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Investigating Biotic Interactions as a Tool to Improve the Growth of Vegetation in a Green Roof

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Investigating Biotic Interactions as a Tool to Improve the Growth of Vegetation in a Green Roof

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Abstract

Green roofs are an integral part of the urban forest and are widely used as tools in urban planning for sustainable development. One of the main reasons for the increased focus on green roofs is their social, environmental and economic benefits. Plant selection is a critical component in green roof design, as the vegetation on a roof influences the benefits obtained. Incorrect plant selection also contributes to some of the drawbacks of green roofs, such as the high ongoing cost of maintaining or replacing plants and increased water/fertiliser usage. Biotic facilitative interactions were investigated in this thesis as a means to improve plant growth on a green roof.

A survey across green roofs in Sydney was used to establish the current building practices of green roofs in relation to their vegetation, substrate and physical characteristics. I also attempted to classify green roofs based on their function and investigated whether current designs provided social, environmental and ecological benefits. This was done by scoring roofs based on surveyable attributes associated with each benefit. Of the 29 green roofs surveyed across Sydney, only two roofs scored high in all three categories. Seven roofs scored high in social and environmental benefits, with four scoring high only in ecological benefits. The focus on social benefits was unexpected since most green roofs specified environmental benefits as one of their key aims. The results indicate there might be a shift in the perception of green roofs from being a tool to ameliorate urban physical characteristics to a tool that can enhance concentration, productivity and mental health in the workplace. However, care should be taken so that the focus on social benefits does not come at the expense of the other benefits.

A 12-month experiment using green roof mesocosms with four different native species pairs (nurse plant and a target ground cover plant) was used to investigate the effect of nurse plants on plant survivability. To examine the effect of shading and competition on plant growth, plant pairs were subjected to four treatments: naturally shaded with a

potential nurse plant shading the target plant; artificially shaded with an artificial plant shading the target plant; unshaded natural, which had a trimmed potential nurse plant providing no shade to the target plant; and an unshaded control with the target plant being the sole occupant of the mesocosm. Overall, shading had a positive effect on plant growth, but I also found a positive influence of growing together with a nurse plant that is not accounted for by shading. The results highlight the complexity of biotic relations between plants and emphasise that a nurse plant's presence can be beneficial to the survival and growth of other species within a green roof ecosystem.

One of the possible interactions not accounted for in the shading experiment was plant-microbe facilitation. A 9-month glasshouse experiment was used to investigate the effect of substrate and inoculation type on mycorrhizal colonisation and plant biomass across two native plant species, *Dichondra repens* and *Viola hederacea*. Four green roof substrates were tested: soil (potting mix), scoria, terracotta and perlite. I also compared a locally sourced inoculant against a commercial inoculant. I found that substrate and inoculation type strongly influenced mycorrhizal colonisation and facilitation of plant growth. Hard substrates, such as terracotta, performed poorly, with terracotta having the lowest mycorrhizal colonisation and biomass increase. Potting mix had the highest biomass increase, with perlite sustaining higher mycorrhizal colonisation across the two species.

Contrary to what I expected, the commercial inoculant performed better, increasing plant growth and improving colonisation compared to the locally sourced inoculant. The experiment highlights how mycorrhizae can be used as a mechanism to improve plant growth on a green roof. Across all treatments, the addition of mycorrhizae increased biomass when compared to the control.

Using what I learned in the previous two experiments, I investigated if plant–plant and plant-microbe biotic interactions varied over at different levels of water abiotic availability. A similar experiment was set up in a factorial design with two native species, *D. repens* and *V. hederacea*. The effect of a nurse plant on biomass, growth and mycorrhizal colonisation was compared under varying water treatments in a 9-month experiment. The water treatment had three levels: 25%, 50% and 100% of current watering practices used on semi-intensive green roofs in Sydney, New South Wales (4 L/m^2 daily, Chapter 2 Survey). I found that the addition of a nurse plant and elevated water levels increased biomass for both species; however, there was no evidence of a significant interaction between increased shading and water levels, which suggests that the facilitatory effect was not improved when conditions became more stressful.

Overall, the experiments substantiate the use of biotic interactions as a mechanism of improving plant growth on a green roof. Nurse plants increase the variety and structure of plants on a green roof, contributing to the benefits of a green roof while reducing ongoing maintenance costs. Both the addition of nurse plants or mycorrhizae are easily implemented and do not require a change to the structure of the green roof.

Acknowledgements

Firstly, I would like to thank my supervisors, Kris French and Sharon Robinson. Kris thank you for encouraging me to start a PhD and allowing me to pursue the topics that I was passionate about. Your open-door policy, patience and constant positive feedback is what kept me afloat through some of the trying times. Sharon, you have been instrumental in helping me finish my PhD and in developing my abilities by giving me an opportunity as an HDR Mentor and with the iMOVE scholarship.

A big thank you to the Kris french lab (both past and present members) for all the great times and discussions in the Lab and for helping me with my experiments. Mitch, thanks for making the PhD journey more enjoyable. It was valuable having someone to bounce ideas off and spend hours procrastinating “building a graph in r” or 3d printing a “necessary” part for the experiment.

Thank you to my friends and family. Mum, Dad thank you for your constant support and weekly calls to see how I am doing. Kent, buddy thanks for your patience and support, I have probably missed out on half the events we planned this year to “finish my PhD”

Lastly an extra special thankyou to my better half Marie, for sacrificing her time on weekends and holidays to read through my dreaded 1st drafts and supporting me though the ups and downs of the PhD

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Certification of Authorship

I, Axton Conrad Aguiar, declare that this thesis submitted in fulfilment of the requirements for the conferral of the degree Doctor of Philosophy from the University of Wollongong is wholly my own work unless otherwise referenced or acknowledged. This document has not been submitted for qualifications at any other academic institution.

Axton Conrad Aguiar

22 September 2020

Publications and Presentations

Publication

Aguiar, AC, Robinson, SA & French, K 2019, ‘Friends with benefits: the effects of vegetative shading on plant survival in a green roof environment’, *PloS One*, vol. 14, no. 11, article e0225078.

Presentations

‘Friends with benefits: effects of vegetative shading on plant survival in a green roof environment’, paper presented at the *Ecological Society of America* (New Orleans, United States), 6-10 August 2018, and *Society of Experimental Biology* (Florence, Italy),2-6 July 2018.

‘Facilitation as a mechanism in adding plant growth and survival on green roofs’, invited presentation to the environment team at Shellharbour and City of Sydney Council.

Scholarship

Scholarship (iMOVE): Worked with an industry partner (Westpac Little Ripper) to enhance machine learning algorithms with the use of drone-collected hyperspectral images to aid in the detection of noxious/agricultural weeds.

Australian Government Research Training Program Scholarship

Thesis Outline

This thesis is organised into four main data chapters, with three written to be published in the journals as listed in Table 1. In each of the papers below, the other authors comprise my supervisors, Kris French and Sharon A Robinson. My supervisors assisted in the conception and design of the experiments, the interpretation of the results and the reviewing of manuscripts.

Because the chapters were written as standalone papers, there is some repetition between the literature review (Chapter 1) and the standalone papers (Chapters 2–5).

Table 1: Outline of the thesis.

Chapter title	Publication	Contributions
Chapter 1		
Literature review	Not written for publication	Axton Aguiar: 75% Kris French: 20% Sharon A Robinson: 5%
Chapter 2: Urban survey		
Australian Focus on Green Roofs: Evaluating Green Roof Design	Not written for publication	Axton Aguiar: 75% Kris French: 20% Sharon A Robinson: 5%
Chapter 3: Plant–plant facilitation		
Friends with Benefits—The Effects of Vegetative Shading on Plant Survival in a Green Roof Environment	Aguiar, AC, Robinson, SA & French, K 2019, 'Friends with benefits: the effects of vegetative shading on plant survival in a green roof environment', *PLOS One*, vol. 14 no. 11, article e0225078	Axton Aguiar: 70% Kris French: 20% Sharon A Robinson: 10%
Chapter 4: Plant–microbe facilitation		
The Effect of Green Roof Substrates on Mycorrhiza–Plant Interactions	Submitted to *Urban Ecosystems*	Axton Aguiar: 70% Kris French: 15% Sharon A Robinson: 15%
Chapter 5: Plant–plant facilitation across an abiotic gradient		
Investigating Biotic Facilitation along an Abiotic Gradient (water) in a Green Roof Environment	Written to be submitted to *Landscape and Urban Ecology*	Axton Aguiar: 70% Kris French: 15% Sharon A Robinson: 15%
Chapter 6		
Discussion	Not written for publication	Axton Aguiar: 80% Kris French: 15% Sharon A Robinson: 5%

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List of Abbreviations

AMF	arbuscular mycorrhizal fungi
ANOVA	analysis of variance
DESA	Department of Economic and Social Affairs
ERC	Ecological Research Centre
HSD	(Tukey's) honestly significant difference
NSW	New South Wales
UHI	urban heat island
SA	shaded artificial
SN	shaded natural
U	unshaded
UN	unshaded natural

Chapter 1: Literature Review

Contributions

Axton Aguiar collated and reviewed the research, identified gaps in the field and wrote the manuscript.

Kris French aided in identifying research gaps, framing research questions and reviewing the manuscript.

Sharon A Robinson aided in identifying research gaps, framing research questions and reviewing the manuscript.

Publication

Not written for publication.

Urbanisation

Urbanisation is one of the fastest-growing land uses worldwide. Urbanisation has been associated with improved economic standing, such as higher prospects of finding a job; improved literacy, education and health; longer life expectancy; and diverse social and cultural activities due to increased opportunity (Department of Economic and Social Affairs [DESA] 2014). These benefits have seen the world's urban population increase exponentially. Globally, 55% of the world's population inhabits cities, which is projected to increase to 68% by 2050 (DESA 2018). This increase in urban population worldwide has resulted in a higher demand on urban resources, increased pollution, increased urban density and the expansion of urban sprawl (DESA 2014; 2018). This change in land use and the increased population density associated with cities cause a host of adverse environmental impacts that have severe consequences for local biodiversity, human health, and well-being. Some of the most prominent environmental effects include increased pollution (Dikareva & Simon 2019), loss of local biodiversity (Sol et al. 2014), increased storm water runoff due to the increase of impervious surfaces (Hamel et al. 2013, Walsh et al. 2005) and the creation of the urban heat islands (UHIs) (Rizwan et al. 2008). The UHI effect is a phenomenon first described by Manley (1958), whereby the urban city experiences warmer temperatures when compared to the surrounding/nearby rural areas. The temperature difference is due to anthropogenic surfaces, such as bricks and roads, that have a higher thermal capacitance when compared to natural surfaces.

As the urban population continues to increase, there has been a need to identify, develop and implement strategies and policies that can help offset some of the negative aspects of urbanisation. This is highlighted in the United Nations Sustainable Development Goals, 'Goal 11: Sustainable Cities and Communities' (UN, 2018). One strategy that has received increasing interest over the last decade is the use of urban vegetation and green spaces to ameliorate some of the negative aspects of urbanisation.

Urban Green Spaces

Green spaces, in an urban environment, incorporate all vegetation within the boundary of the urban area, including street trees, parks, gardens and green roofs. Green spaces in urban landscapes have been actively promoted as a planning tool to provide essential ecosystem services and functions that have environmental, economic and social benefits for the inhabitants of the city.

Environmental Benefits

Green spaces have been shown to reduce environmental degradation caused by urbanisation. The introduction of vegetation has been shown to improve air quality, reduce pollution in waterways and reduce the effect of UHI (Nowak, Crane & Stevens 2006; Paton-Walsh et al. 2019; Robinson & Lundholm 2012; Szota et al. 2018, Santamouris 2014). Urban environments have high particulate matter in the air from traffic emissions and industrial waste and development/construction (Paton-Walsh et al. 2019), which can pose a significant risk to human health. Urban vegetation is not only effective at trapping fine particulate matter but also capturing some of the airborne pollutants, such as ozone, nitrous oxide, carbon monoxide and carbon dioxide (Beckett, Freer-Smith & Taylor 1998). Green spaces have also been shown to delay the egress of stormwater, reducing the burden on stormwater systems (Ercolani et al. 2018; Szota et al. 2017; 2018). By ameliorating some of these adverse effects, urban green spaces also enhance urban sustainability and build resilience for the urban environment to accommodate the effects of climate change.

Social Benefits

Socially, urban vegetation can enhance human health and wellbeing, improve levels of concentration, promote healing, and increase productivity (Hartig et al. 2014; Williams et al. 2019). Green spaces provide recreational opportunities, encourage physical activity, reduce stress and provide a space for social cohesion (Hartig et al. 2014). Such spaces have also been linked to reductions in crime (Burley 2018; Donovan & Prestemon 2010). Burley (2018) found that there was a strong negative correlation between the number of trees planted and violent crimes for suburbs in Portland, Oregon.

Economic Benefits

The economic advantage of vegetation is closely tied to its social and environmental benefits. For example, the associated increase in human wellbeing and mental health leads to higher levels of productivity in businesses. Lee et al. (2018) found that workers who had a view of a green roof outside their window had a higher level of productivity, were more consistent in responding to tasks and made significantly fewer errors.

Another positive economic benefit relates to the power savings due to shade trees and the reduction in the UHI effect. Xang et al. (2014) found that the shading effect of green roofs reduced the energy consumption of a two-storey building by 27%. Green spaces also directly increase the value of real estate. Altunkasa et al. (2004) investigated whether there was a correlation between urban greenness and real estate values. They found that suburbs with street trees and parks had a higher house value when compared to suburbs without.

There is a broader argument for increased urban vegetation stemming from the need to improve the resilience of cities to climate change and improve quality of life and public benefits. Urban green spaces, such as parks and street trees, face complex social and development pressures. One type of urban vegetation not affected by land-use pressures and makes use of underutilised space in an urban environment is vegetated roofs, commonly known as green roofs.

Green Roofs

Green roofs, also known as living roofs or vegetated roofs, are roofs that support the growth of vegetation. This is typically done by incorporating additional layers such as a growing medium and plants to an existing traditional roof system. Green roofs have historically been divided along a spectrum based on the vegetation structure and substrate depth. At one end are extensive green roofs, which are characterised by shallow growing substrates ranging from a few centimetres to 20 centimetres, typically constructed with no irrigation. However, these roofs can be supplemented with emergency irrigation in the summer months. The vegetation typically consists of bryophytes and succulents, often from the *Sedum* genus. At the other end of the spectrum are intensive green roofs, which consist of a deeper growing substrate ranging from 20 centimetres to 1 metre, with irrigation. The deeper substrate and inclusion of irrigation facilitate the use of more complex vegetation such as herbs, shrubs and trees.

Green roofs are not a new concept, iterations of green roofs can be seen throughout history dating back to the ziggurats of Mesopotamia (Osmundson 1999). Some of the earliest examples of widespread green roofs were sod/turf roofs in northern Europe (Peck 2008, Osmundson 1999). These roofs consisted of turf/grass vegetation grown on roofs used for their insulating properties and to grow produce. The modern iteration of a green roof began in Germany, which is one of the leading countries in green roof implementation (Herman 2003). What distinguishes these modern roofs from their historical counterparts is that they are engineered structures designed to be added to existing buildings/houses and are of a layered design incorporating a growing medium, vegetation, drainage and structural layers (Osmundson 1999). Early extensive green roofs (also known as sedum roofs) were constructed with thin substrate layers to enable them to be placed on pre-existing buildings (Getter et al. 2006). These roofs were predominantly built to assist in stormwater mitigation and building heat amelioration (Getter et al. 2006). As green roof technologies advanced, more complex green roofs were incorporated into building design increasing substrate depth and vegetation types used to deliver on additional ecosystem benefits such as biodiversity and wellbeing (Lundholm et al. 2015a). Currently, in many countries, there are strong initiatives to both incorporate green roofs into new developments and retrofit old buildings.

Most of the benefits associated with green roofs are closely tied to the substrate properties and vegetation present (Lundholm et al. 2015a, Lata et al. 2018). Substrate type, composition and depth play an important role on a green roof. The size and type of substrate influence the water holding capacity, albedo and runoff of a green roof (Vijayaragjavan and Raja 2014, Cook and Larsen 2020). Substrate type and depth also play a critical role in determining the vegetation that can be used on a green roof (Cao et al. 2014, Cook and Larsen 2020). Substrate types can affect plant growth and survival by influencing factors such as water availability, soil temperature and nutrient availability (Cao et al. 2014, Reyes et al. 2016 Ampim et al. 2010).

The type, variety and cover of plants play an important role in determining the success of a green roof in providing many of the potential ecosystem services (Lundholm et al. 2015 Cook-Patton et al. 2012, Farrell et al. 2013). Appropriate plants improve stormwater management (Farrell et al. 2013), reduce UHIs (Bevilacqua et al. 2017, Santamouris 2014), provide habitat-increasing biodiversity (Schindler et al. 2011) and reduce energy for heating and cooling (Kumar and Kaushik 2005).

Plant Selection

Plant selection is one of the most important factors when designing a green roof, as a green roof is a harsh environment for a plant to grow in (Nagase and Dunnett 2010). The vegetation on a roof must contend with shallow growing substrates, low water, high environmental stressors (wind and irradiance) and pollution. The substrates, or the growth medium, are often nutrient-deficient, inorganic natural or recycled materials designed to reduce the weight of the substrate and prevent compaction over time, with low nutrients to prevent weed growth and eutrophic run-off (Vijayaraghavan 2016). Due to the specific needs of vegetation for a green roof, much of the early research focused on using succulents, specifically *Sedum* spp., due to their ability to reduce transpiration and conserve water, making them highly resilient to drought conditions (VanWoert et al.

2005, Oberndorfer et al. 2007). However, using only one type of plant has been shown to limit the ecosystem services a green roof can provide (Lundholm 2015; Nagase & Dunnett 2010). Nagase & Dunnett (2010) investigated the effect of plant species, diversity, and structure had on extensive green roof water runoff. Twelve species were selected from three functional groups, forbs, succulents and grasses. They found that grasses were the best at reducing water runoff and succulents the worst. They also found that plant size and structure played a significant role in determining if a plant effectively reduced water runoff. Several studies have suggested that the use of a multi-trophic diverse type of plantation would help maximise the benefits. Species diversity on green roofs has been associated with increased ecosystem benefits (Lundholm 2015; Nagase & Dunnett 2012). However, incorrect selection of vegetation results in mortality, poor growth, increases costs and potentially decreases benefits. The two primary methods of selecting plants for a green roof are based on plant traits or matching habitat traits (Lundholm 2006, Lundholm et al. 2015, Van Mechelen et al. 2014, Farrell et al. 2013). Plant traits can be used to improve survival by selecting plants with traits such as Crassulacean Acid Metabolism (CAM) and leaf succulence (Van Mechelen et al. 2014 Farrell et al. 2013) or traits that improve ecosystem function such as increased flowering or improved water retention (Lundholm et al. 2015, Zhang et al. 2018). The second method is the habitat template approach recommended by Lundholm (2006). It is based on the notion that some species are preadapted to green roof environments due to their natural habitat sharing similar characteristics with a green roof (Lundholm 2018). Following these ideas, the use of theories and practices developed through observing natural communities would be useful for informing plant selection on a green roof.

Vegetation Communities

The species composition of vegetation communities in natural settings is driven by their interactions with biotic and abiotic factors. Historically, abiotic factors (soil moisture temperature, nutrients and pH) were thought to have a greater influence on species establishment and survival (Billings & Mooney 1968) than biotic interactions (e.g., competition and facilitation), which were thought to have a greater role in species coexistence and structure (Callaway 1995; Grime 1973). The intensity or variation of these factors along a gradient is what accounts for the variation both in and between vegetation communities (Brooker & Callaghan 1998). These variations can occur at small spatial scales of a few centimetres to global scales. With an uncertain climatic future, changes in climatic variables will likely play an even bigger role in the distribution and composition of vegetation communities (Ballare et al. 2011; French et al. 2019; Keith, Elith & Simpson 2014).

Changes in climate also affect biotic factors such as competition, predation and facilitation, with changes in local herbivory patterns and microbial communities already identified (Bale et al. 2002; de Sassi, Lewis & Tylianakis 2012). Sassi, Lewis and Tylianakis (2012) found that higher temperatures and elevated nitrogen altered the relative abundance and composition of herbivorous insects. The composition of the herbivorous insects' community was more homogenous in elevated temperature, and its biomass doubled in size with elevated nitrogen and quadrupled with increased temperature. Breeuwer et al. (2009) found that increasing temperatures associated with climate change influenced the competitive strength in some sphagnum moss species. They found that sphagnum moss species such as *Sphagnum balticum* that are adapted to shallow pools (hollow species) would be outcompeted by *Sphagnum fuscum,* which are more adapted to drier habitats (hummock species). Changes in climatic patterns also affect microbial communities in the soil. Research has shown that warming and CO_2 influences arbuscular mycorrhizal fungi (AMF) colonisation (Hodge, Campbell & Fitter 2001; Wilson et al. 2016). Despite these studies, few have considered how changes in the microbial community due to climate change affect their facilitative interactions with the host plant.

While there is abundant literature on the effects of varying abiotic factors on vegetation communities, there is a knowledge gap in understanding the subtleties of how abiotic variables influence biotic interactions among species (plant–plant and plant–microbe interactions). Most of our understanding of plant community–based interactions have been derived from studies that have isolated and analysed how abiotic variables affect specific mechanisms (e.g., competition, predation, facilitation) that, in turn, affect vegetation communities (Callaway 1995; De Bello et al. 2011). However, biological interactions are rarely in isolation from each other but rather represent a web of complex interactions. Using this single-interaction approach means we have been unable to identify how interactions between mechanisms may be important in affecting community structure.

Over the past two decades, there has been some debate concerning the major drivers of community structure. While, historically, positive and negative interactions were identified as contributors to community dynamics (Callaway 1995; Connell 1983), there has been an overemphasis on competition processes at small spatial scales (Bertness & Leonard 1997). Many prominent ecological theories and models, such as niche theory and the competitive exclusion principle, are based on the view that conflict structures the natural world. This has led to almost two decades of research classifying and outlining the importance of competition, predation, disturbance and environmental stresses in shaping communities (Connell 1983; Schoener 1983). While, two decades ago, negative species interactions and physical environments were considered the dominant drivers of species distribution and abundance (Connell 1983), there is now a consensus among ecologists that facilitation, a factor once thought of as anomalous, plays an equally important role in determining community structure (Callaway 1995).

Facilitation and Competition

Competition is an interaction between two organisms in which the fitness of both organisms is reduced (Strobek 1973). Direct competition is where an organism directly interferes with the growth/survival of another organism through either interference or resource use (Lawlor 1979). One of the most prominent examples found on a green roof is resource competition.

Facilitation is defined as an interaction between organisms in which at least one organism receives benefits, and neither are negatively affected by the interaction (Boucher 1982). Facilitation can be expressed in two different ways: mutualism and commensalism.

Mutualism is an interaction in which both organisms receive benefits (Boucher 1982). An Australian example of this is acacia species and their nitrogen-fixing rhizobial symbionts. The rhizobial symbionts use the sugars produced by the plant through photosynthesis to produce fixed nitrogen, which is then used by the host plant for growth. Fungal endophyte – plant symbioses, in which the fitness of the host plant and the symbiont fungi are closely aligned towards mutually beneficial cooperation, are well known (Carroll 1988; Sortibrán, Verdú & Valiente-Banuet 2019). While the direct benefits of the symbiosis are commonly measured, indirect benefits through controlling plant predators have also been found. Hinton and Bacon (1985) found that cattle consuming tall fescue grasses had an elevated level of alkaloid toxicity, linked to alkaloids produced by an endophytic fungus (*Neotyphodium coenophialum*). *Neotyphodium* grass endophytes form a lifelong association in the above-ground section of the host plant. The fungi grow into the developing inflorescence and seeds and thus increase its virulence and dispersal, while the host plant benefits as the toxins contained in the fungi dissuade predation of both the host plant and its seeds (Bacon & Hill 2013; Hinton & Bacon 1985).

Commensalism is an interaction in which one species receives benefits, and the other is unaffected. Dispersal of cobbler's pegs (*Bidens pilosa*) is a form of phoresy, whereby seeds attach to an animal's fur to aid in distribution. The propagule is dispersed through animal movement, with the animal receiving no benefits or small negative impacts. Epiphytic plants, which are a major component of tropical flora (Silva et al. 2010; Zotz & Bader 2009), have developed physiological traits that allow them to grow on other plants, which elevates them from the ground where there is limited light availability. The host plant receives no benefits or drawbacks from this interaction.

However, competition and facilitation do not occur in isolation from each other. These interactions between positive and negative mechanisms have been documented across communities (Brooker et al. 2008; Callaway et al. 2002; Olofsson, Moen & Oksanen 1999). It is the net effect, the sum of positive and negative interactions, that determines the overall interaction between two species (Brooker & Callaghan 1998). Biotic interactions have been shown to vary across abiotic variables (Bertness & Callway 1994). The stress-gradient hypothesis (SGH) is a conceptual model proposed by Bertness & Callway (1994); it states that net positive or facilitative interactions should be more prevalent in communities that are under higher stresses (defined as external abiotic constraints on productivity, Grime 1977) when compared to communities that develop in more benign conditions (Bertness & Callway 1994). Over the past two decades, there has been overwhelming evidence supporting the SGH across a variety of abiotically stressful environments such as deserts (Tielbörger & Kadmon 2000), alpine (Callway et al. 2002), salt marshes (Pennings et al. 2003) habitats.

Research on facilitation in abiotic stressful environments is particularly important for green roofs. Green roofs are stressful environments for plants to grow in (Van Mechelen et al. 2014) and have similar abiotic characteristics to harsh natural environments where facilitative interactions have been shown to be important. For this thesis, the two main areas of facilitation investigated were the benefits of plant–plant and plant–microbe facilitation in relation to plant growth and survival.

Testing the interaction between abiotic variables and species composition can determine the relative importance of competition and facilitation. They are also novel ecosystems in which plants can coexist in ways not normally found in nature. Therefore, we can test various combinations of plants for both facilitation and competition interactions that help shape and delineate different vegetation communities.

Plant–Plant Facilitation

Facilitative interactions among plants can enhance pollination, seed dispersal, seedling establishment and plant growth/survival (Figure 1; Barbosa et al. 2009; Brooker et al. 2008; Filazzola & Lortie 2014; Lortie, Filazzola & Sotomayor 2016; Reid, Lamarque & Lortie 2010). Co-occurring plants with a shared flowering phase contribute to the reproductive success of neighbouring plants by attracting pollinators (Barbosa et al. 2009; Mcintire & Fajardo 2014). Plants can also facilitate the dispersal and establishment of seeds. Similar to pollinators, co-occurring fruiting plants increase fauna visitation rates that may be beneficial to neighbouring plants (Albrecht et al. 2015). For example, the attraction of fauna to a fruiting plant could increase the dispersal of neighbouring plants that rely on a hitchhiking diaspore mechanism, an example of a commensal interaction. Cushion plants in subarctic tundra conditions act as a trap for windblown seeds (Reid, Lamarque & Lortie 2010). Their canopy regulates the temperate and environment under them, providing a suitable habitat for the seeds to germinate and grow (Reid, Lamarque & Lortie 2010).

