

Research Article

The Effect of Soil Management on Olive Yield and VOO Quality in a Rainfed Olive Grove of Central Spain

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Received 5 August 2016; Accepted 24 October 2016

Academic Editor: Nicola Caporaso

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Rainfed olive groves have been traditionally tilled in order to reduce the competition for water and nutrients. In sloping Mediterranean olive groves, this practice leads to high erosion rates, resulting in a reduction in soil fertility. Cover crops have been employed as a sustainable olive grove management strategy, but previous studies found differences in their effect on fruit load and there is scarce information on their influence on the virgin olive oil (VOO) quality. The aim of this study is to evaluate the effect of different soil management strategies on olive and oil yield and VOO physicochemical and sensory characteristics in a rainfed olive grove (238 trees·ha⁻¹) of Cornicabra cultivar, the main in Central Spain. No effect of soil management was found in olive or oil yield along three cropping seasons. VOO quality was mainly influenced by the year, but slight differences were found in the driest year between the studied treatments. Small differences in fruit ripening, pigments, and several fatty acids as well as in sensory parameters were found.

1. Introduction

Olive tree (*Olea europaea* L.) is one of the most drought-tolerant tree crops in the world [1]. They are able to tolerate low availability of soil water by means of morphological and physiological adaptation [2, 3]. Three periods of high sensitivity to water stress can be considered [4]: flowering; fruit growth, 6 to 10 weeks after blooming; and oil accumulation, 18 to 22 weeks after blooming. Water stress in the first period reduces the number of fruits, while water stress in the other two periods reduces fruit fresh weight. A positive relationship between water stress of olive trees and phenolic compounds [5, 6], volatile profile [7, 8], monounsaturated fatty acid levels, and sensory properties [9] of olive oil has been described.

In the Mediterranean basin, rainfall and underground water resources are the only supplies for most of the olive trees [10]. In this area, traditional olive groves were designed with low plant density according to total rainfall and paying

attention to canopy size [3]. Actually around 50% out of 2,500,000 ha of olive groves in Spain is tilled [11] to avoid weed competition for water and nutrients, in order to increase olive tree yield. This conventional practice gives rise to a large area of bare soil that is prone to erosion processes, one of the most important land degradation driving processes in Mediterranean areas [12, 13].

Cover crops in olive groves have proved to be an effective tool in reducing soil erosion [14–16] and improving water storage and physical properties [17], but its effect on olive tree yield is controversial. A negative effect on fruit yield due to cover crops was reported by Gucci et al. [18] in a high-density olive grove and by Caruso et al. [19] in a young and intensive olive grove. Nevertheless, other authors [17, 20–22] did not find a significant reduction in olive yield in mature olive groves.

The influence of cover crops on food quality has been studied in vineyards [23–27], apple orchard [28], eggplants

[29], and sweet cherries [30]. In olive groves, the influence of soil management on some oil quality parameters has been seldom studied [18, 19, 21, 31] and to our knowledge there are no previous studies on the effect of cover crops on sensory characteristics of virgin olive oil.

Cornicabra cultivar is the most common in Central Spain, being the second cultivar in extension in Iberian Peninsula [32] covering over 270,000 ha [33]. Although Cornicabra cv. is considered one of the most drought-tolerant cultivars in Spain [33], small amounts of water increase olive and oil yield [34, 35]. The oil of Cornicabra is valued for its high stability and food sensory characteristics [36], with medium bitterness and pungency mainly due to the content in total phenols [37] and high oleic acid and low linoleic acid content [38]. Phenolic compounds, oleic acid, and monounsaturated fatty acids (MUFA) of VOO have beneficial effects on human health [39], and thus a higher content in total phenols and a high ratio of MUFA to polyunsaturated fatty acids (PUFA) are desirable in VOOs.

In order to recommend a more sustainable practice to rainfed olive growers of Central Spain, we performed a comparative trial in an intensive olive grove of Cornicabra cv. to evaluate the effect of three different cover crops and minimum tillage on olive yield and physicochemical and sensory virgin olive oil parameters.

2. Materials and Methods

2.1. Field Conditions, Climatic Data, and Plant Material. This study was performed in an experimental olive grove (*Olea europaea* L.) located in Central Spain, in southern Madrid (40°4'21"N, 3°31'11"W). The average elevation is 540 m.a.s.l., and the slope ranges from 9 to 12%. The soil is classified as *Haplic Gypsisol* [40], with a xeric moisture regime. The field capacity (FC) was $0.21 \text{ m}^3 \cdot \text{m}^{-3}$ and permanent wilting point (PWP) was $0.08 \text{ m}^3 \cdot \text{m}^{-3}$. This soil has a moderate silt content (29%) and it is low in organic matter (1.1%).

The climate is semiarid Mediterranean, with long hot summers (quite often above 35°C in July and August) and cold winters (2.7°C on average in December). The average annual temperature is 13.6°C and reference evapotranspiration (ET_0) is 1112 mm. The annual precipitation is approximately 390 mm with high inter- and intra-annual variability [41, 42]. During the last 17 years, the total precipitation has strongly decreased (40%) in comparison with a previous period (1933–1969) in the study area [43]. Since it is a rainfed olive grove, as most of the region, the amount and distribution of rainfall and ET_0 are very important. Thus, there is an automatic weather station in the olive grove to record the temperature, rainfall amount, relative humidity, solar radiation, wind speed and direction, and atmospheric pressure every 10 minutes. With the previous data, Penman-Monteith ET_0 was calculated according to FAO [44].

The olive plantation was established in 2004 with trees in $6 \times 7 \text{ m}$ spacings ($238 \text{ trees} \cdot \text{ha}^{-1}$), with an area of approximately 3 ha.

2.2. Soil Management and Treatments. Before the start of the trial, in November 2010, the whole area was tilled with a

chisel at 0.30 m depth following the traditional management. The tillage was performed in 6 m wide interrows. Each treatment was performed in 3 consecutive interrows. The treatments consisted of the following: (1) *barley* annual cover (*Hordeum vulgare* L.) that was seeded each autumn at $70 \text{ kg} \cdot \text{ha}^{-1}$; (2) sainfoin (*Onobrychis viciifolia* Scop.), a *legume* that was seeded each year ($42 \text{ kg} \cdot \text{ha}^{-1}$); (3) purple false brome (*Brachypodium distachyon* L. P. Beauv.), henceforth *Brachypodium*, a permanent grass cover seeded once (the first year) during the study period at $40 \text{ kg} \cdot \text{ha}^{-1}$; and (4) *control*, consisting of one pass per year with a chisel at 0.15–0.20 m deep in mid-November. All of the treatments were mechanically mowed once in the spring (during the first fortnight of May), except in 2013, when the vegetation was mowed twice (the second cut was at the end of May) because of high vegetation growth resulting from the abundant rains of that spring. Plant debris was left on the surface.

2.3. Soil Moisture Measurements. Three EC-5 soil moisture sensors (Decagon Devices Inc.) per treatment were placed in the interrow at 30 cm depth plugged into a data logger, which recorded data each 15 minutes. Data were fortnightly downloaded to a laptop.

