

Seasonal changes of macronutrients concentration in olive trees grown in acid and in alkaline soils

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Abstract

Leaf samples from mature olive (*Olea europaea* L. cv. 'Kalamon') trees were collected monthly from April 2018 to March 2019 from two olive orchards, cultivated one in acid and one in alkaline soil, located in Western Greece. Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) concentrations in the leaves were determined and seasonal variation curves were calculated for each nutrient and orchard. The seasonal concentration patterns of N, P, K, Ca, and Mg nutrients were almost similar in both soils. Seasonal variation nutrient curves independently of soil acidity varied according to vegetation stages and no significant differences in nutrient concentrations were observed at different development stages between olives grown in the acid or the alkaline soils, with only one exception the leaf K concentration. The nutrient concentration measured in wintertime was at a sufficient level for optimum olive growth in both orchards. These concentrations could be used as reference values for leaf analysis interpretation and for developing an optimum fertilization program under Mediterranean climatic conditions.

Keywords: leaf nutrient analysis; macronutrients; *Olea europaea* L. cv. 'Kalamon'; soil reaction

Introduction

Olives (*Olea europaea* L.) have been cultivated in the Mediterranean region since prehistoric times. Olive cultivation has always been one of the most important sources of income among the economic activities of all civilizations that lived in the Mediterranean Basin. The area under olive trees in the EU accounted for about 4.6 million ha in 2017. Spain (55%) and Italy (23%) accounted for over three-quarters of the total EU area under olive trees, followed by Greece (15%) and Portugal (7%) standing out as the countries with the largest cropping acreages. The four other olive-producing countries (France, Croatia, Cyprus, and Slovenia) together accounted for 1% of the EU total olive tree area. In Greece, olive cultivation is one of the most significant agricultural activities, with great social, economic, ecological, and economic importance. In two-decade seasonal periods, average Greek exports of table olives climbed by a noteworthy 66.16%. The olive

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variety 'Kalamon', which is grown in an area of about 22 thousand ha in Greece, has a great economic interest for the world market as a table olive and also the highest-selling rate (Eurostat, 2021).

Generally, leaves constitute a major storage organ, and thus seasonal changes in the composition of leaves must be very important not only for the standardization of the sampling techniques and the understanding of nutrient movement within the plant but also for the assessment of the nutrition potential of the trees (Androulakis, 1987). Leaf analysis is a major tool for determining fertilization schemes. The interpretation of the leaf analysis allows the collection of useful data for the fertilization program to be applied, as well as monitoring the seasonal variation of macro and micronutrient concentrations between different tree species or varieties (Fernández-Escobar *et al.*, 1999, 2018; Paskovic *et al.*, 2013). The genetic background of a variety, the agro-climatic conditions and the optimal fertilization management are key functional factors for olive yielding and product quality (Michelakis, 2002; Michalopoulos *et al.*, 2020; Fraga *et al.*, 2021; Solomou and Sfougaris, 2021). The plant nutrient uptake in different soil types that have different nutrient availability at sub-optimal concentrations limits plant growth (Pasković *et al.*, 2013). Knowledge of seasonal variation of olive plants has been reported in the recent literature (Stateras and Moustakas, 2018; Zipori *et al.*, 2020) but no data existed concerning the nutrient seasonal variations in leaves of *Olea europaea* cultivated in different soil acidity. Therefore, monitoring seasonal changes in mineral nutrients of olive varieties in different soil conditions is essential for the development of a better fertilization program that reduces fertilizer costs and environmental hazards. Studies on seasonal nutrient changes in olive leaves grown at different soil pH, especially for the 'Kalamon' variety, are not sufficient. The current study aims to examine whether leaf nutrient content is affected by soil pH during a growth period.

Materials and Methods

General site data

Two intensively cultivated (irrigated, fertilized, and pruning) olive orchards located in the Agrinio prefecture area, Western Greece, were selected to study the seasonal variation of leaves nutrient's concentration in *Olea europaea* var. *ceraticarpa* 'Kalamata' (or 'Kalamon'). The olive orchard, which consists of 20-year-old olive trees and is grown in acid soil, is located at 38° 37' 55.53" N, 21° 16' 27.28" E and consists of 270 olive trees in the size of 8×7 m². The other orchard, which consists of 15-year-old olive trees and is grown in alkaline soil, is located at 38° 31' 43.47" N, 21° 16' 17.46" E, 800 olive trees in the size of 8×7 m² (Figure 1). Both orchards were irrigated by furrow irrigation with a 10 days threshold period based on a maximum allowed depletion (MAD) of 50% (Allen *et al.*, 1998) according to the weather conditions.

