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RESEARCH ARTICLE

New fungal endophytes in *Bromus valdivianus* Phil. diversity, ecological function, and their relationship with the host in the grasslands of southern Chile

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ABSTRACT

Endophytic fungi play a crucial role in plant-host interactions; however, the endophytic diversity associated with *Bromus valdivianus* Phil. (pasture brome), a forage species of agronomic importance, remains largely unexplored. In this study, we determined the diversity and ecological function of endophytic fungi in *B. valdivianus* in southern Chile. Seeds and leaves were collected from wild plants and cv. Bronco in La Araucanía, Los Ríos, and Los Lagos Regions during 2022-2023. Isolates were grown on agar water and potato dextrose agar media, followed by morphological characterization and molecular identification through polymerase chain reaction amplification of the internal transcribed spacer region (rDNA-ITS), β-tubulin, and elongation factor 1-α. Nineteen species of endophytic fungi were identified: 18 from the phylum Ascomycota and one from the phylum Mucoromycota. *Fusarium proliferatum* was the most abundant species, followed by *Trichoderma harzianum*. Five species of interest were identified: *Chaetomium subaffine*, *Beauveria bassiana*, *Penicillium sanguifluum*, *T. harzianum*, and *T. koningiopsis*. A phylogenetic analysis revealed tissue-specific colonization patterns, with 80.5% infection in leaf samples and 19.5% in seeds. Functional predictions based on FUNGuild database determined five trophic modes distributed across nine ecological guilds. Location, ecological role (endophytes, phytopathogens, saprotrophs), and morphological variables explained 57.1% of species distribution. This research provides the first comprehensive characterization of endophytic fungi in *B. valdivianus*, laying the foundations for understanding plant-endophyte interactions and applications in sustainable grassland management in southern Chile.

Key words: Biodiversity, *Bromus valdivianus*, ecological guilds, endophytic fungi, grass-fungus interaction.

INTRODUCTION

Endophytic fungi are microorganisms that live systemically and asymptomatically within the tissues of a host plant throughout its life cycle. However, the term endophyte is used broadly and often variably, with its reference limited to factors such as host class, taxonomic group, type of colonized tissue, or transmission mechanism (Liao et al., 2025). This biological complexity implies that fungus-plant interactions, under the endophyte-host-environment model, can manifest a spectrum of responses ranging from mutualism to parasitism (Bala et al., 2025).

These endophytic associations have been extensively documented (Iannone et al., 2021; Kivlin et al., 2022). Historically, research has focused on the associations between *Epichloë* endophytic fungi and cool-season grasses (Brachypodieae, Bromeae, Poeae, and Triticeae). This symbiosis is of particular agronomic relevance in species such as *Lolium perenne*, *Festuca arundinacea*, *Dactylis glomerata,* and several representatives of the genus *Bromus*. However, the successful establishment of these associations is strictly conditioned by factors intrinsic to the host, such as its ploidy level, the occurrence of hybridization events, genetic compatibility, and the degree of fungal colonization (Franco et al., 2015).

Despite research on global patterns of these interactions, region-specific reports from South America remain limited. In this region, studies have focused mainly on *Epichloë* endophytic fungi associated with *Bromus* spp. of the Bromopsis section, leaving other sections underexplored. Given the taxonomic complexity of this genus, significant knowledge gaps remain regarding these associations, as numerous species from the Bromus, Bromopsis, and Ceratochloa sections have not been investigated for the isolation and characterization of their endophytic fungi (Iannone et al., 2021).

Moreover, South American grasslands exhibit remarkable diversity of grasses, with native species of the genus *Bromus* standing out for their agronomic value in livestock production in countries such as Argentina, Chile, and Uruguay. Several species are of particular interest for fodder, including *B. auleticus*, *B. burkartii*, *B. catharticus*, *B. setifolius*, *B. stamineus*, *B. valdivianus*, and *B. wildenowii*. In southern Chile, some studies have focused on *B. catharticus*, *B. stamineus*, and *B. valdivianus*, all of which are classified within the Ceratochloa section (Williams et al., 2011).

Among these, the species *B. valdivianus* deserves special attention. A perennial grass native to southern Chile and distributed from Concepción to Puerto Montt (Stewart, 1996), this species has a hexaploid genome; it also self-pollinates and exhibits rapid and uniform growth, reaching a height of 0.5 to 0.7 m (Ordoñez et al., 2017). Its importance for livestock lies in its nutritional quality, resistance to summer drought, and high persistence, which allow it to coexist with other forage species (López et al., 2025).

However, Ortega et al. (2007) mention that *B. valdivianus* lacks nutritional limitations as it is not associated with endophytic fungi of the genus *Epichloë*. Despite its favorable agronomic characteristics and the documented absence of *Epichloë*, a significant knowledge gap exists regarding the diversity of other endophytic fungi potentially associated with this native species, and only a few studies have explored the native *Bromus* genus in central and southern Chile. Therefore, the only known record of *Epichloë* is in *B. setifolius* var. *pictus* (Iannone et al., 2021). This lack of characterization contrasts with studies on other regional forage grasses, where research has focused on specific ecological roles, highlighting the use of *Neotyphodium lolii* in *Lolium perenne* and *Festuca arundinacea* for pest control (Parra et al., 2017) or the identification of non-epicloid endophytes (*Beauveria* and *Metarrhizium*) in *L. perenne* (Vera et al., 2022).

Fungal endophytes play a role that transcends their function as symbionts, positioning them as key elements in sustainable agriculture. In forage grasses, these microorganisms have been documented to confer greater tolerance to abiotic stresses such as drought and salinity, critical aspects in the context of climate change and pasture management in southern Chile. They also act as biocontrol agents by inducing systemic resistance or producing metabolites that inhibit pathogens and repel herbivores, providing an ecological alternative to the intensive use of agrochemicals. Furthermore, they contribute to soil health by promoting plant growth, solubilizing nutrients, and improving soil structure, directly impacting the productivity and resilience of livestock systems. In this context, the characterization of the endophytic community of *B. valdivianus* represents a fundamental step in the development of sustainable management strategies in these agroecosystems.

Since other native and forage grasses in the Southern Hemisphere harbor diverse communities of endophytic fungi with relevant ecological functions, we hypothesized that *B. valdivianus* also has a diverse and complex community of endophytic fungi that includes vertically transmitted endophytes. It is postulated that these endophytes play crucial functional roles in the plant's adaptation to the environmental and agronomic conditions of southern Chile. Therefore, this research aimed to determine the diversity and characterize the ecological function of endophytic fungi associated with the grass *B. valdivianus* in the grasslands of southern Chile.

