



TUM School of Life Sciences

Chair for Forest Growth and Yield Science

**Root Growth, Biomass Allocation and  
Ecosystem Services of a Widespread Central  
European Urban Tree Species in Response to  
Extreme Drought**

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*"Courage is going from failure to failure without losing enthusiasm. "*

Winston Churchill

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## List of Abbreviations

<i>Ac</i>	<i>Acer campestre</i> L. subsp. <i>campestre</i>
<i>Cb</i>	<i>Carpinus betulus</i> L. ‘Fastigiata’
DBH	Diameter at Breast Height
IPCC	Intergovernmental Panel on Climate Change
LAI	Leaf Area Index
PCA	Projected Crown Area
RER	Rainfall Exclusion Roof
SD	Standard Deviation
SLA	Specific Leaf Area
<i>Tc</i>	<i>Tilia cordata</i> Mill. ‘Greenspire’
<i>Tt</i>	<i>Tilia tomentosa</i> Moench. ‘Brabant’
VPD	Vapor Pressure Deficit
VWC	Volumetric Soil Water Content

## Thesis Abstract

In the context of climate change and urbanization, drought is likely to become more frequent and more severe in cities. Urban trees are considered to play an important role in fixing carbon, improving air quality, reducing noise and providing other ecosystem services. However, data on the response of urban trees to climate change, particularly to drought, are still limited, which prevents a comprehensive understanding of the role of urban trees in ameliorating some of the adverse effects of climate change and their ability to cope with it.

To investigate whole-plant responses to drought stress, we selected *Tilia cordata* ‘Greenspire’ Mill, a commonly planted urban tree in Central European cities as our research object and established a drought controlled experiment in 2017. In this research, we measured the root development, leaf area index development and biomass allocation between the above- and below-ground compartments of *Tilia cordata* to see how it coped with water shortage. Besides, we applied a process-based ecological model named ‘CityTree’ to explore how much the ecosystem services including CO<sub>2</sub> fixation, evapotranspiration and cooling were affected by drought stress. Additionally, the root development measurement was also launched for *Tilia tomentosa* Moench. ‘Brabant’ and the biomass allocation measurement was conducted for *Acer campestre* L. subsp. *campestre* and *Carpinus betulus* L ‘Fastigiata’ as well in the same study site, which could be an direct comparison of drought resistance between *Tilia cordata* and these selected urban tree species.

The stagnant growth of total fine root biomass from our root coring campaign suggested that *Tilia cordata* did not enhance its investment in underground process under water shortage, which was also verified by its decreasing root:shoot ratio. Besides, its deep roots had merely an ordinary growth, which was different as a drought-resistant tree species such as *Tilia tomentosa* that substantially increased the deep root growth to absorb water under drought stress. Apart from the passive growth of the total and vertical growth of fine root, it showed an increase of fine root biomass in the outmost area, suggesting a special underground strategy in the horizontal level to alleviate water shortage.

The results from the harvest campaign indicated that *Tilia cordata* was susceptible to drought with a highly reduced biomass in all three compartments: branch (30.7%), stem (16.7%) and coarse roots (45.2%). Compared to other surveyed tree species showing advantages in maintaining their biomass, this great biomass loss of *Tilia cordata* exhibited a comprehensive weakened ability of coping with water shortage.

After being verified for its reliability of simulating biomass growth and ecosystem services, the *CityTree* model also proved *Tilia cordata*'s susceptibility to drought stress. On one hand, the drought stressed trees almost stopped biomass growth completely in comparison to unstressed trees. On the other hand, a strong degradation of providing ecosystem services of the drought stressed trees was predicted, i.e., reduction in CO<sub>2</sub> fixation of 84%, reduction in evapotranspiration of 65%, and reduction in cooling of 64%.

In conclusion, *Tilia cordata* did not adapt to drought stress well so that it was not recommended to be planted in cities with high average temperatures and low annual precipitation amounts as it might face more extreme drought events under future climate change, even in a complex urban environment with bad soil conditions and less rooting space. For use in harsh urban environments in the future, information regarding both above- and below- ground process is essential and we recommend that urban green managers fully take the adaptation of trees to drought stress into consideration, which could be a positive action towards global change and urbanization.



## Original Articles

**This dissertation is based on investigations that were published in the following original articles:**

**Zhang C\*, Stratopoulos LMF, Pretzsch H, & Räder T. (2019). How Do *Tilia Cordata* Greenspire Trees Cope with Drought Stress Regarding Their Biomass Allocation and Ecosystem Services? *Forests*, 10(8), 676. (IF=2.116)**

**Author Contributions:** Data management: C.Z. and L.M.F.S.; Formal analysis: C.Z., L.M.F.S. and T.R.; Investigation: C.Z.; Methodology: C.Z., L.M.F.S. and T.R.; Project administration: T.R.; Resources: L.M.F.S. and T.R.; Software: T.R.; Supervision: H.P. and T.R.; Validation: C.Z.; Writing—original draft: C.Z.; Writing—review & editing: L.M.F.S. and T.R.

**Abstract:** In the context of climate change, drought is likely to become more frequent and more severe in urban areas. Urban trees are considered to play an important role in fixing carbon, improving air quality, reducing noise and providing other ecosystem services. However, data on the response of urban trees to climate change, particularly to drought, as well as the relationship between their below- and above-ground processes in this context, are still limited, which prevents a comprehensive understanding of the role of urban trees in ameliorating some of the adverse effects of climate change and their ability to cope with it. To investigate whole-plant responses to water shortages, we studied the growth of *Tilia cordata* Greenspire, a commonly planted urban tree, including development of its roots and stem diameter, leaf parameters and the harvested biomass. Our results showed that this cultivar was susceptible to drought and had reduced biomass in all three compartments: branch (30.7%), stem (16.7%) and coarse roots (45.2%). The decrease in the root:shoot ratio under drought suggested that more carbon was invested in the above-ground biomass. The development of fine roots and the loss of coarse root biomass showed that *T. cordata* Greenspire prioritized the growth of fine roots within the root system. The *CityTree* model's simulation showed that the ability of this cultivar

to provide ecosystem services, including cooling and CO<sub>2</sub> fixation, was severely reduced. For use in harsh and dry urban environments, we recommend that urban managers take into account the capacity of trees to adapt to drought stress and provide sufficient rooting space, especially vertically, to help trees cope with drought.

**Zhang C\*, Stratopoulos LMF, Chao Xu, Hans Pretzsch, Thomas Rötzer. Development of Fine Root Biomass of Two Contrasting Urban Tree Cultivars in Response to Drought Stress. *Forests* 2020, 11, 108. (IF=2.116)**

**Author Contributions:** Data management: C.Z. and L.M.F.S.; Formal analysis, C.Z.; Investigation, C.Z. and L.M.F.S.; Methodology, C.Z. and L.M.F.S.; Supervision, H.P. and T.R.; Visualization, C.X.; Writing—Original draft, C.Z.; Writing—Review and editing, L.M.F.S., H.P. and T.R.

Global climate change associated with rapid urbanization is projected to cause a worsening of environmental problems such as extreme heat and drought in cities. Urban trees play an essential role in improving air quality, fixing carbon, mitigating environmental degradation, and providing other ecosystem services. However, limited research has been conducted on belowground processes, which hampers a comprehensive understanding of the effect of climate change and urbanization on urban tree growth. Fine roots (<2 - mm diameter) are the primary pathway for water and nutrient uptake by plants, and they considerably contribute to the survival of urban trees under drought stress. In this study, we conducted a controlled experiment on the development of fine roots of *Tilia cordata* Mill ‘Greenspire’ and *Tilia tomentosa* Moench ‘Brabant’ in response to drought stress via soil coring. Our results indicate that the two cultivars have different strategies for coping with drought. *Tilia tomentosa* ‘Brabant’, originating from drier regions, gave allocation to deeper soil parts priority probably to obtain more water. On the other hand, *Tilia cordata* ‘Greenspire’, which is native in Central Europe, showed a negative response to water shortage and preferred a more horizontal development of fine roots rather than a vertical development. Longterm studies are needed to gain a better understanding of the

belowground processes of urban trees to select tree species and cultivars which are appropriate for planting in major cities, particularly with regard to future climate change.

**Zhang C<sup>1\*</sup>, Stratópoulos LMF<sup>1</sup>, Häberle, K-H, Pauleit, S, Duthweiler, S, Pretzsch H, Rätzer, T. Effects of Drought on the Phenology, Growth, and Morphological Development of Three Urban Tree Species and Cultivars. *Sustainability* 2019, 11, 5117. (<sup>1</sup> shared first-authorship) (IF=2.592)**

**Author Contributions:** Data curation, L.M.F.S. and C.Z; Formal analysis, L.M.F.S. and C.Z; Investigation, C.Z.; Methodology, L.M.F.S. and C.Z; Writing, L.M.F.S. and C.Z; Supervision, K.-H.H., S.P., S.D., H.P. and T.R.; Review, K.-H.H., S.P., S.D., H.P. and T.R.

**Abstract:** Under changing climatic conditions, drought may become a critical constraint for trees in urban areas, particularly at roadsides and highly paved squares. As healthy urban trees have proven to be an important mitigation and adaptation tool for climate change as well as a significant provider of ecosystem services, there is a need for planting species and cultivars capable of coping with the limited water supply. However, data on species' and cultivars' response to drought, particularly their water supplying root systems remains rare. To consider the whole plant responses to drought situations, we studied the growth and phenology of three frequently planted tree species and cultivars with a diameter of 5–6 cm during a one-year rainfall exclusion experiment conducted in a nursery field as well as the dry biomass of the compartments branch, stem, and root after excavation. Our results revealed that species' and cultivars' performance were linked to their within-plant carbon partitioning. A high tolerance to drought was noted for *Acer campestre*, with a particularly high ratio of root:shoot ratio, which made it presumably less susceptible to droughts. *Tilia cordata* 'Greenspire' was highly affected by the reduced water availability visible through prematurely leaf senescence, while *Carpinus betulus* 'Fastigiata' suffered from losing a considerable part of its root biomass, which resulted in the lowest root:shoot ratio of all species and cultivars. This study demonstrated the need for investigating the reaction patterns of species and cultivars by considering both the above-and

the below-ground plant parts. We recommend that, for future tree plantings at harsh and challenging urban sites, an important selection criterion should be species' and cultivars' capability to develop and retain strong and dense root systems even under limited water supply, as that is believed to be an important trait for drought tolerance.

## Other Publications

**Zhang C, Zhao Q, et al. How Well Do Three Tree Species Adapt to the Urban Environment in Guangdong-Hongkong-Macao Greater Bay Area of China Regarding Their Growth Patterns and Ecosystem Services? *Forests*, 2020, 11(4): 420. (IF=2.116)**

My contributions: undertaking data curation, formal analysis, and paper writing.

**Stratópoulos LMF, Zhang C, Duthweiler S, et al. Tree species from two contrasting habitats for use in harsh urban environments respond differently to extreme drought. *International Journal of Biometeorology*, 2019, 63(2): 197-208. (IF=2.377)**

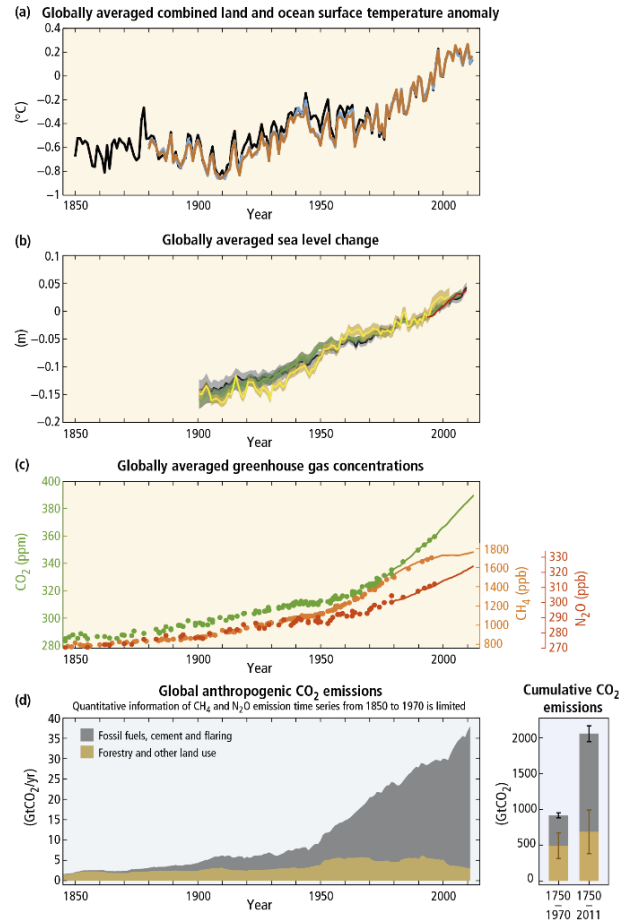
My contributions: participation in experiment design, equipment installation and measurement of tree variables including stem growth, leaf area index, fine root development.

**Rahman MA, Moser A, Anderson M, Zhang C, Rötzer T, & Pauleit S. (2019). Comparing the infiltration potentials of soils beneath the canopies of two contrasting urban tree species. *Urban Forestry & Urban Greening*, 38, 22-32. (IF=3.043)**

My contributions: undertaking the root coring campaign for all the trees and measuring the fine root biomass.

## **1. Introduction**

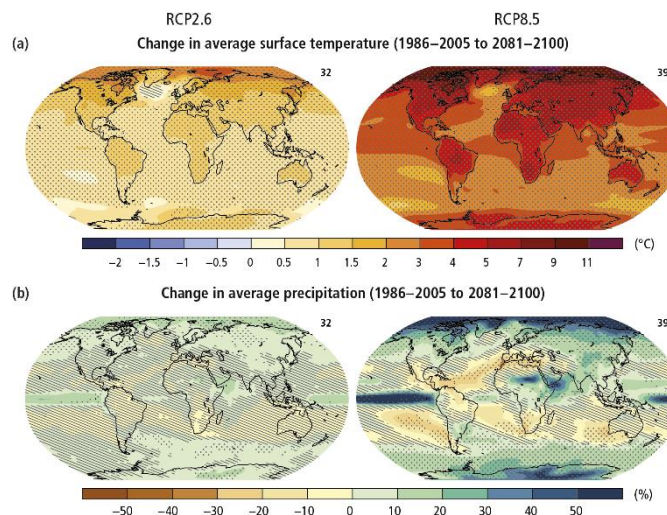
Global change including land-use change, elevated CO<sub>2</sub> concentrations, increased temperature and increased rainfall variability was projected to have increasing impacts on the terrestrial ecosystem (Eisenhauer et al., 2012; Solomon, 2007). It was reported that in recent decades, changes in climate had caused impacts on natural and human systems on all continents and across the oceans, that changing precipitation and melting ice or snow were affecting hydrological systems, following many terrestrial and marine species shifted their physiological activities and living scope (Pachauri et al., 2014) (Fig. 1).



**Figure 1:** Observations and other indicators of a changing global climate system. Observations: (a) Annually and globally averaged combined land and ocean surface temperature anomalies relative to the average over the period 1986 to 2005. Colors indicate different data sets. (b) Annually and globally averaged sea level change relative to the average over the period 1986 to 2005 in the longest-running dataset. Colors indicate different data sets. All datasets are aligned to have the same value in 1993, the first year of satellite altimetry data (red). Where assessed, uncertainties are indicated by colored shading. (c) Atmospheric concentrations of the greenhouse gases carbon dioxide ( $\text{CO}_2$ , green), methane ( $\text{CH}_4$ , orange) and nitrous oxide ( $\text{N}_2\text{O}$ , red) determined from ice core data (dots) and from direct atmospheric measurements (lines). Indicators: (d) Global anthropogenic  $\text{CO}_2$  emissions from forestry and other land use as well as from burning of fossil fuel, cement production and flaring. Cumulative emissions of  $\text{CO}_2$  from these sources and their uncertainties are shown as bars and whiskers, respectively, on the right hand side. The global effects of the accumulation of  $\text{CH}_4$  and  $\text{N}_2\text{O}$  emissions are shown in panel c (IPCC, 2014).

Furthermore, changes in many extreme weather and climate events had been observed since 1950, including a decrease in cold temperature extremes, an increase in warm temperature extremes, an increase in extreme high sea levels and an increase in the number of heavy precipitation events in a number of regions (Pachauri et al., 2014). It is likely that the frequency

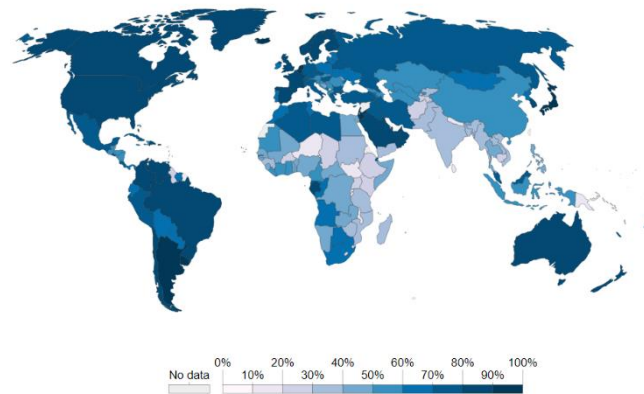
of heat events will increase with changing precipitation in large parts of Europe, Asia and Australia, which increases the risk of exposure of some ecosystems and human systems to global climate change (Fig. 2).



**Figure 2:** Change in average surface temperature (a) and change in average precipitation (b) based on multi-model mean projections for 2081–2100 relative to 1986–2005. The number of models used to calculate the multi-model mean is indicated in the upper right corner of each panel. Stippling (i.e., dots) shows regions where the projected change is large compared to natural internal variability and where at least 90% of models agree on the sign of change. Hatching (i.e., diagonal lines) shows regions where the projected change is less than one standard deviation of the natural internal variability (Pachauri et al., 2014).

Simultaneously, urban areas around the world were expanding rapidly and would have more than 60 percent of the estimated world population by the year 2030 (Angel et al., 2011; Seto et al., 2011) (Fig. 3), and in association with which, extreme heat events had become more prevalent than in previous decades in cities (Alberti, 2005; Breshears et al., 2005), which were due to multiple reasons such as replacement of vegetation, storage of more sensible heat and anthropogenic heat emissions (Oleson et al., 2013; Sailor, 2011; Stone, 2012). This phenomenon might cause a series of ecological problems and increasing costs for cooling demands (Hassid et al., 2000; Santamouris et al., 2011).



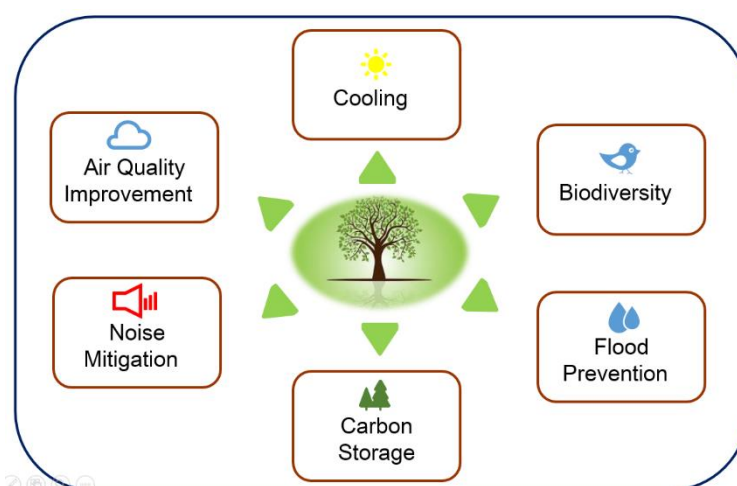


**Figure 3:** Proportion of the total population who live in urban areas all over the world in 2017 (IPCC, 2014).

With a population of 1.5 million, Munich is the third largest city in Germany and 12<sup>th</sup>-largest city in the European Union, which is the most densely populated municipality in Germany that reaches 4500 people per km<sup>-2</sup> (DWD, 2019). It was predicted that a warming of the annual average temperatures by 2 to 4.5 °C for Germany by 2071 to 2100 based on the emission scenario of the ‘Intergovernmental Panel on Climate Change’ (IPCC), following longer dry periods together with less precipitation (IPCC, 2014). Under the background of climate change and urbanization, Munich was predicted to encounter more hot days, longer summer days and more drought events in the next 50 years (DWD, 2019). Hence, it was essential for cities like Munich to find effective solutions as rapid urbanization was destroying natural ecosystems and degrading the environmental quality of towns and cities (Alberti and Marzluff, 2004; Areas, 1997; Gregg et al., 2003).

Urban trees could be a key component in the adaptation of cities to climate change and provide ecosystem services accompanying rapid urbanization (Roy et al., 2012; Tyrvänen et al., 2005), including improving urban air quality, reducing noise, attenuating storm-water flooding and conserving energy, which had drawn increasing interest from researchers (Arthur and Martin, 1981; Burden, 2008; Miller et al., 2015; Nicholas, 2005; Sutton et al., 2004). For example, Nowak et al. (2013) studied how urban trees could filter pollutants that had adverse effects on human health. Konarska et al. (2016) quantified the magnitude of daytime and night-time transpiration of common urban tree species. Sandström et al. (2006) emphasized the importance

of urban green space with natural structures to maintain high ecological diversity. Ross et al. (2011) reported that green infrastructure in cities could provide noise and air pollution reduction. Urban trees and greening are therefore regarded as a multifunctional and comprehensive solution that not only mitigates the negative impacts of urbanization and climate change but also provides ecosystem services and benefits to humans and society. Fig 4 concludes the multiple ecosystem services provided by urban vegetation.



**Figure 4:** Ecosystem services provided by urban vegetation, including providing cooling and biodiversity, storage of biomass, preventing flood, mitigating noise and improving air quality (Moser, 2016).

There is an increasing demand for information on the growth and ecosystem services of urban trees. However, multiple factors could prevent researchers from obtaining profound understandings of urban trees (Moser et al., 2016). These factors can be classified into three types: 1) the cost of researches in cities. It is time-consuming to plant, prune and maintain urban trees and usually expensive for managers and researchers to conduct experiments on them. Besides, harvest campaigns on urban trees are destructive and costly; 2) the complex environment in cities. Urban trees usually grow in streets, parks, public squares, and in front of buildings. These locations in cities might lead to urban trees growing in compact and dense soil (Day et al., 1995; Li et al., 2003), having limited rooting space (Meier and Leuschner, 2008), suffering from harsh pavement (Rowell and Jones, 2006) and even get mechanical injuries (Beatty and Heckman, 1981); 3) complex below-ground process. Yet, these below-ground

impacts are difficult to observe. Most researches are conducted and focused on above-ground processes as it is much easier to obtain information of stems, leaves and branches than to get information from below-ground processes. The limited and compacted soil environment is a great obstacle for researchers to acquire direct and quantitative information on the growth dynamics of roots.

As described above, research for quantitative information of urban trees may face multiple challenges although more and more researchers are trying to fill the knowledge gap about the development and ecosystem services from urban vegetation. Some studies only focused on one city in a certain climatic region which could not be applied in other cities (Moser, 2016). Allometric equations were commonly used to estimate urban tree growth because of the expensive and time-consuming harvesting process of the whole tree (Poudel and Temesgen, 2015) while process-based models were used for ecological forecasts which might involve various uncertainties (Luo et al., 2011). In most cases, less attention was paid to the root development because of the complex below-ground processes which led to knowledge gaps regarding carbon allocation and ecosystem services (Johnson and Gerhold, 2003; Zhang et al., 2019). In addition, a large number of experiments regarding the growth and ecosystem services of urban forests and trees were launched in US cities while there was a lack of data for Central Europe in temperate climatic regions (Rust, 2014; Semenzato et al., 2011). Hence, accurate assessments of the growth of urban trees including both the above-ground and below-ground processes in Central Europe were extremely urgent under the background of climate change and urbanization as there was an increasing demand on the information how well urban trees could adapt to the local environment in European cities (Bigler et al., 2006; Br ěda et al., 2006; Pe řuelas et al., 2001).

Water availability was considered to be an important determinant of tree growth (Williams et al., 2013; Zhao and Running, 2010). A higher frequency of drought events would expose urban trees to more restrictive growth conditions. To enhance the living and improve the quality of ecosystem services of urban trees, i.e., how different urban tree species coped with drought stress, had been commonly discussed (Del R ó et al., 2014). It was reported that stress caused by a water deficit could reduce photosynthetic productivity and tree growth (R ätzer et al., 2012).

Moser et al. (2016) found marked growth reductions during drought periods, but subsequent rapid recovery for *Robinia pseudoacacia*. Furthermore, Stratópoulos et al. (2018) proposed that trees from dry regions, such as *Ostrya carpinifolia* and *Tilia tomentosa* ‘Brabant’, showed a high tolerance to drought stress with the expense of above-ground biomass production. However, comprehensive researches including above- and below-ground processes are still scarce which prevent a profound understanding of key parameters such as fine root growth and root: shoot ratio. This results in lacking crucial information on how urban trees cope with drought stress. Roots, especially fine roots are the primary pathway for water and nutrient uptake. Although fine roots constituted only about 2-3 % of the total biomass, they were also a prominent, possibly the most prominent sink for carbon acquired in terrestrial net primary productivity (Hendrick and Pregitzer, 1993; Neill, 1992; Vogt et al., 1995). In addition, root:shoot ratio was usually given as the ratio of the weight of the roots to the weight of the top of a plant (Harris, 1992) and it is often used to estimate relative biomass allocation between above- and below-ground process (Poorter et al., 2012; Wilson, 1988). Especially under water shortage, the root:shoot ratio could be an important index for a plant’s vitality and growth. For example, some drought-resistant tree species could invest more on root growth to enhance water uptake, which led to an increase in the root:shoot ratio (Lemoine et al., 2013). In conclusion, to obtain precise knowledge of how urban trees respond to drought stress should not ignore the root growth.

