

Dynamics, abundance, and trends of extensive green roof plant communities under different productivity conditions

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Abstract:

1. Roofs are free spaces where living organisms can find an appropriate habitat to colonise. The adding of just a thin layer of growing media allows plants to grow, adapting to the extreme conditions and scarce nutrient availability. The establishment of plant species with different functionality can enhance biodiversity and provide urban ecosystem services. However, drought and nutrient availability can affect the plant development, and the monitoring of plant community dynamics under limiting factors is important to assess possible changes in community structure.

2. The experiment started in 2014 on a roof on which 12 plots of 10 cm depth were set up and filled with three different substrates composed of compost, pelletised paper sludge, and commercial tephra product (Vulcaflor), as follows: VC (Vulcaflor + compost), VPC (Vulcaflor + pellet + compost), and VP (Vulcaflor + pellet), characterized by different level of nitrogen content (productivity). The plants were introduced as seeds or seedlings and grouped into plant functional types (PFTs): annual and perennial forbs, annual and perennial legumes, geophytes, crassulacean acid metabolism (CAM), grasses and bryophytes. In 2019, five years after the planting, the community composition and structure were monitored for two years using the point-intercept method to evaluate the establishment.

3. Besides seasonal fluctuations, nitrogen shaped the composition of the community in terms of PFTs, and CAM species showed high cover values in nitrogen-rich substrates. Annual forbs colonised the plots with a lower nitrogen content. Seasonality affected the community structure. In summer, the number of species fell drastically, and CAM was the dominant functional group in the three substrates, with differences in percentage cover. Seedling recruitment regenerated the community in the cooler season, increasing the diversity in the poor substrate.

4. *Synthesis and applications.* Studying extensive green roofs provides the opportunity to investigate the dynamics of early successional plant communities. The scarcity of nitrogen in substrate led to the development of stress-tolerator annuals in rainy - cool seasons, thus increasing the biodiversity in low productivity substrate. The phenological complementarity increased the diversity under limiting factors,

as annual species constitute a transient seed bank which enables the system to regenerate when rain follows periods of heat and drought.

Keywords: annuals, biodiversity, point-intercept method, functional groups, nitrogen, tolerance strategy, seasonal fluctuation, *Sedum album*.

1. Introduction

Extensive green roofs (EGRs) are a nature-based green infrastructure characterised by shallow growing media and no agronomical maintenance, thus there is almost no disturbance. 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 & Kolasa Więcek, 2019). 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 colonize and cover the free space (Vijayaraghavan et al., 2016), adapting to the novel ecosystem conditions. They should also be able to tolerate extreme climates, 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 (Catalano et al., 2018), 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 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). The limited availability of nitrogen in the 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, and provide opportunities for weak competitors (Bretzel et al., 2016). The establishment of stress tolerant species, which enhance the plant diversity in EGRs, may thus limit the dominance of sedums, through the lack of soil fertility (Salman et al., 2020).

In many cases, the most successful attempts at EGRs are those that establish different species of plant with different functionality and life forms, inspired by ecological models, aimed at constituting a self-sustainable community (MacIvor et al., 2013). The “habitat template” is a fascinating approach to planning urban green areas, through the selection of plant species that occur in habitats characterized by

similar environmental conditions with the urban green areas. However, in cities, the factors that affect the communities' dynamics do not just apply to nature. Pollution, heat island effects, and soil sealing all tend to amplify the stress of natural ecosystems. Consequently, in order to select species for EGRs, adopting the habitat template as a coarse filter and including the entire plant community are likely to be more successful strategies (Lundholm & Walker, 2018).

Plant functional types (PFTs) reveal the ecological assembly of communities and are a useful complementary tool to the traditional taxonomy (Duckworth et al., 2000). CAM plants (sedums) reduce the temperature of the substrate and 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 a Mediterranean climate (Vasl et al., 2017). *Sedum album* and *S. acre* are facultative CAM, which shift from C3 to C4 metabolism under water stress (Sayed et al., 1994; Kluge, 1977). Nitrogen-fixers are another effective functional group for green roofs as they have a pioneer strategy and can fix atmospheric nitrogen. They are thus 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 established on a dynamic green roof is subject of changes, due climate and nutrient availability, among the others, though altering the provided ecosystem services (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 & 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 characteristics (e.g. drought, high irradiance level, reduced soil moisture), such studies also reveal how an early successional plant community can ecologically respond to the climate changes expected in many regions of the world.

The aims of this study were to understand the small-scale processes underlying the response of the EGR plant communities (PFTs) and structure (biodiversity) to substrate fertility. In addition, we evaluated the community assemblage dynamics, in terms of PFTs and diversity, related to seasonal temperature and rainfall, and recovery after drought.

