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Disentangling Dynamics of Green Roof Vegetation Analogue to Dry Grassland Over 3 Years: Plant and Substrate Response to Microenvironmental Variations

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ABSTRACT

Extensive green roofs (ExGR) present an opportunity to support urban native biodiversity. However, most of the existing ExGR in Europe support exotic stonecrop with poor plant diversity. The abiotic conditions of the ExGR substrate are analogous to those of native dry grasslands (poor, shallow and highly drained soil), providing opportunities for diversifying ExGR with native flora. This study characterized vegetation and substrate dynamics of a sown native dry grassland community (29 species) on a 254 m² ExGR in relation to microenvironmental conditions (substrate depths: 6, 12 cm; maximum sun exposure: 3-6h, 6-9h, 9-12h). The plant community taxonomic and functional compositions (%Competitive, %Stress tolerant, %Ruderal Grime strategies) were measured over three years after which the substrate was analyzed. The results indicated a strong effect of time since sowing on plant community composition, associated to effects of environmental factors. The specific richness and plant cover increased over time and were higher in plots with less insolation and greater substrate depth. Plant community functional composition was dominated by stress tolerant strategy but functional composition evolved through time with an increase in competitive strategy. Most substrate characteristics -pH, N, P, K, C/N, and % silt- were significantly influenced by the plant cover after three years. These results highlight the importance of temporal dynamics and microenvironmental variations on plant community outcomes within ExGR.

Key words: *Plant dynamics, native biodiversity, microenvironmental conditions, functional ecology*

INTRODUCTION

Green roofs are repeatedly reported as urban infrastructure solutions for supporting local biotopes and providing habitat for native arthropods and birds populations (Getter and Rowe 2006; Kowarik 2011; Madre et al., 2014). Specifically, extensive green roofs (ExGRs) are targeted to increase biodiversity support in urban areas due to their lower constraint on building as compared to intensive green roofs (Getter and Rowe 2006). ExGRs could support native, local, and even rare species (Kowarik 2011). However, developing biodiversity on ExGRs (substrate depth < 20 cm) is a challenge, due to environmental constraints on plant growth (Olly et al., 2011). ExGRs have shallow substrate depths and therefore limited space for plant root development and water reserves, leading to increases in drought period and temperature fluctuations (Oberndorfer et al., 2007). As a result, ExGRs are often monospecific (Gioannini et al., 2018), composed of generalist species (Thuring and Dunnett 2019) or frequently planted with non-native species (Madre et al., 2014) at the time of installation.

To promote native biodiversity, ExGRs should be considered analogous to natural habitats (Lundholm 2006). Analogous habitats are anthropogenic ecosystems able to support indigenous biodiversity due to their structural or functional resemblance to natural ecosystems, habitats, or microsites that may be present in the region, but not part of the historic ecosystem on a particular site (Lundholm 2006). Harsh environmental constraints of ExGR are similar to those encountered on species rich dry grasslands and rocky environments in temperate Western Europe. In temperate Europe, dry calcareous grasslands are one of the most species-rich ecosystems both botanically and entomologically (Piqueray et al., 2007). They are characterized by high solar radiation, low soil depth (< 20 cm) and high variability in soil water retention capacity, representing a strong analogy with ExGR. Developing ExGR with dry calcareous grassland vegetation provides an opportunity to enhance regional biodiversity (Lundholm and Richardson 2010).

ExGRs are also characterized by local microenvironmental variation impacting the composition and dynamics of plant community (Bradbury 2021; Heim and Lundholm 2014; Roulston et al., 2020). Abiotic factors modifying the microenvironmental conditions of the roof and the dynamic of plant community include depth of the substrate (Brown and Lundholm 2015; Dunnett et al., 2008), microclimatic conditions due to sun exposure (Piana and Carlisle 2014; van der Kolk et al., 2020), building height (Walker 2011), wind speed (Lu et al., 2014), and internal building temperature (Lundholm et al., 2014). Microenvironmental conditions can also be altered by biotic factors such as increased cover of vascular plants and mosses that affect substrate water retention and substrate chemical properties (Anderson et al., 2010; Chenot et al., 2017; McCallum et al., 2018). ExGR plant community is also marked by changes in plant composition over time as ExGRs relate to primary ecological succession conditions (Brown and Lundholm 2015). Understanding variations of ExGR plant community in relation to successional processes and environmental conditions is needed to understand ExGR as habitat analogs.