2.4. Olive Harvesting, Yield and Oil Elaboration, and Oil Content Determination. Fruit olives were harvested in three consecutive cropping seasons: 2011/2012, 2012/2013, and 2013/2014. Ten healthy olive trees per treatment were randomly selected each year and fruits were hand-picked, when the maturity index (MI) was around 3.5. This MI was selected in order to obtain an optimal oil content and quality [45]. Yield per tree was measured in field. Olives collected of the same treatment were mixed up and 3 subsamples of 5 kg ($3 \text{ subsamples} \times 4 \text{ treatments}$) were carried to the laboratory. MI was determined at the laboratory for each subsample of fruits following the method of Beltrán et al. [45] based on skin and pulp fruit colors. Olive oils from each subsample were extracted in the last two years using the Abencor system (MC2 Ingenierías y Sistemas), determining the industrial oil yield [46]. Oil yield per hectare was obtained multiplying fruit yield per tree by industrial oil yield by the number of trees per hectare.

2.5. Analysis of Virgin Olive Oil. In 2012 and 2013, different VOO parameters ($2 \text{ years} \times 4 \text{ treatments} \times 3 \text{ repetitions}$) were analyzed.

2.5.1. Physicochemical Quality Parameters. Free acidity is expressed as percentage of oleic acid; peroxide value (PV) is expressed as milliequivalents of active oxygen per kilogram of oil ($\text{meq O}_2/\text{kg}$); and UV spectrophotometric indices (K_{232} , K_{270} , and ΔK extinction coefficients) were determined following the analytical methods described in the European Commission Regulation 2568/91 and later amendments [47].

2.5.2. Total Phenolic Content. Total phenolic compounds were determined after methanol extraction and subsequent reaction with Folin-Ciocalteu reagent and measured at a

wavelength of 725 nm [48]. Results are expressed as mg of caffeic acid equivalents per kg of oil.

2.5.3. Pigments (Chlorophylls and Carotenoids) and Chromatic Coordinates. Carotenoids and chlorophylls were determined at a wavelength of 470 and 670 nm, respectively, in cyclohexane, according to the method of Minguez-Mosquera et al. [49]. The concentrations of chlorophyll and carotenoids were expressed as mg of pheophytin and lutein per kg, respectively. Chromatic coordinates were measured by the software CINTRAL to obtain the color according to the CIEL $a^* b^*$ method [50].

2.5.4. Fatty Acid Composition. The fatty acid composition of the oils was determined by gas chromatography as fatty acid methyl esters (FAMES). FAMES were prepared by saponification/methylation with sodium methylate according to European Regulations (EEC 2568/91) [47]. A chromatographic analysis was performed in an Agilent Technologies 6850 series II Network GC system gas chromatograph equipped with a 60 m \times 0.25 mm \times 0.20 μ m film thickness fused capillary column Supelco 24111 (Agilent Technologies) coupled to a flame ionization detector. Samples were introduced into the column at 170°C during a period of 30 min; after this time, the temperature was increased by 5°C/min to 200°C and maintained for 12 min. The flow rate of He, used as carrier gas, was 0.5 mL/min. Injector and flame ionization detector temperatures were 230°C and 250°C, respectively. FAMES were identified by comparing their retention times with those of standard compounds.

Fatty acid composition was calculated as the percentage of total fatty acids, after their conversion to methyl esters, according to regulation EEC 2568/91 [47].

2.5.5. Sensory Analysis. Sensory analysis was performed by the “Panel de Catadores de Aceite de Oliva Virgen de la Comunidad de Madrid,” according to the method described in the European Commission Regulation (EC) 640/2008 [51]. This method allows for the classification of VOOs based on the detection of negative attributes (fusty/muddy, musty, winey, rancid, wet wood, and others) as well as the measurement of the intensity of three positive attributes (green or ripe fruitiness, bitterness, and pungency). The panel was constituted at least by 8 trained tasters that scored the descriptors on a normalized sheet (from 0 to 10).

Chemical determinations were performed twice per sample, obtaining the mean for statistical analysis.

2.6. Statistical Analysis. Statistical analyses were performed with the software package SPSS 19 (SPSS Inc.) for Windows. When needed, data were log-transformed before parametric testing. Repeated-measures ANOVA was performed for yield data and MAN(C)OVA analyses were performed on the VOO data to assess the differences between treatments and years, employing MI as covariate. LSD test ($p < 0.05$) was used to establish significant differences between groups.

After MANOVA a Discriminant Function Analysis was performed to assess how the dependent variables discriminate groups.

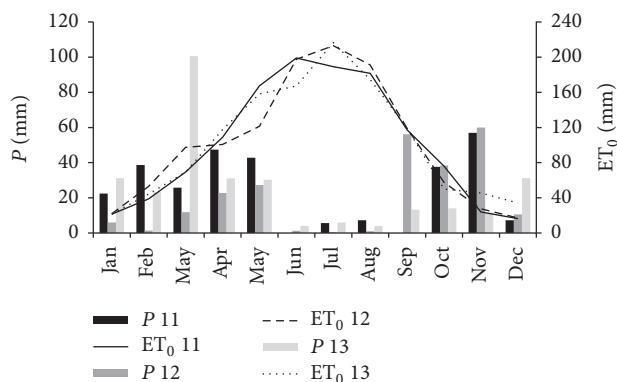


FIGURE 1: Monthly rainfall (P) and reference evapotranspiration (ET_0) for the 3 years of the trial.

3. Results and Discussion

3.1. Meteorology and Soil Water Content. Year 2012 registered the lowest total rainfall amount in the studied period (237 mm) while 2011 and 2013 were quite similar, 292 and 300 mm, respectively. Distribution of the rainfall varied among years: autumn was the rainiest season in 2011 and 2012, while in 2013 spring was the rainiest (Figure 1). Reference evapotranspiration (ET_0) values were similar among the three years: 1209, 1217, and 1218 in 2011, 2012, and 2013, respectively. Summers have been characterized by high ET_0 and scarce rainfalls.

Control was the treatment with the lower soil moisture at 30 cm depth along the three years. During the rainfalls of spring in 2011 and 2013, these differences decreased; the same happened with the autumn rainfall in 2012 (Figure 2). In summer, all the treatments endured several days below the PWP ($0.08 \text{ m}^3 \cdot \text{m}^{-3}$), mainly in 2011, which implied water stress to the olive trees in the phase of oil accumulation [4].

