

# Low productivity substrate leads to functional diversification of green roof plant assemblage

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## ABSTRACT

Green roofs are roof free spaces where living organisms can find an appropriate habitat to colonise. The establishment of plant species with different functionality can enhance biodiversity and provide ecosystem services. However, drought and nutrient availability can affect the plant development. The extensive green roof was set up in Pisa (Italy) in 2014, 12 modules of 10 cm depth were filled with three substrates composed of compost from municipal mixed waste, pelletised paper sludge, and commercial tephra product (Vulcaflor), as follows: Vulcaflor + compost, Vulcaflor + pellet + compost, and Vulcaflor + pellet, characterised by decreasing level of nitrogen content. The species planted in 2014 were chosen from the herbaceous spontaneous vegetation of urban and rural swards not often mowed, plus two sedum species. After the establishment phase, the green roof community was progressively dominated by *Sedum* species and other species were seeded in 2016. In 2018–19 the plant functional types and the community structure were monitored. Besides seasonal fluctuations, nitrogen shaped the composition of the community, and *Sedum* species showed high cover values in nitrogen-richer substrates. Annual forbs colonised the plots with a lower nitrogen content. In summer, the number of species drastically fell, and *Sedum album* was dominant in the three substrates. Seedling recruitment regenerated the community in the cooler season, increasing the diversity in the poor substrate. The scarcity of nitrogen led to the development of stress-tolerator annuals increasing the biodiversity in the rainy-cool season. Annual species constitute a transient seed bank which enables the system to regenerate when rain follows periods of heat and drought.

## 1. Introduction

Extensive green roofs (EGRs) are a nature-based green infrastructure characterised by highly stressful conditions for plants (shallow growing media layer, extreme temperature, extreme drought) and very low disturbance (mowing, trampling). EGRs also have a specific seed bank and ecological relevant trait species, which makes them novel ecosystems (Vanstockem et al., 2018). The study of EGRs involves various important aspects such as the ecosystem services provided to human beings living in urban areas, including thermal insulation, storm water retention, and the increase in urban biodiversity (Suszanowicz and Kolasa Więcek, 2019). The connectivity provided by many small patches of vegetation in urban settings is of great importance (Gaston et al., 2013), so EGRs create new habitats for wildlife and many studies have

demonstrated that even a thin layer of substrate can be colonised by living organisms (Kyrö et al., 2018; Rumble et al., 2018).

The plant species suitable for EGRs should ideally quickly colonise and cover the free space (Vijayaraghavan, 2016), adapting to the novel ecosystem conditions. They should also be able to tolerate extreme weather, temperatures, and scarce nutrient availability, due to the shallow substrate layer and intrinsic material of which it is composed (Oberndorfer et al., 2007). In fact, the scarcity of nutrients in EGR substrates is a necessary requirement to prevent the excessive development of plant biomass. This is because the biomass can provoke dominance and reduce biodiversity, lead to weed development, eutrophic runoff and damage to the technical structures by the overgrown root apparatus (Vijayaraghavan, 2016).

*Sedum* is a plant genus often successfully used in EGRs. Besides its

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resistance to heat and drought (Pérez et al., 2020), new plants are easy and quick to reproduce by cuttings, and the genus includes many species and varieties with high growth indices and suitable plant development (Tuttolomondo et al., 2018). Some species, such as *S. album*, have an abundant flowering in summer which attracts pollinators, and particularly the Halictidae family (Vannucchi et al., 2018). However, *Sedum*-dominated EGRs have limited diversity as some species such as *S. album* tend to rapidly colonise, occupying all the open spaces and reducing the niches potentially available for lower growing stress tolerant species (Nash et al., 2019). To reduce the productivity of EGR growing media reduces the dominance of sedums, thus promoting species richness and the hardiness of the plant community (Vannucchi et al., 2018). In addition, over time there is competition for resources between sedums and other plant species and this reduces annual plant biomass and diversity (Vasl et al., 2017). Stress factors in herbaceous communities hinder succession, limit competitiveness, thus providing gaps in the vegetation that are opportunities for weak competitors to colonise (Bretzel et al., 2016).

In many cases, the most successful attempts at EGRs are those that establish plant species with different functionality and life forms, inspired by the habitat analogue perspective, aimed at constituting a self-sustainable community (Lundholm and Richardson, 2010). Plant functional types (PFTs) reveal the ecological assembly of communities and are a useful complementary tool to the traditional taxonomy (Duckworth et al., 2000). *S. album* and *S. acre* are facultative crassulacean acid metabolism plants (CAM), which shift from C3 to C4 metabolism under water stress (Sayed et al., 1994; Kluge, 1977). They can reduce the temperature of the substrate and act as nurse species during summer water deficit (Butler and Orians, 2011). Annual species increase the N-fixation and decrease the substrate moisture, thereby improving the rain capture efficiency, a combination of the two PFTs can thus improve the green roof functions, under Mediterranean climate (Vasl et al., 2017). Nitrogen-fixers are another effective functional group for green roofs, they have a pioneer strategy and are able to colonise very poor substrate and perform a nurse role towards species that are more demanding in terms of nutrients (Mitchell et al., 2018). In addition, plants with therophytic and geophytic habit avoid the extreme droughts by spending the hot season in form of seeds or bulbs, in Mediterranean grasslands (Guarino et al., 2020).

Few ecological studies are carried out on green roof communities, indeed as green roofs are dynamics systems, the plant community establishment is subject of changes, due to climate and nutrient availability (Rowe, 2015). The monitoring of such plant communities may evidence the possible changes in the ecological structure and composition over time and plant species that respond to environmental conditions at different time and/or re-establish from a seed bank, contribute to improve the long-term performance of green roofs (Cook-Patton and Bauerle, 2012). Therefore, studying the plant dynamics in extensive green roof includes monitoring the co-existence of different PFTs under limiting factors (limited nutrients) as well as the variations in plant composition and recovery after high temperatures and drought. In addition, thanks to the unique habitat spatially isolated and characterised by limiting factors (e.g. drought, high irradiance level, reduced soil moisture), such studies reveal how an early successional plant community can respond to the climate changes expected in many regions of the world, similarly to ecological communities of isolated patches of low-nutrient soil (Damschen et al., 2012).

