Green roofs as analogous of calcareous grasslands ? Species response to substrate heterogeneity

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GREEN ROOFS AS ANALOGOUS OF CALCAREOUS GRASSLANDS ? SPECIES RESPONSE TO SUBSTRATE HETEROGENEITY

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MASTER THESIS SUBMITTED FOR THE MASTER IN FOREST AND NATURAL RESOURCES MANAGEMENT GRADUATION

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PROMOTER : GREGORY MAHY

A ces quelques personnes qui m'ont bien encadré

Pr. Gregory Mahy, promoteur concerné

Entre vos relectures et conseils avisés

A toute cette belle équipe biodiversité

Aide administrative ou écoute bienveillante

A ma chère famille, (j'espère) toujours confiante

A ses nombreuses couleurs, et son unicité

A ma seconde maison, qui a sur me bercer

Dans cette ambiance cocasse, saisons après saisons

A cette nature défiante qui éprouve notre raison

Chaque minute chaque seconde, dans ce monde qui change

Un monde pittoresque, de partage et d'échange

Summary

English

The environmental conditions on green roofs can strongly influence plant growth and survival. However, community scale study has been little conducted. We started an experiment in which green roofs differed in substrate depth and type (variation in organic matter rate, water capacity and draining capacity). The same seed mix were used to assure comparison. This mix consists of 22 species : 4 generalists species and 3 corteges of 6 specialists species. During two months, with no irrigation, the abundance of individuals (seedlings and adults) was calculated in a way to potentially differentiate communities. It appears that the environmental conditions on green roofs resulted in community distinction as in analogous habitat. Both substrate depth and weather affected community evolutionary path. The plant individual dynamics, and consequently their success rate, might help to explain these differences. However, this study could not highlight any influence of their functional traits, especially SLA and Seed mass, that have demonstrated their value in providing information about rapid returns on leaf investments and improved seedling success in poor nutrient soil. As a conclusion, it is recommended to incorporate environment conditions into appropriate plant selection.

Français

Les conditions environnementales des toitures vertes peuvent gravement impacter la croissance des plantes ainsi que leur survie. Cependant, les études à l'échelle de communautés n'ont été menées que très rarement. Nous avons ainsi démarré une expérimentation dans laquelle des toitures se distinguaient par leur type et profondeur de substrat (variation dans le taux de matière organique, la rétention en eau et la capacité de drainage). Le même mélange a été utilisé afin d'assurer la comparaison. Ce mélange consiste en un ensemble de 22 espèces : 4 espèces généralistes (ou compagnes) complétées des 3 cortèges comprenant chacun 6 espèces spécialistes. Durant 2 mois, sans irrigation, l'abondance des individus (semis et adultes) a été calculée afin de différencier de potentielles communautés. Il s'est avéré que les variations de conditions environnementales sur toitures vertes se sont soldées par une distinction entre communautés tout comme sur l'habitat analogue correspondant. La profondeur du substrat et la météo ont toutes deux affecté la trajectoire évolutive de la communauté. Les dynamiques individuelles des plantes, et par extension leur taux de succès, peuvent peut-être aider à expliquer ces différences. Toutefois, l'expérimentation n'a pu souligner aucune influence des traits fonctionnels, spécifiquement la surface foliaire spécifique et la masse des graines, qui ont démontré un intérêt tant au niveau du retour rapide d'investissement foliaire que du succès de germination sur un sol pauvre en nutriments. Pour conclure, il est recommandé d'intégrer les conditions environnementales dans une sélection appropriée des plantes.

Foreword

This master thesis was carried out under the supervision of the Biodiversity and Landscape Unit of Liège University. It is part of the master program and follow a clear framework.

In these circumstances, each student has to establish the review of literature. This part is followed by the resolution of scientific problem on a specific topic.

However, the format of this study is quite different. As an opportunity, I finalised my study in the form of an article. Therefore, to be as rigorous as possible, I splitted my paper into two parts. They are complementary but could be understood separately.

The first part depicts the concepts that will be discussed in the experimentation, from the main subject, green roofs, to restoration and analogous habitats concept. This part in synthesized in the second part. However, it may represent a real asset to catch all the nuances of this last one.

The second part concerns the scientific article itself. It includes the main experiment and the output results. It is the heart of this thesis and explains how this experiment fall within the current studies about green roofs.

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Chapter 1 : Study framework

Concrete represents a large part of cities areas and this situation leads to a poor installation and development of biodiversity. By 2030, more than 60% of the population will live in cities, but she will always be as dependent on nature as before (Bolund, 1999). Yet, anthropogenic actions are partly responsible for the rapid loss of urban biodiversity. Indeed, it is estimated that half or more of the known species are at risk of extinction in the near future (Sax, 2003). However, green infrastructures benefits demonstrate their importance for human well being and decision makers are gradually more receptive to them. Consequently, knowledge on urban green infrastructures has increased tremendously.

To develop urban biodiversity in urban areas, it is necessary to balance the ratio between impervious and green surfaces. On the one hand, it is vital to preserve existing ecosystems with maximum respect and caution. On the other hand, it is essential to take advantage of novel ecosystems with high biodiversity potential. In any event, those ecosystems need to be as resilient as possible.

Whatever the type of green infrastructure developed, the selection of species matters. For instance, native plants should be preferred to support biodiversity by providing shelter and food to local fauna and strengthening regional ecological network and landscape integration.

Green roofs are essential component of urban green infrastructure and can support analogue habitats of natural / semi natural ecosystems. Developing analogous habitats on green roofs need to consider assembly rules that are based on the ecological niches or functional roles of each species. These ecological rules are the base of habitat restoration, providing added value for ecological networks.

Green Roofs

The history of modern green roofs started in Germany in the 1960s, and has since spread to many countries. Green roofs are now recognized for their capacity to deliver multiple ecosystem services. From environmental to aesthetic advantages (Dunnett, 2010) : i) regulation services with improved water quality (Dapolito Dunn, 2010), mitigation of air pollution (Tarran, 2007 ; Dapolito Dunn, 2010 ; Johnson, 1989 ; Arup, 2016 ; Rowe, 2011), reduction of suspended particles (Bernier, 2011), energy savings through shading, insulation, and evapotranspiration (Jim, 2011 ; Jaffal, 2012 ; Li, 2014) mitigating the urban heat-island effect (Dapolito Dunn, 2010 ; Arup, 2016 ; Brack, 2002), improved storm-water management (Dapolito Dunn, 2010 ; Sacré, 2016 ; Fioretti, 2010 ; Nagase, 2011) or attenuating noise (Connelly, 2008) ii) support services, such as increased biodiversity and urban wildlife habitats (Alvey, 2006 ; Cornelis, 2004 ; Oberndorfer, 2007) iii) cultural services such as increased aesthetic value of city buildings (Brack, 2002) and improved life environment (Bernier, 2011 ; Grahn, 2003 ; Lohr, 2010)

Green roofs are classified in three types based on their substrate depth (figure 1) : extensive green roofs with substrate depth lower than 15 centimetres (FLL, 2008), intensive ones over 15 centimetres and semi-intensive rooftops varying from 10 to 20 centimetres (O'Keeffe, 2008 ; Caldwell, 2010 ; Sutton, 2012).