3.2. Fruit and Oil Yield. Olive harvest dates were quite similar, 1 December 2011, 11 December 2012, and 2 December 2013. MI was around 3.5 in 2011 and 4 in 2012. In 2013, we had to harvest with a low MI, about 3, because of frost alert at the beginning of December. Despite the low soil moisture measured during all the studied period in all the treatments, thanks to the olive tree high capacity to cope with water stress [52], especially in Cornicabra cv. [33], olive trees under semiarid climate without irrigation have low fruit production on a year basis. Mean and standard deviation of fruit yield per tree and oil yield per hectare are shown in Figures 3(a) and 3(b). 2013 was the year with the highest yield in both parameters ($9.1 \text{ kg} \cdot \text{tree}^{-1}$ of fruit and $476 \text{ kg} \cdot \text{ha}^{-1}$ of oil on mean), whereas 2012 was the year with the lowest yield ($1.6 \text{ kg} \cdot \text{tree}^{-1}$ of fruit and $90 \text{ kg} \cdot \text{ha}^{-1}$ of oil on mean) being considered as “off” year (low or no yield). Fruit and oil yields were affected by year but not by treatment; no interaction was detected (Table 1). There were statistically significant differences between the three crop seasons in both fruit and oil yield ($2012 < 2011 < 2013$) according to LSD test. This alternate bearing is typical of olive tree, mainly in Cornicabra

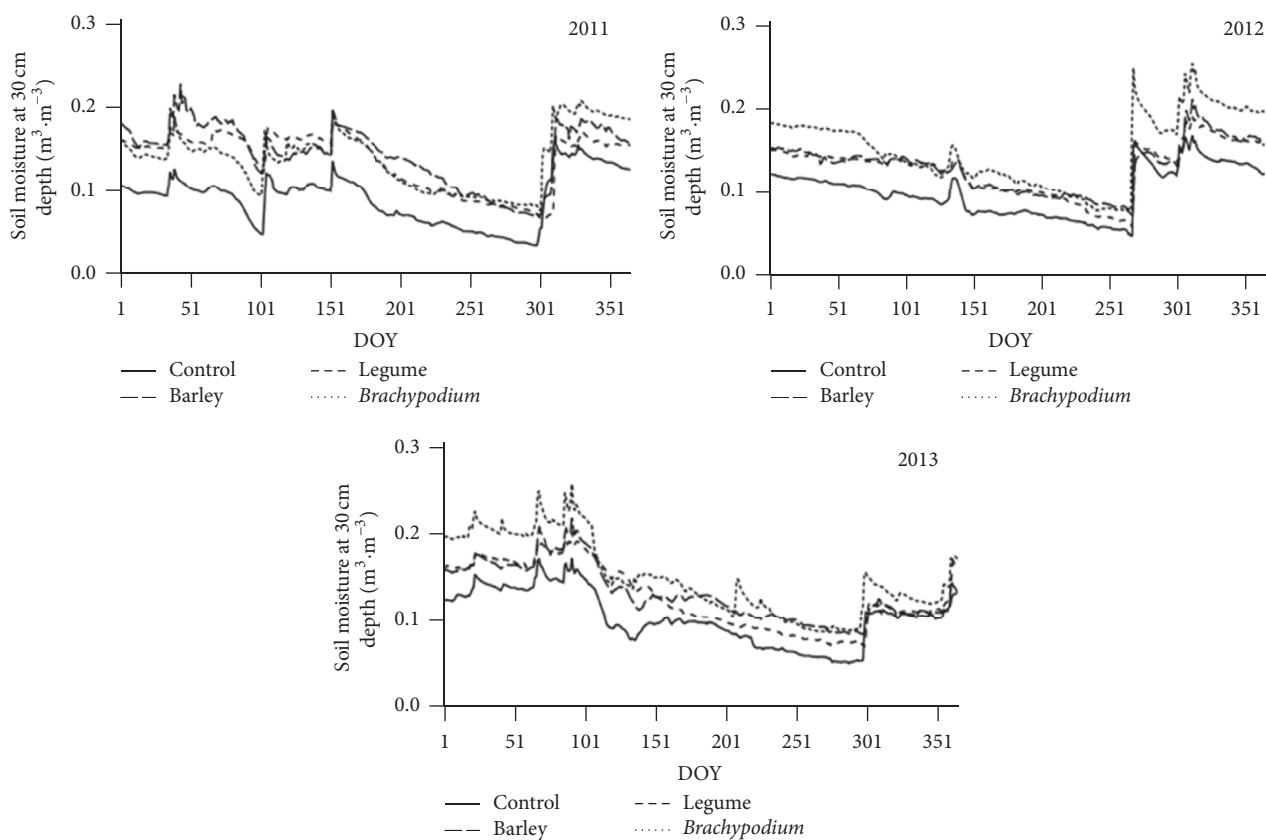


FIGURE 2: Daily soil moisture ($\text{m}^3 \cdot \text{m}^{-3}$) at 30 cm depth in 2011, 2012, and 2013 for the four treatments. DOY: day of the year.

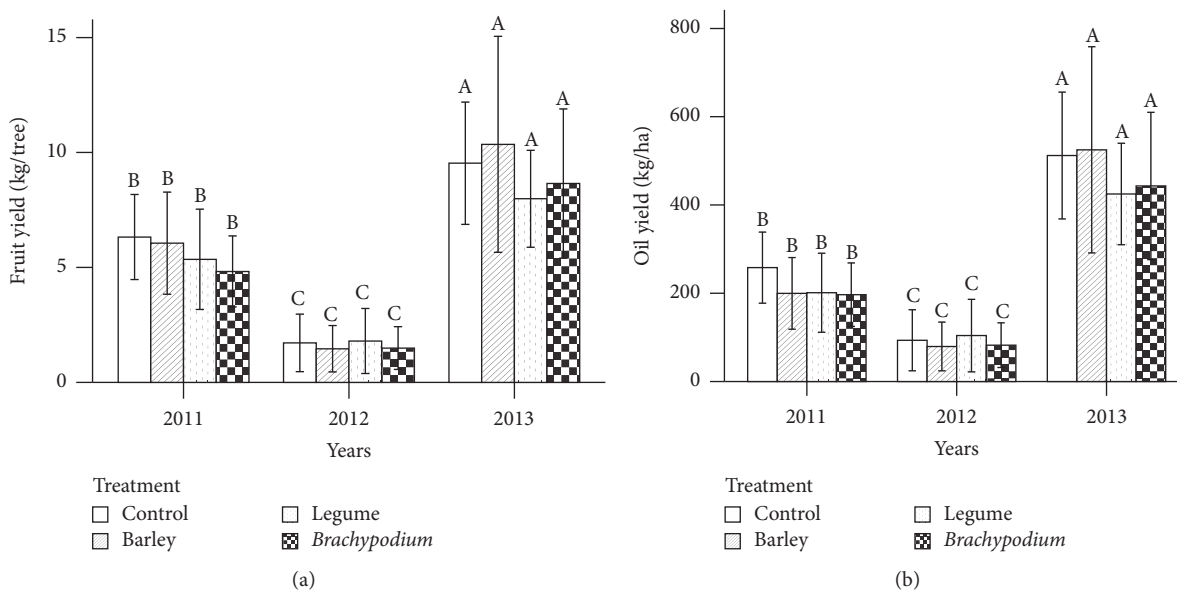


FIGURE 3: Fruit yield (a) and oil yield (b) mean and SD for the three years. Data are means of 10 replicates. Different letters mean statistically significant differences ($p < 0.05$) among years.

TABLE 1: Repeated-measures ANOVA table (prob. > F) for fruit yield per tree and oil yield per ha for the three years ($n = 10$).

	Fruit yield	Oil yield
Treatment	NS	NS
Year	<0.001	<0.001
Treatment \times year	NS	NS

ns: not significant.

cultivar [32], with high yields in the “on” years (heavy yield) followed by “off” years of low yields.

This absence of differences in fruit and oil yield among different soil management systems was also observed by other researchers between tilled and nontilled [17, 20, 22, 53] and rainfed and irrigated olive groves in Cornicabra cv. [35]. By contrast, Moriana et al. [54] found a reduction in Picual cv. under water stress and Caruso et al. [19] reported over 40% olive yield reduction in young trees of Frantoio cv. managed with cover crops regarding tilling management. Corleto and Cazzato [21] found a reduction in fruit yield with perennial species as cover crops but not with annual legume species regarding conventional tillage on Coratina cv. Therefore, proper management of cover crops is crucial to avoid fruit yield reductions, mainly species selection and time of mowing.

