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ARTICLE

Relationships between fungal endophytes and wood-rot fungi in wood of *Platanus acerifolia* in urban environments

Carolina A. Robles, Silvia E. Lopez, Patricia D. McCargo, and Cecilia C. Carmarán

Abstract: Urban forests, which are highly valuable for the urban environment, include trees from city streets that might be susceptible to wood-decay fungi. Fungal endophytes can colonize healthy plant tissues without causing external disease symptoms in standing trees. In this study, the diversity of endophytes isolated from wood of *Platanus acerifolia* (Aiton) Willd. from Buenos Aires, Argentina, is described and related to different levels of urban disturbance, tree age, and presence of wood-decay basidiomycetes. Samples were obtained from 28 asymptomatic trees (1643 wood core fragments) and 75 symptomatic trees (1516 wood sample fragments) from four sites with different levels of human disturbance. Fungal isolates were morphologically identified, and potential wood-decay isolates were phylogenetically analyzed. Twenty-seven endophytic taxa, including five basidiomycetes, were identified. The multivariate analysis suggested that the endophyte community of wood is highly influenced by the level of urban disturbance, followed by tree age and presence of potential pathogenic basidiomycetes.

Key words: basidiomycetes, human disturbance, *Platanus acerifolia*, urban trees, wood endophytes.

Résumé : Les forêts urbaines, qui sont très précieuses pour l'environnement urbain, incluent les arbres le long des voies urbaines qui peuvent être susceptibles aux champignons de carie du bois. Les champignons endophytes peuvent coloniser les tissus végétaux sains sans causer de symptômes visibles de maladie chez les arbres vivants. Dans cet article, la diversité des endophytes isolés dans le bois de *Platanus acerifolia* (Aiton) Willd.) dans la ville de Buenos Aires en Argentine est décrite et reliée à différents degrés de perturbation urbaine, à l'âge des arbres et à la présence de basidiomycètes responsables de la carie du bois. Des échantillons ont été prélevés sur 28 arbres asymptômatiques (1643 fragments de bois de bois de cœur) et 75 arbres symptômatiques (1516 fragments de bois) répartis dans quatre sites soumis à différents nivaux de perturbation anthropique. Les isolats fongiques ont été identifiés sur une base morphologique et ceux qui pouvaient être des champignons de carie ont été analysés sur une base phylogénétique. Vingt-sept taxons d'endophytes, incluant cinq basidiomycètes, ont été identifiés. L'analyse multivariée indique que la communauté d'endophytes du bois est fortement influencée par le niveau de perturbation urbaine, suivi de l'âge des arbres et de la présence de basidiomycètes potentiellement pathogènes. [Traduit par la Rédaction]

Mots-clés : basidiomycètes, perturbation anthropique, *Platanus acerifolia*, arbres urbains, endophytes du bois.

Introduction

Urban areas have a growing impact on local, regional, and global environments. The urbanization of landscapes continuously increases and so does the importance of city forests, which include all of the trees within urban communities (Nowak et al. 2005). The importance of city trees will increase through the 21st century, as they play an essential role in urban ecosystems, providing a wide range of benefits to the environment and to the well-being of people and mitigating many of the impacts caused by the development of cities (Sinclair et al. 2014).

However, urban trees are exposed to several stress factors that can affect their health (Robles et al. 2011). Reduced tree vigor increases the possibility of entry of wood-decay fungi, which can severely decrease their stability and fracture resistance (Schwarze et al. 2000). Due to human safety concerns regarding falling trees and branches, some authors have studied wood rot in city trees (Robles et al. 2011; Schmidt et al. 2012).

One of the most representative street trees is the London plane tree (*Platanus acerifolia* (Aiton) Willd.), a species cultivated as an ornamental tree in cities from Europe, South Africa, Australia, New Zealand (Asturias et al. 2003), the United States, and Canada (DeGraaf and Sendak 2006) due to its fast growth, shading foliage, and good resistance to urban pollution and harsh climatic conditions (Asturias et al. 2003).

Endophytes colonize healthy plant tissues, where they persist for a certain amount of time without causing disease symptoms (Arnold 2007). Most studies on endophytes have focused on foliar endophytes, which are especially diverse and abundant and mainly belong to the phylum Ascomycota (Angelini et al. 2012). However, an increasing number of studies have focused on tree endophytes in woody tissue and the presence of endophytic basidiomycetes (Thomas et al. 2008; Pinruan et al. 2010).

The presence of endophytes in woody tissue suggests that there are wood-decay fungi such as Basidiomycota and Ascomycota that have an endophytic phase in which they do not degrade the xylem (Parfitt et al. 2010). The detection of wood-rot basidiomycetes in an endophytic phase could help to diagnose early stages of decay and improve both human safety and urban forest management.

In a previous study on *P. acerifolia* wood, we explored several factors affecting tree health and found that tree age (estimated from diameter at breast height (DBH, 1.3 m)) and the presence of harmful decay fungi have the greatest influence on landscape planning when making management decisions (Robles et al. 2011).

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C.A. Robles, S.E. Lopez, P.D. McCargo, and C.C. Carmarán. PROPLAME-PRHIDEB-CONICET, Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, PB II, 4to piso, (CP1428EHA) Ciudad Autónoma de Buenos Aires, Argentina.

Corresponding author: Carolina A. Robles (e-mail: carorobles@bg.fcen.uba.ar).

Here, we further examine the woody endophytes and the presence and frequency of endophytic basidiomycetes in wood from *P. acerifolia* trees from Buenos Aires, Argentina.

