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ORIGINAL PAPER

Mycorrhizal status of an ozone-sensitive poplar clone treated with the antiozonant ethylene diurea

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Abstract The antiozonant ethylene diurea is proven to prevent growth reductions in forest trees induced by ozone. The community of mycorrhizal fungi could be useful indicator of environmental stress. In this study, response of mycorrhizal fungi and fine roots to a 4-year exposure to ambient ozone and treatment with antiozonant was investigated in ozone-sensitive poplar clone under field conditions. The community of ectomycorrhizal fungi and root length colonization with ectomycorrhizal, arbuscular mycorrhizal fungi, and root endophytic fungi was analyzed in antiozonant-treated poplar plants and in poplar plants irrigated with water. In general, plants protected by antiozonant showed higher total number of fine roots, number of ectomycorrhizal types, Shannon–Weaver diversity index, and Species richness index compared to the plants treated with water. The ectomycorrhizal community shifted from contact exploration type in the trees irrigated with water to short-distance exploration type in ethylene diurea-treated trees. Ozone protectant may beneficially affect the belowground community of mycorrhizal fungi colonizing roots of ozone-sensitive poplar clone.

Keywords Ground-level ozone · Ectomycorrhizal fungi · Antiozonant ethylene diurea · Fine roots · Root length colonization · *Populus maximoviczii* × *berolinensis*

Introduction

In the atmosphere, ozone (O_3) is generated from oxides of nitrogen and volatile organic compounds reacting in the photochemical oxidant cycle during warm sunny weather. It is an oxidant gas present at the ground level in a background concentration range from 20 to 40 ppb (Vingarzan 2004). When its concentration in the air exceeds the normal background concentration, ozone becomes an air pollutant (Manning et al. 2011).

Nowadays, ozone is considered as the air pollutant of most concern for forests and other terrestrial ecosystems (Serengil et al. 2011). Its concentrations recorded in rural areas are frequently higher than those in the cities (Gregg et al. 2003). In plants, O_3 causes physiological changes in leaves that affect the carbon source strength and the amount of carbon available for allocation to sink tissues (Andersen 2003). Decreased allocation of belowground carbon, due to an increased ozone concentration, can affect fine roots and root symbionts (Grebenc and Kraigher 2007b), as well as rhizodeposition, litter quality and quantity, and the whole soil wood web (Andersen 2003).

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Poplars are widely distributed and well-adapted fast-growing trees with high biotechnology potential (Klopfenstein et al. 1997). They are used in agroforestry systems (Eichhorn et al. 2006), in short rotation forestry (Klašnja et al. 2006), and play a promising role in phytoremediation (Newman et al. 1997). Poplars routinely form functional mycorrhizal associations with ectomycorrhizal and arbuscular mycorrhizal fungi simultaneously (Molina et al. 1992), which can favor establishment and growth in extreme conditions and make poplar suitable for reforestation and reclamation purposes (Khan 2006).

Increase in ozone concentration in the atmosphere has influence on the compatibility of plant and fungus in mycorrhizas, which can induce a shift in fungal morphotypes and species composition (Qiu et al. 1993; Andersen 2003; Grebenc and Kraigher 2007a, b; Matyssek et al. 2010). According to Agerer (2001), functional diversity of ectomycorrhizas, described as exploration types, is a good indicator of site conditions, suggesting a potential relationship between exploration types and their ecological function. Arbuscular mycorrhizal fungi may also react to the elevated ozone concentration mainly by an accelerated colonization and improved colonization rate (Hu et al. 2009). Similarly, it was observed that the colonization of poplar roots by root endophytic fungi was higher at polluted site compared to the unpolluted one (Karliński et al. 2010), but there are no data about ozone influence on this fungal group.

