



Weed flora composition in a young olive orchard as affected by fertilizer types

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Abstract Manure is a useful source of nutrients in olive orchards, but its effect on weed growth in such an ecosystem has not been studied. A field experiment was conducted in a six-year old olive orchard (cv. *Chondrelia Chalkidikis*) in northern Greece to study the effects of two forms of fertilization (inorganic fertilizer and composted cow manure) on weeds abundance and weed flora composition (species diversity and dominance). A randomized complete block design with three treatments (inorganic fertilizer, 150 kg N ha⁻¹ plus 205 kg P₂O₅ ha⁻¹ plus 205 kg K₂O; composted cow manure, 20 Mg dry weight ha⁻¹; and control) replicated three times was used. Inorganic fertilizer and composted cow manure had no significant effect on the number of weed species ($F=0.288$, $P>0.05$), but improved olive fruit yield by 61.6% and 57.1%, respectively, compared with the non-fertilized control. Both inorganic fertilizer and composted cow manure increased the weeds dry biomass by 133.4% and 80.8%, respectively, compared with the non-fertilized control. There was a decrease in the biomass proportion (percentage of the total weed biomass) of annual species with composted cow manure ($\chi^2=6.72$, $P<0.05$), while no significant change in the biomass ratio of grasses and broadleaf weeds was observed. Fertilization increased the growth of *Chenopodium*

album, mainly with the addition of inorganic fertilization; and *Sonchus oleraceus*, slightly more with the addition of composted cow manure. *Centaurea solstitialis* and *Daucus carota* had a high proportion only in the non-fertilized control plots. Composted cow manure can be used as an alternative fertilizer in olive orchards, without enriching weed flora in terms of species number. Long-term experiments would be useful to confirm the influence of composted cow manure on weed flora of olive orchards.

Keywords Organic fertilization · species diversity · species richness

Introduction

Livestock manure is an important resource of organic material, plant nutrients, and useful microorganisms. It is a valuable material for farmers because the nutrients it contains facilitate plant growth and the organic material improves soil quality (Du et al., 2020). Nevertheless, the nutrient content of manure is inherently variable and may not support optimal crop production (Miller et al., 2019; Scotti et al., 2015). Manure is also costly to transport, as several crops may be cultivated at some distance from livestock farms (Kamilaris et al., 2020). In addition, manure often contains pathogenic organisms and releases odors that may offend neighbors. Livestock manure can also affect weed population in farm fields, as it can add viable weed

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seeds into the seedbank, if not properly composted (Tompkins et al., 1998).

Cow manure, preferably the composted form, is a common type of livestock manure that has been used in agricultural fields, especially under organic farming (Alaghemand et al., 2017; Mavandi et al., 2021). For example, cow manure promoted yield and yield components of fenugreek (*Trigonella foenum-graecum* L.) (Alaghemand et al., 2017) and enhanced the total leaf area and dry matter of leaves of lavender (*Lavandula angustifolia* Mill.) by 40% compared to no fertilization (Mavandi et al., 2021). Application of cow manure to cropland improves soil properties (Martinez et al., 2017), while repeated soil applications each year can improve soil fertility and crop yield at levels similar to or even higher than the recommended inorganic fertilization for each crop (Samara et al., 2020). However, attention to the amounts of organic soil amendments added is required, because the application may exceed the nutrients needs of crops (Schlegel et al., 2017) and can contribute to the weed flora of crop fields (Materchera & Modiakgotla, 2006; Pleasant & Schlather, 1994). Most research on manure value as fertilizer has been carried out in areas with different growth conditions related to manure application from those typically take place in the region of the Mediterranean basin (e.g., temperate areas or northern European conditions) (Koutroubas et al., 2016). However, the beneficial effect of manure application on soil fertility and plant growth has been well documented in several field crops under Mediterranean conditions, such as maize (Lithourgidis et al., 2007) and wheat (Antoniadis et al., 2015). In olive orchards, several organic materials, including olive mill wastewater, composted or raw organic manure and chopped pruned material, have been used as a source of nutrients (Zipori et al., 2020). Nevertheless, to the best of our knowledge, there is no information for the influence of composted cow manure on weed growth in olive orchards. Such information would help in the development of better fertilization and weed management strategies in orchard agroecosystems.

Management of competing vegetation has long been a challenge in agricultural production. It is known that some species might be essential for ecosystem functioning and stability, but determining which species have a significant impact on which processes and which ecosystems remains an open

empirical question (Gangantharan & Neri, 2012). Correct weed management and maintenance of adequate orchard biodiversity are crucial for sustainable orchard soil management (Mia et al., 2020a). Although living mulches suppress weeds and enhance orchard biodiversity, selection of less competitive and less pest-attracting species is crucial (Mia et al., 2020a). For example, maintaining vegetation coverage along the tree row in nitrogen vulnerable zone orchards in central Italy supported a balance of soil N and weed biodiversity (Mia et al., 2020b). In addition, the growth of strawberry mulch in the area surrounding vines guaranteed a constant soil cover reducing the risk for soil erosion (Neri et al., 2021).

The objective of this research was to evaluate the impact of inorganic fertilization and composted cow manure on the weed flora of a young olive orchard in a Mediterranean region, where olives are traditionally cultivated. For this purpose, diversity indices that can quantify species diversity, e.g., the Shannon index, the Margalef index, and the Pielou index, were used to characterize the composition of the plant community of the olive orchard. The hypothesis tested in the study is that composted cow manure can support olive growth equally to the common inorganic fertilizer but may enrich the existing weed flora (species diversity and dominance) of the orchard, creating problems for small-scale growers in terms of weed control options. Given that information on the effect of composted cow manure on weed flora of an orchard agroecosystem is scarce, the novelty of the study lies on the lack of a mechanistic link between the type of fertilizer and species diversity of weed flora in an orchard agroecosystem.

