JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Olive (*Olea europaea* L.) Tree Nitrogen Status Is a Key Factor for Olive Oil Quality

Ran Erel,[†,§] Zohar Kerem,[∥] Alon Ben-Gal,[†] Arnon Dag,[‡] Amnon Schwartz,[§] Isaac Zipori,[‡] Loai Basheer,[∥] and Uri Yermiyahu*[,†]

[†]Institute of Soil, Water and Environmental Sciences and [‡]Institute of Plant Sciences, Gilat Research Center, Agricultural Research Organization, Mobile Post Negev 2, 85280, Israel

[§]Institute of Plant Sciences and Genetics in Agriculture, and [∥]Institute of Biochemistry, Food Science and Nutrition, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel

Supporting Information

ABSTRACT: The influence of macronutrient status on olive oil properties was studied for three years. Data were analyzed by a multivariate model considering N, P, K, and fruiting year as explanatory factors. Oil quality parameters were primarily associated with N concentration in leaves and fruits which increased with N in irrigation solution. The effect of P on oil quality was mainly indirect since increased P availability increased N accumulation. The potassium level had negligible effects. The oil phenolic content decreased linearly as a function of increased leaf N, indicating protein−phenol competition in leaves. The overall saturation level of the fatty acids decreased with fruit N, resulting in increased polyunsaturated fatty acids. Free fatty acids increased with increased levels of fruit N. High fruit load tended to reduce fruit N and subsequently improve oil quality. The effect of N on oil properties depended solely on its concentration in leaves or fruits, regardless of the cause.

KEYWORDS: *Olea europaea, mineral nutrition, free fatty acids, polyphenols, fatty acid composition*

INTRODUCTION

Public perception of its gastronomic and health-related benefits has led to continual increases in the global consumption of virgin olive oil (VOO). The growing demand for VOO has led to the intensification of olive cultivation.[1] Unlike most other vegetable oils, VOO is extracted solely by physical means without the use of industrial solvents. Hence, VOO preserves both the unique flavor and beneficial minor compounds. In particular, VOO has been found to reduce the risk of cardiovascular diseases, cancer, obesity, type II diabetes, and metabolic syndrome.[2] The health properties of VOO are related to three major characteristics: the first, a high proportion of monounsaturated fatty acid (MUFA), namely, oleic fatty acid,[3] the second, the existence of powerful antioxidants, mainly phenolic compounds,[4] and the third, the presence of a variety of minor compounds beneficial to health including sterols, carotenoids, squalene, and tocopherols.[5]

Many environmental, horticultural, and genetic factors may alter olive oil properties. The most acknowledged are variety, climate, irrigation, fruit maturity level, pest damage, harvest method, and oil extraction method.[6,7] Mineral nutrition has not traditionally been considered to play a major role regarding oil quality.[7,8] Since proper nutrition is essential for high yields,[9] most of the trials regarding olive mineral nutrition examined nutritional effects on productivity rather than on quality. A number of studies are responsible for the general belief that fertilization has an insignificant effect on oil quality. Inglese et al.[10] and Ferreira et al.[11] did not find any changes in fatty acid composition in response to nitrogen (N), phosphorus (P), and potassium (K) application. Similarly, no effect on oil quality was found subsequent to urea application[12] or for foliar N application on FFA and oil fatty acid composition.[13] In contrast to the above, recent studies indicate that macronutrients may have an effect on some VOO quality properties.[14−17] In a long-term N trial, Fernández-Escobar et al.[14,18] reported that high N fertilization caused a decrease in polyphenol content and thus a decrease in oil stability. In another experiment, using a modern fertigation system, the researchers found that increasing amounts of N, P, and K led to decreased polyphenols, bitterness, and oil stability.[15] The percentage of MUFA decreased, and consequently, the portion of polyunsaturated fatty acids (PUFA) increased with increased fertigation concentrations. Similarly, in a controlled container experiment, Dag et al.[16] reported decreased oleic percentage and polyphenol content in response to high levels of N and P, while K had only a minor effect. Recently, foliar nutrient application was reported to elevate the level of phytosterols and decrease polyphenol content of olive oil.[17] Interestingly, similar to that reported for olive oil, increasing N application was found to reduce phenol content and the oleic portion also in walnut kernels.[19]

The references cited above indicate that present available knowledge regarding the effect of mineral nutrition on oil quality is limited and inconsistent. The different conclusions found in the various studies have a number of explanations. One reason for the inconsistencies may be due to the essential differences between traditional and modern cultivation systems,

Received: July 24, 2013
Revised: September 30, 2013
Accepted: October 30, 2013
Published: October 30, 2013

dx.doi.org/10.1021/jf4031585 | J. Agric. Food Chem. 2013, 61, 11261−11272

Table 1. Annual Means and Statistical Analysis for N Concentration in Leaves in October and N Concentration in Fruit Flesh at Harvest as a Function of N Concentration in the Irrigation Solution[a]

irrigation N		leaf N (%)			fruit flesh N (%)		
treatment	(mg/L)	2007	2008	2009	2007	2008	2009
N1	6	1.17	0.64	0.98	N.D	0.33	N.D
N2	15	1.33	1.52	1.53	1.29	0.78	1.37
N3	25	1.78	1.78	1.68	1.54	0.95	1.73
N4	47	1.70	1.99	1.83	1.67	1.07	1.90
N5	75	1.67	2.12	1.83	1.73	1.14	1.81
N6	109	1.69	2.07	1.89	1.88	1.06	1.78
N7	153	1.78	2.00	1.90	1.78	1.45	2.14
N8	198	1.85	2.24	2.10	2.02	1.58	2.12
year effect		1.62 a	1.79 a	1.72 a	1.70 a	1.15 b	1.84 a
logarithmic regression							
R^2		0.74	0.81	0.88	0.91	0.91	0.78
p value		0.006	0.002	<0.001	0.001	<0.001	0.010

[a]Different letters indicate significant differences between means of annual data ($P \leq 0.05$).

Table 2. Annual Means and Statistical Analysis for P Concentration in Leaves in October and P Concentration in Fruit Flesh at Harvest as a Function of P in Irrigation Solution[a]

irrigation P		leaf P (%)			fruit flesh P (%)		
treatment	(mg/L)	2007	2008	2009	2007	2008	2009
P1	0.2	0.08	0.03	0.03	0.08	0.04	N.D
P2	0.7	0.10	0.06	0.07	0.11	0.07	0.08
P3	1.2	0.11	0.10	0.10	0.12	0.08	0.15
P4	2.0	0.14	0.13	0.17	0.17	0.13	0.23
P5	4.4	0.15	0.15	0.15	0.21	0.15	0.24
P6	9.9	0.15	0.18	0.21	0.20	0.17	0.23
P7	19.3	0.17	0.23	0.24	0.21	0.19	0.30
year effect		0.13 a	0.13 a	0.14 a	0.17 ab	0.13 b	0.20 a
logarithmic regression							
R^2		0.91	0.98	0.95	0.91	0.98	0.79
p value		<0.001	<0.001	<0.001	0.001	<0.001	0.017

[a]Different letters indicate significant differences between means of annual data ($p \leq 0.05$).

for which nutrient availability and uniformity are significantly different. This is especially true when taking into account fertigation compared to traditional methods of dryland fertilization.[20] Another potential complicating factor is the dependence of tree response to mineral nutrition on additional aspects including: soil type,[21] fruit load,[22] and climate. Perhaps the most compelling reason for the conflicting findings regarding mineral nutrition is the way fertilization is being assessed. The traditional way of examining nutritional effects in response to fertilizer application dose is problematic. Minerals taken up and accumulating in the tree may be influenced by a number of factors including fertilizer composition, application methods (fertigation, soil application, or foliar spray), timing, soil physical and chemical properties, tree nutritional status, or environmental conditions. In particular, water availability is highly important for determining plant response to minerals.[23,20] It is therefore necessary to evaluate tree nutritional status rather than application rate when studying response to minerals.

Leaf analysis is considered to be the best method for diagnosing nutritional status in olives.[24,25] However, fruit nutritional level is expected to be more relevant than leaf concentration regarding the examination of nutrient status on oil properties[26] since the fruit is the site of oil biosynthesis. Another level of difficulty in studying the influence of mineral nutrition on oil quality is that interactions between minerals frequently occur. Manipulation of a single mineral can directly or indirectly affect the nutritional level of other minerals,[23] making interpretation challenging. Therefore, assessment of the effect of nutrients on oil quality must include consideration of nutritional levels of each of the main minerals. In a previous paper, we presented an analysis of the effect of single-factors N, P, and K on VOO quality for a single season.[26] The aim of the present study was to further evaluate the effect of the three major nutrients and their interactions on VOO quality over multiple fruiting years. To accomplish this, we investigated the effect of mineral levels in fruits and leaves on oil obtained from trees receiving various fertilizer concentrations in their irrigation solution. The data collected in the first season was reprocessed and integrated with results from the following two seasons. Naturally, during the three examined seasons trees had different fruit bearing levels. A multivariate statistical model was used in order to capture the interactions between N, P, and K nutritional levels in fruits and leaves on the major VOO quality parameters.

