



## Effects of herbaceous covers and mineral fertilizers on the nutrient stocks and fluxes in a Mediterranean olive grove

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### ABSTRACT

The preservation of nutrient capital, soil fertility, and carbon (C) sequestration capacity in Mediterranean olive groves requires evaluation of agricultural practices beyond short-term productivity. We aim to contribute with a mechanistic understanding on the effects that the preservation of herbaceous cover and the use of chemical fertilizers have on the performance of olive trees and on the biogeochemical cycles of the agroecosystem. We compared nutrient fluxes and aboveground leafy stocks in an olive grove that had been organically managed for more than 60 years, in a treatment in which the annual spontaneous herbaceous cover was maintained (H), and after two years of shift to conventional management treatments in which the growth of herbaceous vegetation was avoided by the use of herbicides (NH), and where exclusion of the herbaceous cover is also combined with the supply of mineral fertilizers (NHF). Maintenance of herbaceous vegetation in H contributed to the retention of a high aboveground capital of C and nutrients, particularly nitrogen, (N), phosphorus (P) and potassium (K) that were about 2.9, 3.9 and 7.4 times greater than in NH, respectively. The permanence of herbaceous cover stimulated olive tree leaf litter decomposition rates by about 86 % and increased nutrient release. However, the H treatment led to a 37 % decrease in olive yield and lowered olive foliar N and P content as negative short-term effects. The addition of fertilizers (N, P, K, and Mg) in mineral and solid form in NHF resulted inefficient to improve olive tree nutritional status and olive production, and decelerated olive tree litter decomposition rates by 21 % and nutrient release. The nutrient retention in organic forms in the fast-growing species of herbaceous covers and the progressive nutrient release as litter decomposes may contribute to regulate and better adapt nutrient availability to the nutrient requirements of olive trees.

### 1. Introduction

Olive trees (*Olea europaea* L.) cover around 5.1 Mha in Europe, which accounts for 49 % of the global area of olive cultivation (FAOSTAT, 2022), with a large proportion cultivated on poor or rocky soils of the Mediterranean basin. European olive oil production represents around

76 % of world production, and of this, 75 % derives from Spain (FAOSTAT, 2022). However, the implementation of inadequate conventional soil-management practices in olive groves has led to several environmental problems such as high runoff and erosion rates, soil C and fertility losses (Gómez et al., 2009a, 2009b). The implementation of sustainable agricultural practices in olive groves may potentially derive

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large environmental, social, and economic benefits for Europe (Camarsa et al., 2010). For this reason, the European Union has prioritized agricultural policies designed to mitigate effects of global environmental change in olive groves, such as high-level agreement to reform the Common Agricultural Policy (CAP) and link 30 % of direct agricultural payments to environmentally-friendly farming practices. The assessment of sustainable olive grove management practices is therefore essential for the design of policies that guarantee conservation and delivery of ecosystem services in these agricultural systems, including soil retention, carbon (C) sequestration and biodiversity (FAO, 2004).

The maintenance of spontaneous resident vegetation cover (hereafter referred to as herbaceous cover) has become an effective conservation practice in olive groves if managed adequately (Nieto et al., 2013). The presence of herbaceous cover during autumn and spring reduces soil erosion (Gómez et al., 2009b), ameliorates the soil microclimate (Kairis et al., 2013), improves soil physicochemical properties (Sastre et al., 2018), and increases interception and storage of rainfall water and water availability in deep soil layers (Palese et al., 2014). Greater organic C inputs to the soil associated with herbaceous cover also translate into increases in soil C content (Vicente-Vicente et al., 2016; Morugán-Coronado et al., 2020) and greater levels of ecosystem C sequestration (Aguilera et al., 2015; Chamizo et al., 2017). The increasing availability of organic substrates also enhances microbial activity and biogeochemical cycling, with the consequent improvement of soil structure and fertility (Soriano et al., 2014; Herencia, 2015). Despite their contribution to soil fertility, there is no consensus regarding the effects of vegetation covers on the nutritional status of olive groves (Ferreira et al., 2013; Zipori et al., 2020). Nonetheless, spontaneous vegetation, and leguminous in particular, may contribute to reduce dependency of crops on external nutrient inputs by enhancing soil microbial communities involved in the soil N cycle, leading to improvement of leaf and fruit nutritional status (Rodrigues et al., 2015; Lombardo et al., 2021). Moreover, this practice reduces considerably the management cost and environmental damage related to the repeated use on herbicides, tillage or land-clearing using heavy machinery (Guzmán and Foraster, 2011) and also contributes to higher plant and animal biodiversity (Rey et al., 2019), increasing the resilience of olive groves to pests (Paredes et al., 2019).

However, the presence of herbaceous covers may also have some disadvantages. For instance, the interference with harvesting and other cultural operations and greater damage from spring frosts (Saavedra, 2007a) and the increased risk of fires are some arguments against the presence of herbaceous covers that have been used by olive farmers (Koutsias et al., 2012). Herbaceous covers may also compete with young olive trees for water, resulting into reductions in olive yield (Ferreira et al., 2013). Nonetheless, the net effect on soil water availability is controversial (Ruíz-Colmenero et al., 2011; Palese et al., 2014). It is, as well, not clear whether herbaceous vegetation also competes with olive trees for nutrients, whether yield reductions are extensible to mature trees (Palese et al., 2014; Soriano et al., 2014), and whether this competition also translates into reductions in olive oil quality or production (García-González et al., 2019). Understanding these knowledge gaps may allow optimization of the potential benefits of herbaceous cover maintenance for olive productivity and sustainability.

Olive groves, as agroecosystems, may be framed within the r/K selection theory (Margalef, 1959), where herbaceous vegetation and olive trees have contrasting life-history strategies. Herbaceous vegetation comprises opportunistic with rapid growth rates, short generation times, small sizes and efficient dispersal and colonization strategies useful for unstable, ruderal environments, typical of r-strategists. Olive trees are, in contrast, specialized stress-tolerators adapted to more stable environments, have slow growth rates, longer generation times, and larger sizes, typical of K-strategists (Gunderson and Holling, 2001). Although limitations of this dichotomous r/K selection concept have been highlighted in empirical studies (Roff, 1993), positioning species along an r/K continuum (Jones, 1976) may, nevertheless, be useful to improve

understanding of factors that enhance adaptive capacity and resilience of ecosystems (Angeler et al., 2019). For example, ecosystems characterized by frequent disturbances and low complexity ecological interactions select for r-strategists, such as the ruderal weed species that farmers tend to control in agricultural systems. K-strategists may play an important role in buffering effects of disturbances in agroecosystems, such as during crop harvest, since most of their energy is allocated to vegetative plant parts. A wide spectrum of life-history strategies in ecosystems may thus increase functional diversity and spatio-temporal heterogeneity (Gliessman, 2007), allowing to take advantage of opportunities created by perturbations and to prevent major losses in ecosystem functions and services (Wood et al., 2015). However, an ecological framework for olive groves that accounts for biogeochemical functioning and nutritional strategies of olive trees and herbaceous vegetation remains lacking.

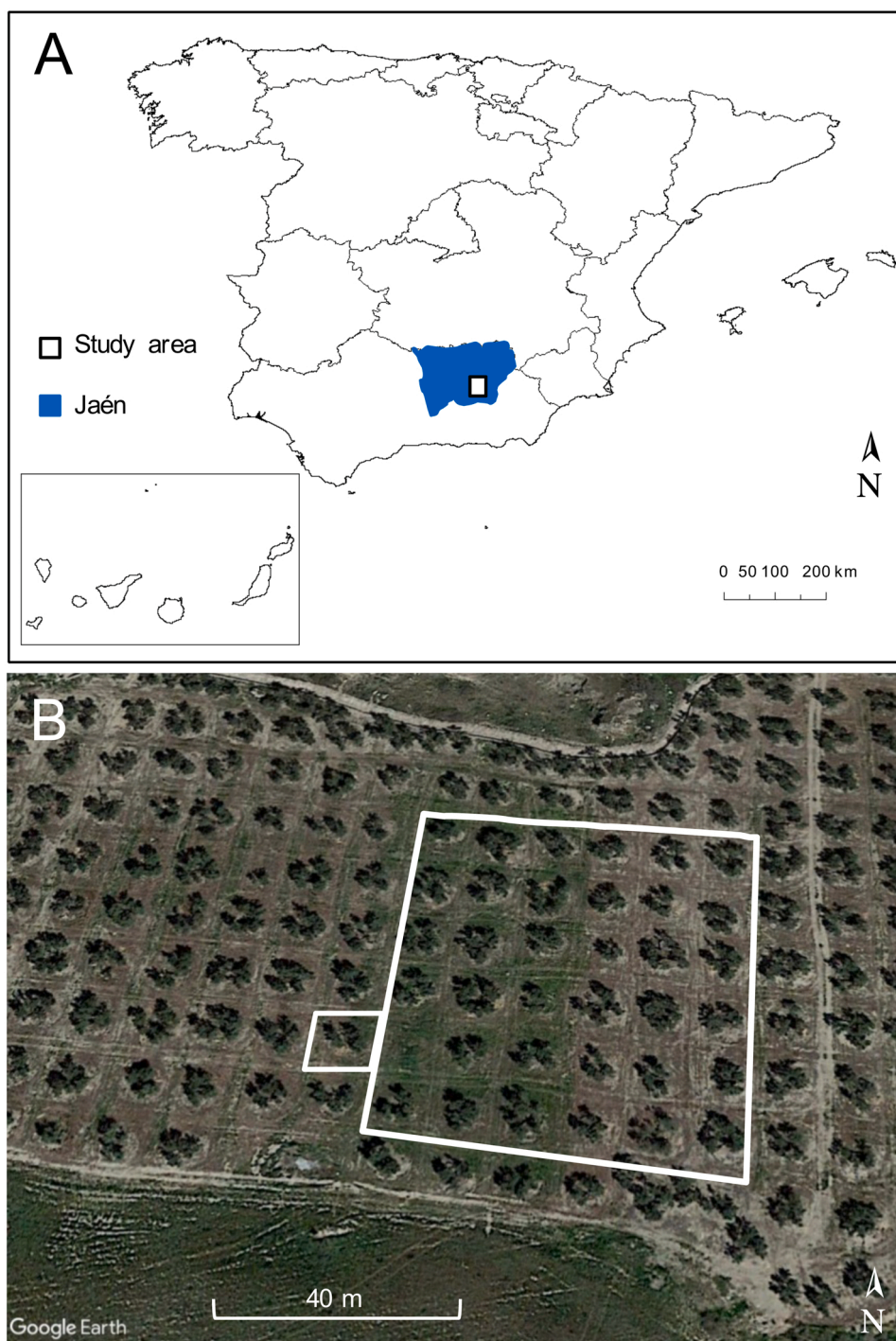
In this study, we aim to contribute with a mechanistic understanding on the individualized effects that the preservation of the herbaceous cover and the use of chemical fertilizers have on the performance of olive trees and on the biogeochemical cycles of the agroecosystem. For that, we compare C and nutrient stocks and fluxes in aboveground leafy litter (herbaceous biomass and olive leaf litter) of olive grove plots with the presence of herbaceous cover (H), with inputs of mineral fertilizers (NHF) and without any herbaceous cover or fertilizers inputs (NH) from April 2015 to October 2017. This allows to 1) assess, for the first time, the direct and indirect management effects of these individualized practices on litter decomposition and on the rates of release or immobilization of C and nutrients, 2) determine the magnitude and relative relevance of C and nutrient pools in the aboveground leafy litter in each of these management treatments, and 3) evaluate the effects of these organic versus conventional management practices on the nutritional status and yield of olive trees. We predict lower nutrient stocks but faster nutrient fluxes in the aboveground herbaceous vegetation compared to the olive leaf litter. As a result, we hypothesize that the coexistence of herbaceous vegetation and olive trees would lead to tighter nutrient cycles in olive grove agroecosystem due to their contrasting and complementary ecological strategies for the acquisition of limiting resources, despite the competition for these resources in the short-term. By contrast, we predict a lower efficiency in the use of nutrients by olive trees when these are provided in the form of mineral fertilizers.