Plant assemblages are traditionally studied at the species taxonomic level, which allows for direct reference to a target species assemblage (Duckworth et al., 2000). Using predictions based on the taxonomic level alone may not allow the detection of differences in functional ecological patterns and the results obtained from a list of species in a given context cannot be extended to all contexts (Keddy 1992; Körner 1994; Woodward and Cramer 1996). Alternatively, ecological or functional approaches help to identify functional patterns and ecological functions independently of the studied pool of species (Keddy, 1992; Körner, 1994; Woodward and Cramer, 1996). Firstly, plant species can be classified into ecological groups based on their ecological niche. In dry grasslands, xerophilic species thrive in shallow soils (generally < 5cm), mesophilic species grow on deeper soils (generally up to 20 cm), and mesoxerophilic species thrive in both (Piqueray et al., 2007). Different ecological groups of species could react differently to microenvironmental conditions. Secondly, Grime's competitor, stress tolerant and ruderal plant strategies (CSR) classification scheme allows for determination of functional patterns and quantification of variation in plant community functional strategies (Caccianiga et al., 2006; Diaz et al., 1992; Keddy, 1992). The C (competitive), S (stress tolerance) and R (ruderal) strategies of plant species are selected according to disturbances and environmental stress gradients (Grime, 1974), two characteristics of the ExGR environment (Nagase and Dunnett, 2010).

This study aims to disentangle the colonization dynamic of a plant community analogous to a dry calcareous grassland by taking into account microenvironmental variation at the scale of an ExGR. We address three questions: (1) Can ExGRs support native species selected based on the analogous habitat hypothesis? (2) Is the taxonomic, ecological group or functional composition of the vegetation influenced by the microenvironmental conditions of a green roof? (3) Are substrate characteristics influenced by microenvironmental conditions and vegetation evolution through time?

MATERIALS AND METHODS

Study Site and Experimental Design

The experiment was conducted outdoors at ambient climate conditions over three growing seasons (2018 to 2020) on the roof of the TERRA Research Centre of Gembloux Agro-Bio-Tech, Belgium (50°33'48" N 4°41'52" E). The climate in the region is classified as a temperate oceanic climate (Cfb) according to Köppen climate classification (Service fédéral belge, 2019). The springs of 2018 and 2020 were characterized by a warmer and drier climate compared to the average climatic variables of the reference period (1981-2010) (IRM).

The ExGR was installed in October 2017 with a commercial substrate for ExGR: lightweight substrate for ExGR composed of a mixture of recycled tiles, bricks and ceramic (Zinco©). The roof included nine ExGR parcels varying from 14 m² to 57 m² (Figure 1). Parcels were characterized by two substrate depths (6 cm and 12 cm) and were oriented East-West. The roof was surrounded by walls on three of its sides (North, East and South), creating differences in sun exposure among parcels. In addition, skylights installed between parcels created an additional fine-scale gradient of shading. Maximum sun exposure during a summer day was modeled with Sketchup Pro toolbox 'De Luminae Sun Exposure' over the ExGR parcels. Three classes of sun exposure were defined: low (3 to 6h), Medium (6 to 9h), and High (9h to 12h).

The two parameters of depth and sun exposure were noted: D1 (6 cm depth), D2 (12 cm depth), S1 (low sun exposure), S2 (medium sun exposure) and S3 (high sun exposure).

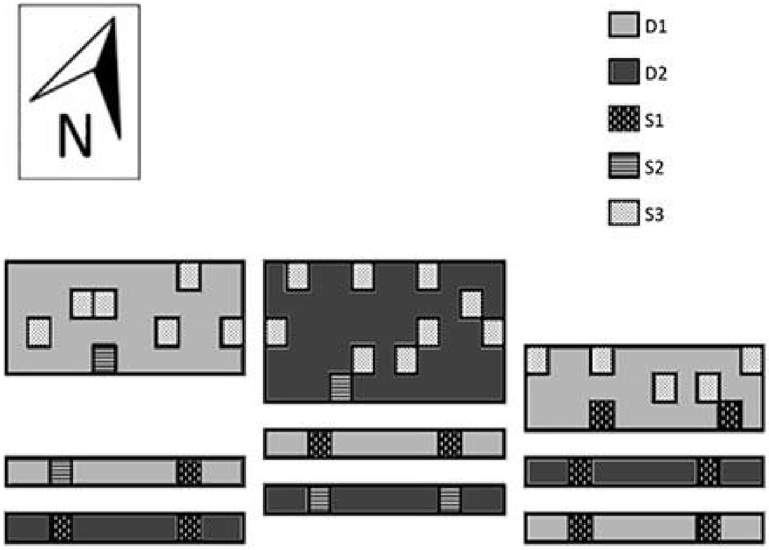

Figure 1. Sun exposure and substrate depth modalities of the plots within the extensive plots of the TERRA building (Gembloux, Belgium). D1: shallow depth 6 cm - D2: deep depth 11 cm - S1: low sun exposure - S2: medium sun exposure - S3: high sun exposure

A set of 28 species typical of dry calcareous grasslands were selected according to their occurrence along the soil depth gradient in Belgian dry grasslands (Piqueray et al., 2007): 7 xerophilic species (shallow soils <5cm), 12 mesophilic species (deepest soils: 5-20 cm), 9 mesoxerophilic species (both conditions) (Table 1). Among xerophilic species, three native Belgian *Sedum* species were chosen because *Sedum* are succulent plants typical of ExGR. The species were sown on 10th November 2017 evenly across the parcels with 830 seeds/species/m² for grass species and 110 seeds/species/m² for other species. No overseeding, watering, nutrients inputs or mowing were applied on parcels during the study period.

