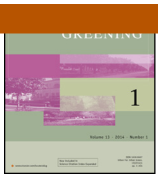
Urban Forestry & Urban Greening 13 (2014) 507–516

Urban Forestry & Urban Greening

journal homepage: www.elsevier.com/locate/ufug



Importance of different components of green roof substrate on plant growth and physiological performance

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ARTICLE INFO

Keywords:
Evapotranspiration
Optimisation
Polyacrylamide gel
Service provision
Substrate components
Water holding capacity

ABSTRACT

Green roof substrate is arguably the most important element of a green roof, providing water, nutrients and physical support to plants. Despite this there has been a lack of research into the role that different substrate components have on green roof plant growth and physiological performance.

To address this, we assessed the importance of three green roof substrate components (organic matter type, brick particle size and water absorbent additive) for plant growth and plant physiological performance. *Lolium perenne* (Ryegrass) was grown in eight substrates in a controlled greenhouse environment with a factorial design in composition of (i) small or large brick, (ii) conifer bark or green waste compost organic matter, and (iii) presence/absence of polyacrylamide water absorbent gel ('SwellGel^{TM}').

We found that large brick substrates had a lower water holding capacity than small brick (−35%), which led to decreased shoot growth (−17%) and increased root:shoot ratio (+16%). Green waste compost increased shoot and root growth (+32% and +13%) shoot nitrogen concentration and chlorophyll content (20% and 57%), and decreased root:shoot ratio (−15%) compared to bark. The addition of swell gel increased substrate water holding capacity (+24%), which increased shoot growth (+8%). Total evapotranspiration (a proxy for potential cooling) was increased by greater shoot biomass and substrate water holding capacity. Overall, this study provides one of the first quantitative assessments of the relative importance of commonly used green roof substrate components. It is clear that substrate composition should be considered carefully when designing green roofs, and substrate composition can be tailored for green roof service provision.

Introduction

Green roofs can have significant beneficial impacts in urban areas including storm water attenuation, urban heat island reduction, passive individual building cooling and provision of urban green space for recreational and aesthetic use (Oberndorfer et al., 2007). Due to these environmental benefits, the green roof industry has experienced a rapid expansion in the last twenty years and green roofs are now a common feature in most western urban areas (Oberndorfer et al., 2007). The amount of empirical green roof research conducted in the last ten years has also expanded, however many aspects of green roof technology and design have still not been fully investigated or optimised, in particular green roof substrate which is arguably the most important component of a green roof. The substrate usually has to perform the role of an artificial soil for plant growth and therefore must provide moisture, nutrients and physical support to plants, whilst also being lightweight, chemically stable, aeratable, and able to drain water freely (Ampin et al., 2010; Friedrich, 2008). These characteristics are vital for the long term survival of green roof vegetation and provision of the benefits (services) that green roofs provide. To date however, there has been little empirical research into the role of substrate on provision of green roof services (Ampin et al., 2010; Olszewski and Young, 2011; Roth-Kleyer, 2005), into new substrate materials (Molineux

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http://dx.doi.org/10.1016/j.ufug.2014.04.007

et al., 2009; Solano et al., 2012), biological properties of substrate (Kolb et al., 1982) or the influence of substrates on green roof vegetation growth (Emilsson, 2008; Farrell et al., 2012; Kotsiris et al., 2012; Nagase and Dunnett, 2011; Rowe et al., 2006). There has also been a lack of research into the effect that each individual substrate component (e.g. mineral content, type of organic matter, artificial additives, mixing ratios) has upon the growth and physiological performance of the vegetation it supports and ultimately the services that it provides (Dvorak and Volder, 2010; Ouldboukhitine et al., 2012).

Most previous green roof substrate research has focused on the effect that substrate depth has on plant establishment, growth and long term survival (Durhman et al., 2007; Getter and Rowe, 2007, 2008; Rowe et al., 2012; Thuring et al., 2010). It is generally agreed that plant growth and physiological performance increases with substrate depth, although substrate depth is not always a limiting growth factor for some green roof species, most notably for hardy succulents (Getter and Rowe, 2008). Increased depth protects plants from temperature extremes and also increases the potential reservoir of water available for plants, reducing the chance of plants experiencing drought stress (Dunnett and Kingsbury, 2010; Thuring et al., 2010). However increasing substrate depth comes at an economic cost (greater volume of substrate required) and also may not be viable due to inadequate strength in the roof to support the greater substrate weight. An alternative is to design substrates to be more efficient and tailored towards specific or multiple services by modifying individual components in order to change substrate properties (e.g. increase water holding capacity or nutrient provision). However in order for this to occur, a full understanding of the effect that all components of green roof substrate have on plant growth and performance must first be gained (Dvorak and Volder, 2010).

Due to the relatively shallow depth and free draining nature of green roof substrates, water stress is one of the most common limitations for plant growth on green roofs (Rowe et al., 2012; Thuring et al., 2010). The water holding capacity of substrates can be increased by decreasing particle size which increases the amount of inner particle pore space, although this can increase the potential of water logging (Graceson et al., 2013; Olszewski and Young, 2011). It has been shown that increased substrate water holding capacity can increase survival of five different succulents during an extreme drought in Australia (Farrell et al., 2012), however it is not fully known how a change in green roof substrate particle size and therefore water holding capacity impacts upon non succulent plant growth and performance during typical growing conditions (Olszewski and Young, 2011).

An alternative to increasing the amount of smaller particles in a substrate, which can have negative effectives on drainage and water logging, is the use of artificial water retention gels. These are often used in horticulture and regeneration of degraded land to increase a soil/substrate's water holding capacity and reduce plant exposure to water stress without the need for large amounts of extra growing media (Agaba et al., 2010; Hüttermann et al., 2009; Kabiri et al., 2011; Williamson et al., 2011). Two previous trials have reported that similar benefits may be possible for green roof vegetation (*Sedum*) by providing longer term storage of water in the substrate (Olszewski et al., 2010; Sutton, 2008). It has also been shown that water retention gels can increase the water holding capacity of green roof substrate, although this does not necessarily translate into benefits for plants during periods of drought as this water may not be available or accessible to plants, and the effectiveness of the gel may be species dependent or vary depending on substrate composition (Farrell et al., 2013).