The trees in both orchards received the same base fertilizer program. So, each tree received 3 kg of 15–15–15 fertilizer at the end of February. Furthermore, each tree in the acid orchard received 1 kg YaraLive Tropicote™ fertilizer (14.1% Nitrate-Nitrogen (NO₃⁻-N) - 1.1% - Ammoniacal-Nitrogen (NH₄⁺-N) and 26.3% CaO) totally in two doses 1st in the middle of June and 2nd in the middle of July. In the alkaline orchard, each tree received approximately 1 kg of NO₃⁻-N in the middle of June. These fertilizer practices are common in Greece, especially for the 'Kalamon' cultivar in the studied area. Yields were approximately 60-80 kg per plant and year independently of soil acidity for the last 10 years considered as the maximum one for 'Kalamon' olive cv.

The climate of the studied area is subtropical-Mediterranean (Papadakis, 1985), characterized by hot dry summers and cold humid winters. The average annual rainfall over 40 years was 1022 mm with 68% falling during the period November to April, and the mean annual temperature was 14.7 °C. The soil moisture and temperature regime were xeric and thermic, respectively (Soil Survey Staff, 1996). The soil of the acid orchard is classified as Rhodic Luvisol and the alkaline one as Eutric Cambisol (World Reference Base, 2014).

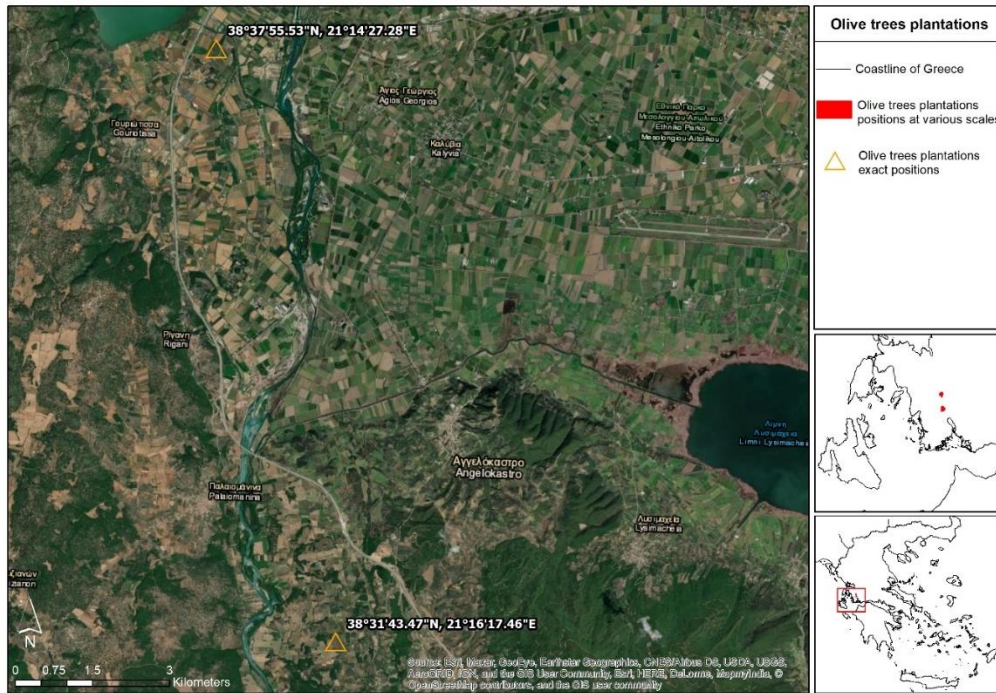


Figure 1. Locations of the studied olive plantations

Plant analysis

For the determination of seasonal variation on macronutrient concentration ten healthy olive trees in each experimental orchard were randomly chosen for sampling. Fifty fully developed leaves from each tree (a total of 500 leaves per orchard) were collected monthly from the central parts of current growing season shoots. Sample shoots were equally distributed all around the canopy at a height of about 2 m (Perica, 2001). Leaf samples were not taken from extremely vigorous or weak shoots. Leaves were stored in paper bags and refrigerated. The leaf samples were transferred quickly to the laboratory, washed with deionized water three times, and dried at 55 °C until reached constant weight. Dried samples were grounded in a stainless-steel Wiley mill, passed through a 150 μm plastic sieve, and stored in covered plastic test tubes until analysis. Total N was determined by the Kjeldahl method (Horneck and Miller, 1998). For other element determinations, 0.5 g of the ground material was dry-ashed in a muffle furnace at 550 °C for 4 h (Miller, 1998). Then the ash was dissolved in 3 ml with 6N HCL and diluted up to 50 ml. In the clear solution the concentrations of Ca and Mg were determined by flame atomic absorption spectrophotometry (AAS) (Varian, A-300; Varian Techtron Pty. Limited, Australia), using an air-acetylene flame while Ca concentration was determined using acetylene-N₂O flame. Potassium was measured using a flame photometer (PG 2000 Instruments). Total P was determined using the Murphy and Riley (1962) method with a PG T60 UV/VIS Spectrophotometer, at 880 nm.

