Trees (2005) 19: 233–250
DOI 10.1007/s00468-004-0374-3

ORIGINAL ARTICLE

J. Sardans · J. Peñuelas · F. Rodà

Changes in nutrient use efficiency, status and retranslocation in young post-fire regeneration *Pinus halepensis* in response to sudden N and P input, irrigation and removal of competing vegetation

Received: 25 March 2004 / Accepted: 29 August 2004 / Published online: 16 March 2005

Abstract The capacity of Mediterranean species to adapt to variable nutrient supply levels in a global change context can be a key factor to predict their future capacity to compete and survive in this new scenario. We aimed to investigate the capacity of a typical Mediterranean tree species, *Pinus halepensis*, to respond to sudden changes in N and P supply in different environmental conditions. We conducted a fertilisation, irrigation and removal of competing vegetation experiment in a calcareous post-fire shrubland with an homogeneous young (5 years old) population of *P. halepensis* in order to investigate the retranslocation and nutrient status for the principal nutrients (N, P, Mg, K, S, Ca and Fe), and the nutrient use efficiency (NUE) of the most important nutrients linked to photosynthetic capacity (N, P, Mg and K). P fertilisation increased P concentration in needles, P, N, Mg and K retranslocations, and NUE calculated as biomass production per unit of nutrient lost in the litterfall. The P fertilisation was able to increase the aboveground biomasses and P concentration 3 years after P fertiliser application. Those responses to P fertilisation were enhanced by the removal of competing vegetation. The N needle and litterfall concentration decreased after P fertilisation and this effect was greater when the P fertilisation was accompanied by removal of competing vegetation. The increase of P availability decreased the P-NUE and increased the N-NUE when these variables were calculated as aboveground biomass production per unit of P present in the biomass. Both P-NUE and N-NUE increased when calculated as total aboveground production per unit of nutrient loss. The results show that it is necessary to calculate NUE on a different basis to have a wider understanding of nutrient use. The irrigation did not change the needle nutrient concentrations and the litterfall production, but it significantly changed the nutrient litterfall concentrations and total aboveground contents (especially P and K). These results show a high capacity of *P. halepensis* to quickly respond to a limiting nutrient such as P in the critical phases of post-fire regeneration. The increase in P availability had a positive effect on growth and P concentrations and contents in aboveground biomass, thus increasing the capacity of growth in future periods and avoiding immediate runoff losses and leachate. This capacity also strongly depends on neighbour competition.

Keywords Mediterranean · Competing vegetation · *Pinus halepensis* · Nutrient use efficiency · Phosphorus

Introduction

The role of nutrients in Mediterranean ecosystems has been studied and debated for decades (Kruger 1979). The importance of nutrients has been appreciated in many experiments of nutrient manipulation by fertilisation in different Mediterranean zones of the world: in Californian Chaparral (McMaster et al. 1982), in South African fynbos (Witkowski et al. 1990), in Australian jarrah and *Eucalyptus* forest (Dell et al. 1987), and in the Mediterranean basin (Mayor and Rodà 1992; Sardans et al. 2004). Frequently the most limiting nutrient is P (McMaster et al. 1982; Witkowski et al. 1990; Sardans et al. 2004). The Mediterranean soils have been considered as nutrient-poor, and the Mediterranean plants share characteristics such as slow growth, sclerophylly and low nutrient contents that are present in the plants of other non-Mediterranean ecosystems adapted to poor soils (Bussotti et al. 2000).

In general, the litterfall production and the nutrient contents are sensitive to nutrient supply changes. In the studies of many natural communities the nutrient availability

J. Sardans (✉) · J. Peñuelas
Unitat d'ecofisiologia CSIC-CEAB-CREAF, CREAF (Center for Ecological Research and Forestry Application), Edifici C, Campus Universitat Autònoma de Barcelona,
Bellaterra (Barcelona), 08193 Spain
e-mail: j.sardans@creaf.uab.es
Tel.: +34-93-5811312
Fax: +34-93-5814151

F. Rodà
CREAF (Center for Ecological Research and Forestry Application), Edifici C, Universitat Autònoma de Barcelona,
Bellaterra (Barcelona), 08193 Spain

correlates positively with leaf concentrations (Seastedt et al. 1991; Rode 1993). Furthermore, the leaf nutrient concentrations are frequently employed as a fertility index of soils (Adams et al. 1987), and also to determine the nutritional status of plants (Chapin 1980), and overall of forest ecosystems (Miller and Miller 1976; McNeill et al. 1988). The sudden increments in nutrient availability following fertilisation treatments generally induce significant increases in leaf concentrations of the applied nutrient, especially when the nutrient is limiting (Harington and Wierman 1989; Björkman et al. 1991). In general, the increase in nutrient supply increases the litter fall and the nutrient losses (Theodorou and Bowen 1990; Diego and Rodà 1992). The species of nutrient rich environments frequently present higher values of leaf nutrient concentrations than species of poor environments when grown under intermediate nutrient availability (White 1972; Clarkson 1978). In general, species adapted to rich environments have a greater capacity to increase nutrient contents in response to a sudden increase in supply than species adapted to a poor environments (Chapin et al. 1986). On the other hand, increases in the leaf nutrient concentrations can vary significantly depending on competition pressure (Mugasha et al. 1991).

A high nutrient use efficiency (NUE) would be advantageous in nutrient deficient soils (Nuñez-Oliveira et al. 1993; Kloeppel et al. 2000). Nevertheless, in some experimental studies lower NUE were found in poor soils than in rich soils (Chapin and Kedrowsky 1983; Lüttge et al. 1991), although higher NUEs have been more often found in nutrient poor environments than in rich ones (Birk and Vitousek 1986; Bridgham et al. 1995). Sometimes NUE was similar at different nutrient supply levels (Folk and Grossnicke 2000). The NUE is difficult to calculate due to the great number of variables that determine it. Consequently the results often depend on the method employed in its determination. A lot of different equations and methods have been employed to determine NUE (Vitousek 1982; Aerts 1989; Kitayama et al. 2000). Higher NUE values have been found in poorer environments in studies which have compared different conditions for a single species and have calculated the NUE as litterfall production divided by the nutrient lost in the litterfall across a gradient of nutrient availability (Vitousek 1982; Bridgham et al. 1995). When the NUE is calculated as the net biomass production divided by nutrient concentration in the biomass (especially the photosynthetic biomass), the results are more contradictory (Wang et al. 1991; Rosati et al. 1999).

Nutrient retranslocation before leaf senescence has been related to NUE capacity in poor environments (Thomas and Grigal 1976) through the capacity to limit the loss of a limiting nutrient (Sharma and Pande 1989) which increases its NUE (Staff and Berg 1981). But when more experimental studies have been conducted, contradictory results have been found. Some have found higher values of nutrient retranslocation in species from rich environments than in species from poor environments (Chapin and Kedrowsky 1983; Nambiar and Fife 1987), while others have found the opposite (Chapin and Moilanen 1991).

In general, in non-Mediterranean areas the results show that evergreen plants have higher P and N NUE than broadleaf plants (Aerts et al. 1999). In Mediterranean species, most of the studies of the nutrients role have been conducted in *Quercus ilex*. Other species such as *Pinus halepensis* that cover wide areas in the Mediterranean basin have received less attention. NUE studies in Mediterranean ecosystems are scarce and have been mainly conducted on shrubs (Nuñez-Oliveira et al. 1993). The studies conducted on *P. halepensis* mostly dealt with growth responses to water and light availability. Less is known about the response of this important forest Mediterranean species to environmental nutrient supply changes.

In Mediterranean ecosystems several global change components are driving changes in nutrient supply. One of these environmental changes is the increase in forest fires (Piñol et al. 1998). Forest fires play a predominant role in Mediterranean ecosystems (Kruger 1979; Terradas et al. 1996). The recurrent fires are important as a key factor in nutrient cycles (St John and Rundel 1976). Fires produce sudden increases of the nutrient availability (Christiansen and Muller 1975; DeBano and Conrad 1978); P and cations are the main nutrients contained in post-fire ashes (Christiansen and Muller 1975). On the other hand, the human pressure on natural ecosystems has strongly increased in the last century in the Mediterranean basin area (Peñuelas and Filella 2002), and it will continue to increase in future decades. The overall warming trend in Mediterranean region during the last century (Peñuelas et al. 2002) has favoured fire recurrence. Global biogeochemical cycles are being strongly altered by human activities. The global N cycle has now reached the point where more N is fixed annually by human-driven processes (fertilisers, fossil fuel combustion) than by natural ones (Vitousek et al. 1997). Peñuelas and Filella (2001) have reported increased input of N to Mediterranean ecosystems in the last decades. Similar changes have been found in the concentrations of other nutrients such as phosphorus emitted from industrial activities, detergent production and agricultural activities. Their emissions have increased in Europe throughout the twentieth century (European Environment Agency 1998) and their concentrations in plants have increased in parallel (Peñuelas and Filella 2001). All these environmental changes will likely produce more frequent, fast and unpredictable changes in nutrient availability in the Mediterranean area. These changes in nutrient supply are frequently related to a change in the density of individual plants and consequently in the intensity of competition pressure, and can interact with changes in the water supply due to global change effects predicted in the Mediterranean basin (Esteban-Parra et al. 1998; Peñuelas et al. 2002). These inputs would be in pulse form (fire ashes) or in irregular steps throughout the time (human driven atmospheric deposition). Therefore the nutrient availability can increase suddenly. In this scenario, the vegetation capacity to take direct profit of this sudden availability with more growth, higher nutrient capture, enhanced NUE and increase in biomass nutrient contents can prevent the loss of these nutrients by retaining them in the ecosystem.

The widespread distribution of *P. halepensis* in the Mediterranean basin, the deficiency of information about NUE in this forest Mediterranean species, the unpredictable variation of the levels of nutrient availability, and the importance of the nutrients in these communities led us to conduct a field experiment with *P. halepensis* with the following aims: (i) to investigate the effects of the increase of nutrients (N + P) supply and water availability and their interactions with the removal of competing vegetation on nutrient contents, litterfall production, nutrient loss in the litterfall and needle retranslocation, (ii) to investigate the separate effects of N and P supply and their interactions with the removal of competing vegetation on these nutrient related variables, and (iii) to investigate the effects of the increase of N and P supply and their interactions with the removal of competing vegetation on the NUE of P, N, Mg and K.