Ethylene diurea (*N*-[2-(2-oxo-1-imidazolidinyl)ethyl]-*N*0-phenylurea), abbreviated as EDU, has been widely used as antiozonant in order to prevent foliar ozone injury and crop losses in crop plants and growth reductions in forest trees (Manning et al. 2011). Although the mode of action of this substance is not well understood, Manning et al. (2011) postulated that EDU might affect O_3 stomatal uptake, plant defense mechanisms against O_3, or both. EDU might also increase the concentration of photosynthetic pigments, participate in direct chemical scavenging, or behave as plant activator through the synthesis of ascorbic acid. Therefore, EDU is an appropriate research tool for diagnosing, under natural conditions, the effect of ozone on plants regarding foliar symptoms, growth, productivity, and understanding the mode of action of ambient O_3 (Manning et al. 2011).

Paoletti et al. (2007b, 2008) showed that 1-year gravitational infusion of EDU into trunks protected adult *Fraxinus excelsior* L. trees from foliar ozone injury and growth reductions. However, number of total root tips and percentage of mycorrhizal infection were not affected, and tree sensitivity to ozone (tolerant vs. sensitive trees) had stronger effect on fine root tips than EDU (Paoletti et al. 2007a). Recently, in a 3-year exposure to ambient ozone, Hoshika et al. (2012) recorded significantly higher number of leaves, lower percentage of injured leaves, and higher total carbon assimilation in EDU-treated poplar trees compared to those irrigated with water. In the same experimental field, Hoshika et al. (2013) discovered that ambient ozone reduced allocation of biomass to leaves, roots, and lateral branches and induced early leaf abscission. Trees irrigated with water showed lower coarse root biomass (−40 %) and fine root biomass (−52 %) compared to trees irrigated with EDU. Nevertheless, the knowledge of EDU effects on belowground, especially ectomycorrhizal types, arbuscular mycorrhizal, and root endophytic fungi, is still limited.

Due to the fact that mycorrhizal and root endophytic fungi may be sensitive to environmental stress, such as ozone, the aim of our study was to investigate how 4-year treatment with EDU affects community of ECM fungi and root length colonization with ectomycorrhizal (ECM), arbuscular mycorrhizal (AM), and root endophytic fungi (END) in the ozone-sensitive poplar clone *P. maximoviczii* Henry × *berolinensis* Dippel when exposed to ambient ozone.

Materials and methods

Study site and sampling

The study was carried out in an experimental field site located in central Italy, near Antella (about 10 km from Florence, Italy), on a post-agricultural land. Soil type is sandy clay loam. Coordinates of the site are 43°44′N, 11°16′E, the altitude is 50 m a.s.l., and the climate is Mediterranean with mean annual temperature of 14.7 °C and total annual precipitation of 1,233 mm in 2010 (Hoshika et al. 2012).

Forty root cuttings of the O_3-sensitive Oxford clone (*P. maximoviczii* Henry × *berolinensis* Dippel) (Marzuoli et al. 2009) were planted in two lines (treated and control). Cuttings were planted in 2007 with a spacing of 1 m between trees along the line and 3 m between trees of the two lines. Every week over the growing seasons 2008 to 2011, each tree was irrigated with 450 ppm EDU solution (EDU, treated line) or with 1–2 L of water (WAT, control line). Concentration of 450 ppm EDU is the most effective concentration for trees, according to Paoletti et al. (2009). Root samples were collected from 5-year-old trees after 4 years of regular EDU/WAT treatment. Ozone concentrations at the plot were continuously recorded during the growing season (April to October), and the average AOT40 in 2010 was 25.8 ppm h, while the maximum hourly O_3 concentration reached 118 ppb (Hoshika et al. 2013), which was 2.5 times the critical ozone level for forests according to WHO (WHO 2000).

In September 2011, five trees in each of the EDU and WAT treatment were randomly selected for fine root and root symbionts analysis. A soil corer of 274 ml volume, reaching 18 cm depth, was used for taking standardized soil samples (Kraigher 1999). For the analysis of ECM community structure and fine root number, four soil samples were taken evenly distributed around each selected poplar tree at a distance of about 50 cm from the tree trunk. Six additional soil samples per plant were taken using the same sampling approach for the determination of root length colonization with ECM, AM, and END fungi.

