RELATIONSHIPS BETWEEN LINDEN TREE DECLINE AND MYCORRHIZAL COMMUNITY IN URBAN ENVIRONMENT – FIRST RESULTS

Claudia Alzetta, Linda Scattolin, Sergio Mutto Accordi
Dipartimento Territorio e Sistemi Agro-Forestali, Università degli Studi di Padova, viale dell’Università 16, I-35020 Legnaro, Padova.

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Abstract: the structure of ECM community is often used as a bioindicator in forest ecosystem. The question raised in this study is whether it can also describe plant fitness in urban environment.

1. Introduction
Among the city’s most prevalent trees species, in Italy *Tilia* spp. has been appreciated for centuries for its large canopy shadow, its good smelling flowers and its assessment ability to urban environment. However, elder trees often reveal decline symptoms: canopy transparency, reduction of foliar mass, ramification’s degree and foliar surface, epicormic twigs, falling of dead branches.

Urban plant fitness is known to be associated to the root systems’ functionality and the urban soil features, often characterized by an elevated pH, increased compaction, high levels of inorganic and organic contaminants, elevated temperatures, interrupted nutrient cycles. The ectomycorrhizal (ECM) symbiosis between soil-inhabiting fungi and plant roots is a mutualistic association widespread not only in forest ecosystems, but also in urban environments: in the extremely difficult urban environment it could help in understanding the ecological bases for a good-developing plant root system and a consequent plant fitness. Unfortunately, even if much is known of the ectomycorrhizal association in forests, little is known of the benefits of it within an urban environment and the challenges unique to the city sites (Garbaye 1996, 1999; Nielsen 1999).

The aim of this ongoing research is to verify if the ECM community composition can be associated to different severity of declining symptoms in urban trees and to the main physical and chemical urban soil features.

2. Materials and methods
2.1 Study site and design
Among the most frequent and representative plantations in public streets, parks and yards of Padova (northeast of Italy), *Tilia x europaea* L. resulted as the most frequent one, by means of the Tree Inventory of Padova Municipality database. The plantation sites were selected according to the most representative trunk diameter (45 cm DBH), plant height (20-25 m), and the presence of the two most characteristic decline classes, classified as “moderately declining” (CL1) and “strongly declining” (CL2). Among these identified sites, 2 *Tilia x europaea* L. plantations [Pio X (PD) and Landucci (LN)] were randomly selected. In each site, 8 trees were selected with a distance of at least 8 m from the nearest tree and coded with a number (1÷16). In PD site four trees (nn. 1-2-4-8) belong to CL1 and the others (nn. 3-5-6-7) to CL2. In LN site all the 8 trees (9÷16) belong to CL1.

2.2 Data collection
Considering each tree as the central point of the 4 main cardinal axes, and considering the four virtual quadrants obtained, 12 cylindrical soil cores (30 cm depth) were collected for each plant, along two different directions: one belonging to the first and/or fourth quadrant (N), the opposite one to the second and/or third quadrant (S). Along each direction 6 soil cores were collected at 3 distances from the tree trunk [100, 150 and 200 cm (Dist)], along two parallel directions having a distance of 20 cm one from each other. These parallel directions are coded as “dx” when situated...
on the right side if facing “N” direction, and as “sn”, when situated on the left side. Soil cores were collected in the last three days of May 2007.

All roots were carefully cleaned in tap water and, among those with Ø≤2 mm and undamaged and fully-developed apical tips, a number of randomly selected root tips, in order to count 20 EM for each sampling point, was differentiated into “non vital” (NV, scurfy surface and easily detachable cortex, with or without remnants of ECM mantle), “vital non-mycorrhizal” (NM, well-developed, turgid and inflated tip, mantle lacking), or “vital ectomycorrhizal” (EM, as above, but with ECM mantle), according to Montecchio et al. (2004). By means of both dissecting and compound microscopes, the 20 EM root tips of each sample were separated into anatomotypes and coded recording colour, type of ramification and features of mantle surface, type of outer, middle and inner mantle, and chemical reactions. Type of emanating hyphae, rhizomorphs, cystidia, laticifers were then observed, according to Agerer (1991). 53 ECM anatomotypes were described through the available literature (Agerer 1987-2002; Agerer & Rambold 2004-2005). All specimens, preserved in FEA (formalin:ethanol 70%:acetic acid=5:90:5) solution and CTAB for molecular analyses, are stored in the herbarium of the TeSAF Dept., University of Padova.

2.3 Data analyses

The number of NV, NM and EM tips was calculated in each soil core, for each plant. The Kruskal-Wallis non-parametric test (P<0.05, Statistica, StatSoft Inc., Tulsa, OK, USA) was used to verify statistical differences in EM among different trees.