## 2. Methods

### 2.1. Study site

The study site, an irrigated orchard of olive trees var. 'Arbequina', was located at Cortijo Guadiana, near Jaén, Spain (37°55'10.13"N, 3°14'24.62"W, Fig. 1a). The site is situated at 370 m above sea level. The climate is classified as Mediterranean, with hot dry summers and mild winters. The highest air temperatures occur in July (mean maximum: 37.4 °C), the lowest air temperatures in January (mean minimum: 0.8 °C), and the mean annual temperature for the area is 16.1 °C. Mean annual precipitation is 466 mm, with the majority occurring in spring and autumn (data obtained from 15-year records at the Agroclimatic Station of Úbeda, located at 7 km from our study site, Red de Información Agroclimática de Andalucía, 2022, <http://www.juntadeandalucia.es/agriculturaypesca/ifapa/ria/servlet/FrontController>).

The olive trees, which were about 20 years old, planted at 12 m average distance among olive trees and at a density of 69.6 trees ha<sup>-1</sup>, were located in a flat area that had been managed using organic agricultural practices for more than 60 years. The study area in the olive plantation was selected due to its representative and well-established, non-perennial herbaceous cover that generally senesced at the start of the summer dry period (May) and re-emerged in autumn following the first post-summer rains (Rey et al., 2019; Tarifa et al., 2021). The most abundant species in the herbaceous cover of the olive grove were *Medicago polymorpha* (61 % frequency of occurrence), *Torilis arvensis* (61 %),



**Fig. 1.** A. Study site location in Jaén province on the Iberian Peninsula, B. Aerial view of the study plots. An olive tree was initially missing from the grid in the selected area, so the area was extended to include an additional tree.

*Scorpiurus muricatus* (56 %), *Bromus madritensis* (47 %), *Anacyclus clavatus* (44 %), *Galium aparine* (39 %) and *Hordeum murinum* (36 %). The management in the selected study area consisted in the maintenance of the spontaneous herbaceous cover from autumn to spring. In spring, herbaceous vegetation was mowed mechanically and left on the surface to avoid competition for water with trees. The olive groves were irrigated using pressure compensating drippers (Netafim, Tel Aviv, Israel) once per week from May to October, at an average rate of  $34 \text{ m}^3 \text{ ha}^{-1} \text{ week}^{-1}$  using water from an irrigation pond. The irrigation water is characterized by low sodium hazard, high salinity hazard and moderate levels of chloride (Zaman et al., 2018) as indicated in Appendix 1. The

only fertilization in the study area was applied as foliar fertilizer. An aqueous solution containing  $60 \text{ g tree}^{-1}$  of NPK fertilizer in a ratio 14:5:25 was sprayed to the leaves with an atomizer twice per year, once in spring and once in autumn. In order to determine the effects of the treatments on the nutritional status and yield of olive trees, foliar fertilization was not further applied over the course of the experiment. Soils were classified as Calcaric Regosols (Table 1).

## 2.2. Baseline soil physicochemical properties

We analyzed pre-treatment soil physicochemical properties from a

**Table 1**

Initial soil variables at two soil depths before establishment of the management treatments. TC: total carbon; TN: total nitrogen; SOM: soil organic matter; SOC: soil organic carbon; DOC: dissolved organic carbon; CEC: cation exchange capacity. Values are means  $\pm$  standard errors from 18 soil samples, collected at three distances from six randomly selected olive trees.

Soil depth	0–5 cm	5–15 cm
Texture (%):	Clay loam	
Sand (0.05–2 mm)	25.65 $\pm$ 0.95	24.90 $\pm$ 1.19
Coarse loam (0.02–0.05 mm)	10.97 $\pm$ 0.52	12.05 $\pm$ 0.34
Fine loam (0.002–0.02 mm)	29.66 $\pm$ 0.61	27.41 $\pm$ 0.92
Clay (<0.002 mm)	33.76 $\pm$ 0.81	35.63 $\pm$ 0.99
Mineralogy (%):		
Quartz	17 $\pm$ 2.31	20.33 $\pm$ 3.84
Calcite	32.67 $\pm$ 3.53	26 $\pm$ 1.53
Muscovite	24 $\pm$ 1.53	28.33 $\pm$ 2.60
Paragonite	4.67 $\pm$ 0.33	5.33 $\pm$ 1.45
Plagioclase	6 $\pm$ 1	6 $\pm$ 1.73
Dolomite	11.67 $\pm$ 1.86	9.67 $\pm$ 1.45
Filosilicate	4 $\pm$ 0.58	4.33 $\pm$ 0.67
Soil fertility parameters:		
pH	8.46 $\pm$ 0.02	8.58 $\pm$ 0.02
TC (%)	6.09 $\pm$ 0.04	5.67 $\pm$ 0.04
TN (%)	0.20 $\pm$ 0.00	0.15 $\pm$ 0.01
SOM (g kg <sup>-1</sup> )	49.65 $\pm$ 2.49	33.68 $\pm$ 2.33
SOC (%)	2.88 $\pm$ 0.15	1.96 $\pm$ 0.14
SOC:TN	14.19 $\pm$ 0.62	12.78 $\pm$ 0.72
DOC (mg kg <sup>-1</sup> )	223.80 $\pm$ 15.29	118.51 $\pm$ 16.85
CaCO <sub>3</sub> (% eq.)	41.46 $\pm$ 0.42	41.85 $\pm$ 0.38
CEC (cmol <sub>c</sub> kg <sup>-1</sup> )	17.35 $\pm$ 0.57	16.49 $\pm$ 0.62
Base saturation (%)	saturated	saturated
Ca (cmol <sub>c</sub> kg <sup>-1</sup> )	11.49 $\pm$ 0.64	11.87 $\pm$ 0.56
Mg (cmol <sub>c</sub> kg <sup>-1</sup> )	2.86 $\pm$ 0.09	3.23 $\pm$ 0.13
K (cmol <sub>c</sub> kg <sup>-1</sup> )	2.85 $\pm$ 0.30	1.38 $\pm$ 0.13
Na (cmol <sub>c</sub> kg <sup>-1</sup> )	0.41 $\pm$ 0.08	0.31 $\pm$ 0.03
P <sub>inorg</sub> (mg kg <sup>-1</sup> )	5.13 $\pm$ 0.47	2.17 $\pm$ 0.45
NO <sub>2</sub> (mg kg <sup>-1</sup> )	11.53 $\pm$ 3.73	4.26 $\pm$ 1.34
NO <sub>3</sub> (mg kg <sup>-1</sup> )	73.10 $\pm$ 550	57.20 $\pm$ 3.62
PO <sub>4</sub> <sup>3-</sup> (mg kg <sup>-1</sup> )	11.22 $\pm$ 4.24	9.45 $\pm$ 4.51

delimited study area of about 5170 m<sup>2</sup> containing 36 olive trees (Fig. 1b). Prior to establishment of the treatments, soil samples were taken at 1, 3, and 5 m from six randomly selected olive trees, where three to four 15-cm deep soil cores were collected using a gouge auger (2.5 cm diameter). Cores were divided into 0–5 and 5–15-cm soil depths and then mixed to create single composite samples by depth and sampling point ( $n = 6$  trees  $\times$  3 distances  $\times$  2 depths = 36 soil samples). Soil samples were air dried, sieved to 2-mm gauge, and stored in dry conditions prior to elemental analyses.

Total C and total nitrogen (N) concentration were determined by combustion at 850 °C (Leco Tru Spec autoanalyzer, St Joseph, MI, USA). Soil organic C concentration was determined from reduced potassium dichromate (Cr<sub>2</sub>O<sub>7</sub>K<sub>2</sub>) with Mohr salt (Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub> 6 H<sub>2</sub>O), following oxidation under excess Cr<sub>2</sub>O<sub>7</sub>K<sub>2</sub>. Soil organic matter concentration was calculated as the product of the soil organic C concentration and the factor of 1.724 (Tyurin, 1951). Dissolved organic C was extracted using the Ghani method (Ghani et al., 2003) and concentration was determined using the Tyurin method (Tyurin, 1951). Concentration of soil calcium carbonate (CaCO<sub>3</sub>) was determined as the amount of carbon dioxide emitted during the reaction of CaCO<sub>3</sub> with hydrochloric acid (HCl) (Bascomb, 1961). Available inorganic phosphorous (P) concentration was determined in sodium bicarbonate extracts using the Olsen method (Watanabe and Olsen, 1965) and measured using a Perkin Elmer 2400 spectrophotometer (Waltham, MA, USA). Concentration of nitrite, nitrate, and phosphate anions was determined using ionic chromatography (DX-120, Dionex, Sunnyvale, USA) with an anionic suppresser (ASRS-II, Dionex, Sunnyvale, USA). Concentrations of calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na) cations were determined from displacement with ammonium acetate using the ammonium acetate method (Soil Conservation Service, 1972). Cation

exchange capacity was obtained after saturation of the soil exchange complex with Na<sup>+</sup> cations, by adding sodium acetate, and then displaced Na<sup>+</sup> cations with ammonium acetate by atomic absorption (Soil Conservation Service, 1972). Soil pH was determined by stirring and settling in distilled water with a pH meter (Crison micropH-2001, Barcelona, Spain), according to (Pansu and Gautheyrou, 2006). Soil texture was determined using the standard pipette methods of Robinson-Köhn or Andreasen (Pansu and Gautheyrou, 2006), and the mineralogy of a subsample of soil that was milled to powder was determined by X-ray diffraction (Whittig and Allardice, 1986) using an X-ray diffractometer (Bruker D8 Advance, Madrid, Spain).

