



Mediterranean ecosystems: the co-existence of plant species depends on soil ammonium concentration

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Along the ecological succession



species characteristic of a particular successional stage must be adapted to the N source available.

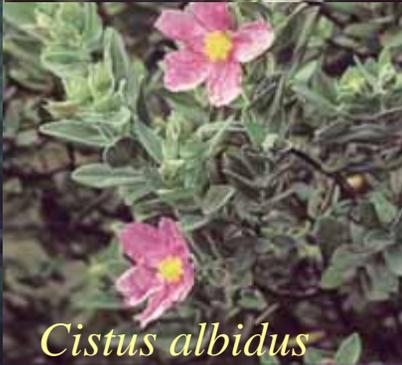
1) how do species co-exist in intermediate phases of succession; and 2) how do Mediterranean ecosystems have high biodiversity despite being limited by nitrogen availability.



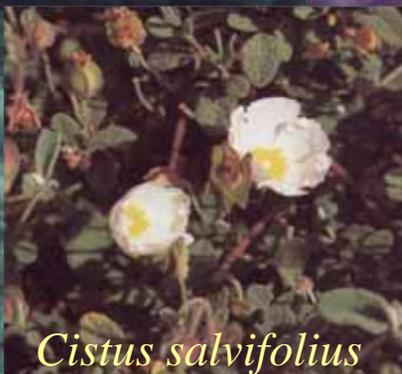
Along the ecological succession



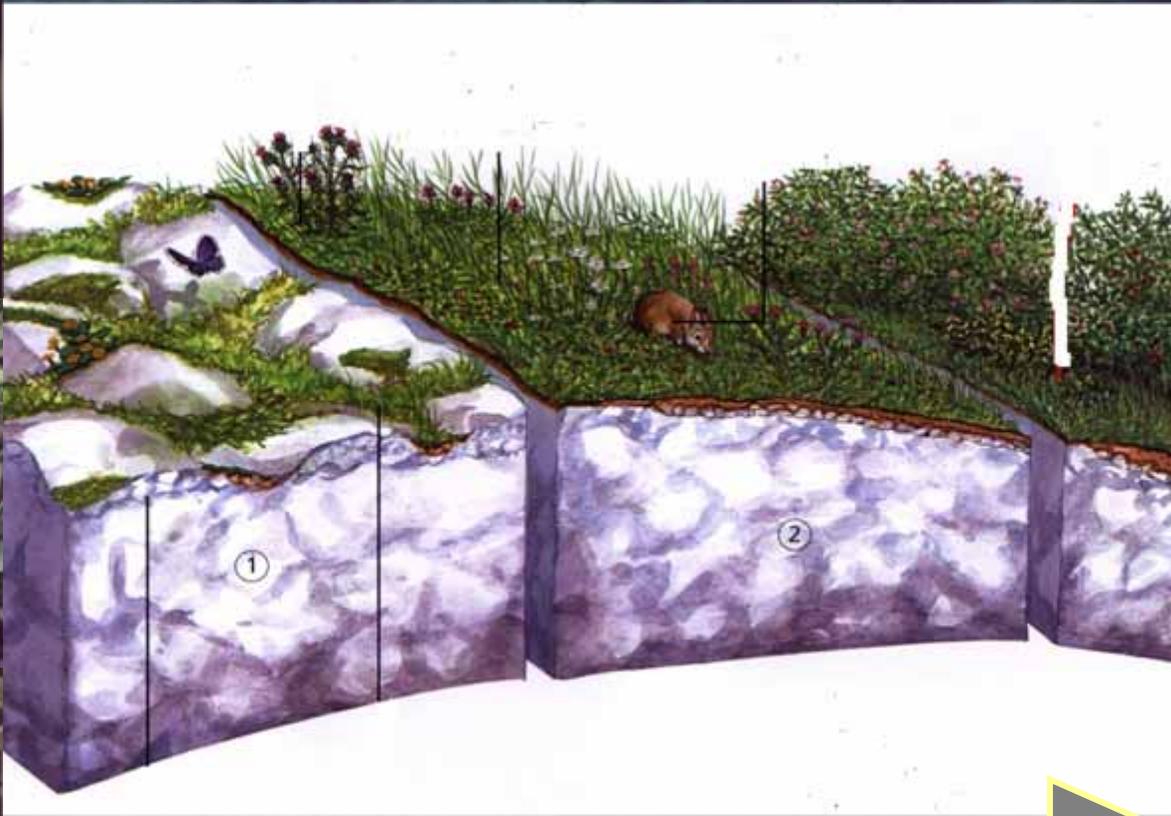
R. officinalis



Cistus albidus



Cistus salvifolius



Arbutus unedo



Quercus



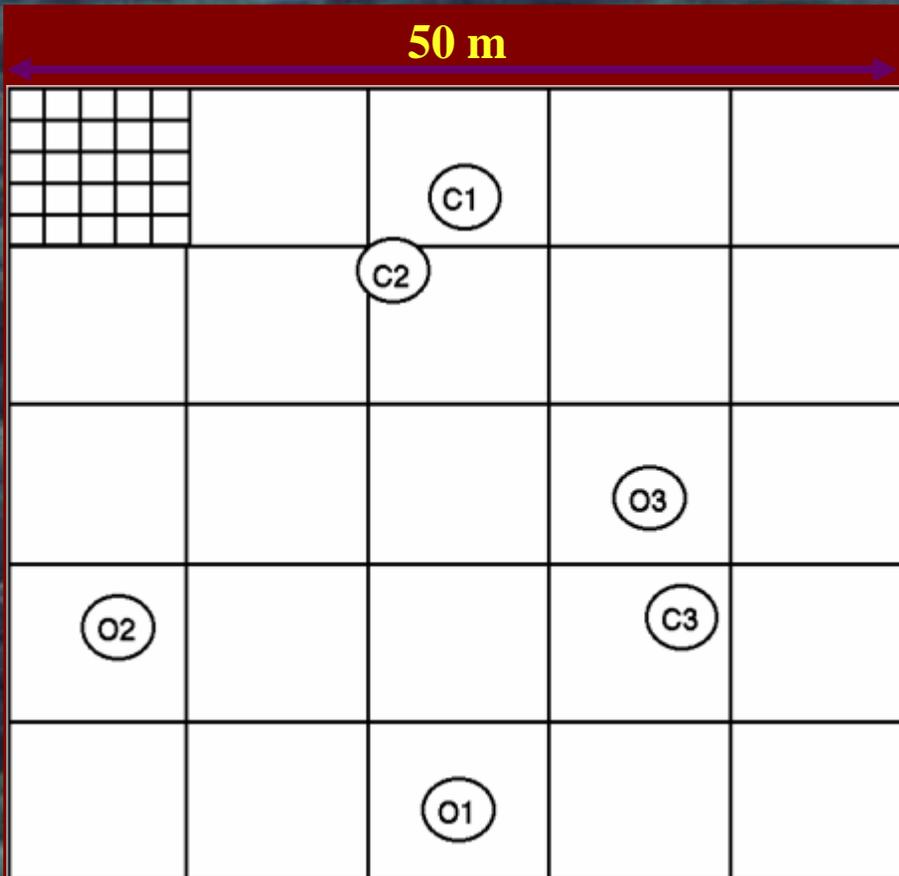
Olea europaea



Driving force



[N] and sources in soil



A) schematic representation of the sampling procedure

B) Sampling procedure in the field.



Frequency of plants (out of 625) per class of inorganic N

Class sizes were as follows:

