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Factors Influencing Endophytic Communities in Poplar Plantations

Jorge Martín-García, Elba Espiga, Valentín Pando and Julio Javier Diez

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The fungal species associated with leaves and twigs from stands of *Populus* × *euramericana* in northern Spain were studied with the aim of evaluating the effects of several factors on endophytic communities in these plantations. Endophyte assemblages were analysed in 12 poplar plantations (clone I-214), chosen according to a factorial scheme with two factors: age and site quality. Crown condition, dendrometric variables and foliar nutrients were recorded in each sampled tree to evaluate their effects on endophytic communities. Fungal species richness and relative isolation frequency (RIF) were higher in young stands than in adult stands. Moreover, the age-related differences depended on site quality, with the lowest richness levels observed in adult stands located in poor sites. At stand level, endophyte assemblages varied among stands according to site quality and, to a lesser extent, stand age. On the other hand, crown discoloration, total height and foliar concentrations of iron and zinc may be key indicators of endophytic communities in poplar plantations, at tree level.

Keywords endophyte, poplar, management, site quality, foliar nutrients, forest health
Addresses *Martín-García*, Sustainable Forest Management Research Institute, University of Valladolid – INIA, Avenida de Madrid 57, 34004 Palencia, Spain, and Forestry Engineering, University of Extremadura, Plasencia, Spain; *Espiga* and *Diez*, Sustainable Forest Management Research Institute, University of Valladolid – INIA, Palencia, Spain; *Pando*, Statistics and Operations Research Department, University of Valladolid, Spain
E-mail jorgemg@pvs.uva.es, jorgemg@unex.es
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1 Introduction

Interest in hybrid poplar plantations is increasing in Spain because of the economic value of the trees. The profits associated with poplar plantations can reach between 1200 and 2400 €/ha/yr on optimum land (Díaz and Romero 2001). Therefore, although the area covered by this species in the region, estimated at about 45000 ha, is relatively low, the trees are a potentially important source of wood products (plywood), non-wood products (fuelwood) and services (shelter, shade and protection of soil, water and livestock). The environmental and economic applications of poplar plantations are therefore driving factors for sustainable forestry and rural development (Rueda et al. 1997, Ball et al. 2005).

Plantations of *Populus*×*euramericana* (Dode) Guinier (*P. deltoides* Marsh. ♀ × *P. nigra* L. ♂) are monoclonal; although several clones are used, clone I-214 is the most commonly planted in Spain, and covers about 70 % of the total area covered by poplar stands (Fernández and Hernanz 2004). Plantations are managed on short rotations (12–16 years), and intensive tillage practices are usually applied (Fernández 1998). Mechanical tillage, logging residue management, pruning and weed control are widely used techniques. The density of poplar plantations is maintained constant during the whole rotation, at about 278–400 stems/ha, depending on the planting distance, 6×6 or 5×5 meters, respectively. This species has a deep rooting system and requires large amounts of water; striplings are thus placed in direct contact with the water table, which is usually at a depth of between 1 and 2.5 meters (De Mier 2001, Fernández and Hernanz 2004).

However, despite the intensive management required, the profitability of poplar plantations varies greatly, as with other types of forest (Ke and Skelly 1994, Ouimet and Camiré 1995, Hallet et al. 2006), and depends, amongst other factors, on the health status of the stand (Camps 2001, Sierra 2001). The importance of forest health has been recognised in recent decades. A forest health monitoring programme has been carried out in Europe since the 1980s within the International Co-operative Programme, ICP Forest (Level I European network). More recently, sustainable forest management programmes have focused huge efforts on assessing forest health. Such programmes have assessed forest health by monitoring crown condition (crown transparency and discoloration), as well as fungal and insect pests. However, other important biotic agents, such as endophytes, have not yet been studied, although many authors have recognised the importance of endophytic communities in forest health (Bettucci and Alonso 1997, Bettucci et al. 1999, Gennaro et al. 2003, Ragazzi et al. 2003, Santamaría and Diez 2005, Zamora et al. 2008, Botella et al. 2010).