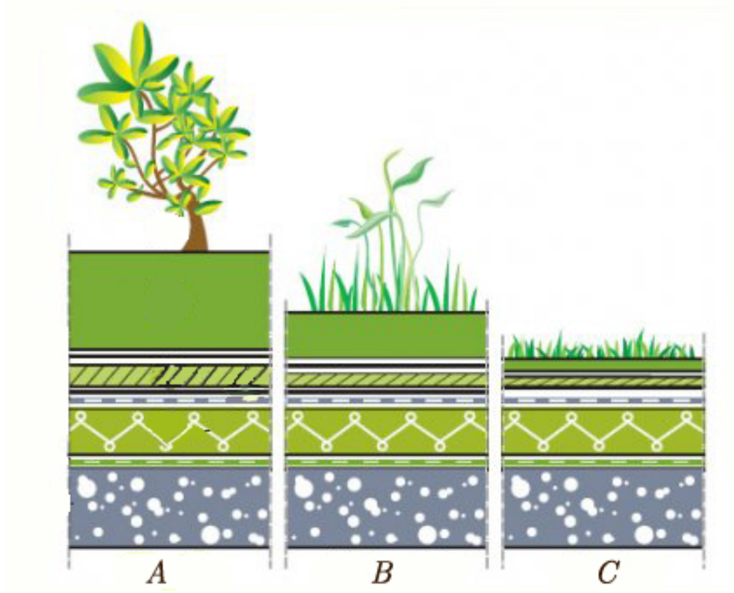

Figure 1 Distinction between green roofs types (http://sandiegosolar.com/roofs.html) - A intensive green roof ; B semi-extensive green roof ; C extensive green roof.

Generally intensive green roofs support a wider variety of plant types (figure 2) but need more irrigation and maintenance than extensive infrastructure (Culnane, 2014), while semi-intensive systems combine aesthetic potential of intensive green roofs and environmental benefits of extensive ones (Dunnett, 2004).

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Figure 2 Left : Extensive green roof ; middle : Semi-intensive design ; right : intensive rooftops

For this reason, and considering the current prevalence of aged buildings in city cores, lightweight substrate (plant weight is negligible in extensive substrate) seems to be the only applicable solution that suits to load-bearing capacity on their infrastructure (Nektarios, 2011).

According to Ondoño et al. (2014), the choice of substrate is also essential by providing support for plant growth (Nektarios, 2011). Availability of nutrients and water depends on the biogeochemical cycling properties and, therefore, on the presence of microorganisms (Roldan, 1994). Adequate capacity of nutrient and water is, so, needed in ideal substrate. Consequently, the value of maximum of 20% organic matter is accepted in all guidelines for green-roof in a way to maintain the balance between lightweight and well-drained material. This ratio enables to save large quantities of nutrients during run-off (Beattie, 2004; Dunnett, 2004 ; Li, 2014) but leads to lower microbial development than 50 organic : 50 mineral compost dose (Ondoño, 2014).

Moreover, plant growth and survival depends on substrate type and depth particularly in unirrigated extensive green roofs (Boivin, 2001; Dunnett, 2007; Durhman, 2007; Getter, 2006; Thuring, 2010; VanWoert, 2005; Nektarios, 2011). Extensive extensive green roof may lead to high draining capacity, in the same way than natural habitats with shallow soils, (Lundholm, 2006) and plant viability risks during during freezing periods (Getter, 2006).

The biodiversity on green infrastructures is inversely proportional to substrate depth. Increasing substrate depth could lead, thus, to less extreme conditions and facilitate the plants establishment (Thuring, 2015). In a way to ensure the colonization of species and their persistence, it is though recommended to replicate faithfully a heterogeneous natural habitat through variation in roof substrate depth (Grant 2006).

Furthermore, the green roofs are generally characterized by hostile environment for plants : high solar radiation or extended shaded time, extreme temperatures and drought, shelter or high levels of wind and decreased moisture (Piana, 2014 ; Walker, 2011 ; Oberndorfer, 2007). All this leads to general stress like disturbance in resources availability to plant communities (Brown, 2015).

The succulent plants are the most adapted species to green roofs. However, the selection are increasingly focused on native plants providing opportunities to restore lost flora in the urban environment (Bousselot, 2011 ; Dunnett, 2010 ; Nagase, 2010). Moreover, their ability to better cope with ecosystem services than sedum spp system is an asset (Lundholm, 2010 ; Oberndorfer 2007). The creation of familiar habitats for the local fauna is an example (Nagase, 2011).

Considering all roof constraints, it is commonly accepted that green roofs offer analogous conditions to natural rocky habitats over shallow soil (Lundholm, 2006). The adaptations of plant species from urban-analogue environments allow them to survive in such harsh conditions.

More specifically, along with low depth and high draining capacity, extensive substrates are relatively calcareous. These conditions are very similar to calcareous grasslands habitats, ecosystem with high biodiversity value that has tremendously decreased in Western Europe.

Therefore, the choice of endemic shrub and herbaceous plants from calcareous grasslands has been accepted to provide added value to green roof design (Choi, 2012).

Calcareous grassland flora may be able to tolerate dry period no matter what the depth and substrate are. Nevertheless, it's important to notice that the correct balance of water is rarely encountered on green roofs (Latshaw K. , 2009). From drought to flood, the circumstances could be problematic and not only for plants.

Analogue Habitat

In contrast with natural ecosystem, there are anthropogenic habitats. They differ substantially from the natural ones they replaced due to Human disturbances such as alterations to resources availability, addition of toxic chemicals, etc. (Kozlov, 2007). According to anthropogenic influence, the habitat may result in functional novelty (novel ecosystem) or stay close to natural ecosystem (analogous habitat).

Generally, the difference between analogous habitat and novel ecosystem stands with the level of management, and thus its non-persistent nature (Hobbs, 2006; Kowarik, 2011; Lundholm, 2010) (figure 3). Green roofs generally belong to the first category, analogous habitat.

The similarities between natural and analogous habitats allow spontaneous spread of species from their endemic habitat to anthropogenic analogues (Ursic, 1997 ; Tomlinson, 2008). More specifically, urban ecosystems are mainly colonized by plants native from local natural ecosystems dominated by rocks or shallow soils (Woodell, 1979 ; Lundholm, 2006).

Therefore, the analogous habitats may sustain the biodiversity in considerably modified ecosystem. In England, for instance, 10% of self-spread invertebrate species are considered as nationally rare. The number of native species that have spontaneously colonized these habitats is quite low, though. It means that analogous habitats in cities could represent natural refuge for rare plant species (Lundholm, 2010) and biodiversity (Larson, 2000).

Management to restore analogous habitats from a state of novelty would be a way to restore ecological connections in the matrix of urban landscapes. Besides, green roofs may contain several analogous habitats from shallow and dry to deep soil and wet soil. These conditions may even coexist through heterogeneity in substrate. Consequently, there is a lot of potential for engineering on roofs.

Increasing species diversity, and consequently many functional group, generally lead to a resources-use complementarity by resident communities to avoid invasive plants to establish (Lundholm, 2010 ; Young, 2009).

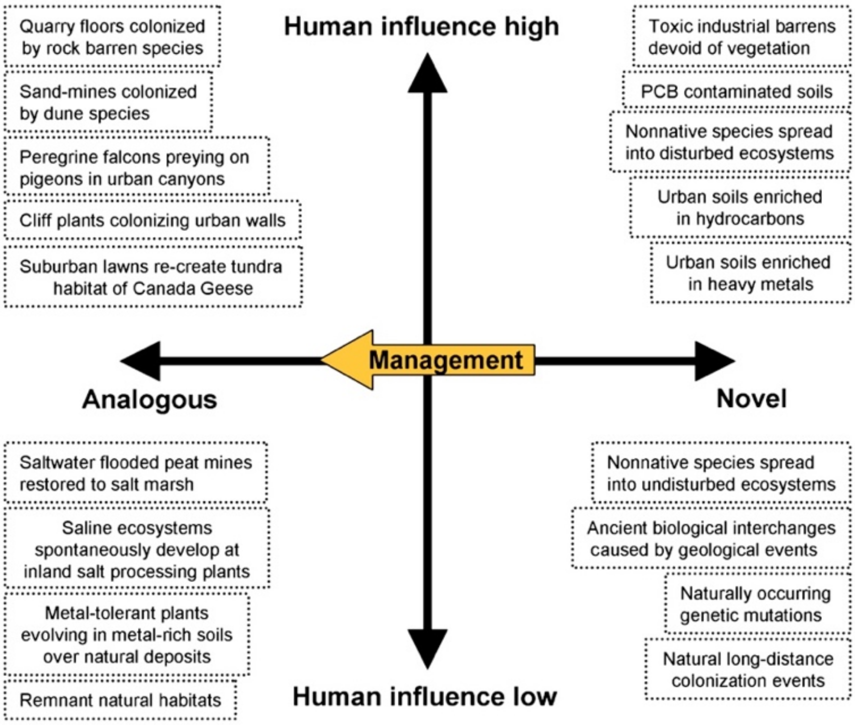

Figure 3 Graphic representation of distinction between novel ecosystem and analogous habitat (Lundholm, 2010).