The type of organic matter used in green roof substrate can also affect water holding capacity due to different absorption properties. However subtle changes to its composition or quantity may have much larger effects on the substrates moisture dynamics due to its impact upon the establishment and long term survival of green roof vegetation (Emilsson, 2008; Nagase and Dunnett, 2011). The vegetation present alters the rate at which a substrate's water reservoir is depleted, as the amount and type of green roof vegetation plays a key role in determining evapotranspiration rates (Berghage et al., 2007; Wolf and Lundholm, 2008). Therefore altering organic matter type and amount in a substrate will also alter green roof performance through influencing plant growth, rate of water use and amount of transpiration.

Despite the potential for substrate composition to heavily influence green roof vegetation and therefore green roof service performance, the extent to which substrate components and their ratios influence green roof vegetation remains unknown. Without this knowledge it is challenging to engineer substrates that are tailored towards providing a specific service and therefore provide an optimised performance e.g. storm water retention at all times of the year.

With these concerns in mind, a pot experiment was established where the growth and physiological performance of the grass *Lolium perenne* (ryegrass) was assessed in controlled environment greenhouse trials. *L. perenne* was grown on green roof substrates composed of factorial combinations of commonly used green roof components of (i) small or large brick, (ii) organic matter as bark or green waste compost, and (iii) presence/absence of a polyacrylamide gel (SwellGel^{TM}). Trials were also undertaken using two substrate depths of 80 and 120 mm.

It was hypothesised that;

1. Small brick would increase the water holding capacity of green roof substrate compared to large brick, increasing evapotranspiration and improving *L. perenne* shoot growth and physiological performance.
2. Green waste compost would increase nutrient availability of the substrate, leading to improved *L. perenne* nutrient status, shoot growth, physiological performance and increases in evapotranspiration.
3. Polyacrylamide gel (SwellGel) would increase water holding capacity of the substrate, leading to greater *L. perenne* shoot growth and physiological performance.
4. In light of these hypotheses, the best performing green roof substrate in terms of *L. perenne* shoot biomass production, evapotranspiration and plant physiological condition would contain small brick, green waste compost and SwellGel.

Methods

Experimental design

The study was undertaken in a temperature controlled greenhouse in a day/night regime of 16 h 20 °C/8 h 15 °C from 28.2.13 to 29.5.13. Where necessary, supplementary lighting was used to ensure the required day length (Helle Lamps, IR 400 HPS, 400 W).

The eight substrates had three component variables: (i) brick size (small brick at 2–5 mm particle diameter; large brick of 4–15 mm diameter), (ii) organic matter type (bark or green waste compost) and (iii) presence or absence of a polyacrylamide gel "SwellGel^{TM}" (www.swellgel.co.uk) (Table 1). Brick was crushed waste red brick, sieved to ensure brick fragments were within the size limits set. Green waste compost (Green Estate, Sheffield, UK) was composed of composted garden waste collected in Sheffield, whilst bark was sourced as common garden mixed conifer bark mulch. SwellGel^{TM} (www.swellgel.co.uk) is a soil additive made of cross linked polyacrylamide which is designed to expand and store

Table 1
Substrate mixes used in the growth trial. Two substrate depths were used (80 mm and 120 mm) and $N = 8$.

Substrate number	Compost type 20% by volume	Brick size Small = 2–5 mm Large = 4–15 mm	SwellGel 1% by volume
1	Bark	Small	Yes
2	Green waste	Small	Yes
3	Bark	Large	Yes
4	Green waste	Large	Yes
5	Bark	Small	No
6	Green waste	Small	No
7	Bark	Large	No
8	Green waste	Large	No

water during high moisture levels and release it slowly back to the plant as moisture levels decline.

The substrate was made up of 20% of either organic matter type (no extra fertilisation was added), with the remaining 80% made up from one of the two crushed brick size categories. Dry SwellGel was then added as 1% of the total substrate volume as per manufactures instructions. Substrate was added to pots (12 cm × 11 cm × 11 cm) with two depths of substrate (80 mm and 120 mm), both of which are commonly used depths on extensive green roofs. The experiment therefore had a fully factorial design of brick size (2–5 mm/4–15 mm), organic matter type (green waste compost/bark), SwellGel (presence/absence) and substrate depth (80 mm/120 mm) (Table 1). Eight replicates of each substrate type and depth were used to give a total of 128 pots.

Plant species and water regime

Although not commonly found on green roofs in the UK, *L. perenne* (Hitchcock and Green, 1929) was used as a phytometer species due to its lower stress tolerance than hardier green roof grasses, and its relatively high growth rate. This was desirable given the primary aims of this project was to detect effects of substrate composition and differences in plant physiological performance between substrates, which would be more readily quantifiable with *L. perenne* than with slow growing green roof species over the duration of the experiment. 1 g of seed (Emorsgate Seeds, Kings Lynn, UK) per pot (approximately 500 seeds) were sown uniformly onto saturated substrate and then watered to saturation every day until two weeks following germination. After this point each pot was subjected to a watering regime of 150 ml per week, spilt over two days (with each day being two watering events of 37.5 ml) in order to make the watering event less intense and to prevent excessive leaching. As a percentage of total pot water holding capacity the weekly watering total was equivalent to 59–122% at 80 mm and 45–95% at 120 mm. This is the equivalent to 50 mm month^{-1} which is average for London, UK during winter months (Met Office, 2010).

Substrate water holding capacity and evapotranspiration

Unplanted substrates were air dried in the greenhouse for three weeks and weighed to quantify substrate dry weight. They were then saturated (in standing water for two days) and allowed to drain for 15 min to reach field capacity, after which they were weighed and the difference in weights given as water holding capacity.

During the experiment, pots were weighed daily as well as 15 min after each watering event. Any reduction in pot weight over time or between watering events was attributed to evapotranspiration (following 15 min draining there was never evidence of further leached losses). Total evapotranspiration of each pot over the duration of the experiment was calculated as the sum of all the weight differences over all time periods. We did not correct for plant biomass in this weight since we did not want to destructively harvest mid-way through the experiment, and plant biomass was less than 1/500th the mass of the evapotranspiration mass.

Plant biomass and shoot nitrogen content

After 16 weeks growth following germination, all above ground biomass was harvested, oven dried at 80 °C for two days and weighed to obtain dry weight. To determine root biomass, material was washed in water to remove all traces of brick and compost. After cleaning, roots with SwellGel still attached were then soaked in water overnight to expand the gel, which was then manually removed using a scalpel. All root material was dried (80 °C for two days) before weighing.