3.3. VOO: Physicochemical Parameters. All the VOO samples had their analytical parameters within the ranges established for the highest quality category “Extra Virgin Olive Oil” in the Regulation 1348/2013 [55]. These thresholds are 0.8% for acidity, 20 meq $O_2 \cdot kg^{-1}$ for peroxides, 2.50 for K_{232} , 0.22 for K_{270} , and 0.01 for ΔK .

MANOVA results are shown in Table 2 for the physicochemical determinations in the VOOs of 2012 and 2013. MI was included as a covariate due the important effect of this index over several VOO parameters [5, 35, 56] but in our study it was not statistically significant on either of the measured variables (data not shown), so it was excluded as a covariate and included as a dependent variable in the analysis. Treatment and year influenced significantly most of the measured parameters (Table 2). There was no statistically significant effect of treatment or year in peroxide index or K_{270} , while K_{232} and ΔK were only affected by the year.

Table 3 shows VOO parameters as a function of the treatments and years. In relation to the effect of the different treatments on the fruit, legume coverage led to earlier olive maturation (3.7) with barley resulting in the opposite effect (3.3). Control and *Brachypodium* VOOs had slightly higher values of free acidity (0.22 and 0.21%, resp.), but these differences were not due to the maturity of the VOOs considering that both treatments had intermediate maturity (3.5). Regarding the pigments, legume VOOs had lower values for carotenoid, chlorophyll, and total pigments because of a higher MI similar to observations by other authors [57, 58]; the highest chlorophyll to carotenoid ratio was found in barley (1.3) while control had the lowest (1.1). As control had the same MI as *Brachypodium*, the differences in pigment

content should be explained through changes in the plant-soil relationship produced by the soil management. VOOs obtained from legume coverage had a significantly higher value of color lightness (L^*) and lower value of greenish color (a^*) than the other treatments, which is due to the lower content in carotenoid and chlorophyll pigments [59].

Comparing the results by year, MI was lower in 2013 than in 2012 despite an earlier ripening date in 2013 due to a higher fruit load of the trees, similar to the results of Dag et al. [60]. In 2012, VOOs had slightly higher acidity (0.26%) than in 2013 (0.15%) probably due to the higher MI. UV absorbance at 270 and 232 nm indicates the presence of oxidized compounds; some authors found that K_{270} and K_{232} increased with water stress [10, 35] as in the present study. The maximum level of total phenols accumulation is reached at veraison [61] that is closely related to the MI of VOOs in 2013, although the higher content in total phenols was in 2012. This contradiction could be related to the scarce precipitation of that year, as a linear relationship has been described between total phenols and water stress by some authors [9, 52]. Nevertheless, Palese et al. [62] stated that the differences in total phenols, K_{232} , and other quality parameters between rainfed and irrigated olive trees were due to the different plant crop load and fruit ripening pattern instead of water availability. VOOs of 2012 had a significantly higher value of color lightness and lower greenish color, with less content in carotenoids, chlorophyll, and total pigments and lower chlorophyll to carotenoid ratio than in 2013, which is related to a higher MI [57, 58].

Figure 4 shows the mean and standard deviation per treatment and year for those parameters with significant interaction. Free acidity (Figure 4(a)) was higher in control in 2012 regarding the other treatments, while in 2013 this place corresponded to *Brachypodium*. These changes were not correlated with MI but they could be related to soil water content (Figure 2) and other changes in plant-soil relationship. Barley had a different behavior regarding total phenols (Figure 4(b)) in both years, the highest content in 2012 and the lowest in 2013. In 2013, no effect of treatment was observed in chlorophyll to carotenoid ratio (Figure 4(c)) and b^* (Figure 4(e)) parameters, while important differences between treatments appeared in 2012. These differences in total phenols and pigments may be responding to a higher water stress of barley on the driest year over the olive trees as was pointed out by Hernández et al. [63]. Regarding a^* parameter (Figure 4(d)), control and barley were more similar to legume in 2012 and to *Brachypodium* in 2013. These differences among treatments should be explained through the effect of cover crops on soil moisture dynamic along the crop season and available nutrients for olive trees, as was described in vineyards in which legume cover crops increased soil N availability and vine N uptake [24] affected must quality.

3.4. VOO: Fatty Acid Composition. Most of the fatty acids were mainly influenced by year (Table 4) and not by soil management, as is the case of oleic acid, for instance, which remained stable across treatments. Similar results were also reported by other authors in relation to water stress [35, 37]

