The role of soil (a)biotic properties on performance of urban plant species

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A thesis submitted in partial fulfilment of the requirements for the degree of Master of Research

Declaration

I declare that this thesis, as a whole or in parts, has not been submitted for a higher degree to any other university or institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

I wish to acknowledge the following assistance with the research detailed in this thesis: Michelle Leishman, Anthony Manea and Jaco Le Roux for guidance and assistance with project planning, experimental design, interpretation, and comments on a draft of this manuscript. Michelle Leishman and Anthony Manea for assistance with statistical analysis and interpretation. Muhammad Masood for assistance in the glasshouse operations.

All other research described in this report is my own original work.

No ethics approval was required for this project.

07/08/2022

Sashini Perera Date

This thesis is written in the form of a manuscript for submission to the *Journal of Ecology*, the majority of the author guidelines have been followed, with some exceptions to meet the requirements of Macquarie University. This includes the Abstract having a 200-word limit, 1.5 line spacing, line numbers and the exclusion of a running title. Further, subheadings are included in the Introduction and Discussion sections, while tables and figures are embedded within the text.

Table of Contents

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Candidate's statement about the impact of COVID-19 changes on the thesis

Dear Examiner,

Many of our HDR candidates have had to make changes to their research due to the impact of COVID-19. Below you will find a statement from the candidate, approved by their Supervisory Panel, that indicates how their original research plan has been affected by COVID-19 restrictions. Relevant ongoing restrictions in place caused by COVID-19 will also be detailed by the candidate.

Candidate's Statement

Initially, there was an aim to study biotic (i.e., microbial diversity and richness) soil properties that characterise urban and non-urban sites in this research by extracting whole genomic DNA and carrying out molecular microbial analysis for the field soil samples and the soil samples after the glasshouse experiment. However, due to COVID-19 lockdown restrictions that were in place last year, the field work got delayed by three months. Extra two months were offered by the university. However, the time was not enough to get the microbial molecular analysis done and get the results back in time for the submission of the thesis. Hence, the microbial data will be used in a publication in the future.

Abstract

The vegetation of urban areas provides many essential benefits. However, urban environments can be challenging for plant growth, with limited space, poor soil quality and low water availability. In addition, many cities are becoming hotter and drier with climate change. This research aimed to test (1) how abiotic properties of urban compared with non-urban soil affect plant performance, and (2) the effect of microbial communities in urban soil compared with non-urban soil on plant resilience to drought stress. To investigate this, I grew three native horticultural tree species (*Angophora costata*, *Callistemon citrinus* and *Syncarpia glomulifera*) in a fully factorial glasshouse experiment. The factors were site (urban streetscape and bushland), soil treatment (sterilised and unsterilised) and water treatment (well-watered and drought). I measured plant stress (Fv/Fm), biomass and allocation. Surprisingly, plants grown in the high nutrient urban soils had greater total biomass than their counterparts grown in non-urban soil, irrespective of the water treatment. Plant biomass was significantly reduced in all three species under water stress treatment. This decline in growth was not negated by the presence of a soil microbiome, irrespective of soil type. These results suggest that soil abiotic properties have a greater effect on plant performance compared to the biotic properties.

Keywords: abiotic properties, plant biomass, plant performance, soil microbes, urban soil.

1. Introduction

Urbanisation is rapidly expanding globally, with this trend projected to continue into the future (Bainard *et al*., 2011). By 2050, it is expected that ~70% of the world's population will live in urban areas (United Nations, 2019), placing increasing pressure on urban green infrastructure. Urban green infrastructure can be defined as street trees, public parks and gardens, remnant bushland, green roofs, and stormwater management systems that incorporate vegetation, such as rain gardens (Lähde & Di Marino., 2019; Ariluoma *et al*., 2021).

Urban green infrastructure provides a range of social, health, economic and environmental services that can substantially improve the liveability of cities and thus human well-being (Feng & Tang, 2017; Song *et al.,* 2018). For example, urban green spaces have been shown to mitigate the urban heat island effect (Chen and You, 2020; Roth, 2007) and extreme temperatures (Ossola *et al.,* 2020) through evaporative cooling and shading (Bowler *et al.,* 2010), increase stormwater capture (Kuehler *et al.,* 2017; Song *et al*., 2018), sequester carbon to mitigate climate change (Nowak and Crane, 2002), absorb gaseous pollutants through plant stomata (Janhäll, 2015), reduce urban noise (Ow & Ghosh., 2017), provide habitat for fauna (Song *et al.,* 2018) and enhance aesthetic appeal (Nowak & Dwyer, 2007). Therefore, the sustainable management of green spaces in urban areas is critical (Cheng *et al.,* 2021; Nowak & Greenfield, 2010).

In recognition of the beneficial services urban green spaces provide, an increasing number of cities around the world are implementing urban greening strategies (Beatley, 2012; Bush *et al*., 2020; Mell *et al*., 2017; Tan *et al*., 2013). For example, the ongoing "Greening our city" program in the Greater Sydney region aims to decrease the impact of urban heat island effect and to increase the resilience of the city towards changing climate by planting one million trees by end of 2022 (NSW Department of Planning and Environment). Further, this program is part of a broader program to plant five million trees by 2030 (NSW Department of Planning and Environment) and achieve 40% urban tree canopy cover in Greater Sydney by 2036. These strategies typically aim to increase canopy cover while maintaining existing canopy cover, identify priority planting areas through tree canopy cover analyses, develop and enhance tree asset databases and raise public awareness of the benefits of urban greening through various programs (Cooke, 2020; McPherson *et al*., 2011; Zhao, 2011).

Challenges posed by urban soils to plant performance

Urban areas globally are facing significant pressures from population growth, environmental degradation, and climate change (Doherty *et al*., 2016; Gasper *et al*., 2011; Leung, 2015). The impact of these pressures on urban areas means that they can be challenging environments for plants to survive and thrive (McDowell *et al*., 2008; McKinney, 2008). In particular, it is the impact of these pressures on urban soils that may have a profound impact on urban greening outcomes (Guilland *et al.*, 2018). In addition to providing the substrate that plants grow in, urban soils provide a range of critical services such as carbon sequestration, nutrient storage and providing habitat for microorganisms, many of which (such as mycorrhizae and rhizobia) may provide benefits to plants (Morel *et al*., 2015; Vasenev & Kuzyakov, 2018). Therefore, soils represent a critical component of urban ecosystems.

The soil in urban areas can represent a significant challenge for the success of urban green spaces. Urban soils are often highly modified when compared to soil found in natural undisturbed environments (Doick & Hutchings, 2013). They are generally highly compacted, anoxic, excessively stony and have a lack of natural soil horizons due to erosion and reduced organic matter content (Cekstere & Osvalde, 2013; Guilland *et al*., 2018; Jim, 2019; Seto *et al*., 2014). These factors result in the structural (e.g., aeration, drainage) and functional degradation of the soil, which significantly alters the abiotic soil properties such as water and nutrient availability, pH, and salinity (Jim, 1998).

Urban soils are often water-limited due to the impervious surfaces (e.g., concrete, asphalt) that characterise urban areas reducing rainfall infiltration into the soil (Kuehler *et al*., 2017; Xiao *et al*., 2000). These surfaces also absorb and retain heat, thus exacerbating the urban heat island effect and water limitation (Brown *et al*., 2015; Golden *et al*., 2008; Stewart & Oke, 2012). In addition to these factors, soil compaction and space constraints in urban areas restrict plant root growth, which reduces the ability of plants to uptake water from the soil (Roberts *et al*., 2006). As plants rely on water to perform photosynthesis as well as transport organic and inorganic molecules through their tissues, reduced soil water availability in urban areas may severely impact the growth and survival of plants directly (McElrone *et al*., 2013).

Soil nutrient patterns in urban areas, are primarily modified by anthropogenic land use and vegetation cover (Su *et al.*, 2022). Reported nutrient patterns of urban and non-urban soils are inconsistent. For example, multiple studies have shown that the deposition of inorganic N (NO_3^- + NH_4^+) is significantly greater in urban areas compared to non-urban areas in the United States

(Lovett *et al.*, 2000; Su *et al.*, 2022; Templer & McCann, 2010). In contrast, Li *et al*. (2013) measured soil nutrients in 405 sites and showed that urban soils had lower nitrogen content than non-urban soils in Hubei, China. Within the Sydney region, Leishman et al. (2004) found that storm water outlets greatly increased soil phosphorus content in urban soils in the Sydney region.

As a result of the weathering of calcareous concrete surfaces, which releases carbonate (Ordóñez *et al*., 2018; Seto *et al.*, 2014), urban soils closer to concrete surfaces tend to have an alkaline pH (Acosta *et al.*, 2015; Hui *et al*., 2017; Nannoni *et al*., 2014; Pouyat *et al.*, 2007; Puskás & Farsang, 2009). This may have an effect on plant performance because it can reduce the availability of nutrients to plants (Vrščaj *et al*., 2008).

Salinity is another soil abiotic property that impacts plant growth. Typically, it is human-associated secondary salinity that impacts urban areas, as opposed to primary salinity, which is the natural occurrence of salts (e.g., salt lakes, tidal zones) in the landscape. Secondary salinity in urban areas is caused by a combination of too much water seeping into the ground from overwatering gardens as well as leaky water supply and sewer pipes, and infrastructure (e.g., retaining walls, roads) acting as barriers to groundwater flow (Nouri *et al.,* 2013). This causes a rise in the water table, which brings salt to the soil surface (Nouri *et al.*, 2013). Increased soil salinity places osmotic and ionic stress on plants, leading to dehydration of plant tissue, which negatively impacts plant growth and survival (Munns & Tester, 2008; Ordóñez *et al*., 2018). High salinity can also inhibit water uptake and damage root cells. For example, Baraldi *et al.* (2019) found that photosynthesis in *Liquidambar styraciflua*, a commonly planted tree species in urban areas, was reduced due to transient salinity stress. Delgado *et al.* (2021) also found a positive correlation between the proportion of Cercozoa and mean annual precipitation and a positive relationship between bacterial and protist richness.

The impacts of climate change on urban green spaces

The challenging plant growth conditions present in urban areas may be exacerbated by climate change (Gillner *et al.*, 2014). For instance, a recent study showed that one-third of commonly planted tree species in Australian urban environments are likely to lose more than 50% of their climatically suitable habitat by 2070 (Burley *et al.*, 2019). In particular, the projected increase in extreme drought events is likely to have a profound impact on plants in urban environments (Naumann *et al.*, 2018; Naylor & Coleman-Derr., 2018; Lesk *et al.*, 2016). For example, Miller et al. (2020) found that urban tree and turfgrass cover significantly declined in Santa Barbara, USA, during the 2012-2016 California drought. Similarly, Tabassum *et al.* (2021) found that many

commonly planted exotic deciduous urban tree species in Penrith, NSW, Australia suffered significant leaf necrosis and mortality in response to prolonged dry conditions and extreme heat experienced in 2019.

Climate change can impact plants in urban green space through multiple pathways, altered precipitation patterns resulting in increased frequency and severity of droughts and flooding (Naumann et al., 2018) and extreme heat (Zhang et al. 2013; Stott, 2016). Climate change may also indirectly impact urban plant growth via changes in phenology and abundance of pathogens, pests, herbivores, pollinators and mutualists such as mycorrhizae.

Interactions between plants and soil microbial communities

Plants interact with a vast number of soil microbial species throughout their lives. These include antagonistic interactions (e.g., pathogens) and mutualistic interactions (e.g., mycorrhizae). These interactions may influence plant ecophysiological responses and, thus, performance. For example, plants employ a range of strategies to ameliorate the impacts of drought stress such as inducing stomatal closure, increasing leaf senescence, and investing in root growth (Chaves *et al*., 2003). Plants may further increase their drought tolerance by forming mutualistic relationships with certain soil microbes that enhance the plant's ability to obtain water and nutrients from the soil in exchange for carbon (Ray *et al*., 2020). For example, arbuscular mycorrhizal fungi have been found to decrease plant drought stress by allowing the plants to explore the soil more thoroughly for water, improving stomatal conductance and increasing antioxidant enzyme activity **(**Augé *et al*., 2015**).** Ectomycorrhizal fungi also provide plants with water, nutrients, and protection from pathogens in exchange for photosynthate (Allen *et al*., 2003; Beiler *et al*., 2015). The importance of soil microbes to plant performance is exemplified by the fact that microbial species richness is generally a strong predictor of plant health and productivity (Van Der Heijden *et al*., 2008; Wagg *et al*., 2011). On the other hand, plant performance can be negatively influenced by the pathogens present in soil (Bever *et al.*, 2012; Yim *et al*., 2013).

Not all microbial taxa are equally represented in soil (Fierer, 2017) and, like all living organisms, their richness and community composition are primarily driven by the (a)biotic environmental conditions they are exposed to (Chai *et al*., 2019). In terms of biotic conditions, the diversity and structure of soil microbial communities are most significantly influenced by the composition of plant communities (Garbeva *et al.*, 2004). In terms of abiotic conditions, soil properties such as

water and nutrient availability, organic carbon, pH, and salinity are the major factors influencing soil microbial communities (Fierer & Jackson, 2006; Terrat *et al.*, 2018).

In natural forests, the underlying geology plays a major role in determining the abiotic soil properties (Augusto *et al.*, 2002). The microbiome and aboveground vegetation that is, the canopy of the forests can mediate soil temperature, the leaf litter and impact the nutrient cycling. (Augusto *et al*., 2002; Uroz *et al.*, 2016). As a result of these factors and the absence of anthropogenic disturbances, soil horizons and resource gradients are formed along the soil profile (Augusto *et al*., 2015; Uroz *et al*., 2016), which in turn can create a diverse and dynamic set of habitats in which microbes can survive (Uroz *et al.*, 2016). In contrast to natural forests, anthropogenic activities (e.g., building infrastructure) can greatly influence the soil abiotic properties and thus microbial diversity and richness in urban environments (Naylo *et al.*, 2019).

In general, microbial biomass is lower in urban soils compared to non-urban soils (Bainard *et al*., 2011; Yang *et al*., 2006; Rai *et al*., 2018; Zhu & Carreiro, 2004; see Huot *et al*., 2017 for exception). However, there is evidence to suggest that this is due to a decline in the richness of mycorrhizae fungi rather than archaea and bacteria (Schmidt *et al.,* 2017). For example, Bainard *et al.* (2011) reported that tree species growing in urban areas in Ontario, Canada had lower levels of arbuscular and ectomycorrhizal fungi colonisation than the same species growing in nearby rural areas. Baruch et al. 2020 observed that, as a consequence of differences in soil traits, above ground vegetation and management practices, the diversity and structure of soil fungal communities among metropolitan urban green spaces indicated a clear difference. Baruch et al. (2020) also suggested that urban green space fungal microbiomes can be restored via planting the native vegetation community. However, studies have shown the health of the urban microbiome is often dependent on the type and design of the green space. In another study, Joyner *et al.* (2019) studied the diversity of soil bacterial communities in five different types of green infrastructure in New York City and found that the most significant driver of community composition was the design of the green infrastructure features. Baruch *et al.* (2021) showed that soil bacterial communities are strongly affected by urban green space type and plant species richness is positively associated with soil bacterial diversity.

Providing cost-effective and sustainable solutions to environmental issues using plants is gaining traction (Chagnon, & Brisson, 2017; Nascimento *et al.*, 2021). Even though these phytotechnologies are plant-based, they need to pay special consideration to the interactions between plants and soil microbes. Yet, despite the profound impact of soil microbiomes on plant

performance in urban environments (Koziol *et al.*, 2018; Molineux *et al.*, 2017; Thrall *et al.*, 2005), our understanding of the urban soil microbiome is still rudimentary. In fact, to date, soil microbial ecology has received relatively little attention compared with other functional ecology research fields (Barrico *et al*., 2018; Guilland *et al.*, 2018) and most of this research relates to agricultural and forest ecosystems in terms of linking plant performance to soil microbial communities (Bahram *et al.*, 2015; Delavaux *et al.*, 2019; Gill *et al.*, 2020; Tedersoo *et al*., 2014). Therefore, there is significant scope for focused research on understanding the functional and biogeographical properties of soil microbiota in urban environments (Baruch *et al*., 2020; Gill *et al.*, 2020).

Research aims and hypotheses

The overall aims of my study were to 1) determine the abiotic properties (i.e. ammonium nitrogen, nitrate nitrogen, available phosphorus, organic carbon, electrical conductivity, pH) that characterise urban and non-urban soils in Sydney, NSW, Australia; 2) assess the impact of abiotic soil properties and soil microbiota on the performance of commonly planted urban plant species in Sydney; 3) examine the role of the soil microbiome on the resilience of urban plant species to drought stress.