Data Collection

A total of 36 permanent 1m² plots were identified on the 9 parcels representing the variation of substrate depth (D1, D2) and sun exposure (S1, S2, S3) (Figure 1). Plant species cover was measured in permanent plots in June 2018, 2019, and 2020 (peak of vegetative development) using the point-intercept method with 100 pins on one square meter (Barbour and Burk, 1987). As bryophyte may play a role in plant species establishment and substrate properties, in June 2020, we recorded the development of volunteer bryophyte cover with the same method.

We collected a composite sample of substrate in each plot in June 2020, after plant removal. A sample of the original substrate was also analyzed. Substrate samples were sieved to 2 mm to remove the remaining roots. The texture was analyzed to estimate the percentage of sand, silt and clay. The pH was measured in 1M KCl with a glass electrode (substrate:solution volume ratio 2:5) after 2h equilibration time (NF ISO 10390). We determined the total amount of organic carbon (%C) using the Springer-Klee method (Springer and Klee 1954). The Dumas method was used to estimate the total amount of nitrogen (%N) (NF ISO 13878) (Nelson and Sommers 1983). The division of the amount of organic carbon by the amount of nitrogen defines the C/N ratio (C/N). We estimated the P and K content (mg/100g) after an extraction with 1 N of $CH_3COONHEDTA$ (pH 4.65) for 30 min (substrate:solution ratio 1:5) (Lakanen and Erviö, 1971) and a quantification by atomic absorption spectrophotometry (PinAAcle 900 F instrument).

Data Analyses

All data analyses were performed in R 4.0.2 (R Core Team, 2020).

To compare ecological groups proportions at the roof scale between 2018 and 2020, we used the McNemar Chi^2 test ("mcnemar.test", stats (R Core Team, 2020)).

To order the taxonomic plant composition variation in relation to microenvironmental conditions (D1, D2 and S1, S2, S3) and over years (2018, 2019, 2020) a global Principal Coordinate Analysis (PCoA) ("cmdscale", stats) was performed on all plots over the three years of surveys. A PERMANOVA analysis ("adonis2", vegan [R Core Team, 2020]) allowed us to determine which factors significantly influenced the separation of plant community. A Pearson correlation was calculated for each species ("cor", stats) to identify species that correlated most with the axes of the PCoA.

The percentage of each CSR strategy of observed species was taken from the Hodgson's Databases (1999), except for *Dianthus carthusianorum* L. and *Bromus erectus* Huds. Are missing from the database for which data were extracted from Kingsbury (2008) and Pierce et al. (2013), respectively. Mean cover weighted C, S, R, percentages for plant community were calculated from percent cover data for all the species in each plot, using the spreadsheet-based tools from Hunt et al. (2004). Mean community C, S, R, percentages were compared using a linear mixed model analysis with repeated measures to determine the effects of year, substrate depth and sun exposure with plot as a random factor. We used the lmer function (lme4 [Bates et al., 2020]) and the Anova function (car [Fox et al., 2020]). Tukey post-hoc tests were performed when necessary ("emmeans", emmeans [Lenth, 2020]). For 2020, the mean strategies of seeded and spontaneous species were compared.

The mean values of the 2020 substrate parameters were compared to the 2017 substrate parameters (original substrate) value using a one-sample t-test ("t.test", stats). To explore correlations among substrate parameters in year 3 (2020), we performed a correlation matrix ("cor", stats) of substrate parameters from plots in 2020: P (mg/100g), K (mg/100g), C (g/Kg), N (%), C/N, pH, clay (%), silt (%), and sand (%). We used a Pearson correlation threshold of 0.7 to determine the collinearity between the substrate parameters. Mean values of uncorrelated parameters were compared with a one-way ANOVA among plots with different sun exposures: P (mg/100g), K (mg/100g), N (g/Kg), C/N, pH, clay (%), and silt (%). We examined the influence of total plant cover after 3 years on substrate parameters with linear regressions ("lm", car).

RESULTS

Floristic Variation of Plant Community

Over the entire roof, a total of 16, 22, and 24 species were observed in 2018, 2019, 2020, respectively. In 2018, 50% of seeded species were observed in the surveys. It increased to 75% and 71% in 2019, and 2020 respectively. The average total cover of vegetation changed from 25.5 ± 19.3% (mean ± sd) in 2018, to 81.1 ± 50.3% in 2019, and 35.4 ± 35.6% in 2020. The number and relative cover of spontaneous species increased over the three years: 2 species - mean relative cover 0.4 ± 1.5 % in 2018, 4 species - mean cover 4.4 ± 16.4% in 2020: *Trifolium arvense* L., *Trifolium pratense* L., *Sonchus oleraceus* L. and *Vicia sativa* L.

