Nitrogen

Article

Influence of an Organic Fertilizer on Agronomic Characteristics and Herbaceous Plant Diversity in a Greek Ecosystem: The Case of Cretan Dittany (*Origanum dictamnus* L.)

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Abstract: In recent years, there has been a growing tendency towards using organic fertilizers instead of chemical ones. This study aimed to evaluate the influence of the organic fertilizer, Prima Humica, on agronomic characteristics and herbaceous plant diversity on *Origanum dictamnus* (Dittany) cultivation. A field experiment was carried out in Kalo Xorio (Lasithi, Crete) and included three different nitrogen organic fertilization schemes: 0 g/plant (T0—unfertilized), 300 g/plot (T1), and 600 g/plot (T2). Plant height, fresh and dry weight, Leaf Area Index (LAI), and plant diversity during the growing season were measured. The results showed that when the T2 treatment was compared with the unfertilized and the T1 fertilizer, the increase in plant height ranged between 9.18% and 40.61%. Moreover, the total fresh and dry weight were positively affected by the T2 treatment. The total fresh and dry weight varied from 111.6 to 239.8 g per plant and from 36.7 to 77.6 g per plant, respectively. Furthermore, LAI was ameliorated using the T2 fertilizer scheme. Concerning plant diversity, a key finding in this study is that the *O. dictamnus* ecosystem favors herbaceous plant species richness and Shannon's diversity index. In total, 18 plant species in the *O. dictamnus* ecosystem were recorded in the study area. The most frequently occurring plants were *Anthemis arvensis* L. and *Piptatherum miliaceum* (L.) Coss. Finally, the highest Shannon's diversity index of herbaceous plants was detected using the T2 fertilizer scheme.

Keywords: medicinal plant; nitrogen; height; yield; Leaf Area Index (LAI); weeds; Crete

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1. Introduction

Greece is a country in the Mediterranean basin with a rich and varied flora across its length and breadth. Such is the diversity that it comprises 6620 taxa, 5758 species, and 1970 subspecies, with 223 of these endemic taxa located on the southernmost island of Crete [1,2]. One of the most well-known medicinal plants native to Crete is *O. dictamnus*, also known as Dittany. With 75% of its species found only in the Eastern regions, the genus Origanum is highly represented in the Mediterranean region [3,4]. However, thus far, *O. dictamnus* has not received much attention from the scientific community, and unlike other Origanum species, not much research has been conducted on it.

Dittany is a perennial plant and member of the Lamiaceae family, recognized for its medicinal properties since ancient times. It grows wild at an elevation of 400–1700 m in phrygana, garigue, rocky areas, and openings in Pinus forests [5]. Nevertheless, nowadays, Dittany can also be cultivated on farms for its pharmaceutical properties [6]. People use the plant's aerial parts as an additive in the food industry [7] and drink its herbal infusion, referred to as "vrastari" in Crete. The word comes from the Greek verb "vrazo", meaning 'to boil'. This infusion is used to alleviate coughs, sore throats, stomach aches, and gastric diseases and to maintain good health [8–11].

The interest in aromatic herbs and medicinal plants is not, however, limited only to Greek culture. Over the last three decades, individuals have been concerned about the physical and chemical composition of the ingredients that are added to foods. In an effort to improve their overall health (both physically and mentally), customers have been increasingly selective about their food choices, leaning towards products derived from aromatic, medicinal plants (MAPs) with promising antioxidative, anti-inflammatory, and antimicrobial properties [12]. As a result, it is estimated that 75% of people on the planet regularly use different aromatic medicinal plants to fulfill a range of medical and health needs [13].

Different nutrient management and fertilization methods can have a beneficial or negative impact on plant development as well as the quality of the produced goods [14]. Nitrogen and phosphorus fertilizer additions can greatly affect plant growth. Fundamental processes in plant physiology, such as photosynthesis and leaf development, are positively affected by nitrogen [15,16]. In contrast, plant cell function and division and the development of their morphological characteristics are regulated by phosphorus [17]. However, prominent studies conclude that the food system is facing a crisis related to land usage, environmental degradation, and the sustainability of agriculture due to excessive fertilizer applications [18,19]. These have provoked numerous environmental and ecological problems, including soil acidification, soil quality degradation, nitrate pollution of surface and groundwater, air and soil contamination through nitrogen leaching, and water eutrophication [20–22]. Research suggests that there is insufficient nitrogen loss from agriculture to the environment to ensure a "safe operating space for humanity" [23]. If there are no major improvements made to the entire food production–consumption chain during the next few decades, factors such as the growing population and the intensified agriculture to cover the population's food demands will increase nitrogen fertilizer applications and exacerbate the trespassing of the "safe operating space" [23].