The goals of this study are to (*i*) characterize the endophytic fungi from wood of urban *P. acerifolia* trees, with an emphasis on the detection of potential wood-decay basidiomycetes, (*ii*) further describe basidiomycetes and Xylariaceae isolates from urban trees using molecular techniques, and (*iii*) analyze whether the biodiversity of the endophytic community is related to predisposing decay factors.

Materials and methods

Study site and sampling

This survey was carried out in Buenos Aires, Argentina (34°36′43″S, 58°25′02″W), throughout 2010 and 2011. This city has an area of 200 km^2 and about three million inhabitants (National Statistics and Censuses Institute (INDEC) 2010). Four sampling sites, 5–13 km apart from each other, were selected within the city: a residential area (site A), a very disturbed site downtown (site B), an industrial and residential area (site C), and a park area (site D). In sites A, B and C, all trees were street trees (Table 1).

The levels of human disturbance (DL) in each of these sites were previously assessed (Robles et al. 2011) according to the following indicators: vehicular traffic and the presence of stores, supermarkets, and gasoline stations. Site D is situated between residential neighborhoods and the natural park of Ciudad Universitaria and did not present any of these indicators of human disturbance.

Five city blocks (ca. 100 m in size) were considered in each of sites A, B, and C. The city blocks were not adjacent and were different from those considered in Robles et al. (2011). In each block, all trees with external symptoms such as cankers or cavities in their trunks were considered symptomatic trees (ST), and wood samples (WS), approximately 5 cm long, were collected from the affected areas by means of an increment hammer, as in Robles et al. (2011). Simultaneously with the sampling of the ST, a maximum of two asymptomatic trees (AT) per block were randomly selected for sampling to investigate the presence and diversity of endophytes. Our survey showed that the total number of trees in the city blocks that was assessed was between 4 and 20, of which between 2 and 13 were ST. In sites A, B, and C, the intensity of pruning was similar, as trees are pruned once a year by the government due to their proximity to buildings.

An area of bark (ca. 3 cm × 3 cm) was removed, and then, wood cores (WC), approximately 10 cm long and visually healthy, were collected from these trees at breast height and (or) at the base of the trunk by means of an increment borer (Suunto, Vantaa, Finland), which was superficially sterilized with 70% ethanol after each use. In site D, which comprised a park area of ca. 0.4 km^2, 19 AT were found, and nine of them were randomly sampled. Each hole made in an AT was sealed with silicone sealer to minimize postsampling infection. WS and WC were sprayed with 70% ethanol and stored individually in sterile polyethylene bags and in 15 mL Falcon tubes, respectively, and processed within 24 h after collection.

During the survey, the DBH of ST and AT was measured and used as an indicator of relative age (Linsen et al. 2005).

Isolation and identification of fungi by morphological and cultural characters

In the laboratory, under aseptic conditions, WS and WC were cut ca. 0.5 cm long fragments, and each fragment was superficially sterilized (50% ethanol for 30 s, 2% mass/volume NaOCl for 1 min, and 50% ethanol for 30 s), flame sterilized, and then placed onto 9 mm Petri dishes containing either 2% malt extract agar (MEA) with chloramphenicol (100 mg·L^{-1}) or a medium that is selective for basidiomycetes (i.e., BDS). The BDS-selective medium that was used was modified from that used by Oses et al. (2008) and contained malt extract (15 g·L^{-1}), agar (15 g·L^{-1}), benomyl (40 mg·L^{-1}), chloramphenicol (100 mg·L^{-1}), and dichloran (20 mg·L^{-1}). A maximum of 24 fragments were obtained from each WC, and up to eight fragments were obtained from each WS. Fragments were incubated in the dark at 24 °C and examined every 2–3 days to detect fungal growth. Isolates obtained from AT were considered as endophytic isolates (Oses et al. 2008).

Table 1. Indicators of relative disturbance in each site sampled.

Indicators	Site A	Site B	Site C	Site D
S	1.4	43.2	17.9	0
GS	0.7	1.2	0.8	0
SU	0.7	1.2	0.8	0
V/min	4	47.6	4.1	0

Note: The values represent the percentage of each indicator out of the total number of buildings. *S*, stores; GS, gasoline stations; SU, supermarkets; *V*/min, number of vehicles per minute. All values in site D are 0, as the trees are located in a park area.

All of the fungal isolates were identified conventionally according to their macroscopic and microscopic features of the somatic and reproductive structures. After determination of their genera (von Arx 1981; Seifert et al. 2011), they were transferred to the specific medium for species identification. Isolates that showed clamp connections and asexual spores that are typical of basidiomycetes were identified using keys for Basidiomycota, based on mycelial characters (Stalpers 1978; Deschamps and Wright 1997). Observations and measurements were carried out on fresh material mounted in a mixture of distilled water, 5% KOH, and 1% phloxine for viewing with an optical microscope. Observations were made using a Zeiss Axioskop microscope (Oberkochen, Germany) that was equipped with an Olympus C-5060 wide zoom digital camera. The online database MycoBank (http://www.mycobank.org) was consulted for currently accepted species names. Identified species were incorporated to the culture collection of the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (BAFCcult).

DNA extraction, amplification, and sequencing

Ten isolates of endophytic and pathogenic fungi, which were only partially identified by morphological characteristics, were subjected to molecular analyses for verification. Eight isolates belonged to four different Basidiomycota taxa (BAFCcult 4361–4363, 4365–4367, 4387, and 4415), and two isolates belonged to the order Xylariales (BAFCcult 4409 and4410). All isolates were obtained from AT, except BAFCcult 4387.