MATERIALS AND METHODS

Sampling sites

Seeds and plants of wild and 'Bronco' *Bromus valdivianus* Phil. were collected randomly at the end of spring and during the summer of 2022 and 2023 in southern Chile (Figure 1). The sampling sites were located in three regions: La Araucanía (Pucón – PUC1, PUC2, Capitán Pastene – CP1), Los Ríos (Valdivia – VAL1, VAL2, VAL3), and

Los Lagos (Puerto Pilar – PP1). At each site, between 10 and 15 *B. valdivianus* plants were collected from polyphytic grasslands. The most frequent accompanying species included grasses such as *Lolium perenne* L., *Holcus lanatus* L., and *Dactylis glomerata* L. and legumes such as *Trifolium pratense* L. and *T. repens* L. The collected plants were stored in plastic bags at 4 °C for processing.

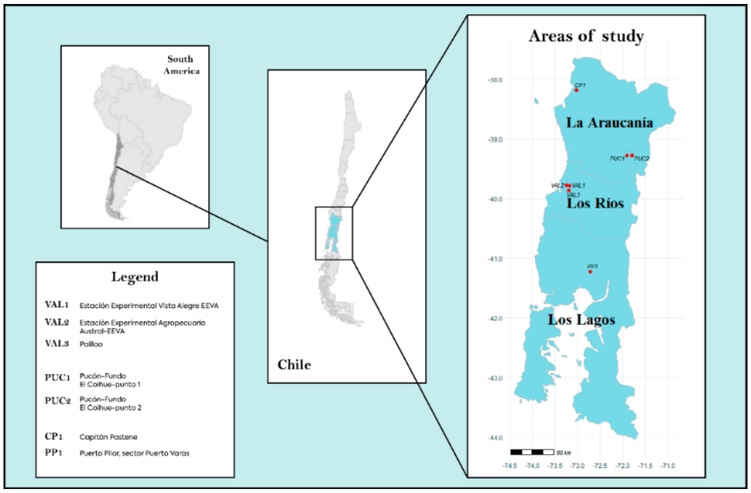

Figure 1. Geographical locations of the sampling sites with *Bromus valdivianus* in grasslands in southern Chile. PUC: Pucón, CP: Capitán Pastene, VAL: Valdivia, PP: Puerto Pilar.

Isolation and characterization of endophytic fungi

Prior to isolation, the outer coat was removed from the seeds, while the leaves were separated into sheaths and blades and cut into 5 mm pieces. All samples were washed with abundant tap water to remove impurities. Surface sterilization was performed by immersing the samples in 70% ethyl alcohol for 10 s, followed by treatment with a 1% sodium hypochlorite solution for 12 min. Finally, they were washed with abundant sterile distilled water until the hypochlorite residues were removed.

The endophytic fungi were isolated from the disinfected samples placed in Petri dishes with water agar (Merck, San Diego, California, USA) and potato dextrose agar (PDA, Merck) supplemented with penicillin and streptomycin (Calbiochem, Merck). The plates were incubated at 25 °C in the dark for 1 to 4 wk, with observations made every 2 d. The isolated fungal colonies were subsequently purified and maintained on PDA medium (Maia et al., 2018).

The endophyte cultures were characterized macroscopically and microscopically; the colony diameter was measured weekly (between first to fourth weeks), and the growth rate was calculated. The microscopic structures of the fungi were visualized with a lactic acid-aniline blue solution and observed under a light microscope (CX31, Olympus Corporation, Tokyo, Japan) with a built-in camera at 400X and 1000X magnification. The conidia and phialides of the isolates with ecological and plant-beneficial importance were measured. Morphological descriptions and identification were performed by consulting the keys of Watanabe (2011).

DNA extraction, polymerase chain reaction amplification, and sequencing

The mycelia of the purified endophytic fungi were collected with a sterile scalpel and placed in Eppendorf tubes with sterile distilled water. The genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega Corp., Madison, Wisconsin, USA), following the manufacturer's instructions. Subsequently, the DNA samples were analyzed by spectrophotometry with NanoQuant Infinite 200 PRO (Tecan Group, Männedorf, Switzerland) at an absorbance of 260 nm, and the purity of the genomic DNA samples was determined at A260/A280 nm.

The genomic DNA from each of the fungal isolates (Table 1), was amplified by a polymerase chain reaction (PCR) of the internal transcribed spacer region (rDNA-ITS). Additionally, some of these fungi were amplified with the β-tubulin (*Bt2*) and Elongation Factor 1-alpha (*EF-1α*) genes. The primers and annealing temperatures used are indicated in Table 2. The PCRs were standardized in a 50 µL volume containing 4.0 µL fungal endophyte genomic DNA; 1.0 µL each primer (Integrated DNA Technologies, Coralville, Iowa, USA); Taq Master-Mix (Promega), which included 0.25 µL GoTaq G2 flexi DNA Polymerase (5 U μL^{-1}); 10.0 µL 5X colorless Gotaq Flexi Buffer; 2.0 µL $MgCl_2$ 25 mM; 1.0 µL dNTP's mix 10 mM (Thermo Fisher Scientific, Waltham, Massachusetts, USA); and 30.75 µL sterile distilled water. The amplification was performed in a T100TM Thermal cycler (Bio-Rad, Hercules, California, USA), and temperature cycles were programmed for each primer following the protocols of White et al. (1990), Glass and Donaldson (1995), and Carbone and Kohn (1999). The amplified PCR products were analyzed by electrophoresis with a 1% agarose gel (Lafken LM, Fermelo Biotec, Santiago, Chile) and visualized on a UV transilluminator (ECX-26M of 312 nm; Labolan, Esparza de Galar, Navarra, Spain).

Table 1. Fungal endophyte isolates obtained from *Bromus valdivianus* and corresponding GenBank accession numbers for the internal transcribed spacer (ITS), β-tubulin (Bt2), and elongation factor 1-alpha (EF-1α) genes.

			GenBank accession number		
Isolate code	Origin host plant *Bromus valdivianus*	Host tissue	ITS	Tub2	Tef
025BV	Wild	Leaves	PV139187		
006BV	Wild	Leaves	PV139175	PV211196	PV339464
028BV	Wild	Leaves	PV139190		
001BV	Wild	Leaves, seed	PV139173	PV389220	PV339462
038BV	Wild	Leaves	PV139193		
010BV	Bronco	Leaves	PV139176		
014BV	Wild	Leaves	PV139180		
026BV	Wild	Leaves	PV139188		
032BV	Wild	Leaves	PV139191		
033BV	Wild	Leaves	PV139192		
019BV	Wild	Seed	PV139182		
011BV	Wild	Seed	PV139177		
021BV	Wild	Leaves	PV139183		
023BV	Wild	Leaves	PV139185		
022BV	Wild	Leaves	PV139184		
013BV	Wild	Leaves, seed	PV139179		
012BV	Wild	Seed	PV139178	PV211197	
017BV	Wild	Leaves	PV139181		
024BV	Wild	Leaves	PV139186		
027BV	Wild	Seed	PV139189		
039BV	Wild	Leaves	PV139194		
040BV	Wild	Leaves	PV139195		
041BV	Bronco	Leaves	PV139196	PV211198	PV339465
005BV	Bronco	Leaves, seed	PV139174	PV211195	PV339463

Table 2. Primers used for amplification and sequencing of the internal transcribed spacer (ITS), β-tubulin (Bt2), and elongation factor 1-alpha (EF-1α) genes.