Materials and methods

Two-hundred-and-forty-two individuals of *P. halepensis* were selected in order to establish a factorial experiment of N and P fertilisation, irrigation and removal of competing vegetation in the field.

Experimental site

The experiment was conducted in a naturally regenerated post fire shrubland that had burnt three times in the past 20 years. The last fire occurred in the summer of 1985, 5 years before the experiment started. The study site was located on a level hill top (slope 0–5%) at 300 m above sea level (41°37′N, 1°50′E) 45 km NW of Barcelona city. Soils were *Lithic hsploxerolls* associated with *Lithic xeroryhents* (Soil taxonomy) developed over calcareous marls. They had a high pH (8.5) and a high proportion of carbonates (56%) and active lime (12%). The climate is Mediterranean with a slight continental component. Mean annual rainfall is 517 mm. The vegetation type is a post-fire Mediterranean shrubland (*Erico-Thymalaeetum tinctoriae*) with young individuals of Aleppo pine (*P. halepensis*) and small regrowths of interior holm oak (*Quercus ilex rotundifoluia*). At the beginning of the study (June 1990) all the Aleppo pines were 5 years old, their overall height was 0.503±0.01 m, the overall pine basal area was 208±12 mm^2 and covered a 27% of total surface of the site, and the total vegetation cover was 75%. At the end of the experiment (October 1993), Aleppo pines overall height was 1.331±0.02 m. The overall pine basal area was 1,162±68 mm^2. Pines covered 30% of total area of the site, and the total vegetation cover was 78%.

Experimental designs

With the aim of investigating the effects of nutrients and water supply and competing vegetation on the leaf nutrient reabsorption and the litterfall production, a factorial experimental design was established. In this first design we analysed the nutrient, water and competing vegetation pressure effects. Thus this design consisted of NP fertilisation (two levels: no fertilisation and fertilisation with 250 kg P ha^{-1} +500 kg N ha^{-1}), irrigation (two levels: no irrigation, and irrigation with 24 mm weekly during the dry season), and removal of competing vegetation (two levels: with and without removal). This design had eight different treatments four for each level of each factor and analysed 88 *P. halepensis* trees (11 per treatment). The treatments were: C, NP, R, NPR, C*, NP*, R* and NPR* (C = control; NP = N fertilisation with 500 kg ha^{-1} plus P fertilisation 250 kg ha^{-1}; R, irrigation; and *, removal of competing vegetation).

We also established a design that allows the separate analysis of the P and N effects in different competitive pressure. This second design consisted of P fertilisation (3 levels: 0, 125 and 250 kg P ha^{-1}), N fertilisation (3 levels: 0, 250, 500 kg ha^{-1}), and removal of competing vegetation (2 levels: with and without removal). This design was established to analyse separately the N and P effects and their interactions. This design had 18 different treatments and analysed 198 *P. halepensis* trees (11 per treatment). The treatments were C, N_1, N_2, P_1, P_2, N_1P_1, N_1P_2, N_2P_1, N_2P_2, C*, N_1*, N_2*, P_1*, N_1P_1*, N_1P_2*, N_2P_1*, N_2P_2* (C = control; N_1 and N_2 = N fertilisation 250 and 500 kg P ha^{-1}, P_1 and P_2 = P fertilisation 125 and 250 kg P ha^{-1}, * = removal of competing vegetation).

To investigate the nutrient supply and neighbour pressure on P, N, Mg and Fe NUE, a third design was established. The NUE estimation requires plant cut off and to avoid excessive destructive sampling we reduced the factor levels to two. This design consisted of N fertilisation (2 levels: no fertilisation, and fertilisation with 500 kg N ha^{-1}), P fertilisation (2 levels: no fertilisation, and fertilisation with 250 kg ha^{-1}) and removal of competing vegetation (2 levels: with and without removal). This design was the same than the second one but with fewer levels of fertilisation. This design had eight treatments and analysed 88 *P. halepensis* (11 per treatment). At the beginning of the experiment ten pines were uprooted to study the root system; we were able to check that the root system did not extend more than 1 m from the stem. Thus we were able to demonstrate that all the pines selected for the experiment were separated from each other at the minimum distance of 4 m. Previously to the treatments application, we conducted several ANOVAs in order to detect possible differences in the average size means (height and basal area) between pines of different treatments; we established the treatments only when there were no differences. The pines were randomly assigned to each treatment with only the condition of minimum distance between them.

Treatment applications

The fertilisers we employed were ammonium nitrate (N) and calcium phosphate (P). The fertilizers were applied in solid form and only once. The fertiliser was applied manually and directly on the soil surface in a single application at the beginning of the experiment in June 1990. We observed the root systems of some individuals of the same size and age as the target experimental individuals at the beginning of the experiment before treatment and saw that the roots did not cover more than an 80 cm diameter; thus we established a 1 m^2 circle around a target where we applied the fertilisation and irrigation treatments. This area was considered sufficient to cover the major part of the root system. The fertiliser was applied in doses equivalent to 125 kg ha^{-1} and 250 kg ha^{-1} in the case of P and 250 kg ha^{-1} and 500 kg ha^{-1} in the case of N. The fertilisation with 250 kg P ha^{-1} and 500 kg N ha^{-1} is a common practice in fertilisation experiments (Xu et al. 2002; Mitchell and Smethurst 2004). These quantities are in the order of magnitude of the possible nutrient liberation during a fire. A typical Mediterranean forest has an average of 335–275 kg N ha^{-1} and 77–56 kg P ha^{-1} in the aboveground biomass and 531–622 kg N ha^{-1} and 41 kg P ha^{-1} in the floor litter (Rodà et al. 1999). Therefore, the quantities of fertilizer employed in the present study are of the same order of magnitude as the nutrient quantities that can be liberated during a fire event in a typical Mediterranean forest.

The irrigation was applied at a weekly dose of 24 mm in the 4 years of the study (1990–1993), in 1990 from July (the beginning of the experiment) to September, and in 1991–1993 from April to September. The irrigation treatment was not applied those weeks with natural rainfalls surpassing 24 mm.

The removal of competing vegetation was conducted by clipping all the competing vegetation for 1 m^2 around the target plant to ground level, a method widely used in this type of experimental design (Connell 1983; Aarsen and Epp 1990). The clipping was repeated every 2–6 months depending on the competing vegetation growth, with the aim of maintaining the soil surface without aboveground competing vegetation, and consequently of maintaining low competition pressure

Needle and litterfall sampling

Needle sampling of all *P. halepensis* trees was conducted in July 1992 and 1993. Two needle fractions were considered in each treatment: the current-year needles and the 1-year old needles. Thus in 1992 we sampled the needle cohort of spring 1991 and spring 1992, 10 and 22 months after fertilisation, and in 1993 we sampled the needle cohorts of spring 1992 and of spring 1993 22 and 34 months after fertilisation application, respectively. To standardise the sampling, the needless of each pine were collected in five groups: one in each cardinal direction and a fifth from the top of the crown.

The litterfall was collected in four 100 cm^2 surface plots placed in each cardinal direction under the crown of each pine. Litterfall was sampled every 3 months and the total litterfall produced by each pine was calculated by extrapolating to the total area of the crown. The total area of the crown was estimated by mean crown projected area. The litterfall was collected for 2 years, the second and the third years after treatment application. These periods comprised from July 1991 to July 1992, and from July 1992 to July of 1993. Aleppo pine litterfall was exclusively formed of needles because other potential litterfall component such as flowers and fruits were not present due the low age of the pines (between 5 and 7 years old). Only the Aleppo pine litterfall was processed.

Chemical analyses

The needles and the litterfall of all *P. halepensis* trees were then chemically analysed. In all the pines (in the 3 experimental designs) P, S, K, Ca, Mg and Fe concentrations were analysed by atomic emission spectroscopy with inductively coupled plasma (ICP-AES). N was analysed in the needles and the litterfall of the 88 pines used in the design for NUE estimation (third design) by the Kjeldahl method (Bradstreet 1991). N was also analysed in the leaves of all *P. halepensis* in the first sampling (July 1992).

Needles and litter were crushed and dried (85°C for 48 h). The ICP-AES analyses were conducted after an acid digestion (HNO_3:$HClO_4$, 2:1, v:v) in a microwave Moulinex Optiquick Duo Y82 using open fluorinated ethylene propylene flasks (Nalge). The concentrations were determined in a Polyscan Thermo Jarrel ASH Model 61 E spectrophotometer. The N concentration analyses were determined in a Kjeltec 1030 Autoanalyzer after acid digestion (H_2SO_4 + catalyser).

Calculation of nutrient loss in the litterfall, retranslocation and NUE

We calculated the total annual loss of nutrients in the litterfall by the product of the litterfall mass and nutrient concentration in litterfall. By knowing the crown area 2 years after the treatment application, we calculated the litterfall losses per unit of crown area.

Retranslocation is the nutrient return from the leaf to the rest of the plant before needle abscission (Chapin 1980; Staff and Berg 1981). We calculated leaf retranslocation as the difference between the needle nutrient concentrations in the living needles of the 1991 leaf cohort sampled in July 1992 (before of its fall period) and the litterfall concentrations between summer 1992 and summer 1993, and then taking this difference from the needle concentration of summer 1992. The needle litterfall of this period corresponded to the needles of the 1991 cohort. The major part of the needles of the 1991 cohort fell during the period between July 1992 and October 1992. Thus, we can consider that when we sampled them in July 1992 they were in a senescence process induced by 1 month of summer drought. This methodology has been widely used by many authors (Sharma and Pande 1986, among others).

$$\%\,\text{Retranslocation} = (N - L)/N$$

where N is the percentage of nutrient in needles before abscission and L is the percentage of nutrient in litterfall.

The retranslocation of P, S, K, Ca, Mg and Fe were calculated in the three experimental designs. The N retranslocation was only calculated in the design with N and P fertilisation treatments and neighbour removal.

