

Article

# Sustainable Growth of Medicinal and Aromatic Mediterranean Plants Growing as Communities in Shallow Substrate Urban Green Roof Systems

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**Abstract:** To date, the evaluation of the performance of Mediterranean native plants under urban green roof conditions has been limited to monoculture studies. However, plants grow naturally in plant communities and therefore it is of interest to evaluate their growth under realistic conditions, including interactions among plant species. The present study aims to evaluate the growth, flowering, and self-reproduction capacity of three artificially created plant communities consisting of native Mediterranean plants, as exemplified in Greece under shallow green roof substrate depths (8 and 15 cm) and two irrigation regimes (high, 20% ET<sub>0</sub> and low, 10% ET<sub>0</sub>). The plant communities (PC) were designed to resemble xerophytic vegetation found either in Chania, Crete (PC-1), as a combined pattern with plants from Attica, Crete, and the Cyclades (Kythnos) (PC-2), or to resemble the coastal vegetation of Attica and Cyclades (Kythnos) (PC-3). Each of the three artificial plant communities (PC-1, PC-2 and PC-3) consisted of nine species and subspecies. The deeper substrate significantly improved the growth, flowering and survival of most plant taxa. The irrigation regime was not significant for all species except one, indicating that minimal amounts of irrigation are required. Four species did not manage to bloom while 15 species were able to self-reproduce.

**Keywords:** endemic; native; pharmaceutical; biodiversity; water stress; drought; artificial plant assemblages; species interaction; ecological planting



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## 1. Introduction

Green roofs are contemporary urban greening techniques for reclaiming the lost flora and fauna within the densely built urban environment. The popularity of green roofs has rapidly increased among architects, landscape architects, and the public due to the positive aesthetic impact and the numerous environmental advantages that they possess. Even in reconstruction projects which are expected to withstand minimal additional loadings, the lightweight nature of extensive green roof systems has provided applicable solutions based on research findings [1–4].

There are several types and construction techniques for green roofing and these are all similar in concept, though they differ in the utilized plant material. More specifically, green roofing is applicable worldwide and is commonly composed of a protection mat, a drainage system, a geotextile, and a growing substrate [5]. However, plant material must be selected meticulously to be adapted to the specific climatic region and provide

sustainable growth in shallow green roof substrates. During the green roof plant selection process, several factors should be taken into consideration including the climatic zone, the microclimatic conditions on the building's rooftop, the stresses expected to occur due to the plants exposure to adverse conditions, the type of green roof as indicated by its substrate composition and the depth and desired level of irrigation [4]. In addition, the selected green roof plant species should exhibit tolerance to temperature extremes and wind gusts, the ability to achieve sufficient substrate coverage and the ability to withstand nutrient and water deprivation.

Apart from the abovementioned adaptations and tolerances, there are further desirable characteristics during the selection of plant species for use on extensive green roof systems. These include a preference towards perennial growth, shallow root systems, low or creeping growth habit, capacity for efficient speed coverage, prolific reproduction and ability to provide sustainable growth with minimal resources and human intervention [6]. The ornamental value of the selected plant species is also of great importance and chosen plants are expected to provide an aesthetically pleasant and appealing visual effect within the urban landscape throughout the year. The morphology, color, shape and texture of the selected plants are integral parts of the overall design concept of the green roof since plant material softens the hard surfaces of the buildings and adds visual interest to them [7]. Green roofs with a greater variety of vegetation and colors and a careful selection of plants with proper flowering planning are preferred over simplified plant design alternatives [8].

Based on these multiple requirements, the selection of plant material suitable for extensive green roofs has pointed toward the exploration of native and endemic species adapted to specific areas and climatic zones [1,2,9–22]. Despite these efforts, there is still a lack of documentation, and a lot of further research is necessary to take advantage of the huge resources offered by native Mediterranean plants in the agricultural, horticultural and landscape industries [23]. Specifically, it is necessary to evaluate native and endemic species for their potential use on green roof systems, due to their adaptation to local environmental conditions, resistance to insects, diseases, drought, high temperatures, wind gusts and other characteristics that could ensure the sustainability of both the green roof and natural resources [9,10,14,16–18,21,24–27].

Plants grow as communities in nature and not as monostands. Thus, it is appropriate to evaluate and select suitable species for extensive urban green roofs under realistic conditions, which include interactions among the participating plants. Habitats that are characterized by extreme climatic conditions, such as drought, high temperatures and strong winds can turn into valuable natural habitats for green roof plant species selection. Greece is characterized by a diverse and intermittent geographical terrain that creates a wide variety of landscapes, which in turn supports a wide variety of habitats. The rocky islands, sandy shores, plains and hills, mountains, canyons, and gorges contribute to the disproportionate size of Greece's biodiversity of native and endemic flora. In addition, climatic differences occur from north to south, with seasonal, irregular rainfall exceeding 1000 mm in northwestern Greece, but reaching less than 400 mm in the Cyclades, Crete, and Attica. This diversity of geomorphology, landscapes, climates, and habitats created ideal conditions for increased plant biodiversity within the Mediterranean basin, providing a wealth of choices for green roof flora selection. Greek flora is rich in endemic species, mainly concentrated in central and southern Greece as well as the Aegean islands and Crete [28–30]. According to Dimopoulos et al. [29] Greek flora is composed of 6620 species and subspecies (1459 endemic species and subspecies), including numerous medicinal and aromatic plants with low demands. These could be considered suitable choices for developing sustainable Mediterranean green roofs, given their minimal needs for water resource inputs [1,2,9,24,31]. Crete is characterized as one of the most prolific biodiversity places among European and Mediterranean regions, with numerous endemic plant species. The diversity of the Cretan flora results from its geological history and geographical location and consists of at least 1820 plant species [32–34], of which 223 (12.3%) are endemic [35]. Various Cretan endemic

species have agro-alimentary, aromatic-medicinal and/or ornamental value [36–38] and attempts are in place to propagate and cultivate them [39–47].

The aim of the present study was to develop three artificial Mediterranean (Greek) plant communities and evaluate the growth of the constituent plants under two extensive green roof substrate depths and two irrigation regimes. The three plant communities comprised 25 native and/or endemic species of the Greek flora, originating from their natural dry habitats in Attica and the islands of Crete and Kythnos (Cyclades). The first plant community resembled the xerophytic vegetation of Chania region in Crete, which is characterized by rocky and stony sites, slits, rock crevices, cliffs, and canyons. The second plant community resembled the xerophytic vegetation of Attica, Crete, and the Cyclades, and the third, the xerophytic coastal vegetation of Attica and Cyclades.

## 2. Materials and Methods

### 2.1. Experimental Setup

The study took place on the rooftop of a building at the Agricultural University of Athens (latitude 37°59', longitude 23°42', 36 m a.s.l.; Figure 1). It was initiated on November 2012 and lasted until February 2015. Sixty experimental plots comprised plastic trays with external dimensions of 1.2 m by 1.2 m (Green Tech Inc., Richmond, VA, USA; Figure 1). Inside each tray a typical extensive green roof layering structure was constructed. Starting from the lower towards the upper part of the plot, the green roof components were: (a) protection cloth (VLS-300, Diadem, Landco Ltd., Athens, Greece), having a thickness of 3.0 mm,  $0.3 \text{ kg m}^{-2}$  dry weight and  $2.7 \text{ L m}^{-2}$  water retention capacity; (b) drainage board (DiaDrain-25, Diadem, Landco Ltd.) made of recycled high impact polystyrene with 25 mm height,  $1.15 \text{ kg m}^{-2}$  weight and water retention capacity of  $10.2 \text{ L m}^{-2}$ ; and (c) a geotextile made of reinforced polypropylene (VLF-150, DiaDem, Landco Ltd.) with a thickness of 2 mm, a dry weight of  $0.15 \text{ kg m}^{-2}$  and a water permeability index of  $90 \text{ mm s}^{-1}$ . The tops of the geotextile halves of the experimental plots were filled with substrate to a depth of 8 cm, while the other halves were filled to a depth of 15 cm. The substrate was a mixture that was created using 75% *v/v* pumice (LAVA, Mineral & Quarry S.A., Markopoulo, Greece), 8% *v/v* peat (Lithuanian sphagnum peat with a corrected pH of 5.5 and an organic matter of 90% (*w/w*)), 7% *v/v* compost from garden waste and dairy manure (L. Cambanis S.A., Koropi, Greece) and 10% *v/v* clinoptilolite zeolite (S & B Industrial Minerals S.A., Athens, Greece). The physical and chemical properties of the substrate are listed in Table 1.



Figure 1. Cont.





**Figure 1.** Panoramic views of the whole experimental setup on the rooftop of the restaurant at the Agricultural University of Athens from above (a) and at eye level (b) and selected plants during flowering period (c).

**Table 1.** Physical and chemical properties of the substrates that were composed of pumice (75% *v/v*), peat (8% *v/v*), compost from garden waste and dairy manure (7% *v/v*), and clinoptilolite zeolite (10% *v/v*). Values represent the mean values of three replications ( $\pm$ SE).

| Measurement                            | Unit                            | Value ( $\pm$ SE)   | Method of Analysis            |
|--|---------------------------------|---------------------|-------------------------------|
| pH                                     |                                 | 8.36 ( $\pm$ 0.02)  | 1:5 ( <i>w/w</i> ) extraction |
| Electrical conductivity                | $\mu\text{S cm}^{-1}$           | 262.7 ( $\pm$ 7.80) | 1:5 ( <i>w/w</i> ) extraction |
| Bulk density at saturation             | $\text{g}\cdot\text{cm}^{-3}$   | 1.30 ( $\pm$ 0.05)  | PVC cylinders [1]             |
| Bulk density at maximum field capacity | $\text{g}\cdot\text{cm}^{-3}$   | 1.20 ( $\pm$ 0.03)  | PVC cylinders [1]             |
| Dry bulk density                       | $\text{g}\cdot\text{cm}^{-3}$   | 0.85 ( $\pm$ 0.03)  | PVC cylinders [1]             |
| Total porosity                         | %                               | 44.4 ( $\pm$ 2.30)  | Calculated (DIN EN 13041)     |
| Hydraulic conductivity                 | $\text{mm}\cdot\text{min}^{-1}$ | 7.56 ( $\pm$ 0.53)  | ASTM Method, F1815-11         |

Treatments included substrate depth and irrigation regime as factors having two levels each. Substrate depth consisted of either 8 cm (shallow) or 15 cm (deep) substrate, and irrigation was either high or low (50% of the high regime). Each treatment was replicated five times.

## 2.2. Plant Species Selection, Propagation, and Planting

To determine the criteria for selecting native and endemic plants suitable for the development of plant communities on extensive green roofs, a systematic literature review was performed. The databases assessed included Science Direct, Scholar, Scopus and

Agricola and the keywords involved array combinations such as (Mediterranean AND plant AND endemic AND green roofs) and (Mediterranean AND flora AND plants AND endemic AND native AND green roofs). The initial plant selection criteria included: (a) the geographical distribution, which had to be limited to Greece and specifically to Attica, Crete and the Cyclades; (b) either xerophytic or succulent species; and c) either native or endemic.

From the formulated initial plant selection, a secondary screening was performed using specific criteria aiming to select suitable plant species for extensive green roof systems. These criteria were based on literature reviews and included: (a) morphological characteristics (evergreen, low height, multi-branched and dense vegetation, crown shape and foliage coloration, leaf surface pubescence, trichomes, spines); (b) aesthetic value (specificity of flower, flowering season, flowering range, shape, color and fragrance of flowers, attraction of beneficial insects); (c) increase of growth (fast, medium, or slow); (d) surface cover (good or moderate); (e) resistance to insects and diseases; (f) recovery capacity (slow, medium, fast); (g) reproduction type (seed, offshoots, rooting stems, rhizomes) and ability to self-reproduce; (h) competitiveness, dominance and allelopathy compared with other plant species; and (i) resistance and tolerance to extreme environmental conditions including drought, extreme temperature fluctuations, increased irradiance and strong wind gusts [1,2,6,10,19,28,48–60].

From the above-mentioned process, 156 plant species were selected and graded. From those, the 25 plant species that belonged to different taxa (species or subspecies) and received the highest grades were used to formulate the three plant communities of the study.

Each plant community consisted of nine plant species, one of which was common for all three of them (*Rosmarinus officinalis* L.). The nine plants were planted within each experimental plot and thus each plant community consisted of a total of 180 plants (20 experimental plots per plant community  $\times$  9 plant species or subspecies per experiment plot = 180 plants). All three communities together reached 540 plants in total (3 plant communities  $\times$  180 plants each = 540 plants). The selected plant taxa are listed in Table 2 (plant nomenclature according to the Euro+Med PlantBase [61] and Flora of Greece [62]).