Materials and methods

Study site

The experiment was conducted on a farmer's field in the north of Alexandroupolis (40° 51' N, 25° 52' E, 49 m above sea level), Greece, during the spring of 2015 and 2016. In the nearby areas, there are many livestock facilities, mainly sheep and goats and, to a lower extent, cow facilities, where the animals graze in the surrounding pastures and in the wheat fields after harvest. The production of manure in these

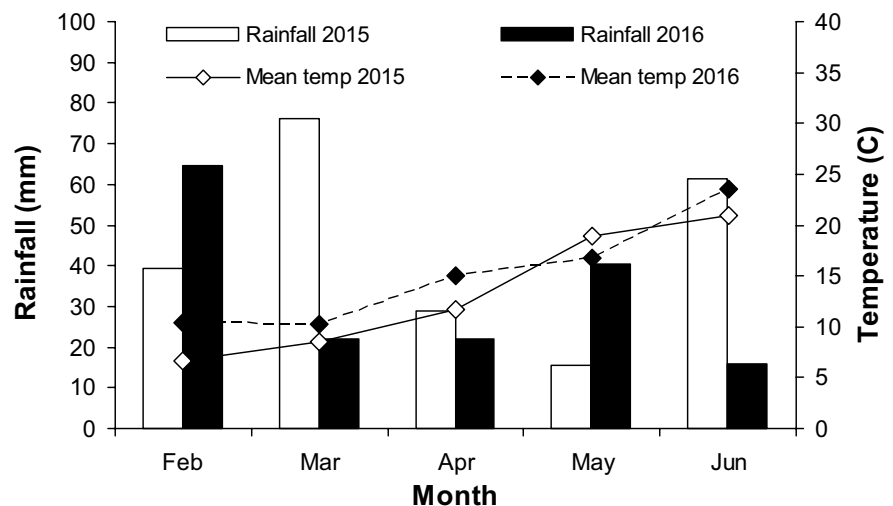
facilities is able to meet the fertilization needs of many crops in the nearby areas, especially of the olive trees of small-scale farmers. The climate of the region is characterized as Mediterranean, with cold and rainy winters and hot and dry summers (mean annual temperature 15 °C, mean annual precipitation 695 mm). The winds usually blow from north or northeast from moderate to strong. The rainfall and the mean temperature during the study period are shown in Fig. 1. The availability of N, P, K and other nutrients at the beginning of the experiment was as follows (units in mg kg⁻¹): 12.7 NH₄-N, 16.3 P (Olsen method), 190.3 available K, 12.6 Fe, 0.3 Zn, 48.5 Mn, 409.2 Mg, 1.6 Cu, and 1.2 B.

Experimental procedure

The experiment was established in a six-year-old olive orchard, with trees of cv. *Chondrelia Chalkidikis* that were planted in rows every 6.5 m with a direction from north to south. Row-to-row distance was 6.5 m. Each experimental plot had dimensions 2 m × 6.25 m, i.e., 12.5 m² and included one olive tree. The limited canopy of the young olive trees did not provide significant shading. The soil of the field is a loam (sand 31.4%, silt 45.9%, clay 22.7%) with pH 6.85, organic matter content 1.65%, and electrical conductivity 0.15 µS/cm. Concerning fertilization history, no fertilizers were applied in the field from the establishment of the orchard. The experiment was set in a randomized complete block design with three replications. Treatments included: i) no fertilization (control), ii)

inorganic fertilizer corresponding to the usual inorganic fertilization for olive trees in the region (150 kg N ha⁻¹ plus 205 kg P₂O₅ ha⁻¹ plus 205 kg K₂O, ammonium and nitrate N form), and iii) composted cow manure (20 Mg dry weight ha⁻¹, approximately corresponding to 150 kg of N ha⁻¹). Annual applications of 80 to 200 kg N ha⁻¹ and even higher, are common in many areas of the Mediterranean basin (Fernández-Escobar, 2011). Composted cow manure was produced aerobically in a nearby livestock facility after the introduction of oxygen to compost piles to allow aerobic microbes to thrive. The decomposition process took place on the ground surface under natural conditions in the spring, with moisture provided through rainfall. The basic characteristics of the composted cow manure were C/N 18.2, P 0.8%, K 1.5%, and moisture content 20%. Prior to the application of the fertilization (inorganic and cow manure), a rotavator was used to break up and aerate the soil in all experimental plots (including non-fertilized control plots). It was assumed that the use of rotavator did not significantly alter the amounts of weed propagules in the plots due to the mild degree of soil tillage (shallow tillage). The fertilizing materials were evenly spread on the plots manually and incorporated into the soil using a rake. Treatments were applied once on March 21, 2015, given that annual application of N fertilizer is not necessary to sustain adequate growth in olive orchards when leaf N is sufficient (Fernández-Escobar & Marín, 1999). Olive trees were grown without irrigation as a common practice in traditional cultivation in the Mediterranean basin, where most of the

Fig. 1 Monthly rainfall and mean temperature during the study periods



world's olive orchards are cultivated (Vossen, 2007). Crop protection products were not used in the growing seasons. In olive-cultivation regions of the Mediterranean area, mineral fertilizers or organic substances are commonly applied in late winter or early spring to take advantage of rainfall events that transport minerals into the root zone (Zipori et al., 2020). The rainfall and mean temperature during the study period (Fig. 1) were taken from the weather station of Alexandroupolis, near the study area (provided by the National Observatory of Athens). In the entire 2015, total rainfall was 473.8 mm with mean air temperature ranging between 5.8 and 26.4 °C, while in the entire 2016 total rainfall was 290.6 mm with mean air temperature ranging between 3.3 and 26.4 °C. No considerable differences between years in rainfall amount and mean temperature during the period of weed growth were recorded.