2. Methods

2.1. Experimental site

The experiment was set up in 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). Twelve ITM (interlock turf module) boxes were set up measuring 1x1 m, 10 cm depth, with 10 cm of underneath drainage. 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, commercial tephra product (Vulcaflor) and pelletized paper sludge. 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 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 [as reported by Vannucchi et al., \(2018\)](#). The initial planting was partially sown and partially seeded, and the species were typical of herbaceous disturbed swards of peri-urban and rural areas nearby. To highlight the ecological strategies, the species were grouped into the following plant functional types (PFTs): annual forbs, annual legumes, perennial forbs, perennial legumes, geophytes, CAM, *graminae* and bryophytes. CAM consisted of *Sedum 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 (H₂O) organic carbon (C_{org}) and total nitrogen (N_{tot}) were analysed (ASA-SSSA, 1996). C_{org} and N_{tot} contents were determined through dry combustion using a Leco CHN analyzer. 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

After five years from the initial planting, a monitoring was carried out to evaluate the plant establishment and to find out the strategies better adapted for the survival on the roof. The contribution of plant functional types (PFTs) and total canopy cover (TCC) were monitored from April 2018 to October 2019, using the point intercept method, with a point frame for botanical surveying composed of ten pins (pin length: 46 cm distance between outer pins: 5cm, leg length: 54cm) (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 number plants touched (Glatzle et al., 1993). 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 from April 2018-March 2019, as the seedling recruitment period, when the number of species was high and in October, 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 & Weaver, 1949), and the evenness of species (J) (Smith & 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^{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_{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) (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, bryophytes reached higher percentages in VPC (6%), compared to VC (0.3%) and VP (0.6%) (Figure 1).

Throughout the experiment PCA was performed to evaluate the PFT composition of plant communities related to different substrate fertility (Figure 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 VP (66%) (Figure 3).

The plant structure in terms of the biodiversity index is shown in Figure 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 (0.5 ± 0.17) and VC (0.4 ± 0.28) ($P = 0.02$). No significant differences were detected between VP (0.6 ± 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 (Figure 5).

The dynamics of PFTs in the three substrates were affected by climate variations (Figure 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 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 (Figure 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 colonized the EGR in the winter until April and disappeared at the beginning of the drought period. *S. vulgaris* reached a 34%, however the maximum height of the plants was 3 cm; the warm-season weed *Portulaca olearacea* L. spread from September - October 2019 (Figure S2).

The seasonal dynamics of the plant community in terms of biodiversity indices are reported in Figure 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 nitrogen 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 availability of substrate nitrogen affected the development of the CAM species *S. album* which colonised the most 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 and increasing the community diversity.

The substrates were designed to gradually create different conditions in terms of productivity. The chemical and physical properties of substrates tended to become uniform, mitigating the differences over time. EC decreased because of the leaching action, C_{org} decreased and the presence of pelletised paper sludge in the substrate led to a serious N deficiency (Eksi et al., 2020).

The differences in substrates in terms of nitrogen content, affected the overall functional composition of the plant community. CAM was dominant in the substrates with higher nitrogen and annuals forbs mainly developed in the nitrogen-poor substrate. The difference in the plant community structure amongst substrates was also highlighted by the biodiversity indices. The vegetation grown in the productive substrate had less 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 enhances large-scale biodiversity (Salman, 2018).

On the other hand, in our study, the reduction in 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 that sedums were weak competitors toward annual species, exerting a reduction in biomass and diversity over time and suffer a reduction in cover and flowering. In our study the spread of *S. album* in the productive substrate limited the development of annual forbs, while the low level of nitrogen content in the unproductive substrate greatly hindered the number of CAM plants, especially *S. album*, thus facilitating colonisation by annuals.

In the study conducted by Emilsson (2008), *Sedum acre* was rare 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 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).

After CAM, annual forbs colonized 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), which ensure a durable plant cover over time (Ondoño et al., 2015). However, in our experiment, the cover of perennials, forbs and legumes was limited. Often the strategy of this PFT to survive in shallow drought environments such as rocky banks, is related to the ability of the roots to go deeper into the cracks in the rock, eventually finding the necessary moisture to grow; however, this is not the case of the EGR substrates (Lundholm & 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 different productivity substrates, by adopting different strategies, however the low level of fertility favoured the ruderals and stress tolerators. Over time these conditions lead to a habitat where the build-up of organic matter is absent or very slow, unlike those commonly found in urban areas, which are rich in organic matter (Ksiazek-Mikenas & Köhler, 2018). *Sedum album* adopted a competitive-stress tolerator strategy and colonised VC. The seasonal regeneration in VP 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 VC and VPC substrates, where the vegetation dominated by *S. album* closed the gaps. *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 substrate composition is important for sustainable plant growth, in fact the presence of high amounts of organic matter can lead to a great development of plant biomass, especially in wet periods, however damage during summer droughts is possible (Nagase & Dunnet, 2011).