For this reason, farmers and regulatory agencies are proposing new agronomic management techniques and cutting-edge sustainable methods for the cultivation of MAPs that come at a low cost to the environment [24–27]. Emphasis is placed on the use of organic fertilizers, as they have been linked to increasing crop development and growth, preserving the quality of the soil and the soil's ability to hold water.

They serve as a productive source of energy for soil microorganisms, enhancing the microbial activity in the soil, resulting in better nutrient cycling, increased levels of organic matter, and an overall proliferated soil ecology, leading to an improved soil structure [28–30]. Organic fertilizers preserve the nutritional balance necessary for crop plants, allowing them to grow healthily by releasing nutrients into the soil solution gradually. Through this gradual release, they lessen potential environmental issues related to synthetic fertilization, such as run-offs, and, unlike synthetic fertilizers, they lessen the need for frequent applications to preserve soil fertility, resulting in higher yields and better quality produce [31]. The use of organic fertilizers in aromatic–medicinal plants (MAPs) cultivation is mentioned in many studies in the literature [16,32–35]. Furthermore, most of the studies related to Dittany cultivation refer to the effect of various factors on the essential oil of the plant. To our knowledge, no research has been reported on the influence of the cultivation of *O. dictamnus* under organic fertilization circumstances. Thus, this should be mentioned as a research gap that should be studied.

In this framework, the objective of this research was to examine the impact of organic fertilizer at different rates on the agronomic characteristics and herbaceous plant diversity of Dittany in the Crete region.

2. Materials and Methods

2.1. Site Description

The experiments were conducted in Crete (Greece), on the campus of the Department of Rural Development and Food, which is located in Istron Kalou Xoriou in the municipality of Agios Nikolaos Lasithiou. The campus has an altitude of 50 m, a latitude of 35°07′28.3″ and a longitude of 25°44′07.8″ (Figure 1).

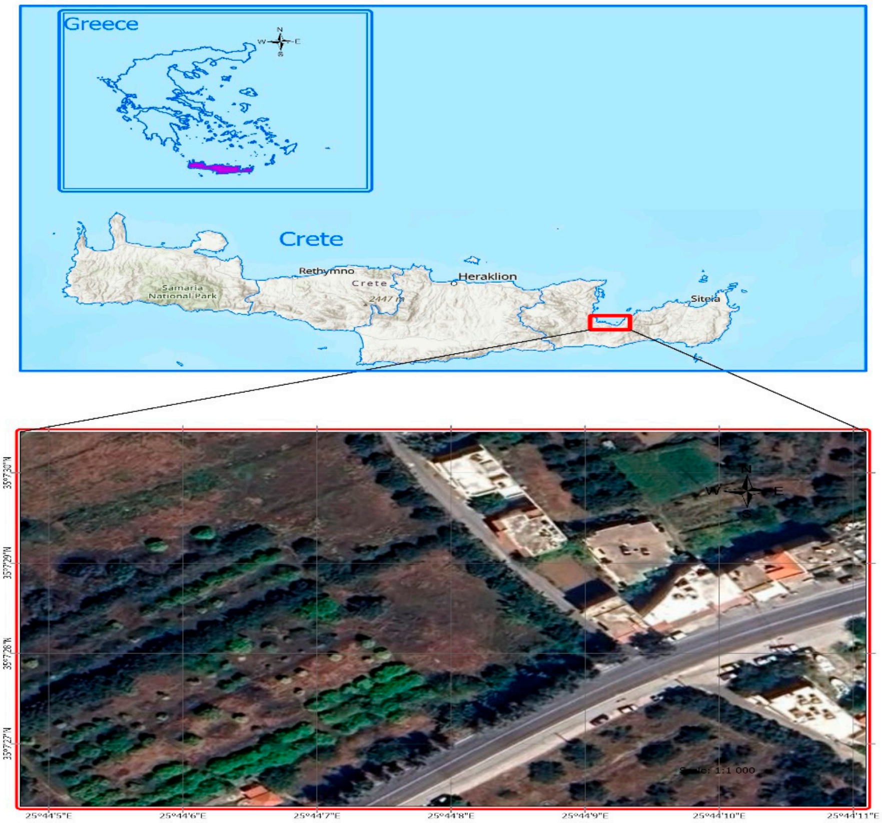

Figure 1. Study area.

2.2. Meteorological Data

The meteorological data were provided by the nearby meteorological station of the Institute of Environmental Research and Sustainable Development at the Hellenic National Observatory of Athens.

