-0) for financial support, the Soil Biology Laboratory (UFSM), the Department of Soils/CCR (UFSM), the Slovenian Forest Institute, and especially Mr. Lailor Garcia for providing the truffles for this research. The authors also thank DDPA/SEAPI (Rio Grande do Sul, Brazil) and Smurfit Westrock Brazil for donating the Pinus seeds used in this study.

## Data Availability Statement

The data sets analyzed or generated during this study are available from the corresponding author upon reasonable request.

## References

AGERER, R., 1991. Characterization of ectomycorrhiza. In: J.R. NORRIS, D.J. READ and A.K. VARMA, eds. *Methods in microbiology*. Berlin: Academic Press, vol. 23, pp. 25-73.

BARROS, S., TURCHETTO, R., MAGALHÃES, J.B., CANEPELLE, E., ANDREOLA, D.S., ROS, C.O., BASSO, C.J., SILVA, V.R. and SILVA, R.F., 2024. Arbuscular mycorrhizal fungi on the development and copper content in corn and sorghum plants. *Brazilian Journal of Biology*, vol. 84, e283238. https://doi.org/10.1590/1519-6984.283238. PMid:39442150.

BONITO, G.M., GRYGANSKYI, A.P., TRAPPE, J.M. and VILGALYS, R., 2010. A global meta-analysis of Tuber ITS rDNA sequences: species diversity, host associations and long-distance dispersal. *Molecular Ecology*, vol. 19, no. 22, pp. 4994-5008. https://doi.org/10.1111/j.1365-294X.2010.04855.x. PMid:21040049.

BRUNDRETT, M., BOUGHER, N., DELL, B., GROVE, T. and MALAJCZUK, N., 1996. *Working with mycorrhizas in forestry and agriculture*. Canberra: Australian Centre for International Agricultural Research. ACIAR Monograph, no. 32.

CHÁVEZ, D., MACHUCA, Á., TORRES-MELLADO, G.A. and PALFNER, G., 2015. Phylogenetic and mycogeographical aspects of *Lactarius* and *Rhizopogon* associated with *Pinus radiata* in south-central Chile. *Phytotaxa*, vol. 226, no. 2, pp. 177-187. https://doi.org/10.11646/phytotaxa.226.2.7.

CORRÊA, A., STRASSER, R.J. and MARTINS-LOUÇÃO, M.A., 2008. Response of plants to ectomycorrhizae in N-limited conditions: which factors determine its variation? *Mycorrhiza*, vol. 18, no. 8, pp. 413-427. https://doi.org/10.1007/s00572-008-0195-0. PMid:18719949.

DELARD, C., LOEWE, V., RÍO, R.D., GONZÁLEZ, M., GREGORI, G., ACEVEDO, M., CARTES, E. and BALZARINI, M., 2024. Effect of *Tuber borchii* inoculation on *Pinus pinea* seedling quality and initial growth in the field. *New Forests*, vol. 55, no. 4, pp. 609-627. https://doi.org/10.1007/s11056-023-09992-8.

DOMÍNGUEZ-NÚÑEZ, J., MARTIN, A., ANRIQUEZ, A. and ALBANESI, A., 2012. The combined effects of Pseudomonas fluorescens and *Tuber melanosporum* on the quality of *Pinus halepensis* seedlings. *Mycorrhiza*, vol. 22, no. 6, pp. 429-436. https://doi.org/10.1007/s00572-011-0420-0. PMid:22068563.

DREWINSKI, M.P., CORRÊA-SANTOS, M.P., LIMA, V.X., LIMA, F.T., PALACIO, M., BORGES, M.E.A., TRIERVEILER-PEREIRA, L., MAGNAGO, A.C., FURTADO, A.N.M., LENZ, A.R., SILVA-FILHO, A.G.S., NASCIMENTO, C.C., ALVARENGA, R.L.M., GIBERTONI, T.B., OLIVEIRA, J.J.S., BALTAZAR, J.M., NEVES, M.A., VARGAS-ISLA, R., ISHIKAWA, N.K. and MENOLLI, N. Jr., 2024. Over 400 food resources from Brazil: evidence-based records of wild edible mushrooms. *IMA Fungus*, vol. 15, no. 1, pp. 1-24. https://doi.org/10.1186/s43008-024-00171-8. PMid:39673069.

FUENTEALBA, C., FERRER, V., ARTEAGA-PEREZ, L.E., SANTOS, J., DELGADO, N., CASAS-LEDÓN, Y., BRAVO-ARREPOL, G., PEREIRA, M., ANDRADE, A., ESCOBAR-AVELLO, D. and CABRERA-BARJAS, G., 2025. Actualized scope of forestry biomass valorization in Chile: fostering the bioeconomy. *Forests*, vol. 16, no. 8, pp. 1-31. https://doi.org/10.3390/f16081208.

