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ISABEL FERREIRA

MARGARİDA ARROBAS

JOSÉ MOUTINHO-PEREIRA

CARLOS M. CORREIA

MANUEL ANGELO RODRIGUES

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The effect of nitrogen applications on the growth of young olive trees and nitrogen use efficiency

Isabel Queirós FERREIRA[1], **Margarida ARROBAS**[1], **José Manuel MOUTINHO-PEREIRA**[2], **Carlos Manuel CORREIA**[2], **Manuel Ângelo RODRIGUES**[2,*]

[1]Centro de Investigação de Montanha – Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal
[2]Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes e Alto Douro, Vila Real, Portugal

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Abstract: Two field trials (FieldExp1 and FieldExp2) and a pot experiment (PotExp) were c0onducted over a period of 3 years to assess olive tree response to nitrogen (N) applications and to estimate apparent N recovery. FieldExp1 was installed in a 3-year-old olive grove. FieldExp2 consisted of a plantation of young rooted plants. Two treatments were applied in both experiments: N application and a nonfertilized control. In PotExp, 4 N rates were used. In FieldExp1, olive yield significantly increased with applied N in only 1 of the 3 growing seasons. In FieldExp2, aboveground dry matter yield significantly increased with N application. In PotExp, total dry matter yield displayed a typical saturation curve in response to N rates. The poor response of olive yield to N application might be due to the reduced amount of N removed in the crop, and also to the negative interaction found between N application and water deficit. Apparent N recovery reached values varying from 13.1% in FieldExp2 to ~100% in PotExp. The results indicate that the olive response to N and N use efficiency seem to be influenced more by the agroecological conditions defining target yield and N loss, rather than by plant species.

Key words: Apparent nitrogen recovery, field trials, *Olea europaea*, olive yield, pot experiment

1. Introduction

Nitrogen is the element usually present in plant tissues in the highest amounts after carbon, oxygen, and hydrogen. Given the limited amounts of N in soils in forms readily available to plants, probably no other nutrient has such a strong influence on the primary production of natural and agricultural ecosystems. In agricultural fields, N is used annually in virtually all crops. However, most of the applied N (50%–60%) is lost from agroecosystems and is not used by plants, which creates several environmental concerns (Scherer and Mengel, 2007; Havlin et al., 2014). The importance of N in agriculture and its relation to environmental damage has led to continued research efforts on improving N use efficiency by adjusting N rates to crop needs.

Olive is a hardy crop traditionally grown in soils of poor fertility and with limited use of fertilizers, although currently better-quality soils are sometimes used. Previous studies have shown that the removal of nutrients by the crop is low (Rodrigues et al., 2012; Fernández-Escobar et al., 2015), which may help explain the relative success of olive cultivation in marginal growing conditions. In traditional rainfed farming systems, pruning is often used to reduce the shoot/root ratio, which keeps the remaining foliage in better watering and nutritional conditions to ensure a minimum level of productivity (Rodrigues et al., 2018). However, nowadays, particularly in irrigated and more intensive farming systems, fertilization is abundant and often excessive (Fernández-Escobar, 2011).

Most of the studies that have been carried out on olive fertilization have been devoted to N, which demonstrates the economic and environmental importance of this element. Unfortunately, the results that have been obtained do not always point in the same direction. The early studies of Hartmann et al. (1958) showed that N may have a marked effect on olive yield in soils with poor fertility but not on soils of higher fertility. Ferreira et al. (1984) reported that only the trees with the highest yield potential, i.e. those with higher nutritional requirements, responded to N fertilization. Bouhafa et al. (2014) recorded an increase in olive yield when N was applied to mature trees (>35 years), but not when N was applied to young trees (7–9 years old), probably because of their lower N requirements. However, there have been studies showing a positive response in

* Correspondence: angelor@ipb.pt

olive yield to N application under a range of agroecological conditions, without any apparent limitations imposed by the age of the crop or the quality of the soil. In a particular study, done in a container with perlite as a substrate (perlite is practically inert), Erel et al. (2008) observed an increase in flowering intensity, fruit set, and olive yield up to moderate N rates. Rodrigues et al. (2011) reported a yield reduction in trees maintained for 4 years without N in comparison to those regularly fertilized. Boussadia et al. (2010), in studies carried out with cultivars 'Meski' and 'Koroneiki', observed that nonfertilized trees responded with lower leaf N concentration, lower chlorophyll content, and, consequently, less photosynthetic capacity. N-deficient plants accumulated carbohydrates (starch, mannitol, sucrose, and glucose) that might have inhibited photosynthesis. As a result, total biomass was strongly reduced in both cultivars. In an intensively managed orchard, Haberman et al. (2019) observed an increase in vegetative growth and oil yields. On the contrary, under low N availability, the trees appeared to be more susceptible to alternate bearing. However, other studies have showed no increase in olive performance with the application of N. Fernández-Escobar et al. (2009a) found no response in tree growth, fruit size, or olive yield to the application of N in a long-term study in southern Spain. In a 5-year study conducted in 4 olive orchards growing in different agroecological conditions, Fernández-Escobar et al. (2009b) also showed no benefits of N application for vegetative growth, fruit size, oil content, and olive yield.

These previous studies on N fertilization in olive groves, although significant, do not provide clear guidance for fertilizer recommendation systems, which makes this subject interesting from the scientific point of view and gives it immense practical importance in the context of olive grove fertilization. In addition, as far as we know, studies estimating apparent N recovery are nonexistent in olive, although abundant for annual crops (Rodrigues et al., 2006; Bouchet et al., 2016; Srivastava et al., 2018). Thus, having in mind the need to improve N fertilizer programs, we concluded that it would be useful to have data on how efficiently the olive tree uses N applied as a fertilizer. The lack of data on olive response to N fertilization is particularly evident for young orchards, since the benefits of fertilizer application in the early years have been poorly demonstrated. Thus, the objective of this study is to evaluate the response of young olive trees to the application of N and to estimate the apparent N recovery as an index of N use efficiency. This is very useful information for fertilizer recommendation systems for young olive orchards. The study was supported by 3 experiments. The main one (FieldExp1) was carried out in a 3-year-old orchard which had already started to produce fruit. In a second field trial (FieldExp2), young rooted plants were purposely installed for the study, and the plants were cut at ground level 3 years after they had been planted to allow the estimation of apparent N recovery in aboveground biomass, as is usual in annual crops. A third experiment was carried out in pots (PotExp), to allow the assessment of N recovery in the whole plant, including the root system.

2. Materials and methods

2.1. Experimental conditions

The study involved 2 field trials and 1 pot experiment, all of them with 'Cobrançosa', the most widely grown cultivar in the region. The field trials were carried out in Bragança at Pinheiro Manso farm (41.807700, –6.733378; 700 m a.s.l.). The region benefits from a Mediterranean climate, with average annual air temperature of 12.7 °C and annual rainfall of 772.8 mm. The average monthly air temperature and precipitation during the experimental period are shown in Figure 1. The pot experiment was carried out in a greenhouse with a double-walled polycarbonate cover, which was aerated by lateral and zenithal windows. The greenhouse was also equipped with an internal refractory screen as a supplementary tool for heat dissipation. The soils of the 3 experiments were analyzed at the beginning of the study. According to the official classification of soil properties in Portugal (LQARS, 2006), all of the soils were slightly acid. Soil organic matter was low in the field trials and mean in the PotExp. Extractable potassium (K) was high in all of the soils, and extractable phosphorus (P) was mean in the field trials and very low in the PotExp. Several other physical and chemical properties are shown in Table 1.

2.2. Experimental designs

The first field trial (FieldExp1) was installed in March 2013 in a 3-year-old olive grove with the trees spaced at 7 m × 6 m. Two treatments, with and without N application, were included in the experimental design, as well as 3 replicates composed of 4 homogenous trees. N was applied at a rate of 48 g tree^{-1} year^{-1} as ammonium nitrate (34.5% N). The second field trial (FieldExp2) was installed in the spring of 2014 and involved the planting of young rooted plants (~20 cm height). Two treatments (N application and a nonfertilized control) and 3 replicates were included. The plants were planted spaced at 1 m in each row and 6 m between rows. Each experimental unit comprised 10 contiguous plants in the row, which corresponds to a fertilized area of 20 m^2 (10 m in the row and 1 m to each side of the plant row). N rate applied in the fertilized treatment was 200 g per experimental unit, as ammonium nitrate. The pot experiment (PotExp) consisted of a 3-year study on crop response to N application by using 4 N rates (N0), 0.4 (N1), 0.8 (N2), and 1.6 (N3) g^{-1} pot^{-1} year^{-1}, as ammonium nitrate, in 6 replicates (6 pots).

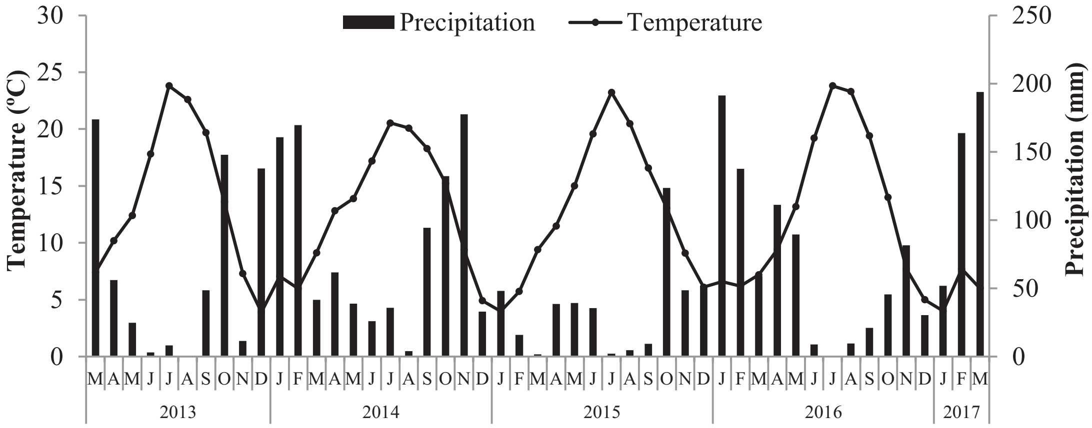

Figure 1. Average monthly precipitation and temperature recorded during the experimental period in the meteorological station of Santa Apolónia in Bragança.

Table 1. Selected properties of the soils used in the field trials (FieldExp1, FieldExp2) and pot experiment (PotExp) from soil samples (0–20 cm) taken just before the trials started.

Soil properties	FieldExp1	FieldExp2	PotExp
pH (H_2O)	5.8	5.5	5.5
Extractable P (mg P_2O_5 kg^{-1})[1]	87.9	93.4	21.4
Extractable K (mg K_2O kg^{-1}) [1]	102.0	114.0	134.0
NO_3^- (mg kg^{-1})[2]	47.2	41.0	25.8
NH_4^+ (mg kg^{-1})[3]	25.6	28.3	17.9
Easily oxidizable carbon (g kg^{-1})[4]	8.7	8.7	13.5
Total organic carbon (g kg^{-1})[5]	27.3	25.6	36.4
Exchangeable K (cmol$_c$ kg^{-1})[6–]	0.2	0.3	0.3
Exchangeable Na (cmol$_c$ kg^{-1})[6]	0.4	0.4	0.3
Exchangeable Ca (cmol$_c$ kg^{-1})[6]	7.2	8.5	3.3
Exchangeable Mg (cmol$_c$ kg^{1})[6]	2.2	2.6	1.0
Exchangeable acidity (cmol$_c$ kg^{-1})[6]	10.7	11.9	5.1
Clay (%)[7]	14.5	14.6	12.2
Silt (%)[7]	27.7	29.2	21.2
Sand (%)[7]	57.8	56.2	66.6

[1]Ammonium lactate; [2]spectrophotometry UV; [3]phenate method for ammonia; [4]Walkley–Black; [5]Incineration; [6]Ammonium acetate; [7]Pipette Robinson.

2.3. Management of field trials and pot experiment

The olive trees of FieldExp1 were managed under rainfed conditions. In addition to the N of the fertilizer treatment, a basal fertilization plan with P, K, and boron (B) was applied. These 3 nutrients were applied at the rates of 70, 133, and 1.2 g tree^{-1} year^{-1} at the end of March. N and B were applied beneath the canopy in an area of 4 m^2 (1 m from the trunk for each quadrant) and P and K in 16 m^2 (2 m from the trunk for each quadrant). The fertilizers used were superphosphate (18% P_2O_5), K chloride (60% K_2O), and borax (11% B). The weeds were controlled with the application of 4 L ha^{-1} of a nonselective glyphosate-based herbicide (360 g L^{-1} active ingredient) applied once a year ~15 days after the application of fertilizers.

The plants of FieldExp2 were watered 3 times during the summer following planting to reduce the risk of plant death. From the second year onwards, the plants were entirely kept under rainfed conditions. N in the fertilizer treatment and a basal fertilization plan with P, K, and B were applied annually, at the end of March. P, K, and B rates were respectively 175, 332, and 6 g per experimental unit, and the fertilizers used were the same reported for FieldExp1.

In PotExp, pots were filled with 3 kg of dry soil (2 mm mesh) and 200 mL of perlite to reduce soil compaction and favor aeration. All pots received a fertilizer supplement with P, K, and micronutrients. P and K were used at the rates of 0.35 and 0.66 g pot^{-1} $year^{-1}$, applied as a liquid compound NPK fertilizer (0:30:20) and a liquid K fertilizer (45% K_2O). Micronutrients, magnesium, and sulfur were supplied as a fertilizer, containing 10% MgO, 0.3% B, 18.5% SO_3, 0.3% Cu, 2% Fe, 1% Mn, 0.02% Mo, and 1.6% Zn, applied at the rate of 0.08 g pot^{-1} $year^{-1}$. The annual rate of fertilizers was divided into 5 monthly applications during the growing season, from April to August, to reduce the risk of saline effect. The pots were kept watered by 1–2 waterings per week depending on environmental conditions to ensure regular plant growth.

2.4. Field and laboratory determinations

In FieldExp1, the diameter of the trunk was periodically measured at 40 cm from the ground and the canopy volume estimated after measuring its maximum height and width in the North/South and East/West directions and assuming an ovoid shape by using the equation $CV = 2/3\ \pi\ R^2\ (L + S)$, where R is the median radius of the canopy at its widest point, L is the distance between the widest point and the top of the canopy (2/3 of the canopy height), and S is the distance between the widest point of the canopy and the base of the canopy (1/3 of the total height of the canopy). The trees were pruned annually in the winter, and the pruned wood used as an index of canopy development. In the autumn, the olive trees were manually harvested and the fresh fruits weighed. Subsamples of 100 fruits were also weighed for fruit size evaluation. From these subsamples, 20 fruits were separated into pulp and pit, weighed, oven-dried at 70 °C, weighed dry, and thereafter used for elemental analysis. Twice a year, in the winter resting period and in summer, leaf samples were taken from current season growth all around the canopy to assess the nutritional status of plants. At the end of the study, soil samples were collected at 3 depths (0–5 cm, 5–10 cm, and 10–20 cm) to evaluate the effect of treatments on soil properties. In September 2016, turnip (*Brassica rapa* var. *Rapa* L.) was sown beneath the canopy where the fertilizers had been applied, and N recovery was used as an index of the soil's available N. The aboveground biomass of the turnip was cut in the following winter and N removal used as an indicator of soil N bioavailability. Chlorophyll *a* fluorescence and fluorescence transient were determined from the dark-adapted protocols F_V/F_M and F_V/F_0 and the advanced OJIP test performed with the fluorometer OS30p+. F_M, F_0, and F_V are, respectively, maximum, minimum, and variable fluorescence of dark-adapted leaves and $F_V/F_M = (F_M - F_0)/F_M$ and $F_V/F_0 = (F_M - F_0)/F_0$. The OJIP test provides basal fluorescence at 20 ms (O), fluorescence at 2 ms (J), fluorescence at 30 ms (I), and maximum fluorescence (F_M). Measurements were taken in the morning (~11:00) on young leaves with fully expanded blades after a period of adaptation to the dark of at least 35 min.

In FieldExp2, twice a year, during the winter resting period and in the summer, leaves from current seasonal growth were collected to assess the nutritional status of the plants. At the end of FieldExp2, in October 2016, the aboveground biomass of 4 inner plants of each plot was cut at ground level and weighed. A subsample was separated into leaves and stems and weighed again. After being oven-dried at 70 °C, the samples were weighed dry. The plant parts were thereafter analyzed for elemental composition.

In PotExp, the aboveground biomass was cut at the end of each growing season in February 2015 and February 2016 at approximately 12 cm above ground level, in order to facilitate the regeneration of the plant in the next growing season. After being cut, the plants were separated into leaves and stems and sent to the laboratory. Leaf gas exchange was measured at midmorning on cloudless summer days in 2015 and 2016 with an infrared gas analyzer (LCpro+, ADC, Hoddesdon, UK), under greenhouse conditions. Net CO_2 assimilation rate (A), stomatal conductance (g_s), and the ratio of intercellular to atmospheric CO_2 concentration (C_i/C_a) were estimated according to von Caemmerer and Farquhar (1981). Intrinsic water use efficiency was calculated as the ratio of A/g_s. In October 2016. the plants were removed from the pots and a homogeneous soil sample was recovered per pot. The roots were then washed with water under gentle pressure and the plant separated into roots, leaves. and stems, and sent to the laboratory. Soil nitrate levels were monitored during the growing season by using anion exchange membranes (AEMs). Briefly, strips of 1 × 2 cm of AEM were saturated with 0.5 M $NaHCO_3^-$ before use. Thereafter, the AEMs were inserted directly into the soil for a period of 7 days. At the end of the incubation period, the AEM strips were removed from the soil and rinsed with distilled water. After the AEMs were washed, they were placed in 20 mL 0.5 *N* hydrochloric acid and shaken for 4 h at 180 rpm. In the extracts, the concentration of nitrates was determined by UV–Vis spectrophotometry.

At the end of the field and pot experiments, soil samples were taken and analyzed to assess the effect of the fertilizer treatments on soil properties. After drying and sieving, soil samples were submitted to analytical determinations: pH (H_2O, KCl); easily oxidizable carbon (C) determined by the Walkley–Black method and total organic C by incineration; cation exchange capacity (ammonium acetate, pH 7.0); extractable P and K (ammonium lactate); extractable B (azomethine-H); and clay, silt, and sand fractions by the

Robinson pipette method (Houba et al., 1989). As indices of easily mineralizable N, extractions by cold and hot KCl were performed. Briefly, 40 mL of 2 M KCl were added to 10 g of soil and placed in an oven at 100 °C for 4 h. After cooling, the suspension was filtered, and the concentration of NH_4^+ in the solution determined. The procedure was repeated using cold KCl. Hydrolyzable NH_4^+ was estimated by the difference between NH_4^+ extracted hot and cold (Arrobas et al., 2015). The concentration of NH_4^+ in KCl solutions was determined by the phenate method, which is based on the development of a blue compound (indophenol) by reaction of ammonia, hypochlorite, and phenol catalyzed by sodium nitroprusside (Clescerl et al., 1998).

Tissue samples (leaves, stems, roots, pulps, and pits) were oven-dried at 70 °C and ground. Tissue analyses were performed by Kjeldahl (N), colorimetry (B and P), flame emission spectrometry (K), and atomic absorption spectrophotometry (calcium, magnesium, copper, iron, zinc, and manganese) methods (Walinga et al., 1989) after tissue samples were digested with nitric acid in a microwave.

2.5. Data analysis

Data analysis was carried out using JMP software. Data was first tested for normality and homogeneity of variances using the Shapiro–Wilk test and Bartlett's test, respectively. The comparison of the effect of the fertilizer treatments was provided by ANOVA ($\alpha < 0.05$). After ANOVA examination, means with significant differences (for factors with more than 2 treatments) were separated by the multiple range Tukey HSD test ($\alpha = 0.05$). In some situations, to facilitate the interpretation of the results and for purposes of graphical representation, the mean confidence intervals ($\alpha = 0.05$) were also estimated.

Apparent N Recovery (ANR) was used as an index of N use efficiency. ANR was estimated according to the equation:

Apparent N Recovery (ANR, %) = 100 × (N recovered in the fertilized treatments – N recovered in the control) / N applied as a fertilizer).

3. Results

3.1. Field experiment 1

In FieldExp1, fertilizer treatment significantly increased olive yield only in the harvest of 2015 (Figure 2). The differences recorded in 2015 resulted in higher accumulated average values in the fertilized treatment group (2.46 kg tree^{-1}), but without significant differences for the control treatment group (1.67 kg tree^{-1}). Some other parameters measured to evaluate tree crop growth, such as the increase in trunk diameter, the canopy volume, and the mass of pruned wood, did not vary significantly between treatment groups.

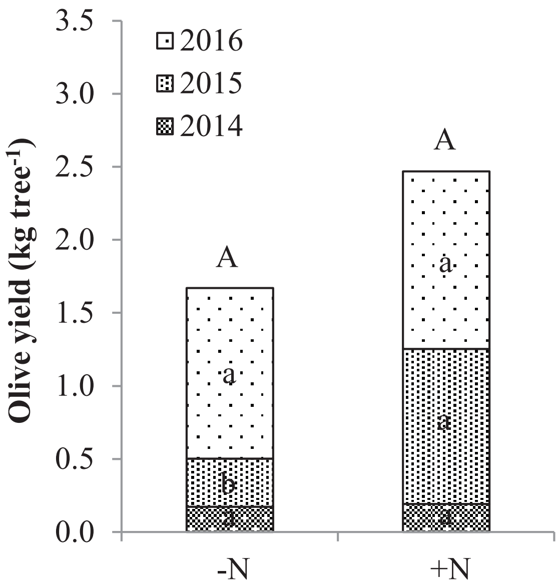

Figure 2. Olive yields as a function of N fertilizer treatments. Capital letters and lowercase letters are the results of analysis of variance ($\alpha < 0.05$), respectively for accumulated and annual olive yields.

Fruit size and pulp/pit ratio also did not vary significantly with N application (Table 2). In 2016, the fruits exhibited a size that was approximately half that of the previous year's fruit, due to the severe lack of summer precipitation (Figure 1). The concentration of N in both the pulp and the pit tended to be significantly higher in the fertilized treatment group. In turn, the concentration of several other nutrients determined in these tissues did not significantly vary between treatment groups.

N concentration in leaves determined in summer and in winter was higher, often with significant differences, in the fertilized treatment group in comparison to the control (Figure 3). However, in both treatment groups, leaf N concentration varied within the sufficiency range as established for this species (15–25 g kg^{-1}).

F_V/F_M, F_V/F_0, and the OJIP parameters revealed reduced sensitivity to the nutritional status of plants from FieldExp1 (Table 3). However, the results of 2016 showed a detail that deserves some attention. In the last reading of 2016, on 10 August, following a particularly dry period (Figure 1) which imposed severe stress on the plants, the photosynthetic performance was lower in the N-fertilized treatment in comparison to the control.

In the soil samples, chemical indices such as easily oxidizable carbon, Kjeldahl N, and KCl extractions did not show significant differences between treatment groups (Table 4). However, a marked gradient was observed from the surface to the deepest layers. In turn, the biological indices that consisted of evaluating dry matter yield, tissue

Table 2. Fresh fruit weight, pulp/pit ratio and N concentrations in pulp and pit in treated (+N, 48 g N $tree^{-1}$ yr^{-1}) and untreated (–N, unfertilized control) plots. Within each year in rows, means followed by the same letter are not significantly different ($\alpha < 0.05$).

	2014		2015		2016	
	-N	+N	-N	+N	-N	+N
Fresh weight (g $fruit^{-1}$)	4.18 a	4.66 a	4.10 a	3.67 a	2.22 a	1.97 a
Pulp/pit ratio (dw)	1.27 a	1.27 a	1.88 a	1.86 a	1.49 a	1.49 a
Pulp N (g kg^{-1})	5.09 b	6.09 a	6.05 a	6.35 a	7.02 b	8.02 a
Pit N (g kg^{-1})	2.63 a	2.75 a	3.27 b	4.62 a	4.81 a	5.41 a

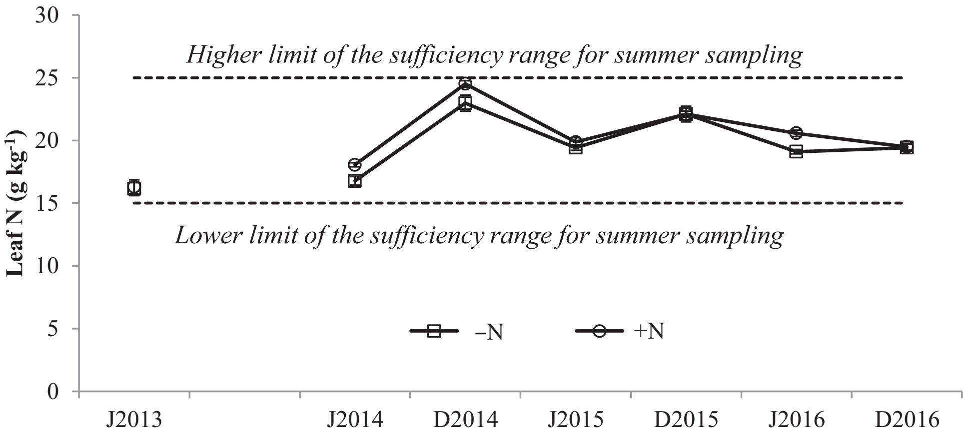

Figure 3. Leaf N concentration from samples taken in July (J), in summer, and in December (D), in the resting period of winter. Error bars are the mean confidence intervals ($\alpha = 0.05$).

Table 3. Basal fluorescence at 20 ms (O), fluorescence at 2 ms (J), fluorescence at 30 ms (I), and maximum fluorescence (P, F_M) and ratio of variable fluorescence and maximum fluorescence (F_V/F_M) and variable fluorescence normalized to minimum fluorescence (F_V/F_0) from measurements taken in 2016 in FieldExp1 (+N, 48 g N $tree^{-1}$ yr^{-1}; –N, unfertilized control). In columns, means followed by the same letter are not significantly different ($\alpha < 0.05$).

Date	N treatment	O	J	I	P (F_M)	F_V/F_M	F_V/F_0
9 June	+N	183.8 a	272.5 a	459.5 a	710.0 a	0.78 a	3.64 a
	–N	198.3 a	304.8 a	500.3 a	751.3 a	0.77 a	3.42 a
12 July	+N	242.0 a	389.3 a	656.8 a	890.8 a	0.79 a	3.85 a
	–N	250.3 a	400.8 a	669.5 a	893.3 a	0.78 a	3.87 a
10 Aug	+N	275.3 a	403.5 a	561.3 a	678.3 a	0.67 b	2.13 b
	–N	262.0 a	413.3 a	654.8 a	803.3 a	0.75 a	3.09 a

N concentration, and N recovery by turnips sowed under the canopy in PVC rings revealed significantly higher values in the fertilized treatment group in comparison to the control.

3.2. Field experiment 2

In FieldExp2, N application increased dry matter yield, tissue N concentration, and N recovery in the aboveground biomass (Figure 4), a clearer response than that observed in the older plants of the FieldExp1. N application had no significant effects on the concentration and recovery of other nutrients analyzed in the leaves and stems (data not shown).

3.3. Pot experiment

In the PotExp, the application of N significantly increased the aboveground dry matter yield in comparison to the control treatment, with the exception of the first cut at the end of

Table 4. Chemical and biological indices of soil N availability as a function of N fertilizer treatment (+N, 48 g N tree^{-1} yr^{-1}; -N, unfertilized control) and soil depth. In columns, separately for N treatment and soil layer; means followed by the same letter are not significantly different by Tukey HSD test (α = 0.05).

	Chemical indices[1]					Biological indices[2]		
	EOC	Kjel N	KCl_C	KCl_H	KCl_{Hyd}	DMY	TNC	NR
N treatment	(g kg^{-1})	(mg kg^{-1})	----- (mg NH_4^+ kg^{-1}) -----			(g plot^{-1})	(g kg^{-1})	(g plot^{-1})
-N	19.7 a	1012.7 a	13.4 a	24.9 a	11.5 a	5.2 b	39.0 b	0.20 b
+N	16.9 a	965.8 a	12.9 a	25.4 a	12.5 a	22.0 a	43.6 a	0.96 a
Soil layer								
0 - 5 cm	24.2 a	1637.7 a	17.3 a	35.9 a	18.6 a			
5 - 10 cm	17.6 b	836.5 b	11.8 b	21.2 b	9.3 b			
10 - 20 cm	13.2 b	493.5 c	10.2 b	18.2 b	8.0 b			

[1]EOC, easily oxidizable carbon (Walkley–Black); Kjel N, Kjeldahl N; KCl_C, cold KCl extraction; KCl_H, hot KCl extraction; KCl_{Hyd}, NH_4^+ hydrolyzable (KCl_C–KCl_H).
[2]Dry matter yield of turnip (DMY); tissue N concentration (TNC); N recovery (NR).

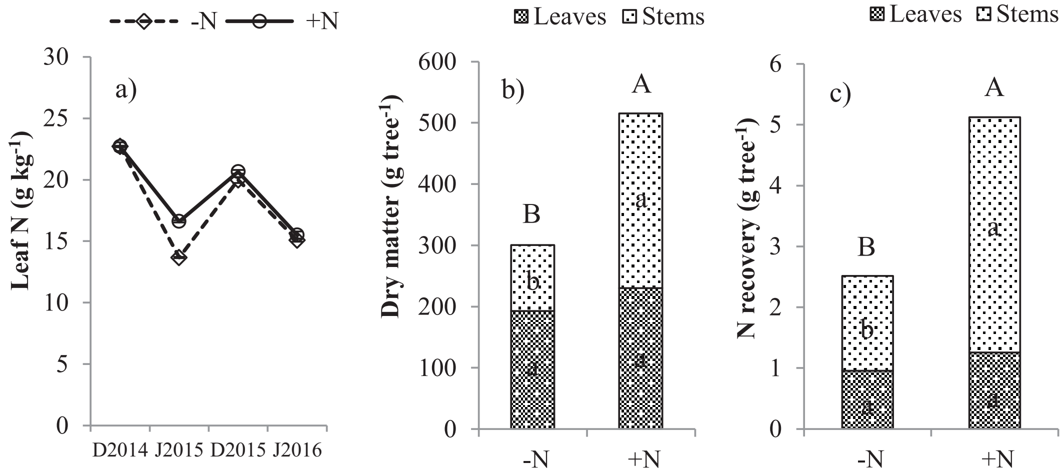

Figure 4. a) Leaf N concentration in December (D) and in July (J), b) dry matter yield in leaves and stems, and c) N recovery in leaves and stems as a function of N fertilizer treatment (–N, +N) in FieldExp2. a) Error bars are the mean confidence intervals (α = 0.05), b) and c) capital letters and lowercase letters are the results of analysis of variance (α < 0.05), respectively for total dry matter yield (stems + leaves) and separately per stems or leaves.

the growing season of 2014 (Figure 5). In 2015 and 2016, aboveground dry matter yield responded with a saturation curve, increasing to low N rates and stabilizing on a plateau, or even decreasing to the higher N rates.

The relationships between the different plant parts showed that the application of N tended to increase the proportion of leaves in relation to stems and roots, as well as the shoots in relation to the roots (Figure 6). The leaves/stems and leaves/roots ratios showed a linear increase with N rate, while the shoots/roots ratio was fit by a second-degree polynomial.

N concentration in plant tissues increased significantly from N0 to N3 in all tissues and at all sampling dates (Figure 7). In 2016, the concentration of N in plant tissues varied between 5.3 and 14.3 g kg^{-1}, 9.5 and 22.0 g kg^{-1}, and 12.4 and 29.8 g kg^{-1}, respectively in the stems, roots, and leaves for N0 and N3 N treatments.

In PotExp, net photosynthesis in N2 and N3 treatments was higher than in N1 and N0 plants in 2016, due to lower nonmesophyll limitations to photosynthesis, as can be deduced by A/gs and C_i/C_a data. On the other hand, stomatal conductance was not significantly affected by N nutrition (Table 5).

Soil pH decreased significantly and very markedly with N application (Table 6). Some indices, such as readily oxidizable carbon, Kjeldahl N, and nitrate-N extracted

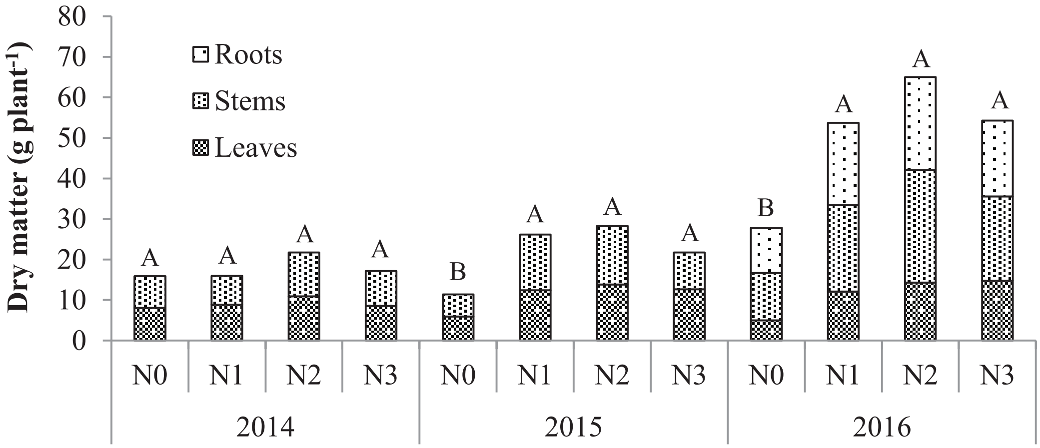

Figure 5. Dry matter yield in leaves and stems (2014–2016) and roots (2016) as a function of N treatment [0 (N0), 0.4 (N1), 0.8 (N2) and 1.6 (N3) g^{-1} pot^{-1} $year^{-1}$] in PotExp. Capital letters above the columns within each year are the result of analysis of variance and Tukey HSD test (α = 0.05) for accumulated dry matter yield (leaves + stems + roots).

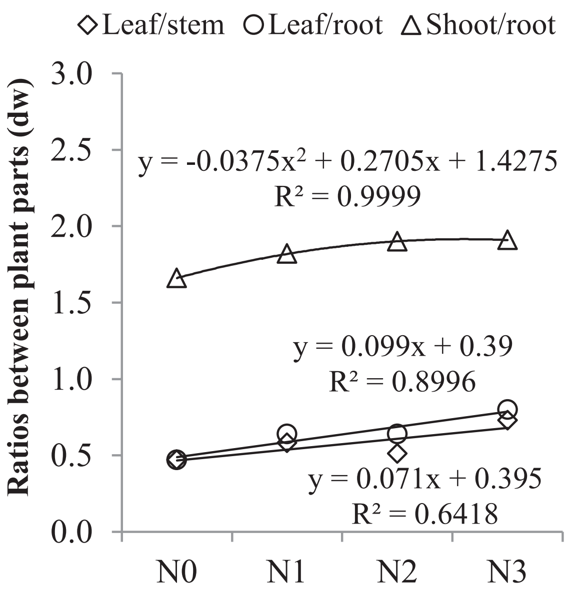

Figure 6. Relationship between different plant parts as a function of N treatment [0 (N0), 0.4 (N1), 0.8 (N2), and 1.6 (N3) g^{-1} pot^{-1} $year^{-1}$].

with anion exchange membranes, showed a significant increase with N rate. NH_4^+ determined in hot and cold KCl and hydrolysable NH_4^+ showed significantly higher values only for the N3 treatment.

3.4. Nitrogen use efficiency in field and pot experiment

At the end of the 3 years, fertilized plants of FieldExp1 recovered 46.9% of the applied N, if both N recovered in the crop (21.0%) and in pruning wood (25.9%) were taken into account (Table 7). The plants of FieldExp2 recovered only 13.1% of the N applied during the 3 consecutive years. In the PotExp, apparent N recovery in total biomass (leaves, stems, and roots) amounted to 100.8%, 78.4%, and 42.3% in the fertilized N1, N2, and N3 treatments, respectively.

4. Discussion

4.1. Growth and yield of olive plants

The results showed a clear response of olive plants to the application of N in pots, where biomass production exhibited a typical saturation curve in response to the application of N. In the field, the interaction with other variables affecting plant growth, such as soil available N and water, may have masked the effect of the applied N. These results are in some ways in agreement with other results reported in the literature. Some authors have recorded significant differences in only part of their experiments or under particular agroecological conditions (Hartmann et al., 1958; Ferreira et al., 1984; Bouhafa et al., 2014). In other studies, olive response to N application seems to have been unequivocal (Erel et al., 2008; Rodrigues et al., 2015, 2019; Haberman et al., 2019), although studies also exist where olive did not respond to N fertilization at all (Fernández-Escobar et al., 2009a, 2009b). Fernández-Escobar et al. (2012) substantiated the lack of response to N application based on a study of N balance in an agrosystem where they estimated that the quantities of mineral N entering the system through mineralization of organic matter and rainwater would be equivalent to N removed in the crop and in pruning wood. N is an important ecological factor, but the response of plants to N applied as a fertilizer depends on crop needs and how the soil can provide the nutrient from its own reserves (Havlin et al., 2014). In this particular study, in FieldExp1, leaf N concentrations did

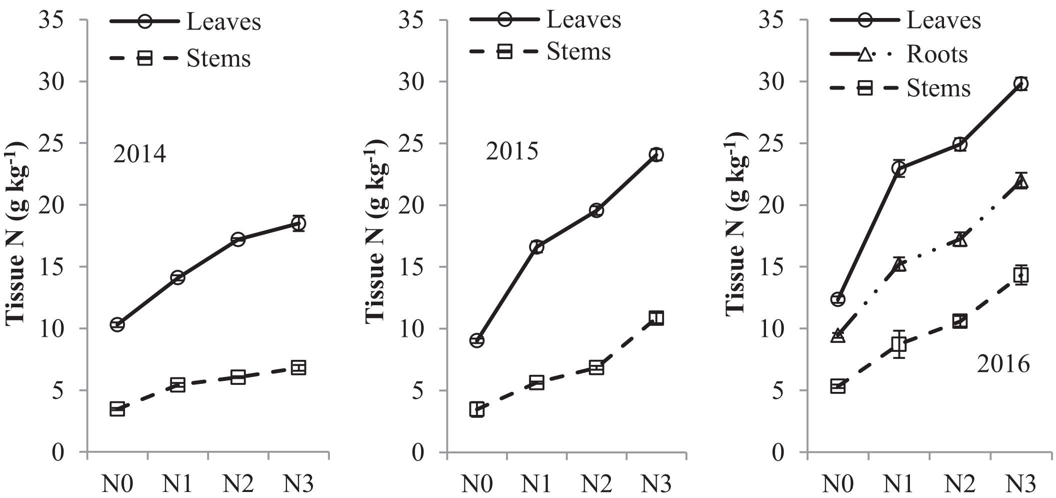

Figure 7. N concentration in plant tissues as a function of N rate [0 (N0), 0.4 (N1), 0.8 (N2), and 1.6 (N3) g^{-1} pot^{-1} $year^{-1}$]. Error bars are the mean confidence intervals (α = 0.05).

Table 5. Net photosynthetic rate (A), stomatal conductance (g_s), intrinsic water use efficiency (A/g_s), and ratio of intercellular to atmospheric CO_2 concentration (C_i/C_a) as a function of N fertilizer treatment [0 (N0), 0.4 (N1), 0.8 (N2), and 1.6 (N3) g N pot^{-1} yr^{-1}] in pot experiment. In columns, within each year; means followed by the same letter are not significantly different by Tukey HSD test (α = 0.05).

		A	g_s	A/g_s	C_i/C_a
Year	N treatment	mmol m^{-2} s^{-1}	mmol m^{-2} s^{-1}	mmol mol^{-1}	
2015	N0	11.5 b	251.9 a	46.2 c	0.763 a
	N1	13.9 a	266.0 a	52.4 bc	0.724 ab
	N2	14.0 a	250.4 a	56.6 ab	0.709 bc
	N3	14.2 a	222.4 a	63.8 a	0.675 c
2016	N0	11.2 c	203.5 a	56.0 b	0.740 a
	N1	13.0 b	167.6 a	78.6 a	0.644 b
	N2	15.4 a	186.6 a	83.3 a	0.625 bc
	N3	15.7 a	172.0 a	92.5 a	0.589 c

not fall below the lower limit of sufficiency range as set forth in Fernández-Escobar et al. (2017), which means that the plants might never have suffered severe deficiency of N even in the control treatment.

The pot experiment revealed that N enhanced the shoot/root ratio and the leaf/stem ratio of olive plants. As soil N availability increases, the plant seems to preferentially redirect photoassimilates to the photosynthetic apparatus rather than to the roots. This ability of plants to allocate resources to priority sinks depending on growth conditions (namely, to increase the proportion of leaves relative to the roots) as soil N availability increases is well documented, and makes the plant more efficient when other soil resources such as water are not limiting (Hawkesford et al., 2012).

4.2. Experimental variability affecting the response of plants to applied N

F_V/F_M, F_V/F_0, and the OJIP test failed to discriminate between N-treated and nontreated plants. Two combined reasons may justify the result: i.e. the reduced sensitivity of these measurements to N nutritional stress (Baker and Oxborough, 2004; Rodrigues et al., 2017), and the fact that the plants of the control treatment never exhibited N concentrations below the lower limit of the sufficiency range (Figure 3). It is important to note that on 10 August 2016, after a period of particularly severe drought stress, the fertilized plants showed values of F_V/F_M and F_V/F_0 lower than those of the control treatment, indicating that water deficit might have reduced the photosynthetic

Table 6. Soil properties related to N dynamic in the soil as a function of N rate [0 (N0), 0.4 (N1), 0.8 (N2), and 1.6 (N3) g N pot^{-1} yr^{-1}] at the end of the pot experiment. In columns, means followed by the same letter are not significantly different by Tukey HSD test (α = 0.05).

	pH	EOC[1]	N Kjel[2]	KCl_C[3]	KCl_H[4]	KCl_{Hyd}[5]	$NO_3^-{}_{AEM}$[6]
N treatment	(H_2O)	(g kg^{-1})	(mg kg^{-1})	---------- (mg NH_4^+ kg^{-1}) ----------			(mg L^{-1})
N0	7.3 a	11.8 b	80.0 c	7.0 b	22.8 b	15.8 b	18.2 b
N1	6.3 b	11.8 b	141.1 bc	7.4 b	14.1 b	6.8 b	59.7 b
N2	5.6 c	26.7 a	234.4 ab	8.4 b	14.3 b	5.8 b	59.2 b
N3	4.7 d	30.2 a	329.3 a	48.3 a	108.5 a	60.2 a	365.8 a

[1]Easily oxidizable carbon (Walkley–Black); [2]Kjeldahl N; [3]cold KCl extraction; [4]hot KCl extraction; [5]NH_4^+ hydrolyzable (KCl_C–KCl_H); [6]NO_3^- in extracts of anion exchange membranes.

Table 7. Apparent N recovery [average (%) ± standard deviation] in the field trials (FieldExp1 and FieldExp2) and pot experiment (PotExp).

FieldExp1[1]		FieldExp2[2]		PotExp[3]	
Fruit	21.0 ± 3.2	Aboveground biomass	13.1 ± 1.6	N1	100.8 ± 7.1
Pruning wood	25.9 ± 2.8			N2	78.4 ± 5.8
Total	46.9 ± 4.7			N3	42.3 ± 4.7

[1]Average values of 3 years (2014–2016); [2]aboveground biomass of the final cut; [3]values of 3 years (2014–2016) including 2 cuts of aboveground biomass and the last cut of total biomass (leaves, stems, and roots).

performance of fertilized plants more than those of the control treatment. Thus, N application might have increased plant susceptibility to drought stress, which may also help to explain the absence of olive yield response to N applied in 2016 when a significant positive response had already been obtained in 2015. A negative interaction between increased available N and water deficit conditions has been frequently found for other crops (Badr et al., 2012; Gheysari et al., 2015; Kiani et al., 2016).

The soil surface layer contained more potentially available N at the end of the FieldExp1 than the deeper layer, in particular in the fertilizer treatment plot. This may hinder N uptake by olive during the summer, since the surface layer usually remains strongly dehydrated, a hindrance to N mineralization and to the movement of nutrients in the soil by mass flow and diffusion (Havlin et al., 2014). This N can leave the system with the oncoming autumn rains by leaching and/or denitrification (Fernández-Escobar et al., 2009a). Thus, summer drought stress may probably have contributed to reduce the differences between N treatments. The available N in the soil at the beginning of the experiments in either mineral or organic form (available after mineralization) (Table 1) associated with the reduced removal of N by the olive (Rodrigues et al., 2012; Fernández-Escobar et al., 2015) and the age of the plants were other likely reasons for the reduced level of response of olive to applied N.

4.3. Apparent N recovery

In FieldExp1, apparent N recovery approached 50% when N removed in the fruits (21.0%) and in pruning wood (25.9%) was taken into account. The value could be even higher if the component of N immobilized in the perennial biomass of the trees had been accounted for. In FieldExp2, plants recovered only 13.1% of N applied, probably due to the combined effect of the reduced N uptake capability of the young plants and the use of excessive N rates in comparison to plant needs, and the exposure to field conditions with high potential for N losses during winter by leaching and/or denitrification. In PotExp, apparent N recovery was very high (100.8%) in the lowest N fertilized treatment (N1), decreasing to 78.4% and 42.3% in the N2 and N3 treatments. These values are not dissimilar from those found for other crops (Fageria and Baligar, 2005; Arrobas et al., 2011; Bouchet et al., 2016); they have revealed that growing conditions rather than particularities of the species are the principle factors determining N use efficiency in olive.

In conclusion, the response to N applied as a fertilizer in the field trials was different from that observed in the pot experiment. In the pots, the response to the applied N was high; in the field, much lower. The results seem to be dependent on natural soil N availability and on the variables that master N uptake and plant growth. Thus, olive may not have a particularly low response to N, but it will be the agroecological growing conditions, mainly those defining the yield potential and nutrient removal, which will dictate the quantity of N to be applied.

The olive plants of the different experiments showed very different apparent N recoveries, varying from 13.1% in FieldExp2 to ~100% in N1 treatment of PotExp. Thus, N use efficiency, as indicated by apparent N recovery, seems also to be dependent on the agroecological conditions defining target yield and N loss rather than on the plant species.

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