Nitrogen availability and plant cover: the importance of nitrogen pools

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Abstract

Despite its great importance to plants' response to changing environments, the soil environment has received much less attention than that above ground. In particular, patterns of nitrogen availability in the ecosystem have been studied in only a few locations and circumstances. The forms of nitrogen most commonly used by plants are nitrate (NO_3^-) and ammonium (NH_4^+), which differ in terms of mobility and assimilation by the plant. Soils differ in their concentrations of NO_3^- and NH_4^+, which also change in response to environmental conditions: temperature, water availability, soil microbial community or successional stages, ecosystem recovery, etc.

In this work we first determined field nitrogen availability (NO_3^- and NH_4^+ concentrations) along with several environmental parameters (soil temperature, water availability, pH and organic matter) and plant species.

Secondly we analysed the nitrogen use strategies of the plant species found in the study area based on: nitrate reductase and glutamine synthetase activities; leaf NO_3^-, NH_4^+ and malate concentrations; and on the variation of these parameters in space (along the twigs) and over time.

In the third stage of this work a plant species of each plant functional group (*Olea europaea* as an evergreen sclerophylous and *Cistus albidus* as a summer semi-deciduous) was grown in pots with several nitrogen availabilities. Our results highlight two distinct strategies in the use of nitrogen by plant species according to their functional group.

Together these results strongly indicate that shifts in soil NO_3^- and NH_4^+ concentrations, due to nitrogen deposition, industrial activities or agricultural

practices, can influence plant species and modify successional trajectories or ecosystem responses in changing environments in different ways.

1 Introduction

Nitrogen (N) supply and acquisition are two of the most important factors that control plant productivity and biodiversity, as growth in natural ecosystems is generally limited by the availability of inorganic N in the soil [1]. Spatial heterogeneity in inorganic soil N forms and concentrations have been suggested as contributors to coexistence of plant species [2] and composition of plant communities. Differences in the spatial and temporal distribution of nutrient transformation and consumption processes result in soil patches of differing concentrations, and the resistance to diffusion and mass flow between regions of high and low concentrations contributes to maintenance of patches [3]. The nutrient status of a soil patch is also affected by a variety of factors including plant cover, plant species composition [4], microbial activity [5] and soil temperature and moisture content [3]. Since factors having the largest influence on soil nutrients may vary over the growing season, the nutrient pool size of soil patches may change depending on which factors are most significant at a particular time [3]. In addition, the dynamics of pool size may be different for nutrients limiting plant growth or microbial activity.

From a biochemical point of view, all plant species should need the same quantity of N to construct a given amount of tissue. However differences do exist in plant tissue N concentration because of heterogeneous distribution of nutrients in the soil and varying uptake efficiency. In addition, plants respond differently to environmental variability, including N storage, and have different physiological needs that cause differences in metabolism and construction [1]. Adaptation to N availability in soils leads to nutrient concentrations that differ widely between organs, species and ecosystems.

Studying ecological succession, Fitter and Hay [2] found that the concentration of NH_4^+ increased from a low level in the pioneer stage to a high value in the climax, contrary to what happens with NO_3^- concentrations. This implies that species characteristic of a particular successional stage must be adapted to the N source available. But it does not explain: 1) how do species co-exist in intermediate phases of succession; and 2) how do Mediterranean ecosystems have high biodiversity [6] despite being limited by nitrogen availability.

We hypothesised that this paradox can in part be explained if variations in nitrogen acquisition in space and time by plant species lead to resource partitioning, decreasing plant competition for a limiting resource.

In order to verify this hypotheses a multidisciplinary approach was followed: 1) characterisation of N sources and concentrations in a Mediterranean ecosystem and relation that with the plant species; 2) characterisation of the chemical and biochemical activities of metabolites and enzymes closely related to the inorganic N metabolism, and 3) characterisation of plant species response to distinct levels of NO_3^- and NH_4^+ in the root medium, under controlled

conditions. Our aim is to integrate the data in order to assess the importance of shifts in soil NO_3^- and NH_4^+ concentrations on local plant cover and species composition.

