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Integrated Vascular Analysis System of Olive Cultivation: Savia Olivar Project

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Olive trees are widely cultivated crops, especially in Mediterranean countries, which requires new practices to maintain productivity, optimize resource use and improve the quality of the environment. Integrated production seeks to achieve these objectives, but this requires rapid and effective methods to plan crop nutrition. Sap extracted using a modified Scholander chamber could provide an accurate method for determining the nutritional status of olive trees. To verify this, two trials were conducted in integrated production systems in southern Spain over two periods of time (2018–1019 and 2022–2024). The trials were carried out in five farms in the provinces of Jaén, Granada and Seville, comparing the nutrient concentrations in sap, leaves and soil from Picual and Hojiblanca olive trees. In the first period (2018–2019), critical times when nutrient flux in the sap increased were identified as spring, early fall, winter, and the first half of July. These periods were selected for sampling in the second period (2022–2024). Sap, leaves and soil were analyzed, determining macro- and micronutrients, pH and electrical conductivity. In the first trial, monthly sampling was successful, although in autumn 2022, a very dry year, little sap was extracted. From April 2023 onwards, the amount of sap recovered, which demonstrated the sensitivity of sap extraction to climatic variations and the phenological state of the olive tree. Soil analyses showed pH from 7.9 to 8.5 and electrical conductivity from 1.1 to 5.9 dS m^{-1}. Nutrient concentrations in leaf were higher than in soil and in soil higher or equal than in sap, except for K, the most abundant element in sap, with concentrations exceeding those in soil. Concentrations of Fe, Cu, Mn and Zn increased in 2022 compared to 2018, possibly due to climatic differences. Sap analysis can complement leaf and soil analyses for a more balanced fertilisation in olive orchards.

Keywords: olive crop, integrated production, macro- and micronutrients, nutritional status, soil, sap, leaves

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INTRODUCTION

Olive tree (*Olea europaea* subsp. *Europaea var. Europaea L.*) is likely the oldest cultivated tree in approximately seventy-seven countries worldwide (El Yamani and Cordovilla, 2024), covering an area of over 11 million hectares (IOC. International Olive Council, 2022). In Europe, it spans the entire Mediterranean region where it originated and remains the dominant cultivated tree (IOC. International Olive Council, 2003).

These vast cultivation areas and conventional agricultural practices have led to intense soil degradation processes (Porta et al., 1999), particularly due to water erosion (Lima et al., 2023), excessive tillage (Aguilera-Huertas et al., 2022), and overuse of chemical fertilizers (Sutton et al., 2013). These processes produce significant losses of organic carbon, nitrogen (Abid and Lal, 2008), soil biodiversity (Morgado et al., 2020; Morgado et al., 2022), fertility (Porta et al., 2008; Domouso et al., 2024), and degradation of soil properties (García-Orenes et al., 2012). Some authors agree that the use of vegetative covers improves soil health (Espejo-Pérez et al., 2013; Sastre et al., 2017).

Integrated production is an agricultural system for food production that optimizes resources and natural production mechanisms to ensure long-term sustainable agriculture. The goal is to use cultivation techniques compatible with social needs, environmental protection, and agricultural production (BOJA, 2008). The Integrated Olive Production Regulation in Andalusia (Junta de Andalucía, 2016) outlines a set of agronomic practices classified into various areas, including fertilization and soil management. In the fertilization section, it is stated that mineral fertilization should take into account crop extractions, soil fertility levels, plant nutritional status, and contributions from other sources (water, organic matter, etc.). To meet these objectives, foliar analyses are required in the first half of July.

Leaf nutrient analysis, which is used to determine the status and fertilization needs of plants, has been widely accepted as a good method for diagnosing deficiencies such as nitrogen (Fernández-Escobar et al., 2011). However, this method is not effective for detecting excess nutrients caused by over-fertilization (Weinbaum et al., 1992). Moreover, changes in crop management can affect nutrient dynamics and reference values. Therefore, integrated production requires periodic reviews of the analyses methods and reference values.

Sap is considered a precise medium for determining plant nutrients (Esteves et al., 2021). However, its use has not been widely adopted because of limitations in the extraction and measurement methods (Esteves et al., 2021). In recent years, various studies (Carella et al., 2016) have helped establish nutrient levels in the xylems of different plants, identifying dynamics related to nutrient supply, plant water status, and soil type. Some authors (Do Amarante et al., 2006) studied nitrogen and amino acid content in the xylem of various legumes under controlled conditions; Cabañero and Carvajal (2007) studied K, Mg, and Ca levels in xylem samples from *Capsicum annuum L.* using atomic absorption spectroscopy. More recently, Larbi et al. (2010) used xylem samples to study Fe levels in *B. vulgaris L.* plants. Guérin et al. (2007) used xylem samples from *L. ovalifolium Hassk* collected in spring to study nutrient mobility and the interaction of N and C compounds in plants with and without fertilization. In olives, xylem morphology is associated with plant–water availability (Bacelar et al., 2007; Rousseaux et al., 2009).

The aim of this study was to assess the effectiveness of sap extraction using a modified Scholander-Hammel chamber for quickly determining the nutritional status of olive trees depending on soil management, as a complementary method to foliar and soil analyses. To achieve this goal, we compared the nutrient concentrations in sap, leaves, and soil from Picual and Hojiblanca olive trees, which are cultivated under integrated production in different provinces of southern Spain, over several years.