MATERIALS AND METHODS

Plant Material and Experimental Design. Olives (cv. Barnea) were grown in containers at the Gilat Research Center, Israel. Two year old plants were planted in February 2006 in 60-L containers filled with type 4 (4−6 mm) granular perlite. Initially, the trees were irrigated excessively via a drip system twice a day with a nutrient

Table 3. Annual Means and Statistical Analysis for K Concentration in Leaves in October and K Concentration in Fruit Flesh at Harvest as a Function of K in Irrigation Solution[a]

irrigation K		leaf K (%)			fruit flesh K (%)		
treatment	(mg/L)	2007	2008	2009	2007	2008	2009
K1	9.9	0.82	0.45	0.31	1.67	1.27	0.74
K2	19.2	1.07	0.74	0.79	2.20	1.61	2.66
K3	28.7	1.18	1.07	0.97	2.57	2.27	2.93
K4	52.1	1.23	1.02	1.15	2.68	2.38	3.09
K5	80.3	1.41	1.30	1.33	3.03	2.88	3.75
K6	102.0	1.27	1.40	1.58	2.75	2.74	3.69
K7	209.0	1.67	1.51	2.02	3.45	3.11	4.24
year effect		1.24 a	1.07 a	1.17 a	2.62 a	2.32 a	3.02 a
logarithmic regression							
R^2		0.91	0.94	0.98	0.92	0.94	0.86
p value		0.001	<0.001	<0.001	<0.001	<0.001	0.003

[a]Different letters indicate significant differences between means of annual data ($P \leq 0.05$).

solution containing 83, 16, and 69 mg/L of N, P, and K, respectively. Differential nutrient-irrigation treatments were initiated in September, 2006. Average nutrient concentrations in irrigation solutions are presented in Tables 1, 2, and 3 for the N, P, and K treatments, respectively. The 20 treatments included eight levels of N, seven levels of P, and seven levels of K, with one of the treatments (N5, P6, and K6) common to all three variables. For each treatment, only one mineral was subject to manipulation. The concentrations of the remaining minerals were set to 70, 10, and 100 mg/L N, P, and K, respectively. The experiment used a randomized block design with six replicates. Following the first harvest on November, 2007, three trees per treatment were transplanted into 500 l containers filled with perlite substrate for two more seasons. Detailed experimental set up information and fertilizer composition are described by Erel et al.[27,28]

Fruit and Leaf Analysis. Fruits were harvested manually during October–November of each fruiting year. Each tree was harvested independently when its maturity index reached ~2.5 according to the IOC method.[29] A sample of fruits was taken for mineral analysis. After the stones were removed, fruit flesh was rinsed for 15 s in deionized water, dried at 60 °C, and ground to powder. Total N, P, and K content was determined after digestion with sulfuric acid and hydrogen peroxide. The concentrations of N and P were determined with an autoanalyzer (Lachat Instruments, Milwaukee, Wisconsin, U.S.A.), and K content was determined with a flame photometer (Corning 400, Corning Inc., New York, U.S.A.). Leaf samples were taken close to the harvest in early November according to the common protocol.[30] The youngest fully developed leaves were collected from the middle portion of nonbearing branches. Sampled leaves were washed, dried, and digested as described for fruits.

Oil Extraction and Quality. Cold-pressed oil was obtained from representative 1 kg fruit samples using an 'Abencor' laboratory mill (mc2 Ingenieria y Sistemas, Seville, Spain) in accordance with the protocol described by Ben-David et al.[31] Determination of free fatty acid (FFA) and peroxide value was carried out according to ISO 660 and 3960, respectively. Total polyphenols, expressed as gallic acid equivalents (mg/kg), were determined with a UV visible spectrophotometer (Beckman Coulter, Inc. Fullerton, CA, U.S.A.) at 765 nm using the Folin–Ciocalteu reagent. Fatty acid composition was determined by gas chromatography (GC) as fatty acid methyl esters (FAMEs). FAMEs were prepared according to AOCS protocol No. Ce 2-66 (97). GC analysis was performed in accordance with ISO 5508, using an Agilent Technologies (model 6890N) GC with a flame ionization detector and the recommended capillary column (60 m, 0.25 mm id, 0.25 μm film thickness; Quadrex Corporation, Woodbridge, CT, U.S.A.).

Data Analysis. Data were analyzed using JMP 10.0 software (SAS Institute Inc., Cary, NC, U.S.A.). The effect of fruiting year on leaf and fruit N, P, and K was tested by Tukey's honest significance test. Initially, the combined relationships of a single mineral level (fruit flesh or leaves), fruiting year, and the measured oil quality parameter were determined using two-way ANOVA. The model includes data composed of all of the measured trees, and each point in the model represents the average of six trees in 2007 and three trees in 2008 and 2009 (sum of 240 measurements). Subsequently, all of the minerals, fruiting years, and interactions were examined in a multifactorial model. Only significant correlations between mineral level and quality parameters were considered. Linear regression of combined data was presented only when there was an insignificant effect of fruiting year; otherwise, regression was presented for each year separately. Interaction between the variables was not significant and therefore not presented. Because of multiple factor comparisons and interactions, the default p value for significance was set for 0.01.

RESULTS

The data incorporate the three years of study and the three elements. Each symbol in Figures 1–5 represents an average of six replicates in 2007 and three replicates in 2008 and 2009. At the lowest N and P levels, missing data is due to the fact that some of the nutrient-deficient trees did not produce enough fruits to obtain oil. Regardless of the nutritional level, in all oils, the FFA, peroxide values and delta-K were well within acceptable IOC thresholds of 0.8%, 20 meq O_2/kg, and 0.01 for extra VOO. No major effect on peroxide value or delta-K was found (see Supporting Information, Tables S4 and S5).

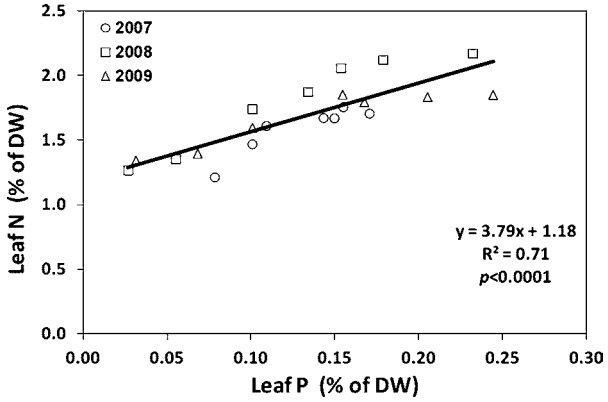

Figure 1. Correlation between leaf P and leaf N in the P treatments (received identical N fertilizer concentration) during the three studied years. Each point represents an average of six replicates in 2007 and three replicates in 2008 and 2009.

Mineral Accumulation in Fruits and Leaves. July is considered to be the accepted period for leaf analysis in

