

Title page**Olive yields and tree nutritional status during a four year period without nitrogen and boron fertilization**

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Abstract.

Nitrogen (N) and boron (B) are mobile elements in soil. Therefore, the application of these nutrients is typically performed annually, as a single dose, or even splitting it into several fractions in the case of N. In olive (*Olea europaea* L.), however, controversial literature has suggested that yearly application of N may not be required. In the case of B some authors indicated that one single application is sufficient for three or four years. Thus, the effect of these elements on olive yield, leaf N and B concentrations, as well as soil available N and B were investigated during a field trial performed in an olive orchard located in NE Portugal, in which N and B were not applied for four consecutive growing seasons. Fertilizer treatments consisted of the following: the control, which was a 'complete' fertilization plan where N and B were included (N+B treatment); -N treatment, with N excluded from the fertilization plan; and -B treatment, with B excluded. Available soil N and B were estimated from a pot experiment with Italian ryegrass (*Lolium multiflorum* L.) and from chemical laboratory extractions. Olive yield decreased significantly in the -N treatment in comparison to the control. A slight yield reduction in the -B treatment in comparison to the control was also observed. Leaf N and B concentrations decreased significantly in the -N and -B treatments, respectively, in comparison to the N+B treatment. Soil available N and B at the end of the experiment were significantly lower in the -N and -B treatments, respectively, in comparison to the N+B control. The results showed a continuous decrease in olive yield and leaf N and B concentrations, which reflected the reduction in soil available N and B in the treatments lacking the respective nutrient. Therefore, it seems prudent the recommendation of adjusted rates of N and B every year to prevent reduction in tree crop performance and improve nutrient use efficiency.

Keywords: Boron fertilization; Nitrogen fertilization; *Olea europaea* L; Soil boron availability; Soil nitrogen availability.

INTRODUCTION

The olive and olive oil sector has a huge social and economic importance in north-eastern Portugal. The orchards of the region are often planted on steep slopes and in low fertility soils. The rainfed orchards comprise 95% of the total harvested area, and low precipitation during the summer season is the main limiting factor for tree crop growth and olive yield. The profitability of rainfed olive orchards is currently marginal and, therefore, production costs should be reduced to the minimum. Although fertilizers may improve olive yields, the economic return of such fertilizing practices is unknown. Nitrogen and B are nutrients that are frequently included in the fertilization programs advised by the regional labs of soil testing and plant analysis. As N and B are mobile elements in soils, fertilization practices should strive to improve the efficiency of N and B utilization by the olive tree.

Nitrogen is the most limiting nutrient for plant growth in natural ecosystems and cultivated fields (Stevenson 1986). Naturally available N in cropped soils is not usually sufficient to ensure the desired productivity. Therefore, the application of N fertilizers to agricultural fields is practiced worldwide. In olive, N is also the most frequent nutrient applied as a fertilizer (Hartmann et al. 1966; Fernández-Escobar 2001; Connell and Vossen 2007). Furthermore, excessive N fertilization appears to be common among olive growers in Spain (Fernández-Escobar 2001). Olive growers in NE Portugal usually apply N fertilizers, but the applied rates are often low, though infrequent excess nutrient amounts may occur.

In a review of earlier studies that were performed in California, Italy, Spain, and other countries during the 1940s and 1950s, Hartmann et al. (1966) indicated that olives significantly responded to N application, particularly in low fertility soils. More recently, Jasrotia et al. (1999b) and Marcelo et al. (2004) also reported positive effects of N fertilizer application on fruit set and olive yield. However, other studies have demonstrated no increase in crop growth or olive yield by N sufficient trees following N fertilization (Fernández-Escobar and Marin 1999; Connell et al. 2002; García-Novelo et al. 2002; García-Novelo et al. 2004). Based on the lack of response to N fertilizers, Fernández-Escobar and Marin (1999) and García-Novelo et al. (2004) concluded that annual application of N fertilizer to olive orchards is not necessary to obtain good productivity when leaf N is above the sufficiency threshold.