Many definitions of endophytes have been reported (Hyde and Soytong 2008); some researchers define endophytes as those fungi that are able to infect their hosts without causing visible disease symptoms (Petrini 1991, Wilson 1995, Schulz and Boyle 2005) and other authors established the term endophyte as synonymous with mutualism (Saikkonen et al. 2004, Backman and Sikora 2008). However, the distinction between pathogenic and endophytic organisms is not clear, and the same fungus or even the same isolate may behave as a saprophyte or pathogen according to the host vigour (Schulz et al. 1999). In the present study we considered those fungi isolated from surface-sterilized samples as endophytes.

Age and environmental conditions have important effects on endophytic communities (Petrini and Carroll 1981, Legault et al. 1989, Carroll 1994, Helander et al. 2006, Kauhanen et al. 2006). More recently, Botella et al. (2010) have demonstrated that several abiotic factors, including water availability, shade, light exposure, age, elevation and mean temperature, appear to influence endophytic communities and forest health in Allepo pine in Spain. However, so far no research has been carried out to determine the effect of these variables on endophytes of poplars. In addition, there is an obvious lack of research designed to clarify the effect of site quality and host nutrient status on endophytic communities.

Taking into account the great importance of endophytic communities and the lack of research on endophytic fungi in *Populus*×*euramericana*, the main goals of this study were: 1) to analyse whether factors such as age and site quality affect endophytic communities at stand level, and 2) to study whether soil nutrient status, dendrometric variables and crown condition could explain endophytic communities at tree level.

2 Materials and Methods

2.1 Site Description and Sampling Procedure

The present study was carried out in Castilla y León (NW Spain). The altitude of the study area ranges between 800 and 900 m. above sea level and in most stands the topography is almost flat. The average annual precipitation varies between 496 and 630 mm and the average annual temperature, between 9 and 11.4 °C (Ninyerola et al. 2005).

The experimental design consisted of a factorial scheme with two factors, stand age (young: 3–7 years old stands, or adult: 8–14 years old stands) and site quality. Stands were assigned a site quality, with rich sites (quality I and II) and poor quality sites (quality III and IV) differentiated on the basis of the site quality curves developed for *Populus*×*euramericana* clone I-214 in the river Duero basin (Bravo et al. 1995). These site indexes are related to a basal area (at the breast height of all trees planted in 1 ha) for stand age up to ten years. The specific values of the site indexes are 20.21, 16.77, 13.31 and 9.87 $m^2\ ha^{-1}$ for site qualities I, II, III and IV, respectively. Three I-214 clonal plantations were sampled, and two trees were chosen within each plantation for each combination of factors. A total of 12 poplar stands and 24 trees were finally selected for study.

The health status of each tree was evaluated during the summer (first two weeks of July) of 2005, on the basis of crown condition (crown transparency and crown discoloration). To avoid possible sources of error due to the subjectivity of human assessment of factors including weather conditions, crown appearance, tree species, tree age and social status (Innes et al. 1993, Ghosh et al. 1995, Solberg and Strand 1999, Wulff 2002, Redfern and Boswell 2004), crown transparency was determined by a more accurate variable, designated Digital Crown Transparency (DCT). This variable is estimated by means of digital photographs obtained by use of a semiautomatic image analysis system, known as CROCO (Mizoue 2002). An automatic thresholding algorithm is used in CROCO to obtain crown silhouette images, where foliage and branches are transformed to black pixels and background sky to white pixels (Mizoue and Inoue 2001). CROCO calculates two fractal dimensions to estimate the crown transparency of the tree silhouette (Ds) and outline (Do). The index of crown transparency, obtained by the CROCO method (DSO), was calculated as the difference between Ds and Do (Mizoue and Dobbertin 2003). DSO was subsequently converted into DCT by means of a calibration equation previously developed for *Populus*×*euramericana* (Martín-García et al. 2009).

Crown discoloration (VCD) was estimated visually and quantified by considering twenty 5%-interval classes, according to Level I of the European network methodology (Eichhorn et al. 2006). Before sampling, the operator took part in an intercalibration session with the Spanish field crew of the European Level I network. Parts of the crown directly influenced by crown interactions or competition were excluded; trees were assessed from a distance of about one tree length, with the observer taking care to avoid looking into the sun (Eichhorn et al. 2006). Biotic damage in the crown was also recorded but there were so few instances of such damage that it was not taken into further consideration.

