Direct Seeding onto Green Roof Substrate Supports Species Rich, High Cover, Novel Grassland

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Front piece: A comparison of vegetation performance parameters- species richness and cover, on two green roofs utilizing grassland species in Melbourne, Australia. Left- The biodiversity green roof at the Burnley Campus, University of Melbourne, 2015; showing a species rich community of local grassland species dominated by grasses, with additional spontaneous colonization, and poor cover. Right- A native species recreational green roof garden, Kangan Institute, Melbourne, 2014; species are of mixed habitat origin and includes a grass species planted as monoculture. In this case, species richness and cover are poor.
Abstract

The vegetation of green roofs is central to their functioning and ability to provide ecosystem services. When vegetation performs well, green roofs contribute to storm water mitigation, thermally buffer buildings, improve biodiversity and provide aesthetic and recreational relief in the grey city landscape. However, poor vegetation performance is common, with a decline in both species richness and cover over time. This decline can in part be attributed to design, failing to consider community assemblage mechanisms that lead to quality vegetation performance. Direct seeding of grassland species could offer a randomness in distribution and abundance of seedlings that supports early community self-organization and co-existence. Comparatively, adult plant establishment does not provide this early opportunity.

This thesis determines, the ability of a scoria based green roof substrate to support the germination and establishment of a species rich, high cover, novel grassland community, and the direct seeding sowing methods to achieve this. Additionally, species richness, and abundance were investigated as potential drivers of cover. A grassland forb only species seed mix was applied in two experiments. Experiment One (n=7), in glasshouse conditions, investigated application of seed with and without a sand bed, and depth of sowing; six treatments. Experiment Two (n=10) in green roof module conditions outside under irrigation, investigated depth of sowing and rate; four treatments.

Main results showed a species rich and abundant germination on scoria based green roof substrate. Results indicated that both depth of sowing at greater than 10 mm and application in a sand bed, reduced species richness and abundance. In green roof module conditions, surface sowing indicated a slight species richness advantage and an abundance disadvantage, in comparison to sowing between 0 to 10 mm depth. Sowing rate approaching that of on ground grassland restoration rates, were shown to be as effective as a doubled sowing rate in producing a species rich, high cover. This study found; no support for species richness as a key driver of cover, however abundance is indicated as an early key driver of cover, and may not act in isolation during rapid cover development. These findings are relevant to management practices.

Quality vegetation performance, achieved at sowing rates approximating on ground restoration, suggest that further investigation into lowering rate and species richness and cover response, is warranted. Long term studies investigating community dynamics, would give insight into this novel community’s ability to continue co-existence as a functional resilient system, as a predictor of ecosystem service potential.
Declaration

This is to certify that:

i. The thesis comprises only my original work towards FRST90077

ii. Due acknowledgement has been given in the text to all other material used

iii. The thesis is between 10 000 and 15 000 words in length, exclusive of tables, figures, references and appendices

Pamela Spencer
June 2016
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Thank you to the inspiring:
Bear Grylls: Never give up.
Lady Gaga: Who would you be and what would you do if you weren’t afraid?
Paul Keating: Brave, creativity changes the world.
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Introduction

Green roofs provide important ecosystem services that are integral city livability (Oberndorfer et al., 2007; Picket et al., 2011). Extensive green roof systems are increasingly adopted worldwide, and belong to a suite of engineered/vegetation strategies which counter reduced ecosystem services within cities (Getter and Rowe, 2006; Tzoulas et al., 2007). Declining ecosystem services, results from increasing population growth with consequential loss of green space, and is magnified by climate change induced extreme meteorological events (Picket et al., 2011). Two key ecosystem services that green roofs provide are; the buffering of buildings against thermal extremes (Bevilacqua et al., 2015, Nardini et al., 2012) and storm water mitigation, through rainfall interception (Beecham & Razzaghamesh, 2015; MacIvor & Lundholm, 2011). It is the character of green roof vegetation and the substrate in which they grow that enable these services. Additionally, green roofs have positive aesthetic potential in an increasingly grey landscape. In this regard green roofs contribute to increased work place productivity, reduced mental fatigue (Tzoulas, et al., 2007), improved health and wellbeing (Jansson, 2013; Kellert & Wilson, 1993; Lee et al., 2014), and affect a sense of community through recreational use (Kim & Kaplan, 2004). As vegetation is diminished within the urban landscape, so too is biodiversity. Green roof vegetation has demonstrated ecosystem service value as a biodiversity provider (Brenneisen, 2006, Sadler et al., 2011) through the establishment of horticultural and spontaneous vegetation (Bates et al., 2013; Lundholm, 2015a). With vegetation selected from a pool of species of habitat origin, green roofs may become a conservation tool, but as pointed out, research is yet to determine equivalence to on ground habitat biodiversity (Williams et al., 2014). However, it has been established that green roof vegetation is capable of serving as a fauna refuge (Butler et al., 2012) and foraging site (Mechelen et al., 2015), with further potential as a seed bank or as a seed rain contributor to on ground vegetation communities.

Underperforming green roof vegetation reduces the functional effectiveness of the system and limits ecosystem service provision. (Hooper et al., 2005; Mechelen et al., 2015b). The repetitive abiotic stressors of the desiccating green roof environment depletes plant health, reducing growth and causing high mortality (Dunnett & Kingsbury, 2004; Nardini et al., 2012). The microclimate of this open habitat is one of strong winds, (Francis, 2010) and high light intensity, which cause plant desiccation and structural damage (Chun & Guilderman, 2004). Compounding this, is that the green roof substrate although designed to anchor plants, can limit plant growth and survival. The substrate properties cause periodic drying, with consequent low water availability to plants (Berretta, Poe & Stovin, 2014; MacIvor & Lundholm, 2011; Rowe et al., 2014)
Extensive green roofs substrates are porous, light weight and shallow to 15cm depth; are engineered to intercept rainwater and yet be freely draining ready to intercept the next rain event, and to thermally buffer buildings (Getter and Rowe 2006, FLL, 2008). However, the porous nature of the substrate while acting as a building insulator, itself accumulates heat, (Olivieri et al., 2013) at temperatures that can be detrimental to plant survival (Dunnett & Kingsburry, 2004). Typically the availability of substrate nutrients to the plants is low, due to low fertilizer input, low organic content (FLL, 2008) and rapid leaching of nutrients, due to the substrate’s free draining nature (Hathaway, Hunt, & Jennings, 2008). The shallowness of the substrate compounds these limiting factors, as small rooting volume further limits resource availability for plant survival and growth (Hathaway, et al. 2008; MacIvor & Lundholm, 2011). Recent studies have demonstrated elevated substrate temperature at shallow depth (Dunnett & Kingsburry, 2010), and their negative impact on plant survival (Zhang et al., 2014) and growth (Dunnett et al., 2008) and cover (Heim & Lundholm, 2014a). Therefore, the extreme green roof environment is the key filter in determining which species survive and grow, and thus the vegetative performance of the green roof system and its ecosystem service provision.

High vegetation performance on green roofs is characterized by a continuance of species rich, high cover (Dunnett et al, 2008; Kohler 2006; Lundholm et al, 2010). Cover is essential to the thermal buffering capacity of buildings and rainfall interception ecosystem services. Plant cover influences total green roof energy balance (Yaghoobian & Srebric, 2015), via a number of mechanisms. Cover increases insulative properties (Lundholm et al., 2010), while plant mortality reduces thermal insulative properties (Speak et al., 2013). Cover reduces substrate surface and sub-surface temperatures, via increased albedo (Buckland-Nicks, Heim & Lundholm, 2016). In a Mediterranean climate Olivieri et al., (2013) demonstrated that, high cover can reduce substrate thermal gain by 60%, in comparison to nonvegetated substrate. Nardini et al., (2012) confirms that vegetated modules outperform non-vegetated modules in their ability intercept rainfall, and showed greater than 90% capture following intense precipitation events. However, while high cover is desirable for these key ecosystem services, recent studies suggest that heterogeneous patchy cover, would support a more diverse fauna, and contribute to biodiversity ecosystem service (Buckland-nicks, Heim & Lundholm, 2016).

Research shows that while cover is the main determinant of thermal building insulation and storm water mitigation, species richness enhances cover and therefore these services. Species richness has a direct positive influence on biodiversity and conservation ecosystem services (Williams, Lundholm & MacIvor, 2014), and in conjunction with cover, could promote aesthetic ecosystem service (Lee et al., 2014; Lundholm, 2011).
Cooke-Pattern & Bauerle (2012), have found that species richness yields greater ecosystem service function than monocultures, and that reduced biodiversity is associated with simple plant systems (Knops, 1999). For example species mix is demonstrated as:- better able to retain cover than monocultures, (MacIvor, 2013; Bevilacqua et al., 2015), and yields greater survivability (Nagase & Dunnett, 2010). Further to this, MacIvor (2013) offers the explanation that species diversity enables positive response to environmental change. The species rich cover advantage has been demonstrated in on ground grassland community dynamics studies, which show that species richness provides an asynchrony in species biomass production over time. This is explained as individual species positively responding to changes in environmental conditions, thus maintaining stable cover over time (Hautier et al., 2014; Moore, 2014). Therefore species richness contributes to stability of cover and ongoing ecosystem service provision.

While green roof species richness is commonly attained through horticultural methods, a growing body of research regards spontaneous colonization of green roofs as a source of species richness. Although colonizing weeds were viewed as a competitive threat to installed species, research now indicates that colonizing vegetation is useful in elevating species richness and in replacing cover lost as plants perish (Bevilacqua et al 2015; Lundholm, 2015a; Nardini et al., 2012). By filling niche space, canopy density is increased, which is positively correlated to storm water interception and thermal buffering (Lundholm, 2015b). Spontaneous colonization benefits are not just limited to cover and species richness, but have been shown to increase abundance (Mechelen et al., 2015a). However, spontaneous colonization is unlikely to apply where dense covers already exist (Miller et al., 2014). Over a four year study Heim and Lundholm (2014b) found that community dynamics change over time as ruderal colonizers dominate the niche gaps, and that as time advances the stress tolerant species become the new dominant groups. These results indicate spontaneous colonization could have a temporal role in maintaining species richness. However, at a seasonal time scale where annuals may be lost and recruited, continuous yearly cover may not be attained (Bevilacqua. et al., 2015).