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 moderate-low productivity substrate, the trend thus fluctuated greatly, the most constant being the intermediate productivity substrate. The increase in plant diversity in the rainy-cold season could be related to the reestablishment of annual species from the seed bank, conferring a resilience towards

seasonal fluctuations to the plant community (Cook-Patton & Bauerle, 2012). In addition, the persistence of a diverse plant community limited the decrease in plant diversity in moderate-low productive substrates, as in September 2018 and in April-May 2019.

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; the same species adopted different strategies. Ruderals and stress tolerators in the summer and in unproductive substrate. *Sedum album*, *S. acre* and bryophytes were shown to be the main stress tolerators. *Calendula arvensis* and *E. cicutarium* the most spread ruderals (Thuring & Dunnet 2019). Stress responses are physiological more than morphogenetic (Grime, 2003), 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. However, in the unproductive substrate, the scarcity of nutrients prevented the production of flowers, which was probably due to the dimensions of the underground organs that are not big enough to sustain the flowering. Synanthous geophytes, which are those that have leaves and flowers simultaneously, there is a high need to allocate resources in order to flower, so in this study the low availability of nitrogen prevented their growth and flower production. Stress tolerators rarely flower and reproduce, in order to prevent the consumption of resources (Grime, 2001).

5. Conclusions

Plant functionality and biodiversity can be improved in EGRs despite the harsh conditions, by reducing the substrate productivity. In addition, the phenological complementarity seasonally enhanced the number of species and functional types. The lower 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 the biodiversity decreased, and CAM was the dominant functional group in the EGR. At the end of the summer drought, there was a plant community recovery, 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 & 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 could focus on CSR strategies, through a plant trait analysis, to further explore the possible changes related to different fertility conditions and seasonality, over the long term.

Author contributions

F.B. conceived the research idea; F.B. and A.B. collected data; F.V and C.C. performed statistical analyses; M.S. performed physical and chemical analyses. F.B., F.V., C.C. and A.B. wrote the paper. All authors discussed the results and commented on the manuscript.

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Table 1. Species on the green roof, the plant functional types (PFTs) were assigned according to Pignatti *et al.* (2017) and Pérez-Harguindeguy *et al.* (2013) and the strategy type according to Klotz *et al.* (2002). AF = annual forbs; A = annual legumes; PL = perennial legumes; BR = bryophytes; PF = perennial forbs; G = geophytes; GR = *graminae*; C = competitive; S = stress tolerator; R = ruderal.

PFT	Species	Family	Growth forms	Strategy type
G	<i>Allium roseum</i> L.	Amaryllidaceae	bulb	-
AF	<i>Alyssum alyssoides</i> (L.) L.	Brassicaceae	Scap/erosulate	SR
PL	<i>Anthyllis vulneraria</i> L.	Fabaceae	Scap/hemirosette	CSR
AF	<i>Blackstonia perfoliata</i> (L.) Huds.	Gentianaceae	Scap/erosulate	SR
AF	<i>Calendula arvensis</i> (Vaill.) L.	Asteraceae	Scap/hemirosette	R
AF	<i>Centranthus macrosiphon</i> Boiss.	Caprifoliaceae	scap	-
PF	<i>Crepis bursifolia</i> L.	Asteraceae	scap	-
PF	<i>Dianthus deltoides</i> L.	Caryophyllaceae	Caesp/erosulate	CSR
AF	<i>Erodium cicutarium</i> (L.) L'Hér.	Geraniaceae	caesp/scap/hemiros	R
AF	<i>Geranium molle</i> L.	Geraniaceae	Scap/hemiros	R
PF	<i>Hypochaeris radicata</i> L.	Asteraceae	rosette	CSR
PF	<i>Lobularia maritima</i> (L.) Desv.	Brassicaceae	Scap/erosul/hemiros	SR
G	<i>Muscari comosum</i> (L.) Mill.	Asparagaceae	Bulb/ros	CSR
G	<i>Ornithogallum umbellatum</i> L.	Asparagaceae	Bulb/ros	CSR
PF	<i>Petrorhagia saxiphraga</i> (L.) Link	Caryophyllaceae	Caesp/erosul	CS
GR	<i>Poa annua</i> L.	Poaceae	Caesp/hemiros	R
AF	<i>Scrophularia peregrina</i> L.	Scrophulariaceae	scap	-
CAM	<i>Sedum acre</i> L.	Crassulaceae	succ	S
CAM	<i>Sedum album</i> L.	Crassulaceae	succ	S
AF	<i>Silene gallica</i> L.	Caryophyllaceae	Scap/erosulate	R
AL	<i>Trifolium arvense</i> L.	Fabaceae	Scap/eros/hemiros	SR
AL	<i>Trifolium campestre</i> L.	Fabaceae	Scap/eros/hemiros	R
AF	<i>Verbascum blattaria</i> L.	Scrophulariaceae	Scap/hemiros	C
BR	<i>Bryophytes</i>			