Soil analysis

Equidistant around the circumference of a circle of radius 0.5 m from the trunk of each selected tree, three samples of soil were taken at depth of 0-35 cm. Then the three samples from each tree were combined and mixed separately, resulting in 1 mixed soil sample from the depth of 0-35 cm. Soil samples were taken in October 2018, transferred to the laboratory, and dried at room temperature, after which they were ground and passed through a 2 mm sieve (fine earth fraction) for the determination of the following properties: the pH was determined in a soil: water (1:1) suspension (Mclean, 1982); soil texture was determined using the hydrometer method (Gee and Bauder, 1986); the organic matter was determined using a modified Walkley-Black method (Nelson & Sommers, 1982); the CaCO₃ equivalent by using the quantity of CO₂ produced on reaction with HCl (Nelson, 1982); the cation exchange capacity (CEC) was determined with ammonium acetate method at

pH 7.0 (Rhoades, 1982); exchangeable bases (Ca, Mg, K), were determined using an NH_4OAc (1N, pH 7) method (Thomas, 1982); total nitrogen was estimated by the Kjeldahl procedure (Bremner and Mulvaney, 1982); extractable P was determined using the Olsen's method (Murphy and Riley, 1962).

Statistical analysis

Statistical calculations were carried out with STATISTICA™ version 8 (StatSoft, 2008). Data were subjected to Tukey's multiple range test for means comparison at $p \leq 0.05$.

Results and Discussion

Soil analysis

The results of soil analysis are shown in Table 1. In acid and alkaline orchards, the pH values were 6.3 and 7.7 and the soil textures were sandy loam and loam, respectively.

Table 1. Physicochemical properties of the studied soils

Soil	S*	Si*	C*	CaCO ₃	OM**	Total N	pH	CEC	P Olsen	exch.Ca ²⁺	exch.Mg ²⁺	exch.K ¹⁺
	%							cmol ₍₊₎ kg ⁻¹				
Acid	53	23	24	0	2.0	0.21	6.3	11.2	38.5	7.4	0.7	0.8
Alkaline	47	36	17	18	1.6	0.3	7.7	16.5	20.6	37	2.67	0.56

*S=Sand, Si=Silt, C=Clay; **organic matter

The equivalent of calcium carbonate content was non-calcareous and very calcareous (Soil Science Division Staff, 2017), respectively for the acid and alkaline soil. Alkaline soil is characterized as marl soil. Organic matter was at low levels in both orchards (2% in the acid soil and 1.6% in the alkaline one). CEC was at medium level in both soils with 11.2 cmol₍₊₎kg⁻¹ and 15.1 cmol₍₊₎kg⁻¹ for the acid and the alkaline soil, respectively. ECE was $<0.4 \text{ S m}^{-1}$ for both soils which account for soil without saline problems. Extractable P quantity is at high levels in acid soil and above very high levels for the alkaline soil (Sibbesen and Sharpley, 1997). Potassium is at high level (0.8 cmol₍₊₎kg⁻¹) in the acid soil and a medium (0.56 cmol₍₊₎kg⁻¹) level in the alkaline soil. Calcium is at a medium level (0.8 cmol₍₊₎kg⁻¹) in the acid soil and at a very high level (7.4 cmol₍₊₎kg⁻¹) in the alkaline soil. Magnesium is at high levels (0.7 cmol₍₊₎kg⁻¹) in the acid soil and at a very high level (2.67 cmol₍₊₎kg⁻¹) in the alkaline soil (Chapman HD and Pratt, 1978). The soil properties of the studied soils were characterized for their fertility status according to those suggesting by International Olive Council (2007) for medium-textured soils. International Olive Council (2007) also referred that no precise testing procedure can be used for nitrogen availability due to losses and gains procedures of nitrogen in the soil.

Leaf analysis

Nitrogen

The leaf nitrogen (N) concentration in olives during the studied period slightly fluctuated as indicated in Figure 2.

