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ORIGINAL PAPER

# Relationship between genotype and soil environment during colonization of poplar roots by mycorrhizal and endophytic fungi

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**Abstract** Poplars are among the few tree genera that can develop both ectomycorrhizal (ECM) and arbuscular (AM) associations; however, variable ratios of ECM/AM in dual mycorrhizal colonizations were observed in the roots of a variety of poplar species and hybrids. The objective of our study was to analyze the effect of internal and external factors on growth and dual AM and ECM colonization of poplar roots in three 12–15-year-old common gardens in Poland. We also analyzed the abundance of nonmycorrhizal fungal endophytes in the poplar roots. The *Populus* clones comprised black poplars (*Populus deltoides* and *P. deltoides* × *Populus nigra*), balsam poplars (*Populus maximowiczii* × *Populus trichocarpa*), and a hybrid of black and balsam poplars (*P. deltoides* × *P. trichocarpa*). Of the three sites that we studied, one was located in the vicinity of a copper smelter, where soil was contaminated with copper and lead. Poplar root tip abundance, mycorrhizal colonization, and soil fungi biomass were lower at this heavily polluted site. The total mycorrhizal colonization and the ratio of ECM and AM colonization differed among the study sites and according to soil depth. The influence of *Populus* genotype was significantly pronounced only within the individual study sites. The contribution of nonmycorrhizal fungal endophytes differed among the poplar clones and was higher at the polluted site than at the sites free of pollution. Our results indicate that poplar fine root abundance and AM and ECM symbiosis are influenced by environmental conditions. Further studies of different site conditions are required to characterize the utility of poplars for purposes such as the phytoremediation of polluted sites.

**Keywords** Populus · Ectomycorrhiza · Arbuscular mycorrhiza · Heavy metals · Fine-root colonization · Depth distribution

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## Introduction

The genus *Populus* (Salicaceae) comprises approximately 40 species widely distributed over the northern hemisphere and present in diverse habitats (Bugała 1973). *Populus* species and natural interspecific hybrids have been important elements of the agricultural landscape for several centuries. Recently, there has been increasing demand for fast growing, stress-tolerant trees, including poplars, that can be used for afforestation of postagricultural lands, recultivation of areas degraded by industry, and production of bioenergy (Sebastiani et al. 2004; Yin et al. 2005; Monclus et al. 2006). To date, poplar research has focused mostly upon the above-ground parts of these trees and their responses to environmental conditions and human management. Less is known about poplar roots and the mycorrhizal associations that play a crucial role in plant nutrition and tolerance to various abiotic and biotic stress factors (Lodge 1989; Gehring et al. 2006).

Poplars are among the few tree genera that can develop both ectomycorrhizal (ECM) and arbuscular (AM) associations; however, variable ratios of ECM/AM in dual mycorrhizal colonizations were observed in the roots of a variety of poplar species and hybrids (Vozzo and Hacskalyo 1974; Lodge 1989; Brundrett et al. 1990; Neville et al. 2002; Khasa et al. 2002; Welc 2004; Gehring et al. 2006).

Different factors have been suggested to influence ECM/AM colonization, such as tree age (Dominik 1958; Gardner and Malajczuk 1988; Paul and Clark 1996; van der Heijden et al. 1999; Chen et al. 2000; Gonçalves and Martins-Loução 1996), fungal inoculum potential (van der Heijden and Vosatka 1999), litter accumulation (Conn and Dighton 2000), N and P fertilization (Baum and Makeschin 2000), and soil moisture (Truszkowska 1953; Lodge 1989; Neville et al. 2002; Gehring et al. 2006). Reports of the influence of soil depth on the ratio of ECM/AM colonization of poplar roots have been inconsistent. Lodge (1989) and Neville et al. (2002) found a negative correlation between the degree of ECM and AM colonization of poplar roots and suggested that there was preferential partitioning of ECM and AM fungi at different soil layers. In contrast, Moyersonen et al. (1998) excluded a clear tendency for horizontal and vertical distribution of ECM and AM and implied a co-occurrence of ECM and AM in the same niche. The influence of host plant genotype on ECM formation was reported by Tagu et al. (2001, 2005), and Khasa et al. (2002) and Gehring et al. (2006) found variable ECM/AM colonization ratios in different poplar species and hybrids. The latter authors, however, suggested that the effect of poplar genotype was minor compared to the influence of environmental factors. Comparatively little information concerning the colonization of tree roots by nonmycorrhizal fungal endophytes (FE) is available in the literature. Neville et al. (2002) revealed that nonmycorrhizal FE colonization correlated positively with AM colonization and negatively with ECM colonization of *Populus tremuloides* roots.

*Populus* species and clones vary in their tolerance to increased concentrations of trace metals in soil and in their ability to uptake and contain metals (Rachwał et al. 1992; Djingova et al. 1999; Sebastiani et al. 2004; Laureysens et al. 2005; Borghi et al. 2008; Krpata et al. 2008; Lingua et al. 2008). As mycorrhizal symbiosis is known to improve plant growth and increase plant tolerance to environmental stresses (Meharg and Cairney 2000), an understanding of the importance of genetic and environmental factors in mycorrhizal development and functioning may provide valuable insight into the selection of poplar cultivars suitable for afforestation of degraded or abandoned lands.

