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Olive tree response to applied phosphorus in field and pot experiments

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ABSTRACT

Phosphorus (P) application in olive orchards is very common in the Mediterranean basin although experimental evidence of crop response to applied P is practically non-existent. In this work soil P and tree P nutritional status of the olive groves of NE Portugal were assessed from a population of 1808 soil and 2252 leaf samples. Plant response to applied P was evaluated from two field and two pot experiments carried out with the cultivar 'Cobrançosa'. The analyses of soil and leaf samples of the olive orchards of the region indicate that P fertilizer recommendations should be based on leaf rather than on soil analyzes, since the latter seems to overestimate the need for P. The field and pot experiments hardly showed any positive response to P applications, which is a sign that the use of P fertilizer in olive can be substantially reduced. Nonetheless, in one pot experiment, P application significantly increased total dry matter yield during three consecutive years, in a strict association with higher tissue P concentrations and enhanced photosynthetic activity, as revealed by gas exchange and chlorophyll fluorescence traits. The experimental results also showed that the roots can uptake and store P when available in the soil, which may buffer the levels of P in the shoots. The acid phosphate activity can provide useful information but deserves caution in the interpretation of results since it depends not only on the availability of inorganic P in the soil, but also on the available organic substrate and pH.

1. Introduction

Phosphorus has prominent roles in plants as a constituent of nucleic acids and phospholipids of biomembranes and in the energy transfer reactions involving adenosine triphosphate (ATP) (Hawkesford et al., 2012; Havlin et al., 2014). P is the second most limiting element to crop growth and yield on a global scale (Li et al., 2016). The availability of P to plant roots is estimated to be limited to approximately 2/3 of the world's soils, causing a major constraint on agricultural productivity (Batjes, 1997; Sepehr et al., 2012). A number of studies have shown the effect of the application of P on the productivity increase of several crops, such as wheat (Brennan and Bolland 2001, Wang et al. 2010), soybean (Watt and Evans, 2003), canola (Brennan and Bolland, 2001) and lupine (Brennan and Bolland, 2001; Watt and Evans, 2003; Wang et al., 2010).

The use of P in agriculture has become of increasing concern due to the fact that it is a finite resource. It is estimated that the phosphate rocks from which P fertilizers are manufactured will be depleted within the next 50 to 100 years if consumed at the current rates (Gilbert, 2009; Hawkesford et al., 2012). On the other hand, the excessive use of P in agriculture can lead to the eutrophication of groundwater (Bai et al., 2016; Dodd and Sharpley, 2016). Thus, for several good reasons, it is necessary to moderate the use of P in agriculture. Different species may need different P fertilization programs since they differ greatly in the ability to use sparingly soluble P. Some species exudate organic acids to the rhizosphere which reduce pH and solubilize P (Wang et al., 2007;Veneklaas et al., 2003) and/or develops cluster roots or proteoid roots which provide enhanced zones for P uptake (Uhde-Stone et al., 2003; Schulze et al., 2006). In trees, for instance, symbiotic relationships between plant roots and arbuscular mycorrhizal fungi can be established, enhancing P uptake in ways that are not readily available to most plants (Smith and Read, 2008; Pereira et al. 2012; Havlin et al., 2014).

In olive, studies showing a positive response of the tree to P fertilizers are practically non-existent (Freeman and Carlson, 2005; Gregoriou and El-Kholy, 2010; Fernández-Escobar et al., 2017). The absence of response may be due to the very low amount of P removed in harvest, with values below 1 kg P per ton of fresh fruit (Rodrigues et al.,

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2012; Fernández-Escobar et al., 2017). Despite the recognized lack of response by the olive tree to applied P, national P fertilizer programs are usually generous in the rates of P fertilizer they recommend. Gregoriou and El-Kholy (2010) reported a summary of the national olive fertilization programs for several countries of Western Asia and North Africa showing annual recommendations frequently exceeding 100 kg P_2O_5 hm^{-2}. In Portugal, an official publication of the Ministry of Agriculture (LQARS, 2006) recommends P rates at an olive orchard installation of 200, 150 and 100 kg P_2O_5 hm^{-2} to soils respectively classified as very low, low and medium in P. For mature trees, LQARS (2006) recommends 40–60 kg P_2O_5 hm^{-2} yr^{-1} when leaf P concentrations are found to be at adequate levels. Notwithstanding, there are no studies in the country showing olive tree response to the application of P.

This work was motivated by the lack of data on olive tree response to P fertilization. Taking into account the large area that olive occupies in the Mediterranean basin, and with phosphate rock being a finite resource, it seems of great importance to use this nutrient more responsibly. The work comprises two parts: i) evaluation of soil P and tree P nutritional status of the olive groves of NE Portugal from high number of soil (1808) and leaf (2,522) samples; and ii) experimental work, consisting of the evaluation of olive tree response to applied P in two field trials and two pot experiments. The hypothesis tested is that P being a primary macronutrient it should be expected that a positive response in tree crop growth and yield to the applied P will be found.

2. Material and methods

2.1. Evaluation of soil and plant P status of olive orchards of NE Portugal

Soil P status of the olive groves was obtained from a population of 1808 soil samples voluntarily delivered by farmers to the soil testing and plant analysis laboratory of the Polytechnic Institute of Bragança in the last 4 years. The P nutritional status of the olive trees was also obtained from 2252 leaf samples sent to the lab by the olive growers in the same period.