Samples were kept stored in refrigerator at 4 °C for up to 1 month before being analyzed. The mycorrhiza and root analyses were performed at the Slovenian Forestry Institute in Ljubljana, in the laboratory of the Department of Forest Physiology and Genetics.

Identification of ectomycorrhizae

Prior to ECM analysis, each soil core was submerged in cold tap water overnight to loosen the soil structure. Roots were carefully washed from soil, and ECM root tips were divided from old non-turgescent, non-mycorrhizal, and herbaceous roots under a stereomicroscope and selected for further identification. Types of ECM were analyzed after morphological and anatomical characteristics by recording relevant identification characteristics under a stereomicroscope Olympus SZX 12 (7.5–64× magnification) and microscope Olympus BX 51 (100–2,000× magnification) as described by Agerer (1991) and Kraigher (1996). Identification was accomplished following the identification keys in Agerer (1987–2008), Agerer et al. (2001–2006), and Agerer and Rambold (2004–2012). Based on the presence and abundance of emanating elements, ECM types were also classified into the exploration types given by Agerer (2001). All fine roots (ECM root tips, old non-turgescent, and non-mycorrhizal roots) were counted under the stereomicroscope.

Identification with molecular approach was based on PCR amplification and sequencing of the complete internal transcribed spacer (ITS) regions in nuclear ribosomal DNA (Gardes and Bruns 1993). DNA extraction was performed with a DNeasy Plant Mini Kit (Qiagen, Germany), and ITS region was amplified with ITS 1f and ITS 4 primer pair (Gardes and Bruns 1993). After separation and excision of the amplified DNA from the agarose gel and purification of the amplified fragments with Wizard® SV Gel and PCR Cleanup System (Promega), sequencing was performed at a commercial sequencing laboratory (Macrogen Inc., Seoul, Rep. of Korea). Species, genus, or family of ectomycorrhizal fungi were determined by comparing the sequence with GenBank (http://www.ncbi.nlm.nih.gov) and UNITE (Abarenkov et al. 2010) database.

Root length colonization

Before evaluation, poplar fine roots isolated from six soil samples were separated from the roots of herbaceous species by means of visual inspection and jointed together in one sample. Extracted roots were gently washed and cleared in 10 % potassium hydroxide and stained with Trypan blue in lactoglycerol according to Kormanik and McGraw (1982) and Karliński et al. (2010). Colonization of poplar roots by ECM, AM, and END fungi was evaluated using the intersection method by McGonigle et al. (1990), modified by Karliński et al. (2010), at 200× magnification. A minimum of 200 line intersections per subsample (microscopic slide) were scored for the presence of AM structures (hyphae, vesicles, arbuscules, and coils), ECM, or END fungi. The results are presented as a percentage of root length colonized.

Data analysis

The ECM community data were used for calculating the following diversity indices: Species richness index, Shannon–Weaver diversity index, evenness, equitability, and Berger-Parker index (Atlas and Bartha 1981). Diversity indices and relative abundance of ECM types were calculated per treatment in the way that ECM community data for the same treatment were pooled. Relative abundance of ECM types was calculated as a ratio between the tips number of individual ECM type and total number of ECM tips.

In order to analyze root colonization by AM, ECM, and END fungi and the abundance of exploration types, the data for samples from the same tree were pooled and used as a composite sample. Thus, the statistical unit was a single tree.

Data were checked for normal distribution by the Shapiro–Wilk test. Since the distribution was not normal, the nonparametric Mann–Whitney *U* test was used to test for significant differences in root colonization by AM, ECM, and END fungi between EDU-treated plants and plants irrigated with water. The Spearman's correlation coefficient R was used to analyze relationships between measured parameters.

The Student's *t* test was used to test for significant differences in the abundance of exploration types between EDU-treated and water-irrigated plants. In order to obtain normal distribution, data were transformed according to arcsine transformation using the Bliss formula (Snedecor and Cochran 1976) and the re-transformed data are presented in the table. Statistical analyses were performed using Statistica12 software (StatSoft).