Foliar sampling was carried out during the first two weeks of September 2005, the period when foliar nutrients are most stable in poplar trees (Bengoa and Rueda 2001). Between 12 and 15 green leaves were removed per tree, from two main branches of the upper third of the canopy (north and south sectors). The samples were transported to the laboratory, stored at 4 °C and processed within 24 hours. The oven-dried (60 °C) samples of leaves were milled (0.25 mm) and digested with HNO_3 in a microwave oven. Total C and N in milled foliar samples were analysed by combustion, with a Leco analyzer (LECO, St Joseph, Michigan, USA). The total concentrations of P, K, Ca, Mg, Fe, Mn, Zn, Cu, B, Ni S, Al, Cr, As, Mo, Cd, Co, Na and Pb in the digested foliar samples were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) (Perkin Elmer, Wellesley, MA, USA).

Finally, diameter at breast height (DBH), total height (TH), pruned height (PH), crown diameter (CD) and crown volume (VOL) were also measured in all trees during autumn in 2005.

2.2 Fungal Isolation and Identification

Leaves and twigs from branches collected for foliar analyses were used for fungal isolation. Surface sterilization of the leaves and twigs was performed by a modified version of the procedure of Kaneko and Kaneko (2004). Samples (both leaves and twigs) were dipped in ethanol (70% v/v) for 60 s, then in sodium hypochlorite solution (2% v/v) for 2 min (leaves) or 3 min (twigs), in ethanol (70% v/v) for 30 s (leaves) or 60 s (twigs), and then washed three times in sterile distilled water.

Twelve pieces of leaves (0.5 × 0.5 cm) and twelve twig segments (0.5 cm diam., 0.5–1 cm thick) from each tree were placed in Petri dishes containing "potato dextrose agar" (PDA) medium. The plates were sealed with Parafilm® and incubated in the dark at 20 °C for one month. The outgrowing fungi were transferred to fresh PDA and grown in pure culture until sporulation. Fungal isolates were identified according to morphological characteristics, using a stereomicroscope and analysing the shape and colour of the colonies, and the main characteristics of fungal structures. Different taxonomic keys were used to identify the fungi (Lanier et al. 1978, Von Arx 1981, McGinnis et al. 1982, Barnet and Hunter 1987, Goidanich 1990, Watanabe 1994, Kiffer and Morelet 1997).

2.3 Statistical Analyses

Univariate statistics The effect of factors (age and site quality) and tissue sampled (leaves or twigs) on species richness of endophytic fungi and on the relative isolation frequencies (RIF) was evaluated by a Mixed Analysis of Variance Model. This model was carried out with three fixed factors in a complete 2^3 factorial design and using different error variances for each of the eight treatments in the model. The RIF were calculated as $RIF = n_{ijk}/N_{ijk}$, where n_{ijk} is the number of isolates recorded for site quality i, age j and tissue k, and where N_{ijk} is the number of samples examined for site quality i, age j and tissue k (Santamaría and Diez 2005). Two linear mixed models (PROC MIXED) were therefore applied by use of SAS (version 9.1) software.

Multivariate statistics Two types of analyses were carried out. Firstly, correspondence Analyses (CA) were carried out at stand level, for the composition of fungal species isolated from leaves only, twigs only and leaves plus twigs, and with 'isolated fungal species composition' as the response variable, in order to assess the influence of both factors, age and site quality, on fungal occurrence. The response variable was transformed by means of log (x + 1) to comply with normality assumptions. Although fungi isolated from only one stand were excluded from these analyses, the downweighting option was also used to reduce the importance of rare species. For presentation in figures, plots were labelled by age and site quality (young/adult and rich/poor respectively).

The second analysis – Canonical Correspondence Analysis (CCA) – was carried out at tree level, to study the influence of the main explanatory variables (nutrient status, dendrometric variables and crown conditions) on the occurrence of fungi. A forward selection procedure with the Monte Carlo test was then applied to determine the significance of the results, with 499 permutations for exploratory analysis and 999 for the final results (Legendre and Legendre 1998). The constrained ordinations were performed with CANOCO software for Windows, version 4.5 (Ter Braak and Smiluaer 2002).