Table 2. Chemical properties of the growing media detected in May 2018. EC= electrical conductivity; C_{org} = organic carbon; N_{tot}= total nitrogen. VP = vulcaflor + pellet; VPC = vulcaflor + pellet + compost; VC = vulcaflor + compost. Data are means of 4 replicates \pm SD. Different letters show a statistical difference for $P < 0.05$ between substrates.

	pH H ₂ O		EC dS/m		C _{org} %		N _{tot} %	
	2018	2019	2018	2019	2018	2019	2018	2019
VC	7.7 \pm 0.06	8.0 \pm 0.06	0.9 \pm 0.04	0.2 \pm 0.03	8.1 \pm 0.85	5.8 \pm 1.08	0.51 \pm 0.18a	0.65 \pm 0.15a
VPC	7.8 \pm 0.09	8.2 \pm 0.14	0.9 \pm 0.12	0.2 \pm 0.02	6.5 \pm 2.08	3.5 \pm 0.37	0.40 \pm 0.14a	0.36 \pm 0.05a
VP	8.0 \pm 0.09	8.5 \pm 0.05	0.8 \pm 0.07	0.2 \pm 0.01	9.0 \pm 0.61	3.8 \pm 0.42	0.28 \pm 0.02b	0.26 \pm 0.01b

Table 3. Total averages of Plant Functional Types (PFTs) not showing significant differences among substrates. 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 \pm SE.