2.2. Field and pot experiments

The study included two field trials and two pot experiments. Field trial 1 (Ftrial1) was installed in March 2013 in a three-year-old 'Cobrançosa' olive grove, with the trees spaced 7 × 6 m, and rainfed managed (41.807665, -6.733173). The second field trial (Ftrial2) began with the plantation of 'Cobrançosa' young trees spaced 6 m between lines and 1 m within the line (41.808259, -6.733402). Planting took place in May 2014. The experimental designs of both Ftrial1 and Ftrial2 included two treatments, P fertilization (+P) and control, without P application (−P), and three replicates. In Ftrial1 the experimental unit consisted of four homogeneous trees, which total 12 trees per treatment and 24 marked trees in the total experiment. In Ftrial2, the experimental unit was composed of 10 trees totaling 60 trees in the experiment. P fertilizer in the + P treatment of Ftrial1 was broadcast in squares of 4 × 4 m around the tree. P was applied at a rate of 70 g P $tree^{-1}$, as superphosphate (18% P_2O_5), which represents 38 kg P2O5 hm^{-2}, a value within the usual recommendations to young orchards in the region when soils present medium P levels. In Ftrial2, P in the fertilized treatment was broadcast in rectangles of 10 × 4 m (2 m both sides of the row), at a similar rate of Ftrial1, which means 175 g P per experimental unit (40 m^2). In both M +P and –P treatments, nitrogen (N), potassium (K), and boron (B) were applied as a basal fertilization plan. K was applied at similar rates of P when expressed as K2O and P2O5, which means 133 and 332 g K, respectively per tree in Ftrial1 and experimental unit in Ftrial2. The fertilizer used was potassium chloride (KCl, 60% K_2O). Due to their higher mobility in the soil, N and B were applied in smaller areas, respectively in 4 m^2 (2 × 2 m, with the tree in the center of the square) and in rectangles of 20 m^2 (1 m for each side of the line) in Ftrial 1 and Ftrial2. N rates were 48 and 200 g applied as ammonium nitrate (34.5% N) in the above mentioned areas in Ftrial1 and Ftrial2. B was applied at the rates of 1.2 and 6.0 g as borax (11% B), respectively per tree and experimental unit in Ftrial1 and Ftrial2. In the year of the installation of the field trials the fertilizers were incorporated in the soil. Thereafter, the soil was no longer tilled and weeds were managed by a non-selective glyphosate-based herbicide (360 g L^{-1} of active ingredient; 4 L of herbicide hm^{-2}) applied once a year in April between rows and complemented by manual weeding close to the trees.

Table 1
Fertilizer treatments of pot experiments 1 (Pexp1) and 2 (Pexp2), rates of nutrients of the fertilizer treatments and basal fertilization plans and fertilizers used.

		Pexp1				Pexp2		
[a]Year	Nutrient	P0	P1	P2	P3	P0	P1	Fertilizer
		g pot^{-1}				g pot^{-1}		
2013	P	0.00	0.35	0.70	1.05			Super (18% P_2O_5)
	K	0.66	0.66	0.66	0.66			KCl (60% K_2O)
	N	0.80	0.80	0.80	0.80			[b]AN (34.5% N)
	Lime	5.0	5.0	5.0	5.0			[c]Lime
2014–2016	P	0.00	0.35	0.70	1.05	0.00	1.05	NP (2:8:0)
	N	0.00	0.20	0.40	0.60	0.00	0.60	NP (2:8:0)
	N	0.80	0.60	0.40	0.20	0.80	0.20	AN (34.5% N)
	K	0.66	0.66	0.66	0.66	0.66	0.66	KCl (60% K_2O)
	Micro	0.08	0.08	0.08	0.08	0.08	0.08	[d]Mixture

[a] Data of 2016 refers only to Pexp2; the rates of 2014–2016 were split into five applications.
[b] Ammonium nitrate.
[c] (88% $CaCO_3$ and 5% $MgCO_3$).
[d] (10% MgO, 0.3% B, 18.5% SO3, 0.3% Cu, 2% Fe, 1% Mn, 0.02% Mo, 1.6% Zn).

The pot experiment 1 (Pexp1) consisted of a completely randomized experimental design with four fertilizer treatments (P0, P1, P2 and P3) and 10 replicates (10 pots) per treatment. The pots were filled with 3 kg of dry and sieved (2 mm mesh) soil mixed with the fertilizer of the experimental design and those of a basal fertilization plan. The rates of nutrients as well as the fertilizers used are presented in Table 1. Semi-hardwood rooted 'Cobrançosa' cuttings, ~20 cm high, were planted in June 2013. In April 2014 a new pot experiment (Pexp2) was installed where the nutrients were applied from liquid fertilizers during the growing season. In that time, it was decided to manage the Pexp1 in a similar way by using the same liquid fertilizers (Table 1). From 2014 the fertilizers were split into five annual applications to reduce salt effect. There was also used a fertilizer consisting of a mixture of macro and micronutrients whose rates were also split into 5 annual applications during the summer growing season. Pexp2 was installed as a randomized complete block design with two fertilizer treatments, with (P1) and without (P0) P application, four different soils (the same as Pexp1 and three new soils) as blocks and six replicates (6 pots) per treatment. Each pot also received 3 kg of dry soil sieved in 2 mm mesh. Previously rooted 'Cobrançosa' cuttings of ~20 cm high were used. The pots of both the experiments were kept in a greenhouse and the fertilizers applied simultaneously with watering. The cover of the greenhouse consists of a double-wall polycarbonate panel. Aeration and heat dissipation in summer relies on lateral and zenithal openings and reflective screen.

Selected properties of the soils of the field trials and those used in pot experiments are presented in Table 2. The climate of the region is of Mediterranean type, with some influence of the Atlantic regime. The average air temperature and the precipitation of the region are respectively 12.7 °C and 772.8 mm.

Table 2
Selected physical and chemical properties of soil samples (0–20 cm) of the field trials (Ftrial1 and Ftrial2), Pexp1 (S1) and Pexp2 (S1, S2, S3, and S4) at the beginning of the experiments.

Soil properties	Ftrial1	Ftrial2	S1	S2	S3	S4
Clay (%)	14.5	14.6	14.9	7.8	9.1	17.1
Silt (%)	27.7	29.2	26.7	9.4	10.2	18.6
Sand (%)	57.8	56.2	58.4	82.8	80.7	64.4
Texture	[h]S-loam	S-loam	S-loam	[i]L-sand	L-sand	S-loam
pH (H_2O)	5.8	5.5	5.8	4.9	5.1	6.9
[a]Organic carbon (g kg^{-1})	8.7	8.7	7.4	4.4	15.1	14.6
[b]P_{AL} (mg P_2O_5 kg^{-1})	87.9	93.4	41.2	14.5	56.3	353.6
[c]P_{Ols} (mg P kg^{-1})	4.8	4.8	9.9	0.0	35.5	57.8
[d]P_{Meh} (mg P kg^{-1})	40.6	45.2	28.7	14.4	173.4	103.8
[e]P_{res} (mg P kg^{-1})	12.7	11.9	12.4	3.4	12.7	55.2
[f]APA (ug L^{-1})	206.4	63.5	162.3	128.1	88.5	450.4
[b]K_{AL}	102.0	114.0	118.0	59.0	53.0	234.0
[g]Exch. K (Cmol$^+$ kg^{-1})	0.2	0.3	0.3	0.1	0.2	0.7
[g]Exch. Na (Cmol$^+$ kg^{-1})	0.4	0.4	0.7	0.3	0.3	0.6
[g]Exch. Ca (Cmol$^+$ kg^{-1})	7.2	8.5	5.5	2.8	0.9	7.4
[g]Exch. Mg (Cmol$^+$ kg^{-1})	2.2	2.6	3.3	1.0	0.4	4.2
[g]Exch. acidity (Cmol$^+$ kg^{-1})	10.7	11.9	11.2	4.6	2.1	13.8

[a] Walkley-Black.
[b] ammonium lactate.
[c] Olsen.
[d] Menlich III.
[e] Resin.
[f] Acid phosphatase acidity.
[g] ammonium acetate.
[h] Sandy-loam.
[i] Loamy-sand.