3 Results

The values of the dendrometric variables (diameter and height) were considerably higher in rich sites than in poor sites. The opposite was true for crown conditions, since DCT and VCD were higher in poor quality sites than in high quality sites, as expected (Table 1).

The fungal species (recovered from at least two poplar plantations) used in multivariate statistical analyses, as well as their relative isolation frequency (RIF), are shown in Table 2. A total of 43 species or morphological types were isolated from 576 plant fragments (288 plant fragments for each tissue), of which the most frequent were *Ulocladium* spp. and *Cladosporium herbarum* (Pers.) Link. ex S.F.Gray. On the other hand, *Glonium*

Table 1. Site characteristics associated with the stands.

Site quality	Age	Coordinate UTM	Diameter (cm)	Height (m)	DCT (%)	VCD (%)	N (mg g^{-1})	P (mg g^{-1})	Ca (mg g^{-1})	Mg (mg g^{-1})	K (mg g^{-1})	Mn (µg g^{-1})	Fe (µg g^{-1})	B (µg g^{-1})	Zn (µg g^{-1})	Cu (µg g^{-1})	N/P
Rich	Young	357.945–4706.111	16.74	15.08	10.47	0.41	31.60	4.30	30.03	3.20	12.11	379.7	163.0	74.9	68.2	7.1	7.3
Rich	Young	364.479–4694.555	17.09	17.16	10.77	5.77	20.80	4.72	22.64	2.98	16.52	42.0	74.5	98.3	152.7	8.4	4.4
Rich	Young	353.909–4711.072	16.73	13.13	8.75	0	36.20	5.51	20.04	2.29	16.98	230.7	117.9	137.3	86.3	11.7	6.6
Rich	Adult	359.952–4701.961	32.20	24.15	15.97	5	20.70	2.58	46.54	4.13	7.78	376.1	155.6	56.7	101.1	8.0	8.0
Rich	Adult	365.346–4693.152	24.20	23.03	12.65	0	15.90	2.76	23.51	2.34	22.86	114.1	177.7	48.5	110.5	4.7	5.8
Rich	Adult	361.242–4699.292	27.54	22.97	12.09	0	19.20	2.08	39.00	3.33	8.87	67.8	110.4	48.3	28.3	6.5	9.2
Poor	Young	356.735–4706.840	11.04	11.03	9.32	0	16.60	2.50	20.34	2.55	8.50	48.5	75.3	14.5	82.4	4.5	6.6
Poor	Young	353.216–4706.840	13.04	10.61	31.93	20.5	28.40	3.48	27.01	3.47	8.44	354.2	62.6	42.3	89.5	6.2	8.2
Poor	Young	366.495–4692.036	9.71	10.34	13.20	0.13	17.50	1.82	15.23	3.14	3.82	41.3	59.1	10.0	70.2	3.6	9.6
Poor	Adult	355.360–4697.636	12.68	10.57	50.70	19.76	22.50	2.86	45.29	4.38	4.20	576.1	62.5	20.8	78.4	5.1	7.9
Poor	Adult	346.405–4696.185	19.59	16.39	44.62	19.02	17.60	1.89	42.21	4.92	3.04	589.0	52.6	29.5	32.9	4.9	9.3
Poor	Adult	359.336–4691.912	16.67	15.33	36.56	17.54	18.30	2.25	23.95	5.34	5.93	263.2	50.7	29.2	51.3	3.7	8.1

DCT: Digital crown transparency. VCD: Visual crown discoloration

spp., *Pestalotia* spp., *Trichotecium roseum* (Persoon) Link. Es S.F.Gray, and several unidentified Deuteromycetes and sterile mycelia occurred at lower frequencies.

The mixed linear model showed that fungal species richness did not differ significantly between site qualities or between tissues (Table 3), but did differ between ages (richness was higher in young stands than in adult stands). Moreover, the differences in richness between ages depend on the site quality, with the lowest richness values observed in old stands located on poor sites (Age × Site quality: $p = 0.03$; Fig. 1). The same pattern was found for RIF values (Fig. 1).

Correspondence Analysis (CA) performed on the relative frequencies of fungi isolated from leaves only, twigs only or leaves plus twigs revealed similar results, although the grouping of the stands according to age and site quality was clearer for leaves plus twigs. For this reason, in addition to the non significant differences found in the mixed lineal model for the variable 'tissue' (Table 3), the individual CA for leaves and twigs are not shown.