Boron deficiency in crops is more widespread than deficiency for any other element (Shorrocks 1997). In olive, B deficiency is also a common nutritional disorder (Hartmann et al. 1966; Tsadilas and Chartzoulakis 1999; Soyergin et al. 2002; Freeman et al. 2005). Boron is usually included in the group of nutrients (N, K, and B) that are frequently deficient in commercial olive orchards (Freeman et al. 2005). Visual symptoms of B deficiency in olive trees are not uncommon in NE Portugal, despite the increase in B fertilizer use during recent years. A previous study by Vale (1988) demonstrated that there is a shortage of B in many soils of NE Portugal.

Boron requirements differ among plant species. Olive is considered to be a crop with a high demand for B (Fernández-Escobar 2001). Olive is also much less sensitive to higher B levels than other commercial fruit tree crops (Freeman et al. 2005). In the majority of plant species, B distribution between plant organs as well as symptoms of B deficiency and toxicity has indicated that B has restricted mobility (Brown and Shelp 1997). Despite the restricted mobility of B in some plant species, B is freely mobile in

other species. Indeed, according to Brown and Shelp (1997), no other element is known to vary as significantly in mobility. Boron appears to be remobilised from leaves to adjacent organs, such as flower buds and fruits, in olive (Delgado et al. 1994; Perica et al. 2002). Evidence also exists that B deficiency could be corrected through the application of foliar sprays (Perica et al. 2002; Connell and Vossen 2007). Therefore, the mobility of B in a species will determine the strategy utilised to supply B to deficient crops.

Boron is distributed in various soil components, including the soil solution, organic matter, and minerals (Tsadilas et al. 1994; Goldberg 1997; Xu et al. 2001). Boron utilization by plants is controlled by the soil solution B level, rather than the total B content of the soil (Yermiyahu et al. 2001). Boron in soil solution is readily available for plant uptake, but this pool constitutes < 3% of total soil B (Xu et al. 2001). Therefore, the strategy chosen for B supply must ensure an adequate B level in soil solution. Since B is a micronutrient element that plants require in trace amounts (Goldberg et al. 2002), some authors have indicated that one single B application should be sufficient for many years (Hartmann et al. 1966; Freeman et al. 2005; Connell and Vossen 2007).

An annual application ensuring a proper absorption rate of these elements is a logical strategy to improve nutrient use efficiency, since N and B are mobile elements that could be leached from soils by winter rains. In this study, the olive yields of groups of olive trees, which were grown without N or B fertilizers for four years, were compared with a control treatment of an annual application of N and B. The effects of fertilizer treatments on leaf N and B concentration were determined. Soil samples were collected at the end of the experiment to determine available soil N and B. For indicators of soil fertility, a pot experiment with Italian ryegrass was performed to

determine biomass yield as well as N and B recovery. The potentially available soil N and B was also determined using chemical laboratory methods.

MATERIALS AND METHODS

Field trial

The field trial was performed in Suçães, NE Portugal (41° 31' N; 7° 12' W), in a commercial dry-farmed olive orchard, cv. Verdeal Transmontana, from October 2003 to January 2008. The mean annual temperature and precipitation in the region are 14.2 °C and 520 mm. The annual rainfall and monthly temperature of the years preceding the five harvests are presented in figure 1. The orchard was planted in a Leptosol derived from schist. In October 2003, soil organic carbon was 6.9 g kg⁻¹, pH (soil/water, 1:2.5) was 4.6, extractable phosphorus (Egner-Rhiem) was 10 mg kg⁻¹, extractable potassium (Egner-Rhiem) was 110 mg kg⁻¹, exchangeable calcium (ammonium acetate, pH 7) was 4.7 cmol_c/kg, exchangeable magnesium (ammonium acetate, pH 7) was 0.7 cmol_c/kg, and soluble B (boiling-water and azomethine-H procedure) was 0.30 mg kg⁻¹.

The orchard was divided into three plots for the different fertilizer treatments. Groups of eight similar trees were tagged before the first harvest in the autumn of 2003. The objective was to reduce experimental variability associated with the different size of the trees, which is a very common feature of dry-farmed orchards. The initial harvest occurred in January 2004, before establishment of the fertilizer treatments. The three fertilizer treatments were: control (N+B), which was the 'complete' fertilization plan; – N treatment, which was the control minus N; and –B, which was the control minus B. The 'complete' fertilization plan included: nitrogen, 520 g tree⁻¹ as ammonium nitrate (26% N); phosphorus, 101 g tree⁻¹ as superphosphate (18%, P₂O₅); potassium, 398 g

