Responses to N deposition in the southernmost European fir forests:

implications for critical loads under a warming climate.

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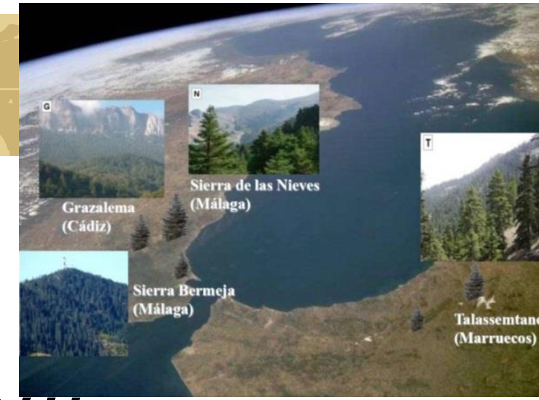
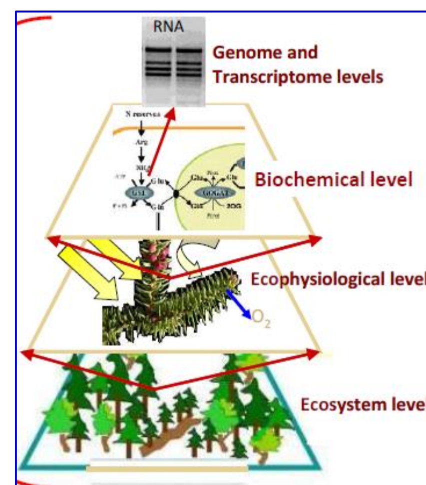
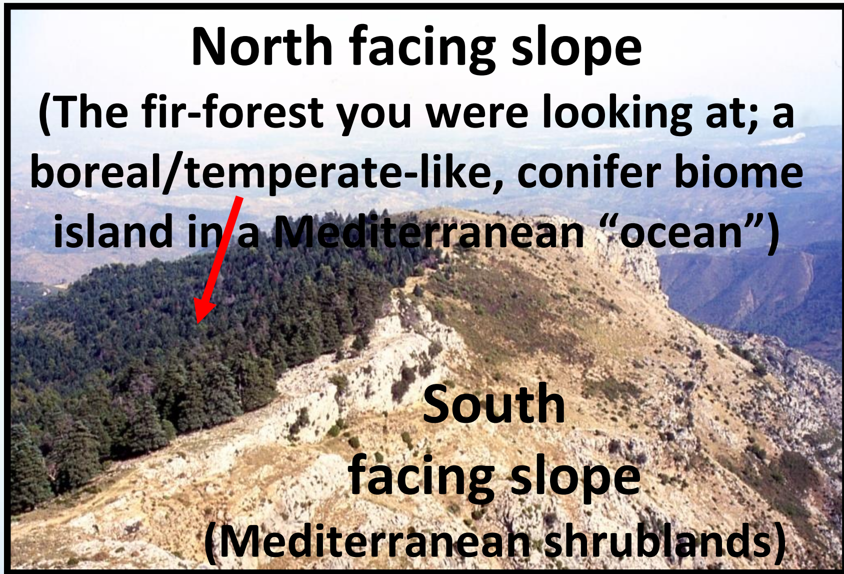
Speech outline



- *Fir-forests in the Gibraltar Strait??!!!*
- *… and along a N deposition gradient!!!*
  - *How do they behave?*

- *Implications for critical loads in the context of a warming Europe:*
  - *"Leaky" N cycles even in N-limited, aggrading forests.*
  - *"Triggering" role of induced P limitation (N to P stoichimetric tensions).*
  - Tree species responses to excess N depend on plant functional traits (e.g., leaf longevity).

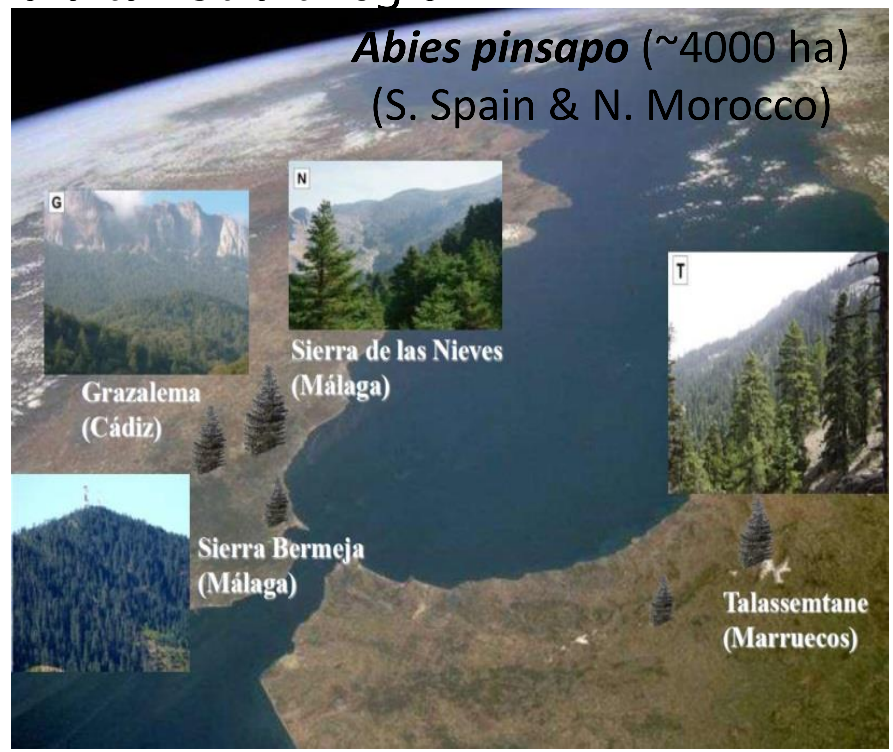


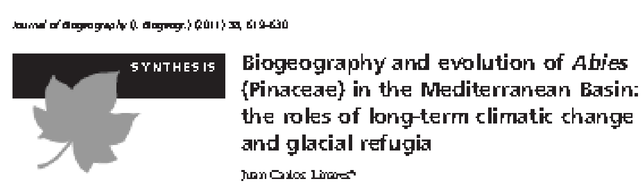


… Re: the warming experiment…
to transplant this foggy, temperate conifer forests to a Mediterranean-type climate location would be ideal…

The Study Model (climatic relic)

- ***Abies pinsapo*** **Fir forests**: paleobiogeographical singularity (climatic relic of temperate-boreal conifer forests currently subjected to Med. seasonality): endemics from Gibraltar Strait region.




Journal of Biogeography (J. Biogeogr.) (2011) 38, 619–630

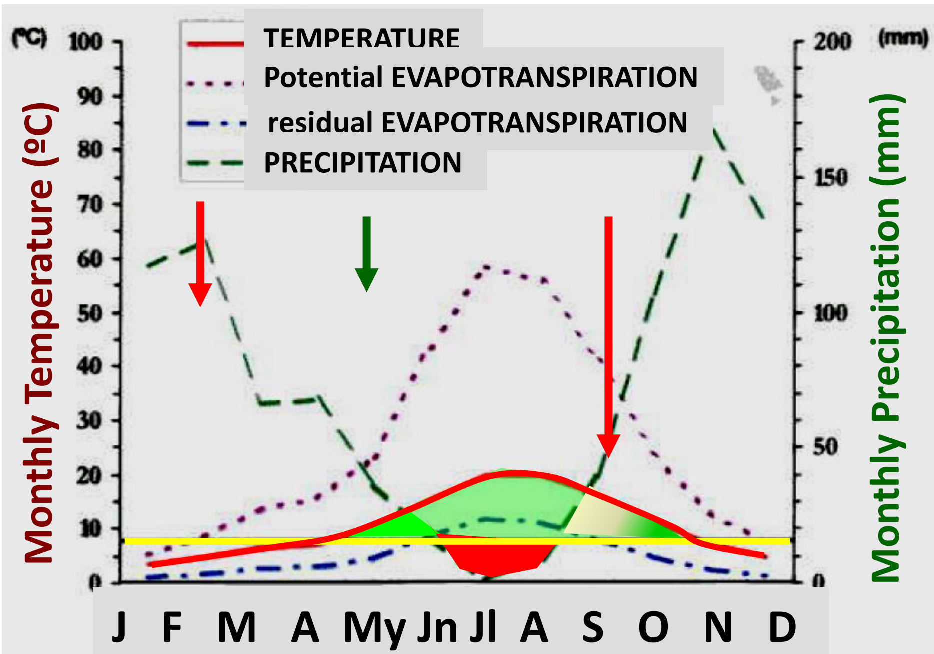
SYNTHESIS

Biogeography and evolution of *Abies* (Pinaceae) in the Mediterranean Basin: the roles of long-term climatic change and glacial refugia

Juan Carlos Linares*

The Study Model (climatic relic)

- **The constraints of Mediterranean-type seasonality:**
  - Typical Bioclimatic-intensity diagram at *Abies pinsapo* sites:



Uncoupling of peaks of plant & microbial activity with peaks of hydrological fluxes

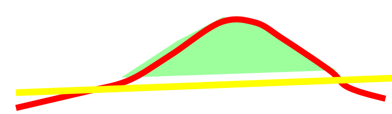
- Growing Degree Days (Tª potential)
- Growing Degree Days (actual)
- Drought intensity (water surplus ≤ residual ETP)
- Conditioned Growing Degree Days

From Reamur's Tª-growth reponse Law:

Site plant-growth supporting capacity: $\int_{t_o}^{t_f} = (Tª_{threshold} - Tª_{ambient})* dt$

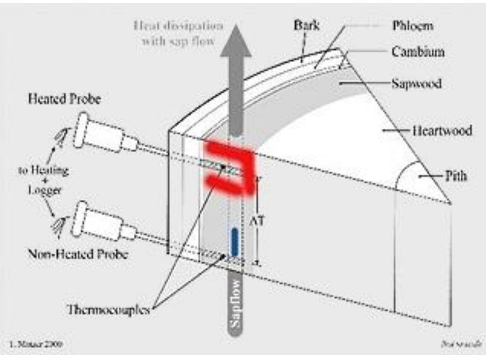
(based solely on ambient Temperature)