2.3. Field determinations

In Ftrial1 the trunk diameter was measured periodically at 40 cm height and the canopy volume estimated by measuring the height of the canopy and the maximum width (NS and EW), assuming that the canopy at this stage has an ovoid shape. The canopy volume (CV) was estimated 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 resting period, and pruning wood used as an index of the growth of the trees. After fresh weighing, a subsample was taken to the laboratory, separated into leaves and stems, and weighed fresh and after drying in an oven at 70 °C. In early winter the olives were hand-picked and weighed separately per tree. Samples of 100 olives were weighed fresh to obtain the unit weight of the fruits. Random subsamples of 20 fruits were separated into pulp and pit and weighed fresh for estimating the pulp/pit ratio. In the winter resting period, and in July at the endocarp sclerification, leaf samples were taken following the standard procedure for this species (Bryson et al., 2014). All tissue samples were dried at 70 °C, ground and analyzed for elemental composition. On May 23, 2016, soil samples were collected at three depths, 0–5 cm, 5–10 cm and 10–20 cm. The samples were sieved in 2 mm mesh, dried at 40 °C and used in the determination of several soil fertility parameters.

In Ftrial2, young fully matured leaves were sampled twice a year. At the end of the study, on 26 October 2016, four random plants per treatment were cut at ground level and weighed fresh. Subsamples were separated into leaves and stems, weighed fresh, oven dried and weighed dry. All tissue samples from this experiment were also ground and analyzed for the elemental composition.

In Pexp2, leaf gas exchange was measured at midday of summer cloudless days of 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), transpiration rate (E) 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. Chlorophyll *a* fluorescence was measured on the same leaves and environmental conditions, as gas exchange, with a pulse amplitude modulated FMS 2 fluorimeter (Hansatech Instruments, Norfolk, England). Minimum fluorescence (F_0) was measured in dark adapted leaves by applying a low intensity light pulse and maximum fluorescence (F_m) was measured after a saturating light pulse (15000 µmol photons m^{-h} s^{-s}) for 0.7 s. Maximum quantum efficiency of photosystem II (PSII) was calculated as $F_v/F_m = (F_m\text{-}F_0)/F_m$. After exposure for 20 s to actinic light, light-adapted steady-state fluorescence yield (F_s) was averaged over 2.5 s, followed by exposure to saturating light (as above) to establish F_m‘. The sample was then shaded for 5 s with a far-red light source to determine the minimal fluorescence in a light-adapted state (F'_0). From these measurements, the following variables were calculated (Schreiber et al. 1994): effective efficiency of PSII (ΦPSII = (Fm‘− − s) /Fm‘), photochemical quenching ($qP = (F'_m\text{-}F_s)/(F'_m\text{-}F'_0)$), and non-photochemical energy dissipation ($\Phi NP = F_s/F'_m$). The apparent electron transport rate (ETR) was estimated as ETR = ΦPSII × PPFD × 0.5 × 0.84, where PPFD is the photosynthetic photon flux density incident on the leaf, 0.5 is the factor that assumes equal distribution of energy between the two photosystems, and the leaf absorbance used was 0.84, a common value for C_3 plants (Björkman and Demmig, 1987). The fraction of PPFD absorbed in PSII neither utilized in photochemistry nor dissipated thermally (P_E), characterizing an excess energy, was calculated as PE = F´v/F´m × (1- qP), according to Demmig-Adams et al. (1996).

In the pot experiments, the aboveground biomass was annually cut, leaving only a small number of basal leaves and buds to allow the regrowth of plants the next growing season. This plant material was oven dried, separated into stems and leaves, weighed, ground and analyzed for elemental composition. After the third and last cut, a soil sample was taken per pot and the root system exposed using water at low pressure. The soil samples were taken to the laboratory for determination of relevant soil fertility properties. The roots were subjected to the same procedures of stems and leaves.

2.4. Laboratorial procedures

After drying and sieving, soil samples from field and pot experiments, including the original samples whose results were presented in Table 1, were submitted to analytical determinations: 1) pH (H_2O, KCl and $CaCl_2$); 2) easily oxidizable carbon (C) determined by the Walkley-Black method and total organic C by incineration; 3) cation exchange capacity (ammonium acetate, pH 7.0). Soil P bioavailability was performed by five methods: 4) Egnér-Riehm or ammonium lactate (P_{AL}); 5) Olsen (P_{Ols}); 6) Mehlich 3 (P_{Meh}); 7) anion-exchange resins (P_{Res}); and 8) acid phosphatase activity (AFA). The Egnér-Rhiem method consists of extracting P with an ammonium lactate solution at pH 3.7. The Olsen method consists of extracting P with a solution of 0.5 M NaHCO3, pH 8.5. The extracting solution of Mehlich 3 consists of a mixture of N ions (nitrate and ammonium) in different combinations with pH 2.5. The resin method consists of extraction of the phosphate ion by exchange with the HCO_3 ion present in an anion exchange resin and then eluted with a dilute acid. The acid phosphatase activity is determined from the conversion of nitrophenylphosphate to nitrophenolphosphate. K was extracted by the Egnér-Riehm solution and by ammonium acetate as a base of the exchange complex. In the initial samples there were also determined 9) clay, silt and sand fractions by the Robinson pipette method. Methods 1–5, 7 and 9 are fully described by Houba et al. (1997); method 6 by Jones (2001) and method 8 by Alef et al. (1995).