To address these aims, I grew three tree species (*Angophora costata, Callistemon citrinus,* and *Syncarpia glomulifera*) that are native to the sandstone-derived soils of the Sydney region and that are commonly planted in urban areas, in both sterilised and unsterilised urban and non-urban soils. After twelve weeks of growth, the plants were exposed to a watering treatment (drought-stressed and well-watered) for a further six weeks. At the end of the watering treatment, stress and growth metrics of the plants were measured.

I hypothesised that the abiotic properties and microbiome of the urban soils would be more impacted by anthropogenic disturbances compared to the non-urban soils, hence, the performance of plants grown in non-urban soils will be greater than those grown in urban soils. I then hypothesised that the plants grown in soil with a microbiome present would show greater resilience to drought stress than plants grown in sterilised soil. The desired outcome of this research is to inform urban land managers on how they can utilise soil microbiota to enhance plant performance in general as well as plant resilience to drought stress, which in turn will lead to improved urban greening outcomes.

2. Material and Methods

Study species and site selection

Three shrub/tree species were selected for this study: *Angophora costata*, *Callistemon citrinus* and *Syncarpia glomulifera* (Table 1- Supplementary material). All three species occur naturally in bushland areas with low fertility Hawkesbury Sandstone-derived soils in the Sydney region, NSW, Australia and are commonly planted in Australian urban streetscapes.

Using the street tree inventories of the local government areas in the Sydney region (Ossola et al., 2020) and field guides, I selected three study locations where the study species occur in both the urban streetscape and neighbouring non-urban (i.e., bushland) sites that were in close proximity. The study locations selected were Turramurra (urban)/Ku-ring-gai Chase National Park (non-urban), Marsfield (urban)/Lane Cove National Park (non-urban) and Hornsby (urban)/Berowra Valley Regional Park (non-urban). These three study locations provided an urban streetscape/non-urban contrast within an area with a similar climate and geology. Plants were located in the road verges in urban locations.

Soil sampling and preparation

Soil sampling was carried out in November 2021. For each study species, I randomly selected eight individual plants within each site type (urban and non-urban) across all the locations. This resulted in 48 total individuals selected for soil sampling (3 locations × 2 site types (urban and non-urban) × 8 individuals). Before collecting soil samples, leaf litter around the base of each individual was cleared. An 8 L sieved (5 mm sieve) soil sample was then collected from within 1 m of the base of each individual. All equipment used for soil collection was sterilised using 80% ethanol between each sample.

In the laboratory, a 250 g sub-sample was taken from each field-collected soil sample for soil abiotic property analyses. Samples were stored at room temperature for 5 days before being sent to the external company. The remaining soil of each sample was then divided evenly into two. One half of the sample was sterilised in the autoclave (GETINGE, Zenith-42477) at 121°C for 1.5 h while the other half was kept unsterilised.

Measurement of soil abiotic properties

Ammonium nitrogen and nitrate nitrogen were measured using Rayment and Lyons method 7C2b. This involved making a solution for each soil sample using 2M potassium chloride. After dilution

of the resulting solution, ammonium nitrogen was measured colourimetrically. Nitrate nitrogen was reduced to nitrite through a copperised cadmium column and measured colourimetrically.

Organic carbon was measured using Rayment and Lyons method 6A1 (Walkey and Black 1934). This involved making a solution for each soil sample using concentrated sulfuric acid and dichromate solution. The chromic ions produced were proportional to the oxidised organic carbon and measured colourimetrically. The heat of the acid-based reaction was used to induce oxidation of organic matter.

pH and electrical conductivity were measured using Rayment and Lyons method 4A1 and 3A respectively. This involved making a solution for each soil sample using deionised water at a ratio of 1:5 before stirring for one hour. pH and electrical conductivity of the solution was measured using a pH and conductivity electrode.

Available phosphorus was measured using Rayment and Lyons method 9B. This involved making a solution for each soil sample using 0.5M sodium bicarbonate (pH=8.5) in a ratio of 1:100. This solution was left for 16 h before being acidified and measured colourimetrically.

Available potassium was measured using Rayment and Lyons method 18C1. Soils were extracted using boiling 1M nitric acid and the extract was read for potassium using atomic absorption spectroscopy.

The analyses of the soil abiotic properties were conducted by a specialist external company (CSBP, Kwinnana Beach, WA, Australia).

Study design

Seeds of the three study species were purchased from a commercial seed supplier who collect from the Sydney region. (Harvest Seeds & Native Plants, Terry Hills, NSW, Australia). The potting mix was sterilised in the autoclave (GETINGE, Zenith-42477) at 121°C for 1.5 h. Seeds were germinated in trays containing sterilised potting mix (Australian Native Landscapes, Terrey Hills, NSW, Australia). The seeds were germinated under 24/19°C day/night temperatures and mist watered thrice daily for three minutes.

Once germinated, the seedlings of each species were transplanted using a pair of forceps sterilised by dipping in to 80% ethanol for 5 seconds, out into a fully factorial glasshouse experiment with

three factors: soil type (urban and non-urban; locations were nested within soil types), soil treatment (sterilised and unsterilised) and watering treatment (well-watered and drought-stressed). For each treatment combination, eight pots had seedlings transplanted into them, with one pot representing each unique field-collected soil sample. This resulted in the experiment consisting of 192 pots (3 species × 2 soil types × 2 soil treatments × 2 watering treatments × 8 replicates). The seedlings were grown in 800 mL pots, which were sterilised prior to the experiment in 20% bleach solution. The pots were spaced 5 cm apart in pot trays to limit soil microbial cross-contamination between the soil treatments. If the seedlings died in the first week after transplanting, they were replaced with another seedling. The seedlings were grown for 12 weeks before the watering treatments were imposed. The positions of the trays were randomized once a fortnight to ensure there is no glasshouse effect (i.e., conditions may vary within a glasshouse, so by moving them around we ensured every plant is exposed to the whole range of potential conditions).

During this establishment period, the seedlings were mist watered thrice daily for 2 minutes and the trays were randomly re-allocated to new positions within the glasshouse on a fortnightly basis. After the 12-week establishment period, the watering treatments were imposed for six weeks (i.e., 18 weeks of total growth). Water treatments were allocated randomly to the trays. The seedlings designated to the well-watered treatment received 30 mL of water thrice daily. The seedlings designated to the drought treatment received 30 mL of water once daily for the first week, followed by 30 mL every second day for weeks 2 to 4 and finally 30 mL twice weekly for weeks 5 and 6. During the watering treatment period, the volumetric soil water content (VSWC) of each pot was measured weekly at a depth of 10 cm using a Hydro-sense II Portable Soil Moisture System (Campbell Scientific Australia Pty Ltd, Garbutt, Qld, Australia).

The glasshouse was set to a 24/19°C Day/night temperature for the duration of the experiment. This temperature regime was continuously maintained by a fan coil unit using a water cooling and heating system. Photosynthetically active radiation (582 µmol $m^{-2}s^{-1}$ at 1400 hr) and relative humidity (73% at 1400 hr) were monitored continuously using a MultiGrow controller system (Autogrow Systems, Auckland, New Zealand).

Harvesting and seedling performance measurements

During the final week of the watering treatment, the maximum potential quantum efficiency of photosystem II (Fv/Fm) of seedlings that were large enough was measured using a mini-PAM-II photosynthesis yield analyzer (Heinz Walz GmbH, Effeltrich, Germany). For each seedling, a fully expanded leaf was dark-adapted for 30 min at room temperature inside a growth cabinet, after

which Fv/Fm was measured on the adaxial surface of the leaf while maintaining light intensity between 500 mV to 600 mV. Measurements were taken randomly across species and treatments. At the end of the experiment, the height of each seedling was recorded. The seedlings were then harvested, rinsed free of soil, and separated into their above and belowground components before being oven-dried at 70°C for 72 hours and weighed using an analytical electronic balance (Mettler Toledo, Port Melbourne, VIC, Australia).

Data analysis

The abiotic soil properties were analysed using two-way ANOVAs, with soil type and location being the fixed factors in the models. When interactions were found, Tukey post-hoc comparisons were carried out to determine which treatment combinations differed.

VSWC was analysed using repeated measures ANOVA. Fv/Fm, total biomass, plant height and R:S were analysed using three-way nested ANOVAs. The fixed factors in these models were soil type (urban and non-urban), soil treatment (sterilised/unsterilised) and watering treatment (well-watered/drought). Location was nested within soil type. When significant interactions were identified, Tukey post-hoc comparisons were carried out to determine which treatment combinations differed.

Before conducting the data analyses, the data were checked to determine if they fulfilled the normality and equal variance assumptions of ANOVA. When the assumptions of ANOVA were not fulfilled, even after log transformation of the data, non-parametric Kruskal-Wallis analyses were used. When a significant difference was found, Mann-Whitney U tests were carried out to determine which treatment combinations differed. All data analyses were conducted at the species-level.

All data analyses were conducted using SPSS 27 (IBM Corporation, Armonk, NY, USA), with the significance level set at 0.05.

3. Results

Field soil chemistry

Ammonium nitrogen

There was no significant interaction between soil type and location for ammonium nitrogen ($F_{2,47}$=0.153; P=0.900) (Fig. 1). Further, there was no significant difference in ammonium nitrogen between the soil types ($F_{1,47}$=0.153; P=0.697) or locations ($F_{2,44}$=1.799; P=0.178) (Figure 1).

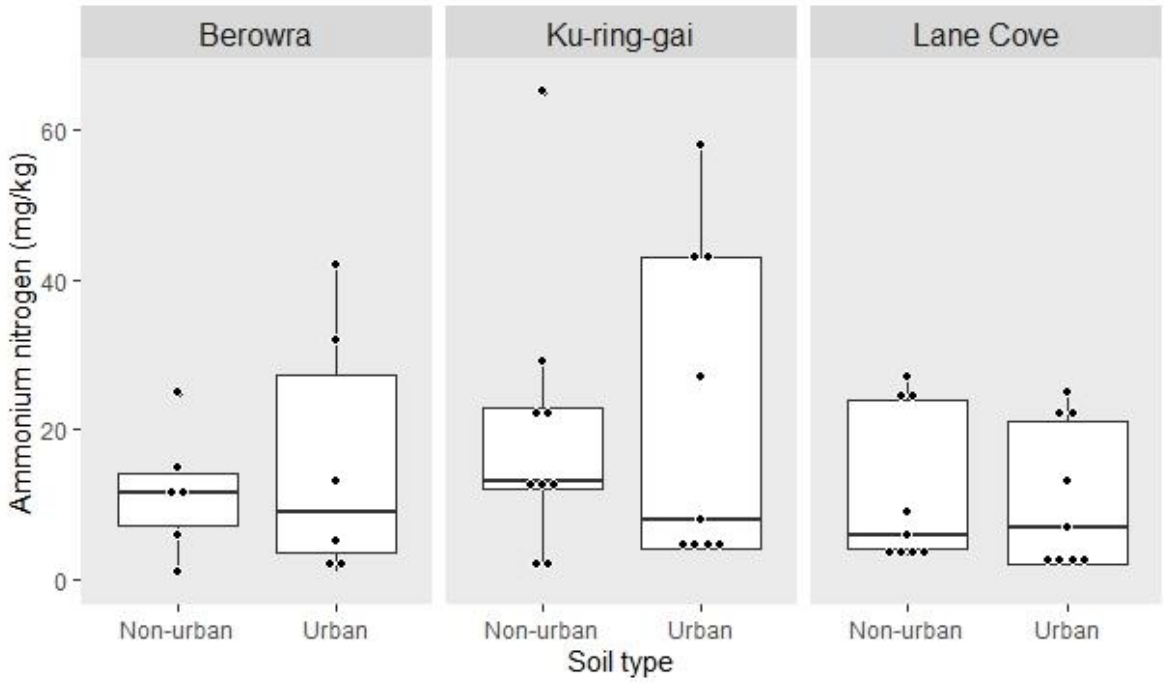


Figure 1 – A boxplot of the ammonium nitrogen concentration for each soil type × location (LC: Lane Cove, KuR: Ku-ring-gai and Ber: Berowra) combination. Boxplot: the box displays the middle 50% of the data (interquartile range, IQR). Within the box, the horizontal bar represents the median value. Vertical bars extend to 1.5 IQR. Dots represent each data point.

Nitrate nitrogen

There was a significant interaction between soil type and location for nitrate nitrogen ($F_{2,44}$=3.838; P=0.029: Fig. 2). Tukey's comparison of means test showed that for Ku-ring-gai and Berowra, urban soils had greater nitrate nitrogen than non-urban soils, while in Lane Cove the difference was not significant.

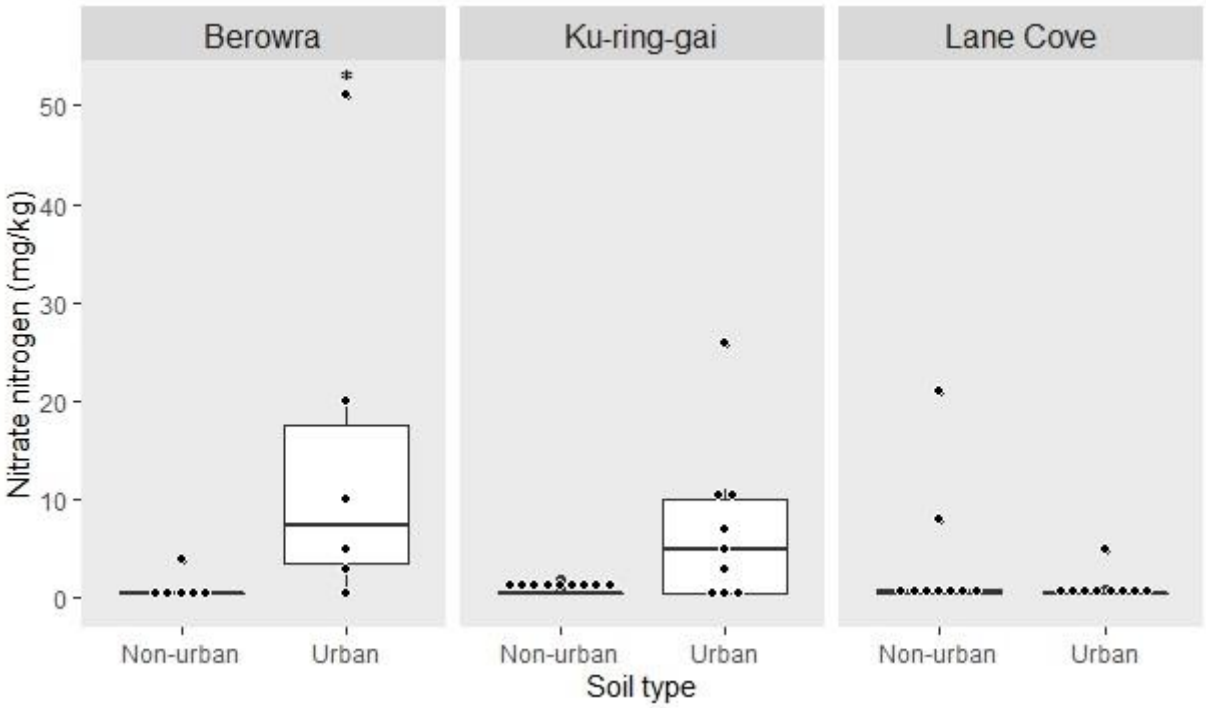


Figure 2 – A boxplot of the nitrate nitrogen concentration for each soil type × location (LC: Lane Cove, KuR: Ku-Ring-Gai & Ber: Berowra) combination. Boxplot: the box displays the middle 50% of the data (interquartile range, IQR). Within the box, the horizontal bar represents the median value. Vertical bars extend to 1.5 IQR. Dots represent each data point.

Phosphorus

There was no significant interaction between soil type and location for available phosphorus ($F_{1,42}$=0.873; P=0.426) (Fig. 3a). However, available phosphorus was significantly greater in the urban soils compared to the non-urban soils ($F_{1,37}$=18.293; P<0.001) (Figure 3b).