Results

Fine roots and ectomycorrhiza diversity

In this study, 25,033 fine roots were recorded in total in the treatment with EDU and 14,246 fine roots in the control irrigated with water, while the total number of vital ECM root tips was 1,714 and 936, respectively (Table 1).

Total values of the number of ECM types, vital ECM roots, total number of fine roots, Species richness index, Shannon–Weaver index, equitability, evenness, and Berger-Parker index were higher in the plants treated with antiozonant compared to the plants irrigated with water (Table 1).

Ectomycorrhizal community structure

Nine ECM fungi were separated and identified based on a combination of anatomorphic and molecular characters: *Hebeloma vaccinum*, *Hymenogaster populetorum*, *Tomentella ellisii*, *Tuber maculatum*, *Tuber excavatum*, *Geopora* sp., *Tomentella* sp. *1*, *Tomentella* sp. *2,* and ECM belonging to Pezizales (Table 2). In the EDU-treated trees, a total of eight ECM types were identified. Four types of ECM dominated with over 90 % of ECM root tips (*T. ellisii*, *T. maculatum*, *Geopora* sp., and *H. vaccinum)*. In soil samples taken from WAT-treated trees, six ECM types were recorded. Among them, *H. vaccinum* made 50 % and *Geopora* sp. 33 % of all ECM tips (Fig. 1).

Colonization of fine roots with ECM, AM, and END fungi

Roots were infected with a dual mycorrhiza, namely ECM and AM, and were also colonized by END fungi. In WAT-treated trees, root length colonization with AM and ECM was higher (50 % and almost 40 %, respectively) than in

Table 1 Total number of ectomycorrhizal (ECM) types, vital ECM roots, old non-turgescent and non-mycorrhizal roots, total number of fine roots, and diversity indices on poplar trees treated with EDU or with water

Treatment	Number of ECM types	Number of vital ECM roots	Number of old non-turgescent and non-mycorrhizal roots	Total number of fine roots	Species richness (d)	Shannon–Weaver index (H)	Evenness (e)	Equitability (J)	Berger-Parker index (BP)
EDU	8	1,714	23,319	25,033	2.16	1.60	1.77	0.77	0.72
WAT	6	936	13,310	14,246	1.68	1.21	1.55	0.67	0.50

Table 2 Ectomycorrhizal fungi on poplar trees treated with ethylene diurea (EDU) or water (WAT), identified on the basis of the similarity with the sequences from Internet databases GenBank and UNITE, and their exploration types

Fungal type	GenBank accession number of best DNA-based hit with the percentage of identity	UNITE accession number of best DNA-based hit with the percentage of identity	Morphological identification	ECM type was recorded in (the treatment with EDU or WAT)	Exploration type
Geopora sp.	Uncultured *Geopora* clone G3p3 GU327417 (99 %)	*Geopora cervina* UDB016155[a]	–	EDU and WAT	Contact
Hebeloma vaccinum	*Hebeloma vaccinum* AY320396 (100 %)	*Hebeloma crustuliniforme* UDB011897 (97 %)	*Hebeloma vaccinum*	EDU and WAT	Medium distance
Hymenogaster populetorum	*Hymenogaster populetorum* isolate zb2028 GU479325 (98 %)	*Hymenogaster luteus* UDB001192[a]	–	EDU	Short distance
Pezizales	Uncultured Pezizales DQ469743 (97 %)	Peziza UDB001572 (97 %)	–	EDU	Short distance
Tomentella ellisii	*Tomentella ellisii* DQ068971.1 (99 %)	*Tomentella ellisii* UDB000219 (96 %)	–	EDU and WAT	Short distance
Tomentella sp. 1	*Tomentella* sp. OTU202 HQ215817.1 (99 %)	*Tomentella subclavigera* UDB003303 94 %	–	EDU and WAT	Short distance
Tomentella sp. 2	Uncultured ectomycorrhiza (*Tomentella*) AJ879644 (99 %)	*Tomentella clavigera* UDB016494[a]	*Tomentella* sp.	EDU	Short distance
Tuber maculatum	*Tuber maculatum* FM205510 (99 %)	*Tuber maculatum* UDB000121 (96 %)	*Tuber* sp.	EDU and WAT	Short distance
Tuber excavatum	*Tuber excavatum* FN433147 (96 %)	*Tuber rufum* UDB016154 (94 %)	–	WAT	Short distance