Our hypothesis was based on the concept that the plant competition is greater on productive substrates, while in unproductive habitats its importance declines (Grime, 2001): so, on infertile substrate the most competitive species would reduce their spread, leaving free spaces to be colonised by slow growing and stress-tolerant plants, in this way the diversity of the green roof plant community would be enhanced. For this purpose, we planted and seeded along with the sedums commonly used in EGRs, other species, belonging to different functional types and life forms, and we focused on processes underlying the response of the EGR

plant communities (PFTs) and structure (biodiversity) to substrate with different percentage of infertile material (i.e. paper sludge). In addition, we evaluated the community assemblage dynamics, in terms of PFTs and diversity, related to seasonal temperature and rainfall, and recovery after drought, and the arrival of different species to eventually define an applicability for the implementation and management of self-sustainable EGRs in Mediterranean climate.

## 2. Methods

### 2.1. Experimental design

The experiment was set up in November 2014 on the flat roof of a ten-meter-high building at the Italian National Research Council in Pisa (Italy), (43°43'9.707" N, 10°25'15.463" E) as reported in Vannucchi et al. (2018). Briefly, twelve ITM (interlock turf module) boxes were set up measuring 1 × 1 m, 10 cm depth plus 8 cm of drainage gravel (1–3 cm Ø). The growing and drainage layers were separated by a 2 cm thick geotextile, to avoid the root and substrate penetrations in the drainage layer. The boxes were arranged in a checkboard pattern with two columns and six rows, and filled randomly with three different substrates, to obtain four replicates each. The substrates were made up of compost (50–70% municipal mixed waste, 30–50% municipal green waste and 0–5% agricultural waste compost), commercial tephra product (Vulcaflor, composed of 30% pumice, 60% lapillus, and 10% peat + compost) and pelletised paper sludge (deinked paper sludge from the production of recycled paper). VC was composed of 80% Vulcaflor +20% compost (productive); VPC of 65% Vulcaflor +27% pellet +8% compost (middle productive) and VP of 40% Vulcaflor +60% pellet (unproductive). The percentages are by volume. The use of paper sludge had the aim to reduce the substrate productivity in terms of nitrogen content and to recycle a waste material, free of pollutants. The initial planting was carried out in 2014, and the species chosen were herbaceous forbs and geophytes growing in herbaceous swards of peri-urban and rural areas nearby, mowed just occasionally, plus two species of sedum. No irrigation or fertilization were performed during the trial. After the plant establishment (Vannucchi et al., 2018), the green roof community was progressively dominated by *Sedum* species, so we carried out a seeding in 2016 with the same quantities in all boxes, some weeds spontaneously colonised (Table 1). To highlight the ecological strategies, the species monitored were grouped into plant functional types (PFTs): annual forbs, annual legumes, perennial forbs, perennial legumes, geophytes, CAM, *graminae* and bryophytes. CAM consisted of *S. album* and *S. acre*, which are commonly used for commercial green roofs (Table 1).

### 2.2. Substrate analysis and climate data

Each substrate was sampled in May 2018 and October 2019. Electrical conductivity (EC), pH (H<sub>2</sub>O) organic carbon (C<sub>org</sub>) and total nitrogen (N<sub>tot</sub>) were analysed (ASA-SSSA, 1996). C<sub>org</sub> and N<sub>tot</sub> contents were determined through dry combustion using a Leco CHN analyzer. The percentage of moisture in each substrate was determined gravimetrically by drying at 105 °C in May 2018 and October 2019 (ASA-SSSA, 1996). Climate data (minimum and maximum temperature, relative humidity and total precipitation) were acquired every hour from a meteorological station, located on the roof of the Italian National Research Council building.

### 2.3. Species composition and biodiversity indices

From April 2018 to October 2019, the counting the number of species was carried out every month, with the point intercept method, a point frame for botanical surveying composed of ten pins (pin length: 46 cm distance between outer pins: 5 cm, leg length: 54 cm) (NHBS Ltd., UK). The PFT and species contributions (%) were calculated as the ratio between the number of PFT/species touched by the pin and the total

**Table 1**  
Species composition of the green roof.

Species	Family	PFT	Growth forms	Strategy type	Introduction in the GR
<i>Allium roseum</i> L.	Amaryllidaceae	G	Bulbous	–	Planted 2014
<i>Alyssum alyssoides</i> (L.) L.	Brassicaceae	AF	Scapose/rosulate	SR	Planted 2014
<i>Anthyllis vulneraria</i> L.	Fabaceae	PL	Scapose/hemirosette	CSR	Planted 2014
<i>Blackstonia perfoliata</i> (L.) Huds.	Gentianaceae	AF	Scapose/rosulate	SR	Seeded 2016
<i>Calendula arvensis</i> (Vaill.) L.	Asteraceae	AF	Scapose/hemirosette	R	Planted 2014/seeded 2016
<i>Centranthus macrosiphon</i> Boiss.	Caprifoliaceae	AF	Scapose	–	Seeded 2016
<i>Crepis bursifolia</i> L.	Asteraceae	PF	Scapose	–	Spontaneously colonising
<i>Dianthus deltoides</i> L.	Caryophyllaceae	PF	Caespitose/rosulate	CSR	Planted 2014
<i>Erodium cicutarium</i> (L.) L'Hér.	Geraniaceae	AF	Caespitose/scapose/hemirosette	R	Planted 2014
<i>Geranium molle</i> L.	Geraniaceae	AF	Scapose/hemirosette	R	Planted 2014
<i>Hypochaeris radicata</i> L.	Asteraceae	PF	Rosette	CSR	Seeded 2016
<i>Lobularia maritima</i> (L.) Desv.	Brassicaceae	PF	Scapose/rosulate/hemirosette	SR	Seeded 2016
<i>Muscari comosum</i> (L.) Mill.	Asparagaceae	G	Bulbous/rosulate	CSR	Planted 2014
<i>Ornithogallum umbellatum</i> L.	Asparagaceae	G	Bulbous/rosulate	CSR	Planted 2014
<i>Petrorhagia saxifraga</i> (L.) Link	Caryophyllaceae	PF	Caespitose/rosulate	CS	Planted 2014
<i>Poa annua</i> L.	Poaceae	GR	Caespitose/hemirosette	R	Spontaneously colonising
<i>Portulaca oleracea</i> L.	Portulacaceae	AF	Succulent scapose	–	Spontaneously colonising
<i>Scrophularia peregrina</i> L.	Scrophulariaceae	AF	Scapose	–	Seeded 2016
<i>Sedum acre</i> L.	Crassulaceae	CAM	Succulent	S	Planted 2014
<i>Sedum album</i> L.	Crassulaceae	CAM	Succulent	S	Planted 2014
<i>Senecio vulgare</i> L.	Asteraceae	AF	Scapose	–	Spontaneously colonising
<i>Silene gallica</i> L.	Caryophyllaceae	AF	Scapose/rosulate	R	Planted 2014
<i>Sochus oleraceus</i> L.	Asteraceae	AF	–	–	Spontaneously colonising
<i>Trifolium arvense</i> L.	Fabaceae	AL	Scapose/rosulate/hemirosette	SR	Planted 2014
<i>Trifolium campestre</i> L.	Fabaceae	AL	Scapose/rosulate/hemirosette	R	Planted 2014
<i>Verbascum blattaria</i> L.	Scrophulariaceae	AF	Scapose/hemirosette	C	Seeded 2016
Mosses (Bryophyta)		BR			Spontaneously colonising