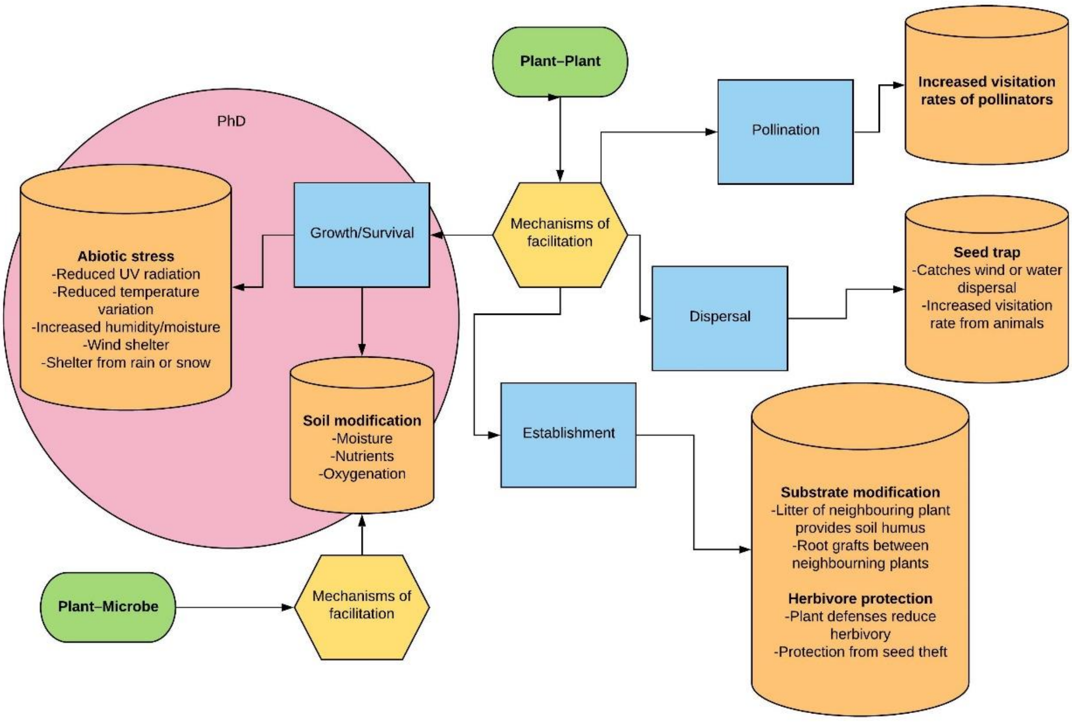

Figure 1: Plant facilitation flowchart outlining the mechanisms of plant–plant and plant–microbe facilitation. Green stadiums outline biotic interactions, blue rectangles outline functions affected by facilitation, and the silos indicate the factors modified. Adapted from Filazzola and Lortie (2014).

There are various mechanisms by which plants can facilitate the growth/survival of neighbouring plants. One commonly studied mechanism is the amelioration of abiotic stresses, such as heat, cold, wind and light. For example, shade provided by co-occurring trees allows for plants that are adapted for low light to grow without the stress induced by direct solar radiation. López et al. (2007) examined the role of nurse plants in the establishment of shrub seedlings and found that nurse plants facilitated plants in all three stages. Windblown seeds were trapped by the nurse plants, the ground below the nurse plant was also nutrient-rich due to the layer of dead leaves providing soil humus and, lastly, the nurse plant also shaded the seedling from high temperatures and radiation.

Nurse plants facilitate the growth and survival of other plants within their canopy by modifying the microclimate and soil (López et al. 2007; Reid, Lamarque & Lortie 2010). The canopy ameliorates abiotic weather conditions by either trapping heat in colder alpine conditions (Molenda, Reid & Lortie 2012) or by providing shade in hotter conditions (Randall et al. 2007); increases soil nutrients by increasing decomposing matter from leaf litter (Anthelme & Dangles 2012); reduces wind (le Roux & McGeoch 2010); decreases soil temperature and increases soil moisture content (Schöb, Butterfield & Pugnaire 2012). Nurse plants also increase species richness (Badano & Marquet 2009) and abundance (Sklenář 2009) and play an essential role in increasing the survivability of species (Badano & Marquet 2009; Molenda, Reid & Lortie 2012; Sklenář 2009).

Nurse plants are ideal for green roof environments as the nurse plants will not only facilitate other plant species but also provides diversity to the vegetative component of the green roof, improving ecosystem services (Lundholm et al. 2014). Facilitation has been shown to improve plant function and survival on a green roof (Butler & Orians 2011, Lundholm et al. 2014). Most of the early experiments exploring the facilitatory benefits of plant-plant interactions on green roofs have focused on succulents. Sedum has been shown to perform well as a nurse plant and facilitate the growth of other plant growth forms by reducing abiotic stresses such as lowering the temperature of the soil and increasing soil moisture (Vasl et al. 2017, Butler & Orians 2011).

Plant–Microbe Facilitation.

Facilitation may also occur between plant species and the microorganisms present in the roots and soil (He, Critchley & Bledsoe 2003; Verbruggen et al. 2013). Plants form associations with two main groups of microorganisms: fungi and bacteria (Brundrett & Tedersoo 2018; Hortal et al. 2017; Lumactud & Fulthorpe 2018). In this thesis, I will be focusing on fungal associations, as they occur across many families of plants (Brundrett 2004; Brundrett & Tedersoo 2018).

The term 'mycorrhiza' is used to describe a symbiotic association between a fungus and a plant. The fungal colonies inhabit the plant's root either within the cells, as in endomycorrhizal fungi (AMF or ericoid mycorrhizal), or extracellularly, as in ectomycorrhizal fungi. Mycorrhizal associations are found in 80%–90% of all terrestrial plants at some stage in their life cycle (Brundrett & Tedersoo 2018; Wang & Qiu 2006), with most occurring as arbuscular mycorrhizae. The association between plants and mycorrhizal fungi is mainly mutualistic: the plants receive minerals and nutrients, and the fungi receive direct access to carbohydrates produced by the plant during photosynthesis (Hampp & Schaeffer 1999). While the plant can directly access some of the macronutrients present in the soil, the fungi have access to nutrients that are chemically or physically immobilised in the soil (Hodge, Campbell & Fitter 2001; Martin, Uroz & Barker 2017). For example, the nutrients in decaying organic matter are unavailable for plant roots to uptake directly; however, mycorrhizal fungi can break down the organic matter, mobilising the nutrients and making them available to the host plant (Hodge et al. 2001). Nutrient transfer from plant–fungi associations can affect plants' growth and survivability in adverse abiotic conditions (Fulthorpe et al. 2018; Nadeem et al. 2014). Porcel et al. (2012) found that, in arid saline environments, plants have developed associations with AMF, which help the host plant combat water loss through mechanisms such as synthesis of solutes to prevent cell dehydration, maintenance of water uptake and regulation of ions.

Plant microbe facilitation could prove a useful and relatively easy way to improve green roof performance (Lundholm et al. 2017). The introduction of mycorrhizae on green roofs have been shown to improve plant performance and survival (Sutton 2008, Molineux et al. 2014, Fulthrope et al. 2018). A study conducted by Young et al. (2015) found that the inoculation of green roof with mycorrhizal fungi improved the flowering time of *Prunella vulgaris*. Mycorrhizal type and substrate play an important role in determining the effect of mycorrhizal facilitation. John et al. (2014) found that commercial green roof media was devoid of any functional mycorrhizal inoculum. Green roofs can be inoculated using either natural inoculant or commercially available inoculant although further research is required to validate the use of commercial products with native species.

Factors that can influence biotic interactions

The outcome biotic interactions can be highly context dependant, it is subjected to plant and mycorrhizae identities, plant growth stage, inoculation type, abiotic variables and substrate properties (Young et al. 2015, McGuire et al. 2013). A positive interaction at one life stage may transform into a negative interaction at a later stage. Nurse plants in alpine conditions provide shade and shelter for seeds, but, as the seeds grow, they start to compete for resources, shifting the relationship from commensalism to competition (López et al. 2007). Wright et al. (2014) found that, in younger pines, when the environmental conditions are severe, facilitation provided by neighbouring plants in the form of protection from environmental extremes was dominant in aiding growth. However, as the plants grew bigger, competition was more substantial than facilitation. These interactions highlight the need to study how biotic interactions vary with changing abiotic factors and time.

The urban environment provides us with an environment in which plants that do not normally coexist are placed together in an enclosed setting in stressful environments. This provides us with an ideal location to test the interaction between abiotic variables and species composition. We can also determine the relative importance of competition and facilitation and whether species from different habitats still respond in the same way and/or provide the same facilitative benefits when in an anthropogenic setting.

Green roofs an Australian context

Australia is one of the most urbanised nations globally, with over 90% of its population inhabiting urbanised areas and concentrated in five major cities (Australian Bureau of Statistics 2017). The population density in urban centres and the hot, dry climate conditions typical to Australia are ideal for realising the benefits of green roofs (Razzaghmanesh et al. 2014). However, the green roof industry in Australia, while growing more popular in recent years, is still in its infancy when compared to North

America and Europe. The main factors limiting the uptake of green roofs in Australia such as high installation cost, no Australian standard for green roofs, lack of governmental support and the lack of local research, were identified by William et al. (2010). In the ten years since that publication, there has been headway in resolving some of the issues, such as increases in experimental green roof research identifying substrates and species for Australian conditions (Farrell et al. 2017, Perkins & Joyce 2012), assessment of the benefits of green roofs (Razzaghmanesh et al. 2014, Farrell et al. 2013, Zhang et al. 2019) and release of local guides: *Growing Green Guide* (State of Victoria. 2014) and the *Sydney City Council Green Roof Design Resource Manual* (City of Sydney Council 2014). There are still areas which need to be improved Australia is still lacking government incentives, overarching industry standards for green roofs, and a centralised body that guides, oversees and maintains green roof information in Australia.

The green roof industry in Australia is primarily run by smaller design and build companies that operate within one of the major cities (with the exception of two larger companies that operate across multiple states). One of the shortcomings of not having green roof standards or an overarching organisation is the lack of consistent green roof terminology used in both the definitions of what is a green roof and the types of green roofs.

Thesis Aim

This thesis aims to investigate whether biotic facilitation through plant-plant and mycorrhizal interactions can improve green roof plant growth.

Objectives

- Identify shortcomings and issues in current green roof terminology and design used in Sydney, Australia. (Chapter 2)

- Investigate the role of nurse plants in providing shade for neighbouring plants as a mechanism to improve survival on a green roof (Chapter 3)
- Investigate the effect of green roof substrates in plant mycorrhizal interactions (Chapter 4)
- Assess whether plant–plant facilitation changes over an abiotic gradient (Chapter 5).

Chapter 2: Australian Focus on Green Roofs: Evaluating Green Roof Design

Contributions

Axton Aguiar conceived and designed the experiment, collected the data, performed the analysis and wrote the manuscript.

Kris French aided in the conception and design, aided in the analysis and helped frame the manuscript.

Sharon A Robinson aided in the conception and design, reviewed the analysis and manuscript.

Publication

Not written for publication.

Introduction

Green roofs are an essential part of urban vegetation and provide social, economic and environmental benefits (Oberndorfer et al. 2007). As one of the few strategies that can be implemented on pre-existing infrastructure, making use of underutilised space in the urban landscape, green roofs are becoming increasingly popular in urban landscapes. Green roofs are also not subject to the complex land-use pressures/conflicts that parks and remnant vegetation are subjected to in the development of new suburbs, roads and infrastructure.

Australia is one of the most urbanised nations globally, with over 90% of its population inhabiting urbanised areas (Australian Bureau of Statistics 2017 and concentrated into five major cities. The Australian climate (Figure 2) is heavily influenced by the Southern Oscillation (ENSO), Indian Ocean Dipole (IOD), and the Southern Annular Mode (SAM) (Power et al. 1999, Cai et al. 2012) These systems are important drivers of hot dry climate across Australia and have been shown to increase annual temperature, drive extreme weather events and multiyear droughts across Australia (Lim et al. 2019, Cai et al. 2012). The population density in urban centres, along with the hot, dry climate), makes these urban centres ideal for the realisation of the benefits of green roofs.

In Australia, the green roof industry is still in its infancy (Williams et al. 2010). Green roofs are yet to be implemented at a scale seen in European countries such as Germany, where regulations and incentives have encouraged the construction of green roofs. The earliest green roof in Australia was created in the 1970s (Williams et al. 2010); however, a focus on developing and implementing green roofs in major cities across Australia has only occurred in the last ten years. Some of the main factors limiting the implementation of green roofs in Australia was summarised in a paper released by William et al. (2010). They identified cost, lack of governmental support, and the lack of local research as the primary barries to the green roof industry in Australia (William et al. 2010)

Since then, some progress has been made in addressing some of these barriers, The City of Sydney was one of Australia's first cities to adopt a green walls and roofs policy in 2014. Additionally, in 2014, a technical manual (the *Green Growing Guide*) was released for Melbourne (State of Victoria 2014), which was developed in collaboration between industry, government and university partners. This guide provided technical advice regarding the design, construction and maintenance of green roofs. The guide filled a significant gap by using Australia-based research and examples. There has also been an increase in green roof research evaluating plants, substrates in Australian conditions (Rayner et al. 2016, Farrell et al. 2012, Razzaghmanesh et al. 2014 Xue & Farrell 2020).

However, some overarching issues remain:

- A lack of an incentives program, green rating program that includes green roofs
- Unlike many international cities, there are no regulatory policies that mandate green roof construction in Australia
- There is yet to be any accepted standards for green roofs across Australia, similar to those outlined by the FLL- German Landscaping and Landscape Development Research Society, Forschungsgesellschaft Landschaftsentwicklung Landschaftsbau, the International Green Roofs Association (IGRA) or the General Service Administration (GSA) USA.
- The lack of a centralised repository for green roof data (location, physical attributes etc.) at either a federal, state or local level.

The green roof industry in Australia is primarily run by smaller design and build companies that operate within one of the major cities (with the exception of two larger companies that operate across multiple states). One of the shortcomings of not having green roof standards or an overarching organisation is the lack of consistent green roof terminology used in both the definitions of what is a green roof and the types of green roofs.

Currently, types of green roofs are most commonly classified as intensive, extensive or semi-intensive (FLL, IGRA, GSA). However, there are no fixed definitions for these categories. For example, FLL classifies intensive green roofs, as roofs consist of a deeper growing substrate ranging from 250 to 2000 mm with irrigation. These roofs are capable of housing more complex vegetation such as herbs and shrubs. These roofs are considered to be more intensive, as they have a higher initial and ongoing maintenance cost. In contrast, the GSA sets this cut off at 12 inches (300mm).

The issues pertaining to the categorisation or classification of green roofs is not a problem that is restricted to Australia (Kotze et al. 2020). A recent paper by Kotze et al. 2020 outlines the shortcomings of using the traditional method of classifying green roofs (intensive, extensive and semi-intensive) based on vegetation structure, substrate depth, and construction factors. The main shortcoming of the traditional way of classifying roofs is the inconsistency in the factors that determine if a roof is intensive or extensive and the lack of function incorporated into the classification. They propose naming vegetated roofs based on their function and vegetation, e.g. “biodiversity roof meadow”.

This study evaluates the benefits of green roofs in Sydney based on their observable characteristics to assess if we can assign a function. We have decided to split green roof function into three categories, which are discussed below: amelioration of urban physical characteristics, improving social value and enhancing biodiversity in the city.

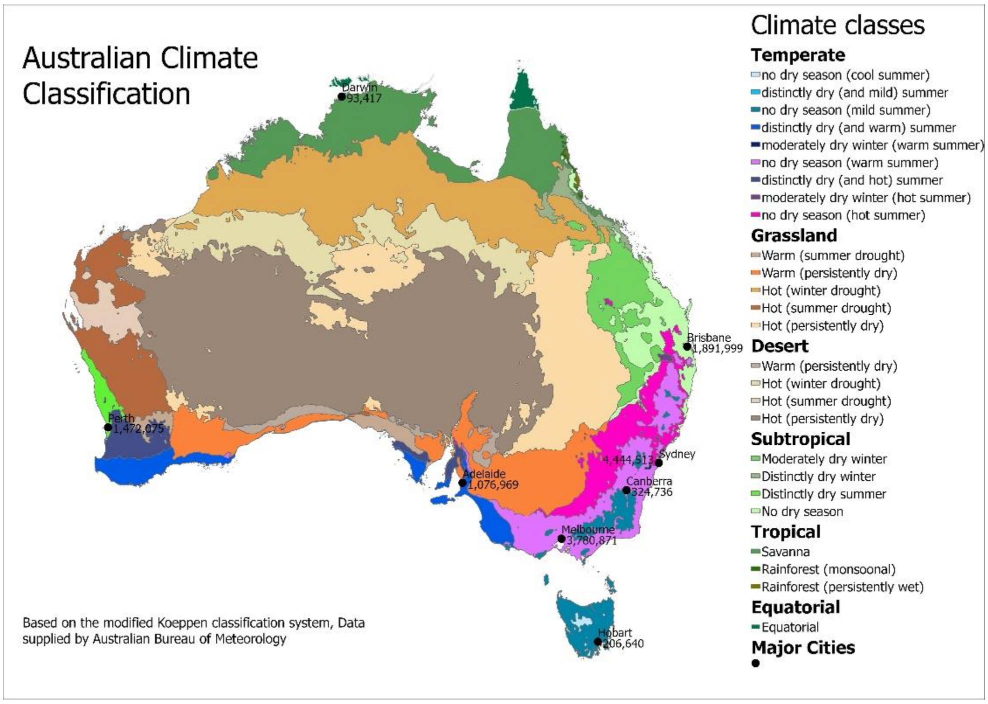

Figure 2: Climatic zones of Australia, based on a modified Köppen-Geiger climate classification system. Adapted from Bureau of Meteorology data.

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Amelioration of Urban Physical Characteristics

A fundamental argument for the development of green roofs is their ability to ameliorate some of the negative physical aspects of urbanisation, such as reducing the UHI effect and (heat, light, air and noise) pollution (Imran et al. 2019). The reduction in heat and UHI is due to the vegetative layer shading anthropogenic surfaces from solar irradiation, thereby reducing thermal energy build-up in anthropogenic surfaces. The vegetative layer also transpires, reducing the atmospheric temperature around a green roof. The shading and insulation of buildings lead to cost-saving in the form of energy reductions in cooling or heating the building (Costanzo, Evola & Marletta 2016). Berardi (2016) found that the installation of an extensive green roof on the main building of the Ryerson at the University of Toronto resulted in a 3% decrease in energy usage.

Another benefit is the ability of green roofs to aid in stormwater retention (Berghage et al. 2009; Farrell, Ang & Rayner, 2013). Urban surfaces are typically impervious and, unlike natural substrates, do not delay the flow of stormwater into creeks and rivers

(Arnold and Gibbons 1996). Due to the change in surface, natural waterways are overcome with stormwater during storms (Bhaduri et al. 2000). Green roofs delay the egress of water into urban stormwater systems (Carter and Ramussen 2006). Retention of stormwater is influenced by substrate properties, green roof design and vegetation present on the roof (Cao et al. 2014, Dunnet et al. 2008, Farrell et al. 2013). Plant composition and vegetation type has been shown to influence stormwater retention by affecting the preferential flow of water through the substrate and influencing evapotranspiration (Zhang et al. 2018, Dunnett et al. 2008, Franzaring et al. 2016). There is also evidence that green roofs act as a filter for rainwater, trapping some of the pollutants (Czemiel Berndtsson 2010; VanWoert et al. 2005)

Improving Social Value

There is a growing body of literature investigating the importance of green roofs for social amenity (Mensah et al. 2016). The natural characteristics of green roofs provide relief from the concrete construction in urban areas and introduces substantial variation to modern architecture. This change has been shown to have a positive psychological effect, reducing anxiety, improving mental wellbeing, helping to reduce blood pressure and lowering heart rate (Getter & Rowe 2006; Lee et al. 2015; Mensah et al. 2016).

Visual sight of green roofs improves productivity and workplace mood and atmosphere (Loder 2014). The presence of community gardens or the act of communally growing produce has been shown to foster the growth of workplace communities and groups, leading to better interpersonal relationships (Harris 2009). The type of vegetation also plays an important role in enhancing the social benefits of green spaces (Jiang et al. 2014, Graves et al. 2017, Hoyle et al. 2017). In a study using pictures of green roofs Lee et al. (2014) found that observers preferred green roofs with flowering plants.

Enhancing Biodiversity

Green roofs are often promoted as ecological refuges in the urban landscape (Oberndorfer et al. 2007; Williams, Lundholm & McIvor 2014). Green roofs provide foraging and roosting habitats for insect and avian species (Fernández Cañero & González Redondo 2010). Wang et al. (2017) evaluated 30 green roofs in Singapore in their ability to support avian and butterfly assemblages. They found that while green roofs had a positive effect on avian and butterfly diversity, factors such as noise, pest control and vegetation cover strongly affected the avian and butterfly communities on green roofs.

Green roofs can be used as ecological corridors connecting fragmented remnant vegetation (Braaker et al. 2014). Additionally, green roofs increase permeability into the city for mobile arthropod species such as bees and weevils, acting like ecological corridors (Braaker et al. 2014). The use of native flora on green roofs has the capacity to improve regional diversity and population abundances, depending on species choice (Cook-Patton 2015, Wang et al. 2017, Madre et al. 2013).

There is also some crossover between benefits, whereby improvements in one benefit directly influence another. For example, improvements in ecological green roofs that lead to increased visitation rates from birds opens up additional social activities such as birdwatching.

In an attempt to better categorise Australian green roofs, I surveyed 29 green roofs in Sydney, New South Wales (NSW). The aim was to establish the current practices used by the green roof industry, specifically the substates used and their depths, types of vegetation on the green roof, water usage and green roof size. I investigated green roofs for their ability to deliver on the three key benefits: amelioration of physical characteristics associated with urbanisation; social aesthetics, which encourage usage of limited space in cities; and biodiversity benefits, which reduce the impacts of city development on local biodiversity.

Methods

Sydney has a warm temperate climate based on the Köppen–Geiger climate classification (Figure 2). In general, summers are warm, with temperatures between 20 °C and 27 °C, and winters between 9 °C and 17 °C. There is no extreme seasonal difference due to the influence of the ocean, and there is no distinct wet and dry season.

Temperatures vary widely across Sydney. The sea breeze moderates the eastern part of Sydney, while the western suburbs are not influenced by sea breezes and generally have higher temperature swings. As a result, the western suburbs have average summer temperatures between 18°C to 29°C. However, in winter, the western suburbs have a high diurnal range from 19 C during the day to 2 C at night with mild to moderate frost (Bureau of Meteorology 2019).

Classification of Green Roofs Based on Their Benefits

To evaluate green roof effectiveness, five characteristics were scored for each of the three categories (Table 2). The characteristics were selected based on literature in which previous surveys or research in each of the categories was assessed. Only observable characteristics that could be easily surveyed were selected. The characteristics, their scoring method and justification is outlined in Table 2. For each green roof, the roof was scored between 0 and 5 in each category. A score of 5 indicates that the roof is effective in the category.

Table 2: Surveyable variables recorded to evaluate the effectiveness of green roofs in three categories.

Measure/Characteristic	Score	Justification
Amelioration of urban physical characteristics		
Percentage of vegetation cover	≥80% = 1 ≤79% = 0	Reduces surface temperature through shading and evapotranspiration (Eksi et al. 2017; Voyde et al. 2010)
Vegetation structure	Complex = 1 Uniform = 0	Filters air by trapping airborne particles (Oberndorfer 2007; Wong et al. 2007)
Substrate colour	Light = 1 Dark = 0	Lighter coloured roofs provide higher thermal insulation (Liu & Minor 2005)

Stormwater aid systems such as water-holding tanks	Presence=1 Absence=0	Reduces stormwater run-off (Berghage et al. 2009)
Substrate depth	≥30 cm = 1 ≤29 cm = 0	Substrate depth plays an important role in water retention and thermal insulation. (Eksi et al. 2017; VanWoert et al. 2005). Depth cut-off selected based on the depth guide for classifying intensive green roofs (GSA 2011, Lata et al. 2018, Shafique et al. 2018)
Improving social values		
Accessibility	Accessible (eg. Walkways) = 1 No accessibility = 0	Accessibility is a desirable trait on green roofs (Teotónio et al. 2020). It promotes socialising and reduces stress.
Sitting area/presence of benches	Yes = 1 No = 0	Reduces stress and provides spaces for socialising (Mensah et al. 2016)
Manicured landscape presence of flowering plants	Yes = 1 No = 0	Manicured green roofs with flowering plants improve the sense of wellbeing and lowers stress (Fernandez-Cañero et al., 2013, Mensah et al. 2016, Jungels et al. 2013).
Ornaments (bird baths, water features)	Yes = 1 No = 0	Improves sense of being in nature; reduces stress (Harting 2014)
Percentage of bare substrate	≥31% = 0 ≤30% = 1	A high percentage of bare substrate or gaps between vegetation reduces aesthetics (Vanstockem et al. 2018)
Enhancing biodiversity		
Presence of green spaces (parks, green roofs or remnant vegetation) within 600 m of green roof	Presence=1 Absence=0	Affects habitat connectivity (Braaker et al. 2014; Fernández Cañero & González Redondo 2010)
Percentage native cover	>80% = 1 <79% = 0	Native foraging/habitat plants attract native fauna (Fernández Cañero & González Redondo 2010; MacIvor & Ksiazek 2015)
Similarities to native habitats	Yes = 1 No = 0	Attracts native fauna (Fernández Cañero & González Redondo 2010)
Presence of nesting/habitat analogues (nest boxes)	Yes = 1 No = 0	Habitat for fauna (Fernández Cañero & González Redondo 2010)
Building height	<5 levels = 1 >5 levels = 0	Affects habitat connectivity (MacIvor 2016)

Survey Methodology

Site Selection

Sites were selected using the City of Sydney's green roof database (City of Sydney Council 2017) and by contacting green roof companies. I collated a list of 96 green roofs around Sydney. Of these, 47 roofs were excluded, as I received no response from the owners, and 20 were excluded due to size/classification issues (e.g., green facade classified as a roof). Across coastal Sydney, 29 green roofs were surveyed.

Prior to the surveys, information such as the aims and benefits ascribed to each roof were collected using publicly available documents and websites. For commercial green roofs, this information was sourced through company websites and promotional brochures. I sourced information from council web pages and council documents such as public consultation documents for council green roofs. Observations on watering type and frequency were collected through consultation or observation.

Roof visits were carried out in the Australian summer (January and February) of 2017. The size of the growing area on the green roof was measured using a tape measure. The growing area on a green roof was defined as an area on a green roof designed to grow vegetation. For large or complex green roofs, aerial images were used to estimate the size.

The following characteristics were recorded as binary data:

- presence of habitat/artificial habitat (nest boxes)
- presence/absence of artificial shade
- presence/absence of natural shade
- sitting area/presence of benches
- flora scaping/presence of aesthetic plants
- presence/absence of ornaments (birdbaths, water features)
- accessibility.

Substrate type was determined visually and confirmed by correspondence with the construction company or owner. All vegetation was identified and recorded. Native vegetation was identified using native keys such as NSW FloraOnline (PlantNET 2019).

