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Abstract The impact of plant species invasions on the abundance, composition and activity of fungal decomposers of leaf litter is poorly understood. In this study, we isolated and compared the relative abundance of ligninocellulolytic fungi of leaf litter mixtures from a native forest and a forest invaded by *Ligustrum lucidum* in a lower mountain forest of Tucuman, Argentina. In addition, we evaluated the relationship between the relative abundance of ligninocellulolytic fungi and properties of the soil of both forest types. Finally, we identified lignin degrading fungi and characterized their polyphenol oxidase activities. The relative abundance of ligninocellulolytic fungi was higher in leaf litter mixtures from the native forest. The abundance of cellulolytic fungi was negatively related with soil pH while the abundance of ligninolytic fungi was positively related with soil humidity. We identified fifteen genera of ligninolytic fungi; four strains were isolated from both forest types, six strains only from the invaded forest and five strains were isolated only from the native forest. The results found in this study suggest that *L. Lucidum* invasion could alter the abundance and composition of fungal decomposers. Long-term studies that include an analysis of the nutritional quality of litter are needed, for a more complete overview of the influence of *L. Lucidum* invasion on fungal decomposers and on leaf litter decomposition.

Keywords Exotic plants · Fungal decomposers · Leaf litter · Lignocellulolytic activities · *Ligustrum lucidum* · Subtropical forest

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Introduction

Most of the plant litter (around 80%) is degraded by microorganisms (Hättenschwiler et al. 2005) and fungi play a key role (Alexopoulus et al. 1996; de Boer et al. 2005; Schneider et al. 2012). Plant litter is composed mostly of cellulose, hemicellulose and lignin that are used by decomposer fungi as a source of carbon and energy (Sánchez 2009). Litter degradation by fungi is possible due to the existence of enzymatic hydrolytic systems (mainly cellulases and hemicellulases) and oxidative extracellular ligninolytic systems

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(composed of laccases, lignin peroxidases and/or manganese peroxidases) (Sánchez 2009).

The abundance, composition and activity of fungi are influenced by the chemical composition and quantity of the litter produced by plant communities (Prescott and Grayston 2013). Particularly, the activity of fungi is influenced by the disposition of the structural components of the litter (Pérez et al. 2002; Wardle et al. 2006). Cellulose is generally present as a crystalline form and a small amount of non-organized cellulose chains form amorphous cellulose. Due to its arrangement, cellulose is more susceptible to enzymatic degradation than other leaf litter components (Pérez et al. 2002). For instance, hemicellulose (composed mainly of xylan) requires more enzymes for its complete degradation since it is a more heterogeneous polysaccharide than cellulose (Malherbe and Cloete 2002). In turn, lignin is a highly refractory and persistent aromatic polymer of complex structure, and is one of the major structural components of leaf litter (Hammel 1997). Lignin arrangement makes the microbial degradation of other components of leaf litter more difficult; thus, decomposition of dead matter is limited by lignin (Berg 2000; Osono 2007; Berg and McClaugherty 2008).

In addition to plant litter quality, the microenvironment and soil parameters influence the structure, composition and activity of fungal decomposers (Pietikäinen et al. 2004; De Angelis et al. 2013). In fact, temperature and soil humidity are the main factors determining fungal activity (Pietikäinen et al. 2004; Lauber et al. 2008). However, it has also been recognized that the chemical characteristics of the soil, such as pH and nutritional properties are important drivers of the structure and activities of fungi (Lauber et al., 2008; Rousk et al. 2010).

Several studies have shown that biotic factors (such as quantity or quality of the substrate reaching the ground) and abiotic factors (e.g. soil properties, light availability) can be altered by the invasion of exotic plants (Ehrenfeld et al. 2001; Ashton et al. 2005; Tateno et al. 2007; Aragón et al. 2014a, b; Slesak et al. 2016). In turn, these changes may alter the composition, structure and functioning of fungal communities (Kourtev et al. 2003; Osono et al. 2006; Broz et al. 2007; Stefanowicz et al. 2016), which can consequently impact on ecosystem functioning (McGuire et al. 2010).

Exotic species are an important component of secondary forests in lower mountain forests ("Yungas") of northwestern Argentina (Grau and Aragón 2000). *Ligustrum lucidum* W. T. Aiton (Oleaceae) ("*Ligustrum*"), specifically, is an important invasive woody species in the Yungas (Aragón and Groom 2003) where it has expanded from 100 to 500 ha approximately in the last 20 years (Montti et al. Unpublished data). Numerous studies report the effects of *L. lucidum* in Yungas ecosystems. The species is a perennial tree which consumes more water than native vegetation and can therefore significantly alter water functioning of the ecosystem (Zamora Nasca et al. 2014). *Ligustrum* forms dense, monospecific stands which modify soil temperature and the contribution of litter biomass to the soil (Aragón et al. 2014a; Ayup et al. 2014). Its litter exhibits differences in chemical composition and stoichiometry and presents a higher decomposition rate than three of the most common native species of Yungas piedmont (Aragón et al. 2014a, b). In addition, *Ligustrum* invasion increases the activity of soil enzymes from microbial communities associated with carbon and nitrogen cycling (Aragón et al. 2014b). However, studies assessing the effect of *Ligustrum* invasion over fungal decomposers of leaf litter have not been reported.