The plant functional types (PFTs) were assigned according to Pignatti et al. (2017) and Perez-Harguindeguy et al. (2013) and the strategy type only for the species present in Klotz et al. (2002). AF = annual forbs; AL = annual legumes; PL = perennial legumes; BR = bryophytes; PF = perennial forbs; G = geophytes; GR = *graminae*; C = competitive; S = stress tolerator; R = ruderal.

number plants touched (Glatzle et al., 1993). The total canopy cover (TCC) was calculated as the ratio between the number of pins contacted by plants and the total number of pins used. A total of 0.5 hits were assigned to PFTs or species present but not hit (Chiarucci et al., 1999). PFT and TCC data were reported monthly and as the total average, for the duration of the experiment. The species contribution was evaluated in April 2018 and March 2019, as the seedling recruitment period, when the number of species was high and in October 2018 and 2019, as the end of the drought period, when the number of species was low.

Biodiversity indices were calculated as the Shannon diversity index ( $H'$ ) (Shannon and Weaver, 1949), and the evenness of species ( $J$ ) (Smith and Wilson, 1996), as follows:  $H' = \sum_{i=1}^k p_i \log p_i$ , where  $k$  is the species number, and  $p_i$  is the fraction of individuals belonging to the  $i^{\text{th}}$  species;  $J = H' / \ln k$  where  $H'$  is the Shannon diversity index, and  $k$  is the species number. In addition, Simpson's index of dominance ( $D$ ) (Simpson, 1949) was calculated to assess the probability that two individuals randomly selected from a sample belonged to the same species.  $D = \sum (n/N)^2$  where  $n$  is the number of individuals of a species, and  $N$  the total number of individuals of all species.

#### 2.4. Statistical analysis

Statistical analysis was conducted using open-source R software (version 4.0.1). Total canopy cover, plant functional type contributions and biodiversity indices (Shannon, evenness and Simpson indices) were monitored over time and compared in different substrates and seasons. The data collected were subjected to the parametric ANOVA test (for homoscedastic normally distributed populations), non-parametric ANOVA, Kruskal-Wallis non-parametric ANOVA test (for homoscedastic non-normally distributed populations) and Friedman's rank sum test (for heteroscedastic non-normally distributed populations). The homogeneity of the variance within populations was verified with Bartlett's test and the Gaussian distribution with the Shapiro-Wilk normality test. Post-hoc comparisons between groups were carried out with the  $t$ -test (or Mann-Whitney non-parametric  $U$  test for heteroscedastic or non-

normally distributed populations), using the Bonferroni adjustment for multiple comparison correction. In all the tests, the  $P$ -value of  $P < 0.05$  was used as the threshold of statistical significance. A principal component analysis (PCA) was performed, to visualize the distribution of the functional groups in the plane identified by the first two main components of the space generated by the substrates.

### 3. Results

#### 3.1. Substrate properties and climate

The total substrate nitrogen content differed significantly between green roof treatments. VP showed lower  $N_{\text{tot}}$  compared to VC and VPC, while VC and VPC had similar values ( $P = 0.049$  in 2018 and  $P = 0.007$  in 2019). The pH was alkaline (7.7–8.5). EC was uniform among substrates and decreased in 2019, in all treatments, from around 0.9 dS/m to 0.2 dS/m. Organic carbon decreased in 2019, especially in the treatments with paper sludge (VPC and VP). The substrate moisture content (%) resulted higher in VC than in VPC and VP in May 2018 ( $P < 0.001$ ) as well as in October 2019 ( $P = 0.007$ ) (Table 2).

In 2018, August was the driest (14 mm of rainfall) and the hottest (35 °C) month, and May had the highest recorded rainfall (107 mm). During 2019 a reduction in rainfall was recorded in June (3 mm) and in August (4 mm). The highest rainfall was in October (339 mm) and July was the hottest month (36.7 °C) in 2019.

#### 3.2. Plant community composition and structure

As the total average of the community composition, annual and perennial legumes, perennial forbs, geophytes, and *graminae* did not differ significantly (Table 3). The CAM functional type was significantly more numerous in VPC and VC ( $P < 0.001$ ) and annual forbs in VP ( $P < 0.003$ ). The highest percentage of CAM was detected in VC (70%), followed by VPC (62%) and VP (25%). Annual forbs showed higher values in VP (19%), compared to VPC (9%) and VC (13%). In addition,