All horticultural variants and exotic species were identified using the horticultural flora for south-eastern Australia (Spencer 2002). Plants were classified into four categories:

- Ground covers (1–10 cm high)—perennial low-lying herbaceous and woody plants with a spreading habitat
- Shrubs (10–100 cm high)— woody plants with several perennial stems that are erect or low to the ground with a diameter no more than 8 cm
- Grasses and grass-like plants—all plants from the family Poaceae and plants with tufting grass-like foliage (e.g., *Lomandra longifolia*)
- Trees (>100 cm)—a woody plant with one erect perennial stem at least 8 cm in diameter.

Results and Discussion

Green Roof Size and Type

The type and size of green roofs in Sydney varied across sites. None of the green roofs surveyed matched the criteria for an extensive green roof under the FLL, IGRA or GSA. For example, in the GSA, extensive green roofs are defined as having 70–100 mm of substrate with no irrigation and comprising mostly succulents such as *Sedum* spp. Only one site had similar characteristics to extensive green roofs. The site was composed of a mix of native and exotic succulents; however, the substrate was much deeper (130 mm) and had irrigation over the summer months. A possibility as to why none of the green roofs surveyed matches the extensive green roofs classification is the cyclic climate experienced on the east coast of Australia. The influence of the ENSO, IOD and SAM results in periods of drought with hot summers and an increase in extreme temperature events (Lim et al. 2019, Cai et al. 2012). These temperature fluctuations and extremes facilitate the need for irrigation and deeper substrate depths to ensure plant survival.

The green roofs surveyed could be broadly classified into three types (Figure 3): Intensive green roofs, semi-intensive green roofs and roof gardens. Because the substrate type, plants used and function are closely related to the broad classifications, each classification will be discussed separately.

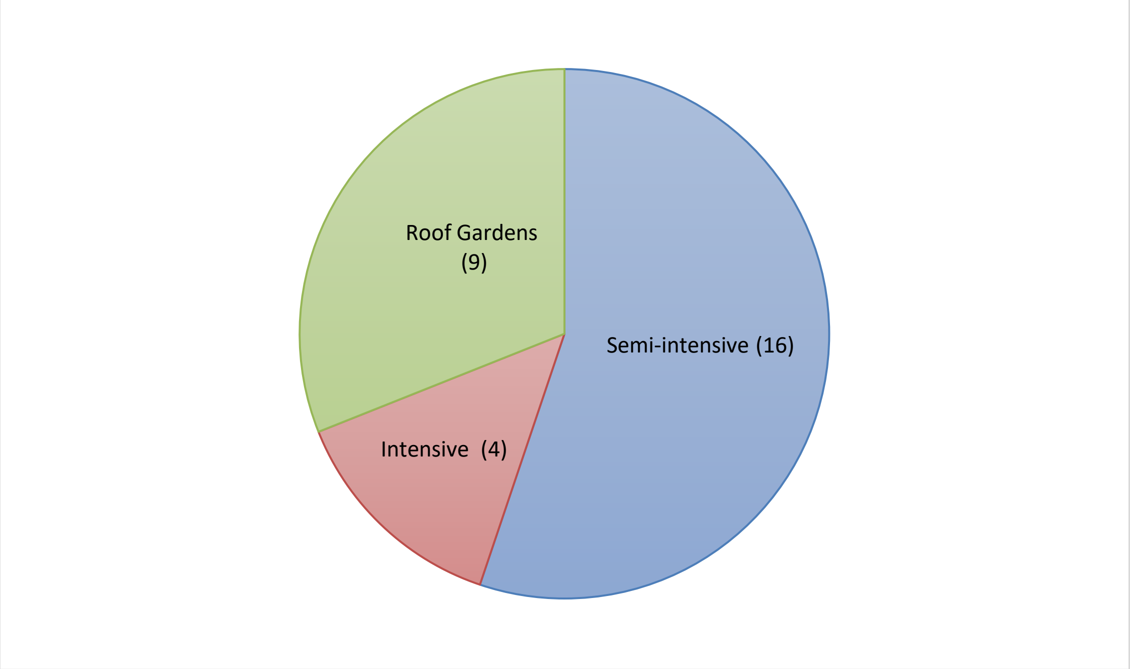

Figure 3: Twenty-nine green roofs surveyed across Sydney are categorised into three different types.

Intensive green roofs (see Figure 4a) were the largest green roofs in our survey. On average, they had a growing area of over 1600 ± 620 m^2 (SD). The primary defining characteristic of this type of roof was its deep substrate, permanent irrigation and full access, with amenities such as park benches. The substrate used for this category of green roofs was typically a soil–sand mix with a depth ranging from 0.5 to 1.5 m. The substrate was generally capped with scoria or mulch around the trees. Due to their large size and deep growing substrate, Intensive green roofs had the highest average number of species recorded (21 ± 8). For example, at the Embarkation Park, 26 species were recorded. These green roofs had the highest ongoing and irrigation costs when compared to other roofs.

Roof gardens (Figure 4b) were the second most common type of green roof. These roofs consisted of large planter boxes (200–1500 L) with an average depth of 630 mm (± 180) and were characterised by being a unit separate from the roof and not built into it. These types of green roofs were found solely in commercial spaces and were typically found accenting outdoor-accessible courtyards and verandas.

Semi-intensive green roofs (Figure 4c) account for most of the green roofs surveyed. The defining characteristic of this type of roof is the shallow growing substrate with subsurface irrigation and limited to no access. This type of green roof had a wide range of growing areas, with some of the smaller examples having a growing area of 20 m^2, the upper range being around 80 m^2 (*Ave*= 35.44 ± 19.22). This category had a mix of owners: eight were private residential; five roofs were private commercial, serving as a community/lunch area for employees; and three were public roofs. The substrate used was largely dependent on the company installing the green roofs. The most common substrate was scoria, although many had soil mixes. The substrate depth ranged from 150 to 400 mm, with most roofs having a growing depth of 200 to 250 mm. All roofs had irrigation; however, the watering period varied across the roofs depending on the substrate media and plants used. Only two green roofs had a growing substrate shallower than 150 mm.

Figure 4: Examples of green roof types surveyed across Sydney—(a) Intensive green roofs, (b) roof gardens and (c) semi-intensive green roofs.

Substrate Type

In our survey, cost and weight were identified as the primary considerations when choosing a substrate for a green roof. Substrate type was mostly dependent on the company that installed the roof, with each company using its own proprietary substrates. These substrates had different additives, and some of the mixes had a capping layer over the growing substrate. In the table below, we score substrates based on what they are primarily composed of. Mixes were defined as substrates where the constituents were mixed in equal or close to equal measures. Figure 5 illustrates the core components of the growing substrates. The capping layer was generally composed of either mulch, scoria or aggregate (Figure 6a & d). Scoria (a natural vesicular volcanic rock) was the most common substrate used; it was found on 14 of the 29 roofs (Figure 6e). Of the 14 roofs, nine used different grades of scoria as the growing medium (2–5 mm), with the other five having scoria mix. Of the mixes, perlite/pumice-soil was the most popular mix with six roofs.

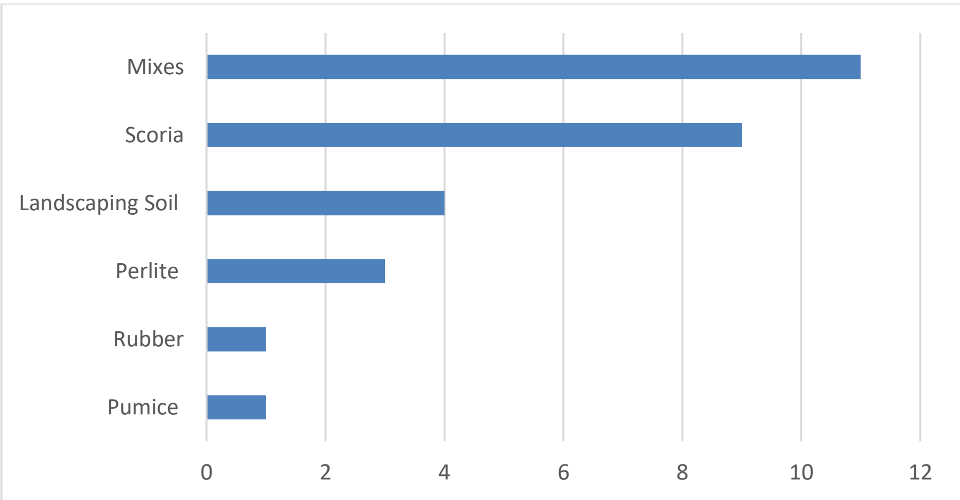

Figure 5: Common green roof substrates recorded in the survey of 29 green roofs across Sydney.

Substrate type was also closely linked to roof type: all four Intensive green roofs had landscaping soil as their growing substrate (Figure 6f), while roof gardens had soil mixed with scoria, terracotta or perlite/pumice (Figure 6c). Semi-intensive green roofs had substrates considered traditional on green roofs: low organic content and high inert media, with a 2–4 mm particle size that would not decompose or compact over time. Scoria and perlite soil mix (Figure 6c) were the most common growing substrates; however, the four newer roofs surveyed indicated that some developers were moving towards lighter media such as perlite (Figure 6b) and recycled rubber.

Another critical factor was the additives applied to the substrate. While I was unable to collect this information from the green roof companies, as some of the mixes were proprietary, I was able to observe some additives, such as water crystals, biochar and coir.

Figure 6: Common substrate types identified in the survey of green roofs—(a) soil capped with pebbles, (b) perlite, (c) soil–perlite mix, (d) soil capped with mulch, (e) scoria (3 mm) and (f) landscaping soil

Vegetation

A total of 62 different plant species were recorded during the survey. All the roofs were polycultures with multiple species present. The most common plant types (Figure 7) were native tussock plants such as *Lomandra longifolia* (Figure 8e), *Dianella revoluta* and *Themeda triandra* variants (Figure 8f). Of the roofs surveyed, 30 had grasses and grass-like plants, and 17 had other ground covers. Trees were present on only seven roofs, either on roof gardens or parks, with none on semi-intensive green roofs. Exotic species were found on 18 green roofs, of which only two roofs had more than a 50% abundance of exotic species. Overall, only three roofs attempted to match native habitats in their plant selection. The remaining roofs contained hardy species or were designed based on aesthetics (e.g., flowering plant arrangements).

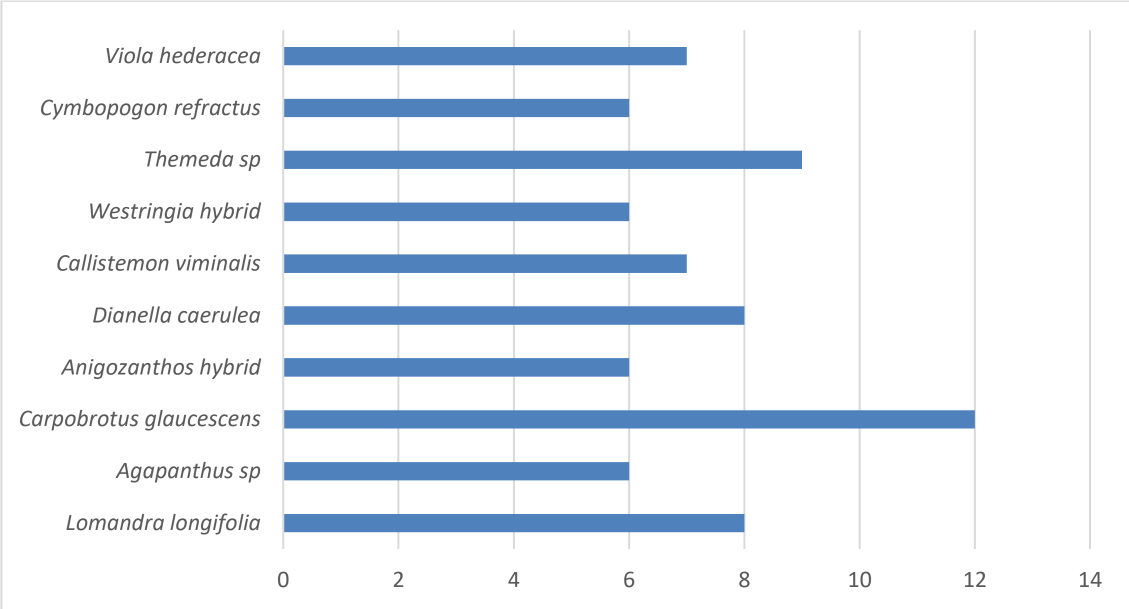

Figure 7: The abundance of the ten most commonly used plant species found on the 29 green roofs surveyed across Sydney.

Figure 8: Images of commonly used plant species—(a) *Carpobrotus glaucescens*, (b) *Viola hederacea*, (c) *Agapanthus sp*., (d) *Grevillea banksii*, (e) *Lomandra longifolia* and (f) *Themeda* hybrid.

Plant selection was influenced by objective, type and access. For example, two roofs with similar physical characteristics (substrate depth and size) had drastically different species selection. One mimicked the coastal environment nearby, while the other used a mix of native and exotic succulents, planted for hardiness rather than mimicking native habitats.

Water Usage and Ongoing Costs

Water usage and ongoing costs were difficult to determine, with all parties (owners and developers) hesitant to release information. Half of the green roofs (14 roofs) surveyed used collected rainwater to irrigate the vegetation; however, I was unable to determine whether rainwater was supplemented by council-supplied water. Semi-intensive green roofs tended to have the highest focus on environmental benefits in relation to water. Eight green roofs in this category collected and filtered water before delivering the water to storage tanks that were later used for other applications such as amenities or watering ground-level parks.

Where I was able to collect specific watering information, I found that the watering regime varied across seasons, with roofs watered once a week in winter but often daily in summer. Typically, 4 L of water per square meter was used. The most popular mechanism to deliver water was drip watering either subsurface, which was the most common in semi-intensive green roofs, or over surface, which was more common in rooftop gardens and parks.

Of the three green roof types, rooftop parks had the highest maintenance costs associated with the maintenance of vegetation (mowing the lawns, weeding) and the upkeep of amenities. Semi-intensive green roof maintenance depended on the company installing the green roof, where visits were either quarterly or half-yearly. Aside from mowing on the intensive green roofs, plant death was the most significant ongoing cost for a green roof (across all green roof types) (Figure 9) and is a reason why green roof companies are hesitant to try new species. Death of plant species on a green roof not only elicits a maintenance call to the green roof company but also the direct cost of replacing the plants, which can be expensive when replacing a tree or a large bush.

Figure 9: Plants undergoing heat and drought stress on a green roof.

Assessment of Green Roofs Based on Their Benefits

I investigated how well roofs performed in terms of the three broad categories (listed in Table 2):

- environmental amelioration of physical characteristics associated with urbanisation
- social aesthetics that encourage social interactions and wellbeing
- ecological—biodiversity benefits to local fauna and flora.

The results of the survey are shown in Table 3.

Table 3: Assessment of 29 green roofs across Sydney sites against their ability to deliver environmental, social or ecological benefits (Measures described in Table 2).

	Environmental ☀ Amelioration of urban physical characteristics					**Social 🧠 Psychological benefits and improving social values**					**Ecological 🍃 Enhancing biodiversity**						
Site	% of vegetation cover	Vegetation structure	Substrate Colour	Stormwater aid systems	Substrate depth	Accessibility	Sitting area/ presence of benches	Flora scaping/ presence of aesthetic plants	Ornaments (birdbath, water features)	% of bare substrate	Presence of green spaces within 600 m of green roof	% native cover	Similarities to native habitats	Presence of nesting /habitat analogues (nest boxes)	Building height	**Function score**	**Total score**
1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	☀:5 🧠:4 🍃:5	14
2	1	1	1	0	1	1	1	1	1	1	0	0	0	0	0	☀:4 🧠:5 🍃:0	9
3	1	1	0	1	1	1	1	1	1	1	1	1	0	1	0	☀:4 🧠:5 🍃:3	12
4	1	1	0	1	1	0	0	1	1	1	1	1	1	0	1	☀:4 🧠:3 🍃:4	11
5	0	1	1	1	1	0	0	1	0	1	1	1	1	0	0	☀:4 🧠:2 🍃:3	9
6	1	1	0	1	1	0	0	1	0	1	1	1	0	1	0	☀:4 🧠:2 🍃:3	9
7	1	1	1	0	1	0	0	1	0	1	1	0	0	1	1	☀:4 🧠:2 🍃:3	9
8	0	1	0	1	1	1	1	1	1	1	1	1	0	1	0	☀:3 🧠:5 🍃:3	11
9	0	1	0	1	1	1	1	1	1	1	1	1	0	0	1	☀:3 🧠:5 🍃:3	11
10	0	1	1	0	1	1	1	1	1	0	1	1	1	0	1	☀:3 🧠:4 🍃:4	11
11	0	1	0	1	1	1	1	1	1	0	1	1	0	0	1	☀:3 🧠:4 🍃:3	10
12	0	1	0	1	1	1	1	1	0	1	0	0	0	0	0	☀:3 🧠:4 🍃:0	7
13	0	1	0	1	1	1	1	1	0	0	1	1	0	0	1	☀:3 🧠:3 🍃:3	9
14	0	1	0	1	1	1	1	1	0	0	0	1	0	1	1	☀:3 🧠:3 🍃:3	9
15	0	1	0	1	1	1	1	1	0	0	0	1	0	0	1	☀:3 🧠:3 🍃:2	8
16	1	0	0	0	1	1	1	1	0	1	1	1	0	0	1	☀:2 🧠:4 🍃:3	9

	Environmental ☀ Amelioration of urban physical characteristics					Social 🧠 Psychological benefits and improving social values					Ecological 🍃 Enhancing biodiversity						
Site	% of vegetation cover	Vegetation structure	Substrate Colour	Stormwater aid systems	Substrate depth	Accessibility	Sitting area/ presence of benches	Flora scaping/ presence of aesthetic plants	Ornaments (birdbath, water features)	% of bare substrate	Presence of green spaces within 600 m of green roof	% native cover	Similarities to native habitats	Presence of nesting /habitat analogues (nest boxes)	Building height	**Function score**	**Total score**
17	0	1	0	0	1	0	0	1	1	1	0	1	0	0	1	☀:2 🧠:4 🍃:2	8
18	0	1	0	0	1	1	1	1	0	0	0	1	0	0	1	☀:2 🧠:3 🍃:2	7
19	0	1	0	0	1	1	0	1	1	0	0	0	0	0	0	☀:2 🧠:3 🍃:0	5
20	0	1	0	0	1	1	1	0	1	0	0	0	0	0	0	☀:2 🧠:3 🍃:0	5
21	0	0	1	1	0	0	0	1	1	1	0	0	0	0	0	☀:2 🧠:3 🍃:0	5
22	0	1	0	0	1	0	0	1	1	0	1	1	0	0	1	☀:2 🧠:2 🍃:3	7
23	1	0	0	0	1	0	0	1	0	1	0	0	0	0	0	☀:2 🧠:2 🍃:0	4
24	0	0	1	0	1	0	0	1	0	0	1	1	0	0	0	☀:2 🧠:1 🍃:2	5
25	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	☀:2 🧠:1 🍃:0	3
26	0	1	0	0	1	0	0	0	0	0	1	1	0	0	0	☀:2 🧠:0 🍃:2	4
27	1	0	0	0	0	0	0	1	1	1	1	1	0	0	0	☀:1 🧠:3 🍃:2	6
28	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	☀:1 🧠:1 🍃:0	2
29	0	1	0	0	0	0	0	0	0	0	1	1	0	0	1	☀:1 🧠:0 🍃:3	4

Environmental

Fifteen of the roofs were likely to have some environmental amelioration reducing urban heat and pollution or water-saving actions (scoring three or higher). Nearly all roofs were likely to have reasonable substrate depths that not only facilitated plant growth but also be a substantial buffer of temperatures and reduce stormwater runoff (Sandoval et al. 2015,Pianella et al. 2017, VanWoert et al 2005). Pianella et al. (2017) investigated the effect of substrate thickness, dry soil conductivity, leaf area index, leaf reflectance and moisture content on green roof thermal performance in the Mediterranean type climate of Melbourne Australia. They found that substrate depth and moisture content influenced the heat flux through the green roof and substrate temperatures. Substrate depth and type also play an important role in stormwater retention (Zhang et al. 2018, VanWoert et al. 2005). VanWoert et al (2005) found that an increase in substrate depth increased the water holding capacity of the substrate and its ability to delay the egress of stormwater.

Only seven roofs scored four or above. This was unexpected, as all the green roofs surveyed ascribed environmental benefits as one of their primary goals. Most of the roofs were watered using council water, with only 14 roofs having a water storage tank to collect and use rainwater. Another characteristic on which most of the roofs scored poorly was thermal amelioration, determined by substrate colour and vegetation cover. There is plenty of evidence that shows that lighter coloured roofs have increased thermal benefits compared to darker roofs (Liu & Minor 2005; Yu & Hien 2006). Darker substrates like scoria and soil are by far the most commonly used substrates on the green roofs surveyed; however, in correspondence with the green roof companies, it was suggested that it is a rapidly developing space with innovations being incorporated into every new roof built. Only nine roofs had high vegetation coverage on the roof: these tended to be coupled with good substrate depth and vegetation structure that would facilitate temperature amelioration and stormwater retention (Zhang et al. 2018). Most roofs were considered to have a poor capacity to ameliorate UHI effects (Low scores in substrate colour and % vegetation cover) and would therefore provide few economic benefits. This result was unexpected, as lower vegetation coverage has been shown to reduce ecosystem services and impact aesthetics (Vanstockem et al. 2018).

Social

Roof garden design was often linked with aesthetic value for social use. Twenty of the gardens had seats and/or ornaments and were available to be used. Of the 29 roofs tested, 19 had scores of 3 or higher, while 10 roofs can be characterised as social green roofs (score ≥ 4). Social benefits, welfare and aesthetics were often listed as one of the main drivers for green roofs, especially in a commercial setting. These findings are contradictory to studies performed in other cities. For example, Nurmi et al. (2013) found that, while green roofs are beneficial in public spaces, they were not cost-effective for commercial/private entities. The prestige of a green roof brought on by its novelty and benefits did not offset the high initial and maintenance costs. One possible reason for this trend towards greater value on social use is that most newer workplaces have an increased focus on employee satisfaction and well-being.

Ecological

Enhancing biodiversity had the lowest scores ($M = 2.1 \pm 1.4$), with eight of the roofs scoring 0 and only three roofs scoring four or higher in this category. Only four of the green roofs surveyed attempted to select local vegetation from native habitats/vegetation communities around Sydney. Of the roofs surveyed, eight green roofs had less than 80% native vegetation cover and two roofs having a majority of exotic vegetation. Mimicking native habitats not only improves local biodiversity by providing a habitat for fauna and invertebrate species but also improves plant growth and survival (Lundholm & Walker 2018; Nagase & Tashiro-Ishii 2018). One of the main limitations of biodiversity benefits is that a full realisation of the benefits often requires coordination across a greater area than an individual green roof. For example, habitat corridors require urban green spaces to be close to each other to allow for the passage of fauna and invertebrates (Williams, Lundholm & McIvor 2014). While these factors cannot be controlled for, restraint should be exercised in ascribing biodiversity benefits to roofs. In this survey, the disconnect between the aims and management/maintenance of the roofs is highlighted in relation to enhancing local biodiversity. For example, two of the green roofs surveyed were actively sprayed or baited with insecticide to control for bees, wasps and ants while still having ‘increasing native insect and bird biodiversity’ and ‘increasing pollinators’ promoted as its core benefits.

Conclusions

The survey highlights the need to revaluate traditional green roof classification in Australia. Roof classification was left up to the developer, and there was no consistency between different companies. Of the roofs we surveyed, six were called extensive green roofs; however, they did not match the criteria under any of the standards we could assess them against (FLL, IGRA, GSA). Additionally, a few recently constructed green roofs had variable depths and irrigation, highlighting some of the challenges in classifying roofs based solely on their physical attributes.

We evaluated different aspects of function to classify green roofs in Sydney based on their observable characteristics. Of the green roofs assessed, we were able to classify the roofs based on their function (Environmental, Social or Ecological). However, the survey highlighted the need for further work and for surveyable attributes to be assessed in the field across a wider number of roofs.

Green roofs are a growing industry in Sydney. While, in the past, green infrastructure was a niche architectural design with environmental benefits (Moghbel & Erfanian Salim 2017), there has been a shift in focus to more social benefits, such as mental health and productivity (Lee et al. 2015; Mensah et al. 2016). Most green roofs covered an area of between 20–80 m^2, with an average growing height of around 190 mm. Grasses and grass-like plants were the most common vegetation used; however, companies tended to use a limited set of native species they had already tried and were hesitant to experiment with new plants. Plant selection remained one of the most critical components of a green roof. All green roof companies recognised research into local plant species and their suitability for green roofs as a significant knowledge gap.

Green roofs for private residential areas are likely to require better plant species diversity and design than currently available. The increased use of large planter boxes provides opportunities for taller vegetation to be used with no modification to current infrastructure and at a relatively lower setup cost. Semi-intensive green roofs have also seen a recent resurgence, with local councils implementing sustainable city schemes such as Sydney 2030. There is a significant need to improve the design of these green roofs to have multiple benefits, including biodiversity benefits. Improvements in plant selection and vegetation structure remain critical gaps in our knowledge.

Chapter 3: Friends with Benefits—The Effects of Vegetative Shading on Plant survival in a Green Roof Environment

Contributions

Axton Aguiar conceived and designed the experiment, collected the data, performed the analysis and wrote the manuscript.

Kris French aided in the conception and design, aided in the analysis and reviewed the manuscript.

Sharon A Robinson aided in the conception and design, reviewed the analysis and manuscript.

Publication

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Introduction

Urban areas have been expanding at an increasing rate (McGranahan & Satterthwaite 2014; DESA 2018). Currently, 4 billion people inhabit urban areas worldwide, with this projected to grow to 10 billion by 2070 (DESA 2014). The consequence of this growth has been a host of negative social, environmental and economic impacts (McKinney 2002; Sol et al. 2014), with increasing pressure assigned to urban green spaces to help ameliorate these impacts (Jim 1998, White et al. 2009). Not only do green spaces provide essential ecosystem services, but they also improve and contribute to human wellbeing (Jennings & Gaither 2015, Niemela et al. 2010, Francis and Jensen 2017).

Green roofs have been actively promoted as refuges for biodiversity in urban environments, as they compensate for vegetation and biodiversity loss associated with urban development (Oberndorfer et al. 2007; Williams et al. 2014). However, due to the harsh conditions on green roofs, vegetative green roof designs have been overly conservative with species selection (Lundholm 2015; Young et al. 2014). The industry currently relies on succulents, given their hardy traits such as drought tolerance, ability to survive high temperatures and low maintenance costs (Monterusso, Rowe & Rugh 2005). While succulents provide some ecosystem services (Zhang et al. 2018), solely using these plants on a roof leads to reduced potential benefits and loss of multifunctionality (Lundholm 2015). Using a diversity of vegetation is critical in maximising performance (Nagase & Dunnett 2012), increasing ecosystem services (Nagase & Dunnett 2012) and building ecosystem resilience (Lundholm 2015).

