Nitrogen Nutrition Diagnosis for Olive Trees (Olea europaea L., cv. Arbequina) Grown in Super-Intensive Cropping Systems

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Keywords:	Olive oil production, oil quality, nitrogen-potassium interaction, alternate fruit bearing

Title Page

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Abstract

The results of a four-year experiment carried out between 2010 and 2013 in a super-intensive grove of Arbequina olive trees grown in calcareous soil in semiarid conditions, combining irrigation, nitrogen, and potassium application, showed that to maintain productivity and tree growth it is necessary to attain a higher leaf nitrogen status than current diagnosis standards. Leaf nitrogen concentration was positively correlated with nitrogen application rate and also showed synergism with potassium. To sustain high oil yield and to avoid excessive vigor and minimize effects on quality, a critical nitrogen concentration was established for olive trees of 1.94% for leaves sampled in July and a critical nutrient range for leaf nitrogen in the interval 1.8-1.94%. A negative relationship was obtained between N leaf concentration and some quality parameters. Above 1.94%, total phenolic content and oxidative stability fell below 220 mg kg^{-1} and 10 hours, respectively, for Arbequina cultivar. In addition, above this N leaf concentration other factors including the alternate bearing index (ABI) and vegetative growth distorted productive and qualitative responses. Moreover, the differential productive response of trees related to alternate fruit bearing status implies that the interpretation of leaf nitrogen content can be more accurate when ABI suggests an ON tree status.

*Additional index words***.** Olive oil production, oil quality, nitrogen-potassium interaction, deficit irrigation, alternate fruit bearing

The olive tree (*Olea europaea* L.) is a widespread crop in arid and semi-arid regions of the Mediterranean basin, mainly due to its high adaptability to water deficit (Connor and Fereres 2004; Fernández 2014). The world market for virgin olive oil has continued to rise over recent years, thus encouraging the establishment of new olive plantations commonly under intensive cropping systems (IOC 2018). In semiarid regions, olive tree productivity is frequently limited by soil and climate constraints and, in addition, alternate fruit bearing commonly appears as a strong penalty for olive crop management (Stroosnijder et al. 2008). Consequently, high investment in technology is necessary to achieve a year-to-year sustainable yield, particularly in intensive crop systems. Globally, the changes are evident in olive crop systems, as shown by the progressive introduction of irrigation – more than 500000 ha in Spain alone (MAPAMA 2017), or the expansion of new orchards worldwide based on irrigation and high and super-high densities which now account for approximately 2% of total olive crop area (AEMO 2012).

Olive tree yield and fruit quality are greatly influenced by a set of non-exclusive factors, such as genotype and nutritional and plant water status. As a result of this influence, traditional olive groves are well adapted to local characteristics (Mora et al. 2007; Aguilera et al. 2014), showing a high degree of plasticity in productive response to environmental variations (Trentacoste et al. 2011; Rossi et al. 2013; Fernández 2014). In contrast, modern cropping systems require intensive use of water and nutrition to improve yield and to sustain oil quality (Metzidakis et al. 2008; Naor et al. 2013). A proper supply of water and nutrients contributes to improving olive tree productivity and olive oil quality, enhancing shoot growth, flowering, leaf chlorophyll content, fruit set and fruit load (Erel et al. 2008; 2013b). However, intensive cropping systems entail risks related to excessive N application or irrigation management, impairing oil quality, decreasing the phenolic and saturated fatty acid content of the oil (Erel et al. 2013a,b), and diminishing bitterness and oil stability (Fernández-Escobar et al. 2009b).

Plant nutrient status and concomitant fertilization needs have commonly been evaluated through the analysis of nutrients in leaves. The earliest studies on the nutritional status of olive trees were carried out by Hartman (1958) and Chapman (1966), who established the diagnostic thresholds for several nutrients which continue to be used today with minor modifications (Jones 2012). Leaf analysis is a useful method for diagnosing nutrient deficiencies and for planning olive orchard nutrition, but interpretation of critical levels for crop response is complex due to cultivar variation (Zipori et al. 2015). Moreover, it is quite common to find discrepancies in the response of plant performance to nitrogen fertilization and plant content. Such discrepancies could be attributed to the complex effects of N nutrition related to N interaction with other nutrients, mainly P, K, and Mg (Dikmelik et al. 1999; Erel et al. 2008; Elloumi et al. 2009; Fernández-Escobar et al. 2009b;Tubeileh et al. 2014), the cultivar (Michelazzo and Sebastiani 2011; Toplu et al. 2009), crop load, leaf sampling data (Gucci et al. 2010), plant water status (Pierantozzi et al. 2013), and the crop system, including soil organic matter (Fernández-Escobar et al. 2009a; Michelazzo and Sebastiani 2011).

Using nutrient content in leaves as a diagnostic method has been criticized lately because of the above factors. In this context, analysis of the fruits (a sink organ) has been proposed on the basis that it is more integrative and efficient to record the effects on the yield and quality of the olive oil (Erel et al. 2013b). However, it should not be forgotten that today leaf nutrient analysis is still the most common method for nutritional diagnosis, constituting over time a database of high value for researchers and technicians. However, given the current widespread intensification of olive oil production, it would appear to be an opportune moment to review this question and consider what might be the limits for nitrogen fertilization in the pursuit of ensuring high production levels whilst sustaining oil quality. In this regard, this present study aims to determine the critical level of leaf N required to sustain yield and oil quality in irrigated Arbequina olive trees under super-intensive cropping systems.

Materials and methods

Experimental site and climate characteristics

The trial was conducted over four years (2010, 2011, 2012 and 2013) at a commercial adult olive orchard (cv. Arbequina) in Torres de Segre (Lleida, Catalonia, Spain). The climate is continental Mediterranean-type. The mean annual rainfall in this region in this period was 324 mm, which was distributed very unevenly, and the mean annual ETo (Penman-Monteith) was 1101 mm. Average rainfall, from April to October, in the area was 192 mm. Accumulated reference evapotranspiration (ETo) in the same period was 912 mm (Table 1).

The trees were planted in summer 2002 at 4.5 m × 2.2 m (1010 trees ha^{-1}) in a moderately deep soil. The soil (classified as Typic Xerorthent) is calcareous with a silty-loam texture, basic pH of 8, organic matter content of 1.5% and an electrical conductivity (EC 1:5) of 1.4 dS m^{-1} (a high value due to the presence of gypsum). Initial soil contents of nitrate-nitrogen ($N\text{-}NO_3^-$), phosphorus (P Olsen), and potassium (K acetate ammonium) were 23, 50 and 131 ppm, respectively.