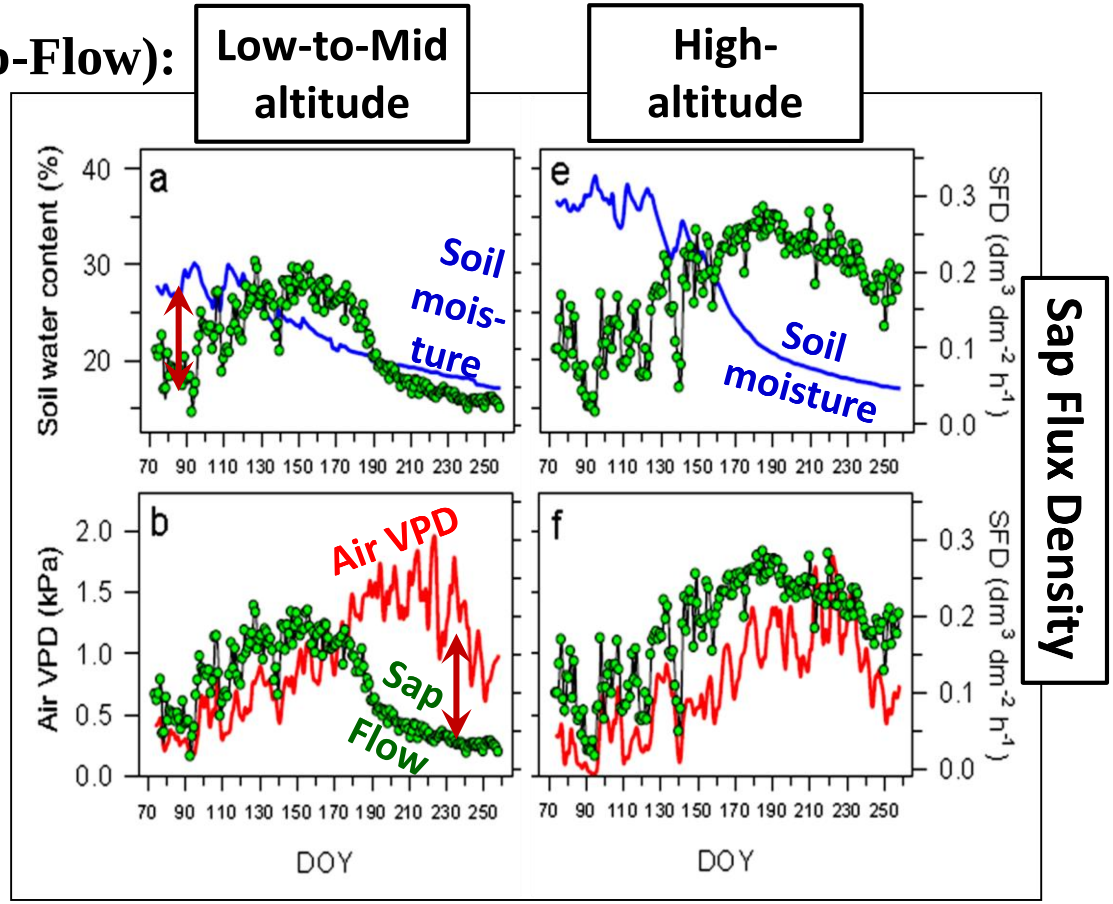
*(GDD or Growing Degree Days, units: ºC*days or bioclimatic intensity units)*

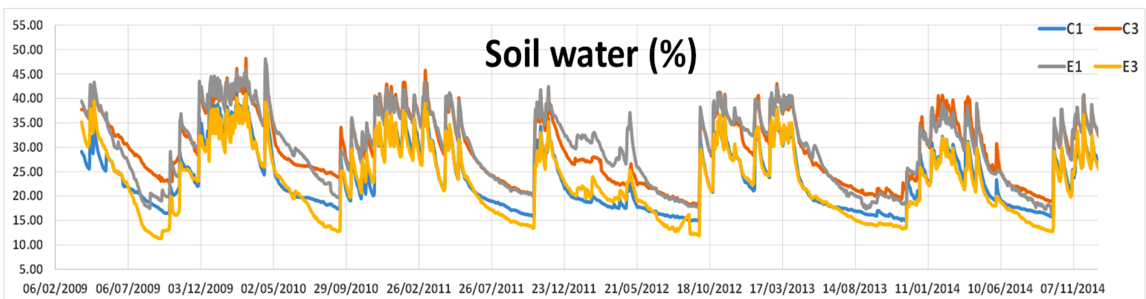


The Study Model (climatic relic)

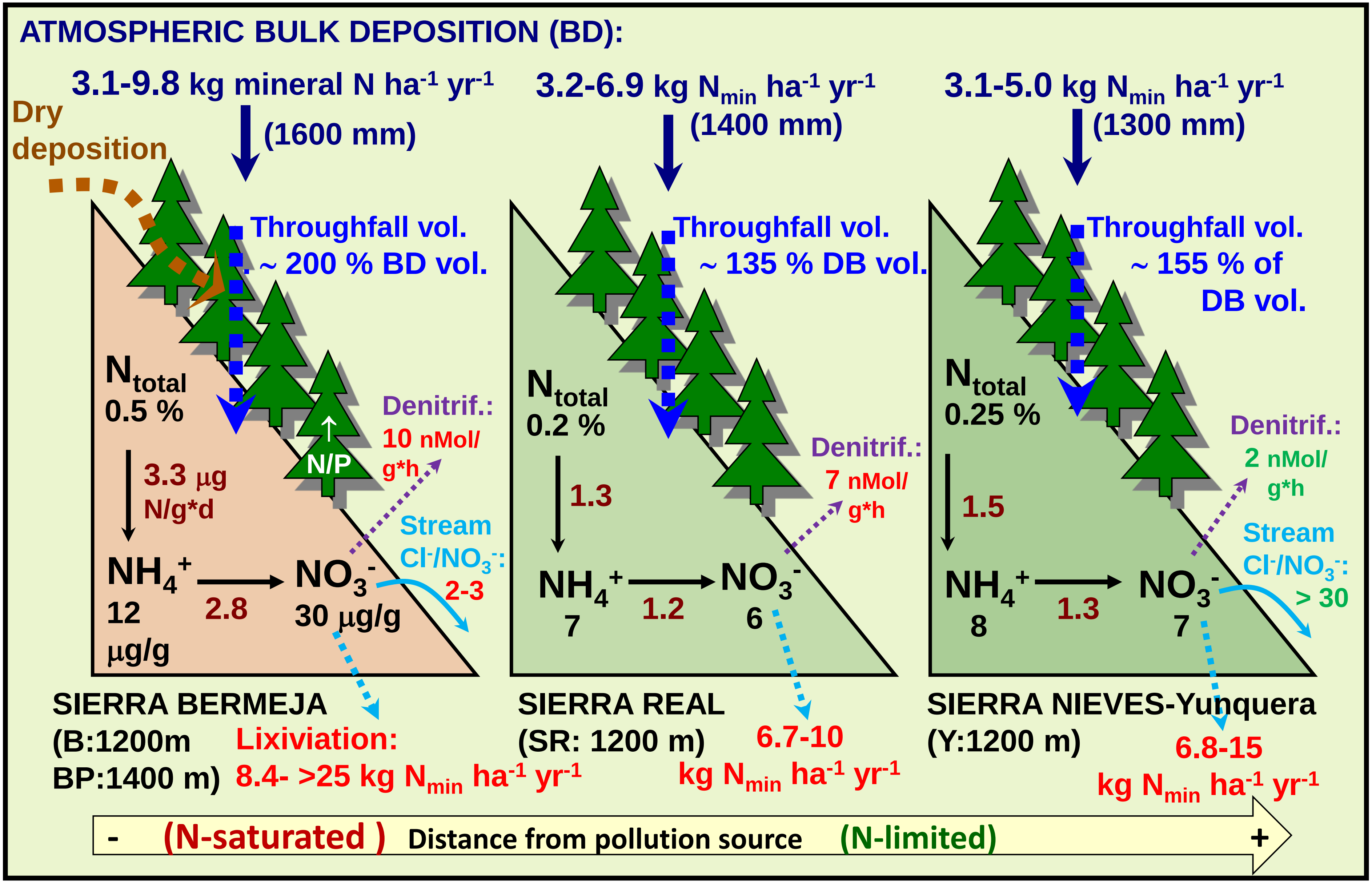
Permanent-Monitoring Plots (Sap-Flow):





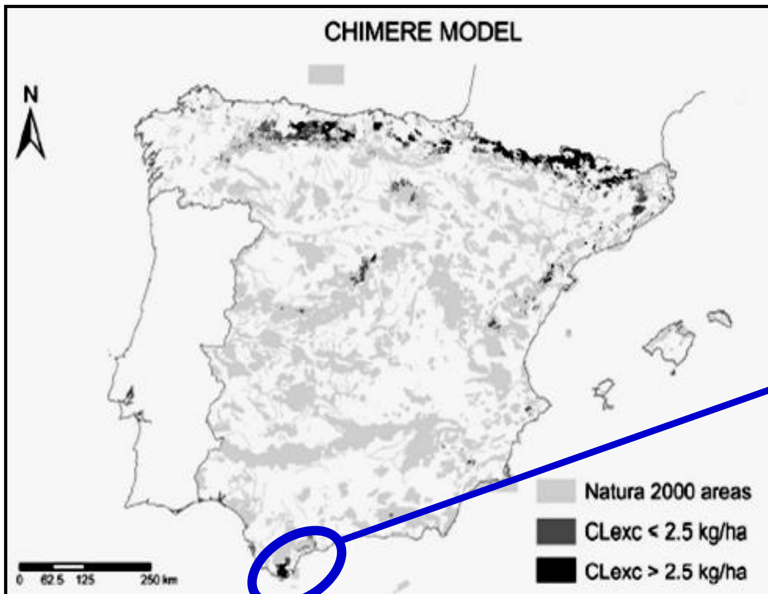


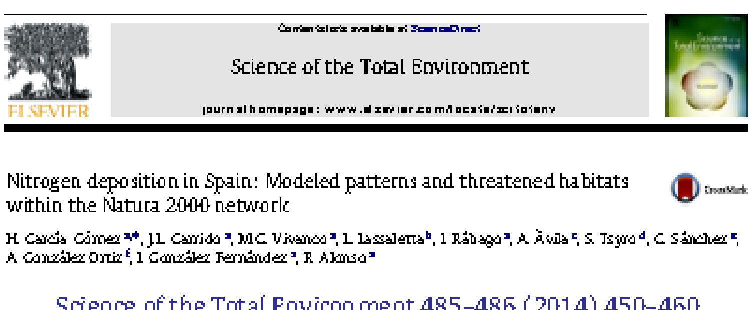
The Study Model (N deposition gradient)



The Study Model (N deposition gradient)

- **Spanish Nature 2000 areas exceeding assigned C.L.**
  (García-Gómez…R. Alonso. 2014. STONTEN485-486: 450-460).