**Table 2.** Composition of the three plant communities with indication of the origin of the plants.

|   | First Plant Community [PC-1]<br>(Western Crete)                  | Second Plant Community [PC-2]<br>(Attica, Crete and Kythnos)                  | Third Plant Community [PC-3]<br>(Attica and Kythnos)                           |
|---|--|---|--|
| 1 | <sup>†,3</sup> <i>Origanum dictamnus</i> L.                      | <sup>1</sup> <i>Hypericum empetrifolium</i> Willd.                            | <sup>1</sup> <i>Limoniastrum monopetalum</i> (L.) Boiss.                       |
| 2 | <sup>3</sup> <i>Helichrysum orientale</i> (L.) Vaill.            | <sup>3</sup> <i>Melissa officinalis</i> subsp. <i>altissima</i> (Sm.) Arcang. | <sup>1</sup> <i>Teucrium brevifolium</i> Schreb.                               |
| 3 | <sup>3</sup> <i>Ballota acetabulosa</i> (L.) Benth.              | <sup>†,3</sup> <i>Ebenus cretica</i> L.                                       | <sup>1</sup> <i>Centranthus ruber</i> subsp. <i>sibthorpii</i> (Boiss.) Hayek  |
| 4 | <sup>†,3</sup> <i>Sideritis syriaca</i> L. subsp. <i>syriaca</i> | <sup>2</sup> <i>Salvia fruticosa</i> Mill.                                    | <sup>2</sup> <i>Limonium graecum</i> (Poir.) Rech. f.                          |
| 5 | <sup>3</sup> <i>Rosmarinus officinalis</i> L.                    | <sup>1</sup> <i>Prasium majus</i> L.  | <sup>1</sup> <i>Suaeda vera</i> J.F.Gmel.                                      |
| 6 | <sup>3</sup> <i>Phlomis fruticosa</i> L.                         | <sup>1</sup> <i>Rosmarinus officinalis</i> L.                                 | <sup>2</sup> <i>Crithmum maritimum</i> L.                                      |
| 7 | <sup>3</sup> <i>Satureja thymbra</i> L.                          | <sup>2</sup> <i>Thymbra capitata</i> (L.) Cav.                                | <sup>2</sup> <i>Sedum sediforme</i> (Jacq.) Pau                                |
| 8 | <sup>3</sup> <i>Origanum onites</i> L.                           | <sup>2</sup> <i>Teucrium capitatum</i> L.                                     | <sup>1</sup> <i>Rosmarinus officinalis</i> L.                                  |
| 9 | <sup>3</sup> <i>Cistus creticus</i> L.                           | <sup>2</sup> <i>Origanum vulgare</i> subsp. <i>hirtum</i> (Link) Ietsw.       | <sup>2</sup> <i>Helichrysum stoechas</i> subsp. <i>barrelieri</i> (Ten.) Nyman |

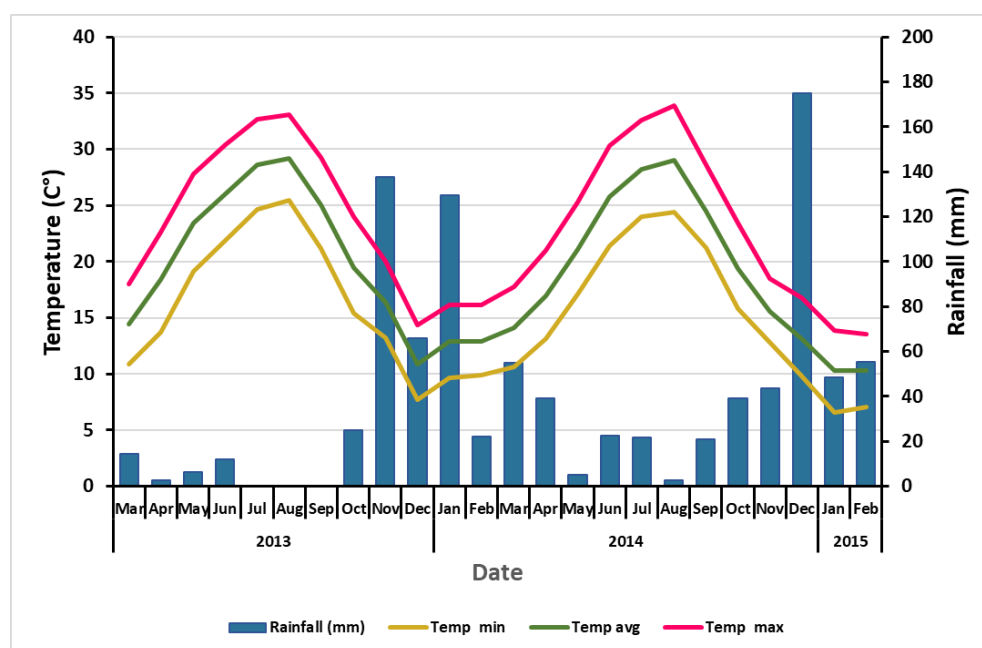
<sup>1</sup> Originating from Attica. <sup>2</sup> Originating from Kythnos Island (Cyclades). <sup>3</sup> Originating from Crete Island.  
<sup>†</sup> Endemic plant of Crete.

Plant propagation materials were obtained from their natural habitats as cuttings from March to April 2012. The cuttings were transferred to the Agricultural University of Athens and were treated with indol-3-butyric acid rooting hormone (Rooton DP) before their insertion in a well-watered 50% perlite–50% sphagnum peat substrate mixture. Treated cuttings were placed under mist for 15 to 25 days, depending on the plant species. Plants

were acclimatized gradually in a greenhouse for 30 days and afterward were placed outdoors for six months. Before transplantation to the final position in the experimental plots, all 540 cuttings were topped uniformly within each species. Topping height varied from 7 to 10 cm, depending on each species' characteristic growth. Plots were divided into nine rectangular quadrats of  $0.4 \times 0.4$  m each. A single plant was planted in the center of each rectangle following a randomization procedure within each plot, to minimize the location influence on the performance of the plants in each community.

### 2.3. Total Water Inputs during the Two Years of the Study

Plants received natural precipitation during the whole study period (Figure 2). From February to May 2013 plants were irrigated to field capacity as needed in order to facilitate their establishment on the rooftop. An automated irrigation system was installed which was composed of a controller, closed-loop pipes, and drippers of two different flow rates, namely  $2 \text{ L h}^{-1}$  (low irrigation) and  $4 \text{ L h}^{-1}$  (high irrigation), placed next to each plant. During the first study year, irrigation was applied from 4 June to 31 July 2013 to ensure the survival and promote the hardening of the plants (Figure 3). In the second study year, deficit irrigation was applied from 30 June to 22 October 2014 and three drought periods (no watering) were included to estimate the drought tolerance of the plants. The three drought cycles were imposed during 20–25 July, 22–31 August, and 23 September–10 October 2014 (Figure 4). To determine the irrigation demands, three evaporation pans of 30 cm internal diameter each, were placed on the roof. Then an irrigation schedule was prepared on a weekly base, according to the prevailing weather conditions, with the aim to approximately apply 20%  $\text{ET}_0$  and 10%  $\text{ET}_0$  for high and low irrigation regimes, respectively.



**Figure 2.** Monthly temperature, minimum, average, and maximum (°C) and precipitation (mm) during the two study years (from March 2013 to February 2015). Data were provided by the Meteorological Station of Gazi, operated by the National Observatory of Athens, which is located 885 m from the study site.

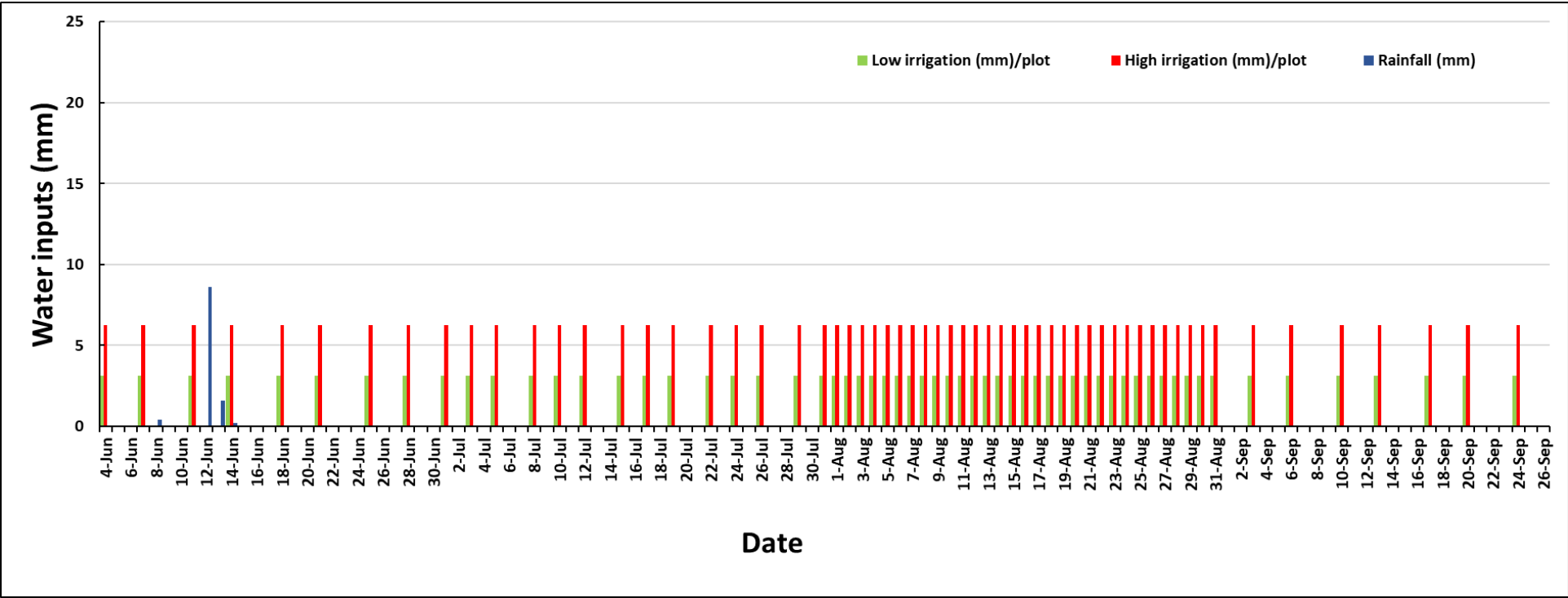
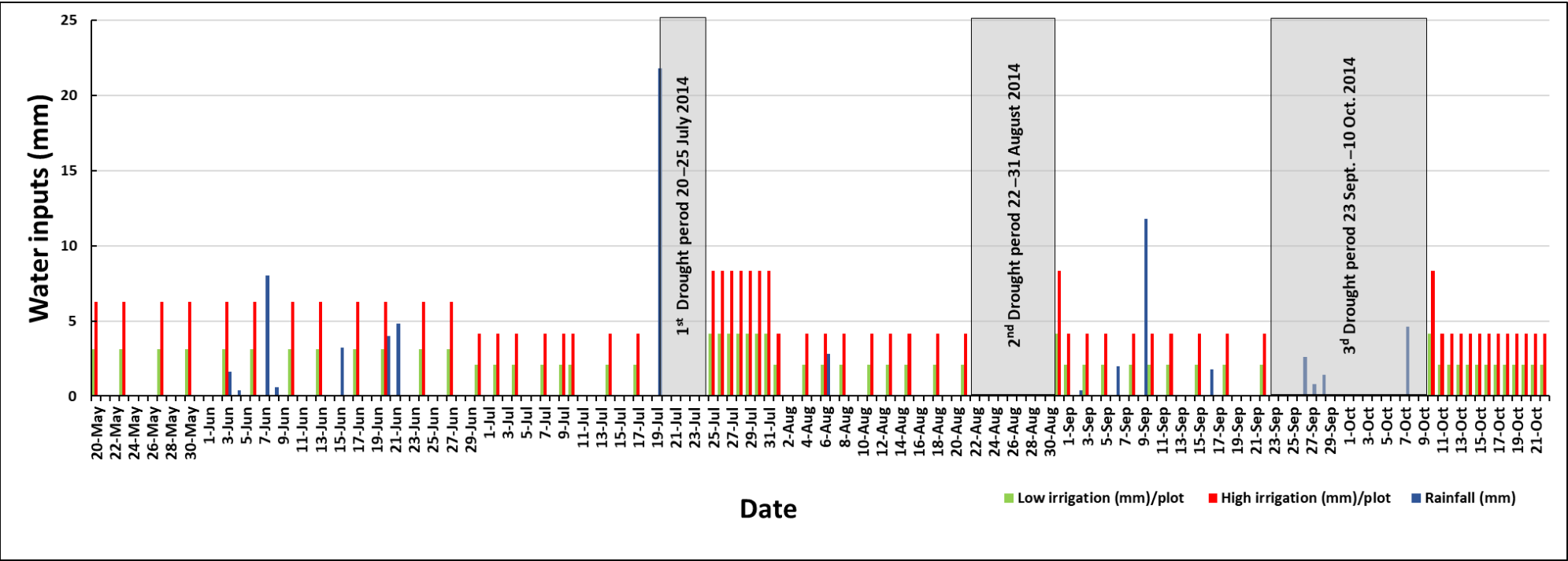


Figure 3. Water inputs by irrigation and precipitation during the summer period of 2013.