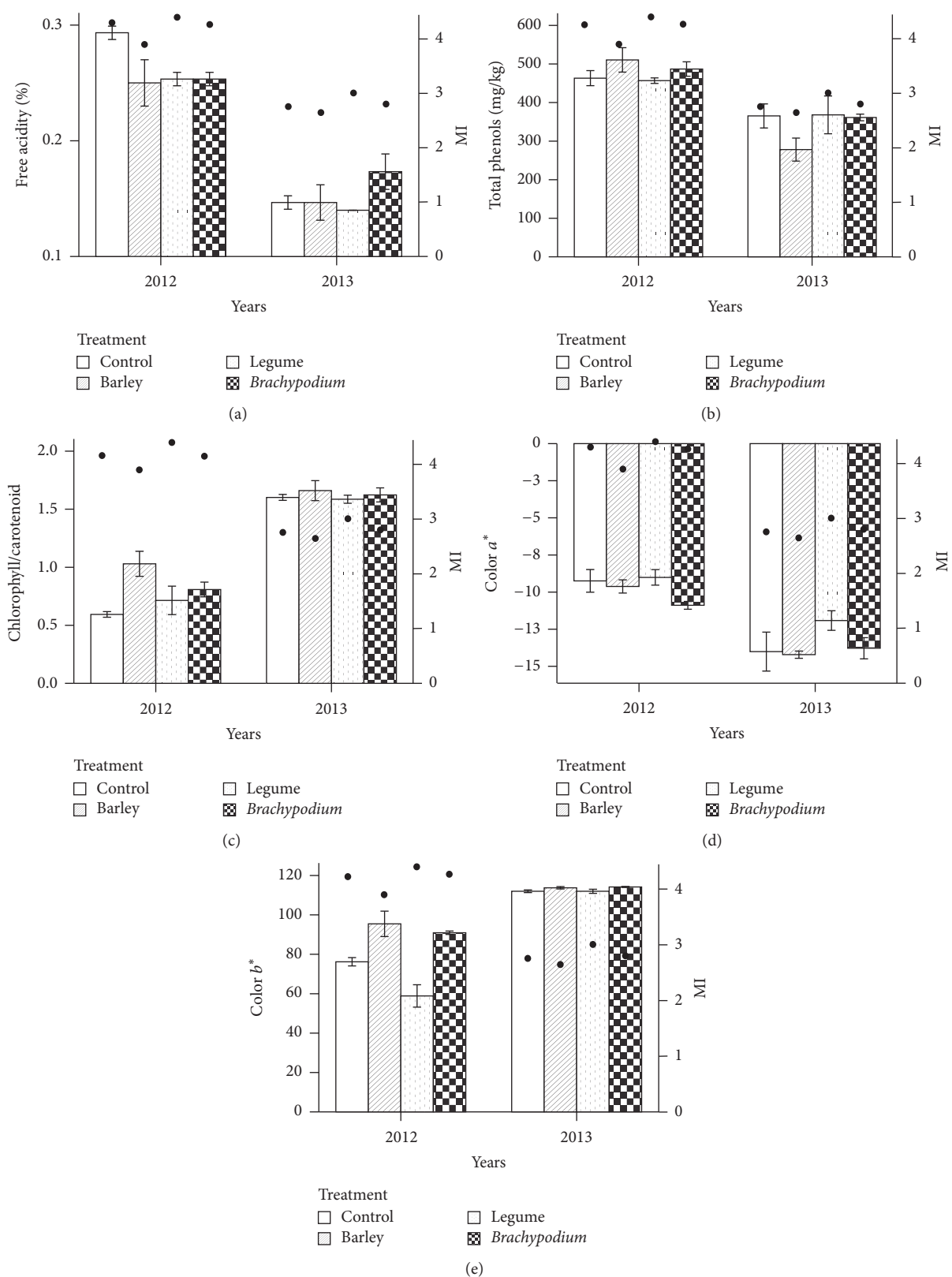


FIGURE 4: Free acidity (a), total phenols (b), chlorophyll to carotenoid ratio (c), color a^* (d), and color b^* (e) mean and standard deviation and maturity index (MI) as dots, of different treatments and years.

TABLE 2: MANOVA (prob. > F) for VOO parameters as a function of treatment and year ($n = 3$).

	MI	Quality parameters					TP	Pigments				Color		
		Ac	Pe	K_{270}	K_{232}	ΔK		CarP	ChloP	Pi	Chlo/Car	L^*	a^*	b^*
Treatment	0.000	0.005	NS	NS	NS	NS	NS	0.000	0.000	0.000	0.000	0.001	0.002	0.000
Year	0.000	0.000	NS	NS	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Tre \times year	NS	0.001	NS	NS	NS	NS	0.001	NS	NS	NS	0.004	NS	0.047	0.000

MI: maturity index; Tre \times year: treatment and year interaction; Ac: free fatty acids; Pe: peroxide index; TP: total phenols; CarP: carotenoid pigments; ChloP: chlorophyll pigments; Pi: total pigments; Chlo/Car: chlorophyll/carotenoid ratio. NS: not significant.

but oleic acid increases throughout fruit ripening involving a reduction in palmitic acid [35]. On mean oleic acid percentage (79.5%) was similar to Cornicabra olive oils described by other authors [36, 37], but with a lower percentage in linoleic acid (3.1%) similar to observations by Gómez-Rico et al. [7]. Margaric, stearic, linoleic, and linolenic acids were influenced by treatment besides the year. MUFA to PUFA ratio and oleic to linoleic acid ratio were influenced by treatment and year and by their interaction. Although these statistically significant differences were observed (Table 4), their magnitude was very low (Table 5).

Brachypodium and barley treatments give rise to oils with higher content of linoleic (3.18%) and linolenic (0.6%) acids, while control had higher content of margaric and stearic acids, 0.05 and 3.09%, respectively (Table 5). The highest rates in MUFA to PUFA and oleic to linoleic acid corresponded to legume, 23 and 27%, respectively, while *Brachypodium* had the lowest, 21 and 25%, respectively. Linoleic acid increases during ripening while stearic [64] and palmitic acids and MUFA to PUFA and oleic to linoleic acid ratios decrease [57, 65]. Nevertheless, these results do not match the MI of the different treatments: barley VOOs had lower MI, followed by control and *Brachypodium* and the most mature legume VOOs. Therefore, barley should have the highest stearic acid percentage and in fact it had the lowest value (2.85%), although this difference with the other VOOs was very low. In the same way, legume VOO should have lower MUFA to PUFA and oleic to linoleic acid ratios, but it was the treatment with the highest value. Gómez-Rico et al. [35] found that MUFA to PUFA ratio was higher in oils obtained under rainfed conditions. Thus, legume could be exerting higher water stress on the olive trees, although it kept more soil water content than control (Figure 2), or legume cover could have a positive effect on the nutrients of the soil [24, 29, 66] influencing VOO fatty acids.

The most mature VOOs of 2012 had higher content of linoleic acid [64] and lower content of palmitic acid [35]. Stearic acid does not accumulate during the ripening process [64], which is the reason why the content in stearic acid in 2012, the year with the higher MI, was significantly lower than in 2013. MUFA to PUFA ratio was expected to be higher in water stressed olive trees [35] but this fact was not observed between the driest 2012 and 2013 probably due to the predominant effect of maturity in olive fruits, as was pointed out by Dag et al. [60].

Figure 5 shows mean and standard deviation for those parameters with significant interaction. *Brachypodium* VOOs had the highest palmitoleic acid content in 2012 (Figure 5(a)),

and control VOOs of 2013 had the highest percentage of stearic acid (Figure 5(b)). The higher value of oleic to linoleic acid (Figure 5(c)) and MUFA to PUFA (Figure 5(d)) ratios was reached in 2013 by legume. The differences in parameters that were not explained by MI or soil water content could be related to changes in plant-soil relationships.

3.5. VOO: Sensory Analysis. As in previous parameters, year had a higher influence on bitterness and pungency than soil management (Table 6). Barley treatment produced slightly less bitter and pungent VOOs, over 1 point below the other VOOs. Fruitness was not affected by treatments. In relation to year effect, in 2013, VOOs had around 3 points less in bitterness and pungency, but with similar fruitiness despite a lower MI in this year. These “sweeter” 2013 VOOs can be explained by the higher rainfall amount that year which produced a reduction in total phenols content (Table 3) as has been observed by other authors [5, 62].

MANOVA results are shown in Table 7. Bitterness and pungency were influenced by treatment, by year, and by their interaction (Table 7). The interaction of treatment and year also was statistically significant for fruitiness.

Figure 6 shows mean and standard deviation of fruitiness (Figure 6(a)), bitterness (Figure 6(b)), and pungency (Figure 6(c)) of the oils for the different treatments and years. Control treatment almost did not change fruitiness, bitterness, or pungency between years, opposite to cover crops VOOs that diminished 2 points in fruitiness in 2013 regarding the previous year. *Brachypodium* reduced less than 1 point of the bitterness and pungency from 2012 to 2013, while legume slightly increased both parameters. In 2012, *Brachypodium* VOOs stood out among the other VOOs for higher bitterness and pungency, while in 2013 barley stood out for less bitter and pungent VOOs. Despite these statistically significant differences, their magnitude was not high enough to state that the VOOs were sensorially different.

3.6. Global Treatment Effect. The MANOVA was followed up with discriminant analysis employing all the variables. As previously mentioned, the main influence of the year on the VOO quality was due to a rainfall amount and crop load higher in 2013/2014 crop season. Due to this important effect of the year on the VOOs quality, discriminant analysis was conducted separately in each year (Figure 7). The most useful variables from all the measured ones to discriminate treatments were MI, free acidity, total phenols, carotenoid and chlorophyll pigments, L^* and a^* color, MUFA to PUFA ratio, and pungency.