Locus	Primer	Position	Sequence (5'–3')	Annealing	Fragment size	References
				°C	bp	
ITS	ITS 5	Forward	GAAGTAAAAGTCGTAACAAGG	52-57	550	White et al. 1990
	ITS 4	Reverse	TCCTCCGCTTATTGATATGC			
Bt2	Bt2a	Forward	GGTAACCAAATCGGTGCTGCTTTC	58-62	500	Glass and Donaldson, 1995
	Bt2b	Reverse	ACCCTCAGTGTAGTGACCCTTGGC			
EF-1α	EF1-728F	Forward	CATCGAGAAGTTCGAGAAGG	52-55	350	Carbone and Kohn, 1999
	EF1986R	Reverse	TACTTGAAGGAACCCTTACC			

The PCR products were analyzed at the Austral Omics Laboratory of the Universidad Austral de Chile, where they were enzymatically purified using a mixture of the FastAP thermosensitive AP and Exonuclease I (ExoI) kits (Thermo Fisher Scientific, Vilnius, Lithuania) following the manufacturer's recommendations. Sequencing of the purified genomic DNA samples was performed with an Applied Biosystems Sanger Sequencing 3500 Series Genetic Analyzer (Thermo Fisher Scientific, Marsiling, Singapore) using the BigDye Terminator v3.1 kit (Applied Biosystems, Thermo Fisher Scientific, Vilnius, Lithuania) with subsequent purification using the BigDye Xterminator kit (Thermo Fisher Scientific, Preston Ct, Bedford, Massachusetts, USA), according to the manufacturer's instructions. Subsequently, the sequence fragments (forward and reverse) were assembled and manually edited using the ProSeq 2.91 software (University of Oxford, UK).

The endophytic fungal sequences were uploaded to the GenBank platform using the NCBI BLAST tool (https://www.ncbi.nlm.nih.gov/) to identify sequences with high similarity indices and find the closest matches to the taxa. Subsequently, all sequences were registered in GenBank with their accession numbers (Table 1). Phylogenetic trees were constructed using the Maximum Parsimony model in the Mega X version 10.1 program (Kumar et al., 2018), employing the subtree-pruning-regrafting heuristic search method with Bootstrap supports at 10 000 repetitions, and edited with the ITOL version 7.2 tool (https://itol.embl.de/).

Functional guilds of endophytic fungi

The identified endophytic fungal species were classified into ecological guilds using the FUNGuild tool and database (Nguyen et al., 2016; available at https://www.funguild.org) and MicoBank (https://www.mycobank.org), accessed on 19 July 2025. For each identified taxon, its trophic condition was assigned (e.g., pathotroph, saprotroph, and symbiotroph), besides its functional guild (e.g., endophyte, plant pathogen, and wood saprotroph). When more than one functional guild was reported for a species, all roles were retained, given the versatility of several fungi in different ecological contexts.

Multivariate analysis of morphological and ecological variation in endophytic fungi based on collection site and source substrate

In this study, morphological, ecological, and origin variables of the endophytic fungal isolates were evaluated to determine whether a variation pattern existed based on the collection site or the substrate (leaf, seeds, or both). Some variables were treated as categorical nominal (colony pigmentation, microscopic characteristics, and ecological function), which, along with colony diameter and growth rate, were analyzed using a principal component analysis (PCA).

Additionally, a principal coordinates analysis (PCoA) was performed using a dissimilarity matrix generated from the same variables. This analysis facilitated the evaluation of the distribution of the endophytic fungal species and their relationship with the ecological and morphological variables considered. All statistical analyses were performed using Google Colab under the R programming environment (https://colab.research.google.com/); the packages used were FactoMineR and Factoextra (Lê et al., 2008).

RESULTS

Diversity, morphological characterization, and phylogeny

This research identified 19 species of endophytic fungi from leaves and seeds of *B. valdivianus*. These findings constitute new records for this forage species and contribute to the characterization of the endophytic fungal community associated with this plant species in southern Chile.

The taxonomic composition of the isolates revealed that 18 of the species belonged to the phylum Ascomycota, grouped into 12 families. The remaining species was classified in the phylum Mucoromycota (family Cunninghamellaceae). The families with the highest number of isolated species were Chaetomiaceae, Phaeosphaeriaceae, Hypocreaceae, and Hypocreaeae, as detailed in Figure 2.

Endophytic fungal isolates were predominantly obtained from leaf samples (80.5% infection) collected at the four locations. In contrast, endophytes isolated from seeds were obtained exclusively from the Valdivia locality, in the Paillao sector, representing 19.5% infection.

In terms of isolation frequency, *Fusarium proliferatum* was the most abundant species with 25 records, followed by *Trichoderma harzianum* with nine records. Four species showed intermediate frequencies:

Chaetomium subaffine (6), *Parastagonospora avenae* (5), and *P. poae* and *Beauveria bassiana* (four records each). The remaining species had lower frequencies, with one to three records for each of them (Figure 3).

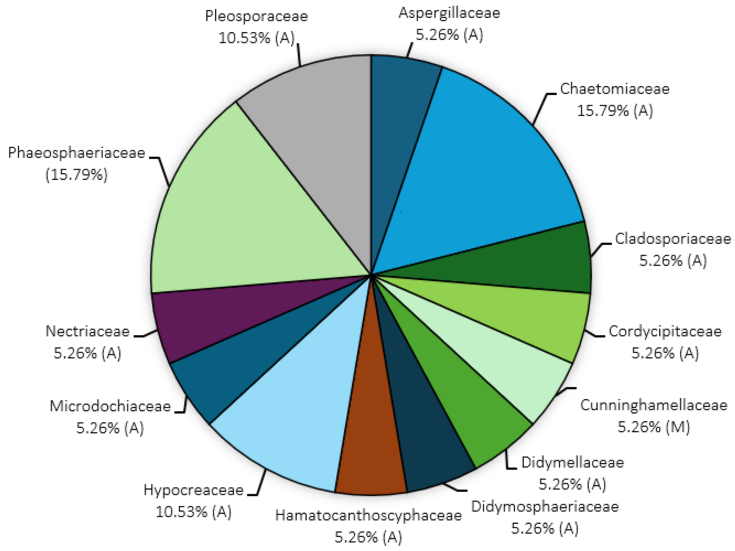

Figure 2. Taxonomic distribution at the family level of endophytic fungi associated with *Bromus valdivianus*. A: Ascomycota; M: Mucormycota.