The goal of this work was to isolate and compare the relative abundance of fungi with lignocellulolytic activity of leaf litter mixtures from a native secondary forests and a secondary forest invaded by *L. lucidum* in the Yungas of Tucuman, Argentina. Additionally, we intended to explore the relationship between relative fungi abundance and physical and chemical soil parameters (temperature, relative humidity and pH) of both forest types. Finally, we identified the lignin degrading fungi and we characterized their polyphenol oxidase activities. We hypothesized that the leaf litter mixtures of native forests are more heterogeneous in terms of species composition and they might provide of more diverse sources of carbon and energy. Therefore, we predicted that leaf litter mixtures from the native forest could have higher abundance of lignocellulolytic fungi than leaf litter from the invaded forest. In addition, the relationship of fungi abundance with physical and chemical soil parameters could be different in each forest type, as well as, the composition and activity of ligninolytic fungi.

Materials and methods

Study sites, sampling collection and soil parameters

The study was conducted in the lower mountain forests of Sierra de San Javier, Tucumán, Argentina (26°70′S, 65°35′W). The study sites are within a protected area that belongs to the University of Tucumán. The native vegetation corresponds to a semi deciduous mountain forest (between 630 and 780 masl) of the Yungas ecoregion (Cabrera 1976). Annual rainfall is 1200 mm (90% in the summer) and average annual temperature is 18 °C (Bianchi 1981). Many areas of the piedmont were deforested for agriculture (mainly citrus and sugar cane crops) and grazing during the early twentieth century (Grau et al. 1997). However, in the last two decades many agricultural fields were abandoned and have initiated forest succession (Grau et al. 2008). Some secondary forests are native while others have been invaded by exotic species, mainly *L. lucidum* (Grau et al. 2008). Both forest types are different in structure and species composition. Native secondary forests present three strata: the canopy, dominated by trees such as *Ocotea porphyria*, *Blepharocalyx salicifolius* and *Cupania vernalis*; the subcanopy, dominated by *Piper tucumanun*, *Eugenia uniflora* and *Allophylus edulis*; and the understory, dominated by the shrub *Psychotria carthagenensis* (Malizia et al. 2010). Meanwhile, forests invaded by *Ligustrum* (from now on "invaded forest") exhibit a lower diversity of trees, shrubs and liana species (Aragón and Morales 2003; Lichstein et al. 2004; Ceballos et al. 2015) and a simplified vertical structure, with the canopy dominated by *Ligustrum* and the understory dominated by *P. carthagenensis* (Lichstein et al. 2004; Easdale et al. 2007). We used a paired design with 5 sites of each forest category (5 Native-invaded forest pairs). Tree density of all the invaded forest sites was higher than 500 ind./ha. Pairs were selected based on a high similarity in age, slope, altitude, soil type, and land-use history. The distance between the members of each pair went from 200 to 500 m. For more details about the location and characteristics of the sites see Aragón et al. (2014a, b). At each site, three samples of mixtures of leaf litter (5 g each one) were collected in March 2015 (wet season). Mixtures of leaf litter from native forests were composed of freshly senesced leaves, mainly of *O. porphyria*, *C. vernalis*, *A. edullis* and *P. carthagenensis*, while 80% of mixtures of invaded forests were composed by leaf litter of *L. lucidum*. At each site a composite sample was obtained (5 g) and stored in plastic vials at 4 °C until processing. Additionally, soil temperature, humidity and pH were registered. For temperature we took five measurements per site using a metallic thermometer in the morning (between 8 and 10 AM) on the same days that soil samples were collected. Prior to soil sampling, the litter and humus layer were removed. Using a hole borer, three samples were taken from the mineral horizon (0–10 cm) at each site, and were stored in polyethylene bags. In the laboratory, samples were oven-dried at 105 °C for 48 h and the gravimetric soil water content was calculated as the difference between wet and dry weights and expressed as a percentage. Soil pH of each site was determined using a pH meter (Cole Parmer, USA).