Genomic DNA was extracted from fresh mycelium grown on 2% MEA using the UltraClean Microbial DNA Isolation Kit (MO BIO Laboratories Inc., Solana Beach, California), following the manufacturer's protocol. The internal transcribed spacer (ITS) region of the isolates was amplified (Schoch et al. 2012) using the universal primers ITS1 and ITS4 under the following PCR conditions: denaturation at 94 °C for 3 min, 50 cycles of denaturation at 94 °C for 45 s, annealing at 57 °C for 45 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min. Reactions were performed in 26.6 μL volumes, containing 10 mmol·L^{-1} of PCR buffer that was supplied by the manufacturer, 50 mmol·L^{-1} of $MgCl_2$, 1 mmol·L^{-1} of each dNTP, 10 mmol·L^{-1} of each primer, 1 U of recombinant Taq DNA polymerase (Invitrogen, Brazil), and 3 μL of fungal genomic DNA. PCR cleanups were performed with the UltraClean kit. DNA fragments were sequenced at the sequencing service of the Facultad de Ciencias Exactas y Naturales.

Phylogenetic analyses

Phylogenetic reconstructions for molecular identification were performed using maximum parsimony (MP) and Bayesian inference. Datasets for the analyses were constructed with sequences

Table 2. Sampling summary.

Site	IT	ST	WS	WS fragments	WS isolates	AT	WC	WC fragments	WC isolates
Site A	72	30	73	704	117 (28)	10	29	696	69 (10)
Site B	52	15	47	320	34 (10)	2	4	96	2 (1)
Site C	54	30	62	492	82 (20)	7	21	504	22 (7)
Site D	26	0	0	0	0	9	16	347	37 (8)
Total area	204	75	182	1516	233	28	70	1643	130

Note: IT, number of inspected trees; ST, symptomatic trees; WS, wood samples; AT, asymptomatic trees; WC, wood cores. Numbers in parentheses indicate the number of trees from which isolates were obtained.

obtained in this study and sequences highly similar to these, according to a BLAST (basic local alignment search tool) search in GenBank (NCBI, http://www.ncbi.nlm.nih.gov/genbank/) (Supplementary Tables S1–S5[1]).

Using NCBI, reference environmental samples from wood were also tested to identify sequences with a high similarity to the sequences obtained in this study (Hibbett et al. 2011). Sequences from environmental samples that showed a max identity and query coverage of ≥97% were considered.

The sequences obtained in this study were aligned with the selected reference sequences using Clustal W v.1.4 for multiple alignment of the BioEdit v.7.0.5.3 program (Hall 1999) and deposited in GenBank under accession numbers KC881187–KC881196. Costs assigned were 15 for gap openings and six for gap extensions. The datasets were analyzed using NONA v.2.0 (Goloboff 1997) for MP. All characters were given equal weight, and gaps were scored as missing data. Parameters used were 1000 replications and a maximum of 10 000 trees. To determine the support for each clade, bootstrap analyses were performed with 1000 replications.

Bayesian inference phylogenies were performed using MrBayes 3.2 (Ronquist et al. 2012). Evolutionary models used for Bayesian analysis were assessed using MEGA v.5 (Tamura et al. 2011). Four Markov chain Monte Carlo searches were run for 500 000 – 2 000 000 generations under a Kimura two-parameter model with gamma distribution, saving one of 100 trees, until the standard deviation of split frequencies was less than 0.01. The first 25% of trees were discarded as burn-in, and then, a 50% majority rule consensus phylogram was computed from the remaining trees. A sequence of *Amanita muscaria* (L.) Lam. (AB096052) was chosen as an outgroup in both MP and Bayesian analyses in all datasets.

Statistical and ecological analyses

The DBH values, as indicators of tree age, were contrasted using a χ^2 test against basidiomycetes from both ST and AT. For the analyses of the endophytic communities, each AT was considered an experimental unit. The relative frequency (*F*) of each endophytic species was calculated as the absolute frequency of the individual species divided by the sum of absolute frequencies of all species × 100. The absolute frequency was considered as the number of experimental units in which a species occurs divided by the total number of experimental units (Horton and Bruns 2001). Species accumulation curves and estimates of total richness of fungal endophytes were inferred with EstimateS v.8.2.0 (Colwell 2006). Diversity of endophytes was measured by Shannon's diversity index (*H*) using EstimateS. The similarity among the studied endophytic communities was assessed with Sørensen's index of similarity using the following formula: $S = 2C/(A + B)$, where *S* is the degree of similarity, *A* and *B* are the number of species at sites A and B, respectively, and *C* is the number of species common to both sites.

Multivariate analysis

Canonical correspondence analysis (CCA) of AT and endophytic taxa was performed using CANOCO for Windows 4.5 (ter Braak and Šmilauer 2002) to evaluate the influence of two quantitative variables (DBH and percentage of WS fragments colonized by basidiomycetes in each site (Bas%)) and one ordinal variable (DL, according to the following scale from the most disturbed site to the least disturbed site: site B, site C, Site A, and site D) on the biodiversity of the endophytic community. A Monte Carlo test with 999 permutations was run to check the validity of the ordination.

Results

From a total of 3159 fragments analyzed (1516 WS fragments and 1643 WC fragments), 363 fungal isolates were obtained (233 from WS fragments and 130 from WC fragments). Details of the sampling are summarized in Table 2. Both AT and ST showed similar numbers of taxa and had between one and eight species per tree. No significant differences were found between the number and identity of strains isolated from the BDS-selective medium vs. the MEA medium.