Correspondence Analysis revealed that the principal coordinate axes 1–2, which explained about 42% of the total inertia, separated two distinct clusters of stands according to site quality. Thus, rich stands corresponded to low and high values on axes 1 and 2 respectively, unlike poor quality sites (Fig. 2a). Such groupings associated with site quality are characterised by a clear gradient in the distribution of fungal species; from species exclusively (*Mste16*) or mainly (*Ccla, Mste18*) isolated from poor quality stands, to those exclusively (*Prsp*) or mainly (*Mste1, Mste6, Mste7*) isolated from rich sites (Table 2, Fig. 2a). Two distinct clusters were identified when plots were considered by age (Fig. 2b), although the grouping was not as clear as that observed for site quality. A weak gradient in the distribution of fungal species was also observed according to age; from species mainly isolated from adult stands (*Cher, Deu2, Mste3, Mste13*), to those exclusively (*Hacr, Mste17*) or mainly isolated from young stands (*Apull, Tvir, Mste2*) (Table 2, Fig. 2b). For other CA plots, such as with the first and third or second and third axes, no groupings were observed for the factors studied.

Five variables were retained in the CCA, three

Table 2. Distribution and isolation frequencies for fungi isolated from at least two poplar plantations.The isolation frequencies for each species are the percentages with respect to the total number of fragments collected in each sample tissue (leaves and twigs) and for each treatment (combinations of age (Y: young and A: adult) and site quality (R: rich and P: poor)). The column labelled "Total" refers to the percentages of isolates for each species with respect to the total number of the fragments cultured throughout the sampling.

Fungi	Code	Leaves				Twigs				Total
		YR	AR	YP	AP	YR	AR	YP	AP	
Alternaria alternata complex. Ness ex Fr.	*Acom*	4.2	-	12.5	4.2	11.1	2.8	5.6	2.8	5.4
Aspergillus niger van Tieghem	*Anig*	1.4	-	-	1.4	4.2	-	-	1.4	1.0
Aureobasidium pullulans Viala & Boyer	*Apull*	1.4	-	2.8	1.4	-	-	-	-	0.7
Chaetomium spp.	*Chsp*	-	-	-	-	1.4	1.4	-	-	0.3
Cladosporium cladosporioides Link. ex Fr.	*Ccla*	4.2	-	5.6	2.8	-	1.4	19.4	-	4.2
Cladosporium herbarum (Pers.) Link. ex S.F.Gray	*Cher*	11.1	16.7	16.7	20.8	6.9	-	4.2	11.1	10.9
Epicoccum nigrum Link.	*Enig*	5.6	2.8	2.8	1.4	12.5	4.2	1.4	6.9	4.7
Harcia acremonoides (Harz) Cost.	*Hacr*	4.2	-	2.8	-	4.2	-	-	-	1.4
Penicillium spp.	*Pssp.*	-	2.8	2.8	2.8	1.4	-	1.4	4.2	1.9
Preussia spp.	*Prsp*	1.4	-	-	-	-	1.4	-	-	0.3
Trichoderma viride Pers. Es S.F.Gray	*Tvir*	-	-	1.4	-	6.9	1.4	1.4	2.8	1.7
Ulocladium spp.	*Ussp*	18.1	12.5	12.5	9.7	30.6	26.4	16.7	6.9	16.7
Deuteromicete 1	Deu 1	4.2	1.4	22.2	1.4	4.2	5.6	22.2	8.3	8.7
Deuteromicete 2	Deu 2	-	1.4	1.4	1.4	-	12.5	2.8	6.9	3.3
Sterile mycelium 1	Mste1	11.1	19.4	1.4	-	2.8	9.7	4.2	5.6	6.8
Sterile mycelium 2	Mste2	-	1.4	4.2	1.4	4.2	1.4	2.8	2.8	2.3
Sterile mycelium 3	Mste3	4.2	15.3	6.9	5.6	2.8	1.4	9.7	4.2	6.3
Sterile mycelium 4	Mste4	4.2	6.9	-	4.2	1.4	4.2	1.4	1.4	3.0
Sterile mycelium 5	Mste5	-	1.4	2.8	-	-	-	2.8	-	0.9
Sterile mycelium 6	Mste6	-	4.2	-	-	-	-	1.4	-	0.7
Sterile mycelium 7	Mste7	1.4	1.4	1.4	-	1.4	1.4	-	-	0.9
Sterile mycelium 8	Mste8	4.2	1.4	8.3	1.4	5.6	8.3	-	1.4	3.8
Sterile mycelium 9	Mste9	-	-	1.4	-	-	1.4	-	-	0.3
Sterile mycelium 10	Mste10	-	-	1.4	1.4	-	1.4	1.4	-	0.7
Sterile mycelium 11	Mste11	6.9	6.9	1.4	-	6.9	1.4	1.4	4.2	3.6
Sterile mycelium 12	Mste12	-	2.8	2.8	-	2.8	1.4	-	-	1.2
Sterile mycelium 13	Mste13	2.8	4.2	2.8	4.2	5.6	5.6	6.9	2.8	4.3
Sterile mycelium 14	Mste14	1.4	-	-	-	4.2	1.4	1.4	-	1.0
Sterile mycelium 15	Mste15	1.4	2.8	1.4	-	1.4	2.8	-	-	1.2
Sterile mycelium 16	Mste16	-	-	5.6	2.8	-	-	1.4	-	1.2
Sterile mycelium 17	Mste17	-	-	1.4	-	2.8	-	-	-	0.5
Sterile mycelium 18	Mste18	1.4	-	1.4	-	1.4	-	1.4	-	0.7

