Macrofungi of urban and anthropogenic influenced habitats of Hungary

Thesis of PhD dissertation

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Introduction

The area of the urban landscapes is continuously expanding, and the urban population is increasing over the years. With the rise of cities and the surrounding agglomeration zones, the habitats of macrofungi could have been modified or even dramatically changed. Only a few information is available concerning the macrofungi of urban habitats, the number of researches in this field is low. Long-term mycological surveys have been performed in urban and anthropogenic habitats in Hungary. This study aims to provide the first comprehensive summary on urban macrofungi of Hungary which expands the understanding of urban macrofungal communities. Furthermore, the study also draws attention to the importance of Hungarian urban territories by describing several surprisingly diverse macrofungal groups.

Aims of the study

The aim of present work is to provide information about the characteristics of fungal communities in the anthropogenic influenced environment by analysing data from long-term mycological surveys and collections in urban areas. In this study our objectives were the following:

- Preparation of comprehensive species list, computer database and fungarium of the collected macrofungi in urban territories.
- Investigation of the macrofungal communities of linden trees (*Tilia* spp.), commonly used ectomycorrhiza-forming species in urban arboriculture.
- Investigation of the most frequent urban ectomycorrhizal (EM) macrofungal group, the family Inocybaceae.
- We performed molecular analyses of morphologically hardly identifiable taxa and phylogenetic reconstructions in the necessary cases.
- Preparing DNA barcode sequences and depositing them in the GenBank international public database.
- Analyzation of taxonomic and spatial distribution of the registered macrofungi in urban habitats.
- Mycological characterization of the common urban habitats of macrofungi.

- Systematic analysis of the registered macrofungi and their habitats based on the functional groups (fungal lifestyles).
- Comparison of the registered macrofungal communities of Hungarian urban territories with macrofungal communities of natural and semi-natural habitats of Hungary. Furthermore, comparison of the registered macrofungal communities of Hungarian urban territories with urban macrofungal communities of other European urban habitats.
- Exploring the possible reasons behind the successful spread of macrofungi in urban areas.

Materials and methods

The data used in this work are provided by the fungarium of the Department of Plant Physiology and Molecular Plant Biology of the ELTE TTK. Macrofungi were collected between 1987 and December 2022, exclusively in urban and anthropogenic habitats. Fungal samplings were conducted throughout the year, mostly during rainy periods, in different urban habitats, most often in Budapest and its agglomeration areas. The majority of the macrofungal data were registered in 11^{th} and in 14^{th} districts of Budapest, mainly in the Lágymányos and Alsorákos quarters of Budapest. Nevertheless, macrofungi were collected in more urban parts of Hungary.

The collected macrofungi were registered with sampling date, location, and the basic description of the habitat. Furthermore, the type of the habitat and the adjacent tree and plant species were also recorded. In case of EM fungi, the potential symbiotic host were specified. While, in the case of wood inhabiting saprotroph and necrotrophic parasite macrofungi the substrate and its condition were recorded. The stages of substrate decomposition were evaluated by a modified seven-level scale which originally used by silviculture management. Most of the collected fungal specimens were dried and deposited in the fungarium of the Department.

Macrofungi were collected in several types of urban habitats which were categorized by us. We distinguished two types of urban habitats, like "primary habitat types" and "secondary habitat types". The primary habitat types include the most common urban habitats with general anthropogenic influence, such as gardens, parks, tree avenues, cemeteries, roadside green belts, roadsides, plantations, etc.. The secondary habitat category refers to the more specific, accurate

sites where the sporocarps were found. Such as mowed lawns, watered and mowed lawns, flower beds, shrubbery areas and groves.

We also categorized the habitats of the registered macrofungi depend on the level of the anthropogenic disturbances. A seven-level category scale were created depending on the anthropogenic influences. We distinguished the following "urban categories":

- URB 1: Extreme urban environment. "Concrete deserts" close to the busiest roads and junctions. The number of green areas in the habitat is low, can be found only in small patches. Trampling activity and vibration effect caused by cars are a continuous phenomenon.
- URB 2: Busy downtown area. Characterized by dense, but discontinuous car and pedestrian traffic. Higher level of concrete cover is usual, but small green areas also can be found. The soil is typically compacted.
- URB 3: This category includes the city parks, gardens, cemeteries and common green areas. Anthropogenic disturbances are present, but not heavy and not general. Artificial and natural elements also present, the level of concrete cover is low. Soil structure and productivity are more favourable for macrofungi.
- URB 4: More natural urban landscapes where the presence of anthropogenic influences is obvious but not significant, rather periodic. Natural elements dominate in the habitat, but traces of human activity are clearly visible. Such as plantations, suburban and rural holiday areas, outskirts of settlements.
- URB 5: The typical anthropogenic influences restricted to land management or touristic use, otherwise the main parts of these areas are mostly natural. For example, forest parks located on the outskirts of cities, where tourism and/or economic activities are actively carried out.
- BOT: The category includes the open-air areas of arboretums and botanical gardens. These areas are characterized by their own special flora, often with exotic plant communities. Although these places were created by humans, but they are often interspersed with natural elements. And moreover, the gardens are usually managed in a nature-friendly way, which is so environmentally conscious.
- URB 0: Special urban category. It refers to the indoor areas of human-made structures and artificial habitats like greenhouses, swimming pools, interior of houses. Typically, significant human impact on the environmental factors is relevant.