Species selection is one of the critical components of a green roof, as it dictates not only the success but also the benefits (Arabi et al. 2015; Lundholm 2015). There have been different methods of selecting species on a green roof, such as the habitat-template approach proposed by Lundholm (2006). This approach is based on the concept that novel artificial ecosystems can be modified to exhibit conditions similar to those in the vegetation's natural habitat, which reduces the novelty of a roof environment for the vegetation (Lundholm & Richardson 2010). Another approach is to use trait-specific selection (Ksiazek-Mikenas & Köhler 2018; Lavorel et al. 2011; Van Mechelen et al. 2014, Rayner et al. 2016), which considers particular plant traits such as plant size, flowering rate, pollinators and aesthetic value (Benvenuti & Bacci 2010). The key assumptions of these models are that abiotic factors are the critical drivers limiting plant growth and survival on a green roof. However, a growing body of evidence highlights the importance of biological interactions in shaping and influencing the survival and growth of vegetation (Brooker et al. 2008; Mcintire & Fajardo 2014).

The species composition of vegetation communities is driven by their interactions with biotic and abiotic factors. Historically, abiotic factors (soil moisture, temperature, nutrients and pH) were thought to have a more significant influence on species establishment and survival (Billings & Mooney 1968), while biotic interactions (e.g., competition and facilitation) were considered to have a more significant role in species coexistence and structure (Callaway 1995; Grime 1973). The presence or absence of these factors along a gradient can be a factor in influencing the variation both in and between vegetation communities (Brooker & Callaghan 1998). These variations can occur at small spatial scales up to global scales.

While negative species interactions and physical environments were initially considered the dominant drivers of species distribution and abundance (Connell 1983), there is now a consensus that facilitation plays an equally important role in determining community structure (Callaway 1995). However, competition and facilitation do not occur in isolation from each other. For example, nurse plants aid in the establishment and survival of surrounding plants by ameliorating the microclimate and altering the soil properties (nutrients and water content; Callaway 2007). Nurse plants in alpine conditions provide shade and shelter for seeds, allowing them to germinate and grow. As the seedlings mature, they compete for resources with the nurse plant, shifting the relationship from commensalism/facilitation to competition (López et al. 2007). The interaction of facilitation and competition also varies with life history. Wright et al. (2014) showed experimentally that facilitation provided by neighbouring plants facilitated the establishment and growth of younger pines in severe environmental conditions. However, this relationship was reversed as the pines matured and exhibited higher levels of competition.

Nurse plants facilitate other plants within their canopy by modifying the microclimate and soil (López et al. 2007; Molenda, Reid & Lortie 2012). The canopy ameliorates abiotic weather conditions by trapping heat in colder alpine conditions (Molenda, Reid & Lortie 2012) or by providing shade in hotter conditions (Butler & Orians 2011); increases soil nutrients by increasing decomposing matter from leaf litter (Anthelme & Dangles 2012); reduces wind (le Roux & McGeoch 2010); and increases soil moisture content (Pugnaire, Armas & Valladares 2004). Nurse plants increase species richness (Badano & Marquet 2009) and abundance (Sklenář 2009), and play an essential role in improving the survivability of species (Badano & Marquet 2009; Sklenář 2009).

Using nurse plants in urban areas is not a new concept. Horticulturally, nurse plants, or ‘buddy planting’, have been used for decades to improve plant survivability, fruiting success and plant growth (Filazzola & Lortie 2014). On a green roof nurse plants/ buddy planting has the added benefit of increasing the diversity of the roof, which has been shown to improve ecosystem services provided by the green roof (Lundholm 2015). Experiments exploring the facilitatory benefits of plant-plant interactions on green roofs have had mixed results (Vasl et al. 2017, Butler & Orians 2011 Ahmed et al. 2017, Heim & lundholm 2014). At present, there is limited information on the mechanisms by which nurse planting may improve growth on green roofs and what influence the competitive effects may be. Butler and Orians (2011) investigated the use of *Sedum album* (Crassulaceae) as a nurse plant to facilitate the growth of *Agastache rupestris* (Lamiaceae) and *Asclepias verticillata* (Asclepiadaceae). They concluded that *S. album* might have acted as a competitor during periods of high water abundance and that, after 3 months, it had a negative impact on the biomass of *A. rupestris* and *A. verticillata*.

We investigated the effects of nurse plants on plant growth and survival in a green roof environment. We aimed to examine the effects of shade and competition on four different plant species in a simulated green roof environment. We predicted that, if the growth of rooftop vegetation is most affected by high irradiance and heat exposure, then exposing vegetation to shade will increase growth. However, if the growth of rooftop vegetation is more strongly influenced by competition, then exposing species to a nurse plant will decrease growth.

Methods

Study Site and Green Roof Array

The study took place at the Ecological Research Centre (ERC) at the University of Wollongong, Australia (34° 25′ 59″ S 150° 52′ 59″ E). Wollongong has an oceanic climate with humid subtropical influences. Rainfall is associated with the orographic lift caused by the escarpment; on average, Wollongong experiences 1300 mm of rain a year, with the wettest months being February and March. Green roof mesocosms were constructed in December 2015 on top of a large concrete slab simulating the roof of a building. All mesocosms were north–south orientated on the slab to ensure that each setup received a comparable amount of exposure to solar radiation.

Each mesocosm was constructed using technical guidelines published by the City of Sydney Council (2014) in an attempt to replicate green roof construction. The frame of the green roof was built using a lightweight timber, which was constructed into a rectangular box (0.5 × 0.6 × 0.3 m; Figure 10). The green roofs were created using a layered design:

- vegetation layer—the species used in the experiment were native sandstone species (forbs, low shrubs, succulents and grasses) found along the coast near Sydney.
- Growing substrate (15-18 cm)— consisting of a mix of organic and inorganic components. We used perlite, vermiculite and soil (Garden topsoil: coir, plant mulch, sand and compost) in a ratio of 4:2:1. Similar mixes in slightly different ratios were used in Vijayaraghavan and Raja 2014, Vijayaraghavan and Joshi 2014, Kotsiris et al. 2012. The amount of organic matter was kept low (approximately 80-85% inorganic to 10-15% organic) to mimic the nutrient-poor sandstone soils native to the region.
- Filter membrane - consisted of a thin membrane that prevented the Growing substrate from falling into the drainage membrane. We used a broad-gauge shade cloth.
- Drainage layer—14 mm crushed terracotta
- protection mat—a thermoplastic sheet layer to provide root and chemical protection
- Waterproofing membrane—we used a liquid applied treatment of bitumen emulsion.

The growing substrate had a water holding capacity of 68% with a dry bulk density of 0.64 g/cm^{-3}

Experimental Design

Testing all the species pairs listed in the local green roof guides was outside the scope of this project due to space limitations. We therefore employed a more general approach by which species were randomly selected from native habitats that experienced harsh abiotic conditions with nutrient-poor soil and high drought stress, such as the foredunes of beaches and exposed sandstone habitats. Accounting for experimental space and replication needed, we were able to test four pairs of plants, each with a potential nurse plant and a target plant (all of which were ground cover/prostrate plants). The potential nurse plants used in this experiment have not previously been assessed as nurse plants. All the plants selected are native to sandstone and shale cliff line, and outcrop vegetation communities found around the city of Sydney NSW (Appendix B) and were purchased from the same nursery and were matched for size.

We tested four groups that were subjected to four treatments, with five replicates per treatment (20 mesocosms per group). The groups were:

- Group A: *Lomandra longifolia* (Nurse plant) and *Dichondra repens* (target plant)
- Group B—*Callistemon citrinus* (Nurse plant) and *Grevillea lanigera* (target plant)
- Group C—*Dianella caerulea* (Nurse plant) and *Carpobrotus glaucescens* (target plant)
- Group D—*Correa alba* (Nurse plant shade) and *Viola hederacea* (target plant).

Each pair was grown for 11 months with three small, equally spaced target plants (5 cm) and one nurse plant (or equivalent) in the middle. The experimental design consisted of 4 planting mixtures that investigated the effect (shading and competition) of the nurse plant on the target plant. The effect of the nurse plant was manipulated by removing, clipping or replacing it (with a plastic plant).

The four different treatments (Figure 10) were:

- Shaded natural (SN) consisted of a nurse plant shading the mesocosm. This treatment emphasised the interaction of both above-ground shading and below-ground root interaction.
- Shaded artificial (SA) consisted of an artificial plant shading a target plant. This treatment isolated the effect of shading by excluding any below-ground/root interactions. The upper section of the plastic shading plant was replaced midway through the experiment as the plastic degraded due to ultraviolet damage.

- Unshaded natural (UN) consisted of a trimmed nurse plant providing no shade to the target plant. This treatment is the opposite of the SA treatment, in which the below-ground interaction was isolated by minimising any above-ground shading effect.
- Unshaded (U) consisted of the target plant being the sole species of the mesocosm. In this control treatment, the target plants had neither interspecific competition nor facilitation (shade).

The mesocosms were set up a month before the start of the experiment to allow plants to acclimatise, reducing the effect of transplant shock. Each mesocosm had two 2.5 L/hour trickle dripper (Netafim Low CNL PCJ Dripper with Tube Spike) placed at a 10cm offset to the east and west from the middle of the mesocosm. All plants were watered twice a day at 7 am and 7 pm with 5 L over 30 minutes. A slow-release fertiliser (Osmocote Exact Standard at 1/3 the recommended dose 20g 3-4M: 7.1% NO_3-N, 8.9% NH_4-N, 9% P_2O_5 and 12% K_2O) was applied twice during the experiment, on Days 1 and 175, to ensure that each treatment received an equal amount of nutrients.

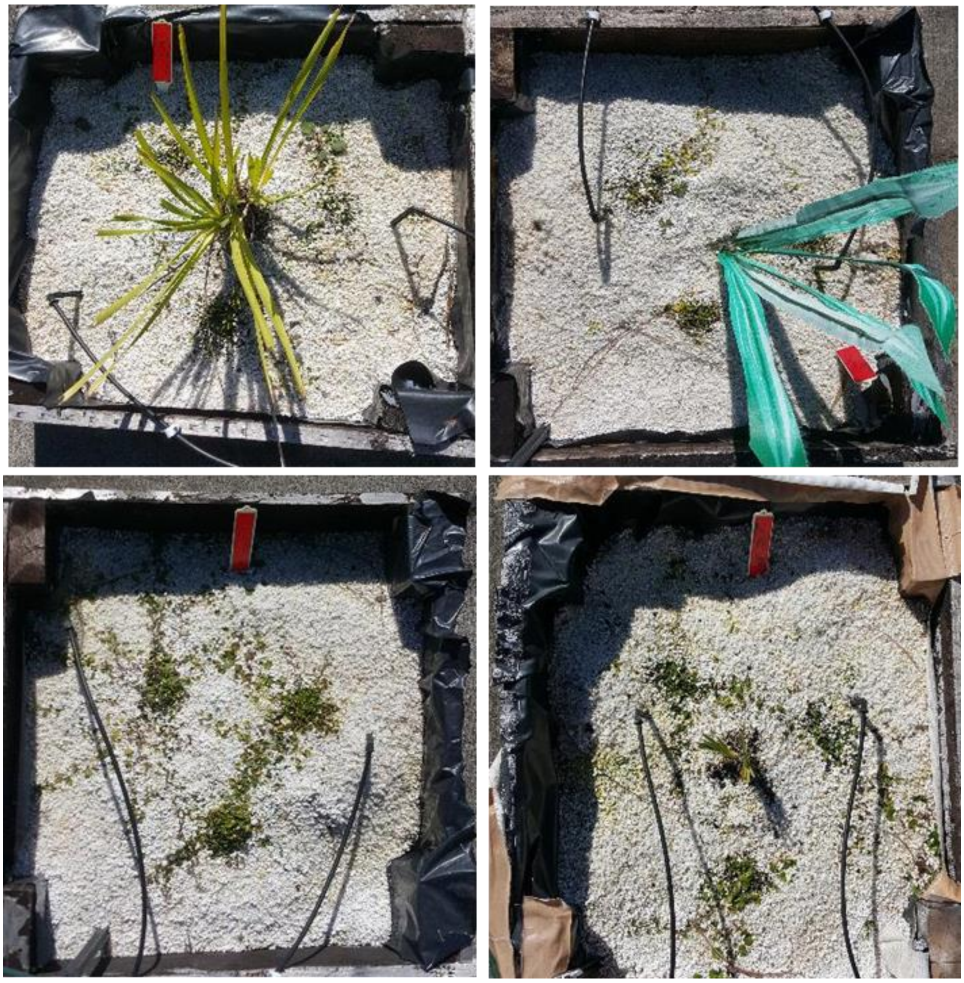


Figure 10: The four treatments used in the experiment. Example of a *Lomandra longifolia* and *Dichondra repens* pair; clockwise from top-left: shaded natural, shaded artificial, unshaded natural and unshaded.

Substrate Moisture Content

To investigate how the change in substrate moisture was affected by a nurse plants, substrate water was measured monthly (the second Friday of each month, 6 hours after the 7 am watering) using a 50 ml container with a diameter of 40 mm (i.e., the gravimetric method described in Gregorich & Carter 2008). The measure was used to identify a change in substrate moisture between watering. Drought stress was not investigated as all mesocosms were well watered. A sample of substrate to a depth of 5 cm (1/3 growing layer depth) was collected from the south side of each mesocosm. Substrate samples were weighed then placed into a drying oven at 80 °C for 48 hours before reweighing. The substrate was then returned to the mesocosm. The volumetric water content in the substrate sample was determined by multiplying the gravimetric water content by the soil bulk density.

Substrate Surface Temperature

To investigate whether nurse plants ameliorate substrate surface temperatures, the temperatures of all mesocosms were logged with a K-type thermocouple connected to a data logger (EasyLog EL-USB-TC) three times daily (at 4 am, 1 pm and 7 pm). Night temperatures (4 am) were used as a control, given that any heat retention should have dissipated by the early morning, and all mesocosms should be around the same temperature. The 1 pm measurement was taken as the primary measurement of shade performance in full sun, and the 7 pm measurement was to evaluate the effect of the UHI on substrate temperatures. The temperature probe was inserted below the top layer of the substrate (3–5 cm deep) on the southern side of the nurse plant.

Biomass Growth Analysis

At the end of the experiment, ground cover plants were harvested, and roots were washed over a sieve to clean them; care was taken to ensure that any fine roots were collected and kept with the sample. For both the growth and biomass measurements, the data from three target plants in each mesocosm were summed because, in the case of *D. repens* and *V. hederacea*, it was impossible to delineate individual plants in some treatments. Growth measurements were collected (as described below), and above-ground and below-ground biomass were weighed separately. Plant material was placed in a drying oven at 75 °C; a subsample was weighed daily until a constant weight was achieved (72 hours).

Growth Analysis

After the roots had been washed for biomass collection, three measures of growth were recorded: 1) length of shoot/runner (for plants with branching shoots/runners, the branches were added to the total length), 2) number of shoots/runners and 3) number of leaves. The number of leaves was not recorded for *G. lanigera* due to the high number of leaves per unit area found all along the stem; instead the stem length was used as a better measure of growth.

Statistical Analysis

Each species was analysed separately. Root and shoot biomass and growth parameters (number of leaves, shoots and shoot length) were compared among treatments using single-factor analysis of variance (ANOVA) after testing for normality (Shapiro–Wilk test) and homogeneity of variance. Significant interactions were further investigated using multiple-comparison tests (Tukey's honestly significant difference [HSD] test). Substrate moisture was analysed using repeated-measures ANOVA after testing for normality (Shapiro–Wilk test) and sphericity (Mauchly's test).

For the substrate temperature analysis, due to the confounding effect of variables such as wind and rain, we decided to use a local weather station to select days that had no rain on the day before measurement, wind speed under 10 km/h and cloud cover below 20%. After filtering days with the above criteria, we then categorised the remaining days into three temperature groups (high = >26 °C; medium = 16–26 °C; low = <16 °C). We only used substrate temperatures collected on these days for our analysis. Data were analysed by ANOVA with replicate mesocosms nested within experimental treatments (shaded unshaded).

Results

Environmental Data

Substrate Moisture Content

Only *G. lanigera* had any statistically significant difference between the treatments for mean substrate moisture (Figure 11; Table 4). The significance was driven by the SN treatment having half the substrate moisture content when compared to the other treatments; all other treatments had statistically similar substrate moisture content.

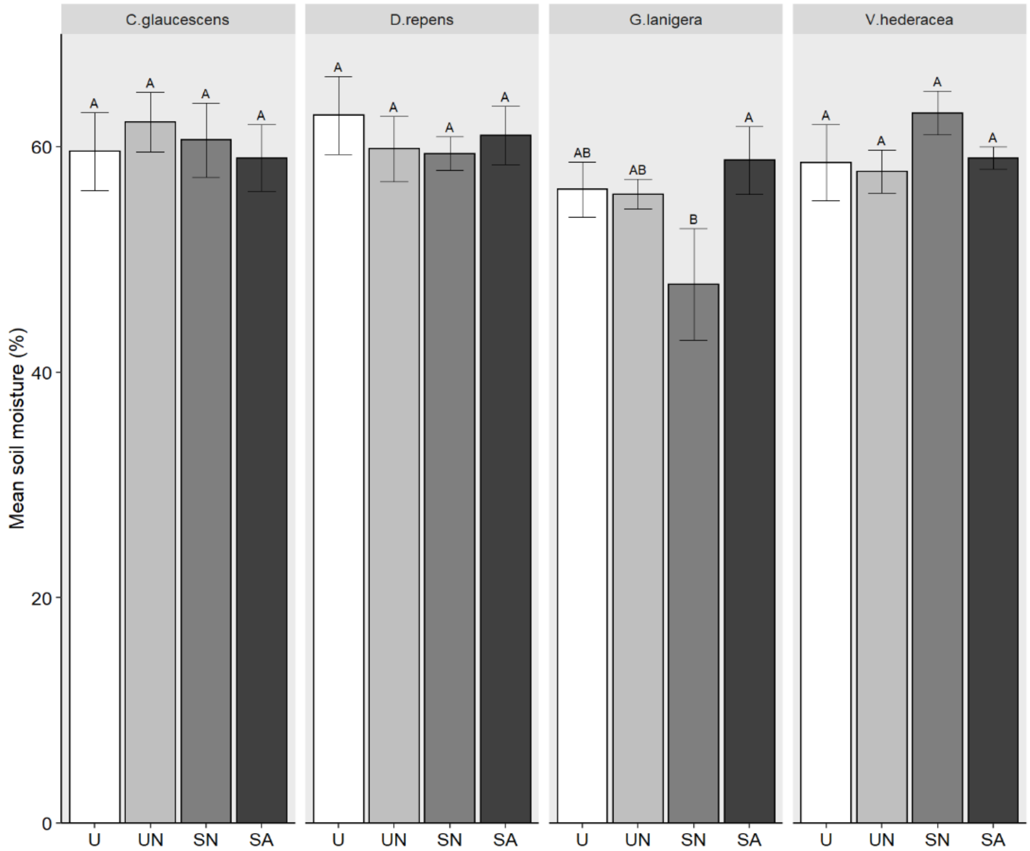


Figure 11: The effect of shading treatment on substrate moisture content (Max water holding content for the substrate = 68%).Mean substrate moisture content across four different treatments. U = unshaded; UN = unshaded natural; SN = shaded natural; SA = shaded artificial. Letters denote statistical difference. Error bars are ±*SE*; *n* = 5.

Substrate Temperature

Shading had a significant effect in reducing substrate temperatures when comparing midday substrate temperatures on high, medium and low days throughout the year (high, $F_{3,304} = 124.16$, $p < 0.001$; medium, $F_{3,304} = 171.84$, $p < 0.001$; low, $F_{3,304} = 207.51$, $p < 0.001$; Figure 12). On average, there was a 15 °C difference between shaded and unshaded treatments. This effect varied with ambient temperature. On hot days, the difference between shaded and unshaded treatments was closer to 25 °C.

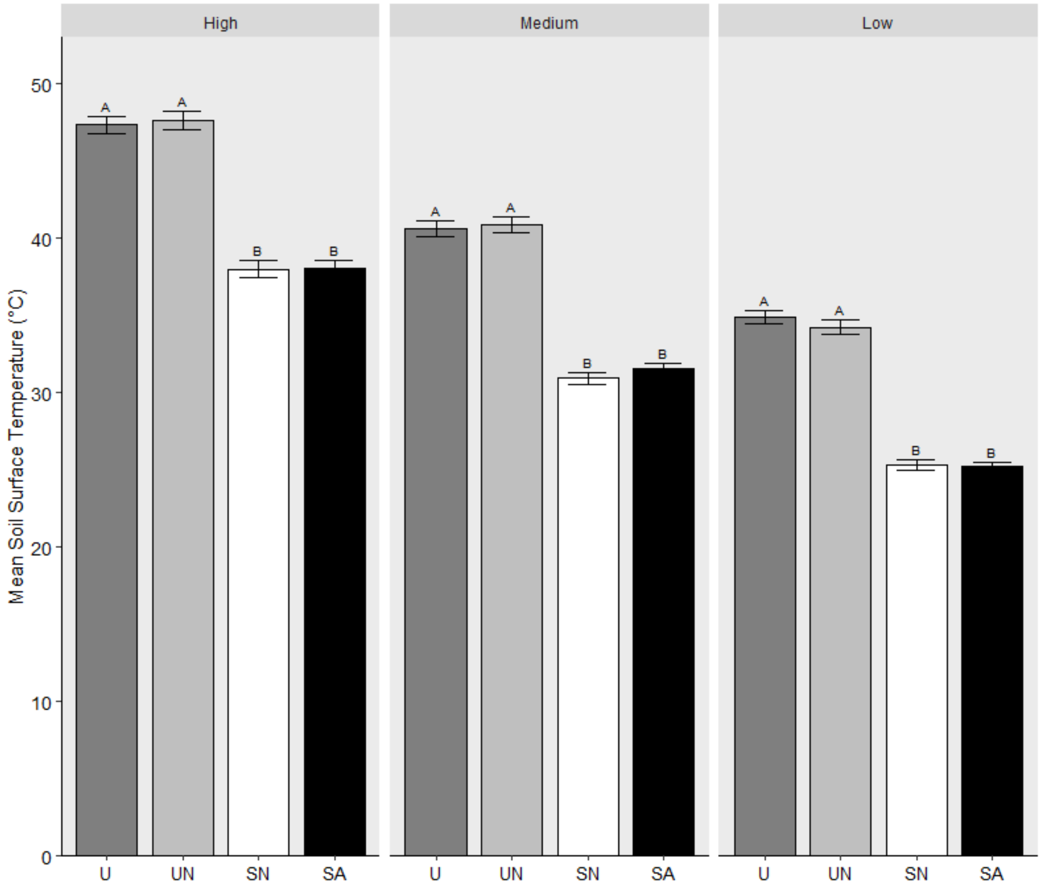


Figure 12: Effect of shading on substrate temperature. Difference between midday substrate surface temperatures across four treatments. U = unshaded; UN = unshaded natural; SN = shaded natural; SA = shaded artificial. On days with high (>25 °C), medium (16–25 °C) and low (<16 °C) ambient temperatures. Letters denote statistical difference. Error bars are ±*SE*; *n* = 20.

Biomass

All treatments, except for *C. glaucescens,* exhibited an increase in both above- and below-ground biomass when compared to the control (Figure 13; Table 4). For *D. repens* and *V. hederacea*, shade increased biomass by at least a factor of 2 (Figure 13). The SN treatment had the highest increase in biomass, with the UN and SA treatments having statistically similar increases in biomass (Figure 13; Table 4). There was thus no evidence of competition; instead, both treatments with a nurse plant exhibited an increase in biomass when compared to U and SA treatments (Figure 13).

The results were more complex for *C. glaucescens* and *G. lanigera*. For *C. glaucescens*, there was a greater increase in above-ground biomass when compared to the below-ground biomass (Figure 13; Table 4). For above-ground biomass, the shade treatments

(SA and SN) exhibited lower or similar biomass to the control treatment (Figure 13; Table 4). For *G. lanigera,* shade had a positive effect on growth. However, this was only observed for above-ground biomass in the treatment with the artificial plant (Figure 13). In contrast, both treatments with the nurse plant exhibited higher below-ground biomass at the expense of above-ground biomass (Figure 13). This effect was strongest in the SN treatment, which had the lowest above-ground biomass and the highest below-ground biomass (Figure 13; Table 4).

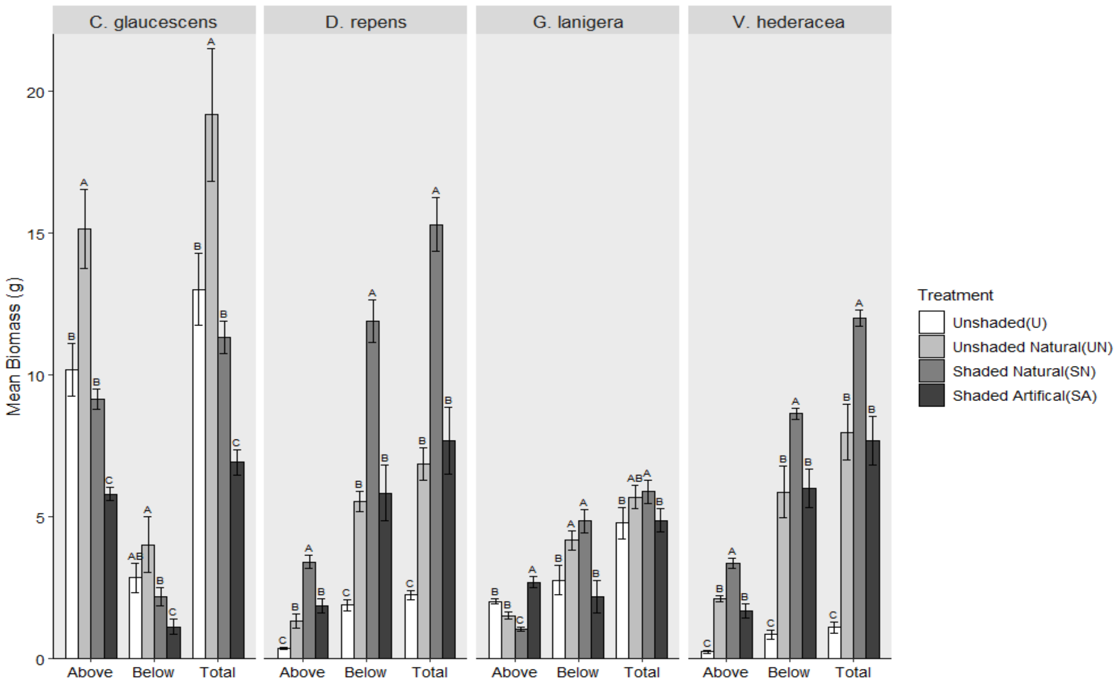


Figure 13: The effect of shading on plant biomass. Comparison of the effect of four different shade treatments on mean pooled dry biomass. Above-ground, below-ground and total biomass for each species were analysed separately. Letters denote statistical difference within factors (above and below). Error bars are ±*SE*; *n* = 5.

Growth

While the overall trend of the growth data was similar to that of the biomass data, each species exhibited subtle differences in the different shade and competition treatments. There was a significant effect of shading on the number of shoots (Figure 14a; Table 4). All four species exhibited a lower number of shoots in the unshaded treatments (U and UN) when compared to the shaded treatments (SN and SA). For *V. hederacea* and *D. repens*, shading doubled the number of shoots (Figure 14a).