The NUE calculation was conducted for the period between July 1992 and July 1993. We calculated the NUE for N, P, K, and Mg. The NUE was calculated by two different equations: as a quantity of biomass built per unit of the nutrient in the biomass (Wang et al. 1991) that correspond to the *A* factor of Aerts (1989) (Eq. 1), and as a quantity of biomass produced per unit of nutrient lost in the litterfall (Birk and Vitousek 1986) (Eq. 2).

$$\mathrm{A} = \mathrm{NUE1} = \text{Increase of total aboveground biomass}/\text{Total nutrient in aboveground biomass} \quad (1)$$

$$\mathrm{NUE2} = \text{Increase of total aboveground biomass}/\text{Total nutrient loss in the biomass} \quad (2)$$

Both calculations require knowledge of increase in total aboveground biomass: (Increase of total aboveground biomass = biomass increment between July 1992 and October 1993 + Litterfall produced in this period).

The total litterfall was obtained by the sum of the litterfall masses sampled in the corresponding 3 month periods, whereas the aboveground biomass increment between July 1992 and July 1993 was obtained as the difference between the biomasses calculated allometrically. In October 1993 the 88 *P. halepensis* of the third design (P fertilisation with 2 levels, N fertilisation with 2 levels, and neighbour removal with 2 levels) were harvested. In each pine, four fractions were considered: needles of the 1993 cohort, needles of the 1992 cohort, stems from 1993, and the rest of the stem fraction. The pines were harvested in October and not in July with the aim of sampling the litterfall from the period between these two dates and therefore of having the same information for these pines as for the pines in the other experiments. Due to the lack of growth between July and October 1993, the biomasses reached in October were considered equivalent to those of July plus the litterfall produced within this period. The biomass that the pines had at the beginning of the period considered for NUE calculations (July 1992) was calculated allometrically through double logarithmic regressions between the biomasses of the different fractions obtained in October 1993 and the basal diameters obtained in the same month. This also allowed the estimation of the biomass in July 1992 through their basal diameter. Nevertheless the possible differences in the allometric relations between the pines that had received different treatments were also tested. With the aim of detecting whether the experimental factors had affected the allometric relations between the trunk basal diameter and the aboveground biomass fractions ANCOVAs were conducted with the experimental treatments as independent variables and the basal diameter of October 1993 (just before harvest) as a covariable on the biomasses of the four fractions obtained. Only the neighbour removal treatment affected the allometric relations at this level. Therefore, different allometric relations were applied to estimate the aboveground biomasses between the pines with and without neighbour removal treatment (Table 1).

Through the allometric relations and with the knowledge of the basal diameter of 1992 of each *P. halepensis* we also calculated the biomass fractions that *P. halepensis* had in the July 1992, and with the sum of the fractions we calculated the total biomass, the fraction biomass and the respective increments between July 1992 and July 1993.

To calculate Eq. 1 (de Wang et al. 1991) it is necessary to know the total nutrient in aboveground biomass present in the total aboveground biomass. Since the *P. halepensis* trees were in an exponential growth phase of their life cycle, we used the mean between the mineral mass contents at the beginning of the period (July 1992) and at the end of it (July 1993). These mineral masses were calculated by the fraction biomasses and the corresponding nutrient concentrations.

To calculate Eq. 2, the equation of Birk and Vitousek (1986), we calculated the total amount of nutrient lost in the litterfall for the study period (July 1992–July 1993). The total quantity of litterfall production between July 1992 and July 1993 was obtained directly through the litterfall samples. The amount for the first period (July–October 1992) was calculated by the nutrient concentrations of the litterfall sampled in the first year of litterfall sampling multiplied by the corresponding litterfall mass. In the last period (October 1992–July 1993), the nutrient lost was calculated by multiplying the nutrient concentrations of the litterfall sampled in the second year of litterfall sampling by the corresponding litterfall mass.

Table 1 Allometric relations between the aboveground biomass fractions and the basal diameter in the 88 pines of the NUE calculation design. The determination coefficients and the signification level (*P*) are shown in each case (*BD*, stem basal diameter)

Pines with competing vegetation	
log Biomass current year leaves = 2.98 log BD−1.17; r^2=0.86	P<0.0001
log Biomass previous years leaves = 2.87 log BD−1.57; r^2=0.88	P<0.0001
log Biomass current year stems = 2.42 log BD−2.13; r^2=0.88	P<0.0001
log Biomass previous years stems = 2.70 log BD−1.65; r^2=0.95	P<0.0001
Pines without competing vegetation	
log Biomass current year leaves = 2.28 log BD−1.35; r^2=0.86	P<0.0001
log Biomass previous years leaves = 2.03 log BD−1.10; r^2=0.89	P<0.0001
log Biomass current year stems = 2.80 log BD−2.73; r^2=0.82	P<0.0001
log Biomass previous years stems = 2.44 log BD−1.22; r^2=0.90	P<0.0001

With all these data we also analysed the absolute increases of nutrients (N, P, K, Mg) in the aboveground biomass, the total quantities of these nutrients lost in the litterfall (period July 1992–July 1993), the increases of the needle, stem and total biomass during the period July 1992–July 1993, and the relation between leaf biomass nutrient contents and stem biomass nutrient contents.

Statistical analyses

The effect of treatments on the studied variables were analysed through factorial ANCOVAS (with the basal diameter as a covariable when it had a significant effect). The litterfall production, the amount of lost nutrients and the NUE values calculated by Birk and Vitousek method (1986) were normalised by ANCOVA analyses through a logarithmic transformation to meet analysis requirements. Since the litterfall began to be sampled 1 year and 3 months after the treatment application, the effects of the fertilisation and neighbour removal could be due to an indirect effect of the treatment though an increment in plant size in the first year after treatment (Sardans 1997). Therefore we also analysed the effects of the treatment factors by ANOVA. Where effects of a treatment factor on one single variable were significant in the ANCOVA, but they were not significant in ANOVA, we deduced that this factor had an indirect effect through plant size. We considered significant the differences between the different levels of the different factors or between the different treatments when $P<0.05$. All analyses were conducted using Super ANOVA (Abacus Concepts, Berkeley).

Results

Needle nutrient concentrations

NP fertilisation, irrigation, removal of competing vegetation

July 1992 sample Fertilised pines had higher P concentration than non-fertilised pines (Table 2). The irrigation increased the Ca concentrations in the needle of the previous years (Table 2). The removal of competing vegetation increased the needle concentrations of Mg, Ca and Fe in the current-year needle (Table 2).

July 1993 sample Fertilised *P. halepensis* trees had higher P needle concentrations than the non fertilised trees, but the difference was not significant. The fertilisation continued to have a negative effect on S and Mg concentrations (Table 2). The irrigation increased the Ca, Mg and Fe concentrations in the 1992 needle cohort (Table 2).

N fertilisation, P fertilisation, removal of competing vegetation

July 1992 sample P fertilisation increased the P concentrations in the 1991 and 1992 needle cohorts (Table 2, Fig. 1).

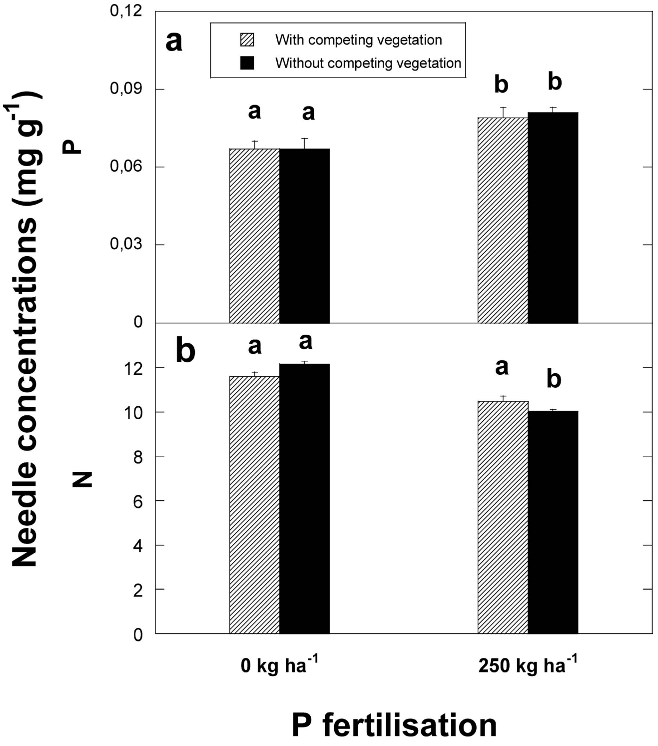

Fig. 1 **a** P concentrations and **b** N concentrations of 1-year-old leaves of the P fertilised (250 kg P ha^{-1}) and the not fertilised Aleppo pines growing with and without removal of competing vegetation sampled in September 1993 in the second design (N fertilisation P fertilisation plus removal of competing vegetation). Different letters indicate significant different values at $P<0.05$

Likewise, P fertilisation decreased the N, S and Mg concentrations in the 1991 and 1992 needle cohorts (Table 2, Fig. 1). N fertilisation increased the N concentrations in the 1991 needle cohort. The removal of competing vegetation increased the Ca and Mg concentrations in the 1992 needle cohort and decreased the concentrations of these two nutrients in the 1991 needle cohort (Table 2).

July 1993 sample P fertilisation increased the P concentrations but only significantly in the 1992 cohort. N fertilisation decreased the S concentrations in 1-year-old needles (Table 2). The removal of competing vegetation decreased the Fe and S needle concentrations (Table 2).

P fertilisation decreased N concentrations in the 1992 and 1993 needle cohorts (Table 2). The N fertilisation increased the N concentrations of the 1992 needle cohort (Table 2). A significant interaction between the removal of competing vegetation and P fertilisation was observed in N concentrations of the 1992 needle cohort (Fig. 1). The N foliar decrease by P fertilisation was greater when it was accompanied by removal of competing vegetation.

