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Linking tree health to soil and root properties in urban trees, Case study of *Acer platanoides* in the city of Vienna

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Abstract

Urban trees in Vienna and cities world-wide are exposed to multiple stresses like soil compaction, restricted root zone, water and nutrient deficiency, excessive heat, and several types of pollution such as de-icing salts. Due to the subsequent decline in vitality, trees must be replaced frequently at the expense of ecological functions and substantial monetary costs. Vienna's most common tree species *Acer platanoides* in two levels of traffic intensity and parks was examined in order to see how tree health and mycorrhization is linked to different soil properties including salt concentration. This study found out that trees from busy streets with high traffic intensity displayed lowest health rates. The soils of these streets demonstrated the highest salt and heavy metal levels. The content of sodium was 4.5 times, of cadmium 3 times and of zinc 2 times higher than in park soils. Crown vitality was negatively correlated with copper content in the soils, relative stem increment showed negative correlation with salt content, which could be due to thereby induced physiological drought. Fine roots of street trees exhibiting larger mean root diameters than park trees, which could be due to succulence of the cortex as adaption to excessive salt intrusion. Furthermore, the fine root distribution over root diameter classes showed large variations between street and park trees within the very thin fine roots of diameter classes 0-0.6mm, which could be due to different root functioning. Endophytic fungal colonization rate (arbuscular mycorrhizae and dark septate endophytes) found little overall colonization, with even less colonization from arbuscular mycorrhizae than from dark septate endophytes. Overall, tree health of urban *Acer platanoides* is probably negatively affected by urban stress, especially de-icing salts and heavy metals, as levels in the soil demonstrated to be proliferated were trees showed decreased vitality.

Affidavit

I hereby declare that I am the sole author of this work. No assistance other than that which is permitted has been used. Ideas and quotes taken directly or indirectly from other sources are identified as such. This written work has not yet been submitted in any part.

Vera Mayr

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Introduction

In 2018 more than half of the global population lived in urban areas and according to the UN World Urbanization Prospects report this trend of urbanization is estimated to continue. In 2050 the proportion of people living in cities is prospected to grow further up to 68% of the world´s population. Nevertheless, the levels of urbanization vary widely from geographic regions (to another). Today the most urbanized geographic regions are Northern America (82%), Latin America and the Caribbean (81%) as well as Europe, with 74% of citizens living in urban areas (United Nations 2019). Considering the ongoing process of climate change and global warming especially cities need to be adapted accordingly/respectively. Extreme weather events, particularly heat waves are predicted to occur more often and last longer (IPCC 2014).

Cities today are already subject to higher temperatures compared to their rural surroundings. This 'urban heat island effect' derives from differences in land-cover such as prevalent usage of the impervious materials asphalt and concrete, that absorb and retain heat rapidly in the case of solar radiation, the densely built structures in combination with surface sealing along with the lack of vegetation (Oke 1973; Bowler et al. 2010; Gillner et al. 2015). Besides 'urban climate' is generally characterized by lower air quality affecting health and quality of life of inhabitants (McPherson et al. 1994).

In this world of growing cities and changing climatic patterns urban vegetation, particularly trees, play a key role in mitigating the pressure of pollution and heat in urban areas in order to create healthy city environments and enhance resilience against global warming (Gill et al. 2007).

Among other functions like having a positive impact (calming and balancing) on human psyche and health due to accelerated regeneration and recovery, trees fulfil important ecological functions (Harris et al. 2004). Those include removing or reducing air pollutants like e.g. ozone, nitrous oxides, sulphur, carbon monoxide while producing oxygen, binding microparticles in their leaves and therefore further enhancing air quality by cleaning from small particulate matter, sequestrating carbon dioxide, being habitat for fauna and flora as well as affecting local climate, e.g. cool the microclimate, hence reduce thermal loads on summer days by shading surfaces and evapotranspiration of water (McPherson et al. 1994, Gillner et al. 2015, Roloff 2016).

The ability of removing pollutions depends on tree species, leaf-surface area and physical condition of the individual tree as the processes come along with the possible side effect of damage. Notably large, healthy trees have a 60 to 70 times higher ability of removing pollution than small trees (McPherson et al. 1994).

City environments in general put multiple pressure on urban trees. Abiotic factors like inappropriate soil conditions in the form of disturbed soils containing a high level of technogenic substrate, i.e. construction waste, debris, ashes and waste, soil sealing which affects water (infiltration) and gas (exchange is negatively affected) households of the soil combined with high rates of compaction limiting pores space for water (field capacity is decreased), oxygen and root growth have a negative impact on the individual plant. Furthermore, urban soils continuously show nutrient deficiency due to the disruption of the nutrient cycling by annual removal of foliage (Braun 1990, Balder 1998). The pH levels in city soils are reportedly higher than in natural forest environments ranging from neutral to slightly alkaline, leading to a reduction in plant availability of nutrient elements like phosphor, boron, iron, manganese, zinc and copper (Braun 1990, Just 1992, Balder 1998, Czerniawska-Kusza et al. 2004, Scheffer/Schachtschabel 2010). Not only the composition of the substrate limits the natural development of trees, but also is the natural habitat, especially rooting space, in most cases limited in

urban areas. Particularly street trees are frequently planted into pits that are by far too narrow calculated to meet the trees rooting demands (Braun 1990, Balder 2002).

Even tough elevated soil temperatures because of high radiation on solidary trees, including reflection from buildings, may promote root growth, the overall higher air temperature reduces the relative humidity leading to higher transpiration, hence higher water demand and in the end inducing drought stress to the tree (Braun 1990).

In addition, city environments are places that cause various forms off pollution. Besides gaseous pollutants like ozone, nitrous oxides, carbon monoxide, hydrocarbons, sulphur dioxide, particle matter and secondary products that potentially accumulate in leaves and consequently decrease photosynthesis or lead to early senescence, there are soil pollutants that negatively impact the health of trees. For example, heavy metals like cadmium, mercury and lead can prevent the growth of fine roots, nevertheless a high pH level immobilizes their solubility (Fathi & Lorenz 1980, Frostegård et al. 1996, Scheffer/Schachtschabel 2010). What is more are selectively high concentrations of phosphorous and nitrogen as a result of dog faeces and urine that can be toxic for microorganisms and roots as well as change soil chemistry. When directly applied on tree tissue the uric acid of the latter may even cause injuries, i.e. trunk/root burn (Balder 1998, Wittig 2002). Finally, notable as potential soil pollutant is sodium chloride (NaCl).

The excessive use of NaCl as de-icing treatment during winter season in temperate and boreal zones is of high relevance acting as a soil pollutant in urban areas (Kleiber et al. 2019). Health, growth and life expectancy of trees have been shown to be negatively affected (Cunningham et al. 2008, Li et al. 2014).The main effects of high salt concentrations in the soil are chemical compaction of the soil due to Na^+ altering the basic texture, i.e. decreasing soil porosity, thereby reducing soil aeration as well as water conductance. Simultaneously do excessive sodium and chloride ions have a toxic effect when dispersing inside the roots or tree tissue. The accumulation disrupts enzymatic structures and other macromolecules, damage cell organelles, inhibit the synthesis of protein and induce deficiency of ions like potassium (Juniper & Abbott 1993). The most problematic effect of high salinity for the tree is the water deficient soil condition that induces the risk of physiological drought. Both saline soils and droughts put osmotic stress on a plant (Porcel et al. 2012). The dissolved sodium chloride diminishes the soils osmotic potential, the tree needs to perform osmotic adjustment in order to prevent water from moving out of the roots including the risk of hydraulic failure by negative hydraulic pressure in the xylem (Choat et al. 2012). Consequently, nutrient imbalances occur because of the restricted nutrient uptake and transport. To conclude salt stress affects photosynthesis and metabolism of the tree negatively. It leads to early senescence of leaves and roots, which reduces the resource capturing abilities. As a result, reducing sugar and starch reserves and inhibiting expansion growth (Braun 1990, Porcel et al. 2012, Rewald et al. 2012). Visible signs of decreased vitality of salt stressed trees are the reduction in biomass (leave size, foliage density, diameter at breast height) in comparison with trees of the same species at the same age as well as chlorosis and necrosis (Czerniawska-Kusza et al. 2004).

As a result of the combination of these various abiotic strains are urban trees prone to biotic stress like deceases (bacteria, fungi, viruses) or parasites from flora or fauna. Especially injuries or damage from often-occurring construction work, parking cars and intentional cuttings from municipal pruning work allow parasitic organisms to settle in the trees tissue (roots, trunk, branches). These pests and pathogens can alleviate the trees vitality further.

Besides the already revealed decreased performance and growth of urban trees, is the overall decreased life expectancy worth to mention (Roloff 2013). Nevertheless, do the estimates in the literature vary from 13-60 years lifespan depending on the site within a city (Skriea & Moll 1992, Balder 1998). This seems highly comprehensible considering the different conditions for roadside trees of high

traffic roads with excessive use of de-icing salts or quiet neighborhoods. Notably these predictions count for young trees that have recently been planted or will be planted today (Dickhaut et al. 2019). In the city of Vienna 2000 new trees are planted every year (Hutsteiner 2017). In the worst case some of them need to be replaced a few years later, which comes with enormous costs for the municipality. So does a survey on the practices of tree establishment in European cities show that the average cost for one tree can be as high as 600€ in Austria, ranging from 250- 1000€ per tree (Pauleit et al. 2002).

When it comes to a freshly planted tree the fast in-growing of roots from the bur-lapped root bales into the surrounding planting pit is very important, because roots as the absorbing organs of nutrients and water play an important role in the tree`s overall health and performance (Tobner et al. 2013). Not only for freshly planted trees, but for trees in general is a steady high fine root density crucial. High amounts of fine roots, i.e. absorptive (the very finest fine roots) and transport fine roots, guarantee the flow of essential nutrients, assimilates and phytohormones due to the capacity to absorb water as well as nutrients and provision of space for (root) symbiosis, thus enhancing adaption to stress (McCormack et al. 2015).

Overall many plants and especially trees need specific symbionts (rhizobia, actinomycetes or mycorrhiza) to prosper. Nevertheless, the isolated locations of planting pits and the isolation during tree breeding impedes the immigration of the required organisms (Balder 1998). Specially, mycorrhizal fungi can enhance a plants tolerance against abiotic and biotic stresses (Porcel et al. 2012). With the associated mycorrhizal symbiosis, the uptake of water and nutrients, like nitrogen, phosphorous and other trace elements, increases. Hyphae of mycorrhizae have the ability to penetrate larger parts, moreover smaller pores, of the soil as hyphae have a smaller diameter and are more efficient in terms of carbon requirements than plant roots. The surface area for absorption of a root system can enlarge by 100-1000 times with an interconnected mycorrhizal hyphae system (Balder 1998). Additionally, are mycorrhizae known for protecting the plants' enzyme activities and facilitating defense reactions. Furthermore, they act as a first barrier for pollutants before entering the roots, to some extent being taken up into the mycorrhizal tissue and not released to the tree (Thangaswamy et al. 2005).

Mycorrhizae can be grouped roughly in ecto- and endomycorrhiza, depending on the way of relationship with the cortical root cells or which formations are built. Ectomycorrhiza (EM) form a characteristic mantle outside the feeder root`s tip as well as surrounding root areas. The second characteristic is the Hartig net which is formed by hyphae growing between the epidermal and cortical cells of the root. Even though EM leads to anatomically changes as the root tip swells and the root loses its root hair, no penetration of the cortical cells appears. The specific mantle has a characteristic structure and color depending on EM species.

Characteristic for endomycorrhiza is the somewhat penetration of cortical root cells. There are different kinds of endomycorrhiza, including arbuscular mycorrhiza (AM) being the most common colonizer colonizing a wide range of plants. The name arbuscular mycorrhiza derives from the typical structures these build in the cortical cells of the associated root- little tree shaped exchange sites. Determined by both plant and fungal partners intracellular hyphal coils can act as exchange sites, which sometimes occur in the absence of arbuscules. Besides, AM can develop vesicles that function as storage sites inside the root, either inside or between the cells. Intraradical hyphae growing between the root cells connect the structures with the external hyphae that are exploring the soil searching for water and mineral nutrients (Smith & Read 2008).

Besides increasing the tolerance of plants against stress from heavy metal pollution, arbuscular mycorrhiza is found to alleviate salt stress (Rewald et al. 2015). The symbiosis under salinity performs its positive outcome due to a combination of nutritional, biochemical and physiological effects. The composition of mineral nutrients, moreover poor mobility nutrients like P, in the plant is enhanced by

increased and selective uptake (Evelin et al. 2009). That means favoring base cations like K^+ and $Ca2^+$ that act as osmotic equivalents while avoiding the uptake or transport of toxic levels of Na^+ to the root (Hammer et al. 2011, Barin et al. 2012). AM can facilitate in compartmentalization of Cl^- ions in vascular membranes, hence impede the negative impact on metabolic pathways in the plant. Another enabling process is the accumulation of compounds like proline regulating osmotic adjustment in the roots. Furthermore, are photosynthesis and water use efficiency shown to be increased with arbuscular mycorrhizal symbiosis (Evelin et al. 2009).

However as mentioned above, do the adverse urban conditions of oxygen deficiency, high pH, salinity due to high use of de-icing salts in soils besides other stressors of many city locations tremendously limit the population of mycorrhizae quantitatively and qualitatively (Balder 2002). Especially the isolation between planting pits or parks, isolated planting material from nurseries, urban climate and excessive deposition of sodium lead to mycorrhizal communities which are patchy distributed, low in diversity and number (Bainard et al. 2011; Tyburska et al. 2014). The pre-inoculation of breeding substrate or on-site root zone with commercially available spores of both AM and EM fungi has been found to increase tree growth in general and is recommended in order to enhance resilience against urban stresses. (Balder 2002, Balliu et al. 2015)

Nevertheless, field studies on tree health associated with mycorrhizal fungi in urban soils are scarce. This research aims to shed light on tree health and linked root characteristics in urban soils, which are contemplated closely and examines the mycorrhizal species living in association with salt-stressed trees. For the study the salt-resistant species *Acer platanoides* has been chosen, as this species is capable of developing symbioses with arbuscular mycorrhizal (Smith & Read 2008). The sampling processes and parts of the laboratory work were combined with another study on urban trees of the salt resistant species *Tilia cordata*. Tree individuals from different urban sites where chosen in consideration of their exposure to de-icing salts. Park trees serve as control as they are presumed as non-stressed from NaCl.

Further details on *Tilia cordata* and the associated ectomycorrhizal fungi can be found in the work of Dylan Goff, with whom the sampling process and parts of the laboratory work of this study were undertaken (Goff 2020).

This study we determined if tree health of *Acer platanoides* and symbiotic activity is found to be different in street trees compared to more protected trees of parks and see if retro- or proactive inoculation of planting pit soil with mycorrhizal spores should be tested. Therefore, it will be of high practical benefit for municipalities in need of cost-efficient and sustainable solutions for the increasingly important work of Urban Horticultural management.

Therefore, the aim of this work is to shed light on the vitality of 20-60-year old *Acer platanoides* trees in the city of Vienna, carefully considering the levels of de-icing salts and traffic, looking at fine root distribution as well as arbuscular mycorrhizal infection rates. Moreover, soil properties, especially macronutrient and trace element status are analyzed, in order to compare the planting pits soil of the street trees to the non-rooting space limiting soils of park trees.

Hypotheses

Hypothesis 1: The higher the urban, i.e. de-icing salt, stress level the lower the vitality of trees. Control trees in parks will have the highest vitality level.

Hypothesis 2: Tree fine roots will be affected by stress. Fine root properties will be different between control trees and roadside trees.

Hypothesis 3: The higher the urban, i.e. de-icing salt, stress level the lower the arbuscular mycorrhiza colonization rate will be. Control trees will have the highest colonization rate.

Material and methods

Selection of trees

Together with the Vienna city garden department MA42 two suitable tree species have been identified for research: *Acer platanoides L.* and *Tilia cordata P. Mill*., which are among the most often replanted tree species in Vienna. Nevertheless, is *Acer* the most common tree genus in Vienna in general with a total number of 25,065 individuals (Magistrat der Stadt Wien 2019). For year 2017, when the sampling took place, the total number of *Acer platanoides* trees in Vienna was 23,638 (Bayr et al. 2017).

Acer spp. are known for regularly forming symbiosis with mycorrhizal fungi, i.e. arbuscular mycorrhiza fungi (Smith & Read 2008).

Acer platanoides or Norway Maple is described as a fast-growing species that can grow on a wide range of soil conditions, shade, radiation or drought and pollution including imissions. Nevertheless, when it matures it becomes more light-demanding and thrives in fertile and moist soils, which are adequately drained and with a sub-acid pH, where it can grow its deep heart shaped rooting system as deep as 1.5m (Roloff 2013). Under these optimal conditions *Acer platanoides* may live for more than 180-250 years, reaching up to 30 meters height. Its native habitat comprises central and northern Europe as well as stretching north-east until the Ural Mountains. However, due to its tolerance against urban conditions and its spreading crowns as well as rapid youth growth, it has been widely used as street tree in many western cities for shading and ornamental purposes (Caudullo & de Rigo 2016). Moreover, it has the ability to re-sprout vigorously after cutting measures and to reduce high levels of emissions. In urban areas the life expectancy of *Acer platanoides* individuals is reduced to less than 150 years, reaching a height of 20 meters and trunk diameter of 1 meter (Roloff 2013). Besides its tolerance to strong calcareous soils, it is rare on acidic soils (pH near 4) and highly sensitive to low soil nitrogen conditions, high evapotranspiration or prolonged drought as well as to excessive infiltration of de-icing salts (GALK 2012, Caudullo & de Rigo 2016). Additionally, young trees are particularly sensitive to sunburn, damage and disease during changing seasons accounting for changing temperatures and weather conditions like frost (Roloff 2013).

To fulfil the requirement of frequent appearance under similar mean altitude as well as soil conditions the investigated area in Vienna was limited to the north-western districts 15 to 20. The mean altitude there is about 200m above sea level. According to the soil map provided by the city of Vienna are the concerned soils of the district 15., 16., 17., 18. and 19. carbonate free cambisols (*carbonatfreie Braunerde*), besides the fluvisols (*Auböden*) of the 20. district (Stadt Wien 2020).

In spring 2017 individual trees of similar ontogenetic stage in the age of 20-60 (1943-1997 planting period) from were chosen to examine. Notably trees with damaged stems and tree pits covered with shrubs or bushes were excluded and a maximum of 4 individuals in close vicinity were selected (Bayr et al. 2017).

The total amount of 59 trees were chosen in three categories: The first category were roadside trees that were supposed to be medium salt stressed, taken from side streets with low traffic. These streets are locations without public transport routes with reduced winter service.

The second category were roadside trees in high traffic streets with public transport. Due to public transport these main streets and neighboring sidewalks were supposed to be treated more regularly and with a higher amount of de-icing salt application, in order to minimize the risk of sliding accidents.

The third and last category were park trees, that were supposed to be non-salt stressed, because no pathways or streets are passing by and therefore there is no risk of any de-icing salt disperse during winter season. These park trees were serving as the control group.

20 suitable trees have been chosen for each category, making 60 trees. Nevertheless, one tree of the main streets turned out to be injured, which therefore was excluded from the study, leaving 59 trees.

The tree selection and health as well as location assessment are the content of a bachelor thesis of five students (Bayr et al 2017).

Field and tree assessment

All trees and their planting environment were visually assessed and got specific gradients in different categories. The Visual Tree Assessment happened from the ground via tour around the respective individual.

The incoming sunlight was estimated from gradient 1 (no insolation) to 10 (full insolation).

The overall tree health was detected according to guidelines of the Vienna municipality MA 42. These combine the Roloff vitality criteria and the ÖNORM L 1122 focusing on crown (treetop) cover, foliation, deadwood, fruit coverage and mean growth (Roloff 1989). Vitality is defined as the vital force of an organism, that is influenced by its age, genes and environmental factors. It manifests in the tree health condition, especially growth, crown structure and foliage, adaptive and regenerative capacity as well as resilience against disease and pests (FLL 2017). Roloffs invented criteria are based on the finding that decreased vitality can modify the branching structures of trees as it is naturally happening while aging when the tree experiences the four stages of exploration, degeneration, stagnation and finally retraction (Roloff 2016). In order to gain more experience how to evaluate the stated criteria, a one-day training with two experts from the Higher Federal Teaching and Research Institute for Horticulture Schönbrunn was held before the assessment. The assessed crown vitality was described with gradients from 1 (lowest vitality) to 10 (highest vitality).