The lowest values 1.4-1.5% dw were observed in summer months (June, July, August) and no statistically significant differences were detected between these months either in the acid (Table 2) or in the alkaline orchard (Table 3).

The summertime period coincides with the time of strong demand for N in developing reproductive and vegetative organs. After that period, a rapid increase in leaf N concentration up to 1.7% dw (November) was observed in the acid and up to 1.6% (October) in the alkaline orchard. This period coinciding with fruit maturity. No significant differences were observed in autumn months between the acidic and the alkaline orchard in N leaves concentration (Table 4). During the resting period from December to early March, N

concentrations in leaves in both orchards were found lower than in the other periods and almost remained constant (1.5% dw). A second peak of N leaves concentration was observed in the middle of spring (April) in both orchards and no differences were detected between them, probably due to the fertilizers added in February-March. Subsequently, a decrease of N concentration was noted due to inflorescence development and vegetation growth until June. Low N values were observed near and after the end of winter (1.5% dw). Finally, we detected two periods of N leaves concentration stability one during the winter with 1.5% dw, and another one during the summer with 1.4% dw. The stability of N leaves concentration in winter and summer used to propose these two periods as the most appropriate for the assessment of the nutrient status of the olive (Perica, 2001).

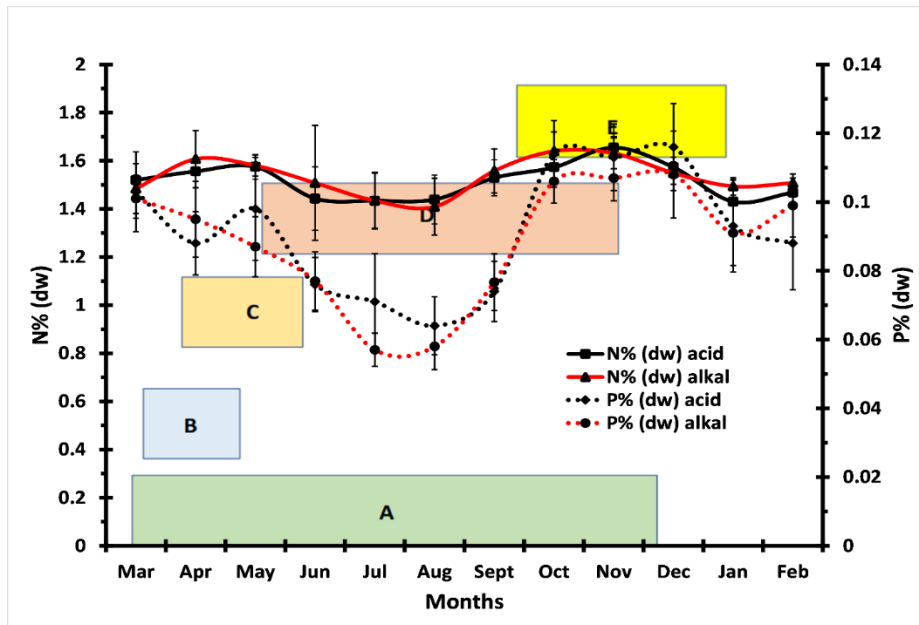


Figure 2. Changes in nitrogen (N) and phosphorus (P) concentration in the leaves of the olive trees grown in acid and alkaline soils during an annual growth cycle
Error bars represent the standard deviation of ten replicates. A, B, C, D, and E correspond to vegetation stages i.e. A=leaf development, B= inflorescence, C= flowering, D= fruit development, E= ripening.

Table 2. Leaf macronutrient (N, P, K, Ca, and Mg) concentrations in olive trees grown in the acid soils during the growth cycle

Month	N	P	K	Ca	Mg
	% (dw)				
Jan	1.4* a	0.09 cd	0.75 bc	1.75 ab	0.13 ab
Feb	1.5 ab	0.09 bcd	0.66 b	1.63 ab	0.11 a
Mar	1.5 ab	0.10 def	0.66 b	1.95 bc	0.14 ab
Apr	1.6 bc	0.09 bcd	0.46 a	1.77 ab	0.13 ab
May	1.6 bc	0.10 de	0.75 bc	3.52 f	0.17 cd
Jun	1.4 a	0.08 abc	0.76 bc	2.38 de	0.14 ab
Jul	1.4 a	0.07 ab	0.83 cd	2.15 cd	0.19 de
Aug	1.4 a	0.06 a	0.90 de	2.31 cde	0.18 d
Sept	1.5 ab	0.07 ab	0.98 de	2.66 e	0.22 e
Oct	1.6 bc	0.11 ef	0.93 de	1.52 a	0.15 bc
Nov	1.7 c	0.11 ef	0.86 cd	1.50 a	0.14 ab
Dec	1.6 bc	0.12 f	0.82 cd	1.47 a	0.13 ab