Irrigation water came from the Segre River and had average conductivity of 0.9 dS m^{-1}, chloride 2.25 meq L^{-1} and sodium 2.14 meq L^{-1}. The water had boron content below 0.15 ppm and a nitrate content of 9 ppm. Soil moisture was monitored continuously with ECH2O-10 capacitance probes (Decagon Devices Inc., Pullman, Washington, USA), which were installed in 2009. Probes were inserted into the soil at a distance of 70 cm from one side of the trunk and at depths of 0.20, 0.40 and 0.60 m, within the wetted soil (for more experiment details see Rufat et al. 2014). One tree from two elemental plots was selected in each irrigation treatment for soil water monitoring purposes.

Experimental design and irrigation and fertilization treatments

Two irrigation treatments were crossed with four fertilization treatments, with four replicates randomly distributed in blocks. Each elemental plot consisted of 18 trees distributed in three adjacent rows, in which the 4 central trees were monitored. The irrigation system consisted of auto-compensated drip emitters (2.3 Lh^{-1}) spaced 0.6 m apart along a dripline. The driplines were placed at a distance of 0.3 m from the tree trunk. The irrigation treatments were FI (Full irrigation), namely irrigation applied as 100% of the water requirements throughout the year, in accordance with the FAO methodology (Allen et al. 1998) and Kc values from Girona et al. (2002); and DI (deficit irrigation), namely restricted irrigation in summer scheduled as follows: March to June, 100% of the requirements; July to September 10, 25% of the requirements; and September 11-30 and October, 100% of the requirements. As a result of these irrigation treatments, the FI plots exhibited a satisfactory water status throughout the experimental period, whereas the DI plots underwent water stress during the periods of imposed restrictions, registering stem water potential values close to -2 MPa (Rufat et al. 2014).

Annual irrigation was, on average, 412 mm for the FI treatment and 246 mm for the DI treatment (Table 1). Actual Kc for the entire season (seasonal Kc) was calculated on the basis of the ETo/(irrigation+ effective rainfall) relationship. The Kc for the FI treatment ranged between 0.47 and 0.54 and for the DI treatment between 0.31 and 0.36. The monthly mean and the range for each month denote similar conditions for ETo and Kc between years.

Fertilization treatments resulted from the combination of two rates of N (0 and 50 kg N ha^{-1}; N0 and N50 respectively) and two rates of K (0 and 100 kg of K_2O ha^{-1}; K0 and K100 respectively). The fertilizers were applied weekly through fertigation from early May to early October as follows: N source was urea ammonium nitrate solution (UAN) N32 (16% amide-N, 8% ammonium-N and 8% nitrate-N) and K was applied as a sulfate potassium solution (15%). P fertilization was not applied during the trial due to the high soil P content.

Plant measurements and leaf analysis

Plant growth was assessed by means of pruning weight (PW) for each individual plot, and canopy volume of a single tree. Trees were manual pruned. A harvest index (HI) was proposed as the ratio between oil yield (OY) and PW.

For nutrient analysis, samples of 60 leaves per plot were taken in July, dried to a constant weight at 60ºC, and then milled to a fine powder. Leaf N concentration was determined using the Kjeldahl method, and the other nutrients were measured by inductively coupled plasma optical emission spectrometry (Agilent 7700X, Agilent Technologies, Santa Clara, CA, USA).

Yield and olive oil quality

Fruit yield, oil yield, and oil content on a fresh matter basis were used as production indicators. Olives were harvested by means of an over-the-row continuous harvester on the following dates: November 25 in 2010; November 28 in 2011; November 30 in 2012; and November 21 in 2013. Olive oil content was determined by nuclear magnetic resonance (NMR) for each plot, using 50 fruits per sample (del Río and Romero 1999). For oil extraction, 3 kg-samples were taken each year and for each plot. Olive oil was obtained using an Abencor laboratory oil mill (MC2, Ingeniería y Sistemas S.L., Sevilla, Spain). Oil samples were placed in dark glass bottles and stored at 4ºC until analysis.

Physico-chemical analyses (free acidity-FFA, peroxide value, UV absorption characteristics at 232 and 270 nm (K232 and K270), were performed following official methods (UE2568/91). The results are expressed as percentage of oleic acid (%), milliequivalents of active oxygen kg^{-1} oil (meq O_2 kg^{-1}) and specific extinction coefficient at 232 and 270 nm. Oil oxidative stability was expressed as the oxidation induction time (hours) measured with a Rancimat-679 device (Metrohm Co, Basel, Switzerland) using 2.5 g of oil sample warmed to 120ºC with an air flow of 20 L h^{-1}. Total polyphenol content was determined by the Folin-Ciocalteu method and expressed as mg kg^{-1} caffeic acid.

The alternate bearing index (ABI) for oil yield was determined for each plot *i* and year *j* using the method proposed by Pearce and Dobersek-Urbanc (1967) (eq. 1). An ABI close to 0 means no alternate bearing conditions, while a value close to 1 indicates full alternate bearing. In addition, the ABI was used to classify the production capacity of each plot as ON or OFF yield response when the ABI inter-annual variation was over 20% (Monselise and Goldschmidt 1982).

$$ABI = \frac{|Y_{i,j+1} - Y_{i,j}|}{Y_{i,j+1} + Y_{i,j}} \quad \text{(eq. 1)}$$

Data analysis

The statistical analysis was performed using JMP-SAS software (JMP, Version 12 Pro. SAS Institute Inc., Cary, NC, 1989–2014). Analysis of variance (ANOVA) and multiple analysis of variance (MANOVA) on repeated measures (year was repeated factor) were carried out for the variable results analysis. MANOVA's significance was determined using the F test or Roy's Maximum Root Criteria ($p \leq 0.05$) for repeated effects, and a contrast test was used ($p \leq 0.05$) to separate the levels of fixed effects.

In addition, a non-linear regression plateau model was used (eq. 2) relating olive oil yield (as relative yield over annual maximum) to leaf N content.

$$Oil\ yield = \beta_0 + \beta_1 (Nleaf - C) + \beta_2\ Nleaf \quad \text{(eq. 2)}$$

The segmented model for variation in olive oil yield to leaf N content was carried out by means of "+function" (eq. 3), adapting the method proposed by Toms and Lesperance (2003) and Shuai et al. (2003) for breakpoint determination (parameter C).

$$Oil\ yield = \beta_0 + \beta_1 * If \begin{bmatrix} Nleaf \leq C \rightarrow\ Nleaf - C \\ else \rightarrow\ 0 \end{bmatrix} + \beta_{2\ Nleaf} \quad \text{(eq. 3)}$$

where β0 is the lowest yield achievable under the experimental conditions, β1 is the slope of yield response under N leaf non-saturated conditions, C is breakpoint of no N-response zone, and β2 defines the maximum oil yield achieved under the experimental conditions. Following the analysis, parameters βo and C were retained to determine the response quadrants by minimizing the sum of squared residuals (Webb 2009).