Elemental analyses of all the tissues (leaves, stems and roots) were performed by Kjeldahl (N), colorimetry (B and P), and atomic absorption spectrophotometry (K, Ca, Mg, Fe, Mn, Cu, Zn) methods (Walinga et al., 1989), after tissue samples were digested with nitric acid in a

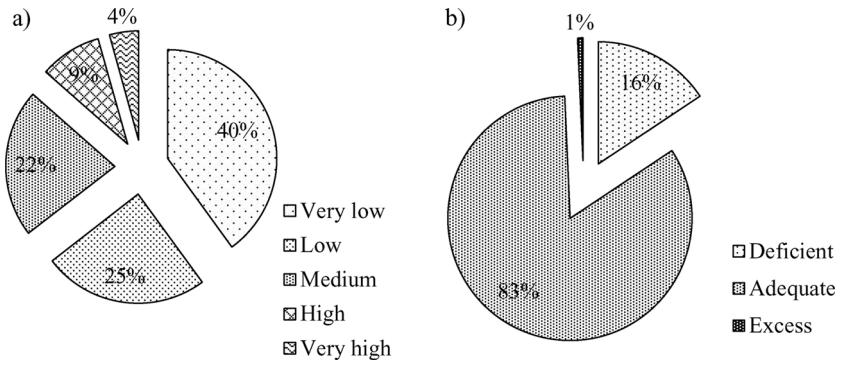

Fig. 1. a) Soil P levels determined by the ammonium lactate method from a population of 1808 soil samples, and b) tree P nutritional status classified in three sufficiency ranges (deficient, $< 1\,g\,kg^{-1}$; adequate, 1 to $3\,g\,kg^{-1}$; and excess, $> 3\,g\,kg^{-1}$), from a population of 2252 leaf samples.

microwave.

2.5. Data analysis

Data was submitted to analysis of variance. When significant differences occurred between treatments the means were separated using the Tukey HSD test ($\alpha = 0.05$). In some situations to improve the understanding of the results and for graphical plotting the means were associated to their confidence intervals ($\alpha = 0.05$).

3. Results

3.1. Soil and plant P status of olive orchards of NE Portugal

The soil P status of olive orchards of NE Portugal appeared very poor as determined by the ammonium lactate method. Very low, low and medium levels of extractable P were found respectively in 40, 25 and 22% of soil samples (Fig. 1a), which means 87% of situations that would lead to a P fertilizer recommendation. From leaf analysis, P nutritional status of the orchards seems much better. Only 16% of the samples showed leaf P concentration falling in the deficiency range (Fig. 1b), which means that soils classified as low or very low in P are supporting trees adequately nourished as determined by leaf analysis.

3.2. Field experiments

In Ftrial1, the young trees did not significantly respond with increased olive yield to applied P in any of the years of the experiment (Table 3). The accumulated olive yields of the three years were also not significantly different between P fertilizer treatments. The average values were 2.19 and 2.47 kg tree^{-1}, respectively to –P and +P plots. Other yield components such as dry weight of fruits and pulp/pit ratio and also pulp and pit P concentrations did not significantly vary with P fertilizer treatments.

The performance of the young trees assessed by periodically measuring the increase in trunk diameter, the volume of the crown on two dates and the yearly prunings are shown in Fig. 2. The results showed no significant differences for any of the parameters evaluated.

Leaf P concentrations did not significantly vary between –P and +P treatments. All average values were found within the lower and upper limits of the sufficiency range (Fig. 3). In this experiment, leaf P concentration showed little sensitivity to the application of P to the soil. Tissue analysis for other macro and micronutrients revealed also no significant differences among P fertilizer treatments (data not shown).

Surface soil layer (0–5 cm) appeared slightly acid in comparison to the lower layers (Table 4). The easily oxidizable organic C significantly decreased along the soil profile, from an average value of 11.7 to 6.1 g kg^{-1}, respectively in the 0–5 cm and 10–20 cm soil layers. All the four methods of P extraction displayed levels of extractable P significantly higher in the P fertilized plot in comparison to the control. Extractable soil P significantly decreased from the upper to the deeper soil layers for all the extraction methods. APA significantly decreased along the soil profile as occurred with extractable P and organic C.

In Ftrial 2, leaf P concentrations were significantly higher in the P fertilized plots in comparison to the control in two of the four sampling dates (Fig. 4). However, dry matter yield after three years of growth were not significantly different between the two fertilizer treatments. P recovery in the aboveground biomass, which is a function of dry matter yield and tissue P concentration, did also not significantly vary with P fertilized treatments.

Table 3
Olive yield, biometric data of fruits and pulp and pit P concentrations in three consecutive harvests (2014–2016) as a function of P fertilization treatments. Within each year and in lines, the same letter 'a' means that no significant differences between fertilizer treatments ($P < 0.05$) were found.

	2014		2015		2016	
	–P	+P	–P	+P	–P	+P
Olive yield (kg tree^{-1})	0.25 a	0.19 a	0.66 a	1.06 a	1.28 a	1.21 a
Fresh weight per fruit (g)	4.45 a	4.66 a	3.65 a	3.67 a	1.72 a	1.97 a
Pulp/pit ratio (dw)	1.36 a	1.27 a	1.94 a	1.86 a	1.45 a	1.49 a
Pulp P (g kg^{-1})	1.38 a	1.22 a	1.19 a	1.16 a	1.18 a	1.16 a
Pit P (g kg^{-1})	0.32 a	0.32 a	0.33 a	0.48 a	0.50 a	0.53 a

3.3. Pot experiments

In Pexp1 dry matter yield did not significantly differ among the four P fertilizer treatments for any of the years and plant parts (stems, leaves and roots) or total biomass (Fig. 5). Average values have even showed a slight but not significantly decrease for the higher P fertilizer rates.

In Pexp1, tissue P concentration significantly increased in all plant parts (leaves, stems and roots) with fertilizer P rate (Fig. 6). The control treatment showed significantly lower tissue P concentrations than the fertilized treatments. Leaves and stems displayed a saturation curve to high P rates, while root P concentrations continued to increase to the higher rates of applied P. Leaf P concentrations were higher than stem P concentration for all P fertilizer rates. No significant differences were found for the other macro and micronutrients analyzed in these plant tissues as a function of P fertilizer treatments (data not shown).

In Pexp1, the lower values of pH (H_2O) and oxidizable C were found in P0 treatment (Table 5). P extracted by the four different methods significantly increased with fertilizer P rate. There were found values which are classified as very low (P0) to very high (P3) for all the extraction methods. APA did not significantly vary with P fertilizer treatments as well as many other chemical properties not shown in Table 5.