TABLE 3: VOO parameters LS means from the MANOVA as a function of the different treatments and years ($n = 3$).

Treatment	MI	Quality parameters				TP (mg caffeic acid/kg oil)	Pigments				Color				
		Ac (%)	Pe	(meq O ₂ /kg)	K ₂₇₀		K ₂₃₂	ΔK	CarP (mg/kg)	ChloP (mg/kg)	Pi (mg/kg)	Chlo/Car	L*	a*	b*
Year															
	Control	3.5 ^b	0.22 ^a	3.8 ^a	0.13 ^a	1.68 ^a	-0.001 ^a	414 ^a	7.9 ^b	10.4 ^b	18.4 ^b	1.1 ^c	84.4 ^{ab}	-11.6 ^b	94.1 ^b
	Barley	3.3 ^c	0.20 ^b	4.2 ^a	0.13 ^a	1.68 ^a	-0.001 ^a	394 ^a	9.4 ^a	13.7 ^a	23.1 ^a	1.3 ^a	82.7 ^c	-11.9 ^b	104.6 ^a
	Legume	3.7 ^a	0.20 ^b	3.5 ^a	0.12 ^a	1.69 ^a	-0.001 ^a	412 ^a	6.9 ^c	9.6 ^b	16.5 ^b	1.2 ^{bc}	85.7 ^a	-10.5 ^a	85.4 ^c
<i>Brachypodium</i>	3.5 ^b	0.21 ^a	4.1 ^a	0.14 ^a	1.70 ^a	-0.001 ^a	424 ^a	9.2 ^a	12.6 ^a	21.8 ^a	1.2 ^b	83.7 ^{bc}	-12.3 ^b	102.6 ^a	
2012	4.2 ^a	0.26 ^a	4.0 ^a	0.13 ^a	1.71 ^a	-0.002 ^b	479 ^a	5.0 ^b	4.1 ^b	9.0 ^b	0.8 ^b	88.4 ^a	-9.7 ^a	80.4 ^b	
2013	2.8 ^b	0.15 ^b	3.8 ^a	0.12 ^a	1.67 ^b	0.000 ^a	343 ^b	11.8 ^a	19.1 ^a	30.9 ^a	1.6 ^a	79.8 ^b	-13.5 ^b	113.0 ^a	

MI: maturity index; Ac: free fatty acids; Pe: peroxide index; TP: total phenols; CarP: carotenoid pigments; ChloP: chlorophyll pigments; Pi: total pigments; Chlo/Car: chlorophyll/carotenoid ratio. Different letters in each column mean differences between treatments or years.

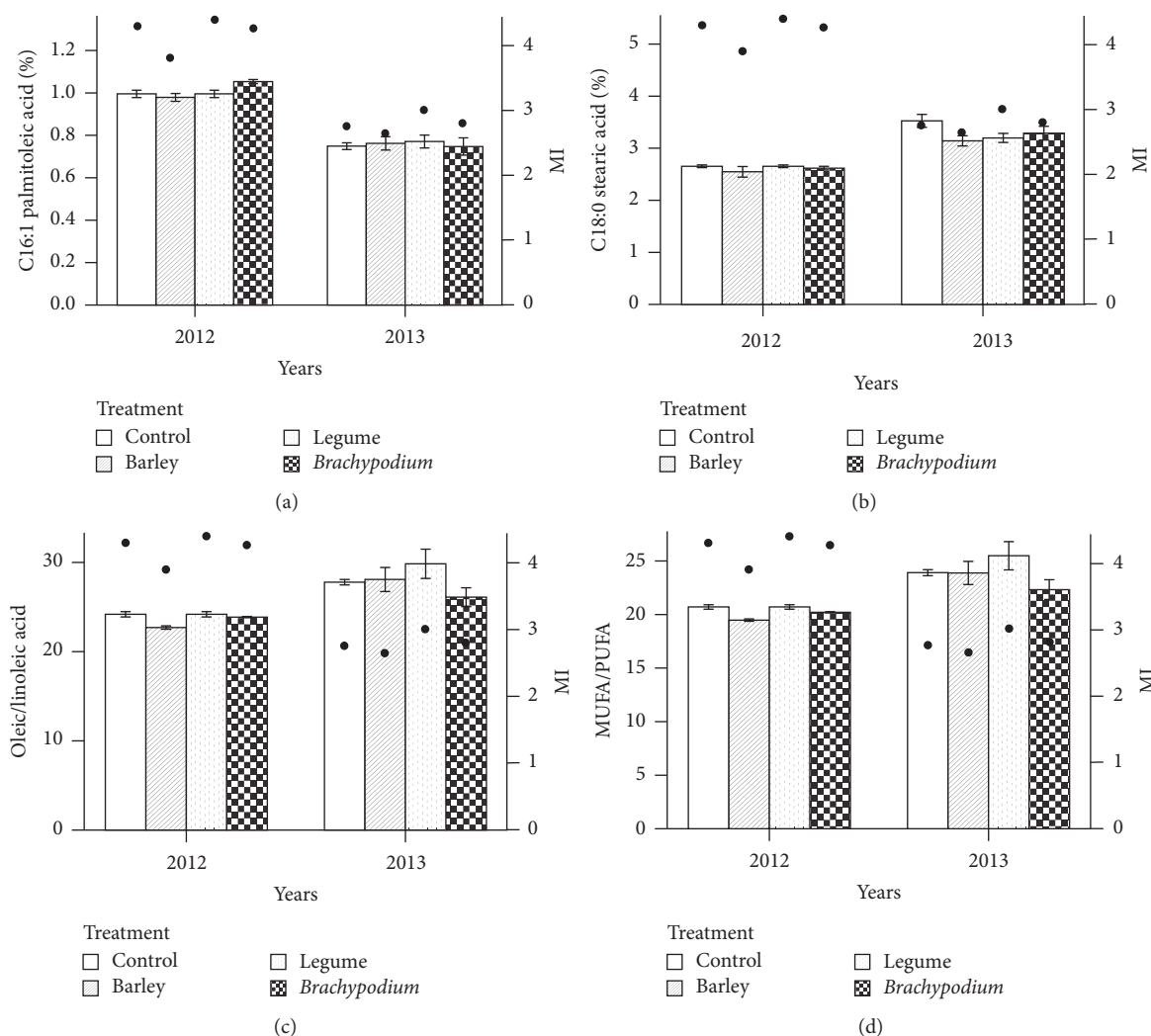


FIGURE 5: Palmitoleic (a) and stearic (b) acids, oleic to linoleic (c) acids, and MUFA to PUFA (d) ratio mean and standard deviation and maturity index (MI) as dots of different treatments and years.

The differences in VOOs between treatments were greater in the driest year, 2012, as can be seen in the longer scale in both functions of this year (± 40 in x -axis and ± 15 in y -axis) regarding 2013 (± 6 in x -axis and ± 6 in y -axis). In 2012 (Figure 7(a)), the first discriminant functions explained 93.3% of the variance, canonical $R^2 = 1.00$, with a cumulative variance of 99.9% with the second, canonical $R^2 = 0.99$. The first axis separates control from barley VOOs. Control VOOs were characterized by high MI and free acidity, while barley VOOs were characterized by low MI and higher content of total phenols and carotenoid and chlorophyll pigments, but a low MUFA to PUFA ratio. The second axis differentiates *Brachypodium* with the greenish VOOs (low a^*) from legume VOOs with higher MI and less content of carotenoid and chlorophyll pigments.

