Final project report: URBANMYCOSERVE

Project acronym		URBANMYCOSERVE
Project title		Understanding and Managing Urban Mycorrhizal Communities
Project coordinator	Person (Title, Full Name)	Prof. Olivier Honnay
	Entity (Company/organization)	University of Leuven
Project period (Start date – End date)		March 2017 – October 2020
Project website, if applicable		www.urbanmycoserve.org

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List of partners involved in the project (company/organization and principal investigator). Please use partner numbers to specify the tasks, work packages and inputs of each partner in sections 4.3, 5 and 6.2 to 6.4.	**Partner 1**: Plant Conservation and Population Biology, Department of Biology, KU Leuven, Olivier Honnay **Partner 2**: Division of Forest, Nature & Landscape, Department of Earth & Environmental Sciences, KU Leuven, Ben Somers **Partner 3**: Escola Superior de Biotecnologia, Catholic University of Portugal, Paula Castro **Partner 4**: INRA, Université Clermont Auvergne, Thierry Ameglio **Partner 5**: Laboratoire des sciences de l'ingénieur, de l'informatique et de l'imagerie, Strasbourg University, Pierre Kasendeuch

1. Short description for publicity


Urban trees provide many ecosystem services to the urban population. Yet, these services may be jeopardized by the typical poor habitat quality of the urban environment which negatively affects tree health. Given their multifunctional role in forest ecosystems, ectomycorrhizal fungi (EcM) may contribute to urban tree health. The objective of this project was to understand and manage urban EcM communities to improve urban tree health. We could first demonstrate that soil sealing and limited root space strongly impeded tree health and accelerated leaf senescence. We then discovered a very high diversity of EcM associated with the urban model tree *Tilia Tomentosa* across three European cities. Local EcM communities were strongly affected by soil acidity and soil organic matter. We found that soil organic matter positively affected tree health, more specifically tree water availability and tree photosynthetic capacity. Yet, we could not directly relate tree health to the local EcM community composition. Controlled experiments revealed that *Russula parazurea* was the most promising EcM species to promote tree growth and mitigate water stress for our model species. Field experiments showed that inoculation of adult trees in an urban context using the mesh bag method was most efficient, as it offered the inoculated fungus a favorable microcosm in terms of soil acidity and soil aeration. Our results also suggest that urban planners should not overlook the importance of soil quality and soil water holding capacity for maintaining the health of urban trees and the ecosystem services they deliver.

2. Summary

Urban trees provide many ecosystem services to the urban population. Yet, these services may be jeopardized by the typical poor habitat quality of the urban environment which negatively affects tree health. Given their multifunctional role in forest ecosystems, ectomycorrhizal fungi (EcM) may contribute to urban tree health. The objective of this project was to understand and manage urban EcM communities to improve urban tree health. We first demonstrated that limited root space and soil sealing strongly negatively affected urban tree specific leaf area and leaf water content, and also resulted in accelerated leaf senescence. We discovered a very high diversity of EcM associated with the urban model tree *Tilia Tomentosa* across three European cities and showed that the local EcM communities were strongly affected by soil acidity and soil organic matter. Heavy metals in the soil, however, did not negatively affect EcM communities. We found that soil organic matter positively affected tree health, more specifically tree water availability and tree photosynthetic capacity. Yet we could not directly relate tree health to the local EcM community composition. Furthermore, when developing and propagating dedicated EcM-inocula, we demonstrated that the five selected inoculum species can grow under a wide soil acidity range. In an ex-situ inoculation experiment, we showed that inoculation had a larger growth promoting effect at an acidic pH, i.e. a more challenging environment for Tilia, which prefers an alkaline soil. The experiment also showed that EcM inoculation mitigates water stress and improves the resilience of the tree. The ex-situ experiment also showed that *Russula parazurea* was the most promising EcM species to promote plant growth and resilience to water stress. Therefore, this EcM species was used in an in-situ inoculation experiment in the three cities (Leuven, Strasbourg and Porto). Our analysis showed that inoculation of adult trees in an urban context using the mesh bag method was efficient, as it offers the inoculated fungus a favorable microcosm in terms of soil acidity and soil aeration, in contrast to the reduced amount of mycorrhiza found in compacted soils. Therefore, this method of inoculating urban trees looks promising to promote urban tree health and the delivery of ecosystems.

3. Objectives of the research

The first general objective of URBANMYCOSERVE was to understand the environmental drivers of the EcM community composition and the EcM diversity of urban trees, and of their role in mediating tree physiology, tree health and (the resilience of) tree ecosystem service provisioning. The second general objective of URBANMYCOSERVE was to explore the possibility of active management of the EcM-community of urban trees, through their inoculation with dedicated EcM-mixtures. We selected a model tree species (*Tilia tomentosa*) occurring in three European cities, covering a N-S gradient (Leuven (Belgium); Strasbourg (France) and Porto (Portugal).

The specific objectives of URBANMYCOSERVE were to:

1. Fully characterize the EcM community composition, EcM richness and diversity of the model tree species, using high-throughput sequencing technology;
2. Identify the environmental drivers of EcM community composition, richness and diversity;
3. Quantify tree health, growth, and ecosystem services provided by the model tree species, and identify the mediating role of EcM community composition, richness and diversity;
4. Develop and propagate dedicated EcM-inocula for urban settings for the model tree species, based on the results of objectives (1), (2) and (3);
5. Test the developed EcM inocula in terms of persistence in the soil, tree growth enhancement and stress protection in ex situ experiments;
6. Test the finally selected EcM inocula in in situ inoculation experiments;
7. Provide and disseminate guidelines for enhancing urban tree health and growth, through actively and passively managing EcM-communities.

4. Project activities and achievements

4.1. General description of activities over the duration of the project

WP1 & WP2: Characterize EcM communities and identify environmental drivers (Coordination: Partner 1)

Tilia tomentosa was selected based on its ectomycorrhizal-dependency and general importance as an urban tree in each of the three cities (Leuven, Strasbourg and Porto). Across these cities, we sampled 175 *T. tomentosa* trees (Leuven N = 52; Strasbourg N = 56; Porto N = 67), growing under a variety of planting conditions, from trees planted in a small soil pits surrounded by a sealed surface, over trees planted in soil strips surrounded by sealed surface, to trees planted within larger green areas. In September 2017, the fine roots of the selected trees were sampled. In parallel with root sampling, we collected a pooled soil sample near each sampled tree for chemical analysis (All partners). A standard chemical soil analysis was performed (Partner 1) on the soil samples measuring pH, organic carbon, moisture content and available nitrogen and phosphorus. Heavy metal concentrations of Cu, Pb, Zn, Cd and Cr in the soil were also measured with ICP-OES (Partner 1).

Following DNA extraction from the root samples, DNA extracts were amplified by PCR targeting the ITS2 region of the rRNA gene using the sample-specific barcode-labelled versions of the primers ITS86F and ITS4 (dual-index sequencing strategy). The amplicon library was sequenced using an Illumina MiSeq sequencer. Generated sequences were processed and clustered into operational taxonomic units (OTUs) using USEARCH following the recommended pipeline (Partner 1).

First, EcM richness was modeled against city, general soil characteristics (moisture content, organic matter, pH, Olsen P, N total), and soil heavy metal concentrations (Cu, Pb, Zn, Cd, Cr) using general linear models (GLM). We used the Bayesian Information Criterion (BIC) to select the most explanatory model (i.e. with the lowest BIC) out of a range of reduced models compared with the full model. Second, EcM community composition was related to city, general soil characteristics and soil heavy metal concentrations using canonical redundancy analysis (RDA). Finally, to disentangle the contribution of city, general soil characteristics and heavy metal concentrations to the variation of the EcM communities, variance partitioning was performed (Partner 1).

WP3: Quantify tree health and growth response provided by the model tree species; and identify the mediating role of EcM diversity (Coordination: Partner 2)

Measure health status and growth of the sampled trees

We selected 187 *Tilia tomentosa* trees growing across different planting conditions (boxed, linear and unlimited) (Partner 1 & 2). We measured leaf reflectance and fluorescence and derived a set of optical traits (Partner 1 & 2). We tested whether these optical traits differed between planting conditions and whether they correlate with leaf functional traits, e.g. specific leaf area (SLA) and leaf water content (LWC), and how spectral signatures can be used to infer urban soil heavy metal pollution (Partner 2). Partner 4 has set up a wireless continuous tree health monitoring system (PépiPIAF) for the selected trees. Partner 5 modelled effects of *Tilia* presence on the local microclimate in Strasbourg.

Statistically model interrelations between tree health status and EcM diversity

Next, the two previously collected datasets were combined: (i) ectomycorrhizal diversity of *Tilia tomentosa* trees as characterized by next-generation sequencing, (ii) tree health data of these trees estimated using a range of reflectance, chlorophyll fluorescence and physical leaf indicators, and tree health indicators were related to soil characteristics and EcM diversity using canonical redundancy analysis (Partner 1).

Develop modeling technique to predict soil heavy mental levels

We hypothesized soil properties affect the role of EcM diversity, which thus is related to certain extent to tree health. We measured leaf total chlorophyll content and the chlorophyll a to b ratio (Chla:Chlb) to evaluate the tree response to soil heavy metal and nutrient parameters (Partner 2 and 1). We developed classification models to predict the occurrence of significant changes in the soil parameters.

WP4: Develop and propagate dedicated EcM-inocula for urban settings for the model tree species (Coordination: Partner 3)

In the literature, several species of fungi are associated with *Tilia* trees. Based on this, species of the the fungi *Paxillus involutus*, *Lactarius deliciosus*, *Boletus edulis*, *Russula virescens* and *Russula parazurea* were selected to be used as inocula in WP5. We isolated two species of *Russula*, as these were not present in the fungi collection from ESB (Partner 3). For this, mushrooms were collected based on their morphological characteristics belonging to the genera *Russula* and used to grow pure fungal cultures. The DNA of the pure mycelium cultures was extracted and the ITS1-5.8S-ITS2 region was amplified and sequenced, following the identification of the species in the UNITE database. Different inocula formulations were tested using the five EcM fungi of interest (Partner 3). These consisted of fungi cultures in liquid medium, fungi cultures in solid medium and mycelium encapsulated in sodium alginate beads. A RT-qPCR assay was also developed and optimized to survey the five selected EcM species to be used for the inoculation.