Results

Irrigation and nutrition effects on olive leaf nitrogen content, oil yield, plant growth, alternate bearing index, and harvest index

The results for olive oil yield (OY), annual bearing index (ABI), pruning weight (PW), harvest index (HI), and leaf nitrogen content (N_{leaf}) are presented in Table 2. According to the MANOVA model, no significant differences due to irrigation treatments were found for OY, and ABI (Table 3). Olive oil yield oscillated between 809 and 3240 kg ha^{-1}, with 2011 the most productive year. The FI irrigation treatment produced significantly higher plant growth (PW) and HI. Both OY and PW increased with N application, whereas the application of K alone had no significant effect on these parameters. The ABI was positively affected by N and K application. The HI was not affected by N and K fertilization (Table 3).

Irrigation strategies had no effect on N leaf concentration (Table 2 and Table 3). Nitrogen and potassium application significantly increased leaf N content (Fig. 1 and 2). The unfertilized treatment (N0) resulted in a significant decline in leaf N content over the study period of 12% on average (Table 2). According to interpretation standards, leaf nitrogen content remained high on all plots over the four years (Table 2). The multivariate repeated measures analysis revealed that K fertilization contributed to maintain leaf N content in unfertilized N plots (Fig. 2 and Table 3). This trend is clearly seen in the Year x Nitrogen x Potassium interaction, indicating that leaf N concentration dropped faster when N and K were not applied (Fig. 2). For the FI irrigation treatment, cumulative OY varied from 6890 kg ha^{-1} for N0K0 to 8710 kg ha^{-1} for N50K100, equivalent to an increase of 24.4%, while cumulative OY for N0K100 was 7115 kg ha^{-1} and for N50K0 7233 kg ha^{-1}, equivalent to respective increases of 3.2% and 5%. This clearly shows the influence of the Year x Nitrogen x Potassium interaction (Table 3).

For the DI irrigation treatment, cumulative OY varied between 6560 kg ha^{-1} for N0K0 to 7901 kg ha^{-1} for N50K0, equivalent to a 20% increase when N was applied, while the cumulative OY for N0K100 was 7384 kg ha^{-1} and for N50K100 7738 kg ha^{-1}, equivalent to an increase of 18%.

In all years, OY was higher for the N50 treatments (Fig. 2 and 3). The only source of N in N0 treatments was the N mineralized from soil organic matter and decomposition of plant residues (Belguerri et al. 2016). N from soil was insufficient to provide the N that the crop needed for high yields. Potassium positively affected OY only in 2012 (Fig. 3). As for plant growth, the results of the seasonal variation of canopy volume and PW were closely related (Fig. 4, R^2=0.84), and plant growth was negatively affected by deficit irrigation and positively by N application over years (Fig. 1 and Table 3). Pruning weight varied between 3.04 kg tree^{-1} (N0) and 9.93 kg tree^{-1} (N50), showing a Year x N interaction related to tree crop load status: in 2011, most trees were in ON status and yield reached its maximum value, limiting vegetative growth for both N50 and N0 (Fig. 1 and Table 3).

ABI gradually declined over the last two years (Fig. 1) and was significantly affected by N and K application, though no effects were observed for irrigation treatments. The HI was significantly affected only by irrigation treatments, meaning that N and K favor the balance between vegetative and reproductive organs over the years enhancing the productive response of fertilized trees (Fig. 3 and Table 3).

Effects of irrigation and N and K treatments on oil quality.

Some oil quality parameters were affected by both irrigation and nutrient application. Nitrogen and potassium negatively affected polyphenol content and oil stability, while irrigation only affected polyphenol content under FI irrigation and ON status. Other oil quality physico-chemical parameters like FFA, peroxides, K232, and K270 were neither affected by the irrigation treatments nor by N and K. FFA was affected only when trees were in "ON" status, when it was significantly lower (Table 4).

Relationships between N_{leaf}, yield and oil quality.

The relationship between leaf N concentration and relative oil yield (relative OY) was apparently randomly scattered when considering the results as a whole (Fig. 5A). However, the classification of plots as "ON" or "OFF" yield status according to eq. 1 allowed a clear relationship to be seen between relative OY and leaf N content. The analysis was carried out assuming as an initial hypothesis that yield in OFF trees is mainly the result of limitations other than the direct factors imposed on this experiment (irrigation and nutrition), in accordance with Lavee (2007). In this way, the piecewise regression model build for oil yield on plots are in ON status (independently of year) elucidate a significant and positive yield response to leaf N concentrations.

According to the model results, it is possible to establish a critical leaf N level at 1.94 g $100g^{-1}$, and a critical nutrient range (Jones 2012) between 1.81-1.94 g $100g^{-1}$ (Fig. 5B). Also, a negative linear relationship was established between leaf N content, polyphenol concentration, and oil stability (Fig. 6). Above this critical leaf N concentration level (1.94%), oil stability and polyphenol content were below 10 h and 220 mg kg^{-1} respectively.

Discussion

Recent scientific studies have established critical N leaf concentrations of 1.5% for rainfed olive trees at very low planting densities (Fernández-Escobar et al., 2009b).

However, from the results of the present study and the literature consulted (Erel et al. 2013b), this level is clearly insufficient for irrigated and fertilized intensive orchards. The trend of N effects on yield is consistent in different scenarios, recording the fact that olive oil yield varies widely year-to-year, from the alternate fruit bearing to the supply of water and fertilizers that must be accommodate to behavior of olive crop in each one of them.