Samplings and measurements

At the beginning of July of 2015 and 2016, weed sampling was performed on all experimental plots. The sampling was random and occupied two areas of 1 m², using a wooden quadrat measuring 1 m×1 m. All the aboveground biomass of the weeds was removed from each quadrat by cutting plants at the ground level, weeds were separated by species using keys of plant identification (guides), all individual plants were counted and weighed by species, and the total fresh weight of the weeds was weighed on each of the eighteen (9×2) quadrats. Plant identification was based on Uva et al. (1997) and Burnie (2000) weed guides. All plants were then stored in separate net sacks for initial drying in an open greenhouse, and their total dry weight as well as the dry weight by species were determined after complete drying (i.e., stabilization of the weight measurements conducted every two days). After sampling, plots were kept free of weeds with shallow soil tillage, as a common grower's practice in the area. An electric digital scale with accuracy of ±5 g was used for weighing and the result was obtained by subtracting the weight of the empty net sack. The analysis of the vegetation composition was done in 2015 and 2016. Olive fruits (green olives) were manually picked in early November to determine olive yield per tree. Initially, olives were harvested by shaking the tree and then the olives of each tree were

'milked' into a sack tied around the harvester's waist using a ladder.

Biodiversity indices

Due to the high variation of natural weed populations usually occurring in field experiments, biodiversity indices were additionally used to tackle this variability. Based on the average proportion (%) of species in each treatment, common biodiversity indices were calculated, including i) the species richness (number of species), ii) the Shannon diversity index, iii) the Margalef abundance index, and iv) the Pielou uniformity index (Magurran, 2004). These indices are mathematical measures of species diversity in a plant community that provide important information on species rarity. The ability of diversity indices to quantify species diversity in this way provides an important tool for understanding the composition of the plant community. The diversity indices in this study were chosen because of their widespread use and easy interpretation.

The Shannon diversity index (H) was calculated as:

$$H = - \sum P_i \ln P_i \quad (1)$$

where $P_i = S/N$ is the total density of weeds in an experimental plot, i.e., S is the number of plants of a species and N is the total number of all plants in the sample. The Shannon diversity index provides an overall assessment of the diversity of weed species, with high values being representative of more diverse communities.

The Margalef abundance index (M) was computed as:

$$M = (S - 1)/\ln N \quad (2)$$

where S is the total number of species and N is the number of plants of a species. High values of the index indicate great diversity.

The Pielou uniformity index (E) was calculated as:

$$E = H/\ln S \quad (3)$$

where H is the Shannon diversity index and S is the total number of species in the sample. The more similar the population sizes of each species, the greater the uniformity of the plant community.

Data analysis

Fresh and dry weight data of the weed flora were subjected to one-way analysis of variance (ANOVA) with years as repeated measures. In this analysis, fresh and dry weight data of the weed flora were the dependent variables and fertilization treatments were the independent variable. Means were separated with the Tukey's test at $P < 0.05$ or otherwise stated. Before the ANOVA, the Shapiro–Wilk test confirmed the normal distribution of data to meet the ANOVA assumption of normality. Moreover, the homogeneity of variances of the dependent variable scores in each year was checked with the Bartlett's test to meet the ANOVA assumption of equal variances. Biodiversity indices were not subjected to analysis of variance. Comparison of the number and the abundance (proportion) of the species was done with the non-parametric test of Friedman (Friedman's test) at $P < 0.05$ or otherwise stated. When a difference was detected, multiple comparisons with Bonferroni control were applied. The data were further classified into various categories (e.g., annual vs. perennial weeds, grasses vs. broadleaf weeds, etc.), which were compared with the chi square test at $P < 0.05$. The olive yield data were subjected to one-way ANOVA and means were separated with the Tukey's test at $P < 0.05$ or otherwise stated.

Results

Composition of weed flora

No treatment by repeated measure (year) interaction was observed in the density of the weed flora. Therefore, the over-year occurrence of weeds per treatment is reported (Table 1). The similar trends in the density of weeds in both years are attributed to the destruction of weeds after sampling (and before seed set) with shallow soil tillage, which prevented enrichment of the seed soil bank. In total, 17 weed species occurred in the experimental plots, of which 13 species were broadleaf weeds and 4 species were grass weeds. Of the broadleaf weeds, 7 were annuals, 3 biennials, and 3 perennials, while all grass weeds were annuals. Three weed species, i.e., *Trifolium arvense*, *Sonchus oleraceus*, and *Centaurea cyanus* occurred only in the experimental plots of the non-fertilized control. Two

Table 1 Species composition in weed flora per fertilization treatment

Species	Fertilization treatment		
	Control	Cow manure	IF
1. <i>Avena sterilis</i> (AG)	+	+	+
2. <i>Daucus carota</i> (BB)	+	+	+
3. <i>Lactuca serriola</i> (AB)	+	+	+
4. <i>Trifolium arvense</i> (BB)	+	-	-
5. <i>Xanthium spinosum</i> (AB)	-	+	+
6. <i>Bromus hordeaceus</i> (AG)	+	+	-
7. <i>Sonchus oleraceus</i> (AB)	+	+	+
8. <i>Sonchus asper</i> (AB)	+	-	-
9. <i>Lolium rigidum</i> (AG)	+	+	+
10. <i>Centaurea solstitialis</i> (BB)	+	+	-
11. <i>Centaurea cyanus</i> (AB)	+	-	-
12. <i>Conyza canadensis</i> (AB)	+	+	+
13. <i>Chenopodium album</i> (AB)	+	+	+
14. <i>Plantago lanceolata</i> (PB)	-	+	-
15. <i>Convolvulus arvensis</i> (PB)	-	-	+
16. <i>Cichorium intybus</i> (PB)	+	+	+
17. <i>Setaria verticillata</i> (AG)	-	+	-
Total number of species	13	13	10

IF inorganic fertilizer, AG annual grass, AB annual broadleaf, BB biennial broadleaf, PB perennial broadleaf; (+) = presence; (-) = absence

of the 17 weed species, i.e., *Plantago lanceolata* and *Setaria verticillata*, occurred only in the experimental plots that received organic fertilization, while *Convolvulus arvensis* occurred only in the experimental plots that received inorganic fertilization.