Contents lists available at ScienceDirect

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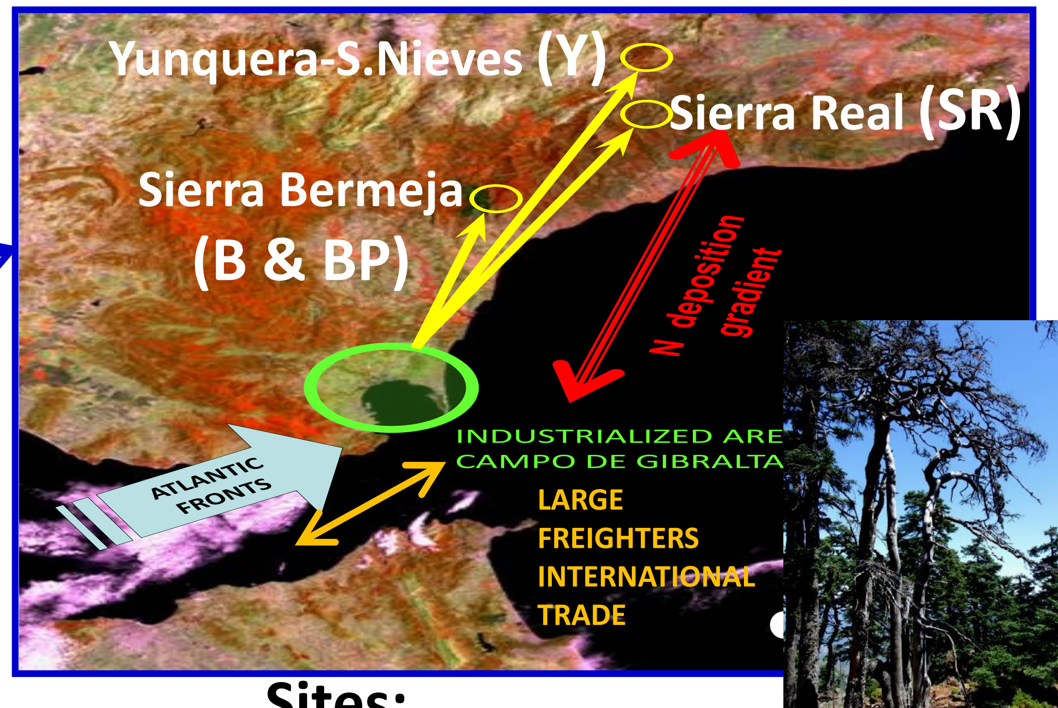
Nitrogen deposition in Spain: Modeled patterns and threatened habitats within the Natura 2000 network

H. García-Gómez [a,*], J.L. Garrido [a], M.G. Vivanco [a], L. Lassaletta [b], I. Rábago [a], A. Àvila [c], S. Tsyro [d], G. Sánchez [e], A. González Ortiz [f], I. González-Fernández [a], R. Alonso [a]

Science of the Total Environment 485–486 (2014) 450–460

Abies pinsapo - Fir Forests

(N-Saturation to N-Limitation gradient):



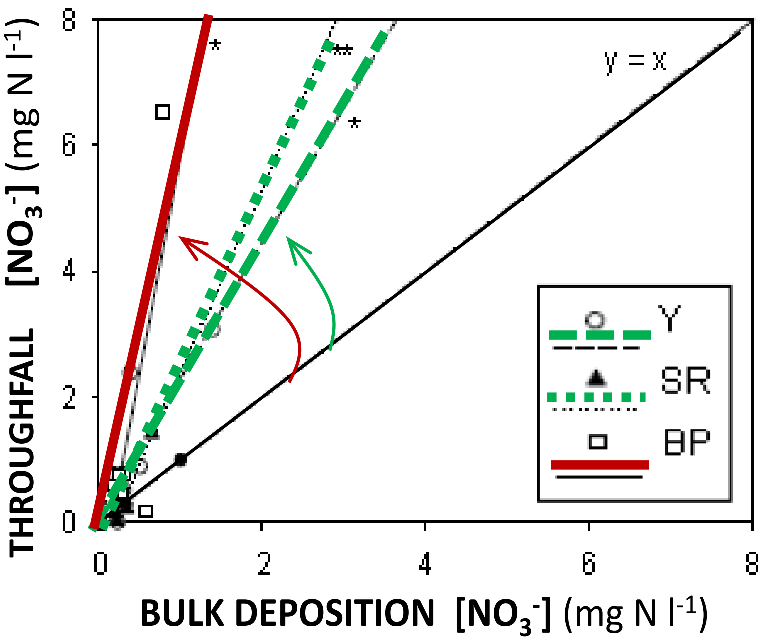
Sites:

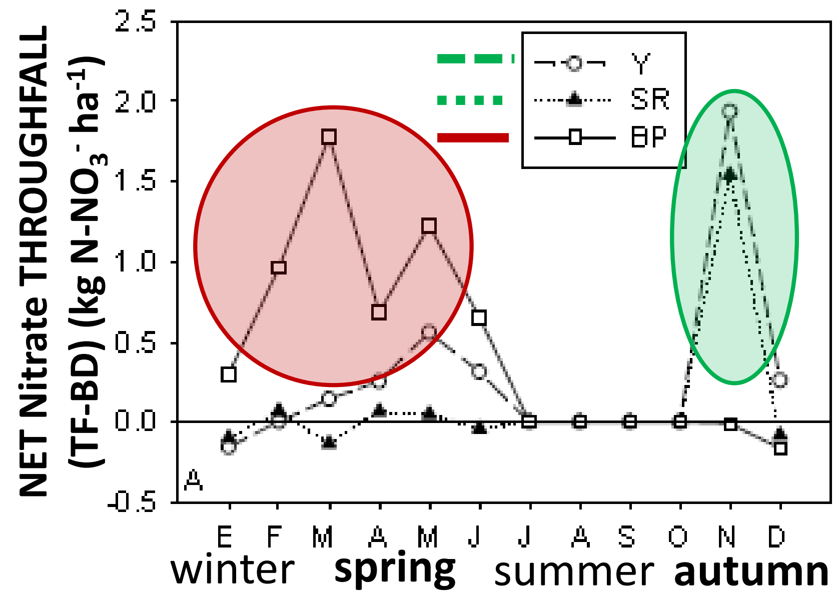
N-limited (S. Real & Yunquera): **SR** (1200 m), **Y** (1200 m)

N-saturated (S. Bermeja): **B** (1400m), **BP** (1200m)

N deposition & *Abies pinsapo* forests

- **Important contribution of dry deposition to N inputs**.
- **Net nitrate throughfall >0 even in N-limited stands** under low N deposition (after summer in autumn; not in spring).
- **Re: C. L.** (N inputs): ***seasonal differences more indicative of N status*** *(between-sites)* ***than year-based figures***.





Sites:

N-limited (S. Real & Yunquera): **SR** (1200 m), **Y** (1200 m)

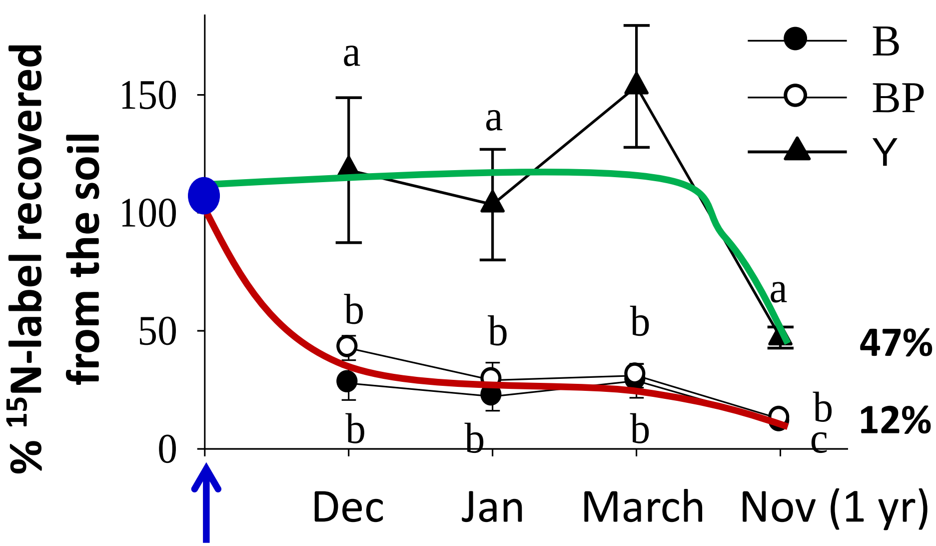
N-saturated (S. Bermeja): **B** (1400m), **BP** (1200m)