In these circumstances, restoration ecology can be based on two main questions. Are green roofs conditions similar enough to natural ecosystem to have potential value for native biodiversity ? Which species functional traits and habitat conditions promote colonization success of area with harsh conditions as green roofs ? (Rehounkova, 2010).

Restoration Ecology

At the dawn of ecology restoration, we can find the Initial Floristics Model of Frank Egler (1954). His model provides keys to establish different types of stables communities based on variation in initial composition. Egler developed that late-successional species already present in the seedbank were the only one to be able to change the course of *community*[2] development due to their sufficient numbers. This last point will later be linked to the priority effect.

[2] ***Community*** *is defined as "an ecological unit composed of a group of organisms or a population of different species occupying a particular area, usually interacting with each other and their environment". Source : biology-online.org/dictionary*

As well explained by Young (2000), there is a profound consistency between restoration ecology and community ecology. Indeed, the greatest challenges and opportunities of restoration ecology is rooted in the restoration of complex communities.

Wherefore, many theories were designed as conceptual bases in community ecology. From these theories, two main conceptual models emerged : *community succession* and *community assembly*. Broadly, community succession model predicts the *climax*[3] and the assembly one developed the existence of many stable states.

The first one is designed to predict changes in species composition after disturbance. The pattern of appearance and disappearance could be therefore determined as the causal mechanism of establishment and persistence of species. It concentrates on the processes that bring back to a pre-disturbance community composition. One approach is to identify the traits that organise the species in a specific stages of a specific sequence. (Young, 2001)

In other words, the development of a specific community could be described as a fluctuation of species associations that replaced each other through time in a specific sequence (Young, 2001).

The assembly theory, one the other hand, is the legacy of Egler's successional model (1954). It focuses on the multiple stable states that can exist instead of the role of transition processes. It provides explanation to composition, the similarities among communities even if it implicitly incorporates process (Young, 2001).

In terms of restoration, the community development is determined by several random parameters : species colonization rates, the likelihood of establishment and persistence of species in the community (Young, 2001).

Therefore, several filters were highlighted to sustain this theory (figure 4). Firstly, dispersal limitation that prevent species from reaching a restoration site (Clements, 1916 ; Egler, 1954 ; Funk, 2008) ; The second filter concerns abiotic site conditions that favour the establishment and survival of species (Hobbs, 2004 ; Cleland, 2013) ; Finally, the biotic concerns inter-specific interactions that limit the persistence and abundance of individual species (Funk, 2008; Cleland, 2013).

In addition, four processes are important : the *Priority effect* is the time of arrival on site. The first species to arrive can become dominant in the community and block invasion particularly for plants with similar niches ; The *Recruitment limitation effect* that occurs during dispersal or establishment stage ; the *Mechanisms of establishment* (Young, 2001) ; and finally, *Facilitation* that occurs

[3] ***Climax*** *is defined as "the last stage in ecological Succession. An ecosystem in which populations of all organisms are in balance with each other and with existing abiotic factors". Source : biology-online.org/dictionary*

when a plant overcomes dispersal barriers thanks to species that change their environment to ease colonization (Bertness, 1994; Callaway, 1997).

Taking all into account, it would be a mistake to simply plant a mix of species a priori appropriated on a particular environment considering that the process of succession will lead the community to a pre-disturbance composition (Holl, 1999).

Currently, as long as structural and functional attributes of the analogous ecosystem match those of the natural habitat, they are considered as identical (Schrader, 2006). Many projects are even using green roofs as habitat compensation (Williams, 2014). It is therefore crucial to evaluate the relevance of these assumptions.

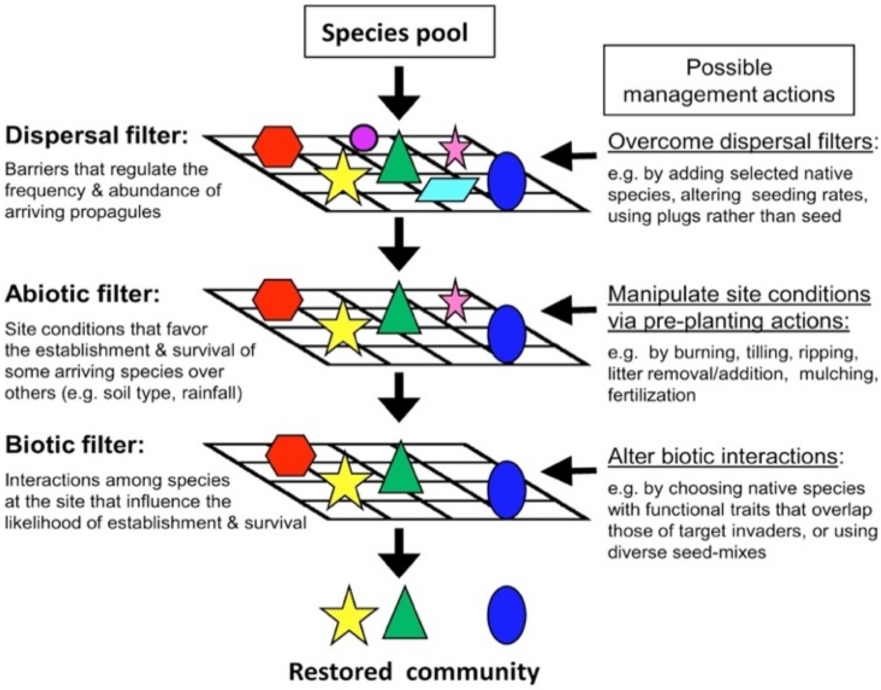

Figure 4 Community assembly filter model (Hulvey, 2014)

In such cases, it is needed to approach the problem the wrong way round. The purpose of this study is the description of communities based on the same seed mix through different environmental variable on green roofs while common studies focus on setting specific parameters to restore specific communities.

Calcareous Grasslands

Roughly, grassland may be defined as "*ground covered by vegetation dominated by grasses, with little or no tree cover*" (Silva, 2008). UNESCO agreed on a limit of ten percent of trees and shrubs cover to be considered as grassland.

From almost desertic Mediterranean grasslands to north humid ones including steppic and mesic ecosystem, Europe gathers many types of grasslands : natural grasslands, semi-natural dry grasslands and scrubland facies, sclerophillous grazed forests, semi-natural tall-herb humid meadows and mesophilic grasslands.

The majority of European grasslands have been degraded and maintained as semi-natural state. Their maintenance is performed by agriculture through grazing and/or cutting regimes which are crucial for this habitat and its species (Silva, 2008). Hopefully, there are still some natural permanent grasslands in Europe.

The value of calcareous (chalky) grasslands concerns their species richness. These natural habitats support a high biodiversity by limiting the establishment and growth of the competitive species in harsh growing conditions. It gives more chance to less-aggressive plant to co-exist (Grime, 2002). These ecosystems could gather up to 80 plant species per square meter and consequently high arthropod diversity per instance.