associated with the nutrient status of the trees (concentrations of iron and zinc, and relation between concentrations of nitrogen and phosphorus), a dendrometric variable (Total height) and another crown condition variable (Visual Crown Discoloration) (Fig. 3). The eigenvalues (λ) for axes 1 and 2 were 0.208 and 0.123, respectively, and the model was significant according to the results of the Monte Carlo test ($F = 1.452$, $p = 0.008$, 499 permutations).

The first axis was positively correlated with VCD and concentration of Zn, and negatively with TH and concentration of Fe. Examination of the CCA plot shows several species associated with high VCD or low concentration of Fe and total height values (*Anig*, *Apull, Pssp, Mste16, Mste18*) and vice versa (*Prsp, Mste1, Mste6*). The second axis was positively and negatively correlated with the N/P ratio and concentration of Zn, respectively. The CCA plot revealed that

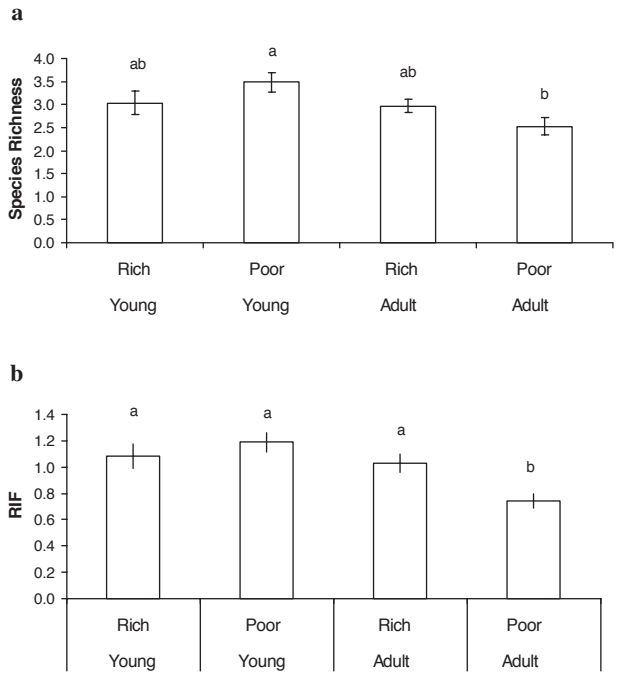

Fig. 1. Mean (± S.E.) (a) Species richness and (b) Relative isolation frequencies (RIF) values per poplar stand for each site quality according to stand age. Different letters above the bars indicate significantly different means (Two-tailed t-test with $\alpha = 0.05$).