[a] Sequence was locked—e.g., not yet published thus having a limited access to available sequence-related information

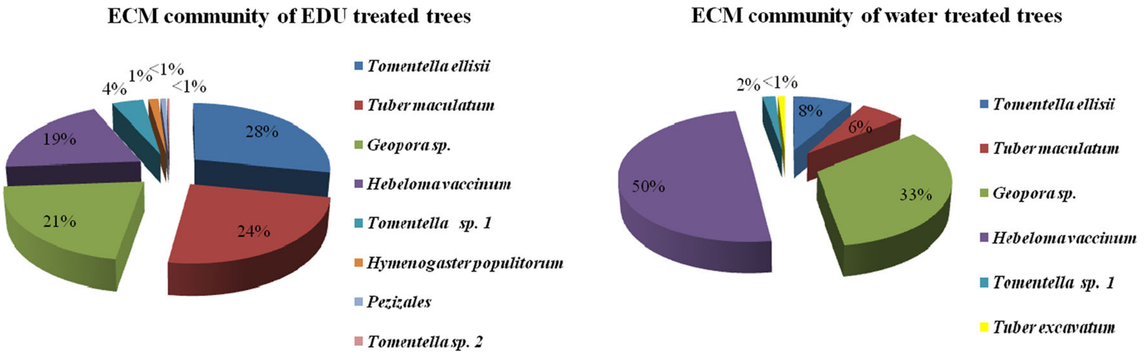

Fig. 1 Ectomycorrhizal fungi recorded on poplar trees treated with ethylene diurea (EDU) or with water (WAT)

EDU-treated trees (Table 3). However, these differences were not statistically significant. Colonization of poplar roots with END fungi was relatively low and was around 2 % in both treatments. In the poplar trees protected with antiozonant, a negative correlation between root colonization with AM and ECM fungi and a positive correlation between colonization with AM and END fungi were recorded. In the poplar trees treated with water, no significant correlation was found for investigated parameters, although Spearman's correlation coefficients had the same sign as in the EDU treatment (Table 4).

Exploration types

Half of the exploration types in the WAT-treated trees were contact type, while short-distance exploration types were the most abundant (67.4 %) in the EDU treatment (Table 5). EDU trees showed a significantly higher abundance of short-distance exploration types, while the abundance of contact and medium distance fringe exploration types tended to decrease compared to the WAT-treated trees (Table 5).

Discussion

Ethylendiurea is a known antiozonant, which reduces ozone visible injury and growth decline in plants (Manning et al. 2011); yet, belowground effects of EDU application are rather scarce. Our study of ECM community and root length colonization with ECM, AM, and END fungi of trees treated with EDU indicated potential changes in the number of fine roots and ECM community structure. The EDU-treated trees showed higher total number of fine roots, number of ECM types, and higher values of diversity indices in comparison with water-irrigated trees (Table 1). Although the variability among individual trees was elevated and the differences were not statistically significant (data not shown), those parameters were always higher in the EDU treatment compared to the not protected control.