Composition of the endophytic community

A total of 130 isolates were obtained from the 1643 WC fragments from AT, and all were considered endophytes. Of these, 69 isolates were recovered from site A (53%), 2 isolates were recovered from site B (1.5%), 22 isolates were recovered from site C (17%), and 37 isolates were recovered from site D (28.5%).

Endophytes belonged to 27 different taxa (Table 3). Most fungal taxa that were recovered were ascomycetes (80%) and represented 68% of the total isolates. The highest number of species belonged to the orders Hypocreales and Pleosporales. The highest relative frequencies were those of *Trichosporon sporotrichoides* (Oorschot) Oorschot & de Hoog (12%) and *Alternaria alternata* (Fr.) Keissl. (10%), as well as *Cladosporium cladosporioides* (Fresen.) G.A. de Vries, *Granulobasidium vellereum* (Ellis & Cragin) Jülich, and *Trichoderma harzianum* Rifai (7% each) (Table 3). *Alternaria alternata*, *Arthrinium phaeospermum* (Corda) M.B. Ellis, *Aspergillus reptans* Samson & W. Gams, *Bipolaris australiensis* (M.B. Ellis) Tsuda & Ueyama, *Trichoderma atroviride* P. Karst., *T. harzianum*, and *T. sporotrichoides* were isolated in more than one site.

The highest species richness was found in site A (15 different species), followed by sites C and D (10 species each). Site B showed the lowest species richness, with only one species, probably due to the low number of AT available and was, therefore, excluded from the diversity analyses. The highest estimated species richness (Michaelis-Menten Means (MM-Means)) was found in site C (24.73), followed by sites A (21.28) and D (20.08). Species accumulation curves for sites A, C, and D are shown in Fig. 1. Shannon's index of diversity was 2.84 when all of the taxa observed were considered. The highest diversity was found in site A (2.36), followed by sites C (1.97) and D (1.87). The similarity (Sørensen's

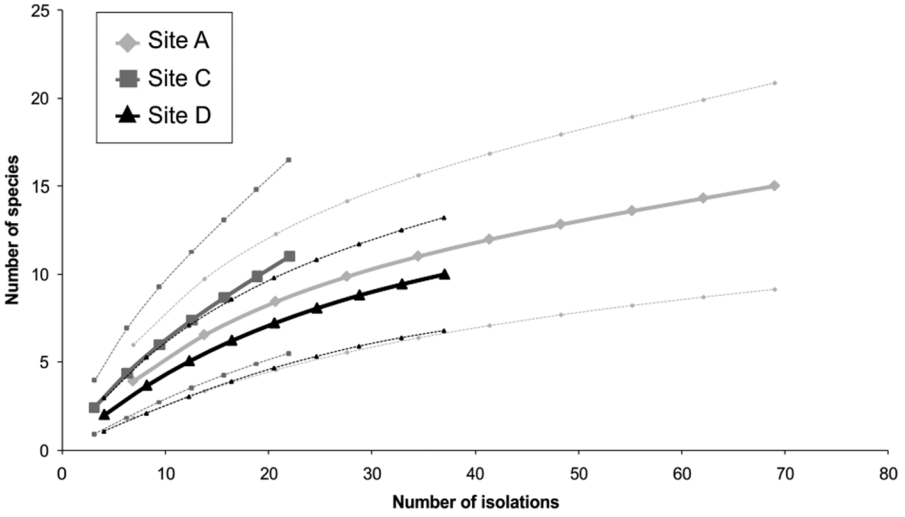
[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjfr-2014-0560.

Table 3. Diversity of fungal endophytes.

Taxa	Number of isolates				*F*
	Site A	Site B	Site C	Site D	
Ascomycota					
Alternaria alternata (Fr.) Keissl. (ALT)	14 (7)	0 (0)	1 (1)	2 (1)	10.8
Arthrinium phaeospermum (Corda) M.B. Ellis (PHA)	2 (1)	0 (0)	6 (3)	0 (0)	5.4
Aspergillus niger Tiegh. (NIG)	0 (0)	0 (0)	1 (1)	0 (0)	1.3
Aspergillus reptans Samson & W. Gams (REP)	1 (1)	0 (0)	1 (1)	0 (0)	2.7
Aspergillus ustus (Bainier) Thom & Church (UST)	1 (1)	0 (0)	0 (0)	0 (0)	1.3
Bipolaris australiensis (M.B. Ellis) Tsuda & Ueyama (AUS)	2 (1)	0 (0)	0 (0)	5 (3)	5.4
Chaetomium funicola Cooke (FUN)	0 (0)	0 (0)	1 (1)	0 (0)	1.3
Chaetomium globosum Kunze (GLO)	0 (0)	0 (0)	2 (1)	0 (0)	1.3
Cladosporium cladosporioides (Fresen.) G.A. de Vries (CLA)	12 (5)	0 (0)	0 (0)	0 (0)	6.8
Curvularia lunata (Wakker) Boedijn (LUN)	0 (0)	0 (0)	0 (0)	3 (2)	2.7
Epicoccum nigrum Link (EPI)	0 (0)	2 (1)	0 (0)	0 (0)	1.3
Fusarium solani (Mart.) Sacc. (SOL)	0 (0)	0 (0)	0 (0)	1 (1)	1.3
Gliocladium penicillioides Corda (PEN)	0 (0)	0 (0)	0 (0)	3 (2)	2.7
Nodulisporium sp. (NOD)	6 (3)	0 (0)	0 (0)	0 (0)	4.0
Pestalotiopsis guepinii (Desm.) Steyaert (GUE)	3 (2)	0 (0)	0 (0)	0 (0)	2.7
Phialemonium curvatum W. Gams & W.B. Cooke (CUR)*	0 (0)	0 (0)	1 (1)	0 (0)	1.3
Phoma eupyrena Sacc. (EUP)	0 (0)	0 (0)	0 (0)	1 (1)	1.3
Phomopsis pittospori S.A. Archer (PIT)	0 (0)	0 (0)	0 (0)	1 (1)	1.3
Pseudallescheria boydii (Shear) McGinnis, A.A. Padhye & Ajello (BOY)	3 (3)	0 (0)	0 (0)	0 (0)	4.0
Sarocladium strictum (W. Gams) Summerb. (STR)	0 (0)	0 (0)	2 (1)	0 (0)	2.7
Trichoderma atroviride P. Karst. (ATR)*	2 (1)	0 (0)	0 (0)	5 (2)	5.4
Trichoderma harzianum Rifai (HAR)	4 (4)	0 (0)	0 (0)	1 (1)	6.8
Basidiomycota					
Coprinopsis cinerea (Schaeff.) Redhead, Vilgalys & Moncalvo (CIN)*	2 (1)	0 (0)	0 (0)	0 (0)	1.3
Granulobasidium vellereum (Ellis & Cragin) Jülich (VEL)*	11 (5)	0 (0)	0 (0)	0 (0)	6.8
Inonotus rickii (Pat.) D.A. Reid (INO)	5 (3)	0 (0)	0 (0)	0 (0)	4.0
Phanerochaete chrysosporium (Burds.) (CHR)*	0 (0)	0 (0)	1 (1)	0 (0)	1.3
Trichosporon sporotrichoides (Oorschot) Oorschot & de Hoog (SPO)*	1 (1)	0 (0)	6 (4)	15 (4)	12.2
Total number of isolates	69	2	22	37	