Abundance of lignocellulolytic fungi from native and invaded forest

For assessing the abundance of lignocellulolytic fungi, leaf litter mixtures of each forest type were sectioned in small pieces. One gram of each mixture was placed in Erlenmeyer flasks containing 15 mL of wash solution (0.5% yeast extract, 0.1% glucose and 0.2% Tween 80) and the flasks were then incubated at 200 rpm, 25 °C for 30 min to separate the fungal cells from the leaf litter. The obtained suspensions were serially diluted and used to estimate the abundance of lignocellulolytic fungi. The abundance of cellulolytic fungi was estimated by spreading 0.1 ml of the suspension in Carboxymethyl cellulose (CMC)-agar [g l^{-1}: 10 CMC, 20 agar, Yeast Nitrogen Base (YNB) (Sigma-Aldrich), pH 3.2–3.6]. The abundance of xylanolytic fungi was similarly estimated in Xylan-agar (g l^{-1}: 5 xylan, YNB, 20 agar, pH 3.2–3.6). Finally, the abundance of ligninolytic fungi was estimated by spreading 0.1 ml of the suspension in Guaiacol-agar (g l^{-1}: 1/10 strength diluted acidic YM agar supplemented with 0.015 guaiacol, pH 3.5). All media were acidified with HCl 0.1 N, to restrict bacterial growth (Kurtzman et al. 2011).

Plates were incubated at 25 °C and examined daily during five days. All colony forming units (CFU) of fungi growing on CMC or Xylan plates were

considered as cellulolytic and xylanolytic, respectively and their abundances (CFU by g of leaf litter) were then calculated. On the other hand, only colonies surrounded by a reddish halo were considered as ligninolytic fungi (Saparrat and Hammer 2006) and were included to calculate their abundance. Colonies that represented different morphotypes of xylanolytic, cellulolytic and ligninolytic fungi were sub-cultured onto acid YM agar medium (g l^{-1}:10 glucose, 5 peptone, 3 yeast extract, 3 malt extract, 20 agar, pH 3.5) and maintained at 4 °C in YM.

To confirm extracellular activities, cellulolytic strains were individually cultured on CMC agar plates. After 10 days of incubation, Petri plates were overlaid with 0.2% Congo red for 5 min and washed with 0.5 M NaCl. Cellulose degradation around the colonies appeared as a yellow or orange area in contrast with the red color produced by undegraded cellulose (Strauss et al. 2001). Similarly, xylanolytic strains were individually cultured on xylan agar plates. After 10 days of incubation, plates were overlaid with iodine stain (0. 25% w/v aqueous I_2/KI) for 5 min. Xylan degradation around the colonies appeared as a yellow-opaque area in contrast with a blue/reddish purple color produced by undegraded xylan (Pointing 1999).

Molecular identification of lignin degrading fungi

Ligninolytic fungi (25 isolates) were individually cultivated in YM agar medium broth at 30 °C for 72 h on an orbital shaker (250 rpm). Mycelia were then collected by centrifugation at 10000×*g* (10 min, 4 °C), re-suspended in 2 M NaCl and finally washed twice with sterile distilled water (Fang et al. 1992). Washed mycelial mats were frozen in liquid nitrogen and grounded to powder by using a sterile pestle and mortar. Ground mycelia were extracted once with phenol: chloroform: isoamyl alcohol (25:24:1) and washed twice with chloroform: isoamyl alcohol (24:1). Two volumes of absolute ethanol and 0.1 volume of 3 M potassium acetate were added to the final aqueous phase in order to achieve DNA precipitation, mixed by inversion and then centrifuged (8000×*g*, 10 min, 4 °C). The pellet was then washed twice with 70% ethanol, dried and finally suspended in sterile water.

Fungal D1/D2 domains of the LSU rDNA gene were amplified by using NL1 (GCA TAT CAA TAA GCG GAG GAA AAG) and NL-4 (GGT CCG TGT TTC AAG ACG G) primers (White et al. 1990). D1/D2 domains were selected over the standard universal fungal barcode since it is easier to amplify, align and analyze, mainly because of its uniform size (See Supplementary Fig. 4 of Schoch et al. 2012). In addition, this sequence outperformed ITS region in the identification of ascomycetous yeast, basal fungal lineages and several *Ascomycota* and *Basidiomycota* taxa (Schoch et al. 2012; Kwiatkowski et al. 2012).

Nucleotide sequencing of the genes was performed by Macrogen (Korea). Sequences were analyzed and edited when necessary, using Invitrogen Vector NTI Advance 10.3.0 software (Invitrogen, San Diego, CA, USA). Strain identification was performed by comparison with sequences from type strains available in GenBank using the provided BLAST tool. Sequences were also compared with species hypothesis in the UNITE database. Arbitrarily, a >98% identity and >99% coverage criterion was employed to identify strains at the species level.