Table 1: vitality rating scheme

Crown vitality level	indicators for crown vitality
1	▪ more than 70% dead branches in crown ▪ visible parasites (e.g. mistletoe) ▪ pale or necrotic leaves ▪ sparely crown ▪ visible disease on trunk

2 .. 3 .. 4	all possible grades in between levels 1 to 5
5	▪ few dead branches in crown ▪ green leaves ▪ crown medium dense (50% view to the sky, when standing under the crown)
6 .. 7 .. 8	all possible grades in between levels 5 to 9
9	▪ no dead branches ▪ no visible parasites ▪ dark green, vital leaves ▪ dense crown (no visible view to the sky, when standing under the crown) ▪ no visible disease on trunk
10	highest vitality due to best possible conditions (unlikely in urban areas)

In addition to the crown vitality rating scheme growth parameters have been collected and calculated respectively: Circumference at breast height (1.3m) was measured in order to calculate the Diameter at breast height (DBH) as well as height of the trees were assessed using the ultrasound measuring system Forestor Vertex digital hypsometer by Haglöf (Langsele, Sweden).

To serve as complementary vitality indicators the following variables have been calculated: growth rate- measured height in relation to age of the tree. Radius growth in relation to planting radius, which was generally assumed to be 5cm at the time of plantation. Relative radius growth in relation to year of plantation. Information for the calculations derived from DBH, height measurements as well as the Vienna City Baumkataster for information about age (Stadt Wien 2017). For 9 out of 59 trees no information about age is recorded. As tree vitality is widely defined as the capacity to survive stress, this study applies: the higher the stem increment, the better the tree vitality over life span (Dobbertin 2005).

Furthermore, gradients were estimated for roadside trees´ traffic, parking lot nearby and vegetation cover. For traffic it was recorded high or low, meaning with or without public transport, i.e. bus. Moreover, it was recorded if there was a parking lot next to the planting pit (yes/no) aa well as if and how densely the planting pit or space was covered with grass or weeds. The pit vegetation categories were no (0), light (1), medium (2) or dense (3).

Coring and sampling

A first round of soil sampling took place in May 2017. In this first period soil was taken on the one hand with a cylindrical tube, on the other hand with the Pürckhauer ground auger in order to analyze the parameters: soil density and electronical conductivity. The cylindrical tube was driven into the surface soil, thereafter, shaved evenly on both ends to produce soil cores with exact volume, which were then removed from the tube to transport for analyses. The Pürkhauer was drilled into the soil at a 50cm distance from the trunk until a depth of 30cm. Notably only the bottom 20cm of the Pürkhauer samples (10-30cm) were collected for further analysis. The collected soil samples were sieved through a metal sieve with a mesh size of 2mm to remove stones and other larger parts (Bayr et al. 2017).

The second field sampling took place in July 2017 and beginning of August 2017, taking core samples with a cylindrical coring tool with a size of appr. Ø 8cm. The corer was placed at 50-100 cm distance on a position where no roots and highly variable vegetational cover were evident. A minimum distance

of 50 cm from the tree stem was kept in order to not injure the tree`s large woody roots and to guarantee a bigger number of fine roots, due to the fine roots` ability to form mycorrhizal symbiosis. The distance was tracked for each sample and if possible, due to different planting pits/strips/environments not always possible, was enlarged up to 100 cm from trunk. The process of coring was carefully conducted by manual hammering in order to notice any resistance that would either indicate a large woody root or an obstacle like stones that would damage the corer. In case of experiencing more resistance than usually the coring process was interrupted and the corer was placed on a new spot. This replacement needed to be done several times during the sampling. As the field sampling took place on urban soils it was not easy to distinguish between resistance due to a real obstacle or if the soil was just highly compacted. Particularly the excavation process took a great deal of manual power due to the high compacted urban soils. The corer was hammered into the soil to a minimum sampling depth of 40 cm- if possible- to 60 cm, tracking the depth for each sample. Thereafter, compaction was measured. Afterwards the corer was removed carefully from the soil and the sample was put into a Styrofoam profile to transport it to the laboratory. For sample cores, where the tree environment differed apparently, notes were taken, e.g. when there was a layer of bark mulch, another tree or shrub nearby.

Specific notes were taken for every sample:

- date
- core distance from trunk
- compaction (comparing inside and outside height of corer)
- sampling depth
- pit, strip or park

The intact cores were stored at 4°C before wet sieving with different mesh sizes in order to separate the *Acer platanoides (AP)* roots from other roots and the surrounding soil (figure 1a & b). By beforehand literature research and training could the contaminating roots of other species be identified by differences in appearance, especially size, shape and color, and put aside (Brundrett et al. 1996). Pictures of the root separation process and differences in root shape and color can be seen in figure 1c-e.

Only the *AP* fine roots <2 mm were kept and separated due to core depths of 0-10 cm, 10-30 cm, 30-50 cm and stored differently for further processing. The roots from the top (0-10 cm) and under most segment (30-50 cm) of the soil core were separated and slowly dried in a heating room at 40°C for several months. The roots in the middle segment from 10-30 cm were stored in 65% ethanol at 4°C in test tubes before treatment. About 45-50g of the sieved soil of the 10-30 cm segment was homogenized, kept in paper bags and slowly dried in the heating room at 40°C for several months.

In October 2017 another soil sampling took place, taking soil from a depth of 15 -20cm at a distance of 50 to 60cm from each tree The soil was sieved to 2mm and homogenized. Part of it was used freshly, whereas the other part was used dried at 105°C.

Figure 1: Soil core processing of soil samples taken from urban Acer platanoides salt stress locations, a. segmentation of intact soil core, b. wet sieving to separate roots from soil, c. manuel sorting of fine roots <2mm, d. separating Acer platanoides roots from alien roots, e. characteristic Acer platanoides root with younger root parts showing orangish, yellowish, greenish color.

The processing and analyses took place in the laboratory of the institute of forest ecology at the University of Natural Resources and Life Science in Vienna.

Root characteristics

The fine roots (<2mm) of the middle segment (10-30 cm) of the soil cores were analyzed with WinRHIZO by Regents Instruments Inc (Quebec, Canada) in order to see if there is a difference in root morphology due to the perceived salt-stress level of the tree locations, overall tree health or other differing soil characteristics (preparation for scanning see figure 2). For the clearing and staining tests about 5cm of some root samples were removed before scanning. From the WinRHIZO data the

variables total root length (cm), total surface area(cm^2) mean root diameter (mm) and the root length and surface area in the specific root diameter classes 0-0.1(mm), 0.1-0.2 (mm), 0.2-0.3 (mm), … , 1.9-2.0 (mm) were of special interest in this study. Note that because of the removal for the clearing and staining tests about 0.5% of total root length and surface area were not recorded. This was classified as a minor measuring error that has no influence on the overall WinRHIZO data.

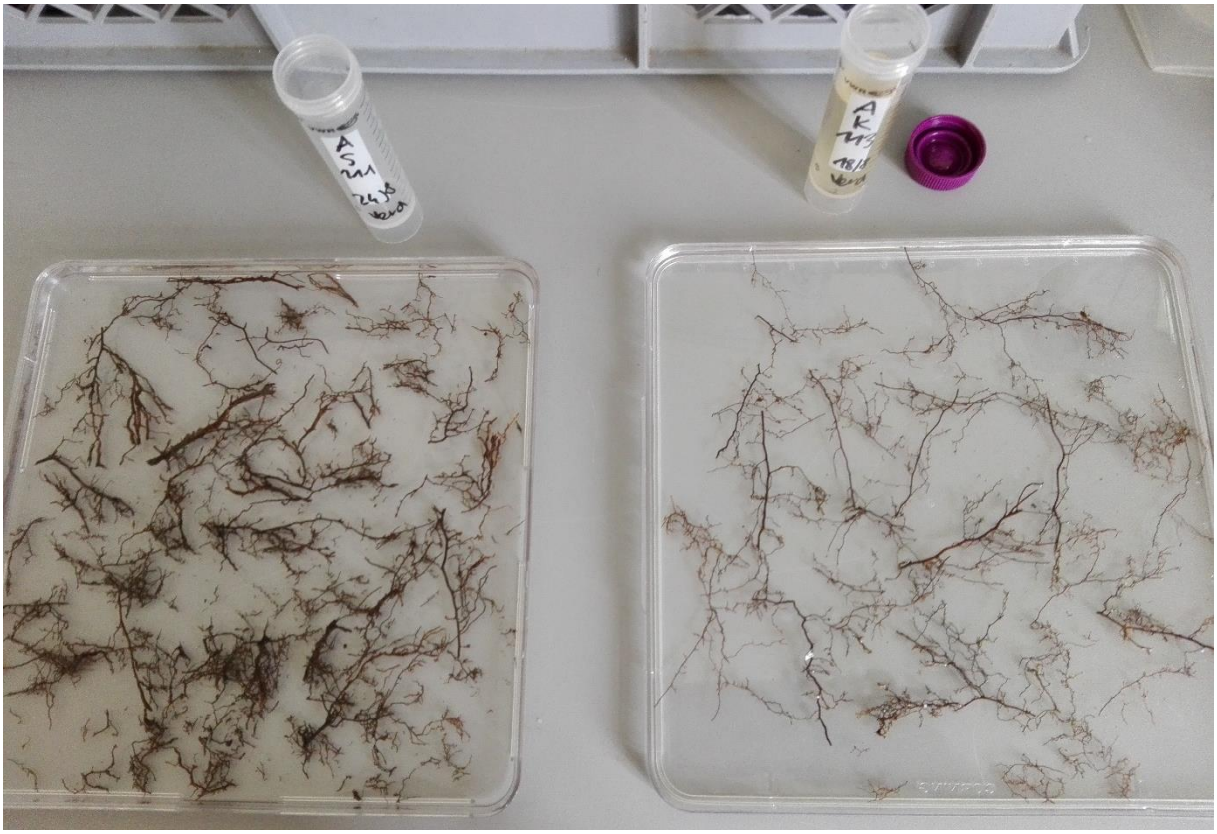

Figure 2: Acer platanoides roots of <2mm size from soil depth 10-30cm prepared in transparent trays filled with water for analysis in WinRhizo scanner, left sample from salt stressed roadside location, right sample from park location

After scanning the roots from depth 10-30cm were slowly dried for several weeks in the heating room at 40°C. Note that just fine roots from soil depth 10-30cm were scanned.

To detect the biomass of the tree fine roots the dried roots of the different core segments, i.e. depth 0-10cm, depth 10-30cm and depth 30-50cm, were weighed. As the root biomass was highly different between samples, for some samples the total weight needed to be determined by summing up the weight from separated subsamples weighed, that were necessary as the complete biomass would not fit into one petri dish.

To compare fine root properties of the trees from different salt stress levels the additional parameters specific root length (SRL), specific root area (SRA) and ratios of distribution in different fine root diameter classes 0-0.1, …, 1.9-2.0 (fine root ratio) were calculated. The required information derived from WinRhizo scan data (scale cm or cm^2) and root biomass data (g). These parameters were calculated for the soil depths of 10-30cm.

$$\mathrm{SRL} = \frac{\text{root length (cm)}}{\text{root biomass } 10-30 \text{ (g)}}$$

$$\text{SRA} = \frac{\text{root surface area (cm}^2\text{)}}{\text{root biomass } 10-30 \text{ (g)}}$$

$$\text{Fine root ratio x} = \frac{\text{root length diameter class x (cm)}}{\text{root length (cm)}}$$

Mycorrhizal experiments

Clearing and staining of tree fine roots

In order to determine the arbuscular mycorrhizal colonization rate in roots there are different possibilities of studies. A common and comparably cost-efficient way is the method of clearing and staining described by Phillips and Hayman 1970. The amount of mycorrhizal infection in form of present fungal tissue in the root cortex is estimated by visual assessment after the root has been cleared and stained. In the clearing step roots are heated in a potassium hydroxide solution to remove the cytoplasm plus most of the nuclei, leading the vascular cylinder to be visible distinctly. Pigmented roots need to be bleached by immersing in an alkaline solution of hydrogen peroxide, then acidified in hydrogen chloride and in the final staining step stained with a staining agent, e.g. ink. As fungal tissue contains chitin and melanin, it is absorbing and eluting stain better than the cleared root tissue. Therefore, the fungal structures are deeply stained showing distinctly against the outlines of the cells in the cortex (Phillips & Hayman 1970, Brundrett et al. 1984).

Nevertheless, the clearing and staining technique is recommended for plant species with roots that have a thick cortex and are not heavily pigmented as fungal structures can be identified easier (Brundrett et al. 1996). A Canadian study highlights the possible difficulties of the clearing and staining occurring with *Acer saccharum,* that can be presumed for other trees of the genus *Acer*. Due to its highly modified root epidermal cell walls, i.e. an exodermis with lignin and suberin in its outer walls as well as tannins in its cortical cells, the roots can stain as intensely as the mycorrhizal structures making the visual distinction impossible (Brundrett & Kendrick 1988). Similar problems have been found for other tree species. Therefore, the method has been adapted further (Chavez-Barcenas et al. 2013).

Considering the difficulties other researchers had, especially with adult trees from field assessment, in this study a set of pre-tests of different method adaptions have been conducted. The following comparison of different studies showed that the concentration of potassium hydroxide, the soaking time and temperature as well as the staining agent are the main factors varying:

Phillips & Hayman (1970)	Koske & Gemma (1989)	Chavez-Barcenas et al. (2013)
<u>Clearing</u>: Heat roots at 90°C in **10% KOH** solution for up to 2h (depending in plant species), wash with fresh KOH	Heat root in **2.5% KOH** at 90°C for 10-30min., or **autoclave** at 121°C for 3min., rinse with water	Submerge roots in **15%KOH** and **autoclave** for **15min**. (at 115kPa), rinse with tap water, **bleach** with **3%H2O2** for **15min**. at room temperature, Rinse with tap water, **Autoclave** in **15%KOH** for **10min**., **bleach** in **3%H2O2** for **20min**. at room temperature, Acidify in 1% HCl for **5min**.
<u>Bleaching and acidify</u>: Immerse at 20°C in alkaline solution of H2O2 10-60min. until bleached, rinse with water, acidify in dilute HCl	Lighten dark roots in H2O2 for 10-45min. (3ml 20% NH4OH in 30ml 3%H2O2), rinse with water, Soak roots in 1% HCl for 1-24h	

Staining: Simmering for 5min. in 0.05% Trypan Blue in **lactophenol**, remove excess stain in lactophenol	**autoclave** roots for **3min**. in 0.05% Trypan Blue in **acidic glycerol** (500ml glycerol, 450ml H_2O, 50ml 1% HCl), de-stain and store in acidic glycerol	Stain roots in 0.05% Trypan Blue in aceto-glycerol in the **autoclave** for **10min**.

In recent years the stain Trypan Blue became suspected of being carcinogenic. Hence Vierheilig et al. found an alternative staining in form of the "ink and vinegar method" that gives equivalent results. After clearing, roots need to be boiled for 3-5 minutes in a 5% acidic acid (household vinegar) and 5% ink (ordinary ink like school children use) solution before the excess dye can be removed by rinsing with slightly acidified water (Vierheilig et al. 1998).

Combinations of methods from the different studies were modified in a pre-testing phase in order to overcome the problems that arose due to either possible insufficient staining of fungal tissue, overstaining of roots or disrupted root material with the standard methods.

Overall, 26 lightly different laboratory protocols with two or three subitems were tested. The tested roots were either from park individuals of *Acer platanoides*, in case of two protocols from a salt-stressed roadside individual. At the beginning roots were either dried or not as well as not soaked or soaked in tap or de-ionized water, in order to let the 65% storage-ethanol leave the tissue. Then the roots were heated in 10% or 15% KOH in the autoclave at 121°C for 10, 15, 20 or 30 minutes, thereafter, let in the autoclave overnight or just for some hours or treated further shortly after autoclaving. Either a second round of autoclaving for 10 or 15 minutes was performed, or the roots were bleached in 3% or in one protocol in 30% H_2O_2 for 10-60 minutes at room temperature, the bleaching step followed all autoclaving scenarios. Then the roots were washed with tap water and acidified in 1% HCl for 5-15 minutes or in slightly acidified water for several hours at room temperature. The subsequent staining was either performed with the described ink-vinegar solution (Schaeffer Black or Pelikan Blue ink) or with 0.05% Trypan Blue in lactoglycerol either autoclaving for 5-15minutes or heating at ~90°C for 10-45 minutes. The de-staining was performed with the advised de-staining solution (acidified water or lactoglycerol) either at room temperature for up to some hours or at 4°C for several days. Finally, the cut pieces have been mounted on slides either with lactoglycerol or polyvinyl alcohol-lactic acid-glycerol (PVLG) (Omar et al. 1979). These slides were visually assessed with a ZEISS Primo Star microscope (ZEISS, Oberkochen, Germany).

Figure 3: De-staining of cleared and stained urban Acer platanoides roots in slightly acified water, staining agent Pelikan Blue, at room temperature for several hours before putting them into the cooling room for further but slower destaining at 4°C for one week.

The detailed 26 protocols can be seen in the appendix I.

Spore extraction- pre-testing of method

In order to deliver a possible spore analysis a pre-testing of spore extraction was undertaken with the soil stored and dried from 6 cores of highly salt-stressed sample trees and the soil of 6 park trees that acted as controls.

With a combination of wet sieving (Gerdemann & Nicolson 1963) and a sucrose centrifugation method (Brundrett et al. 1996) 5g dried soil were put in 200ml tap water and mixed at 550 RPM (revolutions per min.). The mixture was shaken for 5 minutes and let set for 30 minutes. After the sedimentation of the coarse material the suspension was decanted through three interlocked metal sieves with the mesh size of 710 µm, 200 µm, 40 µm in order to separate the debris from fine particles and spores. Water was decanted through the interlocked sieves until the outpouring water was clear. The extracted spores from the finer sieves 200 µm and 40 µm were rinsed with water from a spray bottle into 50ml centrifuge tubes. These were centrifuged for 5min. at 2000 RPM. Thereafter the floating debris was carefully discarded, and the remaining pellet was suspended in a 50% sucrose solution (prepared with white table sugar) by first pouring on a 40µm sieve, secondly rinsing with sucrose solution from a spray bottle into 50ml centrifuge tubes. The tubes were centrifuged for 2 min., before decanting the contents on a 40µm sieve and washing away the sucrose with water. Finally, the spores were rinsed from the sieve onto folded filter papers that have been put onto filtering funnels. After the water droplets disappeared, the filter papers were transferred into petri-dishes to preserve the spores before performing the direct counting method with a ZEISS Stemi 2000.CS dissecting microscope (ZEISS, Oberkochen, Germany).

Bioassay experiment, endophytic fungal potential

In order to shed light onto the endophytic fungal colonization potential of urban soils in Vienna a factorial experiment was conducted on maize seedlings to uncouple difficulties to process *Acer spp.* roots as well as effects of water availability on the growth of arbuscular mycorrhiza (AM) and other endophytic fungi. Spores are known to be just one of the possible sources of inoculum in soils (Smith & Read 2008). Moreover, the ability of colonization by spores differs among AM species. Therefore, colonization of roots by arbuscular mycorrhiza (and other endophytic fungi) can be much higher if all propagules, i.e. spores, sporocarps, infected root fragments and hyphae resting in soil samples can be the source of inoculum. As maize is known for being an excellent experimental host plant because of its uniform and rapid growth along with good staining properties, it was chosen for the experiment that stated in June 2018 (Propster & Johnson 2015). The experiment examined the direct and interactive effects of ambient soil conditions and water availability on the abundance of endophytic fungi and arbuscular mycorrhizae.

141 pesticide-free seeds were put on wet towels, put on a grid tray for aeration, and covered with wet towels and let germinate for several days. The seeds were wetted every day. After 4 days 97 seeds germinated and were put into about 50g of the stored dried soil from the separated *Acer* and *Tilia cordata* roots from another study at the institute of forest ecology. The planting tray was prepared by rinsing with de-ionized water and pieces of teabags acting as a barrier for soil to fall out. The teabags allowed excessive water to drain. The germinated seeds were put randomly into the respective soils, i.e. 59 soil samples formerly surrounding urban *Acer platanoides* and 60 urban *Tilia cordata* trees from different salt stress levels, leaving a set of 10 Acer and 12 Tilia soils without seedlings.