The average number of weed species per fertilization treatment is shown in Fig. 2. Weed species identification revealed no statistically significant differences in the average number of species among treatments, indicating that fertilization did not introduce new weed species, nor did it significantly reduce the weed composition ($F = 0.288$, $P > 0.05$) (Fig. 2). Regardless of fertilization form (organic or inorganic), fertilizer application decreased the proportion of annual weeds and increased the proportion of perennial weeds compared with the non-fertilized control ($\chi^2 = 6.72$, $P < 0.05$) (Fig. 3). No statistically significant changes among treatments in the proportion of grasses and broadleaf weeds were observed ($\chi^2 = 0.360$, $P > 0.05$) (Fig. 4).

Fig. 2 Average number of weed species (over three replications) in each fertilization treatment. Vertical bars indicate the standard errors of the means. Different letters indicate statistically significant differences at $P < 0.05$

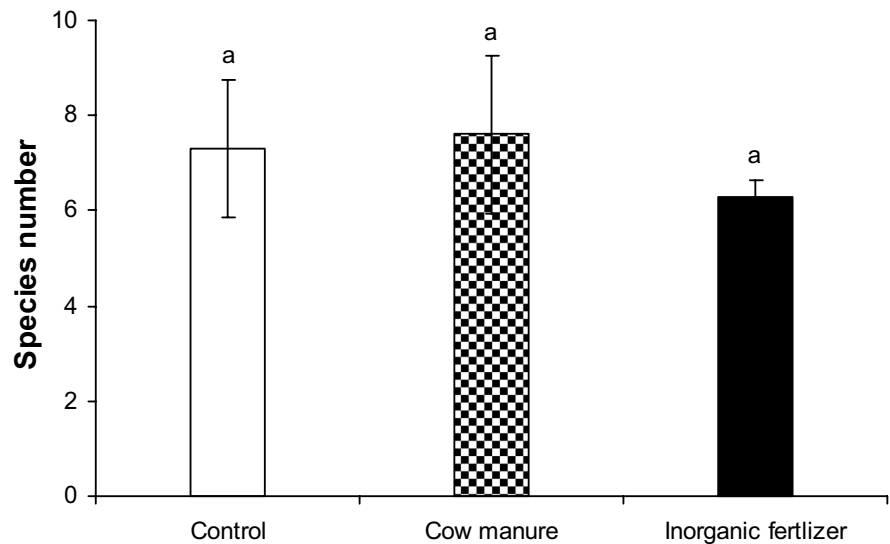
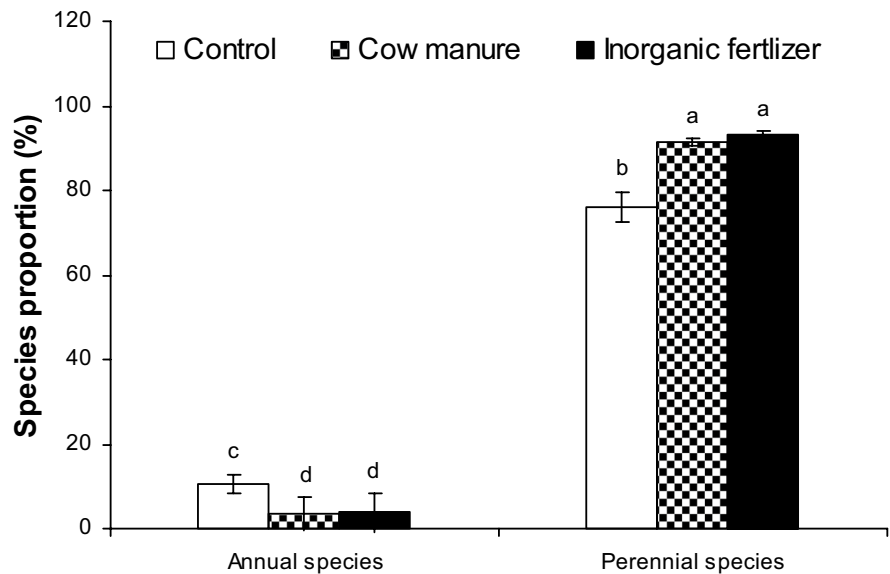


Fig. 3 Annual and perennial species proportion (%) in the weed flora (based on species dry weight) in each fertilization treatment. Vertical bars indicate the standard errors of the means. Different letters indicate statistically significant differences at $P < 0.05$



Biomass of weed flora

There was no treatment by repeated measure (year) interaction in fresh and dry weight of the weed flora (Table 2). Therefore, the means of two years are reported. Apart from the destruction of weeds with shallow soil tillage after sampling and before seed set, the similar weather conditions in terms of rainfall amount and mean temperature during the period of weed growth probably contributed to no differences in the trends of weed flora biomass across years. The total fresh and dry weight of the weed flora and the

dry weight of each weed species per experimental plot as affected by treatments are given in Tables 2 and 3, respectively. Total biomass of the weed flora increased with the addition of fertilization, either organic or inorganic (Table 2). This was mainly due to the biomass of *Chenopodium album* and *Sonchus oleraceus*. *Chenopodium album* showed greater biomass with the addition of inorganic fertilization than with the addition of organic fertilization (Table 3). Similarly, *Sonchus oleraceus* showed greater biomass with the addition of inorganic fertilization (281.7 g), which was almost equal to that achieved

Fig. 4 Grass and broadleaf species proportion (%) in the weed flora (based on species dry weight) in each fertilization treatment. Vertical bars indicate the standard errors of the means. Different letters indicate statistically significant differences at $P < 0.05$