Table 3. Linear mixed models (PROC MIXED) for mean values of Species richness and Relative isolation frequencies (RIF) per poplar stand (N = 12), used to evaluate the effect of stand age, site quality and tissue.

N = 12		Species richness			RIF	
Source	df1	df2	F	Pr > F	F	Pr > F
Age	1	134	6.17	0.01	11.67	<0.01
Site quality	1	134	0.00	0.99	1.51	0.22
Tissue	1	134	0.43	0.51	0.14	0.71
Age × Site quality	1	134	4.70	0.03	7.14	<0.01
Age × Tissue	1	134	0.15	0.70	0.22	0.64
Site quality × Tissue	1	134	1.01	0.32	0.89	0.35
Age × Site quality × Tissue	1	134	1.01	0.32	2.59	0.11

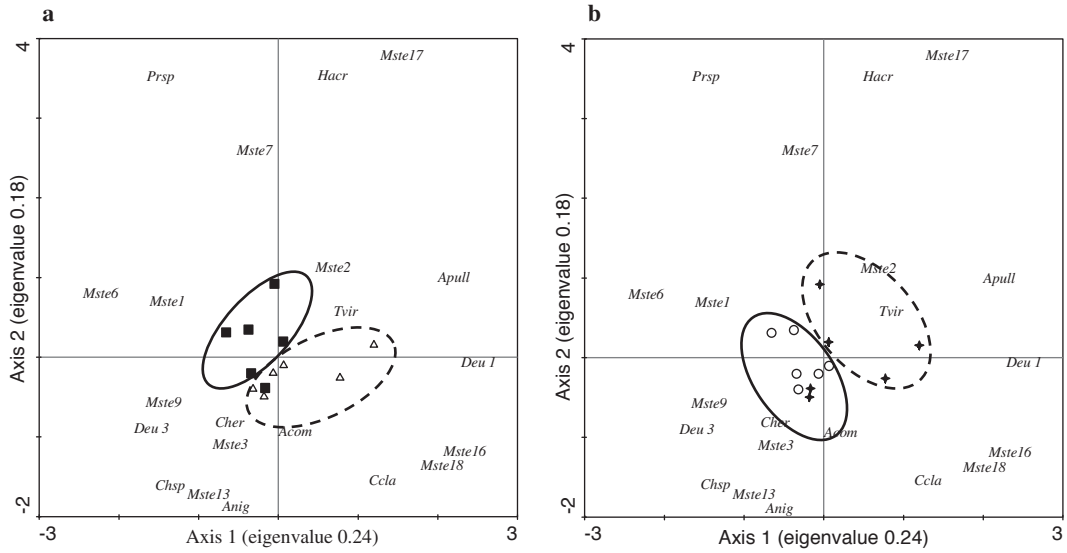

Fig. 2. Correspondence Analysis ordination of the 12 inventoried plots labelled by (a) site quality and (b) age (axes 1 and 2). Plot types: Rich sites (black squares), Poor quality sites (white triangles), Young stands (black stars) and Adult stands (white circles).

several species were associated with high N/P or low concentrations of Zn (*Cher, Hacr, Tvir, Mste15, Mste17*) or vice versa (*Mste5, Mste10, Mste9, Mste14*) (Fig. 3).

4 Discussion

The number of taxa recorded in the present study was similar to the numbers reported in previous surveys on fungal communities associated with other tree hosts under a temperate climate, such as *Populus tremula* (Santamaría and Diez 2005), *Betula pendula* (Green 2004), *Eucalyptus globulus* and *E. grandis* (Bettucci et al. 1999) or several species of pine and oak (Martín-Pinto et al. 2004, Zamora et al. 2008, Botella et al. 2010).