Litterfall production

The litterfall production had a clear maximum in the quarter from July to October in the 2 years of the study (Fig. 2). Since the crown area had a positive effect on the litterfall

Table 2 Needle nutrient concentrations (mg g^{-1}) that presented significant variations due to experimental factors

Sampled July 1992						
NP fertilisation, irrigation, removal of competing vegetation						
Treatments	Needle cohort	Element		Not fertilised	Fertilised	*P* (*n*=44)
Fertilisation	1991	Mg		1.65±0.05	1.41±0.06	0.006
	1991	S		1.35±0.05	1.08±0.04	0.0003
	1991	P		0.5±0.02	0.68±0.04	0.004
	1992	Mg		1.13±0.04	0.96±0.03	0.002
	1992	S		1.13±0.03	0.97±0.03	0.09
				No irrigation	Irrigation	
Irrigation	1991	Ca		7.62±0.42	8.65±0.22	0.022
				With competing vegetation	Without competing vegetation	
Neighbour presence	1992	Mg		0.99±0.04	1.09±0.05	0.051
	1992	Fe		0.43±0.02	0.52±0.03	0.01
	1992	Ca		3.14±0.1	3.97±0.21	0.002
P fertilisation, N fertilisation, removal of competing vegetation						
Treatments	Needle cohort	Element	0 kg ha^{-1}	125 kg ha^{-1}	250 kg ha^{-1}	*P* (*n*=66)
P fertilisation	1991	Mg	1.57±0.05	1.46±0.05	1.42±0.04	0.044
	1991	P	0.49±0.01	0.61±0.02	0.63±0.02	0.0001
	1991	S	1.33±0.04	1.13±0.03	1.08±0.08	0.0001
	1991	N	13.0±0.6	10.1±0.1	10.1±0.2	0.0001
	1992	Mg	1.14±0.03	1.03±0.02	1.09±0.2	0.0001
	1992	P	0.82±0.03	0.95±0.06	1.06±0.03	0.0004
	1992	S	1.1±0.02	1.02±0.01	0.95±0.02	0.0001
	1992	N	12.1±0.3	10.7±0.2	10.9±0.2	0.0001
			0 kg ha^{-1}	250 kg ha^{-1}	500 kg ha^{-1}	
N fertilisation	1991	N	10.3±0.4	11.2±0.6	11.6±0.1	0.02
				With competing vegetation	Without competing vegetation	
Neighbour presence	1991	Mg		1.54±0.03	1.43±0.05	0.02
	1991	Ca		8.44±0.23	7.62±0.2	0.007
	1991	N		11.5±0.01	10.5±0.2	0.02
	1992	Ca		3.16±0.12	4.18±0.12	0.003
	1992	Mg		1.01±0.02	1.1±0.03	0.001
Sampled July 1993						
NP fertilisation, irrigation, removal of competing vegetation						
Treatments	Needle cohort	Element		Not fertilised	Fertilised	*P* (*n*=44)
Fertilisation	1992	S		1.5±0.04	1.14±0.03	0.0001
	1993	S		1.29±0.03	1.1±0.03	0.004
				No irrigation	Irrigation	
Irrigation	1992	Ca		7.67±0.59	9.36±0.41	0.021
	1992	Mg		1.74±0.07	1.98±0.09	0.032
	1992	S		1.27±0.07	1.37±0.06	0.03
				With competing vegetation	Without competing vegetation	
Neighbour presence	1992	Ca		9.26±0.44	7.76±0.59	0.038
P fertilisation, N fertilisation, removal of competing vegetation						
Treatments	Needle cohort	Element	0 kg ha^{-1}	125 kg ha^{-1}	250 kg ha^{-1}	*p* (*n*=66)
P fertilisation	1992	S	1.35±0.04	1.88±0.04	1.05±0.06	0.04
	1992	Mg	1.85±0.05	1.75±0.04	1.65±0.07	0.05
	1993	S	1.20±0.04	1.18±0.02	1.08±0.03	0.016
	1993	P	0.67±0.03	0.78±0.02	0.80±0.02	0.001
			0 kg ha^{-1}	250 kg ha^{-1}	500 kg ha^{-1}	
N fertilisation	1992	S	1.29±0.05	1.14±0.07	1.15±0.04	0.04
	1993	Fe	0.12±0.01	0.09±0.01	0.12±0.01	0.007
				With competing vegetation	Without competing vegetation	
Neighbour presence	1992	Mg		1.68±0.05	1.81±0.04	0.04
	1993	S		1.2±0.004	1.1±0.03	0.008
	1993	Fe		0.08±0.004	0.05±0.004	0.0004

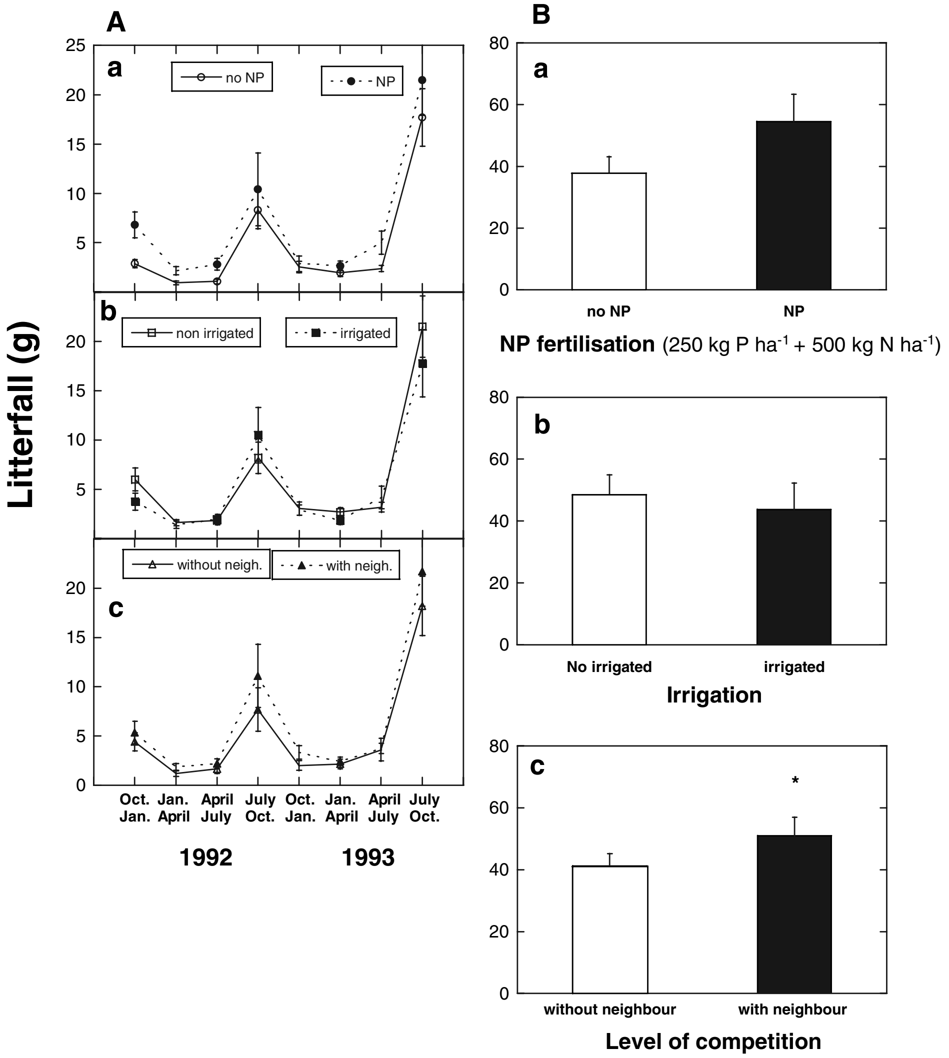

Fig. 2 **A** Mass of litterfall per individual tree sampled every 3 months under different levels of treatment in the experimental design: *a* NP fertilisation, *b* Irrigation, *c* Removal of competing vegetation. **B** Total litterfall production per tree (Mean ± SE) over 2 years (October 1991–October 1993) under different levels of treatment in the experimental design: *a* NP fertilisation, *b* Irrigation, *c* Removal of competing vegetation (* $P<0.05$)

production, this variable was used as a covariable in the ANCOVA test.

NP fertilisation, irrigation, removal of competing vegetation

Neither the fertilisation nor irrigation had any significant effect on litterfall production (Fig. 2). Removal of competing vegetation decreased litterfall production significantly in the 2 years ($P=0.02$, $n=44$ in October 1991–October 1992; and $P=0.03$ in October 1992–October 1993; see Fig. 2). In all litterfall samplings, the pines with removal of competing vegetation treatment produced less litterfall biomass than the pines without removal of competing vegetation (Fig. 2). The crown area was significant in the ANCOVA analysis and thus was maintained in it as a covariable.

N fertilisation, P fertilisation, removal of competing vegetation

Neither N fertilisation nor P fertilisation had any effect on litterfall production. Removal of competing vegetation decreased litterfall biomass production ($P=0.015$, $n=66$ in October 1991–October 1992; and $P=0.05$, $n=66$ in October 1992–October 1993) as in the previous design (data not shown).