Leaf tissue nitrogen (N) content was determined on oven-dried ground samples from the final biomass harvest, following Kjeldahl digestion (Allen et al., 1974). For this approximately 50 mg dry plant biomass was digested in 1 ml concentrated sulphuric acid with 1 microspatular of catalyst (1:10 $CuSO_4$:$LiSO_4$) for 7 h at 375 °C. After a dilution ($N = 1{:}100\ dH_2O$) total nitrogen was determined by Flow Injection Analysis (Burkard FIA Flo2, Burkard Scientific, Uxbridge, UK).

Chlorophyll content

Biomass production and shoot nitrogen content were supported by physiological indicators of plant health. Mean leaf chlorophyll content for each pot was determined through acetone extraction (Cameron et al., 2009). After the last watering event, five grass shoots (0.25–0.5 g fresh weight) from different parts of the pot were harvested and kept on ice in the dark until extraction of chlorophyll (within 1 h to prevent degradation). The grass shoots were ground in a pestle and mortar with acid washed sand to form a paste. 5 ml of ice cold 80% acetone was added and the mixture further ground then transferred to a 25 ml centrifuge tube. The pestle and mortar were rinsed twice with 2 ml ice cold 80% acetone and transferred to the same centrifuge tube then diluted to 10 ml with ice cold 80% acetone. Samples were centrifuged at 8000 × *g* for 5 min and absorbance of the supernatant measured at 645 and 663 nm using a Cecil Ce 1020 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK). Chlorophyll content was calculated using the following equations according to (Arnon, 1949)), and re-expressed as mg chlorophyll per dry shoot weight.

$$\mathrm{Chl}a\ (\mathrm{mg\,l^{-1}}) = (12.7 \times \mathrm{OD}_{663}) - (2.69 \times \mathrm{OD}_{645})$$
$$\mathrm{Chl}b\ (\mathrm{mg\,l^{-1}}) = (22.9 \times \mathrm{OD}_{645}) - (4.68 \times \mathrm{OD}_{663})$$

Statistical analyses

To determine the main factorial effects and interactions of the substrate components (brick size, organic matter type, SwellGel and substrate depth), four-way ANOVAs were performed. Tukey HSD tests were used to determine differences between each individual substrate. All statistical analyses were carried out in R Studio version 2.15.1 (22.6.2012), (R Development Core Team, 2011).

Results

Water holding capacity of substrates

The presence of SwellGel increased water holding capacity by 24% ($p < 0.0001$), whilst large brick reduced water holding capacity

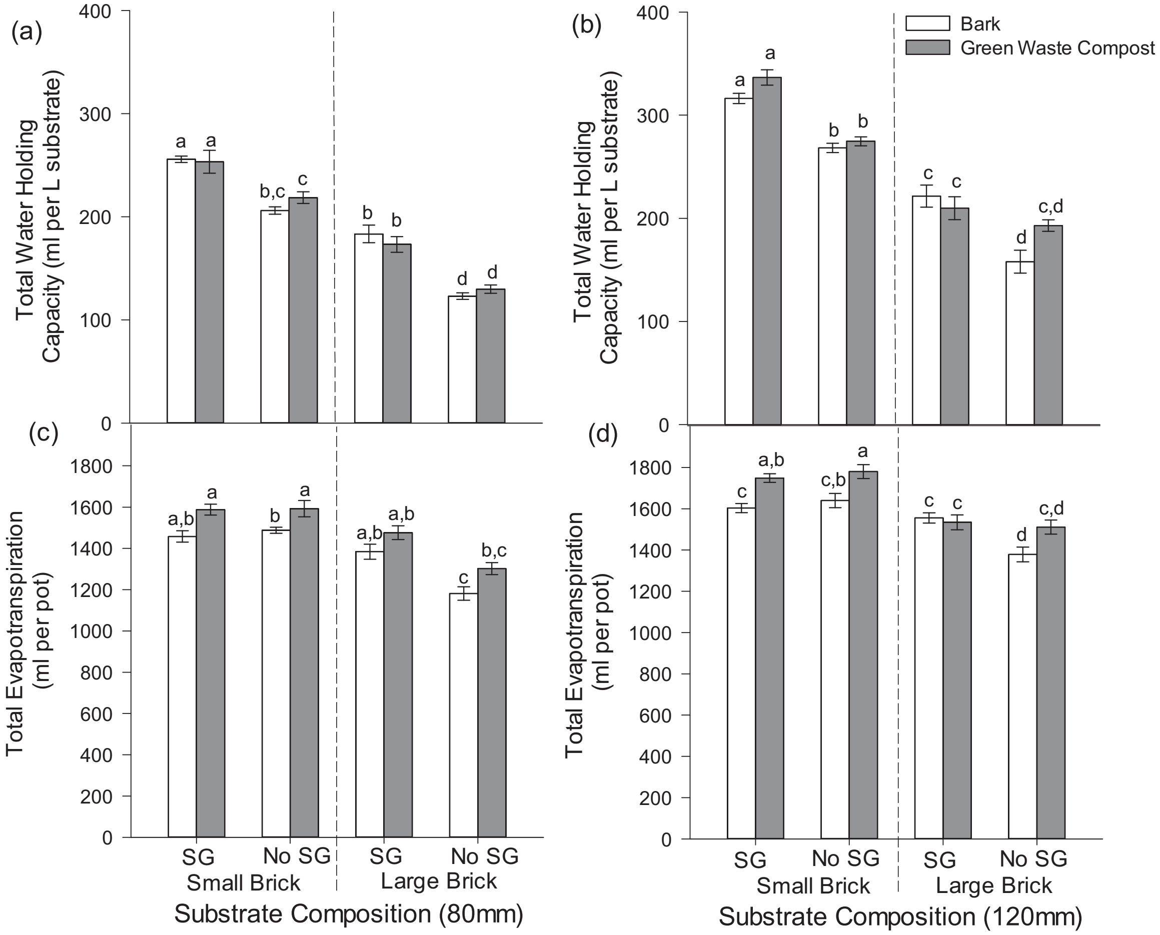

Fig. 1. (a) Water holding capacity (ml per L substrate) of each substrate at 80 mm substrate depth, (b) water holding capacity (ml per L substrate) of each substrate at 120 mm substrate depth, (c) mean total evapotranspiration (ml per pot) at 80 mm substrate depth, (d) mean total evapotranspiration (ml per pot) of at 120 mm substrate depth. Error bars are ± one standard error. Means with same letter do not significantly differ from each other within the same sub-figure (Tukey HSD, $p < 0.05$). Abbreviations are as follows, SG, Swell Gel present; No SG, Swell Gel not present.