As discussed above, it is advisable to apply multiple methods to observe and measure the response of urban trees to water shortage to avoid simple and one-sided estimation of tree growth as well as of the provision of ecosystem services. To acquire a precise knowledge, it is appropriate and beneficial to obtain information of above-ground processes including stem, leaf and branch and of below-ground processes including fine root and coarse root by combining tree coring, image analysis, harvesting campaigns and model simulations.

For the studies of the present thesis, *Tilia cordata* ‘Greenspire’ Mill, a tree species which is one of the most representative urban tree species in Central Europe and can commonly be found in cities, is selected as our research object. To estimate its response to different water conditions, a drought controlled experiment was established, in which stem growth, fine root development

in horizontal and vertical level, biomass allocation between above- and below- ground compartments and root architecture dimension were analyzed via stem diameter measurement, fine root coring campaign, harvest campaign and optical root measurement combined with root analysis program. Moreover, how much the ecosystem services including CO<sub>2</sub> fixation, evapotranspiration and cooling of *Tilia cordata* was affected by water shortage was also analyzed by using the process-based *Citytree* model (Rötzer et al. 2019). In addition, the fine root coring campaign was also launched for *Tilia tomentosa* Moench. ‘Brabant’ and the harvest campaign was conducted for *Acer campestre* L. subsp. *campestre* and *Carpinus betulus* L. ‘Fastigiata’, which trees experienced the same controlled experiment in the same study site, aiming to make a direct comparison of their adaption to drought stress between *Tilia cordata* and these urban tree species. On the basis of these studies the following research questions were addressed:

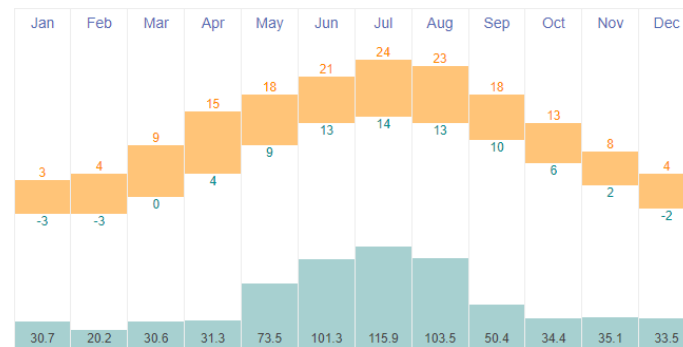
- How did the commonly planted urban tree species *Tilia cordata* develop its fine root biomass under normal conditions and under water shortage?
- What were the patterns of the fine root development of *Tilia cordata* in both vertical and horizontal level?
- What was the relationship between the above- and below-ground processes of *Tilia cordata* under drought stress?
- Compared to other urban tree species, was *Tilia cordata* an adaptive tree species to drought stress?
- Would climate change have a positive or negative impact on *Tilia cordata* in the future regarding its ecosystem services?

## 2. Materials and Methods

### 2.1 Site Description

The drought experiment was performed at the municipal nursery of Munich (48°08'05"N, 11°28'47"E, 534 m a.s.l.), the major city in the southeast of Germany under the marine west coast climate. The 42-ha nursery showed very little variation in terms of topography and microclimatic conditions. The annual means of the temperature and rainfall of the past ten years (2005–2015) from the nearest weather station in the city center of Munich were 9.0 °C and 661 mm, respectively (DWD, 2019). The annual course of temperature and precipitation can be seen in Fig. 5.

The predominant soil types ranged from moderate sandy loam to strong loamy sand, as estimated by a finger test. Interpretation of the water-retention curves provided in the literature indicated a soil moisture tension (pF value) of 1.8 (field capacity) between 29 Vol. % and 33 Vol.-% volumetric soil water content (VWC) and a pF value of 4.2 (permanent wilting point; PWP) at 12 Vol.-% and 16 Vol.-% for the moderate sandy loam and the strong loamy sand, respectively.



**Figure 5:** Monthly means of the temperature ( °C) and precipitation (mm) of the past ten years (2005–2015) from the nearest weather station in the city center of Munich, Germany.

### 2.2 Species Selection

*Tilia cordata* Mill. ‘Greenspire’ (*Tc*), a tree species widely planted in Central European cities, favors good and loamy sites, but can also be found on sandy, infertile soils. This tree species prefers a moist environment and is not thought to be very drought resistant (Gilman and Watson,

1994). As one of the most representative urban tree species in Central European cities and might face severe drought challenges under urbanization and global change, it was selected to analyze the stem growth, fine root development, biomass allocation and ecosystem services under disturbed conditions and under water shortage to estimate its urban adaption ability. Meanwhile, three commonly planted urban tree species with diffuse porous wood anatomy including *Tilia tomentosa* Moench. ‘Brabant’ (*Tt*), *Acer campestre* L. subsp. *campestre* (*Ac*) and *Carpinus betulus* L. ‘Fastigiata’ (*Cb*) were also selected for our controlled experiment to make a comparison between them and *Tilia cordata* on the topic of coping with drought stress.

### *2.3 Drought Experiment*

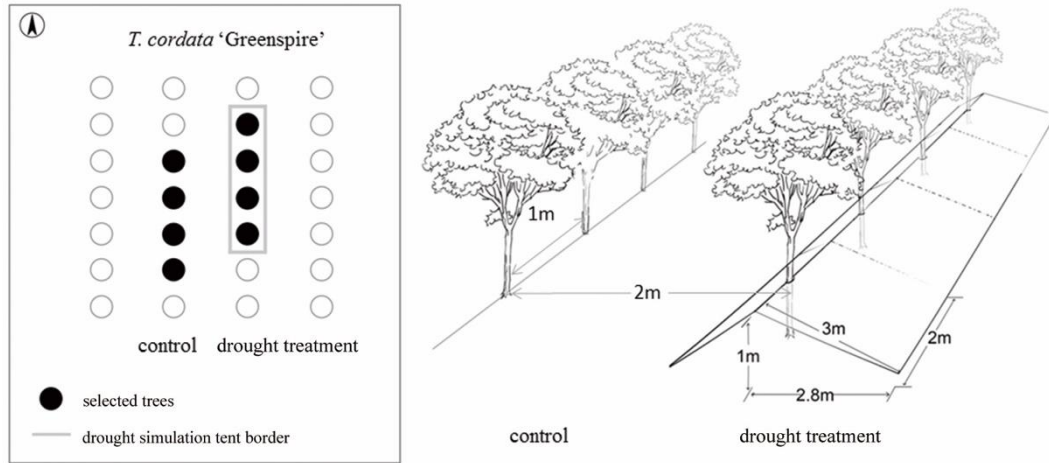
Six to eight trees of the selected tree species with a diameter of 5–6 cm at 1 m height were selected (Table 1). Three or four tree individuals were set as a ‘control’ group and the rest three or four as a ‘drought treatment’ group. Each tree was more than 2 m away from the others to avoid the mutual effect. At the beginning of the drought experiment, the stem diameters between the control and drought treatment groups were not significantly different (Table 1).

**Table 1:** The characteristics of the surveyed trees. Diameter at 1 m height was measured in May 2017.

Species	code	Planted Countries	n		DBH [cm $\pm$ SD]	
			control	treatment	control	treatment
<i>Tilia cordata</i> 'Greenspire'	<i>Tc</i>	EU, Caucasus, N-Iran, W-Siberia	4	4	5.90 $\pm$ 0.10	6.00 $\pm$ 0.16
<i>Tilia tomentosa</i> 'Brabant'	<i>Tt</i>	Balkans, eastern part of Central EU, Syria	4	4	6.57 $\pm$ 0.14	6.39 $\pm$ 0.21
<i>Acer campestre</i>	<i>Ac</i>	EU, Caucasus, N-Iran Morocco, Algeria	3	3	5.60 $\pm$ 0.25	5.66 $\pm$ 0.09
<i>Carpinus betulus</i> 'Fastigiata'	<i>Cb</i>	EU, Caucasus, Iran	4	3	5.30 $\pm$ 0.14	5.61 $\pm$ 0.08

We employed an experimental setting where we simulated the conditions of an enduring drought event to investigate the physiological and growth responses of the trees from April to November in 2017. The trees of the four tree species were divided into two groups (Table 1), one of which was set as the control group that trees were exposed to normal weather conditions, whereas another group of which was set as drought treatment group that the rooting spaces of the trees were covered by a rainfall exclusion roofs (RER) within the whole experimental period. RER was made up of several waterproof tents (2  $\times$  3 m) combined with nylon ropes and installed at a height of 1 m, sloping to the ground in the north and south directions at 3 m from the trees (Fig. 6). RER was totally waterproofed and sufficiently large to create a precipitation-exclusive environment. In the following, we used the abbreviations in the form of 'tree species name' combined with '-c' and '-t' to represent the control and treatment groups for each tree species, e.g., *Tc*-c for the control group of *Tilia cordata* and *Tc*-t for the drought treatment group of *Tilia cordata*.





**Figure 6:** Schematic structure of the drought experiment for *Tc*. The rooting zones of the trees on the right were covered by rainfall exclusion constructions (‘drought treatment’, *Tc-t*), whereas trees on the left were exposed to normal weather conditions (‘control’, *Tc-c*). (Zhang et al., 2019)

## 2.4 Climate and Soils

Climate variables, including temperature and precipitation, were sampled every 10 min with a weather station (Stratopoulos et al., 2019b) located at an unshaded site approximately 200 m away from the experimental plots. Additionally, the precipitation data were calibrated based on the data from the nearest weather station from the German Weather Service (DWD) at the Munich City Centre. VPD (vapour pressure deficit, hPa) was computed from air temperature and humidity data.

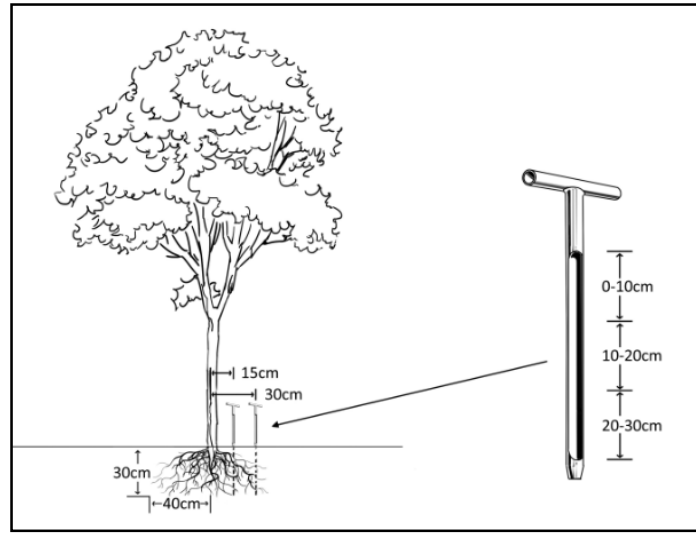
For soil texture, samples were taken with a soil core in three locations around the trees, i.e., 30 cm, 60 cm, and 90 cm to the trunk for replications. Afterwards, they were transported to our laboratory for further analysis. First, stones were removed from the soil samples. Then the samples were dried at 60 °C for 2 weeks to get the dry weight. At last large particles were filtered and the rest was evaluated. To measure the volumetric soil water content (%; VWC) from both the control and treatment groups, HS-10 sensors (Decagon Devices, Pullman, USA) were inserted horizontally at a depth of 30 cm in the horizontal level located 40 cm from the tree stems. For each tree, one sensor was used for one centrally located individual in the row. Given that the continuous measurement devices failed several times probably due to the high soil compaction and drying, we additionally used a portable soil moisture meter (UMP-1, UGT,

Müncheberg, Germany) for point measurements in the upper 10 cm of the soil layer during four days in the summer (Zhang et al., 2019). The readings were made at defined distances from all study trees and the soil moisture data was interpolated spatially for all the tree species (Stratopoulos et al., 2019b).

## *2.5 Growth Measurement*

Growth measurement in this study mainly consisted of three parts: stem diameter, LAI (leaf area index) and fine root biomass. From May to November 2017, the stem diameter at a height of 1 m was measured using a digital caliper for *Tc-c* and *Tc-t* in two perpendicular directions (N–S and E–W) and the data were performed and averaged. LAI ( $\text{m}^2 \text{m}^{-2}$ ) was determined using hemispherical photographs (Nikon Coolpix P5100 camera with a fisheye lens and Mid-OMount, Nikon Corporation, Tokyo, Japan) and analyzed with the program WinSCANOPY (Régent Instruments Inc., Quebec, Canada). Data acquisition was performed under conditions during a uniformly overcast sky in the mid of June, shortly after implementation of the drought experiment.

Fine root coring campaigns were launched for both the control and drought treatment groups for *Tc* and *Tt* in May, September and November, i.e., at the beginning (pre-drought), in the middle and at the end of the growing season. A pre-test coring campaign showed that the range of the root system was similar to a cylinder, with a diameter of 70 cm and a height of 35 cm. Therefore, during every coring campaign, four soil cores were collected for every individual tree: two at a distance of 15 cm from the trunk and two at a distance of 30 cm. The soil was sampled down to a depth of 30 cm using a soil auger with a length of 30 cm and a radius of 3 cm. Each sample was divided into three horizons: soil depths of 0–10 cm (upper layer), 10–20 cm (middle layer) and 20–30 cm (deep layer). Fine roots (<2 mm) were filtered using sieves (2-mm mesh size) and separated by forceps in the laboratory. Then, the samples were washed and dried in an oven at 65 °C for 72 h. Finally, all the samples were weighed using a balance with an accuracy of four decimal places to obtain the dry weight. The fine root biomass at different depths was calculated using the dry weight divided by the cross-sectional area of the auger.



**Figure 7:** Three layers of fine roots in vertical level and horizontal location of the coring campaign at a distance of 15 cm and 30 cm to the trunk (Zhang et al., 2020).

## 2.6 Harvest Campaign and Optical Measurements

In November, a harvest campaign was launched for all the trees of *Tc*, *Cb* and *Ac* because *Tt* trees were unfortunately destroyed and dropped by the workers in the nursery. All the trees were excavated with a tree digger and divided into three parts: branch, stem and coarse roots. All these compartments were dried at 65 °C for 72 h and weighed using a balance with an accuracy of up to four decimal places to obtain the dry weight. Before drying, the root systems were washed to remove the attached soil and stones. Sufficient numbers of points were measured and recorded (7–11 x, z pairs) to describe each of the trees' average crown shape. Geometric data were then processed within the software FV2200 (LI-COR Biosciences, Lincoln, USA) to compute the crown dimensions (volume and projected crown area) by using the model for isolated trees. Similarly, we also measured each trees' average root system dimensions after excavation in winter 2017 (see the following section) and calculated the volumes with the FV2200 software.

Images of the top view of each root system were analyzed by the open source image processing software ImageJ and the plugin DiameterJ (Hotaling et al., 2015), which was a nanofiber diameter characterization tool. First, the scenes were converted into black and white (i.e., binary) images, where the white pixels ideally represented the portions of the original image that were

of interest (root system) and the black pixels represented the background. We compared the results of different segmentation algorithms (auto threshold) provided by the software and selected the appropriate algorithm, which best represented the root system. Inside each binary image, we then selected one rectangular area, where again segmentation most properly displayed the roots and eliminated the features of the image that we could measure (e.g., scale bars or parts of the root system, where segmentation did not work well). This binary segment of the original image scene was used for analysis. The results included summary statistics such as mean root diameter and the percent of porosity.

In addition for *Tc*, an image analysis process including taking high-resolution photos from five views (N, S, W, E and top) was applied to measure the root architecture with the help of the software ‘Root System Analyzer’ (RSA, University of Vienna, Austria). On the basis of a graphical representation of the skeletonized image of the root system as well as segmentation algorithms, RSA was used to describe some of the root traits, including root nodes, width and depth.

## *2.7 Root:Shoot Ratio Calculations and Statistical Analysis*

Three types of root:shoot ratio were calculated for *Tc* to show the relationship between the above- and below-ground process, i.e. (1) fine root:leaf biomass ratio, (2) coarse root:branch biomass ratio and (3) below-ground:above-ground biomass ratio. The leaf biomass was calculated combined with LAI, SLA (specific leaf area) and PCA (projected crown area) as follows:

$$Biomass_{leaf} = \frac{LAI * PCA}{SLA}$$

Fine root biomass was obtained from the fine root coring campaign and biomass of coarse root, stem and branch was from the harvest campaign. Therefore, the below-ground biomass was the sum of fine root and coarse root biomass and the above-ground biomass was the sum of leaf, stem and branch.

The software package R was used for statistical analysis (R Development Core Team, 2011). To investigate the difference between means, two-sampled t-test and analysis of variance (ANOVA) with Tukey's HSD test were used. In all the cases the means were reported as

significant when  $p < 0.05$ . Where necessary, data were log or power transformed in order to correct for data displaying heteroscedasticity.

## 2.8 Model Simulation

The process-based model *CityTree* was used to simulate the biomass of *Tc* trees in both the control and drought treatment groups and predict their ecosystem services (R  tzer et al., 2004; R  tzer et al., 2019). On the basis of the basic measurements of trees (e.g. DBH and tree height), climate and soil data, *CityTree* model calculated their biomass growth and ecosystem services such as CO<sub>2</sub> fixation, evapotranspiration and cooling. The core function of the simulation of a tree's net assimilation is:

$$A = d * \left[ \left( J_p + J_r - \sqrt{(J_p + J_r)^2} \right) - 4 * \theta * J_p * J_r \right] / 2 * \theta \quad (2)$$

where  $A$  is gross assimilation (g C m<sup>-2</sup> d<sup>-1</sup>),  $d$  is mean day length of the month,  $J_p$  is reaction of photosynthesis on absorbed photosynthetic radiation (g C m<sup>-2</sup> h<sup>-1</sup>),  $J_r$  is the Rubisco-limited rate of photosynthesis (g C m<sup>-2</sup> h<sup>-1</sup>), and  $\theta$  is the form factor (=0.7).

$J_p$  is a function of the photosynthetic active radiation (PAR) and the efficiency of carbon fixation per absorbed PAR, which can be calculated on the basis of the intrinsic quantum efficiency for CO<sub>2</sub> uptake, the partial pressure of internal CO<sub>2</sub>, the CO<sub>2</sub> compensation point, the influence of temperature on the efficiency and a species-dependent adjustment function for tree age. The Rubisco-limited rate of photosynthesis  $J_r$  can be estimated by the maximum catalytic Rubisco capacity, the maximum day length, the Michaelis–Menten constant of CO<sub>2</sub> depending on temperature, the inhibition constant of O<sub>2</sub> against CO<sub>2</sub> (temperature-dependent) and the O<sub>2</sub> concentration.

Net assimilation  $A_N$  is calculated as follows:

$$A_N = A - R_d \quad (3)$$

where  $R_d$  is the product of the maximum catalytic Rubisco capacity and the ratio of the maximum catalytic Rubisco capacity and the respiration cost. A fixed share of 50% of the net assimilation is assumed for growth and maintenance respiration (Meier and Leuschner, 2008). The tree growth represented the fixation of carbon, and the fixation of CO<sub>2</sub> was calculated based on the fixation of carbon and the relative molecular mass.

For the ecosystem service evapotranspiration, the central water balance equation from the water balance module was as:

$$prec - int - et_a - ro - \Delta\phi = 0$$

With  $prec$  = precipitation (mm),  $int$  = interception (mm),  $et_a$  = actual evapotranspiration (mm),  $ro$  = runoff (mm),  $\Delta\phi$  = change of the soil content (mm).

Within the module cooling the energy needed for the transition of water from liquid to gaseous phase was calculated based on the  $CPA$  (crown projected area) and the transpiration  $et_a$  sum:

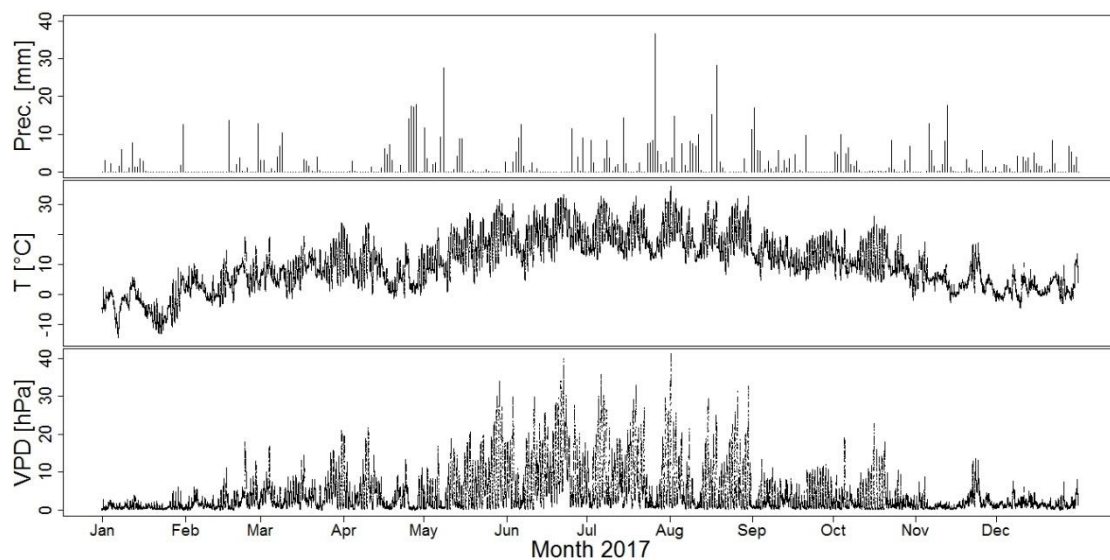
$$E_A = et_a * CPA - (L_O * -0.00242 * temp) / f_{con}$$

With  $E_A$  : energy released by a tree through transpiration (kWh tree<sup>-1</sup>),  $L_O$  : energy needed for the transition of the 1 kg of water from the liquid to gaseous phase = 2.498 MJ (kgH<sub>2</sub>O)<sup>-1</sup> and  $temp$  = temperature in °C,  $f_{con}$  : conversion factor.

### 3. Results

#### 3.1 Climate and Soil Conditions

2017 was a warm year at the site, with a mean temperature of 10.3 °C and total precipitation of 887 mm. The spring of 2017 was warm, sunny and dry. After a strong return to cold weather in April, the temperature started to increase at the beginning of May and remained exceptionally high, in that the average temperature was 19.6 °C from July to August. In this period, monthly precipitation levels of 101 and 123 mm were similar to the long-term records (within 1981–2010) of 122 and 115 mm. The temperature showed a declining trend in early September, and night frost started in the middle of November (DWD, 2019)(Fig. 8).



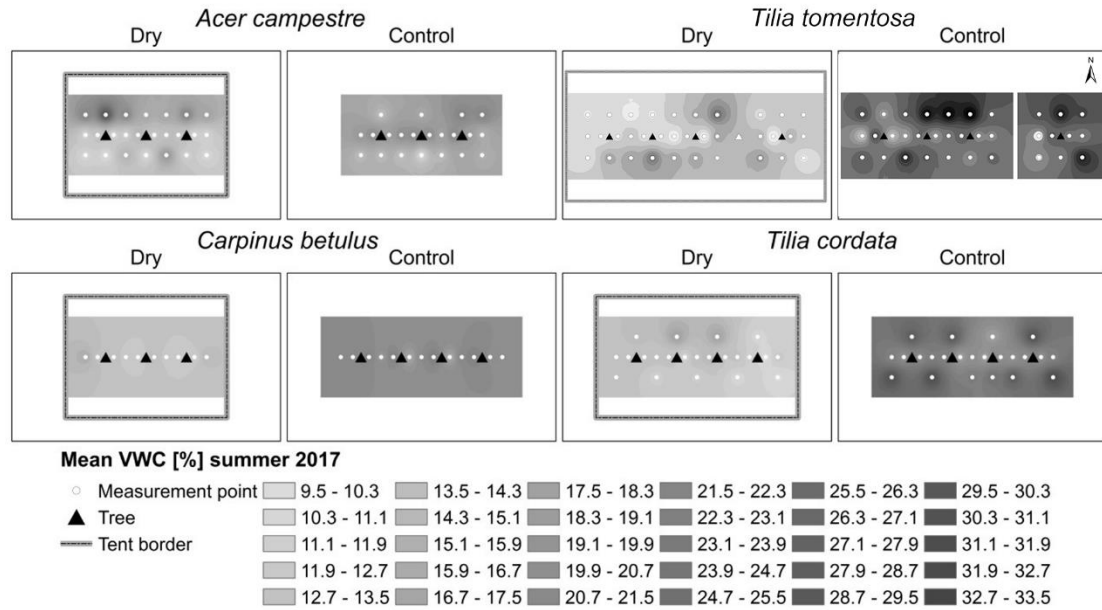
**Figure 8:** Daily amount of precipitation (Prec.; mm), temperature (T; °C), and the vapour pressure deficit (VPD; hPa) in the site for the year 2017. (Stratópoulos & Zhang, 2019)

For both the control and treatment groups, all trees were grown in the similar soil environment ( $P > 0.05$ ), which consisted mainly of medium sand ( $Tc-c$ ,  $27.3 \pm 4.9$ ;  $Tc-t$ ,  $33.4 \pm 4.7$ ), coarse grit ( $Tc-c$ ,  $16.0 \pm 1.2$ ;  $Tc-t$ ,  $12.2 \pm 2.7$ ) and fine sand ( $Tc-c$ ,  $15.8 \pm 3.6$ ;  $Tc-t$ ,  $22.6 \pm 5.9$ ) (Table 2).