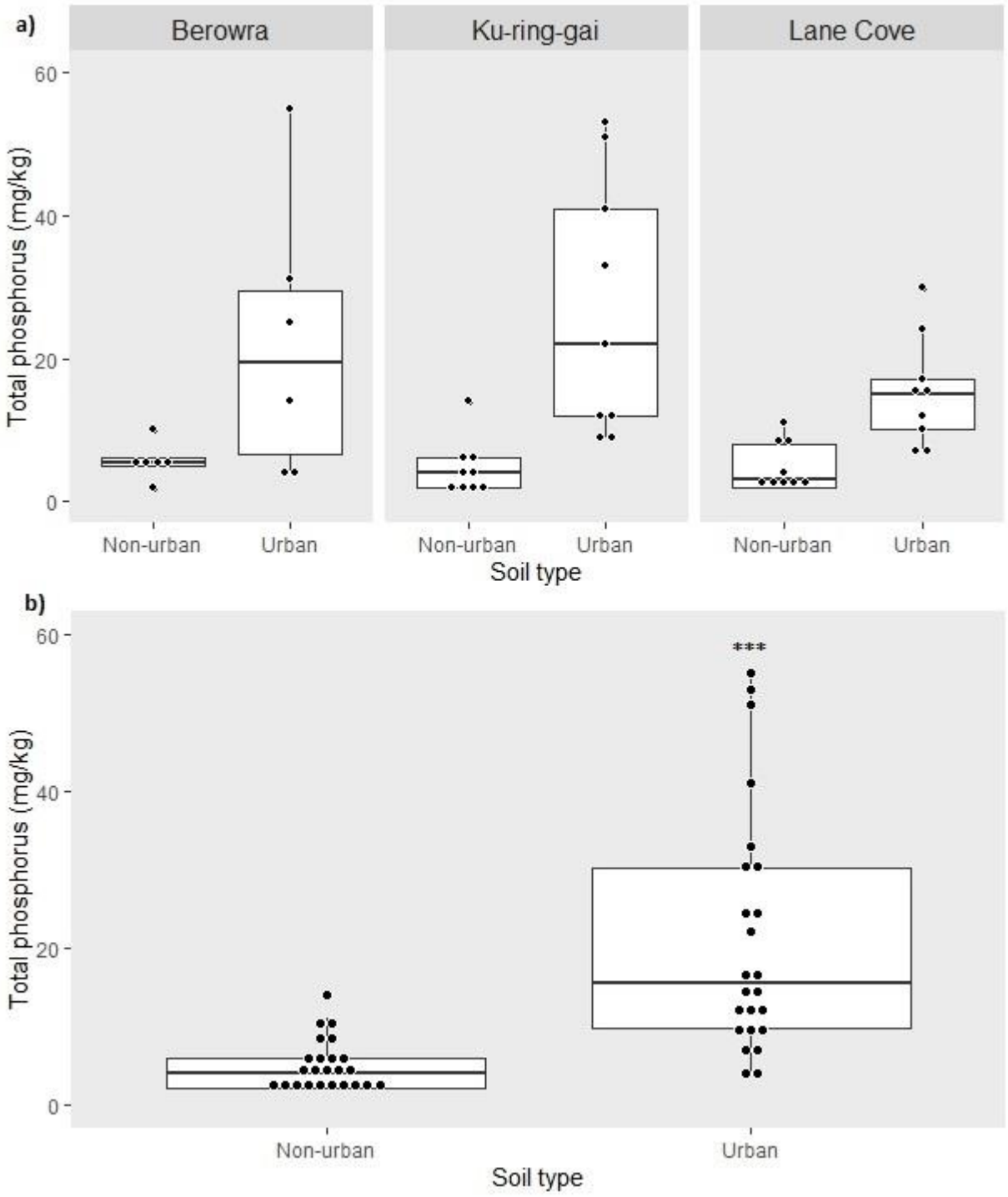
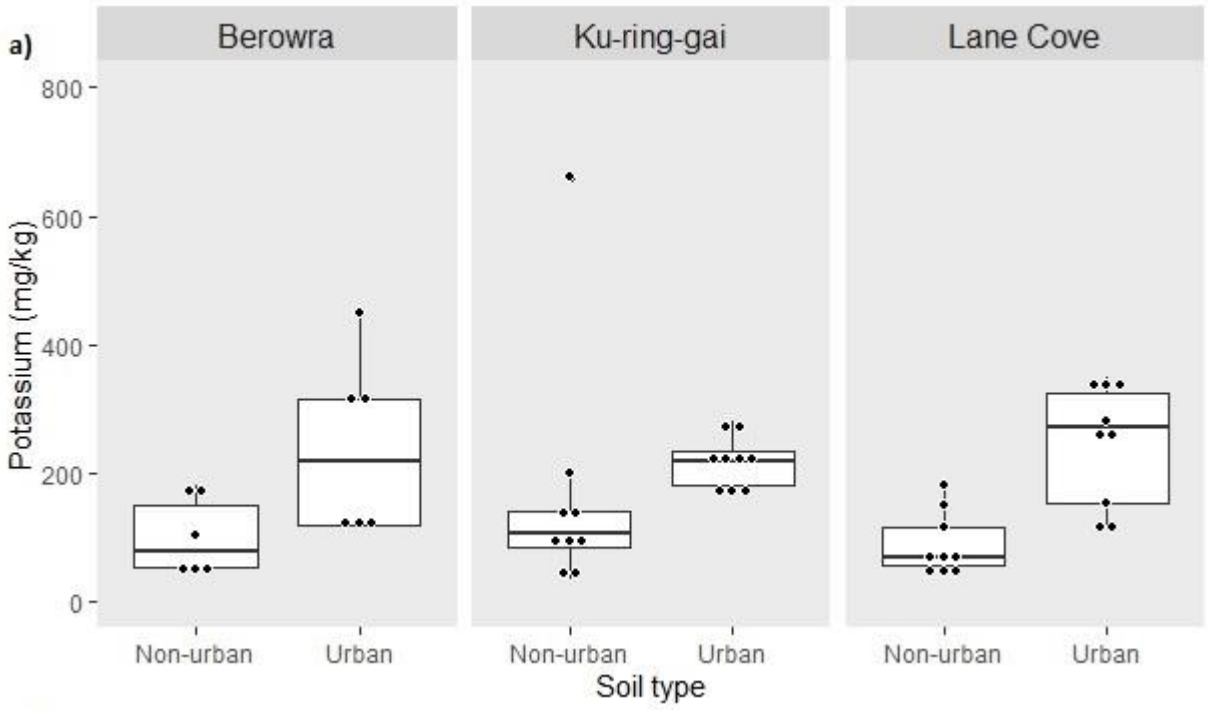


Figure 3 – Boxplots of the total phosphorus concentration for a) each soil type × location (LC: Lane Cove, KuR: Ku-ring-gai & Ber: Berowra) combination and b) each soil type. Boxplot: the box displays the middle 50% of the data (interquartile range, IQR). Within the box, the horizontal bar represents the median value. Vertical bars extend to 1.5 IQR. Dots represent each data point.

Potassium

There was no significant interaction between soil type and location for potassium ($F_{2,44}$=1.183, P=0.316) (Figure 4a). However, potassium was significantly greater in the urban soils compared to the non-urban soils ($F_{1,44}$=12.244, P=0.001) (Figure 4b).



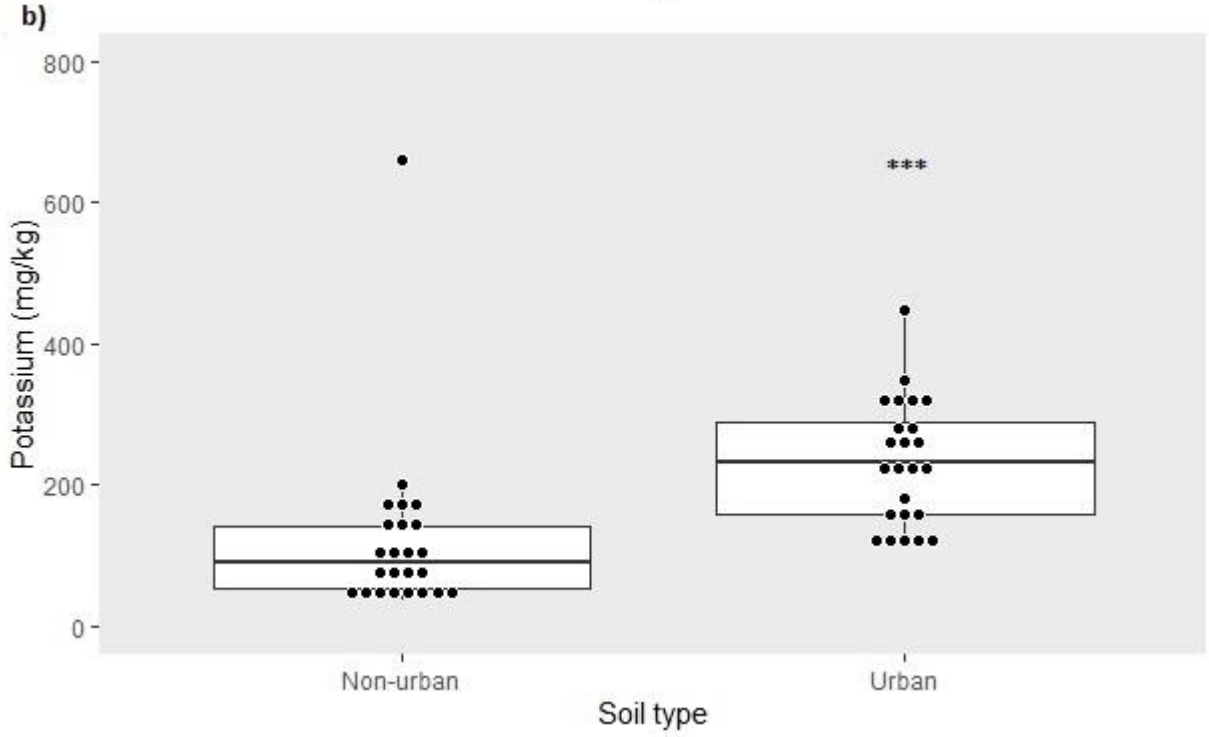


Figure 4a – Boxplots of the potassium concentration for a) each soil type × location (LC: Lane Cove, KuR: Ku-ring-gai & Ber: Berowra) combination and b) each soil type. Boxplot: the box displays the middle 50% of the data (interquartile range, IQR). Within the box, the horizontal bar represents the median value. Vertical bars extend to 1.5 IQR. Dots represent each data point.

Organic carbon

There was no significant interaction between soil type and location for the availability of organic carbon ($F_{2,42}$=0.67, P=0.519) (Figure 5). Further, there was no significant difference in organic carbon between the soil types ($F_{1,47}$=1.85; P=0.180) or locations ($F_{2,44}$=1.75; P=0.187).

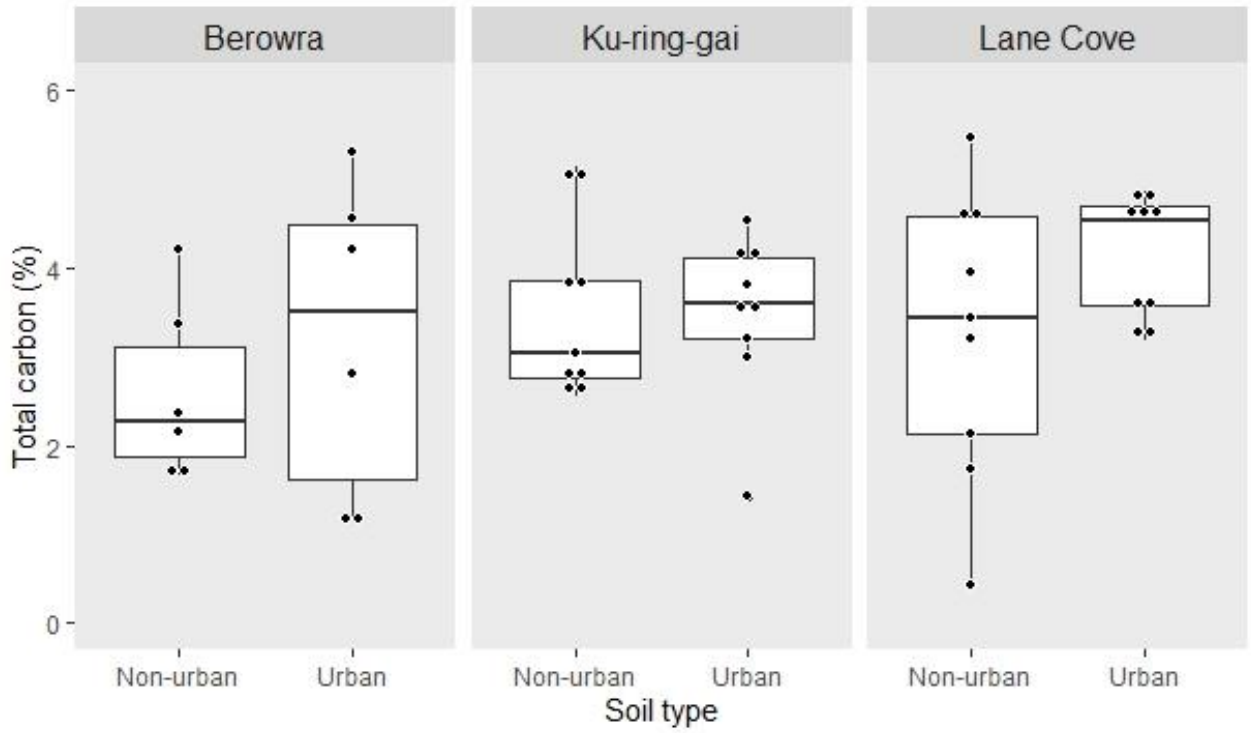


Figure (5) – A boxplot of the organic carbon concentration for each soil type × location (LC: Lane Cove, KuR: Ku-ring-gai & Ber: Berowra) combination. Boxplot: the box displays the middle 50% of the data (interquartile range, IQR). Within the box, the horizontal bar represents the median value. Vertical bars extend to 1.5 IQR. Dots represent each data point.

Electrical conductivity

There was no significant interaction between soil type and location for electrical conductivity ($F_{2,44}$=0.655, P=0.524) (Figure 6a). However, electrical conductivity was significantly greater in the urban soils compared to the non-urban soils ($F_{1,44}$=23.044, P<0.001) (Figure 6b).

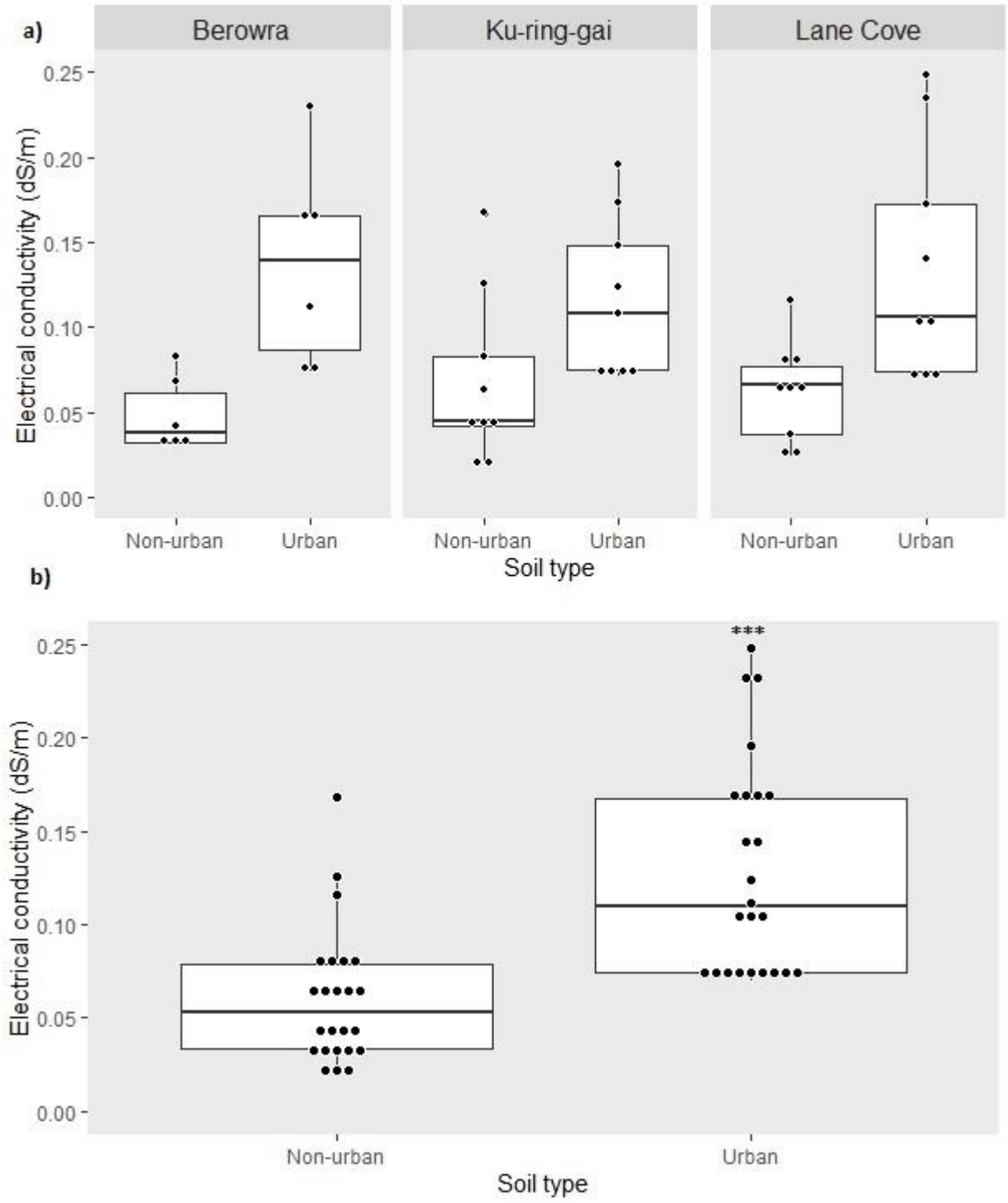


Figure 6a – Boxplots of the electrical conductivity for a) each soil type × location (LC: Lane Cove, KuR: Ku-ring-gai & Ber: Berowra) combination and b) each soil type. Boxplot: the box displays the middle 50% of the data (interquartile range, IQR). Within the box, the horizontal bar represents the median value. Vertical bars extend to 1.5 IQR. Dots represent each data point.

pH

There was no significant interaction between soil type and location for pH ($F_{2,44}$=0.586, P=0.561) (Figure 7a). However, pH was significantly greater in the urban soils compared to the non-urban soils ($F_{1,44}$=5.719, P=0.021) (Figure 7b).