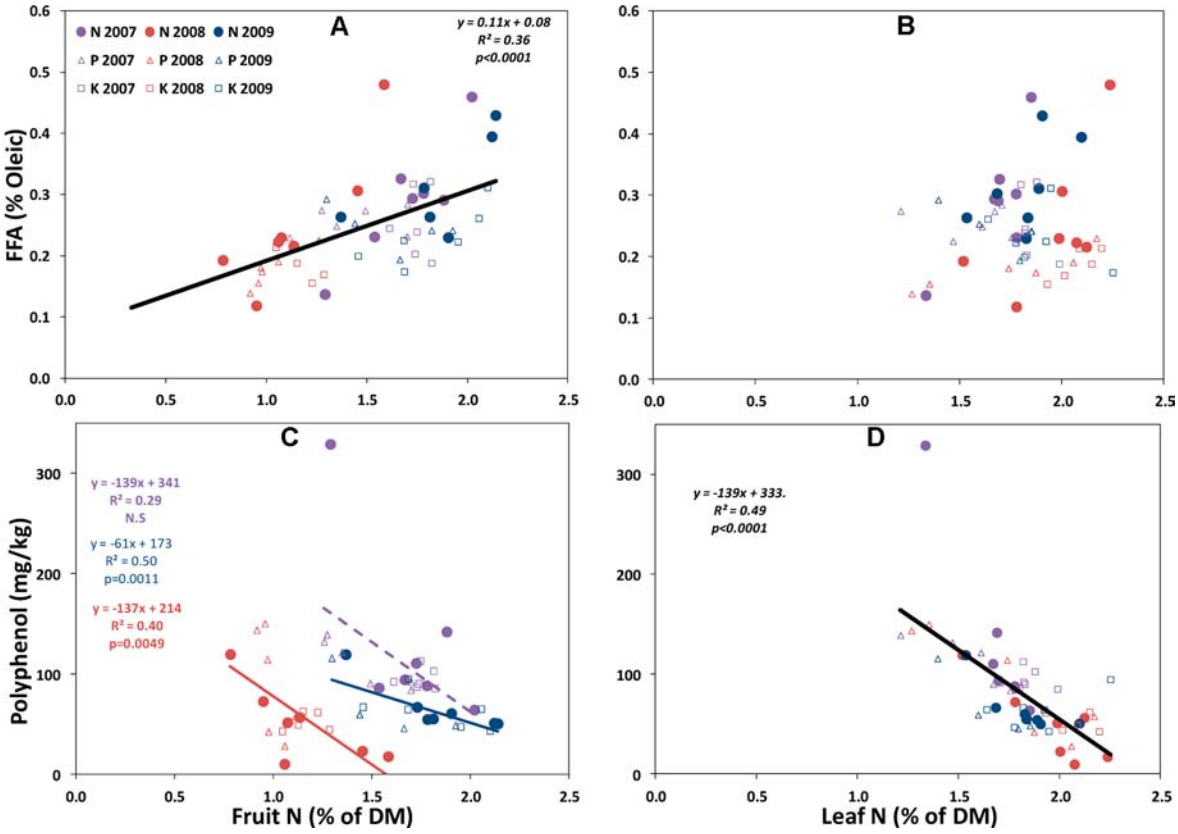

Figure 2. Average free fatty acid (a and b) and polyphenol (c and d) concentration as a function of N concentration in fruit flesh (a and c) and in leaves (b and d) for the three studied years: 2007 (purple), 2008 (red), and 2009 (blue) and the three manipulated treatments: N (●), P (▲), and K (■). Each point represents an average of six replicates in 2007 and three replicates in 2008 and 2009. The statistical parameters of the model are presented in Table 4.

Table 4. Statistical Parameters of the Two Way ANOVA Model (Mineral Level, Year) Presented in Figures 2−7

explanatory variable			model		effect tests		
mineral	plant tissue	responding variable	R^2	significance	year	nutritional status	cross
N	fruit flesh	FFA	0.38	<0.0001	N.S	<0.0001	N.S
N	leaf	FFA	0.21	0.0055	0.0054	N.S	N.S
N	fruit flesh	polyphenol	0.47	<0.0001	<0.0001	<0.0001	N.S
N	leaf	polyphenol	0.58	<0.0001	N.S	<0.0001	N.S
N	fruit flesh	SFA	0.37	<0.0001	0.0024	<0.0001	N.S
N	fruit flesh	oleic C18:1	0.66	<0.0001	N.S	<0.0001	N.S
N	fruit flesh	linoleic C18:2	0.62	<0.0001	N.S	<0.0001	N.S
N	fruit flesh	linolenic C18:3	0.77	<0.0001	N.S	<0.0001	N.S
P	fruit flesh	FFA	0.24	0.0028	N.S	N.S	N.S
P	leaf	FFA	0.17	N.S	N.S	N.S	N.S
P	fruit flesh	polyphenol	0.36	<0.0001	<0.0001	0.0029	N.S
P	leaf	polyphenol	0.29	0.0006	0.0032	N.S	N.S
P	fruit flesh	SFA	0.41	<0.0001	N.S	<0.0001	N.S
P	fruit flesh	oleic C18:1	0.51	<0.0001	<0.0001	0.0015	N.S
P	fruit flesh	linoleic C18:2	0.46	<0.0001	0.0050	0.0005	N.S
P	fruit flesh	linolenic C18:3	0.69	<0.0001	0.0082	<0.0001	N.S
K	fruit flesh	FFA	0.24	0.0024	N.S	N.S	N.S
K	leaf	FFA	0.27	0.0008	N.S	0.0038	N.S
K	fruit flesh	polyphenol	0.28	0.0008	0.0012	N.S	N.S
K	leaf	polyphenol	0.24	0.0029	0.0010	N.S	N.S
K	fruit flesh	SFA	0.08	N.S	N.S	N.S	N.S
K	fruit flesh	oleic C18:1	0.46	<0.0001	<0.0001	N.S	N.S
K	fruit flesh	linoleic C18:2	0.37	<0.0001	0.0006	N.S	N.S
K	fruit flesh	linolenic C18:3	0.41	<0.0001	0.0004	N.S	N.S

dx.doi.org/10.1021/jf4031585 | J. Agric. Food Chem. 2013, 61, 11261−11272

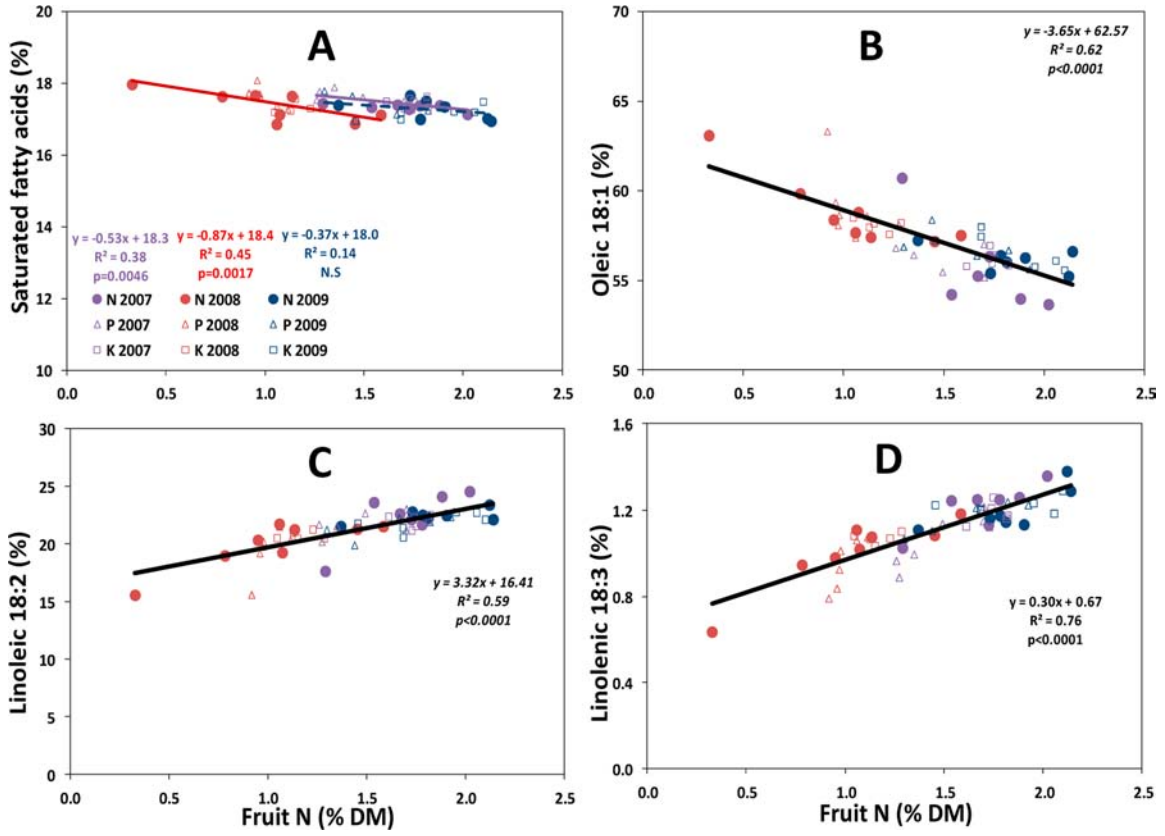

Figure 3. Fatty acid composition as a function of N concentration in fruit flesh for the three studied years: 2007 (purple), 2008 (red), and 2009 (blue) and the three manipulated treatments: N (●), P (▲), and K (■). Each point represents an average of six replicates in 2007 and three replicates in 2008 and 2009. The statistical parameters of the model are presented in Table 4.

olives.[24] However, in order to enable comparison with fruit mineral concentration and oil properties, leaf sampling and analysis were conducted in October each year and are presented in Tables 1−3. Logarithmic regression best described the relationship between mineral concentrations in irrigation solution to those in plant tissue for all 18 comparisons (Tables 1−3). As a function of N concentration in irrigation solution, leaf N ranged from 0.64 to 2.24%. Nitrogen in fruit flesh was comparable to leaf N with the exception of 2008 when N concentration in fruit was slightly lower than leaf N. Leaf P ranged between 0.03 and 0.24%, while P in fruit flesh was inconsistent. Fruit P was higher in 2007 and 2009 and relatively low in 2008. Leaf K ranged from 0.31 to 2.02%, while K in fruit flesh was consistently higher, approximately double that in leaves.

Over the three years, no significant effect of fruiting year on leaf mineral content was found. Conversely, fruit mineral concentrations were lowest in 2008 for each of the three studied minerals (Tables 1−3). The most pronounced fruiting year effect was found for N with fruit levels in 2008 being significantly lower than that in 2007 and 2009 (Table 1). Fruit P was significantly lower in 2008 compared to that in 2009, and fruit K had an insignificant fruiting year effect.

Although only one mineral was subject to change in each treatment, a strong positive interaction between P and N was repeatedly measured. Nitrogen concentration in leaves and in fruit flesh consistently increased with the concentration of P in irrigation solution. The increases in N accumulation with P application persisted in spite of the fixed N concentration in irrigation solution. As a result of this enhanced N uptake in response to P, a linear correlation was found between leaf P and leaf N in the P treatments (Figure 1). The relationship between leaf P and leaf N was consistent over the three seasons. The data clearly indicate that P availability increased N accumulation throughout the range of P investigated. The significant effect of fruiting year on fruit mineral concentrations (Tables 1−2) raises the need to assess the fruiting year as an explanatory factor. Two-way ANOVA model results are presented in Figures 2−7, and the model's statistical parameters are shown in Table 4.