**Table 2:** Soil characteristics in the field site for both the control and treatment groups in 2017.  
(Zhang et al., 2019)

Group	n	Fine sand %	Fine silt %	Grit %	Coarse grit %	Medium sand %	Medium silt %
<i>Tc-c</i>	4	15.8 ± 3.6	6.5 ± 1.1	9.0 ± 2.2	16.0 ± 1.2	27.3 ± 4.9	8.7 ± 3.3
<i>Tc-t</i>	4	22.6 ± 5.9	3.8 ± 1.3	7.7 ± 4.3	12.2 ± 2.7	33.4 ± 4.7	8.1 ± 1.3

In terms of the soil moisture, the drought treatment groups had significantly lower volumetric soil moisture contents (VWC; Vol.-%) in comparison to the control groups ( $P_{Tc} < 0.01$ ,  $P_{Ti} < 0.01$ ,  $P_{Ac} < 0.01$ ,  $P_{Cb} < 0.01$ ), with the roofed *Tt-t* showing the lowest average value of 19.4 % (Fig. 9). In addition, we found that the VWC 1 m from the trees, and therefore closer to the tent border, was significantly higher than that in the center of the roofs ( $P = 0.014$ ). Therefore, the data indicated the overall success of the soil drying experiment.

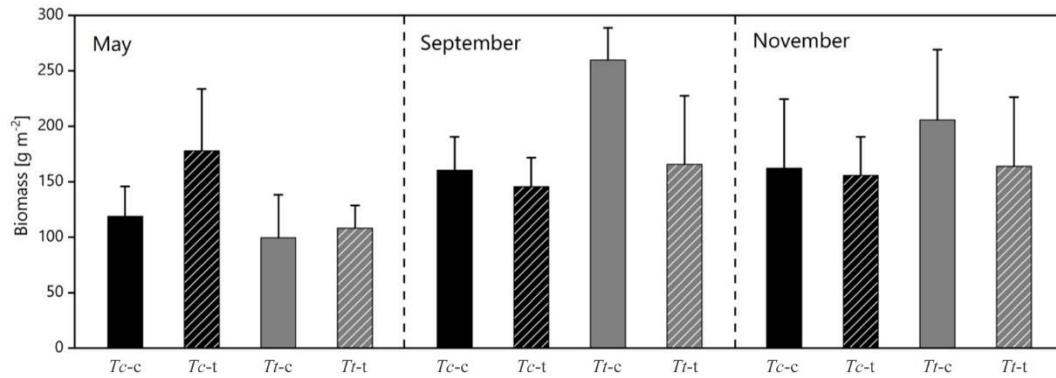


**Figure 9:** Maps (inverse-distance-weighted interpolation) of the volumetric soil water contents (Vol.-%; VWC) at a soil depth of 10 cm averaged over four measurement days in summer 2017 and collected at defined distances from the study trees. (Stratópoulos & Zhang, 2019).

### 3.2 Fine Root Development



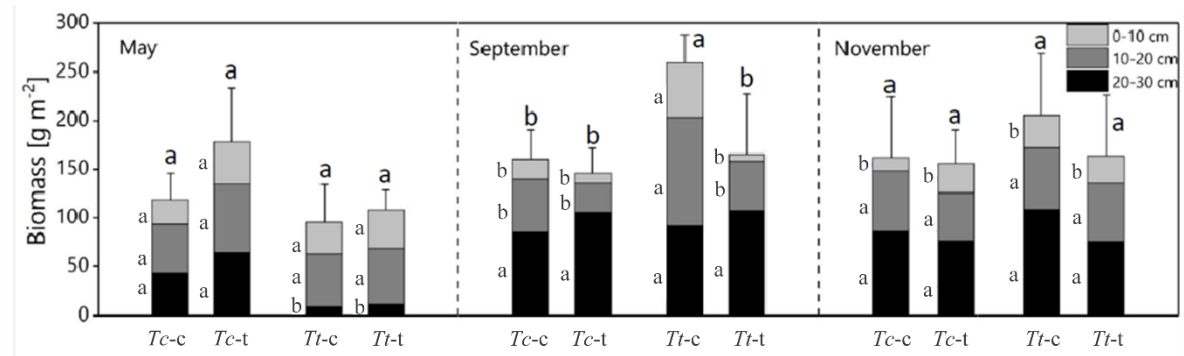
Three types of fine root biomass of *Tc* and *Tt* were measured and discussed: total fine root biomass, horizontal and vertical fine root biomass. For the total fine root biomass of the two species, the growth patterns were significantly different from May to November ( $P < 0.05$ ). During the beginning of the growing season, *Tc* and *Tt* showed no significant difference between the control and treatment groups ( $P_{Tc} = 0.066$ ,  $P_{Tt} = 0.526$ ). The fine-root biomass of *Tc*-c increased by 36.5 % to  $161.4 \text{ g m}^{-2}$  from May to September and remained stable at  $162.2 \text{ g m}^{-2}$  from September to November, while *Tc*-t decreased by 18.1 % to  $145.7 \text{ g m}^{-2}$  from May to September but had a slight growth of 7.0 % to  $155.8 \text{ g m}^{-2}$  from September to November. For *Tt*, the individuals of the control group behaved quite differently from the drought treatment group (Fig. 10). The initial fine-root biomass of the control and treatment groups was nearly similar at  $99.5 \text{ g m}^{-2}$  and  $108.2 \text{ g m}^{-2}$ , but in the course of the experiment, *Tt*-c increased to  $259.8 \text{ g m}^{-2}$  from May to September while *Tt*-t had a moderate increase to  $165.6 \text{ g m}^{-2}$ . Over the period from September to November, *Tt*-c had a remarkable decrease to  $205.7 \text{ g m}^{-2}$  while *Tt*-t remained at  $163.9 \text{ g m}^{-2}$ .



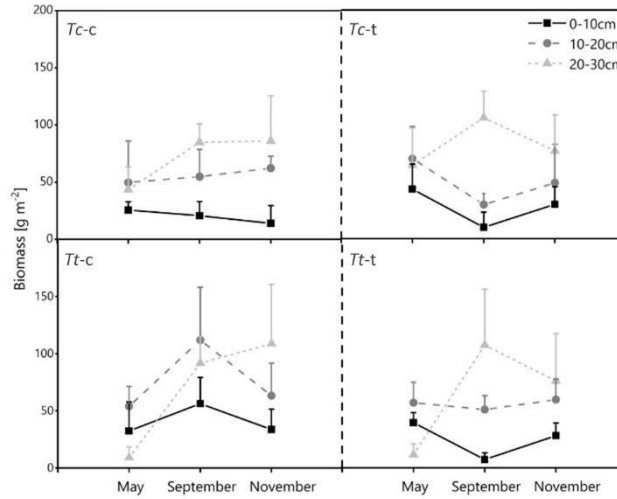
**Figure 10:** Fine-root biomass ( $\text{g m}^{-2}$ ) of the control and treatment groups of *Tc* and *Tt* during May, September, and November 2017. Each panel represents the mean of all the trees per cultivar and treatment. (Zhang et al., 2020)

For the vertical development of the fine root of the two species, the three layers showed different patterns between the control and treatment groups of both tree species (Fig. 11 and 12). For *Tc*, fine-root growth was observed in the 20-30-cm layer from May to September in the control group. In the treatment group, the fine-root biomass from both the 0-10-cm and 10-20-cm layers sharply decreased by 77.1 % and 57.4 %, respectively, meanwhile in the deep

layer we observed contrasting development that it increased to a peak of  $105.8 \text{ g m}^{-2}$  during September and decreased to  $76.8 \text{ g m}^{-2}$  during November. For *Tt*, *Tt-c* continued growing in the 20-30-cm layer while the fine roots from the 0-10-cm and 10-20-cm layers decreased by 40.3 % and 43.4 %, respectively. For *Tt-t*, the fine root in the three layers developed significantly differently from *Tt-c* during the whole period ( $P=0.039$ ). In 0-10-cm depth *Tt-t* decreased from  $39.5 \text{ g m}^{-2}$  to  $7.3 \text{ g m}^{-2}$  during the first four months and increased to  $28.2 \text{ g m}^{-2}$  during the last three months. The roots in the 10-20-cm layer showed only slight fluctuations for the whole period. The fine-root biomass in the deep layer was initially very low ( $11.6 \text{ g m}^{-2}$ ) and steeply increased to  $107.4 \text{ g m}^{-2}$  by September. In contrast, the deep roots of *Tt-c* decreased by 29.3 %.

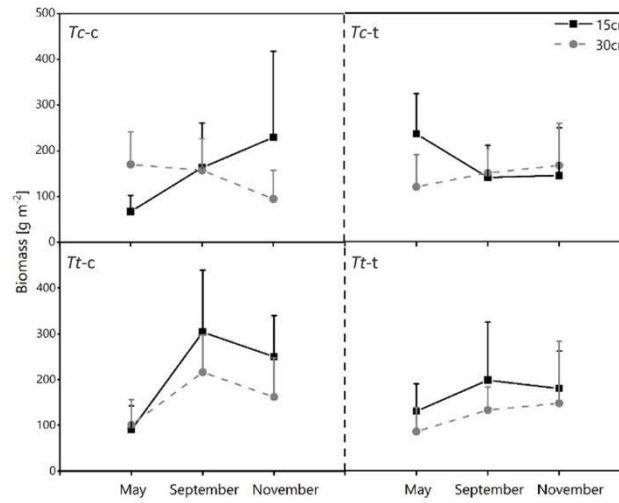


**Figure 11:** Fine-root biomass ( $\text{g m}^{-2}$ ) for the 0-10-cm, 10-20-cm and 20-30-cm layers of the control and treatment groups of *Tc* and *Tt* during May, September, and November 2017. The different letters indicate significant differences ( $P < 0.05$ ) between cultivars and treatments for the overall biomass (black) and different layers (grey). (Zhang et al., 2020)



**Figure 12:** Development of fine-root biomass ( $\text{g m}^{-2}$ ) in the three vertical layers of 0-10 cm, 10-20 cm, and 20-30 cm of the control and treatment groups of *Tc* and *Tt* during May, September, and November 2017. Each panel represents the mean of all the trees per species and treatment. (Zhang et al., 2020)

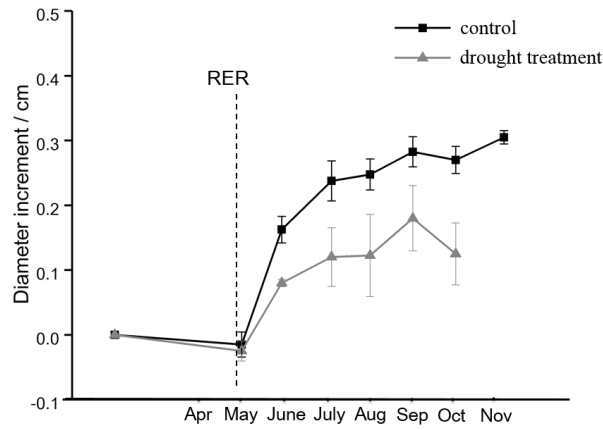
The horizontal development of the fine roots of the two tree species was different when exposed to drought (Fig. 9). *Tc-c* steadily increased fine roots at a distance of 15 cm from the stem, whereas it decreased at a distance of 30 cm from May to November. *Tc-t* decreased its fine-root biomass at a distance of 15 cm from  $235.7 \text{ g m}^{-2}$  to  $140.7 \text{ g m}^{-2}$ , mostly from May to September, whereas the biomass at a distance of 30 cm slightly increased at all measurement times. For *Tt*, the drought had an impact on the fine-root growth from May to September, which suppressed the development by 237.1 % at a distance of 15 cm and 113.9 % at a distance of 30 cm in the control group to 52.2 % and 54.5 % in the treatment group, respectively. From September to November, *Tt-c* decreased from  $303.7 \text{ g m}^{-2}$  to  $249.7 \text{ g m}^{-2}$  at a distance of 15 cm and from  $215.9 \text{ g m}^{-2}$  to  $161.6 \text{ g m}^{-2}$  at a distance of 30 cm.



**Figure 13:** Horizontal development of fine-root biomass ( $\text{g m}^{-2}$ ) at a distance of 15 cm and 30 cm of the control and treatment groups of *Tc* and *Tt* during May, September, and November 2017. Each panel represents the mean of all the trees per species and treatment. (Zhang et al., 2020)

### 3.3 Stem Diameter and Leaf Area Index

The development of the stem diameter growth of *Tc-c* and *Tc-t* was analyzed for the entire year of 2017. At the beginning of the growing season, *Tc-c* and *Tt-t* had similar and slight decreases in diameter. From May to August, distinctly different increases were observed, with the control group exhibiting more rapid growth than the drought treatment group ( $P < 0.05$ ). Despite the similar patterns, in autumn, *Tc-t* had a more obvious and severe decrease in the diameter increment (Fig. 14).



**Figure 14.** Stem diameter growth of the control and drought treatment groups of *Tc*-c and *Tc*-t measured at a height of 1 m from April to November 2017. The panel represents the mean value, and error bars indicate standard deviation. Due to an unexpected failure of the digital caliper, the data for the treatment group in November was missing. (Zhang et al., 2019)

*Tc* was observed to have a quite fast development; the species unfolded its leaves earlier than *Tt* which did not unfold its leaves until the third week of May. In summer, LAI of *Tc*-t under drought was reduced in comparison to *Tc*-c ( $P < 0.05$ ) while the opposite pattern was found for *Tt* that *Tt*-t was higher than *Tt*-c ( $P > 0.05$ ). LAI was lowest for *Ac* in both the treatments (con:  $1.52 \pm 0.11 \text{ m}^2 \text{ m}^{-2}$ , dry:  $1.81 \pm 0.14 \text{ m}^2 \text{ m}^{-2}$ ). *Cb* reached highest values, especially in the drought treatment (con:  $3.01 \pm 0.87 \text{ m}^2 \text{ m}^{-2}$ , dry:  $4.11 \pm 1.49 \text{ m}^2 \text{ m}^{-2}$ ) (Table 3). Additionally in autumn, individuals of *Tc* were leafless while *Tt* showed longer leaf vegetation.

**Table 3:** Leaf area index (LAI;  $\text{m}^2 \text{ m}^{-2}$ ) of the four species from both the control and treatment groups in June 2017. (Zhang et al., 2020)

Species	n		LAI ( $\text{m}^2 \text{ m}^{-2} \pm \text{SD}$ )	
	control	treatment	control	treatment
<i>Tc</i>	4	4	$2.53 \pm 0.49$	$2.13 \pm 0.48$
<i>Tt</i>	4	4	$2.92 \pm 0.21$	$3.11 \pm 0.55$
<i>Ac</i>	3	3	$1.52 \pm 0.11$	$1.81 \pm 0.41$
<i>Cb</i>	4	3	$3.01 \pm 0.87$	$4.11 \pm 1.49$

### 3.4 Biomass Allocation

Due to an unexpected event in November (The nursery workers dug out and threw away the *Tt* samples unintentionally), the harvest campaign was only launched for *Tc*, *Ac* and *Cb*. Tree species and treatment had always a significant effect on biomass, whereas the species was the stronger influencing variable in each of the three cases (Table 4). Generally, the largest proportion of biomass was measured at the trunk section, followed by the root system. The branches had the lowest share.

For the branch biomass, *Ac*-c reached the highest ( $1121.33 \pm 177.46$  g), and *Cb*-t and *Tc*-t lower mean values ( $365.33 \pm 60.34$  and  $308.50 \pm 49.10$  g), respectively. Among the three tree species, *Cb* showed the greatest loss of biomass at 38.4% in comparison to *Ac* at 30.0% and *Tc* at 30.8%. Overall, tree species and treatment strongly influenced ( $p < 0.001$ ) the whole branch biomass of a tree.

For stem biomass, *Ac* showed the highest values in the control group ( $6162.33 \pm 1204.59$  g), and the lowest values were noted for *Tc* in both the control and treatments (*Tc*-c:  $4089.25 \pm 220.16$  g, *Tc*-t:  $3407.00 \pm 322.40$  g). Furthermore, *Tc* lost 16.7% of the biomass in the treatment group which was much higher than *Ac* of 8.3% and *Cb* of 4.2%. The drought treatment effect was a bit smaller ( $P = 0.0146$ ) in comparison to the dry branch and root weight.

For roots, *Ac*, both in the control and drought treatments, had more biomass than any other group (*Ac*-c:  $5280.00 \pm 467.73$  g, *Ac*-t:  $3830.00 \pm 186.62$  g) despite the strong treatment effect. *Cb*-t and *Tc*-t showed the lowest values ( $1338.67 \pm 89.39$  and  $1291.75 \pm 25.66$  g), as was also noted for the branch biomass. Among the three tree species, *Cb* had the greatest biomass reduction at 45.2% while *Ac* and *Tc* were at 27.5% and 30.9%, respectively.

**Table 4:** Dry weight (g) of branches, stem, and root biomass of the three study species grown under the control and drought treatment conditions. (Zhang et al., 2019)

Dry Weight (g)	Group	n	Mean $\pm$ SD (g)	Drought Effect Species Effect	
Branch	Ac	control	3	1121.33 $\pm$ 177.46	P < 0.001 ***
		treatment	3	785.00 $\pm$ 89.50	
	Cb	control	4	593.25 $\pm$ 8.54	
		treatment	3	365.33 $\pm$ 60.34	
	Tc	control	4	445.75 $\pm$ 31.02	
		treatment	4	308.50 $\pm$ 49.10	
Stem	Ac	control	3	6162.33 $\pm$ 1204.59	P = 0.0544 P < 0.001 ***
		treatment	2	5652.50 $\pm$ 127.99	
	Cb	control	4	5226.75 $\pm$ 315.97	
		treatment	3	5007.67 $\pm$ 389.79	
	Tc	control	4	4089.25 $\pm$ 220.16	
		treatment	4	3407.00 $\pm$ 322.40	
Coarse Root	Ac	control	3	5280.00 $\pm$ 467.73	P < 0.001 *** P < 0.001 ***
		treatment	3	3830.00 $\pm$ 186.62	
	Cb	control	4	2440.25 $\pm$ 219.50	
		treatment	3	1338.67 $\pm$ 89.39	
	Tc	control	4	1869.00 $\pm$ 63.59	
		treatment	4	1291.75 $\pm$ 25.66	

### 3.5 Morphology of Crown and Coarse Root

*Tc* was the smallest tree species concerning height (control: 5.13  $\pm$  0.22 m, dry: 5.23  $\pm$  0.18 m) (Table 5), but the narrow and pyramidal shaped *Cb* reached the lowest maximum canopy radius

(control:  $28.50 \pm 1.50$  cm, treatment:  $21.33 \pm 0.94$  cm) and canopy volume (control:  $0.36 \pm 0.09$  m<sup>3</sup>, treatment:  $0.11 \pm 0.55$  m<sup>3</sup>).

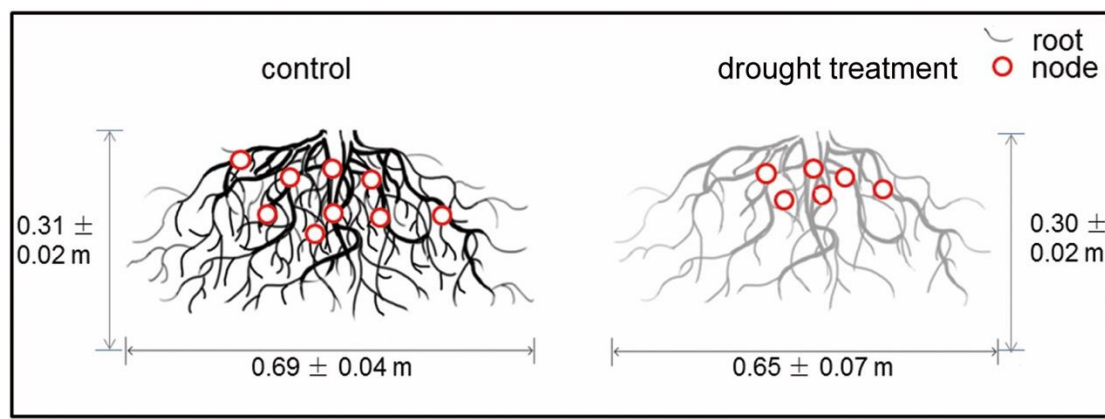
**Table 5:** Above- and below-ground dimensions of the three investigated species and the respective treatments (control and drought treatment) as well as the density of foliage and root system expressed by the means of leaf area index (m<sup>2</sup> m<sup>-2</sup>; LAI) and the percent of porosity of the root system, respectively. (Stratopoulos & Zhang, 2019)

Above-Ground								
Code	Height [m $\pm$ SD]		Max. Canopy Radius [cm $\pm$ SD]		Canopy Volume [m <sup>3</sup> $\pm$ SD]		LAI [m <sup>2</sup> m <sup>-2</sup> $\pm$ SD]	
	control	treatment	control	treatment	control	treatment	control	treatment
<i>Ac</i>	$5.90 \pm 0.14$	$6.00 \pm 0.00$	$48.33 \pm 8.06$	$50.00 \pm 9.63$	$0.64 \pm 0.25$	$0.67 \pm 0.23$	$1.52 \pm 0.11$	$1.81 \pm 0.41$
<i>Cb</i>	$5.90 \pm 0.25$	$5.53 \pm 0.17$	$28.50 \pm 1.50$	$21.33 \pm 0.94$	$0.36 \pm 0.09$	$0.11 \pm 0.55$	$3.01 \pm 0.87$	$4.11 \pm 1.49$
<i>Tc</i>	$5.13 \pm 0.22$	$5.23 \pm 0.18$	$43.00 \pm 17.45$	$52.50 \pm 11.37$	$0.65 \pm 0.20$	$0.68 \pm 0.22$	$2.53 \pm 0.25$	$2.13 \pm 0.24$
Below-Ground								
Code	Rooting Depth [cm $\pm$ SD]		Max. Rooting Width [cm $\pm$ SD]		Root Volume [m <sup>3</sup> $\pm$ SD]		Porosity Root System [% $\pm$ SD]	
	control	treatment	control	treatment	control	treatment	control	treatment
<i>Ac</i>	$30.33 \pm 7.85$	$29.67 \pm 0.47$	$31.00 \pm 2.75$	$29.92 \pm 2.00$	$0.050 \pm 0.019$	$0.056 \pm 0.008$	$0.30 \pm 0.03$	$0.30 \pm 0.05$
<i>Cb</i>	$30.00 \pm 0.00$	$30.00 \pm 0.00$	$32.25 \pm 0.98$	$29.00 \pm 2.01$	$0.057 \pm 0.005$	$0.036 \pm 0.007$	$0.33 \pm 0.05$	$0.34 \pm 0.03$
<i>Tc</i>	$28.50 \pm 1.50$	$29.00 \pm 1.41$	$30.75 \pm 1.75$	$28.67 \pm 2.77$	$0.058 \pm 0.000$	$0.047 \pm 0.009$	$0.24 \pm 0.01$	$0.38 \pm 0.04$

*Ac* and *Cb* showed the deepest rooting system in the control and drought treatment groups and exhibited a medium rooting width. The root system of *Cb*, however, was less dense in comparison to that of *Ac*, especially in the control group, where it showed the highest porosity of all species ( $0.33 \pm 0.05\%$ ). *Tc* exhibited medium rooting depth in both the treatments (control:  $28.50 \pm 1.50$  cm, treatment:  $29.00 \pm 1.41$  cm), but the lowest rooting width of all tree species (control:  $30.75 \pm 1.75$  cm, treatment:  $28.67 \pm 2.77$  cm). For *Tc*, the drought treatment showed the biggest effect on the porosity of the root system (control:  $0.24 \pm 0.01$ , treatment:  $0.38 \pm 0.04$ ) and the rooting density. No effect was noted for *Ac* (control:  $0.30 \pm 0.03$ , treatment:  $0.30 \pm 0.05$ ), while only a minor effect was noted for *Cb* (control:  $0.33 \pm 0.05$ , treatment:  $0.34 \pm 0.03$ ).



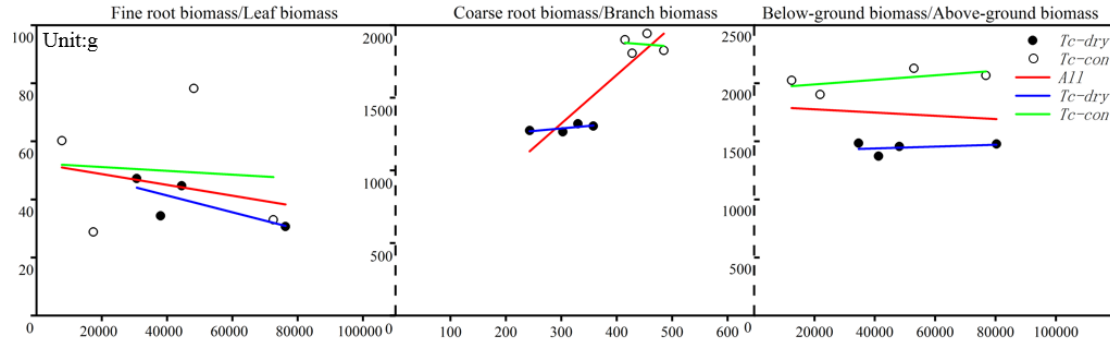
For  $T_c$ , although no significant difference was found between the control and drought treatment groups ( $P > 0.05$ ), the root system in the drought treatment group decreased compared with the control group in terms of width, root number and node number. The numbers of roots and nodes in the drought treatment group were  $1015 \pm 155$  and  $804 \pm 116$ , which were less than the control ones with the number of  $1087 \pm 182$  and  $841 \pm 130$ . Apart from maintaining the rooting depth, the root system in the drought treatment group showed a slight decrease from  $0.69 \pm 0.04$  to  $0.65 \pm 0.07$  m in rooting width (Fig. 15).