In the present study, the effects of irrigation and nutrition were tested using the most widespread strategies to increase oil yield and/or quality. Following this approach, deficit irrigation from July to early September was an efficient method for saving water (up to 40% of FI). The application of irrigation water was substantially modified according to the results of soil water content and the monitoring of plant water status, and in accordance with more recently proposed Kc values for optimal yield and oil quality (Berenguer et al. 2006; Grattan et al. 2006; Ben-Gal et al. 2011; Puppo et al. 2014). The irrigation reduction imposed by DI did not significantly affect short-term oil yield (OY); however, plant growth was affected, thereby suggesting that changes in oil quality were linked to variations in canopy characteristics. Overall, plant growth was positively affected by irrigation and nitrogen. Although the effect of potassium fertilizer alone had no clear effect, a positive interaction was observed of potassium with nitrogen. As expected, N leaf content decreased over the years in the N0 treatments although the values were still relatively high. On the other hand, leaf N concentrations for N fertilized trees remained more or less constant over the years. An ON/OFF·N·K interaction was detected, revealing the positive effect of K on leaf N concentration linked to ON/OFF tree status (Fig. 2). This effect was significant when plots had lower yield (OFF trees), thereby indicating that leaf N content was depressed in conditions of limited K availability after high yielding years (Rufat et al. 2014).

In general, the N·K interaction in crops has been explained in several ways, including the suggestion that a high supply of potassium has a positive effect on ammonium nutrition which is required in high concentrations for protein synthesis (Marschner 1995). On the other hand, it is known that potassium acts as a cation that compensates the ionic balance in the transport of nitrates inside the plant. This is an interesting question for crop N and K management and gains greater relevance with respect to fruit trees characterized by alternate bearing. The year-on-year differences (linked to crop load) in K and N uptake and content in plant tissues has been associated with source-sink changes under different inter-annual fruiting conditions (Erel et al. 2008; Bustan et al. 2013; Rufat et al. 2014). Moreover, under high yield conditions, available K is depleted mainly by fruit, accounting for up to 2/3 of total plant uptake (El-Fouly et al. 2014; Fernández-Escobar et al. 2015).

It was possible to reduce the alternate bearing behavior by simultaneously applying N and K (Fig. 7), showing a significant ON/OFF·N·K interaction (Table 3). This demonstrates the benefits of fertilization in reducing this problem, as Turktas et al. (2013) suggested. These results are in agreement with other studies made in irrigated conditions by El-Sonbaty et al (2012), or by Elloumi et al (2009) in dryland conditions. However, this issue remains controversial since several studies indicate that crop intensification reduces alternate fruit bearing (Moriana et al. 2003; Connor and Fereres 2004), while others indicate the opposite effect (Dag et al. 2010; Ben-Gal et al. 2011).

Concerning oil quality, the results indicate that the oils obtained in all treatments can be classified as extra virgin according to olive oil quality regulation EC/1989/2003. On the other hand, from an extended quality point of view, the phenolic content and olive oil stability were acceptable up to the proposed nitrogen threshold (Barranco et al. 2017).

Generally, the results obtained for oil quality are consistent with those of other research studies (Berenguer et al. 2006; Servili et al. 2007; Erel et al. 2013a; Morales-sillero et al. 2007). In this respect, acute differences in oil quality are often achieved in extreme experimental conditions, such as under severe water or nutrient application deficits which do not conform to common crop standards for intensive olive orchard management (Berenguer et al. 2006; Morales-Sillero et al. 2007; Erel et al. 2013a, 2013b).

Yield response to the nutritional status of the plant is affected by vegetative growth. In fact, the role of light interception and canopy permeability is a current topic in olive tree research (Castillo-Ruiz et al. 2015, 2016). Also fruit canopy position modifies oil quality (Caruso et al. 2017). In recent years, Iniesta et al. (2009) and Pierantozzi et al. (2013) inferred a close relationship between olive oil yield and oil quality and photosynthetically active radiation (PAR) intercepted during spring growth. However, this response is also mediatized by canopy lighting characteristics, which in turn are associated with tree growth (Cherbiy-Hoffmann et al. 2013, 2015). To address this issue, yield response was split according to plant growth class (PW) (Fig. 7). On the basis of oil yield response to leaf N content included in Quadrant IV of Figure 7, the hypothesis is that crop load, yield and oil quality are closely related to canopy characteristics (Cherbiy-Hoffmann et al. 2012; Castillo-Ruiz et al. 2015). Assuming that intensive crop systems are confined in uniform and regular hedgerow dimensions imposed by mechanical harvesting, the growth and spatial arrangement of plants play a prominent role in canopy lighting, modulating yield response to N application and irrigation strategies.

Conclusions

On the basis of the results of this four-year experiment, we established a leaf critical nitrogen concentration for olive trees (cv. Arbequina) of 1.94% for leaves sampled in July. It was also possible to establish a critical nutrient range for N in the interval 1.80-1.94% (in which a 0 to 10% reduction in oil yield occurred). Beyond this range, the stability and total polyphenol content of the oil could be compromised.

Furthermore, an N-K interaction was evidenced by the effect of K on increasing leaf N content and also indicated the positive effect of N-K nutrition on alternate fruit bearing mitigation. Irrigation strategies did not affect leaf N concentration and oil yield.

In summary, the results indicate that high yields can be obtained at levels of leaf nitrogen concentration above commonly accepted thresholds. However, the results also suggest that information about crop load status and plant growth should be included in the nitrogen nutrition diagnosis for olive trees. The role of nitrogen in alternate bearing is limited up to the proposed critical concentration. The results also indicate that changes in canopy characteristics could be drivers of alternate bearing expression.

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Table 1. Monthly ETo, Rain, and Irrigation (mean and range of variation) during the growing season for FI and DI treatments in 2010-2013 years.

	ETo (mm)		Rainfall (mm)		Irrigation FI (mm)		Irrigation DI (mm)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
April	100.3	95-110	31.3	15-52	20	11-37	16.7	7-35
May	145.0	137-151	34.3	13-45	59.3	43-77	48.7	34-64
June	160.3	151-173	30.7	6-70	71	61-83	64.3	51-80
July	174.7	170-183	9.7	2-21	82.7	70-90	30	24-33
August	162.0	154-166	9.3	0-24	97	77-111	24.3	18-29
September	105.7	101-114	29.7	6-45	65.7	63-69	45.7	35-54
October	64.3	54-73	46.7	11-92	16.3	3-34	16.7	4-34
Total	912.3		191.7		412		246.4	
Seasonal Kc (ETo/ (irrigation+rain))					0.52	0.47-0.54	0.34	0.31-0.36

Table 2. Results (mean ± standard error) for olive oil yield (OY, kg ha^{-1}), annual bearing index (ABI, %), pruning weight (PW, kg tree^{-1}), harvest index (HI, OY/PW), and leaf nitrogen content (N_{leaf}, g 100g^{-1}) for irrigation and nutrient treatments by year.