MATERIALS AND METHODS

Study Area

This study was conducted over two periods of time (2018–2019 and 2022–2024) on five integrated production farms located in the provinces of Jaén, Sevilla, and Granada (southern Spain). All farms were under integrated production (Junta de Andalucía, 2016), and the trees were not deficient, as confirmed by leaf analysis data collected in the first half of July before the start of the experiment. All farms were irrigated locally (between 1,500 and 2,200 m^3 ha^{-1}), and soil management practices included vegetative cover between the rows of olive trees. The agronomic characteristics of the study farms are detailed in **Table 1**.

Fertilization practices were very similar across most farms, including soil application, foliar application, and fertigation. Soil was fertilized in February or March by applying nitrogen (either nitrate or ammoniacal) and potassium sulfate. Occasionally, commercial fertilizers containing N, P, K, S, and B were added. Foliar fertilization was usually carried out three times per year: in March, May/June, and December, with N (urea, ammonium sulphate), K (potassium nitrate, potassium chloride), P, and B (monoammonium phosphate, sodium borate).

Fertigation occurred weekly from June to October. Under optimal conditions, when water availability was allowed, the annual water contribution was approximately 1,300 m^3 ha^{-1}. In the irrigation water, ammonium nitrate, potassium chloride, and phosphoric acid were added.

Climatic Characteristics

The climatic data were taken from the agroclimatic stations belonging to the Agroclimatic Information Network of Andalusia (RIA, 2024), selecting those closest to the farms under study. As seen in **Figure 1**, precipitation was higher during 2018 and 2019, with greater amounts in Dílar, Fuensanta, and Osuna than in Luenga/Guadiana, where the differences between periods were smaller. During the second study period (2022–2024), especially in 2022, precipitation was considerably lower.

Experimental Design

In each farm, four plots were established, each consisting of six trees arranged in two consecutive rows. The plots were separated by an intermediate row of olive trees to ensure their independence. All sample types were collected monthly from 2018 to 2019 and quarterly from 2022, 2023, and 2024. To standardize the data treatment, samples were grouped into three-month sets.

Sample Collection and Analyses

Four composite soil samples were collected from each plot of each farm in the area where irrigation was applied for subsequent

TABLE 1 | Agronomic characteristics of the study farms.

Farm	Dílar	Fuensanta	Cortijo Guadiana	Cañada Luenga	Osuna
Location	Granada	Granada	Jaén	Jaén	Sevilla
Olive trees	Picual	Hojiblanca	Picual	Picual	Hojiblanca
Age (years)	25	23	24	24	16
Plantation framework (m)	8 × 4	9 × 9	7 × 7	7 × 7	6 × 7
Fertilization	Cover + Fertigation-cation + Leaf fertilization	Cover + Fertigation-cation + Leaf fertilization	Fertigation-cation + Leaf fertilization	Fertigation-cation + Leaf fertilization	Fertigation-cation + Leaf fertilization
Soil classification (IUSS Working Group WRB, 2022)	Calcaric Cambisols	Haplic Calcisols	Calcaric Regosols	Calcaric Regosols	Calcaric Luvisols

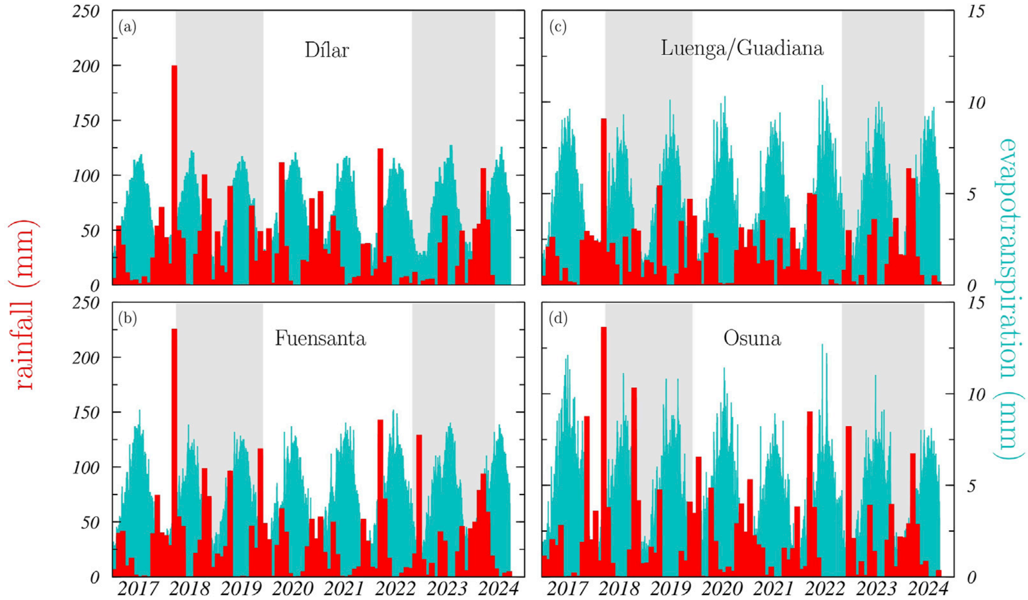

FIGURE 1 | Precipitation and potential evapotranspiration at the four study sites, according to the data extracted from RIA (2024). Shaded areas indicate the sampling periods. Blue lines represent potential evapotranspiration, while red lines indicate precipitation. **(a)** Dílar farm; **(b)** Fuensanta farm; **(c)** Luenga/Guadiana farm; **(d)** Osuna farm.

laboratory analyses. Leaf samples were collected following the guidelines provided by Fernández-Escobar et al. (1999). On each sampling date, 20 leaves were collected from around the crown of each olive tree in the plot. Leaves corresponding to the previous year's growth were collected. The branches used for sap extraction were harvested between 08h00 and 10h00, depending on the time of year and always after sunrise. The branches were placed in dark bags and quickly transported, along with the leaves, to the Olivarum laboratory, which is part of the Fundación Caja Rural de Jaén at the Geolit Technological Park in Mengíbar (Jaén).