2 Materials and methods

Studies were carried out in a mixed sclerophyll scrub whose revegetation started spontaneously after fire, 15 years ago. The site is situated in Serra da Arrábida, 50 km south of Lisbon (38°27'34"N, 9°0'20"W), on a south facing slope of Jaspe Peak, a calcareous elevation with 270 m. The climate is classified as sub-humid, warm variant according to Emberger's pluviometric coefficient with an average annual precipitation of 650 mm, and 16°C mean annual temperature [7]. During the sampling period values of air temperature changed between 5 and 36°C, air relative humidity between 48 and 100%, maximum day light intensity between 340 and 1600 $\mu E\ m^{-2}s^{-1}$, soil temperature between 8.5 and 22.5°C, and soil moisture content between 0.098 and 0.321 ($m^3\ m^{-3}$).

The soil at the site was very thin (max. 20 cm depth) and of calcareous origin. To study soil spatial variability at a medium scale the studying area, 2500 m^2, was divided in 25 squares of 100 m^2 each, each square was divided again in 25 squares of 4 m^2 each, giving a total of 625 sampling points. Samples were taken from the square centres and consisted of three cores of 2 cm in diameter and 15 cm length taken within 1 cm of each other and at 20 cm from the main stem of the nearest plant. Samples were stored at 4°C for analysis. Sampling took place 4 times (February, April, June and November) during the year 2002.

Sieved (2 mm) soil samples were analysed for inorganic N forms and concentrations. NO_3^- and NH_4^+ were extracted from the soil using 2M KCl in a proportion of 1 g soil dry weight to 10 ml of extractant. NO_3^- concentration was determined by electrophilic substitution of salycilate acid; and NH_4^+ concentration using the Berthelot reaction [8]. Soil samples were grouped in 5 classes according to their NO_3^- or NH_4^+ concentration: class 1 - 0 to 90; class 2 - 90 to 180; class 3 - 180 to 360; class 4 - 360 to 720; and class 5 >720 ppm.

Simultaneously a detailed map of the vegetation was prepared, using the same sampling net and considering the dominant species in each 4 m^2 cell. The species found were classified as summer semi-deciduous or sclerophyllous. The summer semi-deciduous species found were: *Cistus albidus, Cistus salvifolius, Cistus ladanifer, Cistus mospeliensis, Lavandula sp., and Rosmarinus officinalis*. The sclerophylous species were: *Ceratonia siliqua, Erica sp., Juniperus phoenicea, Myrtus communis, Olea europaea, Phillyrea angustipholia, Pistacea lentiscus, Rhamnus alaternus, Quercus coccifera, and Arbutus unedo*.

NO3- reductase activity was determined according to Kaiser et al. [9], glutamine synthetase was determined according to Matt et al. [10]. Total soluble amino acids were determined by their reaction with ninhydrin, and malate concentrations were assessed after oxidation of malate to oxalacetate by NAD in the presence of malate dehydrogenase.

In order to check plant response to several N availabilities, plants of *C. albidus* and *O. europaea* were grown in 10 l pots filled with soil from the

studying area with a known N concentration. Higher levels of N availability were obtained by addition of NH_4^+ sulphate. Five levels of N were studied: 180, 360, 740, 1400 and 2800 μmol g^{-1} soil DW, corresponding to a range of concentrations found in the local soils. Plants were watered regularly in order to maintain soil water content of ± 0.3 m^3 H_2O m^{-3} soil. The phenology of plant development was followed and plants were harvested 4 months after the start of the treatments.