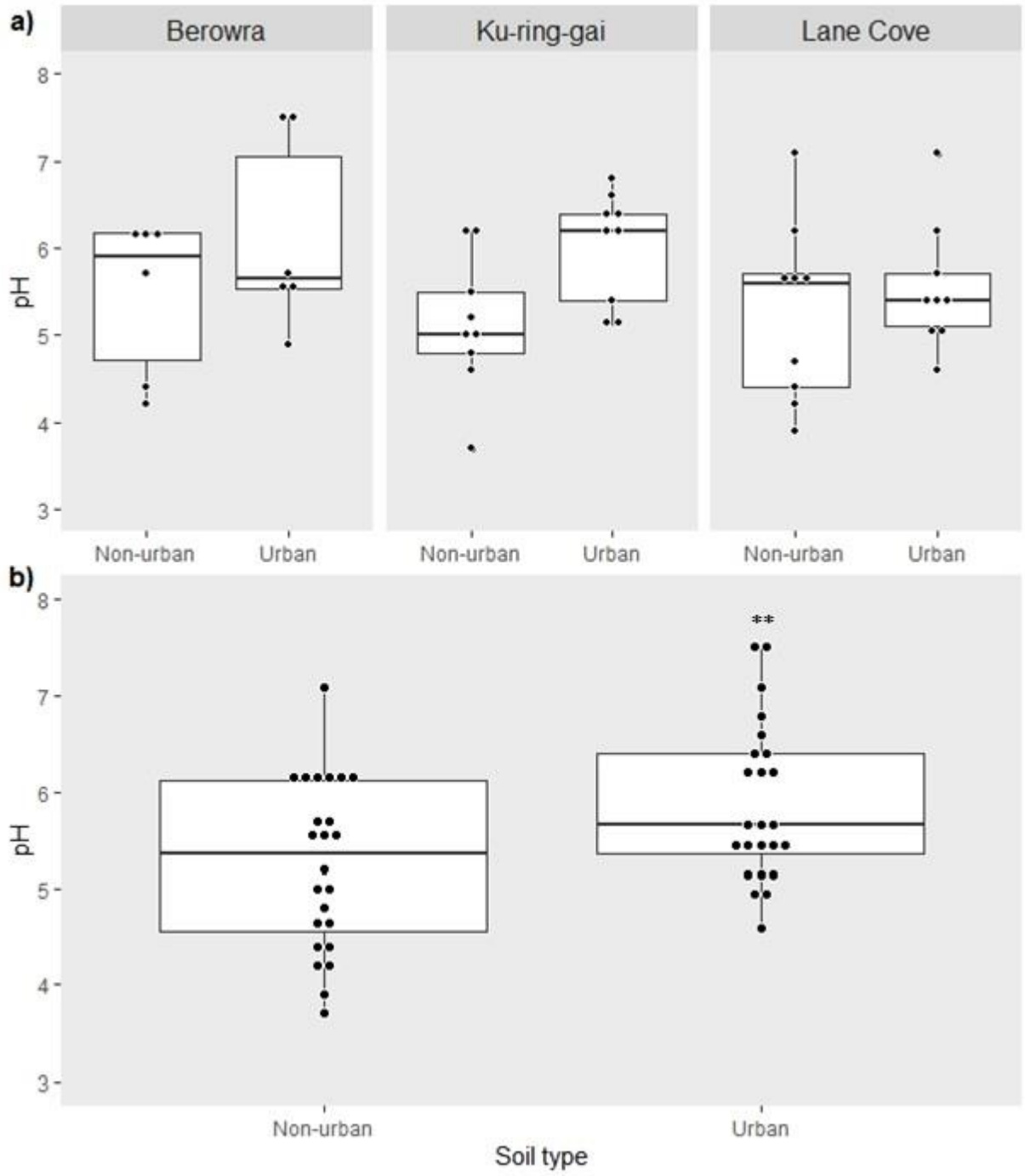


Figure 7a – Boxplots of the pH for a) each soil type × location (LC: Lane Cove, KuR: Ku-ring-gai & Ber: Berowra) combination and b) each soil type (Urban & Non-urban) (b). Boxplot: the box displays the middle 50% of the data (interquartile range, IQR). Within the box, the horizontal bar represents the median value. Vertical bars extend to 1.5 IQR. Dots represent each data point.

Volumetric soil water content (VSWC)

The VSWC of the drought treatment pots was gradually reduced from field capacity (~30%) to a moderate water deficit (~5%) over six weeks. The pots in the well-watered treatment maintained a VSWC of around 30% throughout the six weeks of the experimental treatment period (Figure 8a).

There was no three-way interaction between soil type, soil treatment and water treatment for VSWC across all species. There was no significant effect of soil type on VSWC ($F_{1,1140}$=4.56, P=0.099) (Figure 8b). However, there was a significant effect of soil treatment (Figure 8c), with the unsterilised soil pots having significantly greater VSWC than the sterilised soil pots ($F_{1,1140}$=33.76, P<0.001).

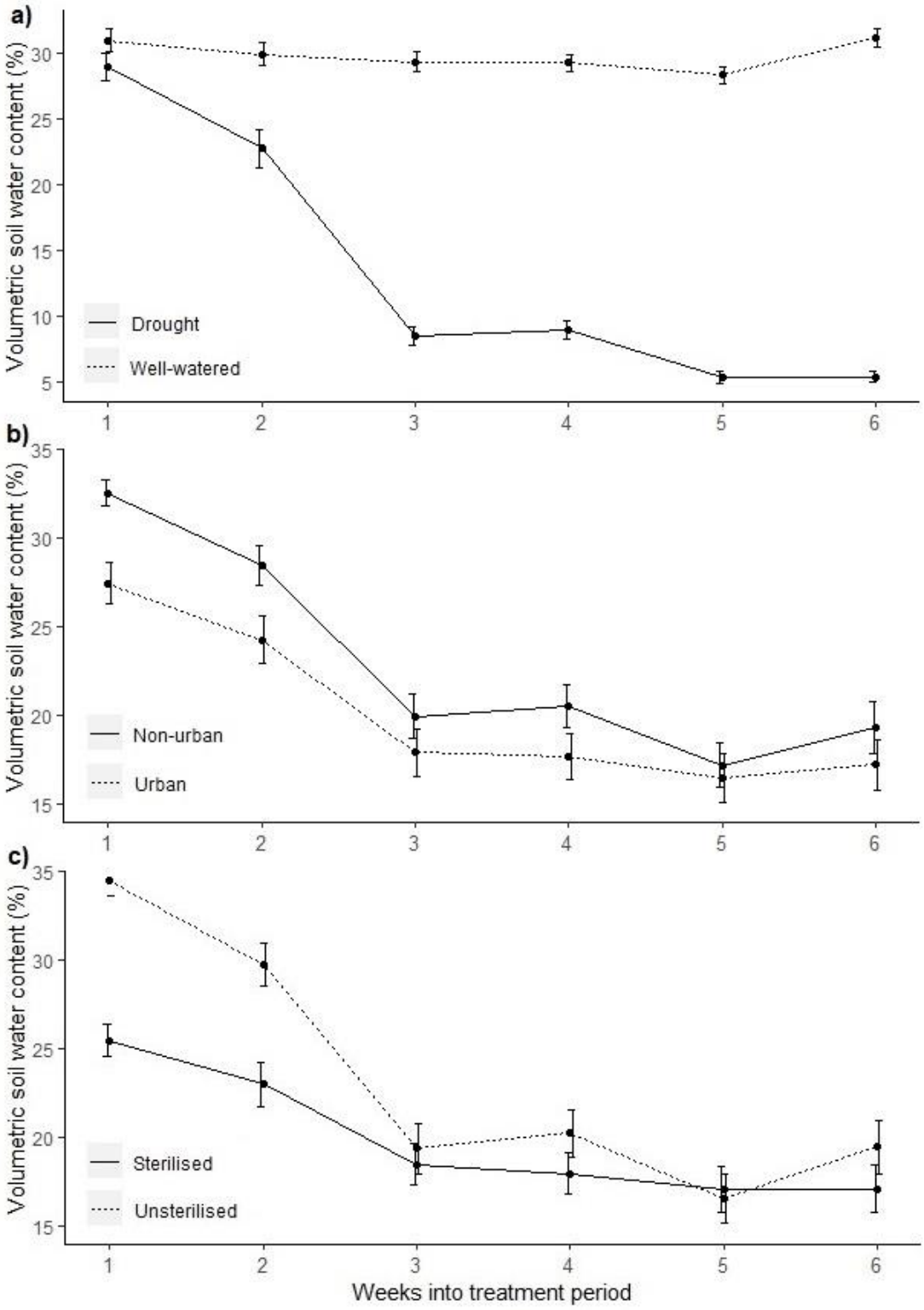


Figure 8: The volumetric soil water content for the different a) watering treatments, b) soil types and c) soil treatments for the 6-week treatment period. Bars represent one standard error.

Fv/Fm Values

There were no three-way interactions between soil type, soil treatment and water treatment for Fv/Fm values in *Angophora costata* ($F_{1,44}$=0.47, P=0.496), *Callistemon citrinus* ($F_{1,32}$=0.01, P=0.943) or *Syncarpia glomulifera* ($F_{1,51}$=0.01, P=0.912). Further, there were no significant two-

way interactions between any of the factors in *Angophora costata* and *Callistemon citrinus.* However, there was a significant difference between watering treatments for *Angophora costata* ($F_{1,44}$=37.59, P<0.001) (Figure 9a) and *Callistemon citrinus* ($F_{1,32}$=55.62, P<0.001) (Figure 9b), with the well-watered plants having greater Fv/Fm values than the drought-stressed plants.

For *Syncarpia glomulifera*, there were significant two-way interactions between soil type and watering treatment ($F_{1,51}$=5.05, P=0.029), and also soil treatment and watering treatment ($F_{1,51}$=6.18, P=0.016) (Figure 9c and Figure 9d). Post-hoc analyses revealed that the well-watered plants grown in urban soils were significantly less stressed than the drought-stressed plants grown in urban soils. Similarly, drought-stressed plants grown in sterilised soils were significantly more stressed than those grown in the other soil treatment × watering treatment combinations.

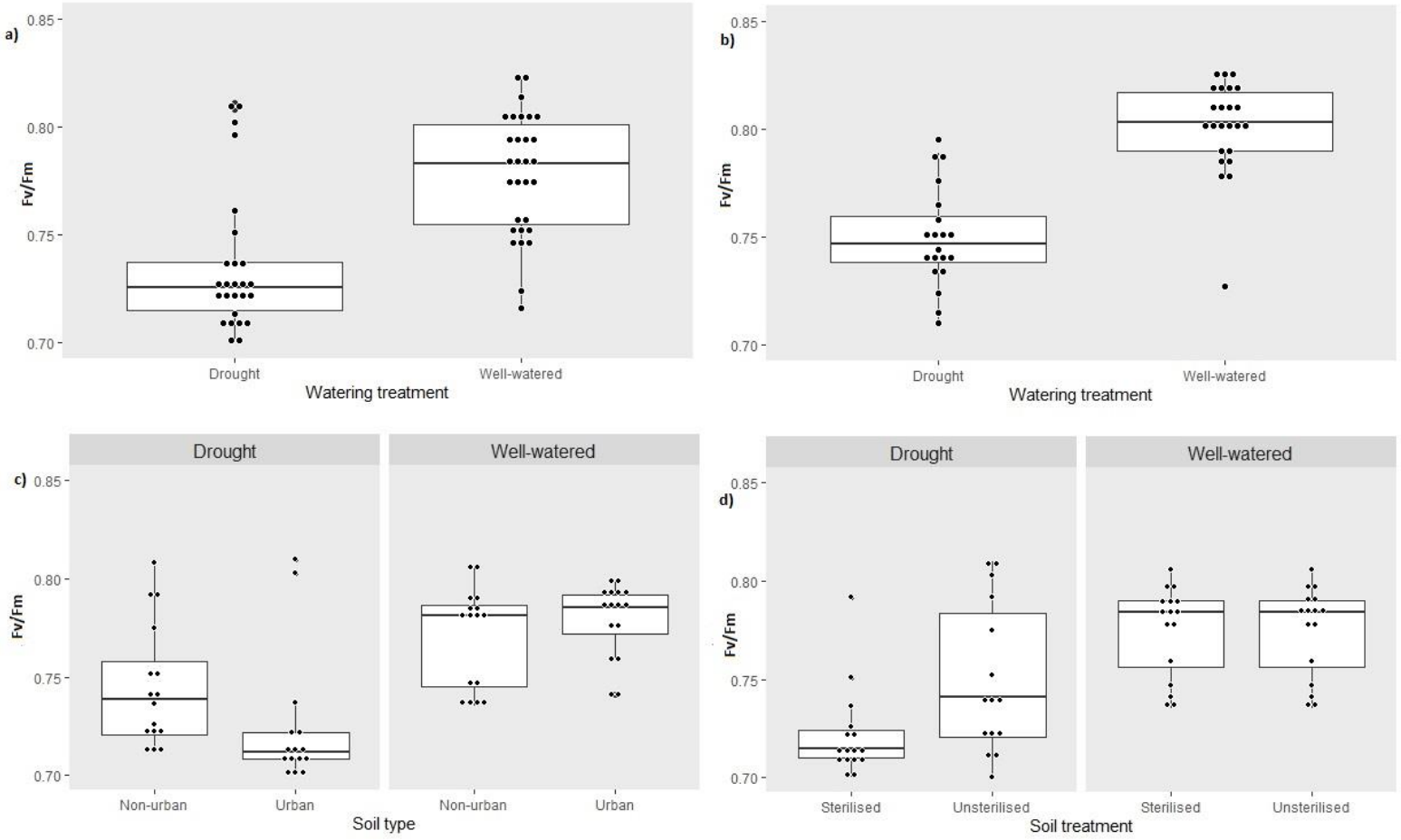


Figure 9: Boxplots of the Fv/Fm values of a) *Angophora costata* and b) *Callistemon citrinus* for each watering treatment, and *Syncarpia glomulifera* for c) each watering treatment × soil type combination and d) each water treatment × soil treatment combination. Boxplot: the box displays the middle 50% of the data (interquartile range, IQR). Within the box, the horizontal bar represents the median value. Vertical bars extend to 1.5 IQR. Dots represent each data point.

Plant growth and allocation

Angophora costata

Total biomass

There was a significant interaction between soil type and watering treatment for total biomass ($F_{1,52}$=5.52, P=0.023), with the plants grown in urban well-watered soils having greater biomass than those grown in the other soil type × watering treatment combinations (Figure 10a). There was also a significant effect of soil treatment on total biomass ($F_{1,52}$=14.45, P<0.001), with plants grown in sterilised soil having greater biomass than those grown in unsterilised soil (Figure 10b).