tree⁻¹ as potassium chloride (60% K₂O); magnesium, 59 g tree⁻¹ as magnesium sulphate (49% MgO); and boron, 16 g tree⁻¹ as borax (11% B). In the first year, calcium (300 g tree⁻¹) was also administered as lime (75% CaCO₃). The fertilizers were ground-applied under the projection of the tree canopies. The first fertilizer application was performed in March 2004. N and B were reduced to half of the pre-defined rate in March 2005, due to the extremely dry winter during the previous year (Fig. 1) and the dry weather forecast for the upcoming months. The fertilizers were applied again according to the 'complete' fertilization plan during 2006 and 2007. In all the years the fertilizers were incorporated by 15 cm depth soil tillage. The harvest was performed in January by a trunk shaker machine and the crops were recorded per individual tree.

Leaf samples were collected in January of 2005, 2006, 2007, and 2008 and in July of 2005 and 2006. Mature leaf samples were collected from the middle portion of the current-season shoots, which were equally distributed around the tree at approximately 1.8 m height. A total of four leaf samples were randomly collected in the eight trees for each fertilizer treatments. The leaf samples were oven-dried at 70 °C and ground. Tissue analyses were performed using Kjeldahl (N), colourimetry (B and P), flame emission spectrometry (K), and atomic absorption spectrometry (Ca, Mg) methods (Walinga *et al.* 1989).

Pot experiment

In October of 2007, soil cores were collected from the three fertilizer plots beneath the tree canopies and from two different depths (0-5 and 5-20 cm). The soil samples were air dried and passed through a 2 mm sieve. Five replicates (five pots) of 1 kg dry soil were prepared for every soil depth from each fertiliser treatment. A total of 50 seeds of Italian ryegrass were sown per pot and the plants grown in a greenhouse for three

months. Two cuts were performed until the crop growth was halted by exhaustion of the available nutrients. The biomass was dried at 70°C and weighed. The dry matter of the two cuts was mixed and analysed for N and B, using the previously described methods for the olive leaf samples.

Chemical nitrogen and boron extractions

The potential soil N availability was determined from the same soil samples collected in the pot experiments using the NaOH- and Kjeldahl-extractable N procedures. The soil B availability was determined by the Azomethine-H method. For NaOH-extractable N, the methodology was almost identical to the procedure described by Sharifi et al. (2007). In summary, 5 grams of soil with 20 ml of NaOH (50%) and 20 ml of distilled water were directly distilled into a Kjeltex Auto 1030 Analyser. The Kjeldahl method consisted of digestion of 1 g of soil with H₂SO₄ and selenium as the catalytic agent in a heated (400°C) aluminium digestion block. After cooling, the suspension was distilled with alkali and the NH₄⁺-N in the digest was titrated with HCl in a Kjeltex Auto 1030 Analyzer (Bremner 1996). Boron was extracted by hot water and determined from a colourimetric procedure. The extraction occurred in sealed pouches with 0.01 M CaCl₂ in order to minimise the positive error due to yellow coloration. The extracts were analyzed by azomethine-H, which is a complexing agent of B(OH)₃ in aqueous media (Keren 1996).

Data analysis

The effects of the fertilizer treatments on olive yield, leaf nutrient concentration, and soil fertility parameters were analyzed by comparing the mean confidence limits ($\alpha < 0.05$) of the measurements determined from each fertilizer treatment.

RESULTS

Initial harvest

The harvest of January 2004, which was performed prior to the first fertilizer applications, produced significant olive yield differences among the plots of the future fertilizer treatments. The olive tree plot, which was thereafter subjected to the –B treatment, produced statistically higher crops than the other treatments. The mean olive yields were 22.1, 21.3, and 26.3 kg tree⁻¹ in the future N+B, –N, and –B treatments, respectively, which indicates that the groups of the pre-selected trees presented dissimilar olive yield potential. Therefore, crops in the following years were expressed in relative yields, i.e., as the percentage of the initial harvest, instead of in absolute terms as kg per tree.