The planting tray was placed in the institute´s conservatory and watered when the soil felt dry.

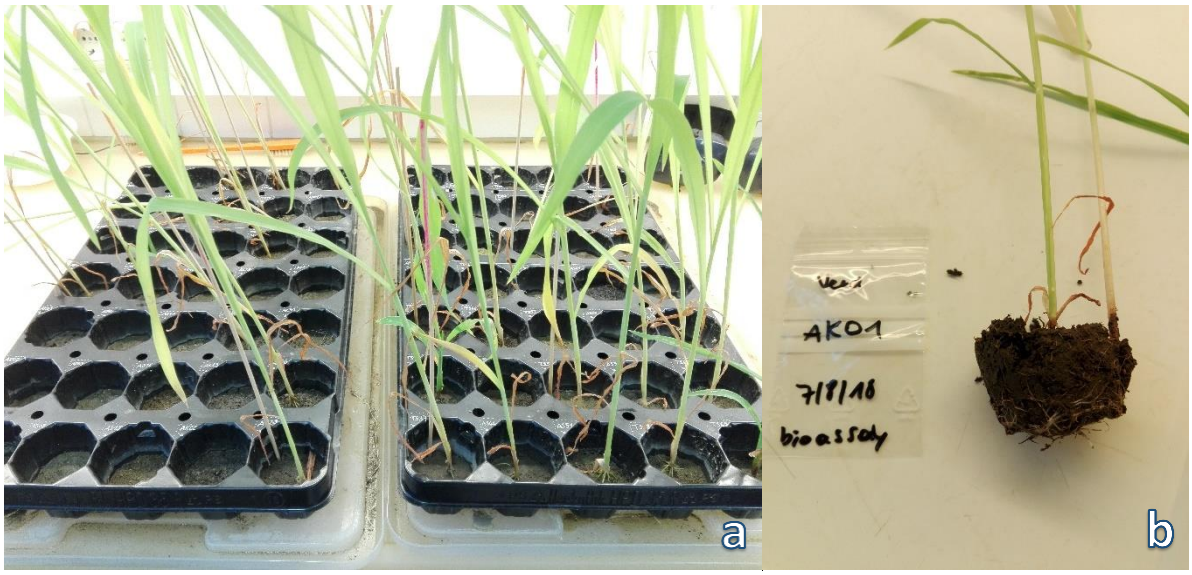

Figure 4: 97 maize seedling grown in ~50g of urban soil formerly surrounding Acer platanoides and Tilia cordata tree roots, a. 47 maize plants grown for 6 weeks, b. harvested to analyze roots for endophytic fungal colonization

47 maize plants have grown out of 97 seedlings. After 6 weeks the maize plants were harvested (figure 4), the roots were separated from the soils, rinsed carefully with tap water and stored in bags with tap water at 4°C before treatment. Roots that have been grown through the teabags have been cut off just before the edge of the teabag and put aside.

Before treatment the lateral roots were cut (figure 5a) and randomly chosen for treatment using an adaption of the ink and vinegar method (Vierheilig et al. 2005). 6 roots of every pot were put into 10%KOH overnight. On the next day, the roots that were still dark were boiled in 10%KOH for 3

minutes. Thereafter the KOH treated roots were rinsed several times with tap water before bringing 10ml of ink/vinegar solution (Pelikan Blue) to boil in small glass beakers for every sample. The respective 6 roots of every sample were carefully put into the boiling ink/vinegar solution and let there for 4 minutes. After boiling, the roots were washed several times in tap water before putting them into slightly acidified tap water for 20 minutes at room temperature. For further, but slower de-staining the roots were kept in acidified water at 4°C for 12 days before microscopic analysis.

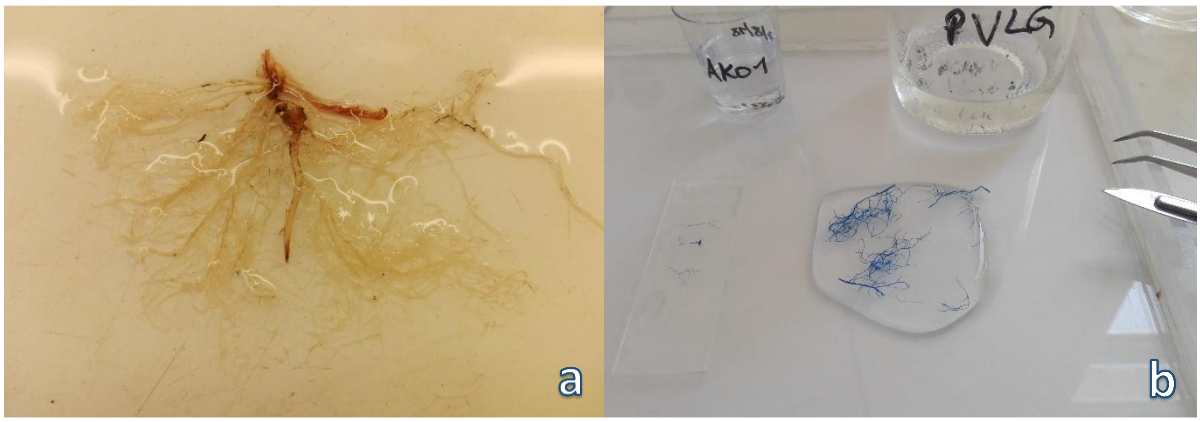

Figure 5: roots from maize plants grown in urban soil formerly surrounding roots of Acer platanoides in a park, a. whole root system, b. cleared and stained roots mounted on analytical slides with PVLG

After this time period the stained roots were mounted on slides with PVLG (Omar et al. 1979). For each sample 3 randomly selected root segments of ~1cm were mounted on one slide (figure 5b).

The mounted roots were carefully analyzed in order to see if signs of colonization of arbuscular mycorrhiza or other endophytic fungi (dark septate endophytes) could be detected with a ZEISS Primo Star microscope (ZEISS, Oberkochen, Germany). AM were recorded when at least one of the species characteristics vesicles, arbuscules or aseptate hyphae could be seen. For dark septate endophytes (DSE) at least one of the associated characteristics of microsclerotia, septate hyphae or chlamydospore-like formations needed to be present (Ahlich & Sieber 1996). Other structures were not taken into account as they could not be clearly identified as a form of endophytic fungi.

Soil analysis

Soil bulk density was calculated with the intact surface soil cores by means of volume of 100.9834cm3 and dry weight of each sample.

For the electronical conductivity (EC) measurement 5g of the Pürkhauer soil samples May 2017 were diluted in 25ml deionized water in test tubes and measured with an EC & TDS Tester Primo 2 produced by Hanna Instruments (Rhode Island, USA).

The pH levels were tested with CaCl2 and deionized water, therefore 5g of the fresh October 2017 soil samples were diluted in 25ml 0.01 Mol CaCl2 and 25ml deionized water respectively. The test tubes were shaken and allowed to rest for over 2 hours, then shaken again 10 minutes before finally measuring the pH value with a Schott pH-meter CG840 (Schott Instruments GmbH, Mainz, Germany).

Total Carbon and Nitrogen were measured with soil collected in October 2017. The soil was sieved to 2mm and dried at 105°C for 24h, thereafter 2.5g were weighed inside enclosed tin capsules and stored in an autosampler. A linear standard calibration curve was created with certified sample material. Then the samples were combusted under a steady oxygen stream at 950°C. Via infra-red detection using a LECO Truspec CN analyzer (LECO Corporation, St. Joseph, USA) CO2 and NOx were determined for carbon and thermal conductivity for nitrogen, expressed as grams of carbon or nitrogen per 100g soil.

No differentiation of inorganic and organic carbon was exerted in this experiment. Data on dissolved organic nitrogen (DON) were taken from this measurement.

Cations were measured with dried soil from the October 2017 samples. 2.5g of soil were extracted for 24h in 50ml ammonium acetate at 1 molar concentration with deionized water, then filtered and finally the filtrate was analyzed using a PerkinElmer® Optima™ 8300 Optical Emission Spectrometer (Perkin Elmer Inc., Waltham, MA, USA). The elements aluminum, arsenic, boron, calcium, cadmium, copper, iron, mercury, potassium, magnesium, manganese, molybdenum, sodium, nickel, phosphorous, lead, sulphur, and zinc were detected. Notably the values for the trace elements were in µg / g dry soil, for the macronutrients (Ca, Mg, K) in mg / g dry soil. For those cases were the levels were below the detection value, a value of 2/3 the minimum detection value was assigned for statistical analysis.

The macronutrients and acid nutrients allowed calculating the cation exchange capacity (CEC) which determines the buffering and adsorption capacity for Na^+ ions as well as the sodium saturation of the soil. Therefore, the ppm values of K^+, Ca^{2+}, Mg^{2+}, Na^+, Al^{3+} were converted to µmol / g values. H^+ values were derived from pH levels, and the CEC was calculated as the sum of the cation values K^+, Ca^{2+}, Mg^{2+}, Na^+, Al^{3+} and H^+. Base saturation (BS) as $K^+ + Ca^{2+} + Mg^{2+} + Na^+$ given as a percentage of CEC, as well as the percentage of each base cation in relation to all four base cations were calculated (e.g. sodium saturation Na%= $[(Na^+ /(K^+ + Ca^{2+} + Mg^{2+} + Na^+))*100]$).

Statistical analysis

All results were collected in Microsoft Excel files. All statistics were computed with IBM SPSS Statistics 26.0, the sunburst diagrams in Microsoft Excel. Outliers in the data variables for the salt stress location categories Side (S), Main (M), Park (P) have been removed, i.e. values were more than two standard deviation away from the mean. A Shaprio-Wilk test was performed for all variables to test for Gaussian distribution. For those cases where normality was met analysis of variance (ANOVA) and post-hoc (Tukey's HSD) were used to test the effects of perceived salt stress levels on vitality criteria and root characteristics of the trees as well as on soil properties of the locations. Respectively a non-parametric test (Kruskal Wallis test and pairwise comparison with a Bonferroni correction) was performed. Correlations were tested with Spearman Rank correlation test: Tree vitality parameters were tested for correlation with aboveground parameters, like sunlight gradient, traffic (high or low), parking space next to tree or not, none, low, medium or dense "pit" vegetation, as well as belowground soil parameters like pH, electronical conductivity (EC), base cations saturation, macronutrients and trace elements and finally belowground root parameters, like root biomass, specific root length, specific root surface area, mean diameter and fine root ratios of diameter classes.

Results

Vitality

Crown vitality and the other health indicators for trees in the perceived salt stress locations side street (S)- medium salt stress, main street (M)- high salt stress, and park (P)- no salt stress, show a general trend that trees from main streets have overall lower values than the other categories (table 2 & 3). Even though main street trees tended to be the oldest, their height tended to be the lowest. For diameter at breast height (DBH) this trend showed significant differences between the main street and park trees, again with main street trees displaying the lowest DBH. Moreover, showed the trees from

main streets the tendency to have the lowest vitality (table 2). Interestingly, trees from side streets show higher crown vitality than expected. For crown vitality and growth rate side street trees tended to have even higher scores than park trees. However, for radius growth, which is related to DBH, park and main street trees have significantly different values, where park trees show the highest radius growth rate (table 3). This is of special interest as trees in the main streets tended to be older, which would be expected to result in higher radius growth than park trees (table 2) The results of relative radius growth follow the same pattern, with trees from park locations trending to have the highest values, followed by trees from side streets and finally trees from main streets with the lowest values (table 3).

Table 2: age, height, diameter at breast height (DBH) and crown vitality (vitality) of urban Acer platanoides trees from the salt stress levels (Side, Main, Park) described by mean value ± standard error, stating n number of contained samples.

salt stress	**age** (years) n=50	**height** (m) n=59	**DBH** (cm) n=59	**vitality** (0-10, 10= highest) n= 59
Side *mean ± SE*	38.2 ± 2.9 a	15.1 ± 1.0 a	31.1 ± 2.5 **ab**	6.40 ± 0.461 a
Main *mean ± SE*	43.2 ± 3.8 a	14.3 ± 0.6 a	27.0 ± 1.5 **b**	5.11 ± 0.535 a
Park mean ± SE	40.7 ± 5.4 a	15.0 ± 0.8 a	35.1 ± 2.8 **a**	6.25 ± 0.383 a

Significant differences between the locations are indicated by different letters (ANOVA with Tukey-HSD, $p < 0.05$)

Table 3: *Growth rate, radius growthnd relative radius growth of urban Acer platanoides trees from the salt stress levels (Side, Main, Park) described by mean value ± standard error, stating n number of contained samples.*

salt stress	**growth rate** (m/years) n=50	**radius growth** (cm) n=59	**rel. radius growth** (cm/year) n=50
Side *mean ± SE*	0.33 ± 0.033 a	10.57 ± 1.245 **ab**	0.28 ± 0.030 a
Main *mean ± SE*	0.31 ± 0.031 a	8.49 ± 0.745 **b**	0.23 ± 0.023 a
Park mean ± SE	0.32 ± 0.032 a	12.55 ± 1.378 **a**	0.30 ± 0.025 a

Significant differences between the locations are indicated by different letters (ANOVA with Tukey-HSD, $p < 0.05$)

Root characteristics

Table 4 displays the mean root diameter, specific root length and specific root surface area of the fine roots at depth 10-30cm. Park trees demonstrated the smallest mean root diameter, followed by main street trees and finally trees from side streets having the highest values, which is significantly different from the park, but not from the main street values. Specific root length (SRL) tended to be highest in main street trees, followed by park trees and displaying lowest values in side street trees. In addition, specific root area (SRA) had the tendency to be 20% higher in trees from main streets. than from side streets and parks.

Table 4: Mean root diameter, specific root length (SRL), specific root area (SRA) of depth 10-30cm of the analyzed urban Acer platanoides trees from the salt stress levels (Side, Main, Park) described by mean value ± standard error, stating n number of contained samples.

salt stress	**Mean root diameter** (mm) n= 58	**Specific root length (SRL)** (cm/g) n= 58	**Specific root area (SRA)** (cm2/g) n= 58
Side *mean ± SE*	0.81 ± 0.018 **b**	1608.4 ± 140.2 a	394.0 ± 27.7 a
Main *mean ± SE*	0.75 ± 0.018 **ab**	2070.7 ± 207.1 a	473.4 ± 42.9 a
Park *mean ± SE*	0.73 ± 0.016 **a**	1730.7 ± 90.6 a	390.7 ± 15.3 a

Significant differences between the locations are indicated by different letters (ANOVA with Tukey-HSD, $p < 0.05$)

The highest fine root biomass tended in the middle soil depth segment of 10-30cm, followed by the deepest segment of 30-50cm, thereafter the topsoil segment of 0-10cm. In the topsoil and middle segments fine root biomass tended to be the highest in side street trees. Significant differences for the stress levels were found in the middle soil segment of depth 10-30cm. There the fine root biomass of the trees from side streets was two times higher than the fine root biomass of main street and park trees. In the topsoil segment main street trees tended to display the lowest biomass. In comparison in the deepest segment of 30-50cm fine root biomass showed a tendency to be highest in park trees, followed by side street and finally main street trees (table 5).

Table 5: Weighed root biomass for 0-10cm, 10-30cm, 30-50cm depth and total biomass of the analyzed urban Acer platanoides trees from the salt stress levels (Side, Main, Park) described by mean value ± standard error, stating n number of contained samples.

salt stress	**biomass 0-10cm** (g) n= 53	**biomass 10-30cm** (g) n= 57	**biomass 30-50cm** (g) n= 57	**total biomass** (g) n= 55
Side *mean ± SE*	0.55 ± 0.10 a	1.36 ± 0.29 **b**	0.62 ± 0.13 a	2.35 ± 0.39 a
Main *mean ± SE*	0.34 ± 0.09 a	0.62 ± 0.10 **a**	0.41 ± 0.07 a	1.31 ± 0.15 a
Park *mean ± SE*	0.40 ± 0.05 a	0.62 ± 0.09 **a**	0.64 ± 0.09 a	1.66 ± 0.16 a

Significant differences between the levels are indicated by different letters (Kruskal-Wallis, pairwise comparison, $p<0.05$)

To display potential root traits the mean proportion of root length of trees from side streets, main streets and parks in the different fine root diameter classes are shown in figure 7. In the relative distribution of the very finest roots big differences can be seen for park and roadside (side and main street) trees. On the one hand park trees have a smaller proportion in the root diameter classes 0-0.1, 0.1-0.2 and 0.2-0.3, on the other hand a far greater proportion in the root diameter classes 0.3-0.4, 0.4-0.5 and 0.5-0.6. The roadside trees show an opposite trend of greater proportions in the root diameter classes 0-0.1, 0.1-0.2 and 0.2-0.3. Hence in the root diameter classes 0.3-0.4, 0.4-0.5 and 0.5-0.6 there is a difference for main street and side street trees. The latter having the smallest proportion. After root diameter class 0.5-0.6 a steep drop in the mean proportions occurs. Nevertheless, do roots from side street trees exhibit the highest proportion in the bigger fine roots with the diameter classes 0.7-0.8 to 1.9-2.0.

Figure 6: Distribution of mean fine root length over the fine root diameter classes in mm for the roots of urban Acer platanoides trees from different salt stress locations (side, main, park), n=58

Figure 8, 9 and 10 illustrate the significant differences in the root diameter classes for salt stress levels (salt status), notably not in all classes the differences, described above, were significant. Most significant differences showed in the finest fine roots (0-0.5) and in the coarser fine roots (1.3-2.0). Notably for the finest roots of root diameter class 0-0.1 and 0.1-0.2 the park trees seem to be a more homogenic group than main street or side street trees, showing less dispersion.

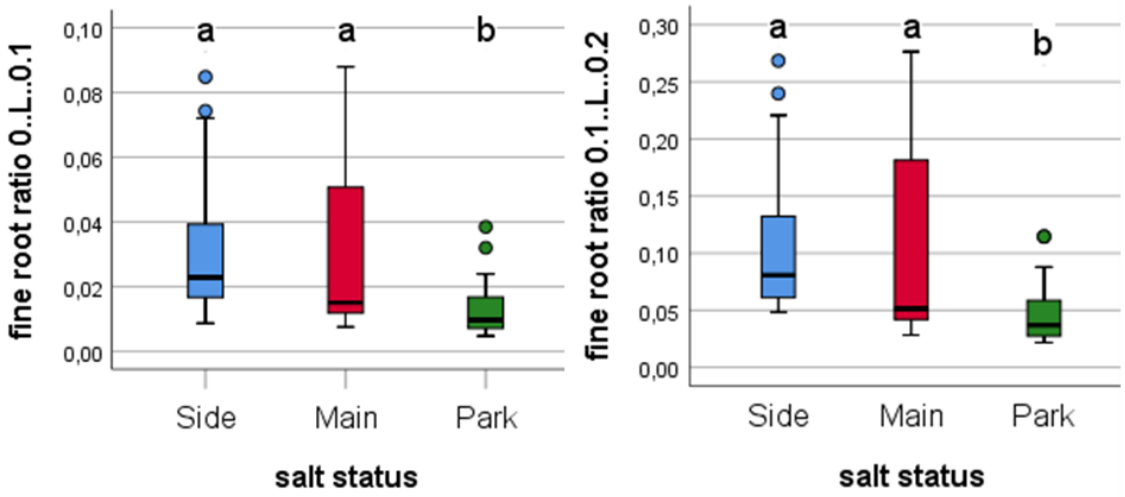

Figure 7: Proportions of fine root length in the root diameter classes 0-0.1 and 0.1-0.2 of the roots of urban Acer platanoides trees with distinct salt status, i.e. from the different salt stress levels (Side, Main, Park), n=58, different letters indicating significant differences between the groups.