### 2.3. Experimental design

In April 2015, three agricultural management treatments, with contrasting herbaceous cover and use of mineral fertilizers, were randomly applied to three plots of nine replicated trees. Treatments comprised the maintenance of herbaceous vegetation without mowing during all the study period (H), use of herbicide to avoid the growth of herbaceous vegetation (NH), and use of herbicide combined with the increase of nutrient inputs by the addition of solid mineral fertilizers (NHF). Since herbaceous cover at the study site was well established, the H treatment did not require any intervention. The NH and NHF treatments were maintained free of herbaceous cover along the complete duration of this study (April 2015 to October 2017). For that, herbaceous vegetation was first cleared using a weeding machine, and then an aqueous mixture of herbicides (24 % oxyfluorfen at 27.8 L ha<sup>-1</sup> and 36 % glyphosate at 66.7 L ha<sup>-1</sup>), similar to that used in conventional management of olive groves (Saavedra, 2007b), was applied evenly over the soil surface using air-carrier sprayers annually in spring. An additional application of preemergence herbicide (oxyfluorfen 24 %, 27.8 L ha<sup>-1</sup>) was also applied annually in autumn in the same way as described above. Annually in spring (2015–2017), we evenly applied 50 kg of solid mineral fertilizer to the 1292 m<sup>2</sup> of the NHF treatment, which comprised 55 kg ha<sup>-1</sup> y<sup>-1</sup> of N (2.9 % NO<sub>3</sub>, 10.9 % NH<sub>4</sub>, 6.2 % NH<sub>3</sub>), 8.2 kg ha<sup>-1</sup> y<sup>-1</sup> of P (10 % P<sub>2</sub>O<sub>5</sub>), 16 kg ha<sup>-1</sup> y<sup>-1</sup> of K (10 % K<sub>2</sub>O), and 4.7 kg ha<sup>-1</sup> y<sup>-1</sup> of Mg (2 % MgO) (García, 2009), to soils between rows of olive trees and under the tree canopies. This allowed to standardize the mineral fertilizer application and to disentangle the effect of the fertilization from the effects of other factors of covariation on soil variables while keeping a feasible number of soil sampling points. Fertilization was synchronized with rainfall events when possible.

### 2.4. Soil microclimate variables

Soil temperature (at 5-cm depth) and moisture (at 25-cm depth) were monitored every 30 min, from February to October 2017, at 1-m distance from six randomly selected olive trees in each of the H and NH treatments using sensors (S-TMB-M002 and 10HS smart sensor S-SMD-M005, for soil temperature and moisture, respectively; Onset Computer Corporation, Bourne, USA).

### 2.5. Litter decomposition

In April 2015, six nylon mesh bags (2 mm in diameter; hereafter referred to as litter bags) containing about 15 g of senescent olive leaves collected from olive trees were placed 1 m from the base of each olive tree in all three treatments ( $n = 9$  replicates  $\times$  6 sampling times  $\times$  3 treatments = 162 olive litter bags). Similarly, four litter bags containing about 15 g of fresh aboveground herbaceous plant material were placed under each olive tree in H ( $n = 18$  replicates  $\times$  4 sampling times = 72 herbaceous litter bags). We included more litter bag replicates for herbaceous litter since they were composed by a more heterogeneous material. Litter bags were sampled more frequently (every 5 weeks) in the early stages of litter decomposition than in the later stages (every 3–6 months) as decomposition slows down at later decomposition stages.

The decomposition period for herbaceous litter was one year, whereas for the more recalcitrant olive tree leaves was 1.5 years (Rodríguez-Pleguezuelo et al., 2009). On each sampling occasion, a single olive leaf and herbaceous litter bag was carefully placed inside a paper bag and transported to the laboratory; then, the leaf and litter material were carefully removed from the bags and dirt was removed using a small brush. The leaf and litter were dried to a constant weight at 60 °C and then ground using a ball mill prior to elemental analysis.

## 2.6. Herbaceous biomass

Herbaceous biomass was sampled annually during peak growing season in spring 2015, 2016, and 2017 from single 100 × 25 cm quadrats placed at 1, 3, and 5 m from the base of olive trees to account for spatial variability in herbaceous vegetation cover in the H treatment (n = 9 replicates × 3 distances × 3 years = 81 herbaceous biomass samples). Aboveground vegetation was non-existent or negligible in the NH and NHF treatments, so was not sampled. All aboveground vegetation in the quadrats was clipped and taken to the laboratory, where it was dried to a constant weight at 60 °C, cleaned with a small brush, weighed, and then ground using a ball mill prior to elemental analysis.

## 2.7. Olive tree litterfall

Two litter traps, comprising two 22.5 cm diameter plastic pots, were hung in different branches of each of the nine olive trees per treatment from June 2016 to June 2017. Pots were positioned at randomized horizontal distances from the tree trunk and hanged below the olive tree canopy. Pots were also leveled horizontally with the soil surface. The base of the litter traps was perforated to prevent water accumulation. On each sampling occasion, collected litter was emptied into a paper bag that was then carefully transported to the laboratory. Sampling frequency (every 3 months during summer and autumn and every 6 months during winter and spring) reflected seasonal litterfall rates. The olive tree litterfall was then dried to a constant weight at 60 °C, cleaned with a small brush, weighed, and then ground using a ball mill prior to elemental analyses. Olives, if present, were discarded prior to weighing and grinding.

## 2.8. Olive tree crown area, olive yield and harvested litterfall

Olive tree crown area was measured at satellite images of high-resolution using Google Earth pro (Google LLC., Mountain View, EEUU). Harvesting of olives was performed as usual, using a combination of manual stirring and specialized machinery, and annual olive yield was determined in November 2016 and 2017 as the fresh weight of biomass harvested per tree (olive fruit plus leaves), minus leaf litter that fell during harvesting operations (hereafter referred to as harvested litterfall). Fresh weight of harvested litterfall per olive tree was also recorded in 2016 and a subsample was collected and processed, as described for olive tree litterfall, to determine moisture content and for elemental analysis. The determination of nutrient stocks and fluxes of tree pruning residues would have required a monitoring period that exceeds the duration of this project as well as our work capacity and economic budget.

## 2.9. Nutritional status of olive trees

At the beginning of the experiment (spring 2015) and 2 years after the establishment of the treatments (spring 2017), fresh olive tree leaves were sampled from the uppermost shoot tip that had elongated during the preceding year. Leaves were processed, as described above, prior to elemental analysis.

## 2.10. Chemical analysis of plant material

Samples of olive and herbaceous litter, olive tree litterfall, and fresh olive tree leaf material were digested in a microwave oven and then extracted using 2 % HCl. We determined Ca, Mg, K, manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn) concentrations using atomic-absorption spectrophotometry. We analysed P concentration using the V/UV spectrophotometry nitro-molybdovanadate method. Concentrations of N and C were quantified using an elemental analyzer (LECO TruSpec CN 2.4., St Joseph, MI, USA). Reported nutrient concentrations are based on dry weight.

## 2.11. Calculation of nutrient stocks and nutrient release

Calculation of annual olive production and harvested litterfall per unit area was based on the olive yield and litterfall per tree in a 12 × 12 m area that equates to the average distance between olive trees in a row. Crown area of olive trees did not vary with management treatment, so there was no need to use this as a correction factor in the calculation of olive production. Calculation of annual herbaceous biomass production was based on the dry weight of herbaceous biomass per unit area. In order to account for the spatial variability of the herbaceous vegetation cover, samples collected at 1, 3, and 5 m from each olive tree were considered representative of concentric circular areas representing the 13 %, 31 % and 56 % of the 12 × 12 m area, respectively. Weighted values of herbaceous biomass per unit area were then summed to obtain the total herbaceous biomass in the 12 × 12 m area. Annual olive tree litterfall by crown area was calculated with the annual cumulative amount of litterfall per tree. Annual nutrient stocks were calculated as the product of biomass stock and the elemental concentrations of the corresponding plant material. The annual release of C and nutrients from aboveground herbaceous vegetation and olive tree leaf litter was calculated based on the annual nutrient stocks and the rates of C and nutrient released (100 - % element remaining after one year of decomposition) in the correspondent decomposing litter (i.e. aboveground herbaceous vegetation or olive tree litter).

## 2.12. Statistical analysis

Main effects of management on tree crown area, olive production (yield), olive tree litterfall, harvested olive tree litterfall, elemental composition of harvested olive tree litterfall (Appendix 2), amount of C and nutrient stocks, and C and nutrient released by olive tree litter, and final concentrations of single elements in olive tree leaf litter and fresh leaves were tested using one-way ANOVAs, with management as a fixed factor. When *P*-values were lower than 0.05, within-treatment effects were analyzed using post-hoc tests, with Tukey correction for multiple testing. The effect of management on the averages of daily soil temperature ranges, daily minimum, maximum and daily mean soil temperatures across days during the senescent and growing periods for herbaceous covers was similarly tested using one-way ANOVAs, with management as a fixed factor. The effect of treatment on olive production and its variation along the time was also explored by repeated measures ANOVA split-plot design, with treatment as main fixed factor between subjects and time and its interaction with treatment as factors within subjects. Differences in elemental composition between herbaceous and olive tree leaf litter were tested using one-way ANOVAs, with litter type as a fixed factor.

The proportion of litter dry weight remaining after the period of decomposition (%) by litter type and treatment was fitted to an exponential model, with the form:

$$X_t = X_0 e^{-kt} \quad (1)$$

where  $X_t$  is the proportion of litter dry weight remaining at time  $t$ ,  $X_0$  is the initial proportion of litter dry weight (100 %), and  $k$  is the instantaneous decay rate. Effects of management treatment on the decay rates of olive tree leaf litter were also tested for individual  $k$  values for

each treatment and replicate using a one-way ANOVA, with management as a fixed factor.

Principal component analyses (PCA) were performed for the elemental composition of herbaceous and olive tree leaf litter at the beginning and end of the decomposition periods, using standardized variables. Differences between initial and final stages of decomposition, and among management treatments for olive tree leaf litter, according to the first two component scores of the PCAs were tested using one-way ANOVAs, as well as Tukey HSD post-hoc tests for management effects (Jolliffe, 2002; Quinn and Keough, 2009). The nutritional status of olive trees was explored using a PCA of the elemental composition of olive tree leaves sampled in spring 2017 (two years after the establishment of the management treatments), using standardized variables. The effect of the agricultural management on the nutritional status of olive trees was then tested for the first two component scores of the PCAs using one-way ANOVAs and Tukey HSD post-hoc tests. The assumptions of normality and homoscedasticity were tested before ANOVAs and data were transformed when required to achieve these assumptions (Quinn and Keough, 2009). Statistical analyses and models construction were performed using JMP®, Version 16.0 (2022).