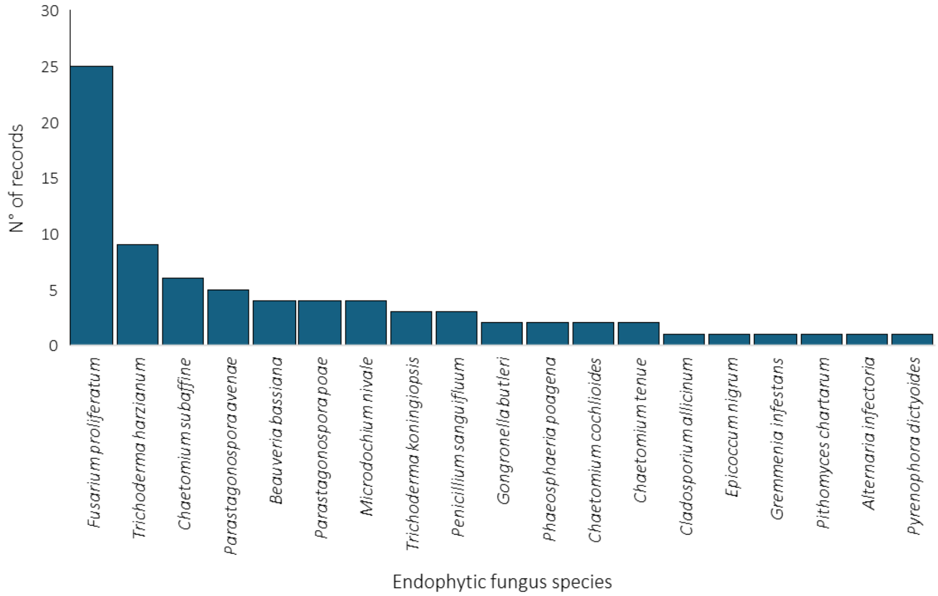

Figure 3. Number of isolates of endophytic fungal species isolated from *Bromus valdivianus*.

A phylogenetic analysis of endophytic fungi isolated from *B. valdivianus* showed that the evolutionary relationships of these fungi are directly associated with the type of plant tissue and the origin of the host. Most species were grouped into clades of the phylum Ascomycota, although their distribution was selective: Some clades were found predominantly in leaf samples, whereas others, such as *Gongronella bluteri* (Mucoromycota), were isolated exclusively from seeds. The study also revealed that both wild *B. valdivianus* plants and those of the commercial 'Bronco' harbor fungal communities, highlighting the importance of these associations between endophytes and their host in different genotypes (Figure 4).

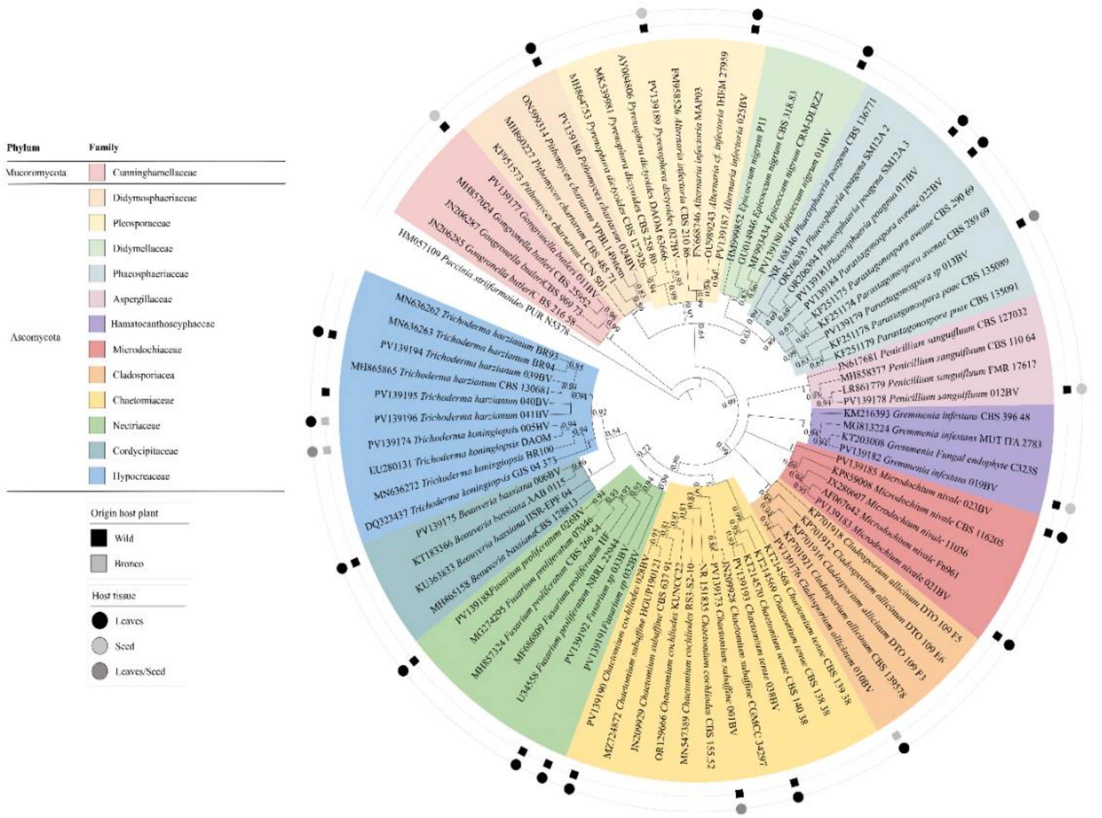

Figure 4. Maximum parsimony phylogenetic tree based on nucleotide sequences from the ITS5-ITS4 rDNA-ITS region of endophytic fungi associated *with Bromus valdivianus*. Fungal species are indicated in each family and denoted by different colors. Squares and circles indicate the origin and tissue of the host, respectively.

Among the species identified in this study, a select group of isolates is of particular interest due to their potential for improving the production and protection of *B. valdivianus*. These isolates include 001BV *Chaetomium subaffine*, 005BV *Trichoderma koningiopsis*, 006BV *Beauveria bassiana*, 012BV *Penicillium sanguifluum*, and 041BV *Trichoderma harzianum*. The taxonomic identification of these species was based on the characterization of specific morphological traits, including colony diameter after 1 wk of culture, as well as the dimensions of microscopic reproductive structures such as conidia, phialides, and ascomata. Detailed morphometric data for each isolate are presented in Table 3, while representative microphotographs of the diagnostic structures and macroscopic appearance of the colonies are shown in Figure 5.

