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Effects of nitrogen deficiency on leaf photosynthesis, carbohydrate status and biomass production in two olive cultivars 'Meski' and 'Koroneiki'

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ABSTRACT

The effects of nitrogen deficiency on CO_2 assimilation, carbohydrate content and biomass were studied in two olive (*Olea europaea* L.) cultivars ('Meski' and 'Koroneiki'). One-year-old plants were grown in pots and subjected to four nitrogen levels for 58 days.

Nitrogen-deficient plants had significant lower leaf nitrogen and chlorophyll *a* contents. They also showed a significant reduction in their photosynthetic capacity. A tolerance difference between cultivars was observed: 'Meski' proved to be more efficient in maintaining CO_2 assimilation rates than 'Koroneiki' under nitrogen deficiency, which was reflected by increased photosynthetic nitrogen use efficiency. Accumulation of carbohydrates, especially starch, mannitol, sucrose and glucose, was observed in nitrogen-deficient leaves. This indicates that both the high carbohydrate and the low nitrogen content inhibit photosynthesis in nitrogen-deprived olive plants. Total biomass was strongly reduced (mainly caused by a decrease in leaf dry weight) under nitrogen deficiency for both cultivars, but root:shoot ratio was hardly affected. Elongation of fine roots was enhanced in 'Koroneiki' under severe nitrogen-deprivation.

1. Introduction

Modern farming requires increasingly fine management of nitrogen (N) fertilisation due to economical and environmental constraints. Moreover, excess fertiliser application is expensive and leads to N losses by leaching with negative impacts on the environment. Fertilisation must, however, be sufficient to provide an optimal final yield and the desired product quality. Thus, the required level of accuracy for fertilisation practices is within 10% (Gastal and Lemaire, 2002).

Olive tree (*Olea europaea* L.) is one of the major crops in the Mediterranean basin with a cultivated area of about 8.2 Mha. Traditionally, fertilisers as well as other crop inputs are applied to olive orchards without considering spatial variability in field characteristics. Such agricultural management might be inefficient due to under-application or over-application of field inputs in specific orchard areas. Under-treated zones will not reach optimum yield levels whereas in over-treated ones there might be a higher risk of environmental pollution and reduced cost efficiency (Bouma, 1997).

N shortage results in a marked decrease in plant photosynthesis in many crops. This is to be expected, because more than half of the total leaf N is allocated to the photosynthetic apparatus (Makino and Osmond, 1991). Photosynthetic capacity and total amount of leaf N per unit leaf area are usually correlated (Field and Mooney, 1986; Sage and Pearcy, 1987; Walcroft et al., 1997).

There is nowadays clear evidence that N deficiency induces sink limitation within the whole plant due to decreased growth (Paul and Foyer, 2001). This leads, in turn, to feedback down-regulation of photosynthesis. N deficiency results in accumulation of carbohydrates (sugars and starch) in the leaves, higher levels of carbon allocated to the roots and an increase in root-to-shoot biomass ratio (Hirai et al., 2004; Scheible et al., 2004; Remans et al., 2006). N deficiency therefore affects, to various extents, primary photosynthesis, sugar metabolism and/or carbohydrate partitioning between source and sink tissues (Paul and Driscoll, 1997; de Groot et al., 2003; Scheible et al., 2004). Although N content in leaves did not correlate with shoot growth, it was negatively correlated with the proportion of carbon allocated to the roots (de Groot et al., 2003; Scheible et al., 2004). Plants indeed constantly sense the changes in their environment and, when mineral elements are scarce, they often allocate a higher proportion of their biomass to the root system (Lawlor et al., 2001). This response is a consequence of metabolic changes

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in the shoot and an adjustment of carbohydrate transport to the roots.

In Tunisia, N deficiency is frequently observed in olive tree orchards, and is responsible for considerable losses of productivity (Braham and Mhiri, 1997). N deficiency is also found to be associated with pistil abortion in olive trees (Morettini, 1950). Nitrogen excess does not increase yield or vegetative growth (Fernández-Escobar et al., 2004), but it negatively affects fruit quality or that of derived products, such as olive oil (Fernández-Escobar et al., 2006). However, in current horticultural practice N is often applied in higher amounts than those needed to ensure a good production (Sánchez et al., 1995).

N fertiliser recommendations for olive trees are traditionally based on soil N status and to a lesser extent on foliar analysis (Boussadia et al., 2006). A rationalization of fertilisation is a prerequisite in the light of the factors up on which productivity depends, namely photosynthesis, translocation of assimilates, growth and production.

To gain a better insight in the optimal use of N fertilisers, one must correctly evaluate the plant's responses to possible deficiencies. This paper therefore aims at evaluating nutritive N deficiency in two olive tree cultivars 'Meski' and 'Koroneiki'. To our knowledge, there is hardly any information available on this functional approach linking olive tree growth with the N status of the leaves. To achieve this goal, repeated measurements were performed in a greenhouse experiment in order to better understand the mechanisms adopted by young olive trees subjected to four levels of N. The effects of N deficiency on chlorophyll concentration, leaf photosynthesis, carbohydrate pools, crop growth and biomass partitioning were quantified in order to assess the differences in response in both cultivars.

2. Materials and methods

2.1. Growth conditions and nitrogen stress treatment

One-year-old olive trees (*Olea* L. 'Meski' and 'Koroneiki') were grown hydroponically in vermiculite (2 l containers) under greenhouse conditions from 22 January till 21 April 2008. Air temperature fluctuated between 20 and 32 °C and the relative humidity of the air ranged between 60 and 70%. Plants were fertigated with a full-strength modified Hoagland's solution (EC = 2.5, pH = 6.5) (Table 1) in a hydroponic system (Hartmann and Brown, 1956). After 34 days of culture, the plants were randomly allocated to four groups and each group consisted of 16 plants (8 plants for each cultivar). Four N levels were provided for 58 days:

100N received a full-strength nutrient solution throughout the experiment (NO_3^- = 23.96 meq l^{-1})
40N reduced N to 40% N (mild nitrogen stress with NO_3^- = 9.58 meq l^{-1})
20N reduced N to 20% N (moderate nitrogen stress with NO_3^- = 4.79 meq l^{-1})
0N reduced N to 0% N (severe nitrogen stress with NO_3^- = 0 meq l^{-1})

Table 1
Modified full-strength Hoagland's nutrient solution (100N) and its adjustments supplied for the N-deprivation treatments (40N, 20N and 0N).

Macronutrients (g/100l)	100N	40N	20N	0N
$Ca(NO_3)_2$	109.6	43.84	21.9	0
KNO_3	27.4	10.96	5.48	0
$MgSO_4$	27.4	27.4	27.4	27.4
NH_4SO_4	13.7	5.48	2.74	0
KH_2PO_4 (MKP)	13.7	13.7	13.7	4.1
EDTA Na Fe	4.1	4.1	4.1	4.1
$CaCl_2{\cdot}2H_2O$	0	48.24	64.34	80.4
KCl	0	11.91	15.88	19.85

All other nutrients were provided as specified by the modified Hoagland's solutions reported in Table 1.

2.2. Gas exchange measurements

Leaf gas exchange measurements were performed weekly on the 8th fully expanded leaf counted from the top of the olive tree using a LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA), along a period of 58 days (from 24 February till 21 April 2008). Measurements took place between 9 and 17 h and were done in 3 replications. The replications were measured, respectively, at 9, 12 and 15 h for each treatment and each cultivar in order to cover as such the daily variability. The photosynthetic active radiation (PAR) was supplied with a 6400-02B LED light source and photosynthetic light response curves were determined by decreasing light intensity from 2000 to 0 μmol m^{-2} s^{-1} in 9 steps. The temperature inside the leaf cuvette was set to 25 °C, and the CO_2 concentration was set to 450 μmol mol^{-1}.

Photosynthetic parameters were obtained by fitting the light response curve to the experimental data of each individual plant according to Drake and Read (1981):

$$A = \frac{A_{max}(I - I_c)\alpha_c}{A_{max} + (I - I_c)\alpha_c}$$

where A and A_{max} are respectively the net assimilation and the maximum net assimilation rate (μmol CO_2 m^{-2} s^{-1}), α_c the quantum efficiency (μmol CO_2 (μmol PAR)$^{-1}$), I the light intensity and I_c the light compensation point (μmol PAR m^{-2} s^{-1}).

The dark respiration rate R_d (μmol CO_2 m^{-2} s^{-1}) was calculated from the relationship describing the light-limited part of the photosynthesis light response curve, characterized by its linear response to increasing PAR intensities:

$$A = \alpha_c I - R_d$$

For $I = I_c$, A equals zero and consequently R_d equals $\alpha_c I_c$ from which α_c can be derived.

After deriving individual plant parameters, mean photosynthetic parameter values for each N treatment level and each cultivar were calculated and used in the analysis.

2.3. Chlorophyll a concentration

Chlorophyll *a* (Chl *a*) concentration was measured at weekly intervals according to Moran (1982). Leaf discs (7 mm × 19.6 mm/plant) of randomly chosen fully expanded leaves for each treatment and cultivar were extracted with N,N-dimethylformamide (DMF) and absorbance was measured at 664 and 647 nm (UV-VIS, Biotek Uvikon XL). Each measurement was done using seven plants selected from each treatment and per cultivar.

2.4. Leaf nitrogen content

Young fully expanded leaves (one leaf per plant) were randomly harvested from eight plants in each treatment and combined into a composite sample, weighed and analyzed. Leaf tissue nitrogen (N) was determined by the Kjeldahl method (Martin-Prével et al., 1984). Photosynthetic nitrogen use efficiency (PNUE) was calculated as A_{max} per unit of foliar N content.

2.5. Leaf carbohydrate concentration

Sugars were extracted with 80% ethanol at 45 °C, followed by centrifugation at 5000 × *g* for 10 min. Glucose, fructose, mannitol and sucrose were analyzed using high pH anion-exchange chromatography with pulsed amperometric detection (Dionex; CarboPac MA1 column with companion guard column; eluent: 50 mM NaOH, 22 °C). The remaining ethanol insoluble material was washed twice with ethanol 80% and the residual pellet was treated with HCl 1 M for 2 h at 95 °C for starch hydrolysis. Starch was determined spectrophotometrically at 340 nm by the enzymatic reduction of $NADP^+$ (UV-VIS, Biotek Uvikon XL).

2.6. Root, stem and leaf dry weight, and specific leaf weight

At the end of the experiment (after 58 days), three plants were randomly harvested per treatment and cultivar. The plants were divided into root, stem and leaf fractions. After measurement of shoot length, length of the longest root and total leaf area (LI-3000C portable area meter, Lincoln, NE, USA), the dry weight (DW) of shoot, leaves and roots was determined after drying at 70 °C for 72 h. Specific leaf weight was calculated as leaf DW per leaf area according to Syvertsen et al. (1980).

2.7. Statistics

Means and standard deviation were calculated. Analysis of variance (ANOVA) was performed using SPSS 16.0; when significant differences occurred, means were separated by the Duncan's multiple range test at $p < 0.05$.

3. Results

3.1. Leaf nitrogen content and chlorophyll a concentration

Leaf N content averaged 2.0% DW for 'Koroneiki' and 1.8% DW for 'Meski' under non-limiting N supply (Fig. 1B and E). For 'Meski', reduced N fertilisation was reflected in the leaf N content 35 days after the start of the treatments resulting in a decrease of respectively 51, 58 and 64% for 40N, 20N and 0N in comparison with 100N (Fig. 1E) at the end of the experiment. For 'Koroneiki', leaf N started to decrease 28 days after reducing the N fertilisation. After 58 days, a decrease of 44, 47 and 55%, respectively, was observed for 40N, 20N and 0N compared to the 100N treatment (Fig. 1B).

Leaf Chl *a* concentration averaged 76 μg cm^{-2} for 'Koroneiki' and 80 μg cm^{-2} for 'Meski' for the 100N treatment (Fig. 1A and D). After 35 days of withholding N from the nutrient solution for 'Meski', the 0N-treated plants started to show significantly lower leaf Chl *a* concentrations compared to the other treatments while it took 49 days for 40N and 20N to decrease significantly from the 100N treatment (Fig. 1D). For 'Koroneiki', Chl *a* concentrations started to decrease after 35 days of N deprivation and declined with 33, 32 and 39% respectively for the 40N, 20N and 0N treatment (Fig. 1A) at the end of experiment.

A significant correlation between the decrease of leaf nitrogen content and Chl *a* concentration was found for the two olive tree cultivars ($R^2_{Meski} = 0.65$, $p = 0.029$; $R^2_{Koroneiki} = 0.67$, $p = 0.025$).

3.2. Leaf photosynthetic response and photosynthetic nitrogen use efficiency

The temporal change in photosynthetic capacity under decreasing N fertilisation is shown in Fig. 1C and F. After 58 days, significant differences between the control and the N-deficient plants for leaf A_{max} were noted in both cultivars (Table 2). Deprivation to 40N reduced leaf A_{max} by 16 and 28% for 'Meski' and 'Koroneiki', respectively, while for 20N leaf A_{max} was 27 and 49% less and for 0N leaf A_{max} was 34 and 55% less than the control plants. Also R_d was significantly reduced by N deprivation for 'Koroneiki', while for 'Meski' R_d tended not to be affected (Table 2). Finally, α_c decreased significantly for both cultivars up to 67 and 31% for 'Koroneiki' and 'Meski' under 0N, respectively (Table 2).

A correlation analysis of A_{max} and N content of the leaves (Fig. 2) showed that for 'Meski' A_{max} was hardly influenced by different leaf N levels varying between 1.7 and 2.5%. Only for values lower than 1.7% A_{max} slightly decreased. For 'Koroneiki', however, a positive correlation between A_{max} and N content levels up to 2% N was found ($R^2 = 0.61$, $p < 0.001$) by a non-linear regression. This linear relation is also reflected in the constant PNUE values between the treatments for 'Koroneiki' (Fig. 3). For 'Meski', however, no linear relation was found in the data which could explain the increase in PNUE compared to control plants (Fig. 3). After 58 days of N deprivation, an average significant increase of PNUE of 45% was found in 'Meski' for the treatments compared to 100N.

3.3. Soluble sugars and starch under nitrogen deficiency

For both cultivars N-deficient leaves accumulated significant higher concentrations of starch (Table 3). 'Koroneiki' also accumulated higher concentrations of mannitol and glucose under N deprivation. Fructose is hardly present in both cultivars and tends to increase under N deprivation for 'Koroneiki'. Sucrose levels in 'Koroneiki' showed a moderate though not significant increase under N deprivation.

3.4. Plant growth and dry matter accumulation

After 58 days of reduced N fertilisation, plant growth was significantly inhibited although cultivar differences were observed (Table 4).

For 'Koroneiki', shoot length did not differ between 100N, 40N and 20N, but plants grown at 0N were 15.6% shorter than control plants ($p < 0.01$). For 'Meski', 20N and 0N significantly reduced shoot length. The root length of 'Meski' was not affected by the treatments, but for 'Koroneiki' root length increased by 52% under severe N deprivation (0N). Total leaf area as well as number of leaves were reduced under N deprivation for 'Meski' compared to control plants. A similar although not significant trend was observed for 'Koroneiki' (Table 4).

Root and stem dry weights were not significantly affected by the imposed N deficiency in both cultivars (Table 5). N deficiency had, however, a pronounced effect on leaf DW of 'Koroneiki'. The 40N, 20N and 0N treatments reduced leaf DW respectively by 29, 37 and 28% compared to 100N. The leaf DW of 'Meski' also decreased significantly to 46% for 0N compared to non-limiting N fertilisation.

4. Discussion

In arid regions, drought stress and N deficiency occur mostly simultaneously and the effects on crop production are often confounded. In this study, we assessed the effect of leaf N on parameters related to photosynthesis and N supply on growth and biomass allocation in 2 olive cultivars grown under non-limiting water supply.

N deficiency affected several components of the carbon metabolism in the olive trees. The response of leaf photosynthesis is largely dependent on the leaf N content. Low levels of leaf N reduced both Chl *a* concentration and A_{max}. These results agree with earlier reports in sorghum (Muchow and Sinclair, 1994) and

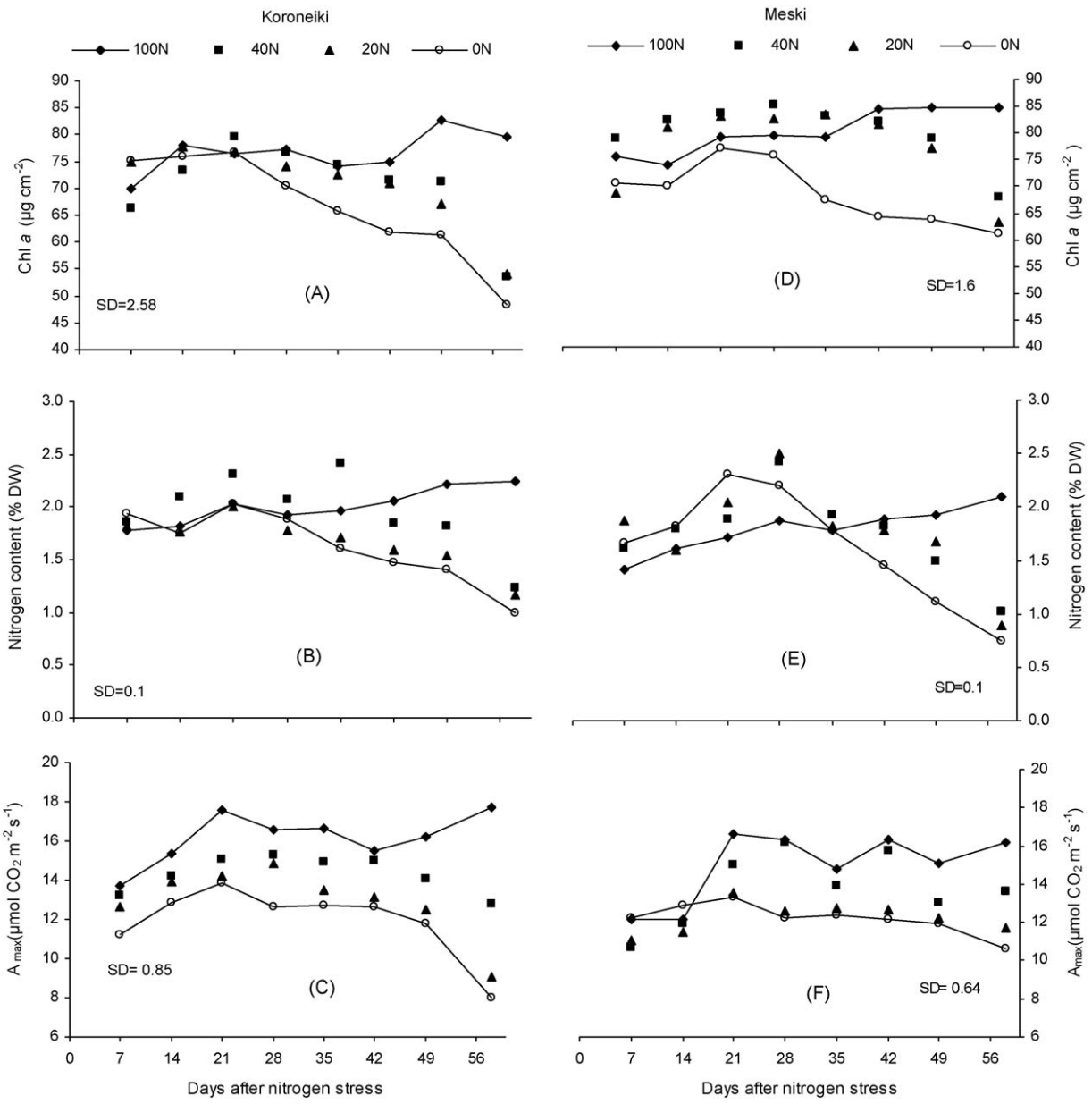

Fig. 1. Change in olive tree leaf chlorophyll concentration (Chl *a*), nitrogen content and net photosynthetic rate (A_{max}) during the nitrogen (N) deficiency treatment, in 'Koroneiki' and 'Meski' olive trees. Values are the means along measurement dates and treatments.

corn (Wolfe et al., 1988; Settimi and Maranville, 1998; Zhao et al., 2003). Leaf N content indeed reflects partly the investment in photosynthetic proteins like Rubisco and the effects of N deficiency on Rubisco are often larger than those on Chl *a*. Effects of N fertilisation on Chl *a* concentration of plants are also reported elsewhere (Ferrar and Osmond, 1986; Evans and Terashima, 1987; Sage and Pearcy, 1987; Seemann et al., 1987; Terashima and Evans, 1988; Sugiharto et al., 1990; Saidana et al., 2009).

In our study N shortage resulted in a marked decrease in plant photosynthesis. The relationship between N content and A_{max} showed that the saturation N level for leaf photosynthesis is found for N content levels above 2% DW and 1.7% DW for 'Koroneiki' and

Table 2
Effects of nitrogen (N) supply on leaf maximum net assimilation rate (A_{max}, µmol CO_2 m^{-2} s^{-1}), dark respiration (R_d, µmol CO_2 m^{-2} s^{-1}) and quantum efficiency (α_c, µmol CO_2 (µmol PAR)$^{-1}$) of olive leaves measured 58 days after the start of the treatment.

	Koroneiki			Meski		
	A_{max}	R_d	α_c	A_{max}	R_d	α_c
100N	17.7 ± 1.12^a	1.17 ± 0.10^a	0.065 ± 0.00^a	16.20 ± 0.80^a	1.12 ± 0.14^a	0.040 ± 0.00^a
40N	12.8 ± 1.04^b	1.02 ± 0.21^a	0.029 ± 0.00^b	13.60 ± 0.64^b	1.43 ± 0.08^a	0.030 ± 0.01^b
20N	9.1 ± 0.44^c	0.53 ± 0.21^b	0.025 ± 0.00^c	11.75 ± 0.53^c	1.38 ± 0.15^a	0.030 ± 0.01^b
0N	8.0 ± 0.80^c	0.36 ± 0.13^b	0.020 ± 0.01^c	10.60 ± 0.53^c	1.56 ± 0.18^a	0.030 ± 0.01^b

Data are mean ± SD of three replicates.
a, b: significantly different – Duncan ($p = 0.05$).

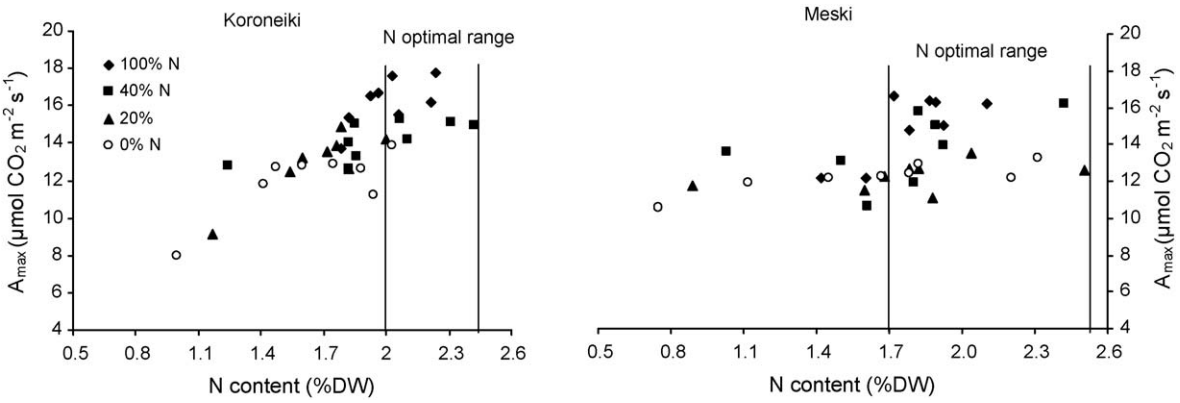

Fig. 2. Relationship between nitrogen (N) content and leaf maximum net assimilation rate (A_{max}) under nitrogen deficiency for 'Koroneiki' and 'Meski' olive trees.

'Meski', respectively (Fig. 2). Also, 'Meski' maintained better a high photosynthetic activity under reduced N.

Substantial differences between both cultivars were found in their PNUE. 'Meski' showed an increase in PNUE when N leaf content was limited (after 58 days, Fig. 3) while PNUE remained constant for 'Koroneiki'. The latter indeed showed a linear relationship through the origin between leaf A_{max} and leaf N up to values of 2%, while the following curvilinear relationship indicated a decrease in PNUE with increasing N availability (Field and Mooney, 1986). 'Meski', hence, showed an improvement of the N budget in its leaves than 'Koroneiki' and might therefore be better adapted to N shortages.

R_d provides energy for growth and maintenance of the existing cell structures (Wilson, 1982). The decrease in R_d of 'Koroneiki' N-deficient plants is more important than the A_{max} reduction (Table 2). Decreased R_d under N stress was also observed for spinach leaves showing N deficiency symptoms (Bottrill et al., 1970). This might explain that no energy remains for new growth, but is directed to maintain reduced leaf biomass for 40N, 20N and 0N plants. R_d for 'Meski', however, tended to increase (although not significantly) which might explain the higher ability of 'Meski' to maintain the leaf biomass of 40N and 20N plants to the same level as 100N.

The starch content was higher in N-deficient than in control leaves for both olive cultivars. This is also observed in other species (Paul and Foyer, 2001). The elevated starch level in N-deficient leaves in this study may result from the decreased demand for carbon in the shoot apical zone due to leaf growth inhibition (leaf area) and leaf initiation, as was the case for 'Meski'. Because starch is the major storage carbohydrate in olive tree leaves (Braham, 1984; Msallem and Hellali, 1988) and because a corresponding, though not significant, increase in specific leaf weight in N-deficient leaves was observed (Table 5) it might be that starch accumulation contributes to the specific leaf weight. Despite feedback down-regulation of photosynthesis, the accumulation of excessive amounts of starch, however, may disrupt chloroplast structure, leading to lower CO_2 assimilation and Chl concentration (Cave et al., 1981). This might also be the case in this study as indicated by the decreased Chl *a* concentration at the end of the experiment (Fig. 1).

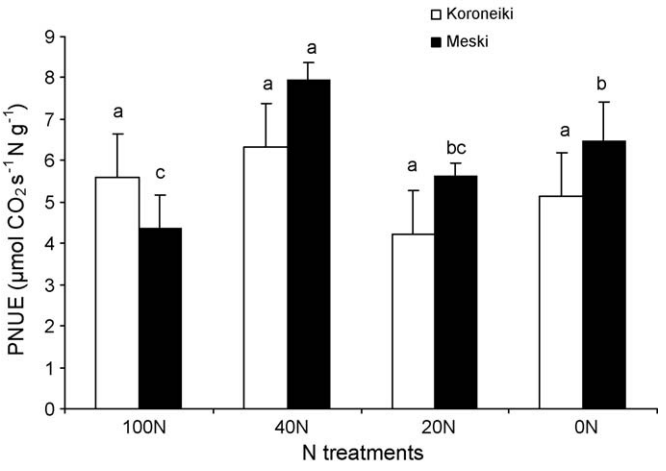

Fig. 3. Effect of nitrogen (N) deficiency on photosynthetic nitrogen use efficiency (PNUE) in 'Koroneiki' and 'Meski' olive trees calculated 58 days after the start of the N supply treatments. Data are mean values ± standard deviations of three replicates. a, b: significantly different – Duncan ($p = 0.05$).

There often exists a more pronounced down-regulation of photosynthesis in starch-accumulating species when sink capacity is limiting (Goldschmidt and Huber, 1992) which is indicated in this experiment by reduced shoot growth and leaf area (Table 4). Previous work on citrus has shown that girdling and defruiting may increase carbohydrates, particularly starch, and decrease photosynthesis (Iglesias et al., 2002). Goldschmidt and Huber (1992) investigated the relationship between feedback inhibition of photosynthesis caused by girdling and the type of carbohydrates accumulated in the leaves of a wide range of vegetable crops. They observed that high-invertase-type species next to starch-accumulating species showed a marked decrease in maximum photosynthetic capacity. In our research not only starch but also glucose, mannitol and sucrose contents were higher in N-deficient than in control leaves for 'Koroneiki'. So, the reduction of photosynthesis in N-deficient olive plants is also a direct consequence of sugar next to starch accumulation, because they both exert metabolite feedback regulation (Koch, 2004; Bläsing et al., 2005).

There is also clear evidence that N deficiency induces sink limitation within the whole plant due to decreased growth (Paul and Driscoll, 1997; Logan et al., 1999; Paul and Foyer, 2001). Decreased plant biomass production due to N shortage was associated with reductions in both leaf area (Saidana et al., 2009) and leaf photosynthetic capacity (Sinclair, 1990). N deficiency suppressed olive shoot growth and dry matter accumulation, especially leaf DW, in both cultivars (Table 5) and this latter was mainly attributed to a reduced total leaf area (Tables 4 and 5). Reduced leaf area was caused by a delay in leaf initiation, but also by leaf abscission visually observed under N deprivation.

Olive trees deficient in N improve their ability to acquire this nutrient by altering their carbon partitioning to favour root elongation as was observed for 'Koroneiki' under severe N deprivation (Table 4). These young roots were very fine and did not influence the total root DW. Therefore no effect on root/shoot ratio was found after 58 day of N-deficient treatment for both cultivars (Table 5).

Table 3
Effects of nitrogen (N) supply on starch, mannitol, glucose, fructose and sucrose (mg (g DW)$^{-1}$) of olive leaves measured 58 days after the start of the treatment.

	Koroneiki				Meski			
	100N	40N	20N	0N	100N	40N	20N	0N
Starch	0.38 ± 0.08^{d}	0.54 ± 0.15^{c}	0.41 ± 0.13^{b}	0.72 ± 0.01^{a}	0.35 ± 0.02^{c}	0.50 ± 0.04^{b}	0.68 ± 0.07^{a}	0.53 ± 0.14^{ab}
Mannitol	1.25 ± 0.10^{c}	1.63 ± 0.07^{a}	1.36 ± 0.02^{c}	1.48 ± 0.03^{b}	1.40 ± 0.18^{a}	1.46 ± 0.10^{a}	1.57 ± 0.14^{a}	1.42 ± 0.06^{a}
Glucose	1.57 ± 0.25^{c}	1.72 ± 0.37^{bc}	2.25 ± 0.30^{ab}	2.42 ± 0.32^{a}	1.42 ± 0.47^{a}	1.32 ± 0.68^{a}	1.47 ± 0.25^{a}	1.36 ± 0.24^{a}
Fructose	0.01 ± 0.01^{b}	0.01 ± 0.01^{b}	0.04 ± 0.01^{a}	0.03 ± 0.01^{ab}	0.04 ± 0.00^{a}	0.03 ± 0.01^{ab}	0.04 ± 0.03^{b}	0.03 ± 0.01^{ab}
Sucrose	0.51 ± 0.12^{a}	0.60 ± 0.04^{a}	0.63 ± 0.04^{a}	0.68 ± 0.10^{a}	0.38 ± 0.03^{a}	0.37 ± 0.02^{a}	0.41 ± 0.05^{a}	0.41 ± 0.04^{a}

Data are mean ± SD of three replicates.
a, b: significantly different – Duncan ($p = 0.05$).

Table 4
Effects of nitrogen (N) deficiency on shoot and root length, leaf area and number of leaves olive plants 58 days after the start of the treatment.

	Shoot length (cm)	Leaf area (cm^2)	Number of leaves	Root length (cm)
Koroneiki				
100N	92 ± 4.0^{b}	1037 ± 396.9^{a}	333 ± 147.8^{a}	32 ± 2.0^{b}
40N	99 ± 9.0^{b}	740 ± 179.7^{a}	299 ± 72.0^{a}	25 ± 4.0^{c}
20N	95 ± 2.2^{b}	587 ± 162.3^{a}	259 ± 94.8^{a}	22 ± 4.0^{c}
0N	78 ± 2.0^{a}	660 ± 37.5^{a}	274 ± 15.6^{a}	67 ± 3.0^{a}
Meski				
100N	85 ± 1.0^{a}	388 ± 45.5^{a}	113 ± 13.2^{a}	23 ± 0.5^{a}
40N	82 ± 1.1^{ab}	342 ± 71.1^{ab}	96 ± 1.4^{ab}	22 ± 1.0^{a}
20N	78 ± 5.0^{b}	267 ± 37.5^{b}	91 ± 1.4^{b}	23 ± 0.5^{a}
0N	60 ± 2.0^{c}	168 ± 44.2^{c}	65 ± 17.1^{c}	24 ± 0.3^{a}

Data are mean ± SD of three replicates.
a, b: significantly different – Duncan ($p = 0.05$).

Table 5
Effects of nitrogen (N) supply on leaf, stem and root dry weight (DW), root/shoot ratio and specific leaf weight of olive plants 58 days after the start of the treatment.

	Root DW (g plant^{-1})	Stem DW (g plant^{-1})	Leaf DW (g plant^{-1})	Root/shoot ratio	Specific leaf weight (g DW m^{-2})
Koroneiki					
100N	13.00 ± 1.41^{a}	12.75 ± 1.06^{a}	16.00 ± 2.83^{a}	0.45 ± 0.01^{a}	143.60 ± 23.12^{a}
40N	12.50 ± 2.60^{a}	13.66 ± 4.37^{a}	11.33 ± 2.57^{b}	0.54 ± 0.27^{a}	153.47 ± 5.59^{a}
20N	11.50 ± 2.12^{a}	14.25 ± 1.06^{a}	10.00 ± 2.12^{b}	0.48 ± 0.15^{a}	165.27 ± 16.50^{a}
0N	10.00 ± 0.71^{a}	12.00 ± 0.71^{a}	11.50 ± 2.12^{b}	0.42 ± 0.00^{a}	171.19 ± 18.12^{a}
Meski					
100N	6.16 ± 0.29^{a}	7.16 ± 0.58^{a}	6.83 ± 0.58^{a}	0.44 ± 0.03^{a}	176.72 ± 9.14^{a}
40N	5.25 ± 2.12^{a}	7.25 ± 0.35^{a}	6.00 ± 0.02^{a}	0.40 ± 0.20^{a}	156.67 ± 1.23^{a}
20N	$5.25 \pm 0.35a^{a}$	6.50 ± 0.71^{a}	5.50 ± 0.71^{a}	0.44 ± 0.03^{a}	223.02 ± 17.89^{a}
0N	4.83 ± 1.04^{a}	6.83 ± 0.29^{a}	3.66 ± 1.44^{b}	0.47 ± 0.12^{a}	212.03 ± 30.60^{a}

Data are mean ± SD of three replicates.
a, b: significantly different – Duncan ($p = 0.05$).

5. Conclusions

N deficiency caused a decrease in leaf N content, Chl *a* and carbon assimilation of olive plants, resulting in a lower dry matter accumulation. Decreased photosynthetic capacity is not only associated with direct effects of N deficiency but also with a negative feedback mechanism from the leaf carbohydrate pool. Indeed both cultivars showed an important increase in starch and for 'Koroneiki' also some sugars (glucose and mannitol) accumulated. Total dry matter was reduced mainly caused by a decrease in leaf DW. The shoot:root ratio was hardly affected although root elongation in N deprived 'Koroneiki' was observed. The ecophysiological and biochemical parameters used in this experiment (A_{max}, PNUE, Chl *a* and carbohydrate accumulation in leaves) suggest that 'Meski' is more efficient than 'Koroneiki' under N deficiency. This approach could be further exploited for cultivar selection.

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