Soil Analysis

The soil samples were initially analyzed at the Department of Soil and Agricultural Chemistry of the University of Granada. During the study, the following analyses were conducted on samples collected from the wet bulb of each tree: pH 1:2.5, soil/water suspension, and electrical conductivity (EC) in soil-saturation extract (U.S. Salinity Laboratory Staff, 1954) were measured using a Crison Basic 20 pH meter and a Crison Basic 30 conductivity meter, respectively; oxidizable carbon was determined using Tyurin's method (Tyurin, 1951); total nitrogen was measured using a LECO C/N analyzer; assimilable phosphorus was analyzed using the Watanabe and Olsen method (Watanabe and Olsen, 1965); exchangeable cations and cation exchange capacity were determined following the Soil Conservation Service Method (1972); soluble K, Ca, Na, and Mg (water-soluble elements in saturation extract) and Cu, Mn, and Zn were extracted using DTPA (Quevauviller et al., 1998).

Leaf Analysis

Leaf samples were analyzed at Olivarum. After washing with Triton 0.1% and distilled water, the leaves were incinerated in a muffle furnace for a minimum of ten hours (Ministerio de Agricultura, Pesca y Alimentación, 1994). They were then

TABLE 2 | Constituents and soil properties in the study farms. Different letters represent the statistical differences between farms (Tukey p ≤ 0.05). Mean value and standard desviation are presented in the table.

Soil's properties	Farms				
	Dílar	Fuensanta	Luenga	Guadiana	Osuna
Sand (%)	55.9 ± 5.1d	28.7 ± 5.1b	15.2 ± 1.0a	11.8 ± 1.5a	40.1 ± 2.0c
Coarse silt (%)	11.1 ± 2.8b	13.3 ± 0.6b	13.8 ± 2.0b	10.1 ± 1.3b	4.0 ± 2.1a
Fine silt (%)	17.8 ± 3.4b	27.3 ± 1.5c	33.9 ± 1.3c	30.3 ± 5.9c	10.1 ± 1.5a
Clay (%)	15.1 ± 1.2a	30.7 ± 1.9b	37.1 ± 2.0b	47.8 ± 6.7c	45.7 ± 0.9c
Texture classification	sandy loam	loamy	clayey	clayey	clayey
pH	7.7 ± 0.5	7.7 ± 0.2	7.9 ± 0.5	8.0 ± 0.4	7.6 ± 0.6
EC (dS m^{-1})	2.1 ± 0.1	1.3 ± 0.6	1.7 ± 0.3	1.7 ± 0.7	1.5 ± 0.5
CEC ($cmol_c$ kg^{-1})	10.9 ± 1.2a	13.0 ± 1.7b	12.7 ± 0.5ab	16.7 ± 2.5c	23.4 ± 0.6d
Na ($cmol_c$ kg^{-1})	0.7 ± 0.2a	0.8 ± 0.3a	1.2 ± 0.3b	1.2 ± 0.3b	0.9 ± 0.3ab
Mg ($cmol_c$ kg^{-1})	8.6 ± 5.4ab	6.1 ± 1.5a	7.6 ± 3.7ab	10.9 ± 4.8c	5.0 ± 1.7a
$CaCO_3$ (%)	13.5 ± 1.1a	40.7 ± 3.8b	58.9 ± 6.0c	63.2 ± 4.4c	13.3 ± 1.2a
Available H_2O (%)	13.7 ± 2.7	13.3 ± 0.5	12.3 ± 0.3	13.6 ± 1.4	10.8 ± 0.7
OC (%)	2.9 ± 0.4b	1.9 ± 0.4a	2.7 ± 0.5b	2.8 ± 0.2b	2.7 ± 0.9b
N (%)	0.18 ± 0.03	0.17 ± 0.10	0.20 ± 0.07	0.17 ± 0.02	0.18 ± 0.05
P_2O_5 (ppm)	64.1 ± 12.1b	73.0 ± 9.4bc	80.5 ± 5.9c	84.1 ± 12.8c	36.0 ± 8.7a
K ($cmol_c$ kg^{-1})	0.6 ± 0.3a	1.1 ± 0.2b	0.6 ± 0.2a	0.8 ± 0.3ab	1.1 ± 0.4b
Mn (ppm)	16.2 ± 5.0	24.1 ± 4.7	13.7 ± 1.4	18.8 ± 5.5	32.7 ± 5.2
Cu (ppm)	22.3 ± 4.9	10.3 ± 2.1	20.3 ± 2.7	26.0 ± 5.8	26.8 ± 4.1
Zn (ppm)	0.9 ± 0.4	0.5 ± 0.1	11.9 ± 1.8	17.8 ± 3.9	1.1 ± 0.8

dissolved in hydrochloric acid, and the concentrations of P, K, Ca, Mg, Mn, Cu, and Zn were measured by optical ICP (Inductively Coupled Plasma Optical Emission Spectrometry).

Sap Analysis

Sap extraction was performed at Olivarum using a modified Scholander-Hammel chamber with an extension that allowed the use of entire branches. Once introduced, the protruding ends of the branches were cleaned with distilled water to prevent possible contamination and were cut, leaving approximately 5 cm with respect to the chamber, to facilitate the exit of the sap. The applied pressure never exceeded 40 bar, and sap expulsion began when the pressure reached 20 bar. The sap was kept as far from light as possible because it is photosensitive. Sap samples were frozen at −24°C until further analyses. Electrical conductivity and pH were measured using glass electrodes, and macro- and micronutrients were measured using optical ICP.