Changes observed in the number of leaves (Figure 14b; Table 4) mirrored the biomass changes with shade treatments (SN and SA), with fewer leaves for *C. glaucescens* and more leaves for *D. repens* and *V. hederacea*. *C.glaucescens* exhibited the highest number of leaves in the UN treatment. For shoot length (Figure 14c), the positive effect of shade (SA treatment) was equal to the positive impact of the nurse plant (UN treatment) for both *V. hederacea* and *D. repens*, with the combination of both, in the SN treatment, achieving the highest shoot length (Figure 14c; Table 4). *G. lanigera* had a significant effect on shoot length for the unshaded treatments, having longer shoots when compared to the shaded treatments.

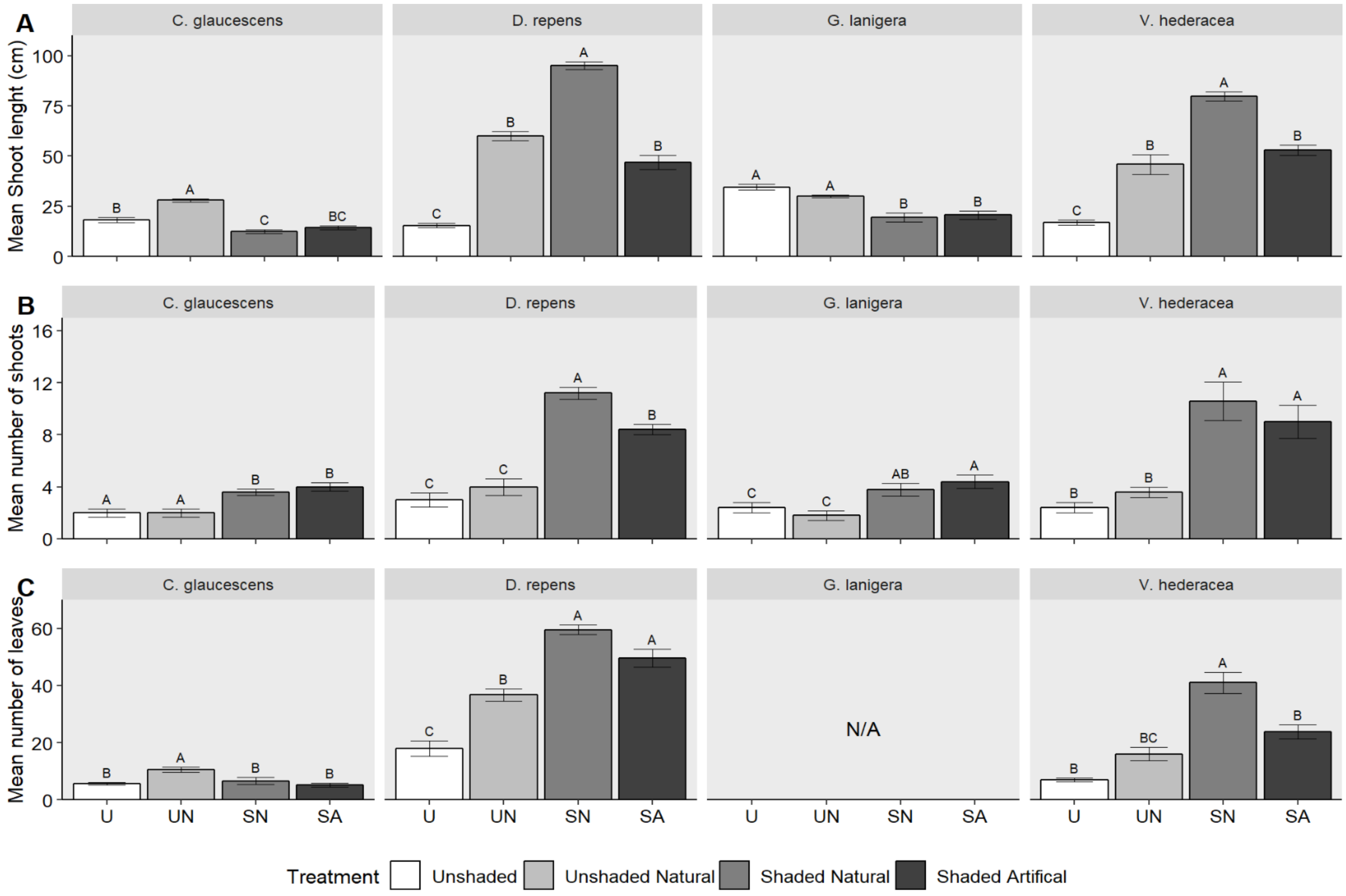


Figure 14: The effect of shading on plant growth. Comparison of the effect of four different shade treatments for four different species: (a) mean shoot length, (b) mean number of shoots and (c) mean number of leaves. Separate analyses of variance and multiple comparisons were performed for each species; different letters signify statistically different means. Error bars are ±*SE*; *n* = 5.

Table 4: Summary statistics from an analysis of variance comparing the effect of nurse plants on biomass, growth and substrate moisture in a green roof environment. * Denotes statistical difference at α = 0.05

Species	**Test**	***F***	***df***	***SS***	***p***
Carpobrotus glaucescens	Biomass above	20.239	3,16	224.822	< 0.001*
	Biomass below	4.2114	3,16	21.961	0.023*
	# of leaves	8.9804	3,16	91.6	0.001*
	# of shoots	12.296	3,16	5.53	0.001*
	Shoot length	44.5	3,16	717.50	< 0.001*
	Substrate moisture	0.2035	3,16	9.78	0.893
Dichondra repens	Biomass above	36.536	3,16	24.57	< 0.001*
	Biomass below	40.388	3,16	257.54	< 0.001*
	# of leaves	51.019	3,16	4832.8	<0.001
	# of shoots	53.46	3,16	220.55	< 0.001*
	Shoot length	194.27	3,16	16286	< 0.001*
	Substrate moisture	0.318	3,16	11.65	0.812
Grevillea lanigera	Biomass above	29.33	3,16	7.56	0.001*
	Biomass below	6.916	3,16	22.82	0.001*
	# of leaves	19.039	3,16	268.32	< 0.001*
	# of shoots	7.266	3,16	7.266	0.003*
	Shoot length	###	###	###	###
	Substrate moisture	3.51	3,16	270	0.040*
Viola hederacea	Biomass above	62.678	3,16	8.2556	<0.001*
	Biomass below	31.707	3,16	158.77	<0.001*
	# of leaves	31.411	3,16	3126	<0.001
	# of shoots	15.76	3,16	241.2	<0.001*
	Shoot length	71	3,16	10031	<0.001*
	Substrate moisture	4.4588	3,16	358.15	0.186

Discussion

In this work, we assessed the performance of four different target plants (ground covers/ prostrate shrubs) under green roof conditions. We focused on the effects of net positive (facilitation) or net negative interactions (below ground competition) of a nurse plant on the focal species. Overall, we identified that facilitation played a more significant role in influencing plant growth compared to competition. Below, we discuss some of the mechanisms affecting plant growth.

Shading

The main facilitative mechanism, shading, positively influenced plant growth, although it differed among species. *V. hederacea* and *D. repens* exhibited increased biomass when subjected to shade in both natural and artificial treatments, suggesting that, for these two species, nurse plants facilitate growth through shading from continuous solar exposure. This is as expected as both *D. repens* and *V. hederacea* natively inhabits communities with abundant shade (open forests) and are able to thrive in shaded communities. *G. lanigera* also exhibited increased biomass when subjected to artificial shade; however, any positive benefit of shading from a nurse plant was overwhelmed by the competitive response to the nurse plant. *C. glaucescens,* on the other hand*,* had a negative reaction to shade.

Shading ameliorates excessive solar radiation and may lower substrate temperatures or reduce water evaporation from substrates. In our experiment, we found no reduction in water loss in shaded substrates, although substrate moisture was reduced in *G. lanigera* due to competitive effects (see below). One limitation of the experimental setup is that the mesocosms were kept in well-watered conditions with daily irrigation and a substrate with a high soil water content. Biotic interactions have been shown to vary across abiotic variables (Bertness & Callway 1994), with net positive or facilitative interactions being more prevalent in communities under higher abiotic stresses when compared to communities that develop in more benign conditions (Bertness & Callway 1994). Future experiments should investigate if the observed facilitative benefits of shading still occur under drought conditions.

Substrate temperatures were affected, which suggests that nurse plants can ameliorate damaging substrate temperatures, particularly on hot days. Substrate temperatures were reduced by 15 °C on hot days but only by 8 °C on days of moderate temperature. This difference may be the main mechanism for facilitating root growth, as high root temperatures are known to be lethal for the roots of plants (Kaspar & Bland 1992).

Excessive solar irradiation is detrimental to plant growth (Jaleel. 2009). Green roofs are typically subjected to harsh environmental conditions (Brown & Lundholm 2015), including excessive irradiation. Getter et al. (2009) found sunlight to be one of the main abiotic constraints on a green roof. The study evaluated the effect of solar irradiation on the growth of six common Sedum species. He found that growth in high solar irradiation was dependant on species with full solar exposure reducing the growth of *S.reflexum* by nearly 90%. Our results also found exposure to limit the growth of our target species, wherein our control treatment had the lowest growth and biomass for three of our species (aside from *C. glaucescens*). High solar radiation leads to reduced photosynthetic activity due to the photoinhibition of photosystem II (Murata et al. 2007; Tyystjärvi 2013), retardation of plant growth (Demmig-Adams & Adams 1992), high leaf temperatures leading to stomatal closure, and inactivation of enzymes leading to a decrease in growth (Zandalinas et al. 2018). Shading helps ameliorate some of the abiotic stresses on a green roof by providing a physical barrier that limits the amount of direct incoming solar irradiation. A gradient of shading is provided by a nurse plant depending on plant characteristics and the nearness of the target plant to the nurse plant, creating areas of varying light intensities. Target plants will likely grow better if this approaches their optimum light range.

For species adapted to high-light environments, neighbouring plants may cause deficient light intensities, which could reduce the rate of photosynthesis. If light intensity drops below the compensation point, plant respiration will use up more photosynthates, including carbon, leading to reduced growth. The addition of shading in our experiment had a negative impact on the growth of *C. glaucescens*. *C. glaucescens* is typically found on exposed seaside cliffs and dunes where it must contend with full solar radiation, high salinity, high winds and low water. It is physiologically adapted to high solar exposure with its succulent leaves and its ability to use the Crassulacean acid metabolism (CAM) photosynthetic strategy

If the shading of a nurse plant is truly facilitative, then the growth habit of plants should reflect a desire to remain within the optimum light range, with a clustered growth habit. In the shade-avoiding species, there is an increase in stem and petiole elongation to cover a wider area to receive more light (Grime 1973. This was observed in *C. glaucescens*; however, increases in stem number and length in *D. repens* and *V. hederacea* were accompanied by an increase in the number of leaves, associated with the overall increase in biomass in the shaded treatments, and there was no evidence of shade-avoiding behaviour.

Competition

The lack of a net competitive effect between some of the pairs of plants was unexpected, as this has been well documented in other experiments that use nurse plants (Fujita & Yamashina 2018; Wright et al. 2015). Some of the positive benefits of nurse plants are offset by the resource competition provided by adding another plant to the habitat (Holmgren, Scheffer & Huston 1997). However, in our experiment, only *G. lanigera* exhibited signs of competition with its nurse plant, *C. citrinus.* Unlike the other species, in treatments with a nurse plant (SN and UN treatments), *G. lanigera* exhibited greater below-ground biomass, while above-ground biomass was reduced. The trade-off effect observed in *G. lanigera* is a resource competition response whereby there is an increase in roots at the expense of shoots. We observed a reduction in available substrate water when a nurse plant was present (SN treatment). Van Noordwijk et al. (2015) found that, under light competition, plants allocated more resources to above-ground growth. In our experiment, we observed that *G. lanigera* was allocating resources below-ground to compete with the nurse plant at the expense of its above-ground biomass.

Below-Ground Facilitation

The other unexpected result was the net facilitative effect that the nurse plant had on the ground covers when they were not providing significant shade. By trimming the nurse plant and limiting the shading benefit, we expected that plants would be under root competition without the confounding positive effect of shade. Interestingly, there was a positive below-ground interaction that increased both above- and below-ground biomass of the target plants. In *D. repens* and *V. hederacea*, the facilitative effect of this below-ground interaction was equal to the positive impact of shade in the artificially shaded treatment (SA). This benefit was also observed in the naturally shaded treatment (SN), where the positive benefit was often above that of the SA treatment.

The two main ways that nurse plants may facilitate plants are by ameliorating abiotic stressors and modifying the substrate (Filazzola & Lortie 2014). Below-ground, hydrological lift may be provided by the nurse plant, increasing water availability for the plants around it (Padilla & Pugnaire 2006). In our experiment, we found no significant differences in the surface substrate moisture content (measured at 1/3 growing depth), suggesting that substrate moisture was not driving the facilitative response.

The other method of facilitation is the modification of the substrate through increasing the nutrients available to the plant through leaf senescence and mycorrhizae (Lanfranco, Fiorilli & Gutjahr 2018). In our experiment, all dead leaves were collected, so contributions from leaf senescence were negligible. There is ample evidence in the literature demonstrating how mycorrhizae increase plant growth and survival (Montesinos-Navarro, Valiente-Banuet & Verdú 2019) and ameliorate abiotic stressors (Filazzola & Lortie 2014). Similar evidence of below-ground facilitation has been found in vines (French et al. 2017). Future studies are required to isolate mycorrhizal facilitation on green roofs and investigate if substrates used in green roofs are able to host mycorrhizal communities.

Conclusion

Our results highlight the effectiveness of shade on a green roof in increasing growth for four species tested. The use of nurse plants is a practical option for improving the diversity of plant selection on a green roof. Not only do the nurse plants enable species that are typically stressed by roof garden conditions to be used, but they also contribute to the biodiversity and aesthetic benefits provided by the green roof. In our experiment, the species pairs were chosen at random with no optimising between nurse plant and target plant. With careful selection of nurse plants that are best at improving abiotic conditions for a target plant, we may be able to increase the nurse plant's effect and reduce some of the competitive effects. Understanding the mechanisms of facilitation, particularly below ground, is essential action for maximising the success of green roofs. We can use nurse plants to optimise the establishment and success of gardens in urban areas to increase social and biodiversity benefits. While green roofs are growing in popularity, care should be taken to carefully select and build green roofs to ensure long-term success.

Chapter 4: The Effect of Substrate and Fertiliser on Mycorrhizae–Plant Interactions

Contributions

Axton Aguiar conceived and designed the experiment, collected the data, performed the analysis and wrote the manuscript.

Kris French aided in the conception and design, aided in the analysis and reviewed the manuscript.

Sharon A Robinson aided in the conception and design, reviewed the analysis and manuscript.

Publication

Submitted to *Urban Ecosystems*.

Introduction

With the rapid growth of urbanisation globally, there has been a shift to develop sustainable cities to maintain ecosystem services. Green roofs are essential components of these strategies, as they provide social, economic and environmental benefits (Cook-Patton & Bauerle 2012; Moghbel & Erfanian Salim 2017; Oberndorfer et al. 2007). Specifically, they can be implemented into pre-existing buildings where they harness underutilised space to help maintain building temperature and prolong the life of roofing materials (Ouldboukhitine et al. 2011). Additionally, they also help ameliorate some of the negative consequences of urban heating (Alexandri & Jones 2008), increase local biodiversity (Cook-Patton & Bauerle 2012) and reduce run-off (Szota et al. 2017).

The ecosystem services provided by a green roof is closely tied to the substrate properties and the vegetation present on the green roof (Lata et al. 2018, Lundholm 2015). Substrates on a green roof consist of organic and inorganic components (Ampim 2010). The inorganic component of a green roof are normally lightweight, stable substrates (e.g., perlite, scoria). The organic component aids in providing nutrients for the biota on the green roof. However, it is normally restricted to 20% total substrate volume to minimize the shrinking of the substrate and leeching of nutrients through decomposition (Ampim 2010).

Ecosystem services are not always a top consideration in green roof design. Designers must contend with weight and cost restrictions; accordingly, green roofs typically have shallow growing substrates, usually 5–15 cm deep and composed primarily of large-diameter mineral aggregates (3-10mm) such as crushed brick, scoria, pumice perlite and some organic matter (not more than 20% by volume) (Ampim et al. 2010; Conn et al. 2020; Chapter 2). These factors, combined with the harsh abiotic conditions such as low water availability, high solar radiation, temperatures and wind present on green roofs (Oberndorfer et al., 2007), form a challenging environment for plants to grow in. These constraints have led to an over-reliance on a few species of plants, mainly succulents, limiting the benefits of a green roof (Lundholm 2015).

One possible way to improve plant survivability on a green roof is using microbial symbionts (Molineux et al. 2015; John, Kernaghan & Lundholm 2017, Xie et al. 2018). Microbial symbionts play an essential role in plant growth and survival in natural (Moora et al. 2014; Powell & Bagyaraj 1984) and agricultural ecosystems to improve yield and survival (Baum, El-Tohamy & Gruda 2015; Trouvelot et al. 2015). Of the microbes found in the soil, mycorrhizal fungi are particularly relevant in improving plant growth and survival on a green roof (Xie et al. 2018). Xie et al. 2018 found that the addition of soil microbial symbionts (*Rhizophagus irregularis* and *Bacillus amyloliquefaciens*) increased plant photosynthetic efficiency and shoot weight.

Between 80% and 90% of all terrestrial plants have mycorrhizal associations during some stage in their life cycle (Schwarzott et al. 2001; Wang & Qiu 2006). In this symbiotic association, plants receive minerals and nutrients, and the fungi gain direct access to carbohydrates produced by the plant during photosynthesis (Hampp & Schaeffer 1999). While the plant can directly access some of the nutrients and macronutrients present in the soil, the fungi have access to distant or chemically immobilised nutrients in the substrate (Talbot, Allison & Treseder 2008). Nutrient transfer from plant–fungi associations affects both growth and survivability of plants in adverse abiotic conditions (Porcel, Aroca & Ruiz-Lozano 2012) through the accumulation of solutes such as K^+, K^+/Na^+ and soluble carbohydrates to prevent cell dehydration (Sheng et al. 2011), maintenance of water uptake (Ruiz-Lozano & Azcón 1995) and regulation of ions (Porcel, Aroca & Ruiz-Lozano 2012).

However, the outcome of mycorrhizal symbiosis can be highly context dependant, affected by plant and mycorrhizae identities, plant growth stage, abiotic variables and substrate properties (Rayment et al. 2020, Crawford 2019, John et al. 2017). Substrate properties are important when introducing mycorrhizae to novel ecosystems such as green roofs. Moisture, pH and substrate particle size affect mycorrhizal community function, distribution and structure (Gaur et al. 2000).

John et al. 2014, found that most commercial green roof substrates are initially devoid of mycorrhizal inoculum. Colonization of green roof substrates can spontaneously increase over time or through deliberate inoculation. Inoculum for mycorrhizae can be sourced either from the field or the use of a commercial inoculum. Commercial inoculant provides an easy method of adding mycorrhizae onto a green roof (Frew 2020). However, inoculum source has also been shown to affect mycorrhizal benefits on a green roof (Klironomos 2003, Rowe 2007).

The objectives of this study were to use a controlled glasshouse experiment to investigate 1) whether mineral aggregates commonly used in green roof substrates can sustain a mycorrhizal community, 2) whether mycorrhizae are effective at facilitating plant growth in novel green roof substrates, and 3) the advantage of using mycorrhizal inoculant sourced from native habitats over commercial mycorrhizal inoculants.

Methods

This study was carried out in a glasshouse at the ERC at the University of Wollongong, NSW, Australia. Two common forb species (two of the four target plants used in Chapter 3) were tested, *Dichondra repens* and *Viola hederacea*. Both the *Dichondra* and *Viola* genus have been shown to have a positive association with Arbuscular mycorrhizal fungi (AMF) (Torpy et al. 1999, Tornin et al. 1999, Bellgard 1991, Castillo 2006). Forbs were used as they have been shown to have the highest rate of colonisation when compared to grasses and shrubs (John, Kernaghan & Lundholm 2017). *D. repens* and *V. hederacea* were grown from seeds in sterilised soil and watered only using reverse osmosis water to avoid mycorrhizal contamination. An automatic drip-watering system was set up to dispense 0.3 L of reverse osmosis water over 15 minutes to each pot twice a day. The daily minimum temperature in the glasshouse across three seasons (winter, spring and summer) ranged from 19 to 33 °C, and the daily maximum temperature ranged from 21 to 38 °C.

Experimental Design

Inside the greenhouse, 120 pots (100 mm) were set up (60 pots for each species) in a factorial design to investigate the effect of substrate composition and inoculation mix on plant growth and mycorrhizal colonisation across the two plant species. Four different substrates were tested: perlite mix (grade 3–4 mm), crushed terracotta (3-5 mm), scoria (4-6 mm) and for the control, a garden soil mix (potting mix; Figure 15). Potting mix was chosen as a control as we needed a substrate that has previously been shown to facilitate mycorrhiza. 20% coir was added to all the aggregates to achieve a composition of 80% inorganic and 20% organic. The aggregates used was based on previous studies using these mixes (Eksi and Rowe 2016, Vijayaraghavan and raja 2014, Vijayaraghavan and Joshi 2014, Kotsiris et al. 2012) and suggestions in the *Sydney City Council Green Roof Design Resource Manual* (City of Sydney Council 2014), Melbourne's *Green Growing Guide* (Francis et al. 2014) The substrate layer was approximately 7 cm deep. All substrates were sterilised in an autoclave oven to ensure no residual soil microbes were present.

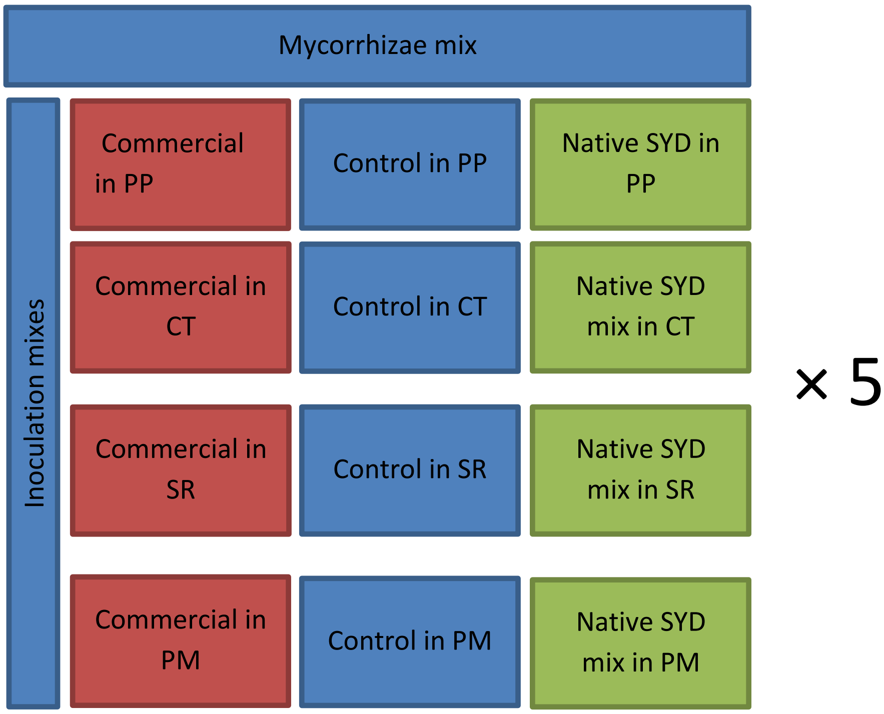


Figure 15: Experimental design to investigate the effect of green roof substrate on mycorrhizal inoculation. The vertical columns represent different mycorrhizal inoculation mixes, and the rows represent the substrate treatments. The design is repeated for each species. PP = perlite mix (grade 3–4 mm); CT = crushed terracotta (5 mm); SR = scoria (6 mm); PM = potting soil mix. SYD= Sydney.

Three mycorrhizal inoculation mixes were applied to randomly allocated pots: locally sourced mycorrhizae, an industrial brand of mycorrhizae (MycoApply Endo/Ecto) and a control with no mycorrhizae spores. Locally sourced mycorrhizae were collected in situ on sandstone soil along the Illawarra escarpment in NSW (Mount Keira: 34° 24′ 14.7″ S 150° 51′ 21.4″ E) using an adapted version of the wet sieving and decanting method described by Gerdemann and Nicolson (1963). In brief, 10 cm^3 of soil was collected from the rhizosphere of native plants, to which 3.5 L of distilled water was added. The mixture was vigorously agitated using a whisk for 5 minutes to break up soil aggregates and to isolate mycorrhizae spores in the solution. The solution was initially passed through a 1 mm sieve to remove debris and then passed through a 40 µm sieve to isolate mycorrhizal spores. A 40 µm sieve was used to collect the spores, as in previous research (Klironomos 2002), spores were successfully extracted using a 45 µm sieve. The 3.5 L filtrate was placed in a 30 L water container, and the process was repeated until 30 L of filtrate had been collected. The filtrate was then split into three 10 L containers to form the base solution for the three inoculate mixes. The naturally collected spores and the commercial spores were placed into Petri dishes and examined under a microscope to ensure a similar spore density of approximately ten g/L^{-1} before adding each to 10 L of the base inoculant. This resulted in three mixes: a natural mycorrhizal inoculation consisting of naturally collected spores mixed with 10 L of base inoculant at a spore density of 10 g/L^{-1}, a commercial mycorrhizal inoculation mixed with 10 L of base inoculant at a spore density of 10 g/L^{-1} and a control, which consisted of 10 L of base inoculant with no mycorrhizal spores.

All pots were irrigated three times a week using reverse osmosis water; each pot received 1 L of water over 15 minutes using an automatic drip system. During the first 12 weeks, two irrigation sessions were replaced by hand-watering using the inoculant treatment. All plots were given a starter fertiliser consisting of Osmocote Native (a controlled release fertiliser with a nitrogen, phosphorous and potassium ratio of 17.9:0.8:7.3) at the recommended rate for the pot size of 8 g/m^2.

After nine months, the plants were harvested from the pots. Roots and shoots were separated, and the roots were washed over a sieve with distilled water to ensure that any fine roots (< 2 mm in diameter) were collected and kept with the sample. The amount collected was kept constant so that all treatments would lose the same amount of weight (5% of total root collected). Procedures outlined by Utobo et al. (2011) were used to visualise fungal colonisation in the roots. In summary, 1 cm fine root segments were placed in Eppendorf tubes filled with 10% potassium hydroxide and heated in a water bath at 90 °C. After 1 hour, the roots were washed in distilled water and immersed in a 1% hydrochloric acid solution for 18 hours. Roots were then submerged in a 2% Parker Quink permanent ink and 1% hydrochloric acid solution and placed in a water bath at 60 °C for half an hour. Roots were then destained by placing them in a 48% glycerol, 48% distilled water and 4% lactic acid solution for two days.

Mycorrhizal colonisation was estimated using the magnified intercept method, described by McGonigle et al. (1990). Ten processed 1 cm root segments were randomly selected for each plant and mounted in two bands of five parallel root sections on a microscope slide. The presence or absence of fungal structures (hyphae, arbuscules and vesicles) were recorded at 100 randomly selected locations along the fine root segments. With no arbuscules evident in the samples I collected, the total of hyphae and vesicles was recorded. The rest of the roots and shoots were placed in a drying oven at 75 °C; a subsample was weighed daily till there was no change in weight (72 hours). The dry weight of all plants was then measured.