Table 2
Main factor effects (four-way ANOVA) for (a) substrate water holding capacity (ml per L substrate) and (b) total evapotranspiration of *Lolium perenne* grown for 3.5 months in eight different green roof substrates. Main factors are brick size (small vs. large), organic matter (bark vs. green waste compost) and SwellGel (absence vs. presence). Main factor means are shown with the % change also shown between the two levels within that factor (e.g. absence vs. presence of SwellGel).

Factor	*Df*	*F*-value	*P*-value	Brick Size		Organic		SwellGel		Depth		% Change (±SE, *n* = 64)
				Small	Large	Bark	GW	No	Yes	80 mm	120 mm	
(a)												
				Main factor means of water holding capacity (ml per L substrate)								
Brick	1	640.6	***	266.2	174.0							−34.6 ± 1.9
Organic	1	3.8	0.053			216.5	223.6					+3.3 ± 3.7
SwellGel	1	168.6	***					196.4	243.7			+24.1 ± 3.8
Depth	1	222.4	***							192.9	247.2	+28.1 ± 4.0
Sw.G:Org	1	4.9	*									
Brick:Depth	1	9.3	**									
Sw.G:Org:Brick	1	4.6	*									
Sw.G:Org:Brick:Depth	1	4.0	*									
(b)												
				Main factor means of total pot evapotranspiration (ml)								
Brick	1	162.9	***	1612.2	1415.5							−12.2 ± 1.2
Organic	1	47.0	***			1461.0	1566.7					+7.2 ± 1.4
SwellGel	1	14.9	***					1484.1	1543.6			+4.0 ± 1.1
Depth	1	108.2	***							1433.7	1594.0	+11.2 ± 1.3
Sw.G:Brick	1	7.8	***									

Significant factorial interactions are also shown. Statistical significances were calculated from four-way ANOVA. Abbreviations for each factor are as follows, Org, organic matter type; GW, green waste organic matter; Sw.G, SwellGel.

[*] Statistical significance of $p < 0.01$.

[**] Statistical significance of $p < 0.001$.

[***] Statistical significance of $p < 0.0001$.

by approximately 35% compared to small brick ($p < 0.0001$) (Fig. 1a and b, Table 2a). Organic matter type (bark or green waste) did not significantly affect water holding capacity (Table 2a). Increasing the substrate depth from 80 mm to 120 mm significantly increased water holding capacity by 28% ($p < 0.0001$) (Fig. 1a and b, Table 2a). Overall substrates containing small brick and SwellGel always had a significantly higher water holding capacity than substrates containing large brick and no SwellGel at both depths regardless of organic matter content (Tukey HSD, $p < 0.05$).

Evapotranspiration

SwellGel and green waste organic matter both significantly increased evapotranspiration by 4% and 7% respectively compared to no SwellGel ($p < 0.0001$) and bark ($p < 0.0001$). Large brick significantly decreased evapotranspiration by 12% compared to small brick ($p < 0.0001$) (Fig. 1c and d, Table 2b). Substrate depth had a significant effect on total evapotranspiration, with evapotranspiration 11% greater from 120 mm depth substrate ($p < 0.0001$) (Fig. 1c and d, Table 2b). At both substrate depths, small brick with green waste organic matter had greater evapotranspiration than large brick with bark organic matter (Tukey HSD, $p < 0.05$).

Shoot biomass

Organic matter type had the largest effect on shoot biomass, with this being 32% greater on green waste than bark substrates ($p < 0.0001$) (Fig. 2a and b, Table 3a). The presence of SwellGel more modestly increased dry shoot biomass by 8% ($p < 0.0001$), and large brick size decreased shoot biomass by 17% ($p < 0.0001$) (Fig. 2a and b, Table 3a). Overall this meant that substrates containing green waste with either brick size or SwellGel presence had significantly greater biomass production than all bark based substrates at both 80 and 120 mm depths (Tukey HSD, $p < 0.05$). Shoot biomass did not differ significantly between 80 and 120 mm substrate depth (Table 3a).

Root biomass

Organic matter type had the greatest effect on root biomass production. Overall green waste significantly increased root biomass by 13% compared to bark ($p < 0.0001$). SwellGel had the next greatest effect on root biomass, decreasing this by 7% overall ($p < 0.001$) (Fig. 2c and d, Table 3b). There was a significant interaction between SwellGel and organic matter type ($p < 0.001$), with bark substrates producing significantly greater levels of root growth when SwellGel was not present. The same interaction occurred between SwellGel and brick size ($p < 0.0001$), with SwellGel significantly decreasing root biomass on small brick, but not on large brick (Fig. 2c and d, Table 3b). Increasing the depth of substrate from 80 mm to 120 mm significantly increased root biomass by 22% ($p < 0.0001$) (Fig. 2c and d, Table 3b). Brick size did not have a significant effect on root biomass.

Root:shoot ratio

Root:shoot ratios with green waste organic matter was significantly reduced by 15% compared to bark ($p < 0.0001$), while large brick significantly increased root:shoot ratios by 16% compared to small brick ($p < 0.0001$) (Fig. 2e and f, Table 3c). The presence of SwellGel reduced root:shoot ratios by 15% ($p < 0.0001$) (Fig. 2e and f, Table 3c). The same factorial interactions observed for root biomass were observed for root:shoot ratios also, with SwellGel reducing root:shoot ratios more when the organic matter was bark rather than green waste ($p < 0.0001$), or small rather than large brick ($p < 0.01$) (Fig. 2e and f). Root:shoot ratios at 120 mm depth were 17% higher than at 80 mm depth at 120 mm depth ($p < 0.0001$) (Fig. 2e and f, Table 3c).