**Figure 15:** Two-dimensional illustration of the root systems in the control and drought treatment groups. Red circles represent the rooting nodes. (Zhang et al., 2019)

### 3.6 Three Types of Root:shoot Ratio of $T_c$

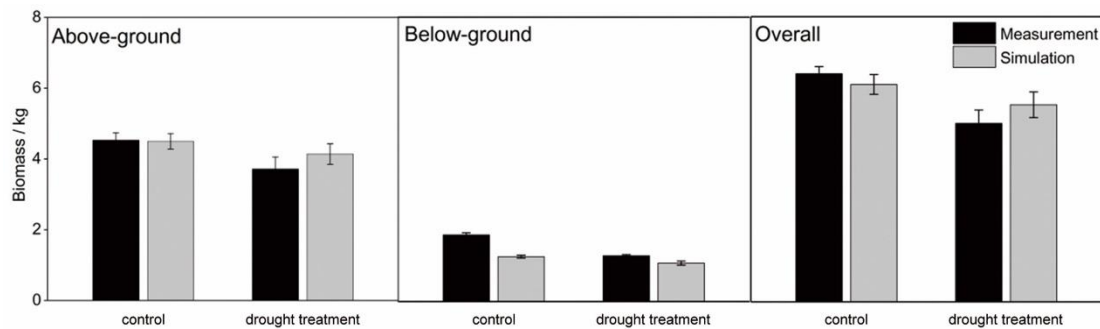
Three types of root:shoot ratio and their fitting regressions were calculated: (1) fine root:leaf biomass ratio, (2) coarse root:branch biomass ratio and (3) below-ground:above-ground biomass ratio. All the root:shoot ratios were significantly different ( $P < 0.05$ ), with roots in the drought treatment group constituting less of a proportion of the whole tree than in the control group (Fig. 16). Among the ratios, the coarse root:branch ratio showed the greatest difference ( $P < 0.01$ ).



**Figure 16:** Three types of root:shoot ratio and their fitting regressions: fine root:leaf biomass ratio, coarse root:branch biomass ratio and below-ground:above-ground biomass ratio. White circles represent the control group, and black circles represent the drought treatment group. Red, green and blue lines are the fitting regressions for all trees, the control group and the drought treatment group, respectively. (Zhang et al., 2019)

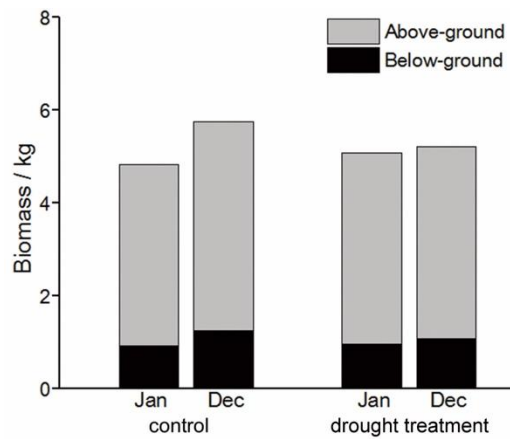
### 3.7 Model's Simulation of Biomass and Ecosystem Services

Using the progress-based urban tree model *CityTree* (Rötzer et al, 2019), the above-ground, below-ground and overall biomasses of *Tc* for both the control and drought treatment groups could be simulated. The measured and simulated biomasses of the trees, including the above-ground, below-ground and overall biomasses, had no significant differences, which showed the model's reliability in prediction ( $P > 0.05$ ). A higher but not significantly different simulation was found in the drought treatment group for the above-ground and overall biomasses (Fig. 17).



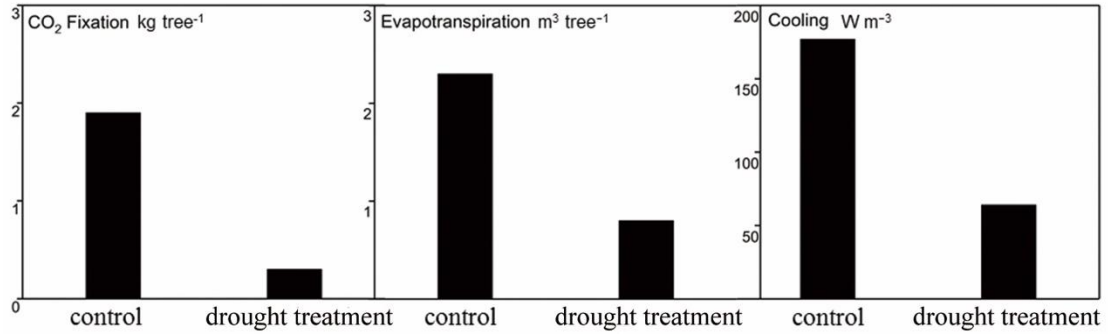
**Figure 17:** Comparison of measurements and simulations for the above-ground, below-ground and overall biomasses of *Tc* from the control and drought treatment groups. (Zhang et al., 2019)

The *CityTree* model also simulated biomass development for the coming next year (Fig. 18). In the control group, for the above- and below-ground biomass, distinct increases of 0.6 kg tree<sup>-1</sup> (from 3.9 to 4.5 kg tree<sup>-1</sup>) and 0.4 kg tree<sup>-1</sup> (from 0.9 to 1.3 kg tree<sup>-1</sup>) were obtained, respectively. In the treatment group, however, after suffering from the drought event in our experiment, trees showed scarce development, in that only a slight increase of 0.1 kg tree<sup>-1</sup> was observed (from 5.4 to 5.5 kg tree<sup>-1</sup>). For the root:shoot ratio, increases were found from 0.23 to 0.29 and from 0.02 to 0.27 for the control and treatment groups, respectively, from January to December.



**Figure 18:** Simulated above- and below-ground biomasses for trees of the control and treatment groups for the coming next year. (Zhang et al., 2019)

The *CityTree* model also helped to provide the simulation of the ecosystem services of the trees, such as CO<sub>2</sub> fixation, water consumption and cooling potential by transpiration for both *Tc-c* and *Tc-t*. Figure 19 gives the values of these variables.



**Figure 19:** Ecosystem services, including CO<sub>2</sub> fixation, evapotranspiration and cooling potential by transpiration, for trees of the control and treatment groups in the year 2017. (Zhang et al., 2019)

The trees in the control group showed significantly higher CO<sub>2</sub> fixation than those of the drought treatment group. Each tree in the control group could fix 1.9 kg CO<sub>2</sub> yr<sup>-1</sup>, which was way more than 0.3 kg CO<sub>2</sub> yr<sup>-1</sup> calculated for trees in the drought treatment group. With a three times higher evapotranspiration (2.3 m<sup>3</sup> tree<sup>-1</sup> compared with 0.8 m<sup>3</sup> tree<sup>-1</sup>), the cooling potential provided by the control group trees was almost trebled compared with that of the drought treatment group trees (177 W m<sup>-3</sup> compared with 64 W m<sup>-3</sup>). In summary, under the intense drought conditions associated with rainfall exclusion, all the ecosystem services of the lime trees were markedly reduced.

## 4. Discussion

### 4.1 Annual Fine Root Development

For the overall fine root biomass, *Tc*-c showed no significant differences with *Tc*-t during the whole study period ( $P > 0.05$ ). *Tc* trees did not behave positively against drought as the fine-root biomass only slightly differed for both *Tc*-c and *Tc*-t. In contrast, *Tt* showed a vigorous fine root growth in both the control and drought treatment groups starting from May, from which we suggested that *Tt* did invest or even give the priority to the belowground process. Additionally, our results showed that the fine-root biomass growth of *Tt*-t decreased compared to *Tt*-c, however, there was still a distinct growth considering the whole growing season. Combined with the result of Stratópoulos et al (2019a), *Tt* originating from dry regions was proved to be a drought-resistant species. This species could cope with water shortage by enhancing the below-ground biomass, in contrast to *Tc*, which exhibited no extra investment in the below-ground process.

For the fine root growth in the vertical soil layers, *Tc* exposed to normal conditions slowly increased their growth in the deep layer and decreased it in the upper layer, which was consistent with some previous research results (Goisser et al., 2016; Leuschner et al., 2004). However, *Tc*-t sharply decreased the fine root biomass in the upper and middle soil layers, while simultaneously, the deep fine roots of *Tc*-t developed stronger than *Tc*-c probably to obtain water from deeper soil parts. From September to November, the fine root development of *Tc* in all the soil layers was completely different compared to estival patterns. The deep roots decreased whereas the roots in the upper and middle soil layer initiated growth. Accounting for a steady total fine root biomass, the preferential development of fine roots in different soil layers might reflect specific allocations to soil layers with reserve water. It was also verified that *Tc* recovered after decreasing growth resulting from a drought event, which could be explained by the resumptive growth of the fine roots in the upper and middle soil layers (Gillner et al., 2014). Less energy was invested by *Tc* in the belowground processes in all the soil layers to obtain

enough growth, especially during a drought event, which might result in a growth balance between the shallow and deep fine roots.

Recent research indicated that deep roots could be of pivotal importance to alleviate water stress in many plants (Gewin, 2010). They particularly played a central role for the drought tolerance in tropical and subtropical environments (Pierret et al., 2016). Schenk (2008) proposed that the rooting profiles of plant communities tended to be as shallow as possible and as deep as needed to meet the evapotranspiration demand. The idea behind this was that plants use less energy for constructing shallow roots than for deep roots. During the beginning of drought events, however, deep roots could uptake more water which was an advantage compared with a shallow rooting system because lower maintenance costs tended to counter-balance the high construction costs of deep roots (Germon et al., 2016; Joslin et al., 2006). For *Tt* in our experiment, fine roots vigorously developed in all the layers in the control group especially for the upper and middle layers. Under water shortage, however, the deep fine roots of *Tt-t* exhibited strong growth, which was even higher than that for *Tt-c*. This was considered as an essential step to adapt to drought for such a drought-resistant tree species like *Tt*, which was not found for *Tc*.

For the horizontal distribution of fine roots, different tree species had different patterns (Leuschner et al., 2004). We found similar results in that under normal conditions fine-root development of *Tc* was more stem-centered whereas *Tt* showed no priority for inner root growth. Under drought stress, this tendency was reversed. The outermost fine roots of *Tc-t* did not decrease any further, whereas its inner fine roots died because of the water shortage. This could be a special strategy of *Tc* trees to expand their root system to acquire more water by decreasing the support of the fine roots at a distance of 15 cm. On the contrary, both the fine roots of *Tt-c* and *Tt-t* had an increase in the innermost area during the first four months and a decrease in outermost fine-root biomass during the last three months, which implied that drought did not affect horizontal expansion greatly and no priority was given to the fine roots in horizontal level. Effects of drought manipulations on root development were very complex (Poorter et al., 2012). Unlike *Tt*, a drought-resistant tree species which developed new fine roots in the vertical direction especially in the deep layer under drought stress, *Tc* showed a minor reaction to

drought in terms of total fine root biomass. Moreover, when the availability of water was high, *Tc* did not show vigorous growth, whereas *Tt* developed abundant deep roots which probably aimed to prevent the risk of drought damage. But we still observed some positive phenomenon that *Tc* invested more on the horizontal fine root growth, which could be its special underground strategy.

#### 4.2 Biomass Allocation

Generally, biomass as well as the root:shoot ratio was significantly affected by drought events. We observed various degrees of above- and below-ground biomass gains and losses under drought, suggesting different strategies and abilities of tree species to cope with this stressor. Under normal conditions, *Tc* exhibited steady growth, especially in spring, which was consistent with previous research (Moser et al., 2016). Since the RER was established, *Tc* showed reduced stem growth in the drought treatment group ( $P < 0.05$ ). Furthermore, a large number of leaves died in the summer of 2017, which was in line with the decreased LAI. From the harvesting campaign, all *Tc* compartments (branch, stem and coarse root) showed significant reductions in their biomass in the drought treatment group compared with the levels in the control group, which was completely from *Ac* originated from a more drought-prone habitat (Kiermeier and Baumschulen, 1995). Unlike *Tc*, *Ac* showed the least losses over the entire plant body under drought. Besides, the high root:shoot ratios of *Ac* and its high rooting depth and root density were assumed to be helpful mechanisms in survival. This could explain why this species did not show any signs of premature leaf senescence and fall in the autumn season and also why it had relatively high resistance values concerning biomass, stem growth, as well as water use (Stratopoulos et al., 2019a). Therefore, all data supported that *Ac* was much more extremely drought resistant than *Tc* (Kunz et al., 2016).

The root:shoot ratio was often used to estimate relative biomass allocation between roots and shoots (Gowda et al., 2011; Poorter et al., 2012; Wilson, 1988). Previous studies have reported that, for some drought-resistant tree species, drought induced root growth to enhance water uptake, which led to an increase in the root:shoot ratio (Asch et al., 2005; Lemoine et al., 2013). Mokany et al. (2006) proposed that an increase in the root:shoot ratio could be a strategy when

facing drought in the long term, whereas water shortage could shift carbon allocation to storage in the short term (McDowell et al., 2008). For *Tc* in our experiment, drought significantly reduced all the three types of root:shoot ratio ( $P < 0.05$ ), i.e., fine root:leaf biomass ratio, coarse root:branch biomass ratio and below-ground:above-ground biomass ratio, which showed an opposite pattern to those drought-resistant tree species. Besides, the early discolouring and fall of leaves together with the biomass decrease in all the three compartments (i.e., coarse root, stem and branch) suggested that carbon assimilation was so strongly reduced by drought that there was insufficient assimilation to facilitate its allocation to the entire plant system. Hence, a high susceptibility to drought was assumed for this tree species.

#### *4.3 Simulated Ecosystem Services of *Tc*: Carbon Storage and Cooling*

Urban trees have been widely researched for their various ecosystem services, from which human beings and society benefited greatly (Roy et al., 2012). Ecological process-based models have been applied widely for ecological issues, including biodiversity, phenology, hydrology and ecosystem services (He et al., 2014). To obtain reliable predictions, however, the process-based models needed to be validated first, in combination with observational data (Parmesan et al., 2013). In our study, the *CityTree* model was first used to simulate the biomass from the below-ground and above-ground biomass of *Tc* trees in the control and drought treatment groups, aiming to see the difference between the simulation and our observation. The results proved the high performance of the model, in that little difference was found for the above-ground, below ground, and overall biomass of all the *Tc* trees between the simulation and the observation ( $P < 0.01$ , Fig. 17). Therefore, the model could be trusted for its reliability for simulating biomass and ecosystem services of all the trees from both the control and treatment groups.

Under normal conditions, *Tc-c* could have a development for both the above- and below-ground biomass in the next coming year based on the predictions from the *CityTree* model (Fig. 18). Nevertheless, the model gave the simulation that the biomass growth of *Tc-t* would be substantially reduced from January to December within the same period, suggesting that the drought stress had implemented an intense impact on tree growth, which might last very long



and lead to a stagnant biomass development of the drought-stressed trees. In addition, the above-ground biomass of *Tc-t* stopped growth completely, which accorded with the sharp decrease of the stem diameter from our measurement. Overall, the *CityTree* model exhibited a pessimistic attitude towards the biomass development of *Tc-t* compared to *Tc-c* in the next year, which most likely resulted from the drought event.

For ecosystem services in detail, Figure 19 exhibited the simulation of CO<sub>2</sub> fixation, transpiration and cooling for both the control and treatment groups of *Tc* within one year. *Tc-c* was distinguished from *Tc-t* which showed a strongly weakened pattern of providing ecosystem services under drought stress. As the predicted distinctly different biomass development between the control and the drought treatment groups, a weakened growth pattern between the two groups was ensured and it was not unexpected that the drought stressed trees exhibited a degraded capability of supplying ecosystem services as common trees. The *CityTree* model predicted a reduction in CO<sub>2</sub> fixation of 84% and in evapotranspiration of 65% in the drought-stressed lime trees compared with the unstressed trees. Generally, for an urban landscape, the size, shape and growth of trees might influence their CO<sub>2</sub> uptake and transpiration (Zipperer et al., 1997). From our observation and measurement, the drought simulation had caused the reduction of fine root biomass, stem diameters and leaf areas in *Tc-t* in comparison to *Tc-c*. In addition, it was also verified by our harvested data that *Tc-t* had substantial losses of branch, stem and root biomass, which meant the overall degradation of all the compartments to keep normal development. Furthermore, the phenomenon that most of the leaves discolored and fell in the early edge of *Tc-t* could provide another perspective of a reduction of respiratory action and photosynthesis.

The cooling provided by urban trees was one of their most important environmental benefits that had been extensively researched (Zhang et al., 2014). Generally, the cooling function of urban trees was through shading and evapotranspiration, which was closely related to their growth and environmental conditions (Rahman et al., 2015). From the model's simulation in our research, along with the declined transpiration, the cooling effect was reduced by 64% for the drought-stressed trees. On one hand, the decrease of LAI of *Tc-t* indicated that the trees were hard to maintain the size, shape and common development of crown under drought stress,

resulting in its weakened shading ability. On the other hand, due to the intensively suppressed evapotranspiration, it could be inferred that the less sap flow within the trees could take away heat. Besides, the raising root:shoot ratios suggested that the below-ground process might not obtain enough investment, which could probably reduce the root transpiration. Overall, comprehensive measurement data proved that *Tc-t* had reduced cooling effect rather than *Tc-c*, which was consistent with the model's simulation.

Last but not least, it could not be ignored that the model predicted higher (albeit not significantly) biomass for the drought-stressed trees than for the control trees (Fig. 17), which suggested that drought stress had a more severe impact in reality than predicted by the model. Taking this conservative prediction into account, the negative impact of drought stress on the ecosystem services provided by *Tc-t* might be more serious. In conclusion, the model's simulation of ecosystem services of *Tc* could be explained by that the drought effect strongly reduced the normal growth of *Tc* trees, leading to their weakened functions compared to normal conditions that they had to expend more energy in seeking water to survive rather than proving ecosystem services.

## 5. Conclusion and Prospects

Studies about how urban trees respond to and cope with drought events may greatly promote our understanding of their growth and ecosystem services under climate change and urbanization in the future. Therefore, we selected a widely planted urban tree species, *Tilia cordata* ‘Greenspire’ Mill, and established a drought controlled experiment to measure its growth patterns under different conditions via stem growth measurement, fine root campaign, leaf area measurement, harvest campaign and root system analysis, which aimed to analyze its adaptability to drought stress. In addition, *CityTree* model was also applied to estimate how the ecosystem services including CO<sub>2</sub> fixation, evapotranspiration and cooling of *Tilia cordata* was affected by water shortage. Furthermore, the fine root campaign was also launched for *Tilia tomentosa* Moench. ‘Brabant’ and the harvest campaign was conducted for *Acer campestre* L. subsp. *campestre* and *Carpinus betulus* L. ‘Fastigiata’ in the same controlled experiment from the same study site. Thus, direct comparisons between *Tilia cordata* and the other selected tree species regarding how well they coped with drought stress could be carried on.

Based on the harvest data, it is obvious that drought implements a severe impact of *Tilia cordata*, i.e., a huge biomass reduction occurs to branches, coarse root and stem which suggests a failure of adapting to water shortage especially compared to other tree species. Besides, the total and vertical fine root development also proves that *Tilia cordata* does not behave like a drought-resistant species such as *Tilia tomentosa* which invests more on deep roots to enhance water uptake. Furthermore, the calculated three types of root:shoot ratios shows no priority is given to the below-ground process which has been proved probably the most effective method for plants to avoid being susceptible to drought stress. Simultaneously based on the reduced stem growth and LAI, the above-ground processes of *Tilia cordata* were seriously affected as well. Its only active behavior against drought effect is a horizontal growth that it develops more fine roots in the outmost area, which is probably the special underground strategy. In conclusion, *Tilia cordata* shows a passive behavior under drought stress and fails to play a role as a drought-resistant tree species as both the above- and below-ground processes are strongly affected.

For ecosystem services, our simulations suggest a strongly weakened forecast for *Tilia cordata* that it will not supply adequate CO<sub>2</sub> fixation, evapotranspiration and cooling effects in the future, which is in line with our measurements. Therefore, we will recommend that *Tilia cordata* should not be considered to be planted in cities with high average temperatures and low annual precipitation amounts as it may face more extreme drought events under future climate change, even in a complex urban environment with bad soil conditions and less rooting space, etc.

Trees should be planted and maintained according to their different strategies and site requirements. For this purpose, knowledge about the general growth habit of urban tree species is needed, including their annual growing patterns and their within-plant carbon partitioning and allocation patterns under changing growing conditions, which affect the timing of their physiological processes. In addition, the respective resource supply in the native habitat appears to be a good proxy for drought tolerance, suggesting to progressively plant (non-native) species from drier habitats at harsh urban sites.

In this study, the drought experiment is conducted within one year. However, tree response to drought stress is very complex and may vary enormously in the long-term due to specific characteristics. For example, some tree species respond to water shortage very promptly and take actions for more water immediately, seeming like acting a drought-adaptive tree species. However, this response may not last long after a few days and the trees may die soon. Instead, some tree species may slowly close their stomata to reduce transpiration at the beginning of drought events, which actions seem not so apparent against drought stress. Nevertheless, these tree species may be still alive after a long time, during which they struggle to develop more deep roots to seek water. This information above should be considered carefully and long-term research should be established in the future to observe and measure the comprehensive adaption of urban trees to drought stress regarding their growth for both above- and below- ground process and their various ecosystem services. The growth patterns of urban trees show whether they adapt to the urban environment under climate change and their ecosystem services tell how well citizens can benefit from them, which is expected to provide important insights for urban green space planning and management, particularly for the selection of suitable tree species and cultivars.

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## **Appendix:**

### **Published Articles**

#### **Article I**

**Zhang C\*, Stratopoulos LMF, Pretzsch H, & Rötzer T. (2019). How Do Tilia Cordata Greenspire Trees Cope with Drought Stress Regarding Their Biomass Allocation and Ecosystem Services? *Forests*, 10(8), 676.**

## Article

# How Do *Tilia cordata* Greenspire Trees Cope with Drought Stress Regarding Their Biomass Allocation and Ecosystem Services?

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**Abstract:** In the context of climate change, drought is likely to become more frequent and more severe in urban areas. Urban trees are considered to play an important role in fixing carbon, improving air quality, reducing noise and providing other ecosystem services. However, data on the response of urban trees to climate change, particularly to drought, as well as the relationship between their below- and above-ground processes in this context, are still limited, which prevents a comprehensive understanding of the role of urban trees in ameliorating some of the adverse effects of climate change and their ability to cope with it. To investigate whole-plant responses to water shortages, we studied the growth of *Tilia cordata* Greenspire, a commonly planted urban tree, including development of its roots and stem diameter, leaf parameters and the harvested biomass. Our results showed that this cultivar was susceptible to drought and had reduced biomass in all three compartments: branch (30.7%), stem (16.7%) and coarse roots (45.2%). The decrease in the root:shoot ratio under drought suggested that more carbon was invested in the above-ground biomass. The development of fine roots and the loss of coarse root biomass showed that *T. cordata* Greenspire prioritised the growth of fine roots within the root system. The *CityTree* model's simulation showed that the ability of this cultivar to provide ecosystem services, including cooling and CO<sub>2</sub> fixation, was severely reduced. For use in harsh and dry urban environments, we recommend that urban managers take into account the capacity of trees to adapt to drought stress and provide sufficient rooting space, especially vertically, to help trees cope with drought.

**Keywords:** biomass allocation; drought; ecosystem services; root:shoot ratio; urban trees

## 1. Introduction

In the future, drought is projected to occur more frequently under the warmer conditions associated with the progression of climate change [1]. Additionally, in association with increasing urbanisation, extreme heat events have become more prevalent than in previous decades, which reduce environmental quality [2–4]. Urban trees can mitigate environmental degradation by storing carbon, purifying the air, reducing storm water and providing other ecosystem services [5–7]. Urban trees can also be a key component in the adaptation of cities to climate change [8]. Hence, in recent decades, the use of urban trees for various purposes has drawn increasing interest from researchers [9]. For example, Nowak et al. [10] studied how urban trees can filter pollutants that have adverse effects on human health. Konarska et al. [11] quantified the magnitude of daytime and night-time transpiration of common urban tree species. In addition, Velasco et al. [12] reported that carbon sequestration depends

on the characteristics of urban trees, and Pretzsch et al. [13] proposed that urban climates can either accelerate or decelerate tree growth.

Water availability is considered to be the most important determinant of tree growth [14,15], and a higher frequency of drought events will expose urban trees to more restrictive growth conditions. To improve the quality of ecosystem services, how different urban tree species cope with drought stress has been commonly discussed [16]. Rötzer et al. [17] pointed out that stress caused by a water deficit could reduce photosynthetic productivity and tree growth. Moser et al. [18] found marked growth reductions during drought periods and subsequent rapid recovery in *Robinia pseudoacacia* L. Furthermore, Stratópoulos et al. [19] proposed that trees from dry regions, such as *Ostrya carpinifolia* Scop. and *Tilia tomentosa* Moench ‘Brabant’, show a high tolerance to drought stress; however, this tolerance can come at the expense of above-ground biomass production.