Litterfall nutrient concentration and total nutrients loss in litterfall

NP fertilisation, irrigation, removal of competing vegetation

Fertilisation increased the Ca, P and S concentrations in the litterfall of the first year, and decreased the Mg concentrations in the first year and the Fe concentrations in both years (Table 3). In spite of these effects on concentrations, fertilisation had no significant effects on total nutrients lost in the litterfall. In general, fertilisation increased the nutrient concentrations in the litterfall but reduced the litterfall biomass. Removal of competing vegetation treatment increased concentrations of Ca, S and Fe in both years, and Mg and P concentrations in the second year

Table 3 Nutrient concentrations (mg g^{-1}) in the needle litterfall (Mean ± SE) for the different levels of the factors that had significant effects ($P<0.05$)

October 1991–October 1992					
NP fertilisation, irrigation, removal of competing vegetation					
Treatments	Nutrient	Not fertilised		Fertilised	P (n=44)
Fertilisation	Ca	14.4±0.5		16.2±0.7	0.007
	Mg	1.03±0.03		0.98±0.03	0.0001
	P	0.12±0.004		0.16±0.01	0.0001
	S	0.93±0.03		1.03±0.04	0.019
		Not irrigated		Irrigated	
Irrigation	P	0.15±0.01		0.13±0.01	0.008
	Fe	0.23±0.01		0.19±0.01	0.0006
		Without competing vegetation		With competing vegetation	
Neighbour presence	Ca	17.2±0.7		13.5±0.4	0.0001
	Mg	1.03±0.02		0.98±0.04	0.0001
	S	1.05±0.03		0.91±0.03	0.003
	K	0.33±0.01		0.44±0.02	0.0001
	Fe	0.24±0.01		0.18±0.01	0.0001
P fertilisation, N fertilisation, removal of competing vegetation					
Treatments	Nutrient	0 kg ha^{-1}	125 kg ha^{-1}	250 kg ha^{-1}	p (n=66)
P fertilisation	Ca	13.4±0.4	12.2±0.5	15.8±0.4	0.0001
	Mg	0.99±0.02	0.81±0.03	0.99±0.01	0.0001
	P	0.13±0.004	0.17±0.01	0.19±0.01	0.0001
	K	0.41±0.02	0.46±0.02	0.49±0.02	0.0002
	Fe	0.20±0.01	0.40±0.07	0.33±0.01	0.0001
		0 kg ha^{-1}	250 kg ha^{-1}	500 kg ha^{-1}	
N fertilisation	Ca	13.1±0.5	13.8±0.4	15.6±0.5	0.027
	K	0.42±0.01	0.53±0.02	0.51±0.01	0.0001
	Fe	0.22±0.01	0.40±0.07	0.32±0.01	0.0001
		Without competing vegetation		With competing vegetation	
Neighbour presence	Ca	15.3±0.3		12.4±0.4	0.0001
	S	1.09±0.02		0.95±0.02	0.0001
	K	0.41±0.01		0.49±0.02	0.0001
	Fe	0.31±0.03		0.23±0.02	0.015
October 1992–October 1993					
NP fertilisation, irrigation, removal of competing vegetation					
Treatments	Nutrient	Not fertilised	Fertilised		P (n=44)
Fertilisation	Mg	1.41±0.05		1.14±0.05	0.0001
	Fe	0.22±0.01		0.18±0.01	0.011
		Not irrigated		Irrigated	
Irrigation	Ca	11.3±0.5		12.9±0.04	0.0045
	K	0.40±0.02		0.45±0.02	0.01
	Fe	0.19±0.01		0.22±0.01	0.03
		Without competing vegetation		With competing vegetation	
Neighbour presence	Ca	12.9±0.2		11.3±0.6	0.0031
	Mg	1.40±0.03		1.15±0.07	0.0001
	P	0.19±0.01		0.16±0.01	0.0003
	S	0.96±0.07		0.79±0.05	0.03
	K	0.41±0.01		0.45±0.02	0.03
	Fe	0.22±0.01		0.19±0.01	0.02
P fertilisation, N fertilisation, removal of competing vegetation					
Treatments	Nutrient	0 kg ha^{-1}	125 kg ha^{-1}	250 kg ha^{-1}	P (n=66)
P fertilisation	Ca	12.8±0.2	16.7±0.3	12.3±0.3	0.013
	Mg	1.29±0.03	1.15±0.03	1.09±0.03	0.0001
	P	0.18±0.01	0.18±0.01	0.16±0.01	0.0001
	S	0.92±0.04	0.83±0.03	0.79±0.03	0.013
		0 kg ha^{-1}	250 kg ha^{-1}	500 kg ha^{-1}	

Table 3

P fertilisation, N fertilisation, removal of competing vegetation					
N fertilisation	Mg	1.33±0.03	1.18±0.02	1.02±0.04	0.0001
	P	0.20±0.01	0.19±0.01	0.14±0.01	0.0001
	S	0.93±0.04	0.86±0.04	0.75±0.03	0.0003
	K	0.51±0.02	0.41±0.06	0.34±0.06	0.0001
	Fe	0.20±0.01	0.18±0.01	0.15±0.01	0.0001
		Without competing vegetation		With competing vegetation	
Neighbour presence	K	0.36±0.01		0.48±0.02	0.0001

Table 4 Nutrient losses (mg per tree and year) in the litterfall (Mean ± SE) in the different levels of the factors that had a significant effect ($P<0.05$) on this variable

October 1991–October 1992					
NP fertilisation, irrigation, removal of competing vegetation					
Treatments	Nutrient	Without competing vegetation		With competing vegetation	P (n=44)
Neighbour presence	P	2.94±1		3.2±0.6	0.038
	K	7.22±2.41		9.8±1.9	0.012
P fertilisation, N fertilisation, removal of competing vegetation					
Treatments	Nutrient	0 kg ha^{-1}	125 kg ha^{-1}	250 kg ha^{-1}	
P fertilisation	P	1.87±0.24	3.09±0.50	4.04±0.72	0.01
	K	5.62±0.71	8.86±1.56	10.13±1.67	0.045
		Without competing vegetation		With competing vegetation	
Neighbour presence	K	6.82±1.06		9.59±1.20	0.048
	Fe	5.08±1.01		4.47±0.57	0.048
October 1992–October 1993					
P fertilisation, N fertilisation, competing vegetation					
Treatments	Nutrient	Without competing vegetation		With competing vegetation	P (n=66)
Neighbour presence	K	11.1±1.9		13.8±1.4	0.003

(Table 3). These increases were less evident for the total nutrient losses due to lower litterfall biomass production of pines with removal of competing vegetation. *P. halepensis* lost significantly less K in the first year of litterfall sampling with removal of competing vegetation (Table 4).

N fertlisation, P fertilisation, removal of competing vegetation

N fertilisation tended to decrease all the nutrient concentrations analysed, and it significantly decreased those of Fe, K, S, P and Mg sampled in the second year (October 1992–October 1993) (Table 3), but the lower litterfall production of N-fertilised pines left without any effect on total nutrient losses. P fertilisation increased the P and K concentrations (Table 3) and its total losses (Table 4) in the first year sampled. Similarly, as in the previous design, removal of competing vegetation increased the Ca, Fe and S concentrations in the first year sampled and decreased the K concentrations in both years (Table 3). Removal of competing vegetation decreased the total K losses in the first year of litter fall sampled (Table 4). Tables 3 and 4 show the values of nutrient concentrations and total nutrient losses in litterfall that have increased or decreased significantly ($P<0.05$, $n=66$) due to experimental treatments.

Litterfall nutrient losses per unit of crown area

NP fertilisation, irrigation, removal of competing vegetation

NP fertilisation increased ($P=0.01$) P loss per unit of crown area in the first year sampled (October 1991–October 1992) (Fig. 3) but it did not have any significant effect on the other nutrient losses per unit of crown area. Removal of competing vegetation decrease P, Mg and K losses during the first year and for the K losses during the second year (Fig. 3).

N fertilisation, P fertilisation, removal of competing vegetation

P fertilisation increased the losses of all six elements analysed during the first year (October 1991–October 1992) (Fig. 3), but in the second year of litterfall sampling this effect was not observed. N fertilisation only significantly increased the Fe losses during the first year (Fig. 3) but had no effect on the other nutrient losses. The removal of competing vegetation treatment, as in the previous design, tended to decrease the nutrient losses, and it did it significantly for P, Mg and K during the first year sampled but only for K losses during the second year of litterfall sampling (data not shown).

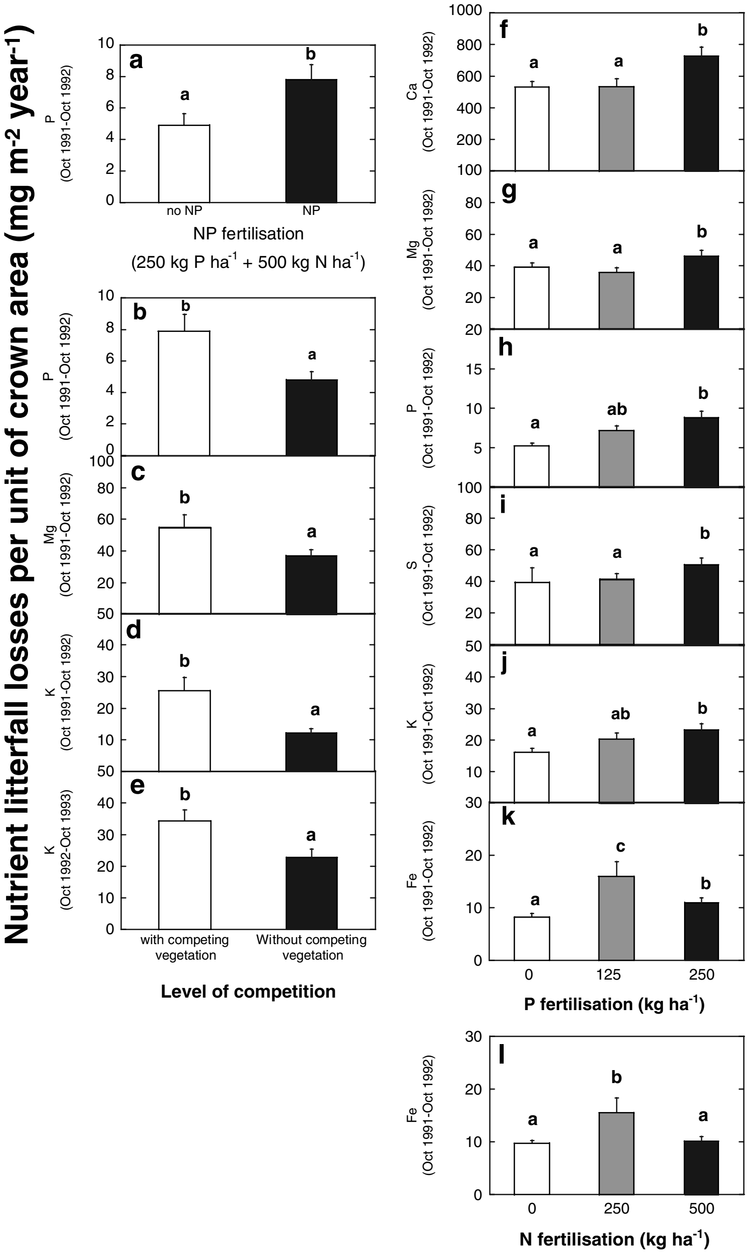

Fig. 3 **a** Litterfall P losses per unit of crown area (mg m^{-2} $year^{-1}$) between the *P. halepensis* with different levels of NP fertilisation in the first experimental design (NP fertilisation, irrigation, removal of competing vegetation). **b** Litterfall P, **c** Mg and **d** K losses (October 1991–October 1992) and losses of **e** K (October 1992–October 1993) per unit of crown area in the *P. halepensis* trees with and without removal of competing vegetation. Litterfall losses of **f** Ca, **g** Mg, **h** P, **i** S, **j** K, and **k** Fe at different levels of P fertilisation and **l** Fe losses at different levels of N fertilisation in the second experimental design (P fertilisation N fertilisation). Different letters above the *bars* indicate statistically different values at $P<0.05$

Retranslocation

NP fertilisation irrigation removal of competing vegetation

NP fertilisation increased P retranslocation (Fig. 4). Remove of competing vegetation treatment decreased Mg and S retranslocation (Fig. 4) and marginally decreased P retranslocation (Fig. 4).