The proportion of xerophilic species (number of xerophilic species / number of total species) in the plant community significantly increased from 7.1% in 2018 to 30.0% in 2020 (Chi² test, $P < 0.001$). The proportion of mesoxerophilic species was similar among years (35.7%, 35.0%, respectively in 2018, 2020) (Chi² test, $P > 0.05$). In contrast, the proportion of mesophilic species significantly decreased from 57.1% in 2018 to 35.0% in 2020 (Chi² test, $P < 0.001$). The relative cover of xerophilic (total cover of xerophilic species / total cover of plant community) and mesoxerophilic species in the community increased with sun exposure for the two substrate depths (D1 and D2) (Figure 2). The relative cover of mesophilic species was higher on D2 than on D1 for any sun exposure conditions. Conversely, the relative cover of mesoxerophilic species was higher in D1 parcels for all sun exposure conditions.

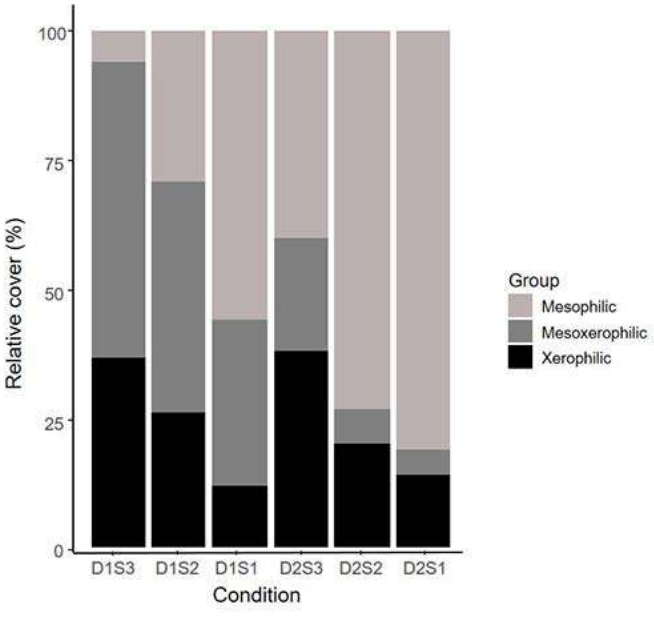

Figure 2. Relative percentage of vegetation cover in 2020 by ecological groups depending on microenvironmental conditions. D1: shallow depth 6 cm - D2: deep depth 11 cm - S1: low sun exposure - S2: medium sun exposure - S3: high sun exposure.

In 2020, the average total plant cover varied from 11.9 ± 10.1% in plots with shallow substrate and high sun exposure (D1S3) to 103.0 ± 57.4% in plots with deeper substrate and low sun exposure (D2S1). Plant community differed according to microenvironmental conditions (Table 1). In the least stressful conditions (D1S1 and D2S1) species richness and cover were higher for seeded species, spontaneous species, and bryophyte. *Anthoxanthum odoratum* L., *B. erectus*, *Echium vulgare* L., *Papaver argemone* L., *Sedum acre* L., and *Sedum album* L. were present in all or almost all conditions and formed a common basis for the plant community on the green roof.

Table 1. Average total plant cover and cover by species (%) in 2020 according to microenvironmental conditions D1: shallow depth 6 cm - D2: deep depth 11 cm - S1: low sun exposure - S2: medium sun exposure - S3: high sun exposure. The number of plots per treatment is indicated. The table is organized according to species cover.