Table 3. Morphological characteristics of endophytes fungal on *Bromus valdivianus*. [a]Taken from seven measurements at 1 wk, mean and standard deviation given. [b]40 conidia examined. [c]16 phialide examined. [d]20 ascomata examined, means and standard deviation of each one are given.

Isolated	Endophytes species	Host	Colony diameter[a]	Conidial dimensions[b]	Phialide[c]	Ascomata[d]
			mm	µm	mm	mm
001BV	*Chaetomium subaffine*	*B. valdivianus*	80.39 ± 1.2	(2.19 ± 0.31) × (1.63 ± 0.21)	(17.07 ± 5.08) × (1.96 ± 0.36)	(71.81 ± 13.29) × (72.89 ± 11.39)
005BV	*Trichoderma koningiopsis*	*B. valdivianus* 'Bronco'	84.48 ± 0.8	(3.02 ± 0.23) × (2.48 ± 0.22)	-	-
006BV	*Beauveria bassiana*	*B. valdivianus*	50.90 ± 1.5	(2.50 ± 0.49) × (2.05 ± 0.31)	-	-
012BV	*Penicillium sanguifluum*	*B. valdivianus*	39.36 ± 0.5	(1.58 ± 0.25) × (1.58 ± 0.24)	(5.42 ± 1.18) × (1.61 ± 0.36)	-
041BV	*T. harzianum*	*B. valdivianus* 'Bronco'	88.19 ± 1.4	(2.54 ± 0.29) × (2.29 ± 0.30)	-	-

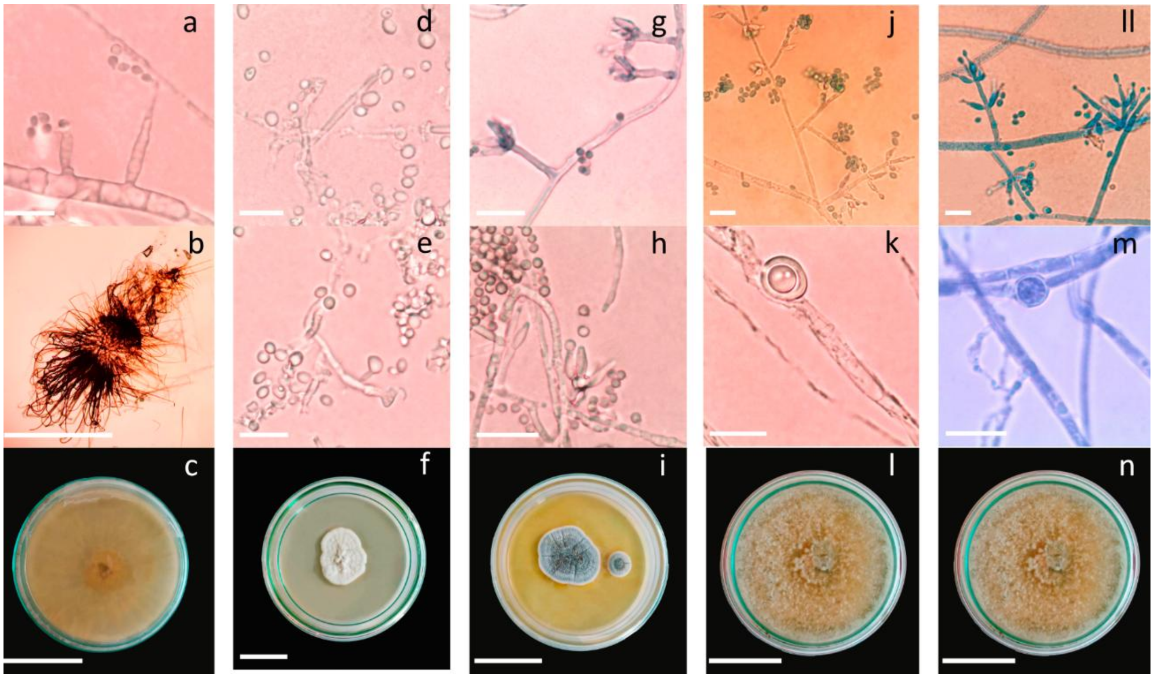

Figure 5. Microscopic morphology and potato dextrose agar medium cultures of endophytic fungi isolated from *Bromus valdivianus*. The reproductive structures and cultures are shown for each species: *Chaetomium subaffine* (a-c), *Beauveria bassiana* (d-f), *Penicillium sanguifluum* (g-i), *Trichoderma koningiopsis* (j-l), *T. harzianum* (ll-n).

Molecular phylogenetic analysis validated the identification of endophytic fungal species using three molecular markers: ITS5-ITS4 rDNA, β-tubulin (Bt2), and elongation factor (EF-1α), besides a combined matrix analysis (Figure 6).

Maximum parsimony trees showed congruent topologies between individual genes and the combined analysis. Each isolate was consistently placed within its respective clade, supported by high bootstrap values. Thus, 005BV *T. koningiopsis* and 041BV *T. harzianum* were grouped within the Hypocreaceae family with high bootstrap support values, while 006BV *B. bassiana* (006BV) was positioned in Cordycipitaceae. Meanwhile, 001BV *C. subaffine* formed a monophyletic clade within Chaetomiaceae, and 012BV *P. sanguifluum* was placed in Aspergillaceae.

The combined analysis of the three genes provided the highest phylogenetic resolution and the most robust support values, confirming the evolutionary relationships among the studied species and validating their correct taxonomic identification at the molecular level.