Average, maximum, and minimum air temperatures and total rainfall during the study period (May to September) are shown in Figures 2 and 3. The average air temperature ranged from 16.6 °C in May to 32.2 °C in July. Regarding precipitation, a total of 60 mm fell during the duration of the experiment. The highest rainfall was observed in June, while the lowest occurred in August.

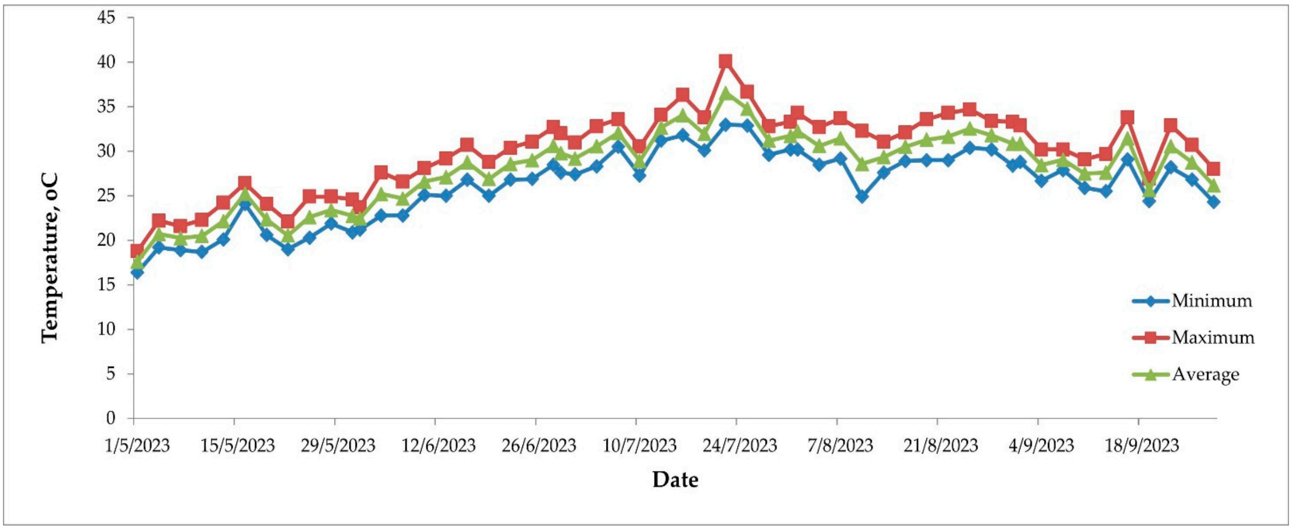

Figure 2. Air temperature (°C) during the study period (May 2023–September 2023).

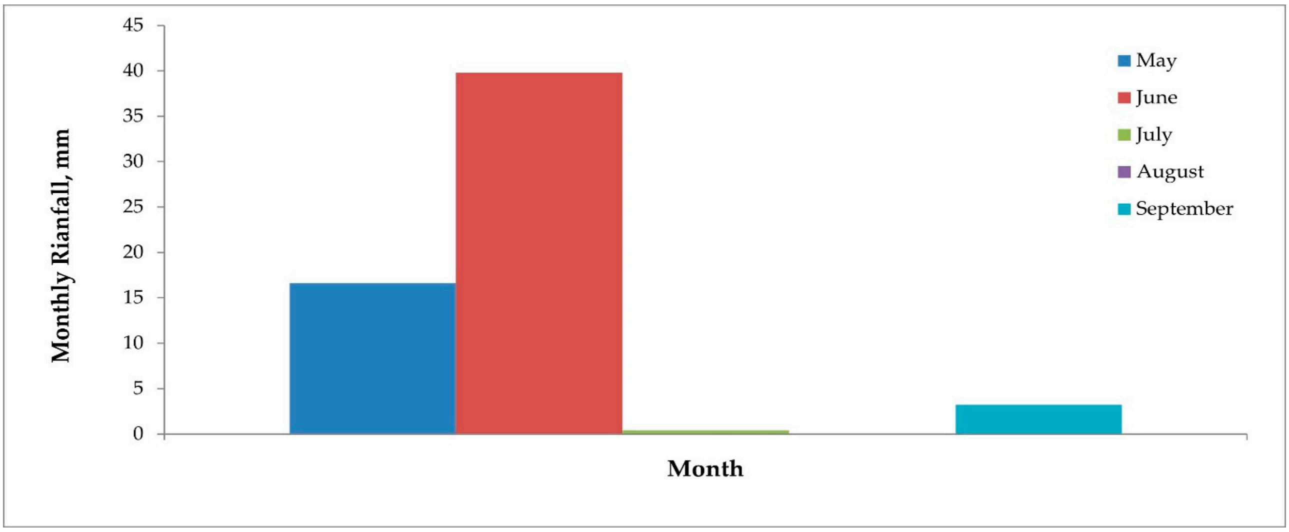

Figure 3. Precipitation (mm) during the study period (May 2023–September 2023).