In Pexp2, P application significantly increased total dry matter yield

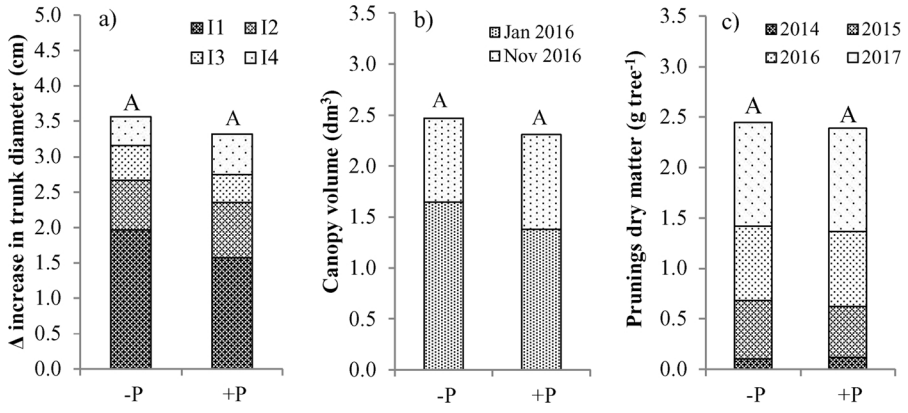

Fig. 2. a) Variation (Δ) increase in trunk diameter in four consecutive intervals (I1, Jun 2013 – Oct 2014; I2, – Jul 2015; I3, – Dec 2015; I4, – Nov 2016), b) canopy volume estimated in two dates, and c) pruning wood in the resting periods of 2014–2017, as a function of P fertilizer treatments. Capital letters above the columns is the result of the analysis of variance (no significant differences between fertilizer treatments,$P < 0.05$) for the sum of all records of each parameter.

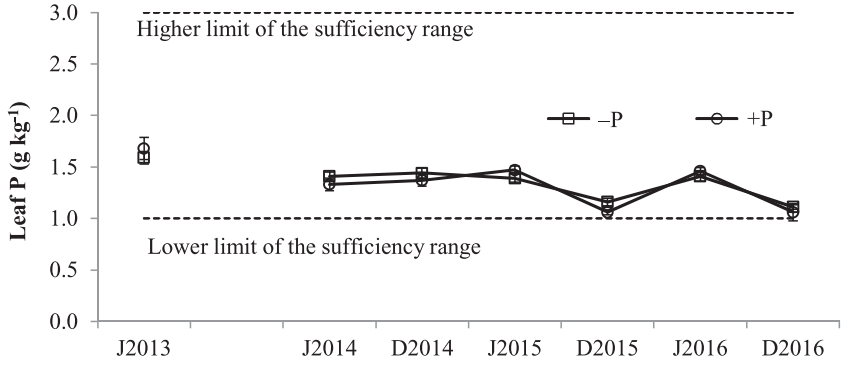

Fig. 3. Leaf P concentrations in July (J) and December (D) in Ftrial1. Dashed lines are the lower and the upper limits of the sufficiency range established for olive. Error bars are the mean standard deviations.

Table 4
Soil acidity pH(H_2O), oxidizable organic carbon (OC), phosphorus extracted by ammonium lactate (P_{AL}), Olsen (P_{Ols}), Mehlich 3 (P_{Meh}) and ion-exchange resin (P_{Res}) methods and acid phosphate activity (APA) as a function of P fertilizer treatment and soil layer. In columns, within P rate or soil layer, means followed by the same letter are not significantly different by Tukey HSD test (α = 0.05).

P rate	pH (H_2O)	OC ($g\ kg^{-1}$)	P_{AL} (P_2O_5)	P_{Ols} (P)	P_{Meh} (P)	P_{Res} (P)	APA ($\mu g\ dm^{-3}$)
				($mg\ kg^{-1}$)			
P0	5.95 a	9.0 a	48.5 b	8.0 b	34.1 b	16.1 b	76.8 a
P1	5.98 a	8.7 a	184.0 a	30.2 a	101.9 a	46.3 a	84.7 a
Soil layer							
0–5 cm	5.65 b	11.7 a	192.3 a	30.5 a	102.8 a	47.3 a	123.5 a
5–10 cm	6.12 a	8.7 b	96.1 b	18.8 ab	62.5 b	28.2 b	104.9 b
10–20 cm	6.13 a	6.1 c	60.3 b	8.0 b	38.7 b	18.0 b	13.8 c

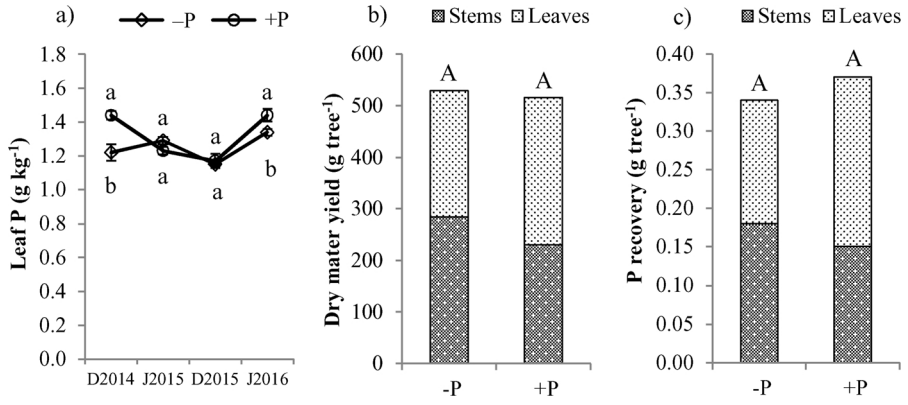

Fig. 4. a) Leaf P concentration [from December (D) 2014 to July (J) 2016], b) dry matter yield and c) P recovery in the above ground biomass as a function of P fertilizer treatments (–P, +P). Lower case letters a) are the result of analysis of variance and Tukey HSD test (α = 0.05), and capital letters above the columns b) and c) are also the result of analysis of variance for the sum of stems and leaves of the respective parameter.

in the three years of study (Fig. 7). Significant differences also often occurred when the dry matter yield of the different plant parts were separately analyzed. In 2014 significant differences occurred in leaves, in 2015 in leaves and stems and in 2016 in leaves and roots. In 2014 no significant differences were found among the different soils in total dry matter yield or in each one of the plant parts. In 2015 and 2016,

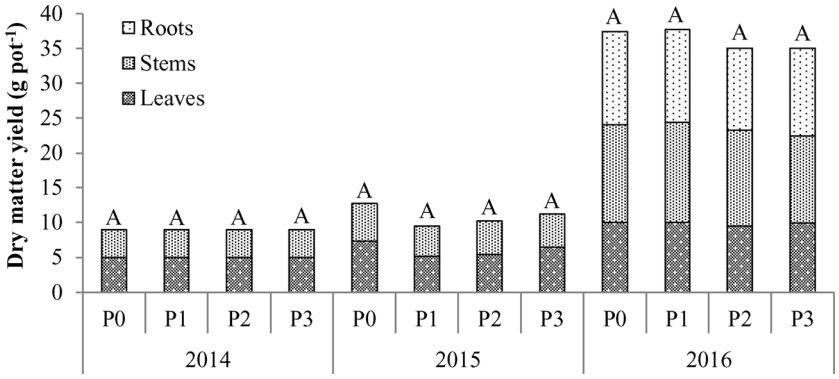

Fig. 5. Dry matter yields separated into the plant parts stems, leaves (2014–2015) and roots (2016) for each of the three growing seasons as a function of the fertilizer treatments. Within each year, capital letters above the columns is the result of analysis of variance (no significant differences among the fertilizer treatments,$P < 0.05$) for the sum of the plant parts.