Statistical Analysis

For the statistical analysis of the samples, IBM SPSS Statistics 19 software (IBM Corp, 2010) and R were used (R Development Core Team, 2017). Normality and homocedasticity were checked prior to all the analyses using the Kolmogorov–Smirnov test and Levene's test, respectively. For cases not meeting the normality or homogeneity requirements, the data were transformed to assume statistical parametric assumptions. ANOVA was performed to establish the differences between the different soil constituents measured on the farms studied. In order to study the differences between the farms studied, a Tukey´s test ($p < 0.05$) was subsequently applied.

For the figures presented, uncertainties include both those type B associated to the measurement procedure and those type A linked to the sample variability. Both were added quadratically. Uncertainty bars correspond to a coverage factor $k = 1$.

As **Supplementary Material**, we have added Pearson's bivariate correlations between the 3 matrices studied: soil to leaf, soil to sap and sap to leaf.

RESULTS

Initial Soil Characterization

The most important soil constituents and properties are summarized in **Table 2**. Soil texture varied among the farms. pH was basic in all cases, but it was higher in Luenga and Guadiana, which also had the highest calcium carbonate content, exceeding 50%. All farms had plant cover due to integrated production, which helped to maintain relatively high organic carbon levels. Similarly, N, P, and K contents were maintained within typical ranges, as regular soil analyses guided fertilization practices.

Notably, Osuna had a significantly lower phosphorous content compared to Dílar, which had the next lowest levels. The difference between farms was particularly evident in the phosphorus values, which were nearly 50% lower at Osuna compared to Dílar, and also lower than those in Luenga and Guadiana, where the highest values were found. These three farms (Osuna, Luenga, and Guadiana) showed the greatest variation between sampling periods.

The soil was deep with no signs of excessive erosion due to gentle slopes, no plowing, and the maintenance of shredded pruning residues along with vegetation cover between rows.

The First Study Period (2018–2019)

Leaf Analysis

Results from the first study period (2018–2019) are presented in **Figure 2**. Data were grouped by trimester, with the first data point representing the average of the five farms during April-June 2018, followed by three additional points for the remaining 2018 and

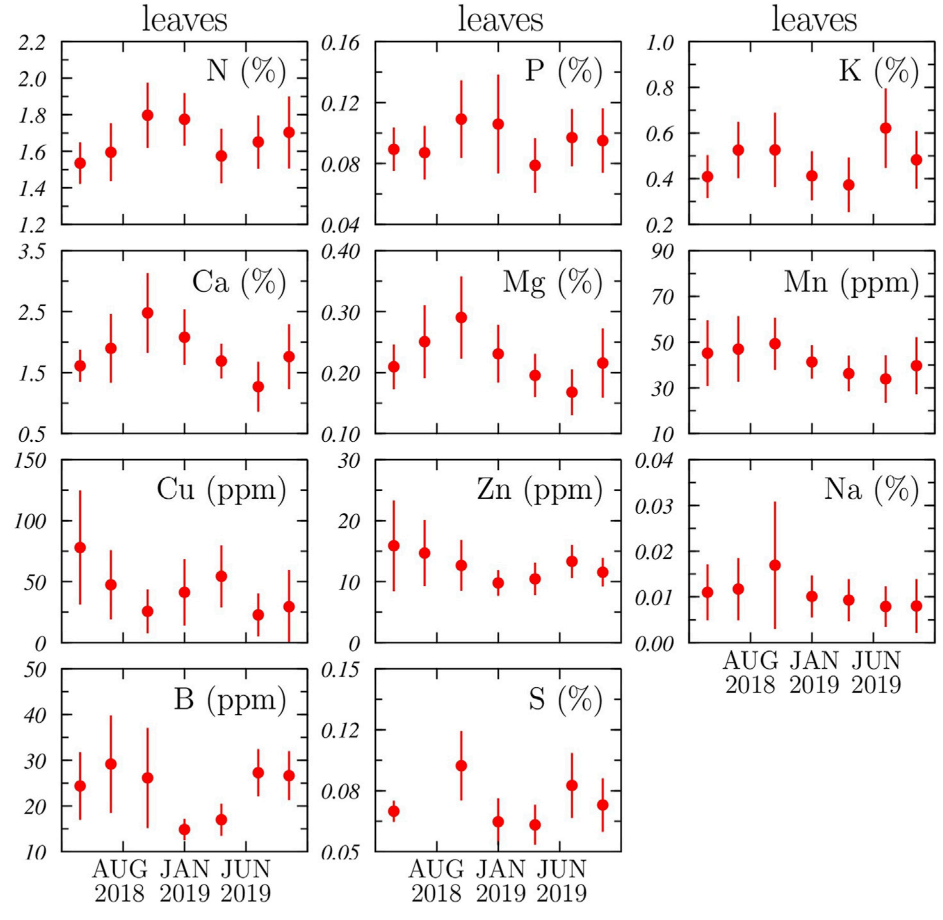

FIGURE 2 | Concentrations of nutrients in leaves for the first study period (2018–2019). Uncertainties include both those type B associated to the measurement procedure and those type A linked to the sample variability. Both were added quadratically. Uncertains bars correspond to a coverage factor k = 1.

2019 periods. The dynamics of N, P, and K in leaves were similar to each other, showing the lowest concentrations in the first trimester (April-June 2018) and the highest in October-December 2018 and January-March 2019.

N and P concentrations were significantly higher during the October-December 2018 and January-March 2019 periods, whereas K showed the opposite pattern, peaking during the last two trimesters of 2019. Ca and Mg exhibited similar dynamics, with the lowest concentrations occurring in the first and second trimesters of both years and the highest in the same period as the N, P, and K peaks (October-December 2018).