Table 3 Means (±standard error) and levels of significance of Mann–Whitney *U* test for the effects of EDU (EDU vs. water-treated trees, WAT) on the root length colonization of poplar trees with ectomycorrhizal (ECM), arbuscular mycorrhizal (AM) and root endophytic (END) fungi

Root length colonization (%)	EDU	WAT	Mann–Whitney *U* test *p* value
AM fungi	16.44 ± 7.93	24.76 ± 9.31	0.391
ECM fungi	15.60 ± 2.23	21.84 ± 6.17	0.540
END fungi	2.08 ± 0.57	1.84 ± 0.25	1.000
AM/ECM ratio	1.41 ± 0.78	1.61 ± 0,75	0.713

Table 4 Spearman's correlation coefficient between root colonization with ectomycorrhizal (ECM), arbuscular mycorrhizal (AM), and root endophytic (END) fungi of poplar trees treated with antiozonant EDU or water (WAT)

	ECM	AM
EDU		
AM	$R = -0.900$	
	$p = 0.037$*	
END	$R = -0.700$	$R = 0.900$
	$p = 0.188$	$p = 0.037$*
WAT		
AM	$R = -0.800$	
	$p = 0.200$	
END	$R = -0.200$	$R = 0.400$
	$p = 0.800$	$p = 0.600$

* significant effect

Since total number of identified ECM fungi, vital ECM roots, and total number of fine roots were higher in plants treated with antiozonant compared to plants irrigated with water, we assume that EDU mitigates the negative effects of elevated ozone concentration on fine roots.

In Loblolly pine seedlings from two families differing in ozone sensitivity and exposed to four ozone concentrations, Qiu et al. (1992) recorded a decrease in foliage dry matter

Table 5 Mean relative abundance and levels of significance of *t* test for ectomycorrhizal exploration types of antiozonant (EDU) or water-treated (WAT) poplar trees

Exploration type	EDU	WAT	*t* test *p* value
Contact	16.466	55.712	0.152
Short distance	67.402	10.767	0.046*
Medium distance	9.418	22.316	0.512
Long distance	0	0	–

* significant effect

and root surface area with increasing ozone concentrations in the ozone-sensitive family. Other studies have reported that twice ambient ozone fumigation decreased root growth in aspen and diminished fine root biomass in birch and young aspen compared to charcoal-filtered air-treated trees (Coleman et al. 1995, 1996). As it was previously mentioned, in a 3-year exposure to ambient ozone, Hoshika et al. (2012) recorded significantly higher number of leaves, lower percentage of injured leaves, and higher total carbon assimilation in EDU-treated poplar trees compared to trees irrigated with water. Also, Hoshika et al. (2013) noted that in poplars irrigated with water, ambient ozone reduced total biomass of poplar trees with shifts in allocation to fine roots (−52 %) and coarse roots (−41 %) in comparison with EDU-protected poplar trees. Much lower number of fine roots in the trees exposed to ambient ozone compared to protected EDU plants recorded in our research was in accordance with their study. Furthermore, Andersen (2003) proposed that roots are not directly exposed to ozone, but they are affected through decrease in carbon allocation, which causes reduction in their biomass.

However, in a 5-year treatment with twofold ambient ozone on adult beech trees, an increase in the number of vital ectomycorrhiza, non-turgescent short roots, and number of ECM types was observed with the most pronounced and significant effect in the number of fine roots per volume unit (Grebenc and Kraigher 2007a). On the other hand, Paoletti et al. (2007a) did not find differences in fine root biomass and length, root tips, and mycorrhizal infection of adult ash trees after 1 year of EDU treatment.

Opposite to the results from adult trees, in beech seedlings, a significant reduction in the number of vital ECM types and number of ECM root tips was observed in the twofold ambient ozone treatment (Železnik et al. 2007). Andrew and Lilleskov (2009) recorded decrease in mean sporocarp biomass of ECM fungi with 10-year-old *Populus tremuloides* due to 9-year treatment with elevated O_3 and ambient CO_2.

Cudlin et al. (2007) suggested that the increased growth of fine root biomass and number of active ECM root tips in adult trees after several years of ozone fumigation might indicate a transient response of a tree to different source–sink relationships in the ecosystem. However, in our experiment, similarly to the work of Železnik et al. (2007) on young plants, exposure to ozone was long enough to generate final response of trees and their symbionts. According to Grebenc and Kraigher (2007a), increased O_3 concentration affected Species richness index and individual ECM species abundance. Community structure of ectomycorrhizal fungi with *Populus tremula*, based on their sporocarps, differed significantly in the treatments with O_3 and CO_2 (Andrew and Lilleskov 2009). Since ECM fungi differ in their requirements for carbohydrates, an ozone-induced decrease in carbon distribution underground could lead to change of compatibility between plant and fungus and therefore to the shift in community of ECM fungi (Andersen 2003). Also, a shift in ECM community can lead to different mycelial networks providing the fluxes of water and nutrients between different components in the ecosystem.