1 - 0 to 90

2 - 90 to 180

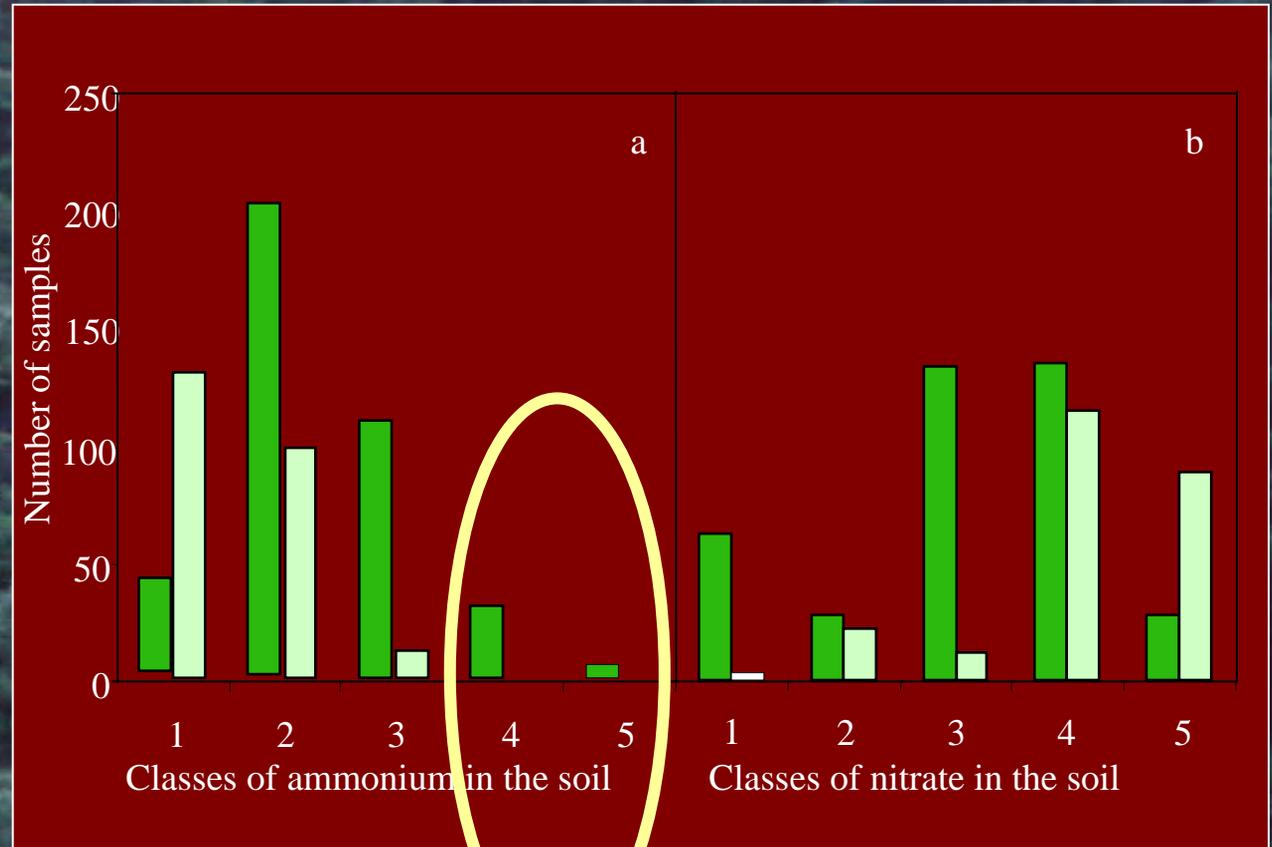
3 - 180 to 360

4 - 360 to 720

5 - > 720 ppm

NO_3^- or NH_4^+ concentrations.

- Evergreen sclerophyllous
- Summer semi-deciduous

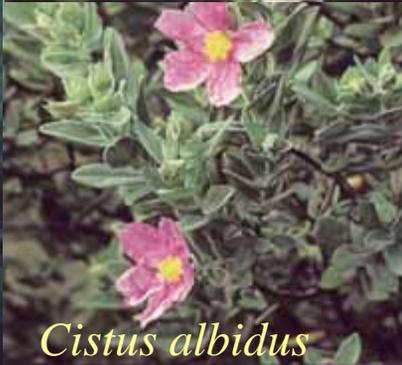




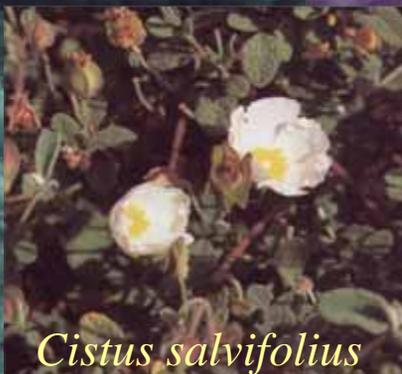
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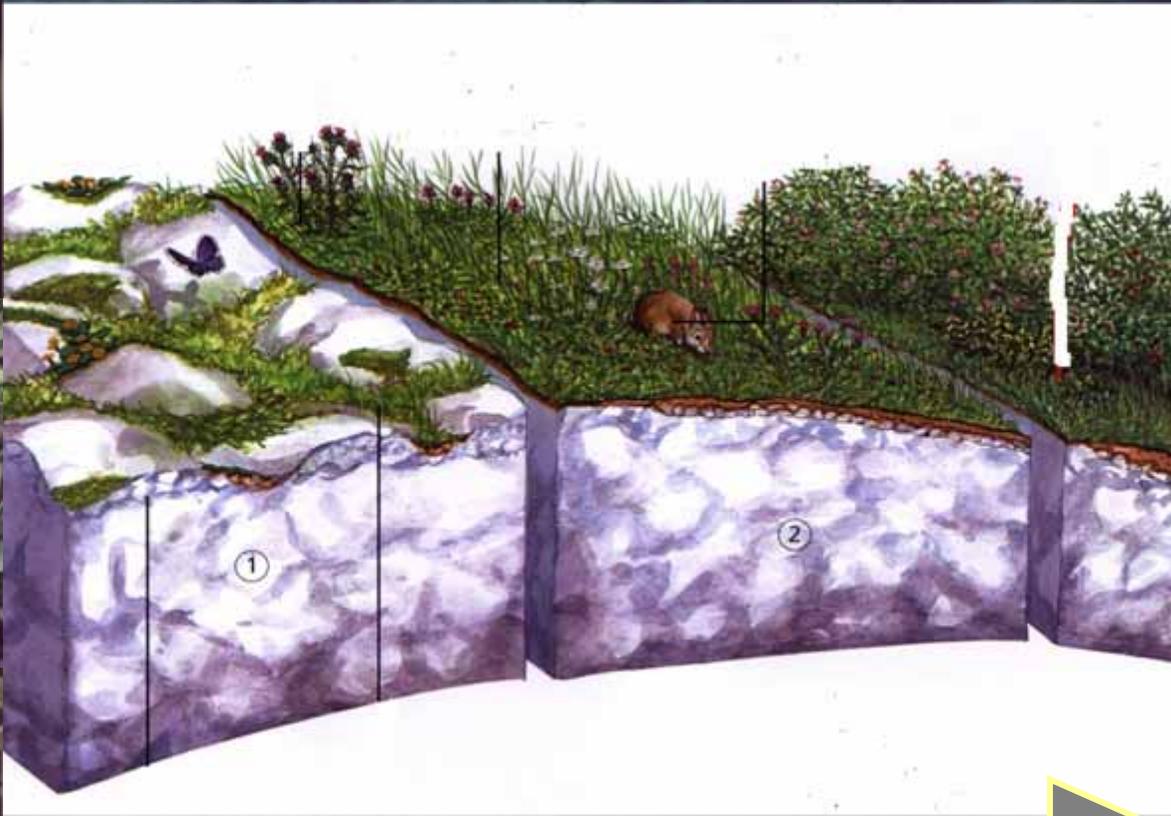
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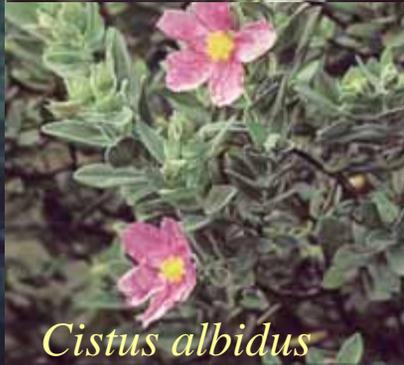
Driving force - NH_4^+ ?



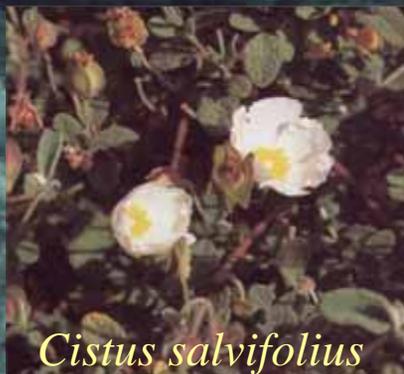
Along the ecological succession



R. officinalis

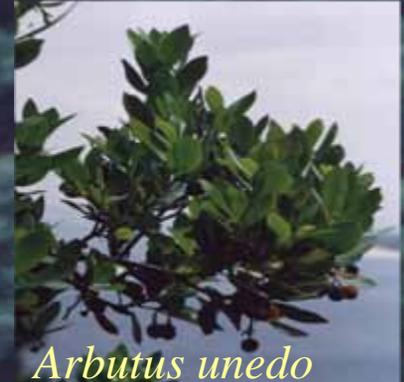


Cistus albidus



Cistus salvifolius

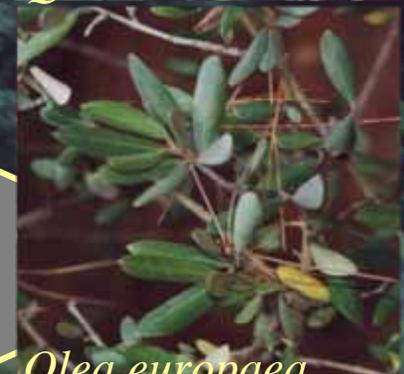
- Leaf NH_4^+ and NO_3^- concentrations
- Carboxylates
- Total soluble carbohydrates
- Nitrate reductase activity
- Glutamine synthetase activity
- Amino acid concentrations