To our knowledge, this is the first study to simultaneously take into account several factors—four host genotypes, three environments (sites), and three rooting depths—that may play roles in determining fine root abundance and colonization by mycorrhizal (ECM versus AM) and nonmycorrhizal endophytic fungi. Replicated common gardens, in which clones of *Populus* species and hybrids representing the same parent material of similar ages were growing in adjacent blocks and managed similarly, provided a unique opportunity to investigate environmental factors. Each of the three study sites differed with regard to environmental characteristics, and one of the sites was under strong anthropogenic pressure. Our study objective was to investigate the importance of internal and external factors on the development of poplar fine roots and root–fungi associations.

## Materials and methods

### Plant material and study sites

We studied four poplar types—*Populus deltoides* (clone S-1-8 “DUNAV”), *P. deltoides* × *Populus nigra* (clone 490-1), *P. deltoides* × *Populus trichocarpa* (clone “DONK”), and *Populus maximowiczii* × *P. trichocarpa* (clone NE-42)—in three common-garden experiments established in 1993 (sites 1 and 3) and 1996 (site 2) based on plant material derived from the populetum of the Institute of Dendrology in Kórnik, Poland.

Selected four poplar genotypes are well-known clones, cultivated in Europe and USA for many years, which represent black, balsamic poplars, and its hybrid—*P. deltoides* × *P. trichocarpa*. At the past, these clones were used in different field experiments located in vicinity of industrial plants, emitters of air pollutants harmful to the environment. Since 1993, the average growth of poplars (expressed as diameter at breast height (DBH)) was monitored. According to these data, all clones grown in polluted area revealed decrease of stem diameter and differentiated tolerance to environmental pollution. Our study is the first evaluation of mycorrhizal status of these clones.

All of the study sites were on postagricultural lands consisting of Cambisol soils (Food and Agriculture Organization), loamy sand in site 1, and sandy loam in sites 2 and 3. Sites 1 and 2 were situated in the Kórnik region (52°15′ N, 17°04′ E) in an area free from the direct influence of industry. Site 1 was located at the edge of a coniferous forest complex in the experimental forest of the Institute of Dendrology (forest compartment 1Ac). In late autumn and winter, this area is partially flooded because of high levels of groundwater. Site 2 was situated in a relatively dry habitat in the vicinity of mature poplar trees on one side and a *Larix* collection on the other side. Site 3 was located in the protective zone of the Głogów copper smelter in Żukowice (51°40′ N, 16°05′ E; compartment 12b) near the Odra River. This site is occasionally flooded. The smelter has been active since 1971. In 1980, it emitted 14,442 tons of dusts and 125,700 tons of $SO_2$ per year. The pollution was gradually reduced to 2,017 tons of dust and 34,100 tons of $SO_2$ in 1990 and then 179 tons of dust and 4,000 tons of $SO_2$ in 2005 (http://www.kghm.pl/). Two years after the poplar common-garden experiment

was established, a layer of unpolluted soil approximately 10 cm thick was spread on the soil surface to support the young poplars. Each of the three common-garden experiments was established according to the same method. Each poplar clone was represented by three plots (four trees per plot) in each common-garden site; plot locations were randomly assigned within each of the three common gardens.

### Sample collection

In November 2007, root samples were collected from each plot using a soil corer (diameter 5 cm, length 30 cm). Soil samples (200 $cm^3$) were taken between individual poplar clone trees at a distance of approximately 1.5 m from the stem base. Three samples were collected from each plot, a total of nine samples for each clone per site. Each soil core was divided into three layers: 0–10, 10–20, and 20–30 cm. In total, 324 samples were analyzed (9 samples × 4 clones × 3 sites × 3 rooting depths). Samples were kept in plastic bags at −10°C until they were analyzed.

### Chemical analyses of soil

The soil pH was determined using soil suspension in water and 0.5 M potassium chloride. Prior to chemical analyses, soil samples (2.5 g dry weight each) were digested in a mixture of spectrally pure concentrated acids—nitric acid and perchloric acid at a ratio of 4:1 (*v*/*v*), diluted with bidistilled water to yield 25 ml. The carbon and nitrogen content in soil was measured using the Elemental Combustion System 4010. Concentrations of copper, lead, zinc, and cadmium were measured by atomic absorption spectroscopy (Varian 220 FS) after being atomized in air with an acetylene flame. The accuracy of the analyses was checked against standard reference materials: standard Chinese soils NCS DC 73322, NSC DC 73322, DX STII#57590, and DX #46070.

### Soil fungi biomass

The biomass of soil fungi was estimated by measuring the ergosterol content. Soil samples were sieved (2 mm diameter openings), lyophilized, homogenized by ball-mill (Retsch 200), and stored at −20°C until they were analyzed. The extraction (1 g dry weight) was performed using the microwave-assisted extraction method described by Montgomery et al. (2000). Ergosterol extracts dissolved in methanol were separated and quantified by a high-performance liquid chromatography (HPLC) device (Waters) fitted with a Nova-Pak C18 3.9×150 mm (WAT 086344) stainless steel and an absorbance detector (Dual Wavelength Absorbance Detector Waters 2487). HPLC was monitored with Waters Millennium32 software. Ergosterol was eluted for 10 min with 100% methanol at a flow rate of 1 ml/min and detected at 280 nm wavelength. The ergosterol peak was identified by comparing retention time of sample with the external standard synthetic 5,7,22-ergostatrien-3β-ol (provitamin D2, Sigma) and by coinjection of sample and standard. Ergosterol content was determined by comparing the sample area peak with a model curve based on a number of external standard solutions. For calculations of fungal biomass, we used the conversion factor of 250 μg of dry fungi biomass per 1 μg of ergosterol (Montgomery et al. 2000).