To investigate whether mycorrhizae affected water loss. At the end of the experiment, plants were watered to saturation on the final watering before harvesting and collection trays were placed under each pot. Each pot was watered with 1 L, and any water lost through the bottom of the pot was collected for 30 minutes after watering. The field capacity of the substrate was calculated by subtracting the run-off from 1 L.

Statistical Analyses

Each species was analysed separately. Biomass, mycorrhizal colonisation and water loss were compared among treatments using two-factor ANOVAs after testing for normality (Shapiro–Wilk test) and homogeneity of variance (Cochran's test). Significant interactions were further investigated using multiple-comparison tests (Tukey's HSD). The control treatment for the mycorrhizal colonisation was not included in the analysis; I only compared native and commercial inoculants.

Results

The Effect of Substrate and Inoculation Type on Mycorrhizal Colonisation

Our control treatment was effective, with no mycorrhizal colonisation detected across any substrate type, except the potting mix treatment in *V. hederacea,* which showed a 3% colonisation rate (Figure 16c).

Both species were colonised by mycorrhiza in all substrate types and for both inoculants, but the percentage of colonisation varied across species (Figure 16 &Figure **17**).

Colonisation rate was higher when commercial inoculant (25–80%) was used compared to native inoculant (25–35%), except in the terracotta substrate (interaction term, $F_{3,32} = 10.573$, $p < 0.001$). In *D. repens,* the substrate influenced the colonisation rate; however, inoculation type had no effect on colonisation rate (substrate, $F_{3,32} = 75.066$, $p < 0.001$; mycorrhizae, $F_{1,32} = 1.409$, $p = 0.244$; interaction, $F_{3,32} = 1.777$, $p = 0.171$). Overall, plants in perlite had the highest hyphal colonisation rates for both species, and plants in terracotta substrates had the lowest colonisation (Figure 16c &Figure **17**c). For *V. hederacea*, substrates of perlite, potting mix and scoria showed similar colonisation—all higher than terracotta (Figure 16c). For *D. repens*, plants in potting mix showed intermediate colonisation rates, with scoria and terracotta substrates both similarly lowest (Figure 17c).

The Effect of Substrate and Inoculation Type on Plant Biomass

There was a significant interaction between substrate and mycorrhizae on final biomass, and this effect differed across species (interaction: *Dic. repens*, $F_{6,48} = 8.561$, $p < 0.0001$; *V. hederacea*, $F_{6,48} = 3.043$, $p = 0.013$). For both species, the control mycorrhizal treatment had the lowest biomass across all substrates, with the potting mix commercial treatment having the highest biomass.

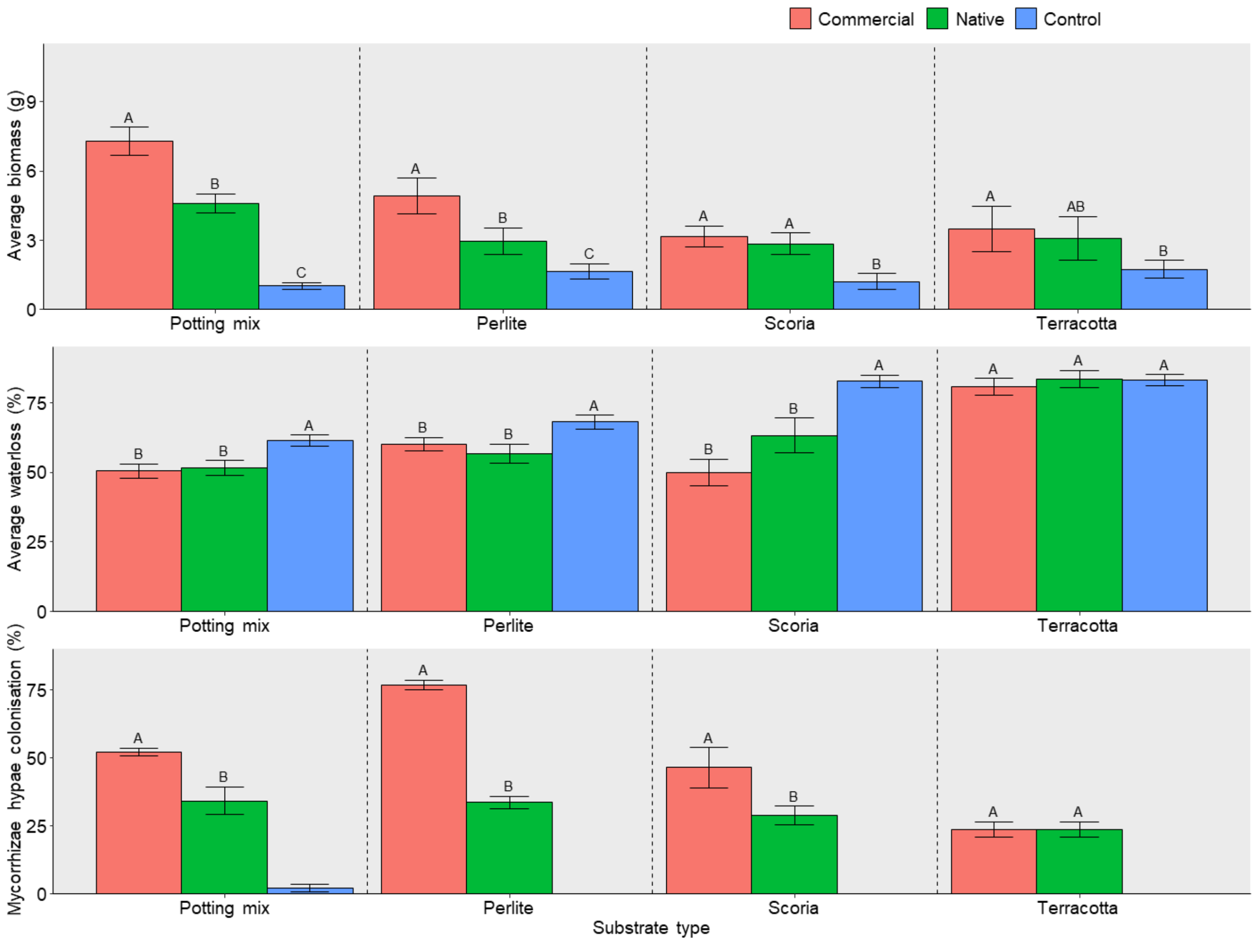
Overall, substrate type influenced the final biomass of both species (main effect: *D. repens*, $F_{3,48} = 20.477$, $p < 0.001$; *V. hederacea*, $F_{3,48} = 5.864$, $p = 0.002$; Figure 16a &Figure **17**a). The inoculation of mycorrhizae more than doubled the biomass in both potting mix and perlite substrates. The main exception to this was found in the terracotta substrate treatments, where *D. repens* control plants grew as well as those inoculated with the commercial mycorrhizae (Figure 17a); for *V. hederacea*, plants showed no difference in biomass gain between control and native mycorrhizae (Figure 16a).

For inoculated plants, plants grown in potting mix generally had the greatest biomass, while those in scoria and terracotta tended to have less. The biomasses of plants in potting mix were 10–50 % greater than those in other substrate treatments.

While there was a marked difference between substrate types for the overall biomass of *D. repens*, the effect was not as strong for *V. hederacea*. Plants grown in potting mix with commercial inoculant had the highest biomass (7.4 g). However, the difference in biomass between inoculate types in perlite was less, while in the other two substrates (scoria and terracotta), biomass did not vary. In the control (no inoculant), terracotta had the highest biomass for *D. repens*, and perlite had the highest biomass for *V. hederacea.*

The Effect of Substrate and Inoculation Type on Water Loss

The effect of substrate type on substrate water loss was influenced by inoculation type (interaction: *D. repens* $F_{6,48} = 3.043$, $p < 0.0132$; *V. hederacea*, $F_{6,48} = 8.355$, $p < 0.0001$; Figure 16b & Figure **17**b). Overall, in all substrates but terracotta, water loss was reduced by mycorrhizal inoculation. For *V. hederacea*, water loss was reduced by 30% in scoria and 10% in both perlite and potting mix. For *D. repens,* water loss was reduced by 20% in potting mix and 10% in perlite; in scoria, water loss was only reduced in the native mycorrhizal inoculation.

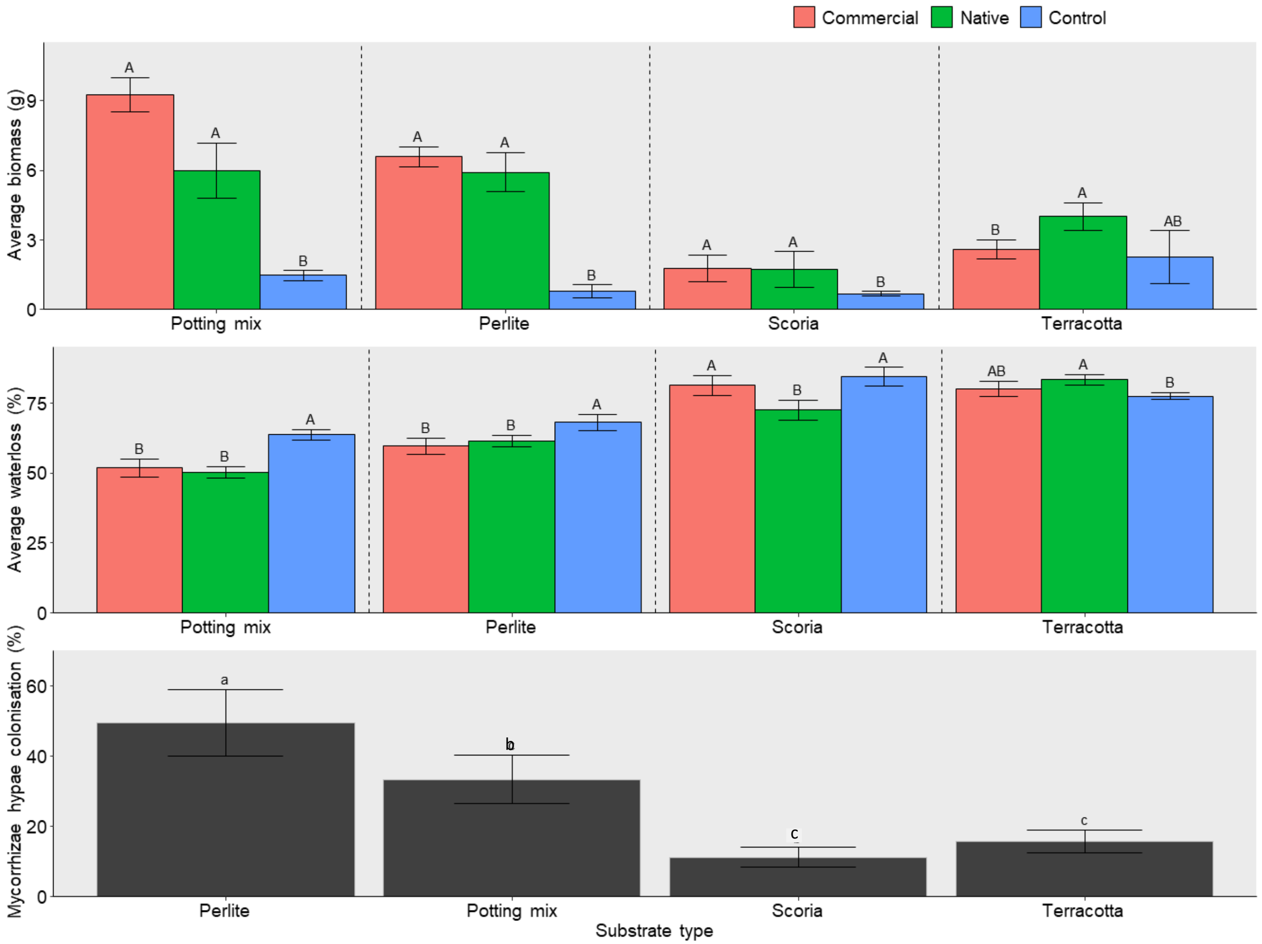


	Comparisons
Commercial	Potting mix>Perlite>Scoria=Terracotta
Native	Potting mix>Perlite=Scoria=Terracotta
Control	Perlite>Potting mix, Terracotta & Scoria intermediate

	Comparisons
Commercial	Terracotta>Potting mix Perlite & Scoria intermediate
Native	Terracotta >Perlite = Scoria > Potting mix
Control	Terracotta = Scoria > Perlite > Potting mix

	Comparisons
Commercial	Perlite > Scoria = Potting mix > Terracotta
Native	Perlite = Scoria = Potting mix > Terracotta
Control	N/A

Figure 16: (a) Average biomass, (b) average water loss and (c) total arbuscular mycorrhizal fungi colonisation of *Viola hederacea* across four different substrate types and two inoculation types. For (a) and (b), each bar represents the average of five individuals. For (c), mycorrhizal inoculation was pooled as there was no interaction term. The tables represent Tukey's HSD comparisons between the substrate types. Error bars represent the standard error of the mean. Letters signify statistically different means.



	Comparisons
Commercial	Potting mix > Perlite > Scoria = Terracotta
Native	Potting mix = Perlite > Terracotta > Scora
Control	Terracotta > Scora, Potting mix & Perlite intermediate

	Comparisons
Commercial	Scora = Terracotta > Perlite > Potting mix
Native	Scora > Terracotta > Perlite > Potting mix
Control	Scora = Terracotta > Perlite > Potting mix

Figure 17: (a) Average biomass, (b) average water loss and (c) total arbuscular mycorrhizal fungi colonisation of *Dichondra repens* across four different substrate types and two inoculation types. For (a) and (b), each bar represents the average of five individuals. For (c), mycorrhizal inoculation was pooled as there was no interaction term. The tables represent Tukey's HSD comparisons between the substrate types. Error bars represent the standard error of the mean. Letters signify statistically different means.

Discussion

I found that all green roof substrates were able to sustain a mycorrhizal community; however, the facilitative effect of the mycorrhizae on plants depended on the substrate and inoculation type. The least effective substrate was terracotta, which had the lowest water-holding capacity, mycorrhizal colonisation and biomass increase. The most effective substrate was potting mix: *D. repens* plants in this substrate exhibited a 300% increase, and *V. hederacea* a 200% increase, in biomass over plants planted in terracotta.

Mycorrhizal Inoculation

I found that substrate type significantly affected mycorrhizal colonisation. Physical properties such as Soil texture, pH and particle size play an essential role in mycorrhizae establishment (Wang et al. 1993). Particle size and structure affect mycorrhizal colonisation by limiting the distance that plant root exudates can travel (Zhalnina et al. 2018). Exudates enhance mycorrhizal growth in the rhizosphere (Broeckling et al. 2008; Zhalnina et al. 2018) and play a vital role in the initial mycorrhizal colonisation of a plant. Soil texture has been shown to influence mycorrhizae by influencing the time it takes for mycorrhizae to establish a symbiotic relationship with a target plant species (Carrenho et al. 2007). Our results support these findings: both the control potting mix and perlite substrates had lower levels of hardness, whereas scoria and terracotta were harder. These influenced the ability for mycorrhizae to establish good substrate hyphal networks, thereby impacting on the strength of the symbiotic relationship.

While substrate type played an essential role in mycorrhiza colonisation, for *V. hederacea*, commercial inoculate resulted in improved colonisation. This was unexpected, as it would be predicted that native mycorrhizae would develop better symbiotic relationships with native plants. One possible reason for this is that commercially sourced mycorrhizal fungi are bred commercially to have a broad ability to colonise a range of species, while native mycorrhizal spores may find novel environments, such as scoria and terracotta substrates, more difficult. Furthermore, while the commercial mycorrhizae offered some benefit for one of our species, it should be noted that the commercial mycorrhizae is a product made overseas (in the United States) and, as such, is not likely to have native spores. Mcguire et al. (2013) found that the microbial communities found on green roofs were similar to those found on disturbed, heavily polluted urban sites, suggesting a strong selection for species able to cope with the harsher conditions found on roofs, which may be better sourced using commercially bred stock.

Another key factor that has not been well studied is the host-specificity of mycorrhizae. While it is generally accepted that the major families of mycorrhizae (Glomeromycota) are generalists (Öpik et al. 2006; 2010), research has shown that, for some species of plants, there is some host-specificity in mycorrhizal associations (Kil et al. 2014). This suggests that fungal spore identity is a critical element in colonisation rate across a range of substrates and species, which requires further investigation using both native and commercially available products.

Mycorrhizal Facilitation of Plant Growth

Mycorrhizae increased biomass across all substrate and inoculation types. Irrespective of substrate type and plant species, plants with no mycorrhizae had lower biomass. There was a 300%–400% increase in biomass in mycorrhizal treatments for perlite and the control potting mix, indicating the value of the symbiotic relationship in these substrates. Mycorrhiza improves nutrient availability through an extensive mycelial network that covers a much larger area than the plant’s roots. This increase in the effective surface area leads to a higher uptake of nutrients that would normally not be available for the plants (Leake 2004).

Substrate types with the highest plant-colonisation rates also had the highest biomass increase. In fact, biomass increases in these substrates far outweighed what would be expected from the increase in mycorrhizal colonisation levels recorded. This could be a result of two processes: improved uptake of nutrients through better development of a mycelial network in the substrate and/or an increase in the water-holding capacity of the substrate, reducing water stress in plants. Mycorrhizae normally improve substrate quality, as their mycelia release humic compounds, polysaccharides and glycoproteins that help bind substrate, promoting water retention and aeration of the substrate (Drinkwater and Snapp 2007). As expected, the control, potting mix had the highest growth and inoculation rates, suggesting that this medium had properties that promoted this symbiosis between the plants and fungi and which are potentially associated with the improved water-holding capacity of these substrates. Alternatively, mycorrhizae may be mining normally unavailable nutrients from the organic matter in the substrate, which were not available in other substrates. As a result, the symbiotic relationship was stronger in the potting mix treatment.

The introduction of mycorrhizae reduced the water loss of both scoria and terracotta. However, this did not translate to higher plant biomass. While there was some association between the plants and mycorrhizae—albeit at relatively low colonisation levels—there may also have been a reduced substrate network of hyphae, resulting in little exchange of nutrients or other facilitatory relationships taking place. Further study is needed to investigate whether the small increase in biomass for scoria and terracotta is due to the improved water-holding effect of substrate brought on by mycorrhizae or the mining ability of the mycorrhizae to improve the transfer of nutrients to the plants.

The reduction of substrate water loss is influenced by the increase in plant biomass: higher biomass leads to a higher level of transpiration. Franco et al. (1994) found that evapotranspiration increased linearly with total leaf surface area as the plant grew. To understand how best to improve survival on green roofs, further work is required to tease apart the interaction of plant biomass, substrate mycorrhizal networks and substrate drying.

Interestingly, the increase in plant biomass associated with mycorrhizal colonisation was in the complete absence of arbuscules. Arbuscules are thought to be the critical pathway of nutrient transfer (Harrison 1997), yet the low level of arbuscule development in this experiment suggests that other non-arbuscule transfers may be important.

Local vs Commercial Mycorrhizal Inoculant

Overall, commercial mycorrhizal inoculant was better than natural inoculant, increasing colonisation, plant growth and reducing water loss. This finding contrasts with other studies comparing local to commercial inoculants (Middleton et al. 2015; Symanczik et al. 2017). Most of the studies (Baum, El-Tohamy & Gruda 2015; Hijri 2016) are performed in agricultural systems, and thus, microbial interactions in the soil are less disrupted than in purely artificial environments. However, in ecosystems such as green roofs, the habitat is artificially created, making it novel to any mycorrhizae added to the system. Furthermore, as I added no other microbial species to the substrate, there was less chance of negative interactions disrupting colonisation. Further, McGuire et al. (2013) found that mycorrhizal communities on green roofs were compositionally distinct from other urban green habitats such as parks and only shared 54% of the microbial taxa. They found that the most abundant fungal taxa were those found in disturbed environments with high resistance to varying abiotic conditions and major substrate contaminants.

Our study was carried out under controlled conditions in a glasshouse, however, further investigation is required to elucidate the interaction between substrate types and mycorrhizal benefits on a green roof which would be subjected to higher variability and extremes of abiotic conditions.

Conclusion

The development of resilient plant communities on green roofs has multiple benefits in improving the benefits obtained from green roofs and reducing costs. Our study highlights how mycorrhizae could be used in novel environments to help increase plant growth and reduce substrate water loss. Commercial inoculant provides an easy alternative to locally sourced inoculant. However, care should be taken in sourcing commercial inoculants, and more research is required to assess the inherent risk of introducing potentially non-native microbiota.

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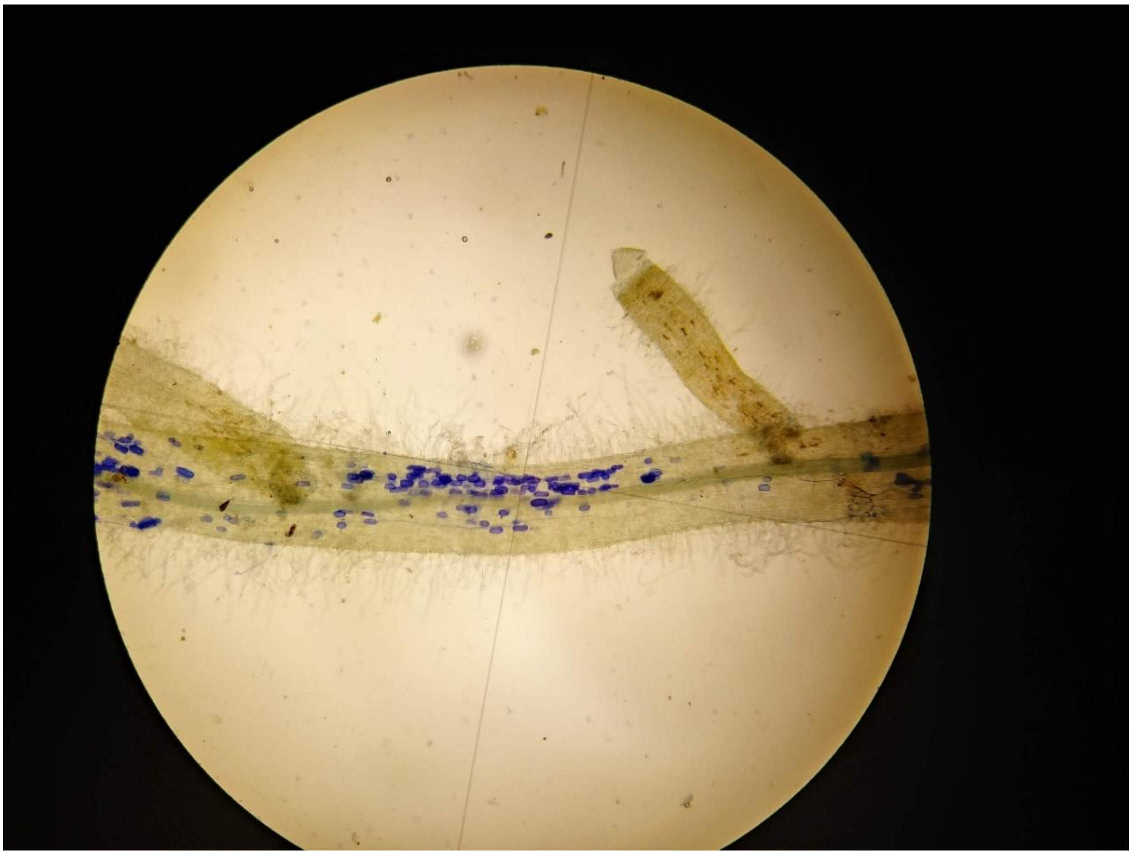
Chapter 5: Investigating Biotic Facilitation along an Abiotic Gradient (Water) in a Green Roof Environment



Contributions

Axton Aguiar conceived and designed the experiment, collected the data, performed the analysis and wrote the manuscript.

Kris French aided in the conception and design, aided in the analysis and reviewed the manuscript.

Sharon A Robinson aided in the conception and design and reviewed the analysis and manuscript.

Publication

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Introduction

Green or vegetated roofs are an important strategy employed in cities worldwide, as they provide essential ecosystem services that help combat some of the negative aspects of urbanisation. For example, green roofs ameliorate UHI effects, reduce stormwater run-off and reduce energy costs associated with heating and cooling (Oberndorfer et al. 2007; Vijayaraghavan 2016). Green roofs can be a challenging environment for plant growth and survival: plants must contend with shallow growing substrates, limited water and high abiotic stress (temperature, light and wind; Eksi et al. 2017; Vijayaraghavan 2016). High abiotic stressors such as high heat and light stress reduce the efficiency of the plant's photosystems (McDonald et al. 1997). One way plants can offset heat stress is by increased water intake (McDonald et al. 1997) however, in a green roof environment, where water is limited, plants are also subjected to drought stress. The combination of heat and drought stress results in a reduction in the plants' electron transport rate (Bukhov et al. 2000), energy synthesis (Tezara et al. 1999), creation and utilisation of photosynthates (Suwa et al. 2010 and the carbon cycle (Sippel et al. 2018). These effects can lead to a reduction in plant growth and eventually plant death.

While studies on drought and heat-induced plant death have been undertaken in established natural ecosystems (Allen et al. 2010; Mueller et al. 2005), there has been little focus on urban plants despite the potential for heat stress to be particularly high (Aguiar et al. 2014). Understanding the extent to which theories of plant stress responses developed in natural ecosystems translate to urban environments will be important for improving survival in urban areas. For example, in natural ecosystems, plant–plant facilitation can increase plant growth and survival (Grant et al. 2014), but this has not been investigated in urban environments.

Nurse plants facilitate other plants within their canopy and surroundings by modifying the microclimate and soil (Lu et al. 2018; Reid, Lamarque & Lortie 2010; Chapter 3). The canopy ameliorates abiotic weather conditions by trapping heat in colder alpine conditions (Molenda, Reid & Lortie 2012) or providing shade in hotter conditions (Randall et al. 2007). Canopies of nurse plants can also increase soil nutrients by increasing decomposing matter from leaf litter (Anthelme et al. 2012), reduce wind (le Roux & McGeoch 2010) and increase soil moisture content (Schöb, Butterfield & Pugnaire 2012). As a result, nurse plants play a vital role in plant community structure (Gavini, Ezcurra & Aizen 2019), abundance (Sklenář 2009) and increasing survival (Badano & Marquet 2009; Brooker et al. 2008; Reid, Lamarque & Lortie 2010; Sklenář 2009; Chapter 3).

The presence of a nurse plant may also be facilitatory through interactions occurring below ground. Mycorrhizal networks are well known to provide benefits to plants by linking resource acquisition among neighbouring plants (He, Critchley & Bledsoe 2003; Lanfranco et al. 2018; Montesinos-Navarro et al. 2017; Chapter 4), and the presence of a nurse plant that forms symbioses with mycorrhizal fungi may have significant benefits for target plants.