Mn and Zn concentrations were higher in 2018 than in 2019, but neither micronutrient showed signs of deficiency (Mn > 40 ppm and Zn between 10 and 20 ppm) (Fernández-Escobar et al., 1999; Nieto et al., 2017). An inverse relationship was noted between K and the concentrations of Ca, Mg, and Mn in leaves.

The Cu levels in leaves were highest in spring and fall, reflecting their use in phytosanitary treatments, and were significantly above the 4 ppm threshold considered typical for July. B levels ranged from 10 to 30 ppm, increasing during foliar applications, especially in July, August, and September, where some farms had concentrations exceeding 40 ppm.

S concentrations in leaves were higher in the October-December period, but there was considerable variability among farms.

Sap Analysis

Results from sap analysis are shown in **Figure 3**. As with leaf analysis, differences between farms were minimal, but significant variations were observed across sampling periods. The pH of sap was generally stable, showing slight increases from July 2018 to early 2019, with a notable decrease in September 2019, possibly due to fertilizer applications.

K concentrations in sap were lower in the last trimester of both years, ranging from 20 to 30 ppm, whereas the highest concentrations (around 60 ppm) were associated with spring fertilization. Nitrate and P levels showed similar patterns in 2018, with P being more stable but with higher standard deviations. Both elements increased during the first two trimesters of 2019, then slightly decreased at the end of 2019.

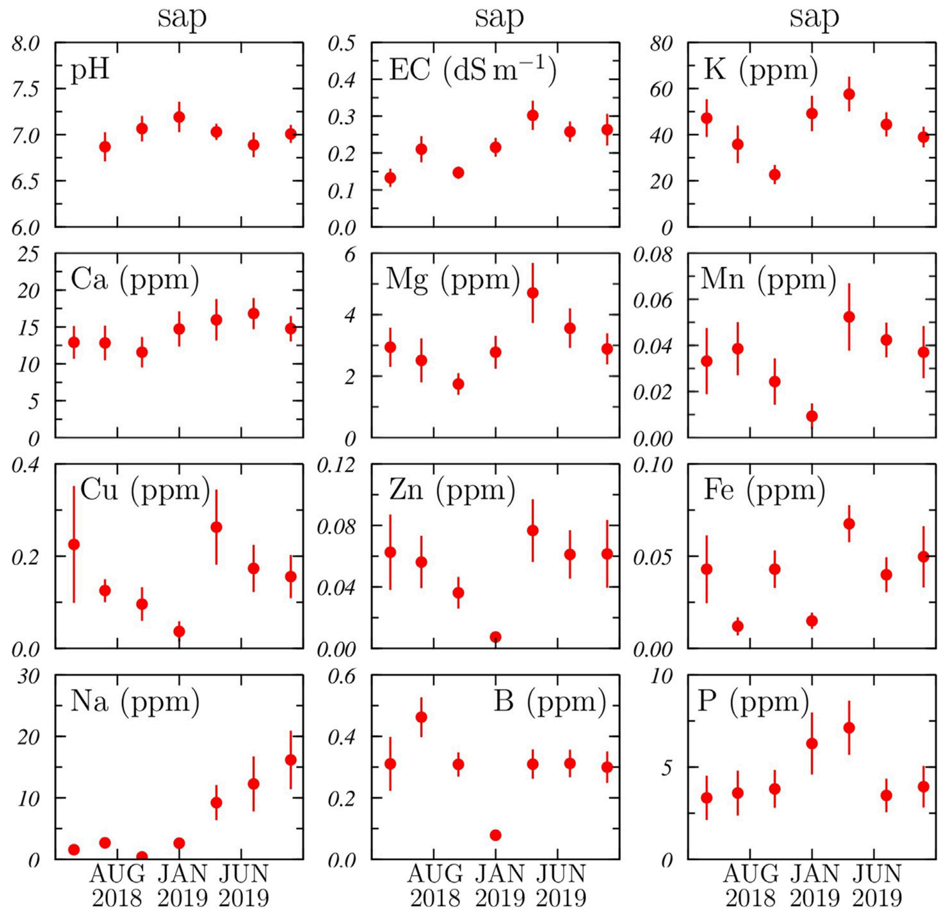

FIGURE 3 | Values of pH, EC and different nutrients in sap for the first study period (2018–2019). Uncertainties include both those type B associated to the measurement procedure and those type A linked to the sample variability. Both were added quadratically. Uncertainty bars correspond to a coverage factor k = 1.

Ca and Mg showed similar dynamics in sap, with a decrease from June to December and an increase in the first months of the year, with magnesium concentrations increasing more sharply than calcium.

Micronutrients, including Mn, Cu, and Zn, had high initial concentrations (April-June 2018), followed by a gradual decline into early 2019. Cu concentrations ranged from 0.2 to 0.6 ppm, Zn concentrations ranged from 0.1 ppm, and Mn concentrations remained low (<0.05 ppm).

The concentrations of chloride, sulfate, and Na were higher in the last three trimesters of 2019, whereas B showed an increase during the second trimester of 2018 and a significant decrease by early 2019.

Soil, Leaf, and Sap Data From the Two Study Periods (2018–2019 and 2022–2024)

The second period of study (2022–2024) showed comparisons of pH and electrical conductivity (EC) of soil and sap (**Figure 4**). pH in soil was significantly higher in Osuna during this period, whereas EC showed no significant differences across the two periods, although larger standard deviations were observed in Luenga, Guadiana, and Osuna.

In sap, EC was more stable, with smaller deviations, except for Guadiana in the second period (2022–2024). As shown in **Figure 4**, pH values are higher in the first study period (2018–2019). However, the EC shows the higher values in the second study period (2022–2024).