Polyphenol oxidase activities

Polyphenol oxidases were detected by the well test (Pointing 1999). Petri dishes containing malt extract agar (MEA) (g l^{-1}: 20 malt extract, 1 yeast extract, 20 agar, pH 6) and lignin modifying enzymes (LME)-agar (g l^{-1}: 0.1[1] peptone, 0.01 yeast extract, 2 glucose, 20 agar, pH 6) were inoculated with five mm agar plugs from fresh fungal cultures and incubated at 25 °C for 10 days. Wells (five mm in diameter) were cut in the agar growth medium, in the actively growing edge of the fungal colony. Wells received 40 µL of a 0.09–0.1% solution of eight test solutions. The following substrates were assayed to detect ligninolytic activities: 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 4-hydroxy-3,5-dimethoxybenzaldehyde azine (syringaldazine, SGZ), 2,6-dimethoxyphenol (2,6-DMP), 1,1′-biphenyl-4,4′-diamine (Benzidine), 1-Hydroxynaphthalene (α-naphthol). The substrates for detecting catechol oxidase and tyrosinase activities were 1,2-dihydroxybenzen (catechol) and 4-methylphenol (*p*-cresol), respectively. All the substrates were purchased from Sigma-Aldrich (standard reagent grade) (Pointing 1999).

Uninoculated plates were included as abiotic controls. Abiotic controls and inoculated plates were both

incubated at 30 °C in the darkness for 10 days, and were examined daily for fungal growth. Tests were performed in triplicate and repeated if necessary. The intensities of the color reactions were registered after 0.5, 1 and 2 h by comparing against negative controls (without substrates) and against *Pycnoporus sanguineus* plates, a well-known laccase producer, included as positive control (Lomascolo et al. 2011).

Data analyses

To compare the relative abundance of lignocellulolytic fungi (CFU g^{-1} leaf litter) between both forest types, we used a paired *t* test ($p < 0.05$). Data were previously transformed to log base 10 to meet *t*-test assumptions. The physical–chemical parameters of the soil were also compared with a paired *t*-test. To analyze the relationship between temperature, humidity and soil pH and the abundance of lignocellulolytic fungi, we conducted a generalized linear model (GLM) with a quasi-Poisson distribution (since data were over dispersed) and a log link function. The number of CFU g^{-1} leaf litter of cellulolytic, xylanolytic and ligninolytic fungi were used as response variables and temperature, humidity and pH were the explanatory variables. We conducted these analyses in two steps as described in Crawley (2007). Firstly, we excluded the variables in a backwards-stepwise fashion based on their p-value. Secondly, for each removed variable, we compared the model with the original model using ANOVAs (*F*-test statistic). The final model was selected based on the pseudo R^2 using the *F*-test. The pseudo R^2 of the final model is reported at a significance level of $p < 0.05$. All statistical analyses were performed using R (version 3.2.1, Development Core Team 2015).

Results

Abundance of lignocellulolytic fungi from native and invaded forest and its relationship to soil parameters

The abundance (log CFU g^{-1} leaf litter) of cellulolytic fungi was significantly higher (t (8) $= -2.66$; $p = 0.03$) in leaf litter from the native ($4.12 \times 10^5 \pm 1.15 \times 10^5$) than from the invaded forest ($2.78 \times 10^5 \pm 5.11 \times 10^4$); as well as the abundance of ligninolytic fungi in leaf litter (t (8) $= -3.29$; $p = 0.01$) from the native ($1.65 \times 10^4 \pm 4.11 \times 10^3$) than from the invaded forest ($1.02 \times 10^4 \pm 2.22 \times 10^3$). In contrast, the abundance of xylanolytic fungi was slightly higher in the invaded forest ($4.36 \times 10^5 \pm 4.98 \times 10^4$) compared to that of the native forest ($3.87 \times 10^5 \pm 6.57 \times 10^4$) but these differences were not statistically significant (t (8) $= 1.39$; $p = 0.20$) (Fig. 1). Among the physical and chemical parameters of the soil, only the pH was significantly different between forest types. In the native forest, the soil was more acidic than in the invaded forest (Table 1). Also, soil pH showed a significant positive association with the abundance of cellulolytic fungi (pseudo $R^2 = 0.60$; $p = 0.007$) while soil humidity associated positively and significantly with the abundance of ligninolytic strains (pseudo $R^2 = 0.45$; $p = 0.042$) (Fig. 2).