Olive yield

The relative olive yields obtained in the three plots corresponding to the different fertilizer treatments are presented in figure 2. The initial harvest corresponded to an ‘on’ year, and the relative olive yields of each plot were converted to 100%. In 2005 the olive yields were poor. The relative olive yields ranged from 25.2 to 34.8% and there were no significant differences among treatments. The season of 2006 was expected to be an ‘on’ year, according to the biennial cycle of olive production. Flowering and fruit set occurred normally in this year. However, the severe drought of the previous growing seasons (Fig. 1) did not allow for normal fruit development. Many small fruits persisted on the tree until the autumn, but these fruits had no commercial value. The production of this orchard was essentially zero in 2006. In 2007 a mean relative olive yield of

95.3% was observed in the control N+B treatment. The relative olive yields in the –B and –N treatments were 88.9 and 74.4%, respectively. The relative olive yield in the –N treatment was significantly lower than that in the control treatment. The 2008 crops were lower than the 2007 crops. The relative olive yields in 2008 were 63.2, 56.5 and 24.6% in N+B, –B, and –N treatments, respectively. The relative yield in the –B treatment was slightly lower than, but not statistically different from the N+B treatment. The relative olive yield in the –N treatment decreased dramatically in 2008 to a mean value that was significantly lower than the value recorded in the N+B treatment.

Tree crop nutritional status

The mean leaf N concentrations in the N+B treatment were in the range of 16.2 to 18.0 g kg⁻¹ throughout the six leaf sampling dates (Fig. 3a). The mean leaf N concentrations in the –N treatment were lower than in the N+B treatment and decreased consistently over time. The mean leaf N concentrations in the –N treatment were 16.9, 15.7, 14.9, and 14.1 g kg⁻¹ for the winter sampling dates in 2005, 2006, 2007 and 2008, respectively. The differences in the mean leaf N concentrations between N+B and –N treatments were even higher in summer samples than in winter. The mean leaf N concentrations in the summer samples of the –N treatment decreased below the adequate range of 15 mg kg⁻¹ (Fig. 3a).

The mean leaf B concentrations in the control treatment ranged from 22.7 and 27.4 mg kg⁻¹ in the winter samples and from 25.6 and 31.7 mg kg⁻¹ in the summer samples (Fig. 3b). The mean leaf B concentrations in the –B treatment were lower than the concentrations of the control treatment. The differences were statistically significant for the winter samples of 2006, 2007, and 2008. The variation over time of the mean

leaf B concentration in the –B treatment were 26.4, 22.7, 19.2, and 20.3 mg kg⁻¹ for the winter samples of 2005, 2006, 2007, and 2008, respectively.

No coherent patterns were observed in regards to leaf P, K, Ca and Mg concentrations, which could be directly related to the fertilization treatments included in the experimental design. Leaf P, K, Ca, and Mg concentrations varied greatly among sampling dates, which was probably influenced by weather variables and alternate bearing cycles of olive. Data is not shown, since there was no relevant trend determined from this analysis.

Pot experiment

Soil from the N+B treatment produced significantly higher Italian ryegrass dry matter than soil from the –N treatment for both the 0-5 cm and 5-20 cm layers (Fig. 4a). Nitrogen concentration in tissues was also higher for the plants grown in the N+B treatment (Fig. 4b). As a consequence of the cumulative effect of the aforementioned results, N recovered by Italian ryegrass was markedly higher in the plants from N+B treated soil in comparison to the –N treated soil (Fig. 4c).

Italian ryegrass dry matter produced in the pots with soil from the N+B treatment was significantly higher than that produced by plants in the soil from the –B treatment for the 5-20 cm layer (Fig. 5a). The B concentrations in tissues were markedly higher in the plants developed in the soil of the N+B treatment than those developed in the soil of the –B treatments for both the 0-5 cm and 5-20 cm layers (Fig. 5b). The B concentration in tissues significantly influenced plant B recovered, which was also significantly higher in the plants that grew in the soil derived from the N+B treatment (Fig. 5c).

Chemical extractions

The soil of the N+B treatment released more N than the soil of the -N treatment when subjected to extractions from the Kjeldahl and NaOH methods (Fig. 6a,b). For the extraction procedures, the upper layer released more N than the deeper layer. This result agreed with N recovered by Italian ryegrass in the pot experiment. Generally, the results from the pot experiments were also in agreement with N concentration in olive leaves and with the yields recorded in the later harvests of the experiment. Boron extracted by the Azomethine-H method was significantly higher in the soil sampled in the N+B treatment than the -B treatment (Fig. 7). Therefore, there was a general agreement in B extracted by the Azomethine-H method, B recovered by Italian ryegrass, B concentration in olive leaves, and olive yields, albeit to a lesser extent.