### Root characteristics

Fine root tips were counted under a stereomicroscope. The lengths of fine roots were estimated using a scanner equipped with WinRhizo 5.0 software. Afterward, the root samples were stored frozen at −10°C until mycorrhizal colonization analysis.

### Fungal colonization of roots

Root samples were cut into 1 cm lengths, and 0.250-g subsamples were collected for staining. Roots were cleared in 10% potassium hydroxide for 2.5 h at 95°C, then for 1 h in alkaline hydrogen peroxide at room temperature, and stained with Trypan blue in lactoglycerol, according to a modified method of Kormanik and McGraw (1982). Root colonization by AM, ECM, and nonmycorrhizal FE was evaluated using the intersection method (McGonigle et al. 1990) at ×200 magnification; a minimum of 100 line intersections per subsample of approximately 7 cm was scored for the presence of AM fungal structures (hyphae, vesicles, arbuscules, and spores), ECM, and mycorrhizal FE. Approximately 200 cm of fine roots in total was analyzed for each sample. The results are presented as a percentage of root length colonized (%RLC).

### Statistical analysis

Three samples collected from one plot were treated as a block. There was no plot effect for each clone in each site (data not shown). Results were analyzed for the overall effect at all three sites (sites 1, 2, and 3), as well as for each site separately. Three- and two-way analysis of variance (ANOVA) was used to examine the levels of significance ($p<0.05$) of the factors site, clone, and depth and their interactions. Mean values of root parameters, fungal biomass, and chemical elements in soil were separated using Tukey's honestly significantly difference test. The data were transformed when neces-

sary to achieve normality. The %RLCs were transformed according to the Bliss formula (Snedecor and Cochran 1976): $x = \arcsin\sqrt{(n\%/\ 100)} \times 180/\prod$, where $n\%$ is the percent value. The nonparametric Kruskal–Wallis test was used to test for significant differences in colonization of roots by mycorrhizal fungi (AM and ECM) and nonmycorrhizal FE. The figures present nontransformed data. The Pearson correlation coefficient ($r_{xy}$) was used to analyze relationships between measured parameters. Statistical analyses were performed using Statistica 5.1 (StatSoft) software.

## Results

### Soil chemistry

Soil pH analyses revealed significantly lower soil pH values in polluted site 3 compared to sites 1 and 2 (Table 1). Site 3 was characterized by the highest soil concentrations of copper, lead, zinc, cadmium, carbon, nitrogen, phosphorus, and potassium and the lowest concentration of $N\text{-}NO_3$. Higher levels of copper and lead were observed in the upper soil layer. The concentration of zinc was relatively stable throughout the soil layers, although the highest level was observed in the 20–30-cm soil layer. There were no significant differences in $N\text{-}NH_3$ and calcium soil concentrations among the three sites or in the carbon/nitrogen ratio; however, the concentration of $N\text{-}NH_3$ in site 3 was slightly lower than in sites 1 and 2.

### Soil fungi biomass

ANOVA revealed significant differences in the biomass of soil fungi as measured by ergosterol content. Fungi biomass differed depending upon rooting depths and sites and interactions between these two factors. The biomass of soil fungi decreased according to depth at each site. In polluted site 3, fungal biomass was significantly lower than in sites 1 and 2 (Fig. 1).

### Fine-root abundance

ANOVA showed that environment had the most significant effect on overall fine-root parameters. A statistically significant effect was also revealed for the site and clone interaction (Table 2). The highest abundance of root tips calculated per 100 cm of root length was found at site 2, followed by sites 1 and 3. An overall comparison of the three study sites did not show a direct, significant influence of poplar clones on root-tip abundance. ANOVA also revealed significant differences in the abundance of fine roots among the soil layers (Table 2). In an overall comparison, the highest abundance of root tips was found in the upper soil layer (0–10 cm) and decreased significantly with soil depth.

In separate analyses of the three study sites, the type of clone was significant only in site 3 (Table 2), where *P. deltoides* × *P. trichocarpa* had a higher abundance of fine roots in the upper soil layer (0–10 cm; Fig. 2a). The overall abundance of this poplar clone's root tips at all rooting depths was higher at the metal-contaminated site 3 than at