The characteristics of calcareous grasslands soil are similar to green roofs : typically thin, light, and dry. It leads to an overlap of environmental conditions that suggests higher results and fewer maintenance requirements (Choi, 2012)

According to FAO (2006), the area of grasslands in the EU declined by 12.8% from 1990 to 2003.

It remains only strongly fragmented areas that leads to extinction of species. Furthermore, anthropogenic activity induced loss in habitat quality of the remaining grasslands (Choi, 2012). The threats are numerous like changes in livestock density, the intensification of grasslands management and mowing by the use of fertilisers, pesticides and phytocides, the lowering of watertable, etc. More specifically, the area occupied by dry grasslands has dramatically drop (Delescaille, 2005) by 90% of their area and 30% of their characteristic plant populations in 90 years (Piqueray, 2011)

Recently, this habitat shows his potential and lead the ecologists to restore this forgotten habitat with the Commission's environment and nature funding programme, LIFE.

Functional traits

As indicated by Sandel (2011), "*functional traits and trait-based community ecology theory can provide a basis for predicting the success of a restoration treatment in a particular community*". Hence, the inter-population restoration capability may be predicted from their traits (Lavorel, 2002 ; Pywell 2003 ; da Silveira Pontes, 2010 ; Roberts, 2010).

According to green roof conditions, Sandel (2011) suggested that inter-population restoration capability may be predicted from their *functional traits*[4], which includes *Specific Leaf Area* (SLA) and seed mass. Therefore, in this study, these two last traits were selected to be representative of plant colonizing abilities on green roofs. SLA is globally a way to evaluate resource saving strategy of plant. Nonetheless, only his aspect of rapid returns on leaf investments is to be considered when soil resources become more limiting (Knops, 2000). On the other hand, seed mass contributes to improved seedling success, in ecosystem poor in nutrients (Milberg, 1998).

4 ***Functional traits*** *are defined as "morphological, biochemical, physiological, structural, phenological, or behavioral characteristics that are expressed in phenotypes of individual organisms and are considered relevant to the response of such organisms to the environment and/or their effects on ecosystem properties" (Violle et al. 2007).*

Chapter 2 : Green roofs as analogous of calcareous grasslands ? Species response to substrate heterogeneity.

Abstract

The environmental conditions on green roofs can strongly influence plant growth and survival. However, community scale study has been little conducted. We have undertaken an experiment in which green roofs differed in substrate depth and type (variation in organic matter rate, water capacity and draining capacity). The same seed mix was used to assure comparison. This mix consists of 22 species : 4 generalist species and three corteges of 6 specialist species. During two months, with no irrigation, the abundance of individuals (seedlings and adults) was calculated in a way to potentially differentiate communities. It appears that the environmental conditions on green roofs resulted in community distinction as in analogous habitat. Both substrate depth and weather affected community evolutionary path. The plant individual dynamics, and consequently their success rate, might help to explain these differences. However, this study could not highlight any influence of their traits, especially *Specific leaf are* (SLA) and *Seed mass*, that have demonstrated their value in providing information about rapid returns on leaf investments and improved seedlings success in low-nutrients environment. As a conclusion, it is recommended to incorporate environment conditions into appropriate plant selection.

Introduction

Concrete represents a big part of urban cities areas and leads to a poor installation and development of biodiversity. However, it is important to balance the ratio between impervious and green surfaces to maximize the possibilities for the development of biodiversity and ecosystems in urban settlements. The history of modern green roofs started in Germany in the 1960s and has since spread to many countries. From that time, many studies have demonstrated green roof benefits, including ecosystem services support (Dapolito Dunn, 2010).

Urban green infrastructures may be developed in a way to support biodiversity and restore ecological networks. Historically, ecological restoration aimed to restore ecosystems to their original state before being impacted. However, in urban context, environmental conditions are drastically altered. In these circumstances, urban ecological restoration would aim to develop analogous ecosystems or to manage novel ecosystem (Lundholm, 2010). Both of them result from Human disturbances (Kozlov, 2007). The analogous habitat stays close to natural ecosystem while the novel ecosystem results in functional novelty (Lundholm, 2010).

Urban restoration ecology can be based on two main questions. Are analogous habitats similar enough to natural ecosystem to have potential value for native biodiversity ? Which species traits and habitat conditions promote colonization success of urban or post-industrial landscapes ? (Rehounkova, 2010). These questions are even prominent in isolated and stressful environment such as on green roofs.

Currently, green roofs are classified in three types based on the substrate depth : extensive green roofs with substrate depth lower than 15 centimetres (FLL, 2008), intensive ones over 15 centimetres and semi-intensive green roofs varying from 10 to 20 centimetres (O'Keeffe, 2008 ; Caldwell, 2010 ; Sutton, 2012). Generally intensive green roofs support a wider variety of plants types but need more irrigation and maintenance than extensive infrastructure (Culnane, 2014) while semi-intensive systems combine aesthetic potential of intensive green roofs and environmental benefits of extensive ones (Dunnett, 2004). However, considering the low load-bearing capacity of aged buildings (Nektarios, 2011), extensive green roofs stays the most used solution.

Precisely, considering hostile roofs constraints (Oberndorfer, 2007), it is commonly accepted in Western Europe that green roofs offer analogous conditions to natural rocky habitats over shallow soil (Lundholm, 2006). Therefore, the species selection is increasingly focused on native plants from calcareous grasslands. They tolerate dry period regardless of substrate, provide allowance to lost flora in the urban environment (Dunnett, 2010) and better cope with ecosystem services than sedum spp. systems (Lundholm, 2010).

Piqueray et al. (2007) identities seven calcareous grassland communities in Southern, Belgium (table 1). Those communities, that differ in plant assemblages, are correlated to environmental variables corresponding to main stress conditions on green roofs. Specifically, the different calcareous grassland plant communities present noticeable differences in soil depth with two main groups : mesophilic and xerophytic grasslands completed by an inter-substrate situation. Soil depth in calcareous grasslands varies between 0,5 and 15 centimetres Harzé (2016), a range similar to extensive green roofs. Furthermore, the variability in environmental conditions results in differences in hydrological status.

Substrate type used on green roofs is also to be considered (Thuring, 2010) along with depth (Ondoño, 2014) to extend the range of environmental conditions. Indeed, water capacity increase with the organic matter content (Patriquin, n.d.). Consequently, extensive substrate leads to low water capacity, as natural habitats with shallow soils (Lundholm, 2006); and high viability risks during freeze period (Getter, 2006).

Table 1 Comparison of mean environmental variables between grassland communities identified on the basis of the TWINSPAN classification. Pairwise comparisons; using Kruskall-Wallis test. mF = pean Ellenberg indicator value for soil moisture; mN = pean Ellenberg indicator values for nutrient status (Piqueray, 2007)

Group	*I*	*II*	*III*	*IV*	*V*	*VI*	*VII*
Soil depth	4.14	8.90	4.67	2.78	2.26	2.90	3.57
pH	4.99	6.27	6.75	6.61	6.47	4.88	4.68
mF	3.58	3.92	3.77	3.09	2.98	3.28	3.12
mN	2.89	3.19	3.08	2.39	2.13	2.86	2.40

Consequently, green roofs may be seen as habitat analogue to calcareous grasslands and may be considered as an opportunity for biodiversity restoration in urban areas. It is viewed in the context of the dramatically shrunk of areas occupied by dry calcareous grasslands (Delescaille, 2005) while their undeniable high value, through their number of plant and animal species per square meter, is acknowledged.

In a way to ensure the colonization of species and their persistence, it is recommended to replicate faithfully a heterogeneous natural habitat through depth variation in roof substrate (Grant 2006).