2.3. Soil and Plant Analysis

Before conducting the experiment, five different sub-samples (0–20 cm) from the studied soil were collected and mixed to form a composite soil sample. The soil sample was then transferred to the Soil Laboratory of Hellenic Republic, Ministry of Rural Development and Food in Heraklion (Greece) for subsequent analysis. The sample was air-dried and passed through a 2 mm sieve. The following physicochemical parameters were determined: pH (1:2.5 d. H_2O), electrical conductivity (1:5 d. H_2O), calcium carbonate ($CaCO_3$) using a calcimeter, the percentage (%) of sand, clay, and silt using the Bouyoukos method [36], and organic matter using the Walkley–Black method [37]. Furthermore, the Kjeldahl method was used for measuring the total nitrogen (N) content [38], while the available soil phosphorus (P) was determined using the Olsen method [39] and analyzed with ammonium vanadomolybdate/ascorbic blue. The phosphorus content was measured in a UV spectrophotometer at 882 nm. Exchangeable potassium (K) was determined using a 1:10 extraction with 1 M CH_3COONH_4 at pH 7 [40] and analyzed using a flame photometer.

The physicochemical properties of the soil are presented in Table 1. The soil used in the experiment was sandy loam in texture and alkaline, with a pH of 7.31 and a calcium carbonate ($CaCO_3$) content of 3.98%. Moreover, according to the soil analysis results, the soil had a 2.25 % content of organic matter.

Table 1. Physicochemical parameters of the soil used in the experiment.

	0.30 cm
pH	7.31
E.C. (mS cm^{-1})	1.04
$CaCO_3$	3.98
Organic matter (%)	2.25
Total nitrogen (%)	0.08
Olsen P (mg kg^{-1})	20.08
Exchangeable K (mg kg^{-1})	162.63
Sand (%)	68.90
Clay (%)	12.20
Silt (%)	18.90

2.4. Establishment of Field Experiment and Measurements

Two-month-old Dittany plants were obtained from a local nursery (named Anagnostakis Ioannis, Sitia Crete) and were immediately transplanted to the field on 2 May 2023. The experimental period lasted from May to September 2023. The distance between plants was 40 cm and between rows 60 cm. Each plot was 0.24 m^2 in size. Fertilization was the primary component in the completely randomized blocks (CRB) experiment design. An organic fertilizer (named Prima Humica Ledra Fertilizers, Thessaloniki, Greece) was applied 10 days after the transplantation and immediately after the first harvest. The experiments included three nitrogen fertilization treatments: T0: 0 g/plot, T1: 300 g/plot, and T2: 600 g/plot of organic fertilizer. The organic fertilizer was composed of 68–78% organic matter, 5% total N, 3% P_2O_5, and 0.0% K_2O. Each treatment consisted of 10 replicates. A total of 30 plants were transplanted in the field.

An automated drip irrigation system with 2 L/h adjustable drippers was used to water the crops. Watering was conducted once every three days.

Measurements were taken to determine each plot's plant height. Two plant material cuttings were collected during the study period on 30 July 2023 and on 20 September 2023. The aboveground biomass was then collected and immediately weighed to record the fresh weight using a portable scale. Subsequently, the harvested plants of each plot were oven-dried at 40 °C until constant weight was achieved. Finally, the dry biomass was calculated.

Moreover, the Leaf Area Index (LAI) was calculated from 15 leaves that were harvested from the plant of each plot, and the measurement was conducted using an automatic LI-COR.

2.5. Herbaceous Plant Composition

Thirty 0.24 m^2 sites were used for the herbaceous plant sample. The abundance and composition of herbaceous plant species in organic *O. dictamnus* were noted in each plot [41].

2.6. Statistical Analysis

The statistical data analysis was performed using the software package "STATGRAPHICS Centurion" software package (v.18.1.01, Statgraphics Technologies, Inc., The Plains, VA, USA), with the LSD test conducted at a significance level of 95% ($p < 0.05$).

The Shannon plant diversity index was calculated using the Species Diversity and Richness IV software [30]. The Shannon index (SH) is a widely utilized index that considers both species abundance and species richness [42,43]. The SH was computed for every sample.

$$H' = -\sum_{i=1}^{s} P_i \text{In} P_i$$

where, s equals the number of species and P_i is the relative cover of its species. The Shannon diversity index value ranges from 1.5 to 3.5 [44,45] (for a detailed description of this index, see Seaby and Henderson [42]).