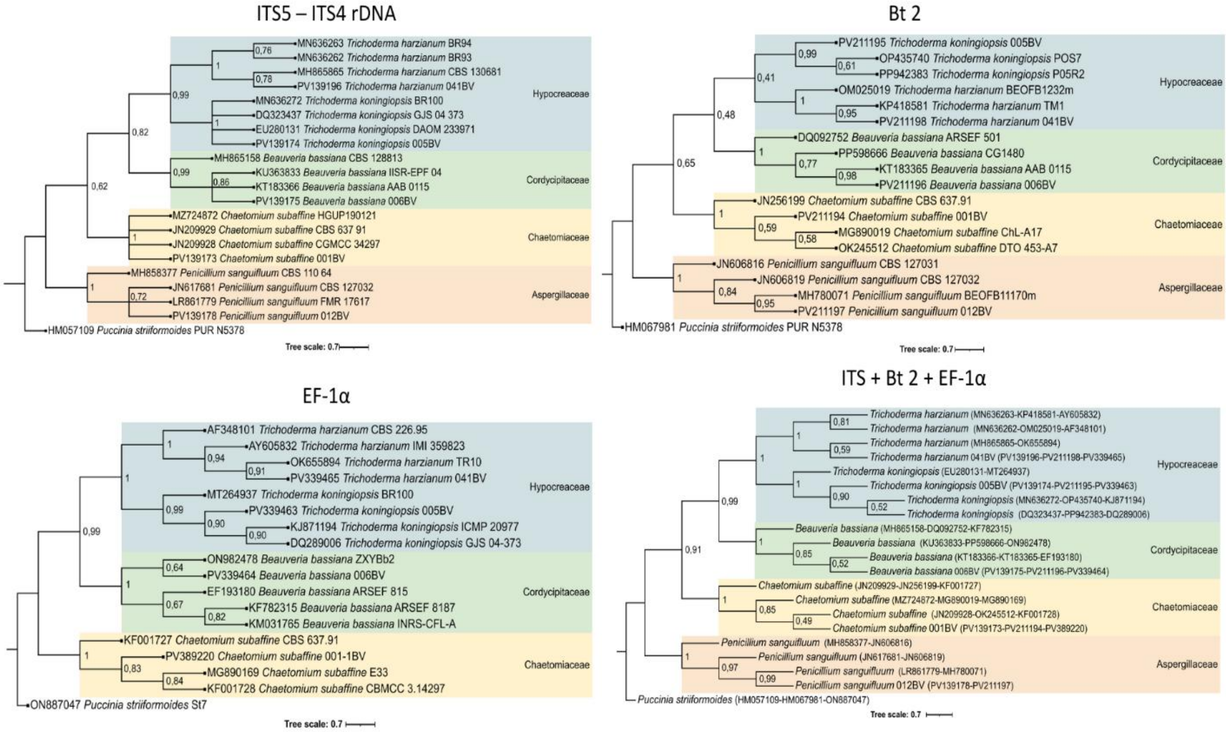

Figure 6. Maximum parsimony phylogenetic trees based on ITS5-ITS4 rDNA, β tubulin (Bt2), elongation factor (EF-1α), and combined analysis (ITS + Bt2 + EF-1α) sequences for the fungal species 001BV *Chaetomium subaffine*, 006BV *Beauveria bassiana*, 012BV *Penicillium sanguifluum*, 005BV *Trichoderma koningiopsis*, and 041BV *T. harzianum*.

Functional guilds

Endophytic fungal species were classified according to their trophic modes and ecological guilds based on the plant tissue of origin and the FUNGuild database (Table 4). Five main trophic modes were identified: Pathotroph-saprotroph-symbiotroph, pathotroph-saprotroph, pathotroph-symbiotroph, pathotroph, and saprotroph. These were distributed into nine different ecological guilds: Endophytes (three species), endophytes-phytopathogens (four species), endophytes-phytopathogens-wood saprotrophs (three species), endophytes-wood saprotrophs (one species), endophytes-epiphytes-wood saprotrophs (two species), animal parasites-pathogens (one species), phytopathogens (two species), phytopathogens-saprotrophs (two species), and undefined saprotrophs (one species).

Morphological and ecological variation in endophytic fungi according to collection site and substrate of origin

A PCA of the morphological, ecological, and origin variables of the endophytic fungus isolates explained 57.1% of the total variance in the first two components (Figure 7). The variables that contributed most to the differentiation were collection site, colony pigmentation, growth rate, and colony diameter. Spatial distribution showed that isolates from the La Araucanía Region exhibited less variability than did those from the Los Ríos Region, where greater dispersion was observed. This difference is related to the greater number of locations sampled in the Los Ríos Region. Likewise, overlap was observed between the regions, suggesting similarities in the diversity of endophytic fungi associated with *B. valdivianus* without clear differentiation at the regional level.

A PCoA identified the determining factors in the grouping of species (Figure 8). Principal coordinate 1 explained 92% of the total variability. Growth rate and ecological function were the most influential variables for grouping fast-growing species with symbiotrophic, saprophytic, and endophytic ecological functions.

Microscopic characteristics contributed to differentiating species with distinctive morphological traits, whereas collection site and substrate type showed complementary effects on group structuring.

Table 4. Trophic modes and ecological guilds of endophytic fungal species isolated from *Bromus valdivianus*. Fungal taxa were classified using the FUNGuild tool and database.

Code	Endophyte taxon	Host tissue	Trophic mode/Guild*
025BV	*Alternaria infectoria*	Leaves	Pathotroph-Saprotroph-Symbiotroph; endophyte-plant pathogen
006BV	*Beauveria bassiana*	Leaves	Pathotroph-Saprotroph-Symbiotroph; animal parasite-animal pathogen-endophyte
028BV	*Chaetomium cochlioides*	Leaves	Pathotroph-Saprotroph-Symbiotroph; endophyte
001BV	*Chaetomium subaffine*	Seed, leaves	Pathotroph-Saprotroph-Symbiotroph; endophyte
038BV	*Chaetomium tenue*	Leaves	Pathotroph-Saprotroph-Symbiotroph; endophyte
010BV	*Cladosporium allicinum*	Leaves	Pathotroph-Saprotroph-Symbiotroph; endophyte-pathogen plant
014BV	*Epicoccum nigrum*	Leaves	Pathotroph-Saprotroph-Symbiotroph; endophyte-plant pathogen-wood saprotroph
026BV	*Fusarium proliferatum*	Leaves	Pathotroph-Saprotroph-Symbiotroph; endophyte-pathogen plant-wood saprotroph
011BV	*Gongronella butleri*	Seed	Saprotroph; undefined saprotroph
019BV	*Gremmenia infestans*	Seed	Pathotroph; plant pathogen
021BV	*Microdochium nivale*	Leaves	Pathotroph-Symbiotroph; endophyte-plant pathogen
018BV	*Parastagonospora avenae*	Leaves	Pathotroph-Saprotroph; grasses plant pathogen-grasses plant saprotroph
013BV	*Parastagonospora poae*	Seed, leaves	Pathotroph-Saprotroph; grasses plant pathogen-grasses plant saprotroph
012BV	*Penicillium sanguifluum*	Seed	Pathotroph-Saprotroph-Symbiotroph; endophyte-wood saprotroph
017BV	*Phaeosphaeria poagena*	Leaves	Pathotroph-Saprotroph-Symbiotroph; endophyte-grasses plant pathogen
024BV	*Pithomyces chartarum*	Leaves	Pathotroph; grasses plant pathogen
027BV	*Pyrenophora dictyoides*	Seed	Pathotroph-saprotroph; grasses plant pathogen-wood saprotroph
039BV	*Trichoderma harzianum*	Leaves	Pathotroph-Saprotroph-Symbiotroph; endophyte -epiphyte-wood saprotroph
005BV	*Trichoderma koningiopsis*	Seed, leaves	Pathotroph-Saprotroph-Symbiotroph; endophyte -epiphyte-wood saprotroph

*Adapted from Nguyen et al. (2016), database available at https://www.funguild.org/ and the Mycobank database (accessed 19 July 2015).