The adaptation of urban trees to drought has been a recent topic of research, but there is still a knowledge gap regarding carbon allocation as well as ecosystem services [20]. Carbon allocation between below- and above-ground biomasses is one of the key parameters towards understanding tree survival, especially under the global change accompanied by urbanisation [21]. Data on the relationship between below- and above-ground processes is limited, which may result in scarce information of the ecophysiology and hydric behaviour of urban trees under climate change [22–24]. The development of below-ground biomass has also been rarely studied because of multiple factors such as soil compaction, limited root volume and harsh paved environments [25–27]. Furthermore, urban trees can make an enormous contribution to mitigating the urban heat islands by providing a cooling effect [28,29], which is considered a feasible option for adapting to climate change [30]. Hence, information regarding how urban trees allocate their biomass and provide ecosystem services such as cooling and carbon fixation is crucial for city planners to implement appropriate management practices [31].

In some previous studies, only allometric equations were used to estimate such allocation because of the expensive and time-consuming harvesting process [32]. This has led to a lack of precise information on carbon allocation, and hence researchers have been prevented from establishing appropriate strategies for managing urban trees. Against this background, in this study, we selected *Tilia cordata* Mill. ‘Greenspire’, a cultivar widely planted in Central European cities, to analyse tree growth and carbon allocation under undisturbed growing conditions (control) and under extreme drought. Combined with a harvesting campaign, we applied the urban tree model *CityTree* to simulate biomass development as well as ecosystem services. The following research questions are addressed in this work: (1) How does *T. cordata* Greenspire respond to extreme drought in terms of growth and carbon allocation? (2) What is the cultivar’s strategy within the root system? (3) Are its ecosystem services severely affected under drought?

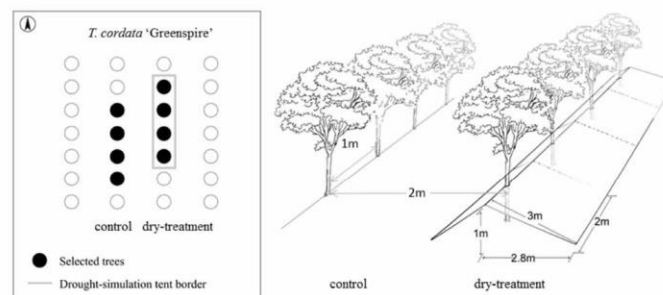
## 2. Materials and Methods

### 2.1. Study Site and Drought Experiment in 2017

This study was performed at the municipal nursery of Munich (48°08′05″ N, 11°28′47″ E, 534 m a.s.l.), the major city in the southeast of Germany under the marine west coast climate. With the predominant soil types ranging from moderate sandy loam to strong loamy sand, the 42 ha nursery shows very little variation in terms of microclimatic conditions. The measurements in this study were performed from April 12 to November 12, 2017. Eight young individuals of *T. cordata* Greenspire with diameters of 5–6 cm at a height of 1 m were selected, four of which were set as a ‘control’ group and the other four as a ‘dry-treatment’ group. Each tree was more than 2 m away from the others to avoid the mutual effect.

For the drought-stress experiment, we used experimental settings in which conditions of an enduring drought event were simulated to investigate the tree growth responses. The rooting spaces of the four trees in the dry-treatment group were covered by a rainfall exclusion roof (RER) from May to November in 2017, whereas the four trees in the control group were exposed to normal weather

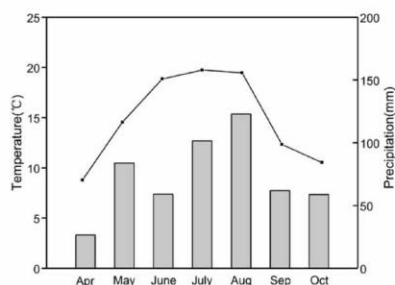
conditions. The RER was made up of several waterproof tents (2 × 3 m) combined with nylon ropes and installed at a height of 1 m, sloping to the ground in the north and south directions at 3 m from the trees (Figure 1).



**Figure 1.** Schematic structure of the drought experiment. The rooting zones of trees on the right were covered by rainfall exclusion constructions ('dry-treatment'), whereas trees on the left were exposed to normal weather conditions ('control').

## 2.2. Measurement of Climate Variables and Soil Moisture

Climate variables, including temperature and precipitation, were sampled every 10 min with a weather station (Davis Vantage Pro2; Davis Instruments, Hayward, CA, USA) located at an unshaded site approximately 200 m away from the experimental plots. 2017 was a warm year at the site, with an mean temperature of 10.3 °C and a total precipitation of 887 mm. The spring of 2017 was warm, sunny and dry. After a strong return to cold weather in April, the temperature started to increase at the beginning of May and remained exceptionally high, in that the average temperature was 19.6 °C from July to August. In this period, monthly precipitation levels of 101 and 123 mm were similar to the long-term records (within 1981–2010) of 122 and 115 mm. The temperature showed a declining trend in early September, and night frost started in the middle of November (DWD, 2018) (see Figure 2).



**Figure 2.** Monthly totals of precipitation (mm; bar graph) and monthly average temperature (°C; dotted line) for the study site from April to October 2017.

We measured the volumetric soil water content (VWC; Vol.%) with a portable tool (UMP-1; UGT, Müncheberg, Germany) at the rooting zones of all the trees four times in the summer of 2017, with the goal of comparing the moisture differences between the control and the dry-treatment groups (see Table 1). The values of VWC between the control and the dry-treatment groups were significantly different for all the four times ( $p < 0.01$ ), which proved the success of the drought simulation.



**Table 1.** Volumetric soil water content (Vol.%; VWC) at 10 cm soil depth for the control and dry treatment groups of *T. cordata* Greenspire four times in 2017.

Group	n	Date			
		July 12	July 31	Aug 16	Sep 7
control	4	16.5 ± 1.3	19.6 ± 0.7	25.3 ± 4.6	21.3 ± 1.7
dry-treatment	4	12.0 ± 1.2	12.1 ± 2.0	12.8 ± 1.0	13.4 ± 1.6

### 2.3. Measurement of Above- and Below-Ground Biomasses

The stem diameter at a height of 1 m was measured using a digital caliper at the beginning of each month from April to November 2017. Measurements in two perpendicular directions (N–S and E–W) were performed and averaged.

Fine root coring campaigns were launched for all trees in May, September and November: that is, at the beginning (pre-drought), in the middle and at the end of the growing season. A pre-test coring campaign showed that the range of the root system was similar to a cylinder, with a diameter of 70 cm and a height of 35 cm. Therefore, during every coring campaign, four soil cores were collected for every individual tree: two at a distance of 15 cm from the trunk and two at a distance of 30 cm. The soil was sampled down to a depth of 30 cm using a soil auger with a length of 30 cm and a radius of 3 cm. Each sample was divided into three horizons: soil depths of 0–10 cm (upper layer), 10–20 cm (middle layer) and 20–30 cm (deep layer). Fine roots (< 2 cm) were filtered using sieves (2-mm mesh size) and separated by forceps in the laboratory. Then, the samples were washed and dried in an oven at 65 °C for 72 h. Finally, all the samples were weighed using a balance with an accuracy of four decimal places to obtain the dry weight. The fine root biomass at different depths was calculated using the dry weight divided by the cross-sectional area of the auger.

In November, a harvest campaign was launched in which all the trees were excavated with a tree digger and divided into three parts: branch, stem and coarse roots. All these compartments were dried at 65 °C for 72 h and weighed using a balance with an accuracy of up to four decimal places to obtain the dry weight. Before drying, the root systems were washed to remove attached soil and stones. An image analysis process including taking high-resolution photos from five views (N, S, W, E and top) was applied to measure the root architecture with the help of the software ‘Root System Analyzer’ (RSA, University of Vienna, Austria). On the basis of a graphical representation of the skeletonised image of the root system as well as segmentation algorithms, RSA was used to describe some of the root traits, including root nodes, width and depth.

The leaf area index ( $\text{m}^2 \text{m}^{-2}$ ; LAI) was determined using hemispherical photographs (Nikon Coolpix P5100 camera with a fisheye lens and Mid-OMount) and analysed with the program WinSCANOPY (Régent Instruments Inc., Quebec, Canada). Data acquisition was performed under conditions of a uniformly overcast sky in the middle of June, shortly after implementation of the drought experiment. Sufficient numbers of points (7–11 x, z pairs) were measured and input into the software FV2200 (LICOR Biosciences, Lincoln, NE) to compute the projected crown area (PCA) of each tree. Combined with the specific leaf area (SLA), the leaf biomass was calculated as follows:

$$\text{Biomass}_{\text{leaf}} = \frac{\text{LAI} \times \text{PCA}}{\text{SLA}} \quad (1)$$

Therefore, the below-ground biomass was the sum of the fine and coarse root biomasses, and the above-ground biomass was the sum of the branch, stem and leaf biomasses.

### 2.4. Simulation of Biomass and Ecosystem Services

A process-based model was used to simulate the wood biomass of *T. cordata* Greenspire and calculate its ecosystem services [33,34]. On the basis of the basic measurements of trees (e.g., DBH (diameter at breast height) and tree height), climate and soil data, this model consists of seven modules

to calculate tree growth and ecosystem services such as CO<sub>2</sub> fixation, evapotranspirational cooling and shading. The core function of the simulation of a tree's net assimilation is as follows:

$$A = d \times \left[ (J_p + J_r - \sqrt{(J_p + J_r)^2 - 4 \times \theta \times J_p \times J_r}) / 2 \times \theta \right] \quad (2)$$

where  $A$  is gross assimilation (g C m<sup>-2</sup> d<sup>-1</sup>),  $d$  is mean day length of the month,  $J_p$  is reaction of photosynthesis on absorbed photosynthetic radiation (g C m<sup>-2</sup> h<sup>-1</sup>),  $J_r$  is the Rubisco-limited rate of photosynthesis (g C m<sup>-2</sup> h<sup>-1</sup>), and  $\theta$  is the form factor (= 0.7).

$J_p$  is a function of the photosynthetic active radiation (PAR) and the efficiency of carbon fixation per absorbed PAR, which can be calculated on the basis of the intrinsic quantum efficiency for CO<sub>2</sub> uptake, the partial pressure of internal CO<sub>2</sub>, the CO<sub>2</sub> compensation point, the influence of temperature on the efficiency and a species-dependent adjustment function for tree age. The Rubisco-limited rate of photosynthesis  $J_r$  can be estimated by the maximum catalytic Rubisco capacity, the maximum day length, the Michaelis–Menten constant of CO<sub>2</sub> depending on temperature, the inhibition constant of O<sub>2</sub> against CO<sub>2</sub> (temperature-dependent) and the O<sub>2</sub> concentration.

Net assimilation  $A_N$  is calculated as follows:

$$A_N = A - R_d \quad (3)$$

where  $R_d$  is the product of the maximum catalytic Rubisco capacity and the ratio of the maximum catalytic Rubisco capacity and the respiration cost. A fixed share of 50% of the net assimilation is assumed for growth and maintenance respiration [35]. The tree growth represented the fixation of carbon, and the fixation of CO<sub>2</sub> was calculated based on fixation of carbon and the relative molecular mass.

For the ecosystem service of evapotranspiration, the central water balance equation from the water balance module was as:

$$prec - int - et_a - ro - \Delta\phi = 0 \quad (4)$$

With  $prec$  = precipitation (mm),  $int$  = interception (mm),  $et_a$  = actual evapotranspiration (mm),  $ro$  = runoff (mm),  $\Delta\phi$  = change of the soil content (mm).

Within the module cooling the energy needed for the transition of water from liquid to gaseous phase was calculated based on the  $CPA$  (crown projected area) and the transpiration  $et_a$  sum:

$$E_A = et_a \times CPA - (L_O \times -0.00242 \times temp) / f_{con} \quad (5)$$

With  $E_A$ : energy released by a tree through transpiration (kWh tree<sup>-1</sup>),  $L_O$ : energy needed for the transition of the 1 kg of water from the liquid to gaseous phase = 2.498 MJ (kgH<sub>2</sub>O)<sup>-1</sup> and  $temp$  = temperature in °C,  $f_{con}$ : conversion factor.

## 2.5. Statistical Analysis

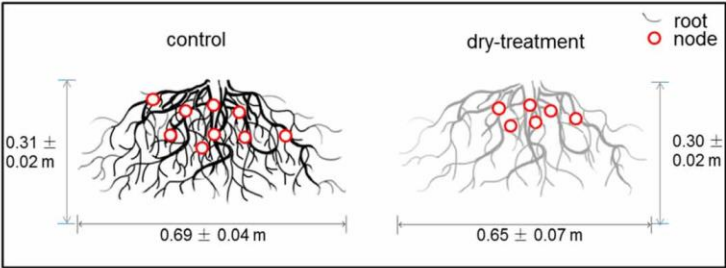
The software package R [36] was used for statistical analysis. To investigate the difference between means, two-sampled  $t$ -test and analysis of variance (ANOVA) with Tukey's HSD (honestly significant difference) test were used. In all the cases the means were reported as significant when  $p < 0.05$ . Where necessary, data were log or power transformed in order to correct for data displaying heteroscedasticity.

## 3. Result

### 3.1. Stem Growth Under Drought

At the beginning of the growing season, the two groups had similar and slight decreases in diameter. From May to August, distinctly different increases were observed, with the control group exhibiting more rapid growth than the dry-treatment group ( $p < 0.05$ ). Despite the similar patterns,

Although no significant difference was found between the control and dry-treatment groups ( $p > 0.05$ ), the RSA software showed that the root system in the dry-treatment group exhibited decreases compared with the control group in terms of width, root number and node number. The numbers of root and node in the dry-treatment group were  $1015 \pm 155$  and  $804 \pm 116$ , which were less than the control ones with the number of  $1087 \pm 182$  and  $841 \pm 130$ . Besides maintaining the rooting depth, the root system in the dry-treatment group showed a slight decrease from  $0.69 \pm 0.04$  to  $0.65 \pm 0.07$  m in rooting width (see Figure 5).



**Figure 5.** Two-dimensional illustration of the root systems in the control and dry-treatment groups. Red circles represent the rooting nodes.

3.3. Biomass Allocation and Root:Shoot Ratio

Generally, the stem biomass had the largest proportion of the tree, followed by the root system, and the branches had the lowest share (see Table 2). From the harvesting campaign, reductions of branch biomass (30.7%), stem biomass (16.7%) and coarse root biomass (45.2%) were observed. The biomasses of all three compartments in the dry-treatment group showed a distinct decrease in comparison to the control group, with the coarse roots in particular showing the largest difference ( $P_{\text{branch}} < 0.01$ ,  $P_{\text{stem}} < 0.05$  and  $P_{\text{coarse roots}} < 0.001$ ).

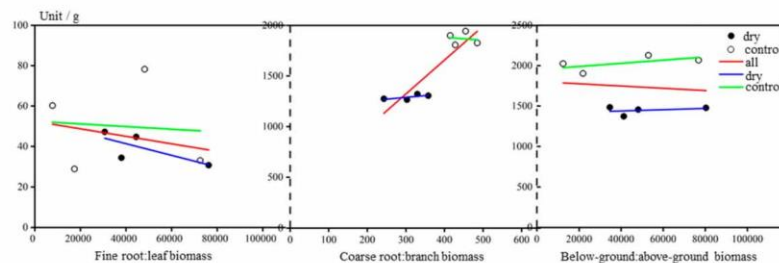
**Table 2.** Biomasses of branch, stem and coarse roots of both the control and the dry-treatment groups from the harvesting campaign as well as leaf area index (LAI) and projected canopy area (PCA) measured in summer 2017.

Group	n	Wood Biomass			Leaf		
		Branch (g ± sd)	Stem (g ± sd)	Coarse Root (g ± sd)	LAI (m <sup>2</sup> m <sup>-2</sup> ± sd)	PCA (m <sup>2</sup> ± sd)	SLA <sup>1</sup> (m <sup>2</sup> g <sup>-1</sup> )
control	4	445.8 ± 31.0	4089.3 ± 220.2	2440.3 ± 219.5	2.53 ± 0.25	0.58 ± 0.04	0.023
dry-treatment	4	308.5 ± 49.1	3407.0 ± 322.4	1338.7 ± 89.4	2.13 ± 0.24	0.58 ± 0.05	

In this study, we did not measure the SLA but used the same value of specific leaf area (SLA) from the literature for both the control and treatment group. <sup>1</sup> According to [34], the SLA of *T. cordata* in urban areas is 23.44 kg m<sup>-2</sup>.

Three types of root:shoot ratio were calculated: (1) fine root:leaf biomass ratio, (2) coarse root:branch biomass ratio and (3) below-ground:above-ground biomass ratio. All the root:shoot ratios were significantly different ( $p < 0.05$ ), with roots in the dry-treatment group constituting less of a proportion of the whole tree than in the control group (Figure 6). Among the ratios, the coarse root:branch ratio showed the greatest difference ( $p < 0.01$ ).

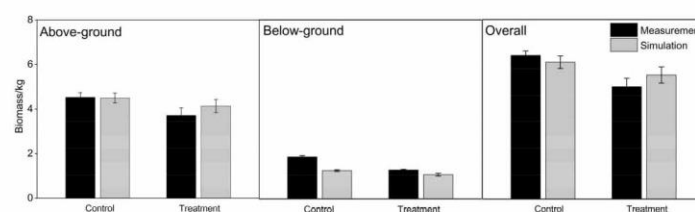




**Figure 6.** Three types of root:shoot ratio: fine root:leaf biomass ratio, coarse root:branch biomass ratio and below-ground:above-ground biomass ratio. White circles represent the control group, and black circles represent the dry-treatment group. Red, green and blue lines are the fitting regressions for all trees, the control group and the dry-treatment group, respectively.

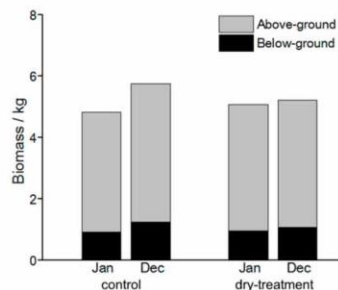
#### 3.4. Simulation of Biomass and Ecosystem Services

Using the urban tree growth model *CityTree* [34], the above-ground, below-ground and overall biomasses for both the control and dry-treatment groups can be simulated. The measured and simulated biomasses of the trees, including the above-ground, below-ground and overall biomasses, had no significant differences, which showed the model's reliability in prediction ( $p > 0.05$ ). Higher but not significantly different simulation was found in the dry-treatment group for the above-ground and overall biomasses (see Figure 7).



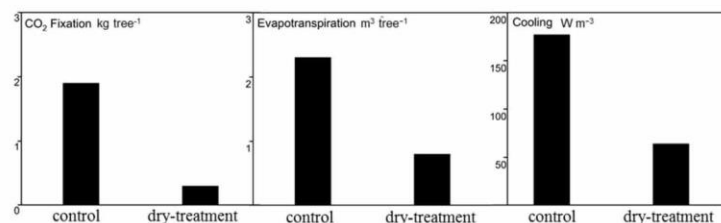
**Figure 7.** Comparison of measurements and simulations for the above-ground, below-ground and overall biomasses of trees from the control and dry-treatment groups.

The *CityTree* model also simulated biomass development for the entire year 2017 (Figure 8). In the control group, for the above- and below-ground biomasses, distinct increases of  $0.6 \text{ kg tree}^{-1}$  (from 3.9 to  $4.5 \text{ kg tree}^{-1}$ ) and  $0.4 \text{ kg tree}^{-1}$  (from 0.9 to  $1.3 \text{ kg tree}^{-1}$ ) were obtained, respectively. In the treatment group, however, trees showed scarce development, in that only a slight increase of  $0.1 \text{ kg tree}^{-1}$  was observed (from 5.4 to  $5.5 \text{ kg tree}^{-1}$ ). For the root:shoot ratio, increases were found from 0.23 to 0.29 and from 0.02 to 0.27 for the control and treatment groups, respectively, from January to December.



**Figure 8.** Simulated above- and below-ground biomasses for trees of the control and treatment groups for the entire year 2017.

By using the model *CityTree*, the ecosystem services of the trees, such as CO<sub>2</sub> fixation, water consumption and cooling potential by transpiration, could also be estimated. Figure 9 gives the values of these variables.



**Figure 9.** Ecosystem services, including CO<sub>2</sub> fixation, evapotranspiration and cooling potential by transpiration, for trees of the control and treatment groups in the year 2017.

The trees of the control group showed significantly higher CO<sub>2</sub> fixation than those of the dry-treatment group. Each tree in the control group could fix 1.9 kg CO<sub>2</sub> year<sup>-1</sup>, which was far more than 0.3 kg CO<sub>2</sub> year<sup>-1</sup> calculated for trees of the dry-treatment group. With a three times higher evapotranspiration (2.3 m<sup>3</sup> tree<sup>-1</sup> compared with 0.8 m<sup>3</sup> tree<sup>-1</sup>), the cooling potential provided by the control group trees was almost trebled compared with that of the dry-treatment group trees (177 W m<sup>-3</sup> compared with 64 W m<sup>-3</sup>). In summary, under the intense drought conditions associated with rainfall exclusion, all the ecosystem services of the lime trees were markedly reduced.

#### 4. Discussion

##### 4.1. Growth Patterns Under Drought

This study aimed to compare and analyse the growth patterns of *T. cordata* Greenspire from the control and dry-treatment groups. On the basis of the significant difference in soil water between the control and treatment groups ( $p < 0.01$ ), our RER proved to be successful at simulating drought stress. The effects of drought manipulations are very complex [37], and the responses of plants to water scarcity are complicated, involving adaptive changes [38]. Under normal conditions, *T. cordata* Greenspire exhibited steady growth, especially in spring, which is consistent with previous research [18]. Since the RER was established, *T. cordata* Greenspire showed reduced stem growth in the dry-treatment group ( $p < 0.05$ ). Furthermore, a large number of leaves died in the summer of 2017, in line with the decreased LAI. From the harvesting campaign, all the three compartments (branch, stem and coarse roots) showed

significant reductions in their biomass in the dry-treatment group compared with the levels in the control group. Overall, the findings showed that *T. cordata* Greenspire did not cope well with a dry period and lost a great deal of biomass, which could be thought of as being associated with a negative water balance due to insufficient water supply. This is also in line with previous research showing that the water management of *T. cordata* Greenspire was disrupted following growth reduction [19,39].

The root:shoot ratio is often used to estimate relative biomass allocation between roots and shoots [37,40,41]. Previous studies have reported that, for some drought-resistant tree species, drought induced root growth to enhance water uptake, which led to an increase in the root:shoot ratio [42,43]. Mokany et al. [44] proposed that an increase in the root:shoot ratio could be a strategy when facing drought in the long term, whereas water shortage could shift carbon allocation to storage in the short term [45]. In our study, drought significantly ( $p < 0.05$ ) reduced all three types of root:shoot ratio (fine root:leaf biomass ratio, coarse root:branch biomass ratio and below-ground:above-ground biomass ratio), which showed an opposite pattern to those drought-resistant tree species. Besides, the early discolouring and fall of leaves together with the biomass decrease in all the three compartments (i.e. coarse roots, stem and branches) suggested that *T. cordata* Greenspire did not have the means to positively adapt to drought. Additionally, the differences in water use efficiency between *T. cordata* Greenspire on the one hand and *Acer campestre* L. subsp. *campestre*, *O. carpinifolia* and *T. tomentosa* 'Brabant' from drier habitats on the other were measured and analysed [19]. The results showed that *T. cordata* Greenspire coped poorly with drought with highly reduced water use, whereas the other three tree species/cultivars maintained higher water use efficiency, possibly because of the better use of carbon for root production at the expense of above-ground biomass. Hence, the growth patterns of stem growth, biomass allocation and root:shoot ratios showed that *T. cordata* Greenspire failed to act as a drought-resistant cultivar.

#### 4.2. Strategy Within the Root System

Fine roots are the main plant component involved in absorbing water and nutrients, whereas coarse roots provide stability [46,47]. Joslin et al. [48] and Germon et al. [49] proposed that the cost of fine root construction could be balanced by the uptake of water under drought stress. In this study, the control group showed gradual development from May to September and maintained its biomass from September to November. In contrast, fine root biomass was reduced from May to September but slightly increased from September to November in the dry-treatment group. Combined with the results of Figure 5, the root system in the dry-treatment group had fewer roots and nodes as well as decreased widths. Taking into account the decreased root:shoot ratio, it was shown that *T. cordata* Greenspire did not invest greatly in the root system.

Nevertheless, we found a particular pattern where the deep fine roots achieved substantial development, whereas the fine root biomass of the upper and middle layers clearly decreased. Gewin [50] showed that deep roots could be crucial to alleviating water stress in plants, especially for plants living in tropical and subtropical environments [51]. This suggests that, within the root system, *T. cordata* Greenspire prioritises deep fine roots to enhance its water uptake capacity rather than the shallow ones, which is in line with previous findings [52,53]. Furthermore, from September to November, it was observed that the deep roots had a decrease while the biomass from the upper and middle layer had an obvious development. This could be implied that the response of fine root to drought had a time difference among layers. Overall, despite inadequate investment in the root system, the dynamics of fine roots in different layers could reflect the positive behavior under drought.

#### 4.3. Simulated Ecosystem Service Provision Under Drought Stress

Ecological process-based models have been applied widely for ecological issues, including biodiversity, phenology, hydrology and ecosystem services [54]. To obtain reliable predictions, however, the process-based models need to be validated first, in combination with observational data [55]. In our study, the *CityTree* model was first used to simulate the biomass from the below-ground

and above-ground processes. The results showed high performance of the model, in that little difference was found between the observation and the simulation. Hence, the model should be reliable for simulating ecosystem services.