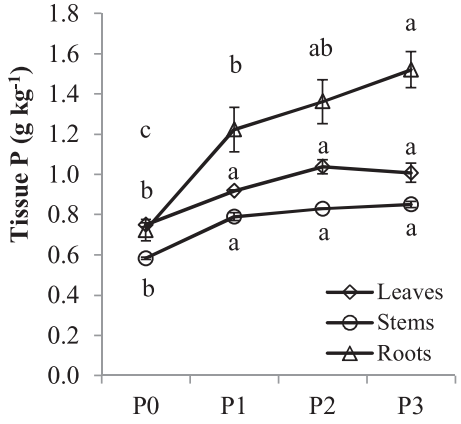

Fig. 6. Phosphorus concentration in leaves, stems and roots as a function of P fertilizer treatments from pot experiment 1. Error bars are the mean standard deviation. Letters are the result of analysis of variance and Tukey HSD test ($\alpha = 0.05$) within each plant tissue comparing the different P fertilizer treatments.

Table 5
Soil acidity pH(H_2O), oxidizable organic carbon (OC), phosphorus extracted by ammonium lactate (P_{AL}), Olsen (P_{Ols}), Mehlich (P_{Meh}) and ion-exchange resin (P_{Res}) methods and acid phosphate activity (APA) as a function of P fertilizer treatment. In columns, means followed by the same letter are not significantly different by Tukey HSD test ($\alpha = 0.05$).

P rate	pH (H_2O)	OC ($g\ kg^{-1}$)	P_{AL} (P_2O_5)	P_{Ols} (P)	P_{Meh} (P)	P_{Res} (P)	APA
			$mg\ kg^{-1}$				($\mu g\ dm^{-3}$)
P0	6.41 b	3.2 b	22.8 d	0.0 c	10.2 d	2.56 c	33.1 a
P1	6.93 a	3.7 ab	442.9 c	17.3 b	328. 0 c	26.11 b	32.0 a
P2	6.93 a	3.8 a	497.1 b	27.4 a	522.2 b	27.95 b	27.7 a
P3	6.88 a	3.8 a	556.6 a	30. 0 a	717.0 a	37.03 a	30.8 a

significant differences were found among the different soils in dry matter yield of leaves, stems and total.

The ratios root/leaf and root/shoot significantly increased with P application in Pexp2 (Fig. 8). The ratio between the aboveground parts (leaf/stem) did not significantly change with P fertilization.

Tissue P concentrations significantly increased with P fertilization in all plant parts (leaves, stems and roots) (Fig. 9). In the control treatment, P concentrations in leaves, stems and roots were quite similar. However, with P application, root P concentration increased much more than P concentration in leaves and stems. Tissue analysis for other macro and micronutrients revealed no significant differences between P fertilization treatments (data not shown).

Net photosynthesis of olive tree increased 27% in 2015 and 31% in 2016 in response to P-supply (Table 6). This effect was accompanied by consistent increases in stomatal conductance and transpiration rate, while A/g_s and C_i/C_a were not significantly affected by P nutrition. Similar trends on leaf gas exchange variables were detected for the two studied soils (S3 and S4), being A, g_s and E higher in S3 provenance, namely in 2016. Chlorophyll *a* fluorescence traits also changed in response to P fertilization (Table 7). The PSII photochemistry variables in light-adapted leaves, ΦPSII, qP and ETR increased, whereas ΦNP and P_E decreased in P1 treated plants. The application of P had no significant effect on the maximum efficiency of PSII photochemistry (F_v/F_m) and no significant differences in photochemical parameters were found among the different soils (data not shown).

The application of fertilizer P significantly increased soil pH(H_2O) and reduced oxidizable organic C (Table 8). There were also found significant differences in pH (H_2O) and oxidizable C among the different soils. P1 treatment showed significantly higher extractable P, as determined by the four methods, and significantly APA in comparison to P0. Soils also significantly differed in extractable P as determined by all the methods and in APA.

4. Discussion

The P status of olive orchards of the region assessed by soil analysis gave a higher percentage of situations in which fertilizer P is recommended in comparison with the use of leaf analysis. It seems that in the conditions of these experiments (acidic to neutral soils) diagnosing by soil analysis may lead to the recommendation of more P than is necessary, which would represent a waste of a resource that is finite. This apparent overestimation of recommended P may be attributed to limitations of the analytical method, since P_{Ols} and P_{AL} do not hold the organic P component of the soil, despite the importance of organic P in crop nutrition (Darch et al., 2016; Arrobas et al., 2018). Furthermore, in tree crops, the arable layer, from which soil samples were taken, represents probably only a small fraction of P available to plants, due to the depth of the root system, which reduces the accuracy of soil analysis if compared to annual crops with shallower roots (Römheld, 2012). On the other hand, trees are able to establish symbiotic relationships with ectomycorrhizal fungi which may enhance the access of trees to sparingly soluble P forms (Pereira et al. 2012; Havlin et al., 2014).