WP5: Test the developed multispecies EcM inocula in terms of ex situ persistence in soil, model tree growth enhancement, and stress protection (Coordination: Partner 3)

To test the developed EcM inocula it a nursery experiment with 3 months old *Tilia tomentosa* was established. Trees were planted in substrate with acid pH and alkaline pH in order to assess the effect of pH in the development of the plant and the performance of fungi. The plants were divided by 24 treatments, including a control non-inoculated treatment, four EcM fungi (*Lactarius deliciosus*, *Paxillus involutus*, *Boletus edulis*, *Russula parazurea*), three fungi carriers (solid, liquid, alginate) and two levels of pH (acid and alkaline), with 12 replicates for each treatment (Partner 3).

A water-stress experiment was also performed in order to assess the stress mitigation effect conferred by the inoculation. The experiment consisted in turning off the watering system for 14 days (stress point). After resuming the watering, the plants were monitored after 14 days to assess recovery from drought (recovery point). Biometric parameters, biomass production, photosynthetic activity, markers of water stress, nutritional parameters and mycorrhizal parameters were measured to test the effect of inoculation on tree growth and stress (Partner 1, 2, 3)

WP6: Test the finally selected EcM inocula in an *in-situ* inoculation experiment on the three model species (Coordination: Partner 4)

Inoculation of Tilia tomentosa urban trees with inoculum developed during WP4 and tested during WP5 (Partner 3)

Tilia tomentosa urban trees from the three cities of the study – Porto, Leuven and Strasbourg - were selected based on the planting system and health status to be inoculated with inoculum developed during WP4 and tested in the nursery experiment of WP5. The selected inoculum showed growth promoting and water-stress mitigation properties. To promote the success of mycorrhization, a method of inoculation using a microcosm for the fungus was developed (Partner 3). Based on the PepiPIAF system, tree health was monitored by Partner 4.

4.2. Table of deliverables

Work Package	Deliverable or Milestone	Lead partner	Planned	Delivered	Comments
WP1 EcM characterization	T1.1 Root, soil and leaf samples of 175 trees	Partner 1	05/2017	09/2017	
WP1 EcM characterization	T1.2 EcM OTU dataset	Partner 1	08/2017	03/2018	
WP1 EcM characterization	T1.3 EcM diversity and community composition	Partner 1	11/2017	06/2018	
WP2 Environmental drivers	T2.1 Chemical, biological and physical environmental variables	Partner 1	08/2017	03/2018	
WP2 Environmental drivers	T2.2 Relations between environmental variables and EcM communities	Partner 1	11/2017	06/2018	
WP3 Tree response	T3.1 Tree health status using leaf reflectance and fluorescence parameters	Partner 2	10/2019	01/2018	
WP3 Tree response	T3.2 Tree ecosystem services	Partner 2 Partner 5	12/2019	02/2019	
WP3 Tree response	T3.3 Tree resilience	Partner 2	12/2019	Not delivered	Time series was not long enough to allow measuring resilience to climate anomalies.
WP3 Tree response	T3.4 Relation between EcM and tree health status	Partner 2	02/2020	11/2019	
WP4 EcM Inocula	T4.1 EcM inocula for *Boletus edulis, Paxillus involutus, Lactarius deliciosus, Russula virescens, Russula parazurea*	Partner 3	04/2018	04/2018	
WP4 EcM Inocula	T4.2 qPCR assay for *Boletus edulis, Paxillus involutus, Lactarius deliciosus, Russula virescens, Russula parazurea*	Partner 3	05/2018	05/2018	
WP5 Test EcM inocula ex situ	T5.1 Greenhouse experiment and tree response to inoculation	Partner 3	01/2020	03/2020	
WP6 Test EcM inocula in situ	T6.1 Field experiment and tree response to inoculation	Partner 4	02/2020	Ongoing	

4.3. Scientific outcomes

WP1&2: Characterize EcM communities and identify environmental drivers (Coordination: Partner 1)

Our results (all Partner 1) showed that Porto had higher EcM richness compared to Leuven and Strasbourg. A higher EcM richness in Porto may be explained by the lower pH levels (mean = 6.58) compared to Leuven and Strasbourg (mean = 7.65 and 8.24, respectively) (Fig. 1). Moreover, most enzymes produced by EcM have a pH optima between 4 and 6, which may explain higher EcM richness in this pH range.

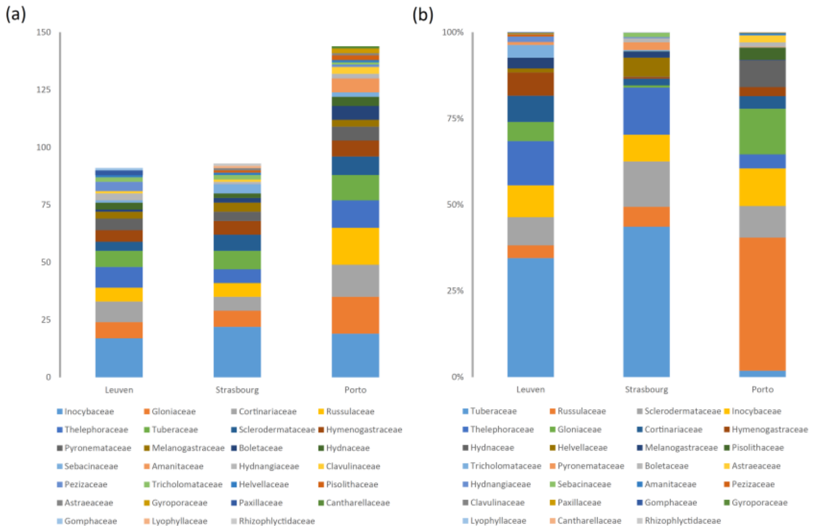

Figure 1 Identified EcM families across Leuven, Strasbourg and Porto per number of EcM OTUs (a) and proportion of EcM reads (b).

Next to differences in EcM richness, our results also revealed strong differences in EcM community composition between cities. Indeed, we detected large differences in abundance of Tuberaceae and Russulaceae between Leuven and Strasbourg on the one hand, and Porto on the other hand. These differences in abundance may also be explained by the differences in soil acidity between the cities. Russulaceae generally prefer more acidic conditions, such as the soils in Porto, while Tuberaceae prefer more alkaline conditions, such as the soils in Leuven and Strasbourg (Fig. 1). Porto experiences high levels of water stress in the summer and, therefore, it was no surprise that *Cenococcum geophilum* was a dominant indicator OTU in this city. *C. geophilum* tolerates a wide range of stressors and is widely known for its ability to tolerate water stress. Additionally, our results show that the heavy metal concentrations in urban soils were not negatively correlated with EcM richness. These results show that the heavy metal concentrations of Cu, Pb, Zn, Cd and Cr in the soil did not limit EcM diversity. Although the level of heavy metal pollution was relatively low, EcM have a range of extra- and intracellular adaptive mechanisms to accumulate heavy metals, possibly making EcM communities more tolerant to heavy metal pollution in comparison to many other groups of microorganism.

As discussed above, soil acidity can impact EcM colonization, EcM diversity and the enzymes produced by EcM. Indeed, also our analysis revealed that EcM communities were strongly related with soil acidity and organic matter (Fig. 2) The direction of change of organic matter was opposite to soil pH, indicating a change in EcM communities when organic matter content in the soil increased and pH levels decreased. Increased levels of organic matter are often associated with lower pH levels as decay of organic matter can buffer soil acidity through a constant supply of protons. Finally, our results revealed a small but significant effect of moisture content in the soil on EcM communities.

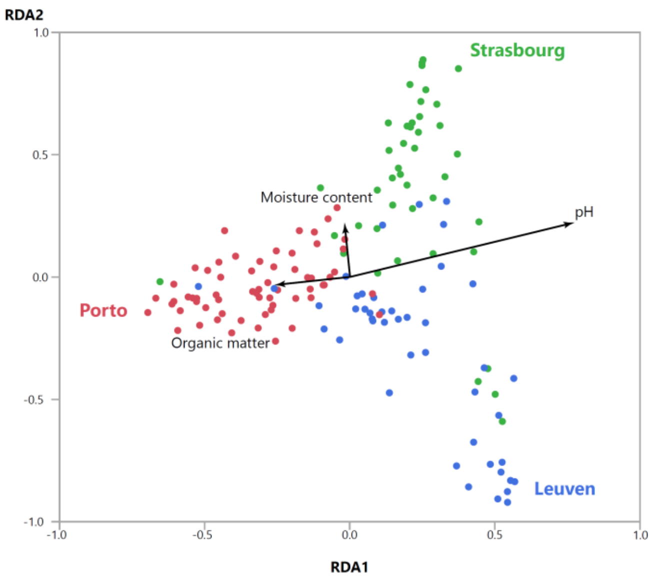

Figure 2 Redundancy analysis (RDA) ordination plot of EcM fungal communities in the roots of *Tilia tomentosa* (N = 150) across Leuven (blue), Strasbourg (green) and Porto (red). Arrows indicate environmental variables explaining a significant proportion of the EcM communities. The arrows represent the direction of the increasing gradient and are proportional to the explained variation in the EcM communities.

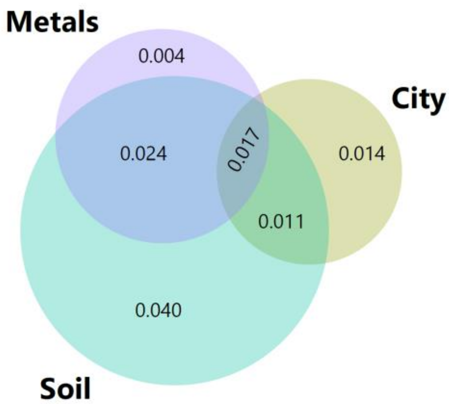

Figure 3 Venn diagrams representing variance partitioning results of EcM communities among three explanatory matrices, i.e. heavy metal variables, soil chemical variables and the variable city. The size of the circles is proportional to the variability in EcM communities as explained by a particular explanatory matrix, while overlap of the circles represents the shared variation among explanatory matrices. Numbers indicate the adjusted R^2 values and thus the variability explained by each partition.