3. Results

3.1. Effect of Fertilization in Plant Height

Plant height data are illustrated in Figure 4. The mean plant height ranged from 6.69 to 9.4 cm in the first cutting and between 6.99 and 11.77 cm in the second. The highest plant height was observed using the T2 fertilization. Statistically significant differences emerged between the control and the different doses of the used fertilizer, both in two cuttings. Notably, over the 2nd cutting, the T2 treatment had statistically significant differences compared to the control and T1 plots. Furthermore, the increase between the dose of T2 and T1 was at a rate of 9.18% during the second harvest. Comparatively, when compared to T1 with control, there was a rise of 34.61% and 40.61% for T2 and T1, respectively.

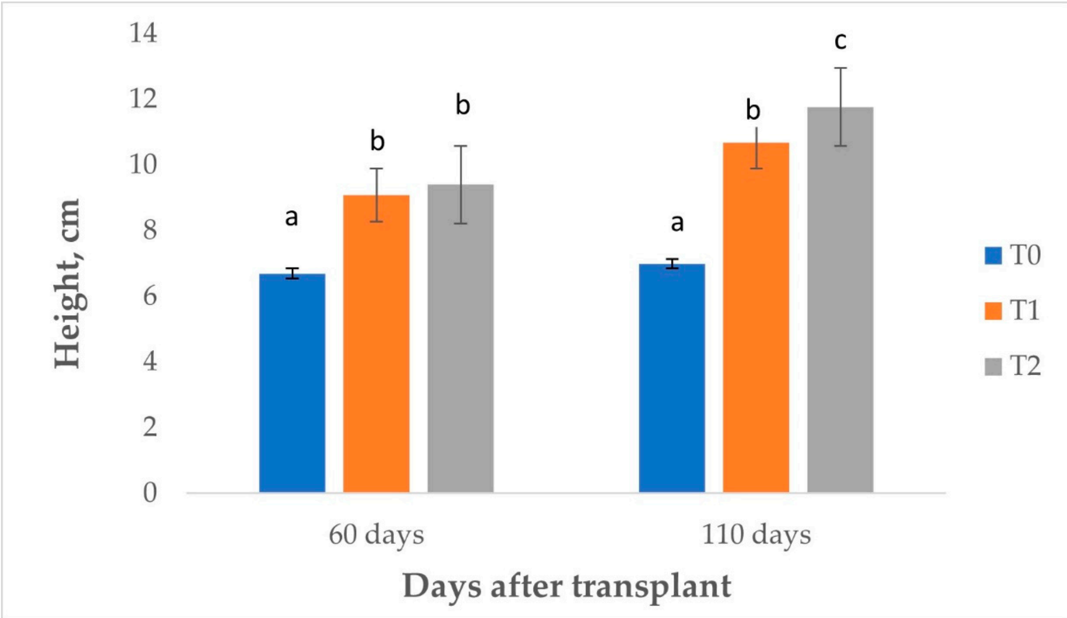

Figure 4. Plant height (cm) during the growing year under the different fertilization types. Different letters indicate statistically significant differences between the different fertilization schemes ($p < 0.05$).

3.2. Effect of Fertilization on Fresh and Dry Weight

Table 2 (fresh weight) and Table 3 (dry weight) show the total fresh and dry weight in two harvest times. The fresh and dry weight of Dittany plants increased significantly as a result of the various fertilization strategies applied. Both of the first two cuttings' fresh weights (120.8 and 119.6 g/plant, respectively) were greater in the T2 treatment. When compared to the control (T0), the yield from the T2 fertilizer increased from 53.5% in the second harvest to 53.6% in the first. Furthermore, compared to the T1 application, the T2 treatment increased the fresh weight by 17.3 to 17.8%. Moreover, a statistically significant distinction was observed between the two harvests, T2 and T1. The dry weight was arranged in the following order: T0 (36.7 g/plant) < T1 (65.2 g/plant) < T2 (77.6 g/plant) being the highest. The two different fertilizer doses, T1 and T2, increased the total dry weight by 52.7% and 43.7%, respectively, over the control (T0). Additionally, T2 increased the dry weight by 16% compared to T1 and T2 treatments. Also, statistically significant variations were noted between T2 and T1.

Table 2. The fresh weight of the Dittany plants (g/plant) under the different fertilization types.

Treatments	Fresh Weight (g/Plant)	
	Days after Transplant	
	60 Days	110 Days
T0	56.0 a	55.6 a
T1	99.3 b	98.9 b
T2	120.8 c	119.6 c
LSD	0.9451	1.0613

Different letters indicate statistically significant differences between the different fertilization schemes ($p < 0.05$).