**Table 1** Soil chemical characteristic of three sites (parts per million)

| | Site 1 | | | Site 2 | | | Site 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0–10 cm | 10–20 cm | 20–30 cm[a] | 0–10 cm | 10–20 cm | 20–30 cm[a] | 0–10 cm | 10–20 cm | 20–30 cm[a] |
| $pH_{[H_2O]}$ | 7.6 | 7.3 | 7.6c | 7.1 | 7.1 | 7.1b | 6.8 | 6.3 | 6.2a |
| $pH_{[KCl]}$ | 7.2 | 7.2 | 7.5c | 6.8 | 6.8 | 6.8b | 6.1 | 5.4 | 5.2a |
| Cu | 7.88 | 9.1 | 4.17a | 5.08 | 8.41 | 4.97a | 2,137.4 | 1,295.2 | 748.8b |
| Pb | 8.47 | 10.78 | 9.47a | 9.33 | 10.02 | 6.69a | 2,154.35 | 518.8 | 515.55b |
| Zn | 20.90 | 18.40 | 18.90a | 30.30 | 32.80 | 25.90b | 94.20 | 71.80 | 114.2c |
| Cd | 0.29 | 0.28 | 0.26a | 0.32 | 0.31 | 0.30b | 0.47 | 0.43 | 0.43c |
| $N\text{-}NO_3$ | 24.69 | 8.75 | 8.51b | 20.59 | 8.66 | 5.76b | 10.29 | 0.95 | 1.34a |
| $N\text{-}NH_3$ | 2.29 | 0.98 | 0.17 | 2.3 | 2.2 | 1.66 | 1.97 | 1.22 | 1.32 |
| P | 492 | 358 | 397.5a | 446 | 438 | 324.5a | 870 | 488.5 | 512.5b |
| K | 1,073 | 820.5 | 750a | 1,617 | 1,616.5 | 1,631.5b | 3,361 | 3,641.5 | 4,333c |
| Ca | 2,521.5 | 2,414 | 3,955.5 | 2,932 | 1,736 | 1,597.5 | 4,220 | 2,526.5 | 2,316 |
| C% | 1.41 | 1.08 | 1.09b | 0.94 | 0.63 | 0.48a | 2.22 | 1.92 | 1.51c |
| N% | 0.13 | 0.1 | 0.11b | 0.07 | 0.04 | 0.03a | 0.19 | 0.1 | 0.12c |
| C/N | 10.97 | 11.23 | 10.18 | 14.09 | 14.95 | 17.02 | 11.46 | 19.09 | 12.48 |

[a] Significant differences between sites are indicated by letters

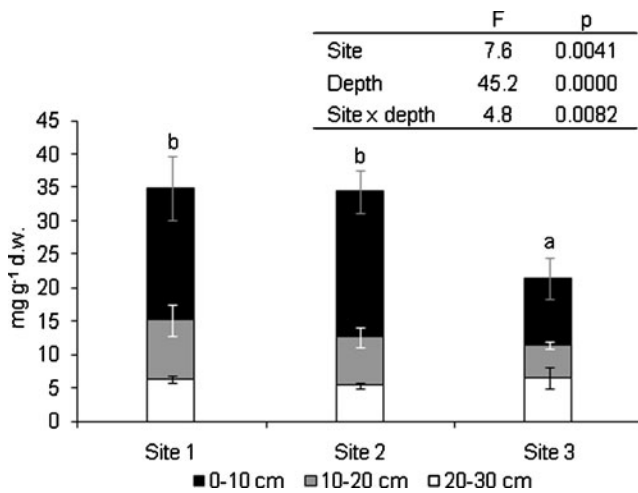


**Fig. 1** Average content of soil fungi biomass (mean ± SE) and results of ANOVA. Differences between poplar clones were analyzed separately for each site. Significant differences are indicated by *different letters* ($p<0.05$, Tukey's test)

the two other sites (Fig. 2a–c). At sites 1 and 2, *P. maximowiczii* × *P. trichocarpa* had a slightly higher abundance of fine roots than the other clones at all three rooting depths (Fig. 2a–c).

Table 2 and Fig. 2a–c show significant differences in the vertical distribution of fine roots at site 1 for all four of the poplar clones studied, although individual clones did not differ in fine-root abundance according to depth. At site 3, the abundance of *P. deltoides* × *P. trichocarpa* root tips decreased significantly with soil depth.

## Fungal colonization

Dual mycorrhizal colonization by ECM and AM fungi was observed in the roots of all poplar clones. The poplar roots were also colonized by nonmycorrhizal FE. The %RLC varied significantly among sites, poplar clones, and soil levels (Table 2; Fig. 3a–i). Generally, the roots of the four poplar clones were dominated by AM fungi, except at site 2, where ECM colonization was more extensive than AM colonization in roots at a depth of 10–20 cm (Fig. 3a–f). The site was the most important factor influencing overall mycorrhizal colonization, although the type of clone and soil depth both had important effects on mycorrhizae within each of the study sites (Table 2).

Nonmycorrhizal FE colonization of poplar roots was relatively low: less than 10% RLC in the upper soil layer (0–10 cm) for all four of the poplar clones at all three of the study sites, up to 30% RLC for *P. deltoides* × *P. trichocarpa* at sites 2 and 3 in the 10–20-cm soil layer (Fig. 3g–i). Soil depths and study sites were the most important factors influencing root colonization by non-