As the three cities sampled in this study largely differ in latitude, we also expected to observe different EcM communities between cities. Although our analysis did indeed reveal strong differences in EcM communities between cities, the variation partitioning showed that city explained 2.85 times less unique variation compared to the general soil characteristics (Fig. 3). Specific spatial analysis also did not explain any additional variation in EcM communities. These results suggest that biogeographical patterns had a limited impact on EcM communities in our dataset and that EcM communities were only significantly related to general soil characteristics. A large part of the variation explained by the general soil variables was shared with the variable city, indicating that effects of soil and city could not completely be disentangled. This was expected as cities also strongly differ in soil characteristics. The heavy metal variables explained very little unique variation in EcM communities, indicating that heavy metal pollution had little or no impact on EcM communities.

WP3: Quantify tree health and growth response provided by the model tree species; and identify the mediating role of EcM diversity (Coordination: Partner 2)

Measure health status and growth of the sampled trees (Partner 2)

We used leaf reflectance spectroscopy, fluorescence and functional traits to evaluate the health status of trees. Results showed that urban soil sealing affects tree leaf functional traits and has negative effects on tree health. Compared to the unsealed trees, sealed trees showed decreased specific leaf area (SLA) and leaf water content (LWC) while increased leaf water per area (LWA). Leaf reflectance and optical traits differed between the unsealed and sealed trees (Fig. 4). These negative effects can be estimated by leaf optical traits, demonstrating the great potential of optical traits in assessing tree health status.

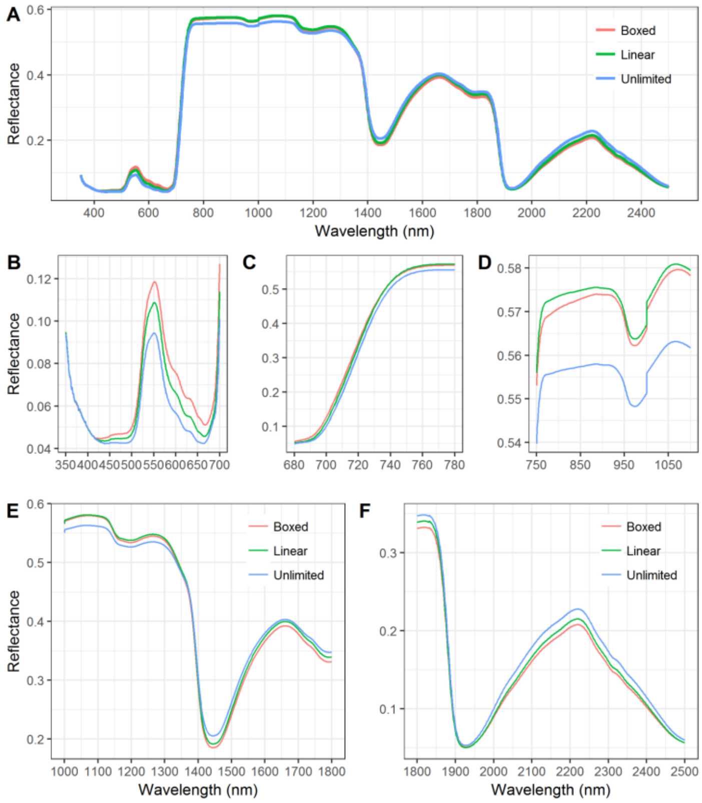

Figure 4 Comparison of leaf reflectance means between the three planting conditions in (A) the full range of 350-2500 nm, (B) visible spectral region, (C) red edge, (D) 750-1100 nm of the near infra-red (NIR), (E) 1000-1800 nm of the short wave infra-red (SWIR1) and (F) 1800-2500 nm of the SWIR2. The mid-row plots share the same legends. Plotted spectra are the means of individual conditions across collections of Leuven and Strasbourg.

Sealed planting conditions also show significant effect on tree phenology. Highly sealed soils accelerated leaf senescence of the sealed trees compared to the unsealed trees, embodied in the temporal trend of optical traits (Fig. 5). Our findings provide insights into facilitating urban green management using optical traits and remote sensing data and will provide input data for further analyses aimed at revealing a link between tree health and EcM communities.

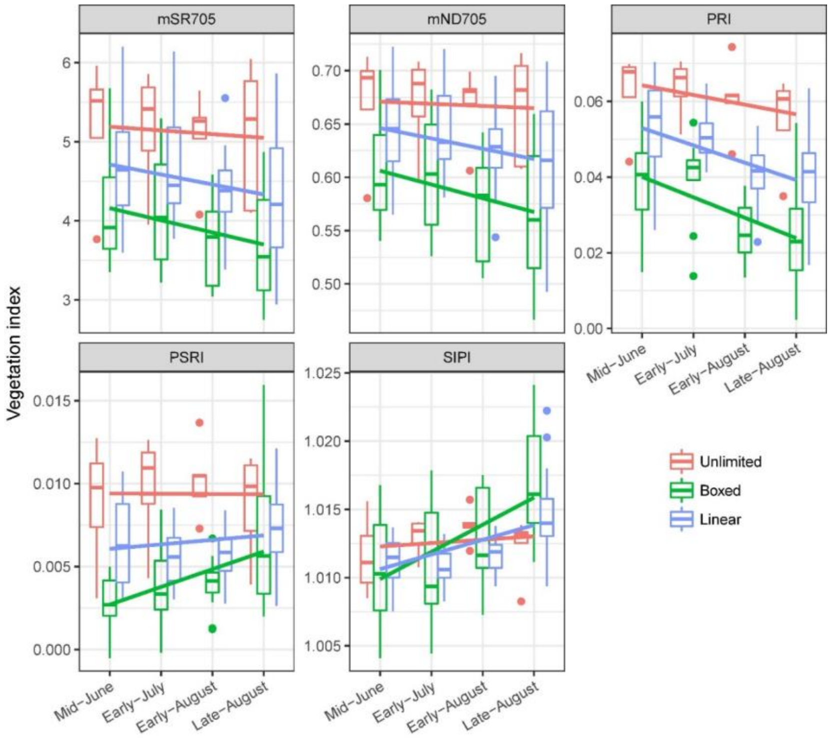

Figure 5 Spectral indices plotted as a function of collection time per planting condition, showing the different changes in phenology between planting conditions.

Partner 4 has set up the PépiPiaf system (developed by INRA (UMR PIAF)), consisting of a LVDT displacement sensor and a wireless data acquisition and transmission unit in the selected Tilia trees. The methodology of continuous measurements of diameter variation by seems sufficiently robust to measure differences in tree growth as a function of their planting constraints, which mainly translate into water stress conditions.

Partner 5 has developed and implement two vegetation modules in the LASER/F microclimatic model (Bournez et al. 2018). The model was used to simulate cooling effects (the most important ecosystem services provided by urban trees) of *Tilia* in the urban park of the University (Strasbourg) (Fig. 6).

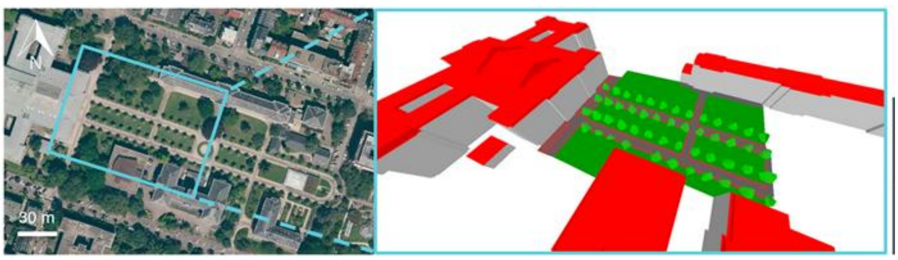

Figure 6 3D model for simulation of cooling effects of Tilia in Strassbourg

Partner 5 also compared the results of the simulation with the measurements obtained during an experimental campaign (measuring the evapotranspiration of grass and *Tilia Tomentosa*). Although there are still many improvements to be made to the vegetation module, the results provided by

LASER/F, such as a tree evapotranspiration simulated with an underestimation of 80 W/m², are promising (Fig. 7).

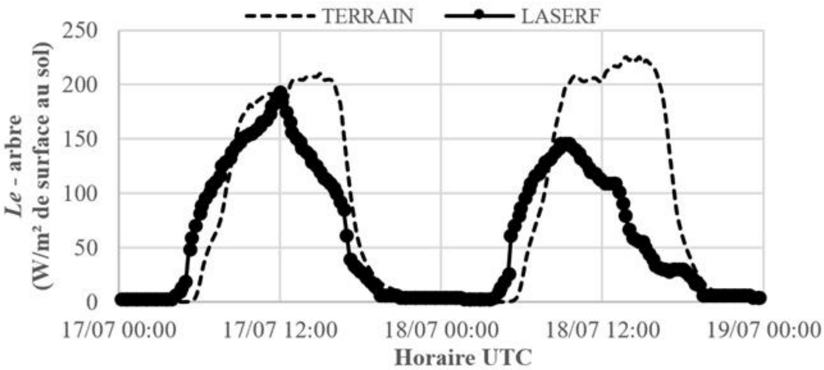

Figure 7 Tree evapotranspiration simulation in Strasbourg.

Statistically model interrelations between tree health status and EcM diversity (Partner 1)

Soil organic matter was the only soil variable that was related to tree health. Our analysis revealed that soil organic matter coincided with leaf water content, indicating that urban trees growing in soils with higher organic matter experienced less water stress (Fig. 8). Soil organic matter was also related to the chlorophyll content in the leaves, photosynthetic light use efficiency and the spectral performance index, indicating better photosynthetic capacity and hence tree health and growth. It has been shown that soil organic matter strongly correlates with the volume of available soil water, as soil organic matter is hydrophilic in nature and promotes soil aggregate formation and the development of soil porosity that enhances infiltration and retention of water. Our results also revealed a strong relation between organic matter and moisture content in the soil, which may explain the relation between soil organic matter and tree water availability, photosynthetic capacity and vitality.