DISCUSSION

Olive yield and plant and soil N availability indices

The crops followed the typical alternate bearing pattern that is widely known for olive (Hartmann et al. 1966; Fernández-Escobar et al. 1999; Sibbett and Ferguson 2002). Weather components may have an important role in olive yields, as reported by Singh et al. (1999). An extreme weather phenomenon was observed during the growing seasons of 2004 and 2005, which resulted in a reduction of olive yields to zero in the harvest of January 2006. Olive yields decreased progressively in the treatment without N application in comparison to the control N+B treatment. The crops from the -N treatment dropped markedly in the last harvest. These results agreed with previous reports by Hartmann et al. (1966), Jasrotia et al. (1999b), and Marcelo et al. (2004), which indicated the positive effect of N application on olive yield. The result is also in

agreement with the general statement that N is the most critical nutrient in olive fertilization (Fernández-Escobar 2001; Freeman et al. 2005; Connell and Vossen 2007).

Leaf N concentrations decreased consistently throughout the experimental period in the summer and winter samples of the trees that were not N fertilized. In the -N treatment, leaf N concentrations decreased below the lower limit of the adequate range of 15 g kg⁻¹. In the N+B treatment, the leaf N concentration was stable within the adequate range in response to regular soil N application. The main goal of N fertilization is to maintain leaf N levels within the adequate range of 15 to 20 mg kg⁻¹ (Fernández-Escobar 2001; Freeman et al. 2005). Therefore, the low olive yields recorded in the later harvests from the -N treatments may primarily result from the poor N nutritional status of olive trees. This result corroborates a previous study by Jasrotia et al. (1999a), which reported a positive relationship between leaf N status and olive yield based on data from several orchards in Greek Islands.

Nitrogen recovered by regrass clearly demonstrated that the soil from the fertilized plots that were not N fertilized released much less N than the soil that was annually supplied with the nutrient. This is an important result, since pot experiments involving annual species with high demand for N have great sensitivity to stress differences between fertiliser treatments (Rodrigues et al. 2006). The data obtained from the chemical methods are also in agreement with the results of the pot experiment. At the end of the experiment, the soil from the N+B treatment presented a higher N release potential than the soil from the -N treatment. The Kjeldahl method produced estimates of the total N (organic + NH₄⁺) in soil. The NaOH-extractable N most likely includes NH₄⁺, α-amino N, and hydrolysable unidentified N fractions (Sharifi et al. 2008). Therefore, this result demonstrated that total N and the most labile N fractions in soil

were significantly lower in the –N treatments compared to the N+B treatments, which influenced N availability for the olive trees.

Olive yield and plant and soil B availability indices

Four consecutive growing seasons without B application did not produce a statistically significant olive yield reduction, in comparison to the regular soil B application. However, the mean olive yields during the two last harvests were slightly lower in the –B treatment. Leaf B concentration was more sensitive to the lack of B supply than olive yield. Throughout the experimental period, the mean leaf B levels became progressively lower in the –B treatment in comparison to the N+B treatment. Soil B application is an efficient means of maintaining or increasing leaf B concentration in olives, as determined by Rodrigues and Arrobas (2008) in orchards of cvs. Madural, Santulhana, and Cobrançosa. During this four-year study, leaf B levels in the –B treatment decreased to the lower limit of the adequate range at 19 mg kg^{-1} .

The ryegrass dry matter yield in the pot experiment was significantly higher in the N+B treatment compared to the –B treatment. Boron recovered in ryegrass dry matter was markedly lower in the –B treatment than the N+B treatment. Boron uptake by plants is controlled by the B level in the soil solution (Yermiyahu et al. 2001; Xu et al. 2001). Therefore, B application as fertilizer seems to prevent depletion of B level in the soil solution, which allows for maintenance of an adequate B supply to plants. Boron extracted by Azomethine-H was significantly lower in the soil of the –B treatment than the N+B treatment, which was in agreement with the results of the pot experiment. Boron extracted by Azomethine-H also provides a measure of B level in the soil solution and the plants responded directly to the B availability of the soil solution (Goldberg 1997).