Fertilizer P did not increase olive yield and other parameters of the performance of the trees in the field trials. In Pexp1, the application of P also did not increase the dry matter yield. These results are in line with an early report of Hartmann et al. (1966), who stated that there were no cases of P deficiency with trees responding to P applications reported from field-grown olive trees. Authors of more recent textbooks are of the opinion that it is not frequent to observe a response to P fertilization in olive (Freeman and Carlson, 2005; Gregoriou and El-Kholy, 2010; Fernández-Escobar et al., 2017). Interestingly, an increase in dry matter yield due to P application was obtained during three consecutive years from Pexp2. Pexp2 differed from Pexp1 in that several P-poor acid soils

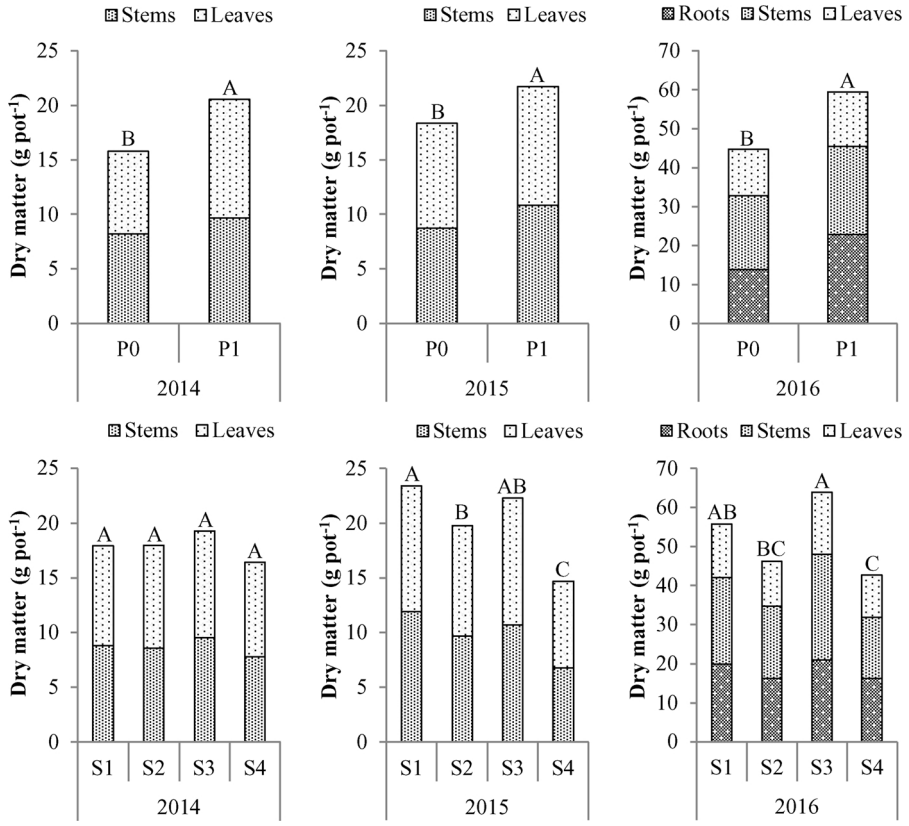

Fig. 7. Dry matter yield in the pot experiment 2 as a function of P fertilizer treatments (top panels) and soils (lower panels). For each individual figure, capital letters above the columns is the result of analysis of variance ($P < 0.05$) and Tukey HDS test ($\alpha < 0.05$) for the sum of the plant parts.

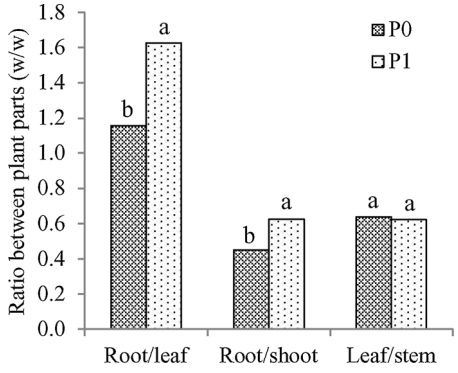

Fig. 8. Ratios between different plant parts as a function of P fertilizer treatment in pot experiment 2. For each ratio, means followed by the same letter are not significantly different by Tukey HSD test ($\alpha = 0.05$).

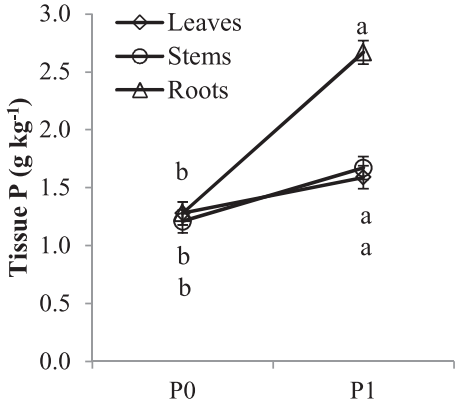

Fig. 9. Tissue P concentration in pot experiment 2 as a function of P fertilizer treatment. Error bars are the mean standard deviations. For each tissue, means followed by the same letter are not significantly different by Tukey HSD test ($\alpha = 0.05$).

were used and the soils were not limed, which will have marked a greater difference in the availability of P between fertilizer treatments. Few studies to date have shown a positive response in olive yield to increased P availability. Erel et al. (2013), from a study with young olive trees grown in containers with perlite as a substrate, found that P application increased flower intensity, fruit set, number of olives per tree and fruit load. It should be noted that perlite is basically an inert substrate, with the plants entirely dependent on external nutrient supply. In a two year study, with 'Picual' and "Arbequina", Centeno and Campo (2011) reported a yield increase with foliar P application in one of the years and only in "Arbequina".

The increase of total biomass in P fertilized plants was correlated with the increase of photosynthetic rate (A), confirming the causal relationship between P nutrition and photosynthesis, as in other studies (Warren, 2011; Veronica et al., 2017). The increase in A due to P-supply was associated with lower stomatal resistance, which increase available internal CO_2 and water loss through transpiration. Nevertheless, since for a given increase in g_s, E rose in a lower extent than A, and the increase of g_s was accompanied by similar values of A/g_s and C_i/C_a there may exist an enhanced capacity for CO_2 fixation in the stroma of chloroplasts of P fertilized plants. Moreover, the analysis of chlorophyll fluorescence data also shows positive effects of P-supply on the photochemical reactions in the thylakoid membranes that also contribute to the higher net CO_2 assimilation rate. In fact, P1 plants presented higher total electron flow through PSII, and thus superior ATP and NADPH production, due to the increase of the proportion of open photosystem II centres (qP). In addition, as a consequence of better photochemistry performance, P fertilized plants did not invest, as P-stressed plants, in thermal dissipation in PSII antennae (e. g. lower ΦNP), a mechanism

Table 6
Net photosynthetic rate (A), stomatal conductance (g_s), transpiration rate (E), intrinsic water use efficiency (A/g_s) and ratio of intercellular to atmospheric CO_2 concentration (C_i/C_a) as a function of P fertilizer treatment and soil. In columns, within P rate or soil and year, means followed by the same letter are not significantly different by Tukey HSD test ($\alpha = 0.05$).