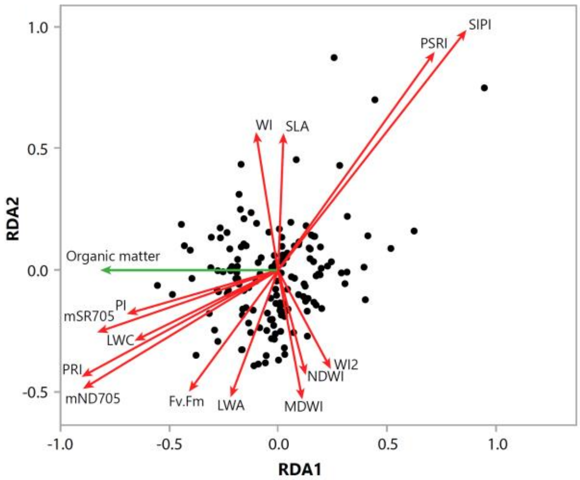

Figure 8 Redundancy analysis (RDA) triplots of the tree health indicators with the variation of city accounted for (partial RDA). Green arrows indicate environmental variables explaining a significant proportion of the tree health indicators, and red arrows indicate tree health indicators. Arrows point out the direction of the increasing gradient in the ordination space. The angles between arrows approximate the correlation between response and environmental variables.

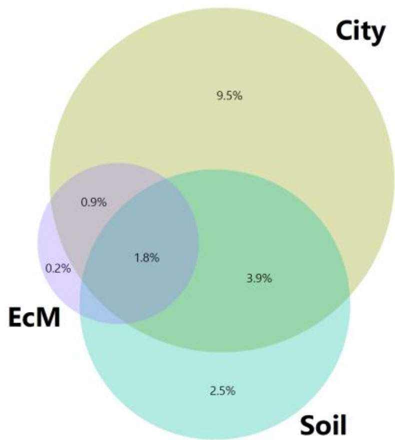

Figure 9 Venn diagram representing variance partitioning results of tree health indicators among three explanatory matrices, i.e. EcM diversity variables, soil chemical variables and the variable city. The size of the circles is proportional to the variability in tree health indicators as explained by a particular explanatory matrix, while overlap of the circles represents the shared variation among explanatory matrices. Numbers indicate the adjusted R^2 values and thus the variability explained by each partition.

To test whether mycorrhizal diversity positively affected tree health status in urban areas, several mycorrhizal diversity measures based on next-generation amplicon sequencing were related to tree health. If mycorrhizal diversity in the roots of urban trees would positively affect tree health, mycorrhizal diversity measures would explain significant variation in the reflectance, chlorophyll fluorescence and physical leaf tree health indicators. Our analysis, however, revealed that, when accounting for soil variables, no significant variation in tree health indicators could be explained by ectomycorrhizal richness or Shannon diversity (Fig. 9). It was also expected that mycorrhizal fungal communities with high phylogenetic diversity, i.e. having more distant mycorrhizal lineages that complementing each other, would better promote tree health in comparison to low phylogenetic diversity. Yet, our analysis also revealed that both Faith's phylogenetic diversity and Faith's standardized phylogenetic diversity did not significantly explain variation in tree health indicators.

Modeling the link between tree health and soil metal levels (Partner 2)

We used tree response as a bio-indicator of elevated levels of soil heavy metal concentration by measuring leaf reflectance and pigment content in tree leaves. Results showed that elevated soil Pb concentrations induced a significant decrease in the ratio of leaf chlorophyll a and b (Chla:Chlb) (Fig. 10), with no significant decrease in the total Chlorophyll content. Elevated soil Cd and Pb levels were also found to be related to contrasting leaf spectral changes, which might be associated with the proportional changes in leaf pigments. These results allow for indirect assessment of ecosystem services of trees in regulating urban environmental pollution.

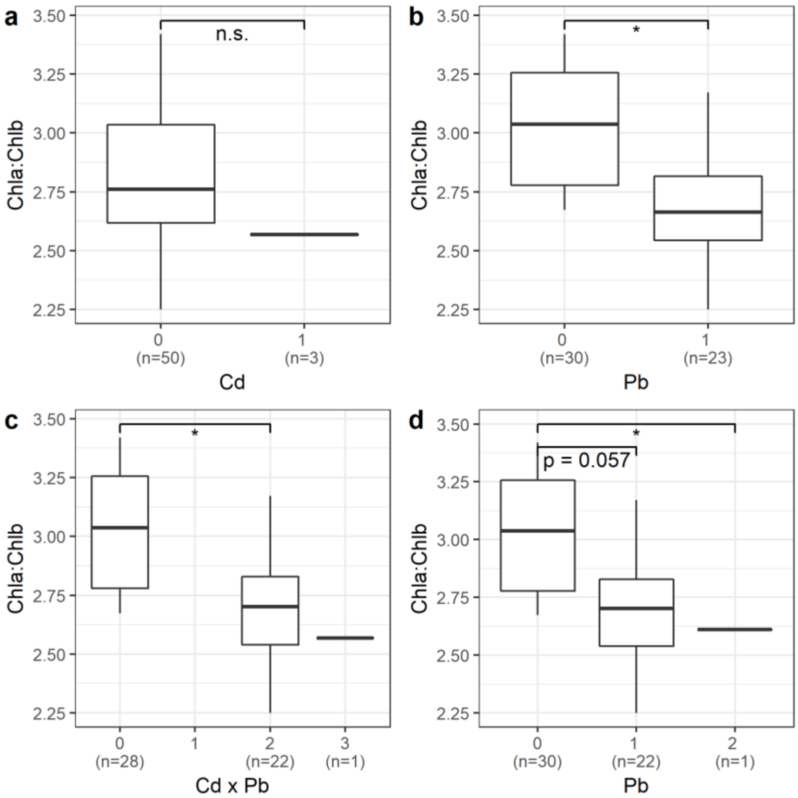

Figure 10 Boxplots with the leaf chlorophyll a to b ratio (Chla:Chlb) differences between the binary classes (0, non-contaminated, 1, contaminated) of (a) Cd and (b) Pb contamination, as well as among multiple classes for (c) Cd _ Pb and (d) Pb contamination. Significance levels are indicated according to the post-hoc Tukey's test of the applied mixed models.

WP4: Develop and propagate dedicated EcM-inocula for urban settings for the model tree species (Coordination: Partner 3)

Taking into account the results of WP1 and WP2, we tested whether the inocula are compatible with the acidity of the soil of the cities under study (Porto, Leuven and Strasbourg). For this purpose, the fungi *Lactarius deliciosus*, *Paxillus involutus* and *Boletus edulis* were grown in solid MMN medium with 4 different pH levels: 5.8 (standard pH used for cultivating EcM in ESB), 6.5 (similar to the average soil pH in Porto), 7.5 (similar to the average pH of the Leuven soil) and 8.5 (similar to the average pH of the Strasbourg soil). The tested fungi demonstrated their ability to grow in the pH ranges used, differing only in the growth rate (all Partner 3).

To optimize the growth conditions of the fungus *Lactarius deliciosus* an experiment was performed using a Box-Behnken design with 3 variables: medium pH, glucose concentration of the medium and incubation temperature (all partner 3). The results showed that the optimal conditions for the maximum biomass production of this fungus are an acid pH (5.5), 5.8 g/L of glucose and an incubation temperature of 15ºC (Fig. 11). These results show that this fungus is well suited for field conditions, since its growth temperature is relatively low, compared to the standard temperature used in ESB laboratories (23ºC).

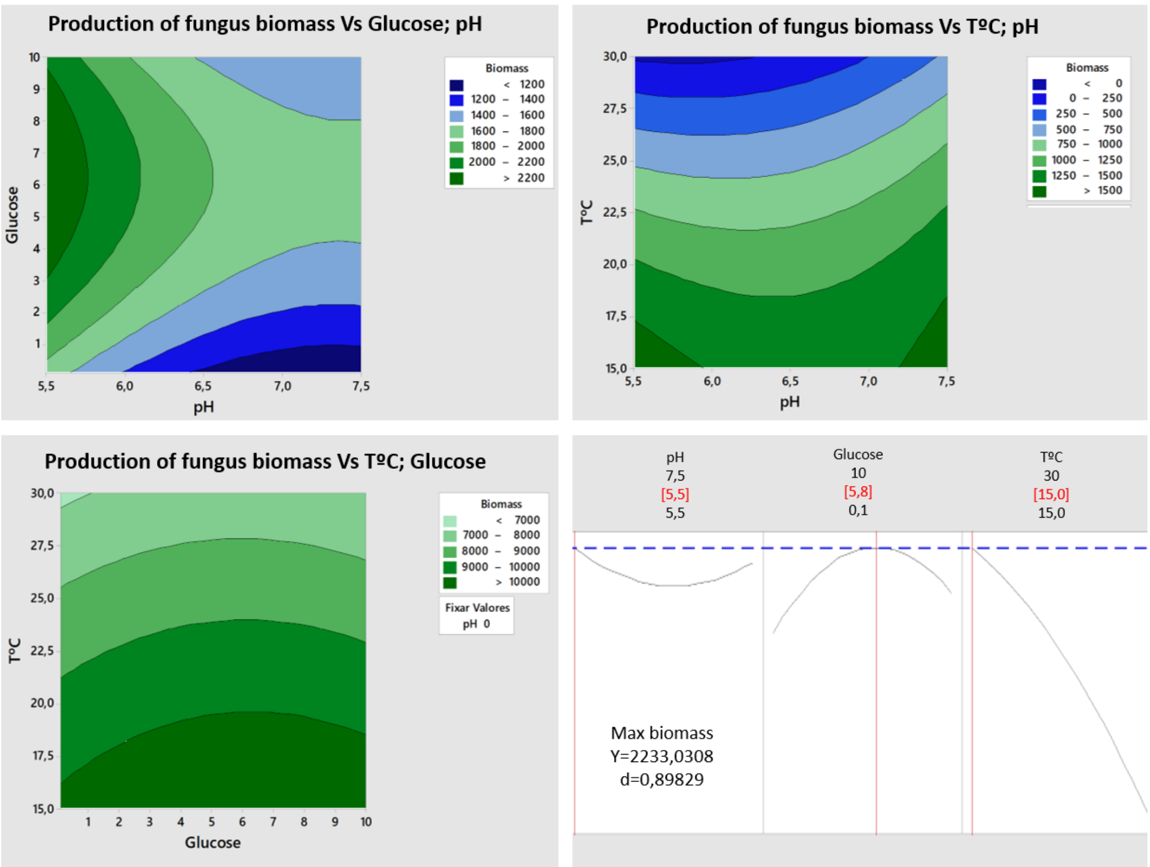

Figure 11 Production of *Lactarius deliciosus* biomass dependent of three variables: medium pH and concentration of glucose and incubation temperature.