Identifying species capable of contributing to high cover, which are able to survive and grow within the limiting abiotic boundaries of the green roof environment, is crucial to a functioning green roof system. Nagase and Dunnett (2010) make the observation that early plant selection studies analyzed individual plant species. Physiological and morphological traits that predict survival and growth under green roof stress conditions are still valuable and commonly involve mature plant trait analysis (Dunnett et al 2008; Graceson et al., 2014; Lundholm et al., 2014a; Mechelen et al., 2014; Nagase et al.,2010). The Sedum genus is widely represented in studies due to their survivorship, rather than their ability to deliver multiple ecosystem services (Bevilacqua et al., 2015). A shift to plant selection based on the plant’s ability to provide ecosystem services evolved. For example, Lundholm, Tran and Gebert (2015) determined, that plant height and specific leaf area were the most
useful traits to predict growth rate and canopy density; and find positive association between plant height and rainfall capture. Farrell et al., (2013) equated vegetative performance to the ability of the plant to take up water when abundant and also tolerate drought conditions, implying not only survival but indicating rainfall capture. Traits have also been investigated with regard to optimizing thermal building buffering. For instance studies found that: - species varied significantly in their influence upon albedo associated with substrate cooling MacIvor and Lundholm (2011) and in ability to insulate from heat (Buckland-Nicks et al., 2016). Many recent studies have investigated collective plant type ability to provide ecosystem services, as a broad base for species section. Studies reflecting this approach find: plant types vary in their potential to cool substrate (Blanusa et al., 2013); grasses perform better for all rainfall interception and thermal buffering functions above those of creeping shrubs and forbs (Van Mechelen et al., 2015); herbaceous plants having higher transpiration rates than Sedum species, and more rapidly remove water from the substrate (Nandini et al., 2012) and plants showing some degree of succulence, are a recommended priority trait for plant selection (Farrell et al., 2013a).

Therefore, green roof plant selection has been greatly informed through research identifying plant traits and types that contribute toward ecosystem services.

However, while making valuable contribution, basing selection upon plant trait and type, on its own does not guarantee a specie rich, high cover plant community. Species selection may not be compatible at a community dynamics level or in delivering ecosystem services. Multiple species mix studies have indicated potential species incompatibility as a reason for diversity loss over time (Dunnett et al., 2008; Lundholm et al., 2010; Rowe et al., 2012). A recent study suggested species mix incompatibility may lead to greater loss of abundance than for species grown as a monoculture (Heim et al., 2014b). In a biotope green roof study in Japan, results indicated loss of forbs due to competition from ruderal colonizing species and from drought (Nagase and Nomura, 2014). While individual species may not deliver multiple ecosystem services, as a collective community, compatible species may. This is illustrated in that, creeping shrubs and tall forbs have greater water capture ability than sedums; yet conversely sedums provide greater substrate cooling benefit than creeping shrubs and tall forbs (Lundholm, Tran and Gebert, 2015). Further, it is advocated that functional diversity between species is a positive contributor to multiple ecosystem services (Hooper et al., 2005; Lundholm, 2015). Mechelen, et al., (2015b), develops the argument that varying functional trait diversity, as varied as flower colour, leaf dry mass, succulence, life form, shoot growth form and drought tolerance, as a selection process would improve functional diversity. A second advantage in a functional diversity approach to
species selection, is that it creates potential for positive facilitative relationships between species, optimizing species richness through increasing species survival (Mechelen et al., 2015b). For example, Mechelen et al. (2015b) found that bryophytes facilitated the establishment of geophytes; and similarly Nagase and Dunnett, (2013) found that sedums improve the emergence of geophytes. These results suggest that the co-existence of species can confer community resilience, through modifying the micro climate conferring species survival; for example, lichen and moss significantly cool the substrate (Heim and Lundholm 2014b). Species responses as indicators of ecosystem services will vary according to climate or region (Zhao et al., 2014). Therefore species selection studies may be most useful at regional climate level. Overall, this research highlights the importance of selecting a mix of species which are functionally diverse, and highlights the need for those species to be able to co-exist.

Recently the species selection process has been placing emphasis on species that are functionally diverse and able to co-exist. The importance of functional diversity is that it imparts multiple ecosystem service delivery; the importance of the species co-existence, is in conferring long term resilience to the vegetative component of the green roof system (Heim and Lundholm, 2015b). The co-existence of species within a community assumes two conditions: - that species are able to compete for the same resources (Cheeson 2000), and secondly that species show resilience under the same environmental pressures (Thorn et al., 2015); to realize long term ecosystem service provision, the plant community must show resilience through co-existence. This requires that the intra and inter species relationships are facilitative or neutral, and negative species interactions such as competitive exclusion are minimized (Thorn et al., 2015). As yet, functional traits do not adequately inform predictions of community assemblage patterns, such as distribution and abundance, (Cardotte et al., 2015a). As green roof species mixes are novel, prediction of community dynamics are largely untested (Carlsile & Piana, 2015), and therefore, green roof resilience unknown. Ecological theory suggests that species emanating from a single habitat pool, have already evolved to co-exist (Walker and del Moral, 2003). Selection of species from a single habitat analogous to green roof abiotic conditions, where the pool of species have evolved to co-exist for limited resources, under the similar environmental constraints, could be a successful strategy for creating a resilient green roof novel community. Mechelen et al., (2014) has developed this selection process in kind, based on pooled species drawn from an analogous habitat and then imposes functional diversity filters. Oberndorfer et al (2007) identifies ideal analogous habitat pools as dry grassland, coastal, and alpine habitats, to this may be added rocky outcrops (Farrell, et al., 2013a). Most recently, species selection based on co-evolutionary relationships rather than species traits has been advanced. This phylogenetic approach could target selection for plant performance (MacIvor et al., 2016) and predict community assemblage patterns (Cadotte, 2015b) although currently is largely unexplored. Overall, engaging a selection approach, which sources a functionally diverse
species mix from an analogous habitat pool, will promote co-existence of species in a novel community and continuance of multiple ecosystem service provision.

A direct seeding application of species mixes, may contribute towards optimal species co-existence, in a novel green roof community. Community dynamics that impart system resilience, is achieved through targeting species from analogous habitats (Dewey, et al 2004; Dunnett & Kingsbury, 2004; Francis, 2010; Hathaway, et al., 2008; Lundholm et al., 2010), however, direct seeding would enable community mechanisms that confer resilient early community assemblage. This is supported in seedling ecology theorizing that, when seedlings dominate vegetation as in a pioneering community, that the seedlings are the major determinant of species spatial heterogeneity and species interactions (Boeken, 2008). In this light, direct seeding has community assemblage advantage over adult plant establishment. The low cost of direct seeding affords abundance of species rich propagules, and in the randomness of distribution, provides for early community dynamics above that of adult plant installation. Direct seeding is a proven establishment method (MacIvor et al., 2013), having many benefits; as a highly cost effective green roof establishment method (Nagase & Dunnett., 2013; Sutton, 2013). Direct seeding of functionally diverse species, pooled from an analogous habitat, provides opportunity for species co-existence and community resilience that can provide multiple ecosystem services. This makes direct seeding onto green roofs a very attractive approach to green roof system establishment.

Dry grassland, meadows and prairies are habitats with abiotic conditions similar to green roofs, and are widely viewed as suitable green roof vegetation (Dewey et al., 2004; Lundholm et al., 2004; Sutton et al., 2012; Williams et al., 2014). Identification of meadows as a preferred green roof aesthetic (Lee et al., 2014), is an additional incentive for green roof grassland establishment. However, grassland establishment and vegetation performance knowledge on green roofs is limited. While grassland graminoid and forb species have been variably used in green roof research; this has rarely been at habitat level; and often in researching functional diversity, utilizing adult plant establishment rather than direct seeding. Mostly, grassland species are mixed with exotics or other natives from unrelated habitats (Lundholm 2015a; Razzaghmanesh, Beecham & Brien, 2014), or as a mix of grassland habitats ( Nagase & Dunnett, 2012; Nagase & Nomura, 2014; Wacker et al., 2007), as wild spontaneous colonizers (Madre et al., 2014; Lundholm et al., 2015a), or tested as monocultures or as few as three species (Heim & Lundholm 2014a; Miller, Heim & Lundholm, 2014), but rarely as a species rich community emanating from a single grassland. These few studies include: - all forbs (Ksiazek et al., 2014), all grasses (Sutton, 2013), as a grass/forb mix (MacIvor et al., 2013). Research lacking at a green roof grassland habitat level, is mirrored in a lack of existing grassland green roofs of single habitat origin. This was shown in Sutton’s (2012) analysis of green roof vegetation habitat origins; where wide use of grassland/prairie species on green roofs was evident, but not as a habitat regional mix. In contrast green roofs in the Toronto
region are commonly direct seeded with grasses and forbs and reportedly successful (MacIvor et al., 2013). Few studies involve testing direct seeding of grassland species in green roof conditions, response is varied and consensus on method has yet to be reached. For example; seed shallowly sown and non-irrigated, showed greatly reduce survival and species richness (Monterusso, et al 2005); and following a two year post direct seedling grass/forb community study, cover was found to be reduced on FLL green roof substrate (MacIvor et al., 2013). In this case a seed mix was evenly applied to the green roof substrate surface and covered with 3 mm of planting media. Seeding was performed twice within a six week period and irrigated up to three times per day. Results from this study indicated species diversity was not affected by green roof substrate, but by irrigation availability. In another study, forbs showed improved growth when grasses were less dense (Sutton, 2013). Seed was applied in a fine sand mix at a depth of 1 inch and in drill lines 4 inches apart. Results showed that plugs reached 80% cover two months earlier than by seeding, and that establishment was not successful due to bird predation, and determined that seedling establishment requires irrigation.

Few long term studies investigate the community dynamics of novel green roof grasslands. More generally, novel green roof community’s assemblage mechanisms have been shown to differ over time, and with resource availability (Meiners et al., 2015; Thorn et al., 2015). A study in Utah, showed evidence of competitive exclusion, as grasses tended to outcompete meadow wildflowers (Dewey et al., 2004). Significantly, species diversity in a novel grassland green roof community was found not to be a driver of cover development (MacIvor et al., 2013). Continuance of ecosystem service provision in the long term is not yet proven, because of sparse research producing varied results. For example, two long term studies show diversity loss over time, however one grassland/forb community continued to provide ecosystem service provision (MacIvor et al., 2013); and in contrast, another study finds resulting poor vegetative outcomes (Kohler, 2006).

Understanding direct seeding grassland techniques on ground, may inform green roof direct seeding method; although these novel green roof habitats may only bare some similarities to on ground habitats from which species are derived (Olly et al., 2011). Local research has made important contribution in identifying on ground grassland seeding technique and community assemblage patterns. In Australian grasslands, perennial grasses as key stone species influence assemblage (Silvertown, 1981). Grasses and forbs make up approximately 50% of the community, and around 50% of forbs are represented in the family Asteraceae, with grasses increasingly dominating the system over time (Gibson-Roy & Delpratt, 2015). An example of grass domination is evident as grasses reducing geophyte growth through competitive exclusion (Hughes, 1986). Soil fertility is reported as promoting a greater growth response for grasses than for forbs (Prober et al., 2005). This implies that grasses in a green roof system could limit species richness, functional diversity, and ecosystem service provision in the longer term. Selection of species mixes with diverse functional groups has been observed to contribute towards
species richness on ground (Gibson-Roy, Delpratt & Moore 2007a). Research shows that, species richness allows for an asynchronous response to environmental changes, yielding stable production (Moore, 2014). In support of this, research has found that synchronous plant growth decreases stable production over time (Hautier et al. 2014); these findings have application towards maintaining constant cover on green roofs.