Since diversity indices, which indicate potential of an ecosystem to react to a changing environment (Atlas and Bartha 1981), were higher under the EDU treatment than in water-irrigated control, we concluded that the treatment with antiozonant positively influenced the ECM species composition and abundance of ECM types. On poplar trees irrigated with water, two ECM types made up the majority of ECM root tips [*H. vaccinum* (50 %) and *Geopora* sp. (33 %)], while on trees protected with EDU, four ECM types participated more equally in the ECM community [*T. ellisii* (28 %), *T. maculatum* (25 %), *Geopora* sp. (21 %), and *H. vaccinum* (20 %)] (Fig. 1). The increased abundance of some ECM fungi in control treatment pointed to their tolerance to the changed physiology of the poplar trees under ozone, while other ECM fungi decreased in abundance or even disappeared. These results are similar to results obtained in Norway spruce on polluted site (Kraigher and Al Sayegh-Petkovšek 2011). On the other hand, the number of low abundance species was higher in EDU treatment, similarly to the drift in adult trees ECM community under $2xO_3$ exposure, which opened new ecological niches for the development of different ECM types (Grebenc and Kraigher 2007b). Majority of ECM fungi recorded on *P. maximoviczii* × *berolinensis* had been previously observed on poplars. Three out of nine ECM types were members of the Thelephoraceae family. ECM fungi from this group are known as diverse and abundant members of ECM communities in drought adapted poplar forests in Hungary (Jakucs 2002; Jakucs et al. 2005). Two Thelephoraceae and one member of the Pezizales were roughly 90 % of all ECM types on aspen clones in the experimental field of Kaldorf et al. (2004). The most frequent taxonomic groups colonizing *P. tremula* on heavy metal contaminated soils were *Tomentella*, *Inocybe*, *Cortinarius*, *Hebeloma,* and *Tuber* (Krpata et al. 2008). In

Populus alba L. plantations, *Tuber* and *Tomentella* species and *H. vaccinum* were frequently found (Katanić et al. 2008, 2010). One of the most abundant ECM type in our study was identified as *Geopora* sp., while an ECM type identified as *Geopora cervina* was frequently recorded on *Populus nigra* × *maximowiczii* cv. Max (Hrynkiewicz et al. 2010).

Overall diversity of ECM types on studied post-agricultural site was low and similar to the one recorded on trees growing in stressful conditions such as heavy metal contaminated sites (7 ECM types, Regvar et al. 2010) or pyrite contaminated sites (6 ECM types, Katanić et al. 2011). Similarly, on clone *P. nigra* × *maximowiczii* cv. Max grown in short rotation coppice, Hrynkiewicz et al. (2010) recorded 5 ECM types identified as *G. cervina*, *Tuber rufum*, *Laccaria* sp., *Inocybe* sp., and Thelephoraceae. Low number of ECM types and structure of ECM community observed in our study were similar to their results. The relatively low numbers of ECM types and vital ECM root tips recorded in our study can be explained by the lack of ECM fungal propagules as a consequence of previous long-term agricultural land use. Karliński et al. (2010) also recorded lower percentage of root length colonization with ECM on poplars growing on post-agricultural lands in comparison with the literature.

Our results showed that in trees irrigated with water, the percentage of root length colonization with AM and ECM fungi was higher (Table 3), while the number of fine roots was lower compared to EDU treatment (Table 1). According to Smith and Read (2008), the rate at which the root system becomes colonized (i.e., the percentage colonization) is influenced by the rate of formation of the infection units and their rate of growth and by the rate of growth of the root system. So, lower percentage of root length colonization with ECM and AM fungi in EDU-treated trees could be explained by higher rate of root growth.