Given the growth rate of *R. parazurea* compared to *R. virescens* and its absence in the *Tília* microbiome in the urban environment, it was selected for inoculum production.

Inoculum of the four selected fungi *Lactarius deliciosus*, *Paxillus involutus*, *Boletus edulis* and *Russula parazurea* was produced. The three types of inoculum produced (Fig. 12) by Partner 3 were:

- **solid inoculum**: one disc with 1 cm of diameter of mycelium pure culture was inoculated in new plates of MMN medium and incubated at 23ºC for one month;

- **liquid inoculum**: one disc with 1 cm of diameter of mycelium pure culture was inoculated in liquid MMN medium and incubated at 23ºC for one month, with occasional shaking of the flasks to promote mycelium growth;
- **alginate beads**: mycelium was encapsulated in sodium alginate polymerized with calcium chloride and were incubated in liquid MMN medium at 23ºC for one month.

The fungus *R. parazurea* did not grow in solid medium, so only liquid and alginate inoculum were produced.

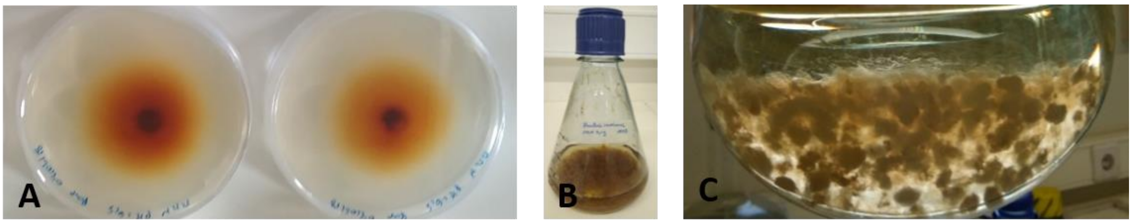

Figure 12 Solid inoculum (A), liquid inoculum (B) and alginate inoculum (C) produced to be used in the inoculation experiments.

Specific primers for the fungi *Paxillus involutus*, *Russula virescens* and *Russula parazurea* were designed using the ITS1-5.8S-ITS2 region as a target. A search was carried out in GenBank to confirm the specificity of the primers designed for the target species. The primers for *Lactarius deliciosus* and *Boletus edulis* were described in the literature by Parladé and collaborators (2007;2017) and were used in this work. Amplification was optimized using gradient PCR to allow a strong amplification of the target and prevent amplification of non-specific products. Using these primers, a qPCR assay was developed for the quantification of mycelium in the soil of EcM fungi species of interest based on the quantification method described by Parladé and collaborators (2007). The qRT-PCR assay allowed the generation of standard curves for the quantification of mycelium in the soil that satisfy the requirements of RT-PCR: the curves obtained showed a high reaction efficiency (close to 100%), a correlation (R^2) close to 1 and the amplification of only one product confirmed through the presence of a single peak in the melting curve of the respective reactions. The curves obtained showed the efficiencies depicted in Table 1.

Table 1 qRT-PCR curve efficiencies for the five fungi in study.

Fungus species	***Curve efficiency (%)***
Paxillus involutus	91.42
Lactarius deliciosus	101.28
Boletus edulis	94.33
Russula virescens	98.00
Russula parazurea	102.80

WP5: Test the developed multispecies EcM inocula in terms of *ex-situ* persistence in soil, model tree growth enhancement, and stress protection (Coordination: Partner 3)

A nursery experiment was established during March 2018 using 3 months old *Tilia tomentosa* that were transplanted to individual pots of 10L of substrate (Partner 3). Acidic pH (~5.8) substrate was obtained by mixing peat moss, perlite and vermiculite and calcium carbonate was added to the mix in order to rise the pH to ~7.5 (alkaline pH). 288 plants were divided by 24 treatments consisting in a control non-inoculated treatment, four EcM fungi, three carriers and two levels of pH, with 12 replicates for each treatment. At this stage the three types of inoculum were produced for the inoculation of the plants during April-May 2018. In September 2018 the experiment of water-stress was performed in the plants that were watered daily (pre-stress point). The watering system was turned off for half of the replicates of each treatment until the phenotype of leaf rolling was visible, indicating that the plants were suffering from water scarcity; the stress point was obtained by turning off the watering system for 14 days. After resuming watering 14 more days were given to the plants to recover (recover point). Leaves were collected at each point of the water-stress experiment and leaves and roots were collected at the end of the experiment.

In November 2018, the analysis of the plants in the nursery experiment was started to quantify the effects of inoculation on the growth and vigor of plants at acid and alkaline pH (partner 1, 2 and 3). The contribution of inoculation to mitigate the effects of water stress was also evaluated. Several parameters were measured until June 2019.

At acid pH, we found that the inoculation had a growth promoting effect on the plants, which were significantly higher than the non-inoculated control plants. The inoculation caused a more evident effect at acidic pH, since it is a more challenging environment for *Tilia*, which prefers alkaline soil. The biomass production of the leaves was also significantly higher compared to the control. The phosphorus content of the root and leaves was higher in the inoculated plants since the EcM fungi confer a greater capacity for absorbing nutrients from the soil. The leaf moisture was significantly lower compared to the control, because mycorrhizal plants have a higher nutrient content, decreasing the solvent-solute ratio. In alkaline soil, inoculation also had a beneficial effect on mycorrhizal and nutritional parameters.

Of the three carriers tested, the liquid carrier had the most notorious effects and will be described in more detail. Regarding the height, in an acidic environment, all fungi promoted plant height relative to the control, with a significant increase by inoculation with *Russula parazurea* and *Boletus edulis* fungi. In contrast, inoculated plants growing in alkaline substrate did not show significant differences in height (Fig. 13). These results can be explained by EcM fungi that have a more notorious effect in more acidic conditions, since the *Tilia* prefers more alkaline soils. Therefore, the benefits of EcM fungi were more pronounced in acidic soils.

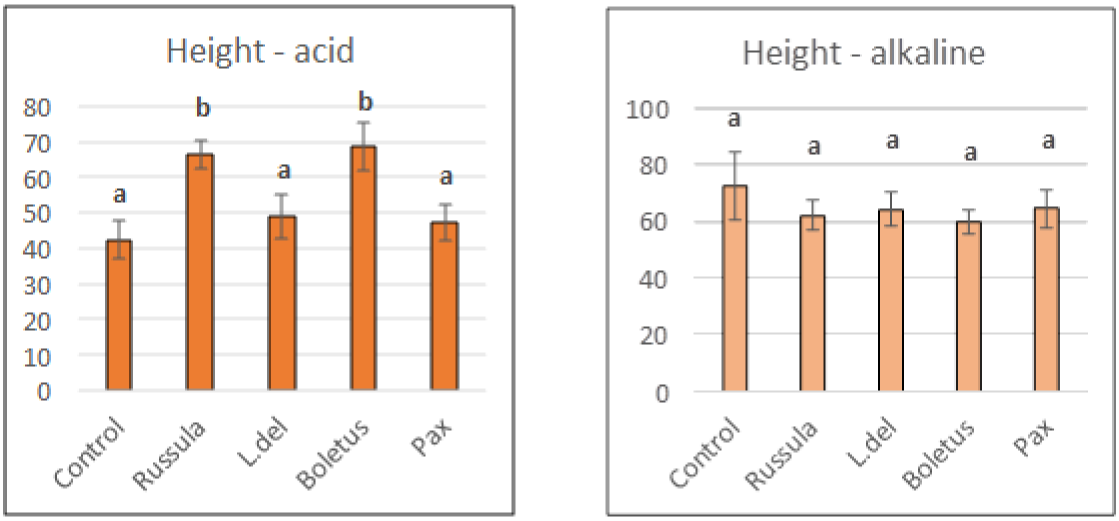

Figure 13 Height (cm) of plants, growing in acid and alkaline pH, inoculated with four EcM fungi compared to a non-inoculated control (Abbreviations: Russula – *Russula parazurea*; L del – *Lactarius deliciosus*; Boletus – *Boletus edulis*; Pax – *Paxillus involutus*). Different letters indicate statistically significant differences ($p<0.05$) between treatments by Duncan's multiple range test.

The diameter of the inoculated plants was higher in both pH scenarios (Fig. 14), being the increase more evident in acid pH for the fungus *Russula parazurea* and in alkaline pH for *Paxillus involutus*.

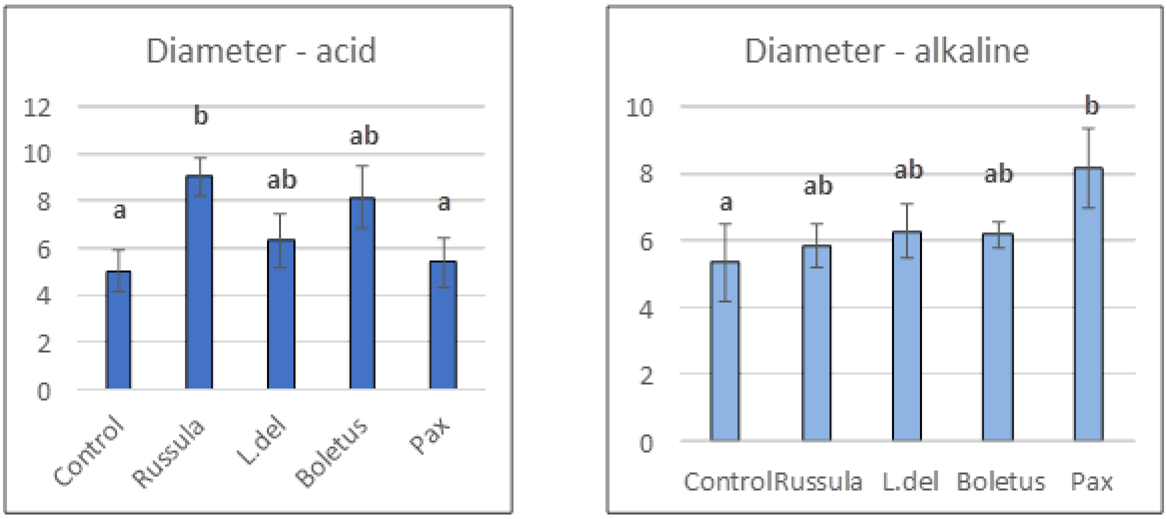

Figure 14 Diameter (mm) of plants, growing in acid and alkaline pH, inoculated with four EcM fungi compared to a non-inoculated control (Abbreviations: Russula – *Russula parazurea*; L del – *Lactarius deliciosus*; Boletus – *Boletus edulis*; Pax – *Paxillus involutus*). Different letters indicate statistically significant differences ($p<0.05$) between treatments by Duncan's multiple range test.