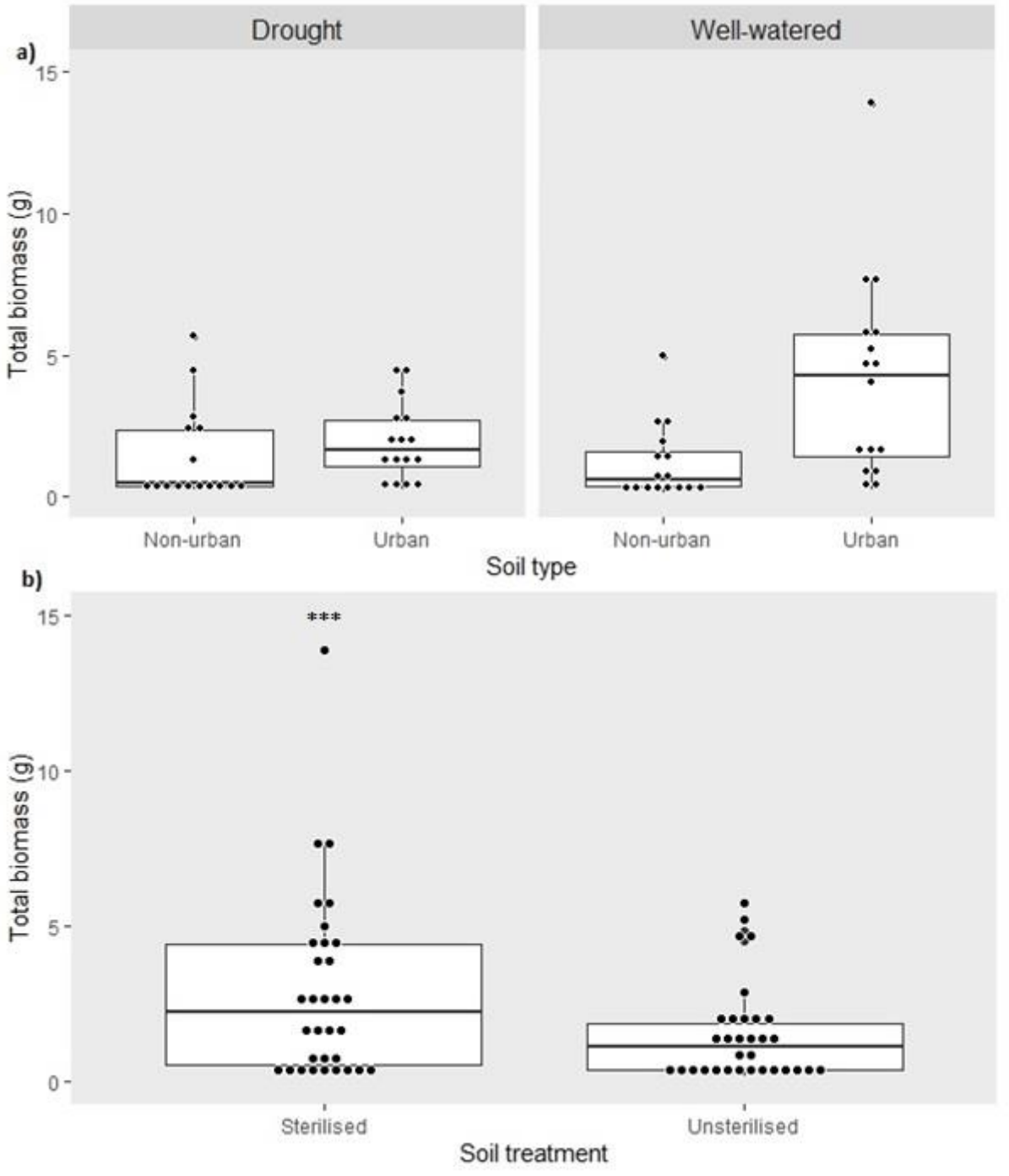


Figure 10: Boxplots of the total biomass of *Angophora costata* for a) each soil type × watering treatment combination and b) each soil treatment. Boxplot: the box displays the middle 50% of the data (interquartile range, IQR). Within the box, the horizontal bar represents the median value. Vertical bars extend to 1.5 IQR. Dots represent each data point.

Root-to-shoot ratio (R:S)

There was a three-way interaction between soil type, soil treatment and water treatment for R:S ($F_{1,52}$=9.29, P=0.004). Post-hoc analyses revealed that drought-stressed plants grown in sterilised non-urban soils had significantly greater R:S, while the well-watered plants grown in unsterilised urban soils had significantly smaller R:S (Figure 11).

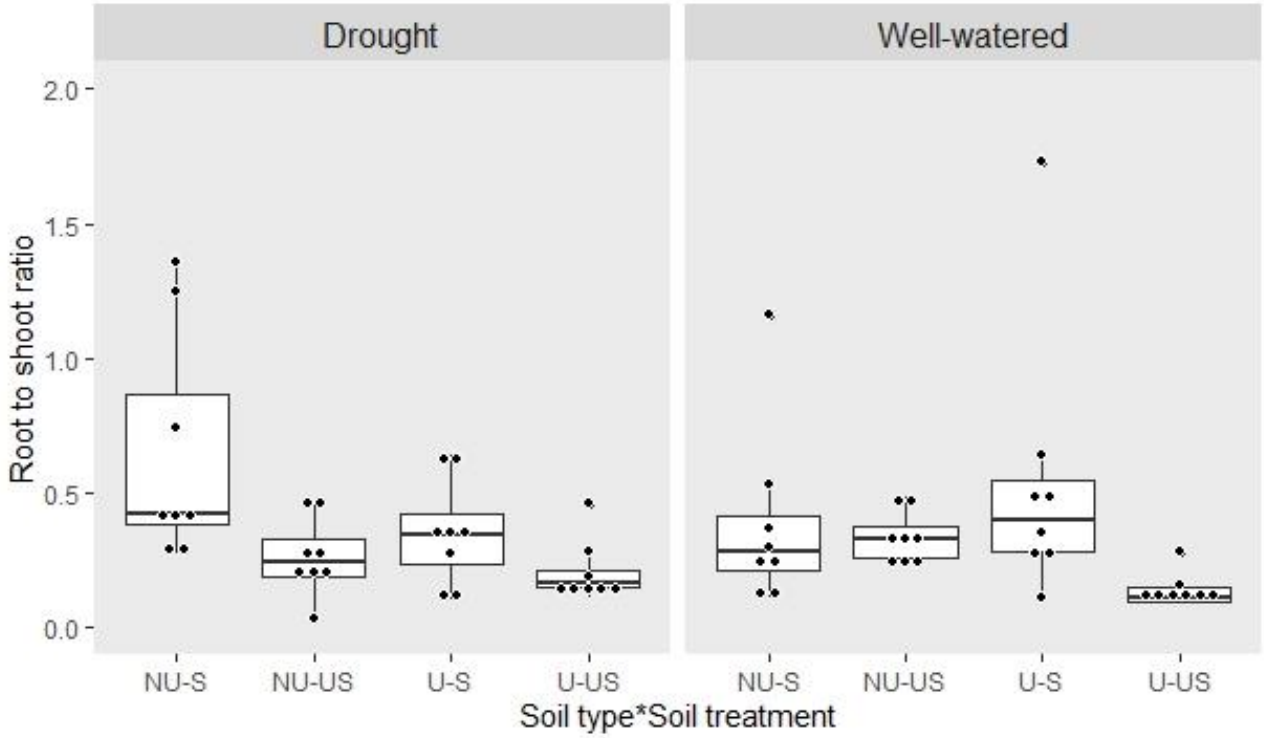


Figure 11: Boxplots of the root-to-shoot ratio of *Angophora costata* for a) each soil type × soil treatment × watering treatment. Boxplot: the box displays the middle 50% of the data (interquartile range, IQR). Within the box, the horizontal bar represents the median value. Vertical bars extend to 1.5 IQR. Soil type*Soil treatment x-axis abbreviations are NU= Non-urban, U= Urban, S= Sterilised and US= Unsterilised. Dots represent each data point.

Plant height

There was a significant interaction between soil type and soil treatment for plant height ($F_{1,50}$=5.97, P=0.018), with the plants grown in sterilised urban soils having the greatest plant height, while plants grown in unsterilised non-urban soil having the lowest plant height (Figure 12a). There was also a significant interaction between soil type and water treatment ($F_{1,50}$=9.40, P=0.003). Post-hoc analyses revealed that the well-watered plants grown in urban soils had greater plant height

compared to the drought-stressed plants grown in urban soils and the well-watered plants grown in non-urban soils (Figure 12b).

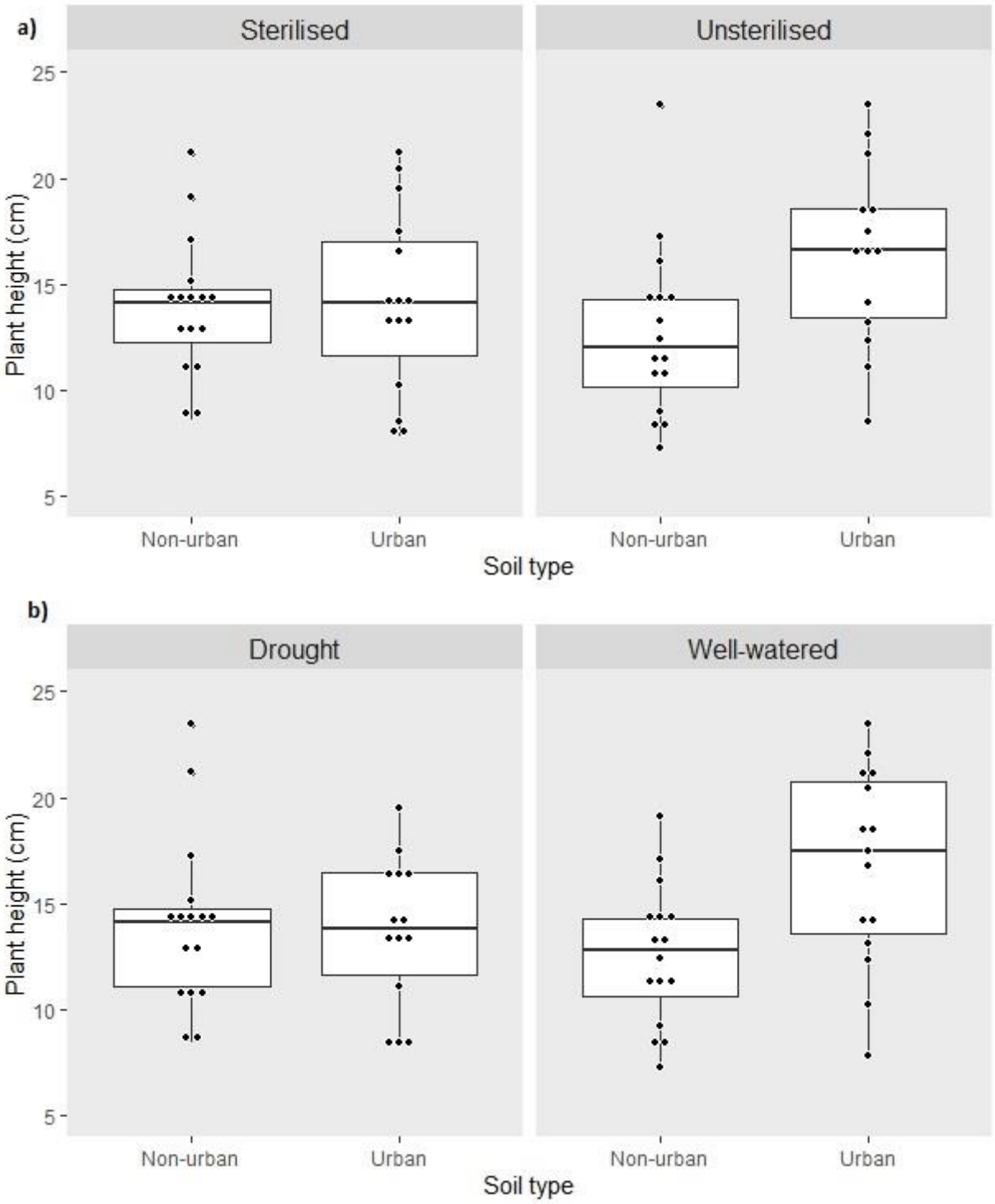


Figure 12: Boxplots of the plant height of *Angophora costata* for a) each soil type × soil treatment combination and b) each soil type × watering treatment combination. Boxplot: the box displays the middle 50% of the data (interquartile range, IQR). Within the box, the horizontal bar represents the median value. Vertical bars extend to 1.5 IQR. Dots represent each data point.

Callistemon citrinus

Total biomass

The total biomass data violated the assumptions of ANOVA, so a non-parametric data analysis was carried out. The analysis found that there were significant treatment effects on total biomass (H=33.75, P<0.05; Figure 13a). Post-hoc analyses revealed that the plants grown in urban soils had significantly greater biomass compared to plants grown in non-urban soils (Figure 13b). Further, plants grown in sterilised soils had significantly greater biomass than plants grown in unsterilised soils (Figure 13c).

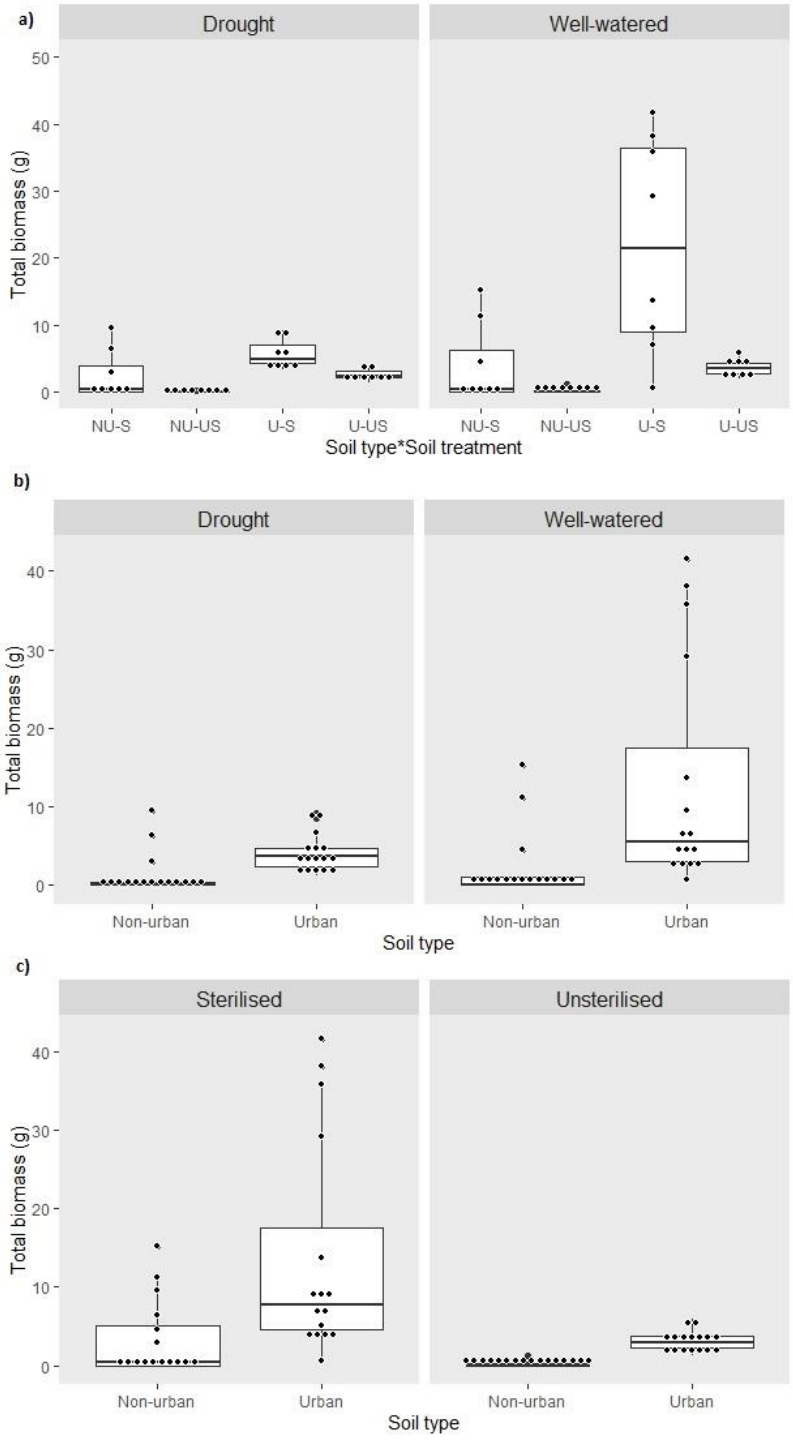


Figure 13: Boxplots of the total biomass for *Callistemon citrinus* for a) each soil type × watering treatment combination, b) each soil type × soil treatment combination and c) each watering treatment × soil type combination. Boxplot: the box displays the middle 50% of the data (interquartile range, IQR). Within the box, the horizontal bar represents the median value. Vertical bars extend to 1.5 IQR. Soil type × soil treatment x-axis abbreviations are NU= Non-urban, U= Urban, S= Sterilised and US= Unsterilised. Dots represent each data point.

Root to shoot ratio (R:S)

The R:S data violated the assumptions of ANOVA, so a non-parametric data analysis was carried out. The analysis found that there were significant treatment effects on R:S (H=32.09, P<0.05). Post-hoc analyses revealed that the plants grown in sterilised urban soils had greater R:S compared to plants grown in the other soil type × soil treatment combinations (Figure 14).