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

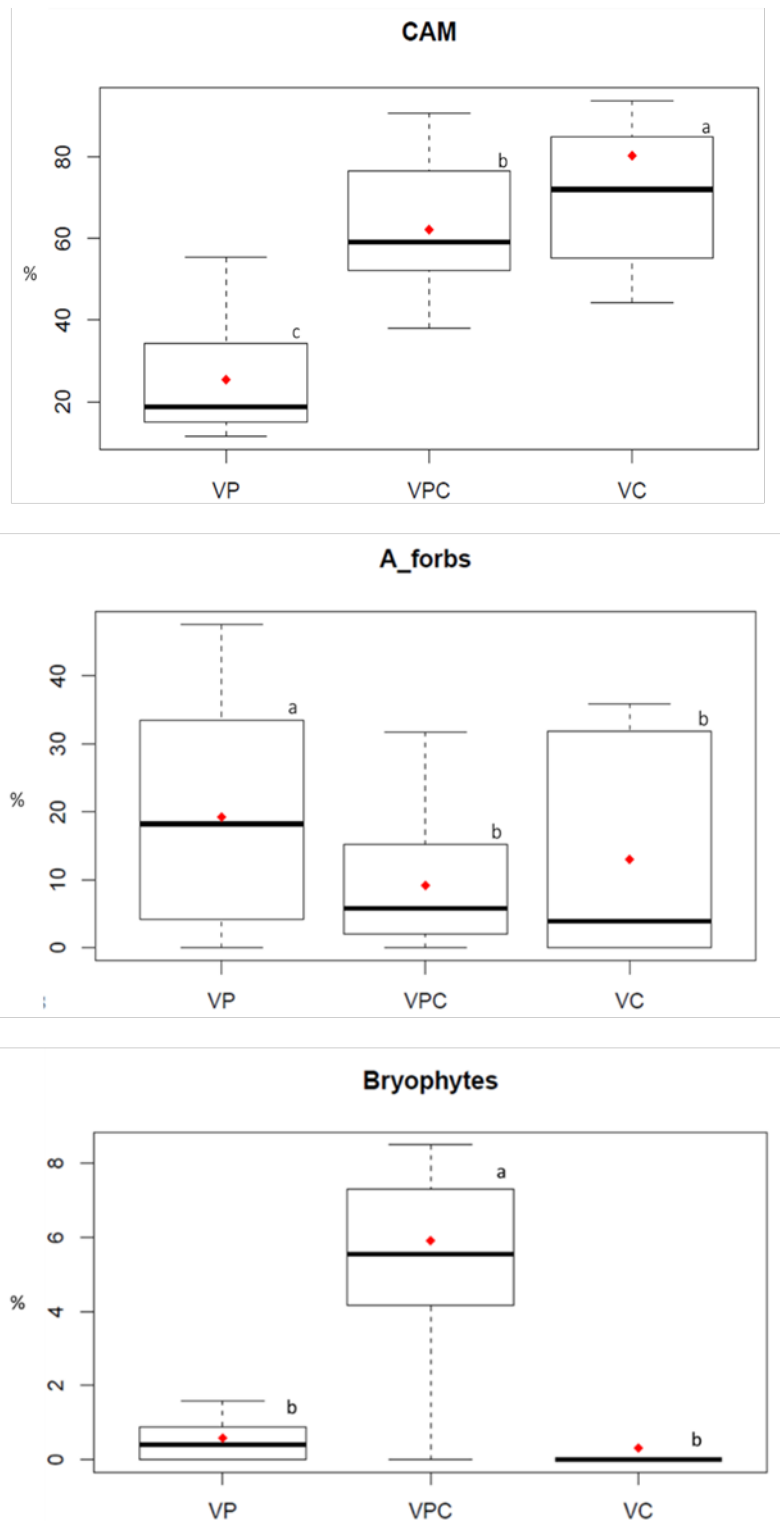


Figure 1. Boxplots of PFT contribution (%) monitored in different substrates. A_forbs = annual forbs. VP = Vulcaflor + pellet; VPC = Vulcaflor + pellet + compost; VC = Vulcaflor + compost. Average values are reported with diamonds. Different letters show a statistical difference for $P < 0.05$ between substrates.

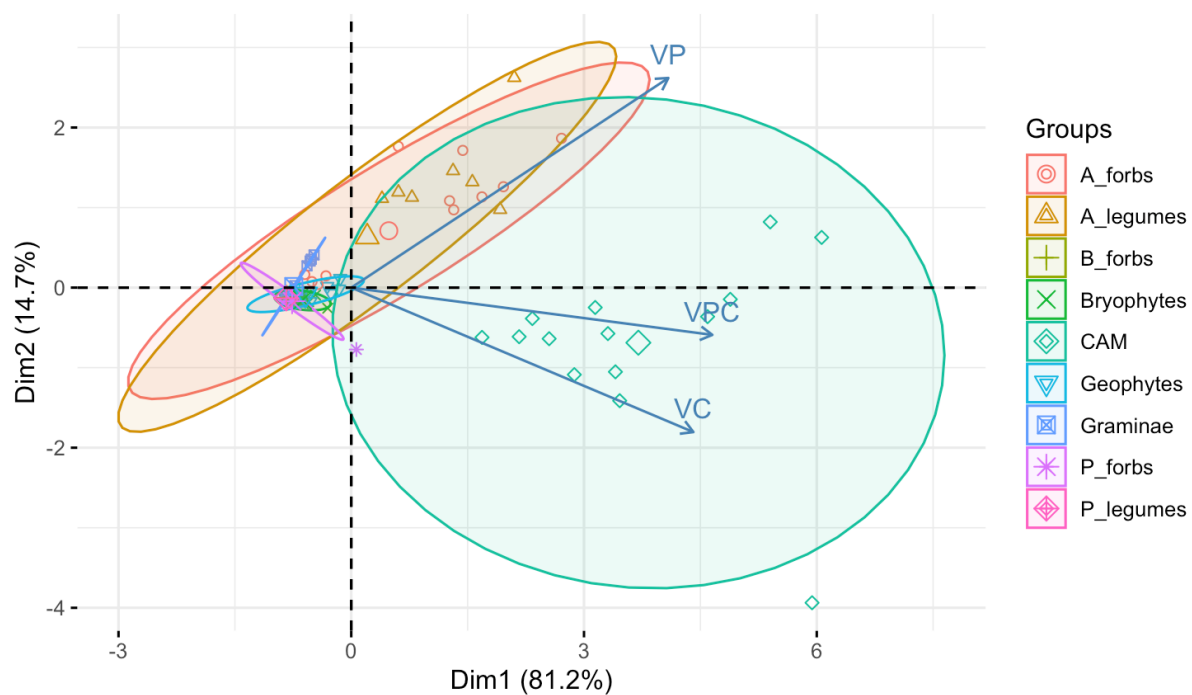


Figure 2. PCA of plant functional types in different substrates. A. forbs = annual forbs; A. legumes = annual legumes; P. forbs = perennial forbs; P. legumes = perennial legumes. VP = Vulcaflor + pellet; VPC = Vulcaflor + pellet + compost; VC = Vulcaflor + compost.