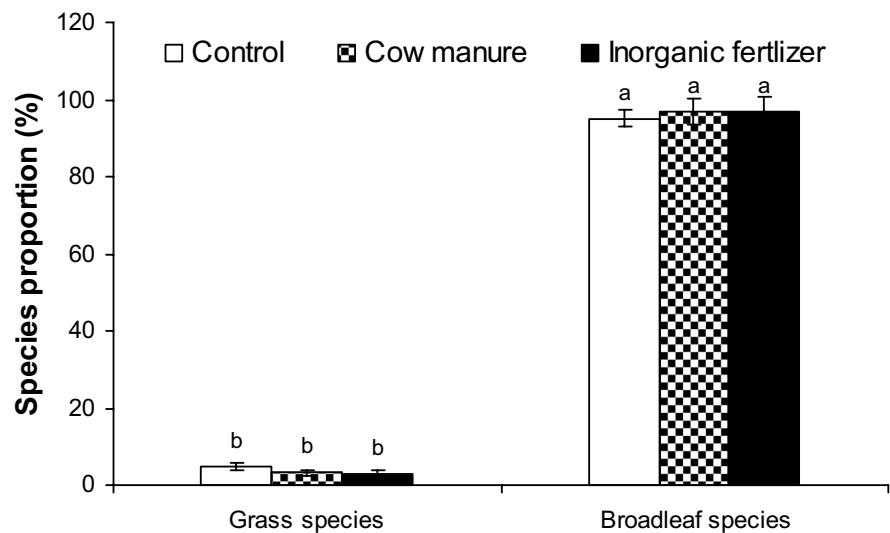


Table 2 Fresh and dry weight of the weed flora in each fertilization treatment (values are means of two years $\pm$ standard error)

Treatment	Fresh weight (g)		Dry weight (g)	
Control	830.3 $\pm$ 178.5		404.2 $\pm$ 74.7	
Cow manure	1809.3 $\pm$ 105.5		730.8 $\pm$ 2.5	
Inorganic fertilizer	1976.8 $\pm$ 222.4		943.3 $\pm$ 79.8	
Source of variation	df	Mean squares		
Year (Y)	1	89,379.9	ns	38,272.2
Treatment (T)	2	2,301,155.2	**	442,568.1
Y $\times$ T	2	6808.6	ns	2593.1

Different letters in each column indicate statistically significant differences at $P < 0.01$

df: degrees of freedom, ns: not significant, ** significant at $P < 0.01$

with the addition of organic fertilization (245.0 g) (Table 3).

The ratio (%) of a weed species dry weight to the total dry weight of the weed flora is given in Table 4. In terms of this ratio, the dominant weed species were, on average, *Chenopodium album* (29.4%) and *Sonchus oleraceus* (24.5%), while *Setaria verticillata* (0.3%) was the least dominant species. However, the species dry weight proportion was dependent on the type of fertilization; *Chenopodium album* dominated in the weed flora with the addition of inorganic fertilization (43.5%) than with the addition of

organic fertilization (31.5%), while *Sonchus oleraceus* showed rather opposite behavior (29.9% vs. 33.5%). As for *Setaria verticillata*, it appeared only when organic fertilization was applied, implying that there were some viable seeds of this weed species in manure, although manure was not tested for weed propagules before application to the plots. Also, two weed species, *Chenopodium album* and *Sonchus oleraceus*, had the highest share in the total dry weight of the weed flora in the experimental plots that were fertilized either with cow manure (31.5% and 33.5%, respectively) or with inorganic (43.5% and 29.9%, respectively) fertilizer (Table 4). Both *Centaurea solstitialis* and *Daucus carota* had a high percentage of dry weight only in the non-fertilized control.

Biodiversity indices

The Shannon diversity index tended to increase in the fertilized plots, especially with the application of organic fertilizer (Fig. 5). In addition, a tendency of decreasing Margalef abundance index was observed in the inorganic fertilization (Fig. 6). Increasing trends in Pielou uniformity index values were found with both organic and inorganic fertilization compared with the non-fertilized control (Fig. 7). Biodiversity indices are quantitative indicators that capture the main trends of weed composition, reflecting the influence of the dependent variable (fertilization).

Table 3 Dry weight (g) per species in each fertilization treatment (values are means of two years $\pm$ standard error)

Species	Fertilization treatment		
	Control	Cow manure	IF
1. <i>Avena sterilis</i>	4.2 $\pm$ 2.95	4.2 $\pm$ 2.95	25.0 $\pm$ 8.90
2. <i>Daucus carota</i>	50.8 $\pm$ 19.16	36.7 $\pm$ 25.93	30.0 $\pm$ 21.21
3. <i>Lactuca serriola</i>	31.7 $\pm$ 13.28	28.3 $\pm$ 20.03	61.7 $\pm$ 22.39
4. <i>Trifolium arvense</i>	6.7 $\pm$ 4.71	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00
5. <i>Xanthium spinosum</i>	0.0 $\pm$ 0.00	15.0 $\pm$ 10.61	20.8 $\pm$ 14.73
6. <i>Bromus hordeaceus</i>	5.0 $\pm$ 3.54	13.3 $\pm$ 5.14	0.0 $\pm$ 0.00
7. <i>Sonchus oleraceus</i>	40.8 $\pm$ 28.87	245.0 $\pm$ 14.14	281.7 $\pm$ 29.04
8. <i>Sonchus asper</i>	16.7 $\pm$ 11.79	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00
9. <i>Lolium rigidum</i>	15.0 $\pm$ 4.08	9.2 $\pm$ 1.18	14.2 $\pm$ 1.56
10. <i>Centaurea solstitialis</i>	95.0 $\pm$ 16.71	35.0 $\pm$ 12.37	0.0 $\pm$ 0.00
11. <i>Centaurea cyanus</i>	4.2 $\pm$ 2.95	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00
12. <i>Conyza canadensis</i>	42.5 $\pm$ 22.57	21.7 $\pm$ 15.32	28.3 $\pm$ 20.03
13. <i>Chenopodium album</i>	53.3 $\pm$ 37.71	230.0 $\pm$ 21.09	410.0 $\pm$ 28.94
14. <i>Plantago lanceolata</i>	0.0 $\pm$ 0.00	5.8 $\pm$ 4.12	0.0 $\pm$ 0.00
15. <i>Convolvulus arvensis</i>	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	10.0 $\pm$ 7.07
16. <i>Cichorium intybus</i>	38.3 $\pm$ 5.62	79.2 $\pm$ 17.98	61.7 $\pm$ 22.94
17. <i>Setaria verticillata</i>	0.0 $\pm$ 0.00	7.5 $\pm$ 5.30	0.0 $\pm$ 0.00
Total dry weight	404.2 $\pm$ 74.7	730.8 $\pm$ 2.5	943.3 $\pm$ 79.8