	IRRI	N	K	2010	2011	2012	2013
OY	**FI**	0	0	1310.7±122.5	2675.9±301.7	931.6±161.4	1972.7±520.3
			100	834.9±236	2756.1±238.7	1641.4±198.8	1882.8±223.9
		50	0	1078.3±175.2	2605.7±489	1451.4±73.5	2098.2±331
			100	1770.1±213.4	3240.8±147.1	1417±332.1	2282.6±279.7
ABI		0	0	0.24±0.07	0.57±0.09	0.44±0.11	0.37±0.02
			100	0.55±0.1	0.55±0.06	0.22±0.07	0.13±0.04
		50	0	0.38±0.14	0.42±0.21	0.21±0.06	0.26±0.09
			100	0.19±0.06	0.5±0.09	0.36±0.16	0.14±0.03
PW		0	0	7.1±1.56	5.42±1.71	7.43±1.77	6.92±1.14
			100	9.85±0.36	5.99±0.79	7.82±1.04	7.33±1.19
		50	0	8.88±0.34	5.98±1.29	9.31±1.8	8.93±1.4
			100	9.93±0.74	6.5±0.57	8.99±0.93	7.83±0.55
HI		0	0	217.52±50.54	730.8±252.08	140.14±23.09	366.35±146.74
			100	87.8±27.97	489.71±88.51	204.38±30.44	292.7±80.01
		50	0	83.85±3.31	479.97±96.31	182.62±46.72	243.01±37.28
			100	177.4±13.02	618.41±150.37	173.49±58.1	288.78±18.09
N_{leaf}		0	0	1.83±0.05	1.72±0.05	1.67±0.08	1.59±0.09
			100	2.04±0.04	1.98±0.09	2.03±0.05	1.9±0.06
		50	0	1.96±0.02	2.13±0.01	2.16±0.06	2.23±0.01
			100	2.12±0.04	2.07±0.03	2.24±0.03	2.17±0.02
OY	**DI**	0	0	818.1±203.7	2628±165.2	894.4±172.8	2220±220.5
			100	809.5±106.8	2366±207.2	1333.5±168.8	1874.2±205.9
		50	0	1172.1±345.8	3000.9±191.2	1438±326	2291.6±252
			100	1528.4±334	2568.1±193.9	1678±324.8	1964.4±180.3
ABI		0	0	0.52±0.1	0.61±0.09	0.44±0.12	0.34±0.05
			100	0.5±0.06	0.48±0.1	0.17±0.08	0.21±0.1
		50	0	0.44±0.14	0.56±0.18	0.3±0.11	0.16±0.08
			100	0.24±0.1	0.34±0.07	0.22±0.11	0.12±0.03
PW		0	0	5.65±0.98	3.24±0.36	4.16±0.92	4.07±0.55
			100	6.08±0.28	3.04±0.22	6.66±0.84	4.06±0.38
		50	0	7.53±1.22	4.55±0.37	7.91±1.31	7.37±0.38
			100	7.63±1.4	4.03±0.89	5.88±0.82	6.63±0.75
HI		0	0	142.88±44.17	828.3±63.63	258.04±76.78	570.46±88.07
			100	136±21.59	788.29±79.11	208.57±42.39	477.42±77.25
		50	0	179.17±57.99	671.37±65.58	207.52±61.51	318.36±50.19
			100	199.26±26.54	741.27±130.51	312.84±92.32	304.81±32.32
N_{leaf}		0	0	1.89±0.04	1.83±0.06	1.75±0.08	1.7±0.11
			100	1.95±0.02	1.88±0.05	1.87±0.07	1.71±0.09
		50	0	2.01±0.05	2.09±0.03	2.09±0.02	2.12±0.04
			100	2.03±0.02	2.17±0.02	2.2±0.04	2.12±0.01

Table 3. Significance of MANOVA mixed model of repeated measures (year was used as repeated factor) for olive oil yield, pruning weight, harvest index, ABI, and N_{leaf} for Year, Irrigation, Nitrogen, Potassium, and interactions.

	Olive oil	**Pruning weight**	**Harvest index**	**ABI**	**N_{leaf}**
Irrigation	0.4800	0.0002[a]	0.0035[a]	0.8574	0.4939
N	0.0164[a]	0.0431[a]	0.2936	0.0265[a]	<0.0001[a]
K	0.4334	0.5547	0.8495	0.0197[a]	0.0013[a]
N*K	0.4600	0.3354	0.1323	0.9900	0.0310[a]
Irrigation * N	0.6656	0.4012	0.1672	0.5343	0.6181
Irrigation * K	0.2531	0.9563	0.8145	0.1782	0.0078[a]
Block	0.8438	0.1762	0.2346	0.3812	0.124
Year	<0.0001[b]	<0.0001[b]	<0.0001[b]	<0.0001[b]	0.0011[b]
Year * Irrigation	0.5233	0.4236	0.0954	0.3294	0.1944
Year * N	0.6476	0.0018	0.0093[b]	0.916	0.0015[b]
Year * K	0.4979	0.2425	0.6601	0.8475	0.0244[b]
Year * N * K	0.0400[b]	0.1956	0.8970	0.0139[b]	0.5721
Year * Irrigation * N	0.8435	0.7045	0.2903	0.9734	0.1157
Year * Irrigation * K	0.4780	0.8006	0.9459	0.2567	0.4721
Year * Irrigation * N * K	0.9123	0.8763	0.8635	0.0812	0.6781

[a] Denotes significant effect as determined by the Tukey HSD test at $p<0.005$.

[b] Denotes significance for interactions with Year as determined by Roy's Max Root criteria ($p<0.05$)

Table 4. Results for olive oil quality for ABI categories (ON-OFF): free acidity (FFA %); peroxide content (meq O_2 kg^{-1}); K232 and K270; oil stability (hours) and polyphenols (mg kg^{-1}). Results were split by irrigation, nitrogen and potassium treatments (mean ± std. deviation). Uppercase letters denote significant effect between columns (ON-OFF state) and lowercase letters denote differences between rows (treatments) as determined by the Tukey HSD test at p<0.005.