Environmental condition		**D1S3**	**D1S2**	**D1S1**	**D2S3**	**D2S2**	**D2S1**
Number of plot		11	2	7	9	3	4
Average total plant cover (%)		11.9 ± 10.1	32.5 ± 6.4	41.4 ± 31.6	25.7 ± 11.8	49.0 ± 8.7	103.0 ± 57.4
Observed seeded species (% cover)							
***Anthoxanthum odoratum* L.**	Mesophilic	0.7	9.5	13.6	2.1	17.0	61.5
***Anthyllis vulneraria* L.**	Mesophilic	0	0	0	7.3	12.0	16.0
***Bromus erectus* Huds.**	Mesoxerophilic	6.4	9.0	7.6	5.2	1.0	4.5
***Sedum acre* L.**	Xerophilic	1.7	4.5	0.6	3.0	0	3.5
***Echium vulgare* L.**	Xerophilic	0.5	0	0.4	3.2	4.7	3.0
***Sedum album* L.**	Xerophilic	2.1	4.0	0.43	2.3	0	0.5
***Sedum rupestre* L.**	Xerophilic	0	0	1.1	1.0	0	6.5
***Briza media* L.**	Mesophilic	0	0	1.6	0.9	1.3	4.5
***Medicago lupulina* L.**	Mesoxerophilic	0.1	4.5	0	0.2	1.3	0
***Silene vulgaris* (Moench) Garcke**	Mesoxerophilic	0	0	0.7	0.1	2.0	0.7
***Papaver argemone* L.**	Mesoxerophilic	0.3	1.0	1.1	0.1	0	0.2
***Dianthus carthusianorum* L.**	Xerophilic	0	0	0	0	1.7	0
***Daucus carota* L.**	Mesophilic	0	0	0.4	0	0	0
***Sanguisorba minor* Scop.**	Mesoxerophilic	0	0	0	0	0.3	0
***Scabiosa columbaria* L.**	Mesoxerophilic	0	0	0.1	0	0	0
***Verbascum* sp.**	Xerophilic	0	0	0	0.1	0	0
Seeded species observed on the roof, not in the plots (% cover)							
***Koeleria macrantha* (Ledeb.) Schult**	Mesoxerophilic	0	0	0	0	0	0
***Primula veris* L.**	Mesophilic	0	0	0	0	0	0
***Rhinanthus minor* L.**	Mesophilic	0	0	0	0	0	0
***Rumex acetosella* L.**	Mesophilic	0	0	0	0	0	0
Non observed seeded species (% cover)							
***Centaurea scabiosa* L.**	Mesophilic	0	0	0	0	0	0
***Hieracium pilosella* L.**	Mesoxerophilic	0	0	0	0	0	0
***Hypochaeris radicata* L.**	Mesoxerophilic	0	0	0	0	0	0
***Leucanthemum vulgare* Lamk.**	Mesophilic	0	0	0	0	0	0
***Lotus corniculatus* L.**	Mesophilic	0	0	0	0	0	0
***Poa pratensis* L.**	Mesophilic	0	0	0	0	0	0
***Thymus pulegioides* L.**	Xerophilic	0	0	0	0	0	0

***Tragopogon pratensis* L.**	Mesophilic	0	0	0	0	0	0
Spontaneous species (% cover)							
***Vicia sativa* L.**		0	0	13.7	0	0	0
***Trifolium pratense* L.**		0	0	0	0	7.3	1.7
***Sonchus oleraceus* L.**		0	0	0	0	0.3	0
***Trifolium arvense* L.**		0	0	0	0	0	0.2
Bryophyte		19.9	80	76.4	14.7	87.3	98.5

The first and second axes of PCoA (Figure 3) based on plot floristic composition over three years explained 27.4% of global floristic variation (PcoA1 = 15.0%, PcoA2 = 12.4%). The PcoA showed an evolution of plant assemblages through years with a strong difference between 2018 and 2019-2020. The effect of time since sowing had a significant influence on vegetation composition (Permanova, $P < 0.001$, $R^2 = 0.085$) and was greater than the effect of substrate depth ($P < 0.001$, $R^2 = 0.032$) and sun exposure ($P < 0.001$, $R^2 = 0.046$) on plant assemblages. Species correlation with PcoA axes indicated that interannual differences were due to the higher presence/abundance of a set of species in year 2 and 3 after sowing than in year 1. *Anthyllis vulneraria* L., *Briza media* L., *B. erectus*, *E. vulgare*, and *Rumex acetosella* L. were more abundant in 2019 and 2020 than in 2018.