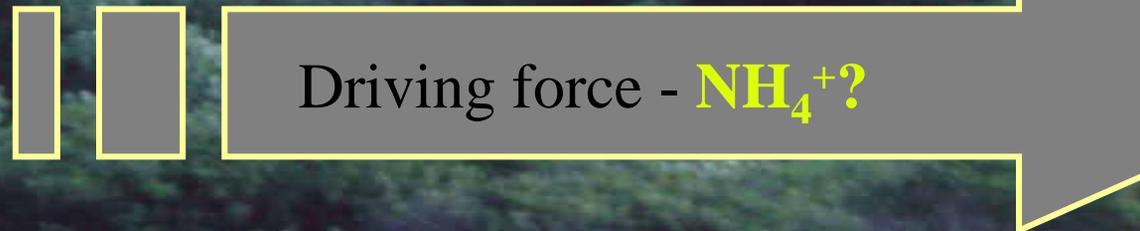
Arbutus unedo



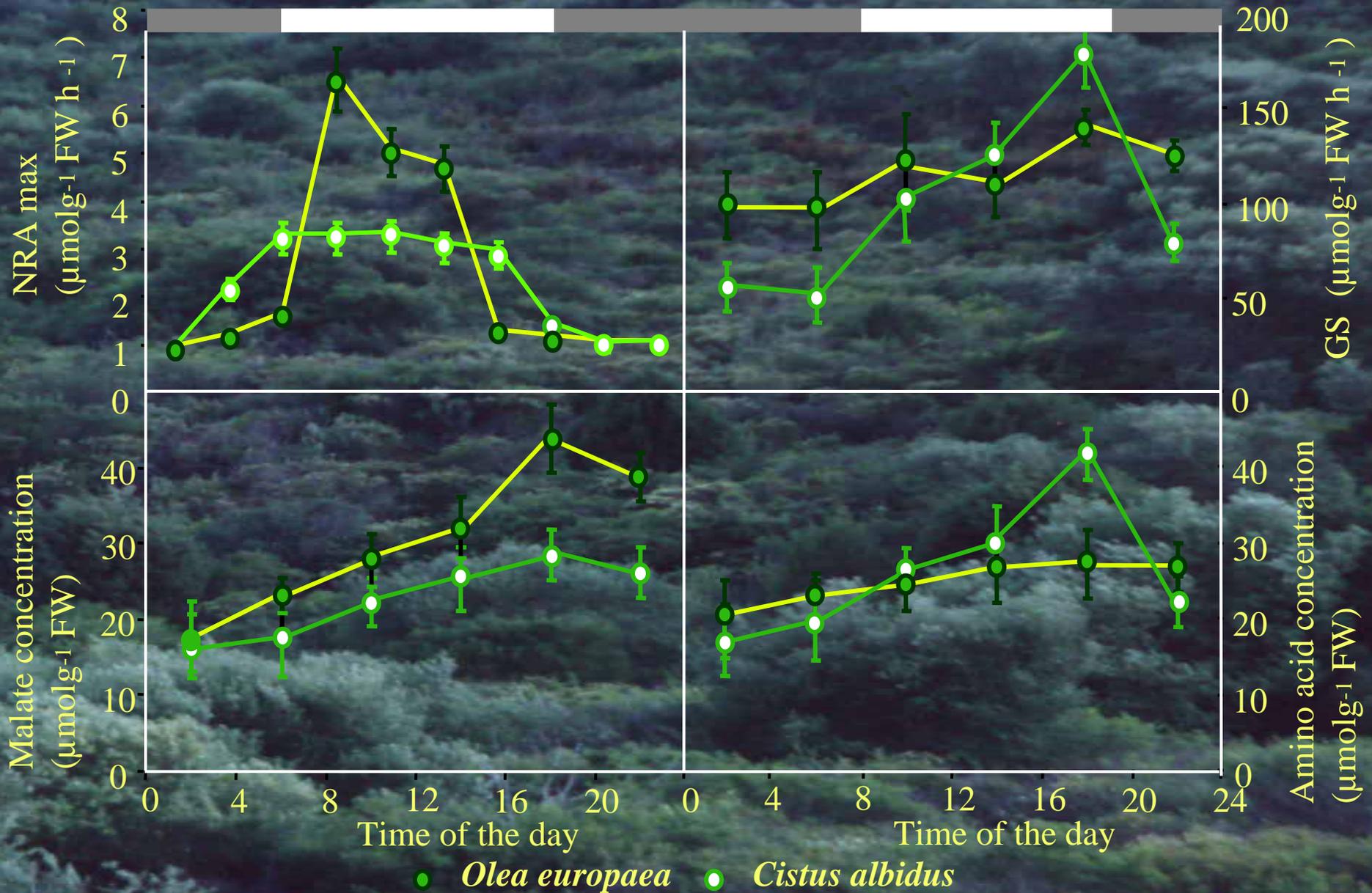
Quercus



Olea europaea

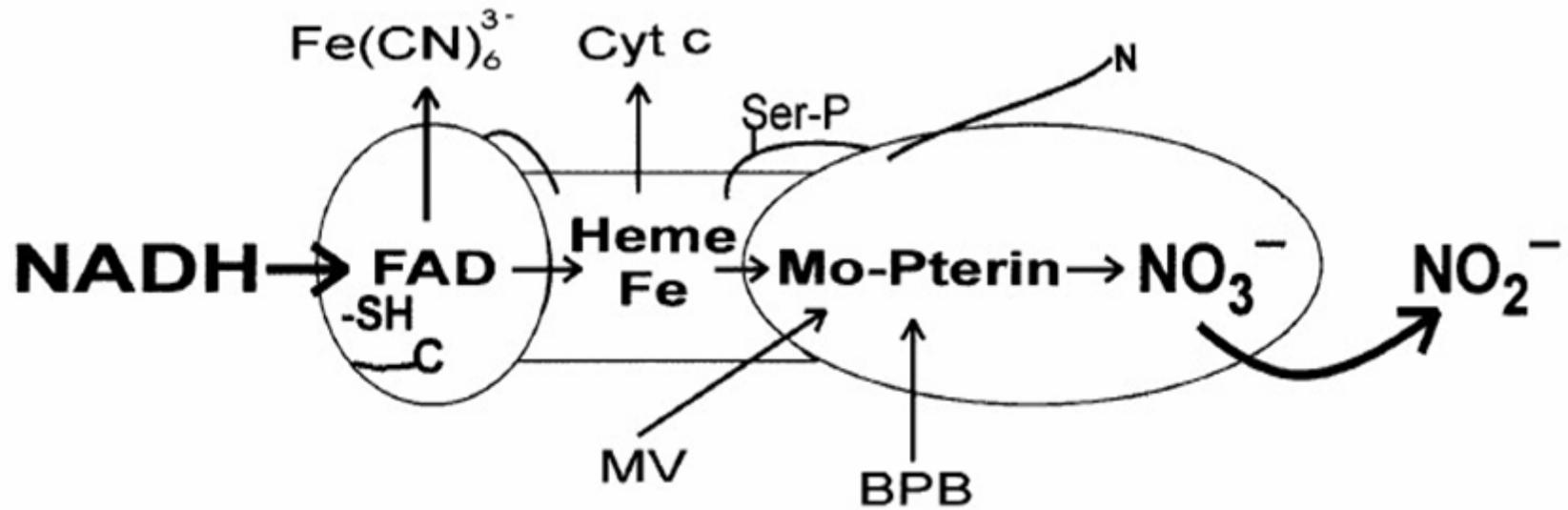


Carbon and nitrogen interaction





Nitrate reductase



Activity is

Induced by nitrate, and
Post translational regulation

Nitrate Reductase (NR)

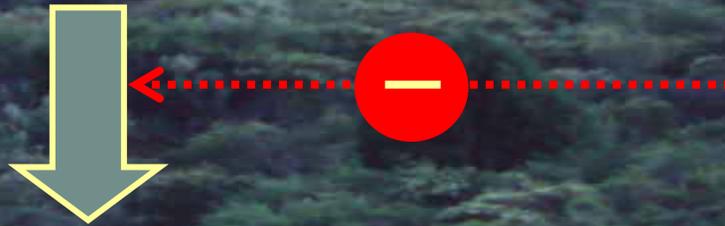


NRA in the field before and after induction by adding nitrate, in species from various soil types (Havill et al., 1974)

Soil	Species	NRA ($\mu\text{mol NO}_2^- \text{ g}^{-1} \text{ FW h}^{-1}$)	
		Before induction	After induction
Calcareous	<i>Poterium sanguisorba</i>	1.11	3.80
	<i>Scabiosa columbaria</i>	1.26	2.12
Neutral	<i>Urtica dioica</i>	7.93	16.10
	<i>Poa annua</i>	4.05	8.40
Acid	<i>Molinia caerulea</i>	0.52	1.50
	<i>Galium saxatile</i>	0.72	3.06
Acid peat	<i>Vaccinium myrtillus</i>	< 0.1	< 0.1
	<i>Drosera rotundifolia</i>	< 0.1	< 0.1