3 Results and discussion

Total N concentrations in Mediterranean soils are, generally, very low [11, 12] and highly heterogeneous [13]. The results here presented show that soil composition is very heterogeneous over space and time regarding NO_3^- and NH_4^+ concentrations (Fig. 1). In February and November NO_3^- concentrations in the soil are very low, since most of the samples (500 out of 625) have less than 10 ppm, and therefore were grouped in soil class 1. This may be related with the winter rainfall regime, leading to a leach out of NO_3^- initially present in the soil. Higher NO_3^- concentrations (classes 4 and 5) were found in April and June. Soil NH_4^+ concentrations were also heterogeneous along the year, but a much smaller frequency of samples display high levels (classes 4 and 5) of NH_4^+.
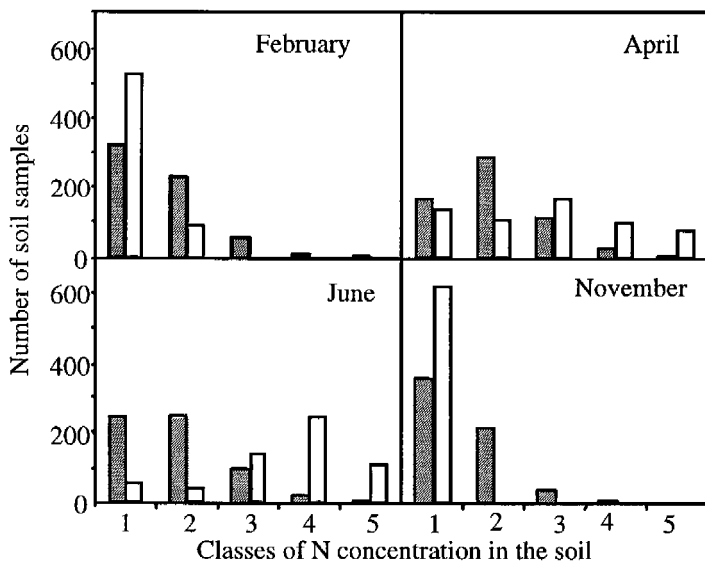

Figure 1: Frequency of soil samples (out of 625) per class of inorganic nitrogen. NO_3^- and NH_4^+ concentrations of the sample were determined and the samples grouped in classes. Class sizes were as follows: 1- 0 to 90; 2 - 90 to 180; 3 - 180 to 360; 4 - 360 to 720; and 5 - > 720 ppm NO_3^- or NH_4^+. ■ and □ represent frequencies of soil samples belonging to a certain class based on their NH_4^+ and NO_3^- concentrations.

Generally NO_3^- and NH_4^+ concentrations were quite variable within the research plot even in close proximity to individual plants, so that the highest and lowest measured nutrient concentrations occurred within the extent of individual plant root systems. The spatial extent of variability was comparable to that found in similar ecosystems [4, 12], but no significant correlation was found between NO_3^- and NH_4^+ ($r^2 = 0.1687$) concentrations in the soil.

The most satisfactory product of the work resulted from the comparison, for each soil sample, of the functional group to which the dominant plant in sampling area ($4 m^2$) belong to and the NO_3^- or NH_4^+ concentrations present in the soil sample (Fig. 2). High NH_4^+ concentrations (classes 4 and 5) were never found in soils from areas dominated by summer semi-deciduous species (Fig. 2a). While a high number of summer semi-deciduous species were associated with soil samples containing high NO_3^- concentrations (classes 4 and 5, Fig. 2b). On the contrary, the presence of evergreen sclerophyllous seem to be rather indifferent to N forms in the soil. Such results suggest that the nutrient cycling properties in natural and recently disturbed systems are spatially complex, and moreover that this complexity may significantly affect plant community structure [13, 14]. The co-existence of plant species at the study site highlights the possibility that variations in soil characteristics leading to variations in competitive abilities may be one of the mechanisms responsible for the dynamic of specific populations and processes occurring at the ecosystem scale, as suggested by Grime [15].
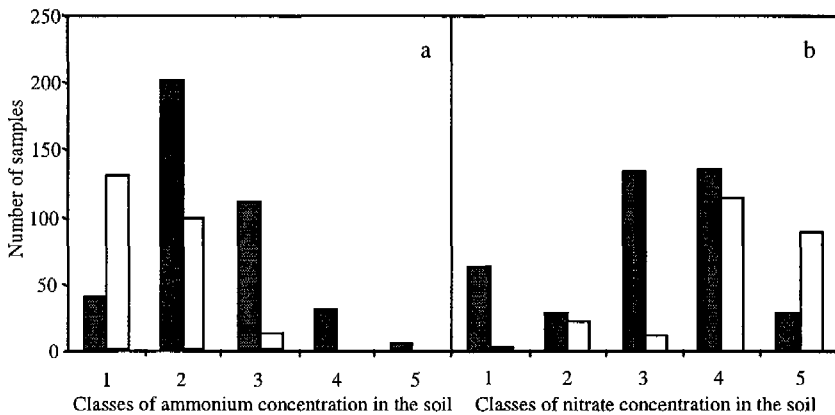