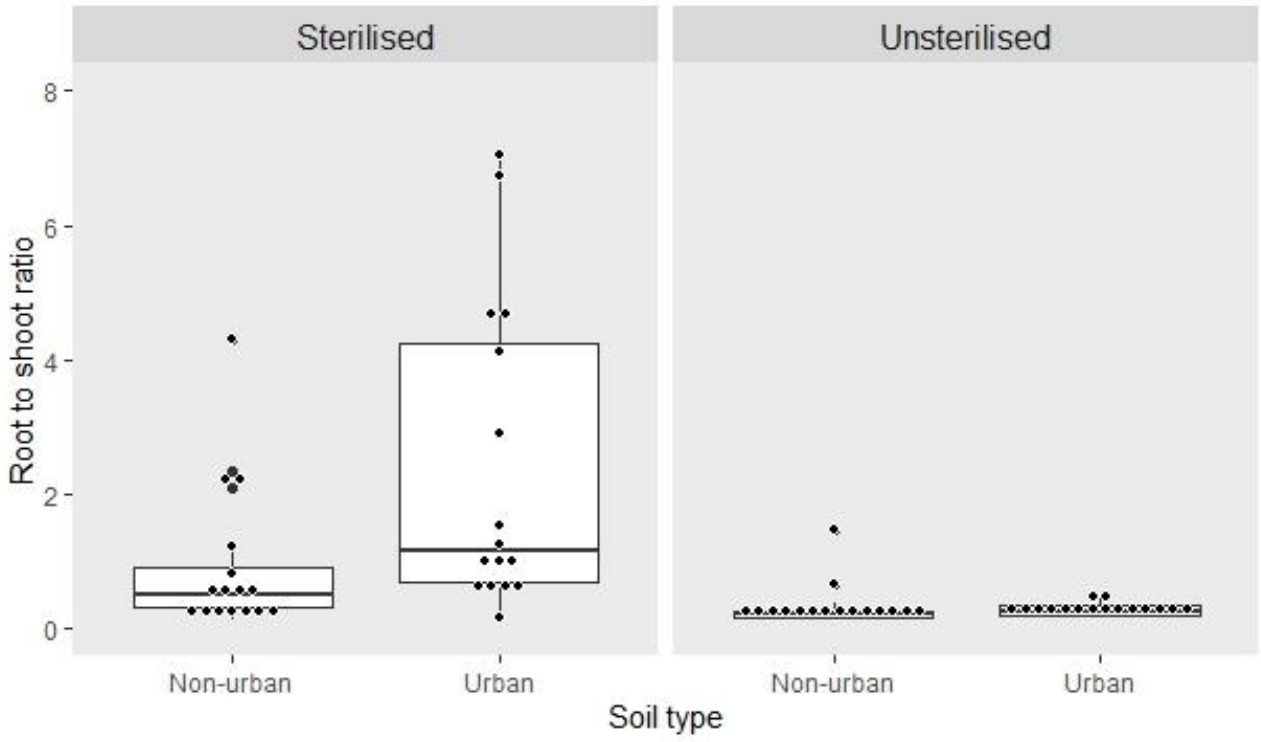


Figure 14: A boxplot of the root to shoot ratio of *Callistemon citrinus* for a) each soil type × soil treatment combination. Boxplot: the box displays the middle 50% of the data (interquartile range, IQR). Within the box, the horizontal bar represents the median value. Vertical bars extend to 1.5 IQR. Dots represent each data point.

Plant height

There was a significant interaction between soil type and soil treatment for plant height ($F_{1,52}$=6.20, P=0.016; Figure 15*)*. Post-hoc analyses revealed that the plants grown in urban soils had greater plant height than the plants grown in non-urban soils (P<0.05).

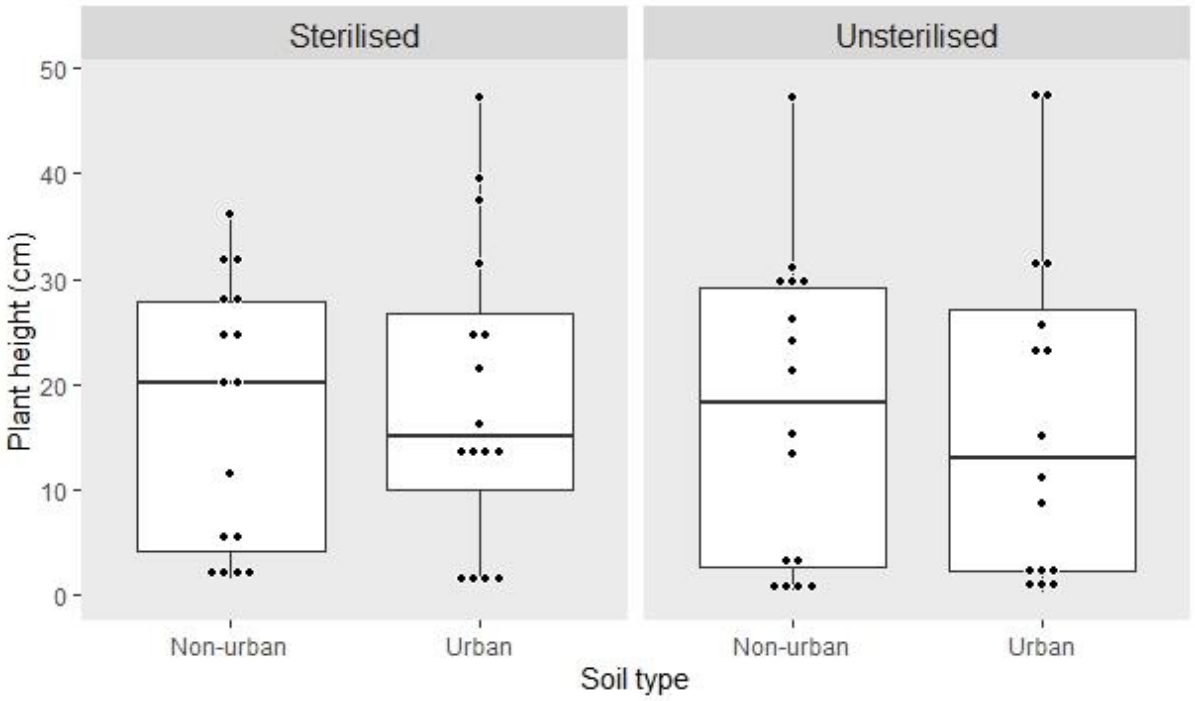


Figure 15: A boxplot of the plant height of *Callistemon citrinus* for each soil type × soil treatment combination. Boxplot: the box displays the middle 50% of the data (interquartile range, IQR). Within the box, the horizontal bar represents the median value. Vertical bars extend to 1.5 IQR. Dots represent each data point.

Syncarpia glomulifera

Total biomass

There were no significant interactions for plant biomass. However, total biomass was greater for plants grown in sterilised urban soils compared to non-urban ($F_{1,52}$=16.77, P<0.001; Figure 16a) and unsterilised soils ($F_{1,50}$=25.50, P= P<0.001; Figure 16b) respectively. Further, the well-watered plants had significantly greater biomass compared to the drought stressed plants ($F_{1,52}$=8.71, P=0.005; Figure 16c).

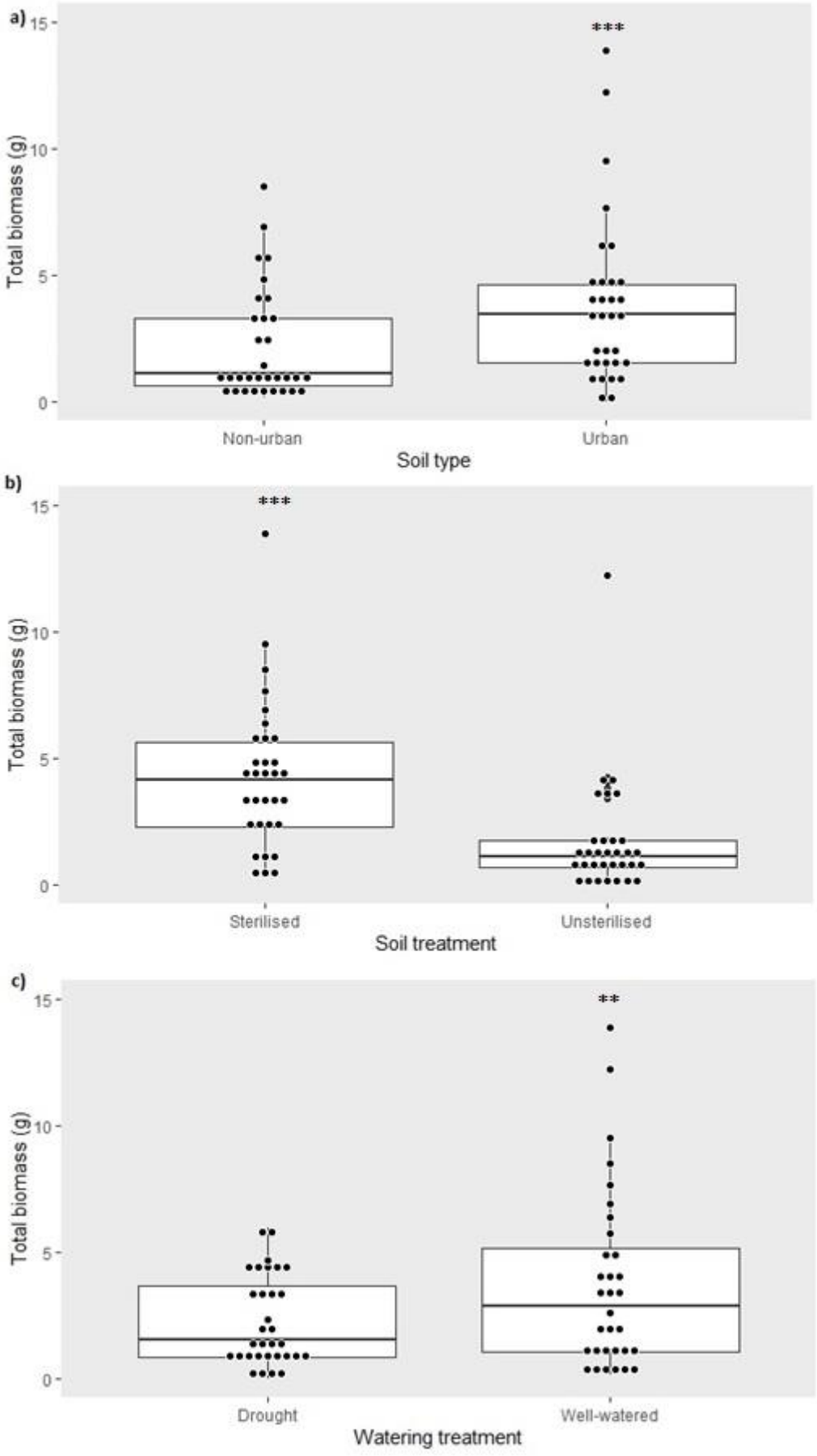


Figure 16: Boxplots of the total biomass of *Syncarpia glomulifera* for a) each soil type, b) each soil treatment and c) each watering treatment. Boxplot: the box displays the middle 50% of the data

(interquartile range, IQR). Within the box, the horizontal bar represents the median value. Vertical bars extend to 1.5 IQR. Dots represent each data point.

Root to shoot ratio (R:S)

The R:S data violated the assumptions of ANOVA, so a non-parametric data analysis was carried out. The analysis found that there were no significant treatment effects on total R:S (H=11.97, P<0.05; Figure 17).

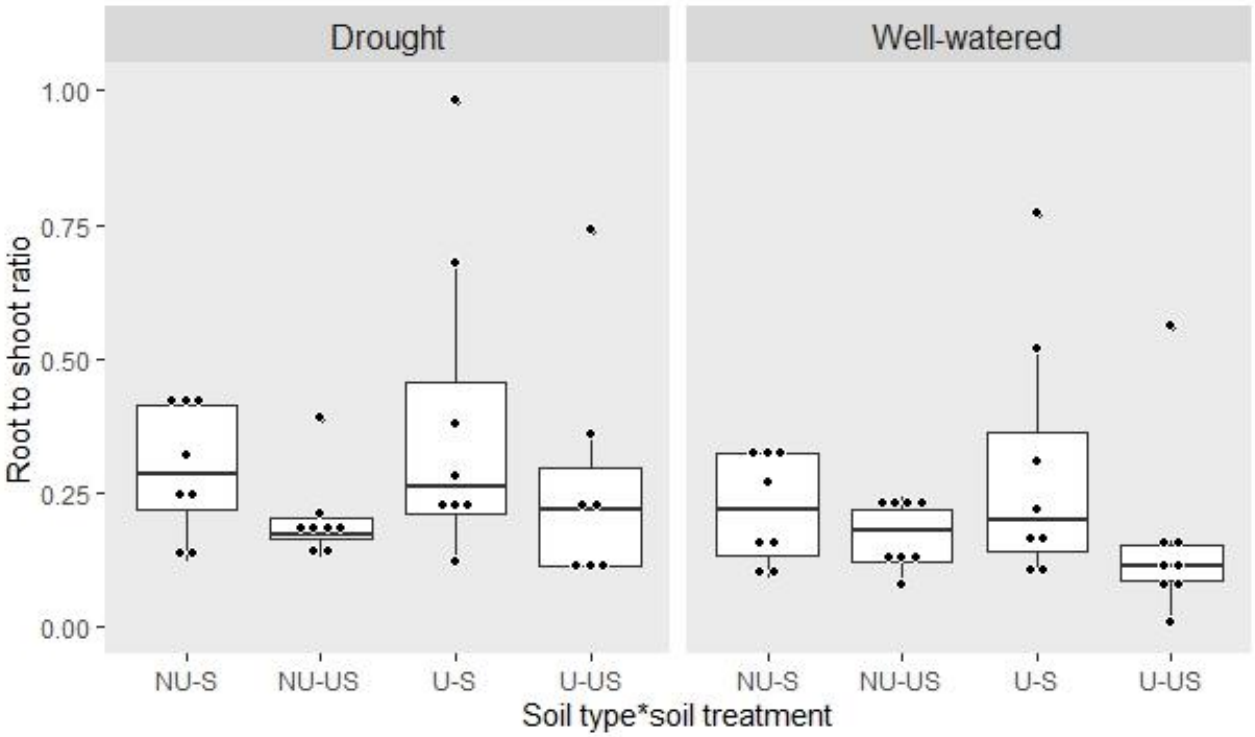


Figure 17: A boxplot of the root to shoot ratio of *Syncarpia glomulifera* for each soil type × soil treatment × watering treatment. Boxplot: the box displays the middle 50% of the data (interquartile range, IQR). Within the box, the horizontal bar represents the median value. Vertical bars extend to 1.5 IQR. Soil type*soil treatment x-axis abbreviations are NU= Non-urban, U= Urban, S= Sterilised and US= Unsterilised. Dots represent each data point.

Plant height

There were no three-way significant interactions for plant height. However, plant height was significantly greater for plants grown in urban and sterilised soils compared to non-urban ($F_{1,52=}$7.22, P=0.01; Figure 18a) and unsterilised soils ($F_{1,50=}$17.31, P<0.001; Figure 18b) respectively. Further, the well-watered plants had greater plant height compared to drought-stressed plants ($F_{1,52=}$7.57, P=0.008; Figure 18c).