The dry weight per leaf was significantly increased in the plants inoculated with the four fungi in acid substrate, and with the fungus *Boletus edulis* in the alkaline scenario (Fig. 15).

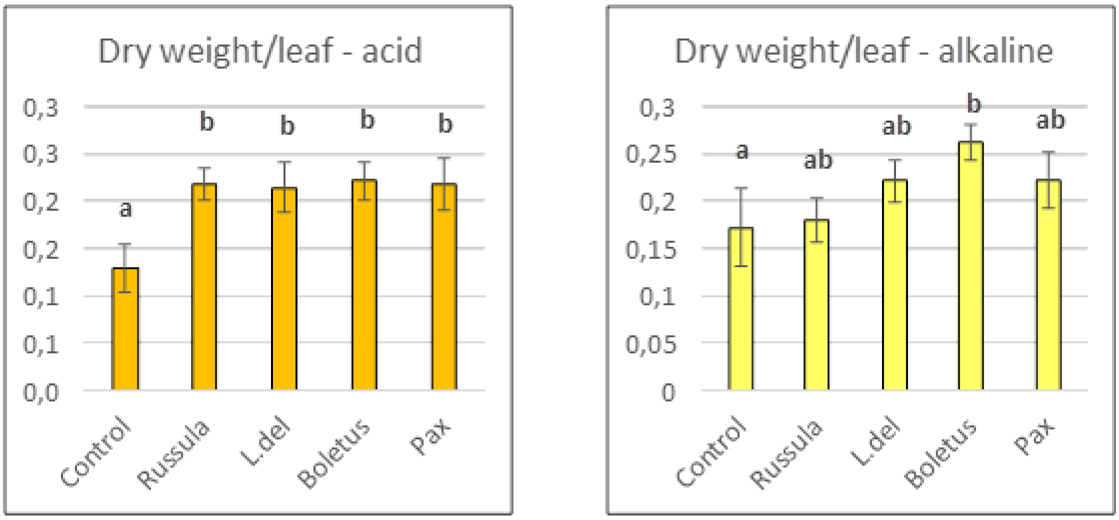

Figure 15 Dry weight (g) per leaf of plant, growing in acid and alkaline pH, inoculated with four EcM fungi compared to a non-inoculated control (Abbreviations: Russula – *Russula parazurea*; L del – *Lactarius deliciosus*; Boletus – *Boletus edulis*; Pax – *Paxillus involutus*). Different letters indicate statistically significant differences ($p<0.05$) between treatments by Duncan's multiple range test.

The SPAD values increased significantly with the inoculation: in acid pH only the fungus *Boletus edulis* and at alkaline pH the fungus *Paxillus involutus* did not promote this parameter significantly in relation to the control (Fig. 16). The SPAD value is indicative of the nitrogen concentration in the plant and its increase is probably due to the accumulation of pigments to increase photosynthesis and consequently increase in biomass production.

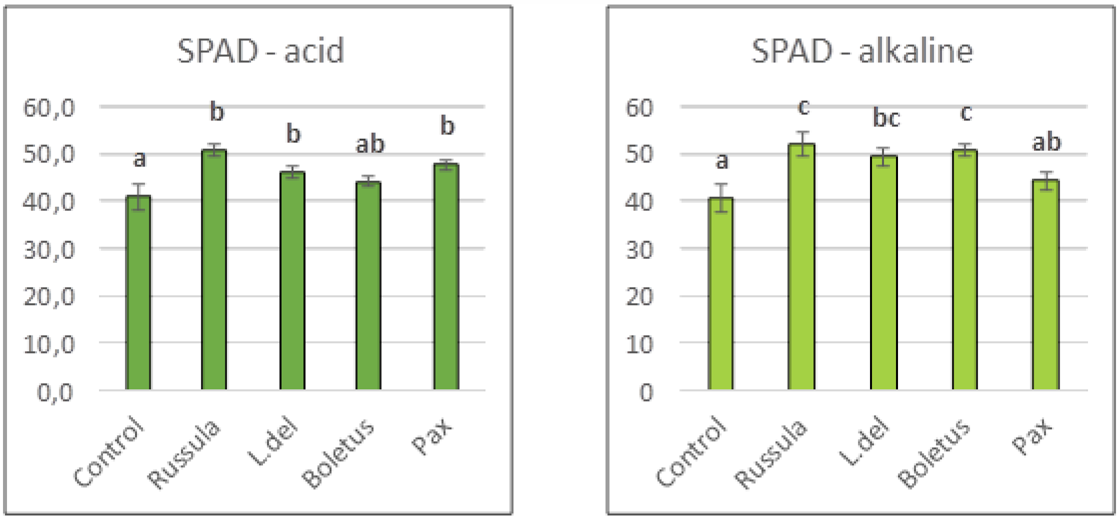

Figure 16 SPAD of plants, growing in acid and alkaline pH, inoculated with four EcM fungi compared to a non-inoculated control (Abbreviations: Russula – *Russula parazurea*; L del – *Lactarius deliciosus*; Boletus – *Boletus edulis*; Pax – *Paxillus involutus*). Different letters indicate statistically significant differences ($p<0.05$) between treatments by Duncan's multiple range test.

The accumulation of solutes such as proline is crucial for the osmotic adjustment of the plant in situations of water stress, allowing it to maintain its normal functions. In plants that were subjected to water stress, at acidic pH (Fig. 17) the plants inoculated with the fungi *Russula parazurea*, *Lactarius deliciosus* and *Paxillus involutus* increased the concentration of proline compared to the control and the state of before-stress, revealing an attempt to protect the plant from the deleterious effects of water scarcity.

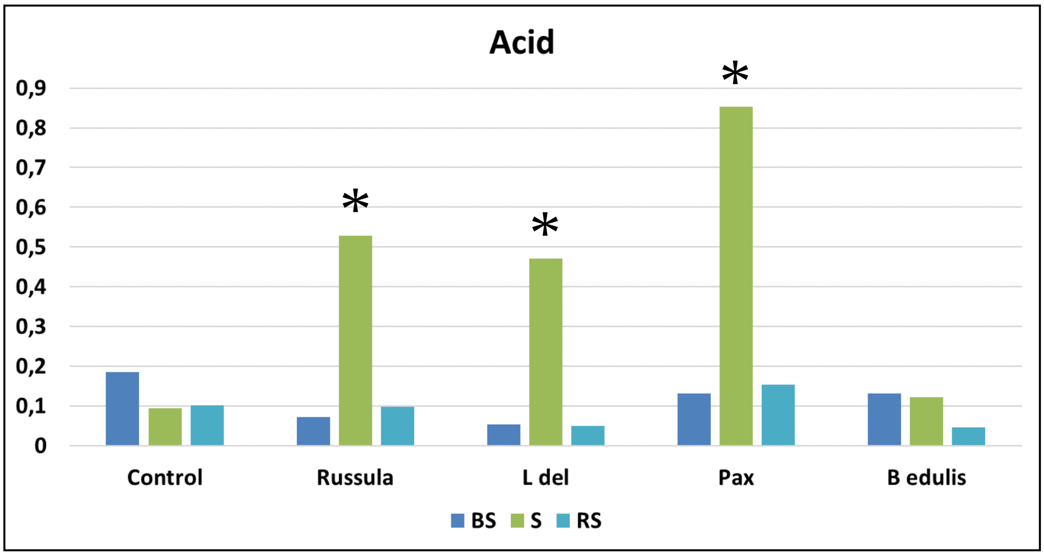

Figure 17 Proline concentration (mg Proline/gr fresh leaf) of inoculated plants with four EcM fungi compared to a non-inoculated control, in acid pH. (Abbreviations. Russula – *Russula parazurea*; L del – *Lactarius deliciosus*; Boletus – *Boletus edulis*; Pax – *Paxillus involutus*; BS- before-stress; S- stress; RS- recovery). The symbol * indicates statistically significant differences ($p<0.05$) between treatments by Duncan's multiple range test.

In the alkaline substrate (Fig. 18), the same behavior was observed in plants inoculated with the fungi *Lactarius deliciosus* and *Paxillus involutus*. This increase indicates a mitigation of the effects of water stress on the plant through a better absorption capacity of water from the soil. It is also observed that after the period of water stress the proline levels stabilize.

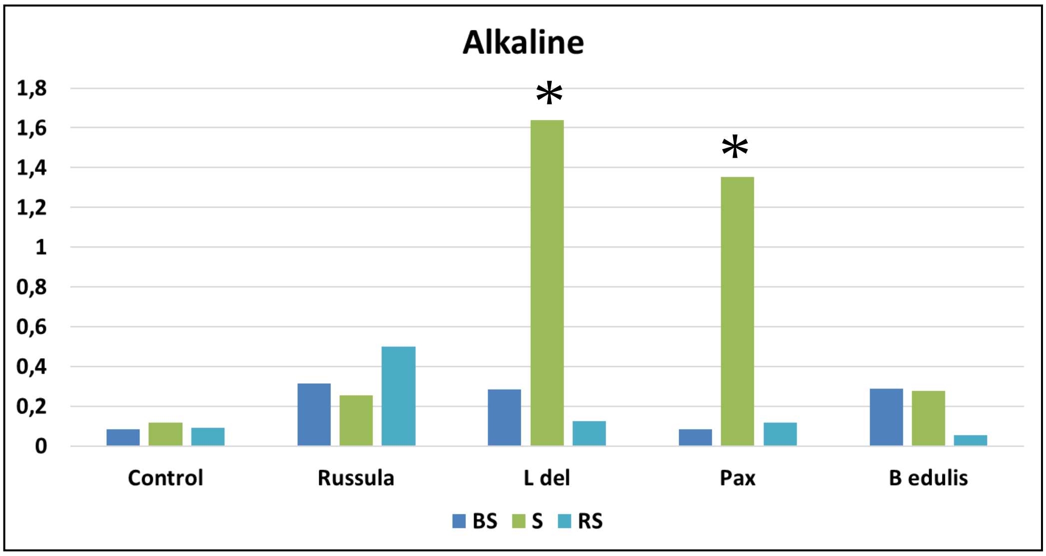

Figure 16 Proline concentration (mg Proline/gr fresh leaf) of inoculated plants with four EcM fungi compared to a non-inoculated control, in alkaline pH. (Abbreviations. Russula – *Russula parazurea*; L del – *Lactarius deliciosus*; Boletus – *Boletus edulis*; Pax – *Paxillus involutus*; BS- before-stress; S- stress; RS- recovery). The symbol * indicates statistically significant differences ($p<0.05$) between treatments by Duncan's multiple range test.