Figure 2:   Frequency of plants belonging to one of the two plant functional types (■ evergreen sclerophylous or □ summer semi deciduous) and the class of NH_4^+ or NO_3^- concentration in the soil to which the soil around them belong. For details on how soil samples were obtained see legend of Fig. 1.

The concentration of NH_4^+ increases from a low level in the pioneer stage to a high value in the climax, contrary to what happens to NO_3^- concentrations [16]. This is in general agreement with our results since plant succession, after fire or

any other perturbation, starts with summer semi-deciduous species and tends to reach a stage where sclerophylous species dominate the vegetation [17].

Species that are adapted to a particular successional stage must be adapted to the available N source. In particular the enzymatic systems for absorbing and incorporating nitrogen should reflect the form of N used. NO_3^- reductase is the enzyme used in the first step of the reduction of NO_3^- into a form that the plant can use [10]. The level of NO_3^- reductase activity in the tissue may therefore be an indicator of NO_3^- availability in the habitat [2].

It may be surprising that nitrate reductase activities (potential and physiological) were higher in sclerophyllous than in summer semi-deciduous species (Fig. 3). These activities were determined in leaves of plants growing where NO_3^- and NH_4^+ may be simultaneously present. Nevertheless it is well known that the intrinsic characteristics of nitrate reductase, including its response to regulation by metabolites such as malate, amino acids, etc, are very species dependent [10]. It is possible that the nitrate reductase of summer semi-deciduous and sclerophyllous species have distinct sensitivities to NH_4^+, with that of summer semi-deciduous species being more inhibited by lower concentrations. In all, the data indicate that summer semi-deciduous species are more abundant than sclerophyllous species at sites that are richer in NO_3^- (Fig. 2) and therefore they have higher NO_3^- - concentrations in their leaves than the sclerophyllous species (Fig. 4).
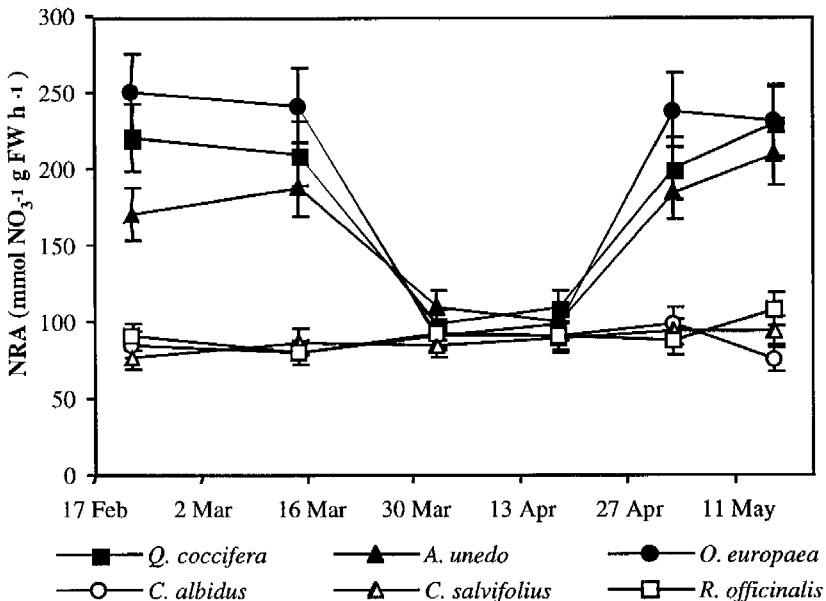

Figure 3: Potential nitrate reductase activity (NRA) in third and fourth pairs of leaves of several species of natural vegetation. Samples were collected between February 2001 and May 2002.