**Table 2** Results of ANOVA testing the influence of site, clone, and depth on fine root abundance (root tips per 100 cm root length) and colonization of fine roots by mycorrhizal fungi (ECM, AM) and nonmycorrhizal FE for overall for all sites and for each site separately

| | Root tips abundance | | ECM %RLC | | AM %RLC | | FE %RLC | |
|---|---|---|---|---|---|---|---|---|
| | *F* | *p* | *F* | *p* | *F* | *p* | *F* | *p* |
| Overall | | | | | | | | |
| Site | 13.8 | 0.0000[a] | 142.1 | 0.0000[a] | 69.5 | 0.0000[a] | 9.9 | 0.0001[a] |
| Clone | 2.4 | 0.0755 | 7.8 | 0.0001[a] | 4.4 | 0.0061[a] | 2.7 | 0.0471[a] |
| Depth | 4.0 | 0.0205[a] | 12.5 | 0.0000[a] | 4.2 | 0.0183[a] | 2.4 | 0.0947 |
| Site × clone | 3.2 | 0.0063[a] | 6.3 | 0.0000[a] | 7.5 | 0.0000[a] | 4.9 | 0.0002[a] |
| Site × depth | 0.3 | 0.9002 | 6.9 | 0.0001[a] | 4.9 | 0.0011[a] | 3.1 | 0.0176[a] |
| Clone × depth | 1.9 | 0.0939 | 7.4 | 0.0000[a] | 2.9 | 0.0128[a] | 12.0 | 0.0000[a] |
| Site × clone × depth | 0.9 | 0.5400 | 1.3 | 0.2241 | 4.1 | 0.0000[a] | 6.7 | 0.0000[a] |
| At each of sites | | | | | | | | |
| Site 1 | | | | | | | | |
| Clone | 2.4 | 0.0846 | 9.8 | 0.0001[a] | 4.0 | 0.0151[a] | 6.7 | 0.0011[a] |
| Depth | 4.1 | 0.0244[a] | 16.9 | 0.0000[a] | 8.6 | 0.0009[a] | 0.1 | 0.9381 |
| Clone × depth | 0.3 | 0.9134 | 2.4 | 0.0451[a] | 2.2 | 0.0697 | 3.8 | 0.0053[a] |
| Site 2 | | | | | | | | |
| Clone | 2.4 | 0.0838 | 4.6 | 0.0076[a] | 5.9 | 0.0023[a] | 2.4 | 0.0825 |
| Depth | 0.9 | 0.4237 | 4.6 | 0.0169[a] | 1.8 | 0.1761 | 2.6 | 0.0885 |
| Clone × depth | 0.7 | 0.6484 | 4.4 | 0.0021[a] | 5.1 | 0.0007[a] | 15.9 | 0.0000[a] |
| Site 3 | | | | | | | | |
| Clone | 4.7 | 0.0073[a] | 4.9 | 0.0061[a] | 10.1 | 0.0001[a] | 2.9 | 0.0472[a] |
| Depth | 2.7 | 0.0835 | 0.0 | 0.9703 | 6.0 | 0.0056[a] | 8.2 | 0.0012[a] |
| Clone × depth | 3.2 | 0.0124[a] | 3.1 | 0.0142[a] | 2.5 | 0.0420[a] | 3.8 | 0.0051[a] |

[a] Significant effect

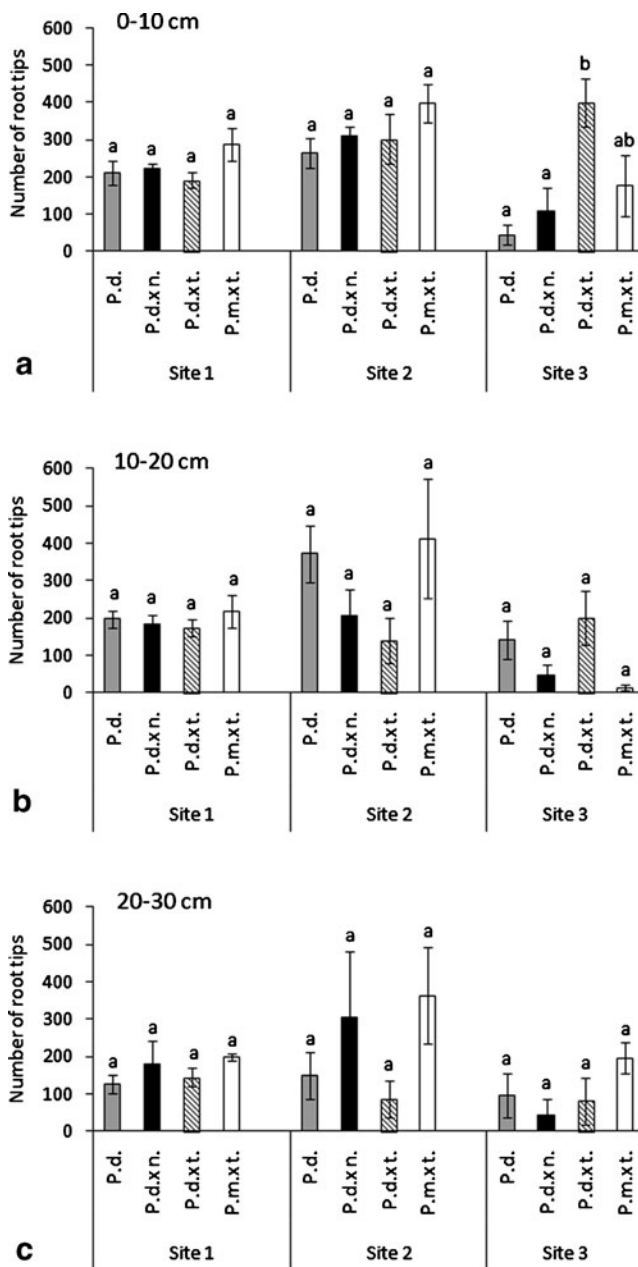


**Fig. 2** **a**–**c** Fine root tips abundance (number of root tips per 100 cm root length) of four poplar clones in three sites at three rooting depths (*P.d. P. deltoides*, *P.d.x n. P. deltoides* × *P. nigra*, *P.d.x t. P. deltoides* × *P. trichocarpa*, *P.m. x t. P. maximowiczii* × *P. trichocarpa*). Differences between poplar clones were analyzed separately for each site. Data are means ± SE. Significant differences are indicated by *different letters* ($p<0.05$, Tukey's test)

mycorrhizal FE; however, poplar clones had significant effects on root colonization in deeper soil layers (10–20 and 20–30 cm; Table 2; Fig. 3g–i).