On ground grassland direct seeding methods have proven successful (Prober et al., 2005). Research has identified successful seeding application method. For instance, a diverse mix of species at 300 seeds/m² yields quick establishment, with fresh seed showing greater germination (Gibson-Roy & Delpratt & Moore, 2007a). The optimal sowing period for the dominant key stone grass species, is air temperature at 25°C, when soil moisture is at field capacity, and with weed competition negated (Cole and Lunt, 2005). Germination success was found to heavily depend upon weed load reduction (Gibson-Roy, Delpratt and Moore, 2010) and well prepared moist seedbed, with good soil –seed contact (Gibson-Roy, Delpratt & Moore, 2007b). Additionally, fresh seed contributes to a synchronous emergence (Gibson-Roy, Delpratt and Moore, 2007a). With regards to depth of sowing, research has shown that many forbs need light to germinate, advancing a case for surface sowing application (Clarke et al., 2000). The on ground grassland knowledge has importance, as informing species selection and as indicators for green roof grassland seeding technique and community establishment. Further, this knowledge may serve as a baseline to evaluate species response when directly seeded onto green roofs.

In general, research of direct seeding as a green roof establishment method has focused three key areas; on comparative benefits to adult plant installation; on germination response to green roof substrate, and species richness and cover response to irrigation and fertilizer applications. Reports of direct seeding success varies. For instance; direct seeding offers wide temporal establishment opportunities (Sutton, 2013), improved time and cost efficiencies in establishment and maintenance (Dewey et al., 2004; Lundholm, 2011; Sutton, 2013), and increase ecosystem function delivery (Mechelen et al., 2015). However, research focusing on the ability of green roof substrate to act as a safe site for germination, shows mixed and at times conflicting results. Many studies find that green roof substrate is a poorly performing germination media. Harp, Chen and Jones (2015) in a trial limited to three species, finds a negative correlation between seed length and width and abundance at germination. The authors of this study suggest that the porous substrate may not allow for the germination or emergence of fine seed, as it could be washed through the substrate. MacDavid (2012) indicates that the free draining nature of the substrate, makes maintaining a moist seed bed difficult, and low germination results. Similarly, a study using native forb grass mixes found that, green roof substrate reduced abundance of seedlings post germination, but did not affect species richness (MacIvor et al., 2013). In contrast, another study found species richness was greatly reduced (Monterusso et al., 2005). Indications of green roof substrate as a poor
germination media, led to studies investigating substrate additives, with a view to achieving more positive germination outcomes. Studies have shown substrate additives as improving germination are; hydrogel (Mechelen et al., 2015), and hydromulch (McDavid, 2012). It is now well accepted that irrigation is required when direct seeding green roofs if seedlings are to survive (Dunnett, Nagase & Hallam, 2008; Sutton, 2013) and that overhead irrigation is the most effective method of delivery (Rowe et al., 2014). While irrigation increases seedling survival, one study finds that irrigation decreases diversity through promoting competition (MacIvor et al., 2013). In relation to irrigation association with cover, a study has indicated that cover varies with manipulation of irrigation and sowing rate. For example, high irrigation requires low sowing rate to achieve high cover, and low levels of irrigation requires high sowing rate, to produce high cover (Nagase & Dunnett, 2013). Additionally, research indicated that fertiliser application is associated with a loss of diversity, due to some species showing a competitive exclusion advantage (Bates et al., 2013). While many studies inform green roof direct seeding methods, much remains unclear and unknown. It must also be considered that direct seeding may not be applicable in all situations. For example; some desired species may only be reproduced via vegetative propagation, or predation of seed and germinants at lower green roof elevation may render direct seeding abundance diminished (Sutton, 2013). Further, direct seeding may be a useful establishment technique in conjunction with selective adult plant trait installation, where adult plant establishment could modify microclimate and substrate conditions and aid facilitation of species germination.

1.1 Research opportunities

Research has greatly contributed towards understanding plant functional traits and types that promote survival and growth on green roofs, as indicators of vegetative performance. As an extension, plant traits have been identified that promote green roof functional effectiveness. Key indicators of functional diversity traits are species richness and high cover. This body of knowledge is large and continues to grow. Recent recognition that a species rich, functionally diverse species mix can promote vegetation performance and green roof system function, is driving a new research focus. Investigation of the ability of species to co-exist and thus confer resilience to the green roof system has become a current theme of research. This requires viewing green roof vegetation as a dynamic community, of species interactions, a product of distribution, abundance, resource availability and time. The novel green roof community must aim for facilitation of relationships towards co-existence; and minimize competitive relationships that decrease species richness and functional diversity.
Wide novel community assemblage dynamics knowledge gaps exist. While potential for grassland green roofs to deliver ecosystem services is accepted, conflicting research exists about the degree of vegetation performance that can be achieved. Community assembly of species and functional groups within green roof grassland systems, is largely unknown.

These knowledge gaps highlight the need to trial a selection of functionally diverse species pooled from grassland habitat, and evaluate community dynamics, over the long term. Such studies are crucial to achieving species rich, high cover vegetation performance in these novel communities, and in determining contribution towards multiple ecosystem service provision. Community assemblage studies are important to informing green roof system design processes. The role of grasses in providing ecosystem services needs to be weighed against grass species dominating forbs over time and resulting diminished ecosystem services. In the short term, the degree to which green roof substrate supports germination and early seedling establishment requires clarification. The absence of direct seeding application methodology to green roofs is vast, and in relation to performance outcomes, is viewed as an impediment to industry implementation (Harp, Chen & Jones, 2015; Nagase & Dunnett, 2013). Sowing method studies should target seeding application, depth of sowing and sowing rates. It is suggested that the effectiveness of sowing methods, be measured against the green roof vegetation performance indicators, species richness and cover; and also in terms of seedling abundance and survival. Therefore it is recommended that, future research in the short term, evaluate green roof substrate as an effective germination media, and identify effective direct seeding sowing methods that yield species rich high cover. In the long term, the novel community assemblage dynamics which determine a species rich co-existence and resilient vegetation performance is advocated as a predictor of ecosystem service provision. This research should be repeated for a number of green roof analogous habitats and across variable climatic regions. Interest in grassland habitat application is evident, and this habitat would be an apt place to begin.

1.2 Aims and research scope

The focus of this research was on determining the ability of green roof substrate to provide safe site conditions for the germination and establishment of a species rich, grassland forb community, without a grass component. As a means for establishing grassland forbs onto green roof substrate, direct seeding methods were explored and evaluated in terms of the vegetation performance indicators:- species richness, abundance/survival and high vegetation cover. With cover a key green roof vegetation performance goal; potential association between response variables species richness and abundance with cover were also explored.
Two experiments were undertaken: Experiment One in stable glasshouse conditions and Experiment Two in green roof modules under variable outside conditions with irrigation.

There are two broad aims of the study. The first aim was to determine the ability of a scoria base green roof substrate, to support the germination and establishment of diverse grassland forb species; and to identify direct seeding methods to achieve this. The second aim investigated association of species richness and total seedling abundance as drivers of cover.

The first three objectives evaluate grassland forb species and community response to:

[1] Sowing application methods and species richness and abundance response (Experiment One)

[2] Depth of sowing and species richness and abundance response (Experiment One and Two) and also cover response (Experiment Two).

[3] Sowing rate under species richness, abundance and cover response (Experiment Two)

2. Methods and Materials

Two quantitative manipulative, experiments, of complete randomized block design, were carried out from September 2015 to March 2016. Both experiments investigated grassland forb species abundance and species richness in response to varying sowing methods over green roof substrate; Experiment One in a greenhouse trial and Experiment Two in variable outside conditions. In addition to varying sowing method, Experiment Two included sowing rate as an independent variable, and the response variable total cover was investigated also. Prior to carrying out the experiments, germination tests were performed in order to identify germinable species available for selection; to determine seed masses required for sowing rates; and to establish a baseline for comparison of species abundance for each sowing method/rate. (Appendix 1). The study site was the Burnley Campus Nursery of the University of Melbourne, South Eastern Australia. (Lat. 37.80 S’, Long. 145.00 E’), elevation 18meters (BOM, 2016 a). The climate is temperate with annual maximum temperature range of 26.6 °C February to 14.2°C July and minimum range 16.0°C February to 7.1°C July; annual rainfall is 602.6 mm (BOM, 2016b).

2.1. Species selection

A habitat template approach to species selection was undertaken and series of four major filters applied. This was an iterative process, where a final selection of 10 grassland forb species were identified for experimental inclusion (Table 1). Species of forbs were selected from local plants represented within the temperate grassland systems of the western volcanic plains, (Vic. Gov., 2016) Forbs are defined as herbaceous flowering plants, not graminoids, rushes and sedges. In order to achieve a species richness response within the limitations of the small experimental unit areas, small forbs approximating less than 30cm height were included; and graminoids which tend to dominate these systems over time were excluded (Gibson-Roy & Delpratt, 2015). Species selection criteria aimed for diversity of taxa. Habitat community assemblage reflects, Asteraceae as the most dominant forb species selected in this study (Vic. Gov., DSE, 2016). Plant life form (Raunkier, 1934) and below ground morphology were also diversified (Figure 1).
An additional, selection criteria of floral - vegetative aesthetic quality was applied (Table 1). The final selection filter, determined availability of quantity and quality of seed through germination tests (Appendix 1). Ten forbs varying in taxonomy, life form and below ground morphology were selected (Table1). This seed was purchased from Western Plains Flora Pty, Wildwood, and Victoria and also provided by Mr. J. Delpratt, University of Melbourne.

Table 1
Perennial grassland forb species selection criteria. Life form persistent bud perennation: Chamaephyte= above ground surface and plant 25 - 30 (cm) tall; Hemicryptophyte= at soil surface; Geophyte= underground, includes shoot apices. Taxa as per APNI (Aust. Gov. ANBG, 2016); *= Applied in Experiment two only.