Based on NR activity determined in vitro (Fig. 3), two patterns of NO₃⁻ reduction were evident: one shown by *Q. coccifera*, *A. unedo* and *O. europaea*, evergreen sclerophyllous species; and the other by *C. albidus*, *C. salvifolius* and *R. oficinalis*, summer semi-deciduous species. The summer semi-deciduous species displayed NO₃⁻ reduction rates more or less constant over the year. In contrast, NO₃⁻ reduction rates in the evergreen sclerophyllous species studied changed along the year in a concerted way. These two patterns of NO₃⁻ reduction activity are coincident with those previously, observed *in vivo* [18].

These results are in agreement with other preliminary studies made in the same area [18] and together suggest that each group is composed of a number of physiologically similar plant species, as suggested by Kumerow [19]. It is not possible to extrapolate each group's response to the expected increased availability of inorganic N [1] from these results.
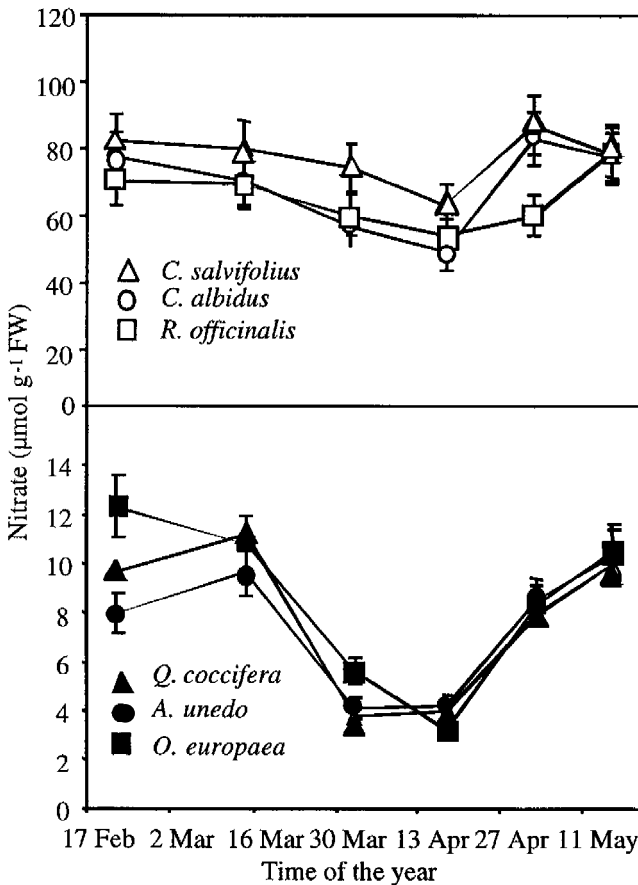

Figure 4: NO₃⁻ concentrations in third and fourth pairs of leaves of several species of natural vegetation. Samples were collected between February 2001 and May 2002.
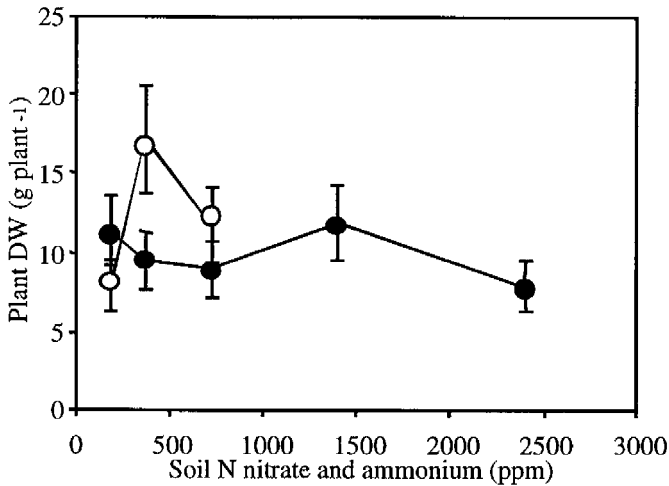
Figure 5:   Biomass production of *O. europaea* (●) and *C. albidus* (○) under distinct root N concentrations. The initial amount of nitrogen in the soil was 90 ppm of N. The other levels of nitrogen were obtained by addition of the adequate amount of $NH_4^+NO_3^-$. Plants were grown for four months. Symbols represent the mean ± standard deviation (n=10).