In 2013 (Figure 7(b)), the first discriminant functions explained 57.2% of the variance, canonical $R^2 = 0.96$, with

a cumulative variance of 88.4% with the second, canonical $R^2 = 0.93$. Legume and control VOOs were quite similar, characterized by a higher content in total phenols and MUFA to PUFA ratio. On the other hand, *Brachypodium* and barley VOOs had higher content of carotenoid and chlorophyll pigments, but *Brachypodium* had more free acidity, greenish color, and lower MUFA to PUFA ratio; and barley VOOs were mainly characterized by low content of total phenols and “sweeter” oils.

The influence of water stress, fruit ripeness, crop load, and nutrient availability and their interactions could explain changes in VOO parameters between soil management treatments [7, 10, 60, 62]. High contents of phenolic compounds, MUFA, and oleic acid have beneficial properties on health [39], but, despite the statistically significant differences found in VOOs between treatments or years, they were too low to get involved in changes in the beneficial effect on health.

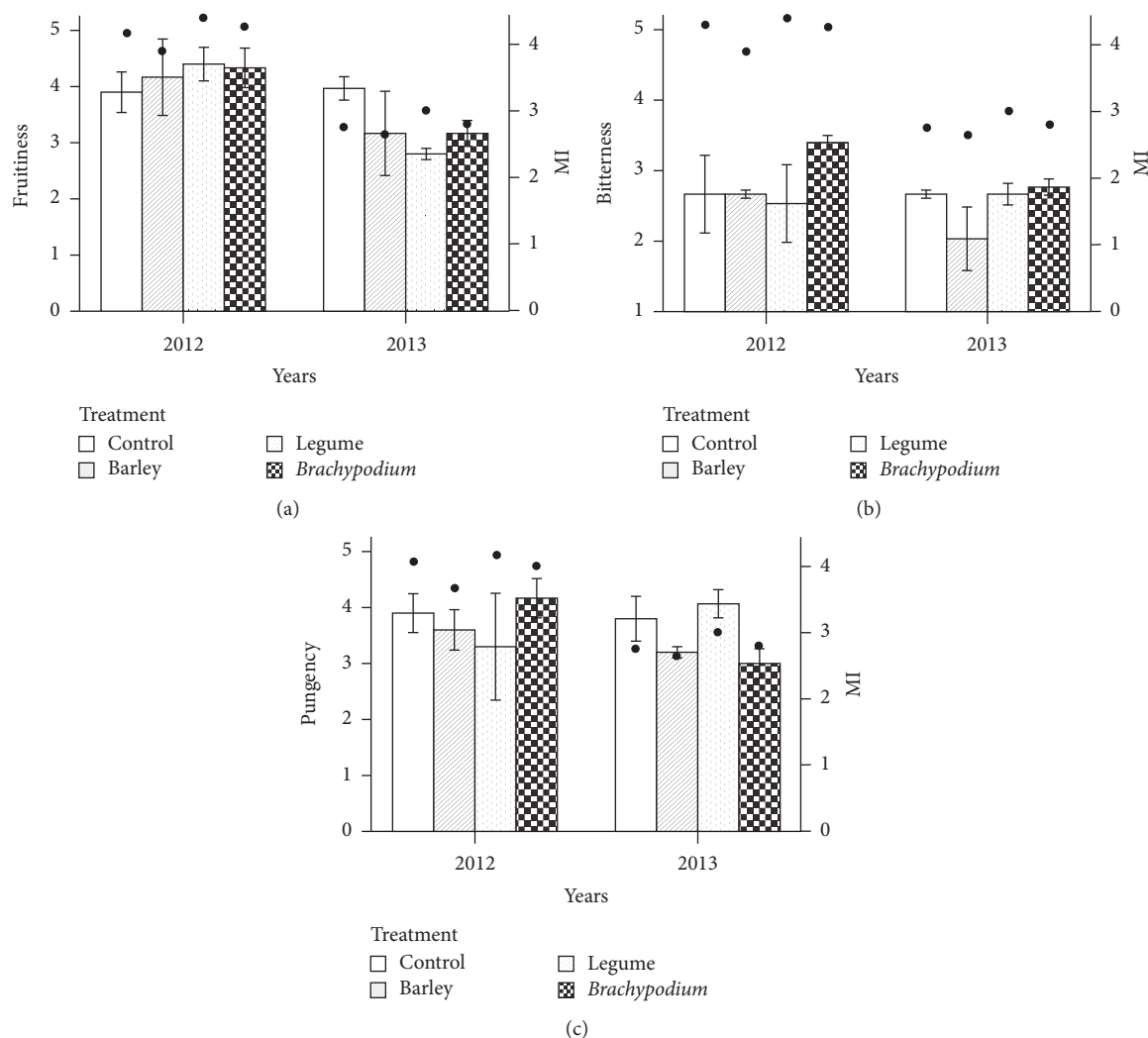


FIGURE 6: Fruitness (a), bitterness (b), and pungency (c) mean and standard deviation and maturity index (MI) as dots of different treatments and years.

TABLE 4: MANOVA (prob. > F) of oil fatty acids (%) as a function of treatment and year ($n = 3$).

	C14:0	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C24:0	MUFA/PUFA	Oleic/linoleic acid
Treatment	NS	NS	NS	0.049	NS	0.002	NS	0.034	0.003	NS	NS	NS	NS	0.005	0.002
Year	0.000	0.017	0.000	0.000	0.000	0.000	0.028	0.000	0.000	0.006	0.000	NS	NS	0.000	0.000
Tre \times year	NS	NS	0.026	NS	NS	0.027	NS	NS	NS	NS	NS	NS	NS	0.010	0.018

Tre \times year: treatment and year interaction. Fatty acids: C14:0 myristic acid, C16:0 palmitic acid, C16:1 palmitoleic acid, C17:0 margaric acid, C17:1 margaroleic acid, C18:0 stearic acid, C18:1 oleic acid, C18:2 linoleic acid, C18:3 linolenic acid, C20:0 arachidic acid, C20:1 gondoic acid, C22:0 behenic acid, and C24:0 lignoceric acid. NS: not significant.

4. Conclusions

Our results support the adoption of cover crops as sustainable management of rainfed olive groves in Central Spain in the drought-tolerant cultivar Cornicabra. Cover crops have not reduced fruit or oil yield either in heavy or in low yield years. Thus, the economic profits of land farmers are maintained, while improving environmental benefits with reducing soil erosion and increasing soil organic carbon.

Virgin olive oil quality was mainly influenced by the year, but slight differences appeared in some physicochemical and sensory characteristics assigned to soil management. In the year with higher rainfall amount, with more soil water content in autumn and high fruit load, there were fewer differences between the treatments of cover crops and minimum tillage. Therefore, slight differences were found in VOOs in the driest year, which could be caused by differences in soil water availability and nutrient uptake

TABLE 5: Oil fatty Acids (%) LS means from the MANOVA as a function of treatment and year ($n = 3$).