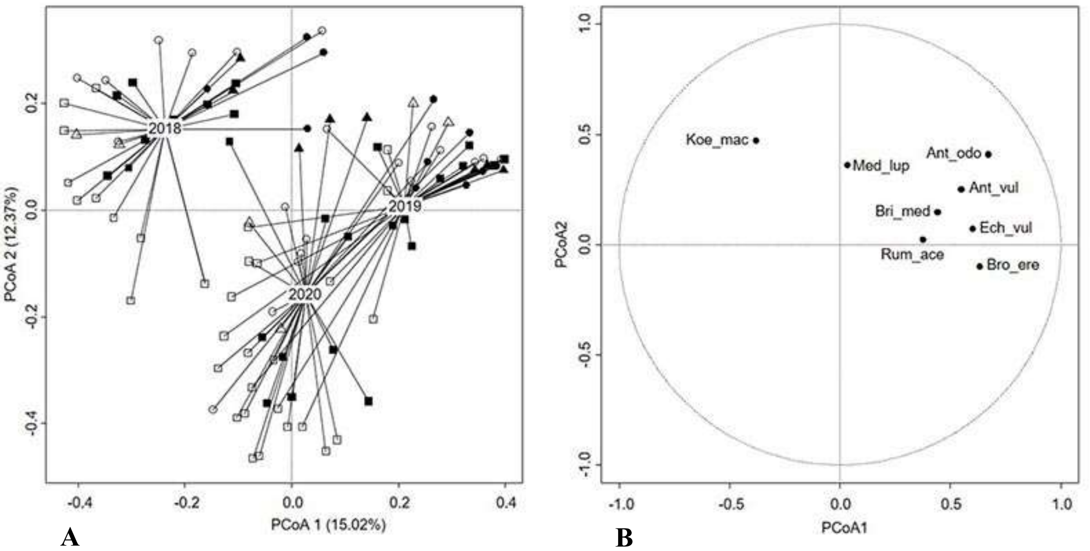

Figure 3. PCoA based on species composition of community A) Ordispiders are pooled by year. Symbols represent sun exposure modalities (circle = S1: low sun exposure, triangle = S2: medium sun exposure, and square = S3: high sun exposure) and substrate depth modalities (white = D1: shallow depth 6 cm, black = D2: deep depth 11 cm). B) Correlation circle of species (correlation > 0.35 in absolute value with one of the two axes of the PCoA). Ant_odo: *A. odoratum*, Ant_vul: *A. vulneraria*, Bri_med: *B. media*, Bro_ere: *B. erectus*, Ech_vul: *E. vulgare*, Koe_mac: *K. macrantha*, Med_lup: *M. lupulina*, Rum_ace: *R. acetosella*.

Functional Variation of Plant Community

Interaction between substrate depth and sun exposure was not statistically significant and neither factors had effect individually on the average values of the CSR community strategies (Table 2). Plant community was globally dominated by the stress tolerant strategy. The year significantly influenced the average value of the competitive (C), the stress tolerant (S) and the ruderal (R) strategies (Figure 4). The average C strategy value was significantly lower (P <0.001) in 2018 (0.14 ± 0.03) than the average C strategy values for 2019 (0.22 ± 0.05) and 2020 (0.23 ± 0.10). The average S strategy value was significantly higher (P <0.001) in 2018 (0.55 ± 0.10) than the average S strategy values for 2019 (0.40 ± 0.04) and 2020 (0.43 ± 0.15). The average R strategy value was significantly higher (P=0.001) in 2019 (0.38 ± 0.09) than the average value for 2018 (0.31 ± 0.08) and 2020 (0.32 ± 0.12).

In 2020, the spontaneous species presented a more ruderal strategy than the seeded species of the plant community: seeded (0.15 ± 0.13 C; 0.48 ± 0.27 S; 0.37 ± 0.22 R) – spontaneous (0.21 ± 0.14 C; 0.14 ± 0.17 S; 0.64 ± 0.21 R).

Table 2. Degrees of freedom (Df) and P-value for comparison of mean community C, S, R, strategies in relation to substrate depth, sun exposure, depth*exposure and year.

	Depth		**Exposure**		**Depth*Exposure**		**Year**	
	Df	P	Df	P	Df	P	Df	P
C	1	0.73	2	0.25	2	0.44	2	**<0.001**
S	1	0.90	2	0.89	2	0.94	2	**<0.001**
R	1	0.41	2	0.19	2	0.71	2	**0.001**

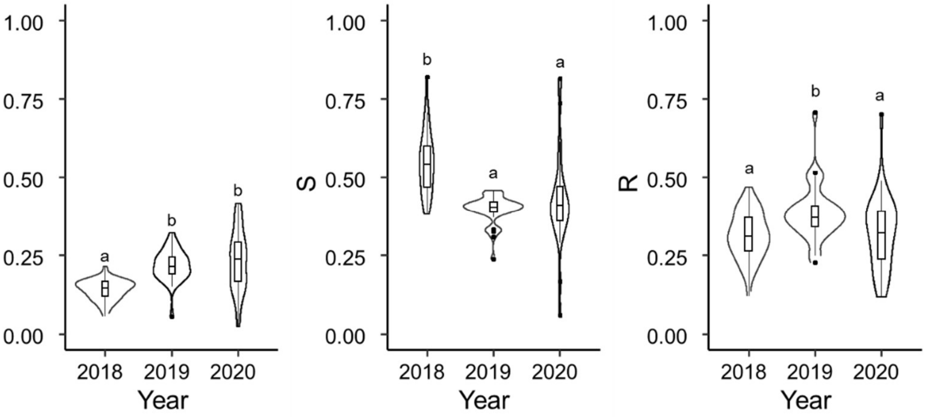

Figure 4. Violin-plot and associated box-and-whisker plot of mean plant community strategies C, S, R, from 2018 to 2020. Different letters indicate significant differences among years.

Substrate Characteristics

In 2020, over all plots, the average P (mg/100g), K (mg/100g) and sand (%) of substrate were significantly higher (P <0.001), while C (g/kg), N (%), C/N, pH, clay (%) and silt (%) were significantly lower (P <0.001), as compared to the original substrate (Table 3).

Table 3. Comparisons of the substrate parameters between the values of the original substrate and the values 3 years after installation. The values shown for 2020 are the means and standard deviations of the values for each plot. The progress indicates whether the parameter values increased (↗) or decreased (↘) after 3 years. P value for one-sample t test.