Note: Number of isolates in each site and relative frequencies (*F*) of endophytic taxa. Three-letter abbreviations of the fungal species used in canonical correspondence analysis are shown in parentheses after the scientific name. An asterisk (*) indicates first records for the country. Numbers in parentheses indicate the number of trees in which the species was recorded.

Fig. 1. Accumulation curves for endophytic species for each sampling site. Dotted lines indicate 95% confidence intervals.



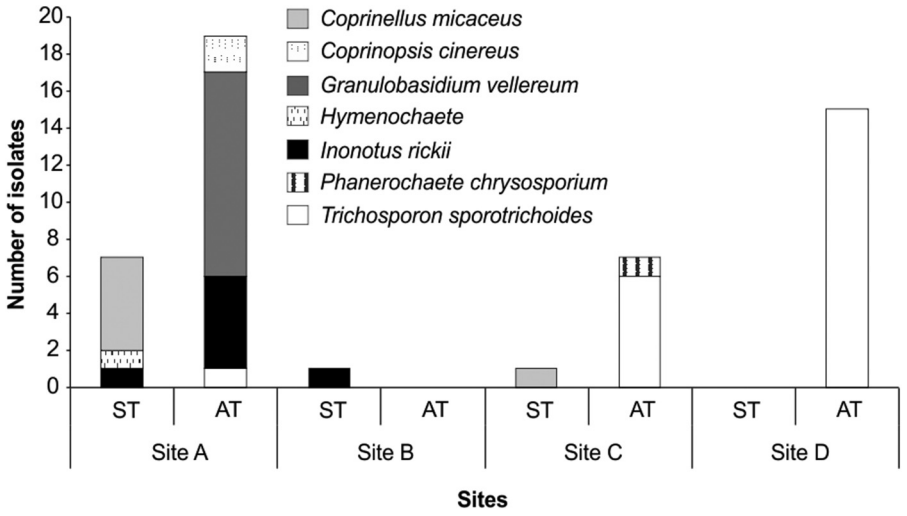
index) of the fungal endophyte taxa was highest between site A and site D (0.4) and lowest between site C and site D (0.2). The similarity between site A and site C was 0.32.

Analysis of total basidiomycetes obtained from ST and AT

Nine and 41 basidiomycetous isolates were obtained from ST and AT, respectively. Twenty-six basidiomycetous isolates were recovered in site A (52%), one in site B (2%), eight in site C (16%), and 15 in site D (30%). The identity, number of isolates, and source of isolation (ST or AT) in each sampling site are shown in Fig. 2. *Inonotus rickii* (Pat.) D.A. Reid was isolated in two sites and was the only basidiomycete detected in both ST and AT. *Trichosporon sporotrichoides* was the most abundant species (22 isolates), followed by *G. vellereum* and *I. rickii* (11 and 7 isolates, respectively).

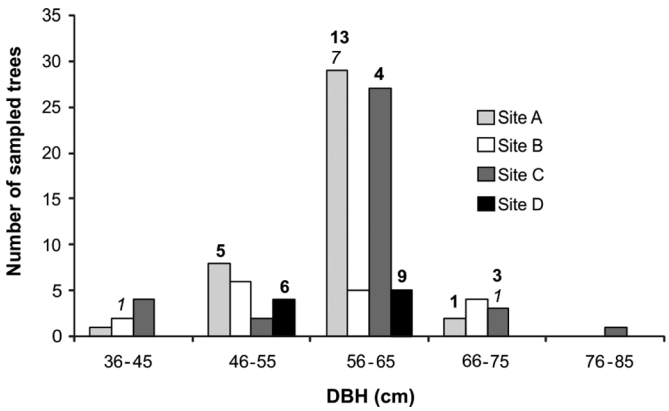
The distribution of DBH for sites A and C (both ST and AT) ranged from 56 cm to 65 cm, suggesting estimated ages of 70–80 years (Linsen et al. 2005). At sites B and D, the DBH of sampled trees ranged between 36 cm and 65 cm, corresponding to estimated