In order to provide a first approach to the question, one species of each plant functional group, *Olea europaea* as a sclerophylous and *Cistus albidus* as a summer semi-deciduous species was chosen and grown with different N availabilities. The lowest and the highest N concentrations chosen, correspond approximately to the lower and upper concentrations found under natural conditions in Mediterranean ecosystems [20].

C. albidus increased their biomass accumulation when exposed nitrogen concentrations up to 360 ppm (corresponding to the presence of 180 ppm of NH_4^+), but all the plants died when exposed to the two highest N concentrations (corresponding to the presence of 360 and 720 ppm of NH_4^+, respectively, Fig. 5). This result clearly shows that *C. albidus* plants are quite sensitive to NH_4^+, in agreement with the observation that under field conditions no summer semi-deciduous plants were found in association with high soil NH_4^+ concentrations (Fig. 2).

Diurnal variations of leaf NO_3^- reductase activity, glutamine synthetase activity, malate and amino acid concentrations were determined for plants of all treatments, although only the results obtained for *C. albidus* and *O. europaea* grown at 720 ppm of N (corresponding to 360 ppm of NH_4^+ and 360 ppm of NO_3^-) will be shown and discussed (Fig. 6).
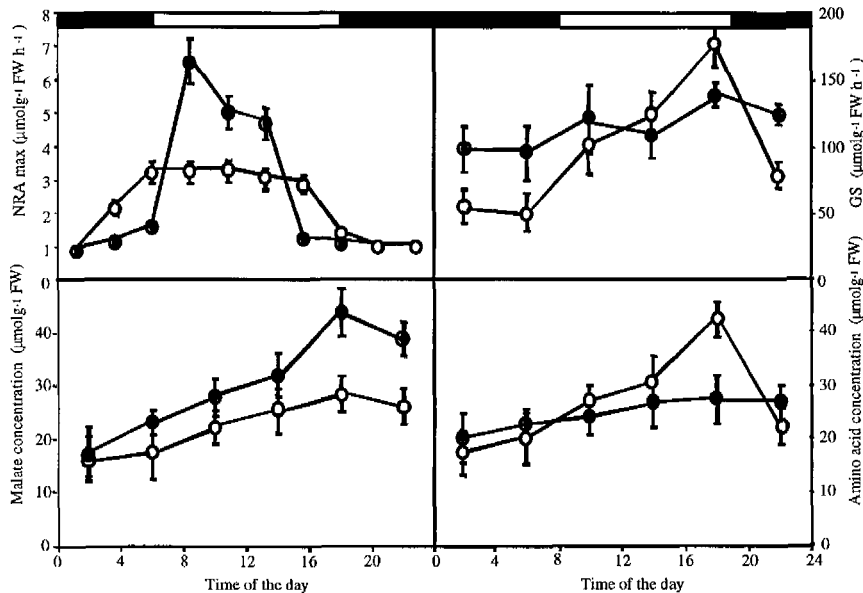
Figure 6: Potential leaf NO3- reductase activity, Glutamine synthetase activity, malate concentratios and amino acid concentrations of *O. europaea* (●) and *C. albidus* (○), grown with 360 ppm NO_3^- and 360 ppm NH_4^+, along the day. Symbols represent the mean ± standard deviation (n=10). The dark bar on the top of the graph represent the dark and the white the light period.

Both *O. europaea* and *C. albidus* displayed relatively low leaf nitrate reductase activities (Fig. 6) in comparison with other plant species (*Poterium sanguisorba* and *Scabiosa columbaria*) characteristic of other calcareous habitats where N is probably more abundant [2]. The pattern of NO_3^- reduction along the day was also distinct for both species. *O. europaea* showed higher activities in the middle of the day (10:00 to 14:00) while activities of *C. albidus* plants were almost constant through the day (Fig. 6). This may be due to complex mechanisms regulating the activity of the nitrate reductase and its interactions with other metabolites [10]. The diurnal pattern of nitrate reductase activity contrasted with that of glutamine synthetase (Fig. 6), since glutamine synthetase activity along the day was almost unchangeable in *O. europaea* while for *C. albidus* it was low during the night but increased during daytime. These differences may have drastic consequences for the partitioning of the newly assimilated carbon. The higher levels of NO_3^- reduction displayed by *O. europaea* during the day, must be associated with high production of organic acids and most probably with high phosphoenolpyruvate carboxylase activities [21], with a consequent increase in the concentration of organic acids, including malate (Fig. 6).