Categorising plant–plant or plant–microbe facilitation provides its own set of challenges, as a positive interaction at one stage or state may transform into a negative interaction at a later stage (Michalet and Callaway 2001, Miriti 2006, Alba et al. 2019). Whether the outcome of biotic interactions is facilitative or competitive is highly context dependant, affected by the identities of the species, growth stages, and abiotic variables (Mastre et al. 2009, Rayment et al. 2020, Armas & Pugnaire 2005).

The Stress Gradient hypothesis (SGH) provides a rationale for deciding the outcome of biotic interactions along an environmental gradient (Bertness and Callway 1994). The hypothesis states that facilitative interactions should be more prevalent in communities under higher abiotic stresses, with competition becoming more prevalent as abiotic stressors decrease and the community becomes more productive. Plant mycorrhizae interactions are a good example of this shift in facilitation (Maestre et al. 2009, Klanderud et al. 2017). When nutrients are scarce mycorrhizal fungi facilitate the uptake of additional nutrients not accessible by the plants; in turn, the plants provide the mycorrhizal fungi with carbohydrates (George et al. 1995, Miransari et al. 2011). However, as the nutrients become more widespread and directly accessible by the plants, the mycorrhizal association becomes less beneficial, and the relationship shifts from mutualism to parasitism. Johnson et al. (1997) proposed that mycorrhizal relationships occur along a continuum ranging from mutualism to parasitism, and the relationship strongly depends on environmental conditions. One area of interest for me is identifying the factors or triggers that switch the plant–plant or plant–microbe relationship between facilitative and parasitic/detrimental.

In this study, I investigated how plant–plant interactions vary with water availability. I was specifically interested in how the relationship between a potential nurse plant and target plant changes at different levels of water availability. If water stress negatively affects nurse plant facilitation, then the presence of a nurse plant in low-water environments may decrease the growth and survival of the target plant when compared to the absence of a nurse plant. Alternatively, nurse plants may facilitate the survival and growth of target plans under water stress by ameliorating direct sunlight or sharing resources via AMF.

Methods

The study took place in the spring and summer of 2017–2018 at the ERC at the University of Wollongong, Australia (34° 25′ 59″ S 150° 52′ 59″ E). Wollongong has an oceanic climate with humid subtropical influences. Rainfall is associated with the orographic lift caused by the escarpment; on average, Wollongong experiences 1300 mm of rain a year, with the wettest months being February and March.

Table 5: Weather over the experimental period measured at the Ecological Research Centre University of Wollongong.

Month	Average temperature	Days above 35C	Number of days with precipitation
Month 1	25.63	2	0
Month 2	24.04	2	3
Month 3	24.16	0	5
Month 4	28.26	5 (one week above 30 between the 18th and 24$^{th)}$	5
Month 5	27.26	1	4
Month 6	27.94	1	3

Green roof mesocosms were constructed on top of a large concrete slab simulating the roof of a building. All mesocosms were north–south orientated on the slab to ensure that each setup received a comparable amount of exposure to solar radiation.

Each mesocosm was constructed following specifications and procedures outlined in Aguiar, Robinson and French (2019). The frame of each mesocosm was built using lightweight treated pine, which was constructed into a rectangular box (0.5 × 0.6 × 0.3 m).

Experimental Design

Testing all the species pairs listed in the local green roof guides was beyond the scope of this project due to space limitations. Instead, I tested two pairs of plants; each pair had a potential nurse plant and a target plant. Both the plants were purchased from the same nursery. Two common forb species, *Dichondra repens* and *Viola hederacea* were used as target plants as they were quick to grow and have previously been shown to increase growth in the presence of a nurse plant (Aguiar et al. 2019; Chapter 3). I matched each with a nurse plant that was natively found in similar habitats, hardy, readily available and slightly larger, to ensure shading. Both the target and nurse plants have previously been shown to form mycorrhizal associations (Chapter 3: unpublished data). The groups were:

- Group A— *Lomandra longifolia* (nurse) and *D. repens* (target)
- Group B—Nurse: *Dianella caerulea* (nurse) and *V. hederacea* (target).

All plants selected are native to sandstone and shale cliff line and outcrop vegetation communities found around the city of Sydney NSW (Appendix B)

The two groups were subjected to three water treatments with five replicates per treatment (30 mesocosms per group). The mesocosms were set up in a factorial design on the concrete slab to investigate the effect of the presence of a nurse plant and three watering levels (25%, 50% and 100% of recommended watering volumes) on plant growth and survival across the two plant species. The substrate was a perlite mix (grade 3–4 mm with 20% Garden mix: coir, plant mulch, sand and compost). The growing substrate had a water holding capacity of 58%, with a dry bulk density of 0.69g/cm^{-3}. The substrate layer was approximately 15 -18 cm deep and was sterilised in an autoclave oven to ensure no residual soil microbial communities were present. A commercial mycorrhizal inoculant (MycoApply), purchased through an Australian supplier, was used. I decided to use commercial mycorrhizae over locally sourced mycorrhizae due to previous research showing both species having higher inoculation and biomass with the commercial mycorrhizal fungi (Chapter 4). MycoApply was purchased in powder form and had a particle size of 250 µm. The inoculant claimed to have a high density of propagules (220,000 propagules/kg) consisting of four members of the *Glomus* spp. The inoculant was added weekly to the mesocosms, based on the instructions from MycoApply (10 g for every 1000 mL of reverse osmosis water), for the first two months of the experiment to ensure that all mesocosms had the same type and amount of soil microorganisms.

The mesocosms were set up a month before the start of the experiment to allow the plants to acclimatise. All plants were watered via a drip system, which allowed me to control the water delivered to each mesocosm. Each mesocosm had a single variable trickle dripper (Pope 100mm trickle dripper) placed at a 10cm offset to the east from the middle of the mesocosm. The water treatment had three levels: 25%, 50% and 100% of current watering practices used on semi-intensive green roofs in Sydney, NSW (4 L/m^{-2} daily; Chapter 2). These values correspond with other studies investigating the effect of irrigation on a green roof (Schweitzer & Erell 2014, Gomes et al. 2016, Jim,2015, Mechelen et al. 2015: Appendix A). A slow-release fertiliser (Osmocote Exact Standard at 1/3 the s recommended dose 20g 3-4M: 7.1% NO_3-N, 8.9% NH_4-N, 9% P_2O_5 and 12% K_2O) was applied twice during the experiment, on Days 1 and 110, to ensure that each treatment received an equivalent amount of nutrients.

During the experimental period, Wollongong was experiencing a drought. The experiment was delayed by a month due to multiple heat events that led to plant death in multiple treatments during the acclimatisation period. On the 12-13th of September 2017, 5 days after translocating plants into the mesocosms, the ambient temperature increased from an average of 17C (10th of September) to 36C, resulting in a loss of 2/3 of the plants. The experiment was reset using backups, and unfortunately, the following week, we experienced another heat event (23rd of September), resulting in further plant loss. The sample size was reduced, and the 12.5% water treatment was dropped to accommodate this loss. The experiment began on the 16th of October and ran for 192 days (~6 months).

Canopy Growth

During the experiment, plant growth was measured monthly. Growth was measured non-destructively using a modified canopy intercept method outlined in Jonasson 1988. In brief, a grid was placed over each mesocosm, with each subdivision covering an area of 5 cm^2. The number of intercepts the plant crossed was counted to create a percentage surface area estimate over the entire mesocosm.

Biomass

At the end of the experiment, target plants were harvested, and their roots were washed over a sieve. Care was taken to ensure that any fine roots were collected and kept with the sample. Above- and below-ground biomass were weighed separately. For both species, the runners, stems, leaves and flowers were considered above-ground biomass, and the roots were regarded as below-ground biomass. Plant material was placed in a drying oven at 75 °C; a subsample was weighed daily until a constant weight was achieved (72 hours).

Mycorrhizae

At the end of the experiment, a small root sample was set aside from each target plant. Mycorrhizal analysis was conducted using the root-staining method described by McGonigle et al. (1990). The amount of root collected was kept constant so that all treatments would lose the same amount of weight (5% of total root collected). Mycorrhizal colonisation was estimated using the point intercept method, which collated counts of both hyphae and vesicles (no arbuscules or hyphal coils were recorded; Newman 1966).

Statistical Analysis

Each target species was analysed separately using SPSS (IBM SPSS). Biomass and mycorrhizal colonisation were compared among watering and shade treatments using a two-factor ANOVA after testing for normality (Shapiro–Wilk test) and homogeneity of variance. Significant interactions were further investigated using multiple-comparison tests (Tukey's HSD). To investigate the type of benefit from a potential nurse plant (shade vs mycorrhizal colonisation), I also ran the biomass models using mycorrhizal colonisation as a covariate. I reasoned that if colonisation levels were higher in plants with the greatest biomass and the effects of shade were non-significant, nurse plants were providing below-ground benefits. Due to the growth data being a measurement over time, I compared the different treatments by analysing the area under the curve in a two-factor ANOVA and presenting the time series graphs for visual assessment.

Results

Biomass

For *D. repens*, both watering level and the presence of a nurse plant affected above- and below-ground biomass (Table 6). Increasing watering significantly increased biomass, with the highest biomass exhibited in the 100% watering treatment (Figure 18a & b), which was ~300% greater than in the 25% watering treatment for both the above- and below-ground biomass. The presence of a nurse plant also significantly increased biomass by 55% (Figure 18c & d) for the above-ground biomass and by 40% for the below-ground biomass when compared to the control. However, the effect of nurse plants on biomass did not change at different watering levels (Table 6).

Table 6: Analysis of variance results investigating the effect of watering and the presence of a nurse plant on biomass and mycorrhizal colonisation for *Viola hederacea* and *Dichondra* repens. Significant *p*-values at 0.05 confidence are indicated with *.

	D. repens			***V. hederacea***		
	df	*F Value*	*p*	*df*	*F Value*	*p*
Canopy cover (area under the curve)						
Watering	2	210.486	0.001*	2	118.019	0.001*
Nurse plant	1	29.926	0.001*	1	35.041	0.001*
Watering × Nurse plant	2	4.486	0.022*	2	3.182	0.059
Residuals	24					
Above-ground biomass						
Watering	2	23.186	0.001*	2	14.292	0.001*
Nurse plant	1	4.608	0.042*	1	2.287	0.143
Watering × Nurse plant	2	0.485	0.621	2	3.286	0.054
Residuals	24					
Below-ground biomass						
Watering	2	31.235	0.001*	2	5.004	0.015*
Nurse plant	1	8.108	0.008*	1	20.202	0.001*
Watering × Nurse plant	2	1.349	0.278	2	0.095	0.090
Residuals	24					
Mycorrhizal colonisation						
Watering	2	5.663	0.009*	2	12.571	0.001*

Nurse plant	1	55.736	0.001*	1	29.345	0.001*
Watering × Nurse plant	2	1.310	0.288	2	3.461	0.047*
Residuals	24					
Mycorrhizal colonisation included as covariate in the above-ground biomass model						
Watering	2	19.751	0.001*	2	15.1122	0.001*
Nurse plant	1	3.925	0.063	1	2.4185	0.137
Mycorrhizae	1	0.102	0.752	1	6.3789	0.021
Watering × Nurse plant	2	0.418	0.664	2	1.4079	0.270
Watering × Mycorrhizae	2	0.066	0.935	2	1.1250	0.346
Nurse plant x Mycorrhizae	1	1.432	0.246	2	0.4573	0.507
Watering × Nurse plant × Mycorrhizae	2	0.383	0.686	2	1.2120	0.320
Residuals	18					
Mycorrhizal colonisation included as covariate in below-ground biomass model						
Watering	2	28.986	0.001*	2	4.008	0.036*
Nurse plant	1	7.524	0.013	1	16.183	0.001*
Mycorrhizae	1	0.236	0.632	1	0.060	0.809
Watering × Nurse plant	2	1.417	0.268	2	0.055	0.946
Watering × Mycorrhizae	2	0.545	0.588	2	0.188	0.830
Nurse plant × Mycorrhizae	1	1.593	0.222	1	0.159	0.694
Watering × Nurse plant × Mycorrhizae	2	0.509	0.609	2	0.335	0.719
Residuals	18			18		

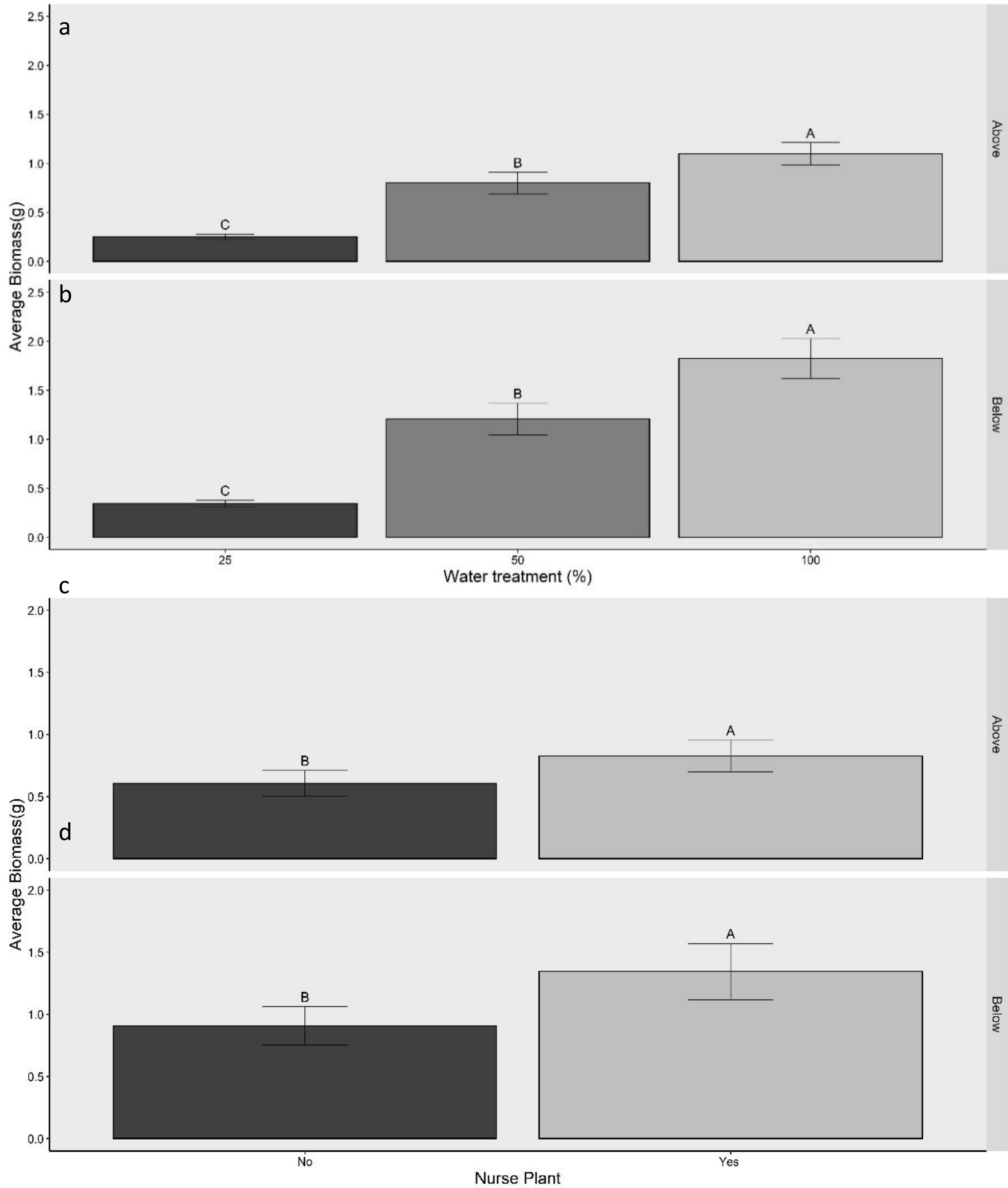

Figure 18: The effect of watering (a & b) and the presence of a nurse plant (c & d) on above-ground, below-ground and total biomass for *Dichondra repens*. Error bars are means M ± *SD*. Different letters signify a statistical difference.

For *V. hederacea*, interactions between factors were more complex. For above-ground biomass, only the watering treatment had a significant positive effect on biomass (Table **6**; Figure 19c & d), with the 100% watering treatment having three times the biomass of the 25% watering treatment. Below ground, both nurse plant presence and watering level affected biomass, with the nurse plant increasing biomass by 37% on average and watering increasing biomass by up to 31% (Figure 19a & b).

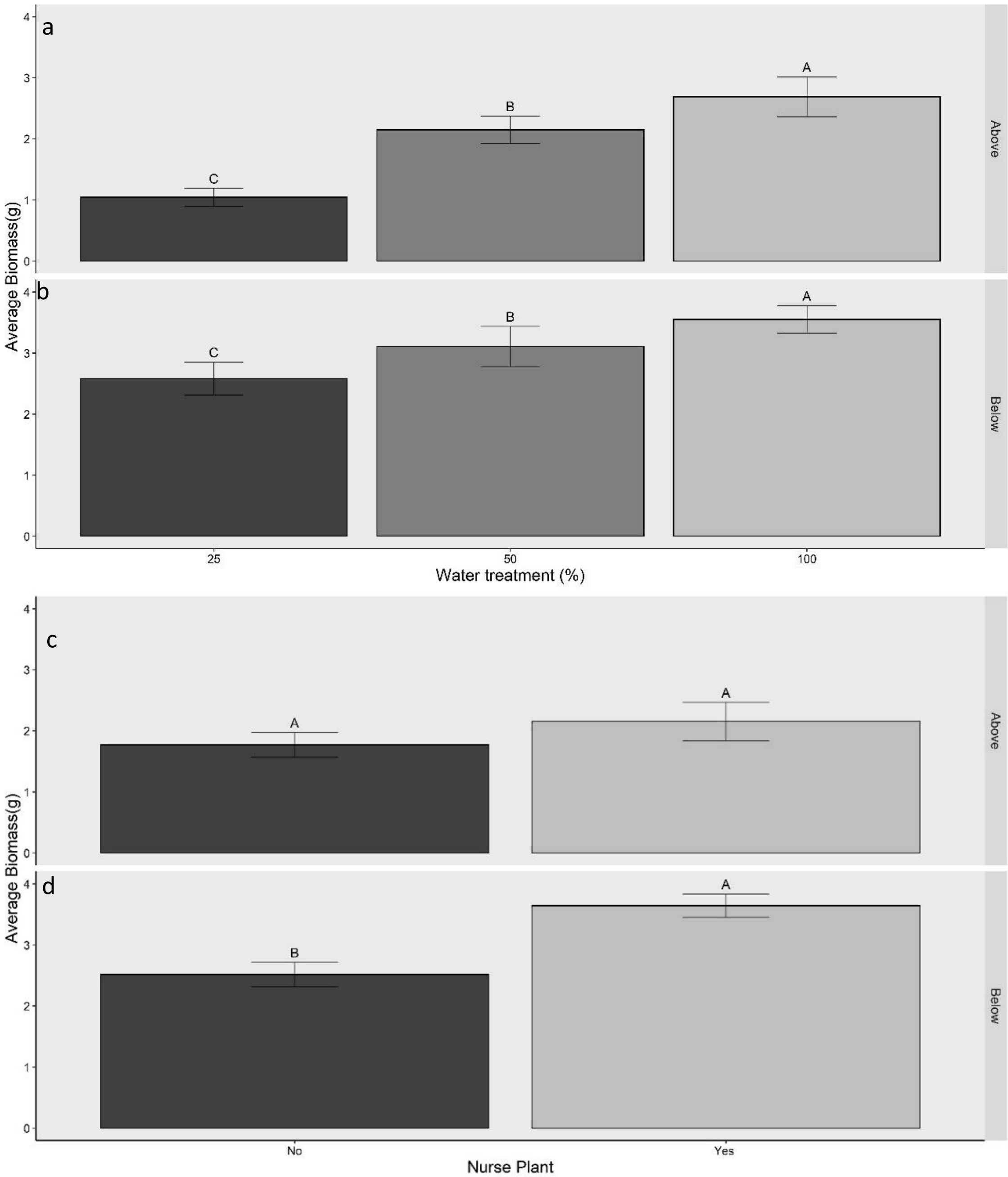

Figure 19: The effect of watering (a & b) and the presence of a nurse plant (c & d) on above- and below-ground biomass for *Viola hederacea*. Error bars are means ± *SD*. Different letters signify a statistical difference.

Changes in Canopy Cover over Time

The effect of nurse plants on canopy cover did not change at different watering levels. While we did not detect an interaction between watering levels and nurse plant presence using biomass as a measure of plant growth, we found that the nurse plant's effect on the canopy cover of *D. repens* was influenced by the watering level (Table **6**; Figure 20). Under low levels of watering, there was no positive benefit of the nurse plant (Figure 20);

however, at 50% and 100% watering levels, the addition of a nurse plant increased the plant canopy by 30% and 20%, respectively, which was maintained throughout the experiment (Figure 20).

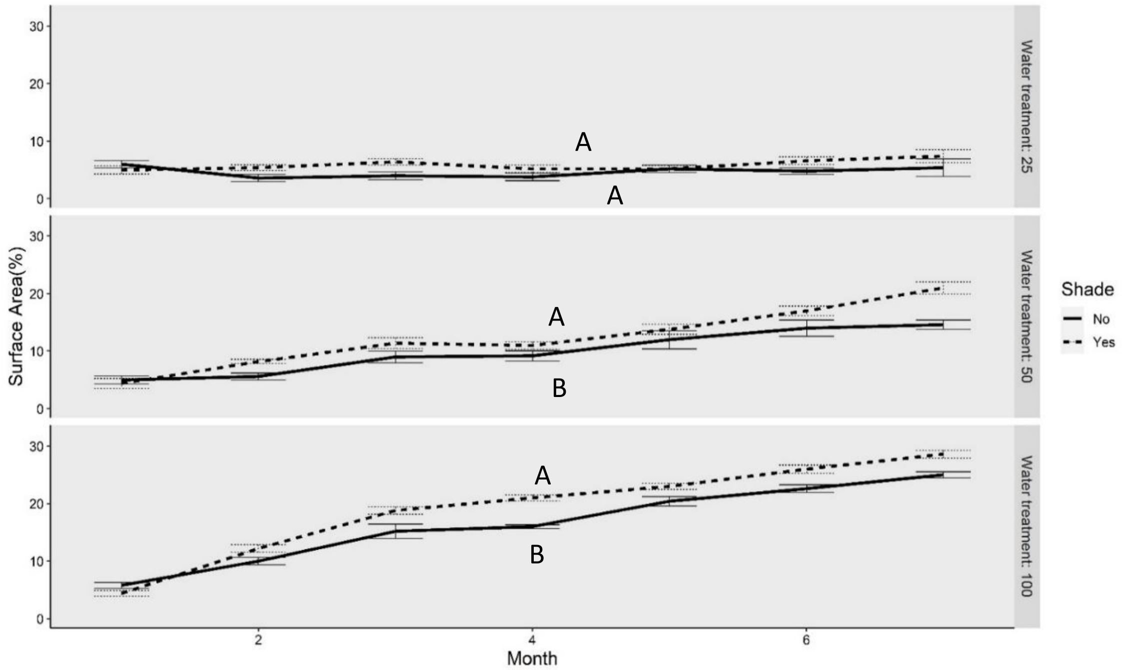

Figure 20: Canopy cover as represented by % surface area of the mesocosm (0.25 m^2) that *Dichondra repens* covered over seven months, with and without a nurse plant, across three different watering treatments. Error bars are means ± *SD*. Different letters signify statistical significance; *n* = 5.

For *V. hederacea,* both nurse plant presence and watering levels influenced plant canopy cover (Table 6; Figure 21); however, there was no significant interaction between the two factors. The addition of a nurse plant improved plant canopy cover by 10%–20% across all three treatments (Figure 21). In the higher watering levels, there was a marked difference in canopy cover between the nurse plant treatment and the no nurse plant treatment, with the 50% watering treatment exhibiting a ~200% increase in canopy cover and the 100% watering treatment exhibiting a ~150% increase after 6 months with the addition of a nurse plant. This increase in canopy cover coincided with the start of the summer season.

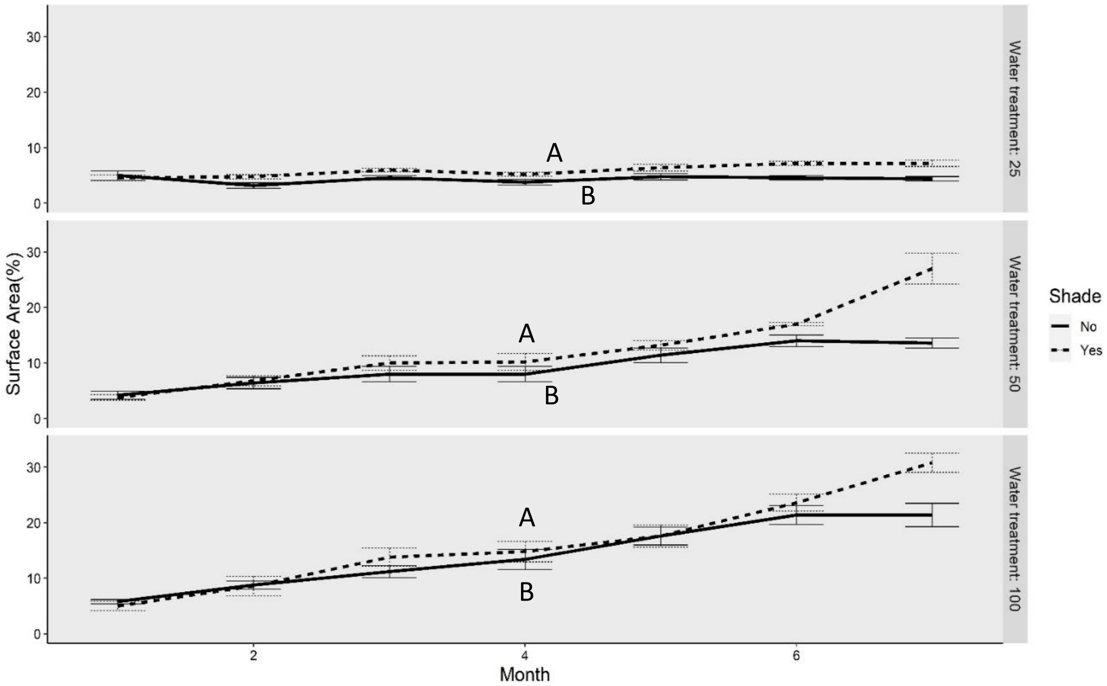

Figure 21: Canopy cover as represented by % surface area of the mesocosm (0.25 m^2) that *Viola hederacea* covered over seven months, with and without a nurse plant, across three different watering treatments. Error bars are means ± *SD*. Different letters signify statistical significance; *n* = 5.

Mycorrhizae

For *D. repens,* mycorrhizal colonisation was influenced by nurse plant presence and watering level; however, there was no interaction between these two factors (Table 6). Treatments with a nurse plant had a 30% higher colonisation rate than when there was no nurse plant (Figure 22b). The watering level did not influence this interaction between nurse plant presence and colonisation rate, but the lowest watering treatment had significantly higher colonisation compared to other watering treatments (Table 6; Figure 22a).