IF inorganic fertilizer

Table 4 Species proportion (%) in weed flora based on species dry weight in each fertilization treatment (values are means of two years $\pm$ standard error)

Species	Treatment			
	Control	Cow manure	IF	Mean
1. <i>Avena sterilis</i>	1.0 $\pm$ 0.73	0.6 $\pm$ 0.40	2.7 $\pm$ 1.00	1.4 $\pm$ 0.45
2. <i>Daucus carota</i>	12.6 $\pm$ 4.61	5.0 $\pm$ 3.58	3.2 $\pm$ 1.86	6.9 $\pm$ 2.03
3. <i>Lactuca serriola</i>	7.8 $\pm$ 2.35	3.9 $\pm$ 2.72	6.5 $\pm$ 2.89	6.1 $\pm$ 0.82
4. <i>Trifolium arvense</i>	1.6 $\pm$ 1.13	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.5 $\pm$ 0.39
5. <i>Xanthium spinosum</i>	0.0 $\pm$ 0.00	2.1 $\pm$ 1.44	2.2 $\pm$ 1.97	1.4 $\pm$ 0.50
6. <i>Bromus hordeaceus</i>	1.2 $\pm$ 0.85	1.8 $\pm$ 0.70	0.0 $\pm$ 0.00	1.0 $\pm$ 0.38
7. <i>Sonchus oleraceus</i>	10.1 $\pm$ 6.92	33.5 $\pm$ 2.03	29.9 $\pm$ 1.36	24.5 $\pm$ 5.14
8. <i>Sonchus asper</i>	4.1 $\pm$ 2.07	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	1.4 $\pm$ 0.97
9. <i>Lolium rigidum</i>	3.7 $\pm$ 0.69	1.3 $\pm$ 0.16	1.5 $\pm$ 0.19	2.2 $\pm$ 0.55
10. <i>Centaurea solstitialis</i>	23.5 $\pm$ 2.11	4.8 $\pm$ 1.69	0.0 $\pm$ 0.00	9.4 $\pm$ 5.07
11. <i>Centaurea cyanus</i>	1.0 $\pm$ 0.71	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.3 $\pm$ 0.24
12. <i>Conyza canadensis</i>	10.5 $\pm$ 3.87	3.0 $\pm$ 2.08	3.0 $\pm$ 1.76	5.5 $\pm$ 1.77
13. <i>Chenopodium album</i>	13.2 $\pm$ 6.62	31.5 $\pm$ 2.99	43.5 $\pm$ 1.06	29.4 $\pm$ 6.22
14. <i>Plantago lanceolata</i>	0.0 $\pm$ 0.00	0.8 $\pm$ 0.56	0.0 $\pm$ 0.00	0.3 $\pm$ 0.19
15. <i>Convolvulus arvensis</i>	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	1.1 $\pm$ 0.75	0.4 $\pm$ 0.25
16. <i>Cichorium intybus</i>	9.5 $\pm$ 1.76	10.8 $\pm$ 2.47	6.5 $\pm$ 2.10	9.0 $\pm$ 0.90
17. <i>Setaria verticillata</i>	0.0 $\pm$ 0.00	1.0 $\pm$ 0.72	0.0 $\pm$ 0.00	0.3 $\pm$ 0.24
Total proportion	100	100	100	

IF inorganic fertilizer

Olive fruit yield

The olive fruit yield was significantly affected by fertilization treatments. Inorganic fertilizer and

composted cow manure significantly improved olive fruit yield by 61.6% and 57.1%, respectively, compared with the non-fertilized control (Fig. 8). No statistically significant differences in olive fruit

Fig. 5 Biodiversity assessment of weed flora with Shannon index (overall assessment of the diversity of weed species). Vertical bars indicate the standard errors of the means

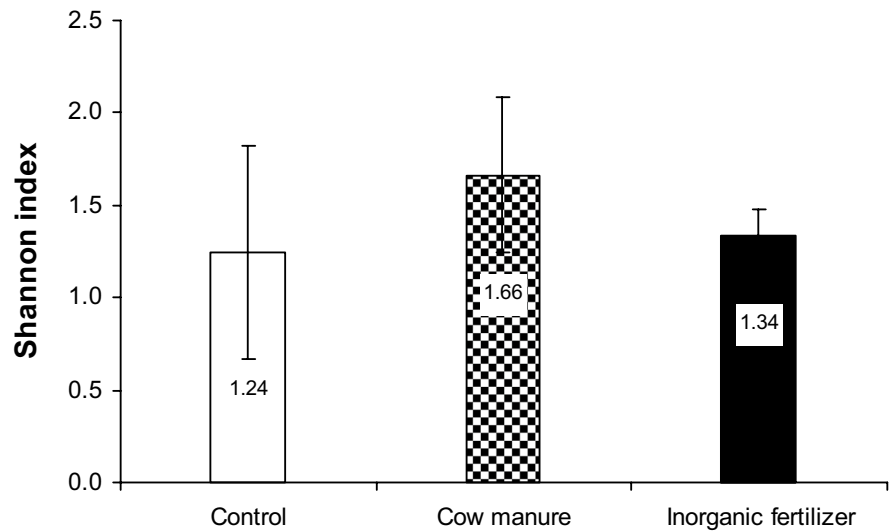
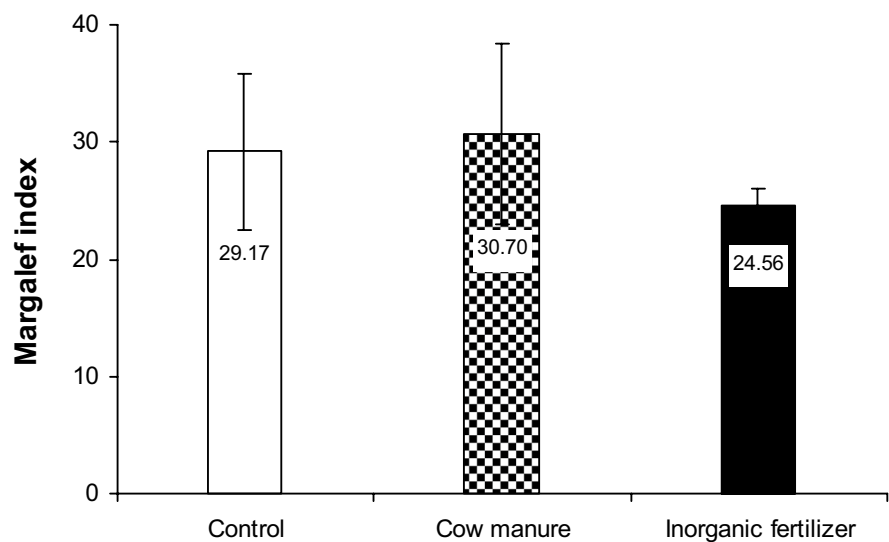