*Values are mean of the ten samples and different letters in the same column are not significantly different according to Tukey's multiple tests at $p \leq 0.05$

Fernández-Escobar *et al.* (1999), studied the leaf seasonal variation of 'Picual' cv. reported that the N concentration reaches the lowest value in August thereafter, showed an increase in N concentration reaching a maximum at the end of the season. They reported that the minimum value was observed in February followed by a rise in April and stabilized thereafter. Leaf nitrogen concentration was high and stable in the winter and decreased to reach the lowest value in summer. These results are supporting to our results. The results of Bouhafa *et al.* (2018) studied the dynamics of macronutrients in olive leaves in the 'Koroneiki', 'Arbequina', and 'Ardosana' cvs. reported the same evolution N leaf concentration curves with our results. Perica (2001) studied the evolution N leaf concentration curve for the 'Oblica' cv. were not following our results. Leaf N concentration value measured in July, was at deficiency level according to Fernández-Escobar (2018) but measured in November was at sufficient level according to Therios (2009), in the studied orchards.

In addition, no significant differences were observed in N leaf concentration between these months in olives grown in an acid and alkaline orchard (Table 4).

Phosphorus

The leaf P concentration curves during the experimental period in the studied orchards are presented in Figure 2. A decrease in leaf P concentration was observed from March (0.1% dw) and reaches the lowest value in August (0.06% dw) in both orchards. This period coincides with the inflorescence and fruit bud's development. No differences existed between leaves P mean concentration in acid and alkaline soil in August (Table 4). After August, a rapid increase of the leaf P concentration value in both soils was detected reaching a maximum in October (0.11% dw) and then remain constant until December. Finally, the general appearance of the evolution curves of the olive leaf P concentration in both orchards is similar to those described for N i.e. leaf P concentration is high in spring drop in mid to late summer and increase again in autumn (Figure 2). Similar results were reported by Fernández-Escobar *et al.* (1999). Stateras and Moustakas (2018) studied the seasonal nutrients variation of the leaves of olive cv. "Manaki" reported that the lowest P-value was observed in May (0.08% dw) and then increased gradually until October (0.15% dw). Chatzissavvidis *et al.* (2005) reported that P concentration in leaves of the olive cv. "Chondrolia Chalkidikis" fluctuated between 0.1% to 0.3% dw during a year. Chatzistathis *et al.* (2010) reported the opposite i.e. the P concentration in leaves of the olive cv. "koroneiki" gradually increased until the middle of summer and then decreased. According to Therios (2009) leaves P concentrations values between 0.09% and 0.11% dw are considered as the optimum ones for olive tree's P mineral nutrition, values between 0.07 to 0.09% dw as relative deficient and values lower than 0.07% dw considered as deficient. In our investigation in October, November, and December the P leaves concentrations were at optimum nutrition level in both orchards. In August and July in acid soil and in August in alkaline soil, the P leaves concentration levels were at deficiency (0.06% dw). In the rest months, the P leaves concentration levels were at relatively optimum ones, fluctuated between 0.07% to 0.01% (dw). Leaf P concentration value measured in July was below the threshold level for sufficiency and above the threshold level for deficiency according to Fernández-Escobar (2018) but measured in November was at sufficiency level in both studied orchards according to Therios (2009).

Potassium

The lowest K leaf concentration in acid and alkaline soil was observed in April with 0.35% (dw) and 0.46% (dw), respectively. After April, the leaves K concentration in both orchards increased gradually until September where the maximum K concentration was obtained with 0.98% dw and 0.86% dw in acid and alkaline orchard, respectively. Then the K leaves concentration decreased gradually until December, remained constant until March, and then drop down to the lowest value in April. Low winter temperatures might have caused this phenomenon, due to the reduced availability and mobility of K (Analogidis, 2000). Significant differences in leaves K concentration were observed in all months except February, June, and December between orchards with leaf K concentration of the acidic orchard being greater than in the alkaline orchard (Table 4).