NRA may be influenced by ammonium

Plant species must be adapted to the nitrogen source available to them

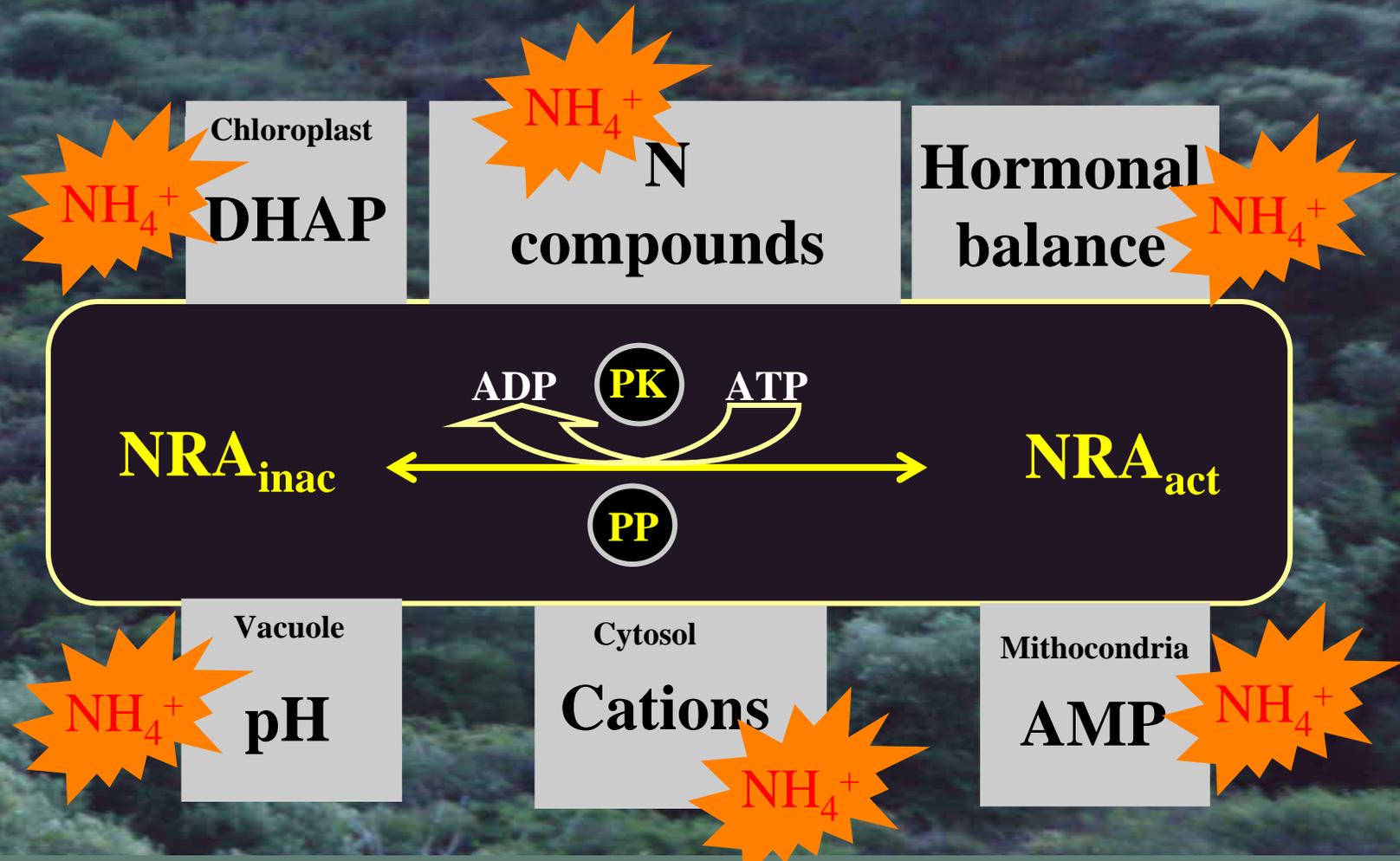


species that use mostly nitrate should have relatively higher levels of NRA

NR converts multiple environmental stimuli into metabolic activity

- Light
- CO₂
- Photosynthesis
- Hypoxia
- Inhibitors of respiration
 - NO₃⁻
- Cytosolic acidification
 - NH₄⁺

NRA may be modulated by ammonium



The modulation of NR gives flexibility to the metabolic systems of many plant species

Nitrate reductase activity reveals the capacity of the plant to use nitrate



Rosmarinus officinalis



Arbutus unedo



Cistus albidus



Quercus coccifera



Cistus salvifolius



Olea europaea

Working hypothesis:

Co-existing summer semi-deciduous and evergreen sclerophyllous plants, the two plant functional types present in Mediterranean ecosystems, have nitrate reductase systems with distinct characteristics according to the distinct richness of their ecological niches in ammonium.

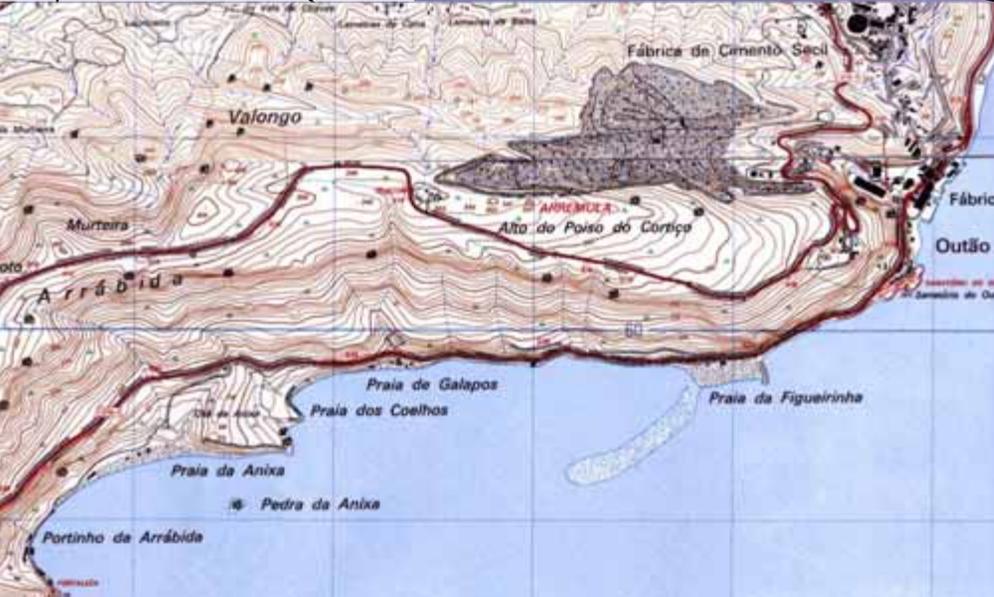
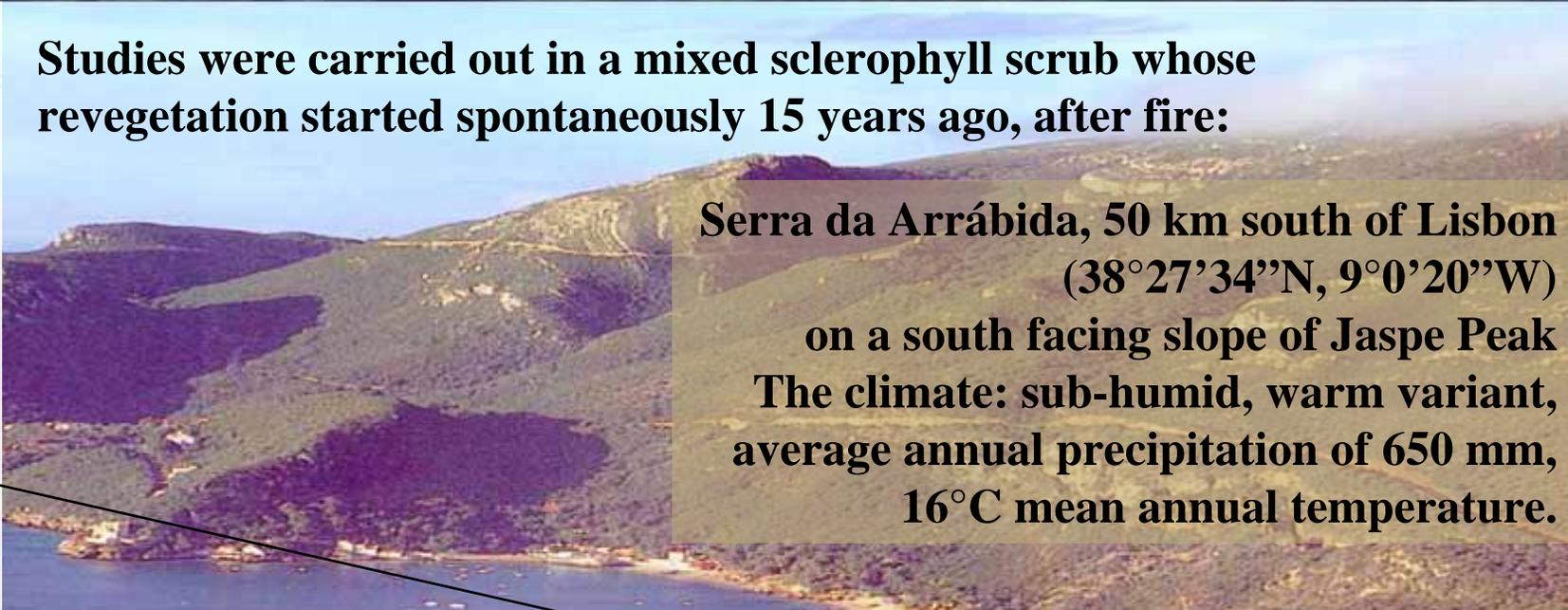
Study area

Studies were carried out in a mixed sclerophyll scrub whose revegetation started spontaneously 15 years ago, after fire:

Serra da Arrábida, 50 km south of Lisbon
(38°27'34"N, 9°0'20"W)
on a south facing slope of Jaspe Peak
The climate: sub-humid, warm variant,
average annual precipitation of 650 mm,
16°C mean annual temperature.

During sampling:

air temperature 5 - 36°C
air relative humidity 48 - 100%
light intensity 340 - 1600 $\mu\text{E m}^{-2}\text{s}^{-1}$
soil temperature 8.5 -
22.5°C
soil moisture 0.098 - 0.321 $\text{m}^3 \text{m}^{-3}$



The plant species pool of the Mediterranean maqui offers an opportunity to assess the importance of the sensitivity of NR to environmental stimuli and its conversion into metabolic activity (the capacity to use nitrate) by co-existing species.



Rosmarinus officinalis



Arbutus unedo



Cistus albidus



Quercus coccifera



Cistus salvifolius



Olea europaea

NRA in leaves was determined in either field plants or in plants grown under controlled conditions.

To assess NRA over the year - measurements were performed each month (3 measurements per plant) using always the third and fourth pairs of leaves.