Fig. 6 Biodiversity assessment of weed flora with Margalef index (assessment of weed species diversity with emphasis on species richness). Vertical bars indicate the standard errors of the means



yield among inorganic and organic fertilization were observed.

Discussion

Consistent rainfall events in late winter or in early spring of both years retained adequate levels of soil moisture in the orchard and apparently facilitated the transport and immediate availability

of minerals into the root zone of the olive trees. Fertilizer application, either in organic or in inorganic form, did not affect the number of species in the weed flora relative to the non-fertilized control. Previous results regarding the effect of fertilization on the biodiversity of the weed flora are contradictory, because weed germination and emergence are dependent on several factors, including weed species and seed source (Sweeney et al., 2008). In addition, weed seed germination

Fig. 7 Biodiversity assessment of weed flora with Pielou index (assessment of weed species evenness). Vertical bars indicate the standard errors of the means

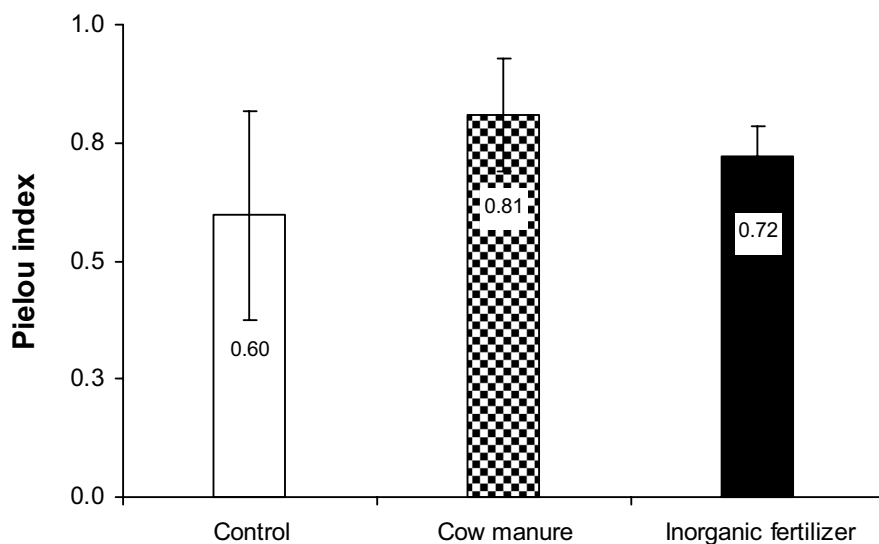
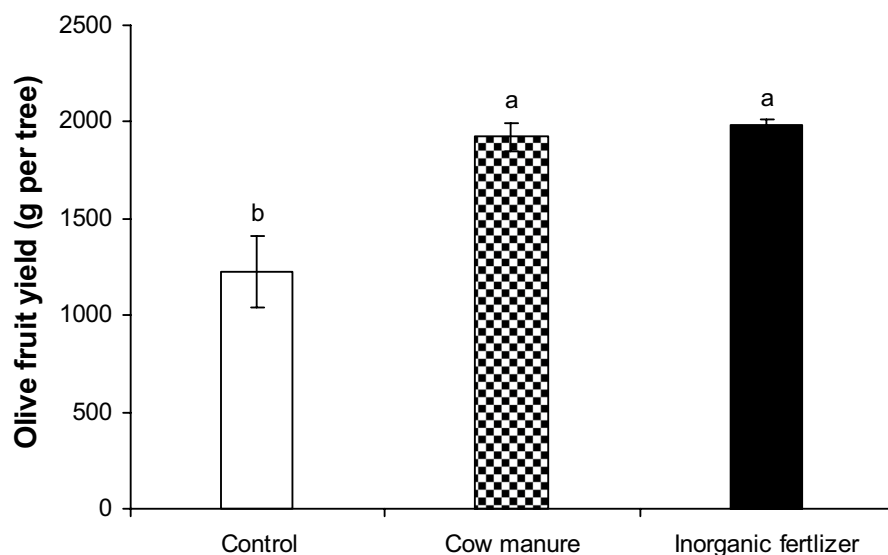


Fig. 8 Average olive fruit yield in each fertilization treatment in the first year of the experiment (mean of three replications per tree). Vertical bars indicate the standard errors of the means. Different letters indicate statistically significant differences at $P < 0.05$



is triggered by soil and environmental parameters, such as soil temperature, soil moisture, light, and N (Booth et al., 2003). Given that data from a similar agroecosystem (olive orchard) are not available in the literature, findings of the present study are compared with similar studies available from field crops (e.g., wheat and rice). In wheat fields, it has been reported that the improvement of soil nutrient status either decreased or had no significant effect on weed species diversity (Gu et al., 2007; Santín-Montanyá et al., 2013). Similarly, spring application of N fertilizer in

wheat fields showed no consistent influence of N on weed emergence (Sweeney et al., 2008). On the contrary, several studies have shown that both the dose and the type of fertilizer could significantly affect the composition of the weed flora in various crops (Banks et al., 1976; Hyvönen et al., 2003; Liu et al., 2020; Tang et al., 2013; Yin et al., 2006). Banks et al. (1976) reported that weed numbers in wheat were increased as with the addition of various nutrients compared with the non-fertilized control. In addition, PK fertilization in wheat favored the weed community

in terms of density and species diversity in wheat fields (Tang et al., 2013). In the present study, the similarity in the number of weed species observed across fertilization treatments hold promise for the feasibility of applying composted cow manure in the olive orchard without risk of increasing weed species, in accordance with Liu et al. (2020). Similarly, no differences in species diversity of the weed flora between inorganic fertilization and manure application were observed in maize (Yin et al., 2006). However, more research is needed to determine the long-term effects of manure application on weed species diversity, because a change in the composition of the weed flora would require a long period (Hyvönen et al., 2003). Perhaps manure from other source farms will have greater abundances of weed seed and therefore pose a greater risk to management, but it seems difficult to draw this conclusion here from only one study.