The reversing trend for the different locations in fine root diameter classes 0.3-0.6 can be seen in figure 9:

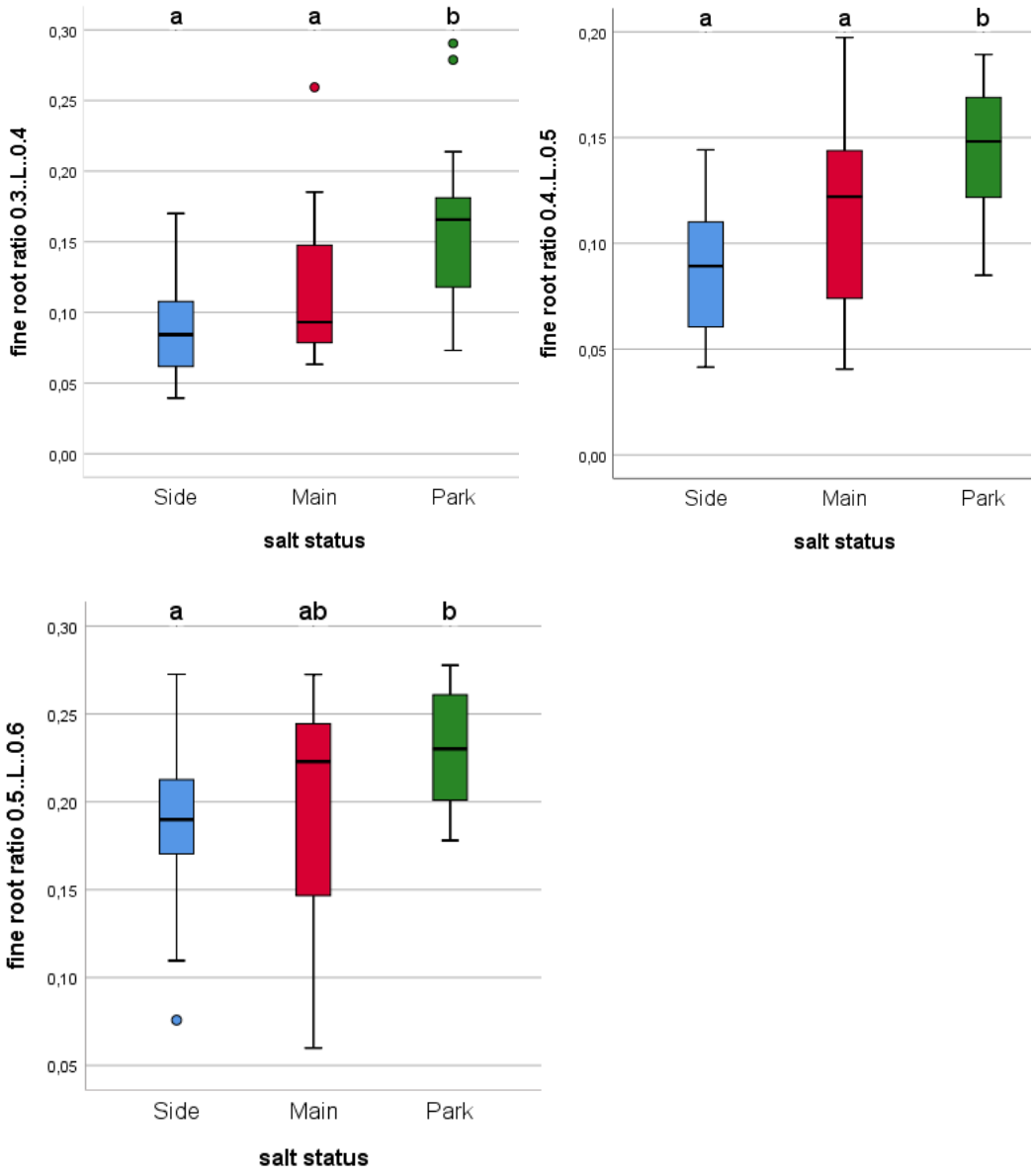

Figure 8: Proportions of fine root length in the root diameter classes 0.3-0.4, 0.4-0.5 and 0.5-0.6 of the roots of urban Acer platanoides trees with distinct salt status, i.e. from the different salt stress levels (Side, Main, Park), n=58, different letters indicating significant differences between the groups

In the root diameter classes from 1.3-1.4 to 1.9-2.0 all differences in fine root diameter distribution were significant. Where in most cases distinction displayed between park and roadside trees, in the case of root diameter class 1.6-1.7 the proportion differed significantly between all salt stress levels (figure 10). The presentation of class 1.8-1.9 and 1.9-2.0 is forgone due to marginal distribution levels.

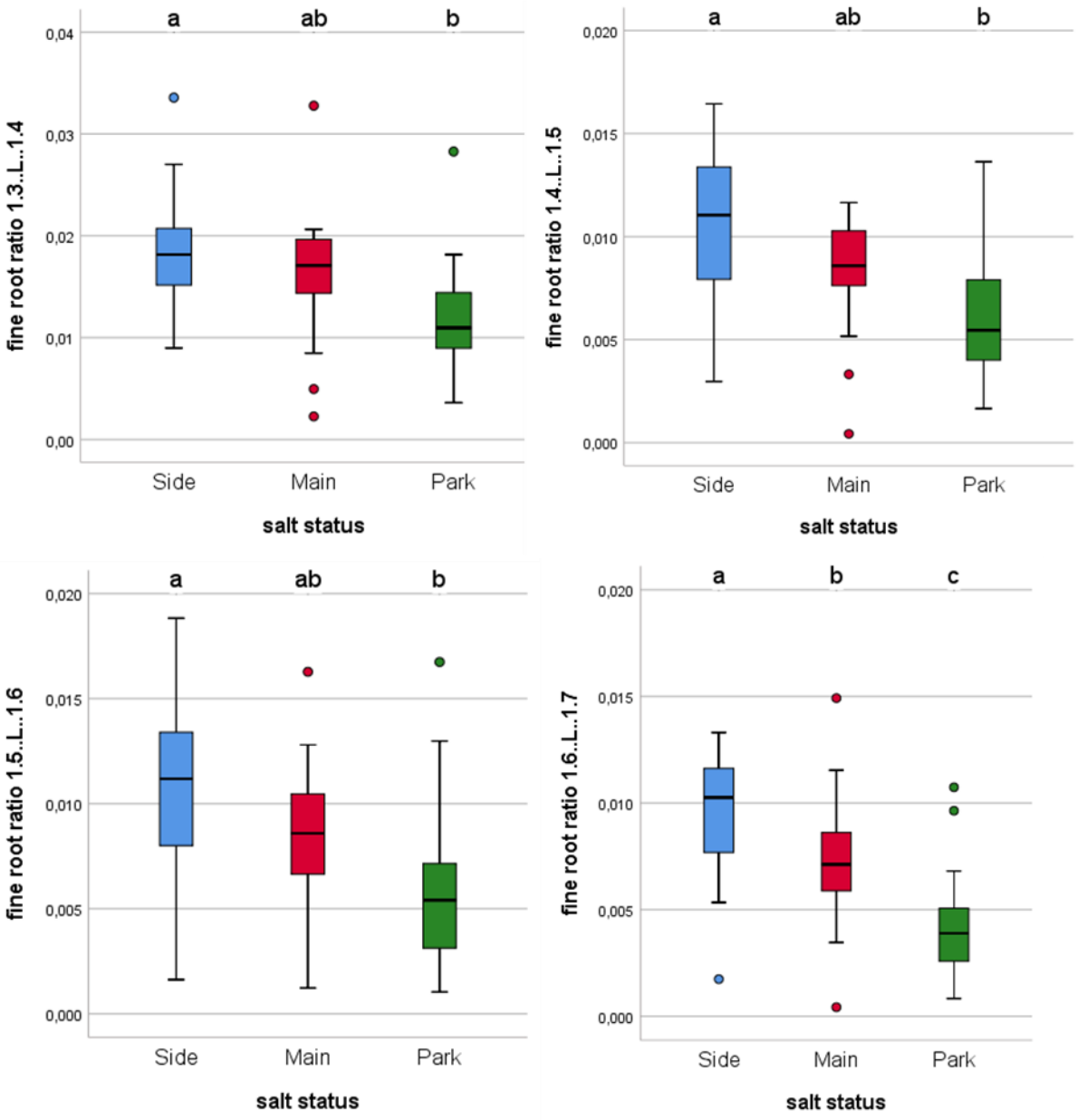

Figure 9: Proportions of fine root length in the root diameter classes 1.3-1.4, 1.4-1.5, 1.5-1.6 and 1.6-1.7 of the roots of urban Acer platanoides trees with distinct salt status, i.e. from the different salt stress levels (Side, Main, Park), n=58, different letters indicate significant differences between the groups

Mycorrhizal experiments

Clearing and staining

In the pre-testing phase no general sufficient modification was found to clear and stain the urban *Acer platanoides* roots. Roots were either to darkly stained or too disintegrated to analyze (figure 11). Some cases revealed very dark tissue with a high number of stained root hair next to parts were all cortical cells were lost (figure 12). No arbuscular mycorrhiza (AM) formations were found, even though in a minor set of samples few mini-vesicular shaped structures, spores, microsclerotia and non-connected septate hyphae could be detected. From visual inspection these fungal structures could be categorized as dark septate endophytes (DSE) but no definite assignment to species was possible. The negligible number concluded further analysis.

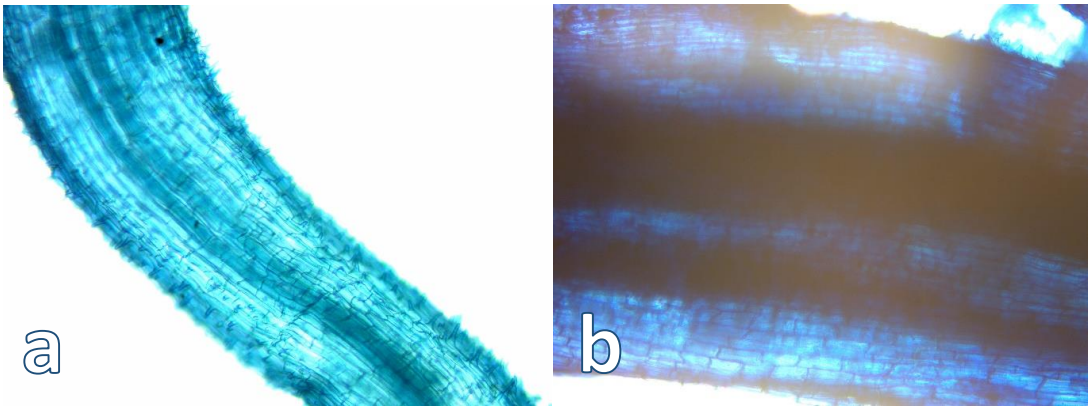

Figure 10: fine roots of urban Acer platanoides cleared, stained with Trypan blue, a. best visibility of all test roots- due to stained root hair not transparent to see through, b. dark root with some cells visible

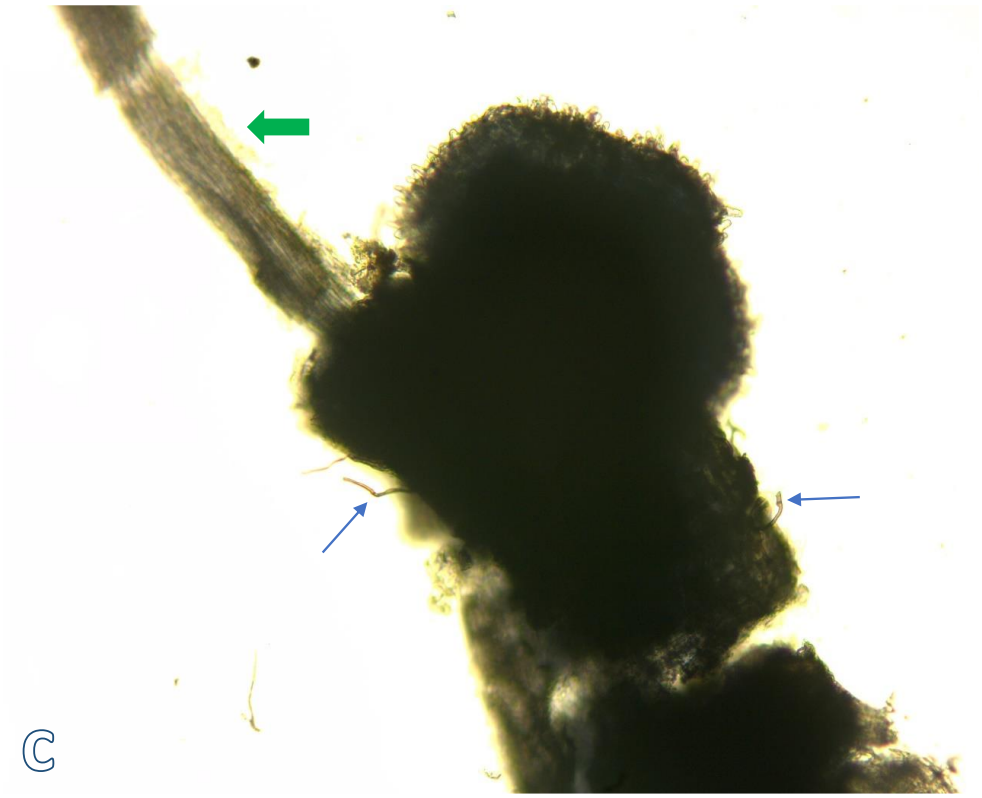

Figure 11: Fine root from urban Acer platanoides cleared, stained with Schaeffer black, showing signs of high disintegration having lost most of cortical cells leaving the steely locally "naked" (big green arrow), displaying septate hyphae of DSE (thin blue arrows)

Spore extraction

There could not be found spores in both kinds of samples, neither in the park soils nor in the perceived salt stressed roadside soils. Further spore separation was therefore not pursued.

Bioassay experiment

As mentioned above from the 97 maize seedlings 47 maize plants grew in urban soils that formerly surrounded *Acer platanoides (AP)* or *Tilia cordata* (*TC*) soils from all salt stress levels (side, main, park). It is worth to note that more seedlings matured in *TC* soils than in *AP* soils, accounting for 66% of all maize plants. Similar percentages can be observed in reference to salt stress, interestingly more plants matured in soils from roadside tree locations than from park soils.

In these 47 maize plants root samples of 26 individuals showed some form of the defined fungal structures. Figure 13 displays examples for categorization by visual assessment.

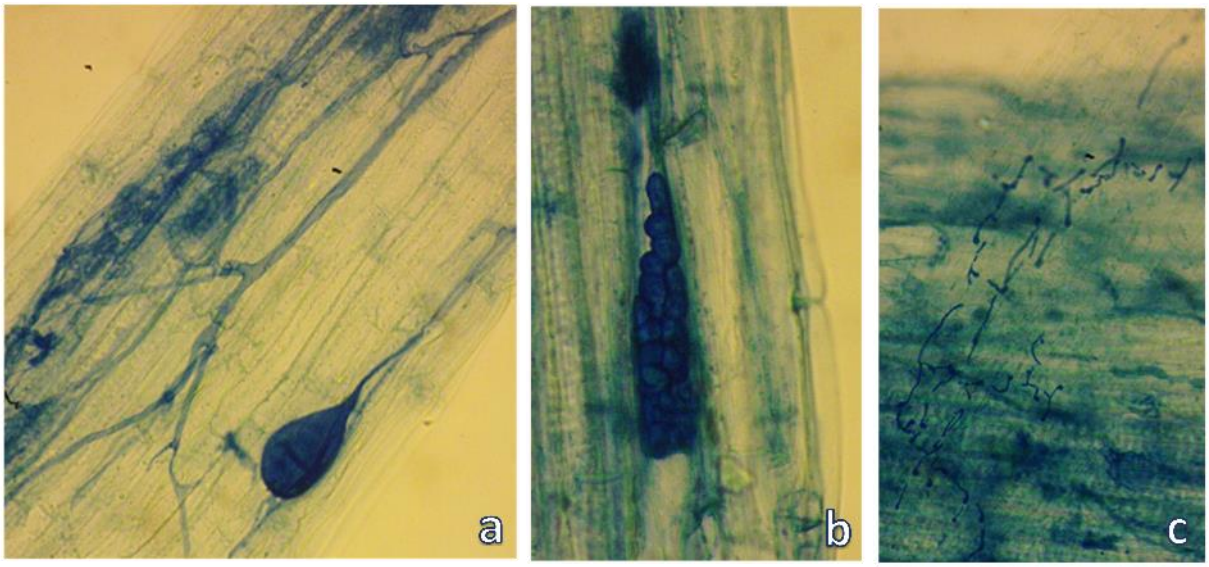

Figure 12: Endophytic fungal structures in maize roots grown in urban soils formerly surrounding Tilia cordata and Acer platanoides roots in roadside or park locations. a. Arbuscular Mycorrhiza: arbuscules, aseptate hyphae and vesicle, b. Dark Septate Endophyte: microsclerotia, c. other structures interpreted as non (endophytic) fungal

Of the 47 maize samples 16 individuals grew in *AP* soils and 31 in *TC* soils. From the roots of those 31 plants that matured in *TC* soils most were not colonized (12 plants), followed by those associated with DSE (10 plants), then colonized by both AM and DSE (5 plants) and finally those living in symbiosis with only AM (4 plants). Likewise of those 16 plants that matured in *AP* soils the majority were not colonized (9 plants), followed by those associated with DSE (5 plants), thereafter those living in symbiosis with solely AM (2 plants) and unlike former *Tilia* soils non were colonized by both AM and DSE. Figure 14 illustrates the findings graphically, with the inner ring for the proportion of maize plants grown in either *Tilia* (brown) or *Acer* (blue) soils and the outer ring showing the respective colonization findings in the maize roots.

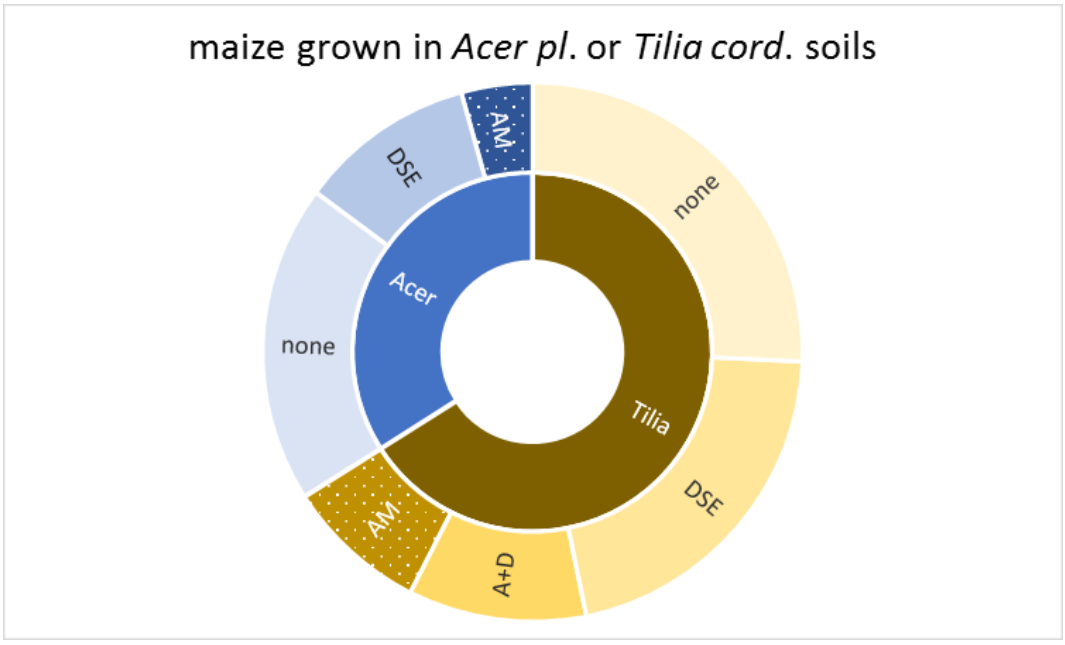

Figure 13: roots from 47 maize plants grown in urban soils colonized by Arbuscular Mycorrhiza (AM), Dark Septate Endophytes (DSE), both (A+D) or none colonized grouped by soils formerly surrounding roots of either Acer platanoides or Tilia cordata

When grouping the endophytic fungal findings according to salt stress levels due to locations the following picture derives (figure 15). The majority of plants grew in roadside soils, i.e. 31 plants opposing 16 plants in park soils. To a great share the plants from roadside soils were not colonized (12 plants), followed by those associated with DSE (9 plants), then those living in symbiosis with solely AM (6 plants) and finally those which were associated with both AM and DSE (4 plants). The maize plants from the former park soils were mostly not colonized (9 plants), followed by those colonized by DSE (6 plants) and one plant was associated with both AM and DSE. Notably no plant that was solely associated with AM was found in the park soil plants.