Figure 9. suggests that *T. cordata* Greenspire trees provided much less ecosystem services under heavy drought than unstressed trees in terms of CO<sub>2</sub> fixation, transpiration and cooling. This can be explained by the fact that, under conditions of water shortage, the lime trees had to expend more energy in seeking out water to survive. However, on the basis of clearly reduced total transpiration, the total biomass growth was markedly reduced, which led to a reduction in carbon fixation of 84% in the drought-stressed lime trees compared with the unstressed trees. Along with the smaller amounts of transpiration, the cooling effect was reduced by 64% for the drought-stressed trees. Besides, Figure 8 shows that the model predicted higher (albeit not significantly) biomass for the drought-stressed trees than for the control trees, which suggests that drought stress had a more severe impact in reality than predicted by the model. Taking this conservative prediction into account, the negative impact of drought stress on the ecosystem services provided by *T. cordata* Greenspire might be more serious.

## 5. Conclusions

In 2014, the IPCC (The Intergovernmental Panel on Climate Change) predicted that extreme climatic events such as severe drought would become more common and severe in the future. The urbanisation accompanying such global change can alter the composition, structure and biogeography of vegetation in cities and surrounding areas [56]. Hence, obtaining comprehensive knowledge about how urban trees react to and cope with dry conditions can be of utmost importance for ecosystem services in cities.

We analysed tree growth and simulated ecosystem services under drought conditions for *T. cordata* Greenspire. The cultivar appeared to be susceptible to drought, in that the biomasses of coarse roots, stem and branches decreased following a decrease in the root:shoot ratio and demonstrated substantial failure to provide ecosystem services. With the loss of much root biomass, it tended to invest in fine roots. At the beginning of drought, investment was given to the deep fine roots and in autumn the shallow fine roots obtained more development. Therefore, urban trees, particularly drought-susceptible tree species and cultivars such as *T. cordata* Greenspire, should be planted in large soil pits so that water shortages are minimised. Intensive maintenance of the trees based on their growth patterns as well as on the site and soil conditions could preserve tree vitality and enhance tree growth and the provision of ecosystem services.

Against a background of global climate change and increasing urbanisation, featuring an enhanced impact of urban heat on tree growth and vitality, *T. cordata* Greenspire will become a vulnerable urban tree species in Central European cities. Especially in temperate cities where currently the precipitation in summer is low and where future climate conditions may feature droughts of increasing number and intensity, tree species and cultivars such as *T. cordata* Greenspire will suffer severely. Long-term drought experiments are necessary to obtain detailed knowledge about the behaviour of urban tree species under intense drought conditions, and such information will be crucial for landscape planners and architects.

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## **Article II**

**Zhang C\*, Stratopoulos LMF, Chao Xu, Hans Pretzsch, Thomas Rötzer. Development of Fine Root Biomass of Two Contrasting Urban Tree Cultivars in Response to Drought Stress. *Forests* 2020, *11*, 108.**



# Development of Fine Root Biomass of Two Contrasting Urban Tree Cultivars in Response to Drought Stress

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**Abstract:** Global climate change associated with rapid urbanization is projected to cause a worsening of environmental problems such as extreme heat and drought in cities. Urban trees play an essential role in improving air quality, fixing carbon, mitigating environmental degradation, and providing other ecosystem services. However, limited research has been conducted on belowground processes, which hampers a comprehensive understanding of the effect of climate change and urbanization on urban tree growth. Fine roots (<2-mm diameter) are the primary pathway for water and nutrient uptake by plants, and they considerably contribute to the survival of urban trees under drought stress. In this study, we conducted a controlled experiment on the development of fine roots of *Tilia cordata* Mill ‘Greenspire’ and *Tilia tomentosa* Moench ‘Brabant’ in response to drought stress via soil coring. Our results indicate that the two cultivars have different strategies for coping with drought. *Tilia tomentosa* ‘Brabant’, originating from drier regions, gave allocation to deeper soil parts priority probably to obtain more water. On the other hand, *Tilia cordata* ‘Greenspire’, which is native in Central Europe, showed a negative response to water shortage and preferred a more horizontal development of fine roots rather than a vertical development. Long-term studies are needed to gain a better understanding of the belowground processes of urban trees to select tree species and cultivars which are appropriate for planting in major cities, particularly with regard to future climate change.

**Keywords:** drought stress; fine root biomass; horizontal and vertical root growth; urban trees

## 1. Introduction

Global change including land-use change, elevated CO<sub>2</sub> concentrations, increased temperature, and increased rainfall variability is projected to have increasing impacts on the terrestrial ecosystem [1,2]. Simultaneously, urban areas around the world are expanding rapidly and will have more than 60% of the estimated world population by the year 2030 [3,4]. In this light, extreme heat is more extensive [5,6] and drought events can be exacerbated in urban areas due to many reasons, such as replacement of vegetation, storage of more sensible heat, and anthropogenic heat emissions [7–9]. Higher temperatures along with shifts in the precipitation rates may severely affect the urban environment

of Central European cities [10]. It is therefore essential for cities to find effective solutions to mitigate the negative effects of climate change and accelerating urbanization [11].

Urban greening and trees can mitigate environmental degradation and provide ecosystem services accompanying global change and rapid urbanization [12]. As rapid urbanization is destroying natural ecosystems and degrading the environmental quality of towns and cities [13], trees can play a key role in improving urban air quality, reducing noise, attenuating storm-water flooding, and conserving energy, which have drawn increasing interest from researchers [14–18]. For example, Pretzsch et al. found that the urban climate can both accelerate and slow down tree growth, depending on the climatic zone of the given city [10]. In addition, Nowak et al. [19] studied how urban trees can filter pollutants that have adverse effects on human health. Furthermore, how urban trees cope with drought has attracted more and more attention. Roetzer et al. [20] showed that water deficit reduces photosynthetic productivity and tree growth. Moser et al. [21] found marked growth reductions occurred after serious drought events for some urban tree species.

Roots, especially fine roots, are the primary pathway for water and nutrient uptake. Although fine roots constitute only about 2–3% of the total biomass, they are also a prominent, possibly the most prominent, sink for carbon acquired in terrestrial net primary productivity [22–24]. It is therefore essential to obtain quantitative information of root development of urban trees as plants spend a considerable part of their energy on the production and maintenance of roots [25]. Multiple studies were developed for the growth of roots in cities, e.g., resource limitation feedback between root and shoot growth [26,27], factors impacting on root growth [28–30], root response to physical constraints [31,32] and to soil chemistry and contaminants [33]. Some researchers reported that fine roots could be invested more to enhance water uptake due to water shortage [34]. Stratópoulos et al. stated that valuable information about root biomass and root: shoot ratio could be important selection criteria for species and cultivars choice [35]. Hence, to acquire more knowledge of the development of fine roots of urban trees will contribute to a better understanding of improving their ecosystem services in cities.

Although urban trees have received increasing attention for their ecosystem services [36–39] and much attention has been paid to drought response of aboveground organs such as stems, twigs, and leaves, little is known about the response of the root system to water shortage [27,40,41]. It is crucial to obtain quantitative information of belowground processes especially in cities as the growth conditions of urban trees are even more challenging to those in natural environments [10,42]. In this paper, we designed a controlled experiment for two contrasting cultivars which are widely planted in Europe to analyze the fine root biomass in response to drought stress, as it was reported that global warming would most likely increase the frequency and duration of summer droughts in Central Europe [43,44]. The objectives of this paper were to (1) explore the development of the fine root biomass of two cultivars during the growing season; (2) investigate the development of fine roots in both the vertical and horizontal directions; and (3) discuss the carbon allocation of the two cultivars under drought.

## 2. Materials and Methods

### 2.1. Site Description and Cultivar Selection

The municipal nursery Munich-Laim (48°08'05" N, 11°28'47" E, 534 m a.s.l.) has an area of 42 ha and is located in the Munich Gravel Plain in southern Germany. The study site provides homogenous microclimatic and edaphic conditions. The annual mean temperature is 9.1 °C and the annual mean precipitation is 959 mm (DWD, 2017). In this study, we selected two cultivars *Tilia cordata* 'Greenspire' Mill and *Tilia tomentosa* 'Brabant' for research because *T. cordata* 'Greenspire' is a local cultivar planted widely in Central European cities while *T. tomentosa* 'Brabant' originates from drier regions of south-east Europe, which was ideal for making a comparison. The cultivar *T. cordata* 'Greenspire' was grafted on the same species and *T. tomentosa* 'Brabant' was grafted on the root system of *Tilia platyphyllos* Scop. This species is often planted in central and south Europe and widely used for grafting, especially hybridized with *T. cordata* as they had similar properties. To avoid the effect of

transplanting shocks [45], trees transplanted in spring 2014 were chosen as their root system should have adequate growth.

## 2.2. Climate and Soil Moisture Measurements, Soil Samples

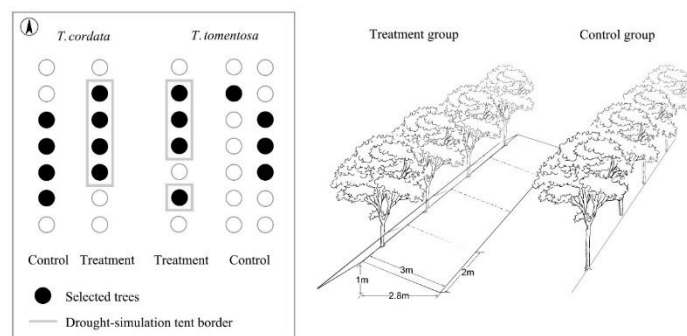
The data of air temperature, relative humidity, and precipitation were recorded with a weather station (Davis Vantage Pro2; Davis Instruments, Hayward, CA, USA) which was placed 450–600 m away from the research plots and sampled every 10 min. In addition, precipitation data were obtained from the nearest weather station at Munich Airport from the German Weather Service (DWD) to calibrate some abnormal values. Vapor pressure deficit (hPa; VPD) was calculated from 10-min time interval air temperature as well as humidity data.

Soil samples were taken with a soil core in several locations around the trees. Afterwards, they were transported to laboratory for further analysis. First stones were removed from the soil samples. Then the samples were dried at 60 °C for 2 weeks to get the dry weight. At last large particles were filtered. The rest was transferred to prefabricated analysis program and evaluated. A portable soil moisture meter (UMP-1, UGT, Muencheberg, Germany) for point measurements in the upper 10 cm of the soil was applied throughout the whole summer. The readings were made at defined distances from all study trees. The soil moisture data was interpolated spatially for the two selected cultivars.

## 2.3. Drought Experiment in 2017

The individuals of *T. cordata* and *T. tomentosa* were planted in separate sites at 2 × 3 m spacing so that all the individuals had enough distance to each other to avoid mutual effect. The selected trees all had stem circumferences between 16 and 18 cm to obtain a similar initial status. In this way, eight random individuals of *T. cordata* (Tc) and *T. tomentosa* (Tt) were selected, respectively.

The trees were divided into two groups, one of which was set as the control group (c) and the other one as the drought treatment (t) group. As a result, four trees form a group: *Tilia cordata*-control (Tc-c), *Tilia cordata*-treatment (Tc-t), *Tilia tomentosa*-control (Tt-c), and *Tilia tomentosa*-treatment (Tt-t). For the control group, all the individual trees were exposed to ambient climatic conditions. For the drought treatment group, rainfall was excluded via rainout exclusion from May to November 2017. Therefore, several waterproof, translucent tarpaulins were connected to build a tent with a size of 2 × 3 m for each tree. This construction was waterproof and sufficiently large to create a totally precipitation-exclusive environment. All of the tents were fixed using several 30-cm iron nails inserted into the ground to prevent wind damage (Figure 1).



**Figure 1.** Schematic structure of the drought experiment and selected trees of *Tilia cordata* and *Tilia tomentosa*. The rooting zones of trees covered by the rainfall exclusion constructions were the drought treatment group, whereas trees exposed to ambient weather conditions for the control group.

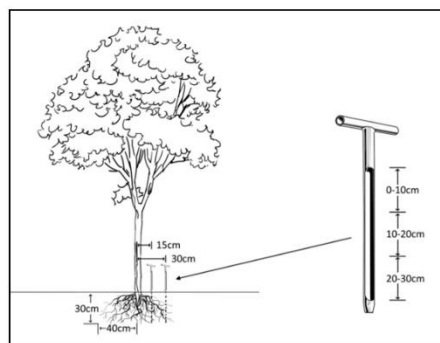
#### 2.4. Stem Growth and Leaf Area Index (LAI)

The diameter at a height of 1 m (nursery workers in Germany measure DBH at 1 m instead of 1.3 m) was measured using a digital caliper at the beginning of each month from April to November 2017. Measurements were conducted in two perpendicular directions (N–S, E–W) and averaged to account for irregularities in the form of the trunks. We focused on the relative rather than the absolute biomass increments to make the data independent of the initial biomass.

The leaf area index ( $\text{m}^2 \text{ m}^{-2}$ ; LAI) was determined using hemispherical photographs (Nikon Coolpix P5100 camera with a fisheye lens and Mid-OMount) and analyzed with the programme WinSCANOPY (Régent Instruments Inc., Quebec, QC, Canada). Data acquisition was performed under conditions of a uniformly overcast sky in the middle of June, shortly after implementation of the drought experiment.

#### 2.5. Coring for Fine Root Biomass

Fine root coring campaigns were launched for all trees in May, September, and November, i.e., at the beginning, middle, and end of the growing season. Due to the homogenous edaphic and climatic conditions in the nursery, we hypothesized that all the trees of the two cultivars had a similar growth condition. A pre-test coring campaign showed that the range of the root system was similar to a cylinder with a 70-cm diameter and 35-cm height. Therefore, during every coring campaign, four soil cores were collected for every individual tree, two at a distance of 15 cm to the trunk and the other two at a distance of 30 cm, i.e., the inner and outer fine roots. The soil was sampled down to a 30-cm soil depth using a soil auger with 30-cm length and 3-cm radius. Each sample was divided into three horizons: 0–10, 10–20, and 20–30 cm soil depth, i.e., the upper, middle, and lowest layers (Figure 2). Fine roots ( $<2 \text{ mm}$ ) were filtered using sieves (2-mm mesh size) and separated by forceps in the laboratory. Then, the samples were carefully washed and dried in the oven at  $65^\circ\text{C}$  for 72 h. Finally, all the samples were weighed using a balance with an accuracy of four decimal digits to obtain the dry weight. Fine root biomass at different depths was calculated using the dry weight divided by the cross area of the auger.



**Figure 2.** Fine roots were analyzed in different vertical and horizontal levels by coring at distances of 15 and 30 cm from the trunk.

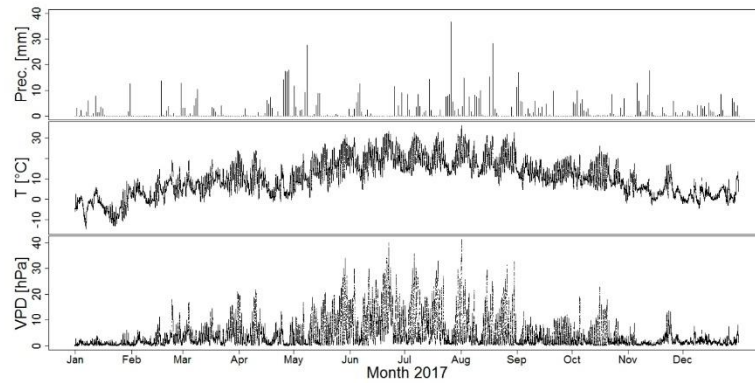
#### 2.6. Statistical Analysis

The software package R was used for statistical analysis [46]. To investigate the difference between means ( $n = 4$ ), two-sampled *t*-test and analysis of variance (ANOVA) with Tukey's HSD test were used. In all cases the means were reported as significant when  $p < 0.05$ . Where necessary, data were log or power transformed in order to correct for data displaying heteroscedasticity.

### 3. Results

#### 3.1. Weather and Soil Conditions

Precipitation varied clearly between April and November in 2017, the total sum of this period was 564 mm. July and August had relatively abundant precipitation reaching 102 and 123 mm, respectively. Other months were relatively dry, particularly during a 13-day period between 13 June and 25 June during which no rainfall was recorded. Precipitation started to increase at the beginning of May and showed a declining trend during early September. The site experienced a continuous warm period from 30 June to 30 August with 19.6 °C average daytime temperature (Figure 3).

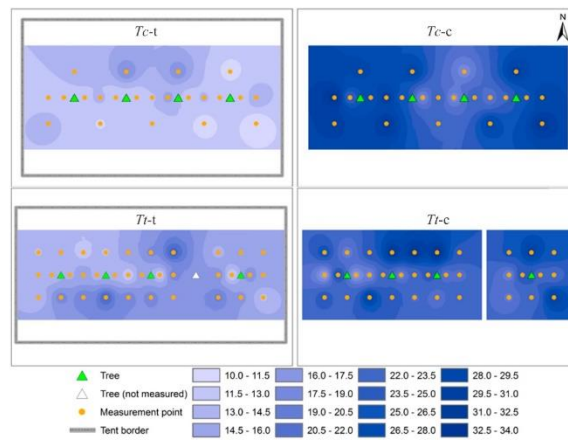


**Figure 3.** Daily amount of precipitation (Prec.; mm), temperature (T; °C), and the vapor pressure deficit (VPD; hPa) at the site for the year 2017.

For both the control and treatment groups of the two cultivars, all trees were grown in the same soil environment, which consisted mainly of medium sand (Table 1). In terms of the soil moisture, the drought treatment groups had significantly lower volumetric soil moisture contents (VWC; Vol.-%) in comparison to the control plots ( $p < 0.001$ ), with the roofed *T. tomentosa* showing the lowest average value of 19.4% (Figure 4). In addition, we found that the VWC 1 m from the trees, and therefore closer to the tent border, was significantly higher than that in the center of the roofs ( $p = 0.014$ ). Therefore, the data indicated the overall success of the drought experiment.

**Table 1.** Soil characteristics for both the control and drought treatment groups of *T. cordata* and *T. tomentosa*.

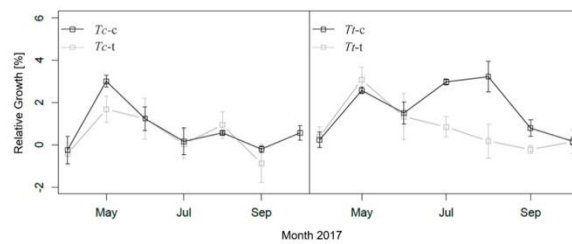
Group	n	Fine Sand %	Fine Silt %	Grit %	Coarse Grit %	Medium Sand %	Medium Silt %
Tc-c	4	15.8 ± 3.6	6.5 ± 1.1	9.0 ± 2.2	16.0 ± 1.2	27.3 ± 4.9	8.7 ± 3.3
Tc-t	4	22.6 ± 5.9	3.8 ± 1.3	7.7 ± 4.3	12.2 ± 2.7	33.4 ± 4.7	8.1 ± 1.3
Tt-c	4	14.2 ± 0.4	5.7 ± 2.5	11.2 ± 0.7	10.8 ± 2.3	23.7 ± 1.8	14.0 ± 4.3
Tt-t	4	14.0 ± 0.7	7.3 ± 0.9	11.6 ± 0.5	12.7 ± 2.3	23.1 ± 2.0	11.2 ± 1.0



**Figure 4.** Maps (interpolated values) of the volumetric soil water contents (Vol.-%; VWC) at a soil depth of 10 cm averaged over four measurement days in summer 2017 and collected at defined distances from the study trees. The rooting zones of the tree individuals in the respective left graphics were covered by the rainfall exclusion constructions ('drought treatment', *Tc-t* and *Ti-t*), whereas the individuals in the right images belonged to the control groups ('control', *Tc-c* and *Ti-c*).

### 3.2. Relative Stem Growth and LAI

For both cultivars, stem growth in the control and treatment groups reached a peak during May. After rapid growth during late spring, a tendency of decreased growth toward autumn for *T. cordata* from both the control and treatment groups with considerably similar patterns was obvious. In contrast, *T. tomentosa* individuals showed different developments between the control and drought treatment groups after June. *Ti-c* had strong growth until September, whereas *Ti-t* was strongly affected by the drought and showed a continuous growth decrease (Figure 5).



**Figure 5.** Monthly relative stem increment (%) of control and drought treatment groups of *T. cordata* and *T. tomentosa* from April to October 2017. Values denote the average of all trees per cultivar and group  $\pm$  the standard deviation.

*T. cordata* showed a quite fast development, i.e., this species unfolded its leaves earlier than *T. tomentosa* that did not unfold their leaves until the third week of May. In summer, LAI of *Tc-t* under drought was reduced in comparison to *Tc-c* ( $p < 0.05$ ) while an opposite pattern was found for *T.*



*tomentosa*, i.e., *Tt-t* was higher than *Tt-c* ( $p > 0.05$ ). In autumn, individuals of *Tc-t* were leafless in the middle of October while *T. tomentosa* had longer vegetation periods (Table 2).

**Table 2.** Leaf area index (LAI;  $\text{m}^2 \text{m}^{-2}$ ) of the two tree cultivars from both the control and treatment groups in June 2017.

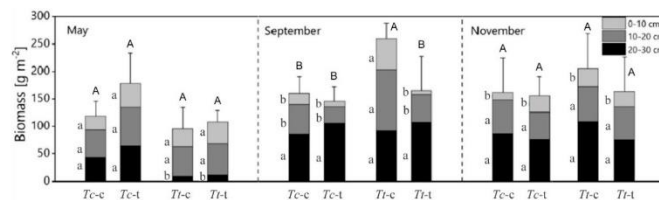
Cultivar	Group	n	LAI ( $\text{m}^2 \text{m}^{-2} \pm \text{SD}$ )
<i>T. cordata</i>	Control	4	$2.53 \pm 0.49$
	Treatment	4	$2.13 \pm 0.48$
<i>T. tomentosa</i>	Control	4	$2.92 \pm 0.21$
	Treatment	4	$3.11 \pm 0.55$

### 3.3. Total and Vertical Fine Root Biomass

Fine root biomass of the two cultivars was different from May to November. During the beginning of the growing season, *T. cordata* and *T. tomentosa* showed no significant difference between the control and drought treatment groups ( $P_{T. cordata} = 0.066$ ,  $P_{T. tomentosa} = 0.526$ ). The fine root biomass of *Tc-c* increased by 36.5% to  $161.4 \text{ g m}^{-2}$  from May to September and remained stable at  $162.2 \text{ g m}^{-2}$  from September to November, while that of *Tc-t* decreased by 18.1% to  $145.7 \text{ g m}^{-2}$  during the first four months (from May to September) but had a slight growth of 7.0% to  $155.8 \text{ g m}^{-2}$  during the last three months (from September to November). For *T. tomentosa*, the individuals of the control group behaved quite differently from the drought stressed group (Figure 6). The initial fine root biomass of the control and treatment groups was nearly similar at  $99.5$  and  $108.2 \text{ g m}^{-2}$ , but in the course of the experiment, *Tt-c* increased to  $259.8 \text{ g m}^{-2}$ , (161.2%) from May to September while *Tt-t* increased to  $165.6 \text{ g m}^{-2}$ . Over the period from September to November, *Tt-c* showed a remarkable decrease to  $205.7 \text{ g m}^{-2}$  while *Tt-t* remained at  $163.9 \text{ g m}^{-2}$ .

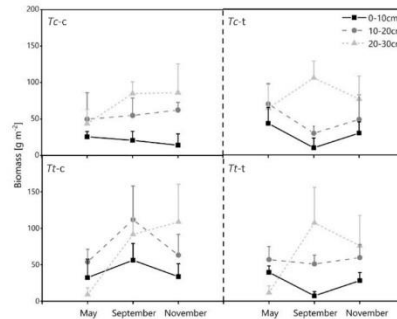
For vertical development, the fine root biomass in the three layers developed differently between the control and drought treatment groups of both cultivars (Figures 6 and 7). For *T. cordata*, fine root growth was observed highest in the 20–30 cm layer from May to September in the control group. In the drought treatment group, the fine root biomass from both the 0–10 and 10–20 cm layers sharply decreased by 77.1% and 57.4%, respectively. In the deep layer (20–30 cm) we observed contrasting development that it increased to a peak of  $105.8 \text{ g m}^{-2}$  during September and decreased to  $76.8 \text{ g m}^{-2}$  during November.

For *T. tomentosa*, *Tt-c* continued growing in the 20–30 cm layer while the fine roots from the 0–10 and 10–20 cm layers decreased by 40.3% and 43.4%, respectively. For *Tt-t*, the fine root biomass in the three layers developed significantly differently from *Tt-c* during the whole period ( $p = 0.039$ ). In 0–10 cm depth the fine root biomass decreased from  $39.5$  to  $7.3 \text{ g m}^{-2}$  during the first four months and increased to  $28.2 \text{ g m}^{-2}$  during the last three months. The fine roots in the 10–20 cm layer showed only slight fluctuations for the whole period. The fine root biomass in the deep layer was initially very low ( $11.6 \text{ g m}^{-2}$ ) and steeply increased to  $107.4 \text{ g m}^{-2}$  by September. In contrast, the same layer in the control group decreased by 29.3%.



**Figure 6.** Fine root biomass ( $\text{g m}^{-2}$ ) for the 0–10, 10–20, and 20–30 cm layers of the control and treatment groups of *T. cordata* and *T. tomentosa* during May, September, and November 2017. The

different letters indicate significant differences ( $p < 0.05$ ) between cultivars and treatments for the overall biomass (capital letters) and different layers (lowercase letters).