The weed flora in the olive orchard was characterized by a high ratio of perennial weeds compared with that of annuals. The composition of the weed community in the olive orchard can be affected also by the soil weed seedbank because competition for nutrients is crucial in low-nutrient environments, with species using more efficiently the limiting nutrients tending to dominate due to a competitive advantage (Koutroubas et al., 2000; Mamolos et al., 1995). Despite the fact that fertilization showed no effect on weed species diversity, both inorganic fertilizer and composted cow manure increased the biomass (dry weight) of the weed flora compared with the non-fertilized control, indicating that weeds responded positively to the improvement of soil nutrient status. However, the response was more apparent in the case of inorganic fertilizer, probably because nutrients were readily available to the plants. Indeed, most conventional fertilizers include chemical forms, which plants can take up or quickly convert upon application to soil. However, the total nutrient content in manure is usually not available the first year and some nutrients can be lost depending on management practices. For manure N and P, there is usually a mix of organic and inorganic materials, which varies among manure sources, production systems, bedding, storage, and handling. This variety in forms of N and P in manure contributes to greater uncertainty in manure nutrient

management compared with conventional fertilizers. These results agree with those of previous studies, which showed that the application of manure and inorganic fertilizer increased the total weed density and biomass in several crops, such as rice (Huang et al., 2013) and quinoa (Kakabouki et al., 2015). In the present study, growth response to fertilization was not similar across weed species. In fact, the growth response of weeds to fertilizer application was dependent on weed ontology (annual vs. perennial), while no differences were observed between weeds with different plant morphology (grasses vs. broadleaf weeds). Compared with the non-fertilized control, the application of fertilizer, either in organic or in inorganic form, decreased the ratio of annual weed species and increased that of perennial species, in terms of dry weight. The preferential increase of perennial weeds was probably due to the earlier and more rapid uptake of nutrients added with fertilization compared with annual weeds.

Some species benefitted considerably from fertilization and prevailed in the weed flora, while others did not. In particular, fertilization significantly increased the growth of *Chenopodium album* and *Sonchus oleraceus*. This response was because both species are nitrophilic, which means they benefit from N fertilization (El Titi, 2003). However, the growth response of these weeds to fertilization was dependent on the type of fertilizer. Indeed, the growth of *Chenopodium album* increased mainly with inorganic fertilization and to a lesser degree with organic fertilization, while *Sonchus oleraceus* showed rather opposite behavior (i.e., increased slightly more with organic fertilization). This differential response could be probably associated with the synchronization between the availability of the nutrients and the species requirements for nutrients. Apparently, this condition was probably better fulfilled by manure for *Sonchus oleraceus* and by inorganic fertilizer for *Chenopodium album*, because *Sonchus oleraceus* occurred later and benefitted from the gradual availability of manure nutrients. Results on weed flora composition were also confirmed by the biodiversity indices, despite non-significant differences that were associated mainly with the high species diversity of natural weeds populations. According to these indices, there was a trend toward greater uniformity with fertilization, with some species predominating over others.

Both inorganic fertilizer and composted cow manure improved olive fruit yield compared with the non-fertilized control, indicating that olive trees responded largely to fertilization. Olive yield per tree with composted cow manure was similar to that obtained with the inorganic fertilizer, suggesting that composted cow manure could be used as an alternative fertilizer in olive orchards. In addition to the supply of nutrients, this management practice is compatible with organic farming protocols and could contribute to the improvement of soil properties, as well as to the increase of soil organic matter (Binder et al., 2002). The latter is of great importance in the case of Mediterranean soils, which generally are characterized by low organic matter content (Antoniadis et al., 2015).

The study provides a mechanistic link between the type of fertilizer (organic and inorganic) and weed flora composition (diversity and dominance) in a young olive orchard for the period of the study. Data on such link have not been reported in a similar agroecosystem in previous research. Yet conclusions on changes of the weed flora composition are restricted by only two years of data, which is a serious limitation of the current study. It might be expected that the impact of fertility on the weeds present might take more than one year to become apparent. This influence would result from increased reproduction in year 1 after fertilization, which would increase population in year 2. This, however, was not the case in this study, given the destruction of weeds after sampling and before seed set with shallow soil tillage.

Conclusion

Following the data of the experimental period of this study, weed diversity was not affected by fertilization type, suggesting the feasibility of applying composted cow manure in the olive orchard without risk of increasing weed species. Weed growth (in term of biomass) was increased with fertilization, with the response to be more evident in the case of inorganic fertilizer. In addition, the application of fertilizer, either in organic or in inorganic form, decreased the biomass ratio of annual weed species and increased that of perennial species compared with the non-fertilized control. More research with long-term experiments would be useful to confirm the influence of composted cow manure on weed flora of olive orchards.

Authors' contributions Conceptualization: [Christos Damalas]; Methodology: [Christos Damalas], [Spyridon Koutroubas]; Formal analysis and investigation: [Petros Platis], [Christos Damalas], [Spyridon Koutroubas]; Writing—original draft preparation: [Petros Platis]; Writing—review and editing: [Christos Damalas], [Spyridon Koutroubas]; Supervision: [Christos Damalas].

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Data availability Available upon request.

Code availability Not applicable.

Declarations

Conflict of Interest No conflicts of interest are relevant to this article.

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