**Figure 4.** Water inputs by irrigation and precipitation during the summer period of 2014. The grey areas represent the three water stress periods where irrigation was not applied.



## 2.4. Measurements

### 2.4.1. Growth Index

This index was assessed by determining the height of the plants, the longest horizontal dimension of each plant's crown, and the length of its perpendicular cross-section. In an effort to produce a landscape architectural growth index and not an absolute horticultural one, it was decided that the flower-bearing stems should be excluded unless they had grown leaves, in which case the height measurement was included up to their highest-grown. Measurements were performed once a month for a total period of 24 months, starting from March 2013 until February 2015. Growth index was calculated as the average of the three measurements according to the formula of Nektarios et al. [1]:

$$\text{Growth Index} = (h + D_{\max} + D_{\text{vert}})/3$$

where:  $h$  = plant height,  $D_{\max}$  = longest horizontal diameter of the crown, and  $D_{\text{vert}}$  = longest perpendicular horizontal dimension to  $D_{\max}$ .

### 2.4.2. Flowering

This parameter was determined by counting the number of flowers in each plant during the flowering period of each species. Measurements were performed every two weeks and the results were pooled in monthly intervals.

### 2.4.3. Self-Reproduction

The ability of each species to reproduce itself within its plant community was determined by counting all emerging new plants produced either by seedlings or offshoots. Reproduction measurements were performed once a month, from March 2013 until February 2015.

## 2.5. Statistical Analyses

The study evaluated the growth of 25 native and/or endemic plant species growing as three distinct plant communities in two different extensive green roof substrate depths (8 cm or 15 cm) and under two irrigation regimes (high or low) during the summer periods. Data were analyzed using two-way analysis of variance (ANOVA) at a significance level of  $p < 0.05$ . In the case of *Cistus creticus* interaction between the two factors was detected, and thus, one-way ANOVA at a significance level of  $p < 0.05$  was performed. Treatment means were separated using least significant difference (LSD), at  $p < 0.05$ . Statistical analyses were performed using the IBM SPSS version 22 statistical software (IBM, Armonk, NY, USA).

## 3. Results

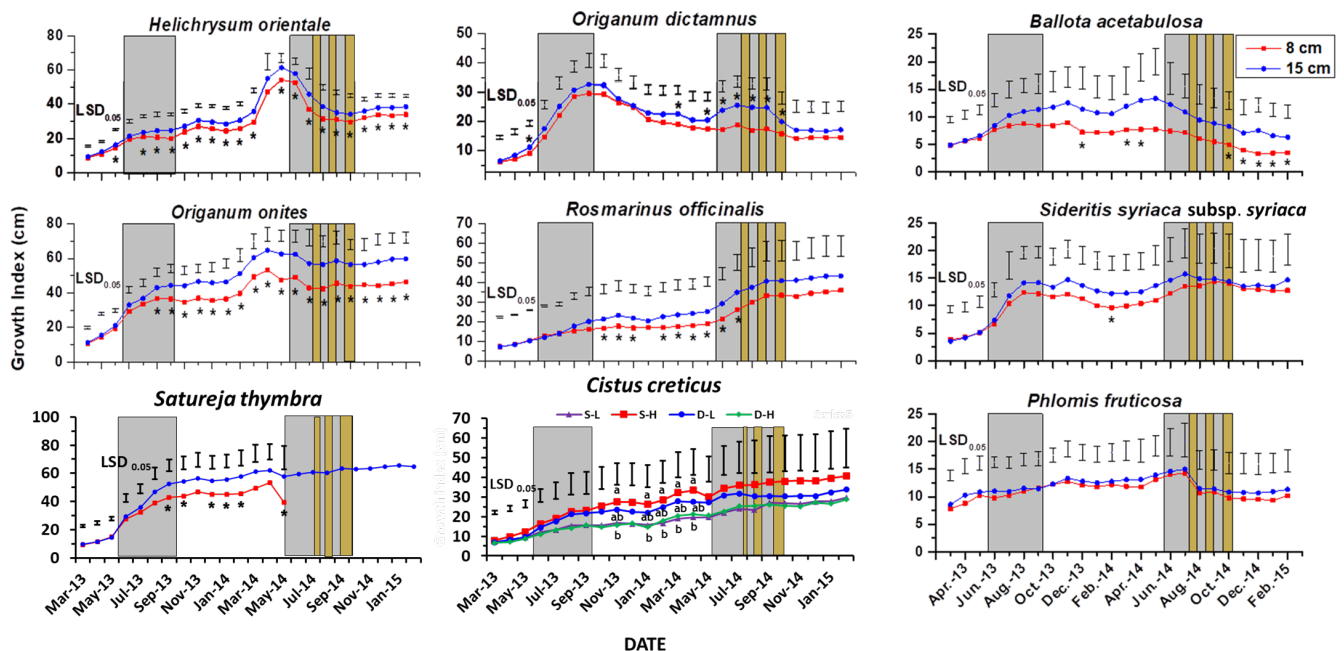
### 3.1. First Plant Community [PC-1]

#### 3.1.1. Plant Growth

The plants in PC-1 were separated into three groups according to their growth index: (a) the first group exhibited low growth indexes of 11–15 cm (*Ballota acetabulosa*, *Sideritis syriaca* subsp. *syriaca*, and *Phlomis fruticosa*), (b) the second group exhibited medium growth indexes of 30–40 cm (*Origanum dictamnus*, *R. officinalis* and *C. creticus*) and (c) the third group exhibited high growth indexes of 60–65 cm (*Helichrysum orientale*, *Satureja thymbra* and *Origanum onites*). However, the reported growth indexes were the result of different growth patterns, because some plants exhibited a prostrate type of growth (*O. dictamnus*, *H. orientale*) while the remaining species exhibited an erect type of growth.

Most plant species participating in plant community PC-1 increased their growth index during the first study year until Oct. 2013 and then either retained their growth unchanged during wintertime (*H. orientale*, *O. onites*, *R. officinalis*, *S. thymbra*, *P. fruticosa*) or reduced their growth index (*O. dictamnus*, *B. acetabulosa*). *Rosmarinus officinalis* and *C. creticus* continued to increase their growth rate almost steadily during the whole study period (Figure 5). In the second study year that water deprivation and drought stress were imposed, the biggest reduction was recorded in *H. orientale*, *B. acetabulosa* and *P. fruticosa*. Some species, including *O. Dictamnus*, exhibited a continuous reduction after the first study

year, even though their growth index increased within the water stress period but only in the deeper substrate. All plants survived during the two-year study except one individual of *O. onites*, one of *C. creticus* and one of *P. fruticosa*.



**Figure 5.** Substrate depth (shallow: 8 cm and deep: 15 cm) effects on the growth index of the plants of the first plant community. The asterisk indicates a significant difference between treatment means based on the least significant difference (LSD) criterion. Bars represent LSD at a significance level of  $p < 0.05$ . The gray-shaded areas indicate the two periods of water stress (4 June–27 September 2013 and 20 May–22 October 2014), while the brown areas indicate the three drought periods (20–25 July, 22–31 August, 24 September–10 October 2014). *Cistus creticus* was analyzed with one-way ANOVA due to interactions between the two factors (shallow /deep substrate and low /high irrigation regime). Letters indicate the mean values being statistically different within the same date, using LSD criterion at a significance level of  $p < 0.05$ .

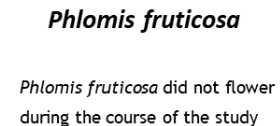
The deeper substrates of 15 cm increased the growth indexes of most plant species except for *S. syriaca* subsp. *syriaca* and *P. fruticosa*, where the observed differences were not significant and *B. acetabulosa* which exhibited minimal differences. In general, the differences in plants' growth indexes between the two substrate depths were more profound in specific periods of the year, except for *H. orientale* and *O. onites* which provided higher growth indexes in 15 cm substrates in an almost continuous pattern.

In *C. creticus* an interaction between substrate depth and irrigation regime was observed in seven out of 24 measurements (October 2013–April 2014). Therefore, the statistical analyses for *C. creticus* were performed as a one-way analysis of variance (Figure 5). Differences between treatments were found in five sampling dates. In those cases, the shallow substrate with high irrigation regime (S-H) exhibited higher growth index compared with the deep substrate with high irrigation regime (D-H) in two cases (November 2013 and January 2014) and compared with the shallow substrate with the low irrigation regime (S-L) in the remaining three cases (February–April 2014). The irrigation regime did not affect the growth of the plants (Figure S1). The only exception was one month in *O. dictamnus* (May 2014) and *O. onites* (June 2014), wherein plants irrigated with the low regime exhibited a higher growth rate than those irrigated with the high irrigation regime.

### 3.1.2. Flowering

Of the nine plant species or subspecies of the first plant community, two did not flower at all (*S. syriaca* subsp. *syriaca* and *P. fruticosa*), three provided minimal flowering during

The irrigation regime provided minimal differences only in *O. dictamnus* for two months just after the water stress period of the second year, and *S. thymbra* in May 2014 (Figure S2). In those two cases, differences coincided with the peak of the flowering, and plants that were irrigated with the high regime reached a higher number of flowers than those that received the low irrigation regime.



**Figure 6.** Substrate depth (shallow: 8 cm and deep: 15 cm) effects on the flowering of the first plant community. The asterisk indicates a significant difference between treatment means based on the least significant difference (LSD) criterion. Bars represent LSD at a significance level of  $p < 0.05$ . The gray-shaded areas indicate the two periods of water stress (4 June–27 September 2013 and 20 May–22 October 2014), while the brown areas indicate the three drought periods (20–25 July, 22–31 August, 24 September–10 October 2014). *Cistus creticus* was analyzed with one-way ANOVA due to interactions between the two factors (shallow/deep substrate and low/high irrigation regime). Letters indicate the mean values being statistically different within the same date, using LSD criterion at a significance level of  $p < 0.05$ .

| Year  | 2013 |   |   |   |   |   |   |   |   |    | 2014 |    |    |    |    |    |    |    |    |    |    |    | 2015 |    |
|---|------|---|---|---|---|---|---|---|---|----|------|----|----|----|----|----|----|----|----|----|----|----|------|----|
| Month   | M    | A | M | J | J | A | S | O | N | D  | J    | F  | M  | A  | M  | J  | J  | A  | S  | O  | N  | D  | J    | F  |
| Month number                                      | 1    | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11   | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23   | 24 |
| 1. <i>Helichrysum orientale</i>                   |      |   |   | ↑ |   |   |   |   |   |    |      |    |    |    | ↑  | ↑  |    |    |    |    |    |    |      |    |
| 2. <i>Origanum onites</i>                         |      |   |   | ↑ | ↑ |   |   |   |   |    |      |    |    |    | ↑  | ↑  |    |    |    |    |    |    |      |    |
| 3. <i>Satureja thymbra</i>                        |      |   |   | ↑ |   |   |   |   |   |    |      |    |    | ↑  | ↑  |    |    |    |    |    |    |    |      |    |
| 4. <i>Origanum dictamnus</i>                      |      |   |   |   |   | ↑ | ↑ |   |   |    |      |    |    |    |    |    |    | ↑  | ↑  |    |    |    |      |    |
| 5. <i>Rosmarinus officinalis</i>                  | ↑    |   |   |   |   |   |   |   |   |    |      |    |    |    |    |    |    |    |    | ↑  |    | ↑  | ↑    | ↑  |
| 6. <i>Cistus creticus</i>                         |      |   |   |   |   |   |   |   |   |    |      |    |    | ↑  |    |    |    |    |    |    |    |    |      |    |
| 7. <i>Ballota acetabulosa</i>                     |      |   |   |   |   | ↑ |   |   |   |    |      |    |    |    | ↑  |    |    |    |    |    |    |    |      |    |
| 8. <i>Sideritis syriaca</i> subsp. <i>syriaca</i> |      |   |   |   |   |   |   |   |   |    |      |    |    |    |    |    |    |    |    |    |    |    |      |    |
| 9. <i>Phlomis fruticosa</i>                       |      |   |   |   |   |   |   |   |   |    |      |    |    |    |    |    |    |    |    |    |    |    |      |    |

**Figure 7.** Visualization of the flowering period with indicative flower color for each plant species participating in the first plant community for the whole duration of the study. The sign ↑ indicates the month of blossom peaking. Cells with only green shades indicate no flowering while the green shade approximates the true color of the leaves of each species.