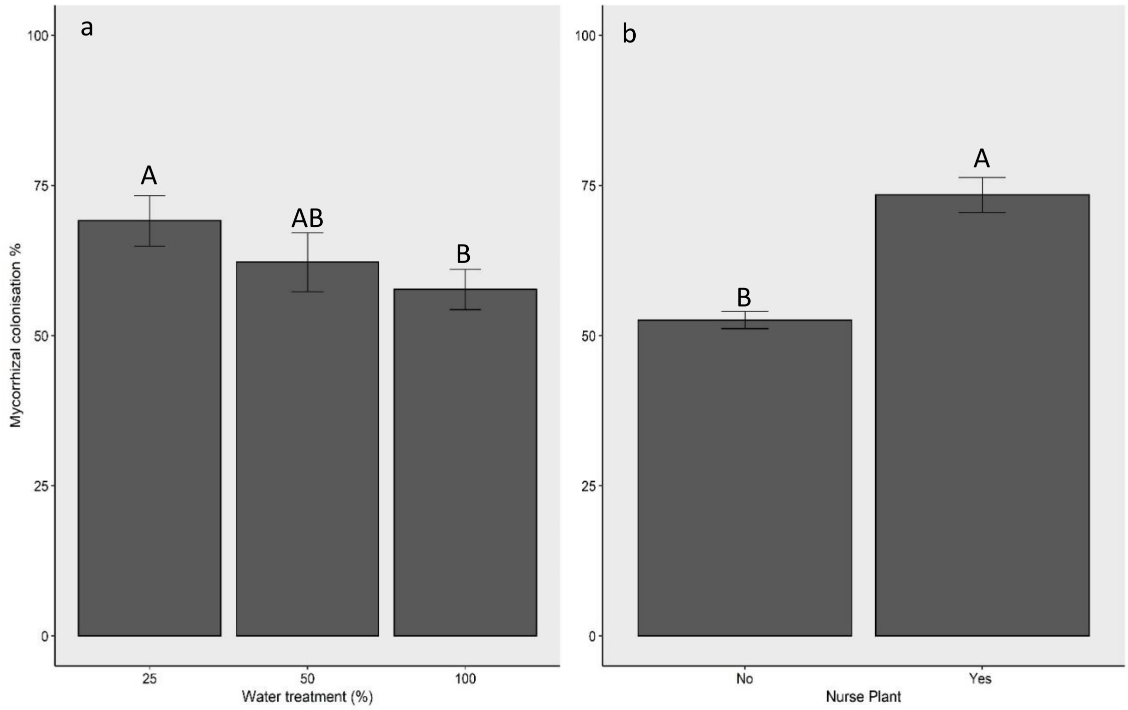

Figure 22: The effect of (a) watering and (b) the presence of a nurse plant on the percentage of mycorrhizal colonisation for *Dichondra repens*. Error bars are ±*SD*. Different letters signify a statistical difference.

For *V. hederacea*, the effect of nurse plant presence on mycorrhizal colonisation was influenced by watering levels (Figure 23a), with lower watering levels having higher mycorrhizal rates (Table 6; Figure 23b). At 25% and 50% watering, the addition of a nurse plant increased mycorrhizal colonisation by 31% and 19%, respectively, while at 100% watering, the difference was not significant (Figure 23c).

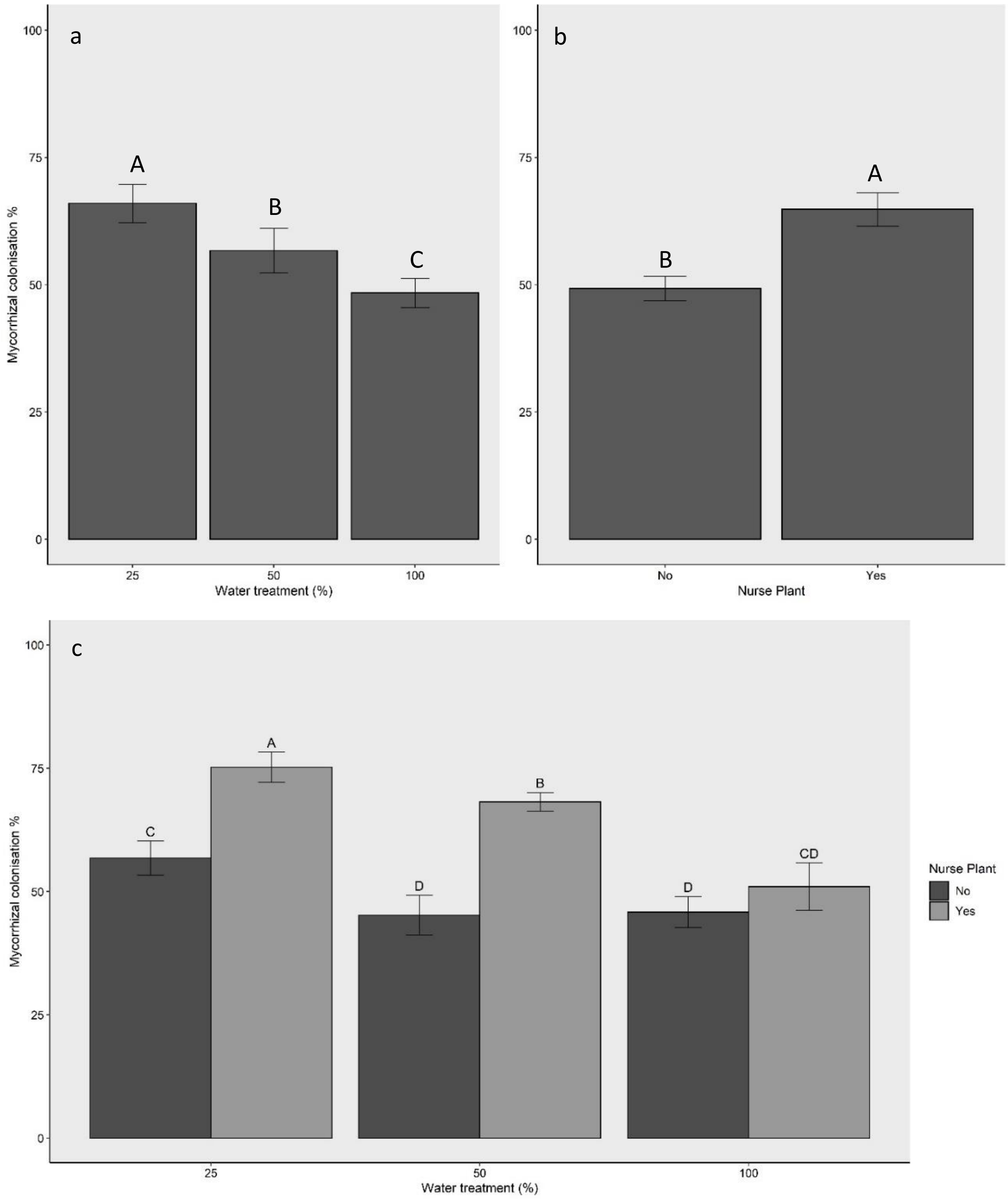

Figure 23: The effect of (a) watering, (b) the presence of a nurse plant and (c) the combination of the two on mycorrhizal colonisation for *Viola hederacea*. Error bars ±*SD*. Different letters signify a statistical difference; *n* = 5.

Mycorrhizal Colonisation as a Covariate in Below-Ground Biomass

Interestingly, the addition of mycorrhizal fungi as a covariate in the biomass model affected the results for both species. In the case of *D. repens,* the effect of the nurse plant was no longer significant, suggesting that the effect of the nurse plant was associated with below-ground effects. However, it did not influence the effect of nurse plant presence on below-ground biomass for *V. hederacea* (Table 6). Furthermore, there were no significant interactions in any of the biomass/mycorrhizae models.

Discussion

This study investigated the effect of biotic interactions with a nurse plant on biomass and mycorrhizal colonisation of two target plants (*D. repens* and *V. hederacea)* across an abiotic gradient (water). I found that both the addition of a nurse plant and increased watering levels increased biomass for both species. However, there was no evidence of a significant interaction between shading and water levels, suggesting that the facilitatory effect was not improved when conditions became more stressful.

Contrary to previous studies in the field, I found no significant difference in facilitation by a nurse plant across an abiotic gradient. Previous studies have found that positive interactions increase as abiotic stressors increase and competition is more prevalent in low-stress environments (Callaway et al. 2002; Michalet et al. 2015). By contrast, across our environmental gradient (water), I did not observe a switch between facilitation and competition between the target plant and the nurse plant. Across all our water treatments, the addition of a nurse plant increased canopy cover and biomass. Interestingly, *V. hederacea* displayed a trend towards increased above-ground biomass when paired with a nurse plant under the high-watering treatment only, but increased below-ground biomass with a nurse plant under all water treatments. Additionally, *V. hederacea* had significantly higher mycorrhizal colonisation under the low-water treatment, suggesting that nurse plant facilitation may have been assisted by increased mycorrhizal facilitation in water-stress conditions.

The importance of mycorrhizal facilitation is well known for plants (e.g., Fulthorpe et al. 2018; Parepa, Schaffner & Bossdorf 2013). Similar to our study, Xie et al. (2018) looked at the synergistic effects of microbes on biomass in green roof environments (Xie et al. 2018). They found that the inoculation of microbial symbionts increased plant biomass and chlorophyll fluorescence for eight species of plants native to Finland (*Antennaria dioica, Campanula rotundifolia, Fragaria vesca, Geranium sanguineum, Lotus corniculatus, Thymus serpyllum, Trifolium repens,* and *Viola tricolor*). I found mycorrhizal colonisation to be highest in the lowest water treatment for both species I tested, with the colonisation rate dropping as water availability increased. Our results agree with the literature. Environmental gradients have been shown to affect mycorrhizal associations with higher levels of mycorrhizal association in stressful environments (Schroder 2019). In environments where plants have access to plentiful resources, reducing the number of associations with mycorrhizal fungi might be a better strategy since the benefits do not outweigh the costs (shared carbohydrates). However, under water stress, mycorrhizal fungi can access water from extensive hyphae, extracting water not accessible to plants (Khalvati et al. 2005). The mechanisms driving the improved colonisation of plants in the presence of a nurse plant are not well understood. Sortibrán, Verdú & Valiente-Banuet (2019) investigated the colonisation of the nurse plant rather than the target plant and found that the mycorrhizal colonisation in the roots of the nurse plant (*Mimosa luisana*) increased with the number of target plant species around the nurse plant. Nurse plants may facilitate a higher diversity of mycorrhizal fungi, leading to higher colonisation levels in target species, or they may form mycorrhizal networks that facilitate the two species (Montesinos-Navarro, Valiente-Banuet & Verdú 2019). Experiments using isotope tracking (^{15}N) and heavy water have shown that plants can exchange both nutrients and water to neighbouring plants using the mycorrhizal network (Walder et al. 2012).

The interaction between watering level and nurse plant presence influenced mycorrhizal colonisation only in *V. hederacea*. For *D. repens,* the presence of the nurse plant was always strongly positive for plant biomass irrespective of water availability; however, for *V. hederacea*, the nurse plants did not improve biomass when water availability was high. Interestingly, the results were switched for canopy growth, with both species exhibiting improved canopy cover at higher watering levels with nurse plants, but less obvious advantages of nurse plants at lower water levels. The strength of the relationship varied among species under improved resources.

Water levels played a key role in increasing plant canopy cover and biomass. Both of the species selected for the experiment, *D. repens* and *V. hederacea*, managed to persist in our 25% watering treatment; however, they did not grow or increase biomass. The effect of drought stress on plants in a green roof environment is well documented in the literature (Brown & Lundholm 2015; Dunnett & Nolan 2004). As plants are subjected to lower water levels, plants undergo physiological changes, such as the closure of stomata to limit water loss through stomatal conductance and transpiration (Radin & Ackerson 1981). Similar to our results, Richard et al. (2011) investigated the effects of drought on 12 different Mediterranean plants. They found that there was a lower limit for water availability at which a plant could survive but not grow or reproduce, and this limit varied between species. Overall, they found that shrub species were more tolerant to lower water levels. Recently, attention has been given to improving the water retention capacity of substrates while maintaining the shallow substrate depths on green roofs via the inclusion of substrate amendments such as seaweed and biochar (Cao et al. 2014; Farrell, Ang & Rayner 2013; Vijayaraghavan & Joshi 2015). Our results highlight that the use of nurse plants is another strategy that can be used in conjunction with these substrate additives.

I found that the addition of a nurse plant for both species increased biomass and canopy cover over all three watering treatments. The results suggest that the facilitation has elements of both ameliorating light stress (as found in Chapter 3) and below-ground processes, which act together to enhance the target plants. Nurse plants help facilitate target plants by acting as a physical barrier, limiting solar radiation beneath their canopy and thus reducing photo-oxidative stress on the plants in the shade (Valiente-Banuet & Ezcurra 1991); they also limit the wind, reducing evapotranspiration (López et al. 2007). At a practical level, the use of nurse plants can be a strategy to minimise the watering required on a green roof. In our experiment, the increase in biomass and canopy cover achieved by the target plant with a nurse plant in the 50% watering treatment was equivalent to a target plant without a nurse plant in the 100% watering treatment.

A limitation to our experimental design was that we did not measure bare substrate between the treatments while we did try to control for this by selecting similar size nurse plants, the quantity of bare substrate could have also influenced growth. Further work is also needed to investigate how mycorrhizal networks between nurse and target plants can be used to enhance survival on green roofs. The experiment conducted was only able to investigate this relationship using one type of substrate and a set of nurse and target plants. Future studies should investigate if similar results are observed across different substrates used on green roofs. Another limitation is the use of mesocosms vs *in situ* trials on a green roof. Green roofs might have a large variety of niches due to changes in abiotic factors such as water availability, shading and microclimatic factors that might further influence mycorrhizal associations.

Our results showed evidence of the value of below-ground processes in the nurse plant – target plant relationship. The influence of mycorrhizal colonisation varied between the two pairs but appeared independent of the effect of water availability: the significance of this main effect was unaffected by the mycorrhizal colonisation level, and there were no significant interaction terms. In contrast, mycorrhizal colonisation reduced the significance of the nurse plant main effect in our models, suggesting that, to some extent, the effect of the nurse plant was influencing mycorrhizal colonisation. Whether nurse plants create substrate environments conducive to fungal growth or whether networks are being developed to link different plant species requires further investigation, but this shows promise as a tool in green roof design.

Conclusion

The use of nurse plants is a practical option for improving the diversity of plant selection on a green roof. Not only do the nurse plants enable the use of species typically stressed by roof garden conditions, but they also contribute to the biodiversity and aesthetic benefits provided by the green roof. However, care should be taken when selecting nurse plants. While nurse plants provide some amelioration to the abiotic factors, they also provide additional competition into an ecosystem that has constraints on space and nutrients, which may become more evident as plants grow. With careful selection of nurse plants that are best at improving abiotic conditions for a target plant, we may be able to increase the beneficial effect of nurse plants as well as reduce some of the competitive effects.

Chapter 6: Discussion—The Use of Facilitation in Improving Plant Growth on Green Roofs

Contributions

Axton Aguiar collated and reviewed the research, summarised the thesis research findings and wrote the manuscript.

Kris French reviewed the manuscript.

Sharon A Robinson reviewed the manuscript.

Publication

Not written for publication.

Research Summary

The thesis demonstrates the use of biotic interactions (plant–plant and plant–microbe) as effective mechanisms to improve plant growth and survival on a green roof. Plant growth and survival are especially important, as most of the benefits of a green roof are derived from the vegetation on the roof. Improper plant selection can lead to additional costs associated with plant upkeep (e.g., water and fertiliser) and maintenance (e.g., trimming and plant replacement).

Historically, the focus has been on identifying and selecting plants that are physiologically adapted to survive harsh environments by having drought avoidance or tolerance traits. However, recently, more focus has been given to ameliorating some of the stressors present on green roofs by modifying environmental conditions to mimic plants' natural ecosystems (e.g., the habitat-template approach; Lundholm 2006; Lundholm & Walker 2018).

This thesis aimed to further develop the habitat-template approach by investigating whether the biotic facilitation observed in natural environments can be used to enhance plant growth and survival on green roofs. Facilitative interactions are some of the driving factors that help shape ecological communities (Mcintire & Fajardo 2014). Nurse plants (plant–plant facilitation) are prevalent in harsh abiotic communities such as deserts and arctic tundra. In these environments, nurse plants help facilitate the establishment and growth of seedlings and surrounding plants by ameliorating the effects of environmental factors, such as heat and wind, in the areas under their canopies.

I investigated four questions:

A. Do green roofs in Sydney deliver on broad social, environmental or ecological benefits? (Chapter 2)

B. Does vegetated shading provided by nurse plants improve plant survival on a green roof? (Chapter 3)

C. What is the effect of green roof substrates on plant mycorrhizal interactions? (Chapter 4)

D. Does plant–plant facilitation or mycorrhizal colonisation change over an abiotic gradient? (Chapter 5)

Research Findings

I surveyed 29 green roofs in Sydney to determine the most common physical attributes of green roofs, such as size, plant and substrate used. Additionally, I used surveyable attributes of the green roofs to assess if it was possible to categorise each roof based on its function. I assessed each roof on its ability to deliver social, environmental and ecological benefits. I found that green roofs in Sydney scored highest in social benefits, followed by environmental benefits, and scored the lowest in ecological benefits. This is contrary to what I expected, as most of the roofs surveyed had environmental benefits listed as one of their main aims. The findings highlight a shift in focus or attitude regarding green roofs. Traditionally, green roofs have been seen as a 'green' technology to help ameliorate some of the environmental effects of urbanisation. However, for most commercial green roofs, I found that there was increased interest in how green roofs can improve the social aspects of a workplace, such as building a sense of community, improving concentration and productivity, and reducing stress. In the commercial green roofs, I found that the design and plant selection were all focused on enhancing the social aspects of green roofs, sometimes at the cost of environmental/ecological benefits.

The survey highlighted the importance of plant selection on a green roof. Water and fertiliser usage and maintenance are closely tied to the type of plants used on a roof. Green roofs are harsh environments for plants, and this has led green roof companies to use a small selection of plants with which they are familiar. The observational data recorded during the survey, specifically of the physical attributes of green roofs, were used to construct mesocosms. These mesocosms were used as the primary basis for the experimental chapters—Chapters 3 and 5—on facilitative interactions to improve plant survival and growth on a green roof.

Plant–plant facilitation, specifically the effect of vegetative shading, was investigated using a factorially designed experiment (Chapter 3). Three of the four species pairs exhibited a positive benefit to shading. Another finding was that the positive benefit of nurse plants was not solely through vegetative shading but also below-ground interactions. This below-ground facilitation was exhibited in the UN treatment, which consisted of a nurse plant that had been trimmed with a target plant. I was not expecting a positive interaction from this treatment, which was initially incorporated into the experiment to account for the competitive interaction between the nurse plant and target (i.e., shaded) plant. However, competition was not observed with the UN treatment, having a higher biomass when compared to the control. I suggested two possibilities for the below-ground facilitation: 1) it assisted in the trapping of additional moisture in the substrate, and the watering level did not put the plants in competition for water, and 2) the presence of nurse plant roots mediated mycorrhizal facilitation.

Mycorrhizae were then investigated as one of the possible explanations for the below-ground facilitation (Chapter 4). Mycorrhizal facilitative interactions have been well established in the literature (Van Der Heijden & Horton 2009). My goal was to establish whether the novel substrates found on green roofs support mycorrhizal colonisation and whether this improved plant growth and survival. I found that irrespective of substrate type, the addition of mycorrhizae increased biomass compared to the control. The facilitative effect of the mycorrhizae depended on substrate type: mycorrhizae colonisation, its facilitative effect was lower in scoria and terracotta than in potting mix and perlite.

This experiment also allowed me to evaluate the use of commercial mycorrhizal inoculation versus local mycorrhizal spores collected from native communities associated with the plants. For one of the two species tested, the commercial inoculate resulted in improved colonisation. This was unexpected, as it would be predicted that native mycorrhizae would develop better symbiotic relationships with native plants. One possible reason for the finding is that commercially sourced mycorrhizal fungi are a mix of mycorrhizal species that have a broad ability to colonise a range of species and environments, while native mycorrhizal spores may find novel environments such as scoria and terracotta substrates more difficult to survive in.

Finally, I investigated whether facilitative interactions varied with increasing abiotic (water) stress (Chapter 5). I found that the addition of a nurse plant and increased watering levels increased biomass; however, there was no significant interaction between the two. I did find, nurse plants facilitated that canopy cover, and this facilitation was increased at higher watering levels. I also found that mycorrhizal colonisation was significantly higher in the low watering treatments, suggesting that the nurse plant facilitation may have been assisted by increased mycorrhizal facilitation in water-stress conditions.

Significance of Findings

My findings significantly highlight how facilitatory interactions can be a useful tool in aiding the growth of plants on a green roof. To my knowledge, the experiments conducted in this thesis are one of the first experiments investigating facilitation and competition using Australian natives in a green roof setting. While a few other studies have investigated the effects of plant-plant interactions on a green roof (Vasl et al. 2017, Heim and Lundholm 2014), aside from Heim and Lundholm 2014, my study is the only other study looking at vegetative shading and isolating the mechanisms of facilitation. Vegetative shading improves plant survival on a green roof, as the nurse plant ameliorates the abiotic conditions present on the roof. While this effect can also be achieved by artificial shading, the nurse plants also contribute to the diversity and structure of the green roof. This, in turn, improves the benefits obtained from the green roof (Lundholm 2015; Nagase & Dunnett 2012). It is a strategy that can be implemented into pre-existing roofs with no modification to the roof itself. However, as highlighted in Chapter 3, vegetated shading is not always beneficial, and care should be taken when selecting nurse plants. Plant selection should be based on pairing fitness rather strictly on aesthetics. More work is needed to identify a range of good native nurse plants.

I have also demonstrated that mycorrhizal inoculation provides another easy-to-implement mechanism for improving plant survival. None of the green roofs tested in our survey used mycorrhizae inoculation. In my experiments (Chapters 4 and 5), the addition of mycorrhizae resulted in an increase in biomass when compared to the control. My results agree with previous green roof research investigating the effects of mycorrhizae on plant biomass (Schroder et al. 2019). While I have specifically focused on the effect of mycorrhizae in improving plant growth, they also deliver additional benefits (John et al. 2017). Incorporating mycorrhizal fungi can also improve nutrient use and leachate quality by reducing the need for additional nutrient inputs (i.e., fertiliser) (John et al. 2017; Richardson et al. 2009).

There are various mechanisms by which mycorrhizae can be added to green roofs (Molineux et al. 2015; Sutton 2008; Young et al. 2015). In my experiment, I decided to isolate spores from field-collected soil and add them to the substrate. Sutton (2008) simplifies the process by directly adding the field-collected soil to the green roof without isolating the spores. Commercial inoculum sources provide an easy delivery mechanism for adding mycorrhizae to green roofs (Young et al. 2015; Chapter 4).

Limitations and Future Research

Future research aiming to improve vegetative shading on a green roof should investigate the pairing fitness of native plants. A limitation of my study was I was only able to test a few pairs of plant species (four pairs in chapter 3 and two in chapter 5). One significant hurdle in selecting plant species is the various combination of plants to be tested. This can be reduced by using a habitat-template approach (Lundholm 2006), wherein naturally co-occurring plants are investigated for their facilitative benefits in green roof environments. Similarly, substrate type and species association have been shown to significantly affect mycorrhizal association (John et al. 2017, Xie et al. 2018). In our study, we were only able to evaluate a subset of native Australian plants and substrates used on green roofs. Substrate composition and mixtures are constantly evolving, with green roof companies developing their own proprietary mixes and additives to green roofs. Further work will be required to evaluate if mycorrhizal associations evaluated in one type of substrate can be generalised for other similar substrates.

While the use of mesocosms is beneficial for experimental setups as the sample size and abiotic conditions can be varied. Further research is required to determine if the results I obtained using irrigated green roof mesocosms are applicable across all green roof types. Focus should also be given to roof gardens (e.g., planter boxes), as they represent some of the biggest growth areas for the industry. Planter boxes are perceived as a low-risk option to having green roofs, as they are not permanent and do not require modification of the building structure. They are also versatile and can be combined to fit various locations. An investigation should be undertaken to determine whether there are any trade-offs or additional benefits of having a planter box design rather than an in-built green roof.

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Appendix A

Survey form used for the green roofs survey across Sydney.

Green roof Survey

Site Number:

Date:

Elevation:

Location:

Description:

Green roof location.

☐ Residential ☐ Commercial ☐ Public

Green roof type.

☐ Intensive ☐ Semi-Intensive ☐ Extensive

Aims

Environmental	☐ Yes | ☐ No	Note:
Social	☐ Yes | ☐ No	
Economical	☐ Yes | ☐ No	

Substrate type

☐ Scoria ☐ Soil ☐ Perlite ☐ Commercial ☐ Other

Note:

Irrigation type

☐ Subsurface drip ☐ Above surface drip ☐ Mist ☐ no irrigation ☐ Other

Note:

Watering frequency

☐ 3-5 times per day ☐ 1-2 times per day ☐ 1-2 times a week ☐ Other

Vegetation

☐ Native ☐ Exotic

List:

1 # Appendix B

Scientific name	Common Name	Family	Type	Description
Lomandra longifolia	Spiny-head Mat-rush or Basket Grass	Asparagaceae	Grass-like	*L. longifolia* is a perennial, rhizomatous herb, that is native to most states in Australia with the exception of Northern territory (NT) and Western Australia (WA). It is frost heat and drought tolerant and is found in a variety of habitats ranging from rocky coastal cliffs, tall open forests and wet swamp/ riparian habitats.
Callistemon citrinus	Crimson bottlebrush	Myrtaceae	Shrub	*C. citrinus* is a small shrub native to New South Wales (NSW), Queensland (QLD) and Victoria (VIC). In its natural habitat it is commonly found in swamps and riparian habitat. It is widely cultivated both in Australia and overseas
Dianella caerulea	Blue flax-lily	Asphodelaceae	Grass-like	*D. caerulea* is a perennial, rhizomatous herb commonly found across the eastern states of Australia and Tasmania (TAS). It is

				commonly found in sclerophyll, woodlands, open forests and mallee- scrublands.
Correa alba	White correa	Rutaceae	Shrub	*C. alba* is a small shrub native to NSW, SA and TAS. It is a hardy species normally found on a variety of coastal habitats ranging from dry malee scrubs to coastal dunes and sea cliffs.
Dichondra repens	Kidney weed	Convolvulaceae	Herb	D. repens is a prostrate herbaceous plant native to eastern Australia and Tasmania. It is widespread and is commonly found as a groundcover in forests, woodland and grassland habitats.
Grevillea lanigera "Mt tamboretha"	Woolly grevillea	Proteaceae	Prostrate shrub	G. lanigera is a small shrub found in NSW, Australian Capitial Territory (ACT) and Victoria. It inhabits a wide range of habitats and is usually found eucalyptius woodlands, coastal scrubs and heaths.

Carpobrotus glaucescens	Pigface	*Aizoaceae*	Succulent	*C. glaucescens* is a prostrate creeping succulent native to the east coast of Australia. It is naturally found growing on coastal cliffs and sand dunes.
Viola hederacea	Australian violet	*Violaceae*	Herb	*V. hederacea* is a short perennial herb commonly found on the east coast of Australia. In its native habitat it is normally found as an ground cover in open forests and coastal scrublands.