Salido et al. Biogeochemistry, submitted

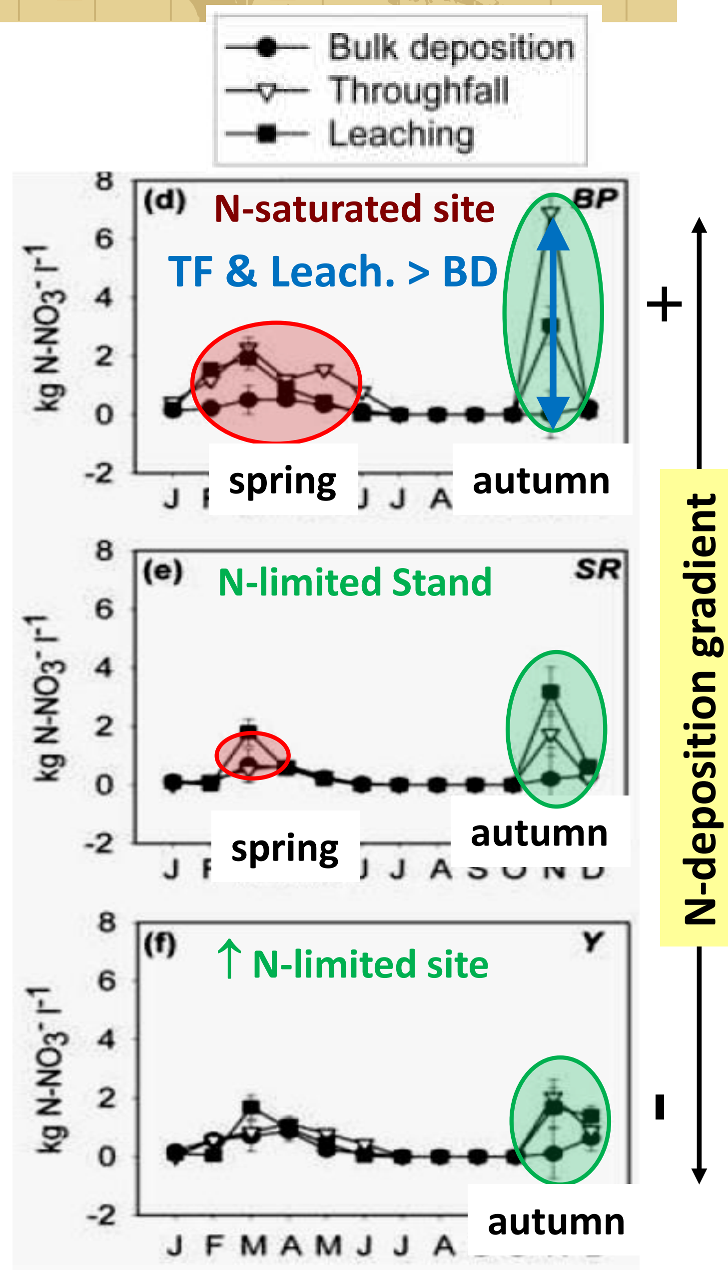
N deposition & *Abies pinsapo* forests

- **N Cycle: intrinsically "Leaky":**
  - ↑↑ **NO_3^- leaching in autumn, independently of N-saturation status**, even in aggrading forest stands.
  - **Low N retention efficiency** (< than typical in temperate/boreal conifer forests).

Manipulative Expt. (+35 kg ^{15}N-labelled NO_3^- ha^{-1}):

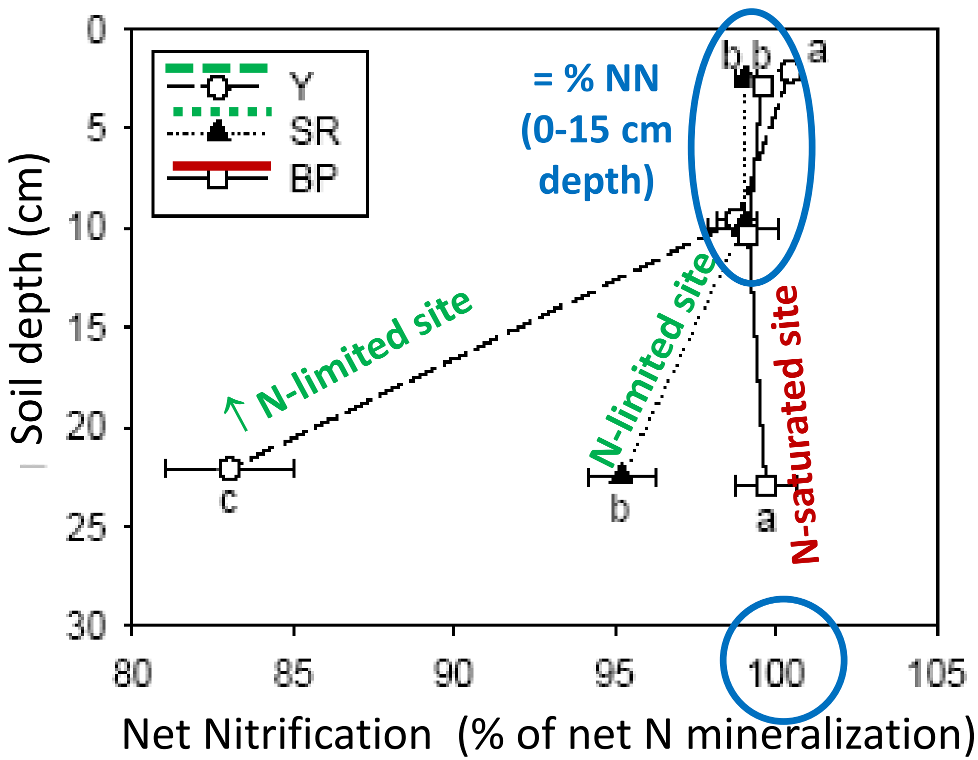


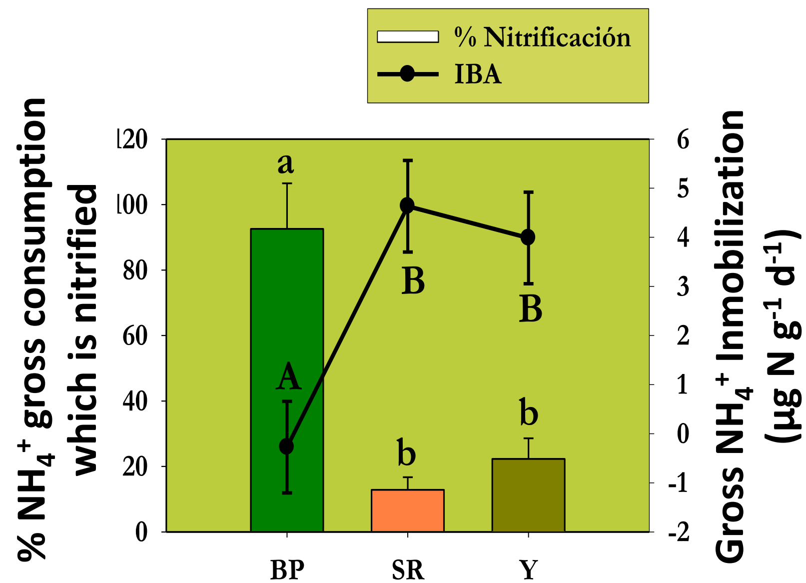
^{15}N addition (October, following first rains)



N deposition & *Abies pinsapo* forests

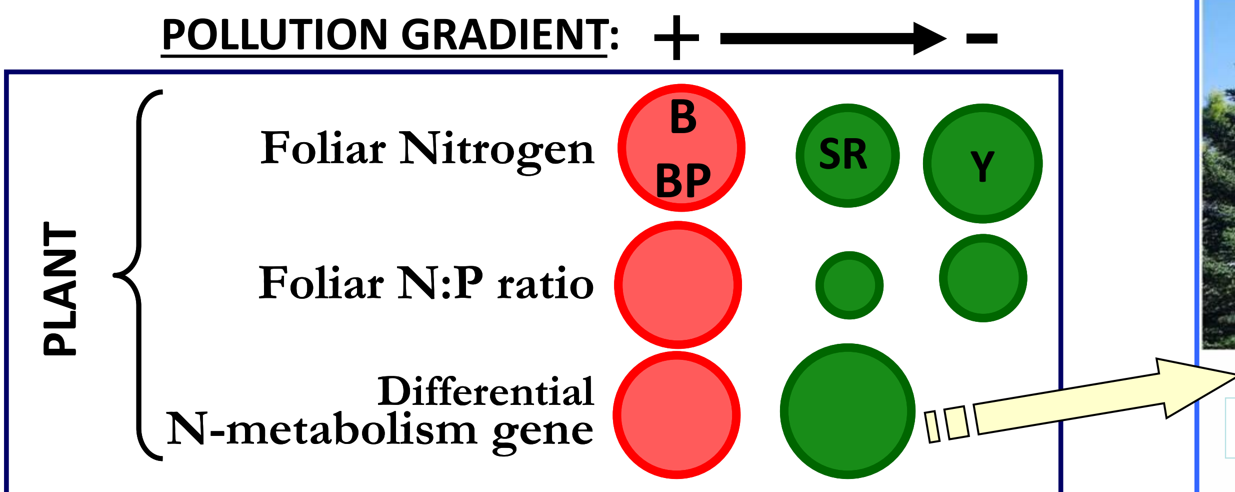
- **N transformations in soil**:
  - **<u>High net nitrification rates</u>** & NN~100% of net N mineralisation, irrespective of the site N-status (except for sub-surface soils).
  - <u>Low N immobilisation</u>: short-term immobilization of added $^{15}NH_4^+$ is 15% (range in temperate forests: 30%-60%).

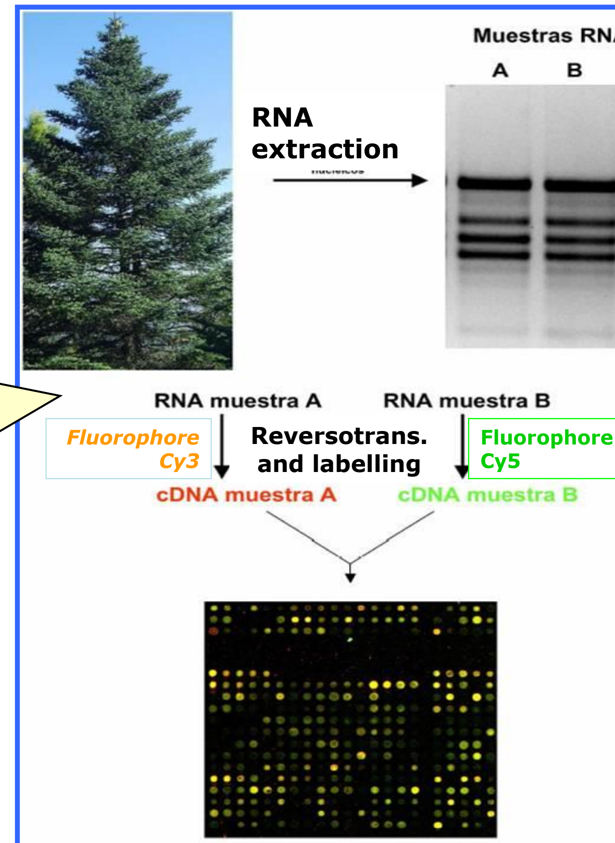




(2) Triggering role of induced P-limitation

- **Inconsistencies between Ecosystem/Soil- *versus* Plant-level indicators of N excess.**
  - Foliar N analyses and transcriptomic (N-metabolism genes expression) failed to differentiate the site-N status.