The results show that proline is a good indicator of the role of EcM in the mitigation of drought. When water stress is induced, inoculated plants significantly increased the levels of proline. Fungi contribute to mitigate the stress induced and improve the resilience of the plant, which accumulate solutes that allow a more efficient absorption of water from the soil. Opposite results are observed in control treatment showing lower defenses comparing to inoculated plants, revealing an incapacity to rise the levels of proline when the stress is induced.

WP6: Test the finally selected EcM inocula in an *in situ* inoculation experiment on the three model species (Coordination: Partner 4)

Inoculation of Tilia tomentosa urban trees with inoculum developed during WP4 and tested during WP5 (Partner 3)

The most promising inoculum used in W5 selected by a preliminary analysis based on plant growth promotion and mycorrhiza formation was *Russula parazurea*. This species was used to inoculate 9 adult trees in the urban environment in the cities of Porto, Leuven and Strasbourg (October 2018); 9 trees were also chosen as a non-inoculated control. These trees were selected taking into account their planting system, the box system, since it was considered the most stressful planting system in the urban context (WP3). Trees referred to as having good and poor health were selected for inoculation. The plants were inoculated in two cardinal points: North and South. Secondary roots were excavated, inoculated with 5 ml of liquid inoculum and wrapped in a permeable mesh bag that contained substrate with acid pH to reinforce the efficiency of the infection by the fungus. The mesh bag was then covered with soil (Fig. 19).

Figure 19 Inoculation of urban *Tilia* trees. Secondary roots were excavated and inoculated with 5 mL of liquid inocula being a mesh bag wrapped around the roots and covered with soil.

After 18 months of inoculation of adult *Tilia* trees in urban context, a preliminary analysis was carried out in Porto by Partner 3 to assess whether the inoculation strategy had a positive effect in the formation of mycorrhizas. It was possible to find the mesh bags previously buried in the boxes of the analyzed trees and the presence of secondary roots of linden was observed both outside and inside the bag, an indication of colonization. The laboratory analysis showed that the secondary roots present had a large number of mycorrhizas, proving that this method of inoculation was efficient and protected the inoculated fungus and probably served as a substrate for other fungi that were already in the soil. Therefore, the mesh bag method is a good method for the formation of mycorrhizae, as it offers the fungus a favorable microcosm in terms of conditions such as pH and aeration, in contrast to the reduced amount of mycorrhizae found in compacted soil.

The mycorrhizas present in the bag were observed, collected and preserved at -20ºC for further analysis of the fungal diversity present. There is an intention to sample the remaining locations following the same procedure.

Taking into account the quarantine situation and social distance regulations due to the Covid-19 pandemic, it was not possible to carry out the sampling and analysis of all the urban trees. Therefore, the sampling has been postponed and the possibility of collection of samples by the partners with the aid of the Portuguese team will be evaluated, as traveling to Leuven and Strasbourg was not possible.

The data analysis of monitoring of the physiological response of the trees by the PepiPIAF system by Partner 4 has not happened yet.

4.4. List of project meetings

Date	Place	Participating partners	Meeting title and object
7 March 2017	Leuven	All	Urbanmycoserve kickoff meeting
30 January 2018	Porto	All	Second annual URBANMYCOSERVE meeting, communicate preliminary results and plan fieldwork and experiments
29 January 2019	Clermont-Ferrand	All	Third annual URBANMYCOSERVE meeting, communicate preliminary results and plan fieldwork and experiments

4.5 Follow up activities and plans for further exploitation of the results

The inoculation in-situ field experiment will be monitored in the next few years to test for the long-term effect of inoculation on the growth and health of urban *Tilia* trees.

5. Stakeholder engagement in the project

5.1 During the project

- The Leuven Greenery Service (Director Jan Vandyck + several local workers) provided sampling locations (WP1-3) and information (planting date, health) regarding the sampled trees. They also assisted with the fieldwork, providing ladders and security close to the road. Finally, they provided a location for the in situ inoculation experiment (WP 6).
- All the field work at Porto had the help of the Greenery Services of the Porto City Council, which provided technical and logistic support.
- Porto: Meeting at the Municipal Council of Porto (April 18th 2017) for identification of sensitive areas in the city and planing the sampling in the different sites.
- The Town and Eurometropole Strasbourg Greenery Service (and specially the Tree service led by Sylvain Leroux) provided the sampling locations and information about the trees disseminated in parks and streets.
- The University of Strasbourg Greenery Service helped to manage the site located within the University where the modelling is ongoing.

5.2 Foreseen after the project's end

A PhD proposal with the title "Ectomycorrhiza as a tool to improve urban trees resilience." based on the project was approved and is ongoing, involving UCP-ESB (Portugal) and PIAF-INRA (France).

A research project for restoration of burned areas in a suburban areas of Porto called reCOVER was submitted to FCT involving the Porto Metropolitan area and a new Municipality as stakeholders. This project involves the Portuguese and Belgian partners.

6. Dissemination of results

6.1 List of peer reviewed scientific publications

- Van Geel M., Yu K., Ceulemans T., Peeters G., van Acker K., Geerts W., Ramos M.M., Serafim C., Kastendeuch P., Najjar G., Ameglio T., Ngao J., Saudreau M., Waud M., Lievens B., Castro M.L.P., Somers Ben. and Honnay O. 2018. Variation in ectomycorrhizal fungal communities associated with Silver linden (Tilia tomentosa) within and across urban areas. *FEMS Microbiology Ecology* 94: 1-11.

- Yu K., Van Geel M., Ceulemans T., Geerts W., Ramos M.M., Sousa N., Castro M.L.P., Kastendeuch P., Najjard G., Ameglioe T., Ngaoe J., Saudreaue M., Honnay O. and Somers B. 2018 Vegetation reflectance spectroscopy for biomonitoring of heavy metal pollution in urban soils. *Environmental Pollution* 243: 1912-1922.

- Bournez E., Landes T., Najjar G., Kastendeuch P., Ngao J., Saudreau M., 2019. Sensitivity of light interception and tree transpiration to the level of detail of 3D tree reconstructions. Urban Forestry & Urban Greening 38:1-10.

- Bensaoud A., Segur F., Améglio T. 2018 - Services écosystémiques des arbres en ville: évaluation du pouvoir rafraichissant par micro-dendrométrie. Irrigazette, 165, 25-31

- Yu K., Van Geel M., Ceulemans T., Geerts W., Ramos M.M., Sousa N., Castro M.L.P., Kastendeuch P., Najjard G., Ameglioe T., Ngaoe J., Saudreaue M., Honnay O. and Somers B. 2018. Foliar optical traits indicate that sealed planting conditions negatively affect urban tree health. *Ecological Indicators* 95: 895-906.

- Van Geel M., Yu K., Peeters G., van Acker K., Ramos M., Serafim C., Kastendeuch P., Najjar G., Ameglio T., Ngao J., Saudreau M., Castro P., Somers B., Honnay O. 2019 Soil organic matter rather than ectomycorrhizal diversity is related to urban tree health. *Plos one* 14: 1-9.

- (Serafim C., Ramos M., Sousa N., Yu K.,Van Geel M., Ceulemans M., Kastendeuch P., Najjar G., JNgao J., Saudreau M., Somers B., Ameglio T., Honnay O., Castro P. 2020 Ectomycorrhizal fungal inoculation of Tilia tomentosa seedlings promotes the resistance and resilience to water stress but depends on substrate acidity. *In preparation*)

6.2. Dissemination of results to scientists and scientific organisations (1-page max)

- Scientific results of the project are communicated through the Twitter account of Olivier Honnay under hashtag #Urbanmycoserve

- Project communication of scientific results through the Research-Gate project website: https://www.researchgate.net/project/ERA-NET-BiodivERsA-URBANMYCOSERVE-Understanding-and-Managing-Urban-Ectomycorrhizal-Fungus-Communities-to-Increase-the-Health-and-Ecosystem-Service-Provisioning-of-Urban-Trees

Posters

- Serafim C, Ramos M, Sousa N, Castro PL 2017. Detection and quantification by Real-Time PCR of Ectomycorrhizal fungi in inoculum formulations for urban trees application, Microbiotec'17 – Congress of Microbiology and Biotechnology 2017, Porto, Portugal, December 2017

- Ramos M, Sousa N, Castro PL, Selection and acclimatization of strains of edible mycorrhizal fungi for improved field persistence, Microbiotec'17 – Congress of Microbiology and Biotechnology 2017, Porto, Portugal, December 2017