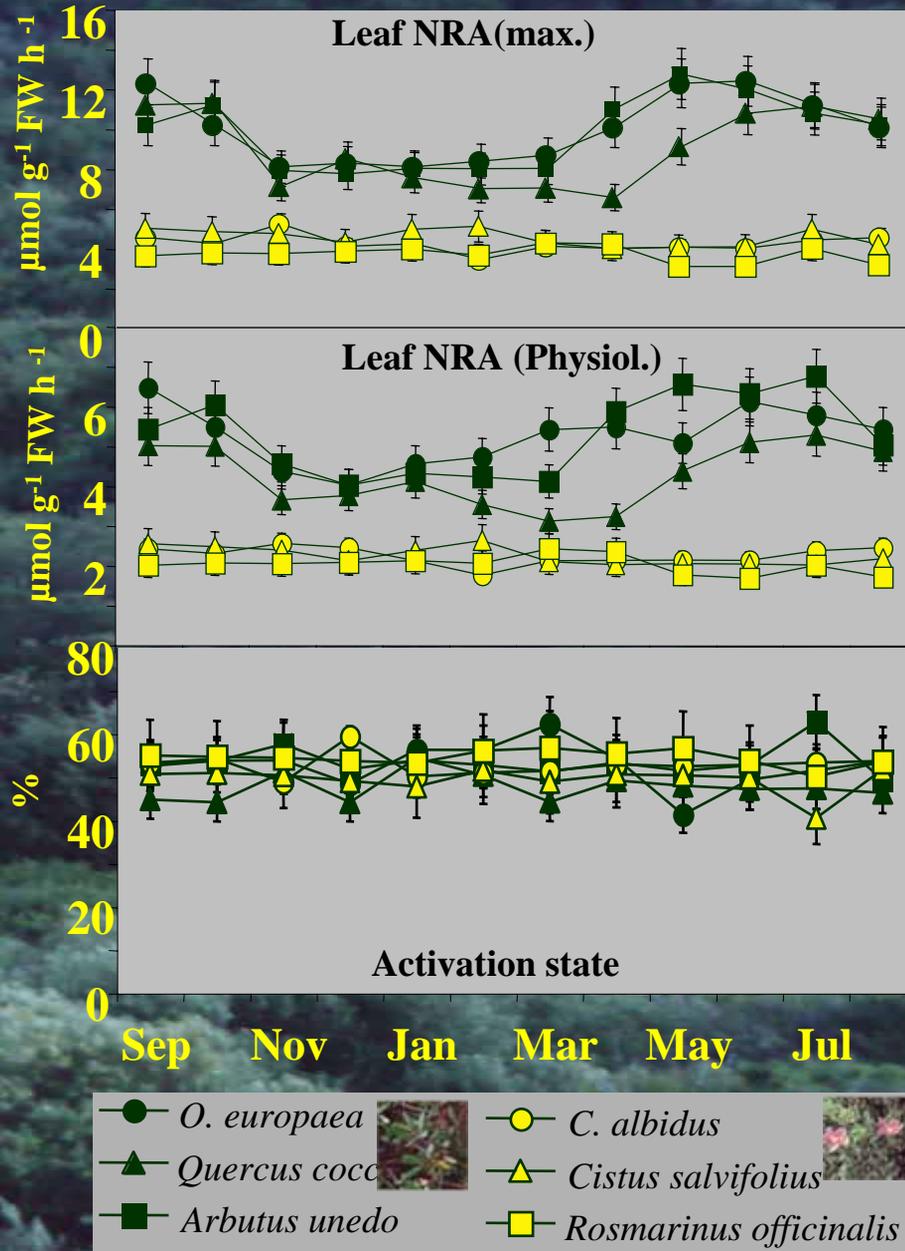
To assess NRA along the twigs - measurements were performed in the youngest 7 pairs of leaves.

To determine the effects of nitrate and ammonium concentrations on the plants' NRA, 100 plants of two plant species were grown in pots with distinct nitrate availabilities and NRA determined in roots and shoots.

To determine the effect of ammonium on NRA - leaves and roots were fed with distinct concentrations of ammonium.

Samples of the soil underneath the plants analysed were always collected and used for nitrate and ammonium determinations.

Leaf NRA in field plants



To assess NRA over the year - measurements were performed each month (3 measurements per plant) using always the third and fourth pairs of leaves.

Two patterns of NRA were evident:

⇒ evergreen sclerophyllous species

Q. coccifera

A. unedo

O. europaea

⇒ summer semideciduous species

C. albidus

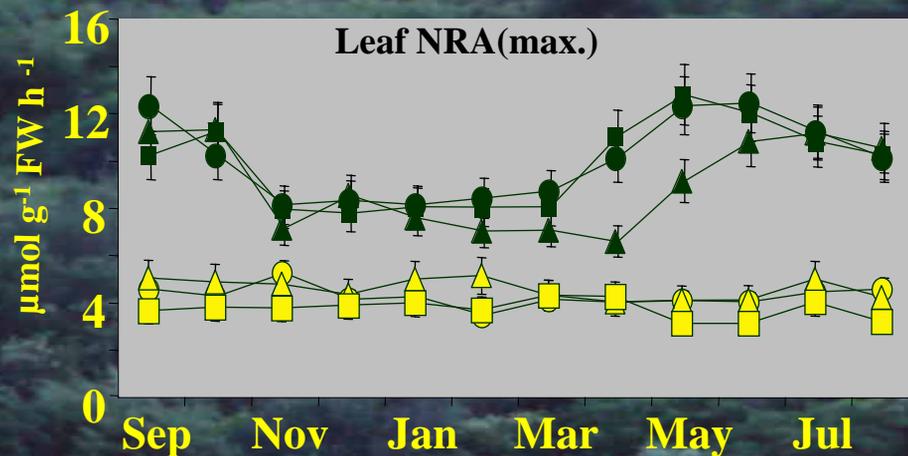
C. salvifolius

R. officinalis

No differences in the activation state of NR

In higher plants NRA is modulated by environmental conditions

It is therefore expected that plant NRA changes over the year, being higher when environmental conditions are more adequate.

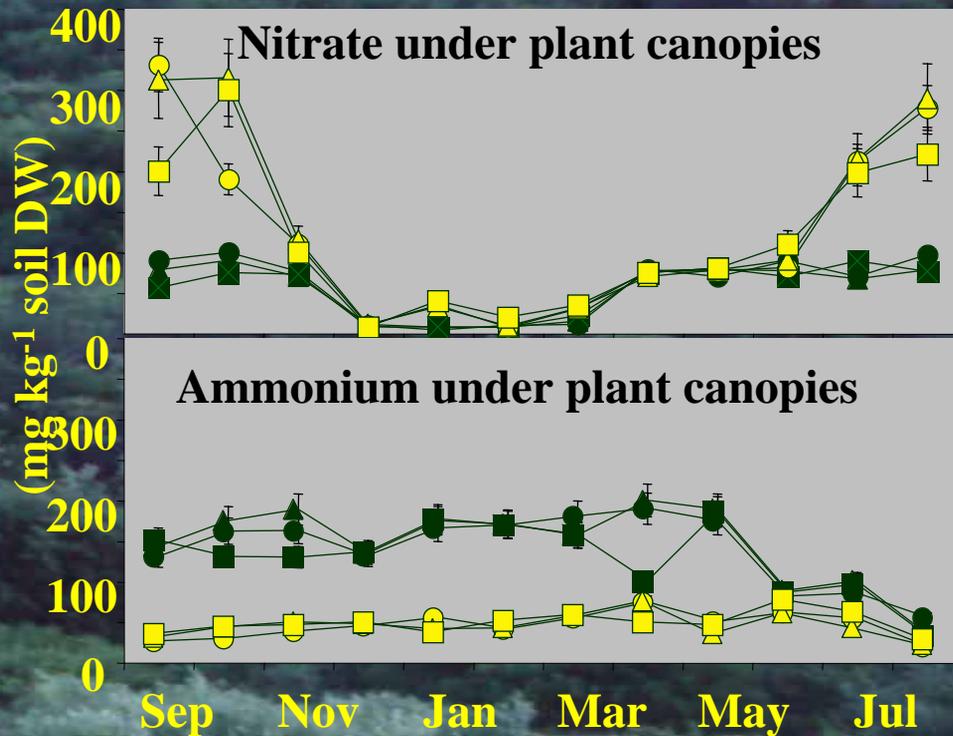


However NRA for summer semideciduous species were almost constant along the year

in contrast with the pattern of NRA found in evergreen sclerophylls



Nitrate and ammonium under plant canopies



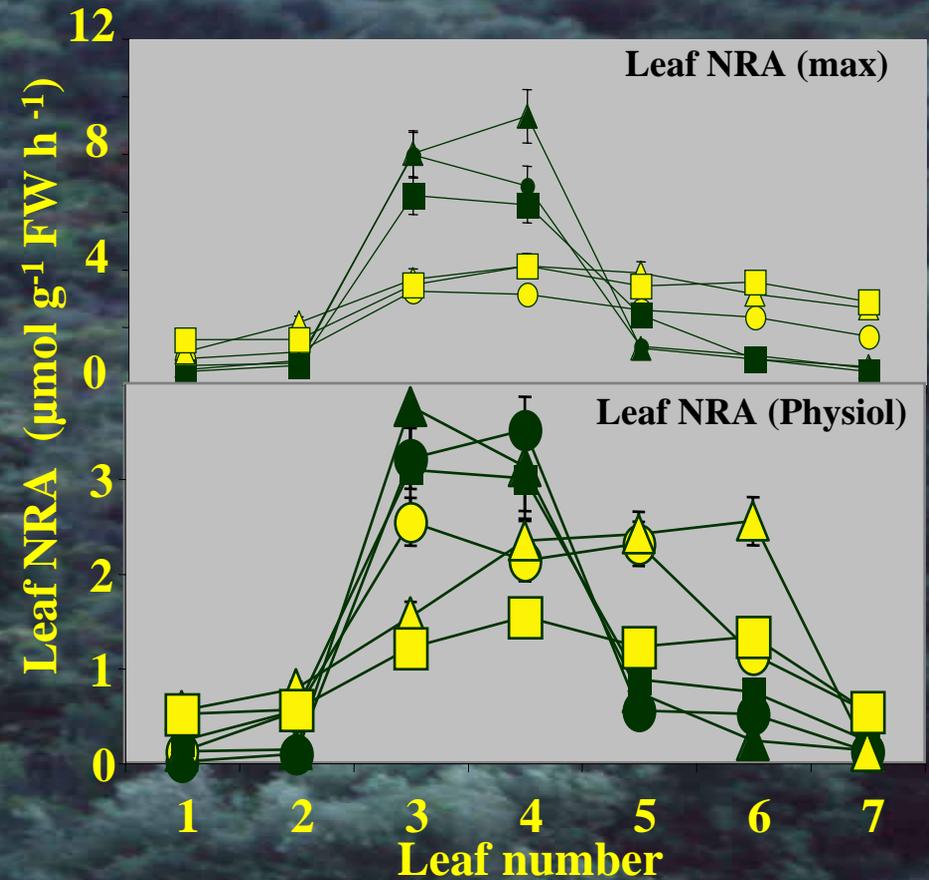
It has been reported that plant species from the later successional stages inhibit the development of nitrifiers (Rice and Pancholy 1972).