<table>
<thead>
<tr>
<th>Species Taxa</th>
<th>Life –form Raunkiaer (1934)</th>
<th>At and Below Ground Morphology</th>
<th>Aesthetic Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocotyledon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arthropodium strictum R. Br. Anthericaceae</td>
<td>Geophyte</td>
<td>Tuberous root</td>
<td>Violet chocolate scented flowers September to December, grass like blades</td>
</tr>
<tr>
<td>Eudicotyledon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calocephalus lacteus Less. Asteraceae</td>
<td>Hemicryptophyte</td>
<td>Stolons and rhizomes</td>
<td>Grey leaves, oblong white flower heads, September to February</td>
</tr>
<tr>
<td>Eryngium ovinum A. Cunn. Apiaceae</td>
<td>Hemicryptophyte</td>
<td>Tap root</td>
<td>Globular flower heads. Metallic blue bracts August to February</td>
</tr>
<tr>
<td>Leptorhynchos tenuifolius F.Muell. Asteraceae</td>
<td>Chamaephyte</td>
<td>Fibrous</td>
<td>Single yellow button flower heads, September to January</td>
</tr>
<tr>
<td>Linum marginale A. Cunn Linaceae</td>
<td>Geophyte</td>
<td>Thick tuberous roots</td>
<td>Narrow blue-green leaves, terminal clusters clear blue flowers, September to May</td>
</tr>
<tr>
<td>*Microseris lanceolata (Walp.) Sch. Bip. Asteraceae</td>
<td>Geophyte</td>
<td>Tuberous root</td>
<td>Bright yellow flower head, July to November + spot flow March</td>
</tr>
<tr>
<td>Plantago gaudichaudii Barneoud. Plantaginaceae</td>
<td>Hemicryptophyte</td>
<td>Tap root</td>
<td>Rusty brown flower spikes August to October but flowering in March</td>
</tr>
<tr>
<td>Podolepis jaceoides (Sims) Voss. Asteraceae</td>
<td>Hemicryptophyte</td>
<td>Thick corm and roots Fibrous</td>
<td>Deep green lanceolate leaves, large bright yellow florets September to October</td>
</tr>
<tr>
<td>Vellia paradoxa R. Br. Goodeniaceae</td>
<td>Chamaephyte</td>
<td>Fleshy tap and rhizomatous roots</td>
<td>Oblanceolate light green leaves, pale yellow orchid like floriferous Spring &amp; Autumn</td>
</tr>
<tr>
<td>Wahlenbergia communis Carolin. Campanulaceae</td>
<td>Hemicryptophyte</td>
<td>Fleshy tap and rhizomatous roots</td>
<td>Long flowering period blue/purple star like flowers with white throats</td>
</tr>
</tbody>
</table>
Figure 1: Habitat pool and diversity selection process. This is an iterative process, with five selection criteria applied to forb species represented in local grassland habitats: Ecological Vegetation Classes EVC 132_61 Heavier Soils Plains Grassland and EVC 132_63 Low Rainfall Plains Grassland (Vic. Gov. 2016). 1= Determine from germinability tests, that seed availability was sufficient to supply both experiments; 2= When germinability was low and seed availability was insufficient, a return to the diversity filters for reconsideration of the species selected.
2.2 Sowing rate and seedling target (ST)

For this study, it was important to calculate the necessary sowing rate to ensure adequate seedling germination and emergence. Sowing rate is 25% higher than on ground grassland restoration rate of 300 seeds/m² (Gibson-Roy et al., 2007b). In consideration of research indicating green roof substrate as negatively impacting germination (Harp et al., 2015; McDavid, 2012), and specifically reducing abundance of grassland /forbs (MacIvor et al., 2013), this higher rate is justified. In order to satisfy statistical analysis in the event of low emergence and high seedling mortality, a target of ten germinable seeds for each of the 10 selected species was determined. The resulting sowing rate was 400 seeds per meter², equating to 100 seeds/ green roof module. *M. lanceolata* was excluded from Experiment One only, as it was not available in sufficient quantity.

Mass of seed or uncleaned seed required, to achieve a minimum of 10 germinable seeds for each species, was determined with sown seed and rolled paper germination tests in August 2015. Sown seed germination tests were carried out for species with seed that was uncleaned, or of small size (Figure 2). Seed was sown onto (pasturised standard nursery grade) seed raising mix, covered with coarse vermiculite and watered. The remaining species underwent rolled paper tests, where 25 seeds of each species were placed onto moist test paper, inside Chux Superwipes. Both tests had four replicates, and were placed into non-temperature controlled glasshouse conditions, with natural light reduced to 70%, upon a heat mat at 24°C, with mist irrigation applied. Glasshouse temperature varied from a minimum 9 to 11.5°C and a maximum of between 20 to 25°C over four weeks. Emergence was recorded at 28 days and percentage emergence for each species calculated. For the 10 final species selected, germination means and coefficients of variation were used to calculate individual species seed masses required to deliver a minimum of 10 seedlings. This seedling target of 10 individuals for each species is the baseline form which abundance within species is compared across sowing methods/rates (Appendix 1).
2.3 Experimental Design

2.3.1 Experiment One - Glasshouse Trial

During September and October 2015, this study investigated species abundance and species richness response to six sowing method treatments over green roof substrate. The substrate meets construction standards (FLL, 2008), is composed of 60% eight (mm) minus and 20% 7mm minus scoria, to which 20% coconut coir was added. Physical properties of the substrate are: - pH = 6.9 ± 0.03; WHC% = 45.9 ±0.3; AFP% = 13.8 ± 0.5; DBD = 1.26 g/cm3 ± 0.01; EC = 0.14± 0.003 dS-2; n=3 (Farrell et al., 2012), and provided by Biogrow, Australia.
The sowing method varied two factors:

1) The amount of sand used to apply the seeds, and
2) The depth of application

Seed was applied with horticultural sand (particle size four to five minus; Daisy’s Garden Supplies) used as either a seed distributor (D) 0.6 mm deep or as a seed bed (B) 5.0 mm deep.

Seed was applied to the scoria substrate at three depths: surface (S); ‘mixed into top 10 mm (M) of substrate; and covered (C) by 10 mm of substrate (Figure 3b). The six sowing treatments and two seed raising mix controls are therefore:

- DS – seed distributed in 0.6 mm deep sand on the surface
- BS - seed distributed in 5.0 mm deep sand on the surface
- DM – seed distributed in 0.6 mm deep sand and then mixed into the top 10 mm of substrate
- BM - seed distributed in 5.0 mm deep sand and then mixed into the top 10 mm of substrate
- DC – seed distributed in 0.6 mm deep sand and then covered by 10 mm of substrate
- BC - seed distributed in 5.0 mm deep sand and then covered by 10 mm of substrate
- DS(R) – seed distributed in 0.6 mm deep sand on the surface of seed raising mix
- BS(R) - seed distributed in 5.0 mm deep sand on the surface of seed raising mix

The (pasturised standard nursery grade) seed raising mix was produced by the Burnley Campus Nursery. One replicate per block was randomly repeated for seven blocks (Figure 3a). The 56 experimental units were ½ tray punnets 32 cm x 13.5 cm x 5 cm deep, filled to a depth of 4 cm. A seed mix of nine native grassland forb species, at ST masses, contained a total minimum of 90 germinable seeds (Appendix 1). The punnets were placed onto heat mats at 24°C, in a non-temperature controlled glasshouse with natural light reduced to 70% and mist irrigation applied twice a day.
Figure 3a left: Experiment One design layout. A complete randomized block design; n=56; The six seeding treatments on the nearest block, clockwise from top left: BC= sand/seed bed covered by 10 mm, BM= sand/seed bed is mixed into top 10 mm, DC= sand/seed distributor covered by 10 mm, DM= sand/seed distributor mixed into top 10 mm, BS= sand/seed bed at surface, BS(R)= sand/seed bed at surface of seed raising mix, DS= sand/seed distributor at surface, DS(R)= sand/seed distributor at surface of seed raising mix. The controls are: DS(R) and BS(R)

Figure 3b right: Sowing depth in punnet. Sowing methods varying depth of sand/seed applications in a punnet: S= at surface, M=mixed into top 10 mm and C=covered by 10 mm.

2.3.2 Experiment Two - Nursery Field Trial

Experiment Two investigated species abundance and species richness, and total cover response to sowing method varying depth of sowing and sowing rate over green roof substrate 150 mm deep in green roof modules. The experiment was conducted at the Burnley Nursery outside facility, during late spring November 27th, 2015 to early autumn, March 4th, 2016. The sowing method levels were ‘S’= seed applied at surface and ‘M’= seed mixed into top 10 mm of substrate. The second factor varies sowing rate, at 100 germinable seeds (10 germinables X’s 10 species), and at 200 germinable seeds (20 germinables x’s 10 species) per module. The four seeding treatments; S100, S200, M100 and M200 were represented randomly once in each of 10 blocks. The 40 experimental units were plastic green roof modules, HDPE boxes, (50 cm x 50 cm x 20 cm depth); fitted with an Elmich drainage liner, overlaid by geotextile liner five mm (Geotextile Australia, Melbourne).
Blocks were placed on wire benches for ease of vegetation survey to reduce weed seed rain burden. Modules were at a slope of between two to four degrees east. Five substrate only modules were placed between groups of two blocks, as controls, (Figure 4).

One litre of water was applied to each module by hand at sowing. During the first week post sowing hand watering at 1 X’s 1.5 L and 3 X’s 0.5 L /module was delivered per day. At the end of the first week post sowing overhead irrigation was applied, delivering 0.6 L /minute for four minutes once per day. However, frequency of irrigation was varied in an attempt to retain seed bed moisture under varying weather conditions. Bird netting tunnels were erected over benches at week two following sowing. On seedling establishment at week 8, netting was removed and irrigation frequency and volume reduced and varied to maintain plants above wilting point. Two slow release pelletized fertilizers applied at 1/3 recommended rate were broadcast over modules: OSMOCOTE PRO for Natives, NPK Mg 15 : 3 : 12 + 3Mg + TE micronutrients Fe Cu Zn Mn B; 12-14 month slow release (low P) applied four weeks post sowing and secondly, OSMOCOTE Exact Patterned Release Fertilser, 3rd Generation, NPK 15:9:12 + 2Mg + TE micronutrients Fe Cu Zn Mn B, (EVERRIS International, The Netherlands), was applied eight weeks post sowing.

Weeds were identified and removed at surface level in experimental units each week, and removed from spontaneous seeded plant modules once identified. Over this time, monthly maximum temperature ranged from 16.0 – 42.2°C and minimum ranged from 8.0 to 25.9 °C (BOM.2016 c).
2.4 Vegetation Survey

2.4.1 Experiment One – Seedling abundance and species richness

Number of individual seedlings was counted at seven day intervals across seven weeks post sowing, from 24\textsuperscript{th} September to 8\textsuperscript{th} November. Cumulative running mean and rate of community population change was calculated each week, and the point at which population plateaued for all eight treatments identified. This point indicated approaching population mean for the seedling emergence phase (Stohlgren, 2007) and species taxa abundance survey was undertaken. Species richness is measured as a count of the total number of taxa present per punnet out of nine taxa.
2.4.2 Experiment Two - Seedling abundance and species richness

Survey was undertaken over two consecutive days each week, and blocks randomly assigned to each day, survey beginning week two post sowing - 22nd December 2015, through to week 18- 2nd March 2016. Surveys took place. This was a visual mapping survey appropriate to the small surface area of the modules. (Gibson D. J., 2002). The quadrat frame (50cm X 50cm) with metal rods evenly spaced created 25 internal quadrants (10cm X 10cm). Species identity and distribution was charted onto consecutive quadrat maps to indicate abundance for each species taxa, weekly from week’s two to eight then fortnightly to week 18. Permanent markers were not used due to potential for root damage as markers pushed down upon large scoria particles. An additional problem with this method would occur when percentage cover is high, and markers become difficult to find (Sutherland, 2006).

