



## Title Stable isotopes in dendroecology

### Module

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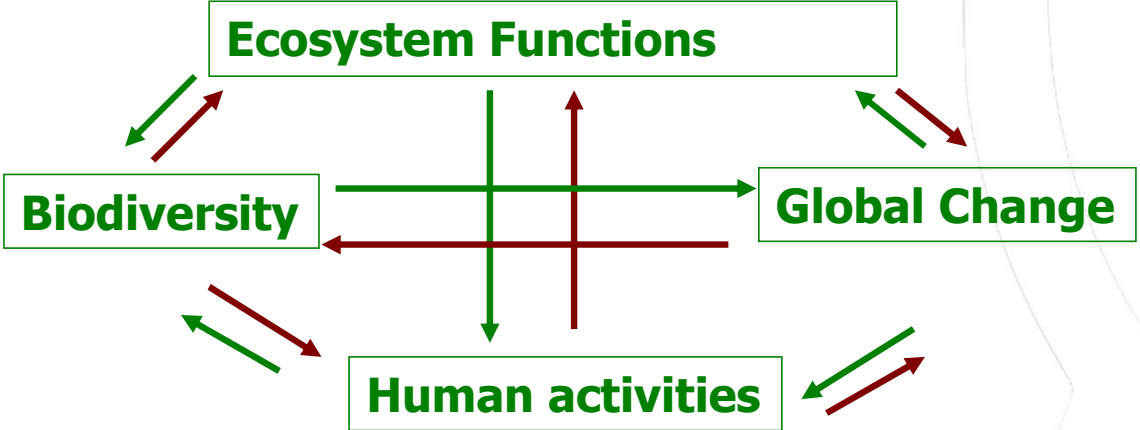
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Mission 4 "Education and Research" - Component 2: "From research to business" - Investment  
3.1: "Fund for the realisation of an integrated system of research and innovation infrastructures"



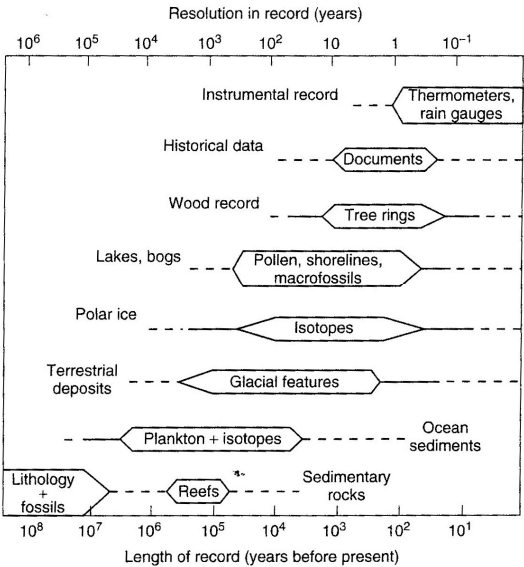
# Outline

- ↓ **Stable isotope: why we should use them?**
- ↓  $\delta^{13}\text{C}$   $\delta^{18}\text{O}$
- ↓ **Applications: global changes and disturbances**
- ↓ **Effects of  $\text{CO}_2$  enrichment on forest**
- ↓ **Mediterranean species and Drought effect**
- ↓ **Fire**
- ↓ **Prospectives**

# Historical perspective



**Time**



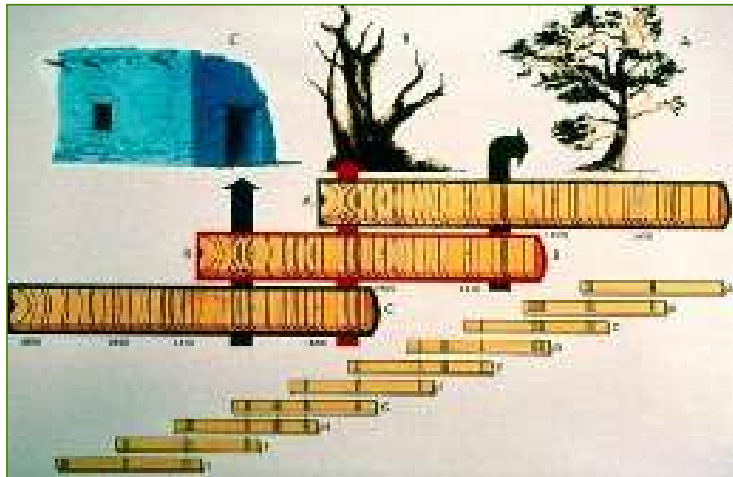
(Webb et al., 1985)

Training current RIS staff and user communities: "Use of Stable Isotopes in Environmental Investigations", Montebretti (Roma)

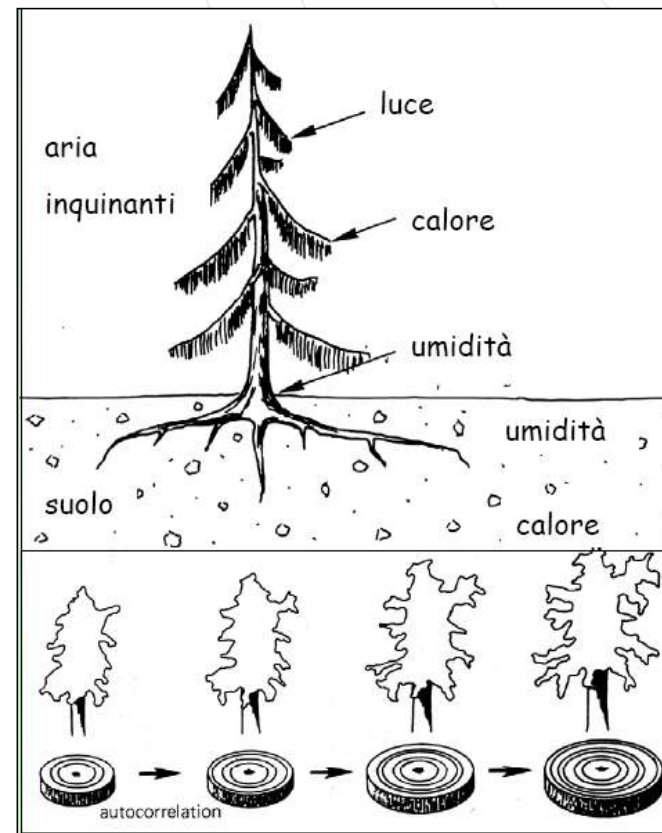
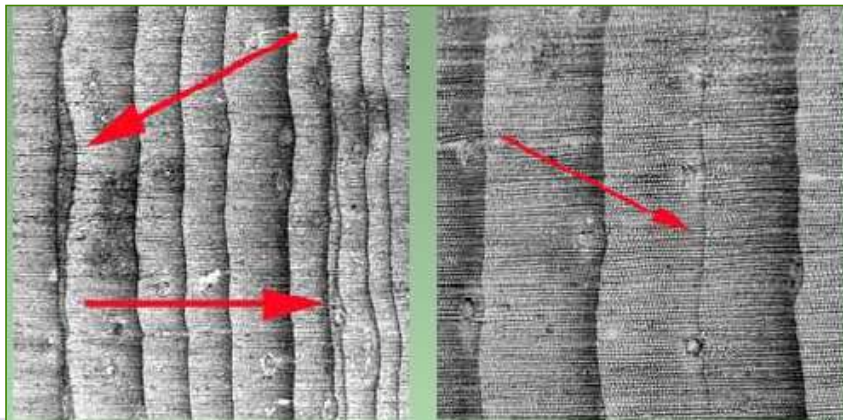
## Dendrochronology

The science that deals with the dating and the study of annual growth layers in wood.

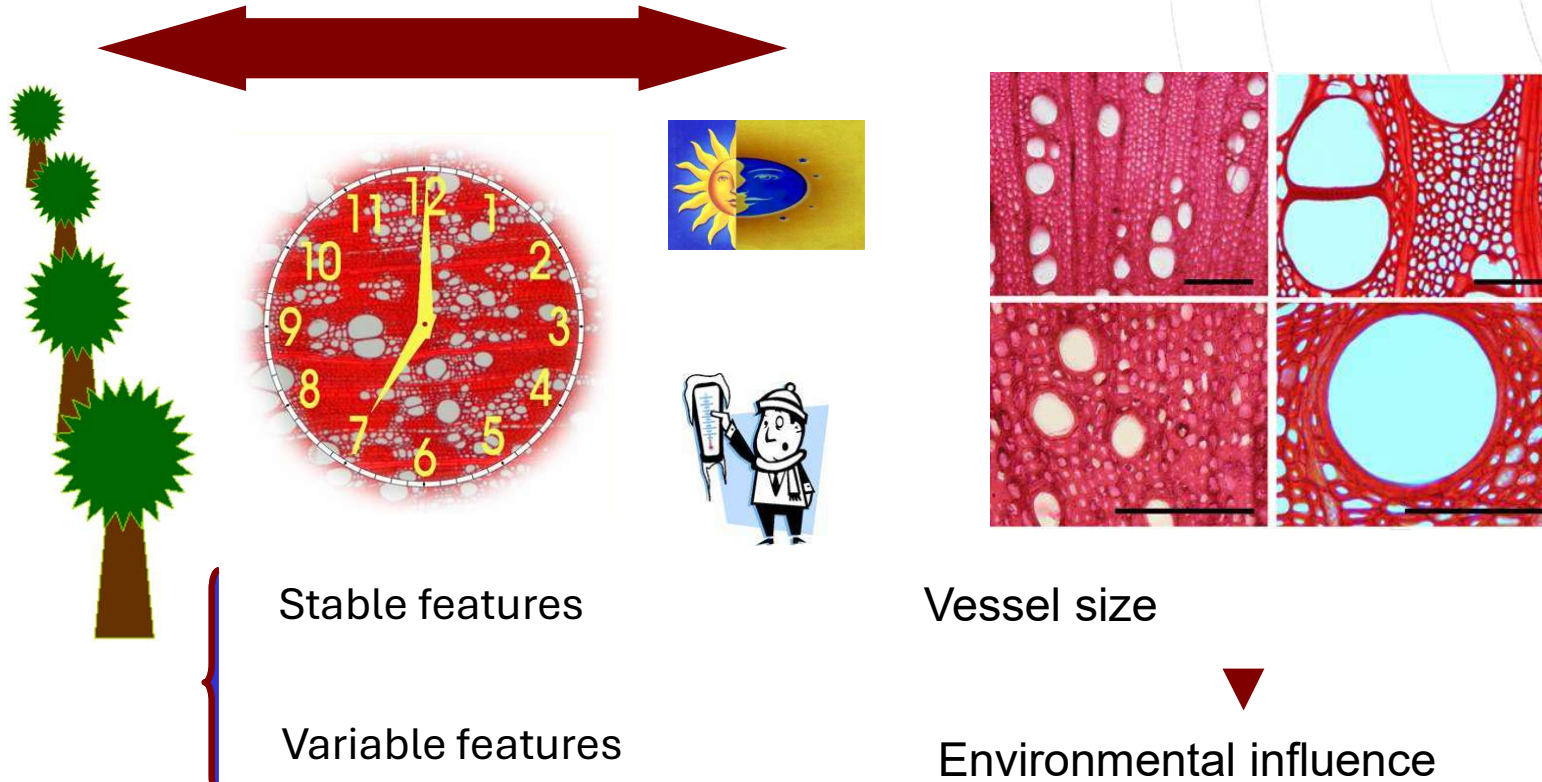
*Fritts, 1976*



- Quantitative variations (ring-width, density)
- Visible signs (scars, callous tissue, resin ducts)
- Changes in chemical composition of wood



# Quantative wood anatomy



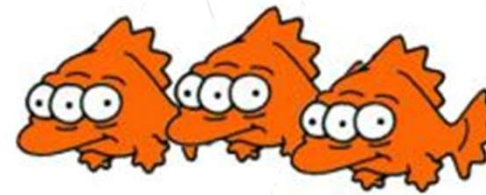
"A methodological approach based on the measurement of wood cell anatomical characteristics, analyzed through time and used to characterize the relationships between tree growth and various environmental factors"

*2<sup>nd</sup> Workshop on QWA, Birmensdorf, Switzerland, May 2007*

I'm a dendrochronologist, so...

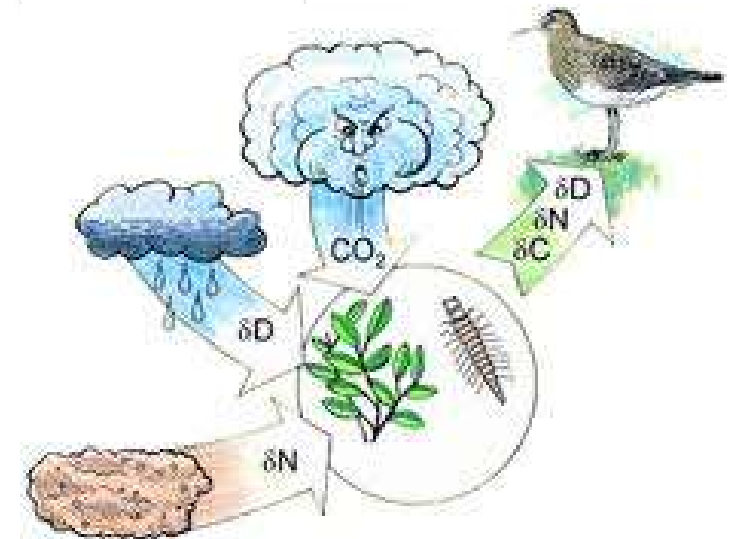
## WHY SHOULD I CARE ABOUT (STABLE) ISOTOPES?

- *they are (quite) expensive*
- *they imply destructive sampling*
- *I can't see them ...*

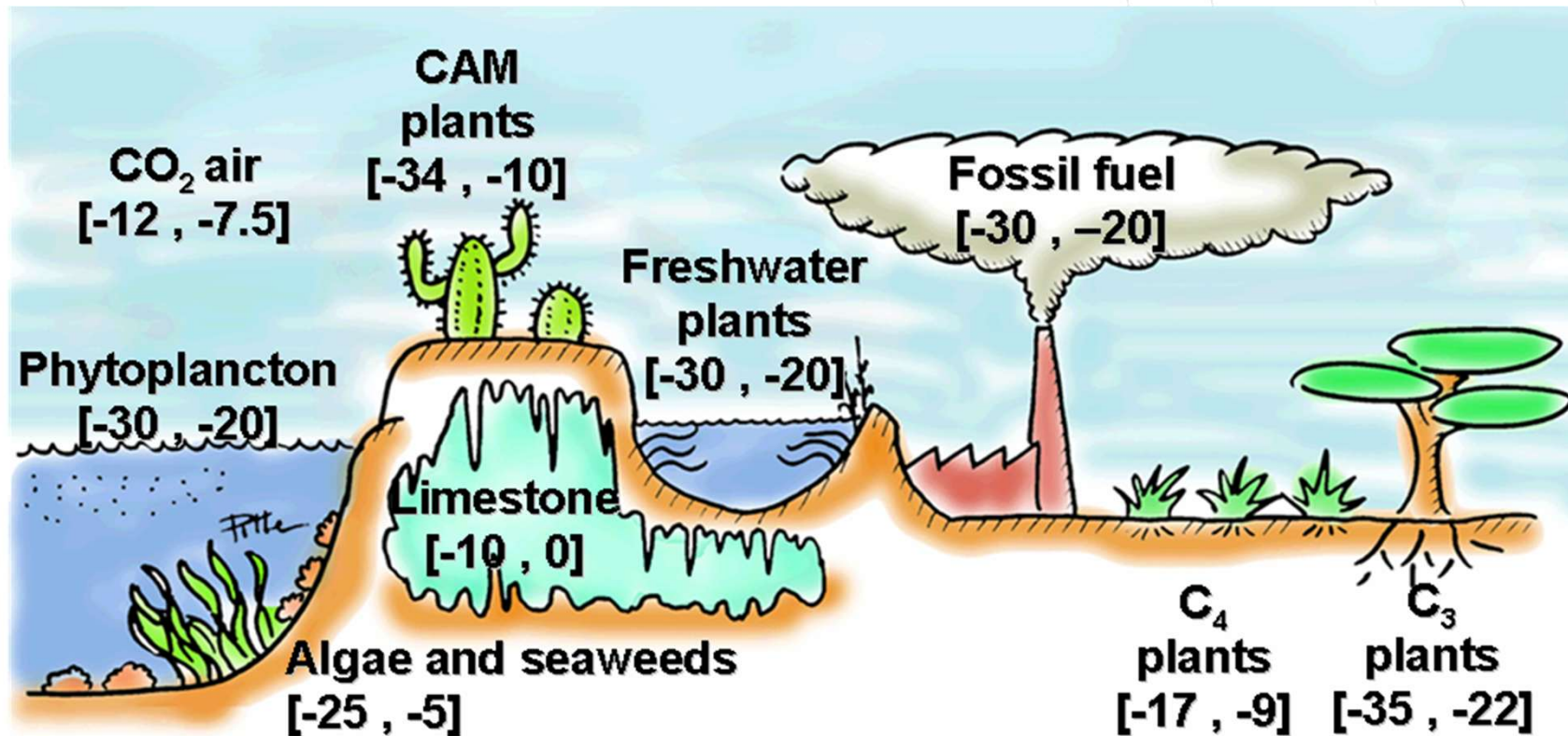


## Stable Isotopes a Valuable Tool in Ecological and Physiological Research

- The isotope abundance is specifically changed in environmental and physiological processes leaving its specific „fingerprint“
- The signal given by the above mentioned processes are well preserved in organic matter, sediments and fossilized materials
- Isotope ratios represent an integrative information over time and space
- Isotopes are ideal tracers