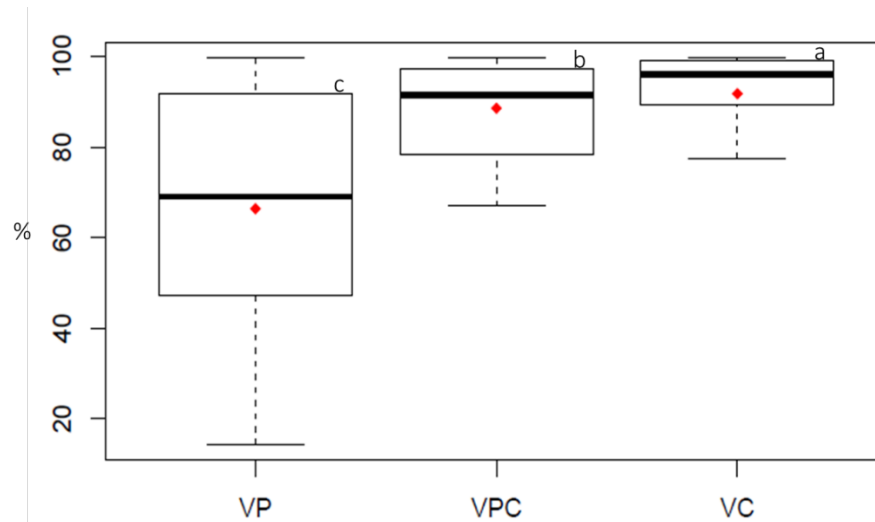


Figure 3. Boxplots of total canopy cover (%) monitored in different substrates. VP = Vulcaflor + pellet; VPC = Vulcaflor + pellet + compost; VC = Vulcaflor + compost. Average values are reported with diamonds. Data are means of four replicates. Different letters show a statistical difference for $P 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

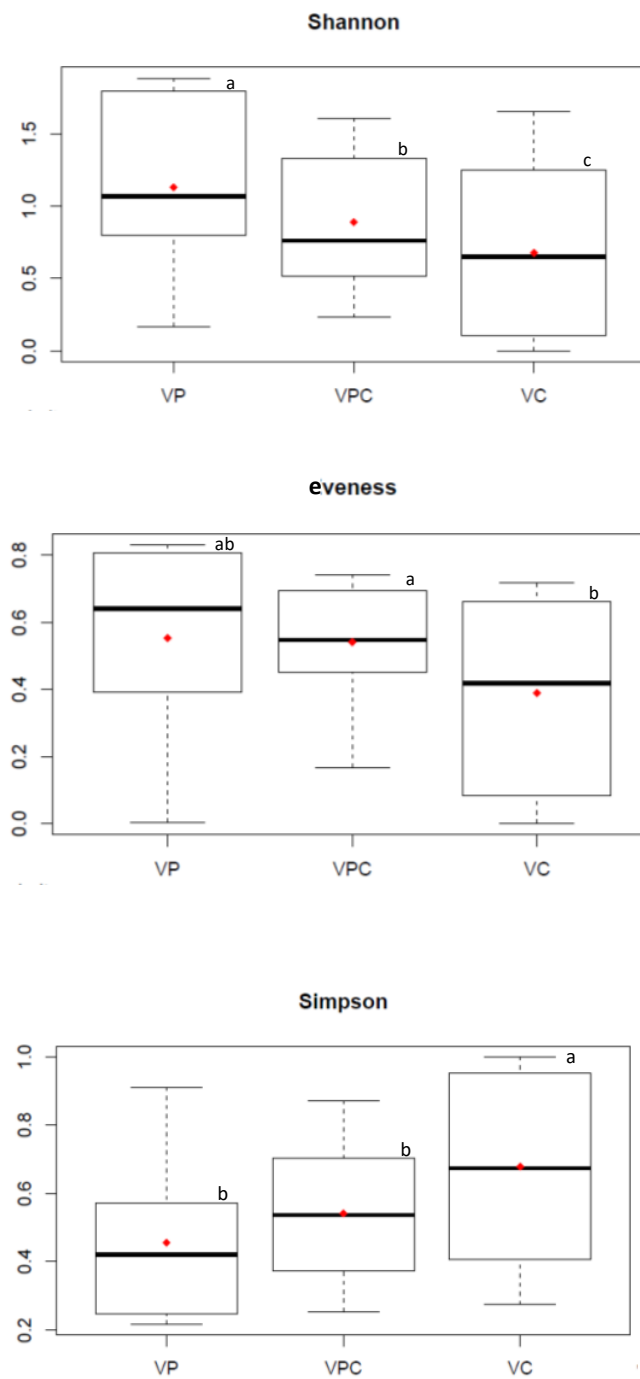


Figure 4. Boxplots of biodiversity indices monitored in different substrates. VP = Vulcaflor + pellet; VPC = Vulcaflor + pellet + compost; VC = Vulcaflor + compost. Average values are reported with diamonds. Data are means of 4 replicates. Different letters show a statistical difference for $P < 0.05$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