Effect of N and Fruiting Year on FFA and Polyphenol Content. For all of the measured treatments and seasons, FFA was relatively low, lower than the threshold of 0.8% for extra VOO. FFA increased continuously from ∼0.15 to ∼0.45% with increased fruit N, while fruiting year had no significant effect (Figure 2a). Four measurements were exceptionally high, exceeding 0.4%. These values were measured for the highest N levels: N8 in 2007, N8 in 2008, N7, and N8 in 2009. In each of the fruiting years, the highest FFA was measured for the highest fruit N. Unlike fruit N, leaf N was not significantly correlated to FFA. In 2008, average FFA values were somewhat lower compared to those in 2007 and 2009 (Figure 2b).

Both fruit N and fruiting year were significantly associated with polyphenol content in oil (Figure 2c). In each year, independently, polyphenol decreased with fruit N, but the effect was inconsistent and significant only in two fruiting years. When examining polyphenol level against leaf N, a robust continuous correlation was found, and no fruiting year effect was discernible (Figure 2d). For the three studied seasons,

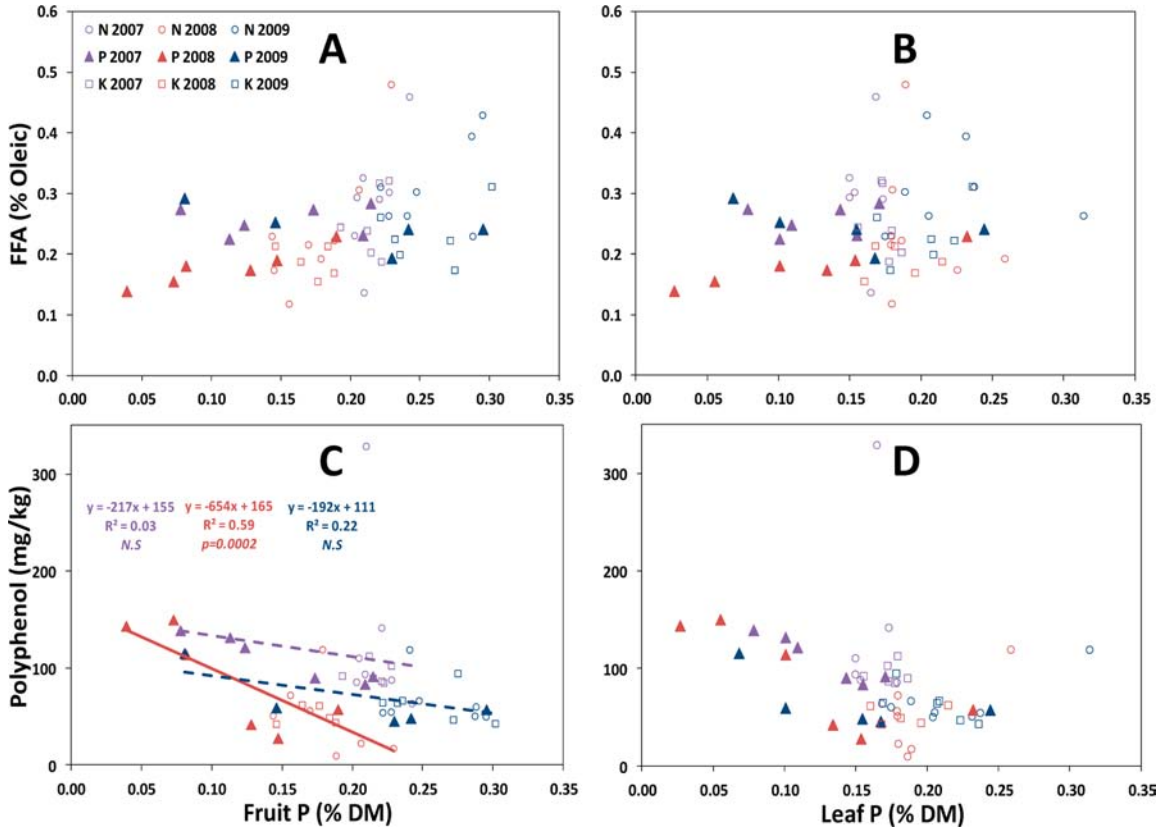

Figure 4. Average free fatty acid (A and B) and polyphenol (C and D) concentration as a function of P concentration in fruit flesh (A and C) and in leaves (B and D) for the three studied years 2007 (purple), 2008 (red), and 2009 (blue) and the three manipulated treatments N (●), P (▲), and K (■). Each point represents an average of six replicates in 2007 and three replicates in 2008 and 2009. The statistical parameters of the model are presented in Table 4.

lowest polyphenol concentration corresponded to the highest leaf N concentration.

Effect of N and Fruiting Year on Oil Composition. The following results refer to the major components of fatty acids: saturated (palmitic C16:0 + stearic C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3). These four components account for 98−99% of the fatty acid profile. Full fatty acid profiles for the different treatments are presented in Tables S1−S3 in the Supporting Information.

The percentage of saturated fatty acids was significantly and negatively associated with fruit N in two out of the three seasons and was significantly affected by fruiting year (Figure 3A and Table 4). Although significant, the range of saturated fatty acid percentage was quite narrow, from 18% for the lowest fruit N to 16.9% in the high fruit N range. Fruit N had substantial negative effect on the main monounsaturated fatty acid, oleic acid. The percentage of oleic acid decreased by 10%, from more than 60% when fruit N < 1% to 53−56% when fruit N > 2%. Solid positive linear responses to increased fruit N were found regarding linoleic (C18:2, Figure 3C) and linolenic (C18:3, Figure 3D) fatty acids. The proportion of linoleic acid ranged between 15.6 and 24.6%, and linolenic percentage increased from 0.6 to 1.4% as fruit N increased.

Effect of P and Fruiting Year on FFA, Polyphenol Content, and Oil Composition. Neither fruit P nor leaf P was found to be correlated to FFA (Figure 4A and B). Although fruit and leaf P greatly changed between the two extreme treatments, P1 and P7 (Table 1), FFA was 0.27−0.28%, 0.14−0.23%, and 0.29−0.24% for P1 and P7 in 2007, 2008, and 2009, respectively. Polyphenol concentration in oil was significantly associated with fruit P only in 2008. Polyphenol in oil was not significantly correlated to leaf P (Figure 4C,D and Table 4).

Fruit P was significantly associated with the four studied fatty acid components (Figure 5). Saturated fatty acid (SFA) percentage in oil slightly decreased as fruit P increased (Figure 5A). Oleic acid percentage in oil tended to decrease with increased fruit P; however, the fruiting year had a highly significant effect (Table 4). Linoleic and linolenic PUFAs increased with fruit P and were also significantly affected by fruit year (Figure 5D,C and Table 4).

Effect of K and Fruiting Year on FFA, Polyphenol Content, and Oil Composition. Potassium had less effect on oil composition compared to that of other minerals. Only FFA was significantly correlated to leaf K (Figure 6b and Table 4) but with a lower p value and lower correlation coefficient compared to those of fruit N (Figure 2a). Polyphenol concentration was entirely independent of leaf or fruit K (Figure 6c,d). Fatty acid composition was also entirely independent of fruit K (Figure 7A−D).

The year was significantly associated with the main unsaturated fatty acids. Most notable was the higher portion of oleic acid and lower linoleic and linolenic acids in 2008 compared to those in 2007 and 2009.