**Table 2**  
Chemical properties of the growing media detected in May 2018 and October 2019.

	pH		EC		C <sub>org</sub>		N <sub>tot</sub>		Moisture	
	H <sub>2</sub> O		dS/m		%		%		%	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
VC	7.7 ± 0.06	8.0 ± 0.06	0.9 ± 0.04	0.2 ± 0.03	8.1 ± 0.85	5.8 ± 1.08	0.51 ± 0.18a	0.65 ± 0.15a	31 ± 2.6a	46 ± 4.8a
VPC	7.8 ± 0.09	8.2 ± 0.14	0.9 ± 0.12	0.2 ± 0.02	6.5 ± 2.08	3.5 ± 0.37	0.40 ± 0.14a	0.36 ± 0.05a	22 ± 4.1b	33 ± 2.4b
VP	8.0 ± 0.09	8.5 ± 0.05	0.8 ± 0.07	0.2 ± 0.01	9.0 ± 0.61	3.8 ± 0.42	0.28 ± 0.02b	0.26 ± 0.01b	19 ± 1.2b	23 ± 3.2c

EC = electrical conductivity; C<sub>org</sub> = organic carbon; N<sub>tot</sub> = total nitrogen. VP = vulcaflor + pellet; VPC = vulcaflor + pellet + compost; VC = vulcaflor + compost. Data are means of 4 replicates ± SD. Different letters show a statistical difference for P < 0.05 between substrates.

**Table 3**  
Total averages of plant functional types (PFTs) not showing significant differences among substrates.

PFTs	VC	VPC	VP	P values
	%	%	%	
Annual Legumes	3.9 ± 1.59	9.2 ± 2.78	15.8 ± 4.75	0.469
Perennial Legumes	0.0	0.03 ± 0.026	0.0	-
Perennial Forbs	2.9 ± 2.78	0.2 ± 0.12	0.3 ± 0.17	0.055
Geophytes	4.2 ± 1.19	2.2 ± 0.73	2.5 ± 0.74	0.435
Graminae	0.02 ± 0.02a	0.0	2.4 ± 0.96a	0.008

Legumes and geophytes did not show significant differences among substrates. Perennial forbs were on the significance threshold. Graminae were not significant different according to post-hoc comparisons. VP = Vulcaflor + pellet; VPC = Vulcaflor + pellet + compost; VC = Vulcaflor + compost. Data are means of four replicates ± SE.

bryophytes reached higher percentages in VPC (6%), compared to VC (0.3%) and VP (0.6%) (Fig. 1).

Throughout the experiment PCA was performed to evaluate the PFT composition of plant communities related to different substrate fertility (Fig. 2). The highest eigenvalues were obtained for two principal components which explained 95.9% of the variability. The first component was strongly determined by VPC and VC, and the second by VP. The PCA of PFTs explains the 95.81% of the total variance. The PCA revealed that CAM correlated with VPC and VC, while annual forbs and legumes correlated with VP.

The average TCC for the duration of the experiment showed different values in relation to the substrate. TCC was significantly higher values (P < 0.001) in VC (92%) compared to VPC (89%) and VC (66%) (Fig. 3).

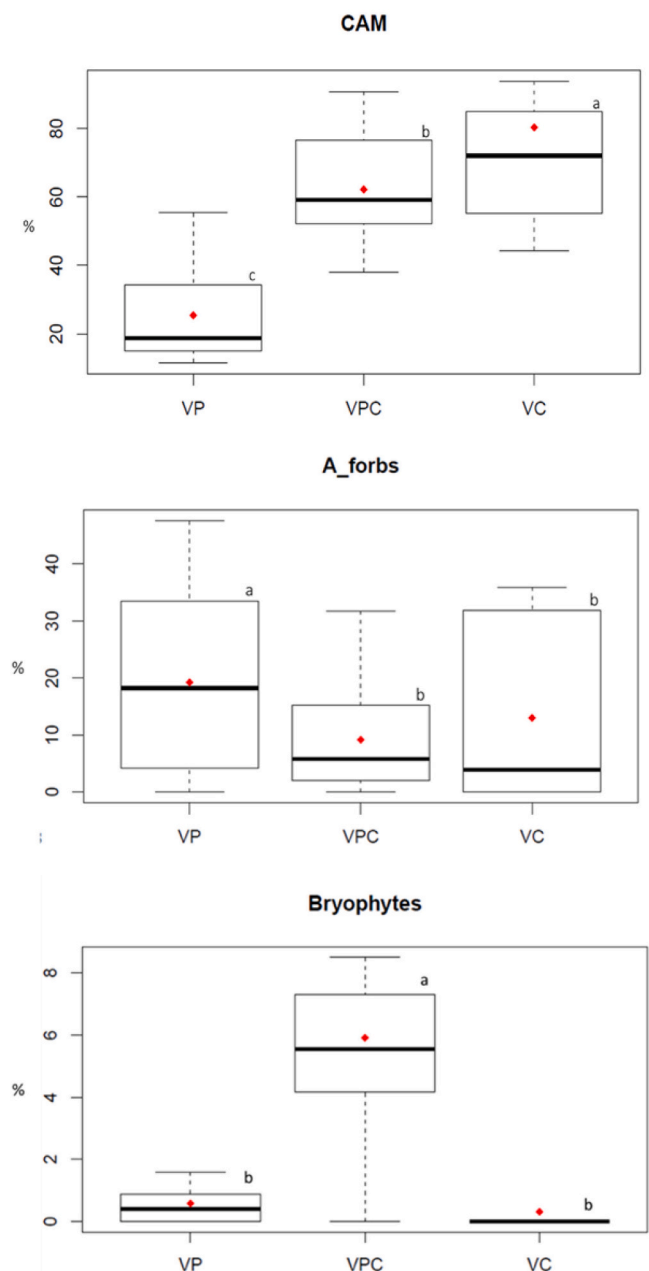
The plant structure in terms of the biodiversity index is shown in Fig. 4. The Shannon index was significantly higher in VP (1.1 ± 0.61) than in VPC (0.9 ± 0.43) and VC (0.7 ± 0.57). The Simpson index showed significantly lower values in VP (0.5 ± 0.25) and VPC (0.5 ± 0.18) than VC (0.7 ± 0.27). In addition, the substrate significantly influenced the evenness index between VPC (0.54 ± 0.17) and VC (0.39 ± 0.28) (P = 0.02). No significant differences were detected between VP (0.55 ± 0.27) and the other substrates.