GARCÍA-MONTERO, L., MORENO, D., MONLEON, V. and ARREDONDO, F., 2014. Natural production of *Tuber aestivum* in central Spain: *Pinus* spp. versus *Quercus* spp. brûlés. *Forest Systems*, vol. 23, no. 2, pp. 394-399. https://doi.org/10.5424/fs/2014232-05112.

GENG, L.-Y., WANG, X.-H., YU, F.-Q., DENG, X.-J., TIAN, X.-F., SHI, X.-F., XIE, X.-D., LIU, P.-G. and SHEN, Y.-Y., 2009. Mycorrhizal synthesis of *Tuber indicum* with two indigenous hosts, *Castanea mollissima* and *Pinus armandii*. *Mycorrhiza*, vol. 19, no. 7, pp. 461-467. https://doi.org/10.1007/s00572-009-0247-0. PMid:19404686.

GROSS, E., CASAGRANDE, L.I.T. and CAETANO, F.H., 2004. Ultrastructural study of ectomycorrhizas on *Pinus caribaea* Morelet. var. *hondurensis* Barr. & Golf. seedlings. *Acta Botanica Brasílica*, vol. 18, no. 1, pp. 1-7. https://doi.org/10.1590/S0102-33062004000100001.

GRUPE, A.C., SULZBACHER, M.A., GREBENC, T., HEALY, R., BONITO, G. and SMITH, M.E., 2018. Tuber brennemanii and *Tuber floridanum*: two new Tuber species are among the most commonly detected ectomycorrhizal taxa within commercial pecan (*Carya illinoinensis*) orchards. *Mycologia*, vol. 110, no. 4, pp. 780-790. https://doi.org/10.1080/00275514.2018.1490121. PMid:30130456.

GUERIN-LAGUETTE, A., CONVENTI, S., RUIZ, G., PLASSARD, C. and MOUSAIN, D., 2003. The ectomycorrhizal symbiosis between *Lactarius deliciosus* and *Pinus sylvestris* in forest soil samples: symbiotic efficiency and development on roots of a rDNA internal transcribed spacer-selected isolate of L. *deliciosus*. *Mycorrhiza*, vol. 13, no. 1, pp. 17-25. https://doi.org/10.1007/s00572-002-0191-8. PMid:12634915.

VAN DER HEIJDEN, M.G.A., MARTIN, F.M., SELOSSE, M.A. and SANDERS, I.R., 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. *The New Phytologist*, vol. 205, no. 4, pp. 1406-1423. https://doi.org/10.1111/nph.13288. PMid:25639293.

HELLER, G., ADOMAS, A., LI, G., OSBORNE, J., VAN ZYL, L., SEDEROFF, R., FINLAY, R.D., STENLID, J. and ASIEGBU, F.O., 2008. Transcriptional analysis of *Pinus sylvestris* roots challenged with the ectomycorrhizal fungus *Laccaria bicolor*. *BMC Plant Biology*, vol. 8, no. 1, pp. 1-13. https://doi.org/10.1186/1471-2229-8-19. PMid:18298811.

JONES, M.D., PHILLIPS, L.A., TREU, R., WARD, V. and BERCH, S.M., 2012. Functional responses of ectomycorrhizal fungal communities to long-term fertilization of lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.) stands in central British Columbia. *Applied Soil Ecology*, vol. 60, pp. 29-40. https://doi.org/10.1016/j.apsoil.2012.01.010.

KAEWGRAJANG, T., KAEWJUNSRI, S., JANNUAL, N. and NIPITWATTANAPHON, M., 2020. Morphological and molecular identification of some *Lactarius* and *Russula* species. *Genomics and Genetics*, vol. 13, pp. 44-58.

KORNERUP, A. and WANSCHER, J.H., 1978. *Methuen handbook of colour*. 3rd ed. London: Eyre Methuen.

LAMIT, L., HOLESKI, L., FLORES-RENTERÍA, L., WHITHAM, T.G. and GEHRING, C., 2016. Tree genotype influences ectomycorrhizal fungal community structure: ecological and evolutionary implications. *Fungal Ecology*, vol. 24, pp. 124-134. https://doi.org/10.1016/j.funeco.2016.05.013.