Analyses of the relationship between ECM and AM colonization in poplar roots revealed a significant negative correlation at site 1 ($r_{xy}=-0.52$, $p=0.0002$) and site 2 ($r_{xy}=-0.49$, $p=0.0004$) and a positive correlation at site 3 ($r_{xy}=0.3$, $p=0.0371$). A significant positive relationship was also observed between ECM and FE colonization at site 3 (Table 3).

## Discussion

According to our results, the study site was the most important factor determining the fine-root abundance of poplar clones and colonization of the roots by mycorrhizal and nonmycorrhizal fungi. A second important factor was the soil depth. However, the effects of these two factors on fine-root abundance and fungal colonization varied with the poplar genotype (Table 2). Of the three common-garden locations, we classified site 3 (located in the vicinity of a copper smelter) as stressful because of high concentrations of copper and lead, which greatly exceeded the maximum allowable limits (100 mg/kg dry weight for copper and lead) used in Poland and other countries (Kloke 1980; Kabata-Pendias and Pendias 1993). The soil nutrient levels of the three study sites were all within the normal range for temperate forests. At the metal-contaminated site, the average abundance of fine roots (analyzed for the four poplar clones together) and their colonization by mycorrhizal fungi, as well the total biomass of fungi in soil, were lower than at the two unpolluted sites. Furthermore, the average growth (expressed as DBH) of the poplars at the metal-polluted site was less than at the two unpolluted sites (Rachwał, personal communication).

Our results also indicated that different poplar genotypes responded to environmental conditions with different "rooting strategies". Individual poplar clones at the metal-contaminated site differed significantly in their fine root-abundance, particularly in the uppermost 10 cm of the soil profile, which had the highest concentrations of copper and lead (Table 1; Fig. 2). The clone *P. deltoides* × *P. trichocarpa* showed a higher tolerance to copper and lead than the other three poplar clones; this poplar clone had significantly more fine roots at site 3 compared to the other poplars. In contrast, at sites 1 and 2, the abundance of fine-root tips did not differ significantly among the four poplar clones. Furthermore, *P. deltoides* × *P. trichocarpa* above-ground parts showed more growth at the polluted site 3 than at the unpolluted sites. The related hybrid poplar *P. trichocarpa* × *P. deltoides* cv. Beaupré was previously reported to be tolerant to zinc (Blaudez et al. 2003). In our study, the hybrid *P. maximowiczii* × *P. trichocarpa* (clone P. "NE-42") showed a tendency toward abundant growth of fine-root systems at the two unpolluted sites and a decreased amount of fine-root abundance at the polluted site, particularly at soil level 10–20 cm (Fig. 2). This clone was previously reported to be sensitive to heavy metals (copper, lead, and zinc) in a pot experiment (Rachwał et al. 1992).