Table 3. Leaf macronutrients (N, P, K, Ca, Mg) concentrations in olive trees grown in the alkaline soils during the growth cycle

Month	N	P	K	Ca	Mg
	% (dw)				
Jan	1.5* abc	0.09 cd	0.70 b	1.58 abc	0.14 ab
Feb	1.5 abc	0.10 cdef	0.60 b	1.79 bc	0.15 ab
Mar	1.5 abc	0.10 def	0.61 bc	1.71 abc	0.16 ab
Apr	1.6 c	0.10 cde	0.35 a	1.64 abc	0.12 a
May	1.6 bc	0.09 bc	0.62 bc	3.82 e	0.17 bcd
Jun	1.5 abc	0.08 b	0.71 cd	2.42 d	0.15 ab
Jul	1.4 ab	0.06 a	0.70 cd	1.91 c	0.19 cd
Aug	1.4 a	0.06 a	0.78 de	2.32 d	0.20 de
Sept	1.6 abc	0.08 b	0.86 e	2.69 d	0.24 e
Oct	1.6 c	0.11 ef	0.85 e	1.45 ab	0.15 ab
Nov	1.6 c	0.11 ef	0.79 de	1.38 a	0.16 bc
Dec	1.5 abc	0.11 f	0.78 de	1.45 ab	0.15 ab

*Values are mean of the ten samples and different letters in the same column are not significantly different according to Tukey's multiple tests at $p \leq 0.05$

Table 4. Differences in mean leaf macronutrients (N, P, K, Ca, Mg) concentration between months during an olive growth cycle grown in acid and alkaline soils

Month		N	P	K	Ca	Mg
		% (dw)				
Jan	acid	1.4 a	0.09 a	0.75 b	1.75 a	0.13 a
	alkaline	1.5 a	0.09 a	0.70 a	1.58 a	0.14 a
Feb	acid	1.5 a	0.09 a	0.66 a	1.63 a	0.11 a
	alkaline	1.5 a	0.10 b	0.60 a	1.79 a	0.15 b
Marc	acid	1.5 a	0.10 a	0.66 b	1.95 a	0.14 a
	alkaline	1.5 a	0.10 a	0.61 a	1.71 a	0.16 a
Apr	acid	1.6 a	0.09 a	0.46 b	1.77 a	0.13 a
	alkaline	1.6 a	0.10 a	0.35 a	1.64 a	0.12 a
May	acid	1.6 a	0.10 b	0.75 b	3.52 a	0.17 a
	alkaline	1.6 a	0.09 a	0.62 a	3.82 a	0.17 a
Jun	acid	1.4 a	0.08 a	0.76 a	2.38 a	0.14 a
	alkaline	1.5 a	0.08 a	0.71 a	2.42 a	0.15 a
Jul	acid	1.4 a	0.07 b	0.83 b	2.15 b	0.19 a
	alkaline	1.4 a	0.06 a	0.70 a	1.91 a	0.19 a
Aug	acid	1.4 a	0.06 a	0.90 b	2.31 a	0.18 a
	alkaline	1.4 a	0.06 a	0.78 a	2.32 a	0.20 a
Sept	acid	1.5 a	0.07 a	0.98 b	2.66 a	0.22 a
	alkaline	1.6 a	0.08 a	0.86 a	2.69 a	0.24 a
Oct	acid	1.3 a	0.11 a	0.93 b	1.52 a	0.15 a
	alkaline	1.6 b	0.11 a	0.85 a	1.45 a	0.15 a
Nov	acid	1.7 a	0.11 a	0.86 b	1.47 a	0.13 a
	alkaline	1.6 a	0.11 a	0.79 a	1.45 a	0.16 b
Dec	acid	1.3 a	0.12 a	0.82 a	1.50 a	0.13 a
	alkaline	1.8 b	0.11 a	0.78 a	1.38 a	0.15 a

Column means followed by the same letter in the same month are not significantly different according to Tukey's multiple tests at $p \leq 0.05$

This difference must be due to the different soils. The soil unit of the acid orchard is classified as Rhodic Luvisol and that in the alkaline orchard is Eutric Cambisol. Rhodic Luvisol is more developed than Eutric Cambisol which is younger, alkaline and contains more soil calcium (Table 1). So, more soil calcium reduces the uptake of K by olive trees as reported by Yassoglou and Gavalas (1979). A significant correlation between leaf K concentration in acid and alkaline orchards existed (Table 5) and is confirmed by the approximately similar seasonal K evolution curves (Figure 3).