  - ¿A problem of the selected tissue sample for diagnose?

-ICP-Forest sampling protocol for foliar analyses:
1-2 yr-old, fully expanded needles

(2) Triggering role of induced P-limitation

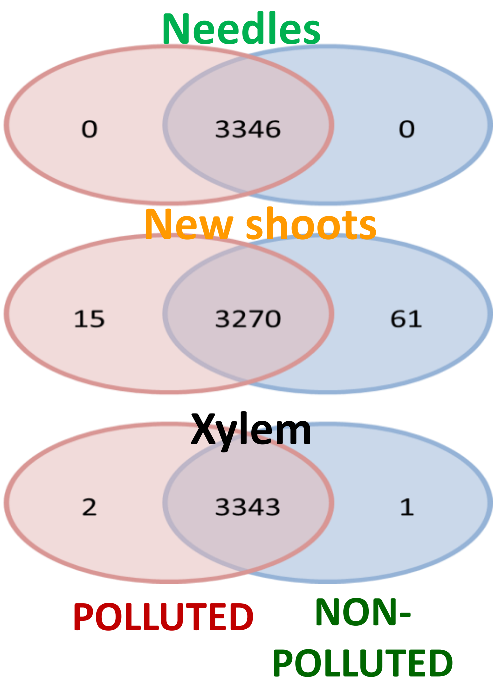
- ***Abies pinsapo*** **(oligotrophic species) seems not to be able to regulate gene expression when subjected to high N availability**:

<u>Venn diagram</u>:

Number of *over-expressed*, *equally expressed*, and *sub-expressed* genes in POLLUTED versus NON POLLUTED SITES.

Needles : No differences.

Xylem : No differences.



New shoots (opening buds):

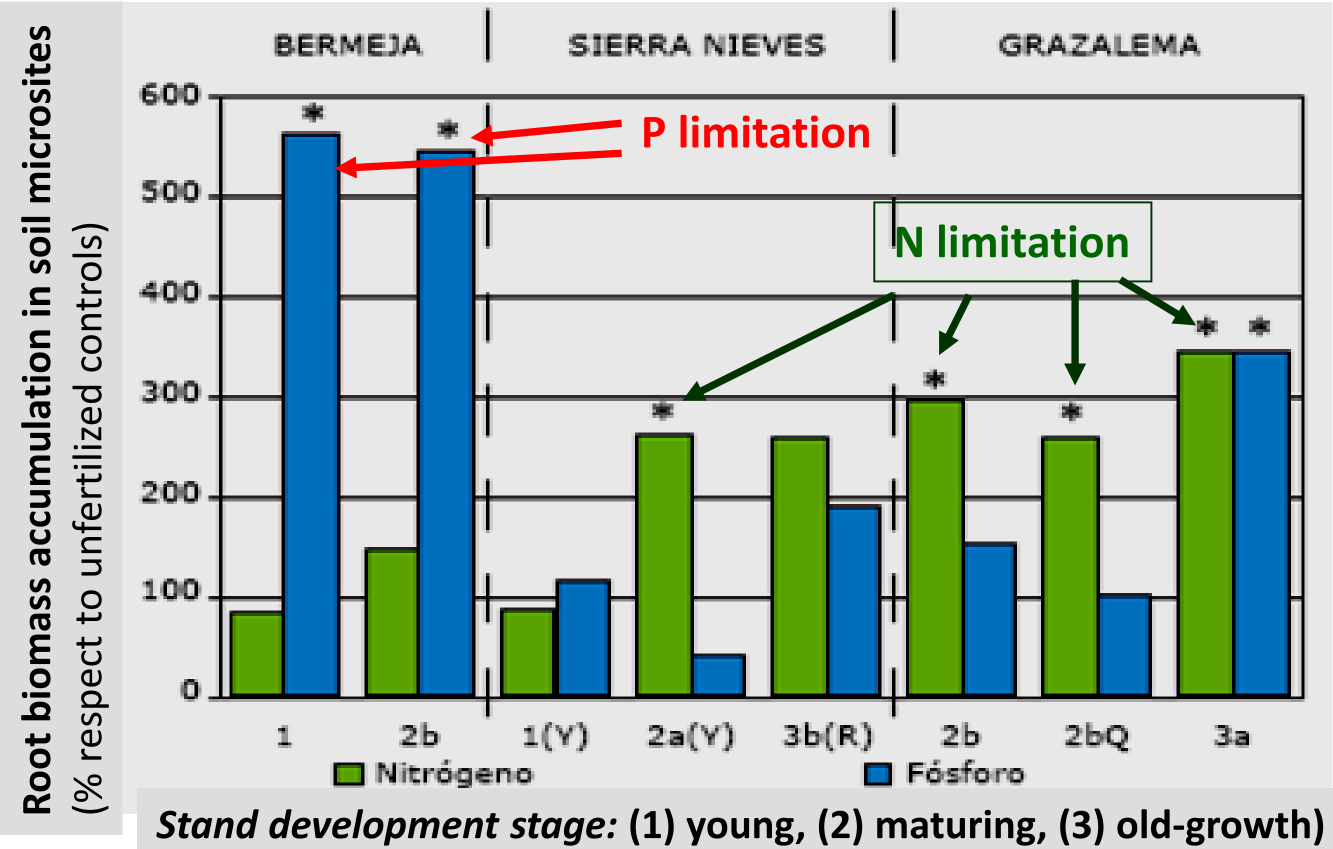
Polluted site: Glycolysis / Gluconeogenesis; pyruvate metabolism, ATP synthase, histones…

Non-polluted site: Rubisco, chlorophyll precursor, chloroplast precursor, cellulose synthase…

Related to differences between sites in phenological phase, **NOT TO Nitrogen METABOLISM!**

(2) Triggering role of induced P-limitation

- Mediterranean-type ecosystems are prone to P-limitation… **thus sensitive to N deposition-induced stoichiometric shifts**
  - All fir-forests are N-limited (irrespective of successional stage; but N & P co-limitation in old-growth ones), except those in S. Bermeja (↑ N Dep.).



Stand development stage: **(1) young, (2) maturing, (3) old-growth)**

Root-ingrowth cores method to assess nutrient limitation:

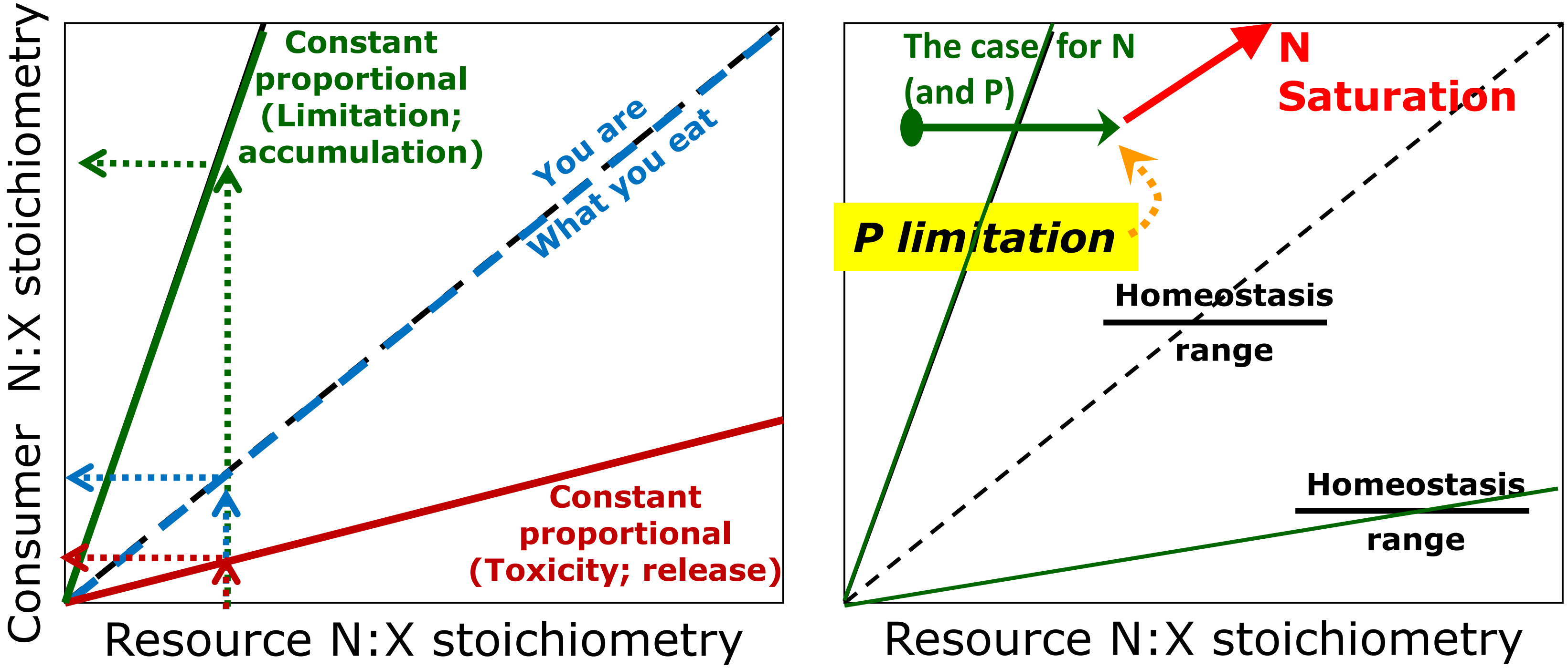
Differential fine-root biomass accumulation in fertilised (N or P,or base cations) and in control unfertilized soil microsites.

Blanes et al. 2012. Plant & Soil

¿Are plant functional traits important?

Consumer to resource stoichiometry theory.