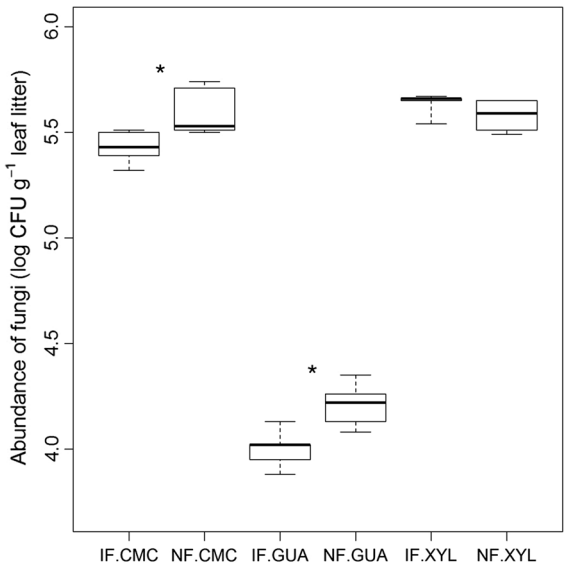

Fig. 1 Abundance of lignocellulolytic fungi (log CFU g^{-1} leaf litter) from native and invaded forests in selective media. *IF* invaded forest, *NF* native forest, *CMC* carboxymethyl cellulose (to isolate cellulolytic fungi), *GUA* guaiacol (to isolate ligninolytic fungi), *XYL* xylan (to isolate xylanolytic fungi). The *boxplot* shows the distribution of the values according to the medians (*central line*), the 25 and 75% quartiles (*box*) and the ranges (*whiskers*). The asterisks on the *boxplots* indicate significant differences between forest types ($p < 0.05$)

Molecular identification of lignin degrading fungi

The identification of D1/D2 fragments by comparision with the UNITE database and with the sequences of the type material in the GenBank, allowed us to identify all of the lignin degrading fungi isolated in this study to the species level (Table 2). Members of fifteen genera belonging to the orders *Hypocreales*, *Sordariales, Capnodiales, Glomerellales, Pleosporales*, *Diaphortales*, *Xylariales*, *Saccharomycetales*, *Russulales* and *Polyporales* were identified. The order *Hypocreales* exhibited the highest richness, followed by the order *Pleosporales*. Strains of the genera *Fusarium*, *Colletotrichum, Trametes* and *Trichoderma* were found in both forest types. Strains of *Alternaria, Corynespora, Flavodon, Peniophora, Phaeoacremonium* and *Phialemonium* were only isolated from the leaf litter of the invaded forest, while strains of the genera *Candida*, *Clonostachys*, *Glomerella, Mycosphaerella* and *Pestalotiopsis* were only isolated from the leaf litter of the native forest.

Table 1 Physical and chemical parameters of the soil of native and invaded forest (n = 5 sites per forest type)

Parameters of the soil	Invaded forest		Native forest	
	Mean	± SE	Mean	± SE
Soil humidity (%)	44.5	8.9	52.7	3.6
Soil temperature (°C)	20.6	0.3	20.5	0.3
Soil pH[a]	6.7	0.2	6.3	0.3

SE standard error

[a] Differences at $p < 0.05$

Polyphenol oxidase activities

LME-agar is especially suitable for the production of lignin modifying enzymes by white rot fungi (Pointing 1999) while MEA is a more general medium for yeast and mold cultivation. Accordingly, isolates such as *Candida homilentoma* S3NG1 and *Alternaria burnsii* S3EG3 did not grow in LME-agar medium, and were only evaluated in MEA medium when they showed negative reactions (Table 3). As expected, LME agar allowed the detection of more positive results for classical laccase substrates such as syringaldazine, benzidine, 2,6-DMP and guaiacol, while MEA plates produced more positive results against catechol and p-cresol, substrates were usually associated with catecholase and tyrosinase activity (Reiss et al. 2013).The strains of *Trametes versicolor* S5NG1, *T. versicolor* S4EG2, *Colletotrichum gloeosporioides* S1EG2 and *Flavodon flavus* S5EG3 oxidized almost every tested substrate on LME and MEA media. On the other hand, the strains of *Phaeoacremonium angustius* S4EG3, *Trichoderma atroviride* S2NG4 and *Mycosphaerella iridis* S2NG1 did not produce the polyphenol oxidases for the specific substrates used.

Discussion

Fungi are part of the naturally occurring microflora of leaf litter and play a key role in its decomposition (de Boer et al. 2005).The abundance, composition and