Relative Contribution of Fruiting Year and Interaction between Minerals. In the previous Results section, all data were analyzed testing only two factors: the studied mineral concentration and fruiting year. This method is suited for

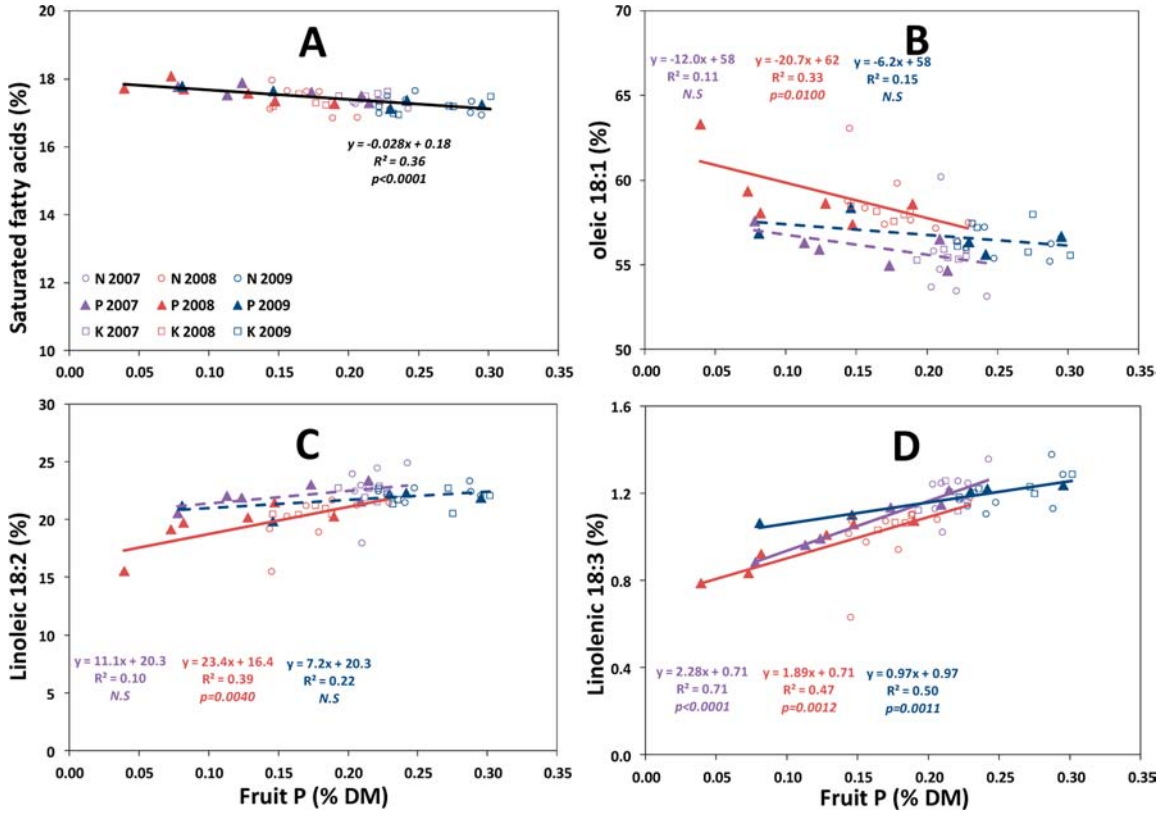

Figure 5. Fatty acid composition as a function of P concentration in fruit flesh for the three studied years: 2007 (purple), 2008 (red), and 2009 (blue) and the three manipulated treatments N (●), P (▲), and K (■). Each point represents an average of six replicates in 2007 and three replicates in 2008 and 2009. The statistical parameters of the model are presented in Table 4.

evaluating the individual effect of each of the elements: N, P, and K. Clearly, the interactions between elements cannot be ruled out, especially in light of the strong correlation between P and N. The results of a multivariable model used to test all minerals together with years and interactions are presented in Table 5. The correlation coefficient of the model regarding polyphenol concentration, oleic, linoleic, and linolenic acid percentage is fairly high (model $R^2 > 0.62$). Especially high are the correlation coefficients for oleic and linolenic acids, which, respectively, explain 71% and 81% of the variation by the four factors: fruit N, P, K, and fruiting year. The effect of minerals and fruiting year on the remaining oil components, FFA and SFA, were highly significant but with relatively low correlation coefficients, implying the involvement of additional factors. Among the minerals, N strongly stands out with the most significant effect on all the oil properties other than SFA. Moreover, N was found to be the only significant factor regarding FFA and linoleic acid percentage. As for P, a relatively weak correlation was found for fruit P on SFA and linolenic percentage in oil. When taking into account all of the factors, fruit and leaf K did not correlate significantly to any of the measured oil properties. The fruiting year, which may contain many hidden factors (climate, pests, etc.), had only a minor effect on polyphenol concentration and oleic acid percentage.

Table 5 presents correlation levels of the explanatory factors with oil properties. In order to evaluate the extent of the effect of each factor, scaled estimates of the main model components are presented in Figure 8. The scaled estimates are defined as the direction (negative or positive) and the extent of change when adjusting half the range of the studied factor. For example, in response to increasing half the given range of fruit N, FFA is expected to increase by 0.13% (Figure 8, upper left). The N level emerges as the factor which had the greatest quantitative impact on all of the studied oil components (Figure 8). Nitrogen level was not only significantly associated with the oil quality parameters but also had a considerable quantitative effect in regard to FFA, polyphenol, and oleic, linoleic, and linolenic acids but not SFA.

■ DISCUSSION

Oil quality is defined by a complex combination of parameters which influence sensory evaluation, nutritional values, health benefits, and oil stability. Mineral nutrition of olives has traditionally been studied in relation to fruit yield and thought to play only a minor role in oil quality.[7] The present study indicates that high nutritional N level is detrimental to major oil quality parameters. Nitrogen concentration in olive fruit flesh was found to be a function of three factors: N application rate, fruit load, and P availability. All three factors should be taken into account when assessing the tree's nutritional status.

Phosphorus and Potassium Nutritional Level and Oil Quality. Potassium nutritional level had only a minor effect on oil quality. This is not surprising since unlike N and P, K is not an intrinsic mineral in oil or in any organic tissue. Phosphorus nutritional level had a minor direct effect on SFA and linolenic acid (Figure 8). Nevertheless, P nutrition enhanced N accumulation in leaves and fruits (Figure 1) and thus indirectly modified the oil properties by increasing the N nutritional level. To the best of our knowledge, the nutritional synergism presented here between P and N in olive is novel, although we have failed to uncover a solid scientific explanation for it. It is very likely that the olive tree has a unique synergism

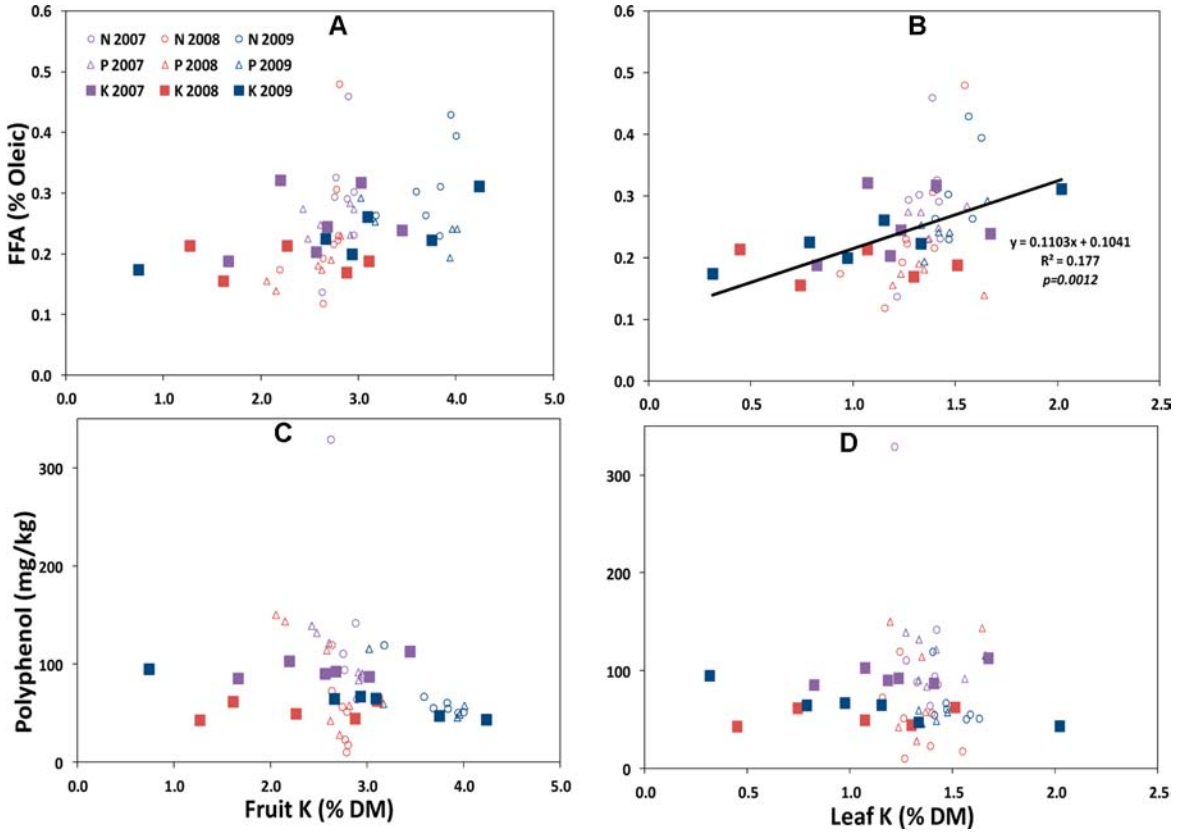

Figure 6. Average free fatty acid (a and b) and polyphenol (c and d) concentration as a function of K concentration in fruit flesh (a and c) and in leaves (b and d) for the three studied years 2007 (purple), 2008 (red), and 2009 (blue) and the three manipulated treatments N (●), P (▲), and K (■). Each point represents an average of six replicates in 2007 and three replicates in 2008 and 2009. The statistical parameters of the model are presented in Table 4.

mechanism in N acquisition. By the end of the experiment, we recorded a continuous increase in root weight with P nutritional level (data not shown). Therefore, the phenomenon of P−N synergism may be the result of P enhancement of root growth and subsequent improved utilization or uptake of N. This stated, we need to question and restrict the conclusion of our previous publication[26] that asserted a direct effect of P nutrition on VOO properties.