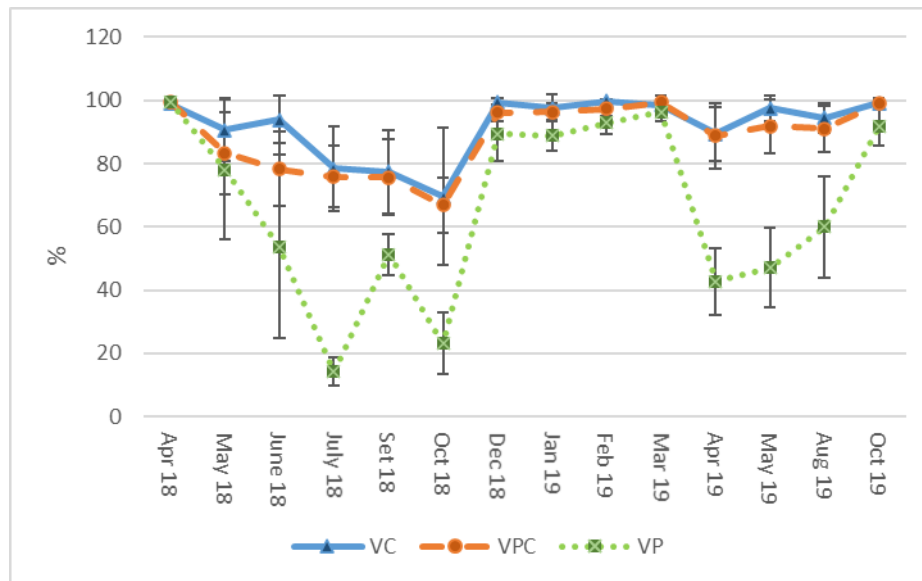


Figure 5. Time course of total canopy cover in VC (Vulcaflor + compost), VPC (Vulcaflor + pellet + compost) and VP (Vulcaflor + pellet). Data are averages of four replicates \pm SD.

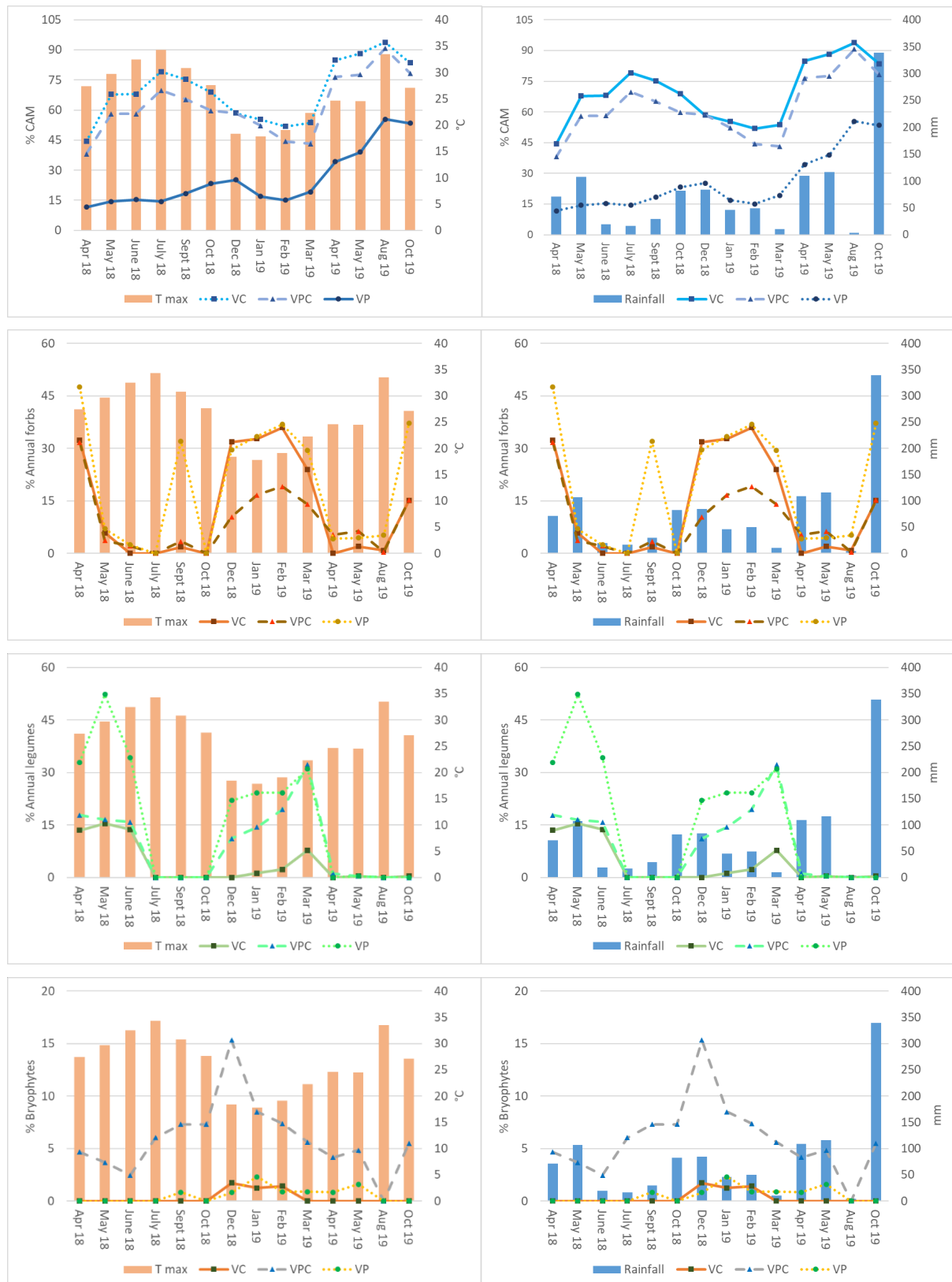


Figure 6. Trends of CAM, bryophytes, annual forbs and legume contributions (%) related to monthly maximum temperature (Tmax) and total rainfall monitored on the green roof, during the experiment, in VC (Vulcaflor + compost), VPC (Vulcaflor + pellet + compost) and VP (Vulcaflor + pellet). Data represent the total percentage of each plant functional type in all replicates of each substrate type.