The direct comparison of each parameter is less clear. In general, the AM and ECM colonization degree in *Populus* spp. shows high variability and also suggests differential host/genotype susceptibility (Khasa et al. 2002; Takásc et al. 2005). The percentage of fine root colonization with fungal endophytes was comparable in the treatment with EDU and WAT and overall lower than in black, balsam, and hybrid poplars analyzed by Karliński et al. (2010, 4.2–9.3 %) or in the roots of *P. fremontii* studied by Beauchamp et al. (2006, 7.4–23.4 %).

In the treatment with EDU, a negative correlation between AM and ECM and a positive relationship between AM and END were observed. A decrease in AM with increasing ECM fungi in the EDU treatment is in accordance with the results of Karliński et al. (2010) who observed significant negative correlation between AM and ECM at two undisturbed sites. Neville et al. (2002) recorded competition between AM and ECM fungi that colonize roots of *P. tremuloides* and they consider that these two fungal groups prefer different soil layers, i.e., different soil conditions. However, on the sites contaminated with heavy metals, Karliński et al. (2010) recorded a positive correlation in colonization with AM and ECM. Also, Saravesi et al. (2011) observed the presence of AM spores in ECM fungal mantle and concluded that both mycorrhizal partners could coexist in the same root.

Positive correlation between colonization with AM and END fungi recorded in treatment with EDU is in accordance with Karliński et al. (2010) who also observed an association between these two fungal groups at sites contaminated with heavy metals.

In roots of poplar trees treated with antiozonant or water, we found the same correlation trends in colonization with AM, ECM, and END fungi (although insignificant in water-treated roots) and it might be concluded that EDU did not affect correlation in colonization with these fungi.

Cudlin et al. (2007) stated that ECM colonization was an unsuitable parameter for environmental change, but fine root length and biomass could be useful. Kraigher et al. (2007) concluded that biodiversity indices of ECM types of spruce could be successfully applied as bioindicators of pollution stress in forest soil ecosystems, while results for beech were controversial. Difference in exploration types could be important finding due to their possibly different ecological function and preference to different soil conditions. The EDU treatment shifted the community from a contact dominated exploration type to a short-distance exploration type, thus changing the key area of nutrient acquisition from a substrate in direct contact with the ECM to a broader area around the ECM (Agerer 2001).

After a 3-year treatment with EDU as soil drench, Hoshika et al. (2013) found no difference in nitrogen content between EDU- and WAT-treated poplars. The improvement of poplar growth due to EDU application was thus interpreted as protective effects of EDU on the O_3 impacts at ambient level. This could either be a direct influence of EDU on the source symbiotic plant through increasing its fitness and carbon allocation belowground (Andersen 2003) or an indirect EDU effect on the rhizosphere structure and function. The relationship between exploration types and soil chemistry was proven by Rudawska et al. (2011) who demonstrated the correlation of a contact type with higher nutrient availability, while medium fringe exploration type was found exclusively on the heavy metal-influenced site. The shift of the ECM community into types that are more diverse and better adapted for distant nutrient acquisition indicates a positive influence of the antiozonant EDU on the plant status. Such plants are likely to be more vital and thus better protected from the environmental stressors such as ozone.

This is the first study about the impact of the antiozonant ethylene diurea on mycorrhizal community structure and colonization of poplar roots with ectomycorrhizal, arbuscular mycorrhizal, and endophytic fungi under elevated ozone concentration. Overall, the number of ectomycorrhizal types, fine roots, and diversity indices were higher in EDU-treated plants than in the plants irrigated with water. Since variability among samples was too high, only a few statistically significant differences were observed. Short-distance exploration type was significantly more abundant in the EDU treatment indicating potentially different ecological roles of ectomycorrhizal fungi in association with poplars protected with EDU relative to the unprotected ones. Our results warrant further studies on ethylene diurea influence on mycorrhizal fungi.

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Conflict of interest The authors declare that they have no conflict of interest.

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