### 3. Results

#### 3.1. Microclimate and stand variables

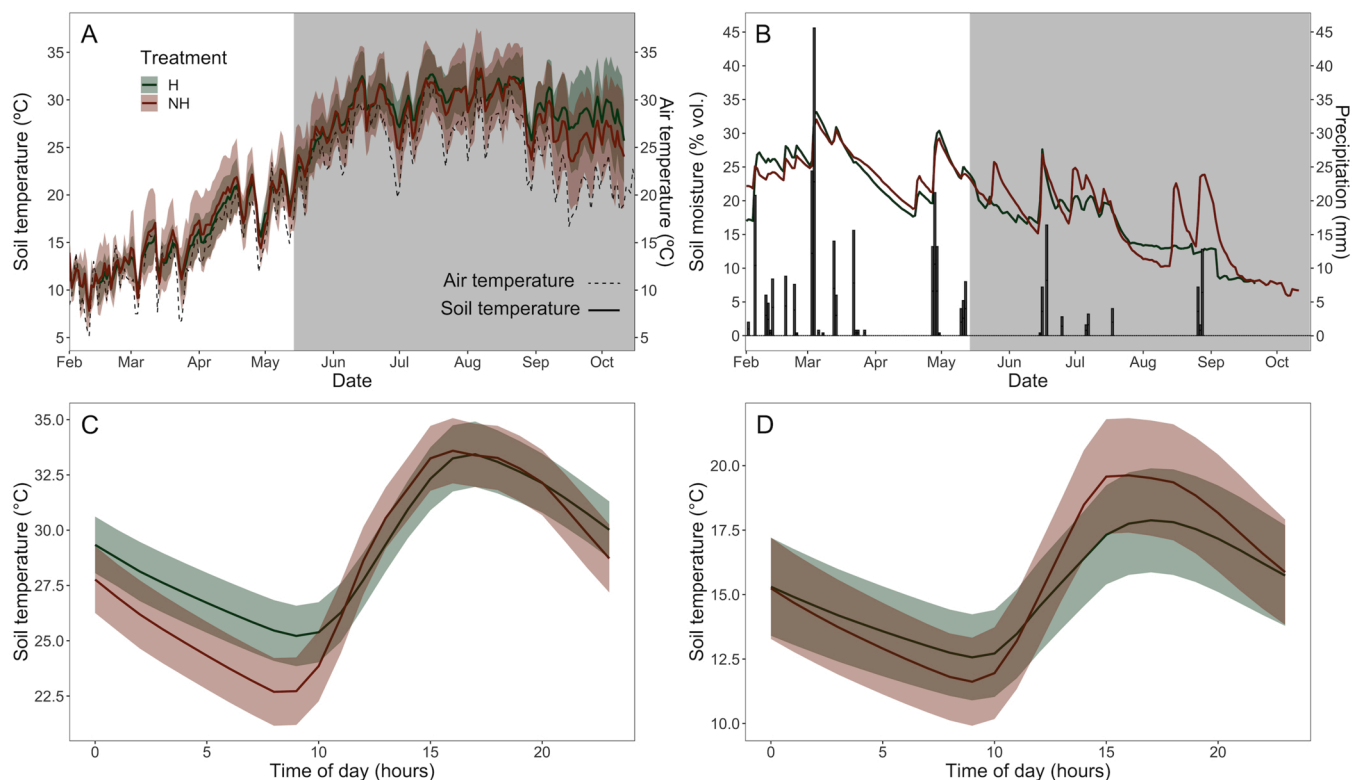
Although overall mean daily soil temperature in the upper 5 cm of soil did not differ visually between areas with (H) or without (NH) herbaceous cover, they were seemingly higher in H from the beginning of September to mid-October (Fig. 2a). In general, soil moisture did not show substantial differences between H and NH visually, but lower levels of moisture were recorded in H after rain events from July to

September (Fig. 2b). The averages of daily mean, minimum and maximum soil temperatures across days did not differ significantly. Nonetheless, the means of daily ranges of soil temperatures were larger in NH compared to H both during the senescent period ( $P = 0.0168$ ) and during the vegetative growing period for herbaceous plants ( $P = 0.0221$ ). This was caused mainly by higher values (although not significant) of minimum daily soil temperatures in H during the senescent period (Fig. 2c) and by lower values (although not significant) of maximum daily soil temperatures in H during the vegetative growth period (Fig. 2d).

Olive tree crown area, annual litterfall, and annual litterfall during harvest did not differ among treatments. However, olive production (olive fresh weight) was affected by management particularly during the second year, where production was lower in H than NH and NHF (Table 2). Overall, the year of harvest did not affect olive production, although the production tended to decrease more in H from 2016 to 2017 ( $P = 0.0015$  for the interaction of year\*treatment).

#### 3.2. Nutrient stocks

Aboveground herbaceous biomass represented an important pool and potential annual input of C and nutrients for the olive grove soil, and accounted for the greatest organic input of C (52 %), N (71 %), P (81 %), K (89 %), Ca (46 %), Mn (54 %), Fe (58 %), Mg (57 %), and Zn (58 %) in the olive grove in the H treatment (Fig. 3). Moreover, herbaceous biomass contained 136 %, 110 %, 96 %, and 6 % of the annual amount of Mg, P, N, and K applied in inorganic fertilizers, respectively. Olive tree litterfall also represented a large potential input of C and nutrients, and accounted for the largest organic input of Cu (79 %) in the H treatment. Stocks of N, K, Mn, Fe, and Cu in olive tree litter that fell during harvesting operations were greatest in NHF. As a result, total C, P, K, Ca,



**Fig. 2.** Soil microclimatic variables in treatments with (H) and without herbaceous cover (NH) from February to October 2017. A. Mean daily soil temperature (5 cm depth) and air temperature. B. Mean daily soil moisture (25 cm depth) and precipitation. C. Diurnal cycles of soil temperature during herbaceous cover senescence periods. D. Diurnal cycles of soil temperature during herbaceous cover vegetative growth periods. Diurnal cycles are the averaged soil temperatures measured every 30 min for each treatment and period. Lines represent the mean of  $n = 6$  sensors per treatment. Grey areas indicate the senescence period. Color shaded areas around lines represent standard deviation.

**Table 2**

Effects of management treatments on main stand variables in the olive grove. Values are means  $\pm$  standard errors. Different letters above means indicate significant differences among treatments according to Tukey post-hoc test, following one-way ANOVA. *F*: Value of the statistic; *P*: P-value. H: presence of herbaceous cover; NH: absence of herbaceous cover; NHF: absence of herbaceous cover and addition of solid mineral fertilizer.

Stand variable	Treatment			<i>F</i>	<i>P</i>
	H	NH	NHF		
Tree crown area (m <sup>2</sup> )	27.2 $\pm$ 2.2	26.0 $\pm$ 2.44	25.1 $\pm$ 2.5	0.19	0.8252
Olive production, 1st year (kg ha <sup>-2</sup> y <sup>-1</sup> )	3463 $\pm$ 571	4151 $\pm$ 313	3744 $\pm$ 311	0.69	0.5120
Olive production, 2nd year (kg ha <sup>-2</sup> y <sup>-1</sup> )	2019 $\pm$ 521 <sup>a</sup>	4649 $\pm$ 554 <sup>b</sup>	5060 $\pm$ 684 <sup>b</sup>	7.34	0.0034
Herbaceous biomass production (kg ha <sup>-1</sup> y <sup>-1</sup> )	2983 $\pm$ 244	N.A.	N.A.	N.A.	N.A.
Olive tree litterfall (kg ha <sup>-1</sup> y <sup>-1</sup> )	1969 $\pm$ 229	2045 $\pm$ 277	1935 $\pm$ 274	0.05	0.9537
Harvested olive tree litterfall (kg ha <sup>-1</sup> y <sup>-1</sup> )	322 $\pm$ 51	388 $\pm$ 32	460 $\pm$ 30	3.14	0.0613

Mn, Fe, Mg, Cu, and Zn pools in aboveground vegetation inputs in the H treatment exceeded the total inputs of these elements in mineral fertilizer-treated NHF plots.

### 3.3. Nutrient release

There were clear differences in initial elemental composition between herbaceous litter and olive tree leaf litter, where olive tree litter contained greater concentrations of C, Ca, Cu, Mn, and C:N, and N:P and C:P ratios, lower concentrations of N, P, Mg, K, and Zn ( $P < 0.0001$ ), and greater Fe concentrations ( $P < 0.05$ ) (Table 3).

Litter type and quality had clear effects on decomposition rates and nutrient release (Table 4, Fig. 4), where there were faster rates of decomposition and release of C, N, P, K, and Mg, and lower retention of Zn from herbaceous litter after the first year of decomposition. In contrast, herbaceous litter retained more Cu than olive tree litter. The agricultural management also affected olive tree litter decomposition rates ( $P < 0.01$ ). The addition of solid mineral fertilizers (NHF) decelerated decomposition rates of olive tree litter to a half-life of 6.31 years (Table 4, Fig. 4a), whereas the presence of herbaceous vegetation (H) accelerated the process (half-life = 2.64 years). The effects of management were also reflected in the release and retention rates of most nutrients, where there were greater rates of release and lower rates of retention in the presence of herbaceous vegetation (Fig. 4).

In general, decomposing litter released progressively C, P and K (Fig. 4), and herbaceous leaf litter was also a source of N and Mg to the soil. In contrast, other meso- and micronutrients, such as Ca, Mn, Fe, Cu, Zn, as well as N and Mg in the case of olive tree litter, were retained in the litter during the decomposition process. Peaks in nutrient retention coincided with periods of greater water availability in early spring and autumn at the end of the decomposition period. Consequently, elemental composition of litter differed between the beginning and end points of the measured decomposition process (after 18 and 12 months for olive tree and herbaceous litter, respectively), according to the first component of the PCAs ( $P < 0.0001$ , Fig. 5). Concentrations of C and K and C:N ratios in olive tree leaf litter decreased ( $P < 0.0001$ ), concentrations of Mg and P and ratios of C:P remained stable, and concentrations of the remaining nutrients and N:P ratios increased ( $P < 0.01$ , Fig. 5a, b) with decomposition. Regarding herbaceous litter, concentrations of N, P, and K decreased ( $P < 0.0001$ ), concentration of C and N:P ratios remained stable, and concentrations of the remaining meso- and micronutrients and C:N and C:P ratios increased ( $P < 0.01$ , Fig. 5c, d). Final nutrient concentrations in olive leaf litter also differed among the olive grove management treatments ( $P = 0.02$ , according to PCs 1 and 2, Fig. 5a), where concentrations of P, K, Mn, and Fe were greater in NHF than in H ( $P < 0.01$ ), but concentrations of C, and ratios of C:N, C:P, and N:P were lower ( $P < 0.01$ ).

Total C and K released by aboveground leafy plant biomass in the H treatment was about 6.6 and 3.7 times greater, respectively, than the total amount of these elements released in the mineral fertilizer-treated NHF plots (Fig. 6). Releases of N, P, K, and Mg from aboveground

herbaceous litter were particularly important, and represented 70 %, 79 %, 575 %, and 61 % of the nutrients provided by fertilizers in NHF. In contrast, there was a net retention of Ca, Mn, Fe, Cu and Zn in decomposing litter. In general, C and nutrient releases from olive tree leaf litter (harvested litter and litterfall) were greatest in H and lowest in NHF. Specifically, immobilized Ca and Mn in H was 42 % and 65 % that in NHF, while immobilization of Cu in H was 3.4 times higher than in NHF.