N fertilisation, P fertilisation, removal of competing vegetation

N fertilisation tended to increase the retranslocation of all six nutrients analysed. This increase was significant for P, K and Fe (P=0.03, 0.01, and 0.03, respectively, n=66) (Fig. 5). P fertilisation tended to increase the retranslocation of the six nutrients. The increase was significant for P and Mg (P=0.016 and 0.006, respectively, n=66) (Fig. 5). The removal of competing vegetation treatment had a similar effect to the previous design.

The values of retranslocation obtained in this experiment have been clearly positive (P 65–75%, Mg 3–32%, S 16–36%, K 77–84%, and Fe 76–84%) except for Ca retranslocation which ranged between −65% and −35%. In the sub-design in which NUE was evaluated, the N fertilisation increased (P=0.03, n=44) the N retranslocation whereas P fertilisation decreased (P=0.0003, n=44) N retranslocation (Fig. 6).

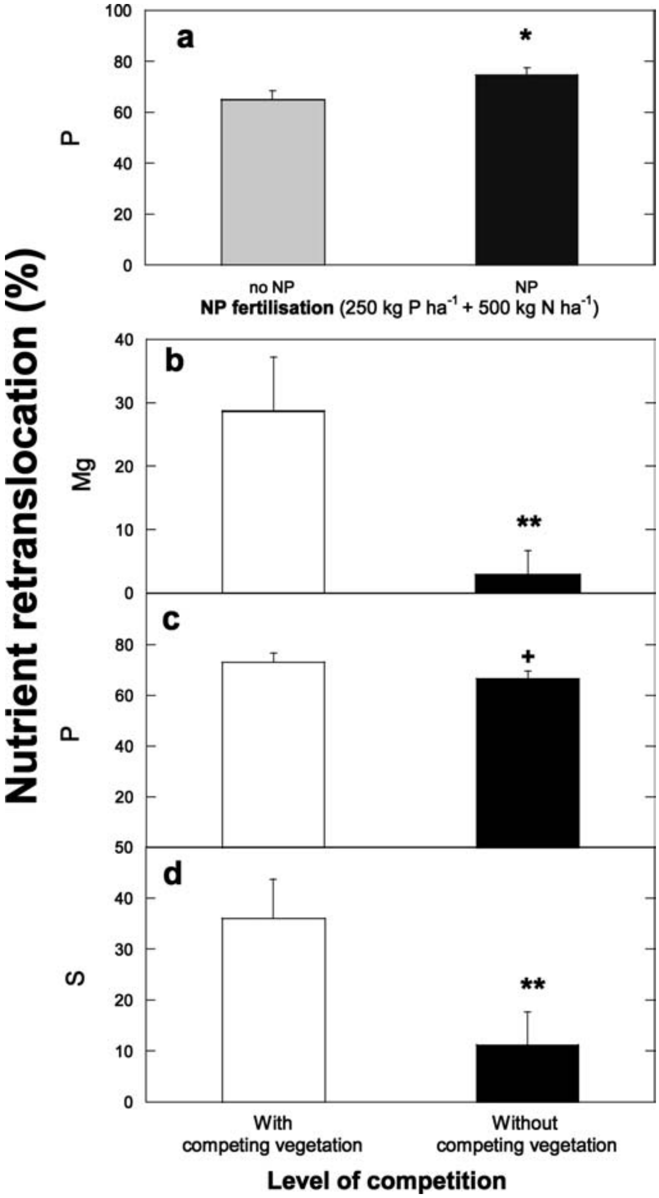

Fig. 4 **a** P Needle retranslocation (%) in the *P. halepensis* trees at different level of NP fertilisation. ($^{*}P<0.05$). **b** Mg, **c** P and **d** S needle retranslocation (%) in the *P. halepensis* trees with and without removal of competing vegetation treatment in the first experimental design (NP fertilisation irrigation remove of competing vegetation) ($^{+}$ $P<0.1$, $^{*}P<0.05$, $^{**}P<0.01$)

NUE and aboveground biomass production

N fertilisation, P fertilisation, removal of competing vegetation

N fertilisation increased the litterfall losses, but did not affect the aboveground biomass growth. Furthermore the total aboveground biomass production (aboveground biomass growth + litterfall production) was higher under N fertilisation but the difference was not statistically significant (Table 5). The N fertilisation increased the losses of N, P, Mg and K in litterfall, but these losses were lower than the increase in total aboveground biomass, leading to an increase in NUE at high levels of N supply according to the Birk and Vitousek equation (Table 5). N fertilisation tended to increase N, P, Mg and K content in the biomass but these increases were not statistically significant. Thus, the N fertilisation increased the nutrient contents in a similar proportion to the total biomass growth and as a consequence there was no effect of N fertilisation on nutrient NUE according to the Wang et al. (1991) equation (Table 5). N fertilisation increased the total N contents in aboveground biomass in the period July 1992 to October 1993 but not significantly, and P fertilisation increased significantly the total P contents in aboveground biomass for the same period (Fig. 7).

P fertilisation increased the net aboveground biomass and the litterfall losses, but these effects were not statistically significant (Table 5). P-fertilised pines had greater P, N, Mg and K contents in aboveground biomass than non-P-fertilised pines, although this increase was statistically significant only for P content (Table 5). P fertilisation significantly decreased N concentrations in litterfall (Table 5). Thus P fertilisation effects in nutrient losses in litterfall had less importance than the increases in aboveground biomass nutrient contents, and consequently there were decreases in the percentage of nutrient losses respect to total nutrient in the aboveground biomass for the four nutrients analysed, although they were statistically significant only for Mg (Table 5). At high P supply, the net aboveground production was proportionally higher than the increases in litter fall losses. These combined effects produced increases in NUE calculated by the Birk and Vitousek equation that were significant for Mg, K and N, and marginally significant for P (Table 5). P fertilisation significantly increased the P contents in aboveground biomass in the period July 1992 to October 1993 (Fig. 7). P contents in the biomass (mineral mass) increased more than biomass in response to the P fertilisation resulting in a significant decrease of P-NUE calculated by the Wang et al. equation (biomass production/P mineral mass) contrasting with the increase of P-NUE (marginally significant, $P=0.1$, $n=44$) when it was calculated by Birk and Vitousek equation (biomass production/litterfall P loss) (Fig. 8a and b). For N-NUE both equations resulted in higher values in response to P fertilisation (Fig. 8c and d).

Removal of competing vegetation had no effects on aboveground biomass production either on the quantity of nutrient in the biomass or on litterfall nutrient losses (Table 5). This absence of effects led to a lack of effects on the NUE equations (Table 5). The pines without competing vegetation from July 1990 to October 1993 significantly increased their needle biomass/stem biomass ratio (Table 5).

Discussion

In general the values of nutrient concentrations found in this experiment were similar to those reported in previous

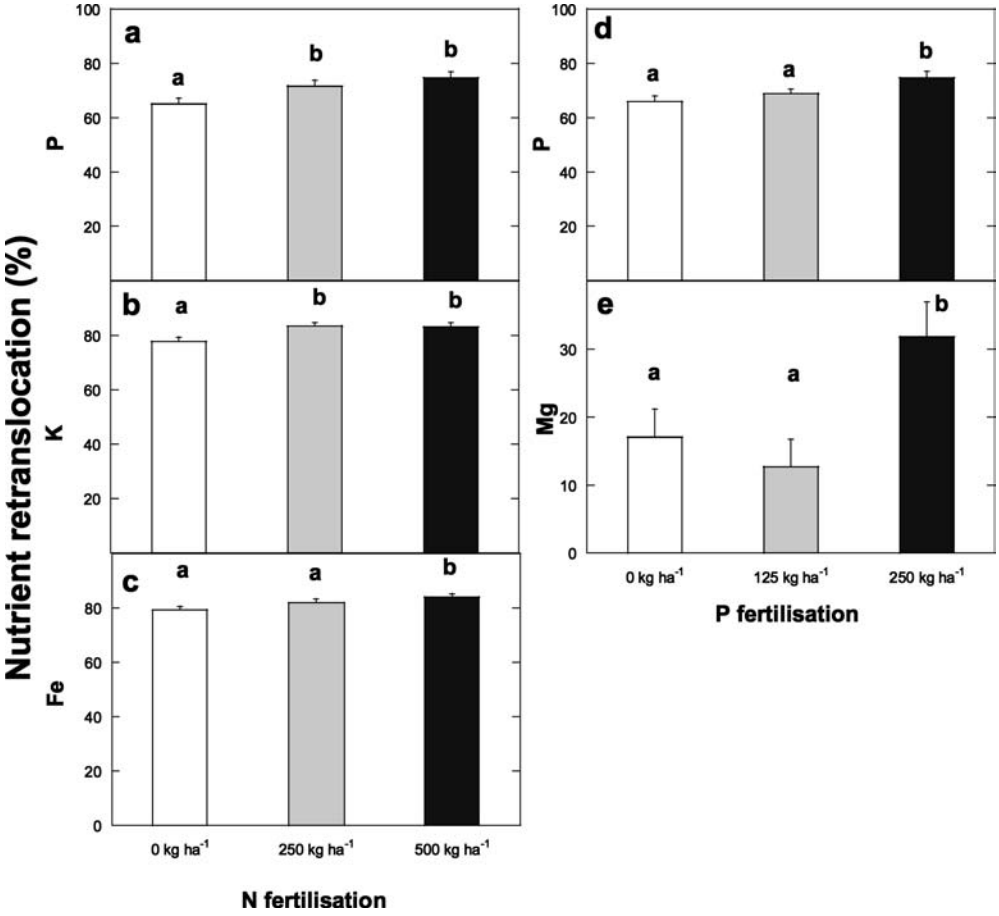

Fig. 5 **a** P, **b** K, and **c** Fe needle retranslocation (%) in the *P. halepensis* trees that received different N fertilisation treatment. **d** P and **e** Mg needle retranslocation (%) in the *P. halepensis* trees that received different P fertilisation treatment in the second experimental design (N fertilisation P fertilisation removal of competing vegetation). Different letters above the *bars* indicate statistically different values at $P<0.05$)