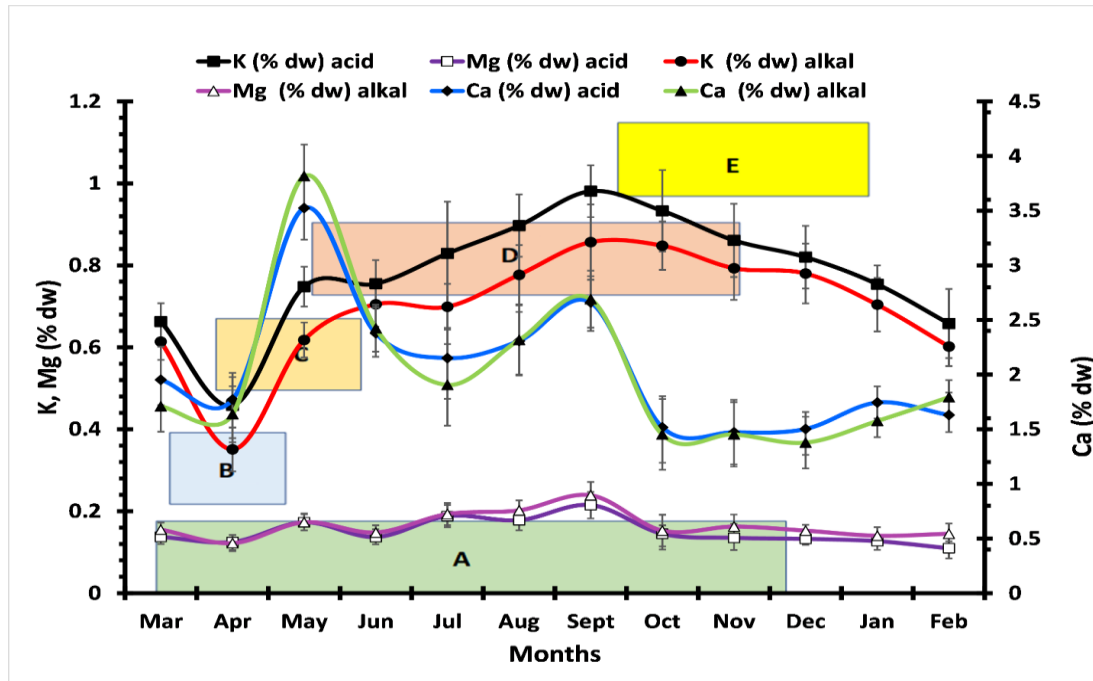


Figure 3. Changes in potassium (K), calcium (Ca) and magnesium (Mg) concentration in the olive leaves grown in acid and alkaline soils during an annual growth cycle
 Error bars represent the standard deviation of ten replicates. A, B, C, D, E correspond to vegetation stages i.e. A=leaf development, B= inflorescence, C= flowering, D= fruit development, E= ripening

Chatzissavvidis *et al.* (2005) and Chatzistathis *et al.* (2010) reported that the maximum leaf K concentration in “Chondrolia Chalkidikis” and “Koroneiki” cvs. respectively, observed during the summer.

Table 5. Correlations between leaf macronutrient concentration in olives grown in acid and alkaline soils

Acid soil		Alkaline soil				
		N	P	K	Ca	Mg
		(% dw)				
N	(% dw)	0.83	0.63	0.245	-0.249	0.088
P			0.921	0.151	-0.708	-0.326
K				0.93	0	0.673
Ca					0.937	0.452
Mg						0.897

Letters in bold indicate a significant correlation at $p \leq 0.05$

Stateras and Moustakas (2018), reported that leaf K concentration in “Manaki” cv. are grown in Eastern Greece, decreased significantly from July to September and increased significantly from May to July then from December to February remained constant and then drop to the lower value (0.9 % dw) in May. The evolution leaf potassium concentration curves between our results and that of Stateras and Moustakas (2018) are approximately similar, but the lower and maximum leaf K concentration value is reached in April and

September, respectively in our experiment and in May and July in Stateras and Moustakas (2018) work (one month difference). This difference as well as the greater K leaf concentration is possibly due to the different climatic conditions. Our studied area belongs to the subhumid climatic zone (Western Greece) and the studied area of Stateras and Moustakas (2018) belongs to the semiarid climatic zone (Eastern Greece). Yassoglou and Gavalas (1979) reported that K deficiency is more frequent in the subhumid than in the semiarid region. Fernández-Escobar *et al.* (1999) mentioned that K concentration in the leaves of olive cv. "Picual" gradually decreased from the beginning of the growing season to August and remained at these levels during autumn. Bouhafa *et al.* (2018) reported that the minimum value for the 'Koroneiki', 'Arbequina', and 'Arbosana' cvs. was recorded in April.