LEE, H., WISSITRASSAMEEWONG, K., PARK, M.S., VERBEKEN, A., EIMES, J. and LIM, Y.W., 2019. Taxonomic revision of the genus *Lactarius* (Russulales, Basidiomycota) in Korea. *Fungal Diversity*, vol. 95, no. 1, pp. 275-335. https://doi.org/10.1007/s13225-019-00425-6.

LEMMOND, B.R., HEALY, R.A., BONITO, G. and SMITH, M.E., 2022. Tuber eburneum and Tuber mujicii: new pine-associated Tuber species from eastern North America. *Mycologia*, vol. 114, no. 3, pp. 575-586. https://doi.org/10.1080/00275514.2022.2037338. PMid:35482507.

LI, X., KANG, W., LIU, S., YIN, H., LYU, Q., SU, Y., LIU, J., LIU, J., FAN, C., CHEN, G., ZHAO, K. and LI, X., 2021. Diversity of ectomycorrhizal fungal communities in four types of stands in *Pinus massoniana* plantation in the West of China. *Forests*, vol. 12, no. 6, pp. 1-17. https://doi.org/10.3390/f12060719.

LIAO, H.-L., CHEN, Y. and VILGALYS, R., 2016. Metatranscriptomic study of common and host-specific patterns of gene expression between pines and their symbiotic ectomycorrhizal fungi in the genus *Suillus*. *PLOS Genetics*, vol. 12, no. 10, pp. 1-24. https://doi.org/10.1371/journal.pgen.1006348. PMid:27736883.

LOEWE-MUÑOZ, V., DELARD, C., DEL RÍO, R., GREGORI, G. and BALZARINI, M., 2024. Effects of *Tuber borchii* inoculation on *Pinus pinea* 3 years after establishment along a latitudinal gradient in the Southern Hemisphere. *Agroforestry Systems*, vol. 98, no. 2, pp. 369-381. https://doi.org/10.1007/s10457-023-00915-2.

MAROZZI, G., SÁNCHEZ, S., BENUCCI, G.M.N., BONITO, G., FALINI, L.B., ALBERTINI, E. and DONNINI, D., 2017. Mycorrhization of pecan (*Carya illinoinensis*) with black truffles: tuber melanosporum and *Tuber brumale*. *Mycorrhiza*, vol. 27, no. 3, pp. 303-309. https://doi.org/10.1007/s00572-016-0743-y. PMid:27838857.

MARTIN, F.M. and VAN DER HEIJDEN, M.G.A., 2024. The mycorrhizal symbiosis: research frontiers in genomics, ecology, and agricultural application. *The New Phytologist*, vol. 242, no. 4, pp. 1486-1506. https://doi.org/10.1111/nph.19541. PMid:38297461.

MARX, D.H., 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. *Phytopathology*, vol. 59, no. 2171, pp. 153-163. https://doi.org/10.1094/Phyto-59-153. PMid:40934401.

MELIN, E. and NILSSON, H., 1957. Transport of C14-labelled photosynthate to the fungal associate of Pine mycorrhiza. *Svensk Botanisk Tidskrift*, vol. 51, pp. 166-186.

MRAK, T., GREBENC, T., FRIEDRICH, S. and MÜNZENBERGER, B., 2024. Description, identification, and growth of *Tuber borchii* Vittad. mycorrhized *Pinus sylvestris* L. seedlings on different lime contents. *Mycorrhiza*, vol. 34, no. 1, pp. 85-94. https://doi.org/10.1007/s00572-023-01135-3. PMid:38236414.

NUYTINCK, J. and VERBEKEN, A., 2005. Morphology and taxonomy of the European species in *Lactarius* sect. *Deliciosi* (Russulales). *Mycotaxon*, vol. 92, pp. 125-168. https://doi.org/10.5962/p.414542.

R DEVELOPMENT CORE TEAM, 2024. *R: a language and environment for statistical computing.* Vienna: R Foundation for Statistical Computing.

RAMOS, R.F., 2026. The soil health-yield trade-off: a critical gap in assessing regenerative organic agriculture. *Ecosystem Services*, vol. 78, pp. 101823. https://doi.org/10.1016/j.ecoser.2026.101823.

RAMOS, R.F., SOBUCKI, L., PAWLOWSKI, E., SARZI, J.S., RABUSKE, J.E., SAVIAN, L.G., KASPARY, T.E. and BELLÉ, C., 2023. Perspective chapter: microorganisms and their relationship with tree health. In: C. BELLÉ, ed. *Current and emerging challenges in the diseases of trees*. London: IntechOpen. https://doi.org/10.5772/intechopen.110461.