	Irrigation	OFF	ON	Potassium	OFF	ON	Nitrogen	OFF	ON
FAA	**FI**	0.18±0.08	0.13±0.05	**K0**	0.1±0.06	0.16±0.09	**N0**	0.11±0.01	0.14±0.07
	DI	0.24±0.05 **A**	0.14±0.07 **B**	**K100**	0.22±0.06 **A**	0.13±0.05 **B**	**N50**	0.23±0.05 **A**	0.13±0.05 **B**
Peroxides	**FI**	8.17±1.72	7.31±1.5	**K0**	6±1.12	6.4±1.34	**N0**	7±1.41	6.5±1.35
	DI	8±1.87	7.25±1.93	**K100**	8.3±1.64	7.5±1.74	**N50**	8.33±1.73	7.8±1.75
K232	**FI**	1.82±0.13	1.76±0.14	**K0**	1.81±0.09	1.77±0.09	**N0**	1.91±0.14	1.79±0.08
	DI	1.76±0.27	1.74±0.15	**K100**	1.79±0.21	1.75±0.15	**N50**	1.77±0.21	1.73±0.17
K270	**FI**	0.09±0.01	0.1±0.02	**K0**	0.09±0.01	0.09±0.01	**N0**	0.09±0.01	0.09±0.01
	DI	0.08±0.01	0.09±0.01	**K100**	0.08±0.01	0.09±0.02	**N50**	0.08±0.01	0.1±0.02
Stability	**FI**	8.0±3.3	9.71±3.27	**K0**	14.3±2.4**a**	13.64±2.22**a**	**N0**	10.95±4.74	13.15±2.61**a**
	DI	6.8±1.46	10.26±3.83	**K100**	6.79±1.31**b**	9.05±3.18**b**	**N50**	6.7±1.35	7.76±2.10**b**
Polyphenols	**FI**	168.0±97.77	197.5±89.61 **b**	**K0**	311±61.2**a**	320.8±58.37**a**	**N0**	228.5±116.67**a**	285.9±76.11**a**
	DI	168.6±10.41	258.56±11.61**a**	**K100**	144.5±32.03**b**	191.92±38.22**b**	**N50**	144.2±35.8**b**	149.71±69.91**b**

Figure Captions

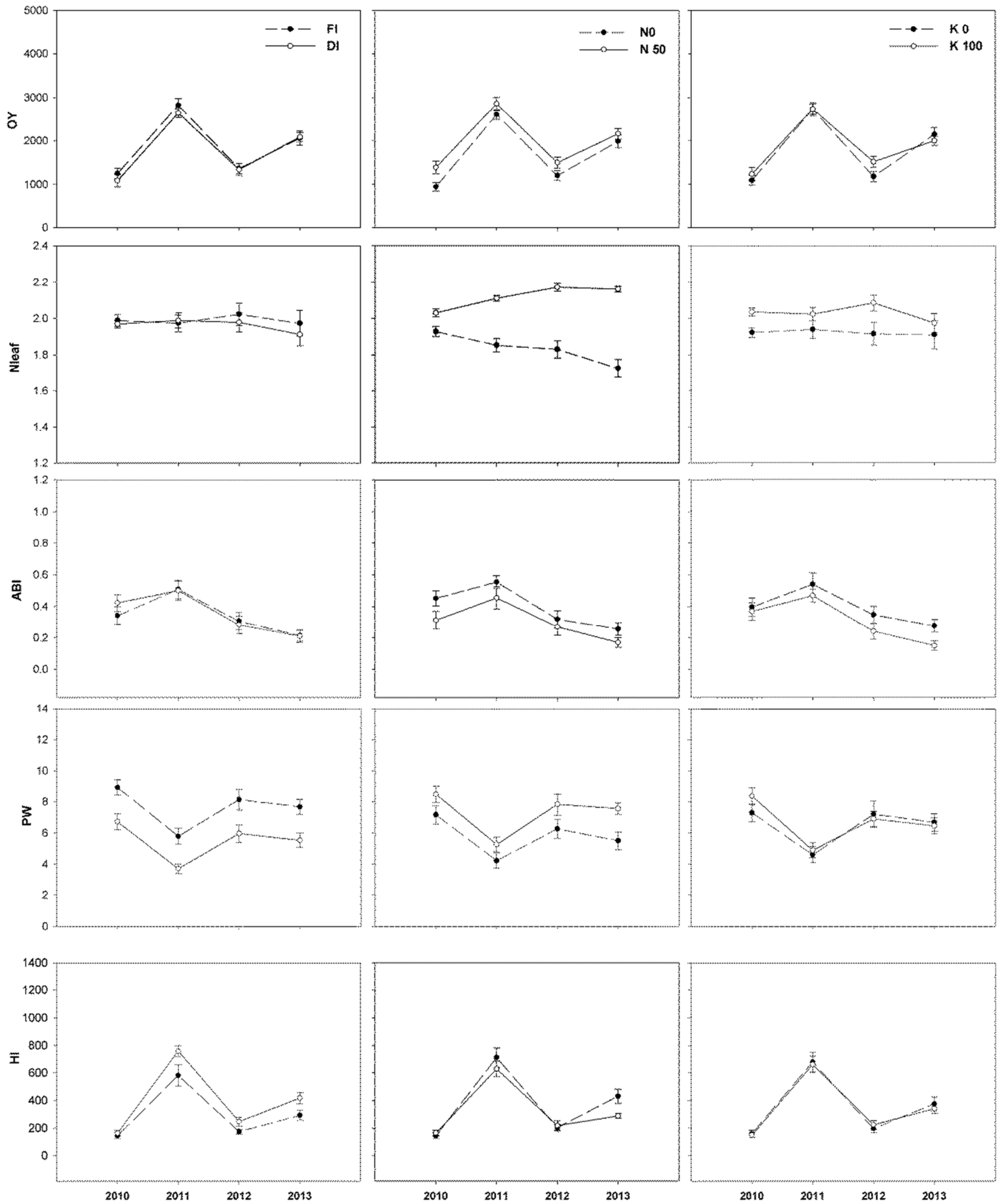

Fig. 1. Annual evolution of olive oil yield (OY, kg ha^{-1}), leaf nitrogen concentration (N_{leaf}, %), alternate fruit bearing index (ABI), pruning weight (PW, kg $tree^{-1}$) and harvest index (HI) for Irrigation, Nitrogen and Potassium treatments (vertical bars are ± standard errors of means)