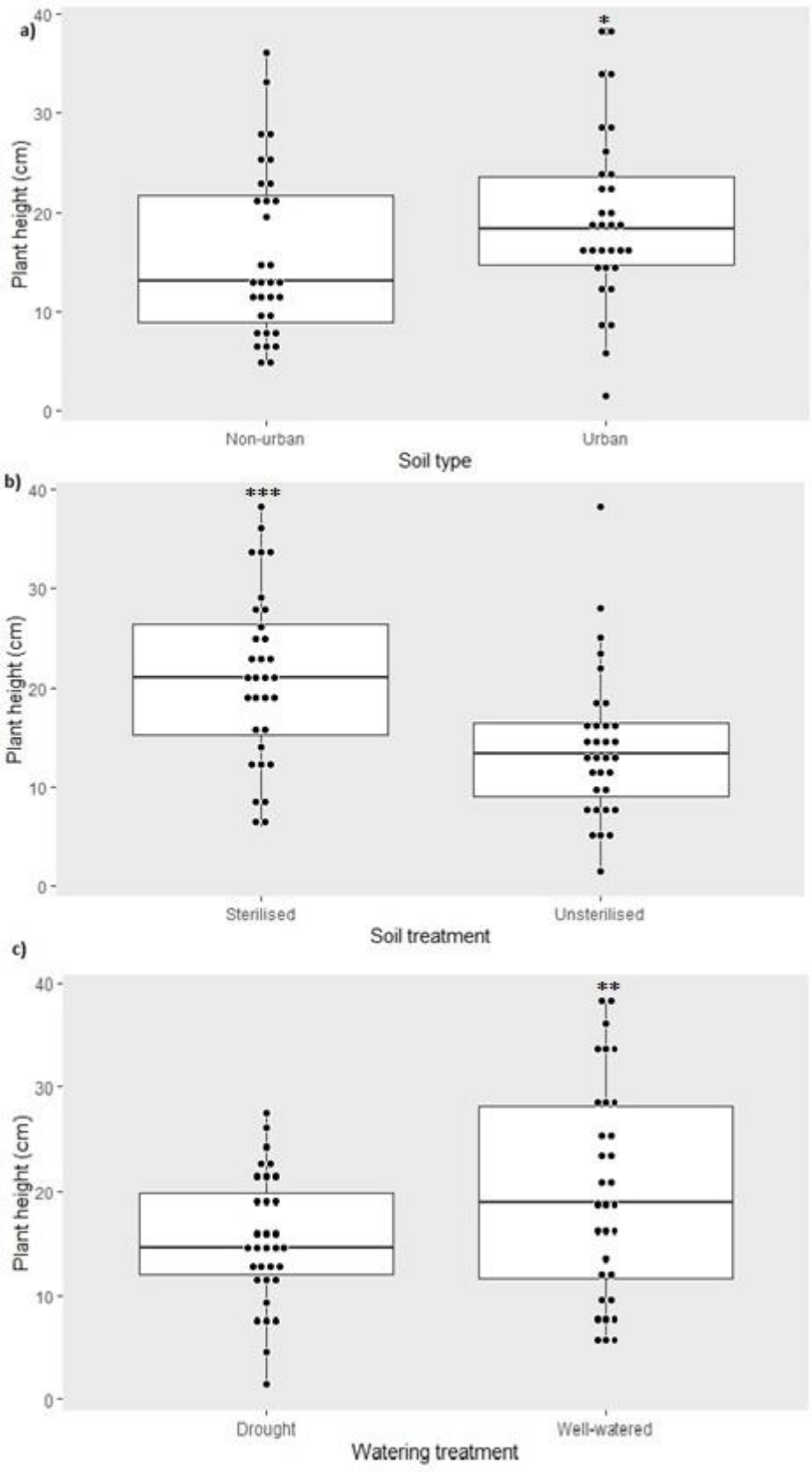


Figure 18: Boxplots of the plant height of *Syncarpia glomulifera* for a) each soil type, b) each soil treatment and c) each watering treatment. Boxplot: the box displays the middle 50% of the data (interquartile range, IQR). Within the box, the horizontal bar represents the median value. Vertical bars extend to 1.5 IQR. Dots represent each data point.

4. Discussion

It is well established that both abiotic and biotic soil factors play key roles in plant responses to stressors such as drought, particularly from research on natural and agricultural ecosystems (Ruiz-Lozano., 2003). Surprisingly, our understanding of this link between soil factors and plant stress resilience in urban contexts is still rudimentary (Guilland *et al.*, 2018). Therefore, this study aimed to determine how the abiotic and biotic soil properties that characterise some urban and non-urban soils impact the performance and drought resilience of three commonly planted urban plant species, in Sydney, NSW, Australia. I hypothesised that the abiotic properties and microbiome of the urban soils would be more impacted by anthropogenic disturbances compared to the non-urban soils, hence, the performance of plants grown in non-urban soils will be greater than those grown in urban soils. Contrary to this hypothesis, I found that the nutrient-enriched urban soils enhanced performance of the three study species, compared to non-urban soils. I also hypothesised that the plants would show greater resilience to drought stress in the presence of soil microbes when compared to plants grown in sterilised soil. However, overall, I found that plants grown in sterilised soil had greater growth performance than those grown in unsterilised soil, suggesting that the soil microbiome negatively impacts plant performance in this study system. This may explain why the presence of soil microbiomes did not improve the drought resilience of the species studied here.

Abiotic characteristics of urban vs non-urban soils

Soil abiotic properties are the key regulators that directly and indirectly, through the soil microbiome, influence plant performance (Prescott & Grayston, 2013). The physical and chemical properties of soil in urban areas are greatly impacted by anthropogenic disturbance and inputs (Morel *et al*., 2015). There are many pathways through which this can occur such as leakage from sewerage pipes, dumping of garden waste, use of introduced soils for the development of streetscapes, and input of stormwater runoff (Leishman *et al.*, 2004). The impact of these factors on nutrient concentrations is notably profound in urban areas developed on low fertility soils, such as the Hawkesbury Sandstone-derived soils of my study area. Hawkesbury Sandstone-derived soils are generally sandy, permeable, low in nutrients (particularly P), and slightly acidic (Grella *et al.*, 2018; Thomson & Leishman, 2004). However, in urbanised areas the abiotic properties of these soils can be dramatically modified, often resulting in soils with a higher organic content and nutrient concentration (Leishman *et al*., 2004).

The results of my study were consistent with previous studies on the abiotic conditions of Hawkesbury Sandstone-derived soils in the urban environment. I found that the soil at urban sites had higher levels of nitrate nitrogen, phosphorus, and potassium than soils at non-urban sites. Further, soil salinity (i.e., conductivity) and pH were also higher at these sites. The causes of these shifts in abiotic soil factors are well understood. The addition of fertilisers to lawns, gardens, and landscape plantings (Law *et al*., 2004), as well as chemical reactions of air pollutants in urban areas resulting in high levels of atmospheric deposition (Lovett *et al*., 2000), are a few of the factors contributing to increased nitrogen levels in urban systems. Leishman et al. (2004) showed that stormwater outlets are the major cause of phosphorus enrichment in urban environments characterised by Hawkesbury Sandstone-derived soils. Similarly, Grella *et al*. (2018) showed that potassium levels were significantly higher in urban soils when compared to non-urban soils on Hawkesbury Sandstone.

In contrast to the soil abiotic properties discussed above, organic soil carbon was not significantly different between the urban and non-urban study sites. However, the organic soil carbon content in urban areas is often highly variable and dependent on the type of green space (Pouyat *et al.,* 2006). For example, Canedoli *et al*. (2020) reported that urban parks in Milan, Italy, store significantly more soil organic carbon when compared to other types of urban greenspaces. In general, due to relatively high carbon to nitrogen ratios in leaf litter, urban soils under tree canopies have been found to have greater amounts of organic carbon when compared to urban soils under turf (Livesley *et al.,* 2016). However, the tree canopy in urban areas is often significantly sparser than in non-urban areas, which means reduced leaf and hence soil organic carbon. This is supported by the fact that soil organic carbon accumulation is strongly related to time since disturbance, and hence leaf litter accumulation, in urban areas (Scharenbroch *et al*., 2005). To date, urban areas have been underutilised in terms of their soil carbon storage capacity, but this is slowly shifting, with recent studies highlighting their potential use to store carbon (Pouyat *et al*., 2006; Lorenz & Lal, 2015; Vasenev & Kuzyakov, 2018).

Plant responses to urban and non-urban soils

The selected species for this study are native to Hawkesbury Sandstone-derived soil and are thus adapted to the abiotic properties that characterise these soils. For this reason, I hypothesised that the plants grown in non-urban soils would perform better than plants grown in urban soils. Contrary to this hypothesis, I found that the plants grown in urban soil had greater growth (i.e., total biomass and plant height) than the plants grown in non-urban soil for all three species. For total biomass, the increase was largest in *Callistemon citrinus* (80%) and smallest in *Syncarpia glomulifera* (40%).

For plant height, the opposite trend was observed with the increase being largest in *Syncarpia glomulifera* (23%) and smallest in *Callistemon citrinus* (5%). These results suggest that the growth of our study species in their natural environment (i.e., non-urban soils) may be limited by either nitrate nitrogen, phosphorus, potassium, or a combination of these nutrients. Given that phosphorus is a critical nutrient for plant growth and is also the most limiting nutrient in Hawkesbury Sandstone-derived soils (Leishman *et al.* 2004), it is the likeliest candidate. However, it has been reported that nutrient addition (N, P and K) to Hawkesbury Sandstone-derived soils results in only a negligible increase in the growth of some native species and increases mortality of many native species (Thomson & Leishman, 2004).

Surprisingly, the higher pH of the urban soils compared to the non-urban soils did not negatively impact the growth of my study species. This is contrary to the literature, with studies reporting that increasing pH can detrimentally impact plant growth (Gentili et al., 2018; Nádasi & Kazinczi, 2011). For example, Gentili et al., 2018 showed that *Ambrosia artemisiifolia* had slower growth rates when grown at pH 7 compared to when grown at pH 5 and pH 6. An explanation for pH not impacting the growth of the study species may be because my urban soils, despite having a greater pH than the non-urban soils, were not actually alkaline. That is, they had an average pH of 5.5, with a range of 4.4-7.5, and may therefore not affect species that are adapted to acidic soil conditions, such as my study species.

The influence of low water availability on plant growth

The intensity and frequency of extreme drought events in southern Australia are projected to increase under a range of climate change scenarios (Naumann *et al.*, 2018; State of the Climate, 2020). Under drought conditions, plants often become stressed and experience a decline in their leaf maximal photochemical efficiency (Fv/Fm), which results in a decline in photosynthesis (Chaves *et al.*, 2003; Lovelock *et al*., 1994; Tyystjärvi, 2013). Plants employ a range of strategies to cope with drought stress (Ngumbi, & Kloepper, 2016). Under drought stress, plants invest fewer resources into producing stems and leaves to decrease water loss (Eziz et al., 2017). Another strategy is leaf shedding, which reduces the overall water demand of the plant (Chaves et al., 2003).

Reduction in Fv/Fm under drought conditions was observed across all three study species. As a result of the drought treatment, total biomass was reduced in the drought-stressed plants compared to the well-watered plants for all three study species. The largest biomass declines were observed in *Callistemon citrinus* (-64%) followed by *Syncarpia glomulifera* (-40%) and the smallest in *Angophora costata* (-37%). Several studies have reported similar results in Australian native plant

species (*Eucalyptus*: McKiernan *et al.,* 2014, 2015, 2017, Manea *et al*., 2021; *Myrtus*: Navarro *et al.,* 2009). Further, Zhang *et al.* (2019) showed that *Tilia cordata* Greenspire, a commonly planted urban tree species, significantly decreases its biomass accumulation under drought conditions.

I also found that the drought-stressed plants were shorter than the well-watered plants for *Angophora costata* and *Syncarpia glomulifera.* Several studies have reported similar findings (Anjum *et al*., 2017; Li *et al.,* 2020; Misra *et al*., 2020; Zhang *et al.,* 2019). A possible explanation for these findings is that during their early growth stages, seedlings require the most water to transport nutrients and as such, a lack of water can severely reduce plant height (Li *et al.*, 2020). Further, cell contraction, increased leaf shedding and reduced mitosis under drought conditions may also result in a reduction in plant height (Yang *et al*., 2021). In contrast to the other study species, the height of *Callistemon citrinus* did not decline in response to drought stress. Even though biomass decline in response to drought stress was greatest in *Callistemon citrinus,* it should be noted that the well-watered *Callistemon citrinus* plants grown in sterilised soils had extremely high growth rates, therefore, exaggerating the biomass decline relative to the other study species. However, studies have reported that both total biomass and plant height for *Callistemon citrinus* decreased under drought stress (Álvarez & Sánchez-Blanco, 2013; Mugnai *et al.*, 2009). Álvarez & Sánchez-Blanco, (2013) showed that *Callistemon* species are particularly resilient to drought stress due to their efficient and adaptive stomatal control, which results in improved water use efficiency. Mugnai *et al.* (2009) and Vernieri *et al.* (2006) also showed that despite declines in growth rates under moderate drought conditions, *Callistemon citrinus* is still able to maintain good overall quality by increasing their R:S and thus, their soil water uptake ability.

In this study, leaf shedding was observed in the drought-stressed plants across all three study species. This leaf shedding may have also contributed to the biomass declines observed in the study species. Another strategy that plants employ to cope with drought stress is increasing their investment in root biomass, which may enhance their water uptake ability (Eziz *et al.,* 2017).

Consistent with previous studies (Álvarez & Sánchez-Blanco, 2013; Asch *et al*., 2005; Eziz *et al*., 2017; Lu *et al.,* 2020; Manea *et al*., 2021; McKiernan *et al.*, 2017; Zhang *et al.,* 2019), I found that *Angophora costata* had increased allocation to root biomass in response to the drought treatment. In contrast, *Syncarpia glomulifera* and *Callistemon citrinus* did not display the same shifts in biomass allocation. An explanation would be that the *Callistemon citrinus* is recorded to be drought resilient due to presence of efficient and adaptive stomatal control, hence it did not shift biomass allocations. However, in *Syncarpia glomulifera,* the total biomass was greatly reduced due to drought stress, and

this would be due to the greatest amount of leaf shedding by this species under drought stress. Hence *Syncarpia glomulifera* species is less tolerant of the drought stress. It is worth noting that the Fv/Fm reduction was small, and this small reduction indicated that the stress level imposed on the plants did not damage the plants severely, instead plants likely adjusted their photochemical efficiency via the xanthophyll cycle.

The influence of the soil microbial communities on plant performance in urban and non-urban soils

Abiotic soil properties are the most critical factors in shaping soil microbial community diversity and composition (Xu *et al.,* 2014), which in turn may significantly influence plant performance (Ruiz-Lozano, 2003). Although I did not examine the soil microbiome directly, I was able to infer its impact on plant performance by comparing plant growth in sterilised and unsterilised soils. Given that the soil microbiome can play an important role in enabling plants to obtain resources from the soil (e.g., nutrients and water), which translates into improved growth and stress resilience (Gupta et al., 2019; Naylor *et al.,* 2018), I hypothesised that the plants grown in unsterilised non-urban soil would perform better than the plants grown in sterilised soil. Contrary to this hypothesis, I found that the plants of all three study species grown in sterilised soil had greater growth (i.e., total biomass and plant height) compared to the plants grown in unsterilised soil. Similar findings have been reported in previous studies (Marschner & Rumberger, 2004, Khaliq & Sanders, 1998; Li *et al*., 2019; Yim *et al*., 2013), with there being a range of possible explanations for this trend.

Sterilisation often alters the chemical properties of soil in addition to eliminating the microbiome (Trevors 1996), in what is commonly referred to as a 'nutrient flush' (Khaliq & Sanders, 1998). Although dependent to a certain extent on the soil type (Hu *et al.,* 2020), it is common for a temporary nutrient flush to occur as a result of sterilisation due to the lysed microbes releasing nutrients directly into the soil and/or eliminating their nutrient uptake (Troelstra *et al*., 2001). For example, it has been reported that soil phosphorus often increases after sterilisation due to the soil microbes lysing (Hu *et al.,* 2020; Li *et al.,* 2019). However, a temporary nutrient flush may not explain why the plants grown in the sterilised soil performed better in this study. *Angophora costata* and *Callistemon citrinus* had greater root-to-shoot ratios when grown in sterilised soil. If a nutrient flush did occur, it would be expected that the plants would shift their resource allocation away from root biomass because nutrient uptake is no longer the limiting factor for growth and there will be stronger effect on low nutrient (non-urban) vs higher nutrient (urban) soils (Van Wijk *et al.,* 2003).

Another explanation for the plants grown in the sterilised soil performing better may be that the impact of pathogens present in the soil outweighed the benefits provided by the beneficial microbes (Troestra *et al*., 2001; Yim *et al*., 2013). Many studies have shown that pathogens can strongly impact plant growth (Delgado-Baquerizo et al., 2020). Beckstead and Parker (2003) showed that there was a significant reduction in growth (up to 81%) of both root and shoot biomass of *Ammophila arenaria* plants grown in unsterilised soil compared to sterilised soil. Ross and Moles (2021) also found greater seedling emergence of *Triodia basedowii* in seeds planted in sterilised soil when compared to seeds planted in unsterilised soil.

The final possible explanation for why my study species performed better in sterilised than in unsterilised soils may be due to mutualist soil microbes, such as mycorrhizal species, not being able to form beneficial associations with the plants during the experiment. Given that urban soils often have very low microbial diversity and richness (Zhu & Carreiro, 2004), it can be suggested that the soil microbiome at the urban study sites may have lacked sufficient amounts of mutualist microbes to enable the formation of beneficial associations with the plants. However, this is unlikely given that within the unsterilised soil treatment, the plants grown in urban soils performed better than the plants grown in non-urban soils.

Many studies have identified a variety of microorganisms, such as plant growth-promoting bacteria or mycorrhiza, that can improve the resilience of plants to drought stress (Begum *et al.*, 2019; Hoch *et al*., 2019; Li *et al*., 2014: Rolli *et al.*, 2015; Staudinger *et al*., 2016). These beneficial associations can lead to positive plant-soil feedbacks (Bever *et al*., 2012). On the other hand, when host-specific pathogens accumulate it may generate negative plant-soil feedbacks (Bennett *et al.*, 2019; Semchenko *et al.*, 2017). Given that I found the soil microbiome to negatively impact the growth of my study species in general, it is unsurprising that it also did not enhance plant resilience to drought stress. Even though *Syncarpia glomulifera* showed lower stress (Fv/Fm values) under drought conditions when grown in unsterilised compared to sterilised soil, this did not translate into greater plant growth, as the plants grown in sterilised soils had greater biomass compared plants grown in unsterilised soils. Therefore, it can be concluded that the soil microbial community had relatively little impact on plant growth compared to the influence of soil abiotic factors such as pH and nutrient content.