Fig. 2. Identity and number of basidiomycetes isolates from symptomatic and asymptomatic trees (ST and AT, respectively) in each sampling site.



ages of 20–80 years (Linsen et al. 2005) (Fig. 3). These results are consistent with those reported by Robles et al. (2011). Basidiomycetes isolates from ST and AT were recorded mostly in large-diameter trees (DBH, 56–65 cm) (Fig. 3). The frequency of ST with different DBH that were colonized by basidiomycetes differed significantly (χ^2 test using Yates' correction, $x^2 = 9.88$, $p < 0.05$, degrees of freedom (df) = 3). The same was observed for the frequency of AT with different DBH that were colonized by basidiomycetes (χ^2 test, $x^2 = 58.14$, $p < 0.01$, df = 3).

Fig. 3. Number of symptomatic and asymptomatic trees sampled according to diameter at breast height (DBH). The number of basidiomycetes isolates from symptomatic (italic) and asymptomatic (bold) trees in each site are indicated above the bars.



Phylogenetic analyses

In all cases, phylogenetic results supported morphological identifications at the genus level. Phylogenetic analyses of endophytic strains of Xylariales BAFCcult 4409 and 4410 (GenBank numbers, KC881196 and KC881195, respectively) showed no clear association with a specific taxon (Supplementary Fig. S1[1]). MP analysis resulted in 27 most parsimonious trees (tree length, 939; consistency index (CI), 0.48; retention index (RI), 0.71).

Both MP and Bayesian analyses showed that the sequence of the endophytic basidiomycete isolate BAFCcult 4361 (GenBank number, KC881188) was located in the same clade with sequences of *Coprinopsis cinerea* (Schaeff.) Redhead, Vilgalys & Moncalvo (Supplementary Fig. S2[1]). MP analysis resulted in 60 most parsimonious trees (tree length, 730; CI, 0.56; RI, 0.76).

Both MP and Bayesian analyses showed that all of the sequences of the endophytic basidiomycetes isolates BAFCcult 4362, 4363, and 4365–4367 (GenBank numbers, KC881192, KC881194, KC881191, KC881190, and KC881193, respectively) were located in the same clade, together with a sequence of *G. vellereum* (GenBank number, AY787674), with a high bootstrap support (100%) and high Bayesian posterior probability (1) (Supplementary Fig. S3[1]). MP analysis resulted in five most parsimonious trees (tree length, 2067; CI, 0.60; RI, 0.81).

Both MP and Bayesian analyses showed that the basidiomycete isolate BAFCcult 4387 (GenBank number, KC881187), obtained from a ST, was located within the genus *Hymenochaete* (Supplementary Fig. S4[1]). MP analysis resulted in nine most parsimonious trees (tree length, 1824; CI, 0.45; RI, 0.70).

Finally, both MP and Bayesian analyses showed that the sequence of the endophytic basidiomycete isolate BAFCcult 4415 (GenBank number, KC881189) was located in the same clade with sequences of *Phanerochaete chrysosporium* Burds., with 88% of bootstrap support and 0.97 of Bayesian posterior probability (Supplementary Fig. S5[1]). MP analysis resulted in one most parsimonious tree (tree length, 882; CI, 0.55; RI, 0.62).

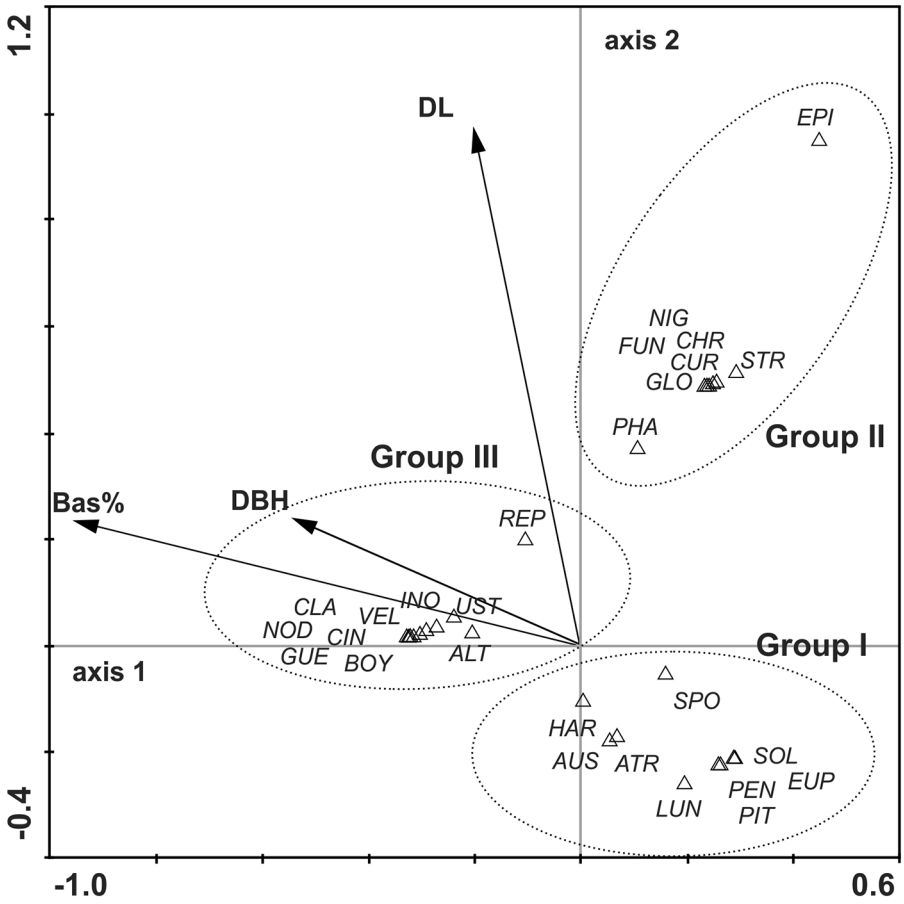
No significant results were obtained when we searched for sequences of environmental samples with high similarity.