### 3.4. Nutritional status of olive trees

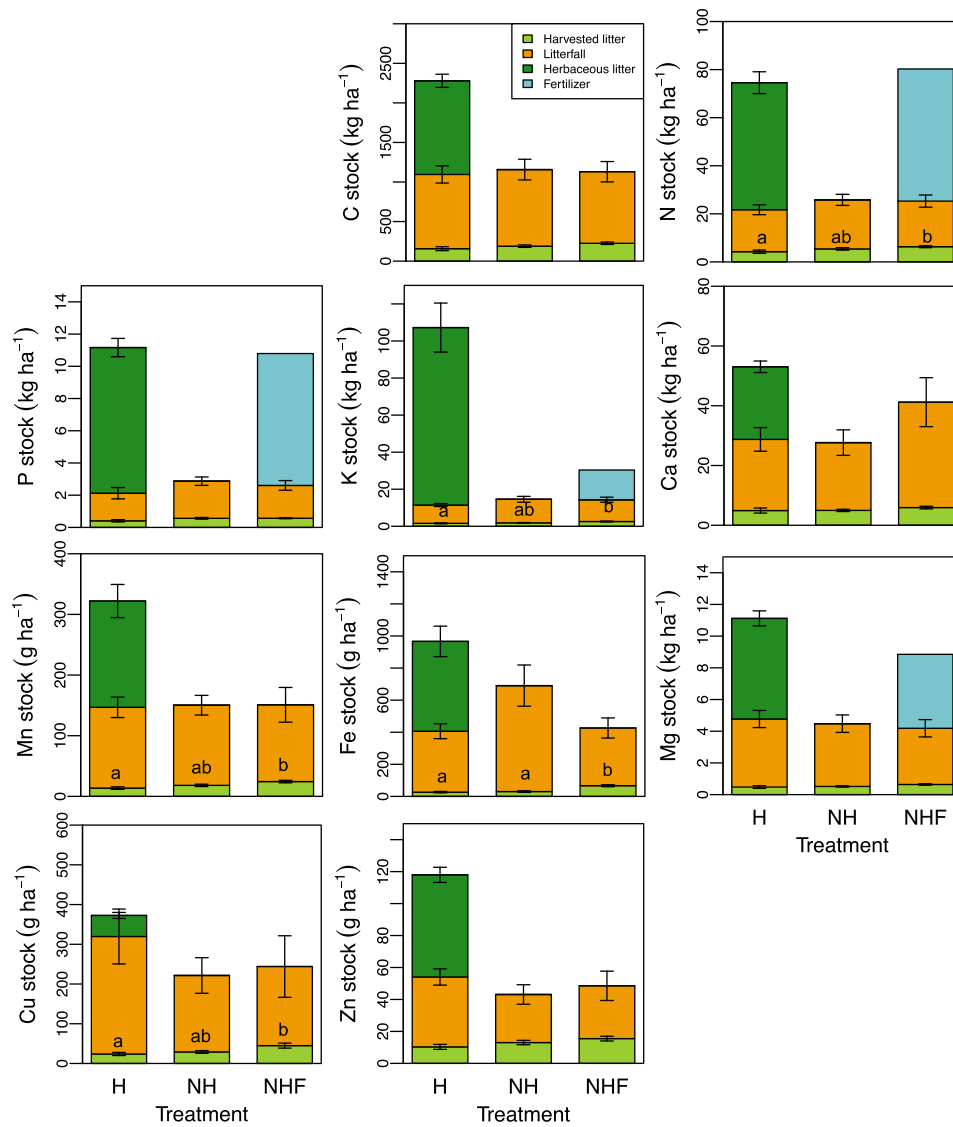
After two years, the olive grove management affected the nutritional status of olive trees ( $P = 0.0004$ , Fig. 7), according to the second component of the PCA. Foliar N concentrations in olive trees were lowest in the presence of herbaceous vegetation ( $P < 0.0001$ , Appendix 3). Phosphorous concentrations followed the same trend, but effects were on the borderline of significance ( $P = 0.048$ ). In contrast, there were no differences on olive tree nutritional status between NH and NHF treatments (Fig. 7).

## 4. Discussion

Allowing spontaneous growth of vegetation in olive groves represents a common agricultural practice that prevents soil erosion and improves soil fertility. This study reveals, for first time, both direct and indirect effects of this practice on litter decomposition. Results here presented also allow the evaluation of the biogeochemical relevance of the herbaceous cover in terms of magnitude of released macro- and micronutrients relative to those provided by fertilizers and to other aboveground plant reservoirs in the agroecosystem. Moreover, this study allows comparison of effects of mineral fertilizer inputs with those of the presence of herbaceous cover on olive yield, nutritional status of olive trees and biogeochemical functioning of an olive grove agroecosystem. Leaving the herbaceous vegetation in situ (H treatment) contributed to the retention of a high nutrient capital in organic forms (Fig. 3) and to faster litter decomposition rates (Fig. 4) than where herbaceous vegetation had been excluded (NH and NHF), indicating a stimulation of microbial activity in H. However, diminished olive production and poorer nutritional status of olive trees in H compared to the other treatments were observed as negative short-term effects. In contrast, the addition of mineral fertilizers in solid form (NHF) resulted inefficient to improve olive tree nutritional status (Fig. 7) and olive production (Table 2) and decelerated rates of litter decomposition and nutrient release (Table 4; Figs. 4 and 5). The relative magnitudes of aboveground C and nutrient stocks and fluxes in leafy litter provided evidence for two contrasting ecological resource acquisition strategies in herbaceous vegetation and olive trees.

### 4.1. Nutrient stocks

Aboveground herbaceous biomass represented the largest potential input of C and nutrients in organic form to the soil of the olive grove,



**Fig. 3.** Effects of management treatments on total annual aboveground capital of carbon and nutrients in litterfall (olive tree litterfall), herbaceous litter (litter from herbaceous vegetation), in mineral fertilizer and in harvested olive tree litter as an additional potential input measured from April 2015 to October 2017. Different letters indicate treatment differences according to Tukey post-hoc tests, following one-way ANOVA. Values are means  $\pm$  standard errors.

**Table 3**  
Initial nutrient concentrations in olive tree and herbaceous leaf litter. Values are means  $\pm$  standard errors. F: Value of the statistic; P: P-value.

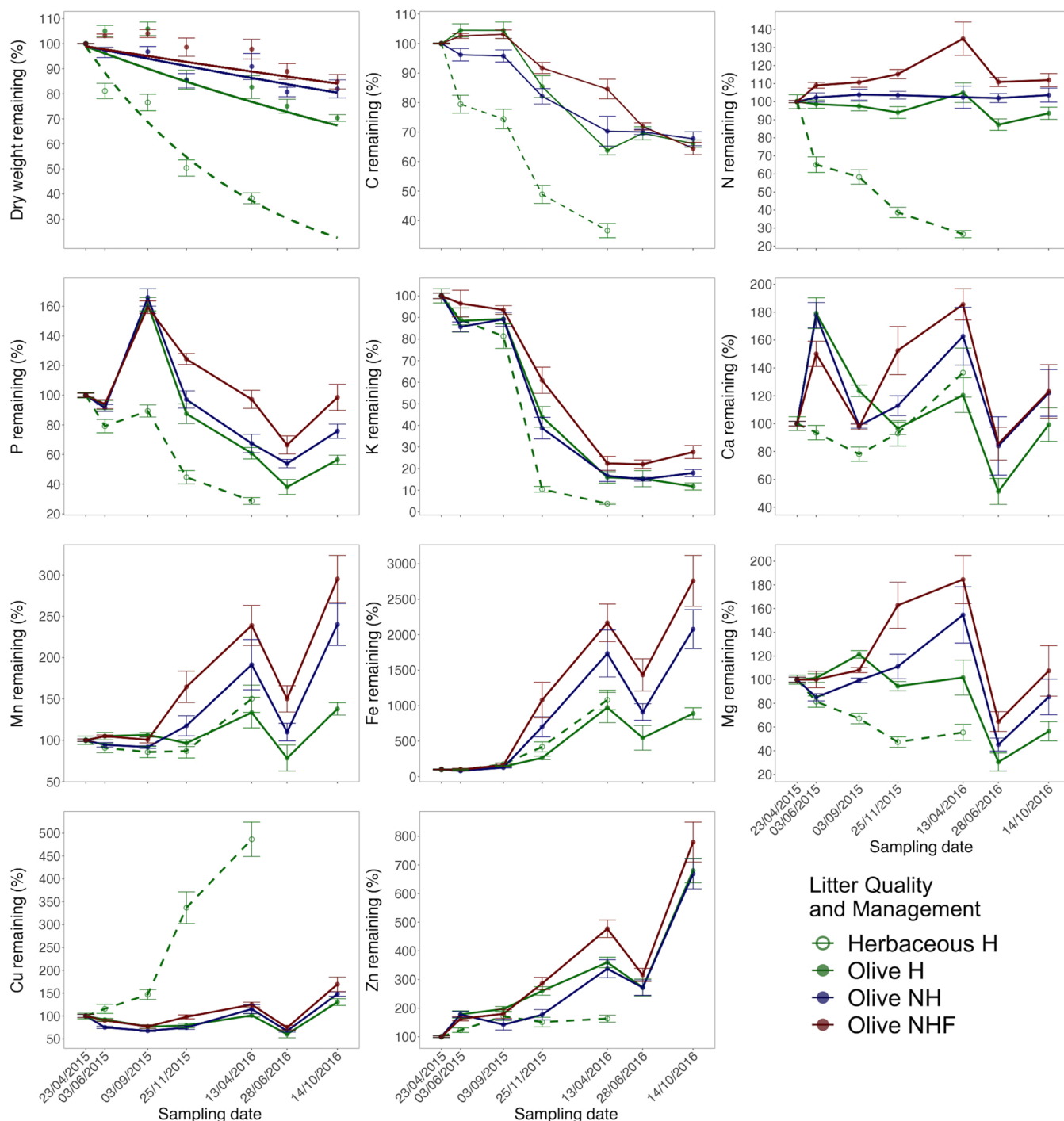
Nutrient concentration	Herbaceous leaf litter	Olive tree leaf litter	F	P
C (%)	42.08 $\pm$ 0.09	48.15 $\pm$ 0.06	3391.82	< 0.0001
N (%)	2.73 $\pm$ 0.1	1.50 $\pm$ 0.01	139.72	< 0.0001
C:N	15.62 $\pm$ 0.58	32.12 $\pm$ 0.19	740.67	< 0.0001
N:P	7.74 $\pm$ 0.32	9.99 $\pm$ 0.14	40.76	< 0.0001
P (mg kg <sup>-1</sup> )	3547.39 $\pm$ 66.50	1503.47 $\pm$ 20.38	863.53	< 0.0001
Ca (mg kg <sup>-1</sup> ) <sup>†</sup>	10420.32 $\pm$ 519.36	22021.90 $\pm$ 375.95	234.33	< 0.0001
Mg (mg kg <sup>-1</sup> )	2432.54 $\pm$ 91.27	1788.61 $\pm$ 38.58	42.23	< 0.0001
K (mg kg <sup>-1</sup> ) <sup>†</sup>	24765.82 $\pm$ 816.07	6258.90 $\pm$ 79.30	1503.74	< 0.0001
Fe (mg kg <sup>-1</sup> )	110.75 $\pm$ 9.86	134.16 $\pm$ 5.32	4.36	0.048
Mn (mg kg <sup>-1</sup> )	38.64 $\pm$ 1.93	53.12 $\pm$ 0.77	48.69	< 0.0001
Zn (mg kg <sup>-1</sup> )	21.22 $\pm$ 0.76	12.74 $\pm$ 0.31	107.14	< 0.0001
Cu (mg kg <sup>-1</sup> )	13.28 $\pm$ 0.82	130.55 $\pm$ 4.54	644.72	< 0.0001

<sup>†</sup>Log-transformed data prior to ANOVA testing

**Table 4**  
Effect of management treatments on the decay rate (k) of litter dry weight (olive tree leaves and aboveground herbaceous biomass), half-life (0.693/k) and turnover time (1/k). Estimates ( $\pm$  standard errors) were obtained by nonlinear regression with Equation 1. Model SE and adjusted R<sup>2</sup> values are reported as measures of goodness of fit. H: presence of herbaceous cover; NH: absence of herbaceous cover; NHF: absence of herbaceous cover and addition of solid mineral fertilizer.