Management implications

The importance of improving depauperate soil microbiomes (e.g., by increasing diversity) through inoculation with beneficial microbes to enhance plant performance has been practised for decades in agricultural and forestry sectors (Kaminsky *et al.*, 2019) and is increasingly employed for

ecological restoration (Koziol *et al.,* 2018; Molineux *et al*., 2017; Thrall *et al.,* 2005). Urban soils are generally low in microbial abundance and diversity hence soil microbial inoculations can have a positive influence in greening of urban areas. To date, the studies that have tested these interventions have delivered mixed results. For example, Fini *et al.* (2011) showed that the inoculation of urban soils with mycorrhizal fungi can improve the drought resilience of plants. Similarly, Schröder *et al*. (2019) showed that arbuscular mycorrhizal fungi inoculation can improve plant performance in urban areas under moderate drought stress but causes earlier wilting under severe drought stress. Appleton *et al.* (2003), on the other hand, showed that mycorrhizal root colonisation in *Quercus palustris* did not increase after a year in response to mycorrhizal inoculations only. However, they did show the inoculations had a benefit for plant growth when combined with soil nutrient addition. It has also been suggested that planting native plant species rather than inoculations could be used to restore fungal microbiomes in urban areas (Baruch *et al.,* 2020).

Given these mixed results as well as the results of this study, it is unclear whether microbial inoculations provide a benefit to plant performance and resilience to drought in urban environments generally. Therefore, recommending it as a reliable management tool to enhance the health and function of urban soil microbiomes and subsequently, the plants growing in them would be inappropriate at this stage, despite their apparent benefits in agricultural systems. This is further supported by the fact that although plants in urban environments have low mycorrhizal colonisation compared to plants in non-urban environments, the plants present in these environments are still often well colonised by mycorrhizal fungi, which indicates that inoculations may not be necessary (Bainard *et al*., 2011).

Study caveats and future research directions

It should be acknowledged that this was a short-term study and longer-term studies are needed to better understand the influence of the soil microbiome on plant performance in the urban context. Firstly, the study was based on a single round of soil sample collection, therefore, there is potential that a lot of seasonal variation was missed. To overcome this caveat, it is critical that future studies are longer-term to examine seasonal trends. However, given the soil microbiome is extremely sensitive to changes in soil abiotic properties (Wardle *et al.,* 2004), it remains to be seen whether finding consistent results within seasons is even possible. Secondly, the fact that the study was only short-term meant that it may have been insufficient time for the plants to establish beneficial associations with the mutualist soil microbes. Thirdly, it is worth noting that the pot size used for this experiment can also have an effect on the root:shoot ratio (Poorter *et al*., 2012).

As a proxy for the soil microbiome health of the samples, I compared the performance of the plants grown in sterilised and unsterilised soils. Ideally, it would have been helpful to quantify the diversity and composition of these microbial communities directly through next-generation sequencing (NGS) analyses, to complement the plant growth study. Studies that use NGS to characterise soil microbial DNA are considered to be the gold standard to determine soil microbiome status and thus its impact on plant performance (Mills *et al*., 2017). In a follow-up to this study, I plan to characterise the bacterial and fungal communities of the urban and non-urban soils, as well as of the sterilised soils, in order to identify if key components of the soil microbial communities differ between the soil types and treated soil. This will facilitate a better understanding of the effect of the different biotic components of urban and non-urban soils on plant performance, and the potential for soil microbial amendments to enhance urban plant performance and green space function.

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Supplementary material

Table 1: Native species and site locations used for soil sampling

Table 2: Analysis of variance for ammonium nitrogen

Location	Species (Common name)	Family	Urban Site	Non-urban Site
Lane Cove	*Callistemon citrinus* (Crimson bottle brush)	Myrtaceae	-33.7660775 S ,151.1326376 E -33.7675196 S, 151.1345376 E -33.764243 S, 151.136529 E	-33.7521688 S, 151.1044687 E -33.7524108 S, 151.1039172 E -33.7521839 S,151.1041341 E
	Angophora costata (Smooth-barked Apple)	Myrtaceae	-33.7666700 S, 151.1335751 E -33.7671589 S, 151.1339841 E -33.7676731 S, 151.1347036 E	-33.7512319 S, 151.1167690E -33.7515098 S, 151.1166513 E -33.7518206 S,151.1169299 E
	Syncarpia glomulifera (Turpentine)	Myrtaceae	-33.772541 S, 151.101368 E -33.772384 S, 151.101613 E -33.773114 S, 151.087862 E	-33.782108 S, 151.087988 E -33.781443 S, 151.087767 E -33.781416 S,151.088377 E
Ku-Ring-Gai	*Callistemon citrinus* (Crimson bottle brush)	Myrtaceae	-33.702344 S ,151.147469E -33.7081525 S, 151.1472778 E -33.7187995 S, 151.1442144 E	-33.6881436 S, 151.156094 E -33.688314 S, 151.156449 E -33.688144 S,151.157274 E
	Angophora costata (Smooth-barked Apple)	Myrtaceae	-33.7008027 S, 151.1481626 E -33.6962680 S, 151.1479309 E -33.6957851 S, 151.1513145 E	-33.688127 S, 151.157328E -33.688237 S, 151.157399 E -33.688303 S,151.157790 E
	Syncarpia glomulifera (Turpentine)	Myrtaceae	-33.702337 S, 151.147146 E -33.7122376 S, 151.1474572 E -33.6903367 S, 151.1520679 E	-33.6888377 S, 151.1550113 E -33.6890823 S, 151.1545798 E -33.6894238 S, 151.1547152 E
Berowra	*Callistemon citrinus* (Crimson bottle brush)	Myrtaceae	-33.680908 S ,151.097883 E -33.69108 S, 151.098562 E	-33.6870676 S, 151.0911338 E -33.06874559 S, 151.0914131 E
	Angophora costata (Smooth-barked Apple)	Myrtaceae	-33.7025569 S, 151.0957382 E -33.7081238 S, 151.1272206 E	-33.6873089 S, 151.0911760 E -33.6863643 S, 151.0910855 E
	Syncarpia glomulifera (Turpentine)	Myrtaceae	-33.6908376 S, 151.1054984 E -33.7128227 S,151.1080505 E	-33.7012309 S, 151.0917182 E -33.7002914 S,151.0906141 E

Source	DF	F-Value	P-Value
Soil type	1	0.153	0.697

Site	2	1.799	0.178
Soil type*Site	2	0.106	0.900
Error	44		

Table 3a: Analysis of variance of nitrate nitrogen

Source	DF	F-Value	P-Value
Soil type	1	6.053	0.018
Site	2	1.794	0.179
Soil type*Site	2	3.838	0.029
Error	44		

Table 3b: Tukey post-hoc analyses for nitrate nitrogen ANOVA

Soil type*Site	N	Mean	Grouping	
Urban-Berowra	6	14.917	A	
Urban-Ku-ring-gai	9	7.056	A	B
Non-urban-Lane Cove	9	3.667	A	B
Non-urban-Berowra	6	1.083	A	B
Urban-Lane Cove	9	1.056		B
Non-urban–Ku-ring-gai	9	0.722		B

Table 4: Analysis of variance for phosphorus

Source	DF	F-Value	P-Value
Soil type	1	18.293	0.000
Site	2	0.954	0.394
Soil type*Site	2	0.873	0.426
Error	37		

Table 5: Analysis of variance for potassium

Source	DF	F-Value	P-Value
Soil type	1	12.244	0.001
Site	2	0.281	0.758
Soil type*Site	2	1.183	0.316
Error	44		

Table 6: Analysis of variance of organic carbon

Source	DF	F-Value	P-Value
Soil type	1	1.85	0.180
Site	2	1.75	0.187
Soil type*Site	2	0.67	0.519
Error	42		

Table 7: Analysis of variance of conductivity

Source	df	F-Value	P-Value

Soil type	1	23.044	0.000
Site	2	0.101	0.904
Soil type*Site	2	0.655	0.524
Error	44		

Table 8: Analysis of variance of pH

Source	df	F-Value	P-Value
Soil type	1	5.719	0.021
Site	2	0.719	0.493
Soil type*Site	2	0.586	0.561
Error	44		

Table 0: Analysis of variance of volumetric soil water content across all species

Source	DF	F-Value	P-Value
Soil type	1	4.56	0.099
Soil treatment	1	33.76	0.000
Watering treatment	1	775.63	0.000
Soil type*Soil treatment	1	1.41	0.235
Soil type*Watering treatment	1	0.52	0.470
Site (Soil type)	4	3.84	0.004
Soil treatment*Watering treatment	1	0.80	0.372
Soil type*Soil treatment*Watering treatment	1	1.63	0.201
Error	1140		

Table 10: Analysis of variance table of Fv/Fm for *Angophora costata*

Source	DF	F-Value	P-Value
Soil type	1	1.23	0.274
Soil treatment	1	0.33	0.568
Watering treatment	1	37.59	0.000
Site (Soil type)	4	1.35	0.266
Soil type*Soil treatment	1	0.59	0.445
Soil type*Watering treatment	1	1.12	0.296
Soil treatment*Watering treatment	1	0.43	0.513
Soil type*Soil treatment*Watering treatment	1	0.47	0.496
Error	44		

Table 11: Analysis of variance of Fv/Fm for *Callistemon citrinus*

Source	DF	F-Value	P-Value
Soil type	1	0.55	0.478
Soil treatment	1	0.68	0.414
Watering treatment	1	55.62	0.000
Site (Soil type)	4	1.31	0.286
Soil type*Soil treatment	1	0.01	0.911
Soil type*Watering treatment	1	0.00	0.986
Soil treatment*Watering treatment	1	0.62	0.437
Soil type*Soil treatment*Watering treatment	1	0.01	0.943

Error	32		

Table 12a: Analysis of Variance of Fv/Fm for *Syncarpia glomulifera*

Source	DF	F-Value	P-Value
Soil type	1	0.33	0.596
Soil treatment	1	6.10	0.017
Watering treatment	1	34.78	0.000
Site (Soil type)	4	1.47	0.226
Soil type*Soil treatment	1	0.01	0.927
Soil type*Watering treatment	1	5.05	0.029
Soil treatment*Watering treatment	1	6.18	0.016
Soil type*Soil treatment*Watering treatment	1	0.01	0.912
Error	51		

Table 12b: Tukey post-hoc analyses for soil type*watering treatment combination of Fv/Fm for *Syncarpia glomulifera*

Soil type*Watering treatment	N	Mean	Grouping		
Urban well-watered	16	0.781	A		
Non-urban well-watered	16	0.770	A	B	
Non-urban drought	16	0.745		B	C
Urban drought	15	0.724			C

Table 12c: Tukey post-hoc analyses for soil treatment*watering treatment combination of Fv/Fm for *Syncarpia glomulifera*

Soil treatment*Watering treatment	N	Mean	Grouping	
Sterilised well-watered	16	0.776	A	
Unsterilised well-watered	16	0.775	A	
Unsterilised drought	15	0.752	A	
Sterilised drought	16	0.717		B

Table 13a: Analysis of variance of total biomass for *Angophora costata*

Source	DF	F-Value	P-Value
Soil type	1	17.52	0.000
Soil treatment	1	14.45	0.000
Watering treatment	1	2.17	0.146
Site (Soil type)	4	20.19	0.000
Soil type*Soil treatment	1	2.06	0.157
Soil type*Watering treatment	1	5.52	0.023
Soil treatment*Watering treatment	1	0.00	0.991
Soil type*Soil treatment*Watering treatment	1	0.14	0.715
Error	52		

Table 13b: Tukey post-hoc analyses for soil type*watering treatment combination of total biomass for *Angophora costata*

Soil type*Watering treatment	N	Mean	Grouping	
Urban Well-watered	16	0.364748	A	
Urban Drought	16	0.085960		B
Non-urban Drought	16	-0.053374		B
Non-urban Well-watered	16	-0.117219		B

Table 14a: Analysis of variance of root to shoot ratio for *Angophora costata*

Source	DF	F-Value	P-Value
Soil type	1	7.70	0.008
Soil treatment	1	19.02	0.000
Watering treatment	1	0.15	0.704
Soil type*Soil treatment	1	1.56	0.217
Soil type*Watering Treatment	1	0.02	0.889
Site (Soil type)	4	3.82	0.009
Soil treatment*Watering Treatment	1	0.35	0.559
Soil type*Soil treatment*Watering treatment	1	9.29	0.004
Error	52		

Table 14b: Tukey post-hoc analyses for soil type*soil treatment*watering treatment combination of root to shoot ratio for *Angophora costata*

Soil type*Soil treatment*Watering treatment	N	Mean	Grouping		
Non-Urban Sterilised Drought	8	-0.256	A		
Urban Sterilised Well watered	8	-0.415	A	B	
Non-Urban Unsterilised Well watered	8	-0.483	A	B	
Non-Urban Sterilised Well watered	8	-0.516	A	B	
Urban Sterilised Drought	8	-0.553	A	B	C
Non-Urban Unsterilised Drought	8	-0.677		B	C
Urban Unsterilised Drought	8	-0.750		B	C
Urban Unsterilised Well watered	8	-0.918			C

Table 15a: Analysis of variance of plant height for *Angophora costata*

Source	DF	F-Value	P-Value
Soil type	1	3.42	0.070
Soil treatment	1	0.78	0.383
Watering treatment	1	1.65	0.205
Soil type*Soil treatment	1	5.97	0.018
Soil type*Watering treatment	1	9.40	0.003
Site (Soil type)	4	8.14	0.000
Soil treatment*Watering treatment	1	1.59	0.214
Soil type*Soil treatment*Watering treatment	1	2.63	0.111
Error	50		

Table 15b: Tukey post-hoc analyses for soil type*soil treatment combination of plant height for *Angophora costata*

Soil type*Soil treatment	N	Mean	Grouping	
Urban Unsterilised	15	16.507	A	
Non-Urban Sterilised	16	14.272	A	B
Urban Sterilised	15	13.808	A	B
Non-Urban Unsterilised	16	13.003		B

Table 16: Kruskal Wallis analysis of total biomass for *Callistemon citrinus*

Method	DF	H-Value	P-Value
Adjusted for ties	7	33.75	0.000

Table 17: Kruskal Wallis analysis of root to shoot ratio of *Callistemon citrinus*

Method	DF	H-Value	P-Value
Adjusted for ties	7	32.09	0.000

Table 18a: Analysis of variance of plant height for *Callistemon citrinus*

Source	DF	F-Value	P-Value
Soil type	1	51.02	0.000
Soil treatment	1	0.10	0.751
Watering treatment	1	1.07	0.306
Soil type*Soil treatment	1	6.20	0.016
Soil type*Water treatment	1	1.51	0.225
Site (Soil type)	4	2.25	0.076
Soil treatment*Watering treatment	1	0.38	0.540
Soil type*Soil treatment*Watering treatment	1	2.90	0.095
Error	52		

Table 18b: Tukey post-hoc analyses for soil type*soil treatment combination of plant height for *Callistemon citrinus*

Soil type*Soil treatment	N	Mean	Grouping	
Urban Unsterilised	16	30.4132	A	
Urban Sterilised	16	23.5257	A	
Non-Urban Sterilised	16	11.7958		B
Non-Urban Unsterilised	16	6.4708		B

Table 19: Analysis of Variance of total biomass for *Syncarpia glomulifera*

Source	DF	F-Value	P-Value
Soil type	1	16.77	0.000
Soil treatment	1	25.50	0.000
Watering treatment	1	8.71	0.005
Soil type*Soil treatment	1	0.06	0.809
Soil type*Watering treatment	1	1.31	0.257
Site (Soil type)	4	7.21	0.000
Soil treatment*Watering treatment	1	0.23	0.633
Soil type*Soil treatment*Watering treatment	1	0.64	0.426
Error	52		

Table 20: Kruskal Wallis analysis of root to shoot ratio for *Syncarpia glomulifera*

Method	DF	H-Value	P-Value
Adjusted for ties	7	11.97	0.101

Table 21: Analysis of variance of plant height for *Syncarpia glomulifera*

Source	DF	F-Value	P-Value
Soil type	1	7.22	0.010
Soil treatment	1	17.31	0.000
Watering treatment	1	7.57	0.008
Soil type*Soil treatment	1	0.80	0.376
Soil type*Watering treatment	1	1.94	0.170
Site (Soil type)	4	4.65	0.003
Soil treatment*Watering treatment	1	0.42	0.519
Soil type*Soil treatment*Watering treatment	1	1.43	0.238
Error	52		