Identification to species level in the majority of species was based on macro- and micro-morphological characters. Additionally, we performed molecular phylogenetic analyses when sporocarps could not be identified with certainty by morphological characters.

Results and discussion

1. During our mycological surveys we have registered the data of 2,269 macrofungal occurrences collected in urban habitats. We demonstrate a species list of the registered macrofungi.

2. We performed successful molecular analyses of 120 macrofungi (114 Basidiomycota, 6 Ascomycota) collected in urban habitats. The sequences were deposited to the NCBI GenBank public database. Sequencing of the ITS gene which generally used for species identification was carried out in most cases and in some cases the LSU gene was also sequenced. Eleven species belonging to the family Inocybaceae were reported for the first time from Hungary, namely *Inocybe alluvionis, I. amelandica, I. caesaraugustae, I. ghibliana, I. pararubens, I. psammobrunnea, I. obscuroides, I. zethi*; *Mallocybe siciliana*; *Pseudosperma aureocitrinum* and *P. permelliolens*.

3. We paid special and extra attention to investigating the macrofungi of the linden tree (*Tilia* spp.), a commonly used species in urban arboriculture, with particular attention to better understanding the macrofungi of the planted linden avenues and park trees. A total of 173 macrofungal records were registered from different Hungarian urban *Tilia* avenues and gardens between 2009 and 2020 as a result of 71 sampling occasions. Two recorded species belong to the phylum Ascomycota and 58 species to the Basidiomycota. We achieved successful molecular analyses in the case of 54 macrofungi. We performed further phylogenetic analyses of morphologically hardly identifiable taxa associated with *Tilia*, such as family Inocybaceae, *Russula insignis*, *Russula pectinata*, and a rare species in Hungary, *Tomentella fuscocinerea* s.l..

4. Molecular phylogenetic analysis of 28 Inocybaceae specimens was carried out in anthropogenically affected habitats of Budapest, Hungary. Using the sequences of the ITS region of nrDNA, we performed a phylogenetic reconstruction in their case. A total of 18 different species belonging to the Inocybaceae family were

registered and identified by genetic analysis. In the case of *Mallocybe* aff. *heimii* (M117) and *Mallocybe* aff. *plebeia* (M118) we demonstrated that they are probably undescribed species. We considered the situation of *Mallocybe* aff. *malenconii* and *Inocybe phaeoleuca* s.l. with uncertain taxonomic position. The most frequently collected *Inocybe* species in urban habitats, like *I. aeruginascens, I. furfurea*, *I. splendens* s.l., *I. alluvionis, I. griseovelata* and *I. semifulva* were discussed.

5. The nrDNA ITS barcoding region of 36 other macrofungal taxa was sequenced. We could identify *Entoloma defibulatum, E. phaeocyathum*; *Gymnopus trabzonensis; Paxillus obscurisporus* and *Xylaria digitata* macrofungal species with scarce published data in Hungary. We also sequenced additional interesting, potentially new-to-science macrofungal taxa, like *Clitocybe* sp., *Hygrocybe* sp., *Lyophyllum* sp., *Mycenella* sp. and *Pluteus* aff. *multiformis*.

6. Macrofungi collected in urban habitats belong to 415 different taxa. 365 taxa belong to the Basidiomycota, while 50 taxa belong to the Ascomycota phyla. The most diverse order is Agaricales containing 24 different families and 271 taxa. Pezizales (7 families, 35 species), Polyporales (8 families, 27 species), Boletales (7 families, 28 species), and Russulales (3 families, 12 species) proved to be also frequently occurring orders. The documented macrofungal taxa belong to 71 family and 165 different genera. Similarly to the distribution of the orders, the family with the highest number of species was Agaricaceae. The other more frequent families were Inocybaceae, Psathyrellaceae, Strophariaceae and Tricholomataceae. The most collected genera were *Inocybe* (24 taxa), *Agaricus* (17 taxa), *Hebeloma* (11 taxa), *Lepiota* (10 taxa) and *Helvella* (9 taxa).