Treatment	k (year <sup>-1</sup> )	Half-life (year)	Turnover time (1/k, year)	Model SE	Adjusted R <sup>2</sup>
Olive tree litter					
H	0.26 $\pm$ 0.03	2.64 $\pm$ 0.26	3.81 $\pm$ 0.38	9.00	0.64
NH	0.14 $\pm$ 0.02	4.84 $\pm$ 0.73	6.98 $\pm$ 1.06	7.33	0.45
NHF	0.11 $\pm$ 0.02	6.31 $\pm$ 1.18	9.11 $\pm$ 1.70	8.15	0.31
Herbaceous litter					
H	1.00 $\pm$ 0.08	0.69 $\pm$ 0.06	1.00 $\pm$ 0.08	14.26	0.70

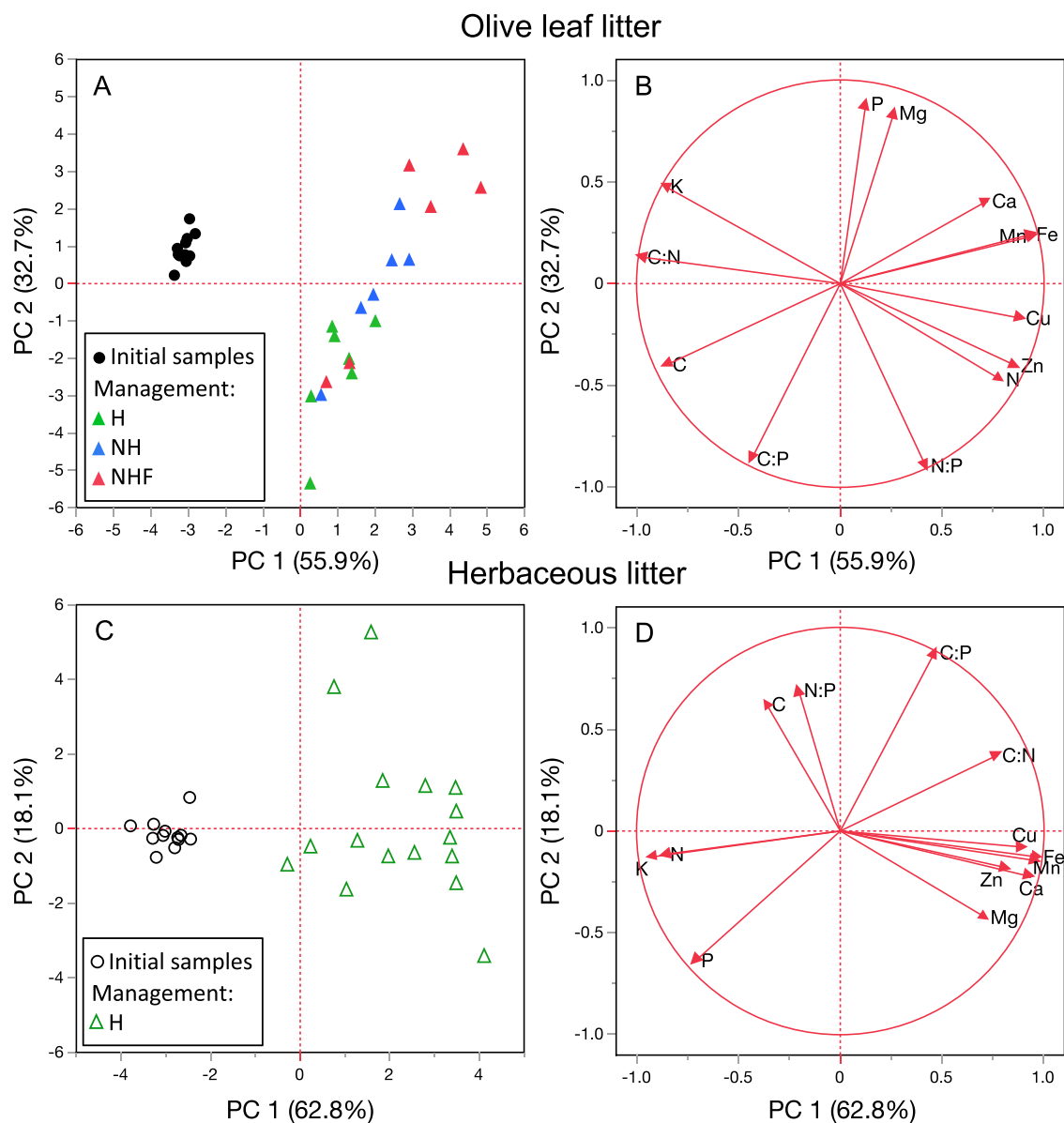




**Fig. 4.** Effects of management treatments on temporal changes in remaining dry matter and nutrient content in decomposing leaf litter from olive trees and litter from herbaceous vegetation from April 2015 to October 2016. Herbaceous vegetation was only present in H, whereas potential inputs from olive tree leaf litter to the soil were present in all three treatments (H, NH, and NHF). Lines in the figure of remaining litter dry weight represent the fitted exponential models for each litter type and treatment (see text for more details). Values are means  $\pm$  standard errors ( $n = 18$  and  $9$  for herbaceous and olive tree leaf litter, respectively).

except for the case of Cu (Fig. 3). Moreover, this pool contained 136 %, 110 %, 96 %, and 6 % of the annual amount of Mg, P, N, and K applied in inorganic fertilizers. This is particularly relevant for the case of N, P and K, which are essential nutrients for plant nutrition and are frequently limiting in agriculture due to the removal of these nutrients in the harvest (Fernández-Escobar, 2019). Herbaceous covers composed by leguminous N fixing species, as was the case in this study, may contribute particularly to increase the soil N inputs and trees nutritional status (Rodríguez et al., 2015; Lombardo et al., 2021). Potassium

deficiency represents the major nutritional disorder in olives growing both in drylands and on calcareous soils, due to its interaction with water shortage and calcium, respectively (Parra et al., 2003; Restrepo-Díaz et al., 2008). Moreover, the high mobility of K in soils with low clay and organic matter content and of  $\text{NO}_3^-$  render them highly susceptible to leaching (Sardans and Peñuelas, 2015). Phosphorous can also precipitate with Ca and be sorbed by Fe- and Al-compounds of clay minerals (Addiscott and Thomas, 2000), and low levels of precipitation limit microbial and plant ability to uptake P in Mediterranean and

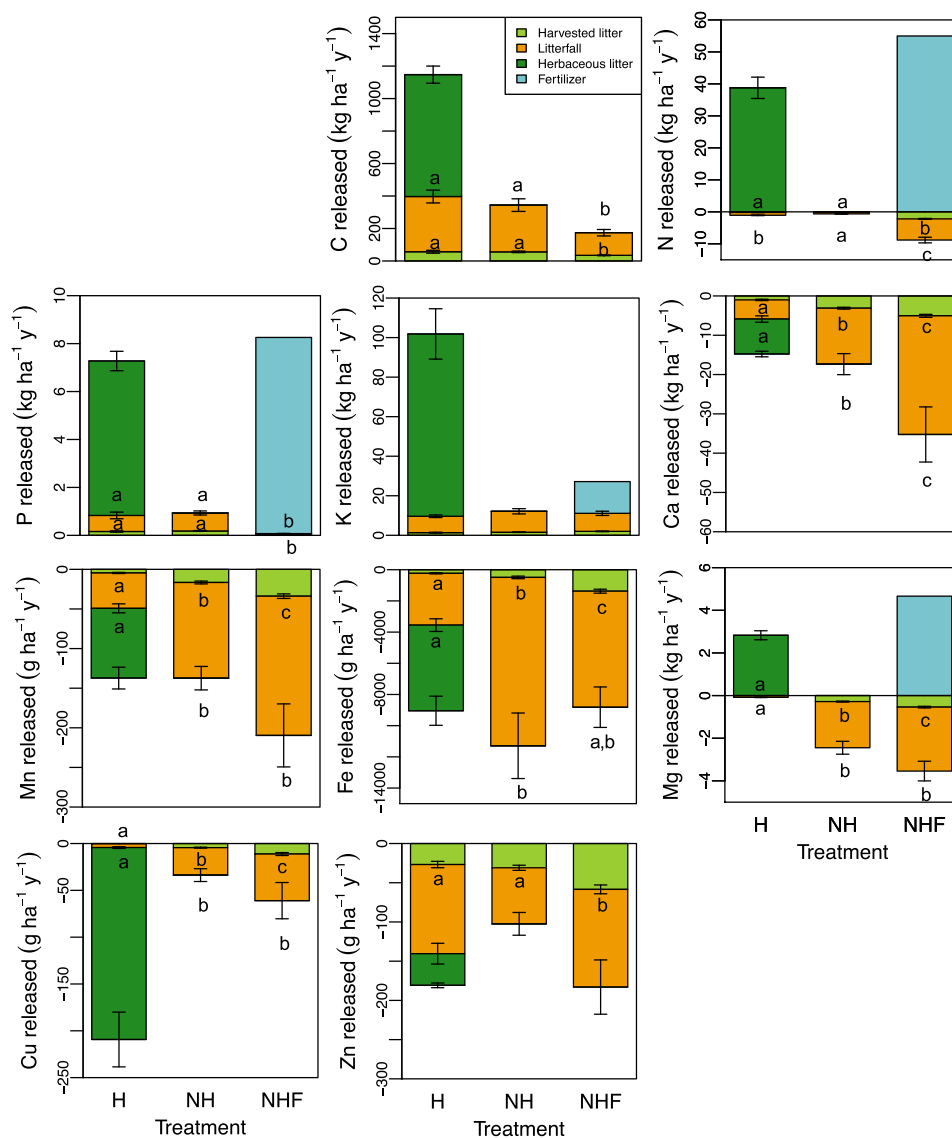


**Fig. 5.** Principal Component Analyses (PCA) of the effects of management treatments on nutrient concentration in olive leaf litter (A and B) and herbaceous litter (C and D) during the decomposition period (April 2015 to October 2016). Symbols in A and C represent the subject scores: initial litter samples (April 2015) are indicated by black circles and final litter samples (October 2016) are indicated by colored triangles. The arrows in B and D represent the variable scores. Percent contribution of the first two principal components to the total variability is shown in parentheses. Herbaceous litter was only present in H.

semiarid regions (Belnap, 2011). In Mediterranean ecosystems, the accessibility of soil nutrients may be also impaired by low moisture conditions, while nutrients can be rapidly mobilized in response to rain events (Nielsen and Ball, 2015). The high N, P and K storage capacity in herbaceous vegetation may contribute to the retention of these nutrients in the agroecosystem following rapid pulses of high availability. The large nutrient pool retained in herbaceous plant biomass implies that a lower proportion of these nutrients are leached out or adsorbed in soil minerals. However, more rapid assimilation of these nutrients by herbaceous vegetation may result in their reduced availability for olive tree nutrition (Lipeccki, Berbec, 1997), at least in the time frame of two years, as it is shown in the H treatment in this study in comparison with NH and NHF treatments.