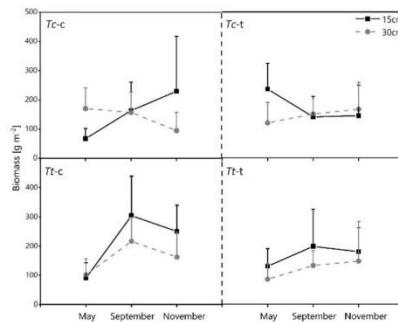


**Figure 7.** Fine root biomass ( $\text{g m}^{-2}$ ) in the three layers of 0–10, 10–20, and 20–30 cm of the control and drought treatment groups of *T. cordata* and *T. tomentosa* in May, September, and November 2017. Values denote the average of all trees per cultivar and group  $\pm$  the standard deviation.

### 3.4. Horizontal Development of Fine Root Biomass

The horizontal development of fine roots of the two cultivars was different when exposed to drought ( $p < 0.05$ ) (Figure 8). The fine root biomass of Tc-c steadily increased at a distance of 15 cm from the stem, whereas it decreased at a distance of 30 cm from May to November. Tc-t decreased its fine root biomass at a distance of 15 cm from 235.7 to 140.7  $\text{g m}^{-2}$  from May to September, whereas the biomass at a distance of 30 cm slightly increased over measurement time.

For *T. tomentosa*, drought had an impact on the fine root growth from May to September. It reduced the fine root biomass by 23.7% at a distance of 15 cm and by 11.4% at a distance of 30 cm in the control groups, and by 52% and 55% in the treatment groups, respectively. From September to November, Tt-c decreased from 303.7 to 249.7  $\text{g m}^{-2}$  at a distance of 15 cm and from 215.9  $\text{g m}^{-2}$  to 161.6  $\text{g m}^{-2}$  at a distance of 30 cm, which had a similar trend in Tt-t.



**Figure 8.** Horizontal development of fine root biomass ( $\text{g m}^{-2}$ ) at a distance of 15 and 30 cm of the control and treatment groups of *T. cordata* and *T. tomentosa* during May, September, and November 2017. Values denote the average of all trees per cultivar and group  $\pm$  the standard deviation.



#### 4. Discussion

In this study, we studied the fine root biomass of *T. cordata* and *T. tomentosa* cultivars in response to drought stress. We found different growth patterns under water shortage between the two cultivars in both the vertical and horizontal directions reflecting clear differences in the carbon allocation within a tree for the two cultivars.

##### 4.1. Total Fine Root Biomass

Effects of drought manipulations are very complex [47]. In this study, we found that drought had a relatively small impact on fine root growth of *T. cordata* and it invested less energy than *T. tomentosa* on belowground growth, the fine root biomass only slightly varied between control and drought treatment trees. Moser et al. [21] found that *T. cordata* showed a continued decrease in aboveground growth after a drought event while our results showed the congruent growth for belowground processes. This implies that the belowground part did not actively react to cope with drought stress.

In contrast, starting from May *T. tomentosa* showed a vigorous fine root growth in both control and drought treatment groups, i.e., *T. tomentosa* invested or gave the priority to the belowground growth. Radoglou et al. [48] also found that *T. tomentosa* with sufficient water supply reacts dynamically with fine root growth. Under water shortage, our results showed that the fine root biomass growth of *Tt-t* decreased compared to *Tt-c* from May to September, however, there was still a distinct growth over the whole growing season. Combined with the results of Stratópoulos et al. [49], *T. tomentosa* originating from dry regions was proved to be a drought-resistant species that could cope well with water shortage. Based on our observations, the main strategy of *T. tomentosa* could be the steady and continuous investment in the belowground growth under drought stress, which could prove the vitality of the fine roots of *T. tomentosa* undertook the ability of water absorption in response to the dry environment.

##### 4.2. Vertical Development of Fine Root Biomass

Despite the fact that the total fine root biomass of the control and treatment groups for *T. cordata* trees were similar, the fine root biomass of the three layers showed different patterns. *Tc-c* exposed to normal conditions slowly increased their growth in the deep layer and decreased it in the upper layer, which was consistent with previous results [50,51]. However, in response to drought, the fine root biomass in the upper and middle soil layers of *Tc-t* sharply decreased. Simultaneously, the deep fine roots of *Tc-t* developed stronger than the fine roots of *Tc-c* probably to obtain water from deeper soil parts. During autumn (from September to November), the fine root development in all the soil layers was completely different compared to summer. The deep roots decreased whereas the roots in the upper and middle soil layer initiated growth. Accounting for the steady total fine root biomass, the preferential development of fine roots in different soil layers may reflect specific allocation to soil layers with reserve water over the course of the season. Gillner et al. found that *T. cordata* recovered after decreasing growth resulting from a drought event, which could be explained by the resumptive growth of the fine roots in the upper and middle soil layers [52]. Less energy was invested by *T. cordata* in the belowground processes in all the soil layers to obtain enough growth, especially during a drought event. This might lead to a growth balance between the shallow and deep fine roots.

For *T. tomentosa*, without a water shortage, fine roots vigorously developed in all the soil layers of the control group during the first four months, whereas it was different for the upper and middle layers in the dry treatment group. The roots in the middle layer showed no growth and the shallow roots almost died because of the drought. However, simultaneously the deep fine roots exhibited strong growth, which was even higher than the growth in the control group. This was considered as an essential step to adapt to drought. During autumn, for both the control and treatment groups, the fine roots in each layer had opposite growth patterns than those earlier in the season. We did not only observe that the shallow roots had a similar growing tendency to that of the fine roots in the middle

layer but also found a dynamic balance for all fine roots, in the way that if the fine roots in the upper and middle layer increased or decreased, the deep fine roots decreased or increased accordingly.

Recent research has shown that deep roots can be of pivotal importance to alleviate water stress in many plants [53]. They particularly play a central role in drought tolerance in tropical and subtropical environments [54]. Our results proved this with the development of the deep fine roots of *T. tomentosa*, which originated from drier regions than those of *T. cordata*. Schenk proposed that the rooting profiles of plant communities tended to be as shallow as possible and as deep as needed to meet the evapotranspiration demand [55]. The idea is that plants need less energy for the construction of shallow roots than of deep roots, although the balance of allocation of carbon to roots and the acquisition of water are central to tree survival. Moreover, soil close to the surface can provide high water and nutrient availability for root growth most of the time. In addition, shallow soils have lower soil strength, which usually avoids the possibility of oxygen deficiency for the roots. Generally, the growth of deep roots can even be a drain for downward hydraulic redistribution [56].

During the beginning of drought events, however, deep roots can uptake more water which is an advantage compared with a shallow rooting system because lower maintenance costs tend to counter-balance the high construction costs of deep roots [57,58]. Fine root turnover increases with soil depth because the roots are probably strongly modulated by a combination of environmental factors, e.g., soil moisture and texture [59,60]. In the study of Stratópoulos et al. [49], *T. tomentosa* showed strong resistance against drought whereas *T. cordata* performed negatively. In this study, the fine roots of *T. cordata* showed a minor reaction to drought. *T. tomentosa*, however, developed new fine roots in the vertical direction especially in the deep soil layers in summer, which could be regarded as an effective solution in coping with drought stress. Moreover, when the availability of water was high, *T. cordata* did not show vigorous growth, whereas *T. tomentosa* developed abundant deep roots which probably aimed to prevent the risk of drought damage.

#### 4.3. Horizontal Development of Fine Root Biomass

Different tree species have different distribution patterns of fine roots in the horizontal direction [51]. Similarly, we found that under normal conditions fine root development of *T. cordata* was more stem-centered whereas *T. tomentosa* showed no priority for inner root growth. Under drought stress, this tendency was reversed. The outermost fine roots did not decrease any further, whereas the inner fine roots died because of water shortage. This could be a strategy of *T. cordata* trees to expand their root system to acquire more water by decreasing the support of the fine roots at a distance of 15 cm. For the *T. tomentosa* individuals of the control group, both the inner and outer fine roots underwent similar growth from May to November, which was also found for the trees in the treatment group. Both the fine roots of *T. tomentosa* in the control and treatment groups had an increase in innermost area during the first four months and a decrease in outermost fine root biomass during the last three months. This implies that drought did not affect horizontal expansion and no priority was given to the fine roots in horizontal direction.

#### 4.4. Carbon Allocation under Drought Stress

Figure 5 displays that the relative stem growth of both *T. cordata* and *T. tomentosa* reached the peak in May which is similar to the results of Rahman et al. [61]. In general, annual stem growth predominantly occurs from May to July [62]. During this period, temperature and water availability are the main determining factors for radial growth [52,63]. However, for different tree species and cultivars, the patterns can be distinct [52]. We found similar trends in stem development for *T. cordata* in the control and drought treatment groups during the whole growing season. This could be explained by the mechanism of anisohydric behavior, i.e., that *T. cordata* reduced leaf water potential under drought stress [64]. There was no significant difference in the stem and root growth between the control and drought treatment groups, because the high levels of stomatal conductance preserved stem and root growth [65]. For *T. tomentosa*, the stem growth in the drought treatment group decreased compared with that in the control group. However, the fine root biomass simultaneously

developed strongly, which implied that *T. tomentosa* prioritized belowground processes to obtain water under drought stress.

## 5. Conclusions

We found that the fine root growth showed different responses to drought stress in two cultivars widely planted in Europe. We hypothesized that both the two cultivars had a similar initial growth condition and the development of their fine root could reflect the underground strategy. *T. cordata* trees showed little growth that similar growth patterns for stem and total fine root biomass were observed under drought stress. However, increased outer fine roots for horizontal expansion could be the potential reason for its living. For *T. tomentosa*, it invested more on roots, particularly deep roots in order to absorb water, which suggests a strategy of prioritizing belowground processes regarding the decrease of the stem growth. In conclusion, the two cultivars displayed different reactions to water shortage that *T. cordata* tended to support the expansion of fine roots horizontally while *T. tomentosa* is assumed to develop deep fine roots.

Thus, under the background of global change with higher temperatures, altered precipitation patterns, and more extreme weather events (e.g., drought), city planners should consider providing enough space when planting trees in streets and squares. The development of fine roots of urban trees should be considered when planting urban trees. The horizontal expansion of fine roots of some tree species and cultivars could alleviate water shortages; however, this could result in damage to pavements. This suggests the necessity of sufficient space for tree species and cultivars such as *T. cordata* ‘Greenspire’. Tree species and cultivars with an active reaction to drought stress could be prioritized in urban management. Those with a deep root strategy such as *T. tomentosa* ‘Brabant’ could be an appropriate and economic choice as they are adaptable to drought stress. Long-term observations of fine root growth patterns are needed to provide more in-depth information and quantify the root development of different trees species and cultivars in urban surroundings.

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### **Article III**

**Zhang C<sup>1\*</sup>, Stratópoulos LMF<sup>1</sup>, Häberle, K-H, Pauleit, S, Duthweiler, S, Pretzsch H, Rötzer, T. Effects of Drought on the Phenology, Growth, and Morphological Development of Three Urban Tree Species and Cultivars. *Sustainability* 2019, *11*, 5117. (<sup>1</sup> shared first-authorship and corresponding author)**

# Effects of Drought on the Phenology, Growth, and Morphological Development of Three Urban Tree Species and Cultivars

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**Abstract:** Under changing climatic conditions, drought may become a critical constraint for trees in urban areas, particularly at roadsides and highly paved squares. As healthy urban trees have proven to be an important mitigation and adaptation tool for climate change as well as a significant provider of ecosystem services, there is a need for planting species and cultivars capable of coping with the limited water supply. However, data on species' and cultivars' response to drought, particularly their water supplying root systems remains rare. To consider the whole plant responses to drought situations, we studied the growth and phenology of three frequently planted tree species and cultivars with a diameter of 5–6 cm during a one-year rainfall exclusion experiment conducted in a nursery field as well as the dry biomass of the compartments branch, stem, and root after excavation. Our results revealed that species' and cultivars' performance were linked to their within-plant carbon partitioning. A high tolerance to drought was noted for *Acer campestre*, with a particularly high ratio of root:shoot ratio, which made it presumably less susceptible to droughts. *Tilia cordata* 'Greenspire' was highly affected by the reduced water availability visible through prematurely leaf senescence, while *Carpinus betulus* 'Fastigiata' suffered from losing a considerable part of its root biomass, which resulted in the lowest root:shoot ratio of all species and cultivars. This study demonstrated the need for investigating the reaction patterns of species and cultivars by considering both the above-and the below-ground plant parts. We recommend that, for future tree plantings at harsh and challenging urban sites, an important selection criterion should be species' and cultivars' capability to develop and retain strong and dense root systems even under limited water supply, as that is believed to be an important trait for drought tolerance.

**Keywords:** allocation; drought tolerance; partitioning; resistance; root:shoot ratio

## 1. Introduction

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During the course of climate change, higher temperatures along with shifts in the precipitation rate are estimated to increase drought severity and frequency [1]. This is also applicable for Central Europe [2] and may severely affect trees planted in paved urban environments in this region. Combined with the urban heat island effect caused by the properties of built structures, the absence of vegetation, and the anthropogenic heat emissions [3–5], the growth conditions of urban trees are even more challenging to those in natural environments [6–8]. As healthy urban trees have proven to be an important mitigation and adaptation tool for climate change as well as a huge provider of ecosystem services [9–12], there is a need for planting species and cultivars capable of coping with extreme climatic events such as prolonged droughts.

It is known that, to some extent drought-survival traits are genetically inherited [13] and those plants cope with drought conditions through structural, functional, and physiological adjustments. Research on selected urban tree species and cultivars revealed higher stomatal sensitivity under drought of trees from relatively dry habitats in comparison to trees from more mesic habitats [14]. It is also known that trees avoid extremely negative water potentials through a combination of internal water storage [15], reduced leaf area, and access to a larger volume of soil via the development of extensive root systems [16]. A recent field study on two linden cultivars [17] showed that trees with compact root systems around the stem tend to develop more fine roots laterally to increase their soil-to-root interface. In the long term, elevated root:shoot ratios can be regarded as an adaptation to dry sites [18] to make trees less susceptible to water shortage [19]. In the short term, however, moderate water stress can shift carbon allocation from growth to storage and resin defense [13], unless drought is severe enough to cause cessation of carbon allocation to all sinks.

Information about species' and cultivars' responses to stress is mainly derived from knowledge about the environmental conditions in their respective natural habitats [20,21]. Only some experimental urban and forest studies have compared the performance of different tree species and cultivars explicitly under dry growing conditions [19,22–24] and in the available urban studies, with the focus solely being on the above-ground processes. In the urban context, knowledge of below-ground biomass and its distribution as well as the changing patterns of carbon allocation to different tree compartments remains limited. In addition, research on the whole plant responses of urban trees needs to consider species- and cultivar-specific seasonal metabolic adaptations because of the superordinate impact on the timing of the physiological processes of trees.

Therefore, we studied the whole plant body when investigating the drought tolerance of different species and cultivars. We provided data on leaf development and the stem growth of trees with a diameter at 1 m height of 5–6 cm during a soil drying experiment of a complete growing cycle as well as values for biomass for the three compartments branches, stem, and roots after excavation of the trees from the field. The specific questions addressed by this study were based on the effect of drought on (1) the phenology, (2) growth, (3) biomass of branches, stems, and roots of three frequently planted native tree species and cultivars, as well as (4) species- and cultivar-specific within-plant carbon partitioning and allocation patterns, respective growth habits and the root system architectures, which may indicate the capacity of tree species and cultivars to respond to drought stress.

## 2. Materials and Methods

### 2.1. Study Site, Species and Cultivars Selection, and Drought Experiment

The study site municipal nursery (48°08'05" N, 11°28'47" E, 534 m a.s.l.) is located to the southwest of Munich, Germany. The long-term (1981–2010) annual means of the temperature and rainfall from the nearest weather station in the city center of Munich are 9.7 °C and 944 mm, respectively [25]. The 42 ha large area is characterized by an almost uniform topography and possible small microclimatic variations. The predominant soil types range from moderate sandy loam to strong loamy sand, as estimated by a finger test. Interpretation of the water-retention

curves provided in the literature indicated a soil moisture tension (pF value) of 1.8 (field capacity) between 29 Vol.-% and 33 Vol.-% volumetric soil water content (VWC) and a pF value of 4.2 (permanent wilting point; PWP) at 12 Vol.-% and 16 Vol.-% for the moderate sandy loam and the strong loamy sand, respectively [26]. This PWP does not represent the true physiological wilting point for our investigated trees; however, it may instead be regarded as an indicator of severe drought stress [19]. This is because we did not determine PWP for each species and cultivar separately, rather obtained it from literature where it is set for agricultural plants only.

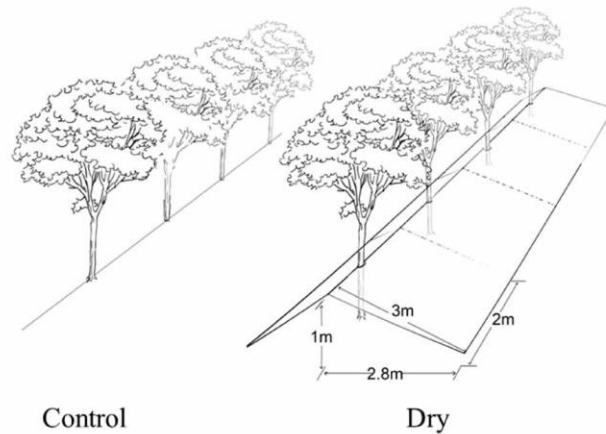
Six to eight trees each of the following temperate deciduous species and cultivars with a diffuse-porous wood anatomy and a diameter of 5–6 cm at 1 m height were selected: *Acer campestre* L. subsp. *campestre* (Ac), *Carpinus betulus* L. ‘Fastigiata’ (Cb), and *Tilia cordata* Mill. ‘Greenspire’ (Tc), which are frequently planted native species or cultivars. The study species and cultivars were assumed to vary in terms of drought and shade tolerance [27] (Table 1). The abbreviations of all the species and cultivars are listed in Table 1 and will be used hereinafter.

**Table 1.** The characteristics of the surveyed trees. Diameter at 1 m height is given for the experimental period May 2017.

	Code	Control <i>n</i>	Dry	Diameter [cm ± sd]		Shade Tolerance <sup>1</sup>	Drought Tolerance <sup>1</sup>
				Control	Dry		
<i>Acer campestre</i>	Ac	3	3	5.60 ± 0.25	5.66 ± 0.09	3.18 ± 0.14	2.93 ± 0.32
<i>Carpinus betulus</i> ‘Fastigiata’	Cb	4	3	5.30 ± 0.14	5.61 ± 0.08	3.97 ± 0.12	2.66 ± 0.16
<i>Tilia cordata</i> ‘Greenspire’	Tc	4	4	5.90 ± 0.10	6.00 ± 0.16	4.18 ± 0.16	2.75 ± 0.15

<sup>1</sup> Extracted from the meta analysis of (Appendix A in [27]), including data of 806 North American, European/West Asian, and East Asian temperate shrubs and trees. Tolerance scales range from zero (no tolerance) to five (maximal tolerance).

We employed an experimental setting where we simulated the conditions of a sustained drought to investigate the physiological and growth responses of the trees [15,28] as well as their phenological timings, the architecture of the root systems, and the carbon partitioning and allocation. The rainfall exclusion experiment started in May 2017 and was conducted throughout the growing period of the year until November. We installed waterproof, translucent tarpaulins (2 m × 3 m) in the middle row of 50% of the individuals (“dry”) at a height of 1 m, sloping to the ground in the north and south directions at 3 m distance from the trees (Figure 1). The tent-like constructions prevented rain infiltration close to the tree stems. A second group was exposed to the prevailing weather conditions and served as a control (“con”). Each plot was made up of three to four plants per species/cultivar and treatment, which were all transplanted in the spring of 2014 in parallel rows at 2 × 3 m spacing. At the beginning of the drought stress study, no significant differences were noticed between the stem diameters of control in comparison to the dry treatment groups (Table 1).

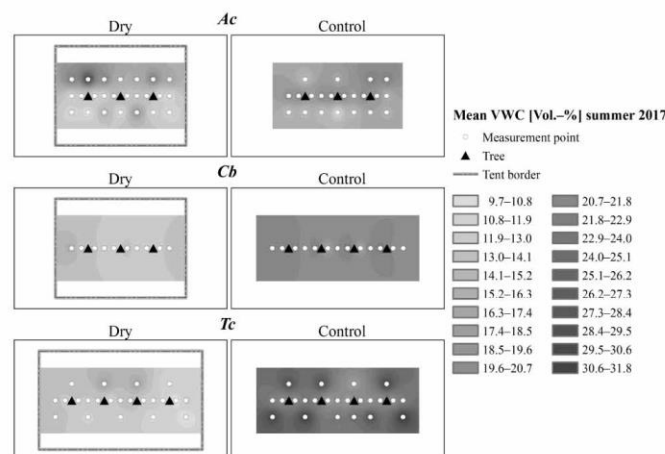


**Figure 1.** Schematic structure of the drought experiment. The rooting zones of the trees on the right were covered by the rainfall exclusion constructions ("dry"), whereas the trees on the left were exposed to the prevailing weather conditions.

## 2.2. Climate and Soil Moisture

A full suite of climatic variables was sampled every 10 min with a weather station (Davis Vantage Pro2; Davis Instruments, Hayward, USA) placed in an unshaded site approximately 200 m away from the experimental plots. Due to equipment failure, the precipitation data were also obtained from the nearest weather station from the German Weather Service (DWD) at the Munich City Centre. The vapour pressure deficit (hPa; VPD) was computed from air temperature and humidity data.

VWC was measured using HS-10 sensors (Decagon Devices, Pullman, USA) inserted horizontally at a depth of 30 cm in the A-horizon located 40 cm away from the tree stems. For each species/cultivar and treatment, one sensor was used for one centrally located individual in the row. Ten-min means were calculated from 1 min readings and logged (CR800 connected to an AM16/32 B multiplexer, Campbell Scientific, Logan, UT, USA and Agilent 34970A Data Acquisition/Switch Unit, Agilent Technologies, Santa Clara, CA, USA). Given that the continuous measurement devices failed several times probably due to the high soil compaction and drying, we additionally used a portable soil moisture meter (UMP-1, UGT, Müncheberg, Germany) for point measurements in the upper 10 cm of the soil layer during four days in the summer. The readings were made at defined distances from all study trees, as shown in Figure 2.



**Figure 2.** Maps (inverse-distance-weighted interpolation) of the volumetric soil water contents (Vol.-%; VWC) at a soil depth of 10 cm averaged over four measurement days in summer 2017 and collected at defined distances from the study trees. For full species and cultivar names see Table 1.

### 2.3. Spring and Autumn Phenology

We classified the spring and autumn phenology according to the BBCH (Biologische Bundesanstalt für Land- und Forstwirtschaft, Bundessortenamt und Chemische Industrie) phenological growth stages and identification keys [29]. All the phenological stages, from the beginning of bud swelling (BBCH 01) until the completion of leaf unfolding (BBCH 19) in spring and from the start of leaf discolouration (BBCH 91) until the end of leaf fall (BBCH 97) in autumn were coded once or twice a week between the end of March and the middle of May and between the beginning of October and the end of November, respectively. In addition, we made observations concerning early wilting symptoms throughout the entire study period.

### 2.4. Optical Measurements of Crown and Root System Architecture

The leaf area index ( $\text{m}^2 \text{m}^{-2}$ ; LAI), which is the one-sided green leaf area per unit ground surface area, was determined using hemispherical photographs (Nikon Coolpix P5100 camera with fisheye lens and Mid-OMount, Nikon Corporation, Tokyo, Japan) analysed with the programme WinSCANOPY (Régent Instruments Inc., Quebec, Canada). Among the available several methods, we used the LAI-2000 generalised method based on the work of Miller [30] and Welles and Norman [31] for deriving the LAI. Data acquisition was performed under conditions with uniformly overcast sky in the middle of June, shortly after implementation of the drought experiment.

We also measured sufficient numbers of points (7–11  $x$ ,  $z$  pairs) to describe each of the trees' average crown shape. Geometric data were then processed within the software FV2200 (LI-COR Biosciences, Lincoln, USA) to compute the crown dimensions (volume and projected crown area) by using the model for isolated trees. Similarly, we also measured each trees' average root system dimensions after excavation in the winter 2017 (see the following section) and calculated the volumes with the FV2200 software.

Images of the top view of each root system were analysed by the open source image processing software ImageJ and the plugin DiameterJ [32], which is actually a nanofiber diameter characterization tool. First, the scenes were converted into black and white (i.e., binary) images, where the white pixels ideally represented the portions of the original image that were of interest

(root system) and the black pixels represented the background. We compared the results of different segmentation algorithms (auto threshold) provided by the software and selected the appropriate algorithm, which best represented the root system. Inside each binary image, we then selected one rectangular area, where again segmentation most properly displayed the roots and eliminated the features of the image that we did not like to measure (e.g., scale bars or parts of the root system, where segmentation did not work well). This binary segment of the original image scene was used for analysis. The results included summary statistics such as mean root diameter and the percent of porosity.

### 2.5. Stem Growth Development, Excavation, and Dry Weight

From April to November 2017, we measured the diameter of the trees at a height of 1 m at the start of each month using a digital calliper. Measurements in two perpendicular directions (N-S and E-W) were performed and averaged.

After leaf fall, all trees were excavated with a tree digger in November 2017 and cut into three compartments: Branches, stem, and roots. The root bales, cut at the root collar, were carefully washed to remove stones and soil and stored in the open air, similar to the other biomass. Fine roots (<2 mm) could not be preserved during this procedure. Subsequently, the biomass was oven dried at 65 °C for 72 h and then weighed using a balance with an accuracy of up to four decimal digits to obtain the dry weight, which is referred to hereafter as “biomass”.