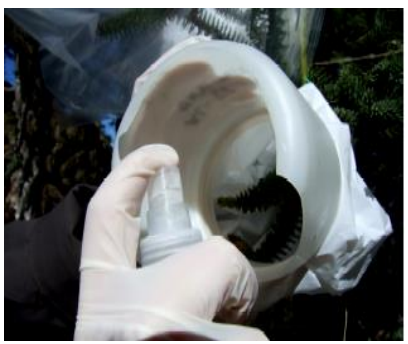
- "You are what you eat" & constant proportional models (toxicity & limitation).
- Consumers need to maintain their stoichiometry (internal homestasis) despite of variable resource stochiometry.
- ***¿are plant functional-traits important in the way consumer homeostatic ability is pressured by chronic N deposition?***



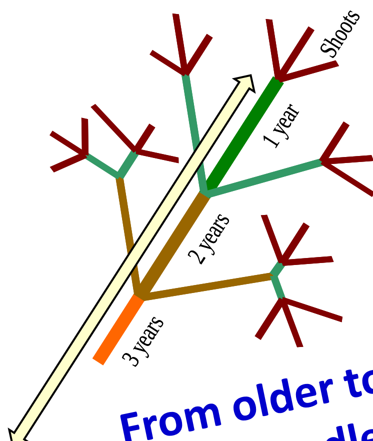
Modified from Sterner & Elser 2002

¿Are plant functional traits important?

- **Failure of leaf tissues as indicators ⇒ leaf longevity?**
  - ***Abies pinsapo***: very ⇑ leaf longevity, up to 15 years
  - ***Pinus pinaster***: ⇓ leaf longevity, (4 years)
    - ***Sampling of all needle cohorts!!!***
      - Foliar ***N:P stoichiometry & Aminoacids profiles***
      - ***In situ ^{15}N needle labelling*** to assess N translocation:
        - Young needles (1+2 yr) / Old needles (4+5 yr), in ≠ branches.



^{15}N label transfer toward older needles in the N-saturated sites??



From older to younger needles in N-limited sites?

The role of Leaf longevity

Foliar nutrients and stoichiometric shifts

- ***Changes along the N deposition gradient.***
  - Long *versus* short leaf-longevity of the tree species. **Foliar Nitrogen**

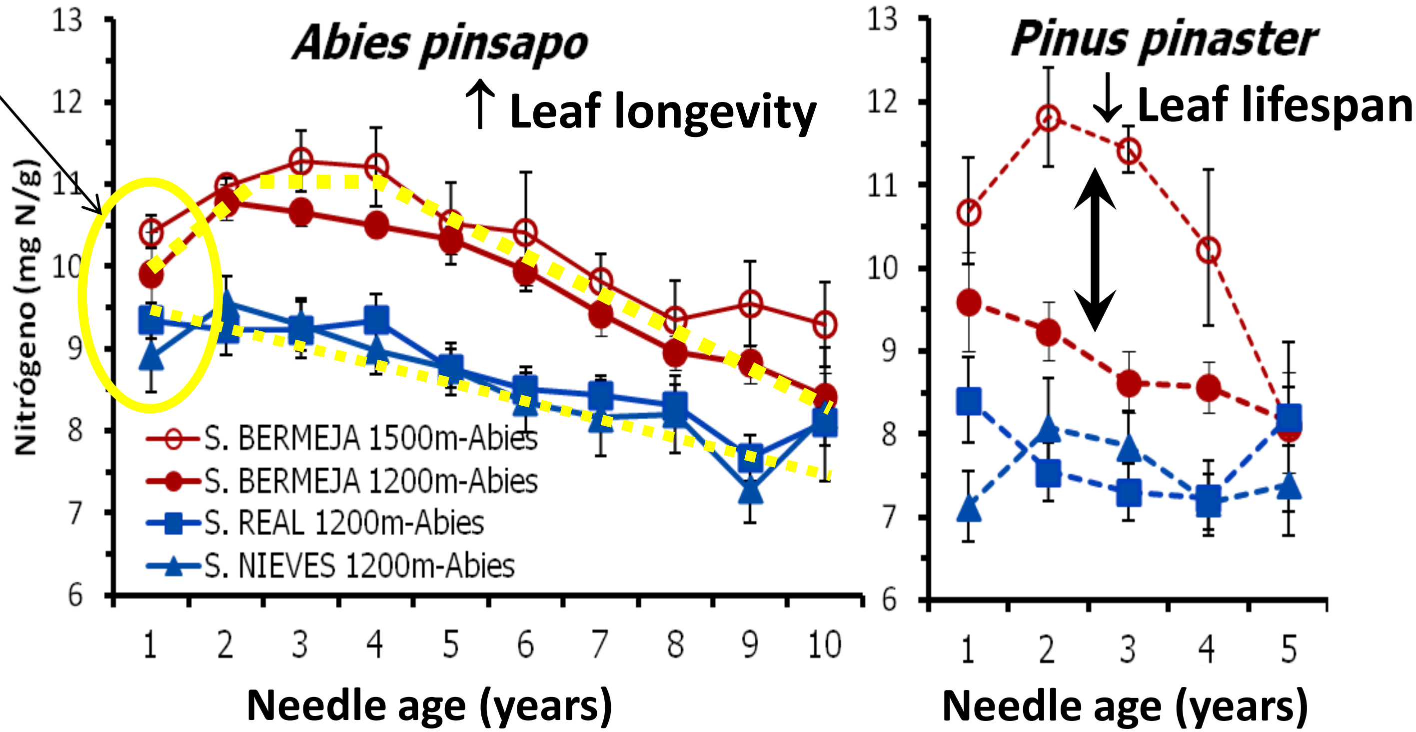
No significant differences for young leaves

N acummulation toward older needles

¿abnormal luxury consumption?

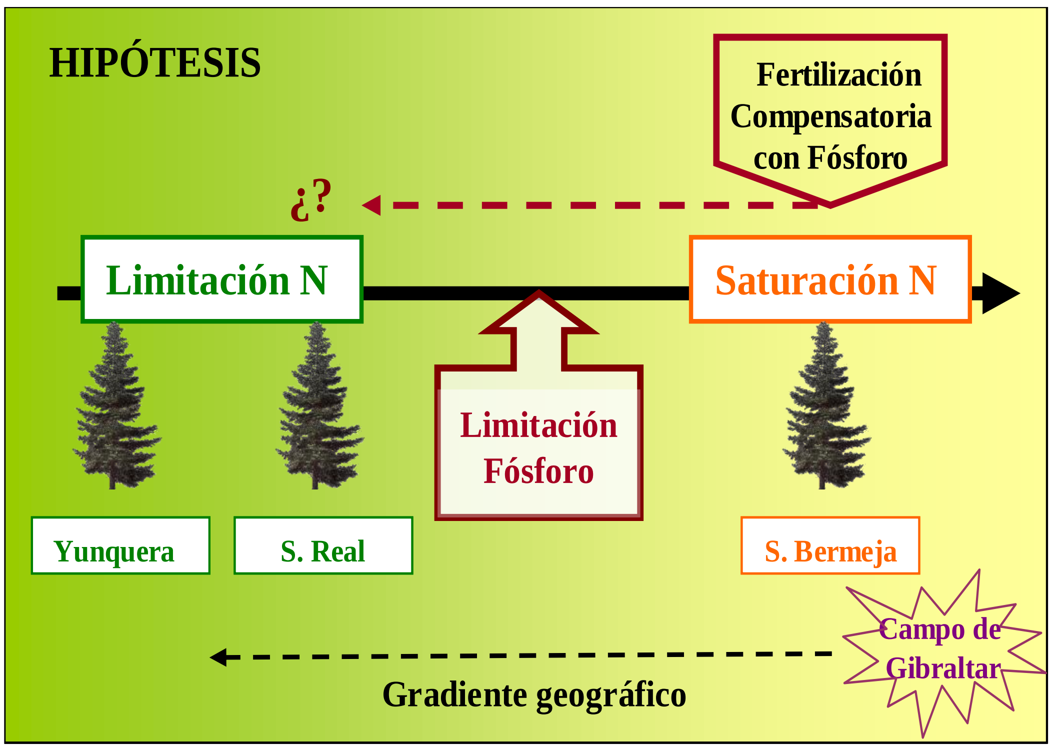
N ↓ with ↑ needle age

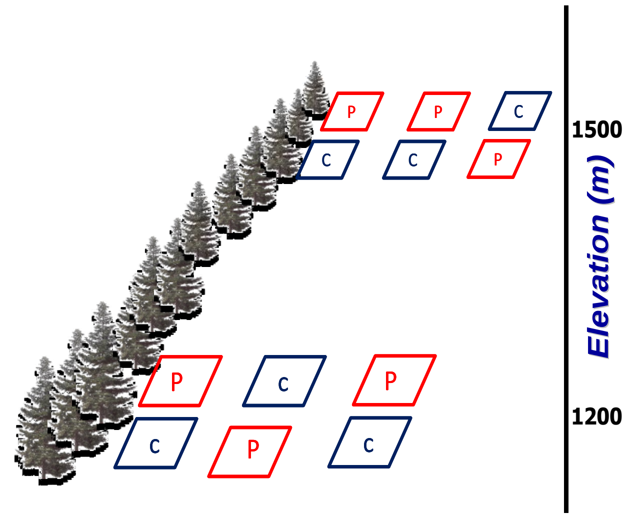
(normal, universal pattern in plants)



Later Work on Med. conifer forests

- **Polluted site: Mitigation measures - compensatory P fertilization experiment in the field**
  - N pollution ⇒ N:P imbalance ⇒ P limitation.
  - … if P fertilization ⇒ ¿back to N limitation/alleviation of N saturation?