Treatment	MI	C14:0	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C24:0	MUFA/PUFA	Oleic/linoleic acid
Control	3.5 ^b	0.010	12.0	0.87	0.05 ^a	0.08	3.09 ^a	79.4	3.07 ^{ab}	0.56 ^b	0.43	0.27	0.12	0.05	22.3 ^{ab}	26.0 ^{ab}
Barley	3.3 ^c	0.010	11.6	0.87	0.04 ^b	0.08	2.85 ^c	79.9	3.18 ^a	0.59 ^a	0.43	0.27	0.12	0.05	21.7 ^{bc}	25.4 ^b
Legume	3.7 ^a	0.009	12.1	0.88	0.04 ^{ab}	0.08	2.93 ^{bc}	79.6	2.98 ^b	0.56 ^b	0.43	0.27	0.12	0.05	23.1 ^a	27.0 ^a
Brachypodium	3.5 ^b	0.010	12.0	0.90	0.04 ^{ab}	0.08	2.95 ^b	79.3	3.18 ^a	0.61 ^a	0.44	0.27	0.12	0.05	21.3 ^c	25.0 ^b
Year																
2012	4.2 ^a	0.014 ^a	11.2 ^b	1.00 ^a	0.04 ^b	0.07 ^b	2.62 ^b	80.1 ^a	3.38 ^a	0.64 ^a	0.42 ^b	0.29 ^a	0.12	0.05	20.3 ^b	23.7 ^b
2013	2.8 ^b	0.006 ^b	12.7 ^a	0.76 ^b	0.05 ^a	0.08 ^a	3.29 ^a	78.9 ^b	2.83 ^b	0.52 ^b	0.45 ^a	0.25 ^b	0.12	0.05	23.9 ^a	28.0 ^b

Fatty acids: C14:0 myristic acid, C16:0 palmitic acid, C16:1 palmitoleic acid, C17:0 margaric acid, C17:1 margaroleic acid, C18:0 stearic acid, C18:1 oleic acid, C18:2 linoleic acid, C18:3 linolenic acid, C20:0 arachidic acid, C20:1 gondoic acid, C22:0 behenic acid, and C24:0 lignoceric acid. Different letters in each column mean differences between treatments or years.

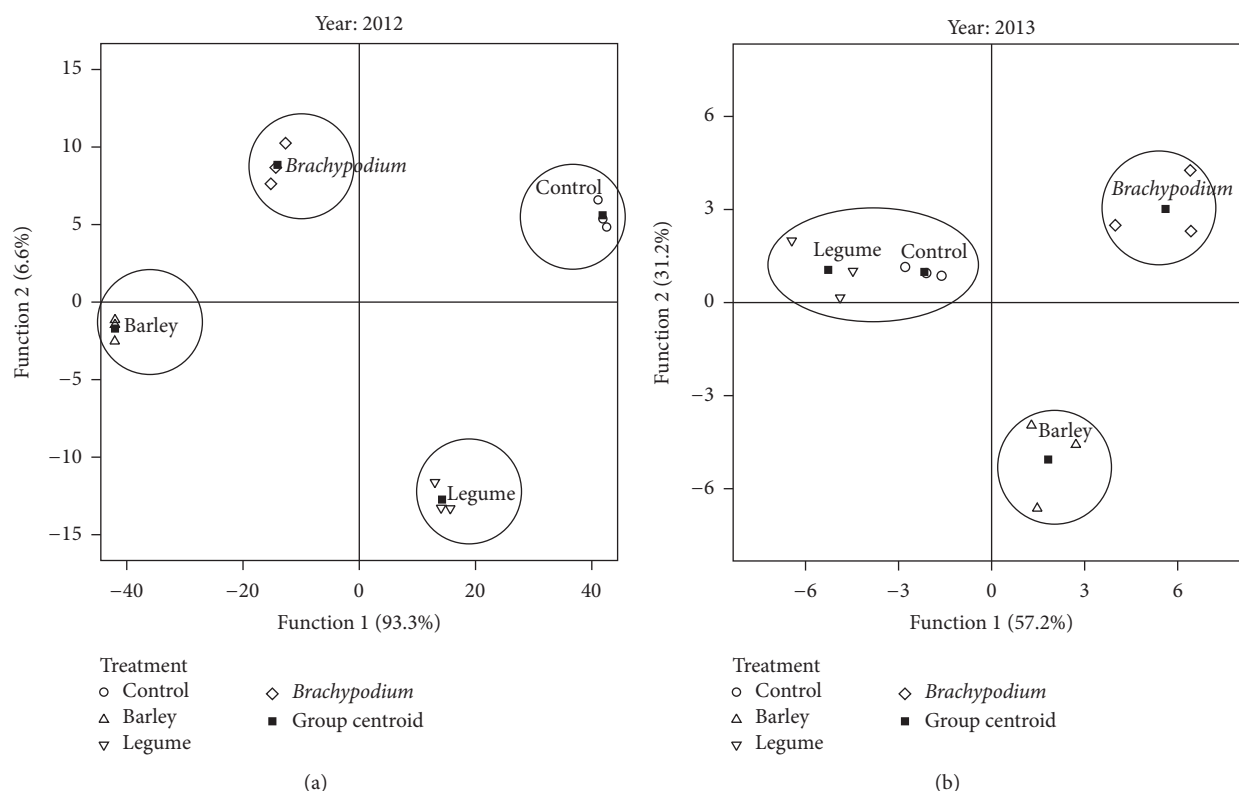


FIGURE 7: Canonical discriminant functions of the VOOs from different treatments in 2012 (a) and 2013 (b). Amount of variability explained by each function in brackets.

TABLE 6: VOO sensory characteristics LS means and standard deviation for treatments and years ($n = 3$). Covariates appearing in the model are evaluated at the following values: maturity index = 3.5.

	Fruitiness	Bitterness	Pungency
Treatment			
Control	3.9 ± 0.2^a	2.7 ± 0.1^b	3.9 ± 0.2^a
Barley	3.6 ± 0.3^a	1.9 ± 0.2^c	2.9 ± 0.2^b
Legume	3.7 ± 0.3^a	3.0 ± 0.1^{ab}	4.1 ± 0.2^a
Brachypodium	3.8 ± 0.2^a	3.1 ± 0.1^a	3.6 ± 0.2^a
Year			
2012	4.4 ± 0.7^a	4.1 ± 0.4^a	5.3 ± 0.6^a
2013	3.1 ± 0.7^a	1.3 ± 0.4^b	2.0 ± 0.6^b

Different letters in each column mean differences between treatments or years.

by olive tree. More research is required to investigate the influence of cover crops on water and nutrients uptake in Mediterranean semiarid olive groves and their influence on the final product. To overcome the year-by-year differences due to the alternate bearing cycle of olive trees and annual variations of Mediterranean climate, more cropping seasons are needed to find out whether the trends seen in this study will consolidate in the future.

TABLE 7: MANOVA (prob. > F) of VOO sensory characteristics as a function of treatment and year ($n = 3$).

	Fruitiness	Bitterness	Pungency
Treatment	NS	0.000	0.024
Year	NS	0.002	0.009
Treatment \times year	0.044	0.049	0.002

NS: not significant.

Competing Interests

The authors declare that they have no competing interests regarding the publication of this paper.

Acknowledgments

This work was supported by the Madrid Institute for Rural, Agricultural and Food Research and Development (IMIDRA) under Grant FP12-CVO and S2013/ABI-2717 AGRISOST Projects.

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