Cumulative running mean and rate of community population change was calculated each week, and the point at which population plateaued for all eight treatments identified, and a seedling abundance survey was undertaken at week 4. These surveys continued fortnightly to week 18. Species richness is measured as a count of the total number of taxa present per module ten taxa.

2.4.3 Experiment Two - Cover

Total vegetative cover is taken as one horizontal layer across the module substrate surface. . The survey was undertaken between weeks eight to week 18, when real total cover is within a range of 15 – 85 %, as accuracy at these percentage covers compares well to objective photographic measurements (Bullock, 2006). The visual cover estimates are well suited to the small surface area of the modules and the small form of species selected in this study, it is repeatable across time and is time efficient. Precision is maximized via photographic assessment, where vertical distance above modules is 40 cm and photos are cropped to 50cm x 50cm (Adobe Creative Cloud Photoshop CC, 2015 Release) A perspex sheet (10 X 10 grid), overlayed the computer screen image. Stohlgren (2007) reports that precision of visual cover estimation is increased in grassland by using large % cover value
and smaller quadrants. Therefore cover is estimated in each of the 100 quadrants, at real scale equal to 2.5 cm²:-
greater than or equal to 50% vegetation occupying a quadrant, scores 1% of cover, and each quadrant with less
than 50% cover scores zero % cover. Quadrant percentage scores are summed and correction made for the area
covered by the module handles to give a total of 88 quadrants/ module.

2.5 Statistical Analysis

In Experiments One (n=7) and Two (n=10), repeated One way ANOVAs (a=0.05), tested between sowing
treatments effects and was repeated for each species. A Fischer Exact Test (a=0.05) was carried out to ascertain
significant difference between treatments as species abundance and species richness responses.
A Linear Regression analysis was conducted to determine: - the relationship between seed dimension and
seedling abundance. All statistical analyses were carried out with Minitab Release 17 software.

3.0 Results

3.1 Experiment One

During the study period September and October 2015, mean weekly temperatures were a minimum of 15.9°C,
and maximum of 29.9 °C.

3.1.1 Emergence

Seedling emergence response was synchronous across sowing methods and controls DS(R) and BS(R). A large
rapid emergence occurred in week two, followed by mortality in week four. A population plateau occurred
across all treatments in week seven (Table 2), indicating mean maximum population of seedlings had been
reached; and an individual species survey was undertaken at this time (Figure 5)
### Table 2: Seedling abundance variation during emergence. Treatments are over green roof substrate unless specified. DS= sand/seed distributor at surface, BS= sand/seed bed at surface, DM= sand/seed distributor mixed into top one (cm), BM=sand/seed bed mixed into top one (cm), DC=sand/seed distributor covered by one (cm), BC=sand/seed bed covered by one (cm); Controls: DS(R) sand/seed distributor at surface over seed raising mix, BS(R)= sand/seed bed over seed raising mix; + an increase, - a decrease, seedling abundance for a given week; * week in which an individual species taxa survey is undertaken. (n=7)

<table>
<thead>
<tr>
<th>Time</th>
<th>Seeding Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DS</td>
</tr>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>+140%</td>
</tr>
<tr>
<td>3</td>
<td>+0.6%</td>
</tr>
<tr>
<td>4</td>
<td>-29.8%</td>
</tr>
<tr>
<td>5</td>
<td>+16.80%</td>
</tr>
<tr>
<td>7 *</td>
<td>+6.1%</td>
</tr>
</tbody>
</table>

### 3.1.2 Species richness

All nine species emerged in sowing methods DS, BS, DM and BM and in the controls DS(R), BS(R). However, for seed burial treatments sand/seed distributor covered by 10 mm of substrate ‘DC’ and sand/seed bed covered by 10 mm of substrate ‘BC’, had significantly lower species richness with only 5/9 species represented. Species which failed to emerge were *A. strictum, L. tenuifolius, C. lacteus* and *W. communis* (Figure 6).

### 3.1.3 Abundance

Generally, seedling abundance significantly varied between sowing application methods and sowing depth, for each of the nine species tested (P<0.001, a=0.05, n=7) and *P. gaudichaudii* (P=0.01) (Figure 5).

There was no significant difference in seedling abundance response for the surface sown methods on green roof substarte and seed raising mix between treatments DS & DS(R) and between BS & BS(R); for 7/9 species
tested (Figure 5). Surface sown DS and its equivalent on seed raising mix DS(R) both achieved the significantly highest ST, with six of these species yielding greater than ST, while an additional two species were within 10% of the ST. The exceptions were, *A. strictum* (P<0.001) with significantly highest abundance response when applied as a sand/seed bed at surface (Treatments BS and BS(R)); and *W. communis* (P<0.001) having a singularly highest emergent response to sand/seed distributor applied to the surface of seed raising mix (Figure 5).

As sowing depth increased, species abundance reduced. 7/9 species reached or exceeded the ST for surface treatments DS & BS and only 4/9 species achieved the ST when mixed into the top 10mm DM and BM (Figure 5). As depth of burial was increased to below 10 mm DC & BC a strong negative response in 8/9 species abundance occurred, with only *P. gaudichaudii* (P=0.01) achieving above 50% of the ST (Figure 5).

Application of seed in a sand bed at surface treatments BS and control BS(R), significantly reduces abundance, in comparison to the sand seed distributor treatments at surface DS and DS(R):- for all but two species *A. strictum* (P<0.001) and *P. jaceoides* (P<0.001) (Figure 2). However, for the remaining treatments, 8/9 species, seed/sand bed treatments were not significantly different from sand/seed distributor treatments with *V. paradoxa* (P<0.001) the exception.
Figure D

Figure E

Figure F
Figure 5: Species abundance response to seeding treatments as a % of the ST achieved. Seeding treatments with varying sowing methods as depth and varying application methods. Figures: A=A.strictum, B=C.lacteus, C=E.ovinum, D=L.tenuifolius, E=L.marginale, F=P.gaudichaudii, G=P.jaceoides, H=V.paradoxa, I=W.communis. --- = seedling target ST of 10 individuals. Species ST to seeding treatments 100% = 10 individuals.
Treatments are over green roof substrate unless specified. DS= sand/seed distributor at surface, BS= sand/seed bed at surface, DM= sand/seed distributor mixed into top 10 mm, BM=sand/seed bed mixed into top 10 mm, DC=sand/seed distributor covered by 10 mm, BC=sand/seed bed covered by 10 mm; Controls: DS(R) sand/seed distributor at surface over seed raising mix, BS(R)= sand/seed bed over seed raising mix. Repeated one-way ANOVAs were used to determine significant differences between treatments. One way ANOVA (n=7, a=0.05); (P < 0.001) for all species except P. gaudichaudii (P = 0.010). Different letters indicate significantly different results, according to the Fisher Exact Test.

![Species richness response to varying sowing depth and application methods.](image)

**Seeding Treatment**

Figure 6: Species richness response to varying sowing depth and application methods. Treatments are over green roof substrate unless specified. DS= sand/seed distributor at surface, BS= sand/seed bed at surface, DM= sand/seed distributor mixed into top 10 mm, BM=sand/seed bed mixed into top 10 mm, DC=sand/seed distributor covered by 10 mm, BC=sand/seed bed covered by 10 mm; Controls: DS(R) sand/seed distributor at surface over seed raising mix, BS(R)= sand/seed bed over seed raising mix. Means and SE from One way ANOVA (n=7, P <0.010, a=0.05, n=7). Treatments with the same letter indicate no significant difference in species richness, according to the Fisher Pairwise Comparison LSD Method (a=0.05)
3.2 Experiment Two

At the study site during late spring (27th October, 2015) to early autumn (4th March, 2016), air temperature varied between a mean maximum range of 16.0 to 42.2 °C and mean minimum range of 8.0 to 25.9 °C (BOM, 2016c) (Figure 3). No heat or rain events were recorded in the first week following sowing, however two significant rain events (12.2 mm & 17.6 mm) occurred mid-week two. In December a series of significant heat events occurred; of note is week eight after sowing, where four days in a row experienced maximums ranging from 35.4 to 41.2 °C (BOM, 2016c).

**Figure 7**: Mean monthly temperature and significant heat and rain events. Melbourne, BOM Station 086338, and 2.6 km from the Burnley field site (BOM Australian Gov. 2016c). On days when temperatures reached or exceed 33 °C, corresponding air temperatures at modules were one to two degrees warmer; this equates to potential plant heat stress defined as ≥ 35 °C (Merilo et al., 2014)). Rain events are defined as ≥ 10mm/day (BOM, 2016c).
Regarding statistical analysis of species abundance data, an extremely small proportion of *W. communis* emerging in week 14 to 18 may have been misidentified as *W. gracilis*, but would not influence statistical results. This is evident as three individuals of *W. gracilis* appeared in three out of five control modules, from week 14 to week 18. Extremely low abundance of *A. strictum* and *E. ovium* prevented statistical assumptions of normal distribution or variance from being met and so these species were excluded from statistical analysis, reducing statistical species investigation from 10 species to eight.

3.2.1 Emergence

Seedling emergence response was synchronous across sowing methods and sowing rates. A plateau in community population growth occurred between weeks four and six post sowing indicating population mean had been reached, and individual species survey was undertaken at week four (Table 3).

Table 3: Seedling abundance variation during emergence and at early establishment. Seeding Treatments are over green roof substrate S100= a minimum of 100 germinable seeds applied to surface; S200= a minimum of 200 germinable seeds applied to surface; M100= a minimum of 100 germinable seeds mixed into the top 10 mm of substrate; M200= a minimum of 200 germinable seeds mixed into top 10 mm of substrate. + = an increase, - = a decrease, in percentage of community population density between survey periods; * week in which the smallest positive increase in community population and population peak occurred for all treatments determining emergence end and survey point. ± SE, n=10, ( ) = Rate of change in abundance per consecutive survey period.