Elsewhere, according to green roof conditions, Sandel (2011) suggested that inter-species restoration capability may be predicted from their traits. Two traits are regularly used as representative of colonizing abilities in a restoration programme, i.e. *Specific Leaf Area* (SLA) and *seed mass*. SLA providing information about rapid returns on leaf investments (Knops, 2000) and seed mass contributing to improve seedlings success, in ecosystem poor in nutrients (Milberg, 1998).

In this study, we test whether plant species environmental conditions variation on green rooftops could lead to the same distinction of plant communities as on natural ecosystems. Specifically, we test the success of installation of plant species representative of the different calcareous grassland plant communities in relation to depth variation of two green roof substrates with different nutritive properties.

Materials and Methods

Study Site

The system was disposed on Gembloux Agro-Bio Tech's campus in Gembloux, Belgium (50°33'52'' N, 4°41'53''). The study site extends over an area of a hundred square meters. According to the Köppen-Geiger classification, the climate is Cfb type. (https://fr.climate-data.org/location/12858/ , 23/3/2017 at 9:30). Gembloux has a warm temperate climate with a year-round temperature of 9.6°C. There is significant precipitation, even during the driest month, with on average 830 mm each year.

Due to infrastructure, site may receive shade for portion of the day during winter along South-East side. In order to consider the potential shadow effects, randomized block design has been chosen. The blocks were oriented north-north-west to south-south-east to comply to constraints of the site.

Green Roof System

We used container units to mimic green roof system. Each one had the same seeded vegetation but differed by their soil environment. Indeed, this study aims at evaluating the two main characteristics influencing plant development on extensive green roofs : two different substrate types (*Substrat leger pour toiture verte Extensif Zinco®* and *Substrat leger pour toiture verte Lavandulis Zinco®*) and three substrate depths (5, 10 and 15 centimetres). The growing substrate consisted in : mixture of recycled tile and bricks, selected ceramic material and adjuvant added to organic material for *light extensive Zinco®* substrate ; mixture of recycled ceramic material and selected additives supplemented with organic material for *Light Lavandulis Zinco®* substrate.

These substrates have been specifically chosen according to their characteristics. They differ under three criteria : organic matter, water flow and water capacity (table 2). Selected substrate depths are within the range of soil depth on calcareous grasslands (Piqueray, 2007 ; Harzé, 2016).

Table 2 Characteristics of Zinco® substrate

	Extensive Zinco® substrate	*Lavandulis Zinco® substrate*
Organic matter rate	*< 65 g/l*	*< 90 g/l*
Water flow rate	*0,6 – 70 mm/min*	*0.3 – 30 mm/min*
Water capacity	*~ 40% volume*	*~ 50% volume*

Each experimental module consisted of "*pallet-case made of sheet steel, reduced height, with solid walls*" (1x0,8x0,3m), draining layer (*Fixodrain XD 20 Zinco®*) that covers a protection layer (*Natte de protection latérale SM-R Zinco®*).

Plant Material

We selected twenty-two species of calcareous grasslands (table 3), all natives from Belgium and available as seed lots among fourteenth European green roof seed mix : ecosem.be, habitataid.co.uk, wildseed.co.uk, ecovegetal.com, vecover.com, optigreen.com, biodiversite-positive.fr, api-site-cdn.paris.fr, greentop-greenroofs.com, ufasamen.ch, greenroofs.com, greenroofplants.com, neu.rieger-hofmann.webseiten.cc, completed by the species mentioned by Min-Sung Choi (2012) in his PhD.

Selected species comprised four generalist species, with wide range of hydrology tolerance, and 18 specialist ones. The 18 specialist species were divided in three groups of 6 species each (table 3). Groups of specialists are representative of the two main calcareous grasslands plant communities prevailing in Belgium (Piqueray, 2007) characterized by different drought stress (*Mesobromion erecti*, *Xerobromion erecti*) and an intermediate association. We hypothesized that the three groups of specialist species will demonstrate a different reaction to green roof substrate depth and type used as a proxy of drought stress gradient.

The experimental design was arranged in five blocks. They are made up of six growing containers for each environmental combination substrate type X depth. These five blocks experimentally stand for five replicates. Within blocks, the containers were located randomly and spaced from each other to limit interactions between them, and to allow walkways for access.

One hundred and ten seeds per species and square meter were seeded on the 21th of April, 2017. Containers were monitored nine times from this date to the 6th of July. It was based on the counting, for each container, of the individuals (seedlings and adults) for any planted specific species. These plants received no water but rain.

Statistical Analyses

Descriptive statistics allowed to visualize differences in species success between substrate depth and type. These basic statistics performed on Excel® include the total sum, mean, minimum, maximum as well as the standard deviation (tableau 3) of the species abundance (number of individuals) according to the three parameters mentioned above : block, substrate type and substrate depth.

General Linear Model (GLM) method were used to identify the relevance of our variables using Poisson distribution. This approach was chosen specifically as common and familiar method unbalanced count data.

Departing from the same seed mix, the nail of the head was the characterization of communities through the abundance of the 18 specific plants. Due to a large proportion of *null* abundance in our data, we selected the principal coordinate analysis (PCoA) as ordination method. This ordination was based on the dissimilarity matrix calculated through Bray-Curtis method suggested for abundance data. It was realised in statistical software, R®. As abundance was quite heterogeneous among species, data were modified through logarithmic transformation in a way to identify gradients while minimising the dominance of specific species in this analysis.

Individual species dynamics was next studied. To that end, we focused on depicting abundance according to time. The Student multiple comparisons procedure ($\alpha = 0.05$) helps us to statistically identify significant differences between mean of abundance per species and substrate depth. This method was realised on the basis of a one-way GLM for each species.

The two last analysis concerned how the functional traits can provide a basis for predicting the success of species as indicated by Sandel (2011). These ones concern one reading according to the seed mass and SLA. Seed mass have to be related to the abundance at beginning of the study (on the 14^{th} of May) while SLA will concern the last survey (on the 6^{th} of July) in accordance with the information they provide. Linear Regression between plants traits and species mean abundance was calculated to determine the relevance of these assumptions.

Our trait analyses were made considering the values of functional traits given by the Universität of Oldenburg's database. This one was a collection of data from other universities and research studies. The mean of every specific trait value was considered since one single value was required.

Results

In regard of germination issue, six of the eighteen specialist species (*A. eupatoria, A. sylvestris, C. glomerata, O. vulgare, P. veris* and *R. lutea*) were removed from the GLM analysis along with the four generalist ones.

*General Linear Model (*GLM*) analysis* – A preliminary global analysis on species abundance was realized in a way to demonstrate variables influence on the abundance of species with four-way mixed crossed model : block, species (plant), substrate type and depth. Blocks show a marginally significant influence (p-value = 0.014148). Therefore, we will not consider this variable to increase the power of the following analyses.

Considering the site climate during the study, a comparison between drought and rainy periods has been added to the analysis. Indeed, according to *IRM* data, the week leading up to the 25^{th} of June was marked by a heatwave (five days above at least 25 degrees, of which at least three days above 30 degrees or more) without rain. On the other hand, the weeks leading up to the 6^{th} of July was marked by lower temperature which did not exceed 26.8°C maximum and daily rainfalls.

In this situation, we realized two GLM with species abundance at the end of the drought period (at the 25^{th} of June), on the one hand, and at the end of the wet period (at the 6^{th} of July), on the other hand. We used a three-way determined crossed model. Interestingly, significant interaction is noticed between species and depth as well as substrate type at the end of the wet period (but not at the end of the dry period) (Table 4), indicating that species did not react in the same way to substrate depth and type variation. Moreover, the interaction between substrate depth and plant, as well as substrate type and plant, is significant. It means that species react differently to substrate depth or type variation. Considering these interactions, it is not possible to study variables separately.