Substrate parameters	2017	2020	Progress	Df	P value t test
P (mg/100g)	13.3	14.9 ± 1.2	↗ 12.6 % ± 8.7 %	6	**<0.001**
K (mg/100g)	53.0	87.5 ± 11.0	↗ 65.1 % ± 20.7 %	6	**<0.001**
C (g/Kg)	50.0	19.7 ± 2.9	↘ 60.5 % ± 6.0 %	6	**<0.001**
N (%)	0.18	0.1 ± 0.01	↘ 44.4 % ± 5.6 %	6	**<0.001**
C/N	28.0	19.3 ± 0.8	↘ 31.1 % ± 2.9 %	6	**<0.001**
pH	8.0	7.9 ± 0.1	↘ 1.4 % ± 1.4 %	6	**<0.001**
Clay (%)	6.6	5.9 ± 0.8	↘ 0.7 % ± 0.8 %	6	**<0.001**
Silt (%)	20.6	16.5 ± 2.9	↘ 4.1 % ± 3.0 %	6	**<0.001**
Sand (%)	72.7	77.6 ± 3.4	↗ 4.9 % ± 3.4 %	6	**<0.001**

C and N were positively correlated (Pearson correlation coefficient 0.96), while silt and sand were negatively correlated (Pearson correlation coefficient -0.97) (Table 4). We retained N and silt for analysis. Table 5 shows the average values for each uncorrelated substrate parameter over plots and statistical analysis of sun exposure and total cover influence. Average value of N (P=0.009), P (P=0.01), K (P=0.007) and silt (P=0.01) were significantly different among plots with different sun exposure, with higher values in S3 plots. pH (P=0.01) increased with total plant cover over three years, while N (P=0.02), P (P<0.001), K (P<0.001), C/N (P=0.001) and silt (P<0.001) decreased.

Table 4. Correlation matrix and Pearson coefficient of substrate parameters in 2020. Significant P values are indicated with * (P<0.05), ** (P<0.01) or *** (P<0.001).

	K (mg/100g)	C (g/kg)	N (%)	C/N	pH	Clay (%)	Silt (%)	Sand (%)
P (mg/100g)	0,58***	0,54***	0,53***	0,19	-0,1	-0,31	0,37*	-0,25
K (mg/100g)		0,35*	0,31	0,26	-0,11	0,05	0,54***	-0,49**
C (g/kg)			0,96***	0,35*	-0,23	-0,12	0,49**	-0,4*
N (%)				0,07	-0,13	-0,08	0,44**	-0,37*
C/N					-0,34*	-0,09	0,31	-0,25
pH						0,42*	-0,08	-0,03
Clay (%)							0,34*	-0,56***
Silt (%)								-0,97***

Table 5. P-value, mean and standard deviation of uncorrelated substrate parameters according to sun exposure modalities (S1: low sun exposure - S2: medium sun exposure - S3: high sun exposure). Regressions between the sum of the cover over 3 years (cover) and uncorrelated substrate parameters at plot scale (P and r^2). Different letters indicate significant differences.

	Sun exposure				Cover		
	S1	S2	S3	P	r^2	P	Relation
pH	7.9 ± 0.1	7.9 ± 0.2	7.9 ± 0.1	0.86	0.15	**0.01**	+
N (%)	0.09 ± 0.02 ^{a}	0.10 ± 0.01 ^{ab}	0.11 ± 0.01 ^{b}	**0.009**	0.13	**0.02**	-
P (mg/100g)	14.3 ± 1.1 ^{a}	14.4 ± 1.2 ^{ab}	15.5 ± 1.0 ^{b}	**0.01**	0.45	**<0.001**	-
K (mg/100g)	81.9 ± 10.0 ^{a}	80.1 ± 13.9 ^{a}	92.4 ± 8.4 ^{b}	**0.007**	0.34	**<0.001**	-
C/N	19.4 ± 0.8	19.0 ± 0.6	19.3 ± 0.9	0.984	0.27	**0.001**	-
Clay (%)	6.2 ± 0.7	5.9 ± 0.7	5.8 ± 1.0	0.45	0.01	0.28	
Silt (%)	14.8 ± 2.7 ^{a}	14.9 ± 2.0 ^{ab}	17.8 ± 2.8 ^{b}	**0.01**	0.26	**<0.001**	-

DISCUSSION

While it is appealing to develop green roofs supporting biodiversity, the analogous habitat hypothesis has been seldom tested so far (Lundholm and Richardson 2010; Sutton et al., 2012). This study suggests that ExGR can act as analogous habitats for native flora of dry calcareous grasslands, habitats with high biological value in Western Europe. However, native plant success and plant community composition varied through time and depend on microenvironmental conditions at the roof scale, with potential feedback on substrate properties.

Time since sowing had a significant influence on plant community composition and was greater than the individual effect of substrate depth and sun exposure. Species richness increased over three years, for both seeded and spontaneous species. This increase in richness during the first year of installation on green roofs has been observed in previous studies (Madre et al., 2014; Piana and Carlisle 2014; Thuring and Dunnett 2019). Plant cover also increased with a peak in the second year following seeding. Increase in total plant cover in the second year is mainly explained by the strong development of grass species in all plots. Maximum average plant cover observed in the second year was equivalent to Belgian mesophilic dry grasslands (84.4%) (Piqueray et al., 2007). The decrease in plant cover in year three may be due to the severe drought observed in April and May 2020 (IRM 2020), which likely caused hydric stress for the plants and growth limitation.