studies on Mediterranean calcareous shrublands (Leonardi and Rapp 1981). The increase of P availability increased those P concentrations. This response indicates the limiting role of this nutrient in these Mediterranean calcareous communities and it is in agreement with growth increases of different species in response to P fertilisation (Sardans et al. 2004). It is interesting that higher levels of P concentrations were found in fertilised pines even 3 years after the fertiliser application. N and/or P fertilisation also produced changes in other nutrient concentrations. Some of them such as Fe and S even decreased as observed in other similar experiments (Ouimet and Fortin 1992). The decreases might be due to the dilution effect consequent to the growth increases or to antagonisms in the absorption process. For example, the low Fe concentrations found in the different aboveground biomass fractions indicate a possible interference in the absorption mechanisms between Fe^{2+} and Ca^{2+}, which is very abundant in these soils.

The irrigation did not significantly decrease the litterfall production. However, the effects of water supply were clear when the litterfall production from October 1991–October 1992 (10.9±2.6 g for each pine) was compared with the litterfall production in October 1992–October 1993 (26±7.5 g for each *P. halepensis*). This great and significant difference can be explained not only by the increase in the size of pines but also by a change in the precipitation during these 2 years. The precipitation was higher in the first year than in the second year (458 mm vs 247 mm during the period of irrigation application, April–August). Previous studies (Voght et al. 1986; Diego and Rodà 1992) have also reported high levels of litterfall production in drier years. The maximum litterfall losses were observed in summer, which is also in agreement with other studies about litterfall production in Mediterranean ecosystems (Escudero et al. 1992; García-Plé et al. 1995). This way, pines have a maximum needle biomass in the spring when the water availability and sunlight allow a positive production balance, and have a minimum needle biomass in the summer when the water stress is maximum. Although fertilisation had no statistically significant effects on litterfall production, it increased the nutrient losses in some periods. The fertilisation frequently induced increases in litterfall production in more mature Mediterranean forests stands (Theodorou and Bowen 1990; Diego and Rodà 1992). The young age of the trees of our experiment can explain the absence of patterns of litterfall production in response to fertilisation. Since the trees were growing exponentially, the majority of nutrient resources were mostly employed in increasing the biomass and less to accelerate the turnover process.

P fertilisation increased nutrient contents, biomass production and P retranslocation to a greater extent when there was removal of competing vegetation, probably as a consequence of the increase in other resources such as light

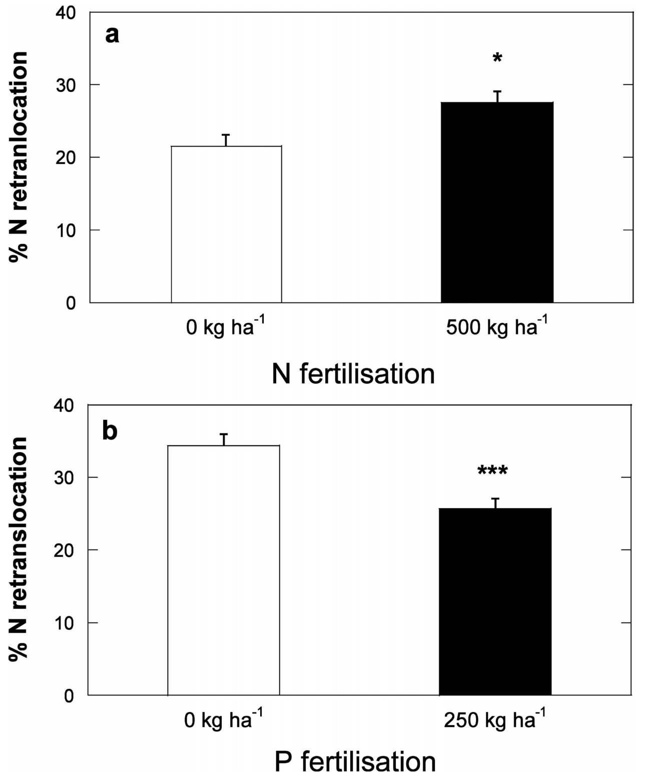

Fig. 6 Needle N retranslocation (%) in the *P. halepensis* trees that received different **a** N and **b** P fertilisation levels in the second experimental design (N fertilisation P fertilisation removal of competing vegetation) (*$P<0.05$, ***$P<0.001$)

and water. The retranslocation would be a mechanism to increase the concentration of limiting nutrients in the biomass (Rapp et al. 1999). The increase of Mg retranslocation as a consequence of P fertilisation may be due to the physiological improvement generated by the P fertilisation-induced increases in the photosynthetic capacity and the consequent Mg retention in leaves. The positive effects of P fertilisation on nutrient contents and biomass growth were only observed when the competing vegetation was removed. In this context, the increase in the P retranslocation observed in the P-fertilised pines can be explained by the increased demands of this nutrient due to the high levels of growth. The general improvement produced by the increase in P availability was more profitable when the competing vegetation had been removed.

The P retranslocation values observed in this experiment (65–73%) are clearly higher than those observed in other Mediterranean species: 46–47% in *Quercus dumosa* and *Q. durata* (Pugnaire and Chapin 1993), 34% in *Q. suber* (Oliveira et al. 1996), or between 30% and 40% in *Q. ilex* (Mayor and Rodà 1992), but are similar with those reported by Wang et al. (2003) in a P deficient mangrove ecosystem. In this experiment P retranslocation tended to increase due to fertilisation with N and P alone or together. It is not clear whether the variations in nutrient retranslocations are positive or negative in response to an increase in nutrient supply (Chapin 1980). The positive effect of fertilisation on retranscolation that was observed in this study can be interpreted as a consequence of the increase of more soluble nutrient organic compounds relative to structural compounds that the better nutritional status produces (Lennon et al. 1985; Negi and Spingh 1992), and perhaps also as a consequence of an improvement in the growth and metabolic status that would demand more nutrients for the new photosynthetic structures. These effects can be especially evident for those nutrients linked to the photosynthetic machinery such as P, N and Mg, as has been observed in our experiment. In this context, we observed that as P fertilisation induced a decrease in needle N concentration and its retranslocation. The decrease of needle N concentrations observed in P fertilised pines can be interpreted as a dilution effect. These data reinforce the limiting role for P in this community and the absence of a limiting role for N. In general, the high levels of P, Fe, K and Mg retranslocation at high P availability have been proportional to high concentrations reached due to fertilisation because the concentrations in the litterfall did not vary between the pines with different P supply. *P. halepensis* did not reabsorb Ca during the needle senescence; on the contrary, the pines allocated more Ca to leaves before leaf fall. Trees can use the litterfall to eliminate the excess of active soluble calcium provided by the high contents of calcareous lime that this soil presents (58%) in an attempt to avoid toxicity problems and/or to maintain the osmotic potentials at the correct levels, this fact have been observed in saline soils with sodium, calcium and magnesium and has been related to a tolerance mechanism to avoid osmotic stress (Pardossi et al. 1999; Wang et al. 2003).

The removal of competing vegetation did not produce great changes in the variables that directly affect NUE, but the lower level of competing vegetation pressure decreased the litterfall production, increasing the needle biomass allocation. This effect could have future implications in the nutrient fluxes and efficiencies and have a positive interaction with P fertilisation by increasing its positive effect on aboveground biomass growth and on some nutrient contents.

P fertilisation had a strong effect on P NUE and NUE of other nutrients. The increase of P availability raised the N, Mg, K and P NUE when this variable was calculated as the total aboveground production per unit of nutrient loss in the litterfall. In contrast, the P supply had a negative effect on P-NUE when this was calculated as total aboveground production per unit of nutrient in the biomass. According to Birk and Vitousek (1986) species from poor nutrient environments have higher production of biomass per unit of nutrient loss than species from rich environments. In our experiment this increase of NUE calculated as a biomass production per unit of nutrient loss would be due principally to the increase of aboveground biomass production that P fertilisation induced. P-fertilised *P. halepensis* strongly increased the P content of the aboveground biomass (66%) in 3 years, and in these 3 years this increase in nutrient content was invested only in part in an aboveground biomass increment. P fertilisation increased both P concentrations and the aboveground biomass and therefore the P content.