Table 3 General Linear Model analyses on species abundance testing the global effect of species, substrate type and substrate depth on species abundances (wet period and dry period separated)

		Df	Deviance	Resid. Df	Resid. Dev	Pr (>Chi)
wet period	*NULL*			359	3006.66	
	Substrate Depth	2	585.64	357	2421.02	< 2.2e-16 ***
	Substrate type	1	2.21	356	2418.81	0.136945
	Plant	11	1533.36	345	885.45	< 2.2e-16 ***
	*Depth*type*	2	2.35	343	883.10	0.308808
	*Depth*plant*	22	235.91	321	647.19	< 2.2e-16 ***
	*Type*plant*	11	43.86	310	603.33	7.701e-6 ***
	*Depth*type*plant*	22	47.23	288	556.10	0.001369 **
dry period	*NULL*			359	2823.02	
	Substrate Depth	2	860.34	357	1962.68	< 2.2e-16 ***
	Substrate type	1	2.99	356	1959.69	0.0840141
	Plant	11	1387.95	345	571.74	< 2.2e-16 ***
	*Depth*type*	2	30.36	343	541.39	2.559e-7 ***
	*Depth*plant*	22	92.70	321	448.69	1.187e-10 ***
	*Type*plant*	11	33.66	310	415.03	0.0004104 ***
	*Depth*type*plant*	22	26.24	288	388.79	0.2415055

PCoA analysis - In the figure 5, an arch gradient is clearly visible. The first axis (*dim1*) explains 35.48% of the variance while the second axis (*Dim2*) explains 12.02%.

First axis clearly differentiates 5 centimetres containers, correlated to negative values of the first axis, from 10-15 centimetres containers correlated to positive values of axis 1. Substrate type is correlated to the first axis but is not significant in regard of depth influence. Therefore, it won't be considered in the following analysis as it demonstrates no pattern influence whatever the period.

Plant assemblages are much more heterogeneous on 5 centimetres depth compared to the 10 and 15 centimetres depth, as demonstrated by the dispersion of 5 centimetres quadrats in the ordination plan. Five centimetres containers plant assemblages are mainly characterized by the absence or low abundance of a group of species : *A. millefolium* (mesophilic), *C. scabiosa* (meso-xerophytc), *O. viciifolia* (meso-xerophytic).

To keep on with comparison of ecological groups, the proportion of the three groups of specialist species (xerophytic, meso-xerophytic and mesophilic) were calculated by dividing the total abundance of species per cortege by the total abundance of species of each substrate depth. The data are based on the mean of the five replicates and types.

Table 4 Seed mix sowed on experimental green roofs. Basic statistics are made regardless the variables. Sum = total number of individuals of the species in the experiment ; Mean = mean number of individuals per container ; Maximum = highest number of indivudal in one container ; Minimum = lowest number of indivudal in one container ; Standard deviation = variability in the number of individuals per container

Latin name	*Supplier*	*Cortege*	*SLA (m_.kg-1)*	*Seed mass (10-3 gr)*	*Sum*	*Mean*	*Maximum*	*Minimum*	*Standard deviation*
Carex flacca	*Rieger hofmann*	*Hygrophilic - xerophytic*	15.0	0.12	0	0.0	0	0	0.0
Festuca rubra commutata	*Rieger hofmann*	*Mesohygrophilic - mesophilic*	-	0.12	0	0.0	0	0	0.0
Hieracium pilosella	*Rieger hofmann*	*Mesophile - mesoxerophytic*	15.3	0.15	1	0.0	1	0	0.2
Teucrium chamaedrys	*Rieger hofmann*	*Mesoxerophytic - xerophytic*	13.0	1.50	0	0.0	0	0	0.0
Achillea millefolium	*Ecosem*	*Mesophilic*	16.7	0.17	412	13.7	32	0	9.9
Agrimonia eupatoria	*Ecosem*	*Mesophilic*	17.0	23.08	0	0.0	0	0	0.0
Anthriscus sylvestris	*Ecosem*	*Mesophilic*	30.0	3.83	0	0.0	0	0	0.0
Geranium pyrenaicum	*Ecosem*	*Mesophilic*	25.8	1.41	309	10.3	26	0	9.2
leontodon autumnalis	*Rieger hofmann*	*Mesophilic*	19.0	1.36	75	2.5	10	0	2.8
Clinopodium vulgare	*Ecosem*	*Mesophilic - mesoxerophytic*	23.6	0.49	14	0.5	4	0	0.9
Centaurea scabiosa	*Ecosem*	*Mesoxerophytic*	18.0	7.46	134	4.5	13	0	3.3
Helianthemum nummularium	*Rieger hofmann*	*Mesoxerophytic*	14.0	1.38	15	0.5	8	0	1.6
Malva sylvestris	*Ecosem*	*Mesoxerophytic*	22.5	3.72	318	10.6	21	0	6.2
Onobrychis viciifolia	*Ecosem*	*Mesoxerophytic*	17.1	20.66	337	11.2	23	0	7.2
Origanum vulgare	*Ecosem*	*Mesoxerophytic*	15.7	0.15	0	0.0	0	0	0.0
Reseda lutea	*Ecosem*	*Mesoxerophytic*	21.0	0.91	0	0.0	0	0	0.0
Allium schoenoprasum	*Rieger hofmann*	*Xerophytic*	10.4	1.18	55	1.8	5	0	1.9
Anthyllis vulneraria	*Rieger hofmann*	*Xerophytic*	13.1	4.87	36	1.2	7	0	1.8
Calendula arvensis	*Ecosem*	*Xerophytic*	31.1	2.60	352	11.7	21	0	6.0
Campanula glomerata	*Rieger hofmann*	*Xerophytic*	22.1	0.17	0	0.0	0	0	0.0
Echium vulgare	*Ecosem*	*Xerophytic*	16.0	2.81	280	9.3	16	1	4.3
Primula veris	*Ecosem*	*Xerophytic*	16.0	1.06	0	0.0	0	0	0.0