### 3.3. Plant community dynamics

The TCC showed significant differences in relation to the substrates and season. In 2018, TCC was significantly lower in VP than VPC and VC, in July (P < 0.001), September (P = 0.014) and October (P = 0.002). This was also the case in 2019. In April (P < 0.001), May (P < 0.001) and August (P = 0.002). The TCC in VPC and VC maintained the same values in 2018 and 2019 (Fig. 5).

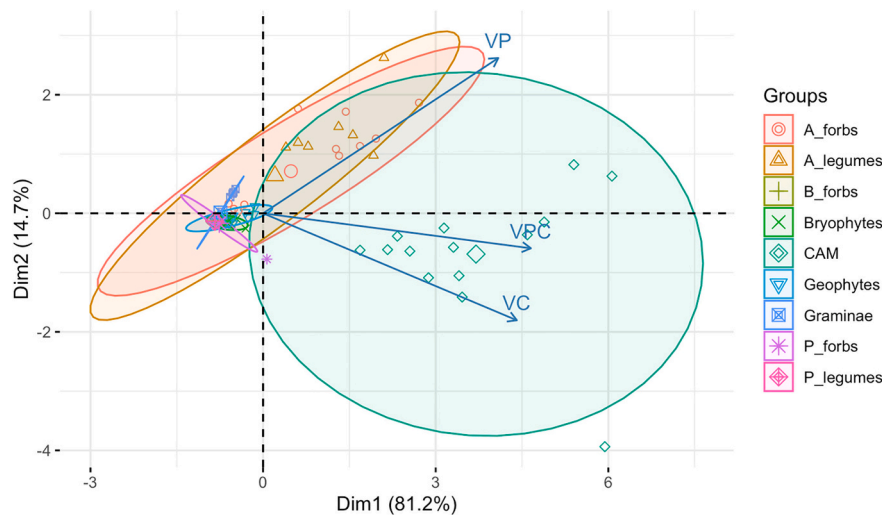
The dynamics of PFTs in the three substrates were affected by climate variations (Fig. 6). In VC, the overall contribution of CAM was more than 40%, reaching 79% in July 2018 and 94% August 2019. Annual forbs reached a maximum contribution of 32% in April 2018 and 36% in February 2019, which decreased in the summers.

The maximum contribution of annual legumes was detected in May 2018 (15%), then the values decreased drastically. Bryophytes did not

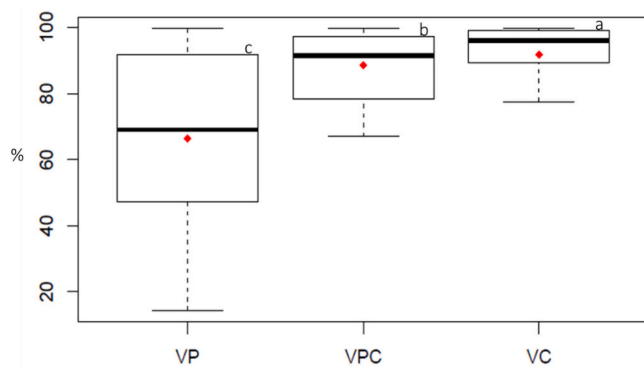


**Fig. 1.** Boxplots of plant functional types (PFTs) contribution (%) monitored in different substrates. A\_forbs = annual forbs. VP = Vulcaflor + pellet; VPC = Vulcaflor + pellet + compost; VC = Vulcaflor + compost. Average values are reported with diamonds. Different letters show a statistical difference for P < 0.05 between substrates.





**Fig. 2.** Principal component analysis of plant functional types (PFTs) in different substrates. A. forbs = annual forbs; A. legumes = annual legumes; P. forbs = perennial forbs; P. legumes = perennial legumes. VP = Vulcaflor + pellet; VPC = Vulcaflor + pellet + compost; VC = Vulcaflor + compost.



**Fig. 3.** Boxplots of total canopy cover (TCC) (%) monitored in different substrates. VP = Vulcaflor + pellet; VPC = Vulcaflor + pellet + compost; VC = Vulcaflor + compost. Average values are reported with diamonds. Data are means of four replicates. Different letters show a statistical difference for  $P < 0.05$  between substrates.

exceed 2% (December 2018). CAM was above 40% in VPC, with a peak in August 2019 (92%), while the annual forbs did not exceed 32%, which was reached in April 2018. In VPC annual legumes reached the maximum contribution in April 2018 (18%) and in March 2019 (32%) and decreased considerably in the summers. Bryophytes reached a peak of 15% in December 2018, showing generally higher values than the other substrates. In VP, CAM ranged between 10% and 55%, reaching the maximum value in August 2019, while annual forbs and legumes did not exceed 50%. In the summer, the cover of annual forbs and legumes decreased. Annual forbs reached 47% (April 2018) and 37% (October 2019) and annual legumes reached 52% in May 2018 and 31% in March 2019. Bryophytes in VP showed a similar trend as VC, reaching 2% in January 2019. Each of the remaining PFTs showed values below 15%, 13% and 8% in VC, VPC and VP substrates, respectively (Fig. S1).

The plant community dynamic, in terms of species contribution, is reported in S2. In VC, *S. album* was the main CAM species, reaching 44% and 54% in April 2018 and March 2019, respectively, and in October 2018 and 2019 it was the only species detected in VC. In VPC and VP, *S. acre* was visible in all the months reported (Fig. S2), with an 8% contribution in VP in October 2019.

The cold-season weeds, *Senecio vulgaris* L and *Sonchus asper* (L.) Hill colonised the EGR in winter until April and disappeared at the beginning of the drought period. *S. vulgaris* reached a 34% of TCC, however the

maximum height of the plants was 3 cm; the warm-season weed *Portulaca olearacea* L. spread from September–October 2019 (Fig. S2).

The seasonal dynamics of the plant community in terms of biodiversity indices are reported in Fig. 7. Overall, spring and winter showed the higher diversity, while summer had the lowest values.

The statistical analysis showed the greater differences in the autumn and spring. The Shannon indices had a similar trend, with VP higher than the other two treatments. The values were significantly lower in VC than in VP, in September 2018 ( $P < 0.001$ ), in April ( $P < 0.001$ ) and May 2019 ( $P = 0.003$ ).