<table>
<thead>
<tr>
<th>Time post sowing (Weeks)</th>
<th>Community abundance and rate of change between consecutive survey period for each sowing method/rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M100</td>
</tr>
<tr>
<td>2 1st survey</td>
<td>36.20 ±2.69</td>
</tr>
<tr>
<td>3</td>
<td>51.5±4.06</td>
</tr>
<tr>
<td>4*</td>
<td>56.6±4.3</td>
</tr>
<tr>
<td>6</td>
<td>47.7±4.58</td>
</tr>
<tr>
<td>8</td>
<td>46.2±5.1</td>
</tr>
</tbody>
</table>

3.2.2 Species richness

The total number of different species represented in each sowing method/rate treatment was high. Species richness did not vary between sowing rates but did between sowing depths, with results showing 10/10 and
9/10 species present in (M100 & M200) and (S100 & S200) treatments respectively, with A. strictum absent (Table 4). Generally all species are presented in each replicate of every seeding treatment at seedling emergence in week four, and closely maintain this level to seedling maturity at week 18 (Table 4). L. tenuifolius’ shows a significant decline in the number within the depth sown replicates (M100 and M200), between week four and week 18. However, A. strictum and E. ovinum show poor representation among the replicates for almost treatments.

Table 4: Frequency of species appearance in 10 replicates for each sowing method/rate treatment. Week four= population mean at emergence end, week 18= final survey at seedling maturity. Seeding Treatments are over green roof substrate S100= a minimum of 100 germinable seeds applied to surface; S200= a minimum of 200 germinable seeds applied to surface; M100= a minimum of 100 germinable seeds mixed into the top 10 (mm) of substrate; M200= a minimum of 200 germinable seeds mixed into top 10 (mm) of substrate

<table>
<thead>
<tr>
<th>Species Tested</th>
<th>Number of replicates each species appears in per treatment.(n=10)</th>
<th>M100</th>
<th>M200</th>
<th>S100</th>
<th>S200</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 4</td>
<td>Week 18</td>
<td>Week 4</td>
<td>Week 18</td>
<td>Week 4</td>
</tr>
<tr>
<td>A. strictum</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>C. lacteus</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>E. ovinum</td>
<td>5</td>
<td>2</td>
<td>7</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>L. tenuifolius</td>
<td>10</td>
<td>7</td>
<td>10</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>L. marginale</td>
<td>10</td>
<td>9</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>M. lanceolata</td>
<td>10</td>
<td>9</td>
<td>10</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>P. gaudichaudii</td>
<td>9</td>
<td>9</td>
<td>10</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>P. jaceoides</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>V. paradoxa</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>W. communis</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>9</td>
<td>10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SPECIES RICHNESS</th>
<th>Total number species represented</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
</tbody>
</table>

One way ANOVA analysis indicates that species richness doesn’t significantly vary between seeding treatments or during weeks four and 18. Although results (P=0.009), a=0.05, n=10., reveal two significantly different species richness responses to seeding treatments at week four, this difference is less than one species; as Fisher Individual Tests for Difference of Means = 0.7±0.311 (P=0.031) a=0.1968, n=10. There was no significant difference between treatments effect on species richness at week 18 seedling maturity (P =0.610), a=0.05, n=10.
3.2.3 Abundance maximum

Community abundance was calculated from week two (10th November 2015) to week eight post sowing (22nd December 2015). Maximum abundance for all treatments occurred at week four, followed by a population decline for weeks seven and eight (Table 3), and community abundance remained stable to week 18, for all sowing depths and sowing rates.

At maximum abundance in week four, 6/10 species ST was closely approximated or exceeded; of note are *C. lacteus* (P<0.001) and *P. jaceoides* (P<0.001) which reached or greatly exceeded ST for all seeding treatments (Figure 8). Treatment S200 yielded the significantly highest abundance for 6/8 species, equaling or exceeding the ST. *M. lanceolata* (P<0.001) and *P. gaudichaudii* (P=0.001) are the exceptions, having significantly lower than the ST for the S200 treatment than for treatment M200. Comparatively, *P. jaceiodes* (P<0.001) showed no significant difference between treatments M200 and S200 (Figure 8).

Generally, mixing seed into the top 10 mm of substrate (M100 & M200) produced a lower abundance at week four than seed sown at surface (S100 & S200) (Table 3 & Figure 9I). For example, community abundance for S100 was 125.2% more abundant than M100, and S200 was 140.3% more abundant than M200 (P<0.001, a=0.05, n=10) (Figure 8I), as evident for 6/8 species (Figure 8).

Both higher sowing rate treatments (S200 and M200) produced greater community abundance at week 4 than the lower sowing rates (S100 and M100) respectively (Table 3 & Figure 9I). Higher sowing rate treatments M200 had 175.2% greater abundance than M100, and S200 had 196.3% greater abundance than S100 (P<0.001, a=0.05, n=10) at week 4 (Figure 9I). Double the sowing rate at when seed is mixed into the substrate to 10mm depth yields a reduced proportional abundance, however, when surface sown an approximate doubling of abundance is achieved. These results were similar at a species level, with higher sowing rates (S200 and M200) more closely approaching the ST than the lower sowing rate treatments (S100 and M100), for 7/8 species. The exception to this is *L. tenuifolius* (P<0.001), where the ST was not significantly different between high and low sowing rates when applied at depth (M100 and M200). Lower sowing rate treatments S100 and M 100 showed no significant difference in the ST for 5/8 species (Figure 8).
Figure 8: Specie abundance response to seeding treatments as a % of the seedling target ST at mean maximum population, week 4. Figures representing species: A= C. lacteus, B= L. tenuifolius, C= L. marginale, D= M. lanceolata, E= P. gaudichaudii, F= P. jaceoides, G= V. paradoxa, H= W. communis; --- = seedling target ST =10 individuals

Seeding treatments are: - surface sown 100 seeds (S100), surface sown 200 seeds (S200), 100 seeds mixed into top 10 mm of substrate, (M100) and 200 seeds mixed into top 10 mm of substrate (M200).

*A strictum* and *E. ovinum* failed to meet statistical variance and normal distribution assumptions, and were excluded from this analysis. Repeated One-way ANOVAs were used to determine significant differences between treatments; (P<0.00, a=0.05, n=10), *P. gaudichaudii* was an exception (P=0.001); Different letters indicate significantly different results, according to the Fisher Exact Test, a=0.05, n=10.
Figures: 9

Figure A

Figures: 9

Figure B

Figures: 9

Figure C

Figures: 9

Figure D

Figures: 9

Figure E

Figures: 9

Figure F
Figure 9: Species and total community abundance from mean maximum population to seedling maturity. (Week four to Week eight). Seeding treatments are: - surface sown 100 seeds (S100), surface sown 200 seeds (S200), 100 seeds mixed into top 10 mm of substrate, (M100) and 200 seeds mixed into top 10 mm of substrate (M200). Figures and Species one way ANOVA:
- A= C. lacteus (a=0.05, n=10, all weeks P<0.001), B= L. tenuifolius (a=0.05, n=10, week4 P<0.001, week8 P=0.005, week18 P=0.002), C= L. marginale (a=0.05, n=10, week4 P=0.000, week8 P=0.001, week18 P=0.02), D= M. lanceolata (a=0.05, n=10, all weeks P=0.000), E= P. gaudichaudii (a=0.05, n=10, week4 P=0.001, weeks 8 & 18 P=0.000), F= P. jaceoides (a=0.05, n=10, all weeks P=0.000), G= V. paradoxa (a=0.05, n=10, all weeks P=0.000), H= W. communis (a=0.05, n=10, week4 P=0.000, week8 P=0.064, week18 P=0.325), I= Community population density (a=0.05, n=10, all weeks P=0.000)
At maximum abundance at week four, there is no evidence of trend reflecting an association between species seed length or width and seedling abundance (Figure 10). This occurred for a wide scale of dimensions; seed length ranged from 9.59 ±1.33 (mm) to 0.58±0.06 (mm) and width ranged from 4.92±0.65 mm to 0.23±0.05 mm, where n=10 (Figure 2). Regression analysis shows strongly suggests that there is no lineal association between abundance and either seed dimensions, for either sowing depth treatments for seed length: - M200 (P=0.527) and S200 (P=0.262) a=0.05, DF=7; or seed width: treatments M200 (P=0.546) and S200 (P=0.538) a=0.05, DF=7.

Figure 10: Seedling maximum abundance comparison to seed dimension: - for high sowing rate treatments a surface sowing method (S200) and sowing method mixing seed into top ten mm of substrate (M200). All species tested for treatments (S200) and (M200) have P values <0.001, a=0.05, n=10. Standard errors for seed width ranges between species from ± 0.65 to ±0.03 and for seed length from ±1.33 to ±0.06 Note species order varies between seed length and seed width. Plotted shapes represent different species.
3.2.4 Abundance and survival

For 6/8 species there was no significant difference between maximum abundance at week four and abundance at seedling maturity week 18, across all sowing depths and rate treatments (Figure 9). Two exceptions to this were *L. tenuifolius* (P<0.005 for all weeks and a=0.05, n=10) and *L. marginale* (P<0.02 for all weeks, a= 0.05, n=10) which both showed a disproportionally greater abundance decline between weeks four and eight for treatment S200 (Figure 9b & c).

As a community however, a small but significant abundance decline occurred between weeks four and eight, for the higher sowing rates only (M200 & S200) a loss of 17.7% and 27.7% respectively (Figure 9I). Both high sowing rate treatments (M200 and S200) produced approximately a 79% and 100% increase in abundance respectively, compared to their equivalent lower sowing rate M100 & S100 abundances (Figure 11). Abundance was reduced by week 14 where both S200 and M200 both were proportionally only 75% greater than the abundances of the lower sowing rate treatments (S100 & M100).

![Figure 11: Abundance proportional difference between surface sown and mixed into substrate sowing treatments. High sowing rate treatments (S200 & M200) community abundance as a proportion of low seeding sowing rate treatments (S100 & M100) over time (n= 10). Treatments: Surface sowing method (S200) and sowing method mixing seed into top 10 mm of substrate (M200). A second germination pulse of *L. tenuifolius* was observed for week 10, and these late germinants had a high mortality recorded by week 12. Seeding treatments are: - surface sown 100 seeds (S100), surface sown 200 seeds (S200), 100 seeds mixed into top 10 mm of substrate, (M100) and 200 seeds mixed into top 10 mm of substrate (M200).](image-url)
3.2.5 Cover

Prior to week 10, cover was <10% for all treatments. In excess of 80% cover was produced for all sowing method depths and rates within 18 weeks of growth post sowing (Figure 12).

Figure 12: High cover, species rich novel grassland forfield community modules at seedling maturity. Experiment Two week 18, University of Melbourne, Burnley campus.