### 3.2. Second Plant Community [PC-2]

#### 3.2.1. Plant Growth

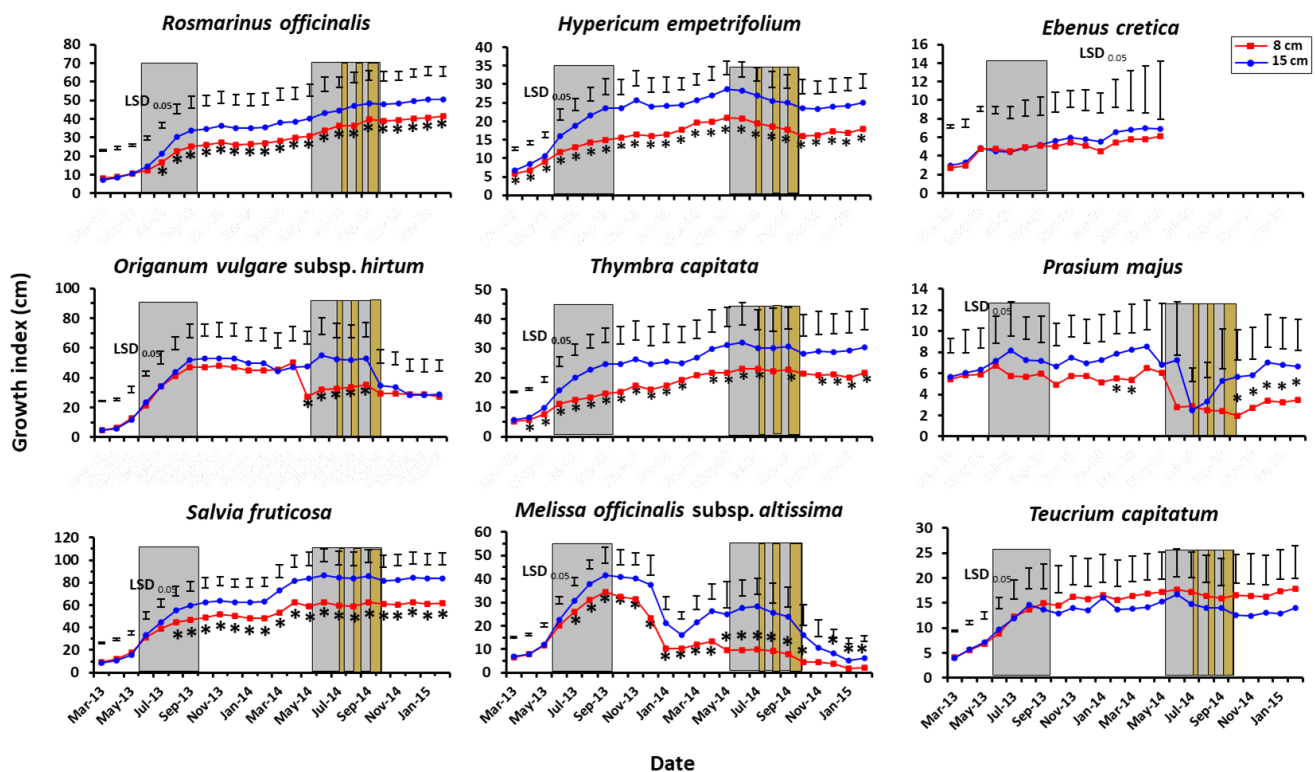
The selected plants were separated into three groups according to their growth index pattern. The first group exhibited low growth indices of 8–18 cm (*P. majus*, *E. cretica*, *T. capitatum*), the second group exhibited moderate growth indices of 29–42 cm (*H. empetrifolium*, *T. capitata* and *M. officinalis* subsp. *altissima*) and the third group exhibited higher growth indices of 50–87 cm (*R. officinalis*, *O. vulgare* subsp. *hirtum* and *S. fruticosa*) (Figure 8).

All PC-2 species possess an erect type of growth except *T. capitatum*, which exhibited a prostrate type of growth. Most plants reached their peak growth during the second year of the study, except *M. officinalis* subsp. *altissima* and *O. vulgare* subsp. *hirtum*. Most plants survived during the whole study period, except for three plants of *T. capitata* grown in the shallow substrate and 18 of the 20 plants of *E. cretica* which were lost in May 2014.

Substrate depth affected the growth of each species in a variable way (Figure 8). *Rosmarinus officinalis*, *H. empetrifolium*, *S. fruticosa*, and *M. officinalis* subsp. *altissima* increased their growth in the 15-cm substrate depth. *Origanum vulgare* subsp. *hirtum* remained unaffected by substrate depth until May 2014 when plants grown in the shallower substrate of 8 cm reduced their growth for the whole period of water stress imposition. *Prasium majus* increased its growth in the deeper substrate depth in seven out of 24 months, while *E. cretica* and *T. capitatum* did not show significant differences between the two substrate depths. *Teucrium capitatum* was the only plant that exhibited a trend of better growth in shallow substrates compared with the deeper ones, but these differences were not statistically significant.

The amount of irrigation did not affect the growth index of most PC-2 plants. Minimal differences in a single month during the course of the two-year study were observed in *E. cretica*, (May 2013) and *P. majus* (August 2014). In contrast, *O. vulgare* subsp. *hirtum* plants grown under the high irrigation regime exhibited a higher growth for eight months towards the end of the second water stress period (Figure S3).

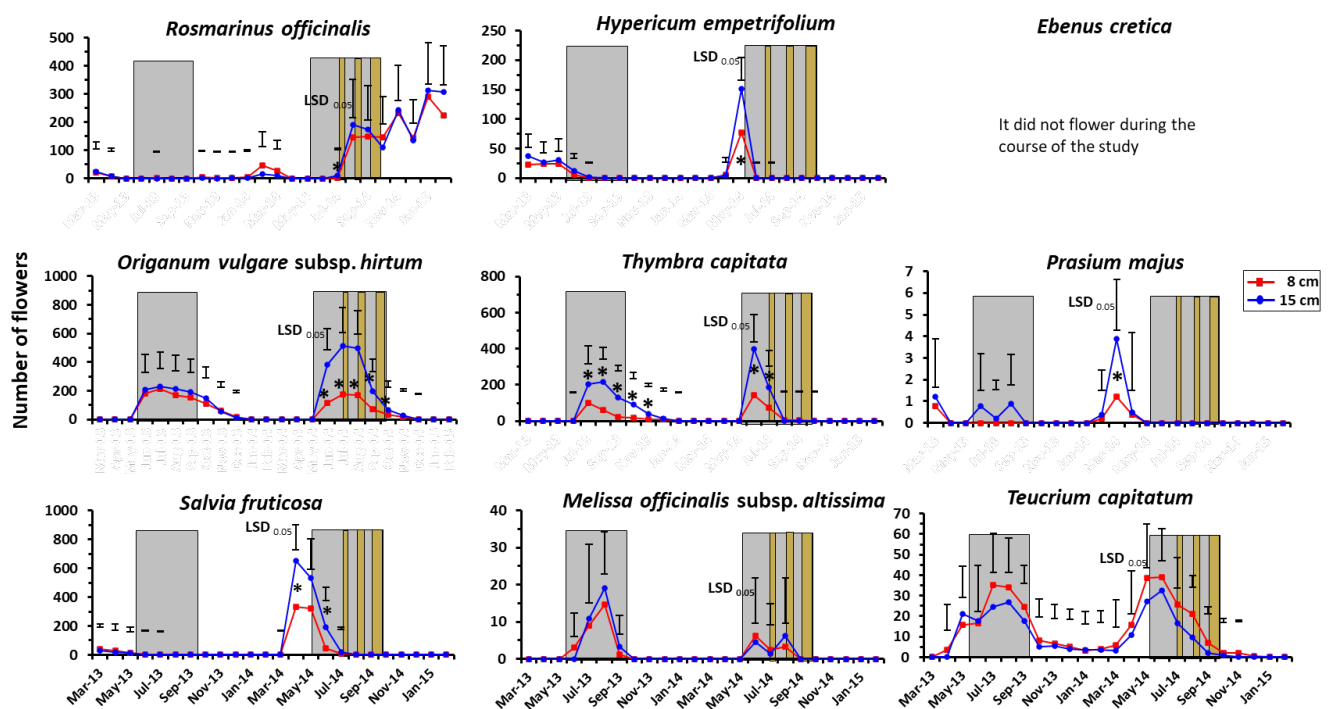




**Figure 8.** Substrate depth (shallow: 8 cm and deep: 15 cm) effects on the growth index of the plants of the second plant community. The asterisk indicates a significant difference between treatment means based on the least significant difference (LSD) criterion. Bars represent LSD at a significance level of  $p < 0.05$ . The gray-shaded areas indicate the two periods of water stress (4 June–27 September 2013 and 20 May–22 October 2014), while the brown areas indicate the three drought periods (20–25 July, 22–31 August, 24 September–10 October 2014). The variation of the growth indices did not follow a specific pattern. *Ebenus cretica* exhibited minimal changes throughout the duration of the study, while *R. officinalis* increased its growth continuously. The growth of *Origanum vulgare* subsp. *hirtum* peaked during the first study year and thereafter did not manage to grow any further. In contrast, the growth of *M. officinalis* subsp. *altissima* significantly declined after the first, but slightly increased during the second study year before declining due to the initiation of water stress periods. The growth of *H. empetrifolium* and *P. majus* steadily increased until the initiation of the water stress periods in the second year of the study, resulting in a small growth decline. The growth of *T. capitata* and *T. capitatum* also steadily increased but remained stable after the imposition of the water stress periods.

### 3.2.2. Flowering

From the nine plants of the second plant community, one (*E. creticus*) did not produce flowers and two provided minimal flowering, *M. officinalis* subsp. *altissima* and *P. majus* (Figures 9 and 10). The remaining plant species were able to produce abundant flowers in both study years. All plants produced flowers mostly in two periods. During the first study year, *S. fruticosa*, *H. empetrifolium* bloomed in spring and summer, *O. vulgare* subsp. *hirtum* and *T. capitata* in summer and autumn while *M. officinalis* subsp. *altissima* bloomed during summer. Flowering occurred for all the above-mentioned species or subspecies in almost similar periods in the second year of the study but in increased flowering numbers except for *M. officinalis* subsp. *altissima*. Though *R. officinalis* bloomed throughout the whole study, a significant increase was recorded after August 2014 and flowering continued at high numbers despite the imposition of the drought periods till the end of the study. Similarly, *T. capitatum* produced flowers almost the whole year round (Figures 9 and 10).



**Figure 9.** Substrate depth (shallow: 8 cm and deep: 15 cm) effects on the flowering of the second plant community. The asterisk indicates a significant difference between treatment means based on the least significant difference (LSD) criterion. Bars represent LSD at a significance level of  $p < 0.05$ . The gray-shaded areas indicate the two periods of water stress (4 June–27 September 2013 and 20 May–22 October 2014), while the brown areas indicate the three drought periods (20–25 July, 22–31 August, 24 September–10 October 2014).

| Year   | 2013 |   |   |   |   |   |   |   |   |    | 2014 |    |    |    |    |    |    |    |    |    |    |    | 2015 |    |
|--|------|---|---|---|---|---|---|---|---|----|------|----|----|----|----|----|----|----|----|----|----|----|------|----|
| Month  | M    | A | M | J | J | A | S | O | N | D  | J    | F  | M  | A  | M  | J  | J  | A  | S  | O  | N  | D  | J    | F  |
| Month number                                   | 1    | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11   | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23   | 24 |
| 1. <i>Rosmarinus officinalis</i>               |      |   |   |   |   |   |   |   |   |    |      | ↑  |    |    |    |    |    |    |    |    | ↑  |    | ↑    | ↑  |
| 2. <i>Origanum vulgare subsp. hirtum</i>       |      |   |   |   | ↑ | ↑ | ↑ |   |   |    |      |    |    |    |    | ↑  | ↑  | ↑  |    |    |    |    |      |    |
| 3. <i>Salvia fruticosa</i>                     | ↑    | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ | ↑  | ↑    | ↑  | ↑  | ↑  | ↑  | ↑  | ↑  | ↑  | ↑  | ↑  | ↑  | ↑  | ↑    | ↑  |
| 4. <i>Hypericum empetrifolium</i>              | ↑    | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ | ↑  | ↑    | ↑  | ↑  | ↑  | ↑  | ↑  | ↑  | ↑  | ↑  | ↑  | ↑  | ↑  | ↑    | ↑  |
| 5. <i>Thymbra capitata</i>                     |      |   |   |   | ↑ | ↑ | ↑ | ↑ | ↑ | ↑  | ↑    | ↑  | ↑  | ↑  | ↑  | ↑  | ↑  | ↑  | ↑  | ↑  | ↑  | ↑  | ↑    | ↑  |
| 6. <i>Melissa officinalis subsp. altissima</i> |      |   |   |   |   | ↑ | ↑ |   |   |    |      |    |    |    |    |    |    |    |    |    |    |    |      |    |
| 7. <i>Prasium majus</i>                        |      |   |   |   |   |   |   |   |   |    |      |    | ↑  |    |    |    |    |    |    |    |    |    |      |    |
| 8. <i>Teucrium capitatum</i>                   |      |   |   |   | ↑ | ↑ |   |   |   |    |      |    |    |    |    | ↑  | ↑  |    |    |    |    |    |      |    |
| 9. <i>Ebenus cretica</i>                       |      |   |   |   |   |   |   |   |   |    |      |    |    |    |    |    |    |    |    |    |    |    |      |    |

**Figure 10.** Visualization of the flowering period with indicative flower color for each plant species or subspecies participating in the second plant community for the whole duration of the study. The sign ↑ indicates the month of blossom peaking. Cells with only green shades indicate no flowering while the green shade approximates the true color of the leaves of each species.

Differences in flowering between shallow and deeper substrate depth occurred only in a few cases, wherein *O. vulgare subsp. hirtum* and *T. capitata* plants grown in the deep substrate (15 cm) produced more flowers than those grown in the shallow substrate (8 cm).

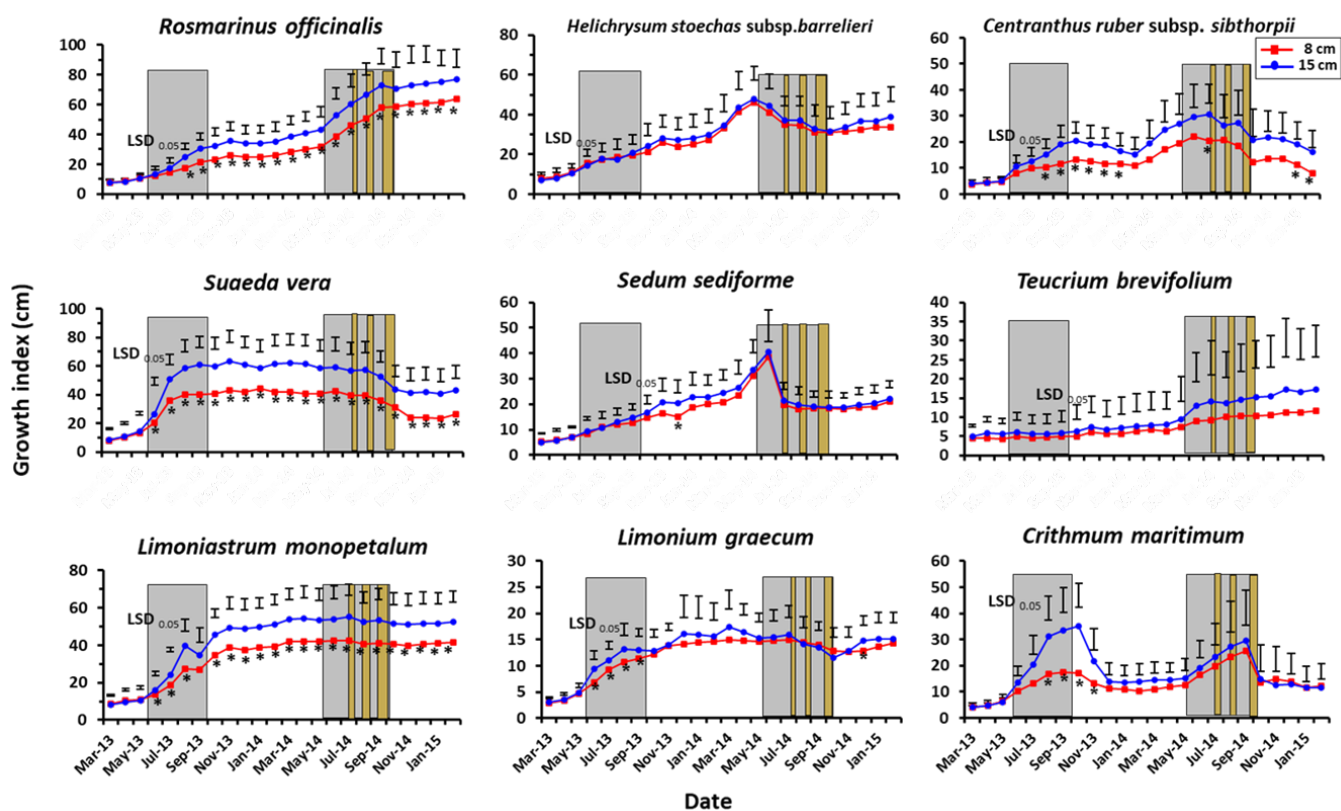
The irrigation regime did not significantly affect the number of flowers of plants except for two months for *O. vulgare subsp. hirtum*, when plants grown under the high irrigation

regime produced more flowers, and one month for *T. capitata*, when only three plants of the twenty were in bloom, two under the low irrigation regime and one under the high irrigation regime (Figure S4).

### 3.3. Third Plant Community [PC-3]

#### 3.3.1. Plant Growth

Similar to the previous plant communities, plants included species or subspecies with low growth index (*T. brevifolium* and *L. graecum*), moderate growth index (*C. ruber* subsp. *sibthorpii*, *C. maritimum*, *S. sediforme* and *H. stoechas* subsp. *barrelieri*) and large growth index (*L. monopetalum*, *S. vera* and *R. officinalis*) (Figure 11). Plants of PC-3 also exhibited different patterns of growth increase. *Rosmarinus officinalis* and *T. brevifolium* managed to grow during the whole study period, the latter at a much lower rate. *Sedum sediforme* and *H. stoechas* subsp. *barrelieri* increased their growth index until May–June 2014 when it began to decline. *Limoniastrum monopetalum*, *S. vera*, *L. graecum* grew quickly after their establishment and thereafter their growth remained more or less constant. In contrast, *C. maritimum* and *C. ruber* subsp. *sibthorpii*, exhibited two growth peaks during the two study years, during the end of summer. Most plants reached their peak growth during the second year of the study. All plants survived except for one plant of *H. stoechas* subsp. *barrelieri* (December 2014) and ten plants of *T. brevifolium* (six were lost in May and four in June 2014).



**Figure 11.** Substrate depth (shallow: 8 cm and deep: 15 cm) effects on the growth index of the plants of the third plant community. The asterisk indicates a significant difference between treatment means based on the least significant difference (LSD) criterion. Bars represent LSD at a significance level of  $p < 0.05$ . The gray-shaded areas indicate the two periods of water stress (4 June–27 September 2013 and 20 May–22 October 2014), while the brown areas indicate the three drought periods (20–25 July, 22–31 August, 24 September–10 October 2014).

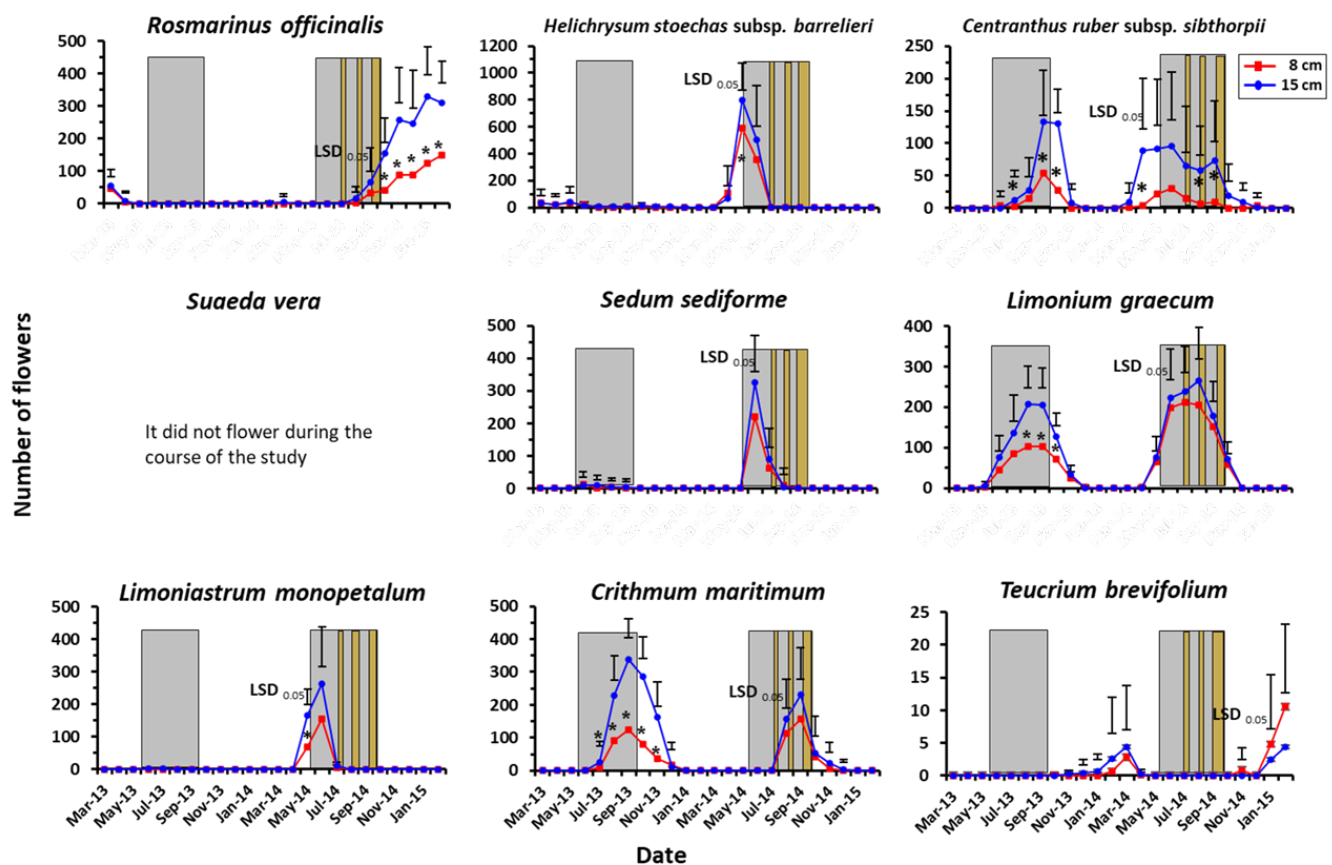
The deeper substrate depth of 15 cm enhanced the growth of three out of the nine species or subspecies (*R. officinalis*, *S. vera* and *L. monopetalum*). Minimal enhancement

was recorded for *C. maritimum*, *C. ruber* subsp. *sibthorpii* and *L. graecum* using the deeper substrate depth. In contrast, the growth of *H. stoechas* subsp. *barrelieri*, *T. brevifolium*, and *S. sediforme* was not affected by substrate depth (Figure 11).

Irrigation regimes did not affect the growth of most plants throughout the study (Figure S5). In limited cases, such as *H. stoechas* subsp. *barrelieri* (two winter months), *S. sediforme* (three winter months) and *C. maritimum* (four months in summer and early autumn), the low irrigation regime favored the growth in comparison to the high irrigation regime.

### 3.3.2. Flowering

All plant species except *S. vera* bloomed naturally. Of the eight plants that bloomed, *T. brevifolium*, provided a low number of flowers (Figures 12 and 13). Three plant species (*C. maritimum*, *C. ruber* subsp. *sibthorpii* and *L. graecum*) flowered equally and abundantly in both study years, demonstrating two peak periods towards the end of summer and the beginning of autumn. The remaining plant species or subspecies, *R. officinalis*, *L. monopetalum*, *H. stoechas* subsp. *barrelieri* and *S. sediforme* flowered in substantial numbers during the second year of the study.



**Figure 12.** Substrate depth (shallow: 8 cm and deep: 15 cm) effects on the flowering of the third plant community. The asterisk indicates a significant difference between treatment means based on the least significant difference (LSD) criterion. Bars represent LSD at a significance level of  $p < 0.05$ . The gray-shaded areas indicate the two periods of water stress (4 June–27 September 2013 and 20 May–22 October 2014), while the brown areas indicate the three drought periods (20–25 July, 22–31 August, 24 September–10 October 2014).



| Year  | 2013 |   |   |   |   |   |   |   |   |    | 2014 |    |    |    |    |    |    |    |    |    |    |    | 2015 |    |
|---|------|---|---|---|---|---|---|---|---|----|------|----|----|----|----|----|----|----|----|----|----|----|------|----|
| Month   | M    | A | M | J | J | A | S | O | N | D  | J    | F  | M  | A  | M  | J  | J  | A  | S  | O  | N  | D  | J    | F  |
| Month number  | 1    | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11   | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23   | 24 |
| 1. <i>Rosmarinus officinalis</i>                        | ↑    |   |   |   |   |   |   |   |   |    |      |    |    |    |    |    |    |    |    |    |    |    | ↑    | ↑  |
| 2. <i>Limoniastrum monopetalum</i>                      |      |   |   |   |   |   |   |   |   |    |      |    |    |    |    | ↑  | ↑  |    |    |    |    |    |      |    |
| 3. <i>Helichrysum stoechas</i> subsp. <i>barrelieri</i> | ↑    | ↑ | ↑ |   |   |   |   |   |   |    |      |    |    |    | ↑  | ↑  |    |    |    |    |    |    |      |    |
| 4. <i>Sedum sediforme</i>                               |      |   |   | ↑ |   |   |   |   |   |    |      |    |    |    |    | ↑  |    |    |    |    |    |    |      |    |
| 5. <i>Crithmum maritimum</i>                            |      |   |   |   |   | ↑ | ↑ | ↑ |   |    |      |    |    |    |    |    |    | ↑  | ↑  |    |    |    |      |    |
| 6. <i>Centranthus ruber</i> subsp. <i>sibthorpii</i>    |      |   |   |   |   | ↑ | ↑ | ↑ |   |    |      |    |    |    | ↑  | ↑  |    |    |    |    |    |    |      |    |
| 7. <i>Limonium graecum</i>                              |      |   |   |   |   | ↑ | ↑ |   |   |    |      |    |    |    |    | ↑  | ↑  | ↑  |    |    |    |    |      |    |
| 8. <i>Teucrium brevifolium</i>                          |      |   |   |   |   |   |   |   |   |    |      |    | ↑  |    |    |    |    |    |    |    |    |    | ↑    |    |
| 9. <i>Suaeda vera</i>                                   |      |   |   |   |   |   |   |   |   |    |      |    |    |    |    |    |    |    |    |    |    |    |      |    |

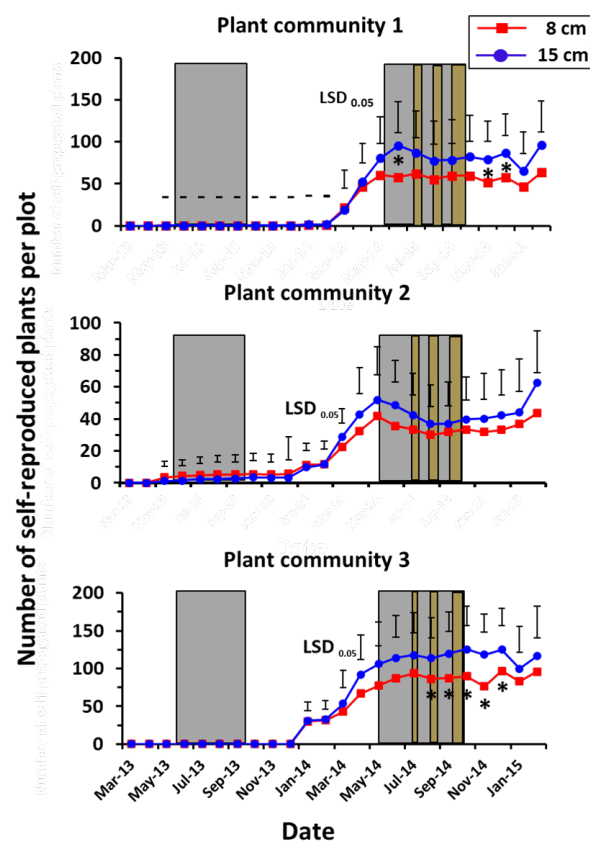
**Figure 13.** Visualization of the flowering period with indicative flower color for each plant species or subspecies participating in the third plant community for the whole duration of the study. The sign ↑ indicates the month of blossom peaking. Cells with only green shades indicate no flowering while the green shade approximates the true color of the leaves of each species or subspecies.

The deeper substrate depth of 15 cm favored the flowering of most plant species or subspecies throughout the study (Figure 13). In contrast, the flowering of *S. sediforme* and *T. brevifolium* was indifferent to the depth of the substrate. The irrigation regime did not significantly affect the number of flowers except for *H. stoechas* subsp. *barrelieri* and *L. graecum*. However, the differences were minimal (Figure S6).

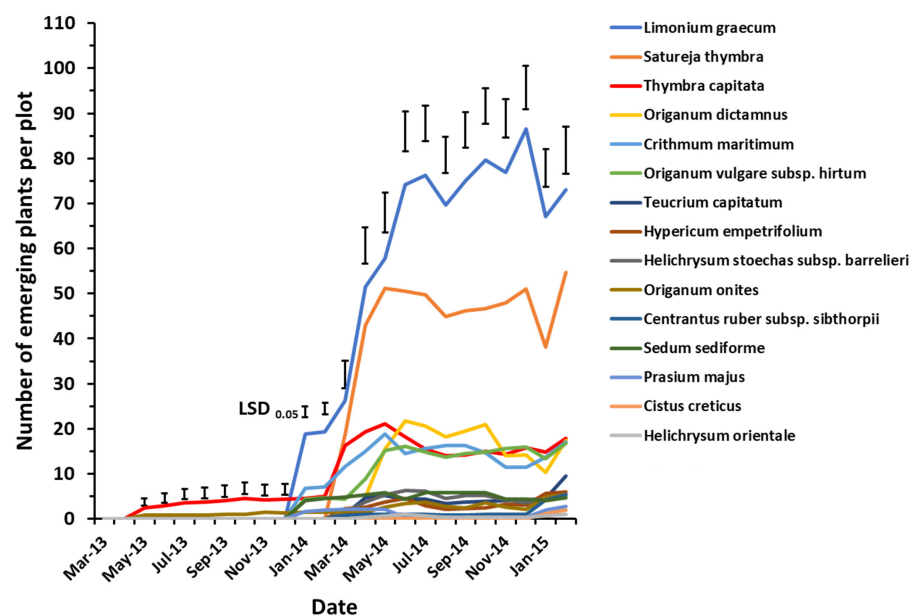
### 3.4. Self-Reproduction within Each Plant Community

Not all plant species or subspecies of the three plant communities managed to self-reproduce. During the first year of the study, only *O. onites* from PC-1 and *T. capitata* from PC-2 managed to provide new plants, starting as early as May 2013 (Figure 14). As seeds were not yet developed, these plantlets were probably derived as offshoots. During the second study year, *O. vulgare* subsp. *hirtum* and *P. majus* from PC-2 and *C. maritimum*, *L. graecum*, and *S. sediforme* from PC-3 followed up and started to produce offspring in January 2014. With the onset of spring in March 2014, *S. thymbra* and *H. orientale* from PC-1, *H. empetrifolium* and *T. capitatum* from PC-2, and *H. stoechas* subsp. *barrelieri* from PC-3, and in April 2014 *O. dictamnus* from PC-1, started to produce plantlets. In total five plant species from the first, five from the second, and five from the third plant community, altogether 15 out of the 25 different plants of all three plant communities, managed to self-reproduce (Figure 15). These new plants originated either from offshoots or through natural dispersion of seeds produced after the first study year.

A clear trend of a higher number of self-propagated plants at the deeper substrate depth was observed, which became statistically significant in selected months in PC-1 and PC-3 (Figure 14). Irrigation level did not affect the self-reproduction process, except for two months in the PC-2 (Figure S7). The highest reproduction rate was achieved by *L. graecum* and *S. thymbra*, followed by *T. capitata* and lastly *C. maritimum*, *O. vulgare* subsp. *hirtum* and *O. dictamnus* (Figure 15).



**Figure 14.** Substrate depth (shallow: 8 cm or deep: 15 cm) effects on the number of self-reproduced plants of the three plant communities. The asterisk indicates a significant difference between treatment means based on the least significant difference (LSD) criterion. Bars represent LSD at a significance level of  $p < 0.05$ . The gray-shaded areas indicate the two periods of water stress (4 June–27 September 2013 and 20 May–22 October 2014), while the brown areas indicate the three drought periods (20–25 July, 22–31 August, 24 September–10 October 2014).



**Figure 15.** The number of new plants emerging as a result of self-reproduction process during the two-year study. The list contains only plant species or subspecies of the three plant communities that managed to self-reproduce in the experimental setting. Bars represent LSD at a significance level of  $p < 0.05$ .

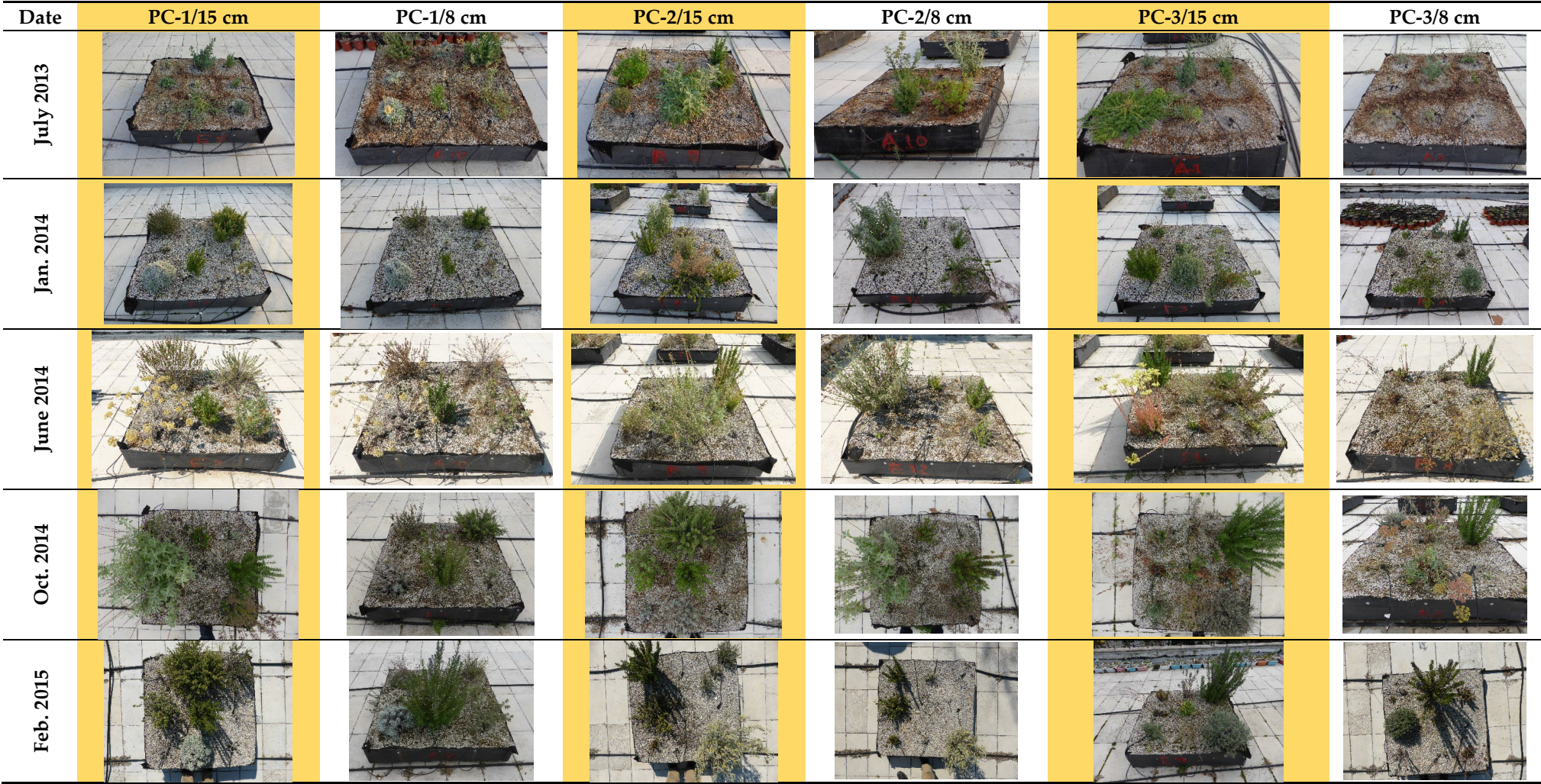
#### 4. Discussion

The current study is among very few that have managed to monitor the growth and flowering of numerous plant species within a plant community on extensive green roof systems and it is expected to be valuable to both researchers and professionals working in this specific area. Monostands rarely possess all the appropriate morphological and physiological characteristics that will aid survival under extreme environmental conditions or fulfill the functional and aesthetic goals of a Mediterranean green roof. In contrast, polystands, comprising diverse plant species groups originating from similar natural habitats are expected to be more resilient and capable of creating plant communities that would resemble natural habitats [63–65]. Polystands composed of native and endemic plant species are expected to create a self-reproducing resilient green roof ecosystem within the urban landscape that will promote biodiversity and enhance fauna [1,2,19,57]. Dunnett and Kingsbury [57] support the concept that the utilization of native vegetation is the most environmentally friendly approach for extensive green roof planting.

However, the existing literature on green roof polystands is limited. Lundholm [66] compared fifteen monocultures comprising succulents, tall, dwarf and creeping forbs and grasses with mixtures of plants from either three or five of the above categories in a four-year study in Canada. He investigated the ecosystem services, namely above-ground production, thermal regulation, stormwater retention and ecosystem functionality. He reports that substrate cooling increased over time, as well as water retention and that these ecosystem services were positively related to the planted species richness. Likewise, Tran et al. [66], installed extensive green roofs with either monoculture of three different species (*Aquilegia canadensis*, *Sedum spurium* and *Sporobolus heterolepis*) or mixtures of these three plant species in three different Canadian cities. They report that the mixture of the three plant species yielded slightly better crown density and soil cover, and increased plant height compared with the monocultures in all three cities. Similarly, Butler and Orians [67] imposed water stress on an extensive green roof study and found increased survival rates in plant communities containing herbaceous species growing together with *Sedum* spp. They concluded that the main advantage of polystands is a cross-species facilitation effect which could provide an easy and low-cost method of developing sustainable urban green roofs. Nagase and Dunnett [58] investigated the effect of vegetation diversity on the survival of plants under drought conditions. Twelve plant species were divided into three groups (broadleaved, *Sedum* spp. and grasses). The experimental design included either monocultures, or mixtures of 4 or 12 plant species, and irrigation frequencies varying from either once, twice or three times per week. The authors report that plant mixtures performed better than monocultures in terms of viability and visual quality under drought conditions. Moreover, when the two mixtures were compared, the one comprising 12 plant species exhibited better viability and higher visual quality compared with that comprising four plant species.

In the present study, though none of the three formulated plant communities was able to provide full coverage of the experimental plots during the two-year period, all of them were able to provide year-round color through their overlapping flowering periods. Each one of the three formulated plant communities included both plants which were successfully established and thrived and plants whose growth and flowering was moderate or poor (Figure 16).





**Figure 16.** Photographic evidence of the study treatments for each plant community (PC-1, PC-2 and PC-3) in the deep (15 cm) and the shallow (8 cm) substrate depth at different time intervals of the study.



Substrate depth proved to be the most significant factor that directly influenced and promoted the growth rate of most plant species. However, within each plant community, the impact of substrate depth was more pronounced on plants that exhibited increased growth. In PC-1, the 15 cm substrate depth promoted *H. orientale*, *O. onites*, *S. thymbra* and *R. officinalis*. In contrast, *B. acetabulosa*, *S. syriaca* subsp. *syriaca* and *P. fruticosa* were either indifferent or had minimal impact by substrate depth. *Cistus creticus* was the only species that exhibited a preference towards shallow depth (8 cm) compared with the deeper ones at the high irrigation regime. According to Filippi [68], *C. creticus* plants develop a double root system to effectively resist drought. In the first stages, the plants quickly develop a long root that reaches a depth that will allow them to obtain the necessary moisture to survive the first summer. During this stage, even though the aerial plant parts grow very slowly with few expanded leaves to minimize water losses, the root system is intensely developing to extract as much soil moisture as possible. Young plants also develop a dense network of secondary surface roots to take advantage of moisture and nutrients close to the soil surface. Due to this dual root system, *C. creticus* plants can maximize the absorption of the available soil water. Under green roof conditions, an extensive secondary surface root system is expected to facilitate water absorption during deficit irrigation and drought conditions.

In PC-2, plants of high and moderate growth exhibited better growth in the deeper substrate depth (*R. officinalis*, *S. fruticosa*, *H. empetrifolium*, *T. capitata* and *M. officinalis* subsp. *altissima*), while *O. vulgare* subsp. *hirtum* and *P. majus* exhibited better growth in the deeper substrate only during the water stress periods of the second study year. *Ebenus cretica* and *T. capitatum* were indifferent to substrate depth but plants of *E. cretica* were lost before the water stress period of the second study year and thus results are considered inconclusive for this species. Similarly in PC-3, the plants that promoted their growth in the deeper substrate depth were those that exhibited increased growth (*R. officinalis*, *S. vera* and *L. monopetalum*). *Chrythmum maritimum*, *L. graecum* and *C. ruber* subsp. *sibthorpii* improved their growth only in limited periods, while *H. stoechas* subsp. *barrelleri*, *S. sediforme* and *T. brevifolium* were indifferent to substrate depth. In a previous study [22] employing native plants in Mediterranean extensive green roofs, *C. ruber* plants grew well and bloomed extensively from May till September receiving deficit irrigation (60% ET<sub>0</sub>). In another study evaluating the agronomic performance of several xerophytes in a simulated dry green roof [9], *C. ruber* subsp. *sibthorpii* was among those showing excellent performance during the hot and dry summer months in terms of survival rates, growth, and vegetation cover dynamics with better results achieved in the deeper substrate depth (20 cm) than the shallower (15 cm). It seems that *C. ruber* subsp. *sibthorpii* requires either a deeper substrate depth and/or higher irrigation regimens than those utilized in the current study.

Based on the above-mentioned pattern, it is obvious that the size of the foliage is the influential factor in demanding deeper substrate depth. Based on Figures 5, 8 and 11, plants with a growth index exceeding 40 cm improved their growth in the deeper substrate depth except for PC-2 where the plants with a growth index close to 30 cm also exhibited a preference towards the deeper profiles. In all other cases, plants with a growth index of 20–30 cm and lower were either minimally influenced by substrate depth or indifferent. Plants with a growth index below 15–20 cm were not influenced by substrate depth.

It seems that the growth pattern of the aerial portion of each plant species indicates the preference for deeper substrate depths with only few exceptions. More specifically, plants with higher growth, and concomitantly increased leaf area, demand more root space to support their upper growth since deeper substrate depth has been correlated with increased water retention. The beneficial effect of increasing the depth of the substrate is attributed mainly to the abilities of the deeper substrate to retain increased moisture and to store more nutrients and to the greater substrate volume available for root growth that can absorb more water and nutrients [1–3,9–11,69–71]. In addition to that, growth in deeper substrates is promoted by the reduction in temperature fluctuations [72]. However, plants that are characterized by restricted aerial growth do not take advantage of the deeper substrate since the demand for water and photosynthates is minimized.

It is also speculated that plants possessing an inherited large or fast type of growth, would retrieve and absorb, in a more efficient manner, water and nutrients from the substrate, at the expense of plant species with slow or low growth habits. Therefore, within a plant community, the expected interactions of the participating plants might have an impact on the growth of each species. In order to support this speculation, it is worth examining the growth of *R. officinalis*, which was the only species in common between the three plant communities. The general growth pattern of *R. officinalis* was similar for all three plant communities since it provided continuous growth with small peaks during the early autumn of the first study year and the summer of the second study year. However, the absolute growth index was significantly different between the three plant communities. In PC-1 (Figure 5), *R. officinalis* increased its growth at a much slower rate, reaching 23 cm and 43 cm in the first and second study year, respectively. In contrast, in PC-2 the growth index was 36 cm and 50 cm and in PC-3 the growth index was 35 cm and 77 cm for the first and second study year, respectively. This would indicate that in PC-1 the participating species would be expected to consume more water compared with the other two plant communities, thus restricting the growth of *R. officinalis* in PC-1 compared with PC-2 and PC-3. Indeed, in PC-1, the participating species *O. onites* has been reported to consume large amounts of water whenever it is available [73]. If this is the case, water stress would be expected to be increased in PC-1 and hinder the growth of the remaining plant species including *R. officinalis*. In PC-2, the same competition effect would be expected to occur due to the presence of *M. officinalis* subsp. *altissima*, but its growth was severely restricted and affected by the imposition of the drought periods and thus could not compete as effectively as *O. onites* in PC-1. In a study on the resilience of native aromatic plants to water stress on extensive green roof systems, Kokkinou et al. [18] report that plants of *M. officinalis* exhibited the least resilience to water stress in comparison with the other four plant species. (*R. officinalis*, *B. acetabulosa*, *S. fruticosa*, *H. orientale*). In PC-3, *R. officinalis* exhibited its greatest growth, indicating that water resources were ampler compared with the other two plant communities. Indeed, in PC-3 succulent plants and plants with CAM metabolic pathways participated (*L. monopetalum*, *S. sediforme* and *C. maritimum*) along with three plants with a low growth index (*C. ruber* subsp. *sibthorpii*, *T. brevifolium* and *L. graecum*), thus permitting *R. officinalis* to utilize more water resources in PC-3 compared with the other two plant communities.

There are numerous studies which have concluded that increasing green roof substrate depth promotes growth, viability and sustainability of the plants. However, there is only a limited number of studies that involved plant communities and reported growth index response. In a recent study [74], the growth and survival of 22 plants, including herbaceous perennials, grasses, and Sedums, were evaluated on a green roof at substrate depths of 4.5, 10 and 20 cm and it has been reported that deeper substrates support a larger variety of species.

During the water stress periods, different reactions were recorded between plant species. Some plants reduced while others retained their growth in both substrates, but *O. vulgare* subsp. *hirtum* (PC-2) reduced its growth only in the shallow substrate depth (Figures 5, 8 and 11). At the initiation of the water stress period, *S. thymbra* (PC-1) lost all individuals growing in the shallow substrate, while *E. cretica* (PC-2) lost all individuals from both substrate depths even before the initiation of the water stress period. *Ebenus cretica* has a drought avoidance mechanism [11] that involves dropping the leaves in order to minimize transpiration during the harsh summer period. However, in our study, *E. cretica* was unable to recover enough to produce new leaves, thus indicating that stress conditions on the green roof system exceeded the limits of its drought tolerance. This is in contrast with the reported results by Nektarios et al. [11], as in their study *E. cretica* grew unobstructed for two years in 7.5 cm substrate depth and irrigation at 30% ET<sub>c</sub>. However, the authors emphasize that its slow growth in green roof systems should be taken into account.

Alongside growth, flowering was also positively impacted by increasing the depth of the substrate (Figures 6, 9 and 12). However, the occurrence of differences in regard to plant species flowering based on the depth of the substrate did not always coincide

with the differences in growth. In PC-1 (Figure 6), flowering increased only for the three species that exhibited increased growth in the deeper substrate depth compared with the shallower ones (*H. orientale*, *O. onites* and *S. thymbra*) and to a lesser extent for *O. dictamnus*. The remaining species or subspecies flowered in similar numbers in both substrate depths while *S. syriaca* subsp. *syriaca* and *P. fruticosa* did not flower. The former, an endemic of the island of Crete, thrives exclusively at high altitudes between 1000 and 2200 m [45,75] and therefore was not expected to flower at climatic zones with prevailing high temperatures. However, despite its low growth and inability to flower, its use in an extensive urban green roof system cannot be excluded due to the aesthetic value and color of its foliage. The growth of *P. fruticosa* was inferior to that of its natural habitat and consequently, it was not able to flower. *Phlomis fruticosa* is a semi-deciduous species and is considered to be able to withstand water scarcity [76] due to its morphological, anatomical, and physiological mechanisms, which include partial leaf shedding during the summer and leaf dimorphism. In spite of all these mechanisms, in our case, it was negatively affected by the imposition of the drought periods [77–80].

In PC-2, all species provided more prolific flowering in the deeper substrate except for *R. officinalis*, *M. officinalis* subsp. *altissima* and *T. capitatum*, which provided similar flower numbers in both substrate depths. Because of the plant loss, *E. cretica* never flowered (Figure 9). This species flowers in Crete typically from April to June depending on the altitude and the local microclimate, grows well in rocky areas or hills and prefers alkaline soils [81]. In a study investigating its potential floricultural use, Vlachos [81] reported that seedlings of *E. cretica*, when grown in natural soil, produced abundant flowering and grew almost three times taller compared with plants grown in 2.4 L pots. He also reported that growth and flowering were improved when seedlings were grown in 12 L pots instead of 2.4 L, indicating that *E. cretica* prefers deeper substrates.

In PC-3, *S. sedifforme*, *L. monopetalum* and *T. brevifolium* flowered in similar numbers in both substrate depths (Figure 12). The two formers are succulents and thus do not stress from shallow substrate depths, while *T. brevifolium* provided very small flower numbers in both substrate depths. *Suaeda vera* did not flower, presumably due to differences between the experimental site and its natural coastal habitat where it thrives as a halophyte. Nevertheless, flowers of this species are hardly visible, due to their small size and pale color and thus its aesthetic value lies mostly in the interesting discoloration of its leaves. Furthermore, due to its variable growth habit (erect and prostrate) could provide good coverage and has been proved to be one of the more successful species of PC-3. The remaining plants flowered more prolifically in the deeper substrate compared with the shallower one, including *C. maritimum* which is also a succulent plant.

In contrast with substrate depth, the irrigation regime had a minimal impact only in *O. vulgare* subsp. *hirtum* growth where the low irrigation reduced the growth of the plants (Figure S3). It seems that either 10% or 20% of  $ET_0$  is adequate for the growth of these plant species when grown in shallow green roof substrates under the Mediterranean climate. These findings support the selection of the specific plant species from the xerophytic habitats of Greece for the composition of the plant communities, which include plant species equipped with resistance mechanisms to water stress. It was calculated that, during the first study year,  $234.6 \text{ L m}^{-1}$  were used for the low irrigation regime and received additionally  $263 \text{ L m}^{-1}$  of natural precipitation (Figure 3). In the second study year,  $188.9 \text{ L m}^{-1}$  were used for the low irrigation regime but received higher natural precipitation, reaching  $576 \text{ L m}^{-1}$  (Figure 4). It was calculated that, following the establishment period, only  $18.9 \text{ m}^3 \text{ y}^{-1}$  would suffice for a  $100 \text{ m}^2$  urban extensive green roof comprising the evaluated endemic and native plant species.

In addition to the increased water stress resistance and tolerance of the selected plants, the green roof layering provides an additional water depot. Previous studies have investigated the influence of different green roof layering types on the amount of water available to plants of *Salvia officinalis* growing in green roof modules in the Mediterranean region [82]. Plants were monitored between early spring and late summer, and findings

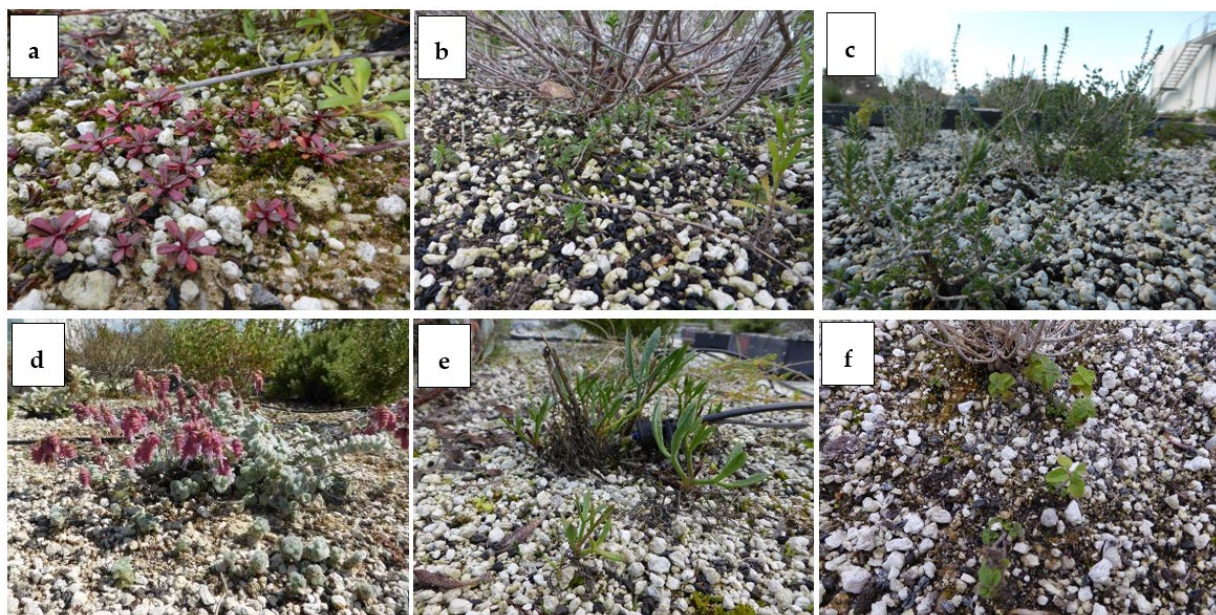


showed that the design of the overall green roof system, and in particular the characteristics of the drainage layer, significantly improved the amount of available water to the plants.

Self-reproduction was positively affected by substrate depth only for very small periods with a time span of a few months in PC-1 and PC-3, while PC-2 had a similar number of new plants emerging between the two substrate depths. Differences in new emerging plants occurred during the second study year and the deeper substrate (15 cm) had a higher number of new plants compared with the shallow one. The observed higher number of new plants in the deeper substrate depth was related to the higher growth rate and the increased number of flowers that occurred in the deeper substrate. It is worth mentioning that, during the water stress and drought periods of the second year, most of the new plants survived and exhibited great resistance to drought.

Attempting a comparison of the three plant communities based on the current two-year study, it was observed that some species managed to grow well at the expense of the others in all three plant communities and therefore growth was not balanced among them in any of the three plant communities. In descending order, the dominating species based on growth index were, *O. onites*, *S. thymbra* and *H. orientale* in PC-1, *S. fruticosa*, *O. vulgare* subsp. *hirtum* and *R. officinalis* in PC-2 and *R. officinalis*, *L. monopetalum* and *S. vera* in PC-3.

Flowering was abundant in all three plant communities. During the first study year, plants were less developed and, concomitantly, flowering was less prolific compared with the second study year. The main flowering season lasted from May to December 2013, when more than ten plant species from all communities (more than one-third of the total number) were blooming (Figure 17). The peak of flowering occurred between June and August with 17, 18 and 15 species blooming, respectively. In the second study year, the main flowering season with 10 or more species blooming lasted from March to October 2014. Peak season occurred again during the months of June, July and August when 16, 17 and 13 species were blooming, respectively. Plant communities PC-2 and PC-3 performed better than PC-1 concerning the duration of flowering and the total number of species that were simultaneously in bloom every month of the study period. PC-2 had four or more plant species blooming for eight months in the first year and seven months in the second year, whereas blooming in PC-3 lasted for seven and nine months, respectively. In contrast, PC-1's flowering period lasted for only four months in the first and four in the second year of the study.



**Figure 17.** New emerging plants of the six most self-reproduced plant species in descending order, (a) *Limonium graecum* (PC-3), (b) *Satureja thymbra* (PC-1), (c) *Thymbra capitata* (PC-2), (d) *Origanum dictamnus* (PC-1), (e) *Crithmum maritimum* (PC-3) and (f) *Origanum vulgare* subsp. *hirtum* (PC-2).



## 5. Conclusions

The present study proves that endemic and native Mediterranean (Greek) plant species may thrive on urban green roofs using only 8 cm substrate depth and with minimal water inputs of 10%  $ET_0$ . The current study demonstrates that the composition of artificial plant communities for extensive green roof systems should also take into account the rate of water use of each participating plant species under the particular green roof conditions and substrate depth. Plants within each community were grown based on their inherited growth pattern but also competed with the plants in vicinity for water resources. Though most of the studied plants managed to survive, the communities became unbalanced since fast-growing plants with higher water consumption rates grew in expense of those with slower growth rates or lower water requirements.

The utilization of endemic and native Mediterranean plant species to formulate plant communities capable of producing resilient and sustainable urban green roofs is a demanding process due to plant species interactions. Based on the findings of the current study, it seems appropriate to re-examine the formulation mentality of the plant communities and group the plant species based on similar growth patterns, flowering and water consumption rate. Unfortunately, to date there is limited information on native and endemic species' water requirements when grown on extensive green roof systems and future research should further investigate this issue.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/su15075940/s1>, Figure S1: Irrigation regime (Low: 10%  $ET_0$  and High: 20%  $ET_0$ ) effects on the growth index of the plants of the first plant community. The asterisk indicates a significant difference between treatment means based on the Least Significant Difference (LSD) criterion. Bars represented LSD at a significance level of  $p < 0.05$ . The gray-shaded areas indicate the two periods of water stress (4 June–27 September 2013 and 20 May–22 October 2014), while the brown areas indicate the three drought periods (20–25 July, 22–31 August, 24 September–10 October 2014). *Cistus creticus* was analysed as One-Way ANOVA due to interactions between the two factors (shallow/deep substrate and low/high irrigation regime). Letters indicate the mean values being statistically different within the same date, using LSD criterion at a significance level of  $p < 0.05$ ; Figure S2: Irrigation regime (Low: 10%  $ET_0$  and High: 20%  $ET_0$ ) effects on the flowering of the first plant community. The asterisk indicates a significant difference between treatment means based on the Least Significant Difference (LSD) criterion. Bars represented LSD at a significance level of  $p < 0.05$ . The gray-shaded areas indicate the two periods of water stress (4 June–27 September 2013 and 20 May–22 October 2014), while the brown areas indicate the three drought periods (20–25 July, 22–31 August, 24 September–10 October 2014); Figure S3: Irrigation regime (Low: 10%  $ET_0$  and High: 20%  $ET_0$ ) effects on the growth index of the plants of the second plant community. The asterisk indicates a significant difference between treatment means based on the Least Significant Difference (LSD) criterion. Bars represented LSD at a significance level of  $p < 0.05$ . The gray-shaded areas indicate the two periods of water stress (4 June–27 September 2013 and 20 May–22 October 2014), while the brown areas indicate the three drought periods (20–25 July, 22–31 August, 24 September–10 October 2014); Figure S4: Irrigation regime (Low: 10%  $ET_0$  and High: 20%  $ET_0$ ) effects on the flowering of the second plant community. The asterisk indicates a significant difference between treatment means based on the Least Significant Difference (LSD) criterion. Bars represented LSD at a significance level of  $p < 0.05$ . The gray-shaded areas indicate the two periods of water stress (4 June–27 September 2013 and 20 May–22 October 2014), while the brown areas indicate the three drought periods (20–25 July, 22–31 August, 24 September–10 October 2014); Figure S5: Irrigation regime (Low: 10%  $ET_0$  and High: 20%  $ET_0$ ) effects on the growth index of the plants of the third plant community. The asterisk indicates a significant difference between treatment means based on the Least Significant Difference (LSD) criterion. Bars represented LSD at a significance level of  $p < 0.05$ . The gray-shaded areas indicate the two periods of water stress (4 June–27 September 2013 and 20 May–22 October 2014), while the brown areas indicate the three drought periods (20–25 July, 22–31 August, 24 September–10 October 2014); Figure S6: Irrigation regime (Low: 10%  $ET_0$  and High: 20%  $ET_0$ ) effects on the flowering of the third plant community. The asterisk indicates a significant difference between treatment means based on the Least Significant Difference (LSD) criterion. Bars represented LSD at a significance level of  $p < 0.05$ . The gray-shaded areas indicate the two periods of water stress (4 June–27 September 2013

and 20 May–22 October 2014), while the brown areas indicate the three drought periods (20–25 July, 22–31 August, 24 September–10 October 2014); Figure S7: Irrigation regime ((Low: 10% ET<sub>0</sub> and High: 20% ET<sub>0</sub>) effects on the number of self-reproduced plants of the three plant communities. The asterisk indicates a significant difference between treatment means based on the Least Significant Difference (LSD) criterion. Bars represented LSD at a significance level of  $p < 0.05$ . The gray-shaded areas indicate the two periods of water stress (4 June–27 September 2013 and 20 May–22 October 2014), while the brown areas indicate the three drought periods (20–25 July, 22–31 August, 24 September–10 October 2014).

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