Olive tree litterfall also represented an important potential input of C and nutrients to the soil (Fig. 3), particularly of Cu. Annual litterfall values in this study (Table 2) about 3.7 times higher than the annual litterfall reported in a rainfed olive grove in drier region (370 mm)

(Almagro et al., 2010). Irrigated olive trees count on less efficient strategies to overcome water stress and generally have higher crown volume and leaf area index than rainfed olive trees (Fernández, 2014). Particularly low precipitations during the collection period (282 mm) compared to the mean annual precipitation in the area (466 mm) may explain the elevated litterfall values found in this study and the particularly low yields during that year in the area. On the other hand, litter nutrients exported from the system during harvest operations represented 3.5–33.3 % of the nutrients contained in annual olive tree litterfall. Exported stocks of N, K, Mn, Fe and Cu were highest in NHF as a result of marginally greater amounts of harvested olive tree litter biomass (Table 2) and higher litter concentrations of K, Mn and Fe (Appendix 2). Olive tree litter is an effective plant residue mulch that increases supply of organic matter to the soil (Malamidou et al., 2018), improves soil microbial activity and biogeochemical cycling, contributes to weed control, and reduces negative impacts associated with herbicide use, such as pollution and soil erosion (Saavedra Saavedra, 2007a).



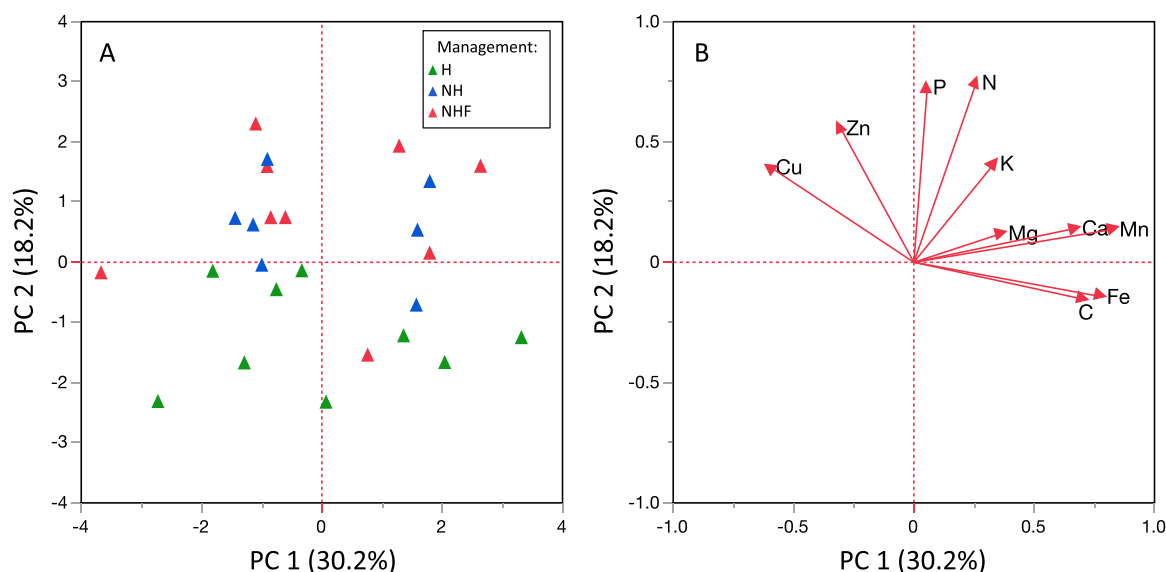
**Fig. 6.** Effects of management treatments on annual releases of carbon and nutrients from litterfall (olive tree litterfall), herbaceous litter (litter from herbaceous vegetation), mineral fertilizer and from harvested olive tree litter measured from April 2015 to October 2017. Negative values indicate a net nutrient retention. Different letters indicate treatment differences according to Tukey post-hoc tests, following one-way ANOVA. Values are means  $\pm$  standard errors.

Therefore, the return of harvested olive tree leaf litter to olive grove agroecosystems and the maintenance of herbaceous cover may represent appropriate approaches to compensate or reduce, at least partially, the nutrient withdrawal that occurs during olive harvesting operations.

#### 4.2. Nutrient release

Despite the large nutrient stocks contained in herbaceous biomass and olive tree litter, not all elements were released to the soil after one year of decomposition. Litter decay rates of olive tree litter in this Mediterranean system were particularly slow ( $k = 0.11 - 0.26$ ) compared with those in subtropical agroecosystems ( $k = 1.18$ ); (Rodríguez-Pleguezuelo et al., 2009), probably due to the much drier conditions at our study site. As reported elsewhere (Gómez-Muñoz et al., 2014; Rodríguez-Pleguezuelo et al., 2009), we found that litter quality strongly determined rates of litter decomposition and nutrient release, where N-rich herbaceous leaf litter, with low C:N ratios, decomposed more rapidly than sclerotic olive tree leaf litter with high C:N ratios and greater lignin content (Table 4, Fig. 4). Additionally, temporal variations nutrient release from litter differed among nutrients; for example,

retention of elements such as Ca, Mg, Mn, Fe, Cu, and Zn was greater during wet periods in spring and autumn, while C, N and the highly mobile K were released in a continuous and progressive way throughout the decomposition period (Fig. 4). These variations may reflect abiotic adsorption of metal elements on humified litter and microbial immobilization of other elements (Rustad and Cronan, 1988). The herbaceous litter function on Cu immobilization may be particularly relevant in organic olive groves, due to the feared effects of copper accumulation in soil (Vitanovic, 2012). Olive grove management affected the decomposition rates of olive tree leaf litter, where the presence of herbaceous vegetation indirectly stimulated decomposition, likely through enhanced microbial activity and ameliorated microclimate conditions (Fig. 2). This result contrasts with observations in a short-term experiment (<60 d), in which there were increases in microbial biomass and respiration following application of glyphosate (Nguyen et al., 2016). Improved microclimatic conditions created by herbaceous cover and higher organic matter inputs from vegetation may have masked any short-term stimulation in microbial activity following the use of herbicides in the NH and NHF in our experiment. By contrast, litter decomposition and nutrient release was slower in absence of herbaceous



**Fig. 7.** Principal Component Analyses (PCA) of the effects of management treatments on carbon and nutrient concentrations of olive tree leaves after two years (from spring 2015 to spring 2017). Symbols in A represent the subject scores, where final leaf samples (spring 2017) are indicated by colored triangles. The arrows in B represent the variable scores. Percent contribution of the first two principal components to the total variability is shown in parentheses.

vegetation cover and with application of mineral fertilizers (NHF). Greater availability of mineral forms of nutrients in the superficial soil of NHF may have reduced the stoichiometric demand of microbes for nutrients during the first stages of litter decomposition (Soong et al., 2020), leading to reductions in the microbial nutrient mining and slowing down litter decomposition rates (Ramírez et al., 2012). Inorganic nitrogen also inhibits lignin-degrading enzymes and the growth of lignolytic fungi (Edwards et al., 2011), explaining the slower degradation of olive leaf litter with high lignin content.

Overall, herbaceous litter was the largest aboveground plant source of N, P, K and Mg, and it released 70 %, 79 %, 575 %, and 61 % of the amount of these nutrients provided annually by mineral fertilizers, respectively (Fig. 6). Due to its rapid growth rate, herbaceous vegetation may be highly efficient in the acquisition of nutrients when they are provided in isolated pulses, such as in response to rain events or to the application of mineral nutrients as inorganic fertilizer. This rapid growth trait allows herbaceous vegetation to intercept and store highly mobile nutrients in its biomass that will then be progressively released at rates more in accordance to those for acquisition in olive trees, contributing to more efficient nutrient cycling. This becomes particularly relevant in Mediterranean and semi-arid regions, where most of olive groves are located. These areas are characterized by drought periods, that limit the nutrient accessibility and uptake by plants, and by an irregular precipitation distribution with isolated rainfall events, during which nutrients are rapidly mobilized (Sardans et al., 2020). Moreover, climate change predictions foresee more irregular precipitations (Vicente-Serrano et al., 2014), exacerbating the irregular patterns of water and nutrient accessibility for plants in these regions. Nutrient immobilization in plant biomass therefore may buffer nutrient availability, by preventing nutrient leaching and sequestration in less available mineral forms during pulses of high-availability, while it facilitates the progressive release of nutrients as organic matter decomposes (Gómez-Muñoz et al., 2014). The presence of micronutrients in organic forms may also improve the capacity for their acquisition in olive trees and reduce immobilization in soil minerals (Chatzistathis et al., 2017).

The slow rates of decomposition and nutrient release from recalcitrant olive tree leaf litter may limit the use of this litter for short-term soil nutrient correction, but its use may be more appropriate to achieve long-term increases in soil fertility (Rodríguez-Pleguezuelo et al., 2009). Nonetheless, the presence of herbaceous vegetation indirectly

enhanced nutrient release from olive tree leaf litter, contributing to more rapid releases of C and P and reduced immobilization of N, Ca, Mn, Fe, Mg, Cu, and Zn in olive tree leaf litter (Fig. 6). Herbaceous vegetation is also a key source of C and organic substrates for soil microbes, contributing to soil mineralization activity and fertility and biogeochemical cycling (Herencia, 2015; Morugán-Coronado et al., 2020). Despite releases of C from decomposing herbaceous litter, the presence of herbaceous vegetation has been shown to increase net C sequestration and reduce the C footprint of olive grove agroecosystems (Palese et al., 2013; Chamizo et al., 2017).

Despite releases of nutrients from herbaceous vegetation and the indirect stimulation of olive tree leaf decomposition rates, the maintenance of herbaceous cover did not improve the nutritional status of olive trees. Instead, olive leaves contained lower levels of N and marginally lower levels of P in H than in NH and NHF (Fig. 7 and Appendix 3), falling behind the sufficiency thresholds of 1.5 % and 0.1 %, respectively (Fernández-Escobar, 2019). Similarly, olive yields were lower in H (Table 2). It is possible that competition between herbaceous vegetation and olive trees for water and nutrients during spring may explain these negative effects on olive tree nutritional status and yield, particularly in this case where foliar N, P and K levels were deficient and where the herbaceous covers were not mowed. Soil moisture actually showed (although not significantly) lower minimum values during summer in the H treatment (Fig. 2). Moreover, leaf levels of K were below the sufficiency threshold of 0.8 % in all treatments (Fernández-Escobar, 2019), as is common in dry and calcareous soils due to its interaction with water and calcium (Parra et al., 2003; Restrepo-Díaz et al., 2008). Potassium plays an important role in the regulation of water status in the olive (Arquero et al., 2006; Fernández-Escobar (2019)). Potassium deficiency may have increased the vulnerability of olive trees to water scarcity and made olive trees less competitive with herbaceous plants for this resource. In addition, K uptake is restricted by both leaf K deficiency and water stress (Restrepo-Díaz et al., 2008), which may explain the inefficiency of solid mineral fertilization to increase leaf K levels in this case (Appendix 3). The preservation of herbaceous covers, however, has demonstrated to contribute to soil protection and fertility in degraded soils or in highly vulnerable soils to degradation, such as in areas of medium to steep slopes (Vicente-Vicente et al., 2016; Gómez et al., 2009a; Soriano et al., 2014). The average slope of the olive orchards in Andalusia is 8–16 %, where around 0.5 million hectares are located under soils > 15 % slope, and 72,000 ha are located in > 30 % of slope

(Unidad de Prospectiva de la Consejería de Agricultura y Pesca de la Junta de Andalucía, 2002). In these areas, the positive effects of the presence of herbaceous covers on soil protection and on the increase of soil organic matter content may compensate any potential negative effects derived from the competition with olive trees for limiting resources (Vicente-Vicente et al., 2016). In these situations, the preservation of herbaceous cover was enough to maintain or even increase the olive yield without the need of additional fertilization (Palese et al., 2014; Sastre et al., 2016). However, the benefits of the presence herbaceous covers on soil organic matter inputs and soil erosion might not be so relevant in highly fertile flat areas (Ferreira et al., 2013), as it is the case of the olive groves in this study.