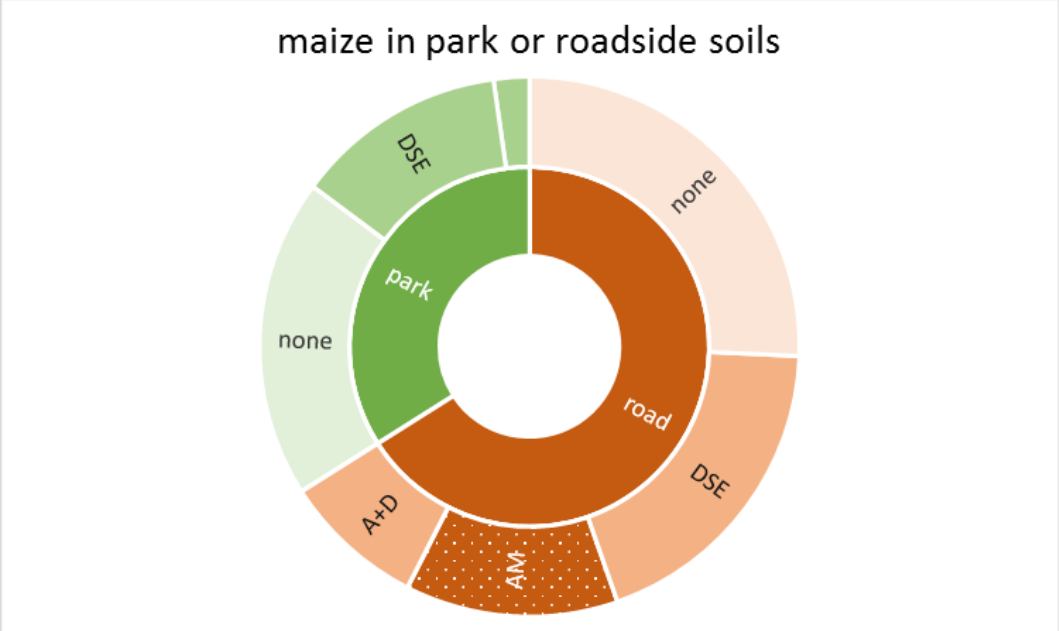

Figure 14: roots from 47 maize plants colonized by Arbuscular Mycorrhiza (AM), Dark Septate Endophytes (DSE), both (A+D) or none colonized grouped by park soils (green) or roadside soils (red)

In the following the graphical grouping is extended by adding the former species rooting in the soil *Acer* or *Tilia*. In figure 16 can be seen that those plants from roadside *Acer* and *Tilia* soils were

associated with all respective fungal categories, whereas plants grown in park soils formerly surrounding *Acer* tree roots did not form symbiosis with AM.

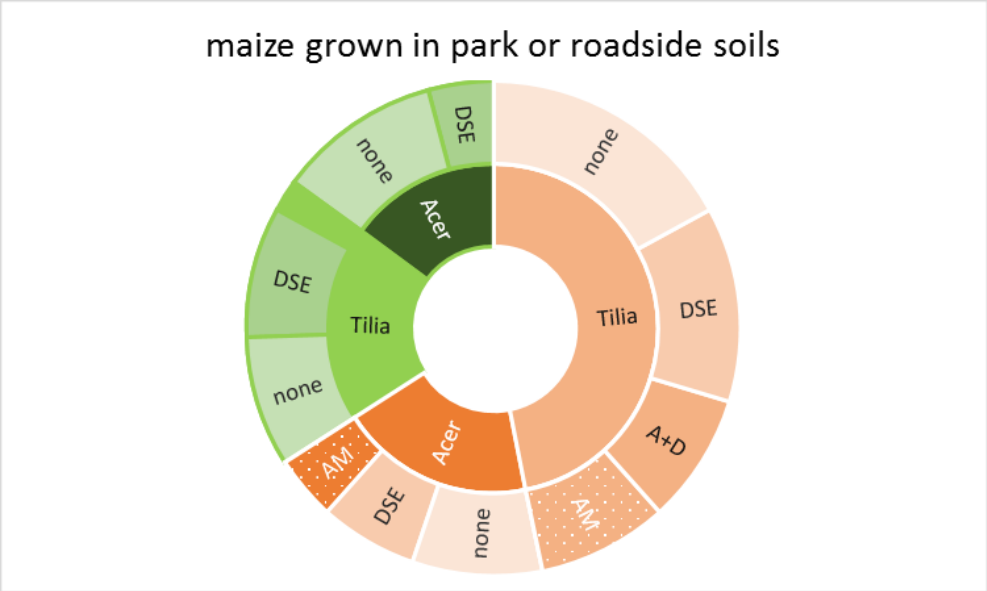

Figure 15: roots from 47 maize plants colonized by Arbuscular Mycorrhiza (AM), Dark Septate Endophytes (DSE), both (A+D) or none. grouped by park soils (green) or roadside soils (orange) and former species Acer or Tilia

In appendix II the list of soils and associated colonization is displayed.

Soil analysis

Characteristics

In general, can the handled soils be classified as typical urban soils, showing great heterogeneity, lacking natural stratification, with topsoils being very hard to penetrate and the deeper soils consisting of bricks and gravel (Braun 1990). Assessment during the process of coring and preparation of root samples displayed physically and visually that these soils clearly fall into urban soil category. In some cores the soil at a depth of about 20cm seemed more like demolition debris, builder´s waste or just sand. In one soil sample an actual solid building brick was cored (figure 17).

Figure 16: Soil core from a park location exhibits high heterogeneity of urban soils by pure visual display, showing a building brick destoyed during coring at the depths of 30cm

Soil nutrients and trace elements

Table 6 demonstrates the values of pH, bulk density and electronical conductivity (EC) for the salt stress level groups (side, main, park). The mean pH values measured with $CaCl^2$ showed slightly acidic to neutral, more precisely pH levels around 6.8 for all salt stress levels. Bulk density tended to be highest in side streets, thereafter in parks and lastly in main streets. EC displayed tendency to be higher in main streets, followed by side streets and then parks. Dissolved organic nitrogen (DON) tended to be highest in soils from side streets, followed by main streets and thereafter by parks. None of these differences were significant.

Table 6: pH ($CaCl^2$), bulk density and electronical conductivity (EC) and dissolved organic nitrogen (DON) of urban Acer platanoides tree locations from the salt stress levels (Side, Main, Park) described by mean value ± standard error, stating n number of contained samples.

salt stress	**pH** ($CaCl^2$) n=59	**bulk density** (g/cm^3) n=59	**EC** (uS/cm) n=57	**DON** (mg/l) n=57
Side *mean ± SE*	6.9 ± 0.08 a	1.09 ± 0.07 a	320.4 ± 30.1 a	7.5 ± 1.08 a
Main *mean ± SE*	6.8 ± 0.08 a	1.03 ± 0.08 a	377.7 ± 33.8 a	7.0 ± 1.17 a
Park *mean ± SE*	6.8 ± 0.08 a	1.06 ± 0.02 a	304.3 ± 20.7 a	5.7 ± 0.48 a

Significant differences between the levels are indicated by different letters (Kruskal-Wallis, pairwise comparison, p<0.05)

The values for sodium (Na) were significantly different for the salt stress groups, precisely for roadside and park locations, Notably Na levels were around two times higher in main streets than in side streets, what is more around five times higher than in parks. There appeared no general trend for the macronutrients according to the salt stress levels. Calcium (Ca), potassium (K) and magnesium (Mg) contents tended to be similar for all groups. Nevertheless, phosphor (P) and sulphur (S) contents tended to be the higher in side street soils, conversely, the second highest content for S tended in park soils, whereas for P in main street soils (table 7).

Table 7: macronutrients, calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), phosphor (P) and sulphur (S) of urban Acer platanoides tree locations from the salt stress levels (Side, Main, Park) described by mean value ± standard error, stating n number of contained samples.

salt stress	**Ca** (mg/g) n=59	**Mg** (mg/g) n=59	**K** (mg/g) n=59	**Na** (µg/g) n=58	**P** (µg/g) n=58	**S** (µg/g) n=59
Side	5.9 ± 0.14 a	0.22 ± 0.02 a	0.25 ± 0.03 a	87.5 ± 15.0 **b**	62.9 ± 10.1 a	48.2 ± 1.2 a
Main	5.8 ± 0.16 a	0.23 ± 0.02 a	0.25 ± 0.02 a	163.4 ± 38.0 **b**	46.5 ± 7.3 a	45.6 ± 0.8 a
Park	6.1 ± 0.14 a	0.21 ± 0.01 a	0.26 ± 0.02 a	35.1 ± 3.0 **a**	35.4 ± 5.9 a	46.7 ± 1.0 a

Significant differences between the levels are indicated by different letters (Kruskal-Wallis, pairwise comparison, p<0.05)

Cation exchange capacity (CEC), base saturation (BS%), calcium saturation (Ca%), potassium saturation (K%) and magnesium saturation (Mg%) showed no striking differences for all salt stress levels. However, the base cation saturation of sodium (Na%) demonstrated significant differences between the salt stress level groups. On the one hand, soils from roadside locations were significantly higher in Na% i.e. main street soils exhibiting almost two times higher saturation than sides streets, on the other hand soils from parks showed a 5 times lower Na% than the main streets (table 8).

Table 8: Cation exchange capacity (CEC), base saturation (BS%), calcium saturation, magnesium saturation, potassium saturation and sodium saturation of urban Acer platanoides tree locations from the salt stress levels (Side, Main, Park) described by mean value ± standard error, stating n number of contained samples.

salt stress	**CEC** (µmol/g) n=59	**BS%** n=58	**Ca%** n=59	**Mg%** n=59	**K%** n=59	**Na%** n=57
Side	321.1 ± 0.008 a	99.88 ± 0.003 a	91.3 ± 0.6 a	5.5 ± 0.4 a	2.0 ± 0.24 a	1.20 ± 0.21 **b**
Main	320.3 ± 0.009 a	99.88 ± 0.004 a	90.1 ± 0.7 a	5.8 ± 0.4 a	2.0 ± 0.16 a	2.12 ± 0.45 **b**
Park	329.5 ± 0.007 a	99.88 ± 0.003 a	92.1 ± 0.4 a	5.3 ± 0.3 a	2.0 ± 0.18 a	0.44 ± 0.03 **a**

Significant differences between the levels are indicated by different letters (Kruskal-Wallis, pairwise comparison, p<0.05)

As well as for macronutrients for the content of the trace elements boron (B), manganese (Mn), molybdenum (Mo), cadmium (Cd), copper (Cu) and zinc (Zn) no general trend could be detected (table 10). Mo content exhibited significantly different for main street and park locations, the latter having the highest mean content. For Cd, Cu and Zn highest contents displayed in main street locations, which was significantly higher than in park locations for Cd (three times) and Zn (two times), whereas for Cu the lowest content in side streets was significantly different from park and main street locations (table 9).

Table 9: trace elements, boron (B), manganese (Mn), molybdenum (Mo), cadmium (Cd), copper (Cu) and zinc (Zn) content of urban Acer platanoides tree locations from the salt stress levels (salt str.- S for side street, M for main street, P for park) described by mean value ± standard error, stating n number of contained samples.

salt str.	**B** (µg/g) n=54	**Mn** (µg/g) n=58	**Mo** (µg/g) n=59	**Cd** (µg/g) n=57	**Cu** (µg/g) n=55	**Zn** (µg/g) n=58
S	0.01 ± 0.000 a	12.1 ± 2.0 a	0.05 ± 0.006**ab**	0.007 ± 0.001**a**	0.6 ± 0. 3 **b**	3.3 ±0.54**ab**
M	0.03 ± 0.007 a	17.4 ± 3.7 a	0.05 ± 0.008 **b**	0.03 ± 0.005**b**	0.9 ± 0.1 **ab**	5.5 ± 1.06 **b**
P	0.04 ± 0.009 a	16.2 ± 2.1 a	0.08 ± 0.012 **a**	0.01 ± 0.002**a**	0.8 ± 0.1 **a**	2.3 ± 0.23 **a**

Significant differences between the levels are indicated by different letters (Kruskal-Wallis, pairwise comparison, p<0.05)

Correlations

Crown vitality was correlated with less parameters than the other vitality indicators of this study. Age, traffic and copper were negatively correlated with crown vitality, whereas dissolved organic nitrogen (DON) was positively correlated. Calcium saturation (Ca%) and pH ($CaCl^2$) were positively correlated to growth rate and relative radius growth. In contrast sodium content (Na) was negatively correlated to relative radius growth (table 10).

Table 10: Spearman correlations of vitality parameters, crown vitality (vitality), growth rate, radius growth, relative radius growth of urban Acer platanoides from different salt stress levels with the aboveground and belowground soil parameters, displaying Spearman´s rho and signifiance level

	vitality	**growth rate**	**radius growth**	**rel. radius growth**
age	-0.259(*)	calc.	0,593**	ns
height (m)	ns	calc.	0,777**	0,499**
sunlight gradient	ns	ns	0,449**	0.265(*)
traffic	-0.240(*)	ns	ns	ns
pH ($CaCl^2$)	ns	**0,292***	ns	**0,310***
bulk density	ns	ns	0,283*	ns
DON	0.254(*)	-0,346*	ns	ns
Ca%	ns	**0,280***	ns	**0,313***
K : Ca ratio	ns	-0,280*	ns	-0,285*
Al (ug/g)	ns	-0,428**	ns	ns
Cu (ug/g)	-0,355**	ns	ns	ns
K (mg/g)	ns	ns	ns	-0,296*
Mo (ug/g)	ns	ns	0,286*	ns
Na (ug/g)	ns	ns	**-0,263***	**-0,321***
Ni (ug/g)	ns	ns	ns	-0,307*

(*) ($p < 0.1$) * ($p < 0.05$) ** ($p < 0.01$)

calc. (calculation components)

ns (no significant correlation)

Concerning the correlations of the fine root ratios of the different root diameter classes it could be shown that for several soil parameters the direction and significance of correlation (positive or negative) is changing due to root diameter class. Particularly this finding is evident in the finest fine roots within diameter classes 0 to 0.5 (table 11), where e.g. correlation with DON, Na, Fe and P is positive for the fine root ratios (f.r.r.) 0-0.1 and 0.1-0.2, hence for the f.r.r. 0.3-0.4 and 0.4-0.5 (for Na also 0.5-0.6) is negative. Notably, from fine root ratio 1.1-1.2 to 1.9-2.0 all are positively correlated with Na and negatively correlated with the base cation ratios Ca : Na and Mg : Na. A more detailed look on the correlations is displayed in appendix III.

Table 11: Spearman correlations of fine root ratios (f.r.r.) of the finest fine roots, i.e. root diameter classes 0-0.5mm, of urban Acer platanoides from different salt stress levels with belowground soil parameters, displaying Spearman´s rho and significance level

	f.r.r.<0.1	**f.r.r.0.1-0.2**	**f.r.r.0.2-0.3**	**f.r.r.0.3-0.4**	**f.r.r.0.4-0.5**	sum < 0.5
pH ($CaCl^2$)	-0,339**	-0,259*	-0,315*	ns	0.233(*)	-0.222(*)
DON	**0,271***	**0,284***	ns	**-0,419****	**-0,402****	ns
Ca %	ns	ns	ns	0,311*	0,275*	ns
K %	ns	ns	ns	-0,349**	-0,340**	ns
K : Ca ratio	ns	ns	ns	-0,358**	-0,350**	ns
Mg : Na ratio	-0,370**	-0,311*	ns	0,518**	0,437**	0,279*
Cd (ug/g)	ns	ns	ns	0.240(*)	ns	ns
Fe (ug/g)	0,288*	0,268*	ns	-0,273*	-0,305*	ns
Mo (ug/g)	-0,267*	ns	ns	0.229(*)	ns	ns
Na (ug/g)	**0,391****	**0,342****	ns	**-0,517****	**-0,437****	ns
P (ug/g)	**0,452****	**0,423****	ns	**-0,431****	**-0,424****	ns

(*) ($p < 0.1$) * ($p < 0.05$) ** ($p < 0.01$)
ns (no significant correlation)

Discussion

Urban stress, especially stress from the excessive use of de-icing salts has been demonstrated to decrease the vitality of trees (Czerniawska-Kusza et al. 2004, Uhrin et al. 2018). In this study sodium levels in the soils of the defined stress levels in main streets, side streets and parks exhibited significant differences. The roadside locations showed significantly higher sodium content than park soils, with main streets having almost 5 times and side streets 2.5 times higher sodium values. These distinct sodium levels were also expressed in significant different base cation ratios of Ca: Na, K: Na and Mg: Na.

For all health indicators the trees from main streets exhibited the lowest values, emphasizing that not only visually assessed crown vitality, but also height parameters and stem increment parameters were probably negatively affected by de-icing salts, as the sodium levels in the main streets demonstrated to be almost 5 times higher than in parks, whereas macronutrient levels displayed no significant differences. Moreover, the main street locations had the highest electronical conductivity levels, and highest cadmium, copper and zinc contents in the soils. Indeed, these findings seem to confirm hypothesis 1 that tree vitality decreases with increased urban stress, i.e. sodium levels in the soils. Not to mention, the higher levels of Cd, Cu, and Zn are another form of urban stress that puts pressure on trees. Those main street locations were associated with public transport and general higher amount of motorized traffic, which is a major source of heavy metals emitted as part of road emissions like engine exhaust gas, possible combustion of lubrication oil, brake emissions (Cu) and tire wear (Zn, Cd) (Chambers et al. 2016).

Accordingly, the parameter for stem increment relative radius growth demonstrated differences for trees from roadside and park locations. For the variable radius growth, in line with DBH, stem increment was even significantly different between main street and park trees, the latter showing 1.5 times higher increment, even though trees from main streets tended to be older, which indeed seems contradicting. This vitality indicator is considered to be one of the first tree-growth reactions to

environmental stress, like drought, and therefore reduces early, when photosynthesis under stress is reduced and carbon allocation is altered (Dobbertin 2005). In this study stem increment showed negative correlation with sodium. This finding of reduced stem increment for trees in high sodium soils, goes in line with the literature, which says that excessive sodium can induce physiological drought on trees (Volkmar et al. 1998).

Nevertheless, part two of hypothesis 1, that park trees would show the highest vitality rates, could not be confirmed for all vitality and health indicators. Interestingly, for the indicator crown vitality, for which i.a. crown structure and foliage abundance were visually assessed, this study showed an unexpected pattern that trees from side streets had the highest mean value, i.e. higher than park trees. One reason for this could be the higher values of the nutrients dissolved organic nitrogen (DON), phosphor (P) and sulphur (S). Nitrogen is the most important inorganic nutrient for plant growth and plant productivity, as it is part of all amino acids and chlorophyll. The subsequent higher amount of photosynthesis assimilates stimulates root growth and development, which leads to greater uptake ability of other nutrients (Brady & Weil 2008). These higher values of DON, P and S could be related to regular input of dog excrements (both urine and feces), as side streets in Vienna tend to be more attractive to dog holders for walking their dogs (Braun 1990). Besides, this study revealed the side street locations significantly lower contents of Cd and Cu than the other locations. The marginally significant ($p > 0.1$) positive correlation of crown vitality with DON and the negative significant correlation ($p > 0.05$) with Cu support this argument. Notwithstanding the high crown vitality of trees from side streets albeit elevated levels of sodium, the question remains why park trees displayed inferior crown vitality. One explanation may be low annual nutrient input due to common park maintenance practices in the form of raking and removal of fallen leaves in late autumn (Lee et al. 2010). A corresponding reason could therefore be higher belowground competition for nutrients as well as higher aboveground competition for sunlight due to more plants and trees directly surrounding in park areas than in roadside planting pits. Studies revealed that temperate forest trees demonstrate horizontal root distribution patterns that can extend for several meters and overlap extensively (Rewald & Leuscher 2009, Borden et al. 2017). Messier et al. 2009 showed that concentrations of nitrogen and phosphor in tree fine roots of sugar maple and ash were lower in the case the trees were grown under competition. A study on young *Acer pseudoplatanoides* growing in competition with young beech and oak trees under a Norway Spruce canopy showed that photosynthetic capacity declined sharply after internal nutrient reserves (especially N) from the nursery became depleted, as *Acer* was not able to replenish the required nutrients from the poorly soils (Kazda et al. 2004). This study revealed the tendency of the assessed park soils to show lower levels of essential inorganic nutrients than roadside trees. DON tended to be almost 30% lower and P 30-50% lower in park soils.