The Azomethine-H method and the pot experiment clearly demonstrated a significant reduction in soil available B associated with the –B treatment. The reduction in leaf B concentration of olive trees in the –B treatment was also evident. However, the productivity of olives was not significantly affected by the absence of B application, since the nutrient would be primarily directed to the most sensitive sinks of the olive trees as the soil available B reduces. However, another year without B fertilization would most likely result in leaf B levels decreasing below the adequate range, and therefore, olive yields would most likely decrease significantly.

CONCLUSIONS

Leaf N and B concentrations and olive yields presented a continuous decrease over time in the treatments without N or B (–N and –B treatments), reflecting the reduction in soil available N and B. Therefore, in rainfed olive orchards with ground-applied fertilizers, the mobile elements must be annually supplied to maintain stable the capacity of the soil to provide the nutrient elements to the tree crop. The application of fertilizers for periods longer than one year assumes the use of higher nutrient rates in each application. Studies in other crops have indicated that the nutrient use efficiency decreases as the fertilizer rate increases (Tyler et al. 1983; Reddy and Reddy 1993). In addition, the application of mobile elements, such as N, is evenly distributed in order to improve nutrient use efficiency for both annual intensive and perennial irrigated crops (Boswell et al. 1985). Thus, excluding situations in which the fertilizer application costs are relevant, N and B are environmentally sound and profitably managed under annual application. Establishment of the most adequate annual rates of N and B for a given orchard must still be determined. The continuous adjustment of the annual rates by

monitoring the leaf nutrient concentration over time is the commonly agreed method to achieve this objective.

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Figure captions

Figure 1. Annual precipitation and mean monthly temperature for the years preceding the five harvests (2003, 04, 05, 06 and 07).

Figure 2. Relative olive yields of the initial harvest, which was performed before the establishment of the fertilizer treatments, and the next four harvests. Error bars indicate the mean confidence limit ($\alpha < 0.05$). The crop yields were approximately zero in 2006, and the olives had no commercial value.

Figure 3. Leaf N and B concentrations for six leaf sampling dates [four winter (w05, w06, w07, and w08) and two summer (s05 and s06)] as a function of the fertilizer treatments. Error bars indicate the mean confidence limit ($\alpha < 0.05$).

Figure 4. Dry matter yield, N concentration in tissues, and N recovery as a function of fertilizer treatments and soil depth for Italian ryegrass. Error bars indicate mean confidence limit ($\alpha < 0.05$).

Figure 5. Dry matter yield, B concentration in tissues, and B recovered as a function of the fertilizer treatment and soil depth for Italian ryegrass. Error bars indicate mean confidence limit ($\alpha < 0.05$).

Figure 6. Nitrogen extracted by the Kjeldahl and NaOH methods from the 0-5 and 5-20 cm soil layers of the N+B and -N treatments. Error bars indicate the mean confidence limit ($\alpha < 0.05$).

Figure 7. Boron extracted with hot water and the Azomethine-H method from the 0-5 and 5-20 cm soil layers of the N+B and -B treatments. Error bars indicate the mean confidence limit ($\alpha < 0.05$).

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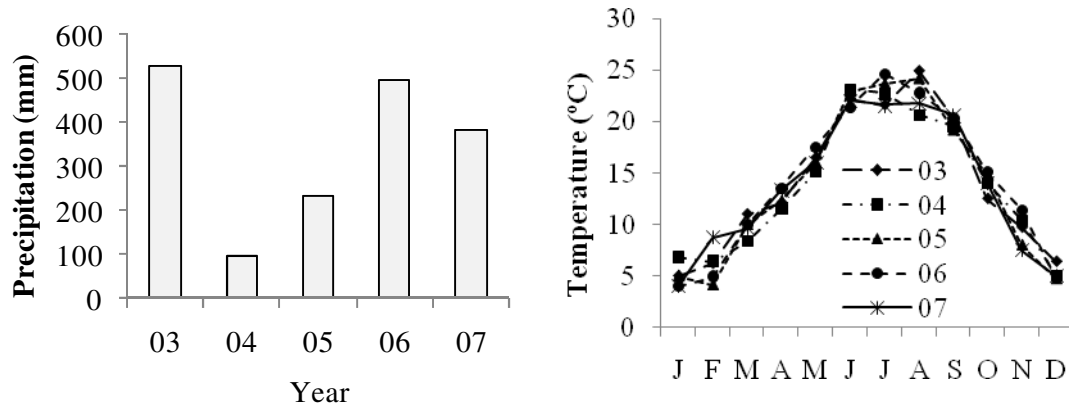