Nitrogen Nutrition and Oil Quality. In general, increasing levels of N in leaves and fruits were found to have an adverse effect on olive oil quality by lowering oleic acid and polyphenol concentrations and by raising the PUFAs linoleic and linolenic acids, and FFA. The sharp decrease in polyphenol concentration and oleic acid percentage and increase in FFA are expected to impair oil stability.[32] Negative effects of N nutrition on a number of oil parameters were reported in previous studies,[14,17,26,33] each focusing on a single piece of the puzzle. The current study offers a comprehensive view on the effect of the individual minerals in relation to their tissue nutritional level.

Nitrogen and Phenol Biosynthesis. The linkage between N nutrition and product quality in various crops was widely discussed in a recent review in which adverse effects of N on overall quality, and specifically on secondary plant metabolites, were presented.[34] Inhibitory effects of N on the biosynthesis of polyphenol were previously reported for olive oil,[14,33] apple,[35] and sphagnum peat.[36] Jones and Hartley[37] suggested a protein/phenol competition model. The model's mechanism is derived from biosynthetic pathways of proteins and phenols which share phenylalanine as a common precursor. A high mineral N level induces the biosynthesis of proteins and hence consumes the limiting precursor. A low N level permits more phenylalanine for the synthesis of secondary metabolites, i.e., polyphenol. Polyphenol content has therefore been proposed to serve as an indicator for N status in woody plants.[38] The olive fruit, due to its commercial importance, has received most of the attention in regard to phenol biosynthesis.[39−41] Very little is known regarding the interactions between biosynthesis sites and transportation of phenols among various plant tissues.[40−42] Ryan et al.[42] suggested that some precursors may be transported from one site to another for subsequent biosynthesis. In the present study, we found a consistent and continuous correlation between polyphenol content in the oil to N content in leaves but a weaker, inconsistent correlation to fruit N (Figure 2c and d). The interaction between leaf N status and polyphenol in the oil implies a possible interaction between leaves and fruit regarding phenol metabolism. Therefore, we tentatively suggest that either phenol precursors or polyphenol itself is being produced in leaves and translocated to the fruit. The N persisting in leaves competes with the upstream precursor and thus reduces its availability. The limiting precursor determines the rate of phenol biosynthesis in the leaf and creates a bottleneck for polyphenol accumulation in fruit.

Nitrogen and Fatty Acid Biosynthesis. Olive oil is composed of ∼98% triacylglycerols that are built from a number of typical fatty acids, mainly oleic acid.[43] A high oleic level is especially desired since it is closely related to oil

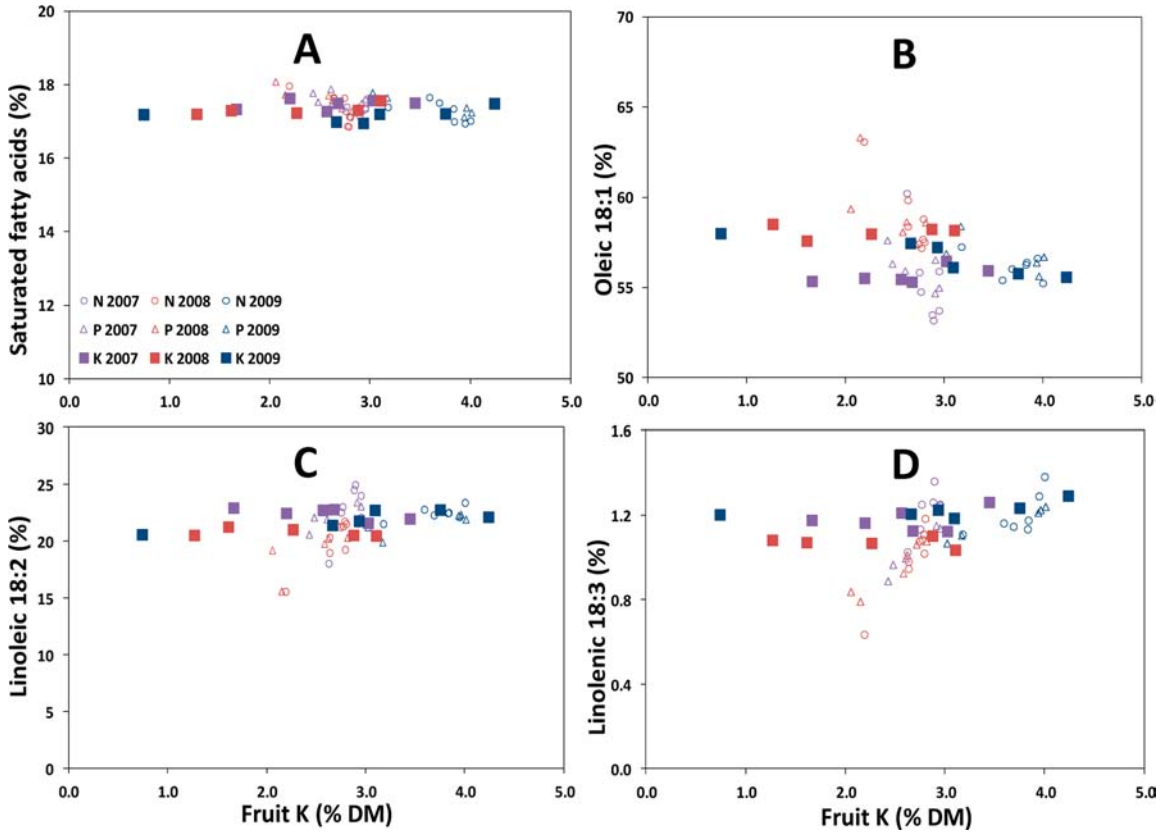

Figure 7. Fatty acid composition as a function of P concentration in fruit flesh for the three studied years: 2007 (purple), 2008 (red), and 2009 (blue) and the three manipulated treatments N (●), P (▲), and K (■). Each point represents an average of six replicates in 2007 and three replicates in 2008 and 2009. The statistical parameters of the model are presented in Table 4.

Table 5. Statistical Parameters of the Multi-Factorial Statistical Model

	FFA	polyphenol	SAT	oleic	linoleic	linolenic
R^2	0.41	0.62	0.47	0.71	0.65	0.81
model sign.	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
source						
N	*0.0034*	<0.0001	N.S	<0.0001	<0.0001	<0.0001
P	N.S	N.S	*0.0057*	N.S	N.S	*0.0028*
K	N.S	N.S	N.S	N.S	N.S	N.S
year	N.S	*0.0052*	N.S	*0.0018*	N.S	N.S

stability[22,32,44] and health promoting nutrition.[2,3] Most of the fatty acid biosynthesis takes place in the plastid which eventually forms oleate (C18) and palmitate (C16). Following desaturation, the C18 chain is converted to oleic acid, which is the main fatty acid in the olive.[45] Down the metabolic pathway, oleic acid can be further desaturated to linoleic and linolenic fatty acids, which is the final step of the process. In the present study, the level of desaturation increased with fruit N for the three major C18 fatty acids, implying enhanced progression of the fatty acid biosynthesis process. All of the C18 unsaturated fatty acids (oleic, linoleic, and linolenic acids) add up to ∼80%. The proportions of the three changed according to the N level. The decrease in oleic was very pronounced, falling below the 55% threshold for authenticity of VOO according to the IOC standards when fruit N was higher than ∼1.5%, in agreement with data previously reported in a study of the combined application of N, P, and K to olive.[33] N fertilization had a similar effect (i.e., increase in PUFA proportion) in various other plants including walnuts,[19] evening primrose,[46] winter mustard,[47] and quinoa.[48]

Although reports regarding the encouraging role of N in desaturation are widespread, no solid linkage between N and oleate desaturase could be found in the literature. Nitrogen level may indirectly affect desaturation via assimilate availability.