This can at least partly justify the lower nitrate concentrations detected under the canopies of the evergreen sclerophyllous species.

NRA along the twigs

Olea europaea

Cistus albidus



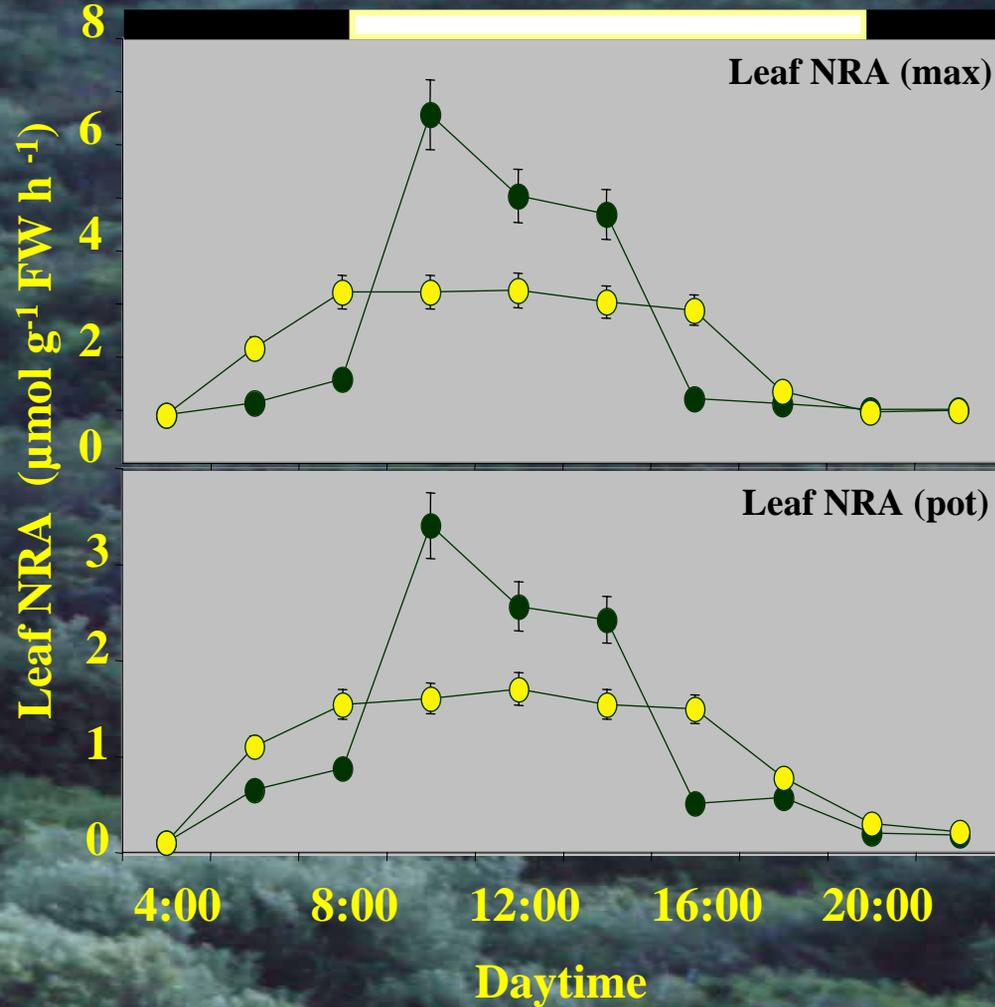
Not all the leaves in the plant canopy behave in the same way

➤ Summer semideciduous species have leaf dimorfism and the lifespan of each leaf is less than a year

➤ Evergreen sclerophyllous have consistently coriaceous leaves with a lifespan of 1 to 2 years



NRA variations through the day



The differences in diurnal patterns will have drastic consequences for the partitioning of the newly assimilated carbon.



To check plant response to several N availabilities:

plants of *C. albidus* and *O. europaea* were grown in pots filled with soil from the study area with a known N concentration.



Higher levels of N were obtained by addition of ammonium nitrate.

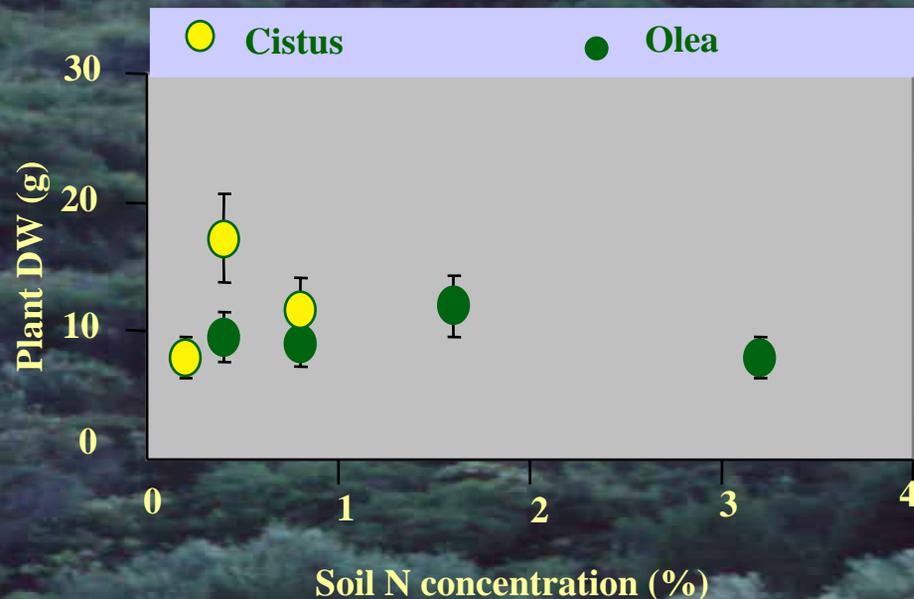
Five levels of N were studied: 180, 360, 740, 1400 and 2800 $\mu\text{mol g}^{-1}$ soil DW

Plants were watered regularly in order to maintain soil water content of $\pm 0.3 \text{ m}^3 \text{ H}_2\text{O m}^{-3}$ soil.

The phenology of plant development was followed and plants were harvested 4 months after the start of the treatments.