Multivariate analysis

The CCA ordinations (Fig. 4) showed the distribution of 27 fungal taxa isolated from 28 AT in relation to three selected variables, i.e., DL, DBH, and Bas%. The first and second CCA axes (eigenvalues, 0.74 and 0.69, respectively) explained 14.6% of the cumulative percentage variance of species data and 74.5% of the cumulative percentage variance of species–environment relationships. The sum of all canonical eigenvalues was 1.92 (20% of total inertia). The three variables considered were negatively correlated with the first axis and showed the following interset correlation (ISC) values: $ISC_{DBH} = -0.52$, $ISC_{Bas\%} = -0.91$, and $ISC_{DL} = -0.20$. At the same time, all three variables were positively correlated with the second axis ($ISC_{DBH} = 0.22$, $ISC_{Bas\%} = 0.21$, and $ISC_{DL} = 0.89$). Both the first axis and all axes were significant (Monte Carlo permutation test, for the first axis, F ratio = 1.71 and P value = 0.03; for all canonical axes, F ratio = 1.7 and P value = 0.001).

The resulting distribution of fungal taxa showed that three different groups could be differentiated in the diagram based on the three selected variables (Fig. 4). Group I included the following taxa: *B. australiensis*, *Curvularia lunata* (Wakker) Boedijn, *Fusarium solani* (Mart.) Sacc., *Gliocladium penicillioides* Corda, *Phoma eupyrena* Sacc., *Phomopsis pittospori* (Cooke & Harkn.) Grove, *T. harzianum*, *T. atroviride*, and *T. sporotrichoides*. This group was associated with a low DL, a low DBH, and an absence of Bas%. Group II included taxa such as *A. phaeospermum*, *Aspergillus niger* Tiegh., *Chaetomium*

Fig. 4. Canonical correspondence analysis ordination biplot (axes 1 and 2) of fungal endophytic taxa (n = 27) from the wood of London plane trees. For an explanation of abbreviations used for the fungal species, see Table 3. DBH, diameter at breast height; DL, human disturbance level; Bas%, percentage of wood samples colonized by basidiomycetes.



globosum Kunze ex Fr., *Chaetomium funicola* Cooke, *Epicoccum nigrum* Link, *P. chrysosporium*, *Phialemonium curvatum* W. Gams & W.B. Cooke, and *Sarocladium strictum* (W. Gams) Summerbell. These taxa were associated with a high DL. Finally, group III included the following taxa: *A. alternata*, *A. reptans*, *Aspergillus ustus* (Bainier) Thom & Church, *C. cladosporioides*, *C. cinerea*, *G. vellereum*, *I. rickii*, *Nodulisporium* sp., *Pestalotiopsis guepinii* (Desm.) Steyaert, and *Pseudallescheria boydii* (Shear) McGinnis, A.A. Padhye & Ajello. This group comprised the highest number of endophytic basidiomycete species (*C. cinerea*, *G. vellereum*, and *I. rickii*). Group III showed a positive association with high DBH and Bas%.

The taxa distribution observed in the first and second axes of the CCA indicates that the fungal assemblage in sites with high DL can differ from the assemblage in sites with low DL. This analysis showed that DL was the main environmental factor affecting the composition of the endophytic fungal community.

Discussion

In this study, 27 different fungal taxa were obtained from wood of asymptomatic London plane trees. To our knowledge, this is the first study on the endophytic fungi from this tree species or members of the Platanaceae and, moreover, from the wood of urban trees, so there are no data available for comparison. However, the richness obtained was lower than that reported from wood of other tree species such as *Acer truncatum* Bunge with a similar sampling effort (Sun et al. 2011).

We characterized the endophytic community of wood from London plane trees. The species accumulation curves from this study are similar to those obtained in cultivation-based studies (Unterseher et al. 2013). As the total of the endophytic species richness was not detected, additional sampling would be preferred. Further studies could involve the use of sampling procedures for DNA-based screening of fungi in wood (Guglielmo et al. 2010). Nevertheless, Unterseher et al. (2013) evaluated the proportion of cultivable endophytic fungi in the entire pool of beech-associated phyllosphere taxa and concluded that biodiversity cultivation-based studies can be considered as a mirror of the "real" biodiversity patterns.

Most fungal taxa and isolates recovered in this survey were ascomycetes. The predominance of Ascomycota as characteristic of endophytic mycobiota and the low recovery of basidiomycetous fungal endophytes in woody and nonwoody hosts, even using selective media, have been previously reported (Gazis and Chaverri 2010). *Alternaria alternata* and *C. cladosporioides* were frequently recovered in this survey and have been recorded as dominant fungal endophytes of wood in other studies (Ragazzi et al. 2003; Sun et al. 2011).

Most genera obtained in this study have been previously reported as wood endophytes in various host trees (e.g., Tejesvi et al. 2005; Sun et al. 2011); however, *A. reptans*, *A. ustus*, and *B. australiensis* are reported for the first time. Additionally, *C. cinerea*, *G. vellereum*, *I. rickii*, *P. boydii*, and *T. sporotrichoides* are reported as endophytes for the first time.