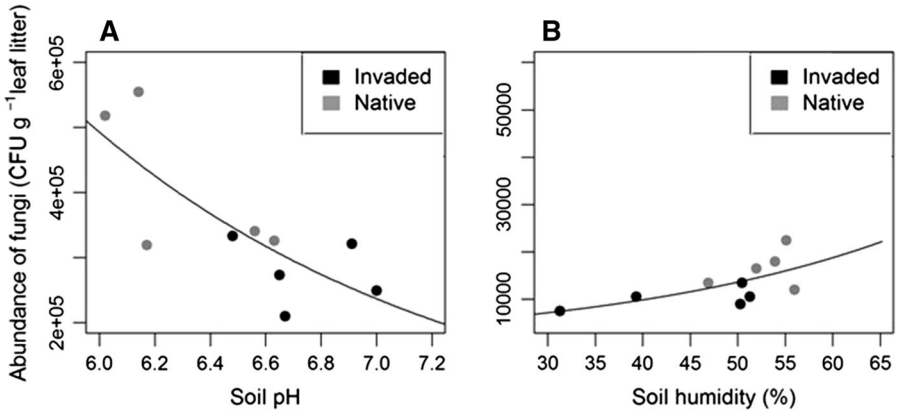

Fig. 2 Relationship between the abundance of lignocellulolytic fungi (CFU g^{-1} leaf litter) and soil physical–chemical parameters of both forest types. **a** Abundance of cellulolytic fungi and soil pH ($p = 0.007$; pseudo $R^2 = 0.60$). **b** Abundance of ligninolytic fungi and soil humidity ($p = 0.042$; pseudo $R^2 = 0.45$). *Fitted lines* are derived from a GLM with quasi-Poisson errors

Table 2 Molecular identification of lignin degrading fungi isolated of leaf litter mixtures from native and invaded forest

Forest type	Strain	GenBank Accession Number	Closest Match (GenBank/UNITE)	Identity (%)	Putative ID
Invaded	S4EG3	KY781967	AB278178/SH214928.07FU *Phaeoacremonium angustius*	99.65	*Phaeoacremonium angustius*
	S3EG1	KY781968	HQ604854/SH188974.07FU *Peniophora aurantiaca*	98.06	*Peniophora aurantiaca*
	S5EG1	KY781970	HM060271/SH211283.07FU *Phialemonium dimorphosporum*	99.44	*Phialemonium dimorphosporum*
	S1EG1	KY781972	KM099499/SH187755.07FU *Trichoderma atroviride*	100.00	*Trichoderma atroviride*
	S2EG3	KY781978	AY188918/SH205225.07FU *Fusarium solani*	99.48	*Fusarium solani*
	S3EG3	KY781981	KR604838/SH215493.07FU *Alternaria burnsii*	100.00	*Alternaria burnsii*
	S4EG1	KY798204	AB278178/SH214928.07FU *Phaeoacremonium angustius*	99.65	*Phaeoacremonium angustius*
	S4EG2	KY781983	KC176344/SH193318.07FU *Trametes versicolor*	98.69	*Trametes versicolor*
	S1EG2	KY781985	EU552111/SH189873.07FU *Colletotrichum gloeosporioides*	98.80	*Colletotrichum gloeosporioides*
	S2EG2	KY781987	gbKF777207.1 *Corynespora torulosa*	99.00	*Corynespora torulosa*
	S5EG3	KY781988	JN710543/SH185167.07FU *Flavodon flavus*	98.14	*Flavodon flavus*
Native	S5NG2	KY781969	HG518666/SH207299.07FU *Glomerella acutata*	100.00	*Glomerella acutata*
	S5NG3	KY781971	KM232462/SH193318.07FU *Trametes versicolor*	98.94	*Trametes versicolor*
	S4NG2	KY781973	EU552147/SH200154.07FU *Pestalotiopsis maculiformans*	100.00	*Pestalotiopsis maculiformans*
	S1NG2	KY781974	HG518666/SH207299.07FU *Glomerella acutata*	100.00	*Glomerella acutata*
	S5NG1	KY781975	KC176344/SH193318.07FU *Trametes versicolor*	98.66	*Trametes versicolor*
	S2NG1	KY781976	AY251089/SH186594.07FU *Mycosphaerella iridis*	99.66	*Mycosphaerella iridis*
	S3NG3	KY781977	KT462721/SH219673.07FU *Fusarium proliferatum*	99.3	*Fusarium proliferatum*
	S2NG2	KY781979	AY188918/SH205225.07FU *Fusarium solani*	99.48	*Fusarium solani*
	S3NG1	KY781980	gbU45716.1 *Candida homilentoma*	99.00	*Candida homilentoma*
	S4NG1	KY781982	EU552110/SH182678.07FU *Clonostachys rosea*	99.64	*Clonostachys rosea*
	S1NG1	KY781984	EU552111/SH189873.07FU *Colletotrichum gloeosporioides*	98.80	*Colletotrichum gloeosporioides*
	S2NG4	KY781986	KC330218/SH190868.07FU *Trichoderma harzianum*	100.00	*Trichoderma harzianum*
	S3NG4	KY798205	KT462721/SH219673.07FU *Fusarium proliferatum*	99.30	*Fusarium proliferatum*

activity of fungi are influenced by the chemical composition and quantity of the litter produced by plant communities (Prescott and Grayston 2013). As expected, in the present study we found a higher abundance of lignocellulolytic fungi in leaf litter mixtures from the native forest compared to the abundance found in leaf litter mixtures from the invaded forest. These results suggest that the leaf litter mixtures from both forest types significantly could differ in their cellulose and lignin content. Differences in the chemical quality between these mixtures are not surprising, since a previous study found that the litter of *L. lucidum* exhibited lower % of C (44.12%) and N (0.82%) than the litter of two of the most abundant species in the mixtures of native forests: *C. vernalis* (49.65% C, 1.83% N) and *O. porphyria* (50.46% C, 1.28% N) (Aragón et al. 2014a). Also, plant diversity is considered one structuring factor of fungal communities because the diversity of compounds in leaf litter could increase the diversity of carbon sources (Mcguire et al. 2012). In our study area, the native forest is composed of several species of trees (Malizia