- 2 elevations:
  - 1400 m^2
  - 1200 m^2
- 3 blocks per elevation
- *2 treatments:*
  - Control
  - P fertilization
  (50 kg ha^{-1} yr^{-1}/3 years)

The role of Leaf longevity

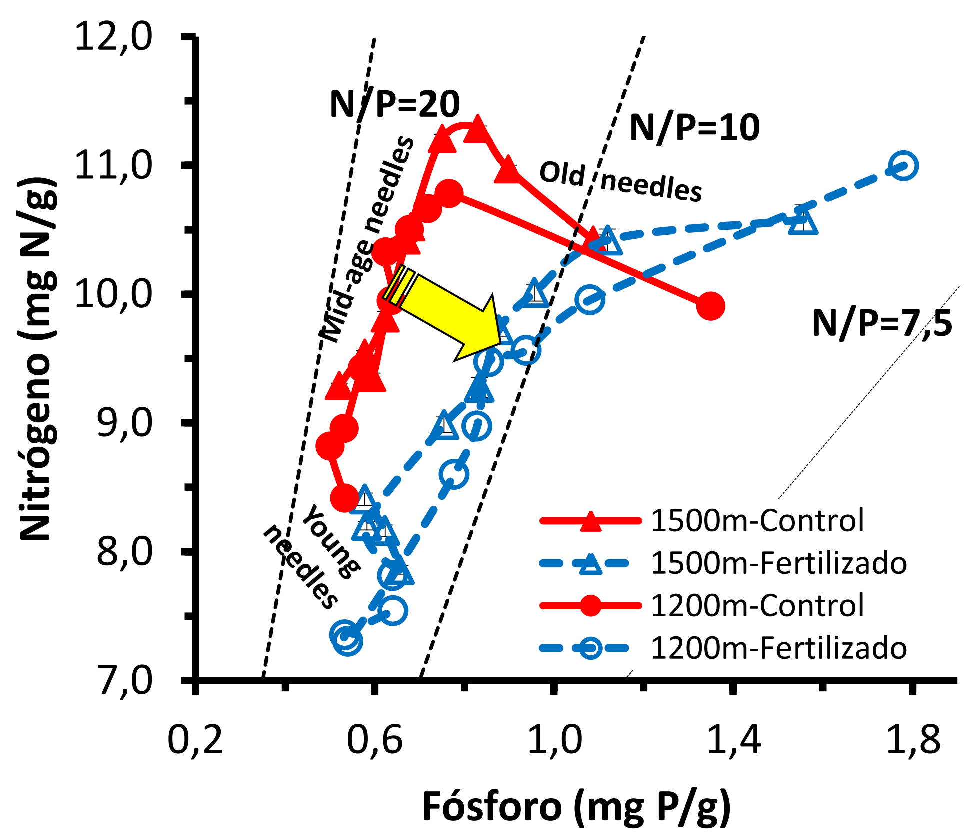
Foliar nutrients and stoichiometric shifts

Effects of Compensatory Phosphorus Fertilization

- *Abies pinsapo* (long leaf longevity), polluted sites. **N:P ratio**

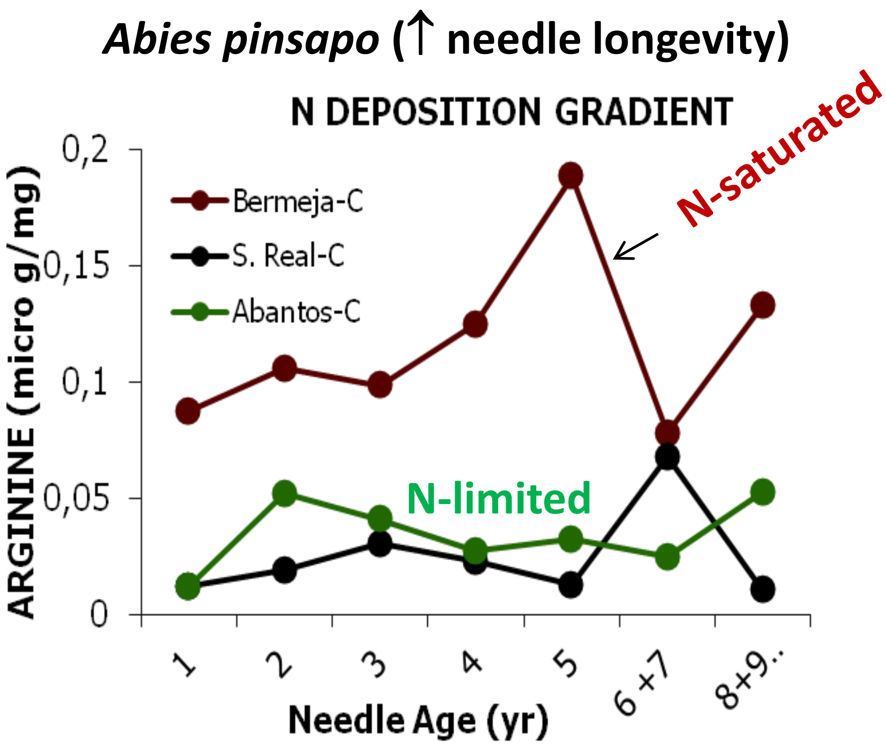
The pattern of very high N/P ratios in mid-age needles dissapeared following compensatory P-fertilization

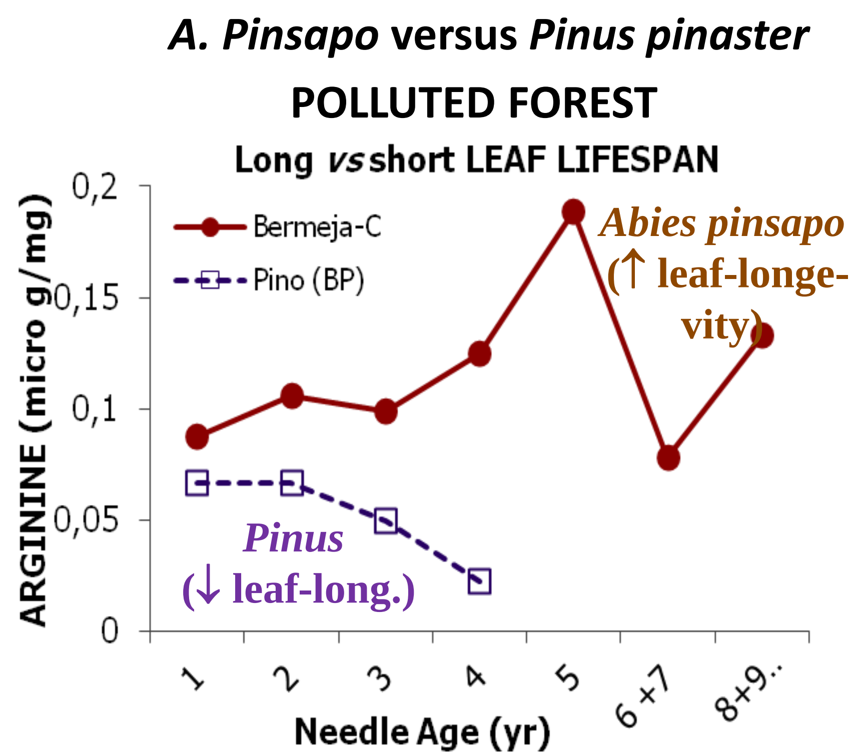
Luxury N consumption – N accumulated in older leaves- can be further metabolized to protein synthesis and growth?



The role of Leaf longevity

- **Free aminoacids - Luxury N consumption**
  - ***Changes along the N deposition gradient.***
    - Abies pinsapo (long leaf longevity), polluted sites. **ARGININE**