Table 3. The dry weight of the Dittany plants (g/plant) under the different fertilization types.

Treatments	Dry Weight (g/Plant)	
	Days after Transplant	
	60 Days	110 Days
T0	18.5 a	18.2 a
T1	32.9 b	32.2 b
T2	39.1 b	38.5 c
LSD	0.3643	0.3678

Different letters indicate statistically significant differences between the different fertilization schemes ($p < 0.05$).

3.3. Effect of Fertilization in Leaf Area Index (LAI)

The findings regarding the Leaf Area Index (LAI) results are shown in Figure 5. LAI varied between 1.76 and 2.26 30 days after transplantation, from 2.05 to 2.91 at the end of July, and from 1.68 to 2.59 in September (110 days after transplant). Throughout the course of the experiment, the highest values of LAI were observed in the T2 fertilization treatment (600 g/plot). Compared to the T1 treatment, an increase was observed in the T2 plots, from 3.48% in the 1st measurement to 11.49% in the 3rd.

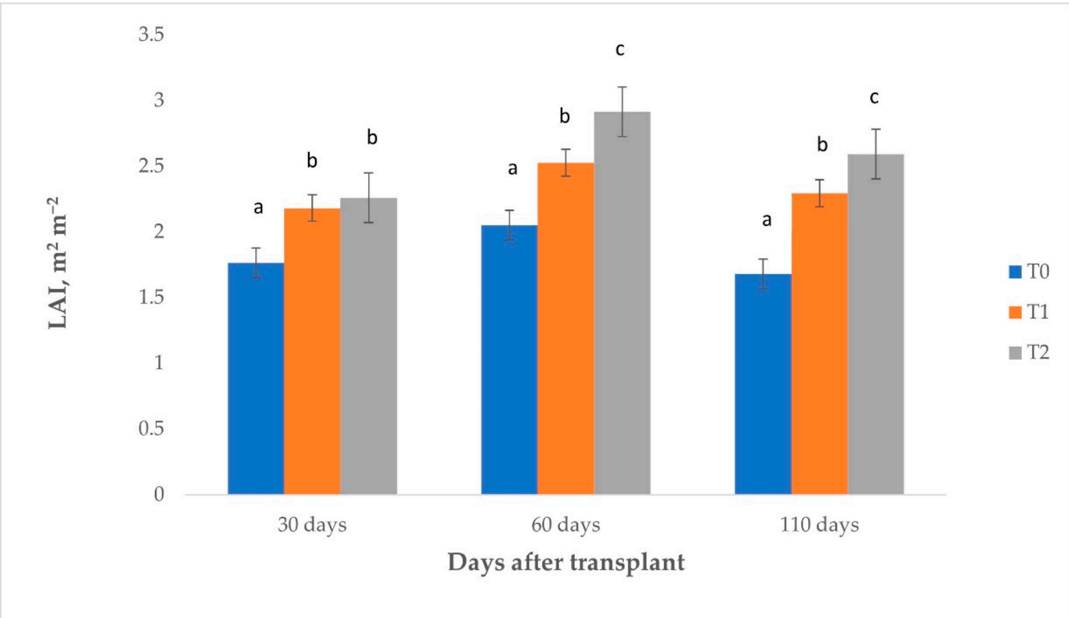

Figure 5. Leaf Area Index (LAI) of the Dittany cultivation during the growing year under the different fertilization types. Different letters indicate statistically significant differences between the different fertilization schemes ($p < 0.05$).

3.4. Herbaceous Plant Composition and Diversity

In total, 18 plant species in the *O. dictamnus* ecosystem were recorded in the plots of the study area (Table 4). The most frequently occurring plants species, which have the same distribution (Figure 6), were *Anthemis arvensis* L. (family: Asteraceae) (status: native; chorology: [Cosmopolitan], European-SW Asian; life-form: therophyte; habitat: agricultural and ruderal habitats) and *Piptatherum miliaceum* (L.) Coss. (family: Poaceae) (status: native; chorology: Mediterranean; life-form: Hemicryptophyte, Chamaephyte; habitat: Xeric Mediterranean Phrygana and grasslands, woodlands and scrub, agricultural and ruderal habitats) in the *O. dictamnus* ecosystem. Also, herbaceous plant species richness and Shannon's diversity index were determined using the T2 fertilizer scheme (Tables 5 and 6).

Table 4. Herbaceous plant species in *O. dictamnus* ecosystem.