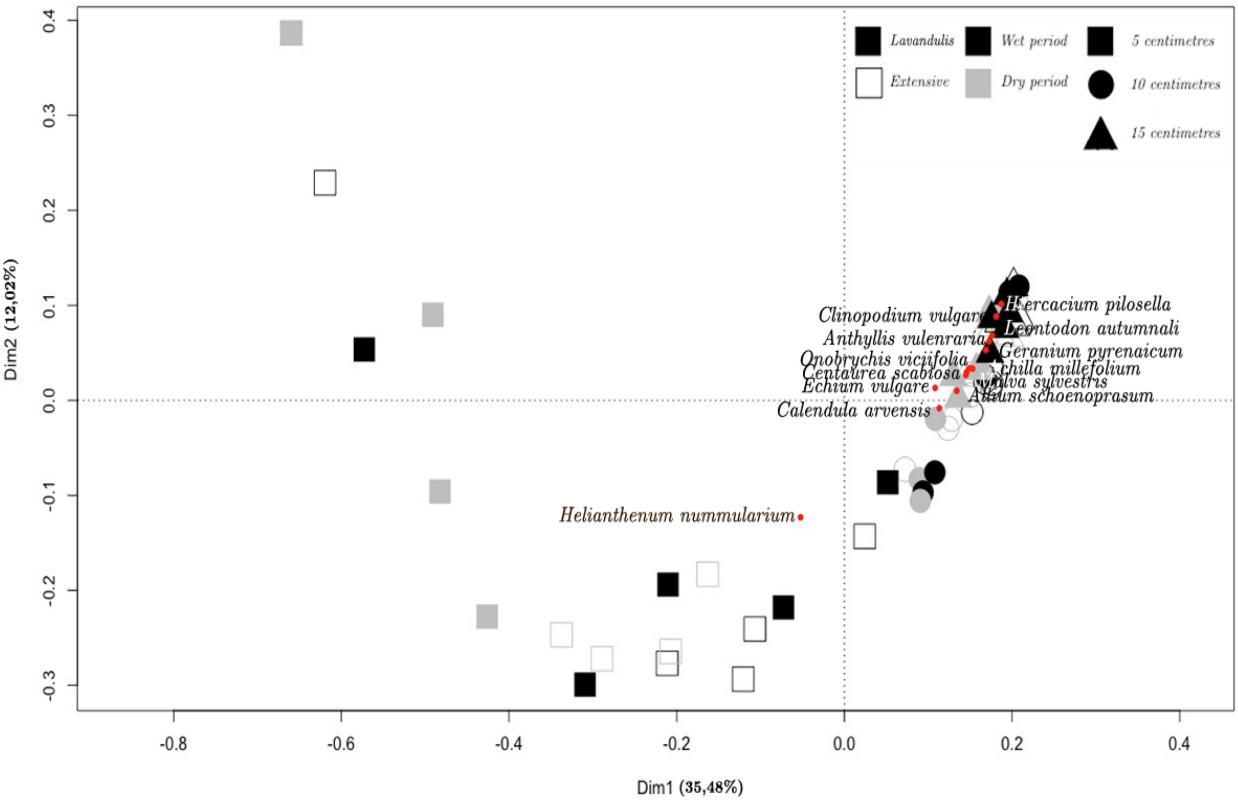

Figure 5 Graphic representation of PCoA analysis on species assemblage in experimental modules. Species assemblages are distinguished between three variables in a way of comparison : wet (species abundance at 6^th of July) and dry period (species abundance at 25^th of June) respectively in black and grey ; Substrate type are distinguished in full shape for Lavandulis and empty shape for Extensive ; Substrate depth is represented by geometrical shape respectively square, round and triangle for 5, 10 and 15 centimetres

Table 5 shows decreasing proportion of xerophytic plants with the substrate depth, while it's the opposite for the mesophilic species. The abundance of meso-xerophytic, on the other hand, is maintained regardless the depth of substrate.

Table 5 Proportion of each group of specialist species according to the depth of substrate.

	Mesophilic	*Mesoxerophytic*	*Xerophytic*
5 cm	0.1	0.3	0.5
10 cm	0.4	0.3	0.4
15 cm	0.4	0.3	0.2

Species dynamic - This topic was split in two parts. The first one (figure 7) is concentrated on the whole experiment period. In a way to be as clear as possible, the results are stated in twelve graphs presenting individual species dynamic. They present the evolution of the mean abundance of the plants through time on the three substrate depths. It can be noticed high difference in abundance patterns especially for the period to first germination events and influence of drought period. Indeed, *C. arvensis* (xerophytic), *E. vulgare* (xerophytic) and *M. sylvestris* (meso-werophytic) double their number of individuals in less than two weeks after drought. On the other hand, we can notice a better relative tolerance to drought for *A. millefolium* (mesophilic), *C. arvensis* (meso-xerophytic), *M. sylvestris* (meso-xerophytic) and *O. viciifolia* (meso-xerophytic) than the others. Indeed, with regard to their abundance, these species lose less than a quarter of their individuals during the drought.

Five species on the twelve tested do not show significant difference in mean abundance among substrate depths (table 6) : *A. schoenoprasum*, *A. vulneraria*, *C. arvensis*, *C. vulgare* and *H. nummularium.* Among other species, *G. pyrenaicum* and *L. autumnalis* show high difference in their abundance according to the three depth modalities, with increasing abundance with increasing depth substrate. Five species show significant differences in abundance between 5 cm and both 10-15 cm depth but no differences between 10-15 cm depth : *A. millefolium*, *C. scabiosa*, *E. vulgare*, *M. sylvestris* and *O. viciifolia.*

Table 6 Student multiple comparisons procedure on mean of abundance per species (final abundance at 6^th^ of July) per substrate depth. Different letters indicate significant differences for α = 0.05

	p-value	*5 cm*	*10 cm*	*15 cm*
Geranium pyrenaicum	0,000***	0,5 a	10,3 b	20,1 c
Leontodon autumnalis	0,000***	0,3 a	2,4 b	4,8 c
Achillea millefolium	0,000***	2,6 a	17,9 b	20,7 b
Centaurea scabiosa	0,000***	1,2 a	5,5 b	7,1 b
Echium vulgare	0,000***	5,1 a	10,3 b	12,6 b
Malva sylvestris	0,000***	4,9 a	12 b	14,9 b
Onobrychis viciifolia	0,000***	2,3 a	14,7 b	16,7 b
Allium schoenoprasum	0,049*	1 a	3 a	1,5 a
Anthyllis vulneraria	0,004*	0,1 a	0,9 a	2,7 a
Calendula arvensis	0,218	9,4 a	14,1 a	11,7 a
Clinopodium vulgare	0,139	0 a	0,6 a	0,8 a
Helianthemum nummularium	0,322	1,1 a	0 a	0,6 a

The second part (figure 6) is a success test considering the two selected traits. Indeed, seed mass is supposed to impact the germination capacity in non-optimal conditions while SLA operate on persistence through time.

Regression between species abundance (mean abundance per module all depth considered) at the 14^th^ of May and the 6^th^ of July, respectively with seed mass and SLA were not significant (seed mass : r : 0.1364, P = 0.1428 ; SLA : r = 0.1174, P = 0.1477).

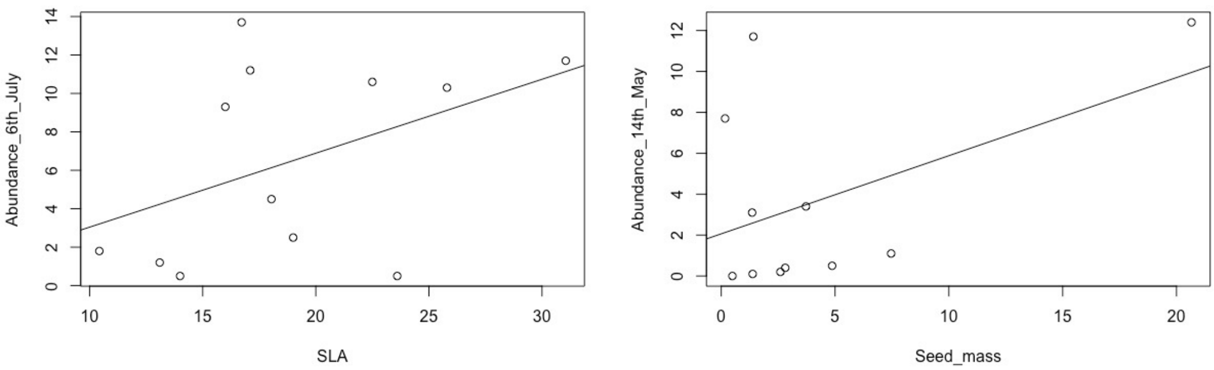

Figure 6 Left graph - Abundance of plant at the 6th of July according to specific SLA ; Right graph – Abundance at the 14th of May according to the specific seed mass.

Discussion

Our experimental design was made in view of technical constraints. The containers were ground level due to a lack of access to experimental roof. Although, we tried to replicate faithfully a high level roof, the containers were quite small and may have induced border effect like preferential water flow or unusual soil drying.

Another point concerns the plant materiel. Four generalist plants were specifically chosen in a way to facilitate the germination of specialist species through facilitation effect. Unfortunately, two of them did not germinate and, consequently, could not play their role of nurse specie. In the future, germination tips have to be studied in a way to assure a high germination rate.

Secondly, as mentioned before, chance of survival is higher in deepest substrate. Moreover, we observed a specific spatial distribution in 10 and 15 centimetres containers. This observation leads us to hypothesize that the bigger individuals play a role of nurse specie. Therefore, it appears that it was sometimes impossible to determine individuals before they perish in the shallowest containers. However, this situation represented maximum 5% of our observations and do not affect the global pattern.