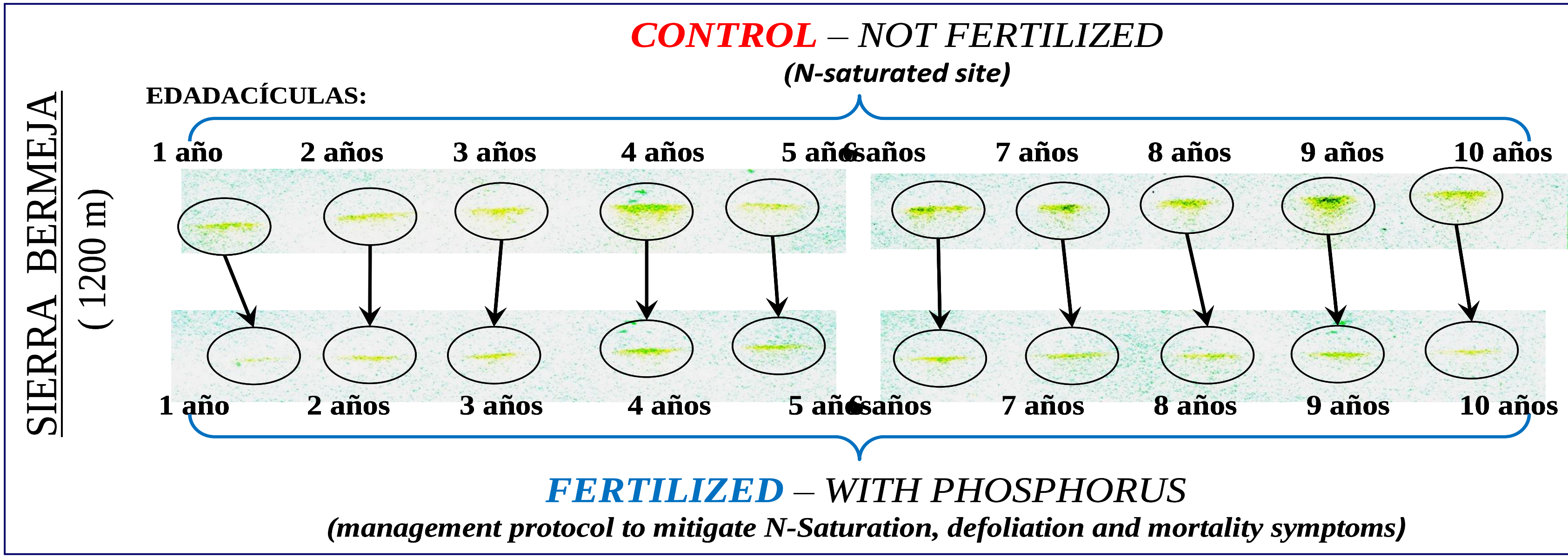


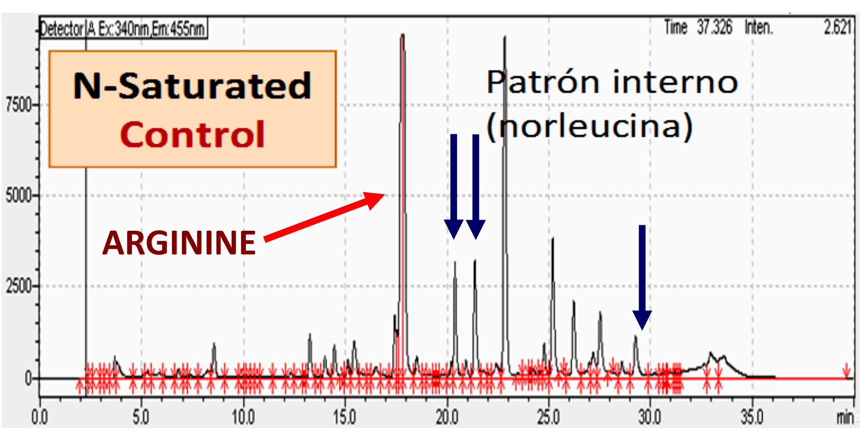


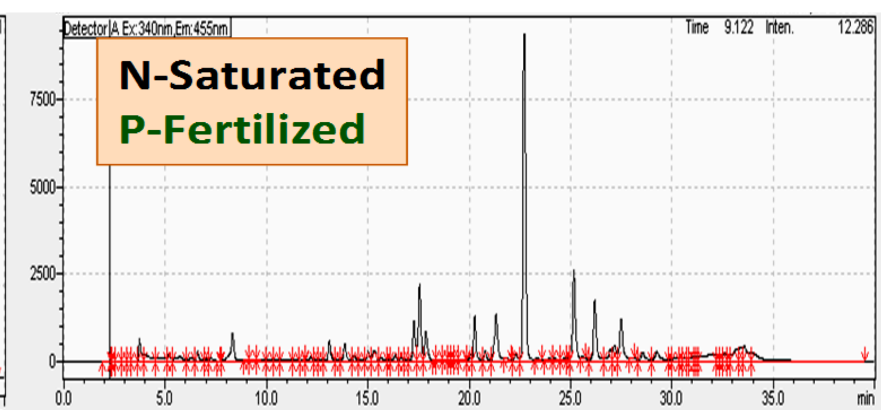
The role of Leaf longevity

Foliar free-aminoacids: paper and HPLC cromatography

* *Abies pinsapo, Polluted site, control vs P-fertilized plots*

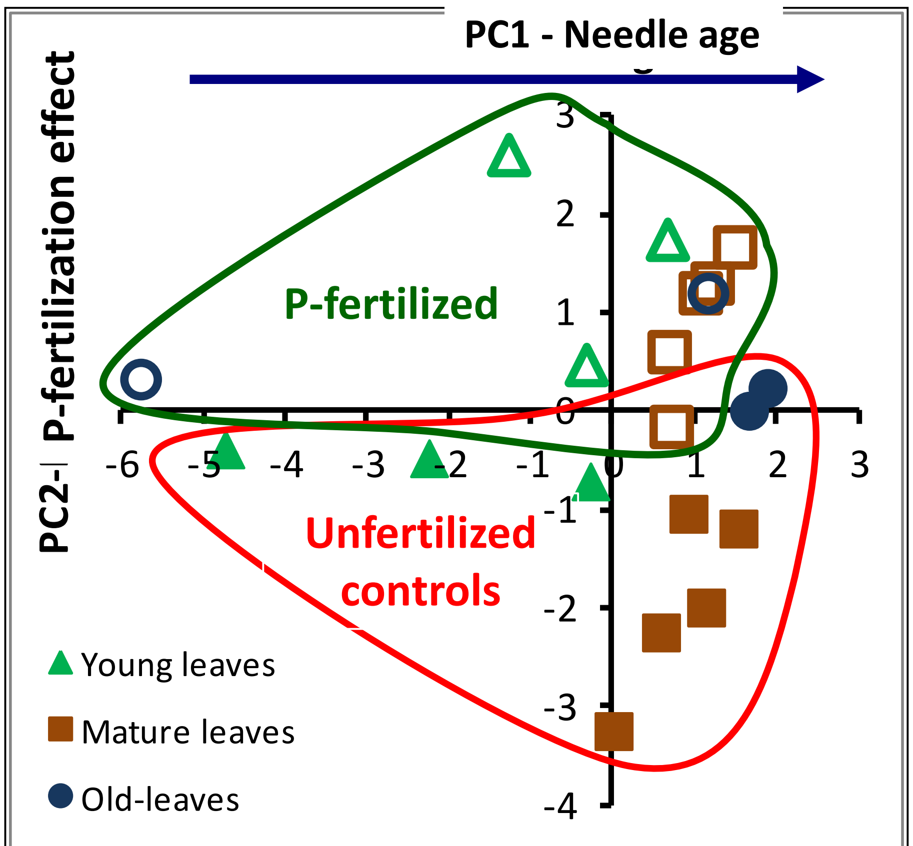


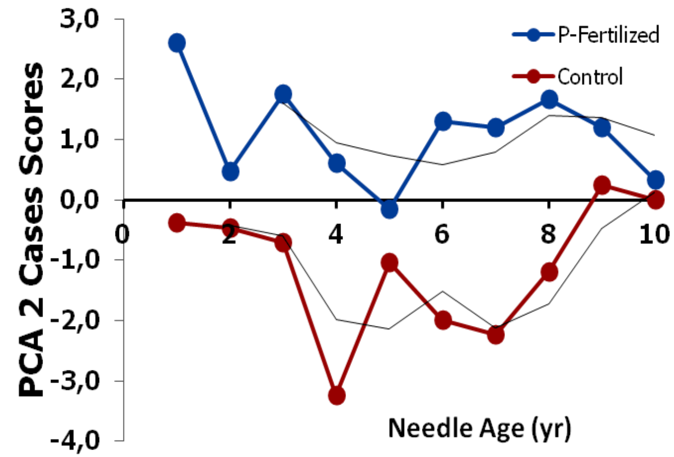




The role of Leaf longevity

- **Needle Aminoacids profiles**
  - ***Effects of P-fertilization – MULTIVARIATE ANALYSIS***
    - *Abies pinsapo* (long leaf-longevity), polluted sites.



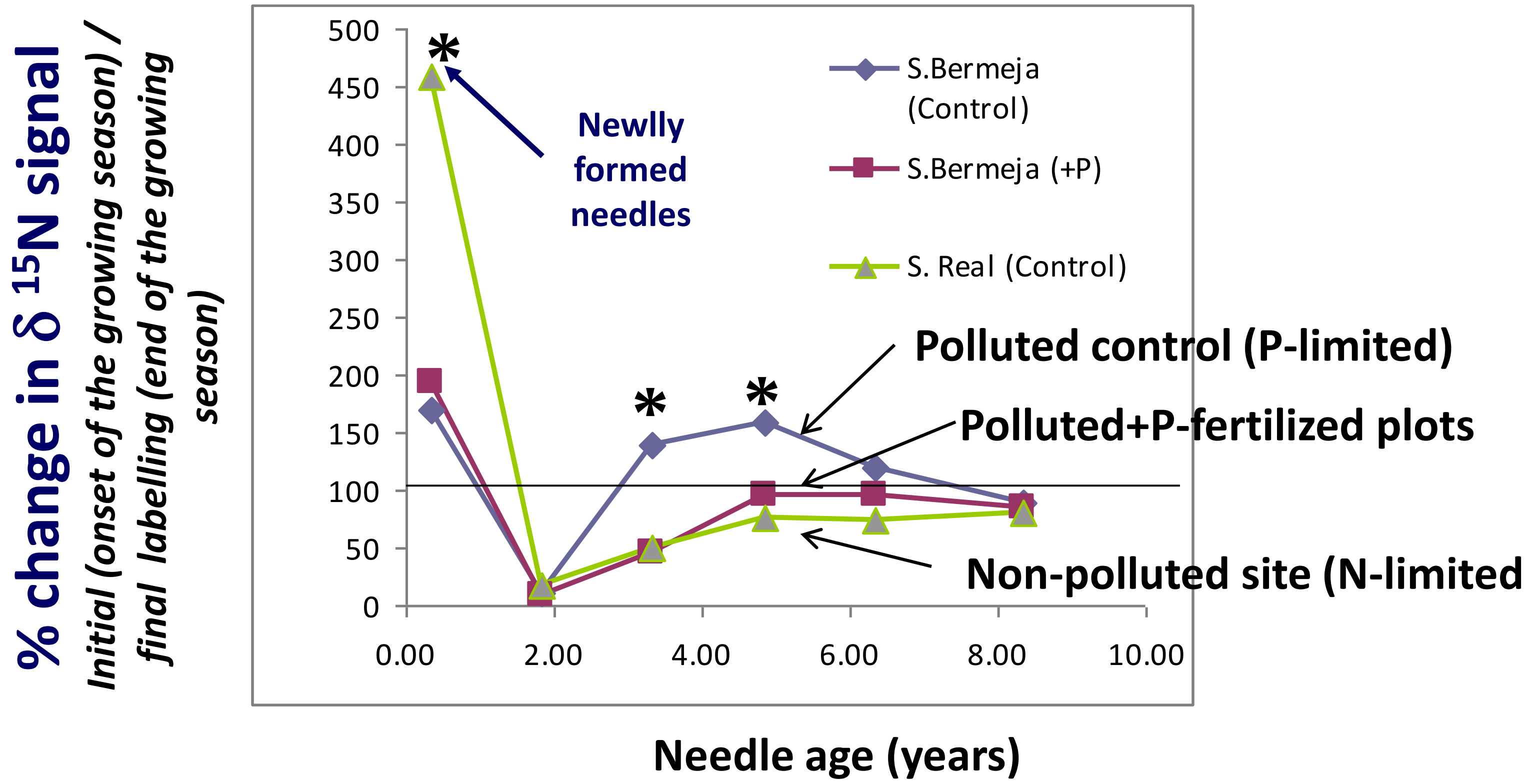


Factor-variable correlations (factor loadings)		
	PCA-1 (Age)	PCA-2 (N-Saturation)
Aspartato	-0,40	0,20
Glutamato	0,49	-0,59
Arginina	-0,25	**-0,80**
Serina	**-0,77**	0,24
Met-Val	**-0,79**	-0,09
Fenilalanina	-0,60	-0,47
Alanina	-0,24	-0,53
Glicina	**-0,96**	-0,04
Histidina	**-0,75**	0,08
Treonina	**-0,79**	-0,12
Tirosina	0,26	**-0,74**

The role of Leaf longevity

Foliar 15-N labelling Experiment

- ***Newly developed needles acts as N atractors:*** much <u>more acute effect in the N-limited forest</u> (S.Real).
- More ^{15}N from young to old leaves in the polluted site.
- Trees from P-fertilized plots in the polluted forests perform like trees in the unpolluted sites.



- **For the future.** Re: N to P imbalances associated to elevated N deposition.
  - Extending the aproach (from ecosystem level to molecular responses)
    - To other plant functional groups and traits.
    - To other types of ecosystems.
  - Project proposal under evaluation (conv. Feder-Junta Andalucía):
    - N deposition impacts on relic tertiary flora from S. Spain: the role of N:P stochimetric stress.
    - With the colaboration of David Elustondo (Univ. Navarra).


Many Thanks!