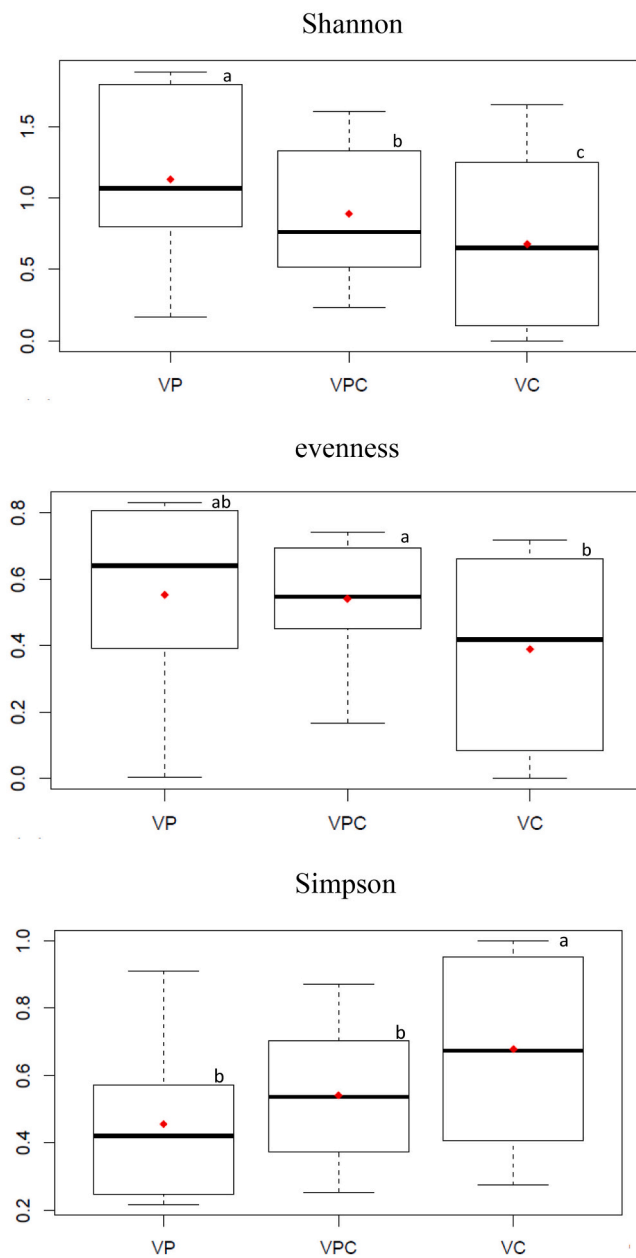
The evenness trend fluctuated and differed significantly among substrates in April ( $P < 0.001$ ), July ( $P = 0.016$ ), September ( $P < 0.001$ ), October 2018 ( $P = 0.024$ ) as well as in May 2019 ( $P < 0.001$ ). VP showed a lower evenness index than VC and VPC in April 2018. The evenness index in VPC had a higher value than VC and VP in July 2018. Evenness reached a peak in VP in September 2018 and the highest value for evenness was detected in VPC in October 2018. In May 2019, evenness showed a significant difference among substrates, which was higher in VP than VC and VPC.

The Simpson index varied significantly in April ( $P < 0.001$ ), May ( $P = 0.003$ ) and September 2018 ( $P = 0.0015$ ), as well as in April 2019 ( $P = 0.0011$ ). The Simpson index decreased in VP, compared to VPC and VC in May 2018. In September 2018, the Simpson index differed between VC and VP, and also in April 2019. In April 2019 a higher Simpson value was detected in VP than VC and VPC.

#### 4. Discussion

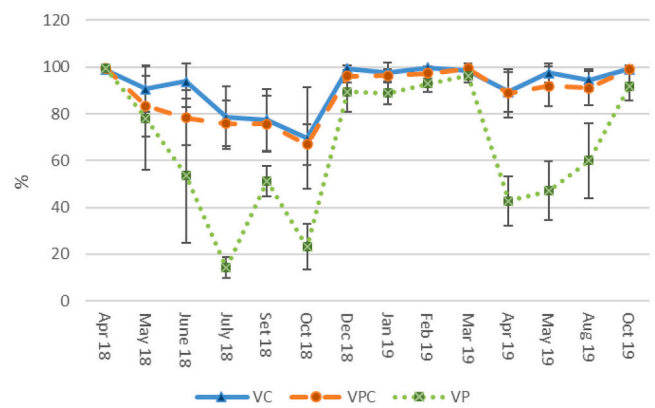
The results highlighted the impact of substrate fertility on the composition and plant diversity of the community functional types. Seasonal temperature and rainfall influenced the community assemblage dynamics as well as the biodiversity in EGRs. The presence of higher values of nitrogen and moisture affected the development of the CAM species *S. album*, which colonised the productive substrates. The unproductive substrate exerted a depressive effect on the spread of *S. album*, the vegetation gaps were thus colonised by annuals and legumes in the cool-rainy season, providing seasonal regeneration through seedling recruitment, increasing the community diversity.

The substrates were designed to provide different conditions in terms of productivity, but some chemical and physical properties tended to converge, mitigating the differences over time. The presence of compost tends to increase the retention of moisture, in fact the substrate richer in compost showed higher moisture content compared to the others as well



**Fig. 4.** Boxplots of biodiversity indices monitored in the different substrates. VP = Vulcaflor + pellet; VPC = Vulcaflor + pellet + compost; VC = Vulcaflor + compost. Average values are reported with diamonds. Data are means of 4 replicates. Different letters show a statistical difference for  $P < 0.05$  between substrates.

as higher values of available water content (Vannucchi et al., 2018). EC decreased because of the rain leaching action,  $C_{org}$  decreased due to the biological activity, while the levels of N were dissimilar over time and the presence of pelletised paper sludge led to a severe lack of N (Eksi et al., 2020). The nitrogen content and moisture in substrates affected the overall functional composition of the plant community. CAM was dominant in the substrates with higher nitrogen and moisture, and annuals forbs mainly developed in the nitrogen-poor substrate. The difference in the plant community structure among substrates was also highlighted by the biodiversity indices. The vegetation grown in the productive substrate had lower species richness and evenness, and the dominance of just a few species (e.g. *S. album*). Providing the EGRs with more resources, such as increasing the substrate depth or improving the growing conditions (shade in the summer), improves the habitat and



**Fig. 5.** Time course of total canopy cover (TCC) in VC (Vulcaflor + compost), VPC (Vulcaflor + pellet + compost) and VP (Vulcaflor + pellet). Data are averages of four replicates  $\pm$ SD.

enhances large-scale biodiversity (Salman et al., 2018). On the other hand, in our study, the reduction of the fertility in the pellet substrate, limited the development of CAM species, increasing the gaps and positively affecting plant diversity (Grime, 2001). The spread of CAM in nitrogen-rich substrate occurs thanks to its rapid spread ability (Nash et al., 2019), which can reduce the colonization by other species (Vannucchi et al., 2018).