However, the overall medium vitality status of the assessed trees is in line with findings from other studies that explain the reduced health of urban trees with deficiency of water and nutrients as well as obstructive alkalized pH (Kleiber et al. 2019). The mixture of calcareous debris like concrete in urban buildings as well as roads, abrasion and finally deposition of alkaline dust is associated with the alkalization of urban soils, including park soils (Czerniawska-Kusza et al. 2004, Greinert 2015, Chambers et al. 2016). Even though, calcium saturation was high in all salt level groups, main streets showed the tendency to have lowest values, which may be explained by the excessive sodium negatively altering the physical properties of the soil by exchanging with Ca (Braun 1990, Cekstere & Osvalde 2013). The availability of P and micronutrients, like iron and manganese, is deteriorated under alkalized soil conditions (Kleiber et al. 2019). Even though, optimal solubility level of P is described at 6.0 - 6.5 pH, soil conditions with high calcium saturation lead to strongly bound P, hence P deficiency is a common problem in calcium-carbonate-rich soils (Scheffer/Schachtschabel 2010, Braun 1990). The present study found mean pH levels at 6.8 in combination with Ca% of 90%, which may decrease the availability

of P markedly for plants and therefore be one explanation for the inferior vitality of the assessed *Acer platanoides* trees in Vienna.

However, the urban stress factor of heavy metal deposition is alleviated by greater immobility at alkalized conditions, e.g. for cadmium at 6.5 pH (Scheffer/Schachtschabel 2010). Moreover, the heavy metal contents displayed in the present study did not exceed recommended threshold levels (Pfleiderer et al. 2002).

In any case, regardless of the soil quality, roots are the organs that acquire the plant essential resources of water and nutrients. According to the functional equilibrium model, entailing that a plant is allocating biomass to structures that best acquire resources that are most limiting, trees should invest more in root biomass and mycorrhizae when belowground resources are limited (Johnson 2010).

In this study total fine root biomass tended to be high in side streets and then parks. Looking at the distribution along soil depths one can see that there are differences for the salt level groups. These differences were significant at soil depth 10-30cm. Side street trees were found to have 2 times higher fine root biomass in this soil depth than park and main street trees. A combination of processes could be the reason for this counter-intuitive finding. On the one hand, trees from parks are not as limited in rooting space, therefore the roots can form the natural up to 1.5m heart-shaped rooting system typical for *Acer platanoides* (Roloff 2013), whereas roadside trees have commonly limited rooting zones, restricted by the spacing of planting pits, not only horizontally, but also vertically, thus, more root biomass can be found in shallower depth (Balder 1998). On the other hand, may the sodium content in main street soils be so high that the trees limit their fine root growth in the shallower depth and penetrate deeper layers. The tendency of park trees having high amounts of root biomass in soil depth 30-50cm, although not significant, seem to support this reasoning.

Furthermore, significant different mean fine root diameter patterns for roadside and park trees were detected. In contrast to park trees, side street trees had the largest root diameter, whereas root diameters of main street trees were in between. For specific root length (SRL) another non-significant picture can be drawn as main street trees tended to exhibit the longest SRL, followed by park trees and finally side street trees. Even though the SRL differences were not significant, it presents in line with the literature that under salinity root system modifications are assumed to be trade-offs between the capacity to exclude excess ions (NaCl) and sustained water or nutrient uptake (Rewald et al. 2013). Small fine root diameters, long specific root length and great specific root surface area are associated with plants that are adapted to drought, while in some plants fine root diameter is stated to increase due to high salinity (Comas et al. 2013). The reason for this is the succulence of the cortex, leading to increased storage capacities for water and dissolved ions (Rewald et al. 2013). To argue for the trees in this study it seems that side street trees with significantly largest fine root diameters may have adapted to enhanced sodium levels in the soil with succulence of the cortex. The trend of longer SRL in main street trees could be interpreted as an attempt to adapt to increased salinity.

Considering the fine root distribution over diameter classes significant differences for the salt stress groups were detected. This finding confirms hypothesis 2, that fine root properties will be affected by the stress levels. Especially the very thin fine roots of diameter classes 0-0.6mm showed large variations between roadside and park trees. In a like manner, all salt stress groups had the highest distribution in the diameter class 0.5-0.6mm, which accounted for about 24% of root length from park trees and about 20% from roadside trees. In contrast, the mean distribution in the thinnest fine roots (0-0.2mm) was highest in roadside trees, while from diameter classes 0.3-0.4mm onwards park trees had higher distribution rates. These diameter classes can be related to different root functions, i.e. absorptive fine roots that are involved primarily in the acquisition and uptake of soil resources as well as the transportive fine roots serving primarily transport functions. Were the distal, unbranched, finest

roots are found to function as absorptive roots (McCormack 2015). Even though for the *Acer platanoides* fine roots of this study no clear separation between absorptive and transportive roots can be made, the fine root distribution findings for the salt stress groups point out that there might be differences in root traits.

As phosphor and sodium content correlated positively with fine root ratio of diameter classes 0-0.2mm and negatively with diameter classes 0.3-0.5mm a relation to soil quality can be assumed. Some studies argue that phosphor (P) uptake declines with decreasing soil moisture content and that P limitation leads to modified root architecture in form of proliferation of lateral roots towards P-rich patches, greater root length as well as thinner roots and root hair abundance (White et al. 2013, Lambers et al. 2015). In addition, increased production of carboxylates (citrate and malate) in order to mobilize inorganic P from the soils was recorded (Wang et al. 2016). With the high calcium saturation of the urban soils of this study little P availability can be assumed.

In general, is the uptake of nutrients reported to increase with mycorrhizal colonization (Smith & Read 2008). The expected infertile soils of the urban sites of this study lead to the assumption that the trees engage in symbioses with mycorrhizae, as symbiosis is widely seen as an important mechanism of plant adaptation to unfavorable environments (Johnson et al. 2010).

In the present study difficulties with the adaption of clearing and staining methods, in order to detect colonization of arbuscular mycorrhizae (AM), for the roots from the field samples of *Acer platanoides* occurred. Still, problems with these methods are stated to include differential tissue penetration by the respective chemicals, especially when the fungal walls reveal to be thick and impermeable as they can be in vesicles. Moreover, the problem of background staining, i.e. root vascular tissue, root meristem tissue and uncleared plant organelles, has been reported (Smith & Read 2008).

Correspondingly to Brundrett and Kendrick 1988 reporting difficulties to analyze the roots of sugar maple trees from a hardwood forest site due to overall staining, the process of bleaching and staining of urban *Acer platanoides* roots in thisstudy did not produce sufficient root material for analysis. The explanation of modified epidermal cells as well as lignified and suberinized outer walls in the exodermis besides abundant tannins in cortical cells of *Acer saccharum* may accordingly serve for *Acer platanoides* roots. Also, may the several urban stresses have modified the primary roots additionally. For further studies on *Acer platanoides* roots from urban sites an altered clearing and staining approach could deliver better results. Indeed, using an autoclave is most time and therefore cost efficient, but it is often too destructive for root material. A less invasive technique would be to soak the fine root material at room temperature for several days in the respective bleaching or staining agent (Hage-Ahmed K. 2018, personal conversation). The Lessons Learnt section in appendix I displays further insights from the conducted mycorrhizal experiments and corresponding conversations. However, the balancing nature of the clearing and staining method needs to be considered, since long bleaching leads to low staining rates of fungi and risks already mentioned dismantling of root tissue. Whereas, too little bleaching leads to dark stained roots were nothing can be detected (or overly be interpreted). For the intact roots of the testing phase in this study any interpretation could have been made, thus being highly speculative. No mycorrhizae in the excessively bleached roots or innumerable arbuscules occluding most of the cells and therefore leading to the dark staining in the roots (Brundrett et al. 1984, Brundrett et al. 1996).

As visual assessment is highly subjective, requiring experienced mycorrhizal diagnosis, bearing the risk of misinterpretation of structures for fungi inside and outside roots as arbuscular mycorrhizae (Brundrett & Tedersoo 2019), alternative detection methods may be of higher scientific value in the case of processing difficulties. For instance, the analysis of the ester-linked phospholipid fatty acid (PLFA) patterns of the soil, i.e. fatty acid 16: lω5 for AM, as the composition of PLFAs varies between

distinct groups of organisms and can be assigned accordingly (Olsson et al. 1995). And DNA extraction of root material and sequencing (applied in Helgason et al. 2014).

Even though, the question remains if less invasive bleaching and staining of the *Acer* roots or alternative detection methods would have revealed colonization of AM, the findings from spore analysis contradict this conjecture. But also, the data from the bioassay experiment point out that arbuscular mycorrhizae play a more than subordinate role in the soils and therefore for the health of urban *Acer platanoides* trees in Vienna. In the latter, maize seedlings have grown to a higher rate in soils formerly surrounding *Tilia* trees than in soils of *Acer* trees. Thus, analyzing differences in soil chemistry could give a reason for this incidence.

From the findings in the present study hypothesis 3, that the higher the salt content in the soils the lower the root colonization with arbuscular mycorrhizae, cannot be confirmed. In contrast, do the results from the bioassay experiment show that the two maize plants colonized by arbuscular mycorrhiza have grown in soil from the salt stress group main street of *Acer platanoides*. Nevertheless, these AM individuals cannot be tracked down to their former associated host, which also could have been any grass or herb that was previously growing near the trees.

Prior studies on arbuscular mycorrhizal infections showed that lower colonization rates were recorded for urban *Acer platanoides* trees than individuals from rural areas and that highly disturbed urban trees displayed even lower rates (Tyburska et al. 2013). This may happen due to the combination of various abiotic stress factors (Bainard et al. 2011). The effect of high input of NaCl, often used as road and sidewalk de-icing salt, on AM status in form of hampered colonization, spore germination and hyphae growth capacity has been summarized in Porcel et al 2012. Whereas, with excessive salt content the colonization by some AM species decreases, other species may be highly salt tolerant existing in saline soils (Evelin et al. 2009). Furthermore, under alkalinized pH conditions AM colonization rates were demonstrated to be significantly reduced in *Acer saccharum* trees and altered to solely specialized AM fungi in Australian wheat (Porter et al. 1987, Kluber et al. 2012). Helgason et al. 2014 discovered that eight different *Acer* species (including *Acer platanoides*) were each colonized by a different community of AM fungi under similar field conditions. Thus, mycorrhizae are a highly adaptive group of fungi and it seems like for every stress there is a specialized species. Nevertheless, in order to benefit from the stress alleviating properties plants must engage in mycorrhizae symbiosis.

Two sides of the same shield could lead to the reduced abundance of arbuscular mycorrhizae in the analyzed soils of *Acer platanoides* in Vienna. Either a very low number of mycorrhizae in the soils. Or the non-engagement or exclusion of mycorrhizal colonization by the trees. During the clearing and staining of the *Acer* roots a high abundance of root hair was calling for special attention, displaying proliferation in some samples. According to Brundrett 2002 this trait along with branched, long roots is associated with plants in habitats with low levels of mycorrhizal colonization. In addition, other authors portray *Aceraceae* as facultatively mycorrhizal plants that retain capacity for mycorrhization, but do not necessarily utilize it (Wiseman & Wells 2009). A German study on dendrochronological analysis of urban trees indicated that *Acer platanoides* is more sensitive to drought than the other tree species analyzed in the city of Dresden (Gillner et al. 2014). In line with the trade balance model this could be an indicator that under water limited conditions the photosynthetic carbon investment cost of mycorrhizal symbiosis would be too high for the analyzed *Acer platanoides* trees in Vienna (Johnson et al. 2010). In this study it can be assumed that the form of water limitation, either high competition due to many plants in park locations, or excessive salt in roadside locations leading to physiological drought symptoms of the trees, may be negligible. Anyway, it seems to impact the trees ability to form symbiosis with possible stress-relieving arbuscular mycorrhizae.

Which role the displayed dark septate endophytes (DSE) play in the urban soils and for the *Acer platanoides* trees needs to be further investigated as less is known about function and occurrence of DSE in urban soils. Even though, it has been proposed that DSE might act as surrogate mycorrhizae in harsh condition environments like polar and alpine habitats, no consensus has been reached on their influence on plant performance (Newsham 2011). If urban habitats, with its increased pH and calcium saturation levels, can be classified as harsh condition environments, in which DSE thrive or at least take over mutualistic functioning of mycorrhizae or if they engage in parasitism of urban trees, needs to be further studied.

At long last, for many years authors have been suggesting that the problematic urban conditions would not have such severe impacts if young saplings were prepared for above described conditions. This can be seen in a number of publications showing that trees that have had a low water supply in their adolescence develop reduced water potential as an adaption asset for possible future drought times (Balder 1998). However, breeding in most tree nurseries do often not adjust the tree seedlings to the altered living conditions there will be in their future in the city. Therefore, the young trees cannot adapt little by little before being finally exposed to simultaneous urban stress. Unlike the widespread use of selective breeding in forest management, planting material for urban sites is highly heterogeneous and often improperly handled in terms of transportation, planning of pits, planting and nursing (Balder 2002, McGrath & Henry 2016, Murer & Schmidt 2019). As arbuscular mycorrhization or dark septate endophytes could help in adapting, though not abundantly displayed in the present study, inoculation of saplings or young trees should be tested in the future.

Conclusion

In this study tree health of urban *Acer platanoides* was linked to soil nutrients and root parameters to examine the effect of de-icing salt stress in different locations. Tree vitality exhibited to be probably negatively affected by excessive sodium content in soils. Interestingly, counter to what was expected, the side street group were not affected proportionally over all vitality indicators. The fact that winter temperatures are predicted to rise, and the excessive use of de-icing salts may not occur as regularly in the future seems promising. Nevertheless, mean temperatures in general are expected to increase, therefore heat waves and drought will occur more often. Moreover, are trees in urban areas, roadside trees as well as park trees, exposed to various biotic and abiotic stress. How the vital *Acer platanoides* in Vienna cope with and which role specific fine root traits play, what factors inhibit colonization with potential stress relieving arbuscular mycorrhizae as well as which role dark septate endophytes as mutualistic symbionts may play in these processes needs to be further investigated.

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Appendix

Appendix I

Clearing and staining modification protocols

test sample	1*	2*	3*	4*	5.*	5L*	6*
storing liquid	stored in tap water ~4°	stored in tap water ~4°	stored in tap water ~4°	stored in tap water ~4°	stored in tap water ~4°	stored in tap water ~4°	stored in tap water ~4°
procedure start	04.07.2017	04.07.2017	04.07.2017	26.07.2017	17.07.2017	26.07.2017	26.07.2017
new		2. autocl. 10 minutes	2 autocl. 15min.				
first step				dry root at 50°C 12h	dry root at 50°C 12h		dry root at 50°C 12h
autoclave	Autoclave (autcl.): 15min. 15%KOH	1. autocl.: 15min. 15%KOH , stored in tap water at 4°C overnight	1. autocl.: 15min. 15%KOH , stored in tap water at 4°C overnight	1.autocl.: 10min. 15%KOH, leaving overnight, bleaching in 3%H2O2 for 15 min.	Autocl.: 15min. 15%KOH	1. autocl.: 10min. 15%KOH leaving overnight	1. autocl.: 10min. 15%KOH leaving overnight, bleaching in 3%H2O2 for 15 min.
step		2. autocl.: 15%KOH 10 minutes	2. autocl.: 15%KOH 10 minutes	2. autocl.: 15%KOH 10min.		2. autocl.: 15%KOH 10min, leaving for 55min., soak 2x in tap water	2. autocl.: 15%KOH 10min.
bleaching	15min. 3%H2O2 at room temperature , stored in tap water ~4° until 10.07.2017	10min. 3%H2O2 at room temperatur e	15min. 3%H2O2 at room temperatur e	2nd: 20min. 3%H2O2 at room temperatur e	a) 45min. 3%H2O2, b)40min. 3%H2O2, c) 35min. 3%H2O2, d) 30 min. 3%H2O2,	A) 50min. 3%H2O2, B) 45min. 3%H2O2 at room temperatur e	A) 50min. 3%H2O2, B) 45min. 3%H2O2 at room temperatur e
acidifiy	stored in tap water ~4° until 10.07 (no acidifying step)	stored in tap water ~4° until 10.07 (no acidifying step)	stored in tap water ~4° until 10.07 (no acidifying step)	5min. In 1%HCl	no	rinse 2x tap water, 1) soak in tap water, B) 5min. 1%HCl	1) 5min. 1%HCl, 2) 10min. 1%HCl
staining	10.07.: cut into pieces, heat for 20min. at 80-90°C in ink-vinegar solution (5% Pelkan Blue ink, 5% acidic acid)	10.07.: cut pieces, heat for 20min.at 80-90°C in ink-vinegar solution (5% Pelkan Blue ink, 5% acidic acid)	10.07.: cut pieces, heat for 20min. at 80-90°C in ink-vinegar solution (5% Pelkan Blue ink, 5% acidic acid)	cut pieces 1.5-2cm, autoclave in in-vinegar solution for 10min.	cut pieces, 25min. in 80°C ink-sol.	cut pieces, heat in ~90°C for x) 20min., *)30min., °) 45min.	cut pieces, autoclave ink-sol. 10min.
stain	ink-vinegar solution (5% Pelkan Blue ink, 5% acidic acid)	ink-vinegar solution (5% Pelkan Blue ink, 5% acidic acid)	ink-vinegar solution (5% Pelkan Blue ink, 5% acidic acid)	ink-vinegar solution (5% Pelkan Blue ink, 5% acidic acid)	ink-vinegar solution (5% Pelkan Blue ink, 5% acidic acid)	ink-vinegar solution (5% Pelkan Blue ink, 5% acidic acid)	ink-vinegar solution (5% Pelkan Blue ink, 5% acidic acid)
destaining	slightly acidified water (MilliQ H2O 400ml +	slightly acidified water (MilliQ H2O	slightly acidified water (MilliQ H2O	slightly acidified water for at least	slightly acidified water for 20min.at	slightly acidified H2O overnight	slightly acidified H2O overnight

	800µl acetic acid) at 4°C overnight	400ml + 800µl acetic acid) at 4°C overnight	400ml + 800µl acetic acid) at 4°C overnight	20min.at room temperatur e	room temperatur e		
mounting liquid	PVLG (polyvinyl alcohol lactic acid glycerol)	PVLG (polyvinyl alcohol lactic acid glycerol)	PVLG (polyvinyl alcohol lactic acid glycerol)	PVLG (polyvinyl alcohol lactic acid glycerol)		lactoglycero l	PVLG
mounting date	12.07.2017	12.07.2017	12.07.2017	27.07.2017	17.07.2017	27.07.2017	27.07.2019
notes:	90°C couldn´t be reached with the heating plate						
***** training tree roots from Türkenschanzpark, KatasterNr. 25004, grafting 1940							