- Yu K, Van Geel M, Ceulemans T, Geerts W, Ramos MM, Sousa N, Castro MLP, Kastendeuch P, Najjard G, Ameglioe T, Ngaoe J, Saudreaue M, Honnay O and Somers B 2017 Urban tree health monitoring with hyperspectral and chlorophyll fluorescence measurements, British Ecological Society; 2017-12; Ecology Across Borders: Joint Annual Meeting 2017, 2017/12/11 - 2017/12/14, Ghent, Belgium
- Ramos M, Serafim C, Sousa N, Castro PL Selection of high performance strains of edible mycorrhizal fungi for improved abiotic stress resistance, ESM2018 - Ecology of Soil Microorganisms 2018, Helsínquia, Finland, June of 2018.
- Yu K, Degerickx J, Van Geel M, Honnay O, Somers B. 2018 Airborne Imaging Spectroscopy for Assessing Soil Sealing Effect on Urban Tree Health. 9th Workshop on Hyperspectral Image and Signal Processing: Evolution in Remote Sensing (WHISPERS)
- Serafim C, Ramos M, Yilmaz T, Sousa N, Castro PML 2019 Non-invasive monitoring of stress response of urban trees inoculated with EcM. Microbiotec19 Congress of Microbiology and Biotechnology 2019, Coimbra, Portugal.
- Bournez E., Kastendeuch P., Landes T., Najjar G., Saudreau M., Colin J., Ngao J., 2018. Simulation du rôle de la végétation d'un parc urbain à partir du modèle microclimatique LASER/F : le cas du jardin du Palais Universitaire à Strasbourg. Colloque AIC, Nice, France, July 4-7 2018
- Bournez E., Landes T., Saudreau M., Kastendeuch P., Najjar G., 2017. From TLS point clouds to 3D models of trees : a comparison of existing algorithms for 3D reconstruction. Conférence 3D ARCH, 7e International Workshop, Nafplio, Greece, March 1-3 2017.
- Bournez E., Kastendeuch P., Landes T., Najjar G., Saudreau M., Colin J., Ngao J., 2018. Simulation of the vegetation in an urban park with the microclimate model LASER/F: the case study of a park in Strasbourg. International conférence on urban climate, 10th symposium, New York, USA, August 6-10 2018
- N. Philipps, P. Kastendeuch, O. Montauban, G. Najjar 2020. Rôle de la végétation et de la géométrie urbaine dans la variabilité spatio-temporelle de l'îlot de chaleur urbain : cas de la ville de Strasbourg, Colloque AIC, Rennes, France, page 6, juillet 2020.
- Ramos MA, Serafim C, Yilmaz T, Yu K, Van Geel M, Somers B, Ameglio T, Honnay O, Castro PML 2019 Development of a biotechnology tool for tree stress mitigation under urban context. 18th Congress of European Mycologists, Warsaw-Białowieża, Poland.

6.3 List of dissemination activities with stakeholders

- All planned activities have been cancelled due to COVID issues

6.4 Dissemination of results to stakeholders (1-page max)

- Website for the stakeholders and general public: **www.urbanmycoserve.org** (c. 1200 unique visitors in total). On this website, news, activities, results and publications are communicated.

Please note that (i) due to language problems, we organized all stakeholder meetings in the three countries separately, and (ii) covid has seriously hampered our dissemination activities.

Belgium

• Personal communication about activities and preliminary results to the Leuven Greenery Service (Director Jan Vandyck + several local workers) in summer 2018.

An article was published in the Belgian local journal 'Groencontact' about urban greening, landscaping, spatial planning and urban development to disseminate the results of this project to a broader audience. The article was also published in the Dutch local journal 'Boomzorg' about urban trees.

Van Geel M, Yu K, Somers B, Honnay O (2020) Vitaliteit van stadsbomen – Het belang van omgeving en bodemschimmels. *Groencontact* 46:2

Portugal

• Prof. Olivier Honnay presented the project at the 2nd PhytoSUDOE stakeholders's workshop in Porto (April 17th 2018). Title: "Understanding and managing urban ectomycorrhizal fungi communities to increase the health and ecosystem service provisioning of urban trees".

• Meeting at the Municipal Council of Porto (February 15th 2018) for dissemination of results as well as for signaling sensitive areas for the growth of *Tilia tomentosa*, namely identification of areas susceptible to water stress. The UrbanMycoServe videos (see website) were send to Municipal Council of Porto (http://www.urbanmycoserve.org/news/urbanmycoserve-researchers-explain-the-project).

• Meeting with Regional Centre of Expertise (CRE) (Portugal) (June 18th 2018) to disseminate the project, results and discussion of future sites for the plantating of mycorrhizal trees produced under the project.

• Meeting with MycoTrend (company http://www.mycotrend.com/pt) (March 5th 2018) to share results in the development of new carriers for application to adult trees in an urban context. Consulting by the company on the level of good practices for inoculation of adult plants and greenhouse cultivation methods.

France

• Personal communication of the preliminary results and activities of the project to Sylvain Leroux (director of the Greenerey Service and Eurometropole Strasbourg).

• Diffusion of the protocol and short report on the processing of available PepiPIAF data by T. Ameglio (August 15th 2018) to the Greenery Service.

• Meeting at the AllEnvi - national French Alliance for Research on the Environment: "Capteurs connectés pour les végétaux", Paris, FRA (2017-11-16 - 2017-11-16). Oral communication presented by Thierry Améglio: « PépiPIAF : Intérêts de l'utilisation des capteurs connectés dans la gestion de la santé des arbres en villes et services écosystémiques qui en découlent ». https://prodinra.inra.fr/record/415830

• Meeting at the CAUE 63 - Advisory service in Architecture, Town and Country Planning and Environment: Aménager les arbres. Concevoir, choisir, entretenir. Billom, FRA (2017-11-23)''. Oral communication presented by Thierry Ameglio: « Biologie, services écosystémiques et adaptation de l'entretien de l'arbre en ville ». https://prodinra.inra.fr/record/415833

• Meeting at the AFB (French Agency for Biodiversity) and CNFPT (National Center for Local Government Service): L'Agence française pour la biodiversité et le Centre national de la fonction publique territoriale Biodiversité et Collectivités Territoriales : Quelles opportunités pour les territoires?, Montpellier, FRA (2018-02-07 - 2018-02-08). Oral communication by Thierry Ameglio: «Le rafraichissement des villes par les arbres : Quantification et modélisation de l'apport des arbres sur le confort thermique des villes». https://prodinra.inra.fr/record/424144

• Citizen conference «Apéros-sciences sur l'Arbre en Ville » – Villa Méditerranée, Marseille, 27 avril 2017, communication presented by Thierry Ameglio: « Les services écosystémiques rendus par les arbres en ville». https://www.ird.fr/toute-l-actualite/colloques-et-manifestations/conferences-grand-public/aperos-sciences-de-la-mediterranee/apero-science-9-aupres-de-mon-arbre

• Bensaoud A., Segur F., Améglio T. 2018 - Services écosystémiques des arbres en ville: évaluation du pouvoir rafraichissant par micro-dendrométrie. *Irrigazette*, 165, 25-31. https://hal.archives-ouvertes.fr/hal-01843738/document.

• Communication: Thierry Ameglio, Jérome Ngao, Marc Saudreau. Atténuer les Ilots de Chaleur Urbains : un service de l'arbre. L'arbre urbain, La FREDON et le CNFPT. Oct 2019, Aix-en-Provence, France. 19 p. https://pastel.archives-ouvertes.fr/PIAF/hal-02310108v1

• Communication: Jérome Ngao, Thierry Ameglio, Marc Saudreau. Variations de diamètres des arbres, un outil innovant pour caractériser la phénologie ? - Un cas d'étude d'arbres en ville. Ecole Thématique FP INRA -InnObs, Nov 2019, Montpellier, France. 39 p. https://hal.uca.fr/PIAF/hal-02444447

• Communication: Marc Saudreau, Thierry Ameglio, Jérome Ngao. Les arbres et les ilôts de chaleur urbains. Journée Technique Echospaysage "Arbres et Sécurité, de la santé des citadins à la gestion des risques de rupture", EchosPaysage Auvergne-Rhône-Alpes., Oct 2019, Lempdes, France. 23 p. http://hal.univ-nantes.fr/PIAF/hal-02394665v1

7. Global Impact assessment indicators

7.1 Synthetic figures for the project publications (including interactions with stakeholders)

Analysis of the *project* publications:

Scientific Journal	***Number***	***Impact Factor (2019)***
Environmental Pollution	1	5.07
FEMS Microbiology Ecology	1	4.09
Urban Forestry & Urban Greening	1	4.02
Ecological Indicators	1	3.98
Plos One	1	2.77

International dimension and multi-partnership for publications

		Number of publications
Multi-partner publications	**Peer-reviewed journals**	4
	Books or chapters in books	0
	Communications (conferences)	6
Single-partner publications	**Peer-reviewed journals**	2
	Books or chapters in books	0
	Communications (conferences)	3
Outreach initiatives including interactions with stakeholders	**Popularization articles**	3
	Popularization conferences	3
	Others	3

7.2. Other scientific outputs

Not applicable.

	Number, years and comments **(Actual or likely outputs)**
International patents obtained	0

International patents pending	0
National patents obtained	0
National patents pending	0
Operating licences (obtained / transferred)	0
Software and any other prototype	0
Company creations or spin-offs	0
New collaborative projects	2
Scientific symposiums	0
Others (please specify)	0

7.4. Assessment and follow-up of personnel recruited on fixed-term contracts (excluding interns)

Identification			Before recruitment for the project			Recruitment for the project				After the project			
Surname and first name	Sex M/F	E-mail address	Last diploma obtained at time of recruitment	Country of studies	Prior professional experience, including post-docs (years)	Partner who hired the person (Organisation and Country)	Position in the project (1)	Duration of missions (months) (2)	End date of mission on project	Professional future (3)	Type of employer (4)	Type of employment (5)	Promotion of professional experience (6)
Yu, Kang	M	kang.yu@kuleuven.be	2014	Germany	3	KU Leuven (B)	Post-doctoral	36	30 April 2020	Open-ended contract	teaching and public research	lecturer-researcher	Yes
Maarten Van Geel	M	maarten.vangeel@kuleuven.be	2016	Belgium	1	KU Leuven (B)	Post-doctoral	36	30 April 2020	post-doctoral position	University	Researcher	Yes
Cindy Serafim	F	cserafim@porto.ucp.pt	2017	Portugal	0	UCP (P)	researcher	36	30 April 2020	PhD student	University	Researcher	Yes

7.5. Data Management and timeline for open access

- All publication preprints (accepted version) are available in the *liras* repository of KU Leuven (**Green Open Access**). https://www.kuleuven.be/english/research/scholcomm/lirias
- All collected soil data and Illumina sequencing data of ectomycorrhizal fungi of the sampled Tilia trees, are available at: *journals.plos.org/plosone/article?id=10.1371/journal.pone.0225714*
- Representative sequences for each EcM OTU were also deposited in GenBank (accession numbers MH801215 - MH801410).
- Survey data of the location (map) and soil sealing of the investigated trees in three cities (Dec 2020) are stored at KU Leuven servers.
- Tree health indicator data derived from leaf reflectance and fluorescence measurements (Dec 2020) are stored at KU Leuven servers.
- Leaf functional traits data including the SLA, LWC and chlorophyll of the investigated trees (Dec 2020) are stored at KU Leuven servers.