7. We evaluated the primary and secondary habitats of the registered macrofungi. The most frequent primary habitats were the gardens (893 data), parks (771 data) and roadsides (318 data) for the urban macrofungi. According to our examinations, these primary habitats were the most suitable for macrofungi in urban territories, 87.5% of the mushrooms were registered in the mentioned primary habitats. In the case of 689 macrofungi records, the secondary habitat type was also listed. The most macrofungal data were registered in the mowed lawns, watered and mowed lawns and in the groves secondary habitat categories.

8. In addition, the habitats of the registered macrofungi were categorized based on the anthropogenic influences/disturbances. 80% of the data registered in

URB 3 category habitats. The URB 4 category was the second most populated habitat type with much lower value. Only 6% of the total macrofungal data were registered in this type. More than a hundred macrofungal data, represent 5% of the total data, were collected in botanical gardens and arboretums ("BOT" category), which belong to also the least-disturbed urban habitats. We registered altogether 113 macrofungal data in extremely and highly disturbed habitats belonging to the URB 1 and URB 2 categories, which represent together around 5% of the total data.

9. Among the macrofungal taxa collected in anthropogenically influenced habitats, most (163 taxa) belong to soil saprotrophic fungal communities. More than a quarter of macrofungi collected belong to EM fungal communities, totally 125 taxa. With a total of 86 registered taxa, the proportion of wood-inhabiting saprotrophic fungi accounts for almost a quarter of the overall taxa. While necrotrophic parasite macrofungi were registered with 23 taxa, representing a total share of 6%. Overall, it can be said that the majority of the taxa collected in urban territories belong to the saprotrophic functional group, almost total of 64% of the registered macrofungi. Saprotrophic functional group includes, besides the mentioned soil and wood-inhabiting macrofungi, other macrofungi which are saprotrophic on other plant remains (6 taxa), coprophilous (2 taxa) and bryophilous (8 taxa) macrofungi.

10. We investigated the distribution of all registered macrofungi (2269 data) in the different primary habitat types based on the functional groups.

In the gardens, which was the most frequent primary habitat type, 39% of the collected macrofungi belong to EM functional group. EM community is closely followed by the group of soil saprotrophic fungi, whose share is 35%. The occurrence data of the necrotrophic parasites and the wood-inhabiting saprotrophic fungi together reach a share of 21.5%.

In the case of parks, the primary habitat category with the second highest number of registered taxa, the proportion of soil saprotrophs is higher, reaching around 40%. While the proportion of EM functional group is 31%. The proportion of necrotrophic parasite and wood-inhabiting saprotrophic fungi is higher in parks than in the gardens. Together they reach 26%, and the number of necrotrophic parasite macrofungi was the highest in the parks (62 data in total).

The proportion of registered EM fungi on roadsides is only 20%, while that of soil saprotrophic fungi is 36%. The proportion of necrotrophic parasites and wood-inhabiting saprotrophic fungi on roadsides is remarkably high, approximately 42%.

11. We investigated the distribution of the registered macrofungi based on the functional groups in habitats which were categorized according to anthropogenic influences (urban categories). Based on the results, it appears that in the more anthropogenically disturbed habitats could register fewer EM macrofungi than in the less influenced habitats.

In extreme urban habitats, URB 1 category, we couldn't register EM fungi at all. All registered macrofungi are saprotroph or necrotrophic parasite.

We could register a few EM fungi in URB 2 category, busy downtown areas but the proportion of the EM fungi is less than 10%. The majority (74%) of the registered macrofungi belong to saprotrophic functional group in this urban habitat type.

The proportion of EM fungi in URB 3 category habitats already reaches 34%, but saprotrophic fungi dominates by far. Totally, 58% of the registered macrofungi belong to saprotrophic functional group. Among the saprotrophs, the soil saprotrophic macrofungi are substantial, the proportion of which is 37% in this urban habitat type.

In the somewhat less disturbed URB 4 category habitats, the proportion of EM mushrooms is only 25%. The reason for this being that the monoculture plantations (often *Robinia* sp.) on the edge of the cities also belonged to this urban habitat category.

The proportion of registered EM fungi was the highest in more natural habitats, categorized as URB 5. In that category, proportion of EM fungi already reach a total of 48%. The proportion of the registered saprotrophic macrofungi is the same as in case of EM group, 48% of total. A much higher proportion of soil saprotrophic macrofungi than wood-inhabiting saprotrophs was collected in this habitat category.

12. We evaluated the registered macrofungi included in the proposed Hungarian Red List of macrofungi (Rimóczi *et al*. 1999) based on their habitats, categorized according to anthropogenic influences (urban category). We registered in urban habitats the occurrence data of 1,020 macrofungi included in the proposed Red

List. Based on the results, it appears similar to the distribution of functional groups in different urban categories. Therefore, in more disturbed habitats could register fewer endangered species than in more natural habitats.