RANGEL, T.S., SANTANA, N.A., JACQUES, R.J.S., RAMOS, R.F., SCHEID, D.L., KOPPE, E., TABALDI, L.A. and SILVEIRA, A.O., 2023. Organic fertilization and mycorrhization increase copper phytoremediation by *Canavalia ensiformis* in a sandy soil. *Environmental Science and Pollution Research International*, vol. 30, no. 26, pp. 68271-68289. https://doi.org/10.1007/s11356-023-27126-7. PMid:37119494.

RUDAWSKA, M., AUČINA, A., NAUGŽEMYS, D., SKRIDAILA, A., KARLIŃSKI, L., PIETRAS, M. and LESKI, T., 2025. Does topography affect the ectomycorrhizal fungal community structure of Scots Pine (*Pinus sylvestris* L.) grown on the sand coastal dunes in Poland and Lithuania. *Forest Ecology and Management*, vol. 589, pp. 1-13. https://doi.org/10.1016/j.foreco.2025.122786.

RWIZI, M., ČĖSNA, V., KROKENE, P. and MAGEROY, M., 2025. Ectomycorrhizal fungi inoculation of conifers increases growth, but not stress resistance: a meta-analysis. *Forest Ecology and Management*, vol. 595, pp. 1-12. https://doi.org/10.1016/j.foreco.2025.122982.

SANTOS, E.A., SILVA FILHO, U.S., BARROSO, G.M., RABELO, J.S., MELO, E.I. and SANTOS, J.B., 2023. Arbuscular mycorrhizal fungi activity in the rhizosphere of tree seedlings subjected to residual herbicides. *Brazilian Journal of Biology*, vol. 83, e242676. https://doi.org/10.1590/1519-6984.242676. PMid:34161454.

SANTOS, V.J., BARROS, G., MOREIRA, T.F., GIOVENARDI, A.R., MAGALHÃES, J.B., STEFFEN, G.P.K., STÜRMER, S.L. and SILVA, R.F., 2024. Occurrence and diversity of arbuscular mycorrhizal fungi in yerba mate (*Ilex paraguariensis* – Aquifoliaceae) cultivation environments. *Brazilian Journal of Biology*, vol. 84, e282738. https://doi.org/10.1590/1519-6984.282738. PMid:39383364.

SARDANS, J., LAMBERS, H., PREECE, C., ALREFAEI, A.F. and PENUELAS, J., 2023. Role of mycorrhizas and root exudates in plant uptake of soil nutrients (calcium, iron, magnesium, and potassium): has the puzzle been completely solved? *The Plant Journal: For Cell and Molecular Biology*, vol. 114, no. 6, pp. 1227-1242. https://doi.org/10.1111/tpj.16184. PMid:36917083.

SEBASTIANA, M., DUARTE, B., MONTEIRO, F., MALHÓ, R., CAÇADOR, I. and MATOS, A.R., 2019. The leaf lipid composition of ectomycorrhizal oak plants shows a drought-tolerance signature. *Plant Physiology and Biochemistry : PPB*, vol. 144, pp. 157-165. https://doi.org/10.1016/j.plaphy.2019.09.032. PMid:31568958.

SILVA-FILHO, A., SULZBACHER, M., GREBENC, T. and WARTCHOW, F., 2020. Not every edible orange milkcap is *Lactarius deliciosus*: first record of *Lactarius quieticolor* (sect. Deliciosi) from Brazil. *Journal of Applied Botany and Food Quality*, vol. 93, pp. 289-299. https://doi.org/10.5073/JABFQ.2020.093.036.

SIMON, C.P., GOMES, T.F., PESSOA, T.N., SOLTANGHEISI, A., BIELUCZYK, W., CAMARGO, P.B., MARTINELLI, L.A., and CHERUBIN, M.R., 2022. Soil quality literature in Brazil: a systematic review. *Revista Brasileira de Ciência do Solo*, vol. 46, pp. 1-21. https://doi.org/10.36783/18069657rbcs20210103.

SMITH, S.E. and READ, D.J., 2010. *Mycorrhizal symbiosis*. 3rd ed. Amsterdam: Academic Press, 800 p.

TAMURA, K., STECHER, G. and KUMAR, S., 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, vol. 38, no. 7, pp. 3022-3027. https://doi.org/10.1093/molbev/msab120. PMid:33892491.

TEDESCO, M.J., GIANELLO, C., BISSANI, C.A., BOHNEN, H. and VOLKWEISS, S.M., 1995. *Análises de solo, plantas e outros materiais.* 2. ed. Porto Alegre: Universidade Federal do Rio Grande do Sul, 174 p. Boletim Técnico de Solos, no. 5.