Nitrate reduction versus soil nitrogen concentration

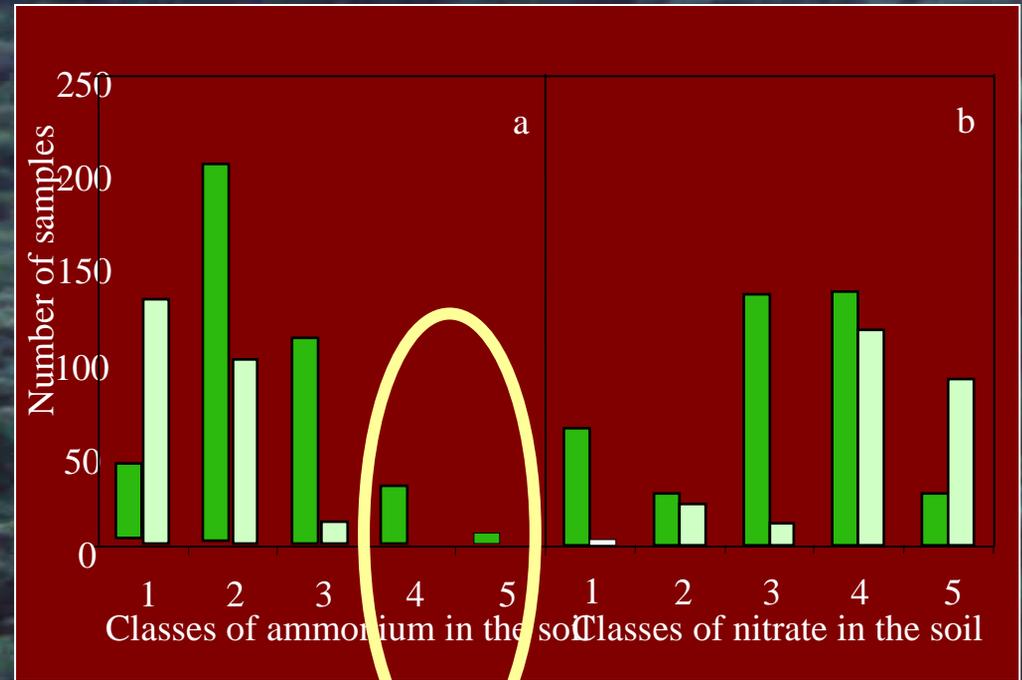
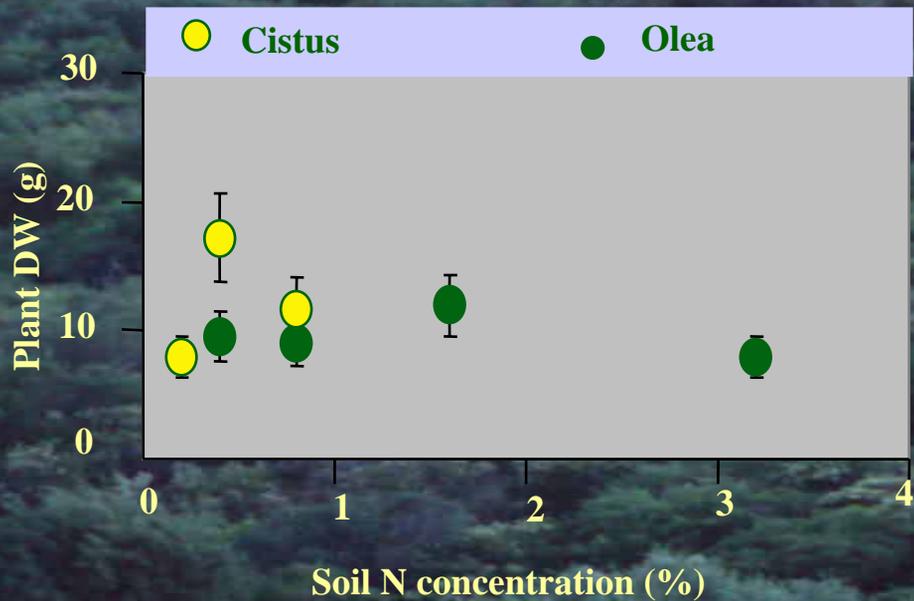
One year-old plants of *Cistus* and *Olea* were grown in pots containing soil to which different concentrations (0.2, 0.4, 0.8, 1.6 and 3.2%) of nitrogen were added:



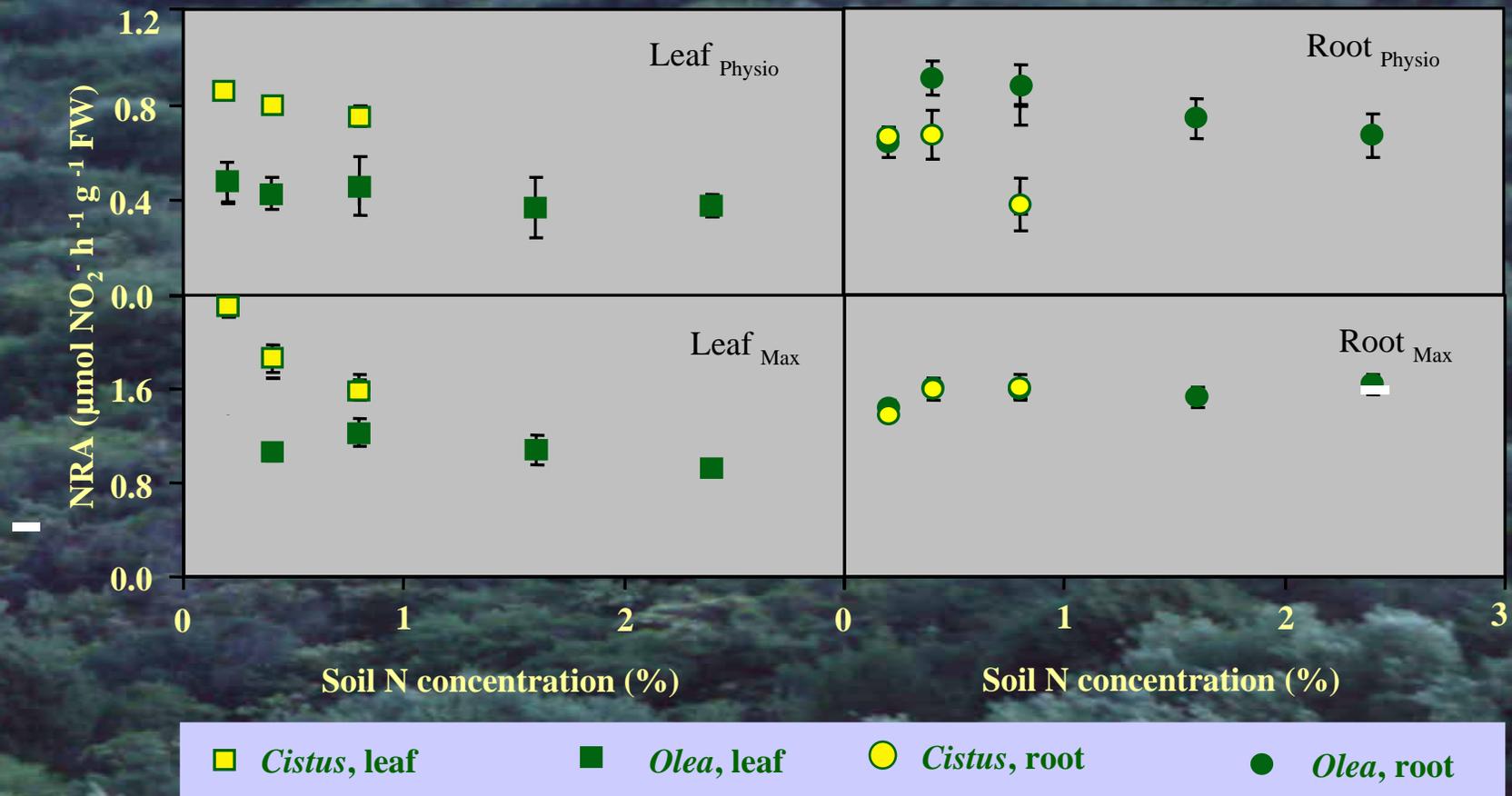
⇒ *Olea* plants developed well under all nitrogen concentrations except for the highest

⇒ *Cistus* plants showed their highest biomass accumulation at 0.4% N in the soil, plants grown with the 2 highest concentrations died before the end of the experiment (3 months)

Nitrate reduction versus soil nitrogen concentration



Nitrate reduction versus soil nitrogen concentration

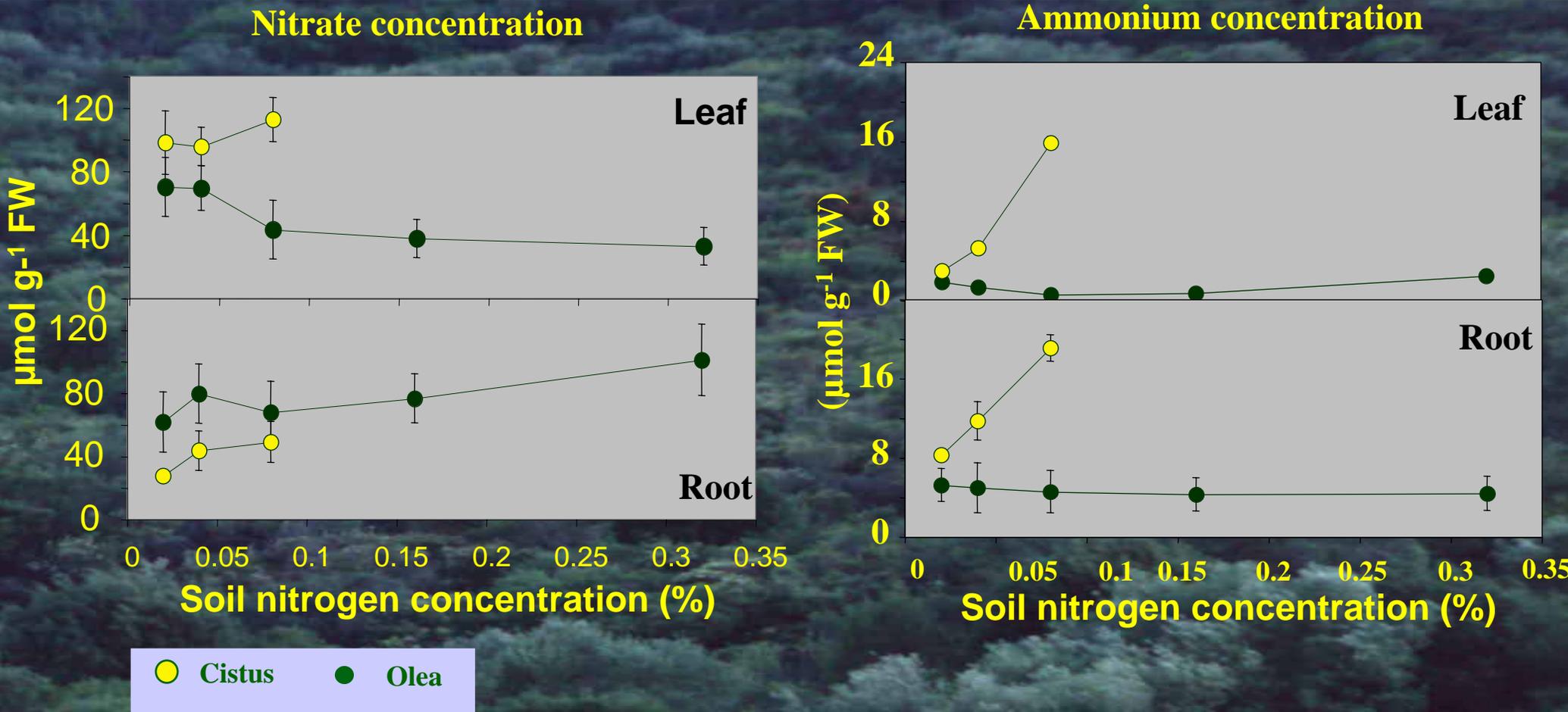


Shoots and roots of both plants displayed distinct capacities for nitrate reduction:

⇒ *Olea* showed higher NRA in the roots than in the leaves, with an activation state very close to 50%

⇒ *Cistus* plants changed their root and shoot capacities to reduce nitrate with nitrogen availability in the root medium

Nitrate and ammonium concentrations in the plant material



Shoots and roots of both plants displayed distinct capacities for nitrate reduction:
⇒ *Olea* showed higher NRA in the roots than in the leaves, with an activation state very close to 50%
⇒ *Cistus* plants changed their root and shoot capacities to reduce nitrate with nitrogen availability in the root medium

NRA as a function of ammonium concentration

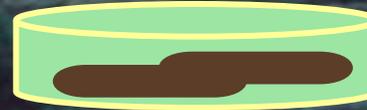
Olea europaea



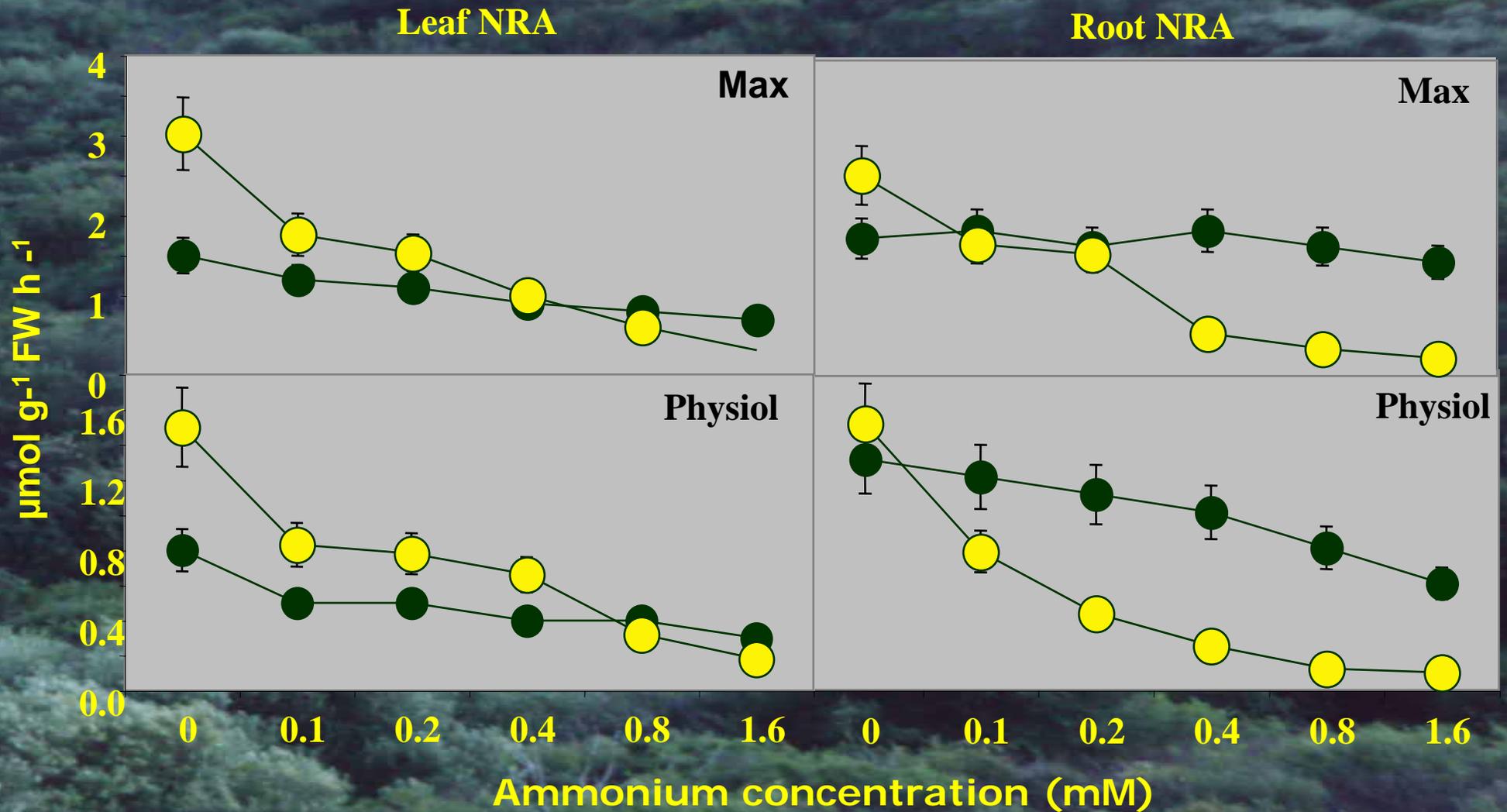
Cistus albidus



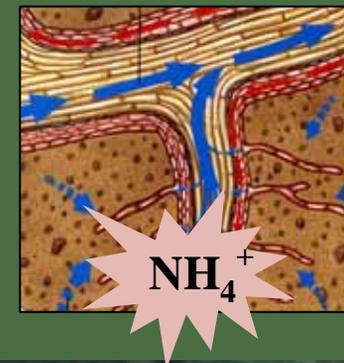
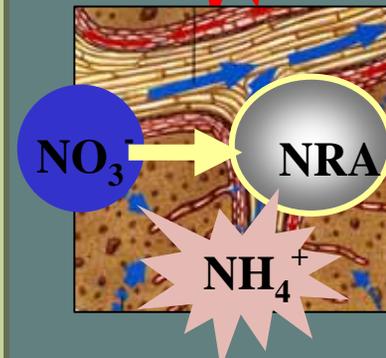
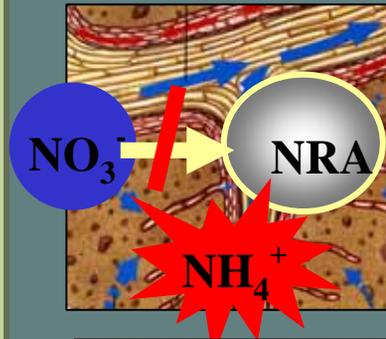
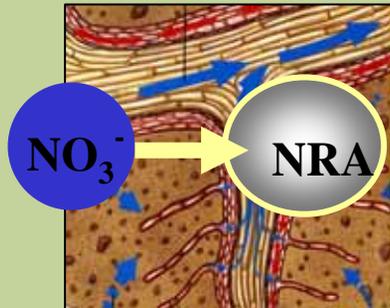
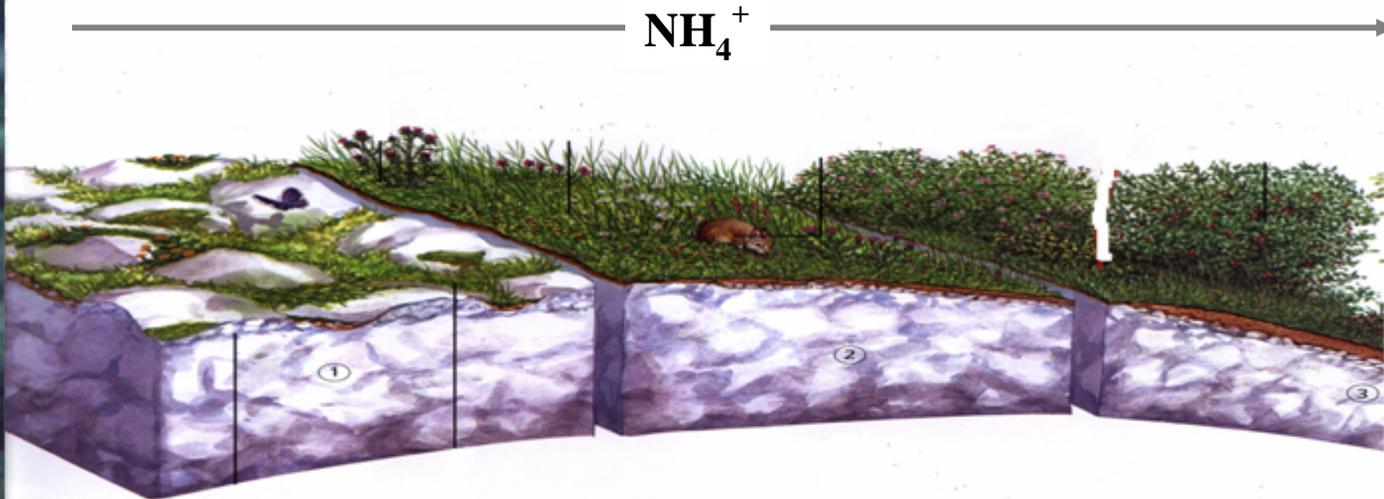
8 h



NRA as a function of ammonium concentration



NRA sensitivity to ammonium may be one of the driving forces for ecological succession....



An aerial photograph of a dense, lush green forest. The trees are packed closely together, creating a textured, undulating surface of various shades of green. The lighting is soft, suggesting a slightly overcast day or late afternoon. In the center of the image, the text "Thank you for the opportunity" is written in a yellow, serif font.

Thank you for the opportunity