Vasl et al. (2017) found a competition between sedums and annuals: sedums reduce annual biomass and diversity, and annuals negatively affect sedum cover and inflorescence production and flowering. In our study there is a correlation between the spread of *S. album* in the productive substrate and the reduction of cover of annual forbs, while the low level of nitrogen content in the unproductive substrate is linked to the reduced number of CAM plants, especially *S. album*, thus likely facilitating the colonization by annuals.

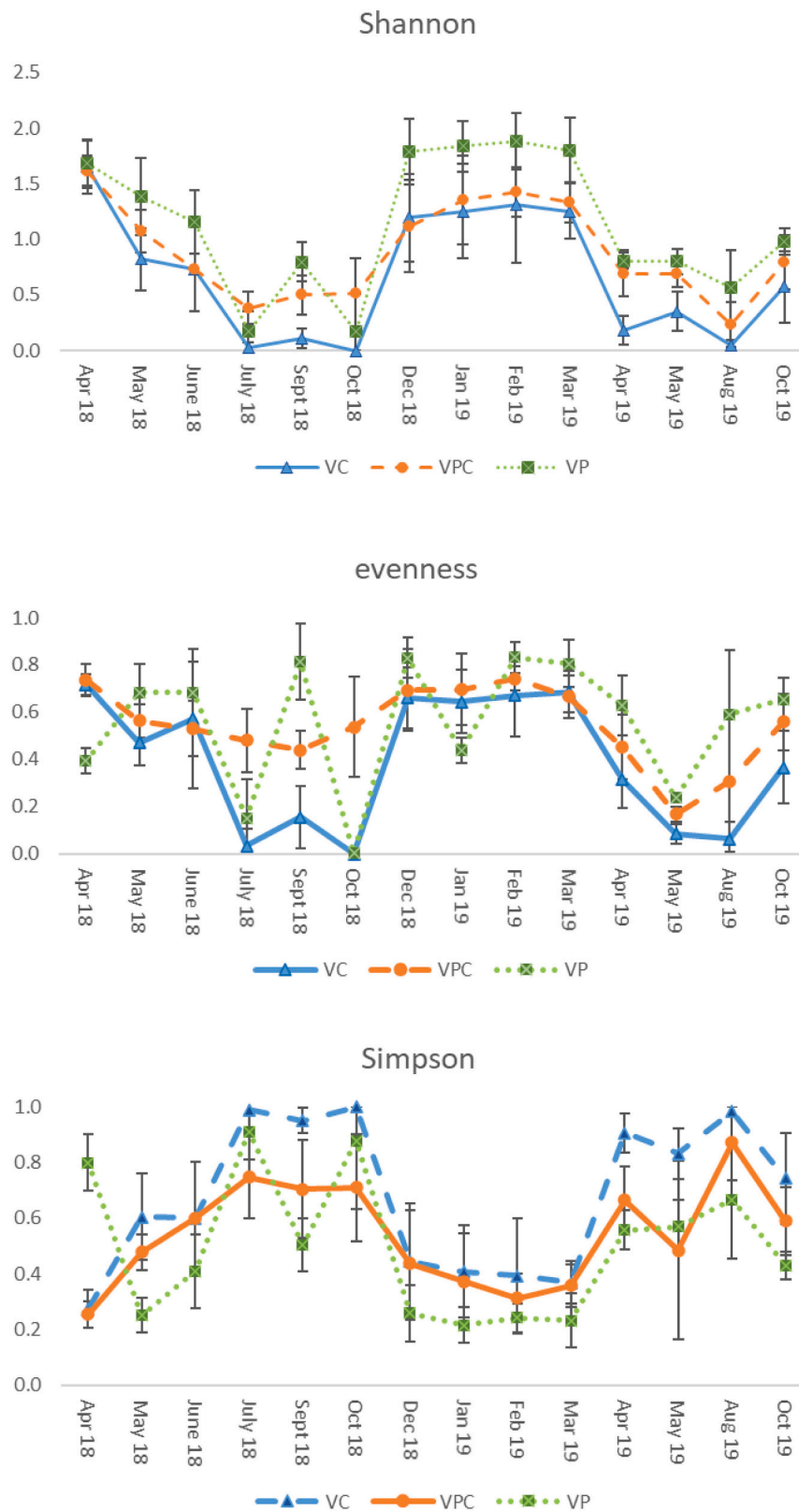
In the study conducted by Emilsson (2008), *S. acre* rarefied after the first few years, but survived in all the substrates; *S. album* competed strongly with *S. acre*. However, the limiting action exerted by the unproductive substrate on the spread of *S. album* enabled *S. acre* to colonise the gaps. The CAM plant type was basically composed of *S. album*, while *S. acre* contributed only by approximately 8%. Drought induces a shift from C3 to CAM in *S. album* (Habibi, 2020) and *S. acre* (Kluge, 1977), thus these species may be the only ones to tolerate the very high stress of EGRs in the summer (June–October).

Besides CAM, annual forbs colonised our EGRs the most successfully. Instead of investing in vegetative development, annuals produce seeds, thus overcoming unfavourable seasons, which in a Mediterranean-temperate climate corresponds to the summer (Grime, 2001). EGRs develop a similar seed bank to those of other novel ecosystems, functioning as a biodiversity reservoir (Vanstockem et al., 2018), as well as preserving species richness, thanks to the persistence of the seedbank (Olly et al., 2011). While not resulting in a significant difference, annual legumes colonised the unproductive substrate and spread into the empty spaces. As a pioneer species, they are able to fix atmospheric nitrogen and create the conditions for survival even in very unproductive soils. This tactic has been observed in trifoliums, which are N-fixing plants, often used in EGRs and play an important role in increasing N in substrates, as well as bryophytes associated with cyanobacteria (Mitchell et al., 2018).