7*	**8* last TKpark**	**9 (AK04**	**10 (AK04)**	**11 (AK04)**	**12 (AK01)**	**13 (AK01)**
stored tap water ~4°	stored tap water ~4°	70% ethanol	65% ethanol	65% ethanol	65% ethanol	65% ethanol
17.07.2017	17.07.2017	04.09.2017	12.09.2017	12.09.2017	19.09.2017	19.09.2017
		no bleaching step			Freshly 15%KOH	fresh 15%KOH
autoclave: 30min. 15%KOH	autoclave: 20min. 15%KOH	1.autoclave: 15min. In 15%KOH at 121°C, overnight stay in KOH+autoclaver	autoclave: for 15min. In 15%KOH (from july) at 121°C, overnight stay in KOH+autoclaver	1.autoclave: for 15min. In 15%KOH (from july) at 121°C, overnight stay in KOH+autoclaver, (D) 13.09.17: 2.autoclave: for 10min. 15%KOH (old from july) at 121°C- overnight stay in acified water at 4°C	1.autoclave: for 15min. In old 15%KOH (from july) at 121°C, stayed in KOH (19.-21.09.17), 2.autoclave (21.09.17): A) for 10min. in fresh 15%KOH at 121°C, B) for 10min. in old 15%KOH at 121°C	1.autoclave: for 15min. In fresh 15%KOH at 121°C, stayed in KOH (19.-21.09.17), 2.autoclave (21.09.17): for 10min. in fresh 15%KOH at 121°C
		2.autoclave: in 15%KOH A) for 15min. (stayed in KOH until B was for autoclaving), B) for 10 min. at 121°C	wash	wash	wash in tap water	wash in tap water
a) 50min. 3%H2O2, b) 45min. 3%H2O2, c) 40min. 3%H2O2, d) 35min. 3%H2O2, e) 25min. 3%H2O2, f) 20min. 3%H2O2	a) 45min. 3%H2O2, b) 40min. 3%H2O2, c) 35min. 3%H2O2, d) 30min. 3%H2O2, e) 25min. 3%H2O2		A) for 50min. in 3%H2O2 at room temperature, B) for 40min. in 3%H2O2	A) for 30min. In 30% H2O2 (like Klironomos 1985 "sugar maple"), B) 20min. In 30% H2O2, C) after overnight stay in slightly acified water at 4°C: 14.09.17: for 15min. In 3ml 20%NH4OH+ 30ml 3%H2O2 (Brundrett book), D) 14.09.17: for 45min. In 3%H2O2	1) for 45min. in 3%H2O2 (old from july), 2) for 50min. in 3%H2O2	A) for 45min. In 3%H2O2, B) for 50min. In 3%H2O2

				- ALL at room temperature		
75min. in slightly acidified H2O	15min. in slightly acidified H2O	acify in 1%HCl for 15min.	for 10min. In 1%HCl, overnight in sliyghlty acified water	for 10min. In 1%HCl	wash, for 10min. In 1%HCl, overnight stay in slightly acified water	wash, for 10min. In 1%HCl, overnight stay in slightly acified water
heat for 20min. in ~90°C ink-vingear solution	heat for 20min. in ~90°C ink-vingear solution	stain in autoclave 121°C for 10min. in ink solution (no 15min. try because roots looked already too smashed)	14.09.17: 1) for 5min. In ink solution at 121°C autoclave, 2) for 10min. In ink solution at 121°C autoclave	14.09.17: for 10min. In ink solution (Pelikan blue) at 121°C autoclave	22.09.17: stain for 10min. in ink solution (Pelikan blue) at 121°C autoclave	22.09.17: stain for 10min. in ink solution (Pelikan blue) at 121°C autoclave
ink-vinegar solution (5% Pelkan Blue ink, 5% acidic acid)	ink-vinegar solution (5% Pelkan Blue ink, 5% acidic acid)	ink-vinegar solution (5% Pelkan Blue ink, 5% acidic acid)	ink-vinegar solution (5% Pelkan Blue ink, 5% acidic acid)	ink-vinegar solution (5% Pelkan Blue ink, 5% acidic acid)	ink-vinegar solution (5% Pelkan Blue ink, 5% acidic acid)	ink-vinegar solution (5% Pelkan Blue ink, 5% acidic acid)
slightly acidified H2O at 4°C for 7 days (17-25.07.17)	slightly acidified H2O at 4°C for 7 days (17-25.07.17)	slightly acified water, no time stated	in slightly acified water at 4°C (overnight)	in slightly acified water at 4°C (overnight)	in slightly acified water at 4°C from 22-25.09.2017	in slightly acified water at 4°C from 22-25.09.2017
			PVLG	PVLG	PVLG	PVLG
25.07.2017	25.07.2017	05.09.2017	15.09.2017	15.09.2017	25.09.2017	25.09.2017
		first tree roots from REAL samples	tried procedure like 6A2, which has been the best looking until 04.09.17			

test sample	**14 (AK01)**	**15 (AK22)**	**16 (AS110b) many roots**	**17 (AS110b)**	**18 (AK119)**
storing liquid	65% ethanol	65% ethanol	65% ethanol	65% ethanol	65% ethanol
procedure start	19.09.2017	21.09.2017			23.10.2017
new	freshly made 15%KOH	first time **Schaeffer black** solution (-> too little acidic acid: 400ml deion. H2O+25ml ink+25ml 5% acetic acid)	before processing roots **1h** in **deionised H2O** MilliQ to get rid of the alcohol in the roots		ink solution readapted with +25ml (96%) acetic acid
first step			1h roots in dein.H2O	1h roots in dein.H2O	1h roots in dein.H2O
next step			scan	scan	scan
autoclave	1.autoclave: for 15min. In old 15%KOH (from july) at 121°C, stayed in KOH (19.-21.09.17), 2.autoclave (21.09.17): A) for	1.autoclave: for 15min. in 15%KOH (19.09.17) at 121°C, overnight stay in KOH+autoclaver, 2.autoclave: 10.min. in fresh	1.autoclave: for **15Min.** In 15% fresh KOH at 121°C, overnight (and longer) stay in KOH, **A)** 2.autoclave: for **10Min.** in 15%KOH at 121°C, **B)** 2.autoclave: for **15Min.** in 15%KOH at 121°C	1.autoclave: for **20Min.** in 15% fresh KOH at 121°C, overnight (and longer) stay in KOH, **A) no 2nd autoclave, B)**	1.autoclave: for **15min.** In 15%fresh KOH at 121°C, overnight in KOH, 24.10.17: wash with tap water **A)**

	10min. in fresh 15%KOH at 121°C, B) for 10min. in old 15%KOH at 121°C	15%KOH (22.09.17) at 121°C		2.autoclave: for **10Min.** in 15%KOH at 121°C, **C)** 2.autoclave: for **15Min.** in 15%KOH at 121°C	2.autoclave: for **15min.** in 15%KOH (from 23.10.17)at 121°C afterwards in deion.H2O, **B)** 11:40-14:16 in deion. H2O, 2.autoclave: **20min.** In 15%KOH (23.10.17) at 121°C
step	wash in tap water	wash	wash thouroughly, min. 3 times (water changed)	wash thouroughly, min. 3 times (water changed)	wash thouroughly, 3 times (water changed)
bleaching	1) for 45min. in 3%H2O2 (old from july), 2) for 50min. in 3%H2O2	3% H2O2 at room temperature: A) for 65min., B) 60min., C) 55min., D) 50min. (planned time was 5min. less each, but as most of the roots were still brown time was expanded)	1) no H2O2 -> overnight in 1%HCl; 2) 35Min. 3%H2O2; 3) 45min. 3%H2O2	1) no H2O2-> overnight in 1%HCl; 2) 35Min. 3%H2O2; 3) 45min. 3%H2O3	for 40min. In 3%H2O2
acidifiy	wash, for 10min. In 1%HCl, overnight stay in slightly acified water	wash, for 10min. In 1%HCl	wash thouroughly, min. 3 times (water change); *) overnight in 1%HCl at 4°C; °) 10min. 1%HCl at room temperature	wash thouroughly, min. 3 times (water change); *) overnight in 1%HCl at 4°C; °) 10min. 1%HCl at room temperature	wash 3 times, for 10min. In 1%HCl
staining	22.09.17: stain for 10min. in ink solution (Pelikan blue) at 121°C autoclave	wash, for 10min. In insuffiently acified ink solution at 121°C autoclave	washed, for 10min. in old (insuffiently acified) ink sol. (Schaeffer black) in 121°C autoclave	washed, for 10min. in old (insuffiently acified) ink sol. (Schaeffer black) in 121°C autoclave	wash, für 5min. In adapted ink solution (5%=25ml Schaeffer black ink+5%=25ml acidic acid+deion.H2O) at 121°C autoclave, (I forgot to put aside roots, that´s why no 10min. try…)
stain	ink-vinegar solution (5% Pelkan Blue ink, 5% acidic acid)	ink-vinegar (5% Schaeffer Black, less than 5% acidic acid- 25ml 5% acidic acid,= 500ml ink solution, 25ml ink Schaeffer Black+ 25ml 5% acidic acid+ 400ml MilliQ)	ink-vinegar (5% Schaeffer Black, less than 5% acidic acid- 25ml 5% acidic acid,= 500ml ink solution, 25ml ink Schaeffer Black+ 25ml 5% acidic acid+ 400ml MilliQ H2O)	ink-vinegar (5% Schaeffer Black, less than 5% acidic acid- 25ml 5% acidic acid,= 500ml ink solution, 25ml ink Schaeffer Black+ 25ml 5% acidic acid+ 400ml MilliQ H2O)	new ink-vinegar solution (5% Schaeffer Black, 5% acidic acid)

destaining	in slightly acified water at 4°C from 22-25.09.2017	in slightly acified water at 4°C from 22-25.09.2017	overnight in slightly acified H2O at 4°C	overnight in slightly acified H2O at 4°C	overnight in slightly acified H2O (freshly made: 2,5ml acetic acid in 500ml deion.H2O MilliQ) at 4°C
mounting liquid	PVLG	PVLG	PVLG	PVLG	PVLG
mounting date	25.09.2017	25.09.2017	11.10.2017	11.10.2017	25.10.2017
notes:		question: bleeched too long- reason why hyphal structures+DSF cannot be seen well?			while "throwing" away used KOH (from first autoclave) it turned red on the metal sieve- happened the first time, beaker were taken out of autoclave and had maybe light contact, 2.autocl. was together with buffer solut. in autoclave… 40min. 3%H2O2 because in 16+17 40min. gave better contrast)

test sample	**19 (AK119)**	**20 (AK31)**	**21 (AK31)**	**22 (AK31)**
storing liquid	65% ethanol	65% ethanol	65% ethanol	65% ethanol
procedure start	23.10.2017	30.10.2017 (roots in tap water at room temperature until 31.10.2017, then stored at 4°C	06.11.2017	07.11.2017
new		tap water soaking before autoclaving: talk with Hans & Boris from 30.10.2017 deion.H2O MilliQ has different osmotic potential- can destroy roots- they may explode, tap water is better, also for destaining, destaining is faster at room temperature, even faster at 40°C		
first step	1h roots in dein.H2O	roots soak in tap water one night, 31.-07.11. in tap water at 4°C	roots soak in tap water one night (6.-7.11.17)	
next step	scan	scan	scan	scan
autoclave	1.autoclave: for **20min.** in 15%fresh KOH at 121°C, overnight in KOH, 24.10.17: wash with tap water **A)** 2.autoclave: for **15min.** in 15%KOH (from 23.10.17)at 121°C afterwards in deion.H2O, **B)** 11:40-14:16 in deion. H2O,	07.11.2017: 1h in 15%KOH at room temperature, then 1st autoclaving 15min., let in autoclave overnight	07.11.2017: 1h in 15%KOH at room temperature, then 1st autoclaving 15min., let in autoclave overnight	07.11.2017: 1h in 15%KOH at room temperature, then 1st autoclaving 15min., let in autoclave overnight

	2.autoclave: for **20min.** In 15%KOH (23.10.17) at 121°C			
step	wash thouroughly, 3 times (water changed)	08.11.2017: wash thouroughly, 3 times, 2nd autoclave: 15%KOH 15min.	08.11.2017: wash thouroughly, 3 times, 2nd autoclave: 15%KOH 15min.	08.11.2017: wash thouroughly, 3 times, 2nd autoclave: 15%KOH 15min.
bleaching		wash with tap water, 35min. 3% H2O2 at room temperature, wash with tap water, stored at 4°C in slightly acidified MilliQ water over night	wash with tap water, 35min. 3% H2O2 at room temperature, wash with tap water, stored at 4°C in slightly acidified MilliQ water over night	wash with tap water, 35min. 3% H2O2 at room temperature, wash with tap water, stored at 4°C in sl. acidified MilliQ water over night
acidifiy	wash 3 times, for 10min. In 1%HCl	10min. 1%HCl, 30min. slightly acidified MilliQ water	10min. 1%HCl, 30min. slightly acidified MilliQ water	10min. 1%HCl, 30min. slightly acidified MilliQ water
staining	wash, für 5min. In adapted ink solution (5%=25ml Schaeffer black ink+5%=25ml acidic acid+deion.H2O) at 121°C autoclave	heat in autoclave for 7min. A) in Schaeffer Black ink solution, B) Pelikan Blue ink solution	heat in autoclave for 7min. A) in Schaeffer Black ink solution, B) Pelikan Blue ink solution	heat in autoclave for 7min. A) in Schaeffer Black ink solution, B) Pelikan Blue ink solution
stain	new ink-vinegar solution (5% Schaeffer Black, 5% acidic acid)	either Schaeffer Black or Pelikan Blue ink solution (5% ink, 5% acidic acid)	either Schaeffer Black or Pelikan Blue ink solution (5% ink, 5% acidic acid)	either Schaeffer Black or Pelikan Blue ink solution (5% ink, 5% acidic acid)
destaining	overnight in slightly acified H2O (freshly made: 2,5ml acetic acid in 500ml deion.H2O MilliQ) at 4°C	1h destaining at room temperature in slightly acidified tap water, microsc. assessment-> more destaining needed, destained from 7.-14.11.17 at 4°C	destained in slightly acidified tap water from 7.-14.11.17 at 4°C	destained in slightly acidified tap water from 7.-14.11.17 at 4°C
mounting liquid	PVLG	PVLG	PVLG	PVLG
m. date	25.10.2017	14.11.2017	14.11.2017	14.11.2017
notes:		fresh 15%KOH 7.11.17, KOH turned red while washing out after first autoclave		22B very interesting

test sample	**23 (Acer Maurer Wald)**	**24 (Acer Maurer Wald)**	**25 (Maurer W.)**	**26 (Maurer Wald)**
storing liquid	65 % ethanol	tap water	A) tap water roots, B) 65% ethanol roots	A) tap water roots, B) 65% ethanol roots
procedure start	04.12.2017	04.12.2017	12.12.2017	12.12.2017
new	Trypan Blue (0.05% Trypan Blue in lactoglycerol)	10%KOH	10% KOH from 04.12.17	15% KOH from 19.09.17
first step			B) wash with tap water	B) wash with tap water
autoclave	autoclave: 15min. in 10%KOH overnight stay	autoclave: 15min. in 10%KOH overnight stay	1st autoclave: 15min. in 10%KOH, let in for 4 h	1st autoclave: 15min. in 15%KOH, let in for 4 h
step	05.12.: wash 3 times tap water	05.12.: wash 3 times tap water	wash with tap water, 2nd autoclave:	wash with tap water, 2nd

			10min. in 10%KOH	autoclave: 10min. in 15%KOH
bleaching	40min. 3%H2O2, wash thouroughly. 2h in slightly acidified tap water	40min. 3%H2O2, wash thouroughly. 2h in slightly acidified tap water	wash with tap water, 50min. 3%H2O2	wash with tap water, 50min. 3%H2O3
acidifiy	10min. In 1%HCl	10min. In 1%HCl	wash with tap water, 15min. 1%HCl	wash with tap water, 15min. 1%HCl
staining	autoclave 10min. in Trypan Blue solution	autoclave 15min. in Trypan Blue solution	wash tap water, autoclave for 10min. In Trypan Blue solution	wash tap water, autoclave for 10min. In Trypan Blue solution
stain	Trypan Blue (0.05% Trypan Blue in lactoglycerol, 5:1:1, lactic acid:glycerol:water)	Trypan Blue (0.05% Trypan Blue in lactoglycerol, 5:1:1, lactic acid:glycerol:water)	Trypan Blue (0.05% Trypan Blue in lactoglycerol, 5:1:1, lactic acid:glycerol:water)	Trypan Blue (0.05% Trypan Blue in lactoglycerol, 5:1:1, lactic acid:glycerol:water)
destaining	destaining in lactoglycerol A) two days at 4°C (5.-7.12.), B) until 11.12. at 4°C (5.-11.12.), C) until 12.12. at 4°C then until 18.12. at room temperature (5.-18.12.)	destaining in lactoglycerol A) two days at 4°C (5.-7.12.), B) until 11.12. at 4°C (5.-11.12.), C) until 12.12. at 4°C then until 18.12. at room temperature (5.-18.12.)	destain in lactoglycerol from 12.-18.12. at room temperature	destain in lactoglycerol from 12.-18.12. at room temperature
mounting liquid	PVLG	PVLG	PVLG	PVLG
mounting date	07.12.2017/11.12.2017/18.12. 2017	07.12.2017/11.12.2017/18.12. 2017	18.12.2017	18.12.2017
notes:			KOH after 2.autoclave of roots from A (tap water) less brown than KOH from B (65%ethanol)	KOH after 2.autoclave of roots from A (tap water) less brown than KOH from B (65%ethanol)

autoclaving is done with 121°C	
15%KOH- 100ml	100ml deion. H2O MilliQ+ 17,46g KOH pallets (85,9%)
3% H2O2- 600ml	540ml deion. H2O MilliQ+ 60ml 30% H2O2
1% HCl-500ml	486ml deion. H2O MilliQ+ 13,50ml 37% HCl
ink solution- 500ml	400ml deion. H2O MilliQ+ 25ml ink+25ml acetic acid

Lessons Learnt

Standard procedures of clearing and staining of roots should be tested on the respective species, of similar age and grown under similar conditions, before starting a project in order to test for feasibility of the study. Moreover, should root orders for the respective species be assessed and training on the categorization of root orders should be undertaken (McCormack et al. 2015).

Roots should be kept in 65% ethanol and not higher concentration (Brundrett et al. 1996 go for 50%) as it may cause dis-integration or they may be harder to bleach. In any case roots that were stored in alcohol should be washed with tap water or let set in tap (not de-ionized!) water for some time (several minutes) in order to let the alcohol leave the root tissue before further treatment.

As stated in the text part Karin Hage-Ahmed indicated that autoclaving may not be the best method to do bleaching or staining. Soaking at room temperature for a longer period could bring a more fruitful output.

For mounting stained roots on analytical slides PVLG is the best solution, cautious application can reduce unwanted oxygen blebs. To conserve the slides for analytical purposes for a longer period, the use of nail polish (transparent) around the PVLG applied slides is recommended, otherwise the PVLG may dry up over time.

If soil for further experiments is kept, it is advisable to keep enough soil, i.e. 1kg - in order to have at least 25g for spore separation and at least 200g soil for bioassay experiments (50g was very little for growing maize seedlings) and other analysis.

According to Karin Hage-Ahmed at least 25g of fresh soil is recommended for spore separation of Austrian soils, even though for peer experiences with Ethiopian soils less soil was appropriate. When there are no experiences with soils from similar environmental conditions keeping more soil is better to be on the safe side (e.g. in Austrian soils arbuscular mycorrhizae are not as abundant as in Ethiopian soils, were little soil mass displays thousands of spores).

For information about AM- colonization potential of soil samples Nancy Collins Johnson recommends bioassay experiments in contrast to spore separation as the number of spores does not necessarily tell something about higher root colonization by the spore forming AM species. Like the following citations N.C. Johnson emphasized that old root parts can act as propagules: "*The density of the spores in soil can be determined but, although this sometimes shows a correlation to the extent of root colonization, this is certainly not always the case. The relationship is complex because, although the extent of colonization may be related to the availability of spores as inoculum, it also influences the capacity of the mycorrhizal root system to produce new spores (see below). Furthermore, the spore assemblage is diverse with respect to species composition, viability and dormancy and sources of inoculum other than spores may play more important roles in the colonization of roots*." (Smith & Read 2008).

Bioassay experiments are best made with fresh or semi fresh soil, 200g for one maize seedling in one pot. Notably, drying, sieving and stirring could damage spores and other propagules. Maize seeds should be surface sterilized in 10 % ethanol for 3 minutes, rinsed in deionized water and soaked over-night- to make sure seeds are not susceptible to mold, which otherwise would definitely occur at least on some seeds. Daily inspection of to-be maize seedlings will reduce their mortality rates due to mold. The treatment pots should be isolated from each other using plastic barriers between treatments. This can prevent cross-contamination of soils and AM fungi due to runoff and reabsorption through the bottom of the pots. See Propster & Johnson 2015 for an example of bioassay experiment set-up.

From the several conversations with different scientists during the present study I encountered that higher inter-institute knowledge transfer and collaboration on a non publicational-only basis would be desirable, especially for research questions that are rarely dealt with at one institute, but more regularly at other (BOKU) institutes. By reinventing the wheel, the "philosopher`s stone" may not be found.