The leaf K concentration measured in July was below the threshold level for sufficiency and above the threshold limit for deficiency in the alkaline orchard and at sufficiency level in acid soil according to Fernández-Escobar (2018) but leaf K concentration value measured in November was at sufficiency level, according to Therios (2009).

Calcium

The seasonal variation of Ca concentration curve for both orchards is presented in Figure 3. The maximum value was observed in May with 3.8% dw and 3.5% dw, in acid and alkaline orchards, respectively. Olive leaves Ca concentration values appeared to increase from July till September up to 2.7% dw in both orchards, this period coincides with the endocarp hardening, thus allowing a large quantity of Ca to accumulate in the leaves. After September, a rapid occurred approach to the minimum value in October and then remained approximately constant until April, in both orchards. The Ca leaf evolution curves were similar for both orchards as illustrated in Figure 3 and as determined by the absence of significant differences in Ca leaves concentration between acid and alkaline orchards (Table 4). The Ca pattern of the seasonal variation curve studied by Stateras and Moustakas (2018) for the 'Manaki' cv. was approximately like our pattern curves, but they differ in the months in which were observed the max and the min values. These differences as mentioned above and for the K concentration may be due to the different climatic conditions. A similar fluctuation pattern was reported by Chatzistathis *et al.* (2010) and Fernández-Escobar *et al.* (1999), who studied the seasonal Ca leaf variation curves in 'Koroneiki' and 'Picual' cvs., respectively.

The leaf Ca concentration was not decreased despite the increased uptake of K, caused by irrigation. Restrepo-Díaz *et al.* (2008) and Steinmetz *et al.* (2015), reported that K is the most closely mineral nutrient to water availability and thus irrigation, so the antagonistic effect (Ca-K) of greater K availability were overcome due to the greater mobility of K. Similar results were reported by Androulakis *et al.* (1997) who studied the concentration of mineral elements in the leaves of 'Koroneiki' cv. concerning irrigation. Significant differences were not observed in leaf Ca concentration values between acid and alkaline orchards except in July (Table 4).

The leaf Ca concentration measured either in July or in November was above the sufficient nutrition level, in both orchards according to Fernández-Escobar (2018) and Therios (2009).

Magnesium

The leaf Mg concentration was at a high level (0.18% dw) during the period of endocarp hardening and fruit maturation (July–November) in both orchards (Figure 3). The maximum values occurred in September with 0.22 and 0.24% (dw), in acid and alkaline orchards, respectively. Subsequently, values drop in October (0.15% dw) and then remained constant until March in the acid orchard and until April in the alkaline orchard. These differences should be due to the easy leaching and increased mobility of Mg, during periods of high and intense rainfall, such as February and March. Fernández-Escobar *et al.* (1999) have reported almost the same results as the ones already mentioned. A similar leaf Mg concentration curve was reported by Stateras and Moustakas (2018) but they differ in the months in which were observed the max and the min values. These differences as mentioned above for K and Ca concentration may be due to the different climatic conditions.

No significant differences were observed in leaf Mg concentration values between acid and alkaline orchards except in November (Table 4).

The leaf Mg concentration measured in July or in November was at sufficiency according to Fernández-Escobar (2018) and Therios (2009) in both orchards. In Table 5 we can see that significant correlations existed between all nutrients in olives grown in acid and alkaline soil.

Conclusions

The olive leaf macronutrient contents are influenced by vegetative growth and varied over time throughout the crop cycle. The evolution concentration curves of leaf N and P in acid and alkaline orchards were almost similar with two stabilizing periods one with the minimum concentrations (winter) and one with maximum concentrations (autumn).

The leaf Ca and Mg evolution concentration curves were almost similar in both orchards and a significant positive correlation existed between leaf Ca and Mg concentration independently of soil acidity. A significant positive correlation was also detected between leaf K and Mg concentration independently of soil acidity. These correlations may be due to the different soil and climatic conditions.

The leaf N, P, Ca, and Mg concentration was not influenced by soil acidity. But significant differences were found between leaf K concentrations in olives grown in acid and alkaline soil.

There were no differences in the concentration of macronutrients during the winter period between olives grown in the acid or the alkaline soils and these values correspond to satisfactory levels for plant growth according to the international literature. On the other hand, the yields of the plants were at high levels (according to the farmers' recordings). Therefore, these values can be used as reference values for leaf nutrient interpretation and the development of an effective fertilization program for healthy growth and satisfactory yield of plants for the specific cultivar under Mediterranean climatic conditions.

Authors' Contributions

Conceptualization, supervision, validation, writing and editing original draft: PB, NM; Methodology, data curation analysis: PV, EK, PB. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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