Relations among environmental variables (PD, LN, dx, sn, Dist, N, S, CL1, CL2) and species abundance of ectomycorrhizae were analysed by Multivariate Ordination Techniques (Jongman et al. 1995) using CANOCO (software for Canonical Community Ordination, 4.5 Version). Detrended Correspondence Analysis (DCA; Hill and Gauch 1980) was performed to obtain estimates of gradient lengths in standard deviation units. The detrending by segments method was applied. Unimodal (DCA and CCA; ter Braak 1986) analyses were performed. DCA considered sampling point as cases, analyzing qualitatively variables (PD, LN, dx, sn, distance, N, S, CL1, CL2). Canonical Correspondence Analysis (CCA) was then done, scaling with a focus on interspecies distances and using a bi-plot scaling type. By means of Forward selection of Environmental variables, the variance singly expressed by each variable [lambda-1 (λ1)], named marginal effect, and the conditional effect, showing the variance in order of their inclusion in the model [lambda-A” (λA)] were investigated, according to ter Braak & Šmilauer (2002).

3. Results

3.1 Root tips ectomycorrhization

The total number of analyzed root tips was 7,944. The total number of NV, NM, and EM resulted as 4,084, 28 and 3,832, respectively. No significant difference (Kruskal-Wallis Test, P<0.05) was found among trees when considering EM root tips (Fig. 1).

Figure 1. Kruskal-Wallis test (P<0.05) on EM root tips (N=192, H=53.50, P=0.0000), no significant differences among the 16 trees.
3.2 Ectomycorrhizal community distribution

The DCA, considering 192 sampling points, showed long gradient lengths (>3*Standard Deviation) and demonstrated that the eigenvalues of axis 1 (horizontally) and 2 (vertically) are 0.86 and 0.82, respectively.

In CCA, the eigenvalues of axis 1 and axis 2 are 0.726 and 0.204, respectively. Fig. 2 shows the CCA bi-plot of ECM species and environmental variables displaying 17.4% of the inertia (=weighted variance) in abundances. The first gradient, with eigenvalues >0.30, indicated strong gradients (ter Braak & Verdonschot 1995), and a high significance of the first axis and all the canonical axes were present when subjected to the Monte Carlo permutations test ($P=0.0020$). By means of the canonical coefficients among variables and axes, we inferred that the first axis is mostly defined by site location and that the second axis is defined by CL1 and CL2. The intra-set correlations of: axis 1 with PD and LN were ±0.988 (pos. value with PD); of axis 2 with CL1 and CL2 were ±0.7966 (neg. value with CL1). ECM species mainly associated to PD and LN site were respectively in the right and in the left area of the diagram; those associated to CL2 and CL1 were respectively concentrated in the upper and in the lower part of the diagram. The distance between species points in the bi-plot scaling (with a focus on species distances) approximated the chi-square distance between the species distributions. The ECM associated to PD site were 2, 32, 35, 17; to CL2 were: 76, 44, 48, 46; to CL1: 12. Many ECM species are associated to LN site: among them, the most important resulted to be: 1, 8, 57, 40, 68, 39, 41.

![Figure 2. CCA bi-plot of the 53 ECM species and the 9 environmental variables (Distance (Dist), site location (PD, LN), direction (N, S) side, (dx, sn), decline class (CL1, CL2)), investigated in 192 sampling points](image)

The marginal effects in CCA demonstrated that the variables that are better suited to explain the model are PD, LN, CL2 and CL1 (respectively $\lambda_i= 0.71, 0.71, 0.30, 0.30$); the conditional effects, showing the environmental variables in order of their inclusion in the model, demonstrated that the most useful features to explain the model are the site location (PD and LN) ($\lambda_A=0.71, P=0.002$), CL2 ($\lambda_A=0.20, P=0.002$), N ($\lambda_A=0.14, P=0.004$).
Comparing the first eigenvalues of both DCA and CCA analyses (0.86 and 0.72, respectively), and as in both DCA and CCA the species-environment correlations of the first axis result as higher, all the environmental variables together are able to explain the main variation of the ECM distribution incompletely (ter Braak 1986).

4. Discussion

The analysis of data collected during 2007 springtime emphasized the presence of few ECM anatomotypes peculiar to a site and the association of specific ECM consortia to different tree decline classes.

Due to this result it is possible to assume, even if as a working hypothesis, that the ECM community structure is a bioindicator that might be used in urban environment in order to improve the urban forest knowledge and consequently its management.

In order to corroborate such presumption, further sampling collections and analyses are being carried on in order to better describe ectomycorrhization and seasonal effects. Moreover, soil chemical and physical analyses are in progress in order to detect the potential environmental nutritional variables that may affect root tips vitality and ECM community structure. Besides, trees health condition will be evaluated, in a objective way, through measures of leaves chlorophyll content.

References


Cairney & Chambers 1999