The adjustment and stabilisation of N metabolism to changed conditions almost always involves modifications of the diurnal variations [22]. This network allows dramatic changes in response to sudden changes in conditions, but in the long term acts as a buffer that stabilises the overall rate of N uptake and assimilation and co-ordinates it with the utilisation of N for growth [21]. A key element in this network may be the excess capacity for NO_3^- assimilation of *O. europaea* which is partially inhibited during most of the diurnal cycle (Fig. 6). During the first part of the diurnal cycle maximal rates are briefly achieved. The rates of NO_3^- assimilation in *O. europaea* are much higher than the rates of NH_4^+ metabolisation by GS, which may lead to depletion of leaf NO_3^- (results not shown) and to the accumulation of downstream products which will exert feedback regulation on the nitrate reductase activity [10]. The high activities of the nitrate reductase observed in *O. europaea* during the light period will prioritise carbon flow to the synthesis of malate for pH regulation, which eventually translates in to daytime malate accumulation (Fig. 6).

In *C. albidus,* nitrate reductase activity was almost constant during the daytime, but the activity of glutamine synthetase increased along the day (Fig. 6). This means that in *C. albidus* carbon flow goes preferentially towards 2-oxoglutarate synthesis in order to provide the carbon skeletons needed for NH_4^+ assimilation, this is in agreement with the higher accumulation of amino acids in *C. albidus* in comparison to *O. europaea* (Fig. 6). These differences in the diurnal variation of enzyme activity (NR and GS) and metabolite accumulation (amino acids and malate) between *C. albidus* and *O. europaea* becomes larger in response to higher concentrations of NH_4^+ in the root medium (results not shown), probably leading to an imbalance between C and N metabolism and therefore to plant death (Fig. 6) in *C. albidus* plants. This suggests that *O. europaea* and *C. albidus* have distinct priorities to the newly fixed C in function of N availability.

It is expected that plants adapted to the same habitats display similar strategies and adaptations to growth constraints [23], and also that co-occurring species exploit distinct ecological niches, in order to avoid competition for limiting resources [24]. These sets of data clearly show that evergreen sclerophyllous and summer semi-deciduous plants have different N use strategies and that N sources, mainly soil NH_4^+ concentration, are a potential driving force in the selection of the plant cover. There is abundant evidence that plant species co-occurring in the Mediterranean ecosystem differ in rooting depth and phenology [17, 20]. These data (Fig. 1) also show that NO_3^- is not the only N source available to plants, but that NH_4^+ is also available and, as occurs in other plant communities [25], species belonging to distinct plant functional groups differ in their preferences for forms of N.

These results are also in agreement with those from northwestern European ecosystems [26], which demonstrated that the response of the vegetation to NH_4^+ inputs was dependent on the status and dynamics of the forest floor, mainly on its capacity to maintain soil NH_4^+ concentrations below certain limits [27]. This is of special importance in view of the forecast of an increase of about 22% in N availability in Mediterranean ecosystems until 2010, mainly as NH_4^+ deposition,

[3]. In particular, the Mediterranean basin itself has for many years been subject to intensive human activities, including forest clearing and grazing, which tend to reduce the accumulation of carbon in these ecosystems and therefore the capacity of soil response [28]. Since water and nutrients, especially nitrogen and phosphorous, are the most limiting factors to plant productivity in these ecosystems shifts in soil NO_3^- and NH_4^+ concentrations due to nitrogen deposition, industrial activities or agricultural practices, can influence plant species and modify successional trajectories. Given that in Mediterranean ecosystems many species are present in small patches a high turnover in the species composition of plant communities along ecological and geographical gradients are observed [29].

Acknowledgements

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