Shoot nitrogen concentration

Green waste, SwellGel and large brick had very similar effects on shoot nitrogen concentration, increasing this by 21%, 20% and 22% compared to bark, no SwellGel and small brick respectively ($p < 0.0001$) (Fig. 3a and b, Table 4a). A significant interaction showed that the increase in shoot nitrogen concentration due to SwellGel was much larger when it was present with green waste rather than bark, although this effect only occurred in small brick ($p < 0.001$) (Table 4a). Substrates containing SwellGel and green waste had significantly higher shoot nitrogen concentrations than substrates without SwellGel and bark based at 80 mm depth (Tukey HSD, $p < 0.05$) and partly at 120 mm. Substrate depth did not significantly affect shoot nitrogen concentration (Table 4a).

Chlorophyll content

Shoot chlorophyll content was most significantly affected by organic matter type and substrate depth, with green waste increasing chlorophyll content by 57% compared to bark, and 120 mm substrate depth increasing chlorophyll content by 40% compared to 80 mm ($p < 0.0001$) (Fig. 3c and d, Table 4b). Increasing brick size from small to large caused a decrease in chlorophyll (−14%) content ($p < 0.01$) (Table 4b). A significant interaction between SwellGel and organic content occurred with large brick only ($p < 0.0001$), with SwellGel increasing chlorophyll content in bark based substrates but decreasing chlorophyll content in green waste substrates (Table 4b).

Discussion

This study is one of the first systematic investigations to quantify the importance of widely used green roof components for plant growth and physiological performance. It is clear that altering the composition/type of the component parts of green roof substrate can have substantial effects on plant physiological performance and water balance. All three substrate composition factors studied (presence of a polyacrymide gel (SwellGel), organic matter and brick size) had significant effects on *L. perenne*, which were largely consistent across both substrate depths, and indeed often had larger effects than the often previously studied substrate depth. Although this trial only assessed initial plant establishment, these findings can therefore begin to inform substrate composition choice depending on plant growth requirements (fast growing/high maintenance/lower drought tolerance vs. slow growing/low maintenance/higher drought tolerance).

Water holding capacity & evapotranspiration

SwellGel increased the water holding capacity of green roof substrates, which explains its benefit to shoot growth and evapotranspiration. In this trial its effect on water holding capacity is less than that of brick size (small brick increased water holding capacity ~50% more compared to adding SwellGel). This does not mean that SwellGel has a limited impact on water holding capacity since it constituted only 1% volume in our substrates compared to 80% brick. Indeed, greater impact of SwellGel could be achieved by increasing the amount used, although there are limitations in the amount that can added due to substrate disturbance from constant expansion and contraction during wetting and drying cycles, physical limitations and negative effects on biomass yield (Farrell et al., 2013). In fact, SwellGel may be more important in times of drought as water stored in it may be released much more slowly to

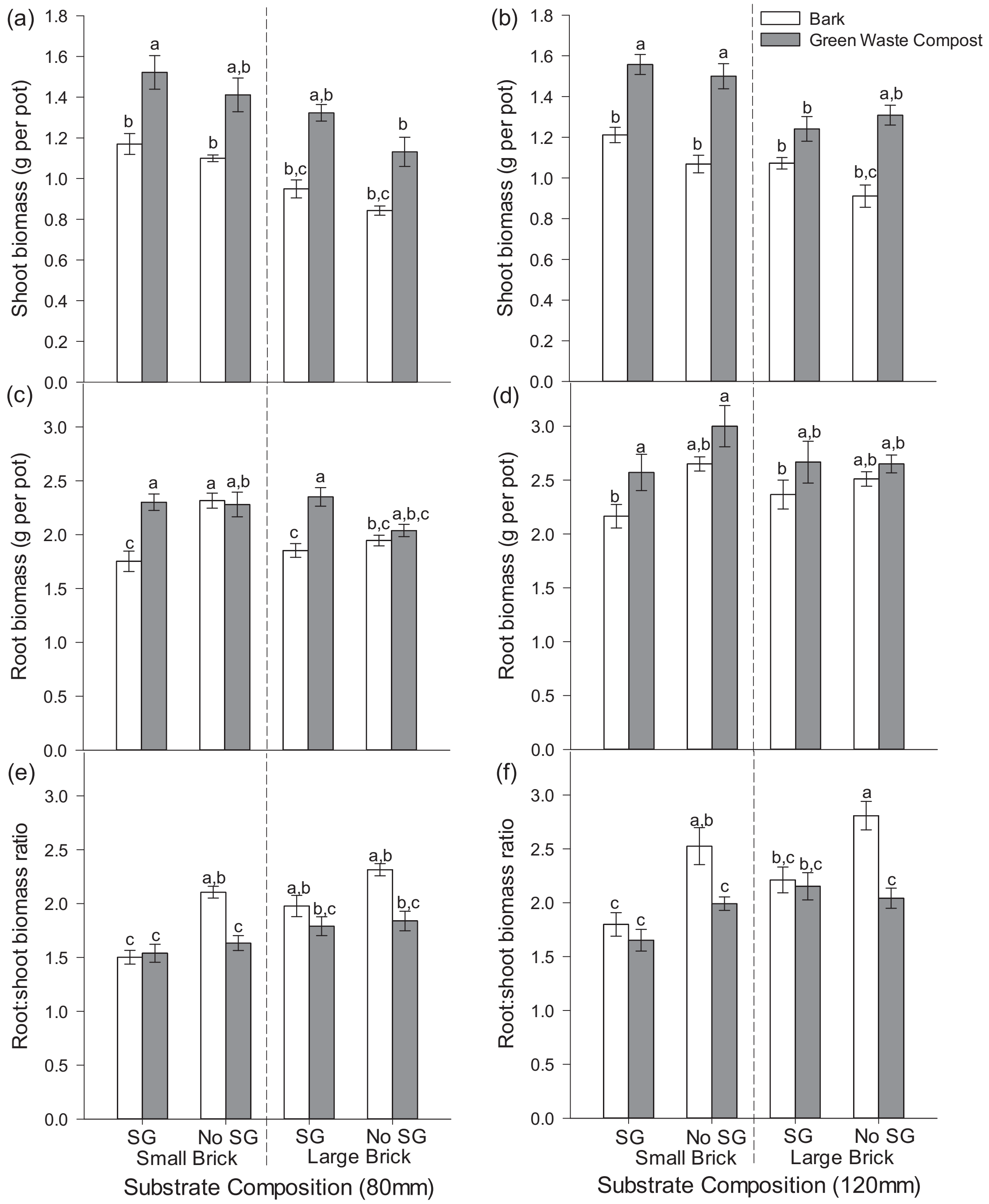

Fig. 2. (a) shoot biomass on 80 mm substrate depth, (b) shoot biomass on 120 mm substrate depth, (c) root biomass on 80 mm substrate depth, (d) root biomass on 120 mm substrate depth, (e) root:shoot ratios on 80 mm substrate depth, and (f) root:shoot ratio on 120 mm substrate depth. Error bars are ± one standard error. Means with same letter do not significantly differ from each other within the same sub-figure (Tukey HSD, $p < 0.05$). Abbreviations are as follows, SG, Swell Gel present; No SG, Swell Gel not present.