The most abundant species (RIF > 3%) observed in the present study are ubiquitous taxa, such as *A. alternata complex*, *C. cladosporoides*, *E. nigrum* and *Ulocladium* sp. The same pattern was also found for *Populus tremula* (Santamaría and Diez 2005), *Salix fragilis* (Petrini and Fisher 1990), *Eucalyptus grandis* (Bettucci and Alonso 1997),

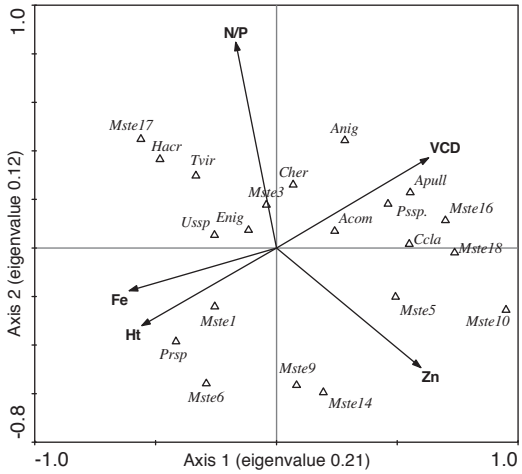

Fig. 3. Canonical Correspondence Analysis ordination biplot (axes 1 and 2), with nutrient status, dendrometric and forest health variables represented by *arrows* and fungal species by triangles. For explanation of abbreviations used for the fungal species, see Table 1.

pine plantations (Zamora et al. 2008) and pine and oak seedlings (Martín-Pinto et al. 2004).

The present findings show that young trees may acquire higher richness and frequency of fungal species than mature trees (especially those in poor sites), contrary to the findings of Kauhanen et al. (2006). There are several possible explanations for this. On one hand, the pruned height of young trees is lower, so that the first branch will be closer to the reservoir of inocula present in the previous year's litter, and therefore fungi may spread relatively rapidly towards the upper third of the canopy. However, multivariate analysis (CCA) showed that pruned height does not affect the endophytic community. On the other hand, several authors (Petrini and Carroll 1981, Helander et al. 1994, Müller and Hallaksela 1998, Collado et al. 1999) indicate that stand density and canopy cover are key factors related to relative humidity, and that these factors may therefore affect the frequency of endophytes in trees. Nevertheless, this does not appear to explain the findings as variables related to canopy cover, such as crown volume and crown transparency, did not have significant effects on endophytic communities, according to the results of the multivariate analysis (CCA). Another hypothesis is that pioneer fungi would quickly colonise young trees and then be replaced over time by more competitive species, as reported by Minter and Millar (1980), who found that *Lophodermium pinastri* replaced other fungi. This appears even more likely when it is taken into account that poplar plantations are subjected to clear cutting, which may eliminate the transmission of inocula of endophytic fungi, as noted by Kriel et al. (2000).

Although several authors have pointed out the importance of edaphoclimatic variables in the development of endophytic communities (Carroll 1994, Sieber et al. 1999, Botella et al. 2010), to our knowledge no specific research has been carried out to study the effect of site quality on fungal assemblages. Korkama et al. (2006) demonstrated that growth rate and size of the host affect the diversity and community structure of ectomycorrhizal species. However, these authors compared eight Norway spruce clones, and therefore could not differentiate between effect of the clone and site quality.

Separation of stands of different site quality according to the associated fungal assemblages has been demonstrated in the present study at clone level (removing the genetic effect of tree host). This may be due to a stress factor caused by nutrient or water deficits in poor quality sites, which appears to be supported by the results of multivariate analysis (CCA), since discoloration, total height and the concentrations of several nutrients were shown to be key variables affecting endophytic communities. It is possible that some endophyte species, such as *Periconiella* spp. (Collado et al. 1999) and *Cytospora* spp. (Bettucci and Alonso 1997, Callan 1998), require trees to be exposed to stress conditions before colonisation.

Although for culturable and sporulating mycelia, identification based on morphology may be of interest, because of the limited number of sequences reported (Kauhanen et al. 2006), the large number of sterile mycelia observed in the present study indicates that sequence-based identification would be advisable in future investigations involving identification of fungal endophytes in poplar plantations. However, taking into account that many fungi (possibly hundreds of thousands) have not yet been classified (Hawksworth and Rossman 1987, Sieber 2007), it would not be surprising if some new species were isolated from *P.* × *euramericana* in the present study.

In conclusion, the present results indicate that several endophytes colonise poplar plantations and that factors such as cutting cycle, selection of land according to site quality or possible fertilization regimes will affect endophytic fungi. These outcomes may be of great interest, not only because of the importance of endophytes as a source of ecological diversity, but also because of their enormous potential as indicators of forest health, owing to their role in acting against forest pests and diseases.

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