Appendix II

List of trees, maize plants grown in soils and associated colonization:

Number	Species	Treatsideent	Kat.nr.	Street	maize growth	endophytic fungi
1	A	PARK	**2031**	Friedrich Engels Platz	1	no
2	A	PARK	**2029**	Friedrich-Engels Platz	0	
3	A	PARK	**2023**	Friedrich-Engles Platz	0	
4	A	PARK	**2**	Park Kapaunplatz	1	no
5	A	PARK	**3**	Park Kapaunplatz	0	
6	A	PARK	**38**	Park Kapaunplatz	0	
7	A	MAIN	**211**	Aignerstraße	0	
8	A	MAIN	**111**	Aignerstraße	0	
9	A	MAIN	**209**	Aignerstraße	0	
10	A	SIDE	**114**	Leystraße	0	
11	A	SIDE	**113**	Leystraße	1	no
12	A	SIDE	**107**	Leystraße	0	
13	A	PARK	**3059**	Forsthauspark	0	
14	A	PARK	**1022**	Forsthauspark	0	
15	A	MAIN	**103**	Forsthausgasse	0	
16	A	MAIN	**102**	Forsthausgasse	0	

17	A	MAIN	**206**	Forsthausgasse	0	
18	A	SIDE	**111**	Forsthausgasse	0	
19	A	SIDE	**116**	Forsthausgasse	0	
20	A	SIDE	**103**	Brigittaplatz	0	
21	A	SIDE	**110**	Brigittaplatz	1	DSE
22	A	PARK	**5009**	Brigittapark	0	
23	A	PARK	**5004**	Brigittapark	0	
24	A	PARK	**5014**	Brigittapark	1	no
25	A	PARK	**28002**	Türkenschanzpark	0	
26	T	PARK	**35013**	Türkenschanzpark	1	no
27	T	PARK	**35009**	Türkenschanzpark	1	DSE
28	T	PARK	**27034**	Türkenschanzpark	1	no
29	A	PARK	**27033**	Türkenschanzpark	1	no
30	A	PARK	**4030**	Türkenschanzpark	1	DSE
31	A	PARK	**29002**	Türkenschanzpark	0	
32	T	PARK	**23018**	Türkenschanzpark	0	
33	A	PARK	**36006**	Türkenschanzpark	0	
34	A	SIDE	**217**	Universumstraße	0	
35	A	SIDE	**105**	Kampstraße	0	
36	A	SIDE	**111**	Kampstraße	0	
37	T	MAIN	**223**	Donaueschingenstraße	1	no
38	T	MAIN	**221**	Donaueschingenstraße	1	no
39	T	MAIN	**219**	Donaueschingenstraße	1	no
40	A	MAIN	**226**	Jägerstraße 18	0	
41	A	MAIN	**220**	Jägerstraße 14	1	**AM**
42	A	SIDE	**124**	Pappenheimgasse	0	
43	A	SIDE	**219**	Pappenheimgasse	0	
44	A	SIDE	**228**	Pappenheimgasse	1	DSE
45	A	SIDE	**245**	Pappenheimgasse	0	
46	T	MAIN	**218**	Kreuzgasse 9	1	DSE
47	T	MAIN	**217**	Kreuzgasse 9	0	
48	T	MAIN	**112**	Kreuzgasse 87 - 89	0	
49	A	SIDE	**103**	Salierigasse 1	0	
50	A	SIDE	**104**	Salierigasse 3	0	
51	A	SIDE	**105**	Salierigasse 5	1	no
52	T	SIDE	**1017**	Herbeckstraße 15	1	no
53	T	SIDE	**2016**	Herbeckstraße 12	0	
54	T	SIDE	**2014**	Herbeckstraße 10	0	
55	T	SIDE	**113**	Scheidlstraße 51	1	no
56	T	SIDE	**108**	Scheidlstraße 47	0	
57	T	SIDE	**106**	Scheidlstraße 45	0	
58	T	MAIN	**208**	Scheidlstraße 48	1	no
59	A	MAIN	**127**	Boschstraße	1	no
60	A	MAIN	**121**	Boschstraße	0	
61	A	MAIN	**113**	Boschstraße	0	

62	A	SIDE	**101**	Anton-Frank-Gasse 5	0	
63	T	SIDE	**210**	Hainzigergasse 28-30	0	
64	T	SIDE	**107**	Haitzingerstraße	0	
65	T	SIDE	**110**	Hainzigergasse 29	1	DSE
66	T	SIDE	**224**	Hainzigergasse 50	**1**	**AM**
67	T	MAIN	**2031**	Czatoryskig. 44	**1**	**AM**
68	T	MAIN	**2033**	Czatoryskig. 44	0	
69	T	MAIN	**1035**	Czatoryskig 49	**1**	**AM**
70	A	MAIN	**1036**	Czatoryskig. 49	1	DSE
71	T	SIDE	**204**	Staudgasse 86	**1**	DSE
72	T	SIDE	**202**	Staudgasse 82	0	
73	T	SIDE	**201**	Staudgasse 82	1	no
74	T	MAIN	**210**	Roggendorfgasse 2 - 4	0	
75	T	MAIN S	**101**	Roggendorfgasse 2 - 4	0	
76	T	MAIN	**217**	Roggendorfgasse 1 - 5	**1**	DSE
77	T	PARK	**85**	Rohrauerpark	0	
78	A	PARK	**69**	Rohrauerpark	1	no
79	T	PARK	**78**	Rohrauerpark	**1**	**AM & DSE**
80	T	PARK	**21**	Rohrauerpark	**1**	DSE
81	T	PARK	**24**	Rohrauerpark	1	no
82	T	MAIN	**238**	Possingergasse	1	**AM & DSE**
83	T	MAIN	**237**	Possingergasse	0	
84	T	MAIN	**236**	Possingergasse	**1**	DSE
85	T	MAIN	**228**	Possingergasse	**1**	DSE
86	T	SIDE	**204**	Demuthgasse	1	**AM & DSE**
87	T	SIDE	**202**	Demuthgasse	1	**AM & DSE**
88	T	SIDE	**106**	Demuthgasse	0	
89	T	SIDE	**104**	Demuthgasse	0	
90	T	MAIN	**2007**	Gallitzinstraße	**1**	**AM & DSE**
91	T	MAIN	**2003**	Gallitzinstraße	0	
92	T	MAIN	**2002**	Gallitzinstraße	0	
93	T	SIDE	**1021**	Gallitzinstraße	**1**	**AM**
94	T	SIDE	**1019**	Gallitzinstraße	1	no
95	A	MAIN	**1004**	Ludo-Hartmann-Platz 8	0	
96	A	MAIN	**1005**	Koppstraße 3	0	
97	T	PARK	**119**	Vogelweid Park	1	DSE
98	T	PARK	**123**	Vogelweid Park	0	
99	T	PARK	**124**	Vogelweid Park	1	no
100	T	PARK	**127**	Vogelweid Park	0	
101	T	PARK	**128**	Vogelweid Park	1	DSE
102	T	PARK	**100**	Märzpark	0	
103	T	PARK	**99**	Märzpark	0	
104	A	MAIN	**141**	Schweglerstraße 33	0	
105	A	MAIN	**144**	Schweglerstraße 37	1	**AM**
106	A	MAIN	**135**	Schweglerstraße 25	0	

107	T	MAIN	**1083**	Hütteldorferstraße 59	0	
108	A	MAIN	**235**	Schweglerstraße 34	1	no
109	A	MAIN	**229**	Schweglerstraße 26	0	
110	A	SIDE	**201**	Stättermayergasse 16	0	
111	A	SIDE	**203**	Stättermayergasse 22	0	
112	T	PARK	**9031**	Auerwelsbachpark	0	
113	A	PARK	**9029**	Auerwelsbachpark	0	
114	T	PARK	**12001**	Auerwelsbachpark	0	
115	T	PARK	**12002**	Auerwelsbachpark	0	
116	T	PARK	**3175**	Auerwelsbachpark	0	
117	T	PARK	**3180**	Auerwelsbachpark	0	
118	A	PARK	**13021**	Auerwelsbachpark	1	DSE
119	A	PARK	**14060**	Auerwelsbachpark	0	

Appendix III

	vitality	**rel. height**	**radius growth**	**rel. radius growth**
f.r. biomass 0-10	0.260 (*)	ns	ns	ns
SRL (cm/g)	ns	ns	-0,313*	ns
SRA (cm2/g)	ns	ns	-0,318*	-0,294*
f.r. ratio L < 0.1	ns	ns	ns	-0.262 (*)
f.r.ratio 0.1..L..0.2	ns	ns	ns	-0.242 (*)
f.r. ratio 0.2..L..0.3	ns	ns	-0,269*	-0.271 (*)
f.r. ratio 0.3..L..0.4	ns	ns	ns	ns
f.r. ratio 0.4..L..0.5	ns	ns	ns	ns
sum < 0.5 (L)	ns	ns	-0,337**	ns
f.r. ratio 0.5..L..0.6	ns	ns	ns	ns
f.r. ratio 0.6..L..0.7	ns	ns	0,335*	ns
f.r. ratio 0.7..L..0.8	ns	ns	0.255 (*)	0.255 (*)
f.r. ratio 0.8..L..0.9	ns	ns	0,328*	ns
f.r. ratio 0.9..L..1.0	ns	ns	0,312*	0.275 (*)
f.r. ratio 1.0.L..1.1	ns	ns	0,352**	0.265 (*)
sum 0.5-1.0 (L)	ns	ns	0,323*	ns

	(g) 0-10	(g) 10-30	(g) 30-50	total (g)	mean diameter	SRL	SRA
bulk density	-0,347*	ns	ns	ns	ns	-0.248(*)	-0.246(*)
EC [uS/cm]	ns	ns	ns	ns	ns	0,354**	0,352**
Carbon%	ns	ns	ns	ns	ns	0.358**	0.360**
Nitrogen%	ns	ns	ns	ns	-0.296*	0.414**	0.403**
C : N ratio	ns	ns	ns	ns	0.226(*)	-0.255(*)	ns
Mg %	ns	ns	ns	ns	ns	0.230(*)	0.225(*)
Na %	ns	ns	ns	ns	0.255(*)	ns	ns
Ca : Mg ratio	ns	ns	ns	ns	ns	-0.235(*)	-0.235(*)
Ca : Na ratio	ns	ns	0.242(*)	ns	ns	ns	ns
K : Na ratio	ns	ns	0,296*	ns	ns	ns	ns
Mg : Na ratio	ns	ns	ns	ns	-0,311*	ns	ns
Ca (mg/g)	ns	ns	0.243(*)	ns	ns	ns	ns
Cd (ug/g)	-0.238(*)	ns	ns	ns	ns	ns	ns
Cu (ug/g)	-0,365**	ns	-0,311*	-0,322*	ns	ns	ns
Hg (ug/g)	0.259(*)	ns	ns	ns	ns	ns	ns
Mg (mg/g)	ns	ns	ns	ns	-0,316*	0,314*	0,287*
Mo (ug/g)	ns	-0.234(*)	ns	-0,281*	ns	ns	ns
Na (ug/g)	ns	ns	ns	ns	0.259(*)	ns	ns
Ni (ug/g)	-0.234(*)	ns	ns	-0,307*	ns	ns	ns
P (ug/g)	ns	ns	ns	ns	ns	ns	0.222(*)
S (ug/g)	ns	0,290*	ns	0.251(*)	ns	ns	ns
Zn (ug/g)	-0,286*	ns	ns	ns	ns	ns	ns

(*) ($p < 0.1$) * ($p < 0.05$) ** ($p < 0.01$)

ns (no significant correlation)

	height	sunlight	traffic	parking	pit vegetation
parking	0,264*	ns	0,408**	1	ns
pit vegetation	0.256(*)	ns	ns	-0,260*	1
bulk density	0,292*	0.227 (*)	ns	ns	ns
EC [uS/cm]	-0.256(*)	ns	0,320*	ns	ns
DON	0.226(*)	0,325*	ns	0,288*	ns
CEC (umol.g)	-0,301*	ns	ns	ns	ns
BS %	ns	ns	ns	ns	ns
Ca %	0,263*	ns	ns	ns	0,303*
K %	ns	0,320*	ns	ns	ns
Mg %	-0.221(*)	ns	ns	ns	ns
Na %	-0.239(*)	ns	0,488**	0,414**	-0,621**
Ca : Mg ratio	0.242(*)	ns	ns	ns	ns
K : Ca ratio	ns	0,302*	ns	ns	ns
Mg : K ratio	ns	ns	ns	-0.228 (*)	ns
Ca : Na ratio	ns	ns	-0,445**	-0,372**	0,571**
K : Na ratio	ns	0,338*	-0,454**	-0,291*	0,407**
Mg : Na ratio	ns	0.237 (*)	-0,426**	-0,414**	0,608**
Al (ug/g)	-0,265*	ns	ns	ns	ns
B (ug/g)	0.253(*)	ns	ns	ns	0.244 (*)
Ca (mg/g)	-0.221(*)	ns	-0.235 (*)	ns	ns
Cd (ug/g)	ns	ns	0,266*	ns	ns
Cu (ug/g)	ns	ns	0,375**	ns	ns
Fe (ug/g)	-0.218(*)	ns	ns	ns	-0,290*
Hg (ug/g)	ns	ns	ns	ns	0.246 (*)
K (mg/g)	ns	0,281*	ns	ns	ns
Mg (ug/g)	-0,341**	ns	ns	ns	ns
Mn (ug/g)	-0.245(*)	ns	ns	ns	ns
Mo (ug/g)	ns	ns	ns	-0.239 (*)	ns
Na (ug/g)	ns	ns	0,441**	0,427**	-0,636**
Ni (ug/g)	ns	ns	ns	ns	-0,387**
S (ug/g)	-0,259*	ns	ns	ns	ns
Zn (ug/g)	ns	-0.236 (*)	0.250 (*)	ns	-0,341**

(*) ($p < 0.1$) * ($p < 0.05$) ** ($p < 0.01$)
ns (no significant correlation)

	height	sunlight	traffic	parking	pit vegetation
f.r. biomass 0-10 (g)	ns	ns	-0,363**	ns	ns
f.r. biomass 10-30 (g)	ns	ns	ns	ns	-0.223(*)
mean f.r. diameter (cm)	ns	ns	ns	ns	-0,399**
SRL (cm/g)	-0,274*	-0,272*	ns	ns	ns
SRA (cm2/g)	-0,298*	-0,304*	0.238(*)	ns	ns
f.r. ratio L < 0.1	-0,263*	ns	0.246(*)	ns	-0,415**
f.r.ratio 0.1..L..0.2	-0.239(*)	ns	ns	ns	-0,404**
f.r. ratio 0.2..L..0.3	-0,302*	ns	ns	ns	ns
f.r. ratio 0.3..L..0.4	ns	ns	ns	-0,286*	0,473**
f.r. ratio 0.4..L..0.5	ns	ns	ns	-0.243(*)	0,475**
sum < 0.5 (L)	-0,279*	ns	ns	ns	ns
f.r. ratio 0.5..L..0.6	ns	ns	ns	ns	0,359**
f.r. ratio 0.6..L..0.7	0,382**	ns	ns	ns	ns
f.r. ratio 0.7..L..0.8	0,270*	ns	ns	ns	ns
f.r. ratio 0.8..L..0.9	0,289*	ns	ns	ns	ns
f.r. ratio 0.9..L..1.0	0.235(*)	ns	ns	ns	ns
sum 0.5-1.0 (L)	0,350**	ns	ns	ns	ns
f.r. ratio 1.0.L..1.1	0,267*	ns	ns	ns	ns
f.r. ratio 1.1..L..1.2	ns	ns	ns	ns	-0,272*
f.r. ratio 1.2..L..1.3	ns	ns	ns	0.233(*)	-0,353**
f.r. ratio 1.3..L..1.4	ns	ns	ns	0.254(*)	-0,368**
f.r. ratio 1.4..L..1.5	ns	ns	ns	ns	-0,367**
sum 1.0-1.5 (L)	ns	ns	ns	ns	-0,349**
f.r. ratio 1.5..L..1.6	ns	ns	ns	ns	-0,445**
f.r. ratio 1.6..L..1.7	ns	ns	ns	0,297*	-0,478**
f.r. ratio 1.7..L..1.8	ns	ns	ns	ns	-0,516**
f.r. ratio 1.8..L..1.9	ns	ns	ns	ns	-0,512**
f.r. ratio 1.9..L..2.0	ns	ns	ns	0,264*	-0,530**
f.r. ratio..L > 2.0	ns	ns	ns	ns	-0,503**
sum 1.5-2.0 (L)	ns	ns	ns	0.227(*)	-0,510**

(*) ($p < 0.1$) * ($p < 0.05$) ** ($p < 0.01$)

ns (no significant correlation)

	f.r.r.0.5-0.6	**f.r.r.0.6-0.7**	**f.r.r.0.7-0.8**	**f.r.r.0.8-0.9**	**f.r.r.0.9-1.0**	sum 0.5-1.0
pH ($CaCl^2$)	0.255(*)	ns	ns	ns	ns	0,261*
EC [uS/cm]	ns	-0,285*	-0,314*	-0,273*	-0.226(*)	-0.226(*)
Mg %	ns	ns	ns	ns	ns	-0.219(*)
Na %	-0.229(*)	ns	ns	ns	ns	ns
Mg : Na ratio	ns	ns	ns	-0,264*	ns	ns
B (ug/g)	ns	0,411**	0.256(*)	ns	ns	0,316*
Mg (mg/g)	ns	-0.220(*)	-0.241(*)	-0,309*	ns	-0.255(*)

(*) ($p < 0.1$) * ($p < 0.05$) ** ($p < 0.01$)
ns (no significant correlation)

	f.r.r.1.0-1.1	**f.r.r.1.1-1.2**	**f.r.r.1.2-1.3**	**f.r.r.1.3-1.4**	**f.r.r.1.4-1.5**	sum 1.0-1.5
EC [uS/cm]	-0.258(*)	ns	ns	ns	ns	ns
DON	ns	0.249(*)	0,289*	ns	0.230(*)	0.241(*)
Na %	ns	0.226(*)	0,316*	0,392**	0,340*	0,328*
Ca : Na ratio	ns	-0.232(*)	-0,313*	-0,361**	-0,299*	-0,318*
K : Na ratio	ns	ns	ns	-0,284*	ns	ns
Mg : Na ratio	ns	-0,299*	-0,360**	-0,405**	-0,323*	-0,360**
Fe (ug/g)	0.221(*)	0,309*	0,334*	ns	0.227(*)	0,298*
Mo (ug/g)	ns	ns	ns	-0,265*	ns	ns
Na (ug/g)	ns	0.241(*)	0,327*	0,405**	0,332*	0,341**
S (ug/g)	ns	0.245(*)	0,264*	ns	ns	0.227(*)

(*) ($p < 0.1$) * ($p < 0.05$) ** ($p < 0.01$)
ns (no significant correlation)

	f.r.r.1.5-1.6	**f.r.r.1.6-1.7**	**f.r.r.1.7-1.8**	**f.r.r.1.8-1.9**	**f.r.r.1.9-2**	sum 1.5-2	**f.r.r.>2**
BS %	ns	ns	ns	-0.259(*)	ns	ns	ns
Na %	0,349**	0,382**	0,387**	0,419**	0,391**	0,398**	0,385**
Ca : Na	-0,300*	-0,337**	-0,346**	-0,370**	-0,343**	-0,352**	-0,332*
K : Na	-0.227(*)	-0.258(*)	-0.228(*)	ns	ns	ns	ns
Mg : Na	-0,330*	-0,375**	-0,395**	-0,368**	-0,383**	-0,373**	-0,337**
Fe (ug/g)	ns	ns	0.231(*)	0,277*	0,272*	ns	ns
Mo (ug/g)	ns	ns	-0.232(*)	-0,289*	-0.235(*)	-0.254(*)	-0,290*
Na (ug/g)	0,338*	0,379**	0,390**	0,408**	0,389**	0,397**	0,384**
P (ug/g)	ns	ns	ns	ns	ns	ns	0.246(*)
Zn (ug/g)	ns	ns	0.222(*)	0.238(*)	ns	ns	ns

(*) ($p < 0.1$) * ($p < 0.05$) ** ($p < 0.01$)
ns (no significant correlation)