plants than water stored in inner particle pore space (Agaba et al., 2010; Hüttermann et al., 2009). However it should be noted that this trial did not assess the effect of SwellGel on plant available water which does not always increase with greater substrate water holding capacity and can be species dependent (Farrell et al., 2013). Where substrates are used in regions with prolonged periods of low rainfall, or where a greater frequency of drought events are predicted from climate change (Coumou and Rahmstorf, 2012), then SwellGel is likely to be an important and beneficial component of substrates. None-the-less, using small instead of large brick size appears to be the simplest (and likely most cost effective) way of improving substrate water holding capacity.

Vegetation plays a major role in increasing evapotranspiration rates from green roofs (Metselaar, 2012; Voyde et al., 2010; Wolf and Lundholm, 2008), and in this trial the presence of *L. perenne* increased total evapotranspiration by between 13 and 57%

Table 3
Main factor effects (four-way ANOVA) for (a) shoot biomass, (b) root biomass and (c) root:shoot ratios of *Lolium perenne* grown in eight different green roof substrates. Main factors are brick size (small vs. large), organic matter (bark vs. green waste compost) and SwellGel (absence vs. presence). Main factor means are shown with the % change also shown between the two levels within that factor (e.g. absence vs. presence SwellGel).

Factor	*Df*	*F*-value	*P*-value	Brick Size		Organic		SwellGel		Depth		% Change (±SE, $n = 64$)
				Small	Large	N	Y	No	Yes	80 mm	120 mm	
(a)												
				Main factor means of dry shoot biomass (g)								
Brick	1	68.5	***	1.31	1.10							−16.7 ± 2.0
Organic	1	157.5	***			1.04	1.38					+32.1 ± 2.7
SwellGel	1	13.2	***					1.16	1.26			+8.3 ± 2.6
Depth	1	3.9	0.05							1.18	1.23	+4.2 ± 2.6
Sw.G:Org:Depth	1	4.3	*									
(b)												
				Main factor means of dry root biomass (g)								
Brick	1	2.2	0.14	2.38	2.30							−3.4 ± 2.1
Organic	1	26.7	***			2.19	2.48					+13.1 ± 2.6
SwellGel	1	9.4	**					2.42	2.25			−7.0 ± 2.3
Depth	1	70.6	***							2.11	2.57	+22.2 ± 2.6
Sw.G:Org	1	7.3	**									
Sw.G:Brick	1	12.1	***									
(c)												
				Main factor means of root:shoot ratios								
Brick	1	35.8	***	1.84	2.14							+16.2 ± 2.8
Organic	1	42.6	***			2.16	1.83					−15.1 ± 1.8
SwellGel	1	43.6	***					2.16	1.83			−15.3 ± 2.1
Depth	1	38.7	***							1.84	2.15	+16.9 ± 3.2
Sw.G:Org	1	22.5	***									
Sw.G:Brick	1	5.0	*									

Significant factorial interactions are also shown. Statistical significances were calculated from four-way ANOVA. Abbreviations for each factor are as follows, Org, organic matter type; GW, green waste organic matter; Sw.G, SwellGel.

[*] Statistical significance of $p < 0.01$.

[**] Statistical significance of $p < 0.001$.

[***] Statistical significance of $p < 0.0001$.

compared to non vegetated substrate (data not shown). The amount of transpiration that *L. perenne* contributed to the total evapotranspiration amount is dependent on the total amount of biomass produced (evapotranspiration and *L. perenne* biomass were significantly correlated; $r^2 = 0.482$, $p < 0.0001$), which in turn is dependent on the nutrient content and water storage capacity of the substrate. Organic matter type did not affect the water holding capacity of the substrate but did indirectly affect the rate at which water was lost from the substrate by influencing biomass production and therefore transpiration. This highlights that water holding capacity should not be the only substrate property that is considered when selecting a substrate for its influence on water dynamics, as vegetation growth also has a considerable influence on this.

Limited evapotranspiration, however, may not always be desirable since this can play an important role in temperature regulation of host buildings (Blanusa et al., 2013; Castleton et al., 2010). Similarly, when designing green roof substrate to promote greater plant growth in order to increase cooling from evapotranspiration, one has to consider the effect that higher evapotranspiration rates may have upon the substrate water reservoir during times of drought. If this is depleted too quickly, leading to water stress and stomatal closure, plants no longer transpire at the same rate, mortality may occur and the net cooling effect of the green roof could be reduced. In addition, by developing a green roof solely for one service, for example building cooling, other green roof services may be compromised, such as biodiversity provision or aesthetic qualities. Therefore such trade-offs must be taken into account when optimising a green roof substrate (Ampin et al., 2010; Lundholm et al., 2010).

Plant growth

Plant biomass was substantially increased when green waste compost was used as the organic matter component instead of bark. Green waste compost will have more nutrients available to plants due to its preconditioned state (composted) and greater diversity of source material. It has been shown previously that increasing the organic fraction of a green roof substrate increases plant growth (Nagase and Dunnett, 2011), although to our knowledge this is the first time that it has been demonstrated that different organic matter types have a significant effect on green roof plant growth. Again, increased plant growth may not always be desirable since it can be detrimental to long term plant survival as plants with more luxuriant growth can be more susceptible to the drought stresses common to green roofs (Bates et al., 2013), and will also require more maintenance compared to slower growing coverage (Nagase and Dunnett, 2011). None-the-less, higher nutrient content (through increased organic fraction or different organic matter type) of green roof substrates increases plant growth (Nagase and Dunnett, 2011; Olszewski et al., 2010) and can improve long term substrate development due to a larger build up of dead biomass, which can also help prevent nutrients from being leached out of the system (Emilsson, 2008).