P rate	Year	A μmol $m^{-2} s^{-1}$	g_s mmol $m^{-2} s^{-1}$	E mmol $m^{-2} s^{-1}$	A/g_s μmol mol^{-1}	C_i/C_a
P0	2015	11.0 b	178.5 b	3.5 b	62.4 a	0.696 a
P1		14.0 a	221.9 a	4.2 a	63.8 a	0.682 a
P0	2016	9.1 b	118.1 b	2.6 b	77.7 a	0.674 a
P1		11.9 a	168.1 a	3.3 a	72.1 a	0.681 a
Soil						
S3	2015	13.2 a	205.8 a	4.0 a	64.6 a	0.687 a
S4		11.8 b	194.7 a	3.6 a	61.6 a	0.691 a
S3	2016	12.3 a	165.2 a	3.2 a	76.7 a	0.664 a
S4		8.8 b	121.0 b	2.6 b	73.1 a	0.691 a

Table 7
Effective photochemical quantum yield of PSII (ΦPSII), coefficient of photochemical quenching (qP), electron transport rate (ETR, μmol e^- m^{-2} s^{-1}), non-photochemical energy dissipation (ΦNP) and fraction of PPFD absorbed in PSII antennae neither utilised in photochemistry nor dissipated thermally (P_E) as a function of P fertilizer treatment. In columns, within P rate and year, means followed by the same letter are not significantly different by Tukey HSD test ($\alpha = 0.05$).

P rate	Year	ΦPSII	qP	ETR	ΦNP	P_E
P0	2015	0.355 b	0.459 b	74.6 b	0.645 a	0.415 a
P1		0.414 a	0.554 a	87.0 a	0.586 b	0.333 b
P0	2016	0.444 b	0.557 b	93.2 b	0.556 a	0.352 a
P1		0.526 a	0.673 a	110.4 a	0.474 b	0.255 b

Table 8
Soil acidity pH(H_2O), oxidizable organic carbon (OC), phosphorus extracted by ammonium lactate (P_{AL}), Olsen (P_{Ols}), Mehlich (P_{Meh}) and ion-exchange resin (P_{Res}) methods and acid phosphate activity (APA) as a function of P fertilizer treatment and soil. In columns, within P rate or soil, means followed by the same letter are not significantly different by Tukey HSD test ($\alpha = 0.05$).

P rate	pH (H_2O)	OC (g kg^{-1})	P_{AL} (P_2O_5)	P_{Ols} (P)	P_{Meh} (P)	P_{Res} (P)	APA (μg dm^{-3})
			(mg kg^{-1})				
P0	5.89 b	1.96 a	109.7 b	20.4 b	57.8 b	12.3 b	256.3 a
P1	6.14 a	1.82 b	448.6 a	63.4 a	174.5 a	42.0 a	108.3 b
Soil							
S1	5.96 b	1.49 b	269.5 b	51.6 b	97.5 b	22.9 a	159.2 b
S2	5.91 bc	1.05 c	164.9 c	17.2 d	75.0 b	25.4 a	63.7 c
S3	5.61 c	2.60 a	273.0 b	32.0 c	193.7 a	24.7 a	75.2 c
S4	6.55 a	2.42 a	409.2 a	66.9 a	98.3 b	35.6 a	431.1 a

that protects against photoinhibition. Higher ΦPSII, ETR and qP and lower ΦNP were also described previously in response to P nutrition (Veronica et al., 2017). Altogether, the changes in PSII photochemistry reported above and the reduction of P_E, indication of a decrease in non-utilized light energy, revealed that P fertilized plants have a low risk of photo-oxidative damage. Similar results applied to nitrogen fertilization were reported earlier (DaMatta et al., 2002; Kato et al., 2003).

Phosphorus application increased tissue P concentration in Ftrial1 and in both the pot experiments. The increase in leaf P concentration as a response to P application has also already been observed in previous studies (Erel et al., 2013; Morales-Sillero et al., 2009). Ftrial1, from which leaf P concentration can be compared with sufficiency ranges of the crop since the leaves were harvested according to the standard procedure (Bryson et al., 2014), helps to justify the difficulty in obtaining a positive response of olive tree to applied P since leaf P concentration never fell below the lower limit of the sufficiency range in the control treatment.

Pot experiments revealed that roots are important tissues accumulating P. The roots registered P concentrations higher than the leaves and stems. Loupassaki et al. (2002) had already recorded higher levels of P in roots in comparison to leaves and stems in a study with six olive cultivars. In Pexp2, the application of P increased not only the concentration of P in the roots but also the root/shoot ratio. Thus, it seems that when soil available P is high, it increases not only the concentration of P in the roots but also the extension of the root system. These results seem to indicate that the roots can store P which may buffer P supply to the shoots, helping to overcome periods of shortage of soil available P, and may also be related to the difficulty in obtaining a positive response of a mature tree to P application.

In Ftrial1, the application of P increased extractable P as determined by the four methods used, whereas APA did not significantly vary. With soil depth, extractable P was reduced, as well as easily oxidizable C and APA. Thus, from this experiment, the organic substrate (i.e., soil organic P) seems to have been more decisive for APA than inorganic P in the soil. There has also previously been demonstrated from other studies the importance of the organic substrate for regulating APA (Turner, 2008; Kitayama, 2013; Zhang et al., 2014). In Pexp1, P application increased extractable P, while APA did not significantly vary. In this experiment the soil was limed which would have reduced APA. It is well documented that high APA generally prevails in acidic soils, whereas the activity of alkaline phosphatase prevails in alkaline soils (Eivazi and Tabatabai, 1977; Nannipieri et al., 2011). In Pexp2, extractable P increased with P application and APA was reduced. This is an expected result since it is also well established that soluble inorganic P inhibits the activity of acid phosphatase (Olander and Vitousek, 2000; Zheng et al., 2015).

5. Conclusions

The diagnoses of the soil fertility and the nutritional status of the olive groves of NE Portugal (soil pH varying from very acid to neutral) suggest that the application of P can be reduced without a high risk of yield reduction and that the diagnosis of the need to apply P should primarily be based on leaf analysis rather than on soil analysis. The experimental results stressed the difficulty in obtaining a response in olive tree growth and yield with soils of this region to the application of P which hardly validates the hypothesis given for this work. However, in Pexp2 P application significantly increased total dry matter during three consecutive years, in a strict association with higher tissue P concentrations and enhanced photosynthetic activity. The olive tree root system seems to have the ability to store P when available in soil which may buffer the P availability to the aerial plant parts. The results also indicate that the information provided from APA should be interpreted with caution since it may depend on variables other than the availability of inorganic P in the soil, namely the organic substrate and pH.

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