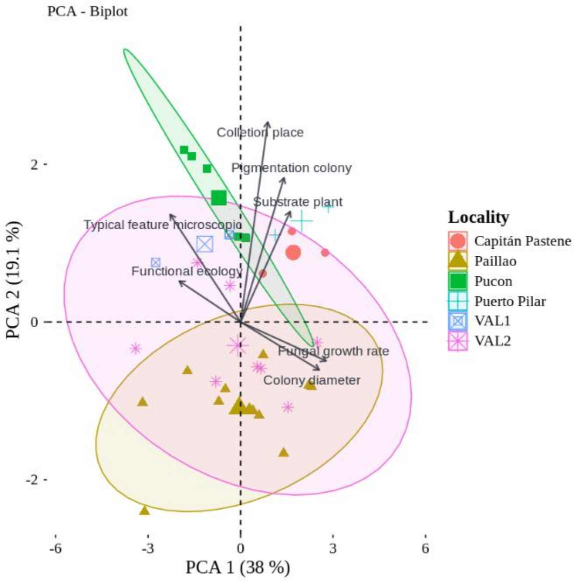

Figure 7. Principal component analysis (PCA) of endophytic fungi isolated from *Bromus valdivianus* according to the collection site in southern Chile. VAL1, VAL 2: Valdivia.

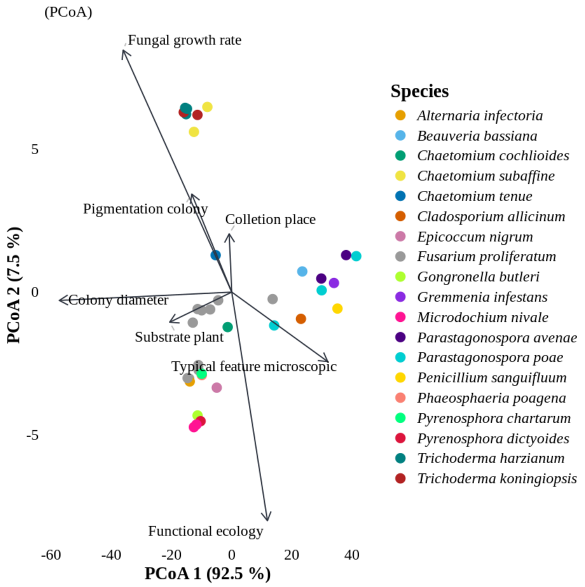

Figure 8. Principal coordinate analysis (PCoA) of endophytic fungi isolated from *Bromus valdivianus* associated with morphological and ecological variables.

DISCUSSION

Diversity and composition of the endophytic community

The identification of 19 species of endophytic fungi in *Bromus valdivianus* confirms our hypothesis about the existence of a complex endophytic fungal community in this forage grass from southern Chile. The fungal diversity recorded is in line with that reported in other temperate grasses. For example, Przemieniecki et al. (2018) identified 19 species of non-symbiotic endophytic fungi in *Lolium perenne* and *Phleum pratense*, while Mc Cargo et al. (2020) reported 24 taxa in *Poa bonariensis*. In both studies, as in ours, a clear predominance of Ascomycota and the recurrent presence of genera such as *Alternaria*, *Epicoccum*, and *Fusarium* were observed, suggesting a common pattern in the composition of endophytic communities of grasses in temperate ecosystems in the Southern Hemisphere.

The high frequency of *F. proliferatum* (25 records) is particularly relevant, as this species is recognized as a soil phytopathogen with the ability to establish itself as a latent endophyte (Sanna et al., 2023), which could represent a potential phytosanitary risk for *B. valdivianus*. Its predominance among the samples implies that this grass harbors asymptomatic pathogens that, under stressful conditions, can become active as disease-causing agents. This pattern coincides with observations in other *Bromus* species, where Gao et al. (2025) also documented the prevalence of *Fusarium* complex species in natural grasslands. Likewise, the endophytic community of *B. valdivianus* showed a clear predominance of the phylum Ascomycota (94.7%). This pattern is consistent with global observations in grasses, which is attributed to the metabolic versatility and ability of this group to establish symbiotic associations (Liao et al., 2025). The most diverse families (Chaetomiaceae, Hypocreaceae, Pleosporaceae, and Phaeosphaeriaceae) (Figure 2) include taxa with a recognized ability to colonize plant tissues, as well as inhabit other niches such as soil or animal tissue (Kandula et al., 2015; Nischitha and Shivanna, 2022).

Our study revealed contrasting colonization patterns: 80.5% infection came from leaf tissue, suggesting a predominance of horizontal transmission, while 19.5% was obtained from seeds, indicating a component of vertical transmission (Rodriguez et al., 2009). Noteworthy in this latter group is the finding of *Gongronella butleri* (Mucoromycota), a terrestrial saprophyte rarely reported as a true endophyte. Its exclusive presence in seeds may be due to a specific colonization strategy during germination or seed development. In this regard, the presence of endophytes in seeds was recorded exclusively in the localities of Valdivia, where the greatest effort was made to sample reproductive material. Although this pattern could reflect greater colonization of reproductive tissues favored by particular environmental conditions or by local genotypes of *B. valdivianus*, its geographical specificity requires further verification. Future studies must determine the relative role of climatic, edaphic, and genetic factors of the host in this distribution pattern.

The distribution of isolates, predominantly foliar (80.5%), may have occurred because of horizontal transmission from the environment. However, the isolation of species such as *G. butleri* and *Penicillium sanguifluum* exclusively from seeds indicates the existence of a vertical transmission component, albeit of a different nature from the original hypothesis. This modifies our initial understanding and suggests that vertical transmission in *B. valdivianus* is mediated by non-Clavicipitaceae fungi, possibly acquired during seed development or germination.

Therefore, determining the modes of endophytic transmission (vertical through seeds *vs*. horizontal from the environment) has critical implications for the management of *B. valdivianus*. Vertical transmission confers stability and heritability to the symbiont, ensuring its generational persistence. If it is confirmed that beneficial strains —such as those that confer drought tolerance— are transmitted vertically, this characteristic would be fundamental for genetic improvement programs, as it would guarantee the presence of the protective trait from germination onwards. Conversely, if horizontal transmission predominates, endophytic colonization would depend mainly on soil and environmental conditions. In this scenario, the establishment of beneficial endophytes would require active inoculation strategies using bioinoculants applied to seeds or soil during sowing or grassland regeneration.