The greater fund of nutrients in green waste is also consistent with the lower root:shoot ratios found in green waste compost substrate compared to bark substrates. This indicates less need for plants to allocate resources to nutrient capturing roots in green waste based substrates, and a greater allocation to the photosynthesising shoots (Hermans et al., 2006). The same response in root:shoot ratios was observed for SwellGel and small brick,

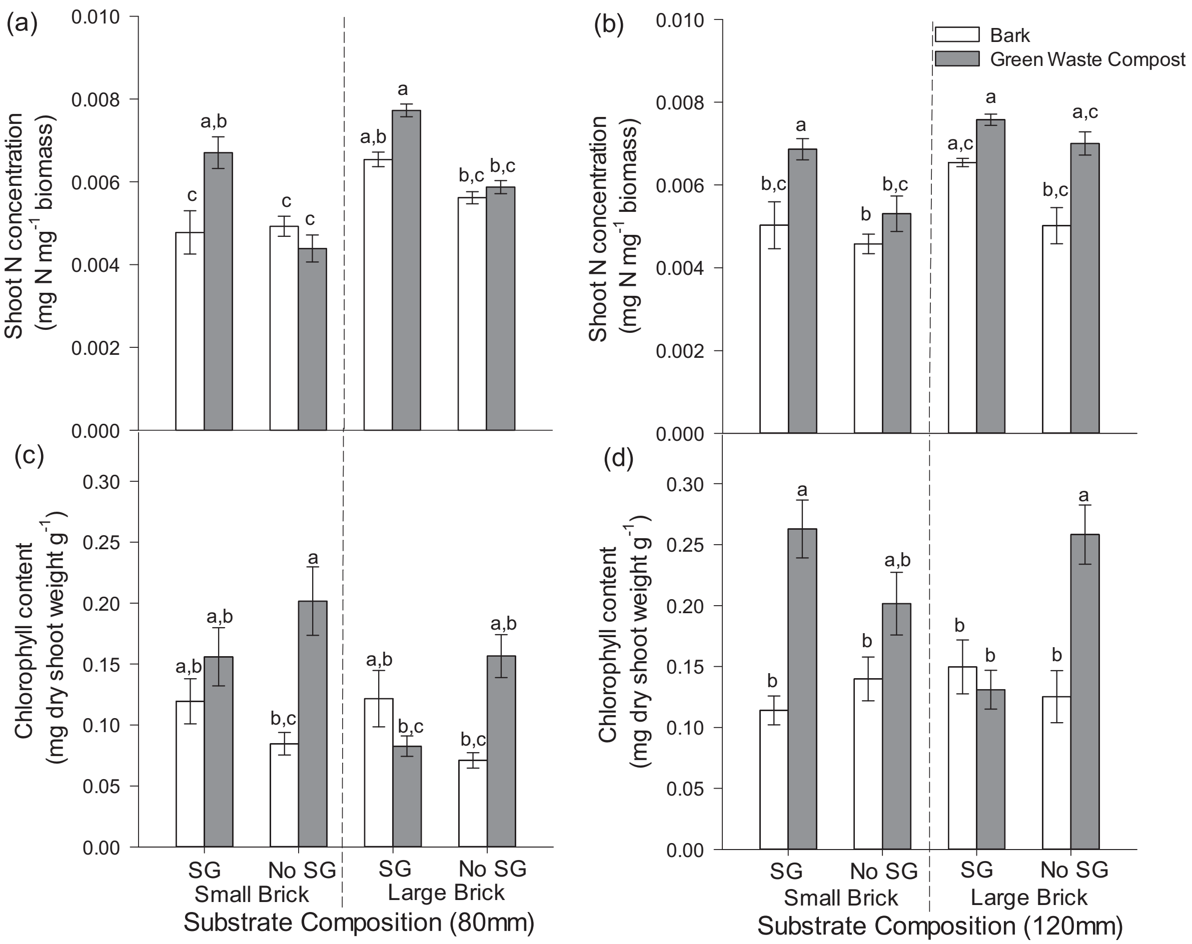

Fig. 3. (a) Shoot nitrogen concentration (mg N mg^{-1} dry biomass) on 80 mm substrate depth, (b) shoot nitrogen concentration (mg N mg^{-1} dry biomass) on 120 mm substrate depth, (c) shoot chlorophyll concentration (mg^{-1} g dry biomass) on 80 mm substrate depth, (d) shoot chlorophyll concentration (mg^{-1} g dry biomass) on 120 mm substrate depth. Error bars are ± one standard error. Means with same letter do not significantly differ from each other within the same sub-figure (Tukey HSD, $p < 0.05$). Abbreviations are as follows, SG, Swell Gel present; No SG, Swell Gel not present.

likely due to the increased availability of water which reduced the need for water capturing root biomass. However substrates that do the opposite and promote a higher root:shoot ratio (i.e. promote resource allocation to roots) may actually be more desirable for green roofs during the establishment phase of plants, especially in areas subject to low precipitation and high temperatures, where greater water capture capacity (roots) and less surface for transpiration (shoots) is desirable (Grossnickle, 2005; Nagase and Dunnett, 2011).

Plant growth was significantly decreased when brick particle size was increased from 2–5 mm to 4–15 mm. This may be due to the poorer water holding capacity of the large brick substrates, as larger particle sizes reduces inter-particle pore space and therefore reduces water holding capacity (Farrell et al., 2012; Graceson et al., 2013). This effect may also be due to the higher amounts of nitrogen leached from large brick substrates throughout the trial, which could have depleted nitrogen stocks in the substrate at a faster rate (see online supporting material).

SwellGel had a relatively small effect on plant growth, although this may be due to the regular watering regime not resulting in great enough water stress for the benefits of SwellGel to be realised. Much larger increases in *Sedum* shoot biomass with polyacrylamide gel amendment has previously been demonstrated, although a higher temperature and less frequent watering regime were used in that study (Olszewski et al., 2010). However different types of water retention amendment seem to differ in their ability to influence green roof plant growth (Farrell et al., 2013).

Depth of substrate had no effect on plant growth, which contrasts with many other studies that have stated this to be a major factor in green roof plant establishment and growth (Durhman et al., 2007; Getter and Rowe, 2007, 2008; Thuring et al., 2010). Past studies have concluded that increased water availability in deeper substrate is one of the most important factors for plant growth (Rowe et al., 2012), but water availability may not have been a major limiting factor in this trial. Increased depth also protects plants from frost damage (Boivin et al., 2001), as well as reducing extreme temperature fluctuations from solar radiation (Butler and Orians, 2011), both of which were not present in the controlled temperature environment of the greenhouse. These benefits of deeper substrate would therefore not have become fully apparent in our study.