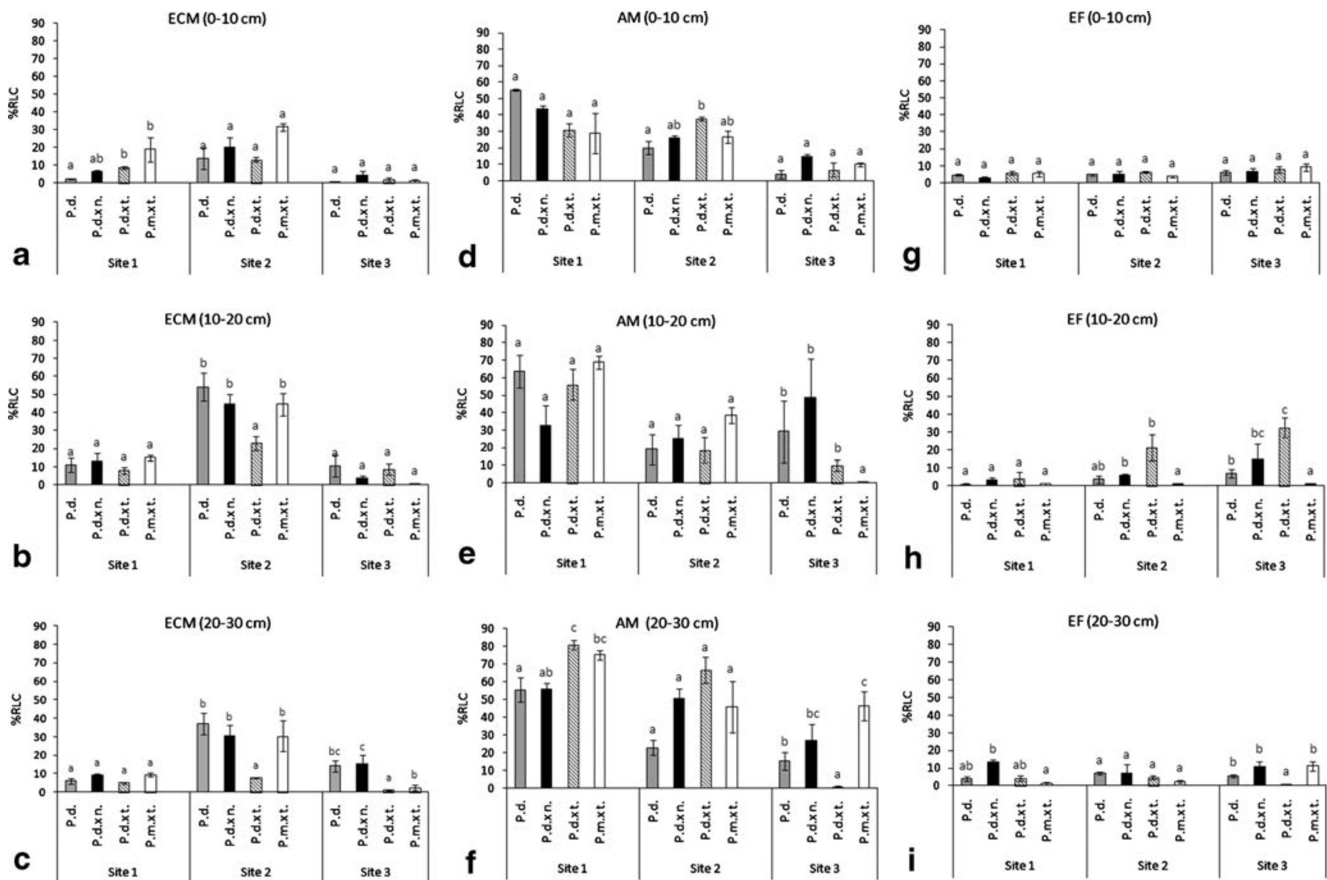


**Fig. 3 a–i** Percentage of root length colonization by ECM, AM, and FE (mean±SE) of four poplar clones in three sites at three rooting depths (*P.d. P. deltoides*, *P.d.x n. P. deltoides* × *P. nigra*, *P.d.x t. P. deltoides* × *P. trichocarpa*, *P.m.x t. P. maximowiczii* × *P. trichocarpa*). Differences between poplar clones were analyzed separately for each site. Significant differences are indicated by *different letters* ($p<0.05$, Kruskal–Wallis test)

**Table 3** Analyses of relationship between ECM, AM, and FE colonization in of roots in three sites

| | ECM | AM |
|---|---|---|
| Site 1 | | |
| AM | $r_{xy}=-0.52$ $p=0.0002$[a] | |
| FE | $r_{xy}=-0.05$ $p=0.7271$ | $r_{xy}=-0.23$ $p=0.1143$ |
| Site 2 | | |
| AM | $r_{xy}=-0.49$ $p=0.0004$[a] | |
| FE | $r_{xy}=-0.23$ $p=0.1141$ | $r_{xy}=-0.17$ $p=0.2482$ |
| Site 3 | | |
| AM | $r_{xy}=0.30$ $p=0.0371$[a] | |
| FE | $r_{xy}=0.30$ $p=0.0360$[a] | $r_{xy}=0.21$ $p=0.1552$ |

[a] Significant effect

In our study, ECM colonization of poplar roots was in the range 5.2–29.2% RLC, depending on the poplar clone and the study site. Similar levels of ECM colonization were found by Baum et al. (2000) for *P. trichocarpa* grown in nutrient-poor soils. However, higher amounts of ECM in the root systems of different poplar species and hybrids were reported by Khasa et al. (2002) in the roots of 28 poplar clones (35–90%), by Neville et al. (2002) in *P. tremuloides* (75%), by Kaldorf et al. (2002) in genetically modified *Populus tremula* × *P. tremuloides* (64–73%), and by Gehring et al. (2006) in *Populus angustifolia* and *P. angustifolia* × *Populus fremontii* hybrids (66–94%). In the present study, the range of colonization of poplar roots by AM fungi was 18–54% RLC. Similar levels of AM colonization were found by Khasa et al. (2002) for different poplar hybrids (20–50%); however, lower AM colonization was reported by Kaldorf et al. (2002; <5%), Neville et al. (2002; 10%), and Gehring et al. (2006; 4–25%). The percentage of nonmycorrhizal FE colonization that we found (4.2–9.3% RLC) was similar to the results of Beauchamp et al. (2005; 7.4–23.4%) in the roots of *P. fremontii*.

The abundance of symbiotic fungi colonizing roots varied among the three different sites. We measured a lower amount of ECM and AM colonization at the polluted site compared to the unpolluted sites. The abundance of mycorrhizal structures in poplar roots positively correlated with the biomass of fungi in soil; however, mycorrhizal abundance did not correlate with the abundance of poplar fine roots. This suggests that the conditions at the polluted site were more harmful to mycorrhizal fungi than to the poplar trees. Heavy metals may affect mycorrhizae by inhibiting fungal growth and by modification of soil chemistry, decreasing the availability of essential nutrients (Gadd 1993, Meharg and Cairney 2000). Heavy metals may influence the abundance of soil bacteria, which are known to be generally more sensitive to pollution than fungi (Kelly et al. 2003). A reduction in soil microbial activity could disturb natural biochemical paths and lead to a decrease in soil fertility (Rossel et al. 1997) or a reduction in nutrient decomposition, resulting in accumulation in the soil (Shi et al. 2002). In our study, the polluted soil at site 3 was characterized by higher total concentrations of carbon, nitrogen, phosphorous, and potassium but lower levels of $N\text{-}NO_3$ and $N\text{-}NH_4$ compared to the unpolluted soils (Table 1).