The concentrations of P, K, Ca, and Mg showed no significant differences between the two periods, except for P in Luenga and K in Fuensanta. However, the mean values of these nutrients in both soil and sap were generally higher in the second period (2022–2024, **Figure 5**).

Despite differences in soil and leaf concentrations, the sap and leaf concentrations of these elements were similar across both periods. For example, in Luenga and Guadiana, which had high concentrations of $CaCO_3$ or clay content in their soils, there were higher soil levels of P and K, but no corresponding increase in sap and leaf concentrations. This finding highlights the role of soil texture and carbonates in nutrient availability.

Micronutrients in Soil, Leaf, and Sap

The micronutrient concentrations of Mn, Cu, and Zn in soil, sap, and leaves were considerably different, with the highest

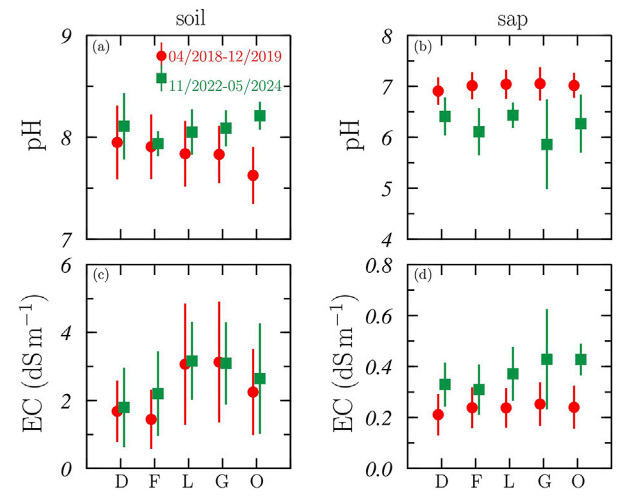

FIGURE 4 | Comparison of pH and electrical conductivity between soil and sap on the five farms selected for the two study periods (2018–2019 and 2022–2024). D: Dîlar, F: Fuensanta, L: Luenga, G: Guadiana, O: Osuna. Labels **a**, **c** correspond to soil and **b**, **d** to sap.

concentrations observed in leaves (Zn up to 20 ppm), lower concentrations in soil (max. 12 ppm), and much lower concentrations in sap (0.1 ppm) (**Figure 6**). The standard deviations were larger for soil concentrations in the first study period (2018–2019).

In sap, the standard deviations were more pronounced during the second period (2022–2024), especially for Cu and Zn, whereas the differences between periods were less significant for Mn and Zn in leaves. These differences are linked to the management of fertilization practices, especially for micronutrients, and changes in environmental factors, such as precipitation and irrigation quality.

DISCUSSION

To understand plant nutrition in general, and olive tree nutrition in particular, it is essential to understand the availability of nutrients in the soil, their concentration in the leaves, and the mobility of elements in the sap. The transport of water and solutes in the tree occurs through the xylem and phloem systems (White and Ding, 2023). Differentiating between xylem and phloem is not straightforward because the exchange of solutes between them is crucial for regulating long-distance transport (White and Ding, 2023). Therefore, comparing the evolution of nutrient concentrations in the soil and leaf is fundamental to understanding what happens in the sap and the overall nutrition of trees.

The pH in sap is very stable, and although it is sensitive to changes related to the application of different anions or cations, these changes are small and quickly reversed (Larbi et al., 2003). In this study, the pH of sap was higher in the first study period (2018–2019) than in the second period (2022–2024), although the values were around 7. In contrast, electrical conductivity was higher in the second study period (2022–2024), coinciding with a drop in pH (with minimum values of pH reaching 5.5) at times. The continued decrease in precipitation from the first (2018–2019) to the second study period (2022–2024) forced farmers to reduce irrigation doses and use lower-quality water. Additionally, some farmers added amino acids and algae as supplementary treatments to strengthen the trees against drought. This also explains the changes in pH and electrical conductivity in soil, which is also a buffered medium (Porta et al., 1999).

The evolution of leaf and sap nutrients was estimated during the first study period (2018–2019), when precipitation was abundant and the trees were in full production. This allowed the study of changes in nutrient concentrations throughout the activity periods of the plantations. The relationship between sap conduction and periods of drought has been reported in other studies (Terral et al., 2025).

The evolution of leaf nutrients studied in 2018 was repeated without significant differences in the same quarters of 2019. The yields during these 2 years (data not presented) were very similar. Some elements such as P, B, and K show different dynamics compared to those observed by other authors (Fernández-Escobar et al., 1999; Nieto et al., 2017). However, other elements such as N, Mg, Ca, Mn, and Zn maintain the same dynamics. This is primarily due to changes in olive fertilization management in recent years. For instance, for N, the evolution of its concentration in leaves has not changed because it was the element that was most carefully applied and dosed. Other elements like K or P no longer decrease in concentration throughout the year, as previously indicated in earlier studies, but their concentrations remain high in leaves for most of the year, with dynamics similar to those of N. On one hand, K plays a fundamental role in nitrate transport in the xylem (Van Beusichem et al., 1988; White, 2012), which is evident in our study by the high similarity of N and K dynamics in leaves (**Figure 2**); on the other hand, these nutrients are currently applied in various and continuous ways: to the soil, in the center of the row, as foliar fertilizers in March, May, June, and December, and in small doses via fertigation throughout most of the year. Additionally, foliar applications prevent K sequestration by soil colloids, especially clays, enhancing its immediate availability throughout the year (Rodrigues et al., 2012). Similarly, studies that have increased P application in the soil have frequently concluded that there is no response from trees or production to these additions (Fernández-Escobar et al., 2017). The type of soil plays a crucial role in this element (Porta et al., 1999). In this study, the farm with the lowest K and P values in the soil (Osuna) also had the lowest concentrations of both sap and leaves during both study periods (**Figure 5**). However, very high K and P values in the soil, as seen in Luenga and Guadiana (**Table 2**; **Figure 5**), do not correlate with higher concentrations in sap and leaves (**Figure 5**). The high $CaCO_3$ and/or clay content in these three farms (**Table 2**) were the factors highlighted by various authors to explain the availability of these elements. Recently, other