TROVATO, V.W., SOUZA, G.G., SANTOS, S.C. and SANTOS, V.C., 2024. Arbuscular mycorrhizal fungi, phosphorus and organic residues in *Peltophorum dubium* (Spreng.) Taub. seedlings. *Brazilian Journal of Biology*, vol. 84, e276160. https://doi.org/10.1590/1519-6984.276160. PMid:38511773.

VALDÉS, R.C., GARCÍA, F.G., RENTERIA, D.F., RENTERIA, L.F., MENDOZA, A.B., TEDERSOO, L. and MORALES, S.G., 2025.

Community of ectomycorrhizae associated with Mexican pinyon pine (*Pinus cembroides*) in northeastern Mexico and its relationships with environmental variables. *Forest Ecology and Management*, vol. 598, pp. 123-237. https://doi.org/10.1016/j.foreco.2025.123237.

WANG, Y.-H., DAI, Y., KONG, W.-L., ZHU, M.-L. and WU, X.-Q., 2022. Improvement of Sphaeropsis shoot blight disease resistance by applying the ectomycorrhizal fungus *Hymenochaete* sp. Rl and mycorrhizal helper bacterium *Bacillus pumilus* HR10 to *Pinus thunbergii*. *Phytopathology*, vol. 112, no. 6, pp. 1226-1234. https://doi.org/10.1094/PHYTO-09-21-0392-R. PMid:35476587.

WARTCHOW, F., MAIA, L.C. and CAVALCANTI, M.A.Q., 2013. Taxonomic studies of *Amanita muscaria* (L.) Lam (Amanitaceae, Agaricomycetes) and its infraspecific taxa in Brazil. *Acta Botanica Brasílica*, vol. 27, no. 1, pp. 31-39. https://doi.org/10.1590/S0102-33062013000100005.

WEI, Z., LIU, L., LEI, Y., XIE, S., MA, J., TAN, Y., TANG, N., YANG, Z. and AI, C., 2024. Establishment of *Pinus massoniana–Lactarius hatsudake* symbiosis. *Forests*, vol. 15, no. 4, pp. 1-18. https://doi.org/10.3390/f15040578.

WHITE, T.J., BRUNS, T., LEE, S. and TAYLOR, J.W., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: M.A. INNIS, D.H. GELFAND, J.J. SNINSKY and T.J. WHITE, eds. *PCR protocols: a guide to methods and applications.* San Diego: Academic Press, pp. 315-322.

XU, H., LV, J. and YU, C., 2023. Combined phosphate-solubilizing microorganisms jointly promote *Pinus massoniana* growth by modulating rhizosphere environment and key biological pathways in seedlings. *Industrial Crops and Products*, vol. 191, pp. 1-15. https://doi.org/10.1016/j.indcrop.2022.116005.

ZHANG, X., CHEN, L., LI, X., ZHANG, L., DEVEAU, A., MARTIN, F. and ZHANG, X., 2025. Ectomycorrhizal symbiosis with *Tuber* spp. enhances host performances in *Pinus* and *Carya* and induces host-specific patterns in defense-related regulation in the leaf transcriptomes. *Mycorrhiza*, vol. 35, no. 5, pp. 53. https://doi.org/10.1007/s00572-025-01227-2. PMid:40928528.

ZHOU, J., GUBE, M., HOLZ, M., SONG, B., SHAN, I., SHI, L., KUZYAKOV, Y., DIPPOLD, M.A. and PAUSCH, J., 2022. Ectomycorrhizal and non-mycorrhizal rhizosphere fungi increase root-derived C input to soil and modify enzyme activities: a 14C pulse labelling of *Picea abies* seedlings. *Plant, Cell & Environment*, vol. 45, no. 10, pp. 3122-3133. https://doi.org/10.1111/pce.14413. PMid:35909089.

## Supplementary Material

Supplementary material accompanies this paper.

**Table 1S.** P-values and coefficients of variation (CV, %) obtained for ectomycorrhizal colonization, growth, mineral nutrition and chlorophyll fluorescence variables in Pinus elliottii.

**Table 2S.** P-values and coefficients of variation (CV, %) obtained for ectomycorrhizal colonization, growth, mineral nutrition and chlorophyll fluorescence parameters in Pinus taeda.

**Table 3S.** Results of the permutational multivariate analysis of variance (PERMANOVA) applied to the set of growth, biomass, mineral nutrition and chlorophyll fluorescence variables of Pinus elliottii and Pinus taeda.

This material is available as part of the online article from https://doi.org/10.1590/1519-6984.305139Ectomycorrhizal compatibility in *Pinus* species