Table 3 In-vitro production of polyphenol oxidases on solid media supplemented with specifics substrates

Strain/tentative ID media and substrates	ABTS[a]		SGZ[b]		Benzidine		α-naphtol		*p*-cresol		2,6 dimethoxyphenol		catechol	
	LME	MEA	LME	MEA	LME	MEA	LME	MEA	LME	MEA	LME	MEA	LME	MEA
Phaeoacremonium angustius S4EG3	−	−	−	ND[c]	−	−	−	−	−	−	−	−	−	−
Peniophora aurantiaca S3EG1	−	−	+	ND	−	+	−	−	−	−	+	−	−	−
Glomerella acutata S5NG2	−	−	+	ND	−	−	−	−	−	−	+	+	−	−
Phialemonium dimorphosporum S5EG1	−	−	−	ND	−	−	−	−	−	+	−	−	−	−
Trametes versicolor S5NG3	−	−	+	ND	−	−	−	−	−	+	−	−	−	−
Trichoderma atroviride S1EG1	−	−	−	ND	−	−	−	−	−	−	−	−	−	−
Pestalotiopsis maculiformans S4NG2	−	−	+	ND	−	−	−	−	−	−	+	−	−	−
Glomerella acutata S1NG2	−	−	+	ND	−	+	−	−	−	−	−	−	−	−
Trametes versicolor S5NG1	+	+	+	ND	+	+	−	+	−	−	+	+	−	+
Mycosphaerella iridis S2NG1	−	−	−	ND	−	−	−	−	−	−	−	−	−	−
Fusarium proliferatum S3NG3	−	−	+	ND	−	−	−	−	−	−	−	−	−	−
Fusarium solani S2EG3	−	−	+	ND	+	−	−	+	−	+	−	−	−	−
Fusarium solani S2NG2	−	−	+	ND	+	−	+	−	+	+	−	−	−	−
Candida homilentoma S3NG1	NG[d]	−	NG	ND	NG	−	NG	−	NG	+	NG	−	NG	−
Alternaria burnsii S3EG3	NG	−	NG	ND	NG	−	NG	−	NG	−	NG	−	NG	−
Clonostachys rosea S4NG1	−	−	+	ND	+	−	+	−	−	−	−	−	−	−
Trametes versicolor S4EG2	+	−	+	ND	+	+	+	+	−	+	+	+	−	−
Colletotrichum gloeosporoides S1NG1	−	−	+	ND	−	−	−	−	−	−	+	−	−	−
Colletotrichum gloeosporoides S1EG2	+	+	+	ND	+	+	−	+	−	+	+	+	−	+

Table 3 continued

Strain/tentative ID media and substrates	ABTS[a]		SGZ[b]		Benzidine		α-naphtol		*p*-cresol		2,6 dimethoxyphenol		catechol	
	LME	MEA	LME	MEA	LME	MEA	LME	MEA	LME	MEA	LME	MEA	LME	MEA
Trichoderma hartianum S2NG4	−	−	−	ND	−	−	−	−	−	−	+	−	−	−
Corynespora torulosa S2EG2	−	−	−	ND	−	−	−	+	−	−	−	−	−	−
Flavodon flavus S5EG3	−	+	+	ND	+	+	+	+	−	−	+	+	−	+

\+ positive reaction, − negative reaction

[a] ABTS: 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)

[b] SGZ: 4-hydroxy-3,5-dimethoxybenzaldehyde azine (syringaldazine)

[c] ND: not detected under these assay conditions. Syringaldazine was not evaluated in MEA agar, due to the production of a dark yellow reaction in abiotic controls

[d] NG: no growth

et al. 2010) and because of this, the native leaf litter mixtures could be more diverse and could offer more carbon sources than the invaded forest mixtures that are composed mostly of *Ligustrum* leaf litter. In addition to diversity, the identity of the species composing the mixtures of leaf litter influences the abundance of microbial decomposers (Wardle et al. 2006). Our results could be related to the observations of Wardle et al. (2006), given that the species from the native forest, *O. porphyria* and *C. vernalis*, produced litter with more cellulose and lignin content compared to that in *Ligustrum* litter (Aragón et al., Unpublished data).