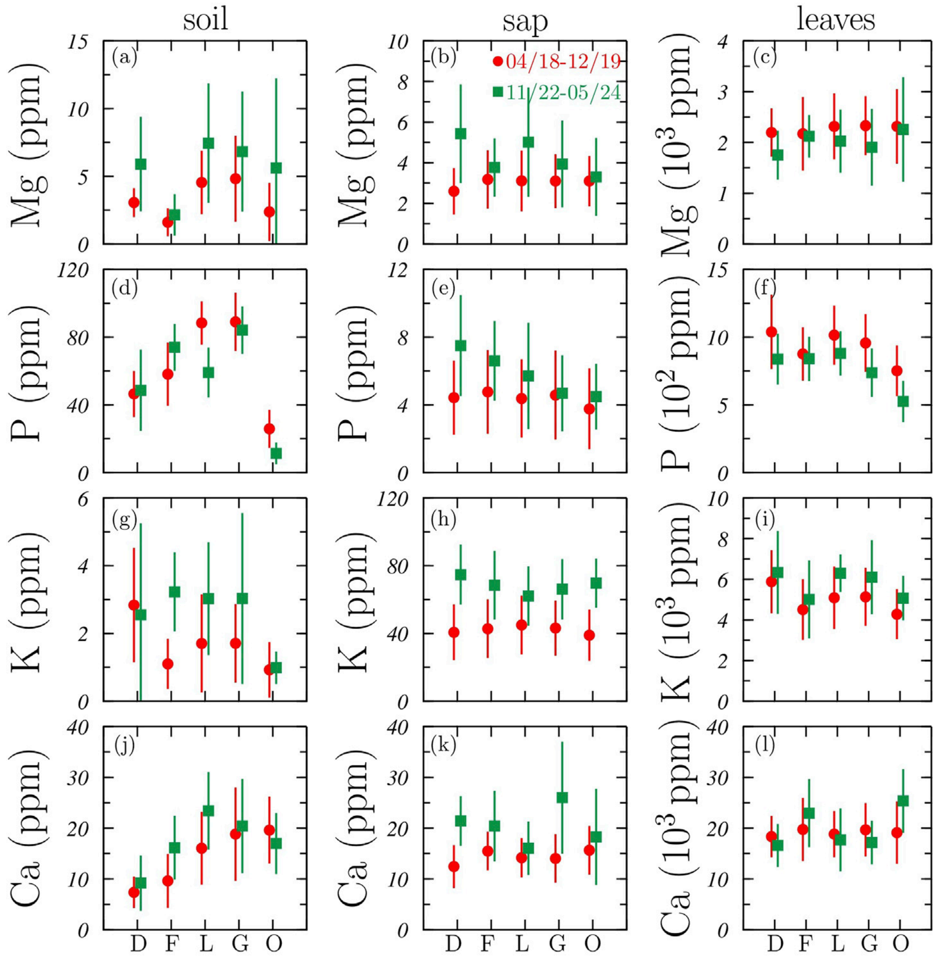

FIGURE 5 | Levels of Mg, P, K, and Ca detected in the three media for the five selected farms in the two study periods. D: Dílar, F: Fuensanta, L: Luenga, G: Guadiana, O: Osuna. Labels **a**, **d**, **g**, **j** correspond to soil concentrations of the above elements; **b**, **e**, **h**, **k** to sap concentrations; **c**, **f**, **i**, **l** to leaves concentrations. Uncertainties include both those type B associated to the measurement procedure and those type A linked to the sample variability. Both were added quadratically. Uncertaintys bars correspond to a coverage factor k = 1.

authors (Ferreira et al., 2018) have reported that when experiments are carried out in inert substrates, a response to P application in the soil is observed.

One element that well reflects this management change is B, whose evolution in previous studies has been described as a progressive decrease in leaf concentration with age, moving toward flowers and fruit (Nieto et al., 2017). However, it is now commonly applied as complexes of B, Mg, and S or as sodium borate via foliar application, which maintains more stable concentrations of B, S, and Na (**Figure 2**). The three elements had their lowest concentrations in January, February, and March of 2019 (point 4, **Figure 2**) and in the following quarter of both years (points 1 and 5, **Figure 2**), after harvest, and before the spring foliar application. The transport of B from the leaf to the fruit has been noted by several authors (Rodrigues et al., 2012; Başar and Gürel, 2016).

Ca and Mg have not changed their application as fertilizers or alongside other fertilization practices, and they share similar dynamics throughout both study periods. These elements had the highest concentrations in leaves and sap, as well as in soils where their concentrations were also higher (**Figure 5**; **Table 2**), such as Luenga (L), Guadiana (G), and Osuna (O). No significant differences were observed between the two study periods for either element, but in general, the standard deviations were higher in the second period (2022–2024, **Figure 5**), especially on the three previously mentioned farms, likely due to differences in precipitation and irrigation water quality during the two periods on these farms.

The availability of Fe, Mn, Cu, and Zn is influenced by soil characteristics such as pH and calcium carbonate content (Marschner, 1993). The dynamics of these elements are very similar to each other (**Figure 2**) and, in general, higher in the second study period (2022–2024) in leaves, and especially in sap (**Figure 6**), compared to the first period (2018–2019). Başar and Gürel (2016) noted that these micronutrients, particularly Zn, are transferred from the leaf to the fruit, which could explain the decrease in Zn concentration in leaves in the third and fourth quarters of both years, when the fruit requires it most (**Figure 2**).