Fruiting Year, Mineral Accumulation, and Oil Quality. Fruit load has previously been reported to significantly affect oil composition and quality.[22,49,50] Specifically, high fruit load was associated with higher oleic[22] and lower FFA.[49,50] Fruiting year significantly affected N and P mineral accumulation in the fruit (Tables 1 and 2). Of the three studied years, 2008 was characterized by high fruit load, while in 2007 and 2009, the trees bore medium-low fruit load.[28] Indeed, high fruit load was coupled with low concentrations of fruit N and vice versa. A similar interaction was reported in a recent field trial where lower fruit N and P levels were found in an "on" compared to an "off" year.[51] Consequently, low fruit N was associated with lower FFA, higher oleic acid, and lower PUFA (Figure 8). The

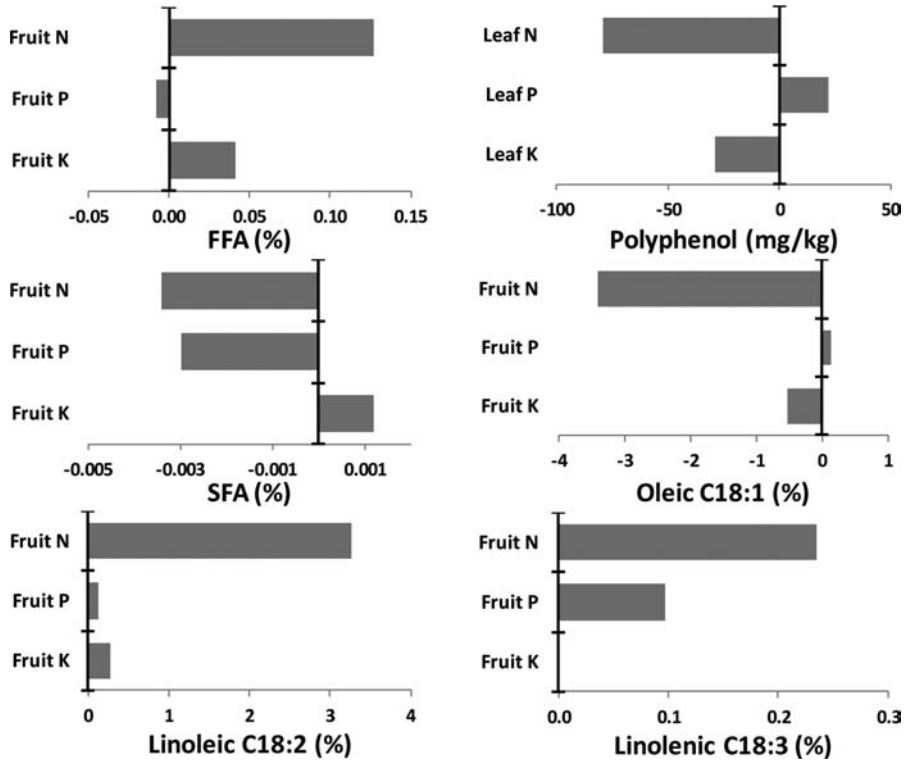

Figure 8. Scaled estimates of the effect of N, P, and K concentration in leaves or fruit flesh on oil quality parameters. Scaled estimates consider the extent of effects after adjusting half of the range of the examined factor. *, significant at $\alpha \leq 0.05$; **, significant at $\alpha \leq 0.001$; and ***, significant at $\alpha \leq 0.0001$.

apparent connection between fruit load, fruit mineral accumulation, and oil quality may partly explain the difference in oil quality among fruiting years found here and elsewhere.[22,49,50] Possibly, the higher fruit load led to lower N in fruit, which consequently resulted in better oil quality.

Nitrogen concentration in leaves was found to be independent of fruit load. Similarly, under field conditions, leaf N concentrations in "on" and "off" trees were fairly similar.[51,52] The levels of polyphenol in the oil were not affected by fruit load. Previous field studies found an inconsistent effect of fruit load on polyphenol.[49,50]

To conclude, olive tree N level was found to have a major effect on main olive oil quality components, including polyphenol content, FFA, and oleic and polyunsaturated fatty acids. Fruit N was influenced by three factors: the first, N concentration in irrigation solution; the second, P availability; and the third, fruit load. The leaf N level was affected solely by N in irrigation and P availability. The effect of N on oil properties depended solely on the absolute N content in leaves or fruits, regardless of the cause. Phosphorus had only minor direct effects on oil quality, while K level had negligible effects. The negative relationship between N and oil polyphenol content may be a result of biosynthesis inhibition of phenols or its precursors in leaves due to protein/phenol competition. Higher N level in fruit resulted in higher desaturation level. High fruit load tended to reduce fruit N and subsequently improve oil quality. The overall effect of high tree N status as found in the current study may lead to decreased oil stability, flat flavor, and lower health benefits. The current study raises questions regarding the role of N in fatty acid biosynthesis and phenol translocation from leaves to fruits. The results highlight the requirement for balanced N fertilization in olives cultivated for oil to ensure good quality. Furthermore, tree N level (and not N and P application rates) should be taken into account when studying the interaction between plant nutrition and oil quality.

■ ASSOCIATED CONTENT

Supporting Information

Detailed fatty acid composition, peroxide values and delta K for the three studied years. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 972 8 9928649. Fax: 972 8 9926485. E-mail: uri4@volcani.agri.gov.il.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Inna Feingold and Shoshana Suryano for technical support in the field and laboratory. We also thank Dr. Hillary Voet for helpful statistical advice.

■ REFERENCES

(1) Vossen, P. Olive oil: History, production, and characteristics of the world's classic oils. *Hortscience* **2007**, *42*, 1093−1100.

(2) López-Miranda, J.; Pérez-Jiménez, F.; Ros, E.; De Caterina, R.; Badimón, L.; Covas, M. I.; Escrich, E.; Ordovás, J. M.; Soriguer, F.; Abia, R. Olive oil and health: summary of the II international conference on olive oil and health consensus report, Jaen and Cordoba, Spain. *Nutr. Metab. Cardiovasc. Dis.* **2010**, *20*, 284−294.

(3) Paniagua, J. A.; De La Sacristana, A. G.; Romero, I.; Vidal-Puig, A.; Latre, J.; Sanchez, E.; Perez-Martinez, P.; Lopez-Miranda, J.; Perez-