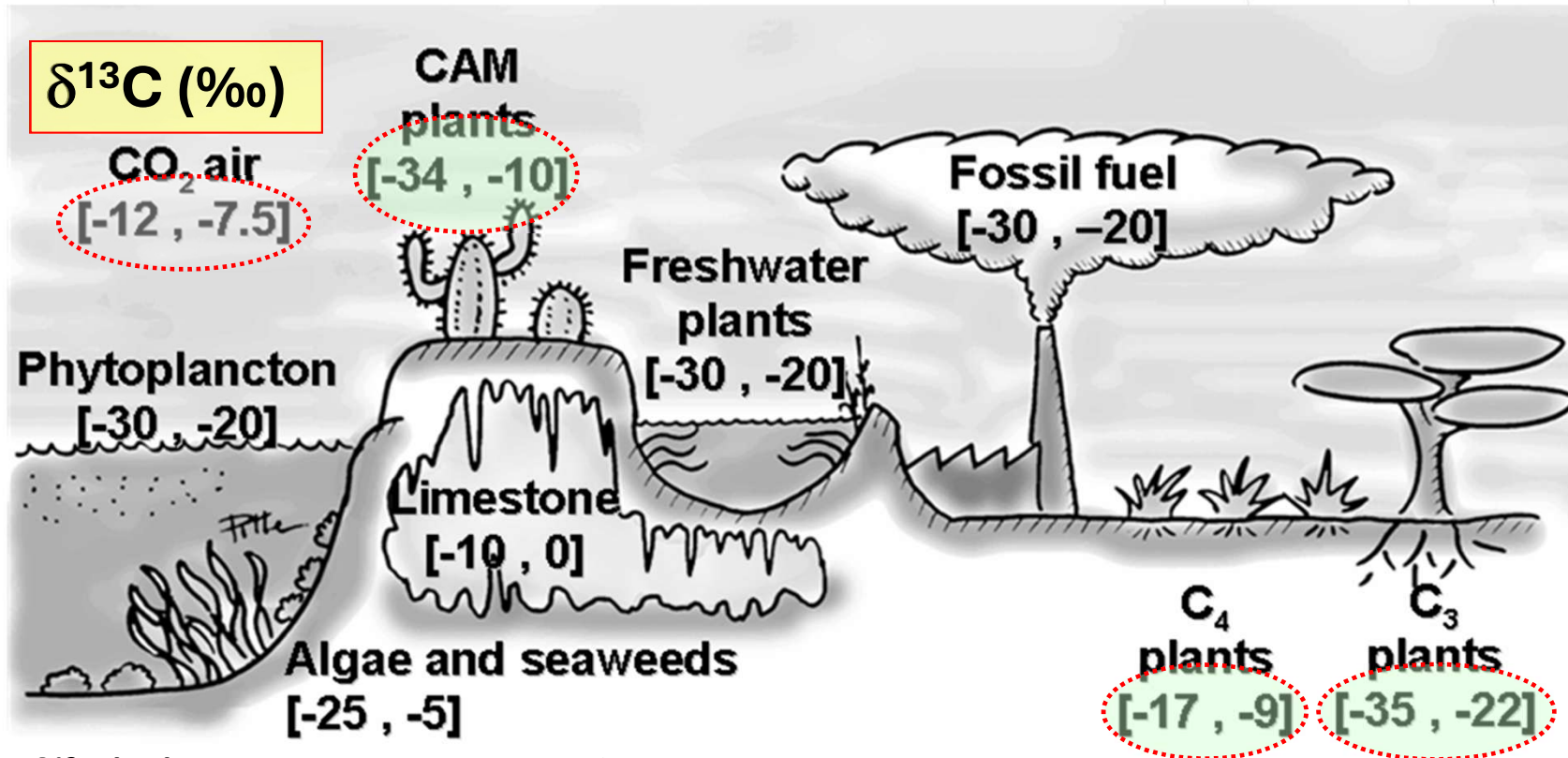


# Carbon isotopes in the biosphere



Ferrio et al., 2005

# What is the range of $\delta$ values for plant tissues:



- $\delta^{13}\text{C}$  (‰) in plants is lower than in fixed  $\text{CO}_2$   
 → Carbon isotope discrimination ( $\Delta^{13}\text{C}$ )
- Discrimination is variable → Phot. pathway

## Basis for $^{13}\text{C}$ variations in plants

There are.....

- ❖ Irreversible steps in the metabolic process, where not all of the substrate is consumed
- ❖ Metabolic branch points
- ❖ Opportunities where diffusion is a fundamental step in the process
- ❖ Secondary fractionation events associated with common pools

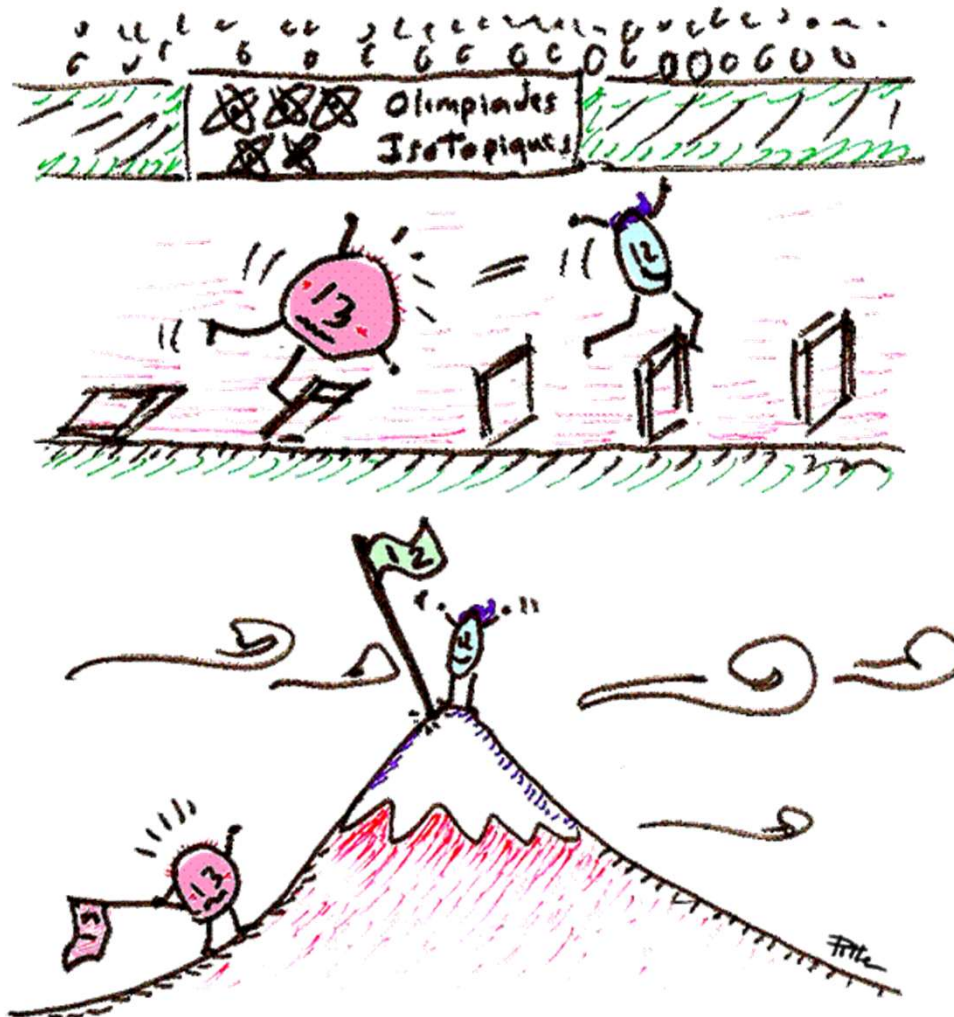
**Table 1** Isotope effects of steps leading to CO<sub>2</sub> fixation in plants.

Process	Isotope effect ( $\alpha$ )	Discrimination (‰)	Symbol	Reference
Diffusion of CO <sub>2</sub> in air through the stomatal pore <sup>a</sup>	1.0044	4.4	$a$	Craig (16)
Diffusion of CO <sub>2</sub> in air through the boundary layer to the stomata <sup>a</sup>	1.0029	2.9	$a_b$	Farquhar (33)
Diffusion of dissolved CO <sub>2</sub> through water	1.0007	0.7	$a_l$	O'Leary (98)
Net C <sub>3</sub> fixation with respect to $p_i$	1.027	27	$b$	Farquhar & Richards (39)
Fixation of gaseous CO <sub>2</sub> by Rubisco from higher plants	1.030 (pH = 8)	30	$b_3$	Roeske & O'Leary (119) <sup>b</sup>
Fixation of HCO <sub>3</sub> <sup>-</sup> by PEP carboxylase	1.029 (pH = 8.5)	20	$b_3$	Guy et al (50)
	1.0020	2.0	$b_4^*$	O'Leary et al (101)
	1.0020	2.0		Reibach & Benedict (117)
Fixation of gaseous CO <sub>2</sub> (in equilibrium with HCO <sub>3</sub> <sup>-</sup> at 25°C) by PEP carboxylase	0.9943	-5.7	$b_4$	Farquhar (33)
Equilibrium hydration of CO <sub>2</sub> at 25°C	0.991	-9.0	$e_b$	Emrich et al (31)
	0.991	-9.0		Mook et al (91)
Equilibrium dissolution of CO <sub>2</sub> into water	1.0011	1.1	$e_s$	Mook et al (91)
	1.0011	1.1		O'Leary (98)

<sup>a</sup>Theoretical value

<sup>b</sup>Data corrected for dissolution of CO<sub>2</sub>

## Carbon isotope discrimination ( $C_3$ )



$^{13}C$  is heavier and less reactive:

- diffuses more slowly through stomata → **Physical fractionation**

- *RuBisCo* fixes preferentially  $^{12}C$  during photosynthesis  
→ **Chemical fractionation**

# Carbon isotope discrimination

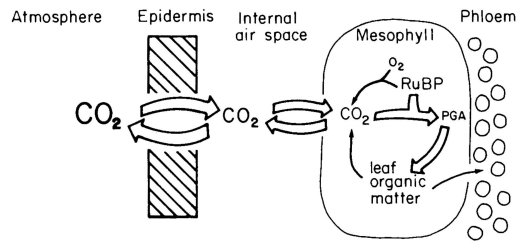
Why do plants contain less  $^{13}\text{C}$  in their leaves than the  $\text{CO}_2$  in the air?

1.  $^{13}\text{CO}_2$  diffuses into leaves more slowly than  $^{12}\text{CO}_2$
2. Rubisco preferentially binds  $^{12}\text{CO}_2$

$$\delta^{13}\text{C}_{\text{plant}} = \delta^{13}\text{C}_{\text{atm}} - (\text{diffusion effect} + \text{enzyme effect})$$

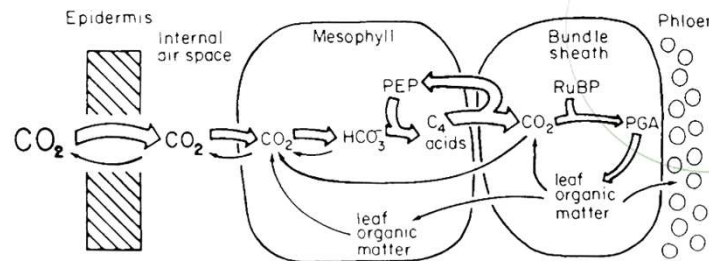
**DISCRIMINATION**

# Different Types of Photosynthesis

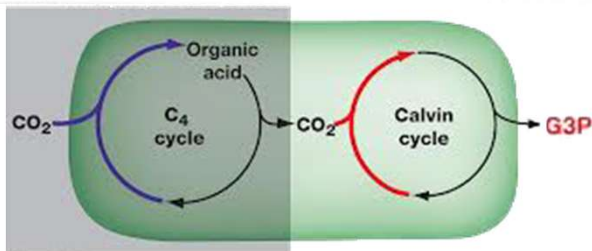


C<sub>3</sub> pathway: First step in photosynthesis converts CO<sub>2</sub> into 3 carbon sugar

C<sub>4</sub> pathway: First step in photosynthesis converts CO<sub>2</sub> into four carbon sugar. Spatial separation of light and dark reactions



CO<sub>2</sub> is stored at night ... .. and used during the day.

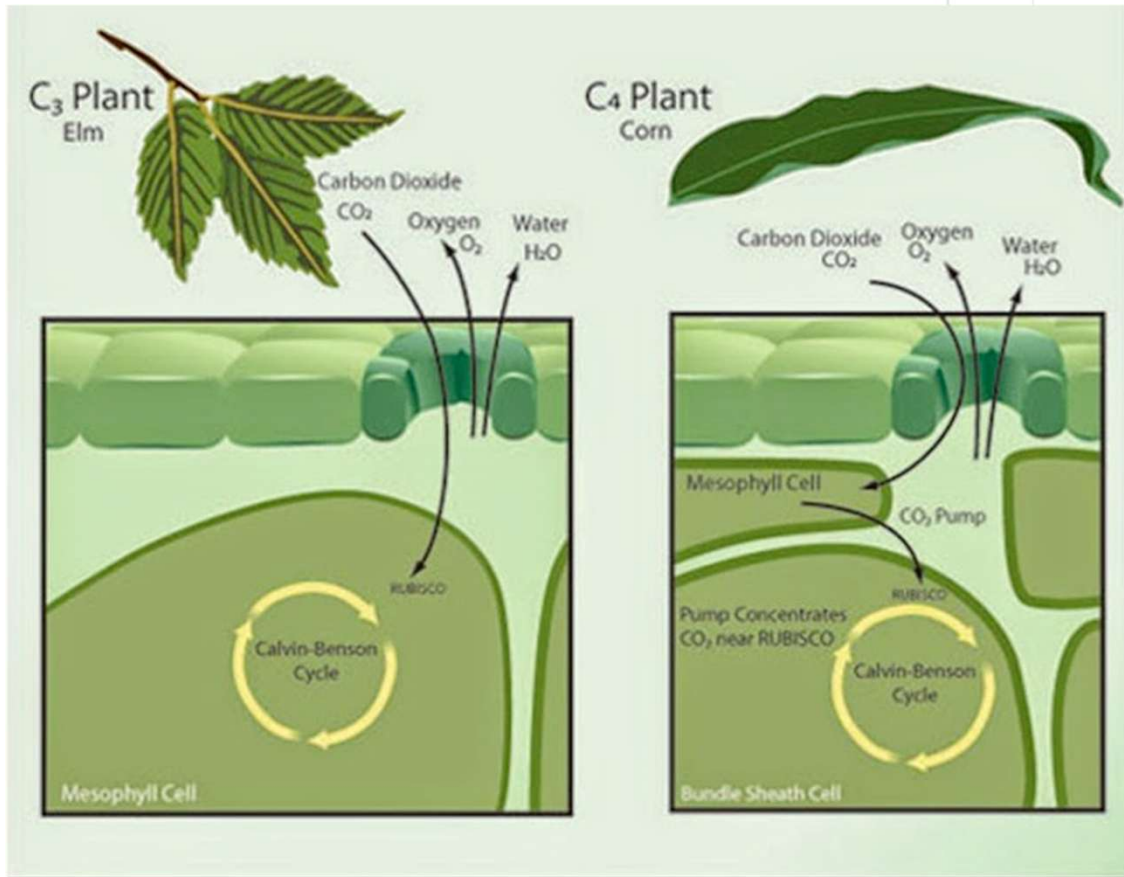


[www.uic.edu](http://www.uic.edu)

CAM pathway: First step in photosynthesis converts CO<sub>2</sub> into 4 carbon sugar  
temporal separation of light and dark reactions

# Variation in $^{13}\text{C}$ are associated with photosynthetic pathway

C3

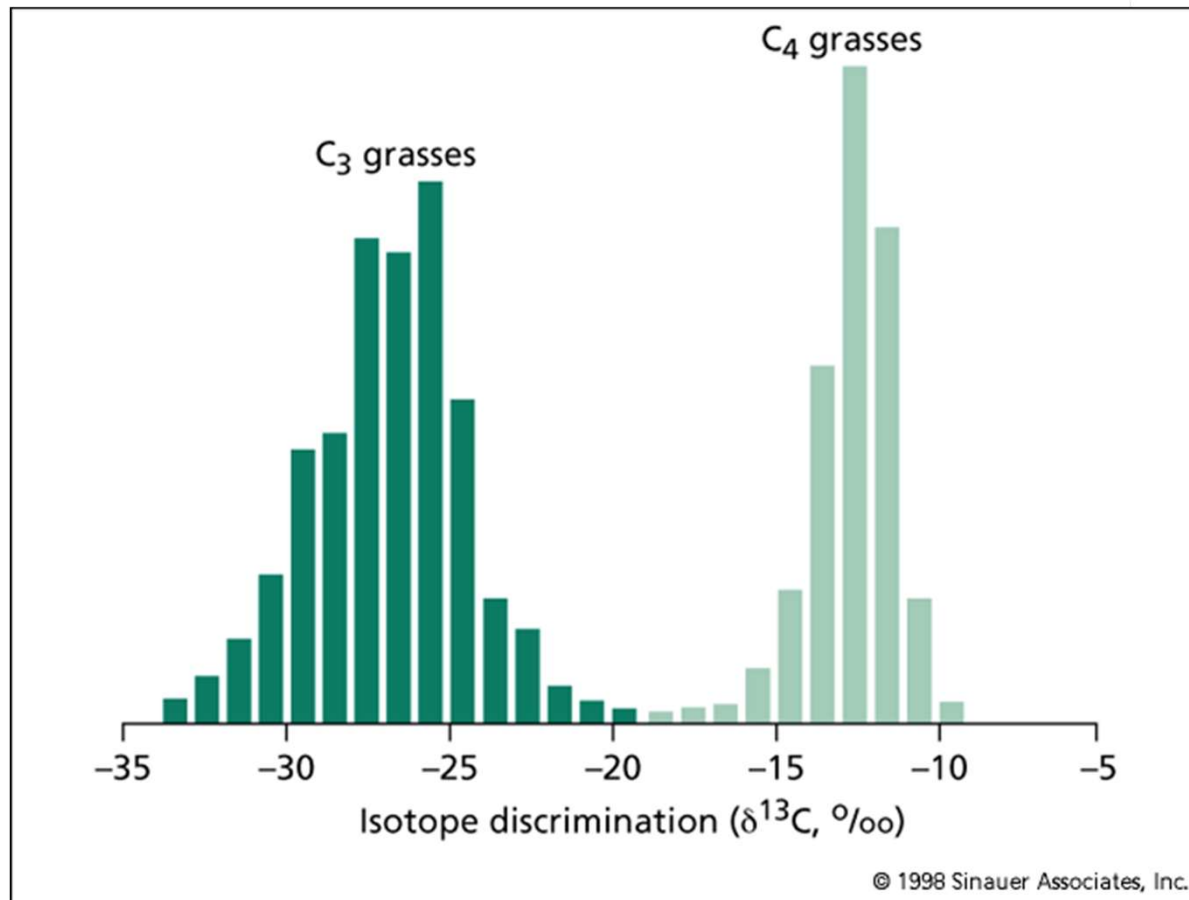


C4

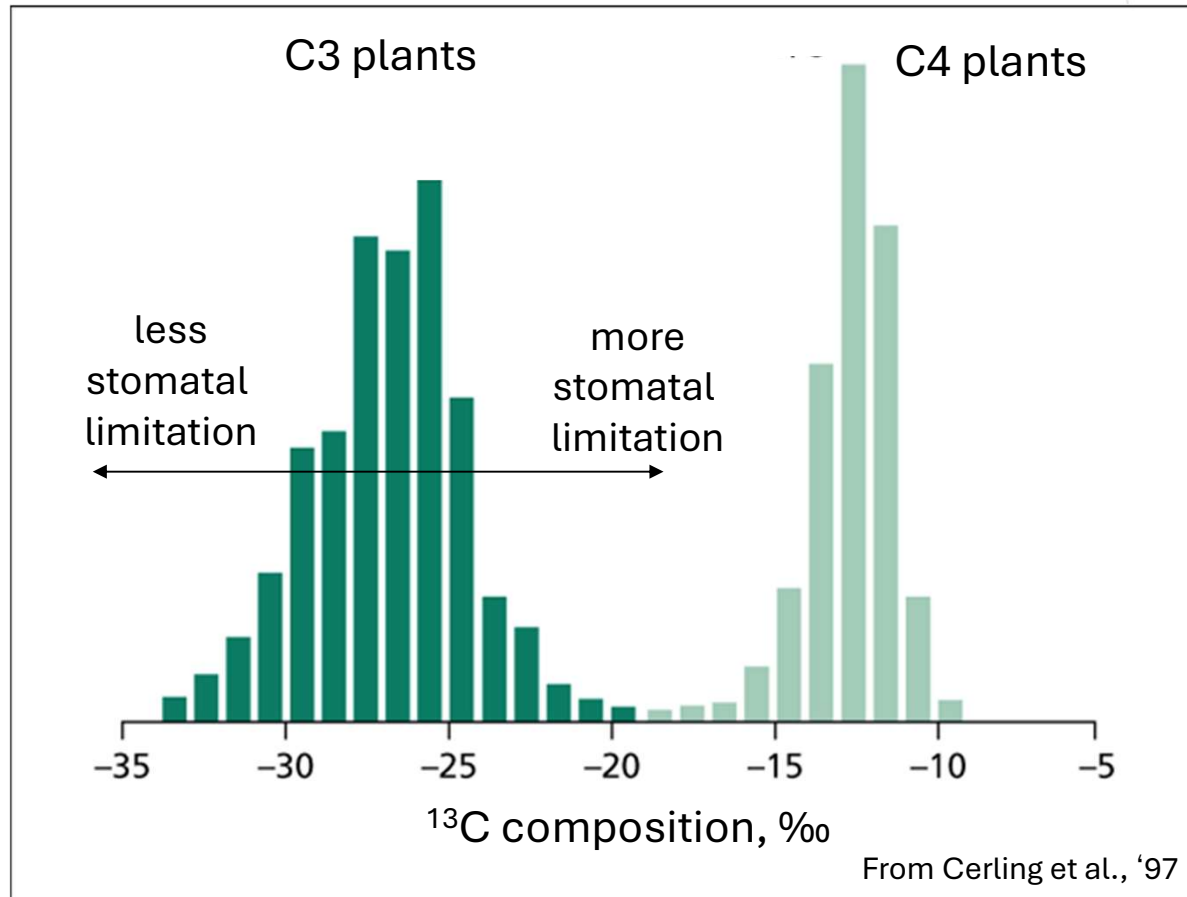
> 95% of all plant species  
70-75% of all productivity (today)

> 5% of all plant species  
25-30% of all productivity (today)

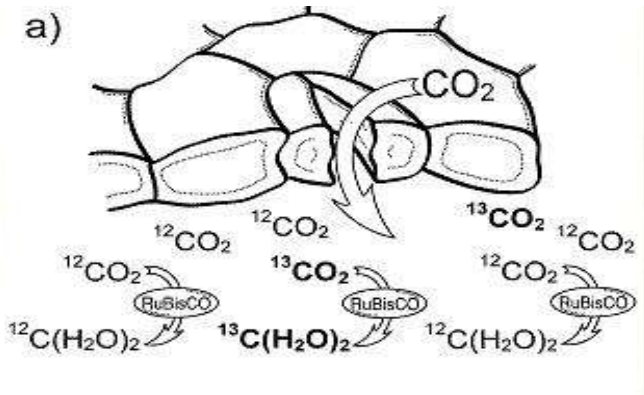
## Why the wide range of $\delta^{13}\text{C}$ values in $\text{C}_3$ plants?



The  $\delta^{13}\text{C}$  of C3 plants reflects how much stomatal conductance limits photosynthesis.



## Relation between C isotope composition and stomatal conductance



High stomatal conductance

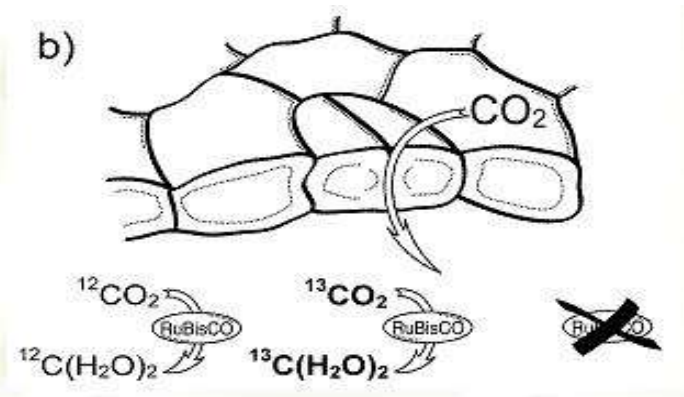


High discrimination



Decrease in  $\delta^{13}\text{C}$

$\delta^{13}\text{C}$  reflects the amount in which the heavier isotope  $^{13}\text{C}$  is discriminated respect the lighter  $^{12}\text{C}$  during the physical and chemical processes involved in the synthesis of plant organic matter (*Farquhar et al. 1989*).



Low stomatal conductance



Low discrimination



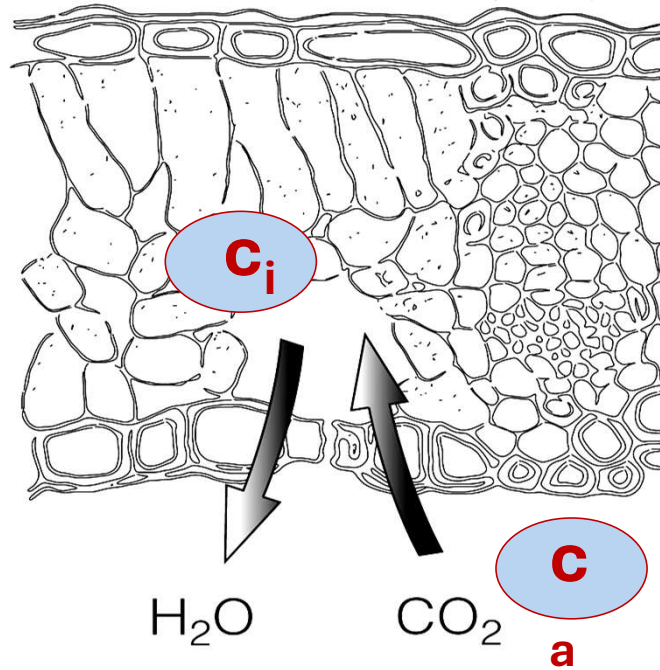
Increase in  $\delta^{13}\text{C}$

## $\delta^{13}\text{C}$ as environmental proxy

$$\delta^{13}\text{C}_{\text{plant}} = \delta^{13}\text{C}_{\text{atm}} - a - (b - a) \cdot c_i/c_a \quad (\text{Farquhar et al., 1989})$$

$\delta^{13}\text{C}_{\text{plant}}$   $\delta^{13}\text{C}_{\text{atm}}$   $a$   $(b - a)$   $c_i/c_a$   
 $-27\text{‰}$   $-8\text{‰}$   $4,4\text{‰}$   $27\text{‰}$   $0,4/0,9$

- $\delta^{13}\text{C}_{\text{plant}}$  = plant  $^{13}\text{CO}_2$
- $\delta^{13}\text{C}_{\text{atm}}$  = atmospheric  $^{13}\text{CO}_2$
- $a$  = fractionation in the gaseous phase
- $b$  = fractionation by RuBisCO
- $c_i$  =  $\text{CO}_2$  concentration in the leaf intercellular spaces
- $c_a$  =  $\text{CO}_2$  concentration of the ambient air



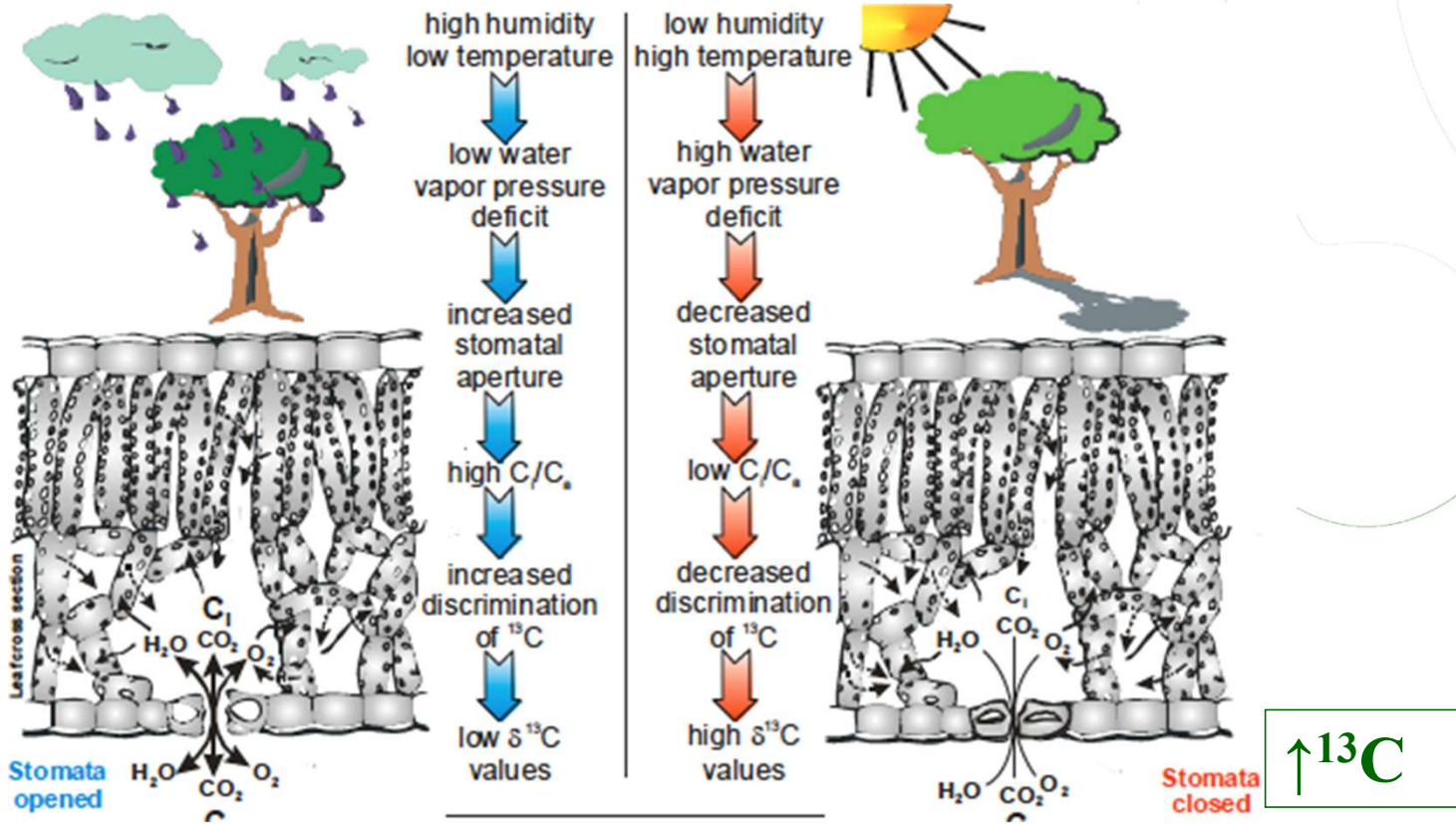
**$^{13}\text{C}$  Discrimination**

$c_i/c_a$

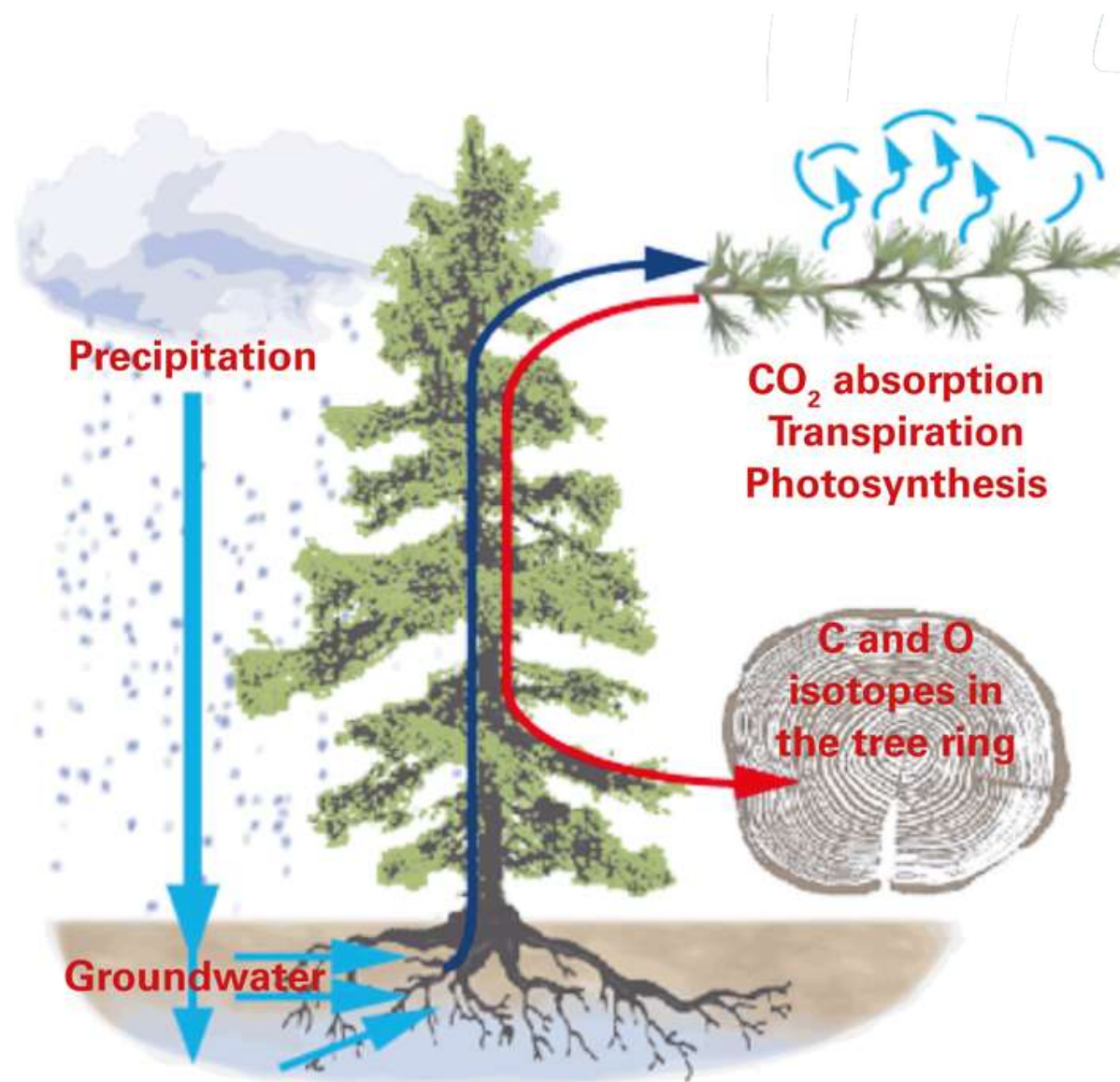
**Stomatal  
conductance**

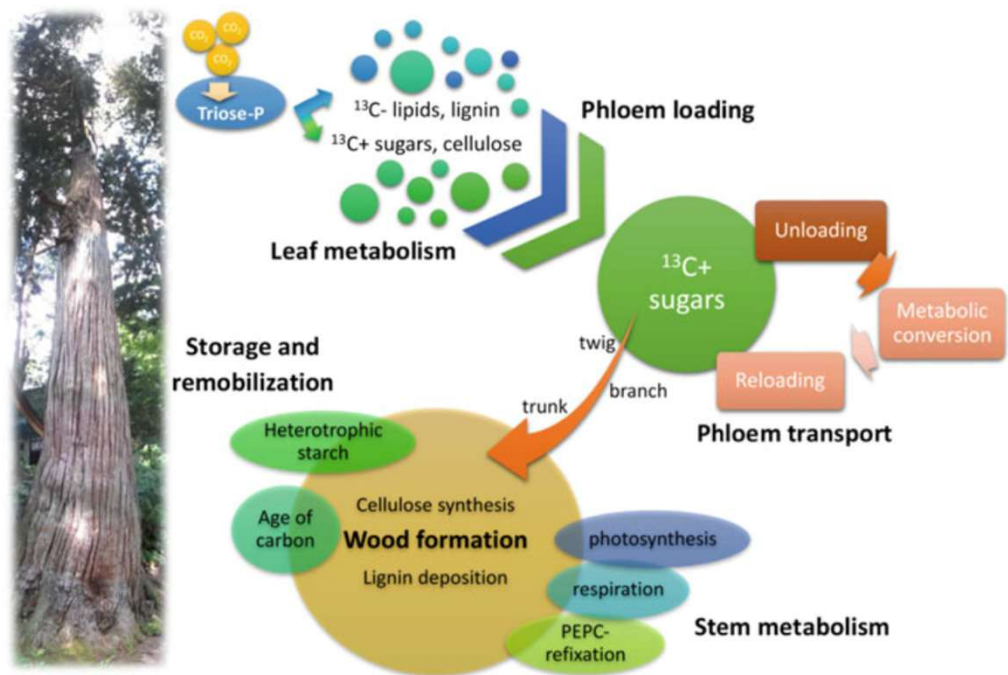
**Photosynthetic  
activity**

# Environmental conditions reflected in $\delta^{13}\text{C}$



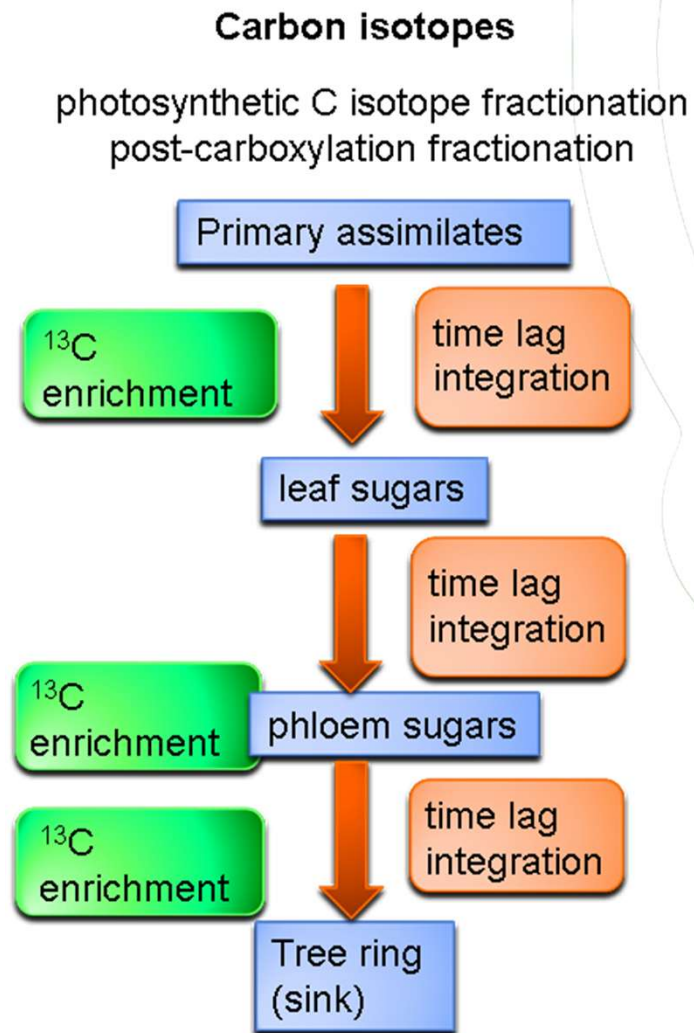
The determination of  $\delta^{13}\text{C}$  in plant tissue is widely used to integrate the influence of a range of environmental factors on plant performance (Leavitt & Long, 1991).





**Fig. 13.1** Conceptual scheme summarizing the main processes in the way from primary assimilates (triose-P) to the wood that may cause fractionation at time scales relevant to tree-ring archives. The first fractionation processes occur already within the leaf, where various isotopic effects during metabolic pathways and phloem loading cause lipids and lignin to be depleted, and sugars and cellulose to be enriched in  $^{13}\text{C}$ . For some species, a basipetal enrichment in phloem sap from twigs to basal stem has been described, potentially associated with the continuous unloading and reloading of sugars during phloem transport. This sugar would be partially exposed to metabolic reactions (e.g. production of lignin) in stem tissues, causing an enrichment of the fraction that is reloaded in the phloem. Wood formation could also be affected by fractionations occurring during stem metabolism. Bark photosynthesis, respiratory fractionation, or PEPC  $\text{CO}_2$  refixation processes are known to produce fractionations, but their direct effect on tree rings is not clear. Finally, the source of carbon used for wood formation could show seasonal and inter-annual variations. In particular, remobilization of stored carbon is crucial for leaf development and early stem growth in deciduous trees but could also play a key role during stress episodes. See text for further details

# Temporal integration



# Carbon Fractionation *within* Individual Plants



## $C^{13}$ *within* Plants

🌐 Does  $C^{13}$  vary within individual plants?

🌐 Is there a difference in internal fractionation between C3 and C4 plants?

Does  $C^{13}$  vary *within* plants?

 YES!

 Carbon fractionation within plants can be described by differences in:

- Plant organs
- Plant compounds

## Fractionation in Plant Organs

- 🌐 Differences in bulk  $\delta^{13}\text{C}$  of different plant parts (leaves, roots) are common.
- 🌐 Since most  $\delta^{13}\text{C}$  measurements are made on leaves, it is important to indicate the plant part measured.
- 🌐 Fractionation in plant organs differs among C3 and C4 plants .

**Table 3** Isotopic differences between leaves and other plant components

Species	Value (‰)	Reference
<b>Grain – leaves (C<sub>3</sub>)</b>		
Oats	3.3, 3.6	Winkler <i>et al.</i> (1978)
Wheat	1.2, 2.0	Yoneyama <i>et al.</i> (1997)
Wheat	2.9, 4.6	Winkler <i>et al.</i> (1978)
Legumes (7 species)	0.9 ± 0.2	Yoneyama & Ohtani (1983)
<b>Grain – leaves (C<sub>4</sub>)</b>		
Maize	1.5	Lowdon (1969)
Maize	1.4	Gleixner <i>et al.</i> (1993)
<b>Roots – leaves, woody vegetation (C<sub>3</sub>)</b>		
Citrus	2.4	Syvrtsen <i>et al.</i> (1997)
Eucalyptus	0.9	Handley <i>et al.</i> (1993)
Fagus	1.5	Fobelli <i>et al.</i> (2003)
Pinus	1.5	E. Hobbie & J. Colpaert (unpublished)
Populus	2.5	Ineson <i>et al.</i> (1996)
Pseudotsuga	1.8	Hobbie <i>et al.</i> (2002)
<b>Roots – leaves, nonwoody vegetation (C<sub>3</sub>)</b>		
Barley	0.4 ± 0.2	Hubick & Farquhar (1989)
Barley – stressed	0.5 ± 0.3	Hubick & Farquhar (1989)
Beta (sugar beet)	1.2	Gleixner <i>et al.</i> (1993)
Desmodium	1.5	Schweizer <i>et al.</i> (1999)
Legumes (seven species)	1.0 ± 0.1	Yoneyama & Ohtani (1983)
Peanut	0.5	Hubick <i>et al.</i> (1986)
Plantago	1	Staddon <i>et al.</i> (1999)
Ricinus	0.6	Handley <i>et al.</i> (1993)
Tomato	1.1	Bradford <i>et al.</i> (1983)
Wheat	1.2 to 1.4	Cheng & Johnson (1998)
Wheat	1.8	Lichtfouse <i>et al.</i> (1995)
<b>Roots – leaves (C<sub>4</sub>)</b>		
Four species	-0.2 to 0.2	Trouve <i>et al.</i> (1994)
Brachiaria	-0.5	Schweizer <i>et al.</i> (1999)
Pennisetum	-1.8	Deslardins <i>et al.</i> (1994)
Saccharum	0.4	Spain & Le Feuvre (1997)
Zea	-1.4	Qian & Doran (1996)

## Organs: C3 versus C4

### C3 Plants

- Roots are typically enriched by 1–3‰ relative to leaves.
- Grains enriched by 1–4‰ relative to leaves.

### C4 Plants

- Roots similar or slightly lower in  $\delta^{13}\text{C}$  relative to leaves.
- Grains enriched by  $\approx 1.5\text{‰}$  relative to leaves in maize.

(Hobbie and Werner, 2003)

# Compounds: C3 versus C4

## C3 Plants

- Alkanes and lipids 4–6‰ depleted (Collister et al., 1994).

## C4 Plants

- Alkanes and lipids 8–10‰ depleted (Collister et al., 1994).
- In C4 plants, lipid concentration was found to be about half that in C3 plants (Chikaraishi and Naraoka, 2001).
- Isotopic enrichment of cellulose relative to lignin is slightly greater in leaves of C4 plants.

## Conclusions

🌐 Isotopic composition can vary by:

- Plant organ measured
- Plant organic compound measured

🌐 Degree of fractionation variable among C3 and C4 plants

🌐 Variations in plant organ  $\delta^{13}\text{C}$  correspond to isotopic variations in plant organic compounds (metabolites).

Gessler et al. 2014.  
*Tree Physiology* 34:  
 796-818

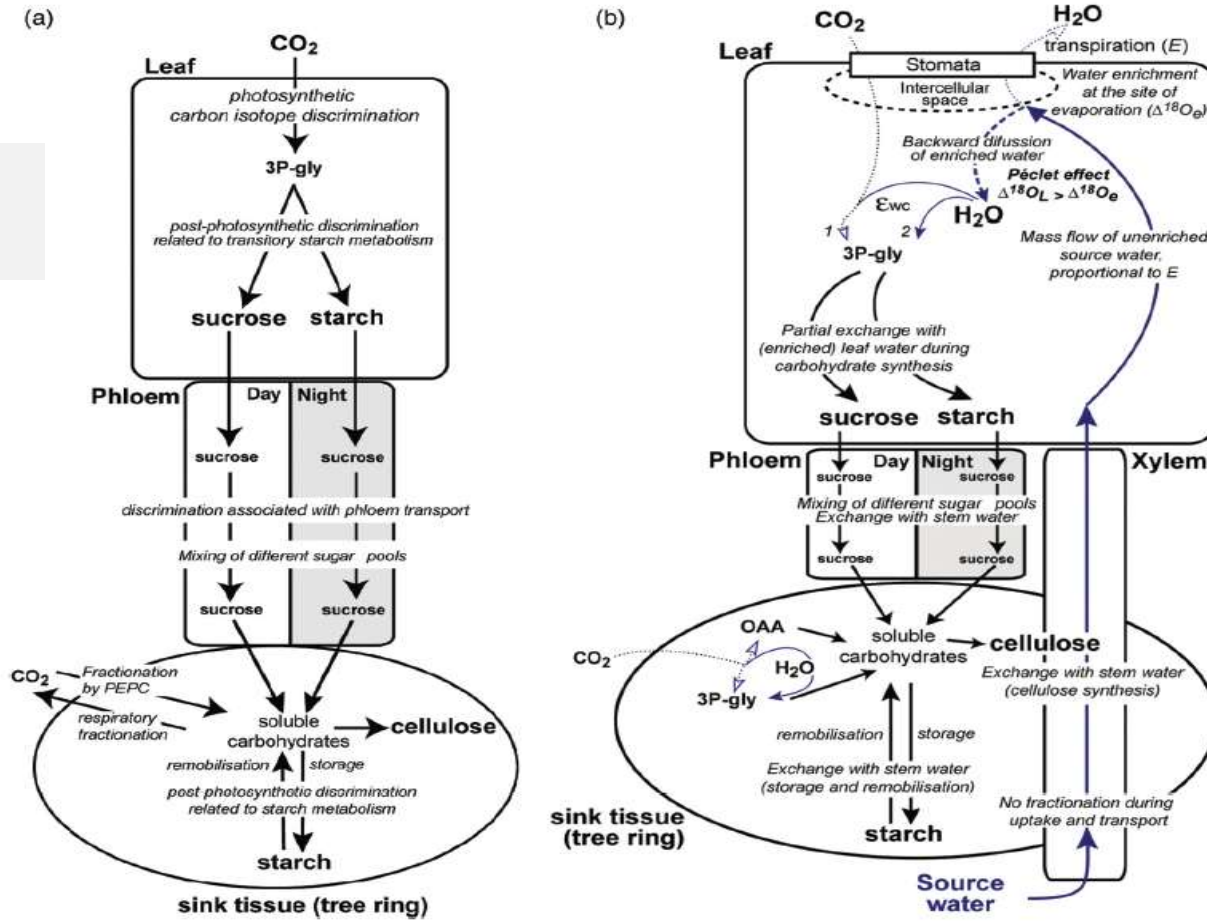
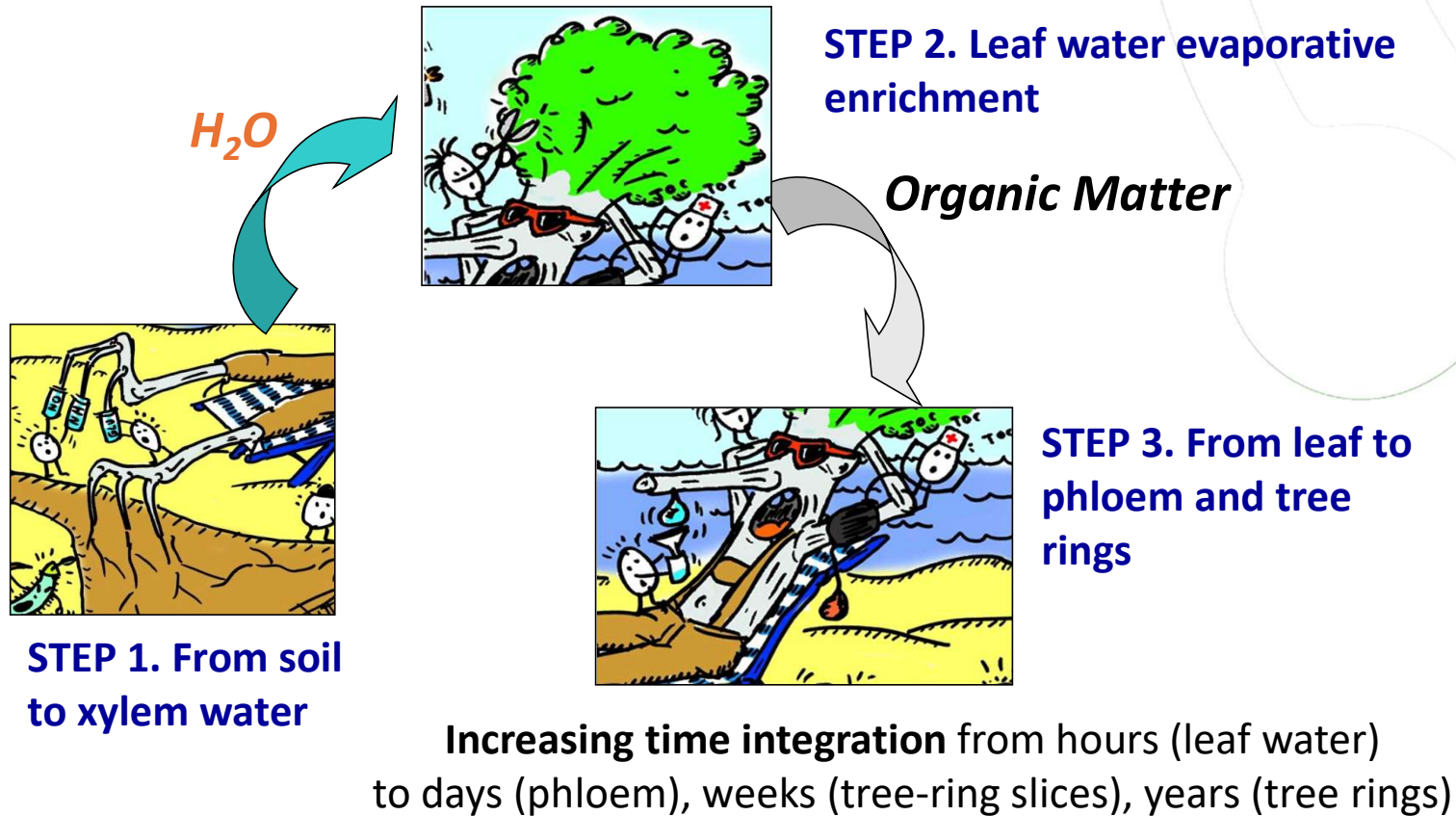


Figure 1. Overview of the different processes influencing the carbon (a) and oxygen (b) isotope signature, from primary sources (CO<sub>2</sub> and H<sub>2</sub>O, respectively) to tree-ring cellulose, going through different organic and inorganic pools. PEPC, phosphoenolpyruvate carboxylase; Δ<sup>18</sup>O<sub>e</sub> and Δ<sup>18</sup>O<sub>L</sub>, isotopic enrichment of water at the site of evaporation and of mean lamina leaf water, respectively; ε<sub>wc</sub>, equilibrium fractionation factor between oxygen in water and carbonyl groups; OAA, oxaloacetate; 3P-gly, 3-phosphoglycerate.

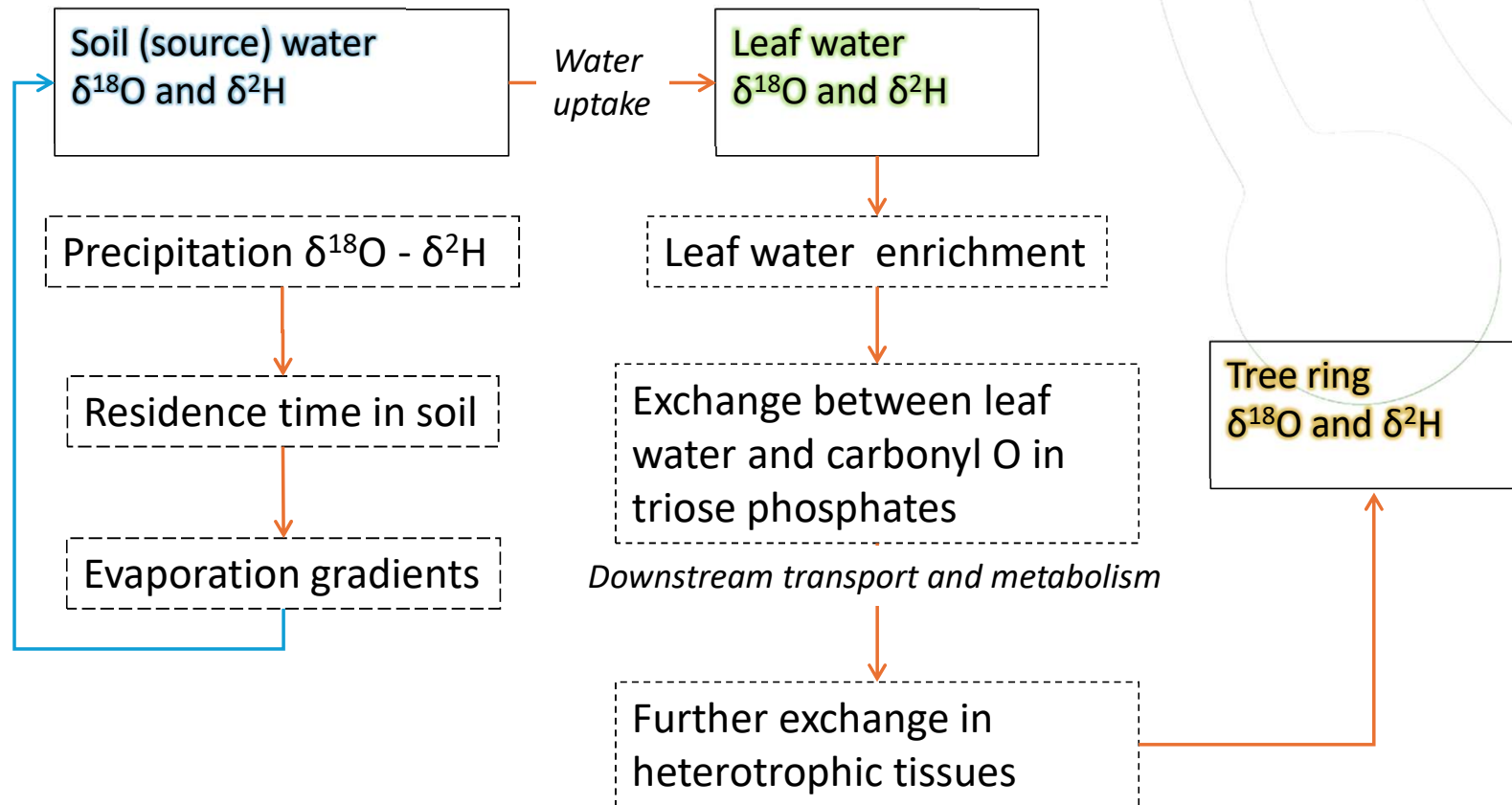
# Oxygen (and hydrogen) isotopes in tree rings

- The primary source: stable isotopes of water in the biosphere
- Sources of variability for stable isotopes in trees
  - ✓ From soil moisture to xylem transport through water uptake
  - ✓ Leaf evaporative enrichment
  - ✓ Transfer of the isotopic signal of leaf water into organic matter and tree-ring cellulose
- Link between isotopic fractionation and long-term tree-ring archive

# Oxygen and hydrogen isotopes in tree rings

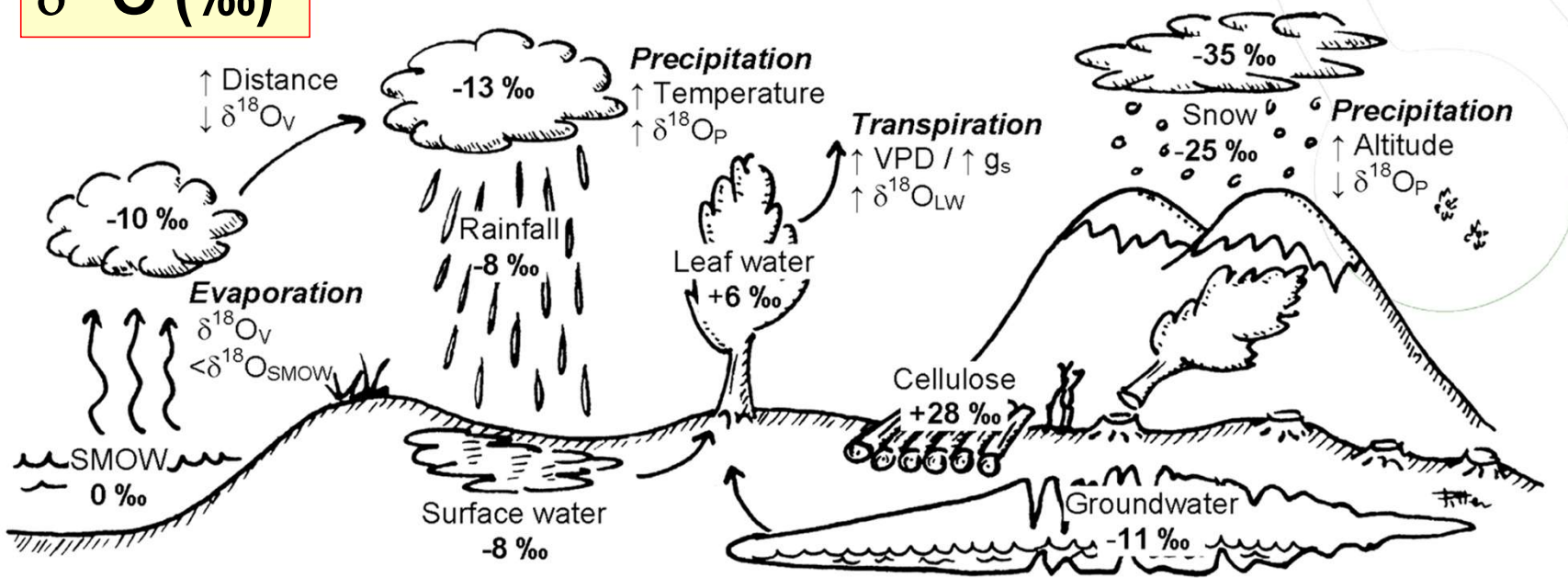


# Oxygen and hydrogen isotopes in tree rings



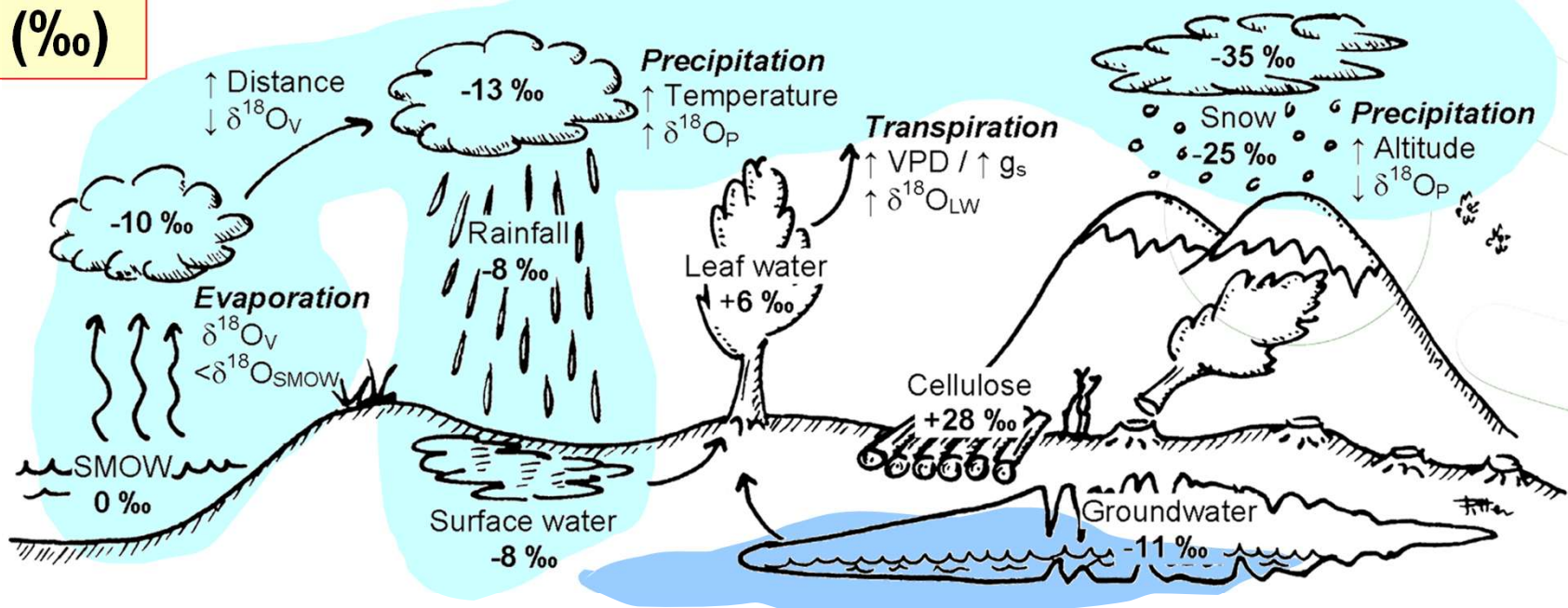
# STEP 1. From soil to xylem water

$\delta^{18}\text{O}$  (‰)



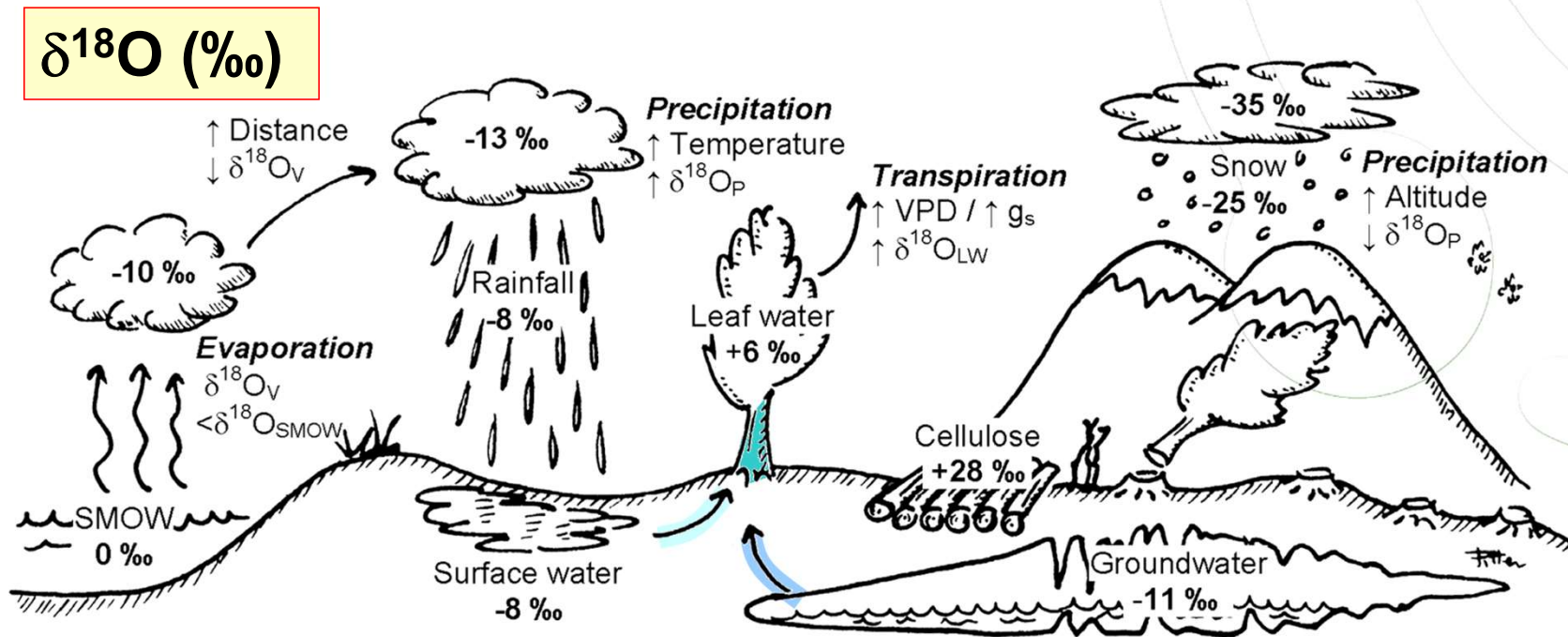
# STEP 1. From soil to xylem water

$\delta^{18}\text{O}$  (‰)



$\delta^{18}\text{O}$  and  $\delta^2\text{H}$  (‰) change in the hydrological cycle owing to a number of fractionation processes

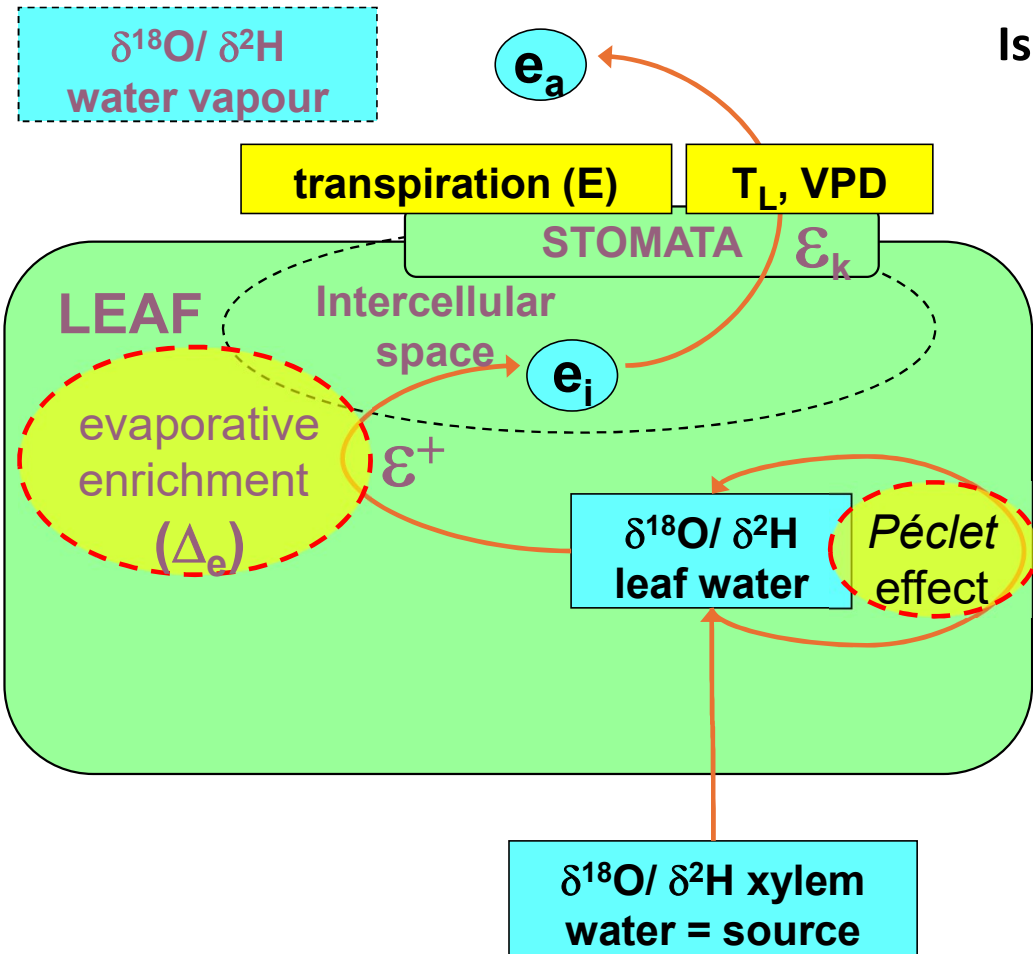
# STEP 1. From soil to xylem water



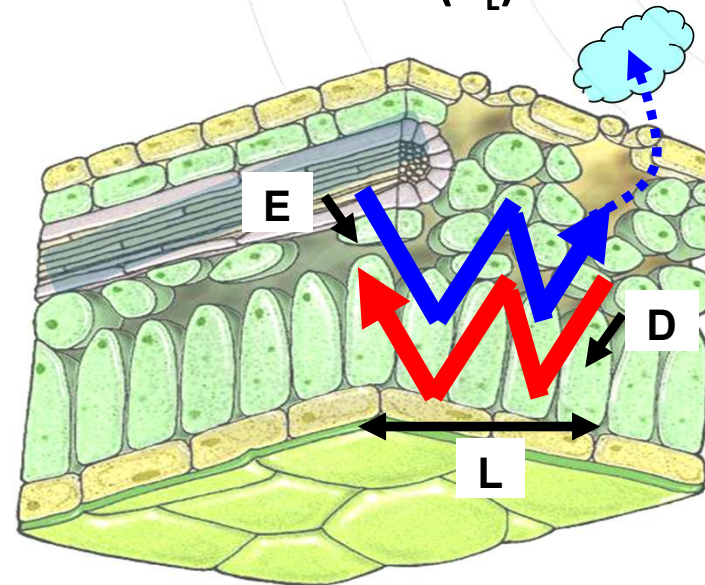
$\delta^{18}\text{O}$  (‰) changes in the hydrological cycle

$\delta^{18}\text{O}$  (‰) in xylem water reflects the origin of source water

# Step 2. Leaf water evaporative enrichment



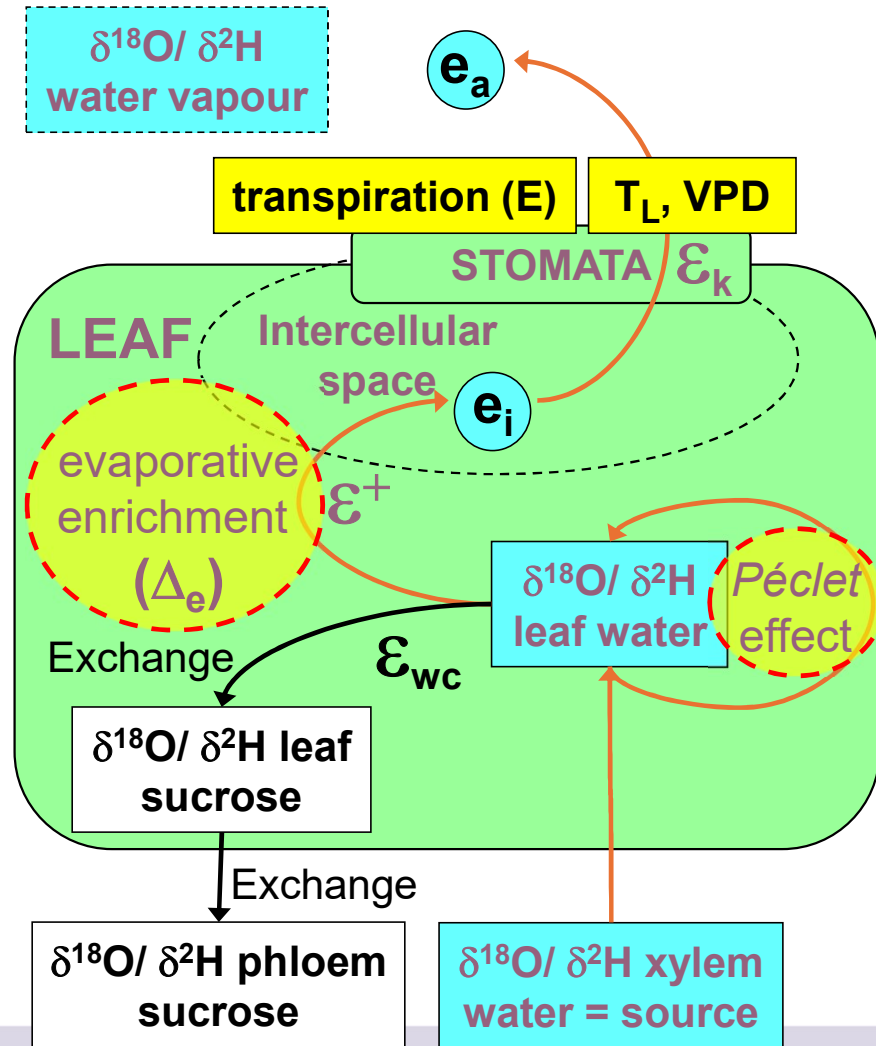
Isotopic enrichment above source water at the whole leaf ( $\Delta_L$ )\*



$E$  = transpiration  
 $L$  = effective pathlength  
 $D$  = self-diffusivity

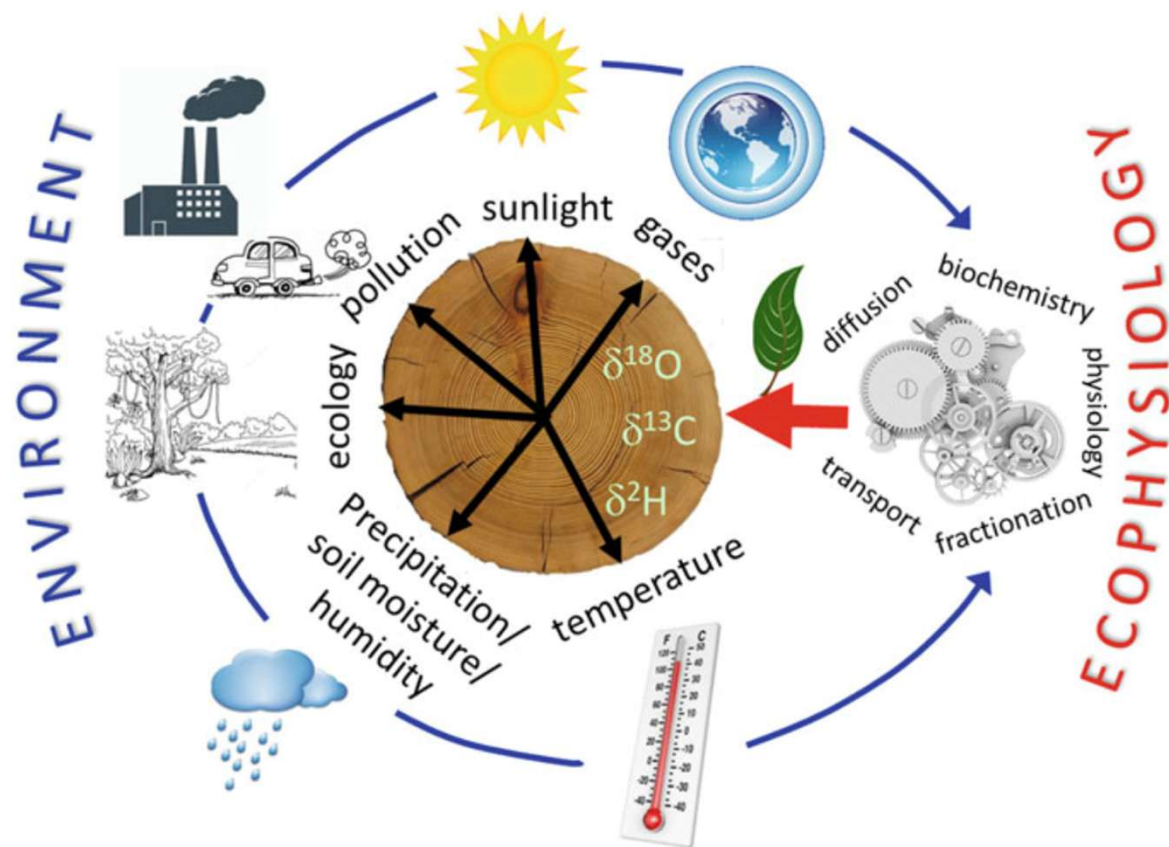
\*Farquhar and Lloyd, 1993

# Step 3. From leaf to phloem and tree rings



In the case of cellulose, which is the main component of wood, about 50% of the enrichment signal is further exchanged with xylem water

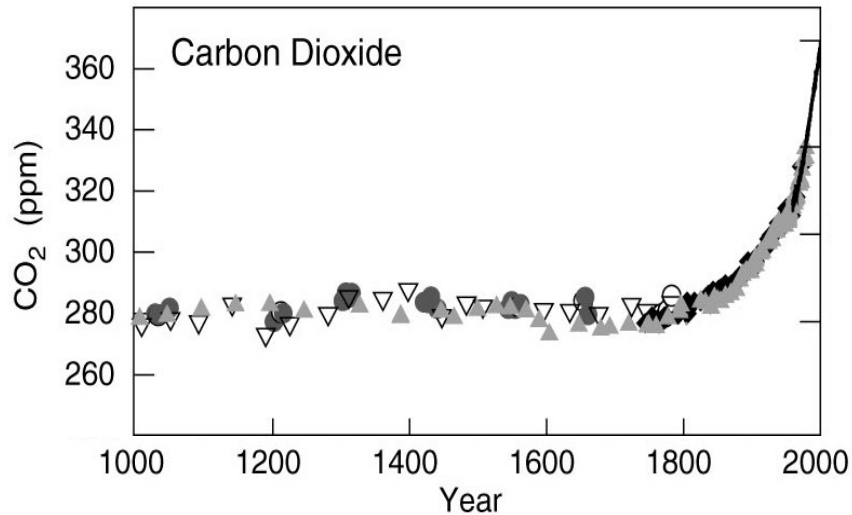
The  $\delta^{18}\text{O}$  signal in wood is a mixture, in varying proportions, of source water  $\delta^{18}\text{O}$  and evaporative enrichment at the leaf level ( $\approx$  evaporative demand modulated by stomatal conductance)



**Fig. 1.1** Schematic representation of interplay between environmental factors and ecophysiology that produces tree-ring C-H-O isotope composition. Environment can influence the ecophysiological, biochemical, and physicochemical mechanisms responsible for producing the isotope composition and consequently isotope dendrochronology can be used to infer environment and ecological events affecting ecophysiology (e.g., insect outbreaks). Tree rings allow identification of changes through time, but if a network of sites is sampled, the tree-ring isotope results can also reveal changes in space (isoscaes)

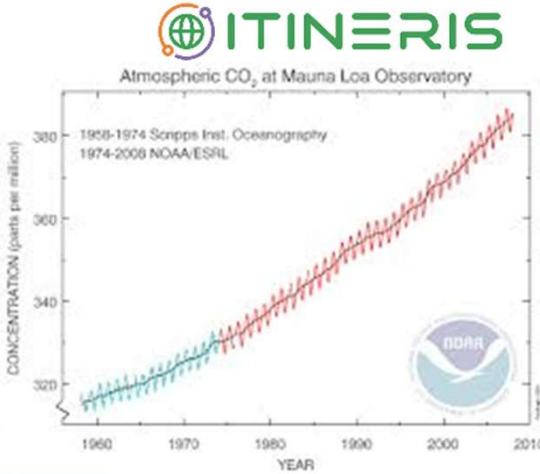
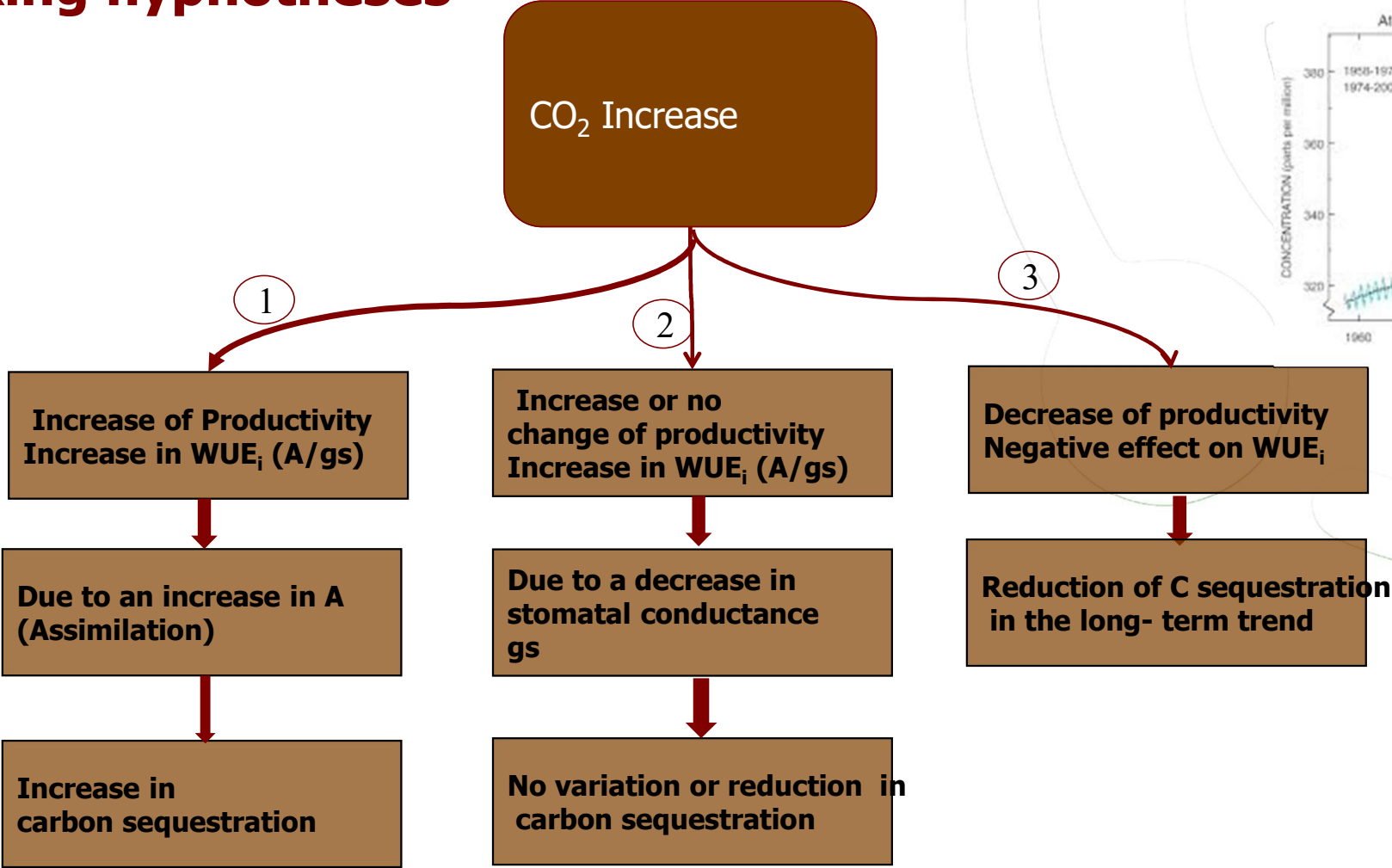
# **Effects of CO<sub>2</sub> enrichment on forest**

For over a millennium these old trees grew in an atmosphere with nearly unchanging  $[CO_2]$  ( $\sim 280$  ppm)



***Photosynthetic assimilation of  $CO_2$  is central to the metabolism of plants. As atmospheric concentrations of  $CO_2$  rise, how will this affect the plants we depend on? And how this will affect Carbon sequestration?***

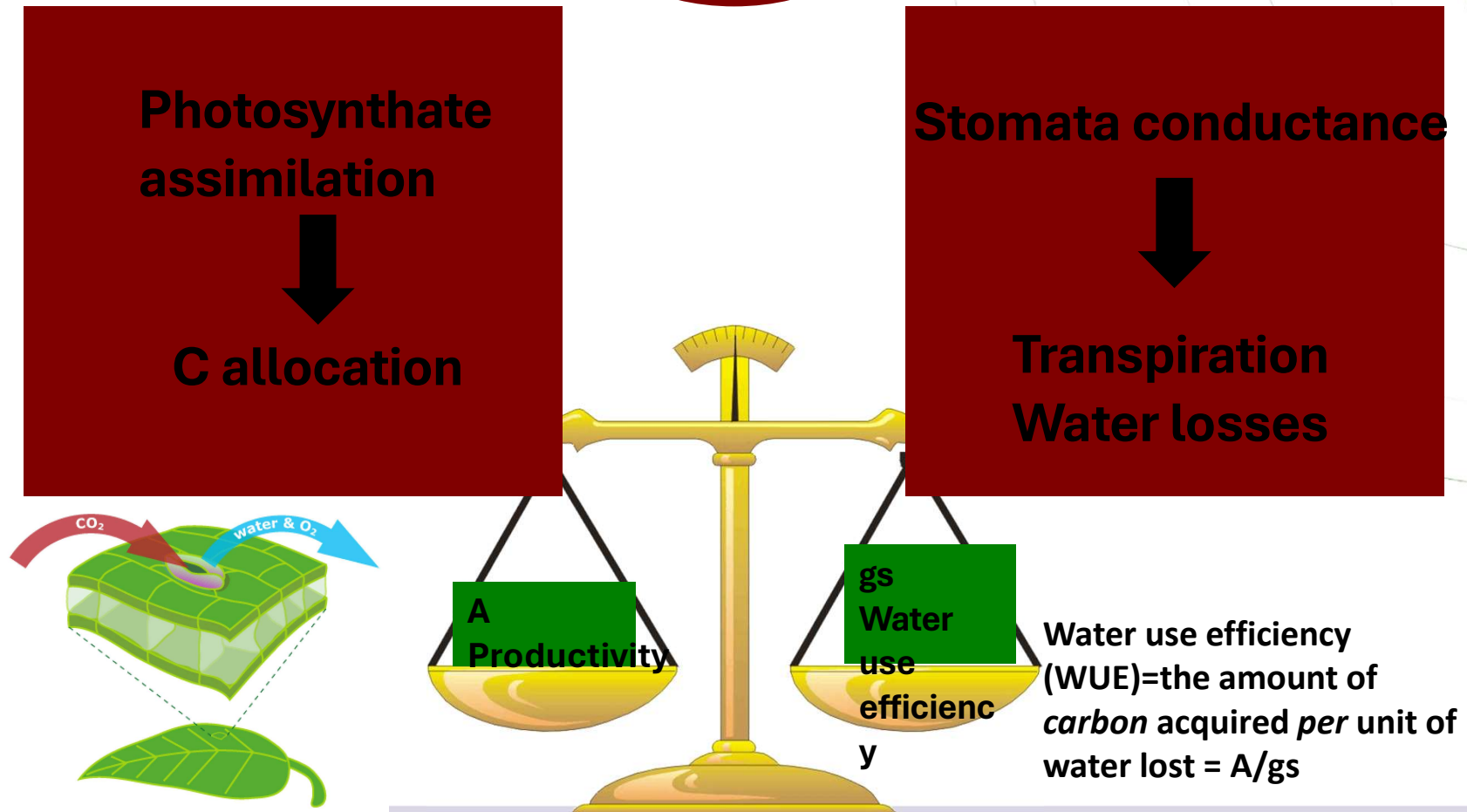
# Working hypotheses



# CO<sub>2</sub> productivity and C sequestration

CARBON FIXATION

CO<sub>2</sub> STOMATA



## Intrinsic Water use efficiency

Carbon isotope discrimination is a measure of Intrinsic water-use efficiency ( $WUE_i$ )

$WUE_i$  is the ratio of carbon assimilation to stomatal conductance  $A/g_s$

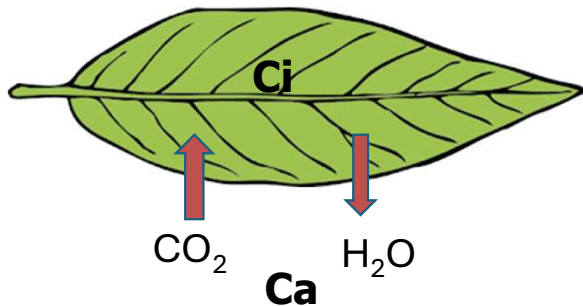
$$WUE_i = A/g_s = \frac{c_a - c_i}{1.6}$$

1.6 = the ratio of diffusivity of water and  $CO_2$  in air

$$\delta^{13}C_{plant} = \delta^{13}C_{air} - a - \frac{(b-a) c_i}{c_a}$$



$$c_i = c_a \frac{\delta^{13}C_{air} - \delta^{13}C_{plant} - a}{b-a}$$



$$WUE_i = \frac{c_a}{1.6} \frac{b - \delta^{13}C_{air} + \delta^{13}C_{plant}}{b-a}$$

## Elevated CO<sub>2</sub> increases tree-level intrinsic water use efficiency: insights from carbon and oxygen isotope analyses in tree rings across three forest FACE sites

Giovanna Battipaglia<sup>1,2</sup>, Matthias Saurer<sup>3</sup>, Paolo Cherubini<sup>4</sup>, Carlo Calzavara<sup>5</sup>, Heather R. McCarthy<sup>6</sup>, Richard J. Norby<sup>7</sup> and M. Francesca Cotrufo<sup>8</sup>

<sup>1</sup>Environmental Science Department, Second University of Naples, 81100, Caserta, Italy; <sup>2</sup>Centre for Bio-Archaeology and Ecology, Ecole Pratique des Hautes Etudes (PALCO EPHE), Institut de Botanique, University of Montpellier 2, F-34090, Montpellier, France; <sup>3</sup>PSI Paul Scherrer Institut, 5232, Villigen, Switzerland; <sup>4</sup>WSL Swiss Federal Institute for Forest, Snow and Landscape Research, 8908, Birmensdorf, Switzerland; <sup>5</sup>IBAF-Institute of agro-environmental and Forest Biology, CNR, Perugia, Italy; <sup>6</sup>Department of Microbiology and Plant Biology, University of Oklahoma, Norman, OK, USA; <sup>7</sup>Oak Ridge National Laboratory, Environmental Sciences Division, Oak Ridge, TN, USA; <sup>8</sup>Department of Soil and Crop Science, Colorado State University, Fort Collins, CO, USA

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Received: 25 July 2012  
Accepted: 4 October 2012

New Phytologist (2013) 197, 544–554  
doi: 10.1111/nph.12044

Key words: climate change, dendro-ecology, *Liquidambar styraciflua*, *Pinus taeda*, *Populus alba*, *Populus nigra*, *Populus x euramericana*.

### Summary

- Elevated CO<sub>2</sub> increases intrinsic water use efficiency (WUE) of forests, but the magnitude of this effect and its interaction with climate is still poorly understood.
- We combined tree ring analysis with isotope measurements at three Free Air CO<sub>2</sub> Enrichment (FACE, POP-EUROFACE, in Italy; Duke FACE in North Carolina and ORNL in Tennessee, USA) sites, to cover the entire life of the trees. We used δ<sup>13</sup>C to assess carbon isotope discrimination and changes in water-use efficiency, while direct CO<sub>2</sub> effects on stomatal conductance were explored using δ<sup>18</sup>O as a proxy.
- Across all the sites, elevated CO<sub>2</sub> increased <sup>13</sup>C-derived water-use efficiency on average by 73% for *Liquidambar styraciflua*, 77% for *Pinus taeda* and 75% for *Populus* sp., but through different ecophysiological mechanisms.
- Our findings provide a robust means of predicting water-use efficiency responses from a variety of tree species exposed to variable environmental conditions over time, and species-specific relationships that can help modelling elevated CO<sub>2</sub> and climate impacts on forest productivity, carbon and water balances.

**AIMS:**  
How the elevated CO<sub>2</sub> will affect forest productivity and WUE in 5 species across 3 forest stands exposed to ~550 ppm CO<sub>2</sub> for 3-8 years



Oak Ridge FACE, USA  
*L. styraciflua*

# Forest FACE Synthesis Project



PAUL SCHERRER INSTITUT  
PSI



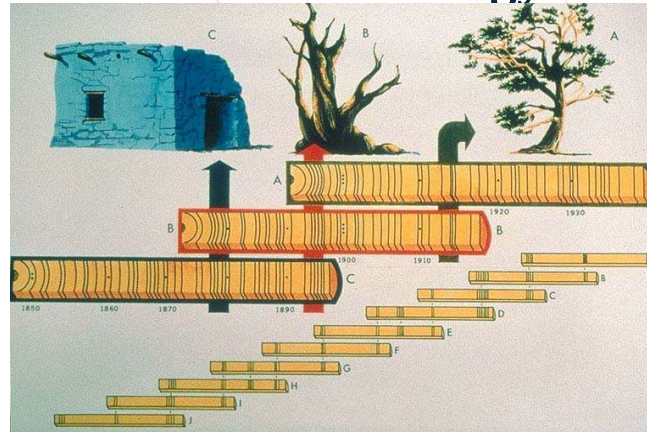
Duke FACE, JSA  
*P. taeda*  
*L. styraciflua*



POP-EUROFACE, I\_P. alba, P. nigra, P. x. euramericana



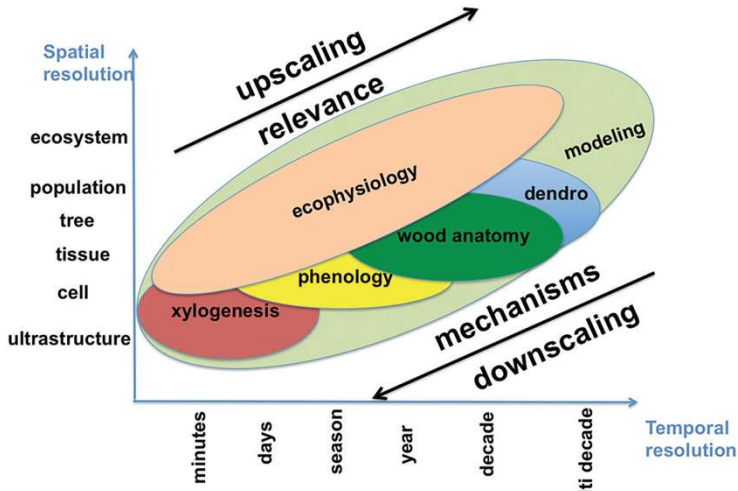
## ➤ Dendrochronology



<http://www.ncdc.noaa.gov/paleo/treering.html>

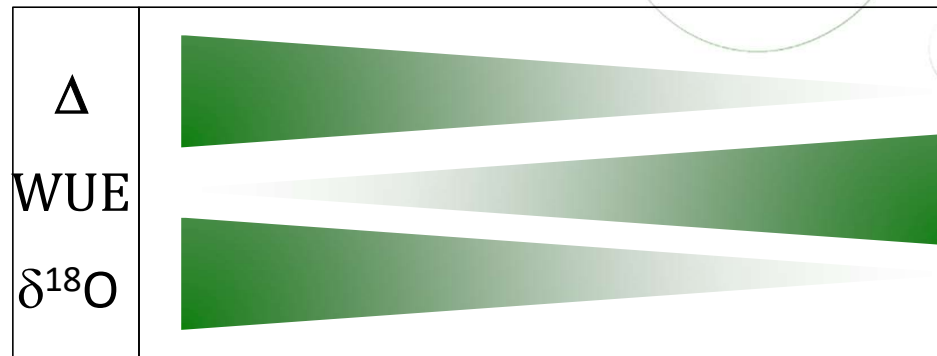
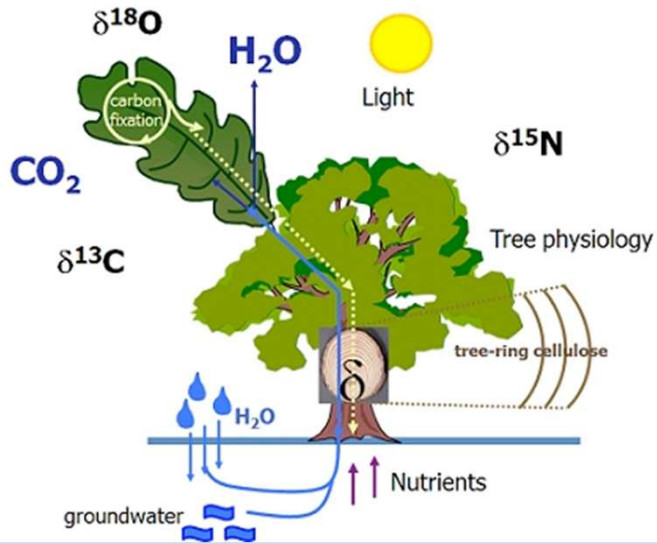


## Methods



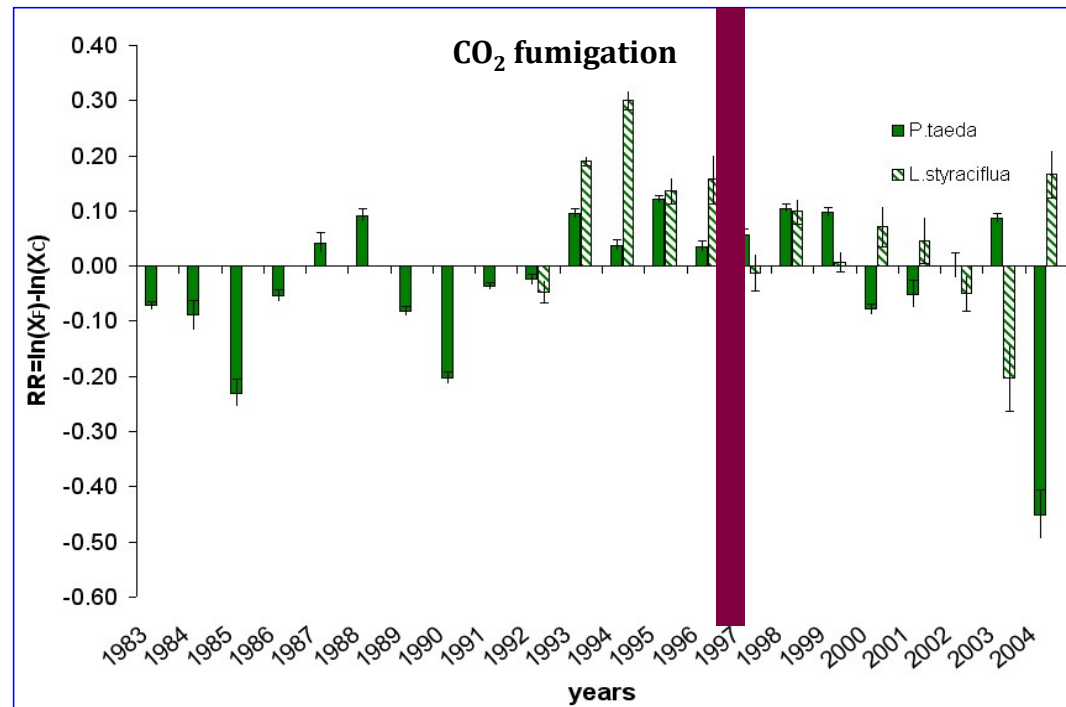
Battipaglia et al. 2014, Tree physiology, 34: 787-791.

## ➤ Stable isotopes



$\Delta^{13}\text{C}$  as proxy of Water use efficiency  
 $\delta^{18}\text{O}$  as proxy of stomatal conductance

## Productivity: CO<sub>2</sub> fertilization effect?



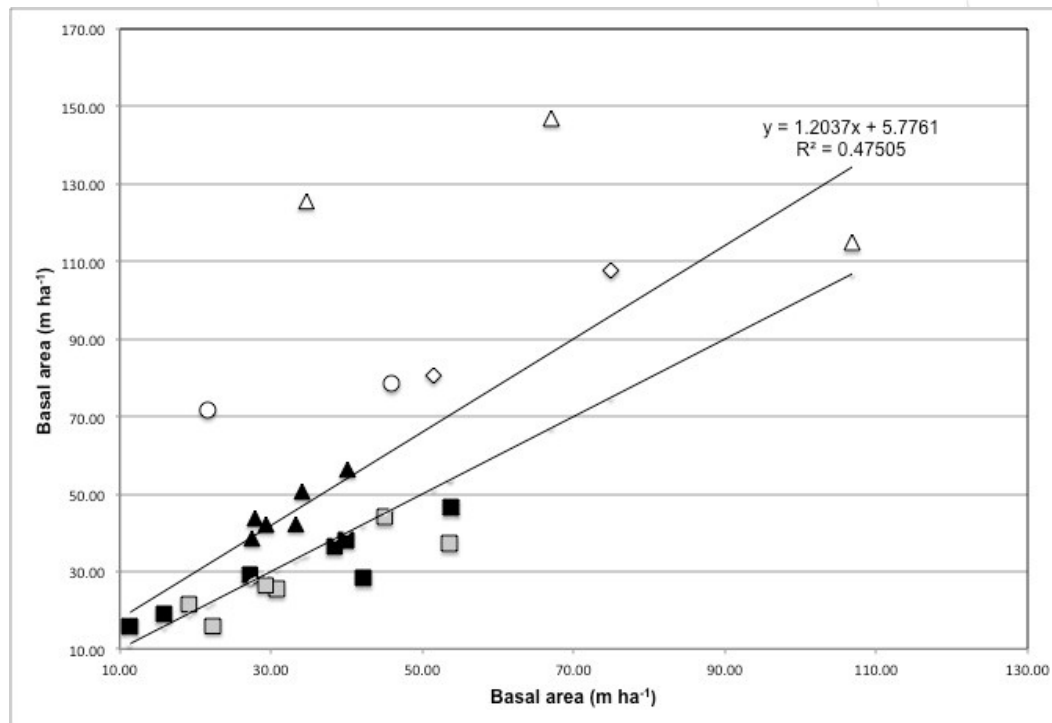
Before the fumigation, the growth of the species were quite variable



No clear BA increase in the fumigated plots

## Productivity: CO<sub>2</sub> fertilization effect?

Regression is significantly different from 1:1 line



- △ P. nigra
- P. alba
- ◇ P. euro
- ▲ P. taeda
- L. styraciflua duke
- L. styraciflua ORNL

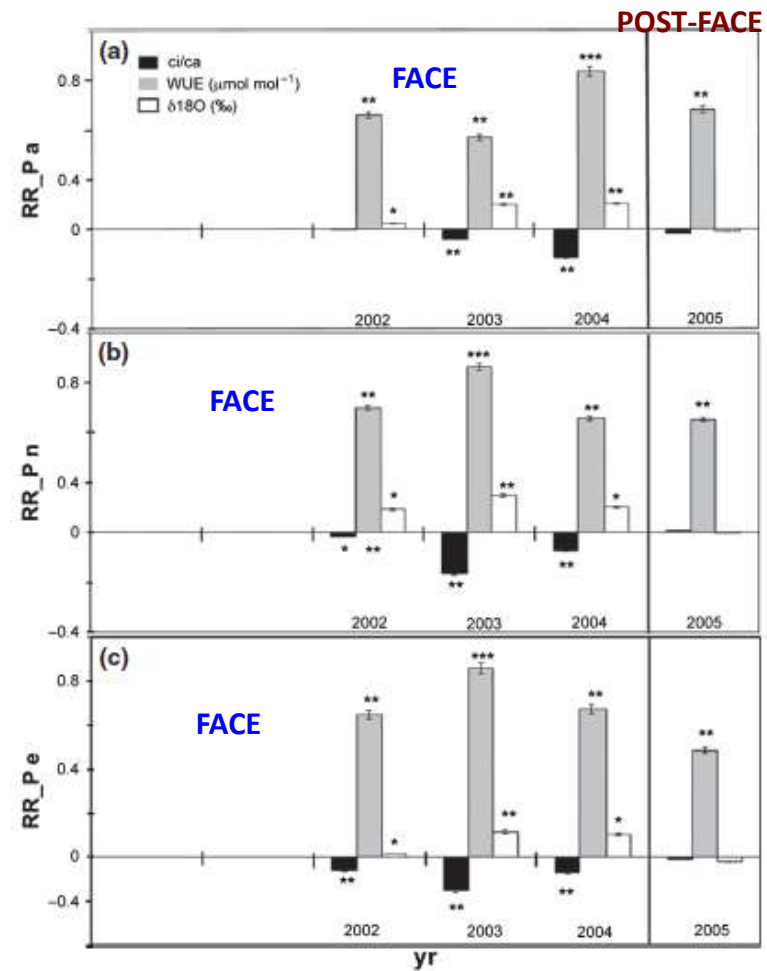
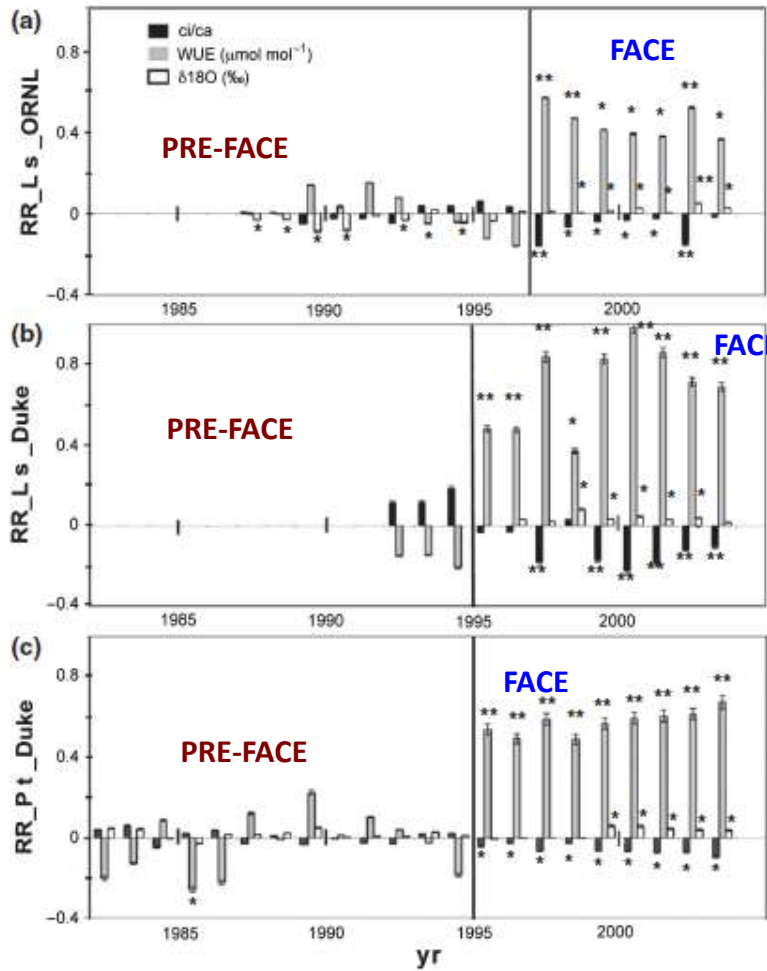
**Growth Response is species specific!!**

**WUE<sub>i</sub>**: the amount of carbon acquired per unit of water lost



$$WUE_i \approx A/g_s$$

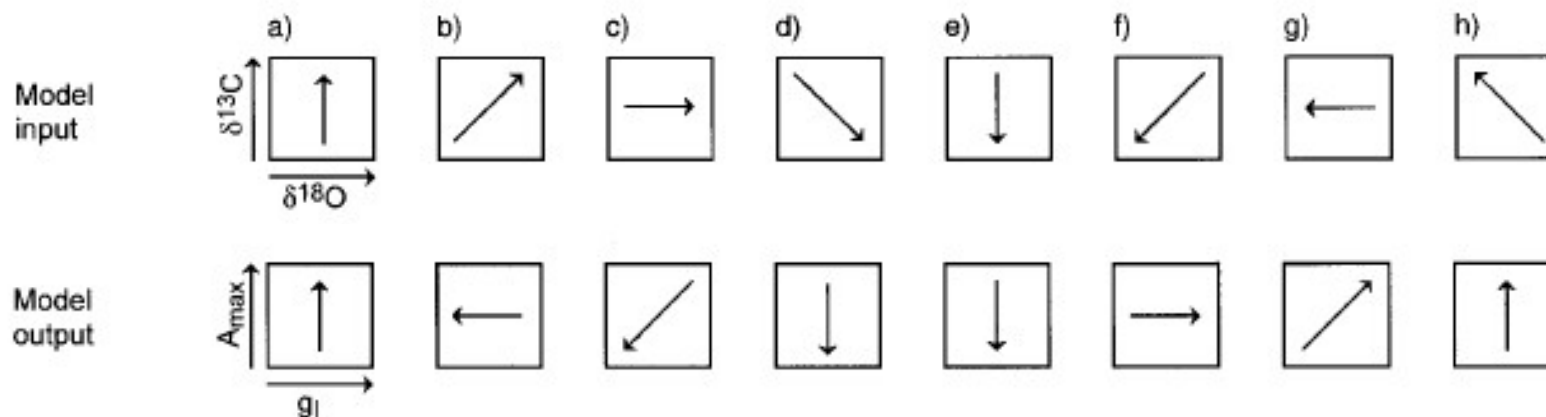
■ ci/ca  
 ■ WUE ( $\mu\text{mol mol}^{-1}$ )  
 □  $\delta^{18}\text{O}$  (‰)