Herbaceous Plant Species	Family	Relative Abundance
Aegilops geniculata Roth	Poaceae	5.26
Amaranthus graecizans L.	Amaranthaceae	1.75
Anagallis arvensis L.	Primulaceae	3.51
Anthemis arvensis L.	Asteraceae	21.05
Avena sterilis L.	Poaceae	5.26
Capsella bursa-pastoris (L.) Medik.	Brassicaceae	7.02
Convolvulus arvensis L.	Convolvulaceae	3.51
Daucus carota L.	Apiaceae	7.02
Hordeum murinum L.	Poaceae	1.75
Lolium rigidum Gaudin	Poaceae	1.75
Oxalis pes-caprae L.	Oxalidaceae	1.75
Papaver rhoeas L.	Papaveraceae	1.75
Piptatherum miliaceum (L.) Coss.	Poaceae	17.54
Setaria viridis (L.) P. Beauv.	Poaceae	1.75
Silybum marianum (L.) Gaertn.	Asteraceae	3.51
Sinapis arvensis L.	Brassicaceae	3.51
Sorghum halepense (L.) Pers.	Poaceae	5.26
Urtica pilulifera L.	Urticaceae	7.02
Total: 18	Total: 10	Total: 100

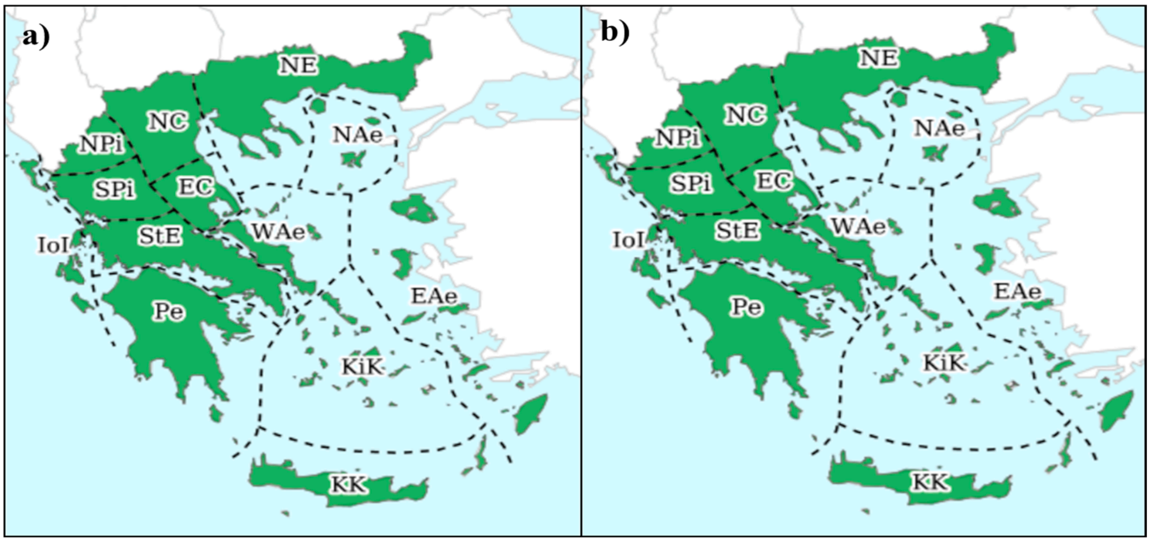

Figure 6. The distribution of plant species with the highest relative abundance in Greece. (**a**) *Anthemis arvensis* L. (left) and (**b**) *Piptatherum miliaceum* (L.) (right). (IoI: Ionian Islands, NPi: North Pindos, SPi: South Pindos, Pe: Peloponnisos, StE: Sterea Ellas, EC: East Central Greece, NC: North Central Greece, NE: North-East Greece, NAe: North Aegean Islands, WAe: West Aegean Islands, Kik: Kiklades, KK: Kriti and Karpathos, EAe: East Aegean Islands) [46].

Table 5. Herbaceous plant species in each treatment.

Treatments	Number of Herbaceous Plant Species
T0	9
T1	14
T2	18

Table 6. Herbaceous plant species diversity in each treatment.

Treatments	Shannon Diversity Index
T0	1.83
T1	2.14
T2	2.41

4. Discussion

4.1. Effect of Organic Fertilization in Plant Height, Fresh and Dry Weight, and in LAI

In this research, the effect of an organic fertilizer on plant height, fresh and dry yield, Leaf Area Index (LAI), and herbaceous plant diversity of Dittany (*O. dictamnus*) cultivation was investigated. In recent years, there has been a growing awareness of agriculture practices that offer not only quality products but also demonstrate a commitment to environmental responsibility and the use of environmentally friendly fertilizers [47].