Rate of cover change varied through cover establishment as distinct phases (Table 5). Cover for all treatments more than doubles between weeks 10 and 12 and similarly between weeks 12 and 14. Rate of cover change increase was reduced between weeks 14 and 16; with S200 and S100 showing a +19.86% and +32.54% to cover increase respectively. Cover continues to increase for all treatments into week 18, but comparatively slowly. S200 shows higher cover at week 10 (mean17.01±2.89) which was significantly different from M100 (mean10.17±1.12). However, there is no significant difference in cover between treatments M100 or S100 and M200 (P=0.095, a=0.05, n=10) at this time (Figure 13). In week 12, S200 (mean 38.12 ± 8.8) had a significantly higher cover than all other treatments which showed no significant difference between them.
Table 5: Percentage cover rate of increase for each seeding treatment. Survey taken at consecutive fourteen day intervals, Week 10 to week 18 post sowing. Seeding treatments are: - surface sown 100 seeds (S100), surface sown 200 seeds (S200), 100 seeds mixed into top 10 mm of substrate, (M100) and 200 seeds mixed into top 10 mm of substrate (M200).

<table>
<thead>
<tr>
<th>Consecutive weeks post sowing</th>
<th>Seeding treatments % rate of cover change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M100</td>
</tr>
<tr>
<td>10 - 12</td>
<td>120.16</td>
</tr>
<tr>
<td>12 - 14</td>
<td>178.92</td>
</tr>
<tr>
<td>14 - 16</td>
<td>29.29</td>
</tr>
<tr>
<td>16 - 18</td>
<td>12.88</td>
</tr>
</tbody>
</table>

Between weeks 14 and 16 treatments form two groups that are significantly differentiated on the basis of sowing rate; high sowing rates (S200 & M200) have 15% greater cover than the lower sowing rate treatments (S100 & M100). The difference of means between these two significantly different groups at week 14 for M200 and M100 is 9.14% cover ±5.07 at CI=80.32% (P=0.080), Fisher Individual Tests for Difference of Means. This difference of means is slightly reduced to 7.94% cover ±3.74, CI =80.32% (P=0.041). By week 18 there is no significant difference between any treatment (P=0.633) with means ranging from S200 mean cover % =94.72 ±1.12 to M100 mean cover% = 92.27 ±2.34.
Figure 13: Community cover response to sowing method depth/rate treatments with time. Seeding treatments are: - surface sown 100 seeds (S100), surface sown 200 seeds (S200), 100 seeds mixed into top 10 mm of substrate (M100) and 200 seeds mixed into top 10 mm of substrate (M200). The results are derived from repeated One way ANOVA’s $a=0.05$, $n=10$, at each time interval. Week 10 ($P=0.095$), week 12 ($P=0.000$), week 14 ($P=0.005$), week 16 ($P=0.002$) week 18 ($P=0.633$). At week 8 total cover for all seeding treatments was $<10\%$ and at week 18 $>85\%$, these are the limits of precision for cover estimate methodology.
4.0 Discussion

High species richness and cover optimize delivery of green roof ecosystem services (Bevilacqua et al., 2015; Lundholm, 2015b). This study investigated the efficacy of direct seeding methods in the establishment of grassland forbs onto green roof substrate in yielding a species rich, high cover, novel community. Three key findings were revealed. Firstly, that green roof substrate is an effective media for grassland forb species germination and seedling establishment, supporting a species rich, high cover community. Secondly, the application of a sowing rate approaching that of on ground restoration rates is as effective as a doubled sowing rate in achieving high species richness and cover, is pertinent to industry. Thirdly in green roof module conditions application of seed at depth 0-10mm produces greater species richness than at surface, but with less abundance; however, either of these treatments produces excellent vegetation performance outcomes measured as species richness and high cover. An additional finding supports recent research that species richness does not drive cover (MacIvor et al., 2013), and suggests that abundance is an early driver of cover, and may act with additional factors to drive growth towards rapid total cover.

4.1 Sowing depth

4.1.1 Species richness

A high species richness response to sowing at surface and when mixed into the substrate between 0 to 10 mm depth, was evident in Experiments One and Two (Table 6). In Experiment One, all species were represented between 0 and 10 mm depth. Conversely, a strong negative species response to sowing depth below 10 mm of substrate, reduced taxa by 4/9 species. The reduced species richness may be due to absence of a germination cue/s or a mechanism preventing emergence. For instance, deeper burial of seed could limit light exposure as a germination cue. This is supported by a past native grassland forb germination study which found that, half of the forbs species tested required light enhanced germination (Clarke et al., 2000). Deeper burial is a mechanism that prevents emergence, by causing seed with small reserves to run out of energy for growth of the epicotyl, prior to emergence (Philipp, 1992). Similarly, Experiment Two results confirm that high species richness is achievable at depths between 0-10 mm with the loss of 1/10 species and no reduction in species richness for surface sowing, in variable and at times extreme outside climate conditions. As germination is the result of multiple germination cues being met, including interaction between light, water availability, oxygen concentration, supply of nutrients and temperature (Busso, ed., 2013); the results suggest that green roof
substrate provides safe site cues in a variable outside environment. Direct seeding in establishing species richness on green roofs, confers functional diversity to the system (Heim and Lundholm, 2014b), and as an early indicator of a community assemblage co-existence, positively conferring resilience to the community (Kitayama, 1996), and therefore could promote continuous ecosystem service provision.

Table 6: Summarized community response to sowing method depth, media and sowing rate.

<table>
<thead>
<tr>
<th>Seeding Treatment</th>
<th>Community Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Species Richness</td>
</tr>
<tr>
<td><strong>Experiment One Glasshouse</strong></td>
<td></td>
</tr>
<tr>
<td>Seed raising mix seed bed</td>
<td>++</td>
</tr>
<tr>
<td>Green roof substrate seed bed</td>
<td>++</td>
</tr>
<tr>
<td>Seed in sand bed at surface</td>
<td>++</td>
</tr>
<tr>
<td>Seed at surface</td>
<td>++</td>
</tr>
<tr>
<td>Seed mixed into top10 mm of substrate</td>
<td>++</td>
</tr>
<tr>
<td>Seed covered by 10 mm of substrate</td>
<td>_ _</td>
</tr>
<tr>
<td><strong>Experiment Two Outside</strong></td>
<td></td>
</tr>
<tr>
<td>Seed at surface</td>
<td>++</td>
</tr>
<tr>
<td>(1sp. lost)</td>
<td></td>
</tr>
<tr>
<td>Seed mixed into top10 mm of substrate</td>
<td>++</td>
</tr>
<tr>
<td>125% rate @ 10 seeds/ species/0.25m²</td>
<td>++</td>
</tr>
<tr>
<td>250% rate @ 20 seeds/species/0.25m²</td>
<td>++</td>
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</tbody>
</table>
4.1.2 Abundance

Synchrony in emergence of different species taxa was evident at all sowing depths, where maximum abundance occurred at the same time, reflecting on ground direct seeding establishment observations (Gibson-Roy, Delpratt, Moore, 2007a). Interestingly, maximum abundance was three weeks earlier under the variable outside conditions of Experiment Two, than under more stable ideal germination conditions of Experiment One. Through major on ground local grassland restoration research, Gibson-Roy, Delpratt and Morgan, reported that many of these species had uniform germination (Williams, Marshall & Morgan, eds. 2015). Synchrony is an important seedling survival mechanism in dry land conditions, enhancing seedling establishment prior to soil moisture depletion (Leck, Parker & Simpson, 2008). The results indicate that perhaps these grassland forbs have this early seedling survival mechanism, an advantage in the desiccating green roof environment (Dunnett & Kingsbury, 2004).

For Experiment One, increased depth of burial negatively impacted seedling abundance (Table 6). Seed is mixed into the top 10 mm of substrate, produced significantly fewer individuals than for surface sown treatments. With increased sowing depth at below 10 mm, abundance was greatly reduced for all species other than P. gaudichaudii. Clarke and Davidson (2004), reported that some grassland forb species have a slightly elevated response to diurnal light (surface sowing) but still produce good germination response in dark conditions. This may provide some explanation as to P. gaudichaudii’s showing little abundance response variation, to depth of sowing. In contrast, surface sowing in Experiment Two has a similarly higher species abundance result as for Experiment One; but a comparatively reduced abundance loss result for depth of sowing between 0 to 10 mm than Experiment One. Inference may be drawn that, in variable outside conditions where extreme weather events occurred, sowing at depth may buffer germinants and early seedlings against; higher radiant temperatures (Zhao et al.,2014) and substrate temperatures (Buckland – Nicks et al., 2016), which have the potential to desiccate and destroy germinating seed and germinants (Busso, 2013). Another depth of sowing advantage could include an increased imbibing opportunity at depth, not afforded on a periodically dry surface, on substrate that experiences comparatively elevated evaporation (Heim & Lundholm, 2014b). Further, abundance over time was not affected by depth of application. Overall results indicate that, in outside conditions that surface sowing has an abundance advantage at germination over depth of sowing. Populations are stable irrespective of sowing depth and this higher abundance is carried forward to seedling establishment.
Experiment One results show a high species richness response, when seed was applied to green roof substrate, seed raising mix and in a sand bed. Green roof substrate in the outside conditions of Experiment Two also produced high species richness (Table 5). Therefore it is evidence that green roof substrate is an effective germination media, irrespective of seed size, contrary to past assertions (Harp et al., 2015; McDavid 2012), and provides safe site conditions for seedling establishment. Secondly, these results indicate that direct seeding grassland forbs onto green roof substrate can produce an early species rich community.

This study establishes that green roof substrate effectively supports species rich seed germination, including small seed/fruit propagules. Therefore, it may be inferred that fine seed is not washed through the porous substrate to render emergence unviable. Species may have adaptive mechanisms, that resist seed being washed through gaps within cracking clays and fragmented soils of rocky outcrops, characteristic of grassland habitat (Vic. Gov., 2016). Possibly, these adaptive benefits are transferred to the porous green roof substrate. For instance, the high attraction static forces noted in *W. communis*, and the pappus of *M. lanceolata* trapping the seed upright in substrate cracks at the edge of the modules, may have prevented seed being washed through the green roof substrate or blown away. Such adaptations to trap seed are crucial to germination success in exposed habitats, (Houle & Phillips, 1989) and could be of similar advantage in the green roof environment.

Experiment One results established that seed applied to green roof substrate produces equal abundance to seed raising mix, for most species. These results demonstrate that green roof substrate under glasshouse conditions doesn’t negatively impact abundance. However under these conditions, application of seed in a sand bed significantly reduces abundance for most species. A possible explanation is that the sand having smaller particle size, reduced porosity and a higher density, (comparatively to green roof substrate and seed raising mix), may have imposed a mechanical resistance greater than the emergence force of the radicle (Liptay and Geier, 1983), thus negatively impacting abundance (Philipp, 1992). Experiment Two results show scoria based green roof substrate to be an effective germination media when surface sowing in outside variable conditions, and when mixed into the top 10 mm of substrate, and is similar to high abundance levels for most species under glasshouse conditions (Table 5). In addition, results indicating no trend between seed size and abundance, is
evidence that application of seed onto this green roof substrate supports abundance irrespective of seed size. Following a small but significant mortality during early seedling establishment for two species, most species showed no significant change to their abundance between weeks four to 18, demonstrating that scoria based green roof substrate supports seedling establishment.