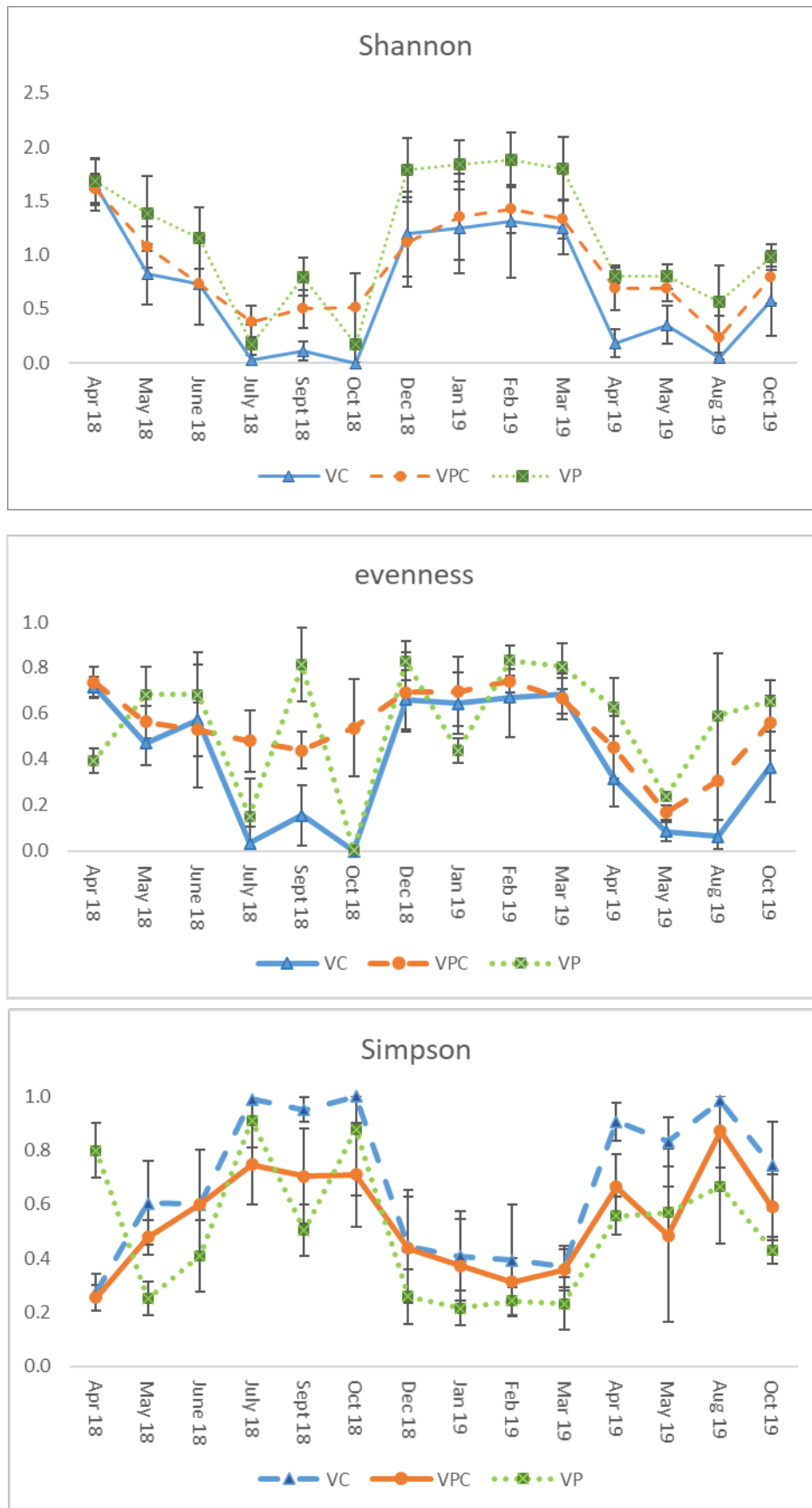


Figure 7. Time course of biodiversity indices in VC (Vulcaflor + compost), VPC (Vulcaflor + pellet + compost) and VP (Vulcaflor + pellet). Data are averages of four replicates \pm SD.