On the other hand, the addition of solid mineral fertilizers elicited no effects on the nutritional status (Fig. 7) or yield (Table 2) of olive trees. Application of the fertilizers in our study occurred just after rain events, which generally favors the incorporation of nutrients to the soil and enhances the nutrient uptake by olive trees (García, 2009). Despite this, it is likely that a high proportion of mineral nutrients, which were applied as a pulse and in solid mineral form, were volatilized before entering the soil (García, 2009), immobilized by soil microbial biomass or leached out in the soil solution before they could be taken up by olive trees. It is, nonetheless, also possible that these parameters may need longer time periods to experience appreciable differences in response to management changes.

Olive groves with resident vegetation cover can be considered agroecosystems in which two distinct plant life-history strategies coexist. Herbaceous vegetation, which comprises fast growing species with rapid turnover rates, is characterized by high rates of resource (nutrient and water) acquisition and nutrient release. Large fluxes in energy and elemental turnover may sustain relatively low nutrient pools and biomass (Gunderson and Holling, 2001). In contrast, olive tree growth, resource acquisition and turnover rates are much lower, but they represent much larger nutrient and total biomass pools in the agroecosystem. These two divergent ecological responses to resource availability, and the duration and frequency of periods of resource scarcity will likely determine the outcome of their competition. Pulses of nutrient and water availability, such as those triggered by fertilization and precipitation events in Mediterranean ecosystems, will favor fast growing r-strategist herbaceous species. In contrast, conditions of scarcity will favor the maintenance of slow growth rates and survival of the large K-strategist, competitor olive trees thanks to their evolved adaptive structures, such as sclerotic leaves and deep roots (Sardans and Peñuelas, 2013). Combining the strengths and advantages of both strategies in the management of a single crop may increase the resilience and stability of the agroecosystem (Gliessman, 2007).

## 5. Conclusions and implications for management

In the short-term, herbaceous vegetation may outcompete olive trees for resources when they are available in short pulses, such as after precipitation or fertilization events. Nonetheless, the maintenance of herbaceous covers may be beneficial for the sustainability of the olive grove agroecosystem in the long term, improving nutrient retention and availability, enhancing soil microbial mineralization activity and biogeochemical cycling, increasing C sequestration and reducing nutrient losses and erosion (Milgroom et al., 2007; Gómez et al., 2009a; 2009b). These benefits are particularly significant in olive groves located in degraded areas or at medium to steep slopes, as it is the case of most of the olive plantations in Andalusia, where the only the preservation of herbaceous covers may be enough to maintain or even increase the olive yield (Palese et al., 2014; Vicente-Vicente et al., 2016; Sastre et al., 2016). In other flat areas, as it is the case of this study, the organic management of olive groves may also benefit from a well established herbaceous vegetation cover, particularly if it is composed by leguminous species, combined with a slow and continual provision of additional sources of water and organic nutrients that reduces the negative

effects of competition for these resources between herbaceous vegetation and olive trees. The addition of fertilizers should be sufficient to meet the specific nutrient requirements of the olive crop to reduce unnecessary costs and prevent nutrient leaching and eutrophication problems. To achieve this, periodic soil and plant nutrient status analyses are highly recommended. Mowing the herbaceous cover crops in spring and leaving the residues on the ground as mulch can also contribute to reduce the potential negative effects of the competition for water (Palese et al., 2014).

Framing these results within ecological theory and principles allows understanding and prediction of the outcomes of ecological relations between tree crops and herbaceous cover crops. These results also highlight the need to consider fertility not just as the amount of nutrient that the soil contains, but as the function of the ecosystem to provide nutrients to plants at the right moments, and safely immobilize them when plants do not need them. This longer-term perspective on ecological functioning of agroecosystems, can assist on the decision making of optimal agricultural practices beyond short-term maximization of productivity and contribute to ensure long-term ecological stability and sustainability of the olive grove agroecosystem. The adoption of an organic olive grove management system, based on enhancement of ecological interactions among vegetation species, may also contribute to agricultural climate change adaptation in a desertification-prone area, such as the Mediterranean basin.

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## CRedit authorship contribution statement

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix 1: Variables related to the quality of the irrigation water. EC: Electric conductivity, SAR: Sodium absorption ratio

Variable	Value
pH	8.12
EC ( $\mu\text{S cm}^{-1}$ )	1641
SAR	2.847
HCO <sub>3</sub> <sup>-</sup> (mg l <sup>-1</sup> )	157.20
CO <sub>3</sub> <sup>2-</sup> (mg l <sup>-1</sup> )	0.00
F <sup>-</sup> (mg l <sup>-1</sup> )	2.73
Cl <sup>-</sup> (mg l <sup>-1</sup> )	200.64
Br <sup>-</sup> (mg l <sup>-1</sup> )	0.49
NO <sub>2</sub> <sup>-</sup> (mg l <sup>-1</sup> )	0.23
NO <sub>3</sub> <sup>-</sup> (mg l <sup>-1</sup> )	0.89
PO <sub>4</sub> <sup>3-</sup> (mg l <sup>-1</sup> )	Non detectable
SO <sub>4</sub> <sup>2-</sup> (mg l <sup>-1</sup> )	329.43
Li <sup>+</sup> (mg l <sup>-1</sup> )	Non detectable
Na <sup>+</sup> (mg l <sup>-1</sup> )	153.59
NH <sub>4</sub> <sup>+</sup> (mg l <sup>-1</sup> )	Non detectable
K <sup>+</sup> (mg l <sup>-1</sup> )	4.07
Mg <sup>2+</sup> (mg l <sup>-1</sup> )	62.98
Ca <sup>2+</sup> (mg l <sup>-1</sup> )	117.20
Sr <sup>2+</sup> (mg l <sup>-1</sup> )	2.63
Ba <sup>2+</sup> (mg l <sup>-1</sup> )	Non detectable

## Appendix 2: Effects of management treatments on nutrient concentrations in harvested olive litterfall. Values are means $\pm$ standard errors. Different letters above means indicate treatment differences according to Tukey post-hoc test, following one-way ANOVA. F: Value of the statistic; P: P-value

	Treatment			F	P
	H	NH	NHF		
C (%)†	48.9 $\pm$ 0.19	48.7 $\pm$ 0.12	49.0 $\pm$ 0.17	0.8651	0.4337
N (%)	1.32 $\pm$ 0.03	1.38 $\pm$ 0.03	1.38 $\pm$ 0.03	1.3318	0.2836
P (ppm)	1194 $\pm$ 38.9 <sup>a</sup>	1454 $\pm$ 48.8 <sup>b</sup>	1204.5 $\pm$ 44.1 <sup>a</sup>	11.1822	0.0005
C:N	37.06 $\pm$ 0.69	35.36 $\pm$ 0.65	35.70 $\pm$ 0.75	1.6276	0.2182
N:P	10.48 $\pm$ 0.67	9.75 $\pm$ 0.37	11.46 $\pm$ 0.26	3.0116	0.0698
C:P	386.6 $\pm$ 21.1 <sup>a,b</sup>	338.0 $\pm$ 12.2 <sup>a</sup>	411.3 $\pm$ 15.0 <sup>b</sup>	4.9521	0.0163
Ca (ppm)	14769.9 $\pm$ 524.2	13051.8 $\pm$ 741.4	12883.6 $\pm$ 442.4	3.2031	0.0585
Mg (ppm)	1496.5 $\pm$ 53.8 <sup>a</sup>	1339.2 $\pm$ 39.3 <sup>b</sup>	1381.3 $\pm$ 34.9 <sup>a,b</sup>	3.5540	0.0460
K (ppm)	4629.4 $\pm$ 210.6 <sup>a</sup>	4808.6 $\pm$ 166.8 <sup>a</sup>	5734.7 $\pm$ 230.8 <sup>b</sup>	8.3772	0.0020
Fe (ppm)	86.23 $\pm$ 6.86 <sup>a</sup>	75.15 $\pm$ 7.84 <sup>a</sup>	143.09 $\pm$ 5.56 <sup>b</sup>	28.5837	< 0.0001
Mn (ppm)	42.93 $\pm$ 2.00 <sup>a</sup>	45.24 $\pm$ 1.01 <sup>a</sup>	52.78 $\pm$ 2.15 <sup>b</sup>	8.0475	0.0022
Zn (ppm)	32.68 $\pm$ 1.02	33.26 $\pm$ 1.26	36.43 $\pm$ 1.85	2.0367	0.1533
Cu (ppm)‡	76.06 $\pm$ 5.45	74.12 $\pm$ 4.03	96.24 $\pm$ 10.65	2.1758	0.1354

†Log-transformed data before ANOVA testing

‡Inverse-transformed data before ANOVA testing

## Appendix 3: Effects of management treatments on the nutritional status of olive leaves of olive tree leaves after two years. Values are means $\pm$ standard errors. Different letters above means indicate treatment differences according to Tukey post-hoc test, following one-way ANOVA. F: Value of the statistic; P: P-value

Nutrient concentration	Treatment			F	P
	H	NH	NHF		
C (%)	48.2 $\pm$ 0.17	48.0 $\pm$ 0.16	48.1 $\pm$ 0.15	0.2163	0.8070
N (%)	1.42 $\pm$ 0.02 <sup>a</sup>	1.68 $\pm$ 0.02 <sup>b</sup>	1.76 $\pm$ 0.05 <sup>b</sup>	27.3223	< 0.0001
P (ppm)	956.5 $\pm$ 55.2	1124.5 $\pm$ 46.0	1123.1 $\pm$ 53.6	3.4756	0.0480
C:N	34.03 $\pm$ 0.53 <sup>a</sup>	28.58 $\pm$ 0.50 <sup>b</sup>	27.54 $\pm$ 0.75 <sup>b</sup>	33.4573	< 0.0001
N:P	15.23 $\pm$ 0.92	14.79 $\pm$ 0.87	16.01 $\pm$ 1.01	0.4238	0.6596
C:P‡	516.9 $\pm$ 29.4	432.4 $\pm$ 18.2	438.6 $\pm$ 26.3	3.5635	0.0449
Ca (ppm)†	10631.8 $\pm$ 337.4	11014.5 $\pm$ 211.3	10385.5 $\pm$ 255.1	1.4993	0.2435
Mg (ppm)	1192.2 $\pm$ 54.9	1175.0 $\pm$ 30.4	1227.4 $\pm$ 64.2	0.2661	0.7686
K (ppm)	5865.9 $\pm$ 263.2	5883.7 $\pm$ 227.3	5864.8 $\pm$ 247.1	0.0018	0.9982

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Fe (ppm)	104.91 ± 3.98	103.57 ± 4.87	96.61 ± 6.46	0.7322	0.4913
Mn (ppm)	43.61 ± 2.42	47.39 ± 1.99	46.19 ± 2.43	0.7089	0.5022
Zn (ppm)	16.35 ± 0.89	18.50 ± 1.22	18.62 ± 0.81	1.6784	0.2078
Cu (ppm)‡	96.72 ± 6.57	86.09 ± 3.48	91.42 ± 5.19	0.9925	0.3854

‡Log-transformed data before ANOVA testing

‡Inverse-transformed data before ANOVA testing

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