The calculation of abundance wasn't that easy. The first weeks has been managed without a problem. The last survey was nevertheless quite challenging. The number of species remains low, though. Indeed, to facilitate the recognition only six species per cortege were selected but are not sufficient to assure perfect comparison with natural habitats.

Environmental conditions gradient as explanation for the differentiation of communities - It appears that calcareous grasslands could be a potential source of native species for green roofs in Southern Belgium. As explained by Sutton (2012), they may be an alternative onto green roofs to Sedum spp. However, quite a few studies developed this side of the problem.

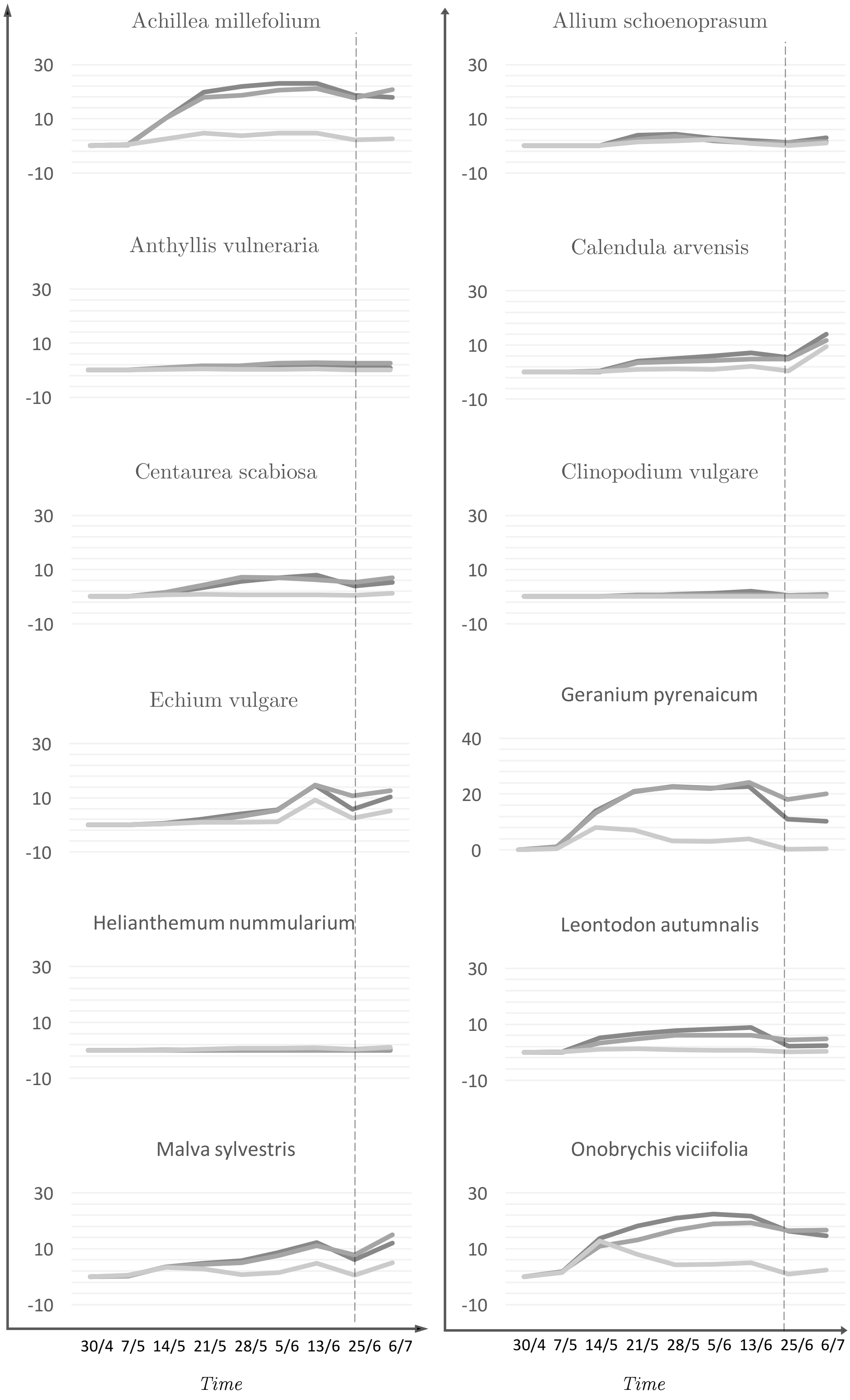

Figure 7 Evolution of plant mean abundance according to time. Lightest line - 5cm substrate ; Darkest line – 10cm ; Last one – 15cm. The two dashed lines put the light on the after drought survey.

Thuring (2010) demonstrated *"how appropriate species selection in the design of unirrigated extensive green roofs may be directed by factors such as substrate type and depth, as well as anticipated drought conditions"*. Our paper goes further by answering positively to our main question : Yes, there is a foreseeable differentiation of seeded plant communities among environmental conditions on green roofs. More globally, specific corteges react on green roofs and natural habitats in the same way. These differentiations are mainly lead by climate and substrate depth. Indeed, each depth corresponds to specific proportion of the three corteges of calcareous grassland plant communities : while shallowest soil possesses highest proportion of xerophytic plants, the deepest containers proportionally contain more mesophilic plants. It concurs with the conclusions of Thuring (2015) and Brown (2015). However, we could not concur with Thuring (2010) on the effect of substrate type on species abundance.

Currently, European seed mix are developed in two format, extensive and intensive one. Our experiment challenges these options, though. Indeed, these mix are too general and only partially germinate. Calcareous grasslands follow a gradient from 0 to 15 centimetres soil depth, seed mix should thus be adaptable to green roof environmental conditions, especially depth in this case.

Functional traits as success marker - If I may paraphrase Sandel (2011), plants behaviours seem to be linked to traits. SLA and seed mass, generally chosen to explain quick start and rapid returns on leaf investments on low-nutrients soil, are our two markers. These assumptions could not be confirmed through our experiment as shown by the low coefficient of determination of the regression lines. Considering the short period of development, we have to be cautious with these results, though. Furthermore, some species did not germinate neither in greenhouse for germination test nor outside : specific conditions of germination are apparently required even if they are not resumed in literature.

Conclusion

The overall objective of this experiment was to reproduce environmental conditions heterogeneity on green roofs as a way of differentiation of communities as on natural soil. This assumption was lead on the basis of three groups of 6 specific species and 4 generalist ones. The abundance of individuals (seedlings and adults) was the indicator of individual dynamic of species.

This study shows that, the reproduction of heterogeneity on green rooftops gives good results.

First, globally, substrate depth is a significant variable in differentiation of communities. While the shallowest soils demonstrate a higher proportion of xerophytic species, the deepest ones got more mesophilic plants.

This conclusion still needs to be seen as results on young green roof ($<$1 year). We guess that the future will lead these communities to greater analogy with natural ones.

With regard to the strategy of species, no statement could be made. Neither *Specific Leaf Area*, nor *Seed mass* seems to explain their success rate. However, those results have to be seen in the knowledge that we evaluated only eighteen species. Moreover, the specific characteristics could have played in germination issues.

Therefore, it would be interesting first of all to carry the same study with less diversity in species characteristics. Sowing seeds who differ only from their SLA could be a way to identify traits influence.

It would also be interesting to lead this study on real experimental rooftops and to check whether those conclusions could be acknowledged on real roof conditions.

In the future, this study deserves to be continued as it founds its interest in developing new perspectives for green roofs in Belgium. These green infrastructures, generally seen as a constraint, are mainly realised with sedum spp. to be as autonomous as possible while concept of biodiversity is ousted. Calcareous grasslands could be the perfect autonomous solution with shallow soil and high biodiversity rate. Moreover, heterogeneity is linked to high biodiversity rate. We could expect, in these circumstances, higher environmental and aesthetic benefits.

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