### 2.6. Statistical Analysis

We tested for significant differences for dry branch, stem, and root weight as well as the root:shoot ratio among the three species and cultivars. First, the datasets were tested for a normal distribution (Shapiro–Wilk test). Wherever necessary, data were log transformed in order to correct for skewness. To determine the effects of any treatment and species/cultivar, all data were subjected to two-way analysis of variance (ANOVA) for unbalanced designs with a significance level of  $\alpha = 0.05$ . Further comparisons were made for the species/cultivar effect, since it contained more than two levels. To check for differences in the means of the species and cultivars we used one-way ANOVA for normally distributed data and the Kruskal–Wallis H as the non-parametric alternative and applied post-hoc tests (Tukey’s post-hoc test and Dunn–Bonferroni test) for follow-up comparisons.

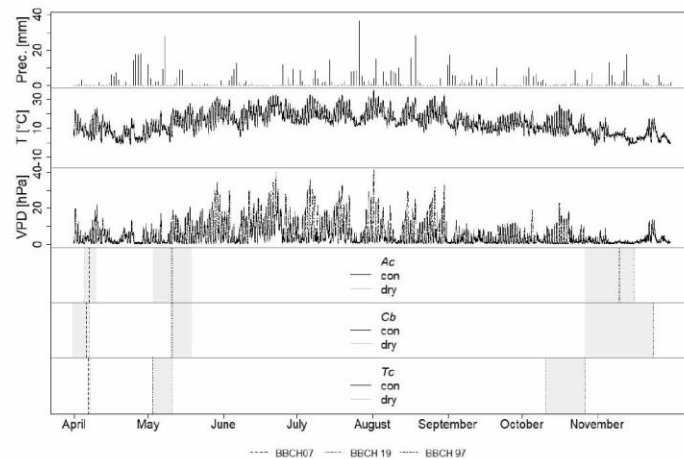
The resistance ( $R_L$ ) to drought was quantified as the ratio between the physiological performance during drought and normal conditions, as represented by the control plants [19], and the root:shoot ratio was calculated as the ratio between the root and above-ground dry mass. The above-ground biomass did not include the leaves of the trees and below-ground biomass not the fine roots (<2 mm), thereby the root:shoot ratio did not reflect investment of photosynthates between the whole above- and below-ground organs [18]. For all statistical analyses and their visualisation the software package R [33] was used. Measurements of VWC in 10 cm soil depth were processed and visualised with the help of ArcGIS, version 10.2.1 (Esri, Redlands, USA) [34]. To create a map from the discrete data, we compared the results of different interpolation algorithms provided by the software. The inverse distance weighted (IDW) method was selected as the most appropriate approach evaluated by visual inspection.

## 3. Results

### 3.1. Environmental Conditions

After a strong return to cold weather in April, a midsummer-like temperature of 14.5 °C on average was measured in May. The month of June was also exceptionally warm, dry, and sunny with VPD values of > 30 (Figure 3) after strong precipitation events that occurred at the beginning of the month. As compared to the long-term (1981–2010) average, July and August with precipitation amounts of 137.9 mm and 139.3 mm, respectively were extremely rainy, alternating

with high sunshine levels. In autumn, it was cold, rainy, and considerably cloudy for most of days. Snow fall and night frosts started in the middle of November [25].



**Figure 3.** Daily amounts of precipitation (mm), temperature (°C), and vapour pressure deficit (hPa) was noted for the entire study period in 2017, as well as the phenological timing of the three species and cultivars under drought treatment (grey) and normal conditions (black). The vertical lines indicate the dates for the beginning of bud burst (BBCH 07), the completion of leaf folding in spring (BBCH 19), and the end of leaf-fall in autumn (BBCH 97), respectively, and represent the median of the observed trees per species/cultivar and treatment. The grey bars mark the time periods during which the phenological progresses occurred. Gaps in the meteorological data were replaced with the data from the German Weather Service (DWD; available online) measured in the Munich City Centre. For full species and cultivar names see Table 1.

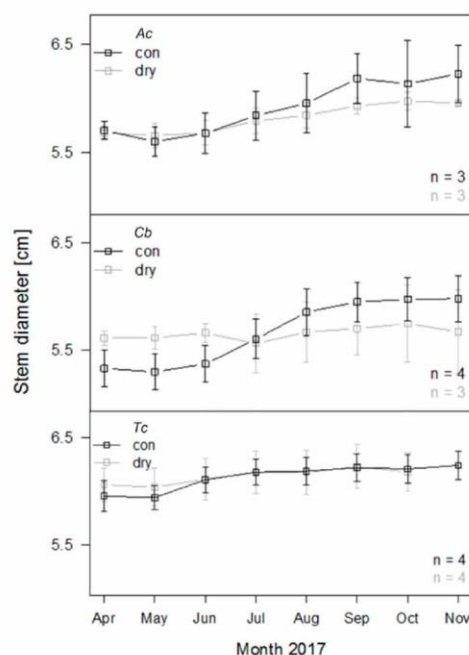
The analysis of the soil moisture data confirmed a high correlation ( $r_s = 0.8$ ) between the measurements at 10 and 30 cm, with the VWC being an average of 28.4% higher in the deeper soil layer. In 10-cm soil depth, the dry treatment plots showed significantly lower soil moisture contents ( $P = 0.004$ ) in comparison to the control plots (Figure 2). Here, a minimum VWC value of 10.1 Vol-% was measured for *Tc*-dry at the end of July (drop below the PWP). At 30 cm, the differences between the control and treatment groups for all the three species and cultivars were also significant ( $P < 0.001$ ), which indicated the overall success of the rainfall exclusion experiment.

### 3.2. Leaf Phenology

*Ac* was less uniform concerning its timing of bud burst (BBCH 07) in comparison to *Cb* and *Tc* (Figure 3). For all the three tree species and cultivars, the completion of leaf expansion (BBCH 19) was observed during the second week of May. The fastest developing species/cultivar was *Tc*, where some individuals unfolded their leaves already at the beginning of May. In autumn, the timing of leaf fall among the different species/cultivars and treatments spanned several weeks. Individuals of *Tc*-dry were leafless (BBCH 97) in the middle of October but the onset of leaf discolouration (BBCH 92) was already observed in July (data not shown). In contrast, the drought conditions caused no difference in the timing of autumn phenology in *Cb* and *Ac*-dry kept its foliage even longer than *Ac*-con.

### 3.3. Stem Growth

Stem diameter growth in the control plot and in relation to water shortage was analysed for the entire year of 2017 (Figure 4). For *Tc*, the growth in both the treatments had a distinct maximum value in May and almost stagnated toward autumn. Concerning the yearly diameter growth, this cultivar showed medium resistance to drought ( $R_{ic} = 0.46$ ). *Ac* showed the maximum growth later in the year in the months of August and showed lowest biomass losses of all species and cultivars. *Cb*-con exhibited its highest growth in the months of June and July. The growth of *Cb*-dry was strongly impacted in June, which was exceptionally warm and dry (Figure 3). Its stem diameter decrease in this month was the highest from all species and cultivars, which was the main reason for its extremely low resistance value calculated for the entire whole year ( $R_{ic} = 0.21$ ).



**Figure 4.** Stem diameter growth of all investigated species/cultivars and treatments (dry and control) for the entire study period in 2017 measured at a height of 1 m at the beginning of each month. The panel represents the mean of all trees per species/cultivar and the treatment and error bars indicate standard deviation. For full species and cultivar names see Table 1.

### 3.4. Biomass Partitioning and Allocation

Species/cultivar and treatment had always a significant effect on biomass, whereas the species/cultivar was the stronger influencing variable in each of the three cases (Table 2, Figure 5). Generally, the largest proportion of biomass was measured at the trunk section, followed by the root system. The branches had the lowest share.

For the branch biomass, *Ac*-con reached highest ( $1121.33 \pm 177.46$  g), and *Cb*-dry and *Tc*-dry lower mean values ( $365.33 \pm 60.34$  and  $308.50 \pm 49.10$  g), respectively. Species/cultivar and treatment strongly influenced ( $p < 0.001$ ) the whole branch biomass of a tree.

For stem biomass, *Ac* showed the highest values in the control group ( $6162.33 \pm 1204.59$  g), and the lowest values were noted for *Tilia* in both the treatments (con:  $4089.25 \pm 220.16$  g, dry:  $3407.00 \pm 322.40$  g). *Ac* and *Cb* exhibited higher resistance ( $R_{lc} = 0.92$  and  $0.96$ , respectively) in comparison to *Tc* which lost more biomass due to the rainfall exclusion ( $R_{lc} = 0.83$ ). The treatment effect was a bit smaller ( $P = 0.0146$ ) in comparison to the dry branch and root weight.

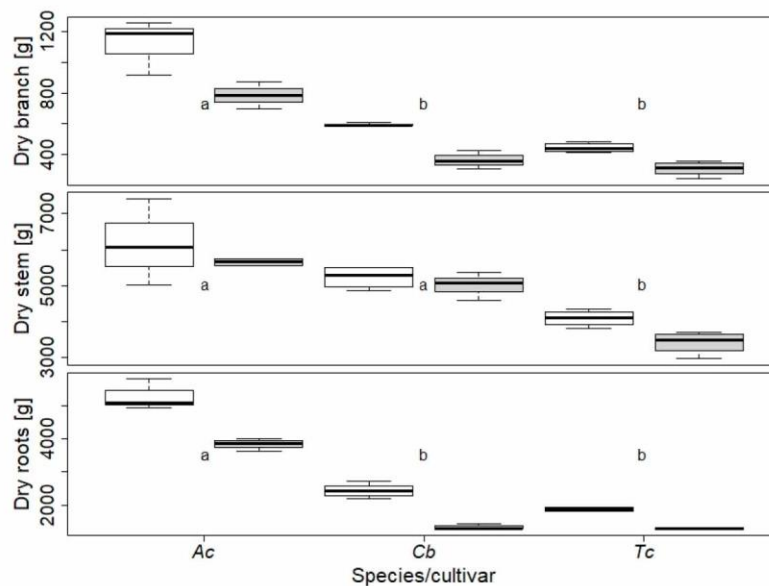
For roots, *Ac*, both in the control and dry treatments, had more biomass than any other group (con:  $5280.00 \pm 467.73$ , dry:  $3830.00 \pm 186.62$  g) despite the strong treatment effect. *Cb*-dry and *Tc*-dry showed the lowest values ( $1338.67 \pm 89.39$  and  $1291.75 \pm 25.66$  g), as was also noted for the branch biomass.

For the root:shoot ratios, a very high ( $P = 0.005$ ) treatment effect was observed. The roots of *Ac*-con and *Ac*-dry reached the highest values ( $0.73 \pm 0.08$  and  $0.59 \pm 0.02$  g g<sup>-1</sup>), and *Cb*-dry and *Tc*-dry showed the lowest average values ( $0.25 \pm 0.01$  and  $0.35 \pm 0.03$  g g<sup>-1</sup>), respectively.

**Table 2.** Weight (g) of dry branch, stem, and root biomass of the three study species and cultivars grown under the control and treatment conditions. Descriptive statistics and resistance with respect to the control ( $R_{lc}$ ) are given as well as results from two-way ANOVA with treatment and species/cultivar as the fixed effects. P-values are given for a significance level of 0.05. n.s. indicates not significant, \* statistically significant ( $P < 0.05$ ), \*\* highly significant ( $P < 0.01$ ), and \*\*\* very highly significant ( $P < 0.001$ ). For full species and cultivar names see Table 1.

Dry Weight (g)	Group	n	Mean	±SD	Min	Media	Max	R <sub>lc</sub>	Drought Effect Species/Cultivar Effect	
Branch	Ac	con	3	1121.33	177.46	920	1189	1255	0.70	P < 0.001 *** P < 0.001 ***
		dry	3	785.00	89.50	696	784	875		
	Cb	con	4	593.25	8.54	588	590	606	0.61	
		dry	3	365.33	60.34	309	358	429		
	Tc	con	4	445.75	31.02	415	442	485	0.69	
		dry	4	308.50	49.10	243	317	358		
Stem	Ac	con	3	6162.33	1204.59	5015	6055	7417	0.92	P = 0.0544 <sup>n.s.</sup> P < 0.001 ***
		dry	2	5652.50	127.99	5562	5652	5743		
	Cb	con	4	5226.75	315.97	4862	5276	5492	0.96	
		dry	3	5007.67	389.79	4592	5066	5365		
	Tc	con	4	4089.25	220.16	3817	4102	4336	0.83	
		dry	4	3407.00	322.40	2962	3480	3707		
Roots	Ac	con	3	5280.00	467.73	4938	5089	5813	0.73	P < 0.001 *** P < 0.001 ***
		dry	3	3830.00	186.62	3628	3866	3996		
	Cb	con	4	2440.25	219.50	2193	2422	2725	0.55	
		dry	3	1338.67	89.39	1271	1305	1440		
	Tc	con	4	1869.00	63.59	1807	1863	1943	0.69	
		dry	4	1291.75	25.66	1266	1290	1321		
Root:Shoot Ratio	Ac	con	3	0.73	0.08	0.68	0.70	0.82	0.81	P = 0.005 ** P < 0.001 ***
		dry	3	0.59	0.02	0.58	0.59	0.60		
	Cb	con	4	0.42	0.02	0.40	0.41	0.45	0.60	
		dry	3	0.25	0.01	0.24	0.25	0.26		
	Tc	con	4	0.41	0.03	0.39	0.40	0.45	0.85	
		dry	4	0.35	0.03	0.33	0.34	0.39		





**Figure 5.** Box plots for dry branch, stem, and root weight (g) for the three study species and cultivars of the control (white box plots) and dry treatments (grey box plots). Small letters indicate the results of the follow-up comparisons for the species/cultivar effect; means are significantly different ( $P < 0.05$ ) when indicated by different letters. For full species and cultivar names see Table 1.

### 3.5. Species/Cultivar-Specific Adaptation and Changes of Tree Shapes with Drought

*Tc* was the smallest species/cultivar concerning height (con:  $5.13 \pm 0.22$  m, dry:  $5.23 \pm 0.18$  m) (Table 3), but the narrow and pyramidal shaped *Cb* reached the lowest maximum canopy radius (con:  $28.50 \text{ cm} \pm 1.50$  cm, dry:  $21.33 \pm 0.94$  cm) and canopy volume (con:  $0.36 \text{ m}^3 \pm 0.09$  m<sup>3</sup>, dry:  $0.11 \pm 0.05$  m<sup>3</sup>). LAI was lowest for *Ac* in both the treatments (con:  $1.52 \pm 0.11$  m<sup>2</sup> m<sup>-2</sup>, dry:  $1.81 \pm 0.14$  m<sup>2</sup> m<sup>-2</sup>). *Cb* reached highest values, especially in the dry treatment (con:  $3.01 \pm 0.87$  m<sup>2</sup> m<sup>-2</sup>, dry:  $4.11 \pm 1.49$  m<sup>2</sup> m<sup>-2</sup>). *Ac* and *Cb* showed the deepest rooting system in the control and dry treatments and exhibited a medium rooting width. The root system of *Cb*, however, was less dense in comparison to that of *Ac*, especially in the control treatment, where this cultivar showed the highest porosity of all species/cultivars ( $0.33 \pm 0.05\%$ ). *Tc* exhibited medium rooting depth in both the treatments (con:  $28.50 \pm 1.50$  cm, dry:  $29.00 \pm 1.41$  cm), but the lowest rooting width of all species/cultivars (con:  $30.75 \pm 1.75$  cm, dry:  $28.67 \pm 2.77$  cm). For *Tc*, soil drying showed the biggest effect on the porosity of the root system (con:  $0.24 \pm 0.01$ , dry:  $0.38 \pm 0.04$ ) and, therefore, the rooting density. No effect was noted for *Ac* (con:  $0.30 \pm 0.03$ , dry:  $0.30 \pm 0.05$ ), while only a little effect was noted for *Cb* (con:  $0.33 \pm 0.05$ , dry:  $0.34 \pm 0.03$ ).

**Table 3.** The above- and below-ground dimensions of the three investigated species and cultivars and the respective treatments (control and dry) as well as the density of foliage and root system are expressed by the means of leaf area index ( $\text{m}^2 \text{m}^{-2}$ ; LAI) and the percent of porosity of the root system, respectively.

Above-Ground								
Code	Height [ $\text{m} \pm \text{sd}$ ]		Max. Canopy Radius [ $\text{cm} \pm \text{sd}$ ]		Canopy Volume [ $\text{m}^3 \pm \text{sd}$ ]		LAI [ $\text{m}^2 \text{m}^{-2} \pm \text{sd}$ ]	
	Control	Dry	Control	Dry	Control	Dry	Control	Dry
Ac	5.90 $\pm$ 0.14	6.00 $\pm$ 0.00	48.33 $\pm$ 8.06	50.00 $\pm$ 9.63	0.64 $\pm$ 0.25	0.67 $\pm$ 0.23	1.52 $\pm$ 0.11	1.81 $\pm$ 0.41
Cb	5.90 $\pm$ 0.25	5.53 $\pm$ 0.17	28.50 $\pm$ 1.50	21.33 $\pm$ 0.94	0.36 $\pm$ 0.09	0.11 $\pm$ 0.55	3.01 $\pm$ 0.87	4.11 $\pm$ 1.49
Tc	5.13 $\pm$ 0.22	5.23 $\pm$ 0.18	43.00 $\pm$ 17.45	52.50 $\pm$ 11.37	0.65 $\pm$ 0.20	0.68 $\pm$ 0.22	2.53 $\pm$ 0.25	2.13 $\pm$ 0.24
Below-Ground								
Code	Rooting Depth [ $\text{cm} \pm \text{sd}$ ]		Max. Rooting Width [ $\text{cm} \pm \text{sd}$ ]		Root Volume [ $\text{m}^3 \pm \text{sd}$ ]		Porosity Root System [% $\pm$ sd]	
	Control	Dry	Control	Dry	Control	Dry	Control	Dry
Ac	30.33 $\pm$ 7.85	29.67 $\pm$ 0.47	31.00 $\pm$ 2.75	29.92 $\pm$ 2.00	0.050 $\pm$ 0.019	0.056 $\pm$ 0.008	0.30 $\pm$ 0.03	0.30 $\pm$ 0.05
Cb	30.00 $\pm$ 0.00	30.00 $\pm$ 0.00	32.25 $\pm$ 0.98	29.00 $\pm$ 2.01	0.057 $\pm$ 0.005	0.036 $\pm$ 0.007	0.33 $\pm$ 0.05	0.34 $\pm$ 0.03
Tc	28.50 $\pm$ 1.50	29.00 $\pm$ 1.41	30.75 $\pm$ 1.75	28.67 $\pm$ 2.77	0.058 $\pm$ 0.000	0.047 $\pm$ 0.009	0.24 $\pm$ 0.01	0.38 $\pm$ 0.04

## 4. Discussion

### 4.1. Drought Resistance and Within-Plant Carbon Partitioning and Allocation

In our study, we observed various degrees of above- and below-ground biomass gains and losses under drought, suggesting different strategies and abilities of tree species and cultivars to cope with this stressor. Generally, biomass as well as the root:shoot biomass dynamics and the root densities were significantly affected by the strong drought applied.

Among the species and cultivars, *Acer campestre* showed the most uniformly distributed losses over the entire plant body under drought, not as *Carpinus betulus* 'Fastigiata', for example, which showed more specific allocation patterns. The high root:shoot ratios of *Ac*, although it could not maintain its normal root production patterns as evident by its relatively low resistance value (Table 2), its high rooting depth and root density were assumed to be helpful mechanisms in survival. They could explain why this species did not show any signs of premature leaf senescence and fall in the autumn season and also why it had relatively high resistance values concerning biomass, stem growth (Table 2), as well as water use [15]. All data supported the assumption that this species was extremely drought resistant [15,19].

With a southern distribution margin, *Ac* originated from a more drought-prone habitat in comparison to *Cb* and *Tc* [35,36]. The extremely potent root system of *Ac* (Table 2 and 3) and its extremely low hydraulic demand due to low LAI (Table 3) could be due to its origin from a drier habitat [18], which suggested a high drought tolerance of this species, also supported by the drought-tolerance ranking of Niinemets and Valladares [27] (Table 1).

*Cb* showed low resistance to drought with high losses in the branch and the root biomass and hence a decreasing root:shoot ratio; the latter suggests that under drought, this cultivar does not allocate much carbon to the development of the root system. Instead, with its very densely leafed canopy (Table 2; [14]) it has a high area of hydraulic demand in relation to a low amount of supplying root biomass, which makes it susceptible to drought [13]. This assumption is also in line with the species ranking of Niinemets and Valladares [27] (Table 1), giving the species *Carpinus betulus* the lowest drought-tolerance value among all study species and cultivars.

Under the rainfall exclusion experiment, *Tilia cordata* 'Greenspire' lost branch and root biomass at high rates but could maintain a relatively high root:shoot ratio in combination with a considerable increase in the density of the root system (Table 2). This observation indicates that carbon assimilation was so strongly reduced by drought that there was insufficient assimilation to facilitate its allocation to the entire plant system. Due to the high overall losses in biomass and the extremely early discolouring and fall of leaves (Figure 3), a high susceptibility to drought was assumed for this cultivar. This finding is in line with the results of Stratópoulos [15] who conducted their study at the same location and in the same year and showed that *Tc* had much lower resistance in terms of water use in comparison to *Acer campestre* and *Acer platanoides* and is still considerably lower than that of *Cb*.

### 4.2. The Role of the Root System in Sustaining Drought

Within the below-ground biomass component, organs perform a range of functions. Coarse roots mainly provide stability, transport, storage, and conduction functions while the fine roots mainly absorb and conduct water and nutrients [37,38]. Generally, plants may increase their soil-to-root or rhizosphere conductance by adjusting their fine-root density, fine root hydraulic conductance, rooting depth, and other root characteristics [13].

Recent research has shown that deep roots can be of pivotal importance to alleviate water stress in several plants [39]. They particularly play the central role for drought tolerance in the tropical and subtropical environments [40]. In our study, we saw a slight increase in the rooting depth of *Tc* under drought conditions (Table 3). Only in *Ac*, both the width and depth of the root system were slightly reduced under decreased soil moisture, while the root biomass of *Ac*-dry was still higher than that of any other species/treatment combination, except for that of its own control

group. Moreover, it had the densest root system of all species and cultivars in the dry treatment. In another study [17], it was observed that *Tc* developed its fine roots more in the horizontal than in the vertical direction to cope with drought. Contrarily, we recorded a decrease in the width of the root system (coarse roots) under drought conditions. A possible explanation for this could be that *Tc* did invest less carbon in developing their coarse root system for stability, and rather gave priority to the development of fine roots in order to enhance its water uptake capacity. Such similar phenomenon of reducing coarse roots was also noted for *Ac* and *Cb*.

It should be considered, however, that for growing, particularly the soil conditions in the nursery in the study under which the root systems developed are fundamentally different from those in the real city environment. Here, the underground site conditions usually don't allow unhampered root development making knowledge about genetically determined types of root systems for species and cultivars applicable only to a limited extent. A study on city trees [41] has shown that an important criterion for spatial root development is the existence of loose and permeable soil parts, toward which trees develop their roots. This high influence of the absence and presence of oxygen on the root system architecture leads to extremely untypical growing patterns in the urban environment [41]. It suggests that the spatial extensions measured in this study under almost unlimited rooting space should become less of a concern in species and cultivar choice in comparison to that in the species- and cultivar-specific root system densities and biomass amounts.

## 5. Conclusions

Predictions suggest a future increase in climate extremes, such as severe drought, due to climate change [1]. Consequently, limited availability of water will be an important cause for tree stress or even complete desiccation that leads to cellular death with significant implications for functions and ecosystem services provided by the trees. The presence or absence of specific structural and functional adjustments and response capacity to cope with the dry conditions may impact species' and cultivars' survival and mortality. In our study, we measured several indexes of three species and cultivars under a controlled experiment and a harvest campaign was applied for the biomass of stem, branch, and root. Valuable information about the root:shoot biomass relations of different tree species and cultivars are provided, and this could be an important selection criterion in species and cultivars choice.

It is assumed that non-lethal droughts can promote drought resilience or the ability to survive droughts because of the acclimation of plant traits [13], future studies should therefore monitor species' performances over several years, including through pre-drought stages and wet periods, where morphological and physiological adaptations clearly manifest themselves according to the respective resource supplies. In an advanced development stage of the trees, this aspect could be achieved by using permanent girth tapes at the stem and root [24] or through retrospective increment core sampling [42].

In this study, *Cb* and *Tc* coped less well with drought than *Ac*. Therefore, they should be rather planted in park-like environments with sufficient water supply, while *Ac* may be more suitable for sites with chronic, severe droughts. Trees should be planted and maintained according to their different strategies and site requirements. For this purpose, we need knowledge about the general growth habit of a species/cultivar, its within-plant carbon partitioning and allocation patterns under changing growing conditions, and the annual growing and wilting cycles, which affect the timing of their physiological processes. In addition, the respective resource supply in the native habitat appears to be a good proxy for drought tolerance, suggesting to progressively plant (non-native) species and cultivars from drier habitats at harsh urban sites [14,15].

We suggest that special attention should be paid to the below-ground biomass and density, which, until date, has received limited research attention. Moreover, sufficient space for the expansion of root growth is necessary to defend drought risk. Future experimental studies are supposed to reduce or even close the disparity in the knowledge between the below-ground processes in comparison to the above-ground processes, which are until date, reasonably better understood. Corresponding knowledge is expected to provide important insights for urban

greenspace planning and management, particularly for the selection of suitable tree species and cultivars.

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