On the other hand, the absence of species of the genus *Epichloë* in *B. valdivianus* coincides with information previously reported by Ortega et al. (2007). This result is particularly significant considering that this species, belonging to the Ceratochloa section, exhibits polyploidy, a characteristic that could limit the establishment of highly coevolved associations with specialized systemic endophytes (Williams et al., 2011). This pattern contrasts sharply with other South American diploid *Bromus* species, where associations with *Epichloë* are common and evolutionarily important (Iannone et al., 2021), suggesting that the genomic complexity of polyploid genomes could affect the molecular interactions required to establish obligate symbiosis.

Complex functional network of the endophytic community

Among the identified endophytes, five species are of particular biotechnological relevance to *B. valdivianus* (Figures 5 and 6). Among these, *Chaetomium subaffine* (and its anamorph *Acremonium* sp.) showed the ability to colonize both leaves and seeds, suggesting a close association that could include vertical transmission. This species, previously reported as an endophyte in cereals and tropical grasses, is known to produce bioactive metabolites with antifungal activity (Wang et al., 2016; Nischitha, 2024). *Beauveria bassiana* was detected in leaf samples from grasslands in La Araucanía. Although its presence could be related to pest control applications, its establishment as an endophyte coincides with previous reports demonstrating its ability to colonize grasses and confer abiotic stress tolerance (Kuzhuppillymyal-Prabhakarankutty et al., 2020). Its identification as an endophyte in the leaves of *B. valdivianus* (a finding that positions it as a leaf endophyte) opens up a promising line of research for the direct protection of the aerial part of the grass against herbivorous insects (such as defoliating pests). This mechanism of protection in the foliage is essential for the persistence of the pasture and offers a cutting-edge application in plant protection. The exploitation of these native strains could lead to specific solutions for the challenges of grassland management in southern Chile.

Trichoderma species (*T. harzianum* and *T. koningiopsis*) represent the most versatile components of the identified endophytic community, which aligns with their recognized ecological plasticity that includes saprophytic, endophytic, and mycotrophic lifestyles (Scott et al., 2023). Their presence in *B. valdivianus* is consistent with previous reports in other South American grasses, where they have been identified as common components of the endophytic microbiota (Depetris et al., 2020). The presence of *Trichoderma* species is of particular agronomic importance. *Trichoderma* species are globally recognized as effective biocontrol agents against plant pathogens, in addition to their ability to promote plant growth by producing phytohormones and improving nutrient absorption. Their discovery suggests a direct potential for the development of specific bioinoculants for *B. valdivianus* that can improve pasture vigor and protect it against disease.

On the other hand, *P. sanguifluum*, traditionally considered an edaphic fungus, demonstrated the ability to colonize *B. valdivianus* tissues. This species has previously been reported as a rhizosphere endophyte with functions in P solubilization and phytohormone production (Herrera et al., 2019). Besides demonstrating potential for bioremediation of contaminated soils (Soldi et al., 2020), this suggests that the species plays a multifunctional role in association with grasses. In contrast to previous records, *P. sanguifluum* was isolated from wild seeds of *B. valdivianus*. Its presence in this substrate could be explained by its metabolic versatility and saprophytic capacity, which would allow it to take advantage of the nutrient-rich microenvironment of the seeds. Additionally, studies such as that by Savković et al. (2021) demonstrate that fertilized soils favor the incorporation of *Penicillium* species during germination, suggesting a potential colonization mechanism for this taxon.

The FUNGuild analysis applied to leaf and seed samples of *B. valdivianus* revealed remarkable functional diversity, with five trophic modes distributed across nine ecological guilds (Table 4). This complexity implies the existence of balanced fungal communities, predominantly classified as endophytes, endophytes-phytopathogens, and endophytes-saprotrophs. Thus, the distribution of guilds in *B. valdivianus* differs from that reported in other grasses. While Garnica et al. (2020) observed a predominance of saprotrophs in wheat, our study recorded a higher proportion of true endophytes and taxa with symbiotrophic potential, reflecting specific ecological adaptations of these grasslands. Although agronomic factors such as fertilization and cutting frequency influence the structure of endophytic communities (Wemheuer et al., 2019), their specific evaluation in our system requires further study.

Geographic variation and local factors: A central or specific community?

Multivariate analyses (Figures 7 and 8) indicate that the structure of the endophytic community is mainly influenced by the interaction between the collection site and physiological traits such as growth rate. The correlation between a rapid growth rate and symbiotrophic ecological functions may provide a competitive advantage for tissue colonization. This relationship suggests that fast-growing isolates have competitive advantages during colonization, supporting the usefulness of physiological traits as predictors of their ecological role (Przemieniecki et al., 2018).

Although some overlap was observed between regions, indicating a common "core" of endophytes, the greater dispersion in the Los Ríos Region may mean that factors such as climate, soil, and management contribute to modulating community composition. This variation is observed in other grasses (Nischitha and Shivanna, 2020), which implies that the differences observed in *B. valdivianus* may reflect not only geographical gradients but also microclimatic fluctuations. Taken together, these results demonstrate that the assembly of the endophytic community is governed by the interaction between multifactorial environmental factors and the intrinsic physiological attributes of fungal isolates that determine the final diversity.

CONCLUSIONS

In conclusion, our research reveals that *Bromus valdivianus* supports a diverse and multifunctional endophytic community dominated by non-Clavicipitaceae fungi. The community is structured by a combination of horizontally transmitted endophytes and, significantly, some with the potential for vertical transmission through seeds. The diversity of trophic guilds identified supports the notion that these fungi form an integral consortium that likely contributes to the resilience and adaptability of *B. valdivianus* to the environmental and agronomic conditions of southern Chile.

The results of this study not only fill a gap in ecological knowledge, but also lay the foundation for translational science in grassland agronomy. As a key future direction, we propose that studies focus on experimental validation in vivo and under field conditions of the ecological functions of the identified strains. This should include key isolates such as *Chaetomium subaffine* and *Trichoderma* spp., and those associated with guilds of mycoparasites and protective endophytes, to determine their ability to confer resistance to environmental stresses (e.g., drought) and protection against region-specific pests. These findings should guide the development of bioinoculants based on native strains of *B. valdivianus*, offering farmers a biotechnological tool to improve the resilience and productivity of their pastures. Finally, this research directly influences regional management strategies, suggesting the possibility of recommending specific fungal strains to promote more sustainable and resilient forage management in southern Chile.

Author contribution

Sample collection: J.A.L.-F., O.B. Isolations, morphological identification, and diversity analysis: J.A.L.-F. Molecular characterization: J.A.L.-F., E.F., O.M. Drafting of the original manuscript: J.A.L.-F. Revision and editing: J.A.L.-F., O.M.-V., O.B. Project administration: O.B. Funding acquisition: O.B. All authors have read and accepted the published version of the manuscript.

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