Jimenez, F. Monounsaturated fat−rich diet prevents central body fat distribution and decreases postprandial adiponectin expression induced by a carbohydrate-rich diet in insulin-resistant subjects. *Diabetes Care* **2007**, *30*, 1717−1723.
(4) Servili, M.; Selvaggini, R.; Esposto, S.; Taticchi, A.; Montedoro, G.; Morozzi, G. Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and technological aspects of production that affect their occurrence in the oil. *J. Chromatogr., A* **2004**, *1054*, 113−127.
(5) Covas, M. I.; Ruiz-Gutiérrez, V.; Torre, R.; Kafatos, A.; Lamuela-Raventós, R. M.; Osada, J.; Owen, R. W.; Visioli, F. Minor components of olive oil: evidence to date of health benefits in humans. *Nutr. rev.* **2006**, *64*, S20−S30.
(6) Aparicio, R.; Luna, G. Characterisation of monovarietal virgin olive oils. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 614−627.
(7) Uceda, M.; M, H.; Aguilera, M. P. Olive Oil Quality. In *Olive Growing*, 5th ed.; Barranco, D., Fernández-Escobar, R., Rallo, L., Eds.; RIRDC: Pendle Hill, Australia, 2010; pp 619−646.
(8) Fiorino, P.; Alessandri, S.; Cert, A.; Dikmen, I.; Rahmani, M. Técnicas agronómicas y características del aceite de oliva. In *La Enciclopedia del Olivo*; COI Plaza & Janés: Barcelona, Spain, 1996; pp 195−222.
(9) Therios, I. N. *Olives*; CABI: Wallingford, U.K., 2009; Vol. *18*, Crop Production Science in Horticulture.
(10) Inglese, P.; Gullo, G.; Pace, L. Fruit growth and olive oil quality in relation to foliar nutrition and time of application. *Acta Hortic.* **2002**, *586*, 507−509.
(11) Ferreira, J.; Uceda, M.; Frias, L.; Garcia, A.; Fernandez, A. *Influencia de Los Fertilizantes en el Rendimiento en Aciete del Fruto y en la Composicion de Acidos Grasos del Aceite Optenido*; Colloquie Internationale Oleicole: Bergamon, France, 1978.
(12) Alcubilla, M.; Gracia, M.; Romero, M.; Espada, J.; Betrán, J. E. M. Fertilización nitrogenada en el olivo cv. Empeltre II. Composición polifenólica de hojas y frutos y calidad del aceite. *Comunicaciones al IX Simposio Ibérico sobre nutrición mineral de las plantas*; Institución Fernando el Católico: Zaragoza, Spain, **2002**; pp 243−246.
(13) Wiesman, Z.; Ronen, A.; Ankarion, Y.; Novikov, V.; Maranz, S.; Chpagain, B.; Abramovich, Z. Effect of olive-nutri-vant on yield and quality of olives and oil. *Acta Hortic.* **2002**, *594*, 557−562.
(14) Fernández-Escobar, R.; Beltran, G.; Sanchez-Zamora, M. A.; Garcia-Novelo, J.; Aguilera, M. P.; Uceda, M. Olive oil quality decreases with nitrogen over-fertilization. *Hortscience* **2006**, *41*, 215−219.
(15) Morales-Sillero, A.; Jiménez, R.; Fernández, J. E.; Troncoso, A.; Beltrán, G. Influence of fertigation in 'Manzanilla de Sevilla' olive oil quality. *Hortscience* **2007**, *42*, 1157−1162.
(16) Dag, A.; Ben-David, E.; Kerem, Z.; Ben-Gal, A.; Erel, R.; Basheer, L.; Yermiyahu, U. Olive oil composition as a function of nitrogen, phosphorus and potassium plant nutrition. *J. Sci. Food Agric.* **2009**, *89*, 1871−1878.
(17) Tekaya, M.; Mechri, B.; Bchir, A.; Attia, F.; Cheheb, H.; Daassa, M.; Hammami, M. Effect of nutrient-based fertilisers of olive trees on olive oil quality. *J. Sci. Food Agric.* **2013**, *93*, 2045−2052.
(18) Fernández-Escobar, R.; Marin, L.; Sánchez-Zamora, M.; García-Novelo, J.; Molina-Soria, C.; Parra, M. Long-term effects of N fertilization on cropping and growth of olive trees and on N accumulation in soil profile. *Eur. J. Agron.* **2009**, *31*, 223−232.
(19) Verardo, V.; Riciputi, Y.; Sorrenti, G.; Ornaghi, P.; Marangoni, B.; Caboni, M. F. Effect of nitrogen fertilisation rates on the content of fatty acids, sterols, tocopherols and phenolic compounds, and on the oxidative stability of walnuts. *LWT-Food Sci. Technol.* **2013**, *50*, 732−738.
(20) Bar-Yosef, B. Advances in fertigation. *Adv. Agron.* **1999**, *65*, 1−77.
(21) Hartmann, H. T. Some responses of the olive to nitrogen fertilizers. *Proc. Am. Soc. Hortic. Sci.* **1958**, *72*, 257−266.
(22) Beltrán, G.; del Rio, C.; Sánchez, S.; Martínez, L. Influence of harvest date and crop yield on the fatty acid composition of virgin olive oils from cv. Picual. *J. Agric. Food Chem.* **2004**, *52*, 3434−3440.
(23) Marschner, H. *Mineral Nutrition of Higher Plants*. 2nd ed.; Academic Press: London, 1995.
(24) Fernández-Escobar, R. Fertilization. In *Olive Growing*, 5th ed.; Barranco, D., Fernández-Escobar, R., Rallo, L., Eds.; RIRDC: Pendle Hill, Australia, 2010; pp 267−297.
(25) Fernández-Escobar, R.; Parra, M. A.; Navarro, C.; Arquero, O. Foliar diagnosis as a guide to olive fertilization. *Span. J. Agric. Res.* **2009**, *7*, 212−223.
(26) Dag, A.; Ben-David, E.; Kerem, Z.; Ben-Gal, A.; Erel, R.; Basheer, L.; Yermiyahu, U. Olive oil composition as a function of nitrogen, phosphorus and potassium plant nutrition. *J. Sci. Food Agric.* **2009**, *89*, 1871−1878.
(27) Erel, R.; Dag, A.; Ben-Gal, A.; Schwartz, A.; Yermiyahu, U. Flowering and fruit set of olive trees in response to nitrogen, phosphorus, and potassium. *J. Am. Soc. Hortic. Sci.* **2008**, *133*, 639−647.
(28) Erel, R.; Yermiyahu, U.; Van Opstal, J.; Ben-Gal, A.; Schwartz, A.; Dag, A. The importance of olive (*Olea europaea* L.) tree nutritional status on its productivity. *Sci. Hortic.* **2013**, *159*, 8−18.
(29) IOOC *Document 6*; International Olive Oil Council: Madrid, Spain, 1984.
(30) Beutel, J.; Uriu, K.; Lilleland, O. Leaf Analysis for California Deciduous Fruits. In *Soil and Plant Tissue Testing in California*, Reisenauer, H. M., Ed.; University of California: Berkeley, CA, 1983; Bul. 1879; pp 15−17.
(31) Ben-David, E.; Kerem, Z.; Zipori, I.; Weissbein, S.; Basheer, L.; Bustan, A.; Dag, A. Optimization of the Abencor system to extract olive oil from irrigated orchards. *Eur. J. Lipid Sci. Technol.* **2010**, *112*, 1158−1165.
(32) Aparicio, R.; Roda, L.; Albi, M. A.; Gutiérrez, F. Effect of various compounds on virgin olive oil stability measured by Rancimat. *J. Agric. Food Chem.* **1999**, *47*, 4150−4155.
(33) Morales-Sillero, A.; Jiménez, R.; Troncoso, A.; Fernández, J. E.; Beltrán, G. Influence of Fertigation in 'Manzanilla de Sevilla' Olive Oil Quality. *HortScience* **2007**, *42*, 1157−1162.
(34) Stefanelli, D.; Goodwin, I.; Jones, R. Minimal nitrogen and water use in horticulture: Effects on quality and content of selected nutrients. *Food Res. Int.* **2010**, *43*, 1833−1843.
(35) Leser, C.; Treutter, D. Effects of nitrogen supply on growth, contents of phenolic compounds and pathogen (scab) resistance of apple trees. *Physiol. Plant.* **2005**, *123*, 49−56.
(36) Bragazza, L.; Freeman, C. High nitrogen availability reduces polyphenol content in *Sphagnum* peat. *Sci. Total Environ.* **2007**, *377*, 439−443.
(37) Jones, C. G.; Hartley, S. E. A protein competition model of phenolic allocation. *Oikos* **1999**, 27−44.
(38) Meyer, S.; Cerovic, Z.; Goulas, Y.; Montpied, P.; Demontes-Mainard, S.; Bidel, L.; Moya, I.; Dreyer, E. Relationships between optically assessed polyphenols and chlorophyll contents, and leaf mass per area ratio in woody plants: a signature of the carbon−nitrogen balance within leaves? *Plant Cell Environ.* **2006**, *29*, 1338−1348.
(39) Ryan, D.; Antolovich, M.; Herlt, T.; Prenzler, P. D.; Lavee, S.; Robards, K. Identification of phenolic compounds in tissues of the novel olive cultivar Hardy's Mammoth. *J. Agric. Food Chem.* **2002**, *50*, 6716−6724.
(40) Ryan, D.; Prenzler, P. D.; Lavee, S.; Antolovich, M.; Robards, K. Quantitative changes in phenolic content during physiological development of the olive (*Olea europaea*) cultivar Hardy's Mammoth. *J. Agric. Food Chem.* **2003**, *51*, 2532−2538.
(41) Gutierrez-Rosales, F.; Romero, M. P.; Casanovas, M.; Motilva, M. J.; Mínguez-Mosquera, M. I. Metabolites involved in oleuropein accumulation and degradation in fruits of *Olea europaea* L.: Hojiblanca and Arbequina varieties. *J. Agric. Food Chem.* **2010**, *58*, 12924−12933.
(42) Ryan, D.; Antolovich, M.; Prenzler, P.; Robards, K.; Lavee, S. Biotransformations of phenolic compounds in *Olea europaea* L. *Sci. Hortic.* **2002**, *92*, 147−176.
(43) Conde, C.; Delrot, S.; Gerós, H. Physiological, biochemical and molecular changes occurring during olive development and ripening. *J. Plant Physiol.* **2008**, *165*, 1545−1562.

(44) Gutierrez, F.; Jimenez, B.; Ruiz, A.; Albi, M. Effect of olive ripeness on the oxidative stability of virgin olive oil extracted from the varieties Picual and Hojiblanca and on the different components involved. *J. Agric. Food Chem.* **1999**, *47*, 121−127.
(45) Harwood, J. L. Recent advances in the biosynthesis of plant fatty acids. *Biochim. Biophys. Acta* **1996**, *1301*, 7−56.
(46) Ghasemnezhad, A.; Honermeier, B. Yield, oil constituents, and protein content of evening primrose (*Oenothera biennis* L.) seeds depending on harvest time, harvest method and nitrogen application. *Ind. Crop. Prod.* **2008**, *28*, 17−23.
(47) Zheljazkov, V. D.; Vick, B.; Ebelhar, M. W.; Buehring, N.; Astatkie, T. Nitrogen applications modify seed and oil yields and fatty acid composition of winter mustard. *Ind. Crop. Prod.* **2012**, *36*, 28−32.
(48) Thanapornpoonpong, S. Effect of Nitrogen Fertilizer on Nitrogen Assimilation and Seed Quality of Amaranth (*Amaranthus* spp.) and Quinoa (*Chenopodium quinoa* Willd). Ph.D. Thesis, Georg-August-University of Göttingen, Göttingen Germany, 2004.
(49) Ben-Gal, A.; Dag, A.; Basheer, L.; Yermiyahu, U.; Zipori, I.; Kerem, Z. The influence of bearing cycles on olive oil quality response to irrigation. *J. Agric. Food Chem.* **2011**, *59*, 11667−11675.
(50) Dag, A.; Kerem, Z.; Yogev, N.; Zipori, I.; Lavee, S.; Ben-David, E. Influence of time of harvest and maturity index on olive oil yield and quality. *Sci. Hortic.* **2011**, *127*, 358−366.
(51) Bustan, A.; Avni, A.; Yermiyahu, U.; Ben-Gal, A.; Riov, J.; Erel, R.; Zipori, I.; Dag, A. Interactions between fruit load and macroelement concentrations in fertigated olive (*Olea europaea* L.) trees under arid saline conditions. *Sci. Hortic.* **2013**, *152*, 44−55.
(52) Gucci, R.; Caruso, G.; Sebastiani, L. Seasonal changes in leaf nitrogen of olive trees grown under different irrigation regimes and crop level. *J. Plant Nutr.* **2010**, *33*, 1849−1859.