In addition to the chemical quality of leaf litter, the microenvironment and soil parameters influence the composition, abundance and activity of fungi (Lauber et al. 2008; Rousk et al. 2010). Invasion by exotic plants modifies the microenvironment conditions (Ashton et al. 2005; Tateno et al. 2007; Aragón et al. 2014a; Slesak et al. 2016) and therefore may alter the abundance and activity of fungi. In this study, humidity and soil temperature were similar in native and invaded forests and both parameters were within the range considered optimal for the activity of the microbiota (Pietikäinen et al. 2004). These results contrast with those reported by Aragón et al. (2014a), who found differences in soil humidity (with invaded forest exhibiting drier soils).The shorter sampling period (only one month) could potentially explain the contrasting results found in the present study. Furthermore, soil humidity and temperature were recorded during the rainy season (summer) of an atypical year in which rainfall was almost twice as normal (Estación Experimental Agroindustrial Obispo Colombres 2015). This situation might also explain the lack of association between temperature or humidity and the abundance of cellulolytic and xylanolytic fungi and the weak association found between the abundance of ligninolytic fungi and soil humidity. Regarding soil pH, the native forest was slightly more acidic than the invaded forest and this variable was only associated with the abundance of cellulolytic fungi. These results agree with those reported by Korniłłowicz-Kowalska et al. (2003), who found an increased abundance of *Penicillium* cellulolytic strains in soils with low pH. Although the difference in pH between native and invaded forests was statistically significant, the pH values in both forests were within the range considered optimal for

the growth activity of fungi in general (Rousk et al. 2010). The absence of a significant association between pH and abundance of xylanolytic and ligninolytic fungi could be due to the fact that the effect of pH on fungi metabolism tends to be species-specific (Bachelot et al. 2016).

The colonization and abundance of fungi also depends on the state of decomposition of leaf litter (Osono et al. 2006). At early stages of decomposition, leaf litter presents low abundance and diversity of fungi (Voříšková and Baldrian 2013). However, at advanced stages of decomposition, the abundance and diversity of fungi increases due to the fact that other compounds are released from the cell walls and are used by species with different nutritional requirements (Voříšková and Baldrian 2013). In our study, the diversity of fungi (15 genera) was low compared to that found in other similar forests (Bills and Polishook 1994; Paulus et al. 2006; Araujo Costa and Pascholati Gusmão 2015). However, it is important to highlight that we only identified ligninolytic fungi from mixtures composed of leaf litter that were in the early stages of decomposition; i.e., when the relative abundance and diversity of ligninolytic fungi is low (Osono 2007). Four genera were common between both forest types; while strains of six genera were isolated only from the invaded forest and strains of five genera only from the native forest. Despite the limited sampling period, this result suggests that the fungal community is different in leaf litter of the native forest compared to that of the invaded forest.

Most of the identified ligninolytic fungi belong to the phylum *Ascomycota*. This result is consistent with studies carried out in tropical and temperate forests, where *Ascomycota* are dominant at the early stages of leaf litter decomposition and are even present in living leaves (Osono 2007; Schneider et al. 2012; Kerekes et al. 2013; Voříšková and Baldrian 2013). In addition, the low occurrence of *Basidiomycota* fungi might be due to the method of fungal isolation used, which favours fast-growing Ascomycetes species (Osono et al. 2009). Although this study was performed at a local scale and a low number of ligninolytic fungi was isolated, all the fungi orders identified in this work are consistent with the orders of fungi reported for the Argentine Yungas (Geml et al. 2014).

Fungi play a key role in forests ecology, since they are the main decomposers of lignin, which is considered the limiting compound of litter decomposition (Berg and McClaugherty 2008). Fungi degrade lignin through the production of extracellular enzymes such as laccases, catecholases, and tyrosinases (Osono 2007; Sánchez 2009). In this study, most of the ligninolytic fungi isolated from both forest types showed laccases activity. Particularly, strains of *T. versicolor* showed laccase and tyrosinase activities, and their laccases have been widely studied in different substrates (Moredo et al. 2003; Cabuk et al. 2006; Márquez et al. 2007; Tong et al. 2007). The different expression of these extracellular enzymes between fungal isolates might be due to the substrate they colonize (Colpaert and Van Laere 1996). This could explain the negative reactions of the strains of *P. angustius, T. atroviride* and *M. iridis* for all the substrates used in this study.

Conclusions

To our knowledge, this is the first study documenting culturable decomposer fungi of leaf litter from native and invaded forests in a Yungas ecosystem. The results found in this study suggest that *Ligustrum* invasion alters the abundance and composition of fungi involved in the degradation of leaf litter. This could impact upon the decomposition rate of invaded forests and consequently, nutrient cycling. However, the limited sampling period of the present study should be taken into account. Long-term studies including also the analysis of the nutritional quality of litter are needed for a more complete overview of the influence of *Ligustrum* invasion over fungal decomposers.

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Conflict of interest The authors declare no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study

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