4.3 Sowing rate

4.3.1 Species richness and abundance

Experiment Two results show that sowing rates at 25% above those of on ground restoration rates and double this rate, all produce high species richness. Abundance however, does vary with sowing rate. This was demonstrated by maximum abundance increases when sowing rate was doubled, producing increases between 79% when surface sown and 100% when mixed into the substrate at 0 to 10 mm depth. These increases in abundance at higher sowing rates were not sustained, following a small but significant loss of abundance between weeks four and week eight. This mortality may have been caused by a severe heat event at this time, in which four days experienced maximums of 35.4°C to 41.2°C; or through negative intra and/or inter species competition (Meiners et al., 2015). Abundance for both depth of sowing applications was reduced to 75% (Table 5). This shows that abundance reduction was greater at the higher sowing rate than when mixed into the substrate. A possible explanation is offered as species interaction, through self-thinning and/or competitive exclusion which may have had a role in determining abundance reductions at the higher sowing rates where abundance is greater (Thorn et al., 2015).

4.4 Cover response

4.4.1 Sowing depth and rate

In Experiment Two, the novel grassland communities produced greater than 80% cover within 18 weeks. This was achieved irrespective of sowing depth or sowing rate. This demonstrates that sowing at surface and at a sowing rate approaching on ground restoration rates is adequate to produce high cover rapidly.
4.4.2 Association with species richness and abundance

High species richness remaining stable, while cover rapidly increased, is an indication that species richness was not the key driver cover for these treatments. This result supports MacIvor (2013) findings that, species diversity (established as a seeded grass/forb mix) isn’t the principle driver of cover.

However, results indicate that abundance is a driver of early high cover levels, but ceases to be the key driver as rapid cover increase occurs. Comparison between treatments show that, high early cover of (S200) may be due to a 20% greater abundance than closest cover response treatment (M200); that is more individuals cover more area. At week 14 and 16 sowing depth is no longer a cover advantage, and two significantly different cover groups exist, which are differentiated based on sowing rate and also by abundance. This is evident as the higher sowing rate treatments (S200 & M200) have a 15% significantly higher cover, than the lower sowing rate treatments (S100 & M100). Yet there is no significant difference in cover between (S200 & M200) even though abundance varies by 20%. Conversely to this within individual treatments, abundance remains stable for each treatment from weeks eight to 18, while cover increase accelerates for all treatments, suggesting that abundance is not a key driver of cover. These disparities between abundance and cover association over the rapid growth phase, could be an indication that abundance does not drive cover in isolation. At week 18 any significant difference in cover ceases to exist between any treatments. This was illustrated with a slowing of cover increase for treatments that have already attained higher cover by week 16; while treatments with the lower cover are slowing less, and therefore catch up. All treatments achieving high cover almost synchronously, could be explained in terms of niche space availability and resource opportunity (Meiners et al., 2015). For instance, high cover has reduced niche space and limited resources, slowing growth and therefore rate of cover increase. Conversely treatments with less cover, have greater niche space available and therefore more resources available for growth, and cover increase proceeds more rapidly.

Achieving high cover early in the desiccating green roof environment, could impart elevated survival. Early high cover is an assemblage character common to dryland communities (Angert et al., 2007) and is a mechanism that increases survival (Silvertown & Wilson, 1994). Therefore, early high cover could impart similar benefit to the novel green roof community. This study was carried out under desiccating conditions but desiccation prevented through irrigation, this is an accepted establishment method. It is contended that creating high cover early could act as a protective mechanism against desiccation, when irrigation is reduced as the community is hardened off. For example, there may be a critical point reached in cover that affects cooling of substrate (Buckland-Nicks et al., 2016), with increased soil moisture (Berreta, 2014), and improved
micro climate conditions (Heim & Lundholm, 2014). In reducing plant stress, plants increase rate of photosynthesis reactions (Merilo, 2014) and therefore increase energy available for growth (Hosey et al., 2003) in cover. It may be hypothesized that, a community with high cover in modifying abiotic conditions which reduce plant stress, could positively feedback to maintain species richness, abundance and cover. These three community characters are essential to a functioning system (Dunnett et al 2008; Lundholm et al., 2010) that provide ecosystem services (Cooke-Pattern & Bauerle, 2012; MacIvor & Lundholm, 2011).

5.0 Application and Recommendations

This research conveys to industry that, direct seeding the surface of a scoria based green roof substrate is an effective installation method for the establishment of a functioning green roof system. Further, that plant community selection based on diversifying plant taxa, life form and morphology, drawn from a local analogous grassland habitat (with the exclusion of grasses), is a successful approach to establishing a species rich novel ‘forbfield’ community. The fact that irrigation is necessary for this establishment should not detract from this method, as it is well accepted that irrigation is central to adult plant establishment also (Rowe et al., 2014). Given the lower cost of direct seeding establishment over adult plant establishment methods (MacIvor et al 2013; Sutton, 2013) and a wider window for establishment (Sutton, 2013), direct seeding can deliver time and cost efficiencies to industry that installation with adult plants do not.

Additionally it is conceivable that as cover is rapidly achieved with low mortality, that additional cost efficiencies would be realized through direct seeding in comparison to adult plant installation. For example, early high cover suggests there is potential to reduce irrigation earlier and reduce maintenance costs comparatively to adult plant establishment. In green roof environments where species richness and cover typically degrade over time (MacIvor et al., 2013), early high cover is not only essential to establishment of a functioning green roof system, but to the ongoing functioning of the system, and thus ecosystem service provision. This places importance upon research that would improve understanding of the key drivers of cover and their interaction at varying phases of community cover establishment. Such research should be long term and so inform horticultural practices in the establishment and maintenance of these novel communities, and inform understanding of dynamic community assemblage mechanisms.
The success of a sowing rate at 25% greater than on ground restoration rates, proving more than sufficient in achieving an abundant species rich high cover; implies that sowing rate could further be reduced, with this high end outcome still obtainable. Undertaking further research in relation to sowing rate and correlation to species richness, abundance and cover, would clarify this, and contribute additional cost efficiencies to industry. In this regard, a comparative cost benefit analysis between establishment methods of direct seeding, adult plant installation and perhaps combinations of the two, would put direct seeding establishment into an economic context from which industry could form decisions with confidence.

6.0 Conclusion

This research confirms that direct seeding onto green roof substrate supports species rich, high cover novel grassland community establishment. This study collectively demonstrates that establishment is rapid in variable outside conditions, under extreme climate events, with irrigation. Green roof substrate is indicated as an effective germination media in producing an abundant, species rich, forb community, that accommodates fine seed emergence, and is a safe site for seedling establishment. This study shows that, similarly to on ground restoration practice and glasshouse results, that sowing seed at depth 0-10 mm reduces abundance in green roof modules conditions in variable outside conditions, when compared to than surface sowing, and with slightly increased species richness. Irrespectively, both sowing depths produce excellent vegetation performance outcomes measured as species richness and high cover. Sowing rate approximating on ground restoration rates was identified as being more than adequate in producing a species rich, high cover. In consideration of the importance of achieving high cover early, this study supports that species richness is not a key driver of cover, and that abundance is associated with early cover development, but may not act in isolation as cover rapidly increases. These findings have practical management relevance and suggest further investigation focus upon refining sowing method /rate and upon informing long term novel community assemblage dynamics.
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### 9.0 APPENDICES

#### APPENDIX 1

Germination test results for the 10 species taxa selected. Calculation of mass required to produce a seedling target ST = minimum of 10 individuals. * Indicates species achieving < 50% germination in the sown tests; all other species achieved >50% germination. ² = this species was only included in Experiment 2.

<table>
<thead>
<tr>
<th>Species selected for Experiments</th>
<th>Mean Germination/ (g)</th>
<th>Coefficient of Variation %</th>
<th>Mass to yield 10 germinable seeds (g)</th>
<th>Mass (g) to yield a minimum of 10 germinable seeds (ST) = Mass 10 germinable seeds + CV % (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sown Trials</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calocephalus lacteus</td>
<td>1473</td>
<td>35</td>
<td>0.0068</td>
<td>0.0092</td>
</tr>
<tr>
<td>Eryngium ovinum *</td>
<td>74</td>
<td>26</td>
<td>0.1351</td>
<td>0.1702</td>
</tr>
<tr>
<td>Leptorhynchos tenuifolius *</td>
<td>217</td>
<td>92</td>
<td>0.0375</td>
<td>0.0783</td>
</tr>
<tr>
<td>Microseris lanceolata ²</td>
<td>358</td>
<td>74</td>
<td>0.0279</td>
<td>0.0485</td>
</tr>
<tr>
<td>Podolepis jaceoides</td>
<td>292</td>
<td>14</td>
<td>0.0342</td>
<td>0.039</td>
</tr>
<tr>
<td>Wahlenbergia communis</td>
<td>10415</td>
<td>15</td>
<td>0.00096</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Rolled Paper Trials</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arthropodium strictum</td>
<td>299</td>
<td>11</td>
<td>0.0334</td>
<td>0.0371</td>
</tr>
<tr>
<td>Linum marginale</td>
<td>667</td>
<td>11</td>
<td>0.0171</td>
<td>0.019</td>
</tr>
<tr>
<td>Plantago gaudichaudii</td>
<td>360</td>
<td>6</td>
<td>0.0278</td>
<td>0.0295</td>
</tr>
<tr>
<td>Velleia paradoxa</td>
<td>136</td>
<td>14</td>
<td>0.0735</td>
<td>0.0838</td>
</tr>
</tbody>
</table>

Of the initial 20 species selected that underwent germination tests:- four species yielded > 50% germination in the rolled paper tests, making the final selection, with *Acaena echinata, Rumex dumosus* and *Bulbine bulbosa* at < 50% germination excluded. In the sown tests the six species with highest emergence were selected. *E. ovinum* and *Leptorhynchos tenuifolius* were selected, despite yielding low germination percentage in the sown tests, as there was sufficient masses to provide for the experiments; however *Brachyscombe dentata, Chrysocephalum apiculatum, Ptilotus spathuatus* and *Vittadinia muelleri* did not, and were excluded. Additional exclusions include: - *Arthropodium minus*, in order to maximise phylogenic diversity, with preference given to *Arthropodium strictum* showing greater germination. *Microseris lanceolata* was omitted from Experiment 1 as insufficient quantity was available, however was included in Experiment 2 when availability prevailed. *Solenogyne dominii* was found to be contaminated with a graminoid and so was discarded from the germination test.
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Spencer, Pamela

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