13. Comparative analyses of the registered urban macrofungal communities with communities of more natural habitats examined in the surveys of National Biodiversity Monitoring System (Benedek 2011; Pál-Fám 2001c; Pál-Fám *et al*. 2007; Siller 2004; Siller *et al*. 2013) were carried out. We found a different functional spectrum and species distribution between the natural/near-natural young and middle-aged forests included in the comparison, and the examined urban areas. In the case of examined older, undisturbed forest stands in Hungary, we found also different functional spectrum and species distribution compared to the urban areas. The more natural areas included in the comparison share with urban areas mainly wood inhabiting macrofungi such as *Bjerkandera adusta*, *Fomes fomentarius*, *Mycena galericulata*, *Schizophyllum*. *commune*, *Stereum hirsutum* and *Trametes versicolor*. We have discussed the mentioned common species.

14. Comparison of the registered macrofungi in Hungarian urban territories with the urban territories of two other European cities was performed. Macrofungal communities and functional spectra recorded in the territories of Coimbra (Barrico *et al*. 2012) and Sicily (Ferraro *et al*. 2022) show similarities with those experienced in Hungarian urban territories. Several macrofungal species were registered in all three urban studies.

15. Considering the functional traits described in the summary work of Halbwachs & Bässler (2021), we tried to reveal relations between the successful urban spread of certain macrofungi taxa and their traits. Such functional traits are, for example, the vitality of mycelium, as well as other physiological traits. Such as the EM host specificity of the mycelium or substrate specifity. Biodegradation ability, or the lengths of the vegetation and fructification period. Furthermore, the size and structure of the sporocarps, the size and shape of the spores, and the way they spread.

References

Barrico L., Azul A.M., Morais M.C., Coutinho A.P., Freitas H., Castro P. (2012): Biodiversity in urban ecosystems: Plants and macromycetes as indicators for conservation planning in the city of Coimbra (Portugal). Landscape and Urban Planning 106: 88-102.

Benedek L. (2011): A Központi-Börzsöny nagygombái. PhD disszertáció, Szent István Egyetem, Budapest.

Ferraro V., Venturella G., Cirlincione F., Mirabile G., Gargano M., Colasuonno P. (2022): The checklist of sicilian macrofungi: second edition. Journal of Fungi 8: 566. https://doi.org/10.3390/jof8060566

Halbwachs H. & Bässler C. (2021): Functional Traits of Stipitate Basidiomycetes. Encyclopedia of Mycology vol 1. pp. 361-377. https://doi.org/10.1016/B978-0-12-819990-9.00047-0

Pál-Fám F. (2001c): A Mecsek hegység nagygombái. Mikológiai Közlemények, Clusiana 40(1-2): 5-66.

Pál-Fám F., Siller I., Fodor L. (2007): Mycological monitoring in the Hungarian Biodiversity Monitoring System. Acta Mycologica 42(1): 35-58.

Rimóczi I., Siller I., Vasas G., Albert L., Vetter J., Bratek Z. (1999): Magyarország nagygombáinak javasolt Vörös Listája. Mikológiai Közlemények, Clusiana 38(1-3): 107-132.

Siller I. (2004): Hazai montán bükkös erdőrezervátumok (Mátra: Kékes Észak, Bükk: Őserdő) nagygombái. PhD disszertáció, BKÁE, pp. 113.

Siller I., Kutszegi G., Takács K., Varga T., Merényi Zs., Turcsányi G., Ódor P., Dima B. (2013): Sixty-one macrofungi species new to Hungary in Őrség National park. Mycosphere 4(5): 871-924. http://dx.doi.org/110.5943/mycosphere/4/5/3

Publications based on the results presented in the dissertation

Csizmár M., Cseh P., Dima B., Assamere A., Orlóci L., Bratek Z. (2023): Contribution to the taxonomic knowledge of the family Inocybaceae in Budapest, Hungary Applied Ecology and Environmental Research 21(1): 409-420. http://dx.doi.org/10.15666/aeer/2101_409420

Csizmár M., Cseh P., Dima B., Orlóci L., Bratek Z. (2021): Macrofungi of urban *Tilia* avenues and gardens in Hungary. Global Ecology and Conservation, 28: e01672. https://doi.org/10.1016/j.gecco.2021.e01672

Csizmár M., Tóth A., Bratek Z. (2018): A városi környezet nagygombavilága - fajösszetételének és változásainak jellegzetességei. Természetvédelmi Közlemények 24: 59-66. http://dx.doi.org/10.20332/tvk-jnatconserv.2018.24.59