Shoot nitrogen and chlorophyll concentration

Shoot N concentration was increased by SwellGel, however the mechanism behind this is unclear. It may be due to the SwellGel degrading to form acrylamide and then ammonium or nitrogen oxides (Holliman et al., 2005; Smith et al., 1997), or by SwellGel absorbing nitrogen from the substrate. Alternatively it could be due to increased microbial activity around the moisture pockets created by the SwellGel as it has been shown that fungi and bacteria can readily colonise polyacrylamide gel and utilise the nitrogen held within it (Holliman et al., 2005; Kay-Shoemake et al., 1998). As chlorophyll content was not significantly affected by

Table 4
Main factor effects (four-way ANOVA) for (a) mean shoot nitrogen concentration (mg^{-1} g shoot biomass), (b) mean chlorophyll content (mg g^{-1} dry shoot biomass) of *Lolium perenne* grown in eight different green roof substrates. Main factors are brick size (small vs. large), organic matter (bark vs. green waste compost) and SwellGel (absence vs. presence). Main factor means are shown with the % change also shown between the two levels within that factor (e.g. absence vs. presence SwellGel).

Factor	*Df*	*F*-value	*P*-value	Brick Size		Organic		SwellGel		Depth		% Change ($\pm$SE, $n = 64$)
				Small	Large	Bark	GW	No	Yes	80 mm	120 mm	
(a)												
				Main factor means of total nitrogen shoot concentration (mg^{-1} g shoot biomass)								
Brick	1	53.897	***	0.0053	0.0065							+21.9 ± 2.8
Organic	1	43.866	***			0.0054	0.0064					+19.6 ± 3.1
SwellGel	1	50.765	***					0.0053	0.0065			+21.2 ± 3.4
Depth	1	1.148	0.29							0.0058	0.0060	+2.9 ± 3.0
Sw.G:Org	1	7.815	**									
Org:Depth	1	4.707	*									
Sw.G:Org:Brick	1	8.014	**									
Sw.G:Org:Depth	1	6.490	*									
(b)												
				Main factor means mean chlorophyll content (mg g^{-1} dry shoot biomass)								
Brick	1	5.4	*	0.16	0.14							−14.4 ± 5.7
Organic	1	44.4	***			0.12	0.18					+56.7 ± 8.9
SwellGel	1	1.7	0.20					0.15	0.14			−8.2 ± 5.8
Depth	1	24.3	***							0.12	0.17	+39.5 ± 8.0
Sw.G:Org	1	11.7	***									
Org:Brick	1	6.7	*									
Sw.G:Org:Brick	1	12.9	***									
Sw.G:Org:Brick:Depth	1	6.1	*									

Significant factorial interactions are also shown. Statistical significances were calculated from four-way ANOVA. Abbreviations for each factor are as follows, Org, organic matter type; GW, green waste organic matter; Sw.G, SwellGel.

* Statistical significance of $p < 0.01$.

** Statistical significance of $p < 0.001$.

*** Statistical significance of $p < 0.0001$.

SwellGel but did show significantly higher levels in plants grown in green waste compost substrates, it could indicate that any additional nitrogen supplied through the presence of SwellGel was not a significant factor in chlorophyll production. Green waste compost increased shoot nitrogen concentration, probably by increasing the amount of nitrogen available for plant uptake (supported by KCl plant available nitrogen analysis of substrates, see online supporting material). This is also the likely reason for significantly higher chlorophyll content on green waste as it has also previously been shown that higher chlorophyll content in temperate grasses is correlated with high shoot nitrogen concentration (Gáborčík, 2003). The higher concentration of shoot N in large brick is, in contrast, likely to be caused by a negative growth dilution as brick size did not have a significant effect on total tissue nitrogen stocks (data not shown), but did reduce shoot growth, and so potentially “concentrating” the nitrogen in the smaller shoot biomass.

Depth of substrate

Increasing the depth of green roof substrate generally improves green roof plant growth and survival by increasing water and nutrient availability, especially during times of drought (Durhman et al., 2007; Getter and Rowe, 2007, 2008; Thuring et al., 2010). Although this trial did not show such dramatic improvements to plant growth and physiological performance with depth as previous trials, it was conducted under controlled temperature conditions and therefore plants did not experience some of the environmental extremes that roof top trials encounter.

Conclusions

This study has shown that altering the characteristics of commonly used green roof substrate components can significantly alter the initial growth and physiological performance of the plants grown upon them. This is especially important for green roofs because vegetation plays a core role in provision of green roof services (Oberndorfer et al., 2007).

All four hypothesises were supported by the experimental data. By looking at each substrate component in turn it is clear that organic matter type was found to have the most influence on plant growth and health. Increasing plant available nutrients by switching from bark to green waste compost significantly increased *L. perenne* shoot N concentration, chlorophyll content and shoot and root biomass, which in turn increased total evapotranspiration. However by also reducing *L. perenne* root:shoot ratio, green waste compost potentially reduced this plant’s ability to survive drought stress. The effectiveness of SwellGel to provide water storage during drought was not thoroughly tested in this trial due to the absence of drought conditions. However, SwellGel still improved plant growth and substrate water holding capacity. Brick size had a larger effect than SwellGel on shoot growth and water holding capacity, however SwellGel may be more effective at providing water to plants during a drought stress, although more studies on the plant availability of water stored in SwellGel must be conducted.

Therefore our fourth hypothesis which predicted that substrates containing small brick, green waste compost and SwellGel would be the best performing substrate in terms of shoot biomass production, evapotranspiration and plant physiological condition was correct. However this does not necessary mean that this mixture of substrate components will be the optimum for every green roof, with designers needing to consider the particular environmental stresses at that location and the core reason why that green roof is being built (e.g. high rainfall areas will not need high water retention for plant growth, but may need it for storm water mitigation). Clearly, compositional changes in green roof substrates – even among commonly used substrate materials – can have large influences on the properties and physiological performance of the vegetative component of the roof, and emphasises the fact that substrate composition should be considered carefully when designing green roofs for optimal provision of particular green roof service.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ufug.2014.04.007.

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