The findings indicated that the T2 fertilization scheme (600 g per plot) resulted in higher values in plant height, total fresh and dry weight, and Leaf Area Index (LAI). Concerning the plant height in the 1st cutting, no significant reference was observed between the T2 and T1 treatments. On the other hand, in the 2nd harvest among the T2 and T1 plots, statistically significant references were noticed. The organic fertilizer has the advantage of releasing the nutrients gradually. This slower release process of nutrients facilitates the plants to assimilate more effectively the nutrients in a natural way, promoting a balanced and sustainable growth of plants without the negative effects associated with excessive nutrient uptake [48,49].

Organic fertilizer affected positively the total yield (fresh and dry) of plants, evident across two cutting periods. Both T2 and T1 treatments increased the yield compared to the control. Specifically, the T2 fertilizer dose led to a 16.0% increase in total fresh yield and a 17.3% increase in total dry yield compared to T1. This increase in yield, observed with the application of 600 g per plot, can be attributed to the higher number of branches observed in these plots.

Only a few investigations have studied the cultivation of Dittany (*O. dictamnus*) by applying organic fertilization, Bilias et al. [14] studied five different fertilizer schemes (two by foliar and three by soil application), and according to their results, the conventional inorganic fertilizer by soil application scheme is in agreement with our data concerning the yield of *O. dictamnus*. Moreover, Martini et al. [6] assessed the development of Dittany under different urban locations and the safety for human consumption was investigated. Hence, it can be inferred that the use of an organic fertilizer can positively impact the yield of Dittany cultivation.

The Leaf Area Index is considered to be one of the most important variables in the process of photosynthesis and respiration [50]. Moreover, LAI is recognized as a fundamental characteristic of vegetation that can directly affect plant growth and yield [51]. As for the Leaf Area Index (LAI) in Dittany cultivation, there is a lack of prior research. In this study LAI was affected by the doses of fertilizer and the results revealed that the T2 treatment has significantly increased LAI. The positive effect of the organic fertilizers in LAI in other cultivations has been recorded by other investigations [52–55].

4.2. Herbaceous Plant Composition, Species Richness and Diversity

The agricultural landscape represents a cultural milieu shaped by human activities over time. Agroecosystems, constituting fundamental elements of rural landscapes, harbor diverse floral populations, thereby conferring upon them the status of agricultural systems with pronounced ecological significance, denoted as high-nature-value farming systems. Among these, the prevalent flora within the *O. dictamnus* ecosystem primarily comprises *A. arvensis* and *P. miliaceum*, as identified by Dimopoulos et al. [46]. Notably, these plant species typify agroecosystems. Encroachments by *A. arvensis* and *P. miliaceum* have already been documented in several conducive regions of Greece.

Also, the highest herbaceous plant species richness and Shannon diversity index were detected using the T2 fertilizer scheme. According to [56], the primary determinants shaping herbaceous plant species composition are management practices and environmental variables. It is worth highlighting that plants serve as pivotal indicators of environmental well-being, acting as conduits between the soil and the atmosphere. While plants predominantly assimilate nutrients from the soil, their physiological processes are directly influenced by atmospheric conditions, as they serve as collectors of gases. Consequently, assessing the chemical makeup of plants thriving in compromised environments compared to those in pristine conditions serves as a crucial method for evaluating contamination levels in areas of plant growth.

Based on Tichý [57] and Zolotova [58], the plant species *A. arvensis* and *P. miliaceum* exhibit ecological indicator values that elucidate the environmental conditions conducive to their growth. More specifically, they are plants that generally prefer light and fertile soils. Also, they have moderate moisture requirements and are indicators of biotopes with moderate availability of nutrients. This information is very important for farmers regarding crop management decision making.

5. Conclusions

In this study, the cultivation of *O. dictamnus* was investigated under different organic fertilization schemes.

In general, T2 nitrogen fertilization (600 g per plot) resulted in higher values of plant height, fresh and dry weight, and LAI. This implies that the optimal dosage of an organic fertilizer for *O. dictamnus* cultivation corresponds to a quantity of 600 g/plot.

As for the plant diversity in Dittany cultivation, in this research it was noticed that within the *O. dictamnus* ecosystem, the *Anthemis arvensis* L. and *Piptatherum miliaceum* (L.) Coss species are promoted. Also, the highest herbaceous plant species richness and Shannon diversity index were detected using the T2 fertilizer scheme.

The data from this study can be used for the development of a healthier fertilization program under the framework of organic practices. Furthermore, the information in this research provides a base for sustainable and eco-friendly agricultural systems.

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