After three years, 71% of native seeded grassland species established on the green roof. Interannual differences in seeded species composition were due to the higher presence/abundance of a set of species in year two and three after sowing: *A. vulneraria*, *B. media*, *B. erectus*, *E. vulgare*, and *R. acetosella*. The contribution of spontaneous species to the plant community also grew over the three years, with an increase in ruderal spontaneous species richness and the cover ratio of spontaneous species to seeded species. Madre et al. (2014) also observed the colonization of ExGR by spontaneous native species.

The plant community was globally dominated by the stress tolerant strategy which is consistent with the dominant S strategy in dry grassland species (Ejrnæs and Bruun 2000). The use of native S strategists in plant community on green roofs is consistent with earlier studies that have found the same preponderance of the stress-tolerance strategy in ExGR plant community (Catalano et al., 2016; Thuring and Dunnett 2019; Van Mechelen et al., 2014). However, the functional composition of the plant community also evolved over the study period with a decrease in the S strategy component and an increase in the C strategy component. The increase in the competitive strategy component in plant community is consistent with a primary succession dynamic, with the plant community dominated by the stress-tolerance strategy in initial stages and with competitive components becoming more important with time (Brown and Lundholm 2015; Ecke and Rydin 2000; Grime 1988). While the spontaneous species presented a more ruderal strategy than the sown species community, consistent with other research (Thuring and Dunnett 2019), it had little effect on the ruderal component of plant community over the study period due to the low cover of spontaneous species.

Besides the global temporal dynamic of plant assemblage, plant community composition also differed among plots with different combinations of substrate depths and sun exposure, reflecting increasing temperature and water stress. These microenvironmental conditions likely induced a gradient of water stresses that led to the development of different plant combinations, also observed by Li et al. (2020). These combinations differed in their taxonomic composition, their plant cover and the ecological groups of species. The plots with low depth (6 cm) and high sun exposure were characterized by low plant cover (11%) whereas plots with deeper substrate had high plant cover (103%) dominated by mesophilic grasses (*A. odoratum*: 61%, *B. media*: 4%) and typical mesophilic species (*A. vulneraria*: 16%) limited to the deepest, lowest exposed plots. The differentiation of plant combinations on the ExGR was analogous to differentiation of plant combinations in dry grassland with the emergence of xerophilic community in the most stressful microenvironment and typical mesophilic grassland in the less stressful microenvironment (Piqueray et al., 2007).

Spontaneous species and moss cover were also structured according to the microenvironmental conditions. The moss spontaneous colonization was lower in the most exposed areas, suggesting that the roof's water supply played a role in establishment. Few studies have been conducted to investigate the influence of green roof micro-scale parameters on moss colonization (Studlar and Peck, 2009). The impact of moss canopy development has controversial effects on the development of vascular plants. Mosses buffer extreme environmental conditions (Heim et al., 2014), increase water retention of the substrate (Anderson et al., 2010) and can facilitate seedling development and emergence (Schröder and Kiehl, 2020) but can also form a dense layer that prevents seed germination (Drake et al., 2018; Lönnqvist et al., 2021).

Not only plant communities evolved over time on the study ExGR but also substrate properties. The developmental paradigms for natural ecosystems predict total nutrient stocks either increasing or declining based on the status and trajectory of plant biomass and whether the ecosystem develop along a primary (from bare soil) or a secondary (from disturbed vegetation) succession. Previous studies that have examined time-evolution of nutrient stocks on ExGR were mostly based on a pre-planted green roof corresponding to secondary succession situations. In these situations, it is hypothesized that the nutrient content of the ecosystem would initially decline as a result of mineralization and leaching of the organic material in the substrate but contrasting patterns were found among studies and for the different nutrients stocks (Buffam and Mitchell, 2015; Mitchell et al., 2021).

Our green roof ecosystem developed from a seeded bare soil, corresponding to a primary succession, a situation rarely explored. As for other ExGR studies, at the scale of the ExGR, we observed a variation of substrate nutrient stocks over time with mean increases in P and K and mean decreases in C (Bouzouidja et al., 2018) and N. Besides this general pattern, N, P, K substrate stocks after four years also decreased with increase in total vegetation cover, resulting in higher substrate nutrient stock in plots with more constraining conditions (higher sunny exposition and lower vegetation cover). This suggests different rates of evolution of nutrient stocks through time in relation to environmental conditions. Disentangling the drivers for such a complex pattern is difficult, given that we did not simultaneously assess nutrient stocks in vegetation, nor fluxes such as decomposition rates or leaching in runoff water. Our results call for more detailed studies on nutrient dynamics on ExGR in order to understand the long term evolution of those ecosystems and its implication for environmental services of ExGR.

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