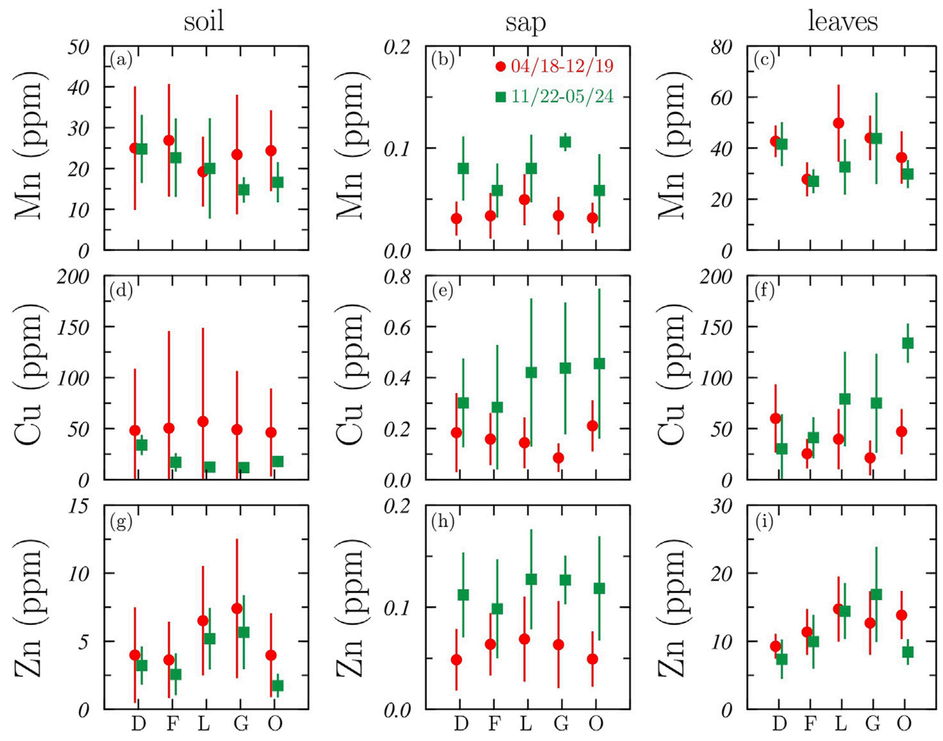

FIGURE 6 | Microelement concentrations (Mn, Cu and Zn) in the three media analyzed in the five farms for the two study periods (2018–2019 and 2022–2024). D: Dílar, F: Fuensanta, L: Luenga, G: Guadiana, O: Osuna. Labels **a**, **d**, **g** correspond to soil concentrations of the above elements; **b**, **e**, and **h** to sap; **c**, **f**, **i** to leaves. Uncertainties include both those type B associated to the measurement procedure and those type A linked to the sample variability. Both were added quadratically. Uncertainty bars correspond to a coverage factor k = 1.

This would also explain the decreases in Mn and Zn in the leaves and the increase in sap during the same period (**Figure 6**). Furthermore, the similarity in Cu dynamics in both years (**Figure 6**), which is only applied to leaf and sap, seems to confirm these results.

The correlations between nutrient concentrations, pH, and EC in leaf, sap, and soil are presented in **Supplementary Figures S1–S3** (**Supplementary Material**). In general, the most frequent correlations are observed within the same matrix (leaf-to-leaf, sap-to-sap and soil-to-soil), especially in sap. Nutrient correlations between sap and soil are positive but statistically insignificant ($R^2 < 0.5$). Additionally, the correlations between pH, EC, Mn, and Cu in soil are negative with respect to other elements, both in soil and sap.

These results are likely influenced by the sampling design, where fertilization practices by farmers were not considered. A study with more rigorously controlled conditions would likely yield more robust correlations.

CONCLUSION

In order to know the nutrition of olive trees and to carry out a fertilisation that generates an adequate production while protecting the environment, it is convenient to know the availability of nutrients in the soil, their concentration in the leaves and the mobility of the elements in the sap. Water and nutrients in the sap are transported from the soil by the xylem and then distributed through the phloem according to the nutritional needs related to the phenological stages of the tree.

The dynamics of the concentrations of many elements in leaf and sap, for example, phosphorus, potassium, calcium or magnesium, follow inverse patterns: in periods when concentrations are increasing in the leaves, they are decreasing in the sap and *vice versa*, as corresponds to a transport medium such as the sap and a nutrient store such as the leaf.

Nutrient concentrations in soil, leaf and sap varied seasonally with tree phenology, fertiliser application and climate. Nutrient concentrations in leaf were higher than in soil and in soil higher or equal than in sap, except for K, the most abundant element in sap, with concentrations exceeding those in soil.

In the first study period (2018–2019) values with smaller standard deviations were observed than in the second study period (2022–2024). Low rainfall and poor irrigation water quality affected the availability and mobility of nutrients. Elements that are not usually applied as fertilisers, such as Ca and Mg, showed higher stability in the two study periods.

Tree sap showed sensitivity to changes in nutrient concentrations. These results indicate that sap extraction using a modified Scholander-Hammel chamber is an effective method for providing additional information to foliar and soil analyses. Therefore, it can contribute to improving the determination of the nutritional status of the olive tree.

DATA AVAILABILITY STATEMENT

The data used in this study are available on demand. Requests to access the datasets should be directed to antagarc@ugr.es.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/sjss.2025.14233/full#supplementary-material

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