The co-existence of perennials and annuals is supported by the suitable physicochemical conditions (e.g., high porosity and water holding capacity) of substrates with pellets (Vannucchi et al., 2018), moreover perennials such as *P. saxifraga* are sensitive to early drought and prefer a deeper substrate (120 mm), (Thuring et al., 2010). In our experiment, the cover of perennials, forbs and legumes was limited: many perennial species survive in dry environments, deepen their roots



**Fig. 6.** Trends of CAM, bryophytes, annual forbs and legume contributions (%) related to monthly maximum temperature (Tmax) and total rainfall monitored on the green roof, during the experiment, in VC (Vulcaflor + compost), VPC (Vulcaflor + pellet + compost) and VP (Vulcaflor + pellet). Data represent the total percentage of each plant functional type in all replicates of each substrate type. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



**Fig. 7.** Time course of biodiversity indices in VC (Vulcaflor + compost), VPC (Vulcaflor + pellet + compost) and VP (Vulcaflor + pellet). Data are averages of four replicates  $\pm$ SD.



into the cracks, eventually finding the necessary moisture to grow (chasmophytes), but the shallow EGR substrates do not give this opportunity, being more colonisable by chomophytes, such as moss, which can grow on the detritus (Lundholm and Walker, 2018). Bryophytes, which showed higher contribution in the middle productivity substrate, play an important role in alleviating the stress suffered by vascular plants due to the harsh conditions of roofs, although in some cases they can inhibit seed germination (Drake et al., 2018).

The three types of substrates had a visually different vegetation cover. VC showed no gaps, the cover was thick and continuous; VPC presented some gaps; and VP showed empty areas, which were larger than the vegetated areas, where sedums, especially *S. acre*, adopted a circular vegetation pattern, typical of arid ecosystems (Rietkerk et al., 2002).

Most of the species were able to grow in the three level of productivity substrates, by adopting different strategies, however the scarcity of nutrients favoured the ruderals and stress tolerators. The build-up of organic matter over time was absent or very slow, unlike those commonly found in urban areas, which are rich in organic matter (Ksiazek-Mikenas and Köhler, 2018). *S. album* adopted a competitive-stress tolerator strategy and colonised the productive substrate. The seasonal regeneration in unproductive substrate was mainly due to small-seeded annuals such as *S. gallica*, *P. annua*, *T. campestris*, while species with large seeds such as *C. arvensis*, are less dependent on vegetation gaps (Grime, 2001), and thus they were able to spread in the closed vegetation dominated by *S. album* in productive and middle productive substrates. *Erodium cicutarium* was able to spread in both situations thanks to its particular ballistic dispersion system (Bretzel et al., 2020). Annuals developed in all the substrates by adopting a ruderal-stress tolerator strategy.

The plant diversity fluctuations generally showed the same trends in all substrates. In the spring-summer period, the Shannon index decreased and the Simpson index increased, while from October to March, Shannon increased and Simpson decreased. Evenness is related to the uniformity of the number of individuals per species and does not depend on rare species, that do not contribute much to biodiversity. In the productive and unproductive substrates, the trend thus fluctuated greatly, the most constant being the middle productive substrate. The increase in plant diversity in the rainy-cold season could be related to the establishment of annual species, weeds and introduced, from the seed bank, conferring a resilience towards seasonal fluctuations to the plant community (Cook-Patton and Bauerle, 2012).

Seasonal variations also determined the plant strategies for competitive, stress tolerator and ruderal species, which were competitive when resources were available (i.e. in the rainy season and in productive substrates), ruderals and stress tolerators in the summer and in unproductive substrate. *S. album*, *S. acre* and bryophytes were the most spread stress tolerators, *C. arvensis* and *E. cicutarium* ruderals (Thuring and Dunnett, 2019). Stress responses are physiological more than morphogenetic (Grime, 2001), therefore *E. cicutarium*, *C. arvensis*, *A. alyssoides*, *L. maritima*, *C. macrosiphon* grew very little in unproductive substrates, and showed phenological plasticity, adapting to the scarcity of resources by flowering and reproducing at the early life stages, allowing the regeneration (Aronson et al., 1992). Geophytes were present in all the substrates, but in the unproductive substrate the scarcity of nutrients prevented the production of flowers, which was probably due to the low allocation of resources in the underground organs. Synanthous geophytes, that have leaves and flowers simultaneously, have high need to allocate resources in order to flower, so in this study the low availability of nitrogen limited their growth and flower production. Stress tolerators rarely flower and reproduce, in order to prevent the consumption of resources (Grime, 2001).

## 5. Conclusions

Reducing the substrate productivity can improve plant functional

types and biodiversity in EGRs, despite the harsh conditions. The low fertility in the substrate limited the development of CAM species, thus positively affecting plant diversity through coexistence with annuals. Seasonality affected the composition and structure of the plant community. During summers, the biodiversity decreased, and CAM was the dominant functional group in the EGR. At the end of the summer drought, the plant community recovered, thanks to the seedling recruitment by annuals. EGRs remain in the early successional plant stage without reaching the next stages of succession, because of stress factors (Ksiazek-Mikenas and Köhler, 2018). Annual plant species play an important ecological-functional role in regenerating the vegetation community in EGRs under Mediterranean climates as in natural ecosystems. Further studies may focus on CSR (competitors, stress tolerators, ruderals) strategies, through a plant trait analysis, to further explore the possible changes related to different fertility conditions and seasonality, over the long term.

## Credit author statement

Conceptualization and methodology FB and FV; Data collection: FB, MS and AB; Formal analysis: MS, FV and CC; Software: CC; Writing - original draft: FB and FV; Writing - review & editing: FB, FV and AB. All authors contributed critically to the drafts and gave final approval for publication.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecoleng.2022.106547>.

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