Table 5 Nutrient use efficiency and its components. Data correspond to the period between July 1992 and July 1993 (between 2 and 3 years after fertiliser application and removal of competing vegetation treatment beginning). Data from the third experimental design (N fertilisation, P fertilisation, removal of competing vegetation). Competing vegetation presence: *With* with competing vegetation, *Without* without competing vegetation. *NUE1* Biomass production/nutrient lost in litterfall. *NUE2* Biomass production/nutrient in biomass. *Biom. Incre* Aboveground biomass increment. *Litterfall prod.* Litterfall production. *ABP* aboveground biomass increment + Litterfall production. Significant differences ($P<0.05$) are in italic type

Factor	Level	Biom. Incre. (g/year/tree)	Litterfall prod. (g/year/tree)	ABP (g/year/tree)	Total N (g/tree)	Total P (g/tree)	Mg losses (g)	K losses (g)	N losses (mg)	P losses (mg)	
N (kg ha^{-1})	0	402±54	18.4±2.9	421±56	3.45±0.38	0.27±0.03	22.3+3.8	8.99+1.5	127±20	3.45±0.43	
	500	456±49	33.2±7.2	490+52	4.44+0.39	0.33+0.03	27.3+4.6	10.8+1.8	227+38	4.06+0.79	
		P=0.46	P=0.066	P=0.37	P=0.075	P=0.16	P=0.64	P=0.73	P=0.069	P=0.98	
P (kg ha^{-1})	0	381±56	20.1±2.8	401±58	3.69±0.42	*0.23±0.03*	23.4+3.5	8.99+1.5	157±22	3.41±0.47	
	250	478±46	31.5±7.3	510±49	4.22±0.36	*0.37±0.03*	26.1+4.8	10.8+1.8	197±34	4.09±0.84	
		P=0.21	P=0.15	P=0.16	P=0.34	*P=0.0009*	P=0.69	P=0.91	P=0.92	P=0.53	
Comp. veg. presence	With	449±60	26.4±4.2	475±62	3.85±0.43	0.27±0.03	25.8±4.1	10.9±1.8	198±34	4.00±0.68	
	Without	410±42	25.2±6.7	434±45	4.05±0.35	0.33±0.03	23.8±4.3	8.91±1.49	155±38	3.51±0.69	
		P=0.59	P=0.88	P=0.59	P=0.72	P=0.96	P=0.67	P=0.93	P=0.58	P=0.90	
Factor	Level	NUE1 N	NUE1 P	NUE2 N	NUE2 P	Mg biomass (g)	K biomass (g)	NUE1 Mg	NUE1 K	NUE2 Mg	NUE2 K
N (kg ha^{-1})	0	4,450±669	155,200±20,704	115.1±6.8	1,549±95	0.76±0.09	1.75±0.21	25,736+3,852	64,173+3,852	536+29	237+15
	500	4,712±115	258,800±43,445	104.1±5.0	1,484±74	0.89±0.08	2.01±0.18	37,002+7,344	103,000+19,812	234+11	252+29
		P=0.34	P=0.19	P=0.18	P=0.58	P=0.26	P=0.35	P=0.36	P=0.33	P=0.85	P=0.98
P (kg ha^{-1})	0	*3,402±530*	165,600±21,818	*100.2±5.4*	*1,654±88*	0.73±0.09	1.69±0.2	*21,435±2,937*	*60,755±9,550*	517±26	223±12
	250	*5,758±1,064*	251,390±43,445	*119.2±6.2*	*1,380±78*	0.92±0.08	2.06±0.18	*41,303±7,552*	*106,500±19,625*	548±28	248±13
		P=0.024	P=0.1	*P=0.023*	*P=0.024*	P=0.11	P=0.16	*P=0.005*	*P=0.032*	P=0.42	P=0.17
Comp. veg. presence	With	3,998±923	200,700±39,808	115.8±6.6	1,545±89	0.84±0.10	1.81±0.2	29,808+6,722	73,492±16,632	541+27	249+14
	Without	5,164±781	216,300±29,166	103.4±5.2	1,487±81	0.82±0.08	1.94±0.18	32,854±4,992	93,725±14,807	525±27	221+11
		P=0.26	P=0.58	P=0.13	P=0.62	P=0.84	P=0.79	P=0.62	P=0.38	P=0.67	P=0.12

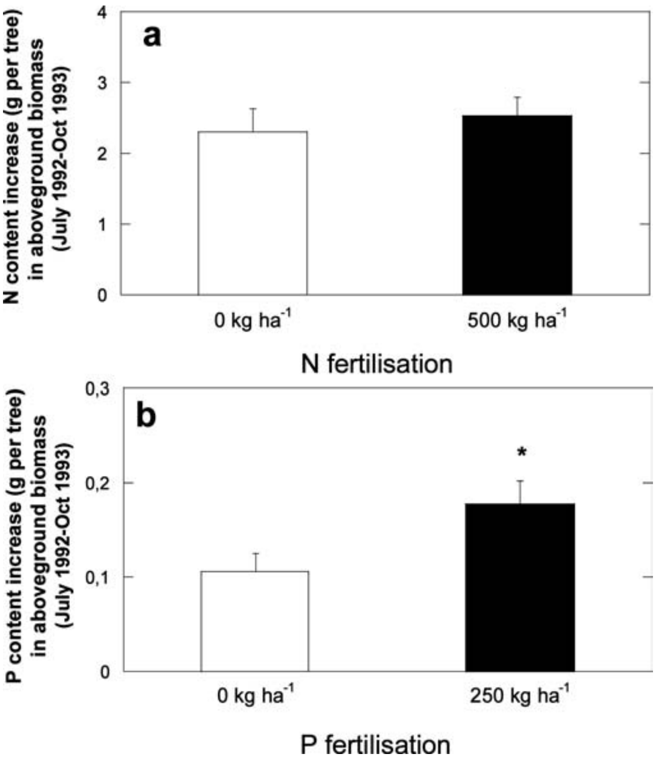

Fig. 7 **a** N and **b** P contents increase in the aboveground biomass during July 1992–July 1993, in the *P. halepensis* trees under different N (**a**) and P (**b**) fertilisation levels in the third experimental design (N fertilisation P fertilisation removal of competing vegetation) (*$P<0.05$)

Thus the decrease in the P-NUE in the P-fertilised pines when this variable was calculated as aboveground biomass production per unit of nutrient present in the biomass was due to the increase in the P concentration. If the increment of biomass is divided only by the P leaf contents, no significant differences are observed between the fertilised and non-fertilised pines. The values of this P-NUE observed in this experiment are high; ten times higher than the values that Nuñez-Oliveira et al. (1993) obtained in a *Cistus* Mediterranean shrubland, probably due to the high capacity of growth of the young Aleppo pines.

In summary, these results show that *P. halepensis* has a strong capacity to quickly respond to the sudden increase of a limiting nutrient such as P. The P concentrations and contents increased in leaves and stems. The percent P retranslocation and the aboveground growth increased. These responses to P fertilisation were enhanced when the vegetative competition was removed, showing the high competing pressure for the nutrients with shrubs in the first phases of forest regeneration in this Mediterranean forest ecosystem. These responses of young pines also show a capacity to retain nutrients such as P in the ecosystem and avoid the immediate drastic erosion losses. The increased P retranslocation together with the increased biomass growth produced an increase of P-NUE calculated as a biomass production per P loss in litterfall. On the other hand, when NUEs were calculated as aboveground biomass production per unit of P present in the biomass, the increase of P availability decreased the P-NUE because of increased P concentrations and increased the N-NUE because of decreased N concentrations. These results emphasize the need to be very careful when discussing NUE results because this variable strongly depends on the equation used in its calculation and the nutrient limiting role. Consequently, it is important to calculate NUE by different equations to have a wider understanding of the variables involved in NUE changes. N has no limiting role in this stand. Despite the fact that it increased its own concentration after

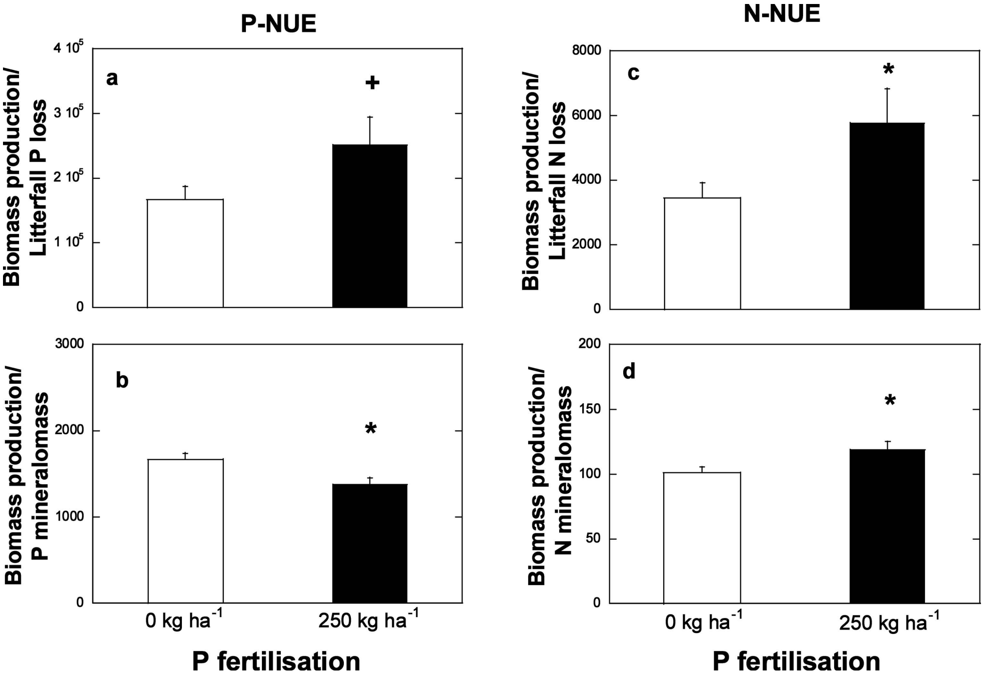

Fig. 8 P-NUE defined as **a** Biomass production/Litterfall P loss and as **b** Biomass production/P mineral mass at different levels of P fertilisation. N-NUE defined as **c** Biomass production/Litterfall N loss and as **d** Biomass production/N mineral mass at different levels of P fertilisation in the third experimental design (N fertilisation, P fertilisation, removal of competing vegetation) (*$P<0.05$, $^{+}P<0.1$)

fertilisation, it did not increase the aboveground production of its own NUE or litterfall production.

Acknowledgements This research was supported by MCYT projects AMB 95-0247, REN2003-04871 CGL 2004-01402/BOS, and the European project ALARM (Contract 506675)

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