Most of the taxa recovered (70%) were represented by two or more isolates. This result could indicate that the wood endophytic mycobiota of London plane trees is dominated by frequent species, rather than occasional taxa (singleton isolates). This pattern

has been observed in other endophytic communities such as that reported from wood from *Vitis vinifera* (González and Tello 2010).

In this study, seven taxa of basidiomycetes were obtained: two from ST (*C. micaceus* and *Hymenochaete* sp.), four from AT (*C. cinerea*, *G. vellereum*, *P. chrysosporium*, and *T. sporotrichoides*), and one from both AT and ST (*I. rickii*). *Inonotus rickii* and *C. micaceus* have been previously recorded on London plane trees (Robles et al. 2011). To our knowledge, *C. cinerea*, *G. vellereum*, *T. sporotrichoides*, and the genus *Hymenochaete* are recorded for the first time on this tree species. *Granulobasidium vellereum* is commonly associated with a white rot of angiospermous logs, slash, and living trees (Nakasone 1990), *C. cinerea* is usually associated with dung and woodchips (Keirle et al. 2004), and *T. sporotrichoides* is mainly found in the soil (van Oorschot 1980).

It is interesting to notice that both *I. rickii* and *P. chrysosporium* are white-rot fungi that were previously found affecting several angiosperms (Stalpers 1978; Dai et al. 2010). However, in this study, both species were detected in the wood of AT, as reported in other studies for members of the genera *Inonotus* and *Phanerochaete* (Crozier et al. 2006; Oses et al. 2008). These results and the detection of members of Xylariaceae (*Nodulisporium* sp.), which are also wood-rot fungi (Pointing et al. 2003), in AT suggest that asymptomatic, urban London plane trees are colonized by potential wood-decay fungi. Several authors have obtained similar results in other tree hosts (e.g., Oses et al. 2008; Parfitt et al. 2010) and suggested that latent infection exists in apparently healthy xylem tissue. Our findings agree with this hypothesis, and further studies will aim to explore the degradative ability and the ecological role of these isolates.

The London plane tree is an extensively cultivated species in cities throughout the temperate regions of the world (Asturias et al. 2003). The contribution to the knowledge of the relationships between fungal communities and decay factors in urban trees can help with management decisions concerning urban environments. This study constitutes the first systematic survey of wood endophytes on urban trees, with special interest in the relationship between the abundance and identity of endophytes, presence and identity of basidiomycetes from ST, and factors such as tree age and DL. The highest DBH values corresponded with the highest values of estimated endophytic richness, indicating a positive association between tree age and estimated endophytic richness. Sieber (2007) noticed a similar pattern in the needles of hosts such as *Picea mariana* (Mill.) Britton, Sterns & Poggenb and *Pinus strobus* L., where the level of endophyte infection increased with the aging of the leaves. In this study, no relationship between endophytic richness and presence of wood-decay fungi was detected. Nevertheless, the combined data of the identity and number of basidiomycetes (Fig. 2) and the number of trees sampled according to their DBH (Fig. 3) suggest that low DL together with a particular tree age (estimated ages of 70–80 years) could determine a high level of diversity of basidiomycetes.

The ordination by CCA allowed for the separation of three groups of endophytic taxa based on DBH, Bas%, and DL. These groups corresponded mainly to a gradient of human disturbance, previously assessed according to different indicators of disturbance. On the other hand, the presence of basidiomycetes in nearby ST, indicated by the variable Bas%, and the DBH of AT did not cause a clear separation of a group of species.

At site D, the least disturbed site, no ST were found. This could be partially explained by the fact that the pruning intensity (another indicator of human disturbance) in this site is lower than in the rest of the sites. Several studies have found that a high incidence of wood decay is associated with pruning wounds (Wardlaw and Neilsen 1999; Barry et al. 2000). Pruning intensity on sampled trees was not quantified in this study, but further investigation into this factor could reveal the actual influence of pruning on the relationships between fungal communities.

Another important factor when studying fungal communities is the water content in wood. The development of fungi in wood is affected by low water availability or by high water content and moisture effects on gaseous conditions (Rayner and Boddy 1988). High moisture levels can affect endophyte colonization by favoring high infection rates (Swart et al. 2000). In this study, differences in moisture levels in the sampling area were considered similar as the four sampling sites share the same climatic conditions. Also, WS were taken from standing trees belonging to the same species, and all WC were the same length.

The influence of human disturbance on the abundance and composition of fungal communities has been observed in previous studies, most of them in relation to species richness (among others, see Beena et al. (2000) and Ochimaru and Fukuda (2007)). Hossain and Sugiyama (2011) found that agricultural land use has a strong influence on the soil fungal community structure, and Matsumura and Fukuda (2013), studying the leaf endophyte community, hypothesized that forest isolation in urban areas affects the endophytic community. Our data agree with these studies, suggesting that human disturbances could have a strong influence on the establishment of fungal species assemblages.

Robles et al. (2011) suggested that tree age is an important factor on the abundance of pathogenic basidiomycetes in *P. acerifolia* trees. In this study, our results suggest that different fungal endophytic communities could coexist in urban London plane trees, determined mainly by the level of disturbance and, to a lesser extent, by tree age. The results related to group III of fungal taxa (CCA) showed a positive association with tree age and the presence of wood colonized by basidiomycetes, suggesting that some particular endophyte assemblages could be related to the presence of wood-decay fungi.

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