In three sites, AM fungi generally predominated in mycorrhizal colonization. Variabilities in ECM and AM colonization and in the AM/ECM ratio were determined mostly by the site and the soil depth. Site 1 promoted mostly AM colonization with a low contribution of ECM (AM/ECM=9.8). At site 2, AM and ECM colonization in poplar roots was more balanced (AM/ECM=2.3). At the polluted site 3, AM predominated over ECM fungi (AM/ECM=8.6). These results indicate that although total mycorrhizal colonization was significantly decreased by environmental pollution, the proportion of two mycorrhizal types was strongly influenced by other environmental conditions.

Soil conditions were previously suggested to be an important factor in dual mycorrhizal colonization (Gonçalves and Martins-Loução 1996, Smith and Read 2008). In our study, humidity may have been a factor differentiating sites 1 and 3, which were characterized by higher soil moisture, from the drier site 2. The importance of moisture in dual mycorrhizal colonization was previously suggested by Lodge (1989), Gehring et al. (2006), and Querejeta et al. (2009). In general, mycorrhizal fungal growth is inhibited at very low and very high water potentials (Mexal and Reid 1973, Lodge 1989). Higher rates of AM colonization are found in moist soil than in very dry or flooded soils (Lodge 1989; Miller 2000; Entry et al. 2002). This seems to be universal for different plant species. An increase in AM colonization of roots growing in soil that flooded periodically was observed by Truszkowska (1953) in *Alnus*, by Lodge (1989) in *Salix nigra*, and by Miller and Bever (1999) in the grass *Panicum hemitomon*. Dry or moist, but well-aerated, soil conditions were found to be more favorable for root colonization by EM fungi (Truszkowska 1953; Lodge 1989). Querejeta et al. (2009) revealed positive correlation between EM colonization, soil hyphal density, and soil moisture potential in the upper rhizosphere of oak woodlands.

Colonization of poplar roots by nonmycorrhizal FE was significantly higher at the polluted site than at the unpolluted sites. These fungi appeared to be more tolerant of high concentrations of heavy metals and to compete with AM fungi in poplar root colonization. These observations are in line with the findings of Routsalainen et al. (2007) and Jumpponen and Trappe (1998), who observed an increase in the abundance of dark septate endophytes in the roots of plants at polluted areas. The contribution of nonmycorrhizal FE to root colonization also differed among the poplar hybrids. *P. deltoides* × *P. trichocarpa* had significantly higher nonmycorrhizal FE colonization than *P. maximowiczii* × *P. trichocarpa.*

The influence of *Populus* genotype on mycorrhizal colonization was significantly pronounced within the individual study sites. Variable ECM, AM, and FE colonization of roots suggested differing susceptibilities of the poplars to symbiotic fungi. Likewise, Khasa et al. (2002) revealed that there were variable degrees of ECM and AM colonization among 28 poplar clones. The role of host genotype in the ability of plants to form mycorrhizal relationships was also pointed out by Smith and Read (2008), Barker et al. (2002), and Tagu et al. (2001, 2005). Furthermore, Gehring et al. (2006) found that host genotype played a minor role in comparison to the influence of environmental conditions on poplar mycorrhizal colonization. Our results indicate that the genotype effect was modified by environmental conditions.

Mycorrhizal colonization of fine roots revealed significant differences among the rooting depths that we analyzed. ECM colonization of roots was the most abundant in the 10–20-cm soil layer. In the two unpolluted sites, we found a significant negative correlation between AM and ECM colonization of poplar roots. A similar tendency was observed by Neville et al. (2002) in 3-year-old *P. tremuloides* growing in boreal clear-cut forest. The authors proposed that EM and AM are preferentially partitioned at different soil depths, coinciding with different soil conditions. Our results appear to be in agreement with this hypothesis. Although both AM and ECM colonization decreased in the polluted site 3, accompanied by a lower total fungal biomass in soil, this was a consequence of environmental pollution. Furthermore, the increase in nonmycorrhizal FE colonization found at site 3 suggests that there was competition between nonmycorrhizal FE and

AM. A similar relationship between FE and AM during root colonization has been suggested for different plants in harsh environments (Jumpponen and Trappe 1998; Postma et al. 2007).

In conclusion, our study indicated the significance of factors influencing the symbiotic relationship between mycorrhizal and endophytic fungi and poplars. Our results support the importance of poplar genotype in the pattern of fine-root distribution, the ability of poplar roots to establish symbiotic relationships with mycorrhizal fungi, and tolerance to heavy metal pollution. However, genetic effects were strongly modified by environmental conditions, which were important for fine-root development and colonization by mycorrhizal and nonmycorrhizal fungi. Toxic levels of heavy metals significantly reduced fungal biomass in soil and decreased mycorrhizal colonization; however, effects from other environmental factors, such as soil moisture, cannot be excluded. Our results underline the importance of conducting studies in different environments when characterizing the ecological plasticity of poplar trees and their fungal symbiotic partners to determine their utility in afforestation of abandoned and degraded lands.

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