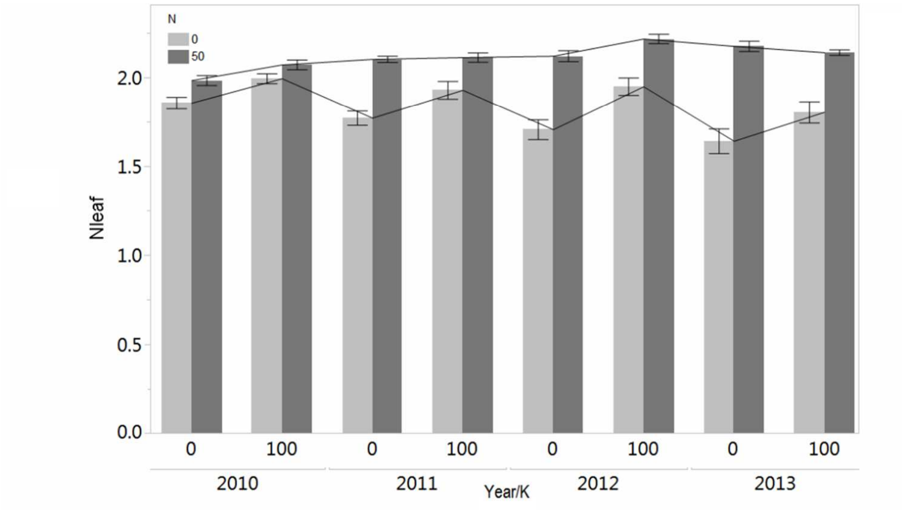

Fig. 2. Interaction Year/N/K. (Vertical bars are ± standard errors of means)

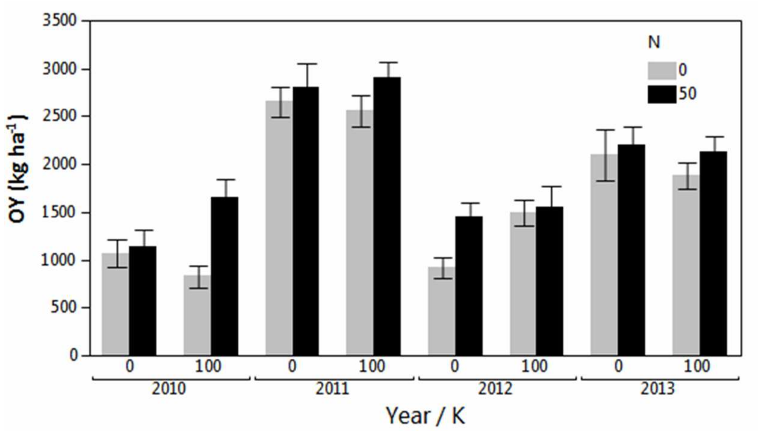

Fig. 3. Interaction Year/N/K. (Vertical bars are ± standard errors of means)

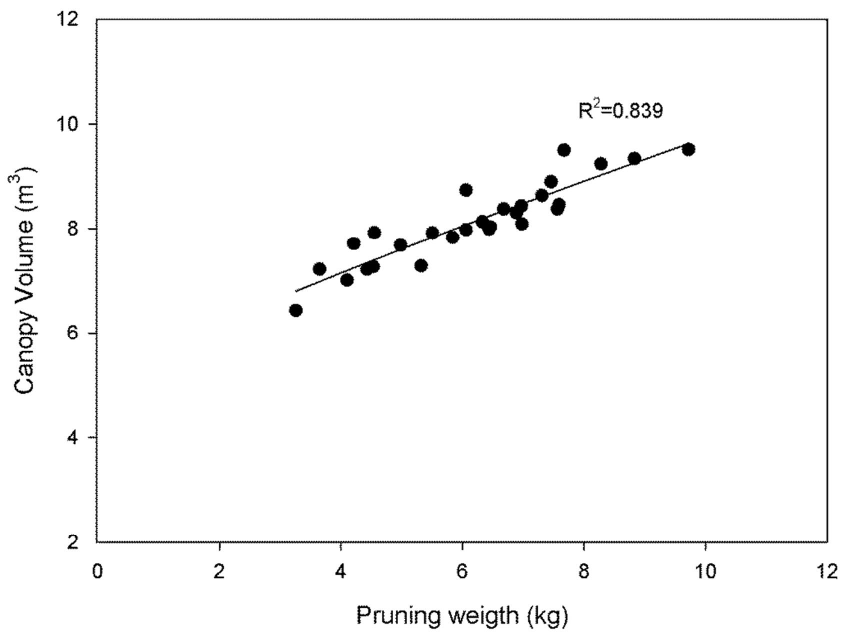

Fig. 4. Relation between pruning weight and canopy volume. Points are the means of each treatment (Irrigation, Nitrogen and Potassium) and each year.

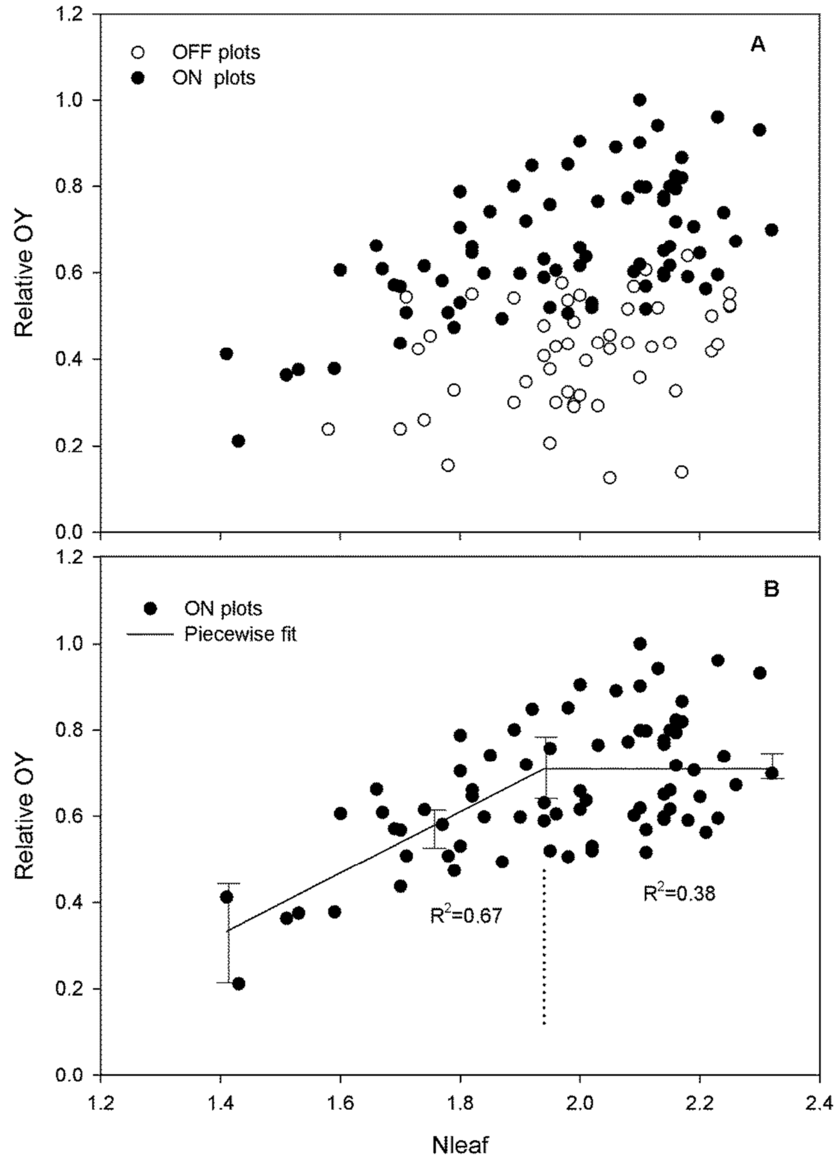

Fig. 5. (A) Plot of relative olive oil yield vs. leaf nitrogen concentration for ON and OFF plots classified according to Pearce's criteria (1967). (B) Piecewise regression between nitrogen leaf concentration and relative olive oil yield for ON plots. Parameters: b0 = 0.71, b1= 0.71, b2=0, C= 1.94. Std.Err: b0= 0.01809, b1= 0.15609, b2=0, C= 0.0590. Lower and upper CL: b0= [0.67-0.81], b1=[0.52-1.16]; C=[1.80-2.13].

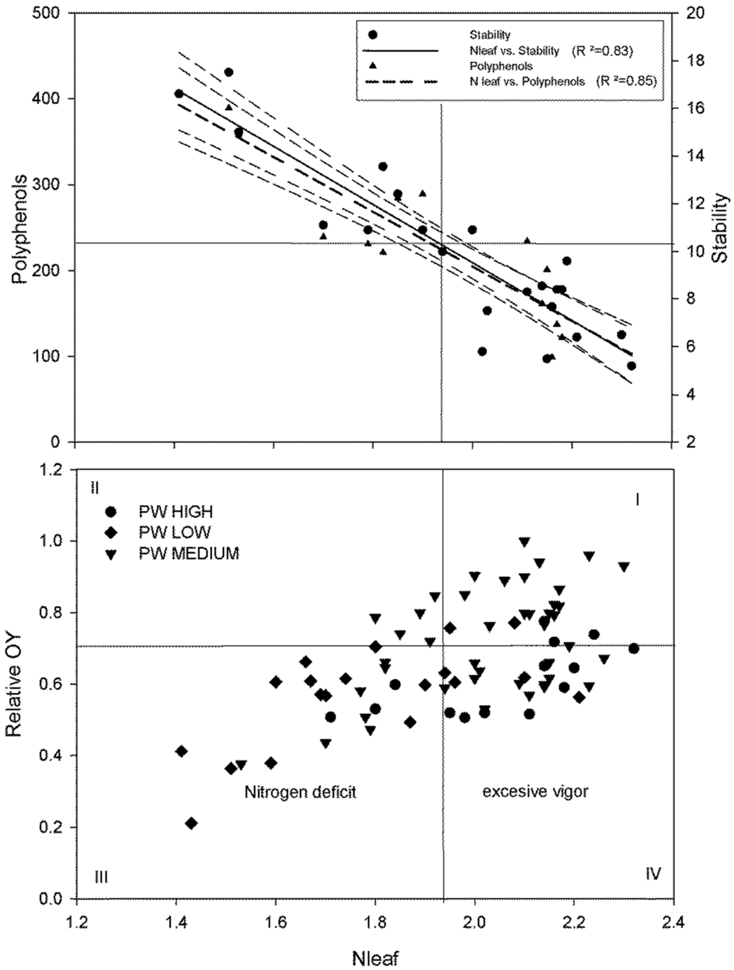

Fig. 6. Relative olive oil yield vs. leaf nitrogen concentration for ON plots classified on the basis of plant vigor (pruning weight) as LOW (1st quartile: <4.5 kg PW), MEDIUM (2nd and 3rd quartiles: 4.5-8.5 kg PW) and HIGH (last quartile: >8.5kg PW).

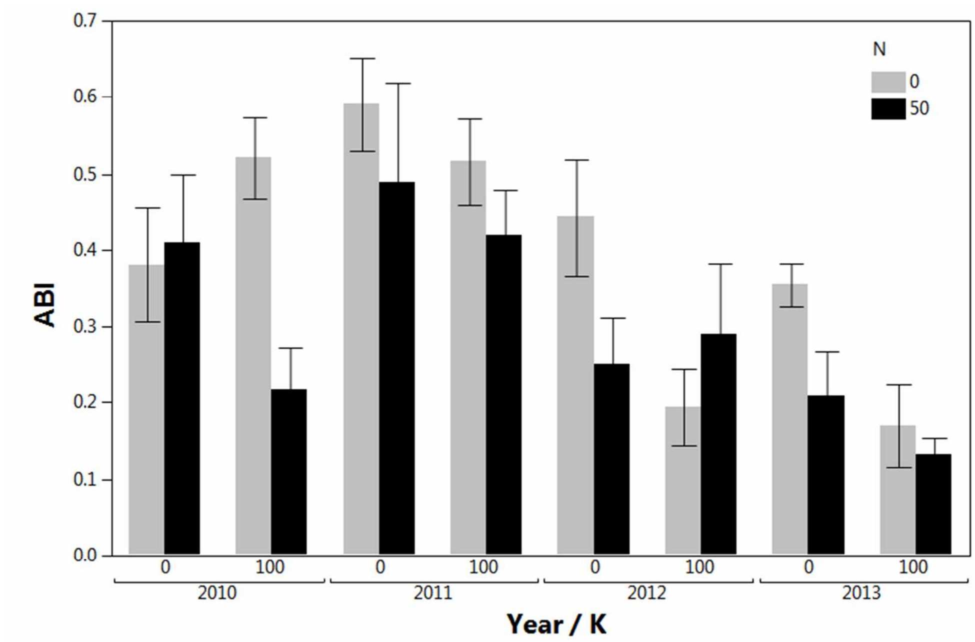

Fig. 7. Alternate bearing index among years split by nitrogen and potassium doses. (vertical bars are ± standard errors of means)

Article title

Nitrogen Nutrition Diagnosis for Olive Trees (Olea europaea L., cv. Arbequina) Grown in Super-Intensive Cropping Systems

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