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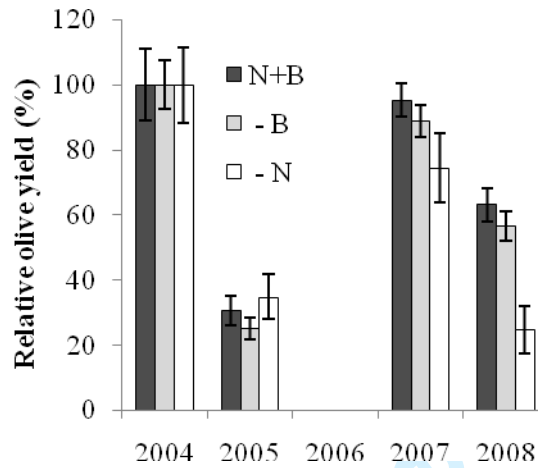


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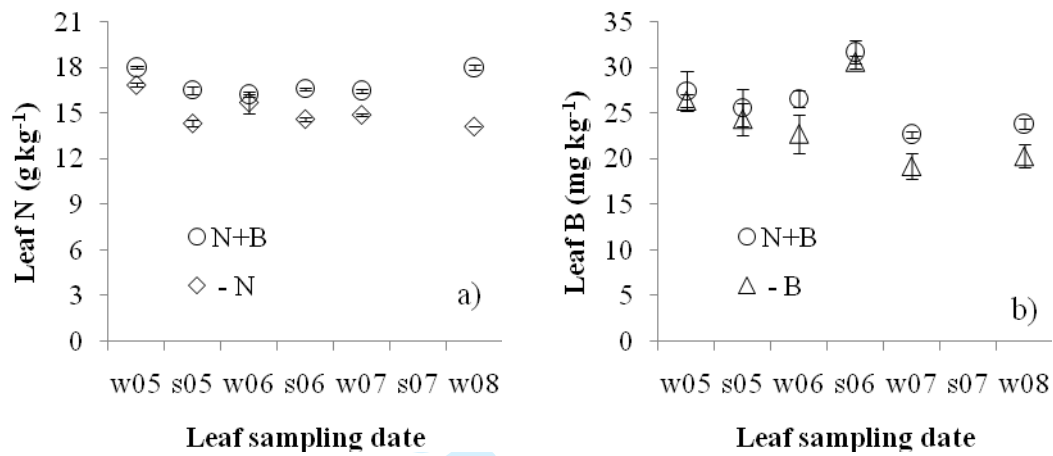


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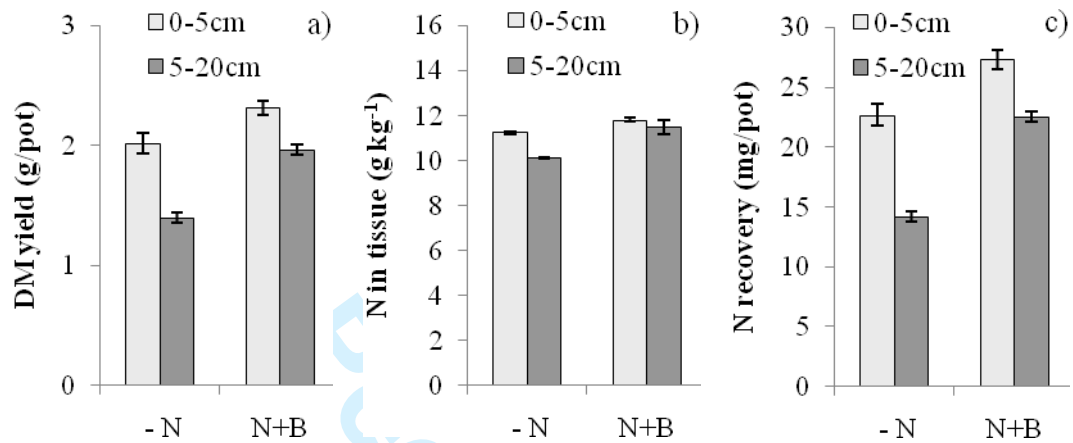


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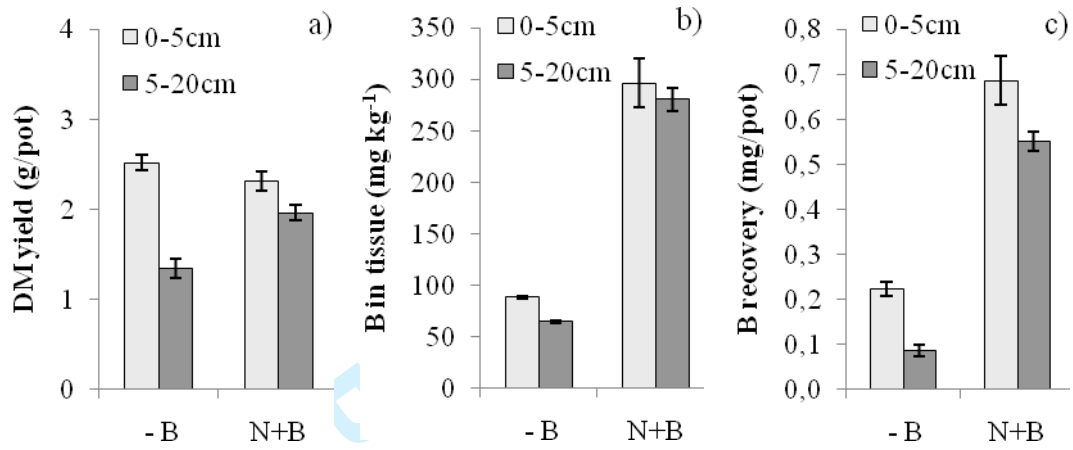


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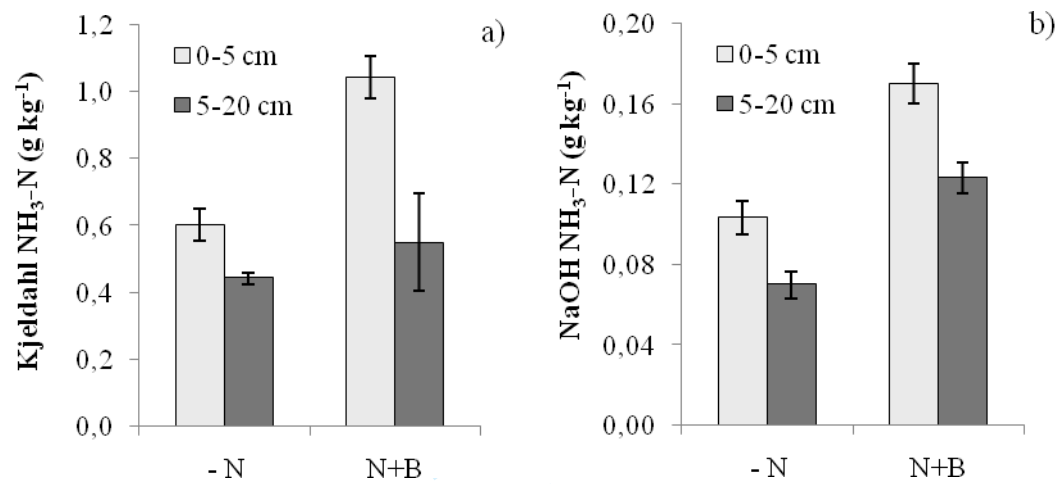


Figure 6. Nitrogen extracted by the Kjeldahl and NaOH methods from the 0-5 and 5-20 cm layers of the soils of the N+B and -N treatments. Error bars indicate mean confidence limit ($\alpha < 0.05$).

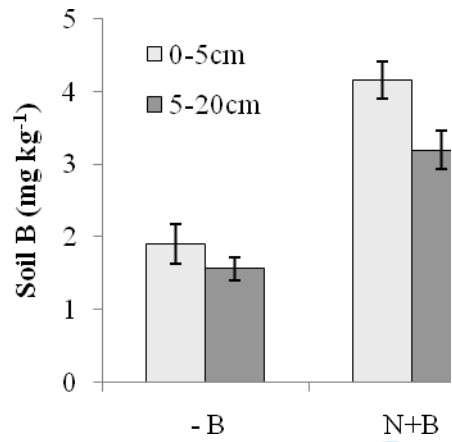


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