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Green Roof Management in Mediterranean Climates: Evaluating the Performance of Native Herbaceous Plant Species and Green Manure to Increase Sustainability

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Abstract: The benefits of ecosystem services provided by urban green systems have been highlighted in research on spatial and landscape planning, and the need has emerged for an integrated approach to urban green planning aiming at increasing climate mitigation and urban resilience. Research indicates that plant selection and substrate management are vital for optimizing the most important performance of green roofs, like building thermal insulation, urban heat reduction, air quality improvement, and stormwater management. In Mediterranean climates, it is essential to investigate sustainable management solutions for green roofs like the growth potential of native, low-maintenance forbs adapted to thermal and water stress on specific substrates. Medicinal species may be suitable, provided that interactions with pollutants are controlled. This study evaluates the performance of *Melissa officinalis* and *Hypericum perforatum* on experimental green roof modules under controlled conditions, comparing chemical fertilization and three different treatments with biomass from *Trifolium repens* used as green manure. The key metrics of fresh and dry biomass, plant cover ratio, and chlorophyll content are measured. Results show significantly higher values of cover and biomass for these two species treated with green manure in comparison to chemical fertilization, with no significant differences in chlorophyll content, indicating that *T. repens* is a useful source of green manure in green roof management. Overall, the results are consistent with the research goals of suggesting sustainable solutions for green roof management, since low-maintenance vegetation and green manure contribute to the elimination of chemicals in urban green.

Keywords: green roofs; green manure; native plants; sustainable management; urban environment; impact mitigation; nature-based solutions

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1. Introduction

Cities in the world are facing growing challenges in relation to the increase of artificialization that generates several critical issues affecting urban areas, in particular the urban heat island effect, air pollution, high energy consumption, and reduced access to nature. These critical issues are further exacerbated by climate change.

In this context, increasing importance is given to green infrastructures and nature-based solutions for the contribution they can make to the resilience and improvement of the environmental quality of cities, through a wide range of provisioning, regulating, supporting, and cultural ecosystem services [1,2].

In the field of green infrastructure, green roofs are recognized as having an important role as a nature-based solution for improving urban sustainability, especially in intensely artificialized and densely built areas, where the possibility of intervening with green areas and green solutions on the ground is limited. The role of green roofs as a crucial component of buildings in relation to NBS (Nature-Based Solutions) in urban areas has been recognized by many authors. In particular, López-Silva et al. [3] maintained that all green roof categories are suitable to reduce the ecological footprint of buildings; according to Ulubeyli and Arslan [4], green roofs represent a passive technique to reduce the level of energy requirement and improve the thermal-energy performance in buildings; Korniyenko [5] emphasized that green roofs can act as an important component to create more sustainable, biopositive, and energy efficient buildings. In fact, there is evidence that green roofs contribute to saving cooling and heating energy, controlling runoff and water pollution, promoting biodiversity, and providing aesthetic and health benefits [6,7]. Moreover, the vegetation of green roofs, if appropriately chosen among the various available species, including perennial herbaceous and aromatic plants, can contribute to the mitigation of the negative effects of urban air pollution [8].

The planning and design of green roofs faces various challenges and must adapt to various contexts and characteristics, not only of the urban fabric, but also of the climate and the environment.

One of the main challenges of the implementation of green roofs in urban areas that has been recognized by many studies is their complex (and often expensive) maintenance [3–5,9–13]. Matos Silva et al. [11], in particular, argue that substrate and plants are amongst the system components most affected by maintenance challenges in Mediterranean areas; moreover, the same authors highlight the need for maintenance strategies adapted to the specific challenges of the Mediterranean climate. For these reasons, a limited-maintenance sustainable management, which may provide a good level of plant growth and ensure the aforementioned benefits and, therefore, the provision of ecosystem services, is one of the topics of greatest interest to scholars; it can also be useful for the diffusion of the green roofs, which still struggle to spread in the real world.

In particular, several research works have addressed the topic of component design optimization of green roof substrate layers with respect to their performance [12,14–16]. Even in cases where construction solutions and substrates that have already become standard in the green roof industry sector are implemented, the development of cultivation strategies with a low environmental impact is of great importance; they combine the choice of plants well adapted to the local conditions, easy to root in the construction phase, with a planting and management plan that provides a reduced input of chemical substances, replaced by nature-based solutions. These strategies would be able to guarantee good levels of aesthetic yield and growth, and, therefore, provide ecosystem services. In this respect, several studies have investigated, in detail, the development of plants in low-maintenance and unfertilized extensive green roofs [17].

The choice of the most suitable plants based on the type of green roof (extensive or intensive, construction type, and substrate) and on its urban and climatic context is a thriving area of research in many countries around the world [18,19]. Significant remarks regarding the study of vegetation related to green roof performance have been made by several authors, who enumerate the required features of plant species particularly suitable for the conditions of urban green roofs: they stress the ability to survive with minimal maintenance and minimal nutrients in the substrate, grow quickly, and reach good ground coverage in a short time [5,13,20,21].

A growing number of studies related to extensive green roofs and their beneficial ecosystem services deal with the choice and performance of wild, native plant species and of suitable plant mixtures (even not commonly used in green roofs) compared to industry-standard plant species [22] to improve the resilience and sustainability of green roofs across continents, spanning from temperate Atlantic climates [23] to continental climates [24] and Mediterranean climates where drought and heat are challenging factors for green roof design [25,26]. Indeed, while the use of extensive green roofs is increasing worldwide, Mediterranean conditions complicate their design due to the long, dry summers with intense solar radiation [27], and uniform, extensive vegetated roofs with a high dominance of succulent species have limited value for plant biodiversity [17].

At the same time, with the aim of increasing the resilience of green roofs, several studies have considered the benefits obtainable through the use of a combination of species that mimic the synergies and interactions of natural communities, rather than monocultures, showing that complementary planting mixtures can promote species interactions and facilitation between species, increasing the number of plant species that can survive on extensive green roofs [28], and that ecosystem services from green roofs can be improved by planting certain life-form groups in combination, directly contributing to climate change mitigation and adaptation strategies [29].

The use of plants to create multifunctional green roofs is a remarkably interesting option. In this sense, the use of plants that combine an aesthetic and environmental value with a medicinal interest is very promising and innovative [30].

Among these plants, we can mention St. John's wort (*Hypericum perforatum* L.), with its well-known antibacterial and antioxidant properties [31,32], and the common balm (*Melissa officinalis* L.), a medicinal plant rich in biologically active compounds (flavonoids, terpenoids, phenolic acids, tannins, and essential oils) which is used worldwide for its therapeutic effects related to them [33] and for which recent studies have shown that fertilization with organic manure can enhance growth and accumulation of citral, total phenols, flavonoids, and the chemical composition of essential oil [34].

The use of clovers (genus *Trifolium*) in extensive green roofs has been studied by many authors due to several ecophysiological traits peculiar to this genus, in particular the ability to perform N fixation, the rapid growth, and the high resilience of some species in limiting soil conditions and dry Mediterranean climates [19,35,36]. *Trifolium repens* has been reported as one of the wild plants growing on green roofs in urban landscapes [37]. Other than sharing the abovementioned traits of clovers' genus, it is known for its phenolic compounds and for anticholinesterase and antioxidant activities, as well as for anti-inflammatory, antiseptic, analgesic, antirheumatic, and antimicrobial properties [38,39], with concentrations of bioactive compounds that have been shown to depend on the growing conditions [40]; moreover, it is well known for its nitrogen fixation capacity, which has also been shown to be modulated by the availability of sulfur in the substrate [41].

While the use of medicinal plants in urban green systems is regarded as innovative, it is important to highlight that the cultivation of plants in urban environments is suitable for food and medicinal purposes, provided that the cities in question are environmentally friendly. Many authors have studied the topic of pollutants and medicinal plants, highlighting the complex relationships among pollutant absorption, bioaccumulation, and effects on plants' metabolic profiles [42–44]. Although this experiment did not deal with the study of this specific topic, their contrasting desirable abilities of removing pollutants and producing useful metabolites imply that the risks and benefits of growing medicinal plants in urban areas should be carefully evaluated.

Many studies have investigated the characteristics and effects of the various types of substrate components that can be used in green roofs, since the substrate represents one of the most important elements, both for the correct growth and the physiological performance of plants (providing them with water, nutrients, and physical support) and for the thermal and hydraulic performance of the roof itself. In particular, studies have shown that the addition of green waste compost to the substrate can remarkably increase shoot and root growth, shoot nitrogen concentration and chlorophyll content, and decrease the root:shoot ratio compared to bark [45]. The reduction of chemical fertilization is evaluated in our research as the main positive output of the addition of green manure to the green roof substrate. Reducing chemical fertilization has been specifically related to the reduction of high nutrient levels in green roof runoff water by several authors [5,9,21]. Lopez-Silva et al. [3] also highlighted this relation and suggested the use of biostimulants and biofertilizers to help with the reduction of chemicals; moreover, they cited other authors [20,46,47] supporting their opinion that using native plants significantly reduces maintenance needs and associated costs, since native vegetation is adapted to the local climate and can better resist pest impacts, reducing the need for pesticide treatments.

This research originated from the abovementioned evidence, with the aim of experimenting with the use of associations of plants of both ornamental and medicinal value suitable for green roofs in southern European cities, in combination with cultivation strategies capable of reducing the input of chemical fertilizers: the experimental test and results presented in this paper suggest a possible answer to the complex and expensive maintenance of green roofs that is one of the main challenges of their implementation in urban areas, therefore increasing both their economic and environmental sustainability.

The aim of the experiment was to test the growth performance of novel, native, low-maintenance forbs (*Melissa officinalis*, *Hypericum perforatum*)—with both ornamental and medicinal values—grown on an experimental green roof, in substrates enriched with different green manures made from *T. repens* biomass, in order to verify the possibility of obtaining good levels of cover and biomass in the first stages of growth as an alternative to chemical fertilization.

The specific goals of the research are as follows:

- Studying the growth performance of *Melissa officinalis* and *Hypericum perforatum* under three different treatments with green manure made from white clover (*T. repens*) biomass.
- Studying the growth performance of *M. officinalis* and *H. perforatum* in the presence of mowed *T. repens* previously grown on the same experimental substrate.
- Quantifying the growth performance of the two species through measurements of biomass, cover ratio, and chlorophyll content.

2. Materials and Methods

2.1. Setting Up the Experimental Green Roof Structure

The experimental test was carried out from 21 March 2024 to 11 June 2024 in the greenhouse of the Imola headquarters of the Department of Agricultural and Food Sciences of the University of Bologna (lat 44°20′14.6″ N, long 11°43′07.1″ E). In particular, the test was set up in a controlled environment by reproducing the structure of a green roof on benches in the central span of the greenhouse.

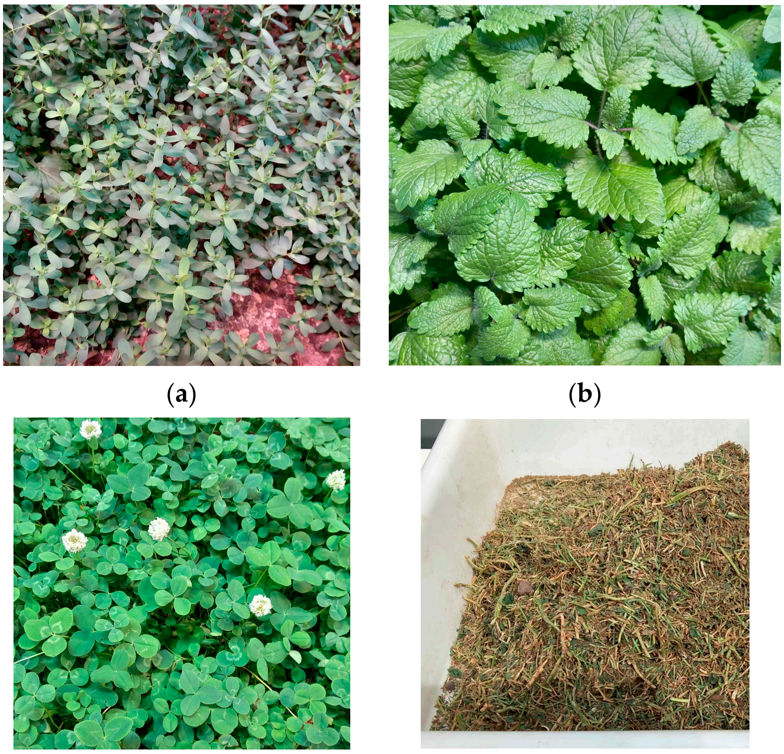
Each bench was divided into eight plots (125 cm × 60 cm) by using an opaque, waterproof polypropylene honeycomb panel. In each plot, a green roof covering was laid out with the following structure (from bottom to top): protection and accumulation felt; an accumulation, drainage, and aeration layer (2.5 cm); a filter sheet; a non-commercial green roof substrate, designed for structural durability, with a high C/N ratio, compacted (15 cm). Both the structure and the thickness values of layers used for this experimental covering were chosen following the ones specifically required by the producer. In particular, 15 cm is the value indicated by the producer to optimize the performance of the substrate, specifically designed for extensive green roofs in Mediterranean climates.

Uniform, supplementary lighting was provided by using white-spectrum LED lamps with dimmers (C-Led, Imola, Italy) placed above the bench. The supplementary lighting period was set to 16 h per day, from 6:00 to 22:00, to keep a constant summer-like photoperiod. After a series of preliminary measurements, the light intensity of the lamps was set to 14,400 lux, the average difference between the values of natural light intensity measured inside and outside the greenhouse. This ensured a light intensity comparable to that found in outdoor environments. The main environmental data were monitored throughout the test by means of a data logger specifically designed for greenhouse experiments [48,49].

2.2. Plant Selection

The experiment was carried out using three species of plant with ornamental, aromatic, and medicinal value (Figure 1):

- *Hypericum perforatum* L., St John's wort (in tables and figures also H), as the tested species.
- *Melissa officinalis* L., lemon balm (in tables and figures also M), as the tested species.
- *Trifolium repens* L., white clover, cultivar Rivendel (in tables and figures also T), as the green manure in three different treatments.



(**a**) (**b**) (**c**) (**d**)

Figure 1. The three species of plants used in the experiment. *Hypericum perforatum* (**a**), *Melissa officinalis* (**b**), *Trifolium repens* (**c**), biomass from *T. repens* used as green manure (**d**).

All the species used in the experiment are either spontaneous or naturalized since ancient times in Italy [50] and have interesting phytochemical profiles and pharmacological properties [31,33,38]. *H. perforatum* and *M. officinalis* have been tested because they might be suitable plants for a multi-purpose green roof, due to their ecophysiological traits and pharmacological properties. Moreover, recent studies carried out in controlled conditions on a green roof experimental substrate have shown that *H. perforatum* and *M. officinalis* can respond well to chemical fertilization and to biostimulation in terms of growth, cover, and metabolite production, but they are more sensitive to a lack of nitrogen in the first three months after seeding; on the contrary, *Trifolium repens* is able to reach full cover in short times, even with no fertilization, but it can be sensitive to water stress during the hot season. In fact, *Trifolium repens* is a nitrogen-fixing species, hence a pioneering, fast-growing plant which might become a nuisance for green roof maintenance; however, these features may be useful in the first steps of green roof management when following an ecological approach [51,52]. On these premises, *T. repens* was chosen as green manure in this experiment, specifically testing three different options that can be followed in managing a green roof on which clover may have grown either as a weed or because sown on purpose.

1. The first option is letting the roots and cut stumps in place after mowing and removing the clover growth.
2. The second option is letting the whole aboveground biomass (leaves, stems, and flowers) in place, together with roots and cut stumps, after the mowing.
3. The third option is adding the aboveground biomass resulting from the mowing to a fresh, new substrate instead of using chemical fertilization.

In order to replicate the conditions commonly found in green roof life cycle management, the plants in the test were all grown starting from certified seeds purchased from specialized producers (*H. perforatum* and *M. officinalis*: Franchi sementi, Italy; *T. repens* var. Rivendel: Four sementi, Italy).

2.3. Preparation of Substrates

- In four of the eight plots of each bench, white clover (*Trifolium repens*) had been grown on an identical green roof substrate as a control for a previous test, for about seven months before the start of the experiment: in these plots, only water had been used for growing the clover plants.
- The remaining four plots of each bench were cleaned and set up with the fresh substrate (in nomenclature also S), with identical physical and chemical properties to the older substrate used for growing the clover in the first four plots.
- In all four plots with the white clover and old substrate, the plants were cut at ground level after about seven months of growth, simulating the mowing of the fully grown vegetation that may be performed during green roof maintenance. The cut aboveground fresh biomass (composed of leaves, flowers, and part of the stems—in nomenclature also B) was weighed, then dried out for ten days by leaving it on the substrate surface and turning it daily.
- The ground-level and belowground biomasses (roots) of the clover were let in place: for this reason, they were not weighted.
- In each bench, after ten days of drying, all the cut biomass was collected, minced, weighed, divided into equal parts, and scattered uniformly both on the surface of two plots with clover roots and on the surface of the two plots with fresh substrate (average weight per plot: 661.125 g; average weight per area unit: 796.5 g; average plot area: 0.83 m^2).
- In the remaining two plots with the fresh substrate, a mineral-based, slow-release fertilizer (Slow Green®, Bottos, Italy, NPK 22.5.10 + 2MgO) was added following the producer's instructions: one single dose of 30–35 g/m^2 (32.5 g/m^2 on average) at the start of the test, that is 27.0 g per plot, since the plot area was 0.83 m^2.

2.4. Design of the Experiment

Two species were independently tested (H and M, see Section 2.2); for each species, three treatments and one control test were performed, as follows (see Figure 2):

- Fresh substrate + slow-release fertilizer (control test; HCK, MCK).

- Substrate + clover roots (MRO, HRO).
- Substrate + clover roots + dried aboveground biomass from the clover (MRB, HRB).
- Fresh substrate + dried aboveground biomass from the clover (MBN, HBN).

(**a**) (**b**)

Figure 2. Layout of the experimental green roof structure on three benches in the greenhouse (**a**). Scheme of the experimental setting, with three treatments and one control test repeated on three benches (**b**). See the abbreviations for the extended names of treatments.

2.5. Sowing of Plants

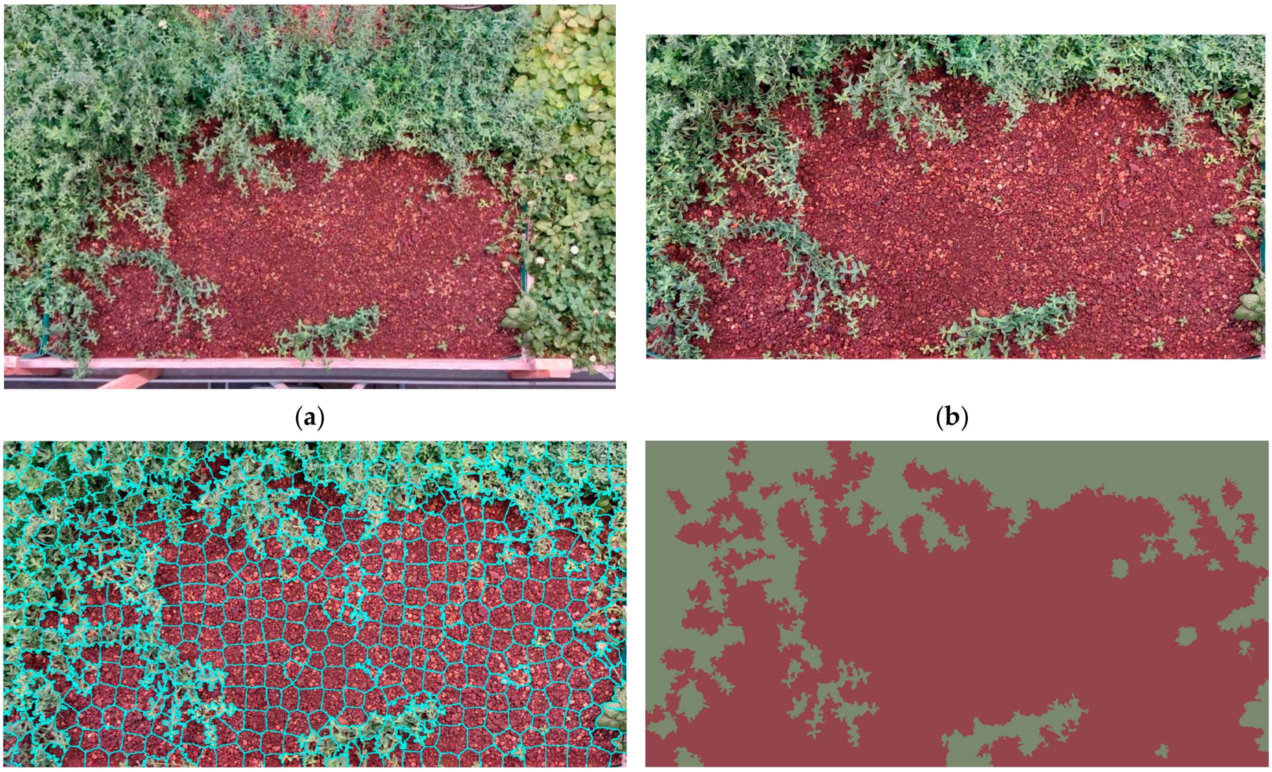
Before sowing the plots, their substrate was irrigated until percolation occurred, with the use of an average quantity of water varying between 10 and 15 l per plot. On 21 March 2024, *Hypericum perforatum* (1 g of seeds) and *Melissa officinalis* (2 g of seeds) were sown on the whole surface of each plot according to the instructions given by the producers. The seeds were broadcasted by hand on the surface of the substrate; each species was sown singularly (see Section 2.4).

After sowing, irrigation was performed by nebulization every 2 days for the first 15 days. After the first true leaves appeared on most of the seedlings, irrigation was performed thoroughly with a water sprayer, starting from two times a week, then reducing the frequency to once a week, until the end of the test.

2.6. Monitoring of Plant Cover

The evolution of plant cover for each treatment was monitored during the experiment through image analysis. After about 50 days from sowing, when a minimum cover of at least 5% was reached in all plots, photographs were taken from above each plot on 14, 20, 27 May and 11 June 2024 (hereinafter T1, T2, T3, and T4, respectively). The pictures were straightened with the software RDF 4.0 (analytic method), pre-processed in Gimp 2.10 [53], then analyzed using the software MatLab R2024a [54] to evaluate the degree of plant cover, as explained below. The illustrative sequence of the procedure is reported in Figure 3.

A MATLAB script was developed for classifying the ground cover percentages of different plant species through the application of image processing techniques. The script classifies areas within an image by segmenting it into various classes, such as LAB color space, superpixel segmentation, and K-means clustering. The input RGB image was converted into the LAB color space using the "rgb2lab" function in order to boost the segmentation and effectiveness of analysis based on color features.



(**a**) (**b**) (**c**) (**d**)

Figure 3. Basic steps of the image processing method. Original image (**a**), result after cropping (**b**), resulting boundaries (**c**), final output (**d**).

Subsequently, to ease subsequent image analysis, segmentation of the LAB image was performed by using the "superpixels" algorithm that separate nearby pixels within images into uniform, relatively homogeneous regions. This was achieved by the "boundarymask" function, producing a label matrix as the output. For more advanced analysis on the segmented regions, the label matrix was converted into a cell array of linear indices by using the "label2idx" function that allows the calculation of the average color per superpixel region; the mean values of the LAB channels in each superpixel were calculated and stored in an array. Image segmentation into a required number of colors was then achieved by the K-means clustering method based on the mean color values of the superpixels: the segmented regions were classified into vegetation and ground categories. Finally, the percentage cover for the clusters' categories was calculated on a proportion of pixel measures.

This method provides several advantages with respect to the use of the LAB color space, such as its ability to separate luminance from color information, making color-based segmentation more accurate. Superpixel segmentation reduces computational complexity, yielding a structured and perceptually meaningful organization for image analysis. The approach of combining such techniques with K-means clustering further provides effective classification of vegetation and ground regions, resulting in accurate quantification of cover percentages. The technique proposes robust and reliable segmentation that is highly important for its applications in agricultural and environmental monitoring.

2.7. Final Measurements

The test was concluded after about three months from sowing, on 11 June 2024. The final measurements were taken for all the species and for all experimental plots. Even though *T. repens* was technically the source of green manure and not a tested species, in fact, its growth contributed to the final cover and biomass: for this reason, the measurements were taken also for this species. In particular, the following parameters were measured (instruments in brackets):

- Physiological measurements:
  - Chlorophyll content in SPAD units (MC-100 Chlorophyll Meter®, Apogee Instruments, North Logan, UT, USA)
- Biomass measurements:
  - Average fresh and dry weight of the plant biomass per unit area (Electronic balance Goldenwall, max = 5000 g, d = 0.1 g)

Chlorophyll content measurements were performed for each treatment on 30 randomly selected leaves for each species. Fresh and dry biomass was measured for each treatment using the total aboveground parts of the plants, distinguishing each species; the dry biomass was measured after keeping the samples at 70 °C until reaching a constant weight.

3. Results

The results of the monitoring of plant cover (Section 2.6) and of the final measurements (Section 2.7) are presented, in particular:

- Plant cover (variation in the percentage cover of each plant species over time).
- Plant biomass (dry and fresh weight of each plant species per area of the plot unit).
- Chlorophyll content (SPAD units).

Moreover, two comparisons between some values measured at the final time of the test are presented, specifically:

- Plant cover versus plant biomass.
- Plant water content versus plant dry biomass.

3.1. Plant Cover

Figure 4 shows the evolution of percentage cover of the three plant species as they grew under each treatment over time; the pictures for percentage cover measurements were taken on four different dates (see Section 2.6).

M. officinalis performed better when growing with clover roots (MRO, Figure 4a) than with clover roots and biomass (MRB, Figure 4b); moreover, it performed better when growing alone on the fresh substrate with clover biomass (MBN, Figure 4c) than with low-release fertilizer (MCK, Figure 4d). Overall, *M. officinalis* responded with a positive growth trend to all four treatments; in the fresh substrate with clover biomass, the final cover was complete (Figure 4c). In co-cultivation (Figure 4a,b), *M. officinalis* showed a clear trend toward reducing the area covered by clover, particularly at T1 and T2.

Compared with *M. officinalis*, *H. perforatum* showed a lower covering performance; only in the substrate with clover roots the growth trends and cover percentage values of the two species were comparable (HRO, Figure 4e). In Figure 4e, the cover percentage values of *H. perforatum* were the highest, while the lowest percentages were in the control (HCK, Figure 4h). In the other two treatments (Figure 4f,g), *H. perforatum* showed very similar trends and cover values over time; in the treatment with clover roots and biomass (HRB, Figure 4f), it did not seem to reduce or keep constant the area covered by clover as in the substrate with clover roots (HRO, Figure 4e). Overall, in no treatments *H. perforatum* managed to reach a 100% cover, neither alone nor coupled with *T. repens*.

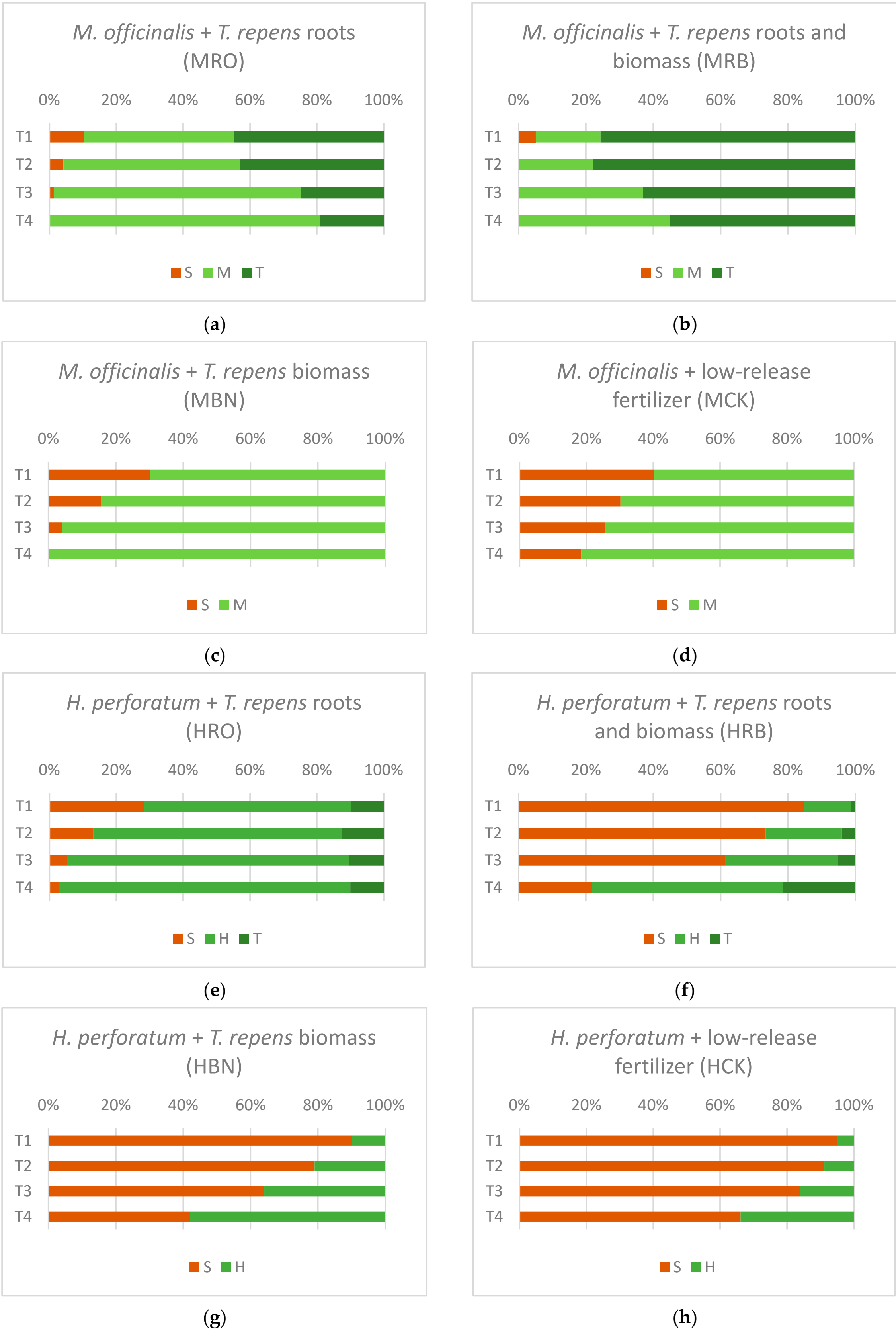


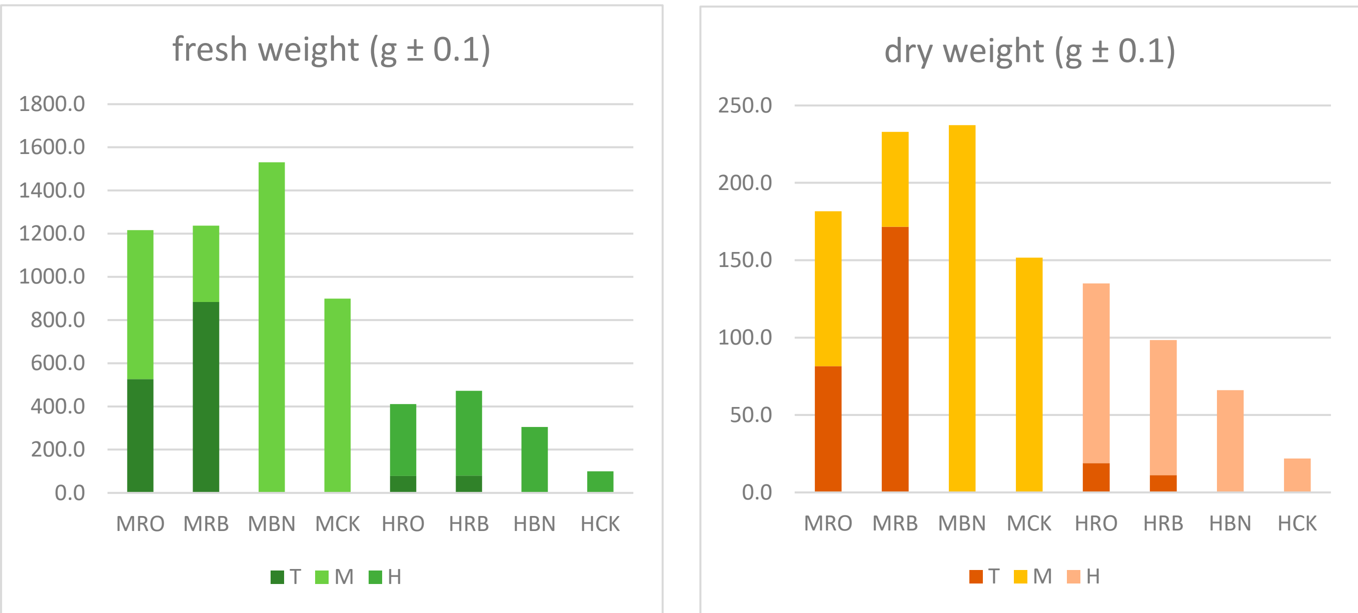
Figure 4. Variation in cover percentages (%) of the three plant species under each treatment over time (T1, T2, T3, T4: times of measurements, see Section 2.6). Each chart shows the cover percentage of plants and uncovered substrate surface (if present) at the four times of measurement, under the specific treatment indicated in the subfigure title (**a**–**h**). S = substrate, H = *H. perforatum*, M = *M. officinalis*, T = *T. repens*. For details about treatments, see Section 2.4 and abbreviations.

T. repens, in addition to being the source of green manure, also contributed to the total biomass and cover (see Section 2.7) and showed the most diverse trends and cover values across treatments and over time. When growing with common balm (Figure 4a,b), it tended to expand its covering at first (T1–T2), then its cover shrank as common balm's cover increased (T3–T4). The same trend was observed either with or without the addition of dried biomass; the only differences occurred in relative cover values, since, in the treatment with the biomass (MRB, Figure 4b), the cover percentage of *T. repens* was higher than that of *M. officinalis* in all four measurements, while, in the treatment without the biomass (MRO, Figure 4a), *M. officinalis* always showed higher values of cover. When growing with *H. perforatum*, *T. repens* showed two contrasting trends. In the treatment without the biomass (HRO, Figure 4e), it maintained a low cover percentage over time, with only a slight increase followed by a decrease, and very similar values at T1 and at T4 (slightly less than 10%). In the treatment with the added biomass (HRB, Figure 4f), it started with a cover less than 5%, then this percentage showed a slow increase until the final T4 measurement, with a sharp increase up to around 21%. This pattern follows that of *H. perforatum* in the same treatment, but with definitely lower values.

Overall, the best cover results were shown by treatments involving the substrate with the clover, either as roots or as roots plus aboveground biomass. For each species, the highest uncovered percentages at T4 occurred when the fresh substrate with the fertilizer was used (Figure 4d,h), while intermediate values at T4 were shown by *H. perforatum* in treatments with substrates containing roots only (Figure 4f,g).

3.2. Plant Biomass

Figure 5 shows the fresh weight and the dry weight of the total aboveground biomass of each plant species in all the treatments at the end of the test.



(**a**) (**b**)

Figure 5. Fresh weight (**a**) and dry weight (**b**) of the total aboveground biomass of each plant species and treatment at the end of the test. See the abbreviations for the extended names of the treatments and plants.

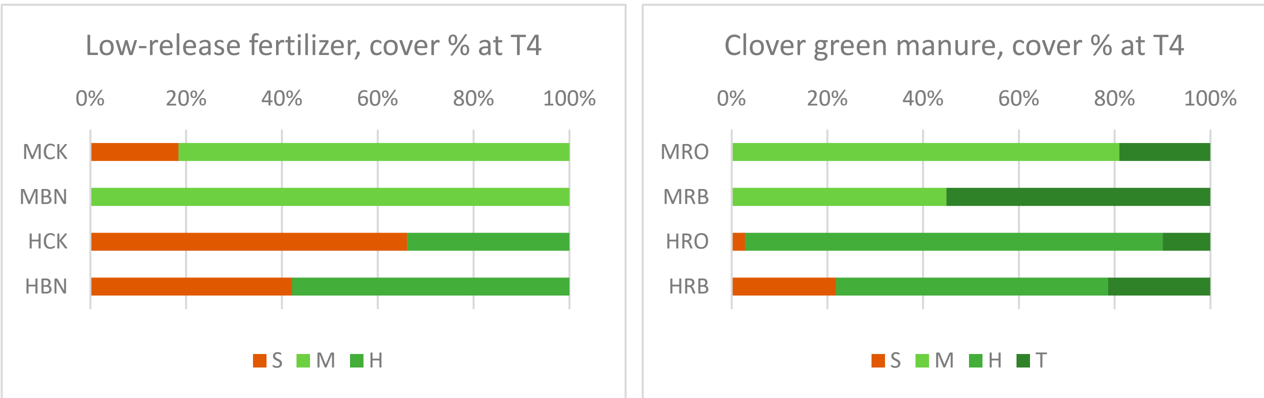
Overall, *M. officinalis* showed the highest values of fresh and dry weight for plants grown on the new substrate (MBN and MCK). Interestingly, here, the highest value occurred when the biomass was added rather than the fertilizer. The opposite was true for the old substrate with the clover roots: there, lower values were shown when biomass was added (MRB).

H. perforatum showed the lowest values of fresh weight and dry weight for plants grown on the new substrate when the biomass and the fertilizer were added, separately (HBN and HCK, respectively), with values slightly higher in HBN than in HCK. Higher values were registered when *H. perforatum* was grown on substrates with clover: here, the highest fresh weight value was measured where biomass was added (HRB), while the highest dry weight value was shown in the treatment without the added biomass (HRO).

Fresh and dry biomass showed higher values in *T. repens* when grown with *M. officinalis*: both values were higher in treatments where aboveground biomass was added (MRB). This was not the case when clover was grown associated with *H. perforatum* (HRO and HRB): here, far lower biomass values were measured, with little difference between the two fresh weights, while the dry weight showed lower values when aboveground biomass was added (HRB) compared to when it was not (HRO).

3.3. Plant Cover vs. Plant Biomass at T4

In Figure 6, all the cover values at T4 are summarized to allow for a comparison between plant cover and plant biomass (see Figure 5 in the previous section for plant biomass).



(**a**) (**b**)

Figure 6. Comparison of cover percentage of all plants at T4 (end of the test, see Section 2.6) grown under treatments with chemical fertilization (**a**) and with green manure (**b**). Cover percentages: S = substrate, H = *H. perforatum*, M = *M. officinalis*, T = *T. repens*. For more details, see the abbreviations.

Qualitatively, the same relative differences seen in the cover percentage were found as corresponding relative differences in fresh biomass, both among different treatments and among different plants inside each treatment. The same was true for dry biomass, apart from the HRB and HRO treatments, where the latter showed slightly higher values than the former. Since a linear relationship between surface plant cover and total aboveground biomass has been demonstrated [55], the observed qualitative similarity at T4 suggests that the two parameters support each other; however, a further experimental test involving quantitative measurements and absolute cover should be conducted to test the validity of the linear relationship in our specific case.

3.4. Water Content Versus Dry Biomass at T4

In Figure 7, the percentage ratio of dry biomass to water content is shown, calculated for the total aboveground biomass of each plant species and treatment at the end of the test.

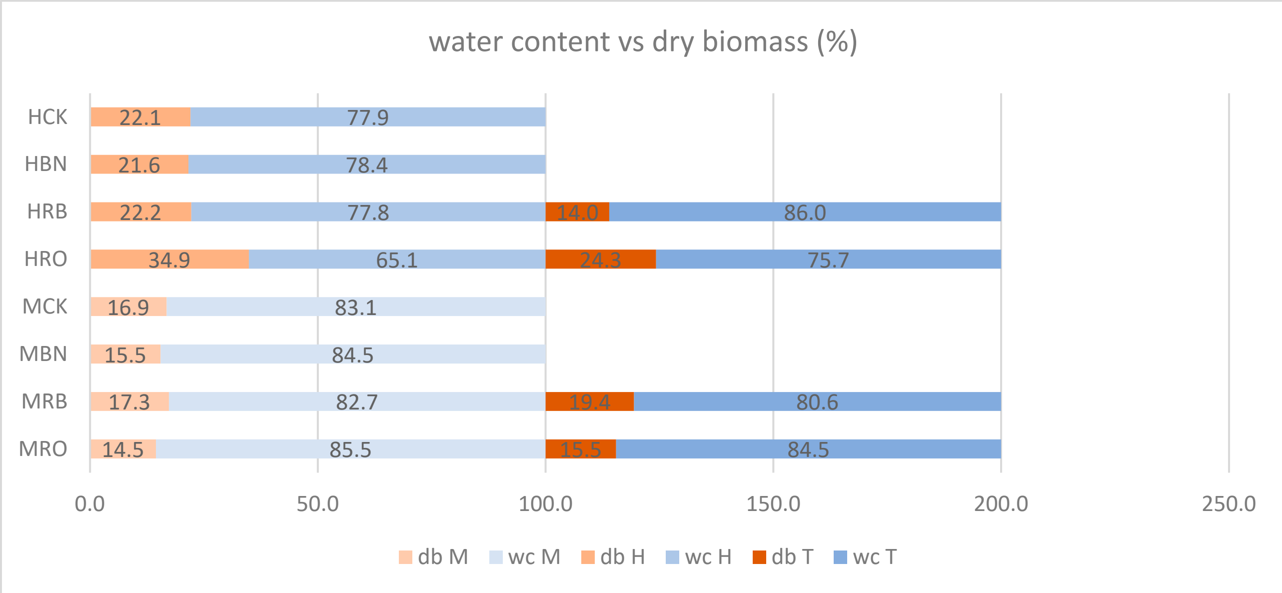


Figure 7. Biomass and water content ratios of the total aboveground biomass of each plant species grown under different treatments at the end of the test. See the abbreviations for the extended names of treatments and plants.

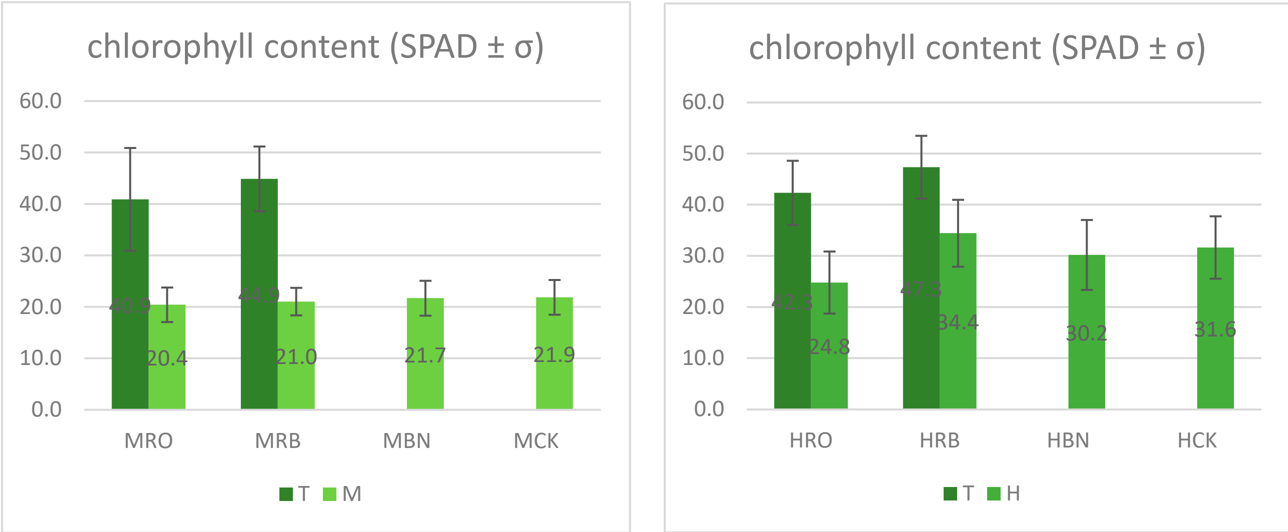
Overall, the water content showed higher values in *M. officinalis* and lower values in *H. perforatum*; comparing these two species, the lowest value was shown by *H. perforatum* grown on the substrate with clover roots (HRO), and the highest value was shown by *M. officinalis* in the same treatment (MRO). *T. repens*, when present, reached intermediate average values relative to the other plants; however, its performance was less uniform among treatments, since the highest water content was 86% in HRB, comparable to the highest value for *M. officinalis* (MRO), and the lowest was 76%, comparable with the average value of *H. perforatum*. Interestingly, the lowest water content values were in the HRO treatment for both *T. repens* and *H. perforatum*: this coincides with the lowest cover percentage of *T. repens*, but with no other corresponding low values for *H. perforatum* parameters.

3.5. Chlorophyll Content

Figure 8 shows the average chlorophyll content of all the plant species in each treatment at the end of the test. The content is given in SPAD units and was calculated as the average of 30 random measurements per species taken on the whole plot canopy, without considering individual plants.

Overall, no species showed significant differences among treatments. *M. officinalis* reached slightly higher values in treatments with the fresh substrate (MBN and MCK), but with no difference between the treatment with the biomass and that with the fertilizer. The chlorophyll content of *H. perforatum* showed the highest value in HRB and the lowest in HRO, while the treatments with the fresh substrate produced intermediate values (HBN and HCK), with a slight, non-significant difference between the two fertilizations. *H. perforatum* reached higher overall values than those of *M. officinalis* as well as a wider range of values, with significant differences between the HRB and the HRB treatments and between the HCK and the MCK treatments, respectively.

The chlorophyll content values of *T. repens* were comparable in all the treatments where it was present, slightly higher in the plots where clover biomass had been added (MRB and HRB). *T. repens* was the only species showing significantly higher values than the other two in all treatments, with by far the highest chlorophyll content in the whole test.



(**a**) (**b**)

Figure 8. Chlorophyll content of the different plant species in each treatment (*M. officinalis* and *T. repens* (**a**), *Hypericum perforatum* and *T. repens* (**b**)) (SPAD units) at the end of the test. See the abbreviations for the extended names of treatments and plants.

Interestingly, the chlorophyll content of plants in treatments with added clover biomass was comparable to that of the plants treated with the chemical fertilizer or even higher, as in HRB versus other *H. perforatum* treatments. Since chlorophyll content is related to nitrogen nutrition [56–59], this implies that no significant differences in N intake were shown by *M. officinalis* and *H. perforatum* at the end of the two treatments.

4. Discussion

The growth performance of *Melissa officinalis* and *Hypericum perforatum* on experimental green roof substrates enriched with green manures made from *T. repens* biomass confirmed the possibility of obtaining good levels of cover and biomass in the first stages of cultivation by reducing the use of chemical fertilization. Overall, biomass, cover ratio, and chlorophyll content measurements indicate that the species studied performed better when treated with green manure than when treated with low-release fertilizer.

M. officinalis performed best in terms of fresh and dry biomass when grown on fresh substrates, producing higher biomass in treatments with added clover biomass than in treatments with the fertilizer. It showed also a good performance in cover rates when grown with clover roots, with a trend that suggests the ability of this species to limit the expansion of *T. repens* over time. *H. perforatum* showed lower overall values of biomass and cover percentage; however, it performed better when growing on substrates with clover roots, suggesting some positive effects of the green manure in those cases. While not as good as *M. officinalis* in cover performance, it seems to be able to withstand the expansion of *T. repens* over time.

The calculated water content showed no significant pattern correlated with the different treatments. All values were higher than 65%, indicating a high potential for heat mitigation performance in all species and treatments, since water content is a measurement of water reserves in the plant and contributes to the total water balance following the equation:

$$B = (A - E) + W$$

where B is the water balance in the plant, A is the amount of water absorbed by the roots, E is the liquid loss during evapotranspiration, and W is the water reserves in the plant [5].

However, further studies testing the response to water stress and heat stress in terms of evapotranspiration might better clarify which are the best species for use in Mediterranean green roofs.

The chlorophyll content, measured in SPAD units, showed clear different ranges in the three species, with the highest values in *T. repens*, the lowest in *M. officinalis*, and intermediate values in *H. perforatum*; however, the absence of significant differences among treatments for each single species indicates that the nitrogen intake was not affected by the treatment. This may seem in contrast to the observed differences in cover percentages and biomass: more specific studies can be conducted to explain this result.

Overall, the percentage cover results discussed above suggest that, in the first stages of a green roof setting, sowing a fast growing, N-fixating plant like *T. repens* and then mowing it and using the cut biomass as a green fertilizer might be a convenient alternative to conventional fertilization. Adding biomass from mowed green roofs or from other green urban areas may be a solution also for disposing of cut biomass by using it as fertilizer and/or soil conditioner. In addition to its low release of nitrogen and enrichment in moisture and organic matter, biomass from clovers like *T. repens* may also be a source of microbiological enrichment of the substrate. Even in those cases where the presence of white clover could be seen as a weed by those in charge of green roof maintenance activities (due to its rapid growth, particularly in challenging edaphic and microclimatic conditions), mowing it and using the derived biomass as green manure, as the results of this experiment suggest, may be a far preferable and cheaper alternative to uprooting or to the use of herbicides. Moreover, in those same cases, limiting the vigorous growth of white clover can allow or enhance the first stage of development of other plants that, in later stages, can withstand the clover's competition while taking advantage of its nitrogen-fixating activity. The comparable chlorophyll content of the plants treated by adding clover biomass and the plants treated with chemical fertilizer further supports the use of *T. repens* as a valid alternative to low-release chemical fertilization.

While the corresponding differences observed in percentage cover and total biomass at T4 (Section 3.3) are only qualitative, they suggest some interesting remarks about the efficacy of the two plant species with respect to green roof performance. Plant cover on green roofs is involved in direct heat reduction through surface shading, while fresh biomass is related to cooling through both physical properties (thermal mass and high specific heat capacity of water) and physiological processes (evapotranspiration) of living plants. Thus, the good cover percentages and the corresponding biomass values of the two species confirm their potential value for cooling and heat reduction.

5. Conclusions

- The main evidence provided by this experimental test is that treatments with green manure produced better performances than treatment with low-release fertilizer in the tested plant species: this suggests that green manure can be profitable for green roof management, both when the aim is to use a single, fast-growing species like the white clover to obtain the highest biomass and when the aim is to use other species like lemon balm or St. John's wort coupled with white clover to obtain the highest cover. The last option has the advantages of allowing a total cover in a shorter time and of managing the growth of white clover that can be integrated into a green roof's vegetation in case it starts growing as a weed; this may happen in extensive green roofs due to their specific conditions of substrate and microclimate.
- The above suggested management of white clover can be a model for dealing with other weeds and makes a contribution to the elimination of chemicals in green urban systems, both as fertilizers and as herbicides. It can also be applied to similar condi-

tions in different countries with Mediterranean-like climates, as well as to analogous conditions in different climates, since *T. repens* is a species which has a wide native range and has been introduced in many countries. In countries where *T. repens* is either absent or regarded as an invasive species, like in Australia, the management suggested by the results of this test can be applied by replacing clover with local, native species with corresponding ecomorphological traits.

- The same can be told for *M. officinalis* and *H. perforatum*: they can be used in regions where they are spontaneous or naturalized, otherwise they can be replaced by other species with similar traits. However, regarding all medicinal plant species, it is important to highlight that the balance between risks and benefits of cultivating these plants in urban areas should always be evaluated very carefully, due to their contrasting properties of being both efficient bioaccumulators of pollutants and sources of useful substances used for human consumption. In order to grow these plants in safe conditions for human use, contamination from pollution sources should be prevented, and proper techniques for monitoring environmental pollutants, testing their presence inside the plants, and extracting and purifying the metabolites should be adopted. Alternatively, medicinal plants should be grown in locations with no significant presence of industrial enterprises or man-made pollution, and with a minimal number of vehicles.
- The present experimental test deals with the first stage of vegetation development in controlled, experimental green roof modules. Further studies can show what might be the growth trends of the selected species over longer time periods (one or more full growing seasons), as well their response to conditions different from those of a controlled environment: this last point is essential to evaluate the feasibility and practicalities of the suggested management model in full-scale urban green roof systems.

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Abbreviations

The following abbreviations are used in this manuscript:

db	Dry biomass
wc	Water content
H	*Hypericum perforatum*
M	*Melissa officinalis*
S	Substrate
T	*T. repens*

MRO	*M. officinalis* sown on substrate with *T. repens* roots
MRB	*M. officinalis* sown on substrate with *T. repens* roots and aboveground biomass
MBN	*M. officinalis* sown on new substrate with *T. repens* aboveground biomass
MCK	*M. officinalis* sown on new substrate with low-release fertilizer
HRO	*H. perforatum* sown on substrate with *T. repens* roots
HRB	*H. perforatum* sown on substrate with *T. repens* roots and aboveground biomass
HBN	*H. perforatum* sown on new substrate with *T. repens* aboveground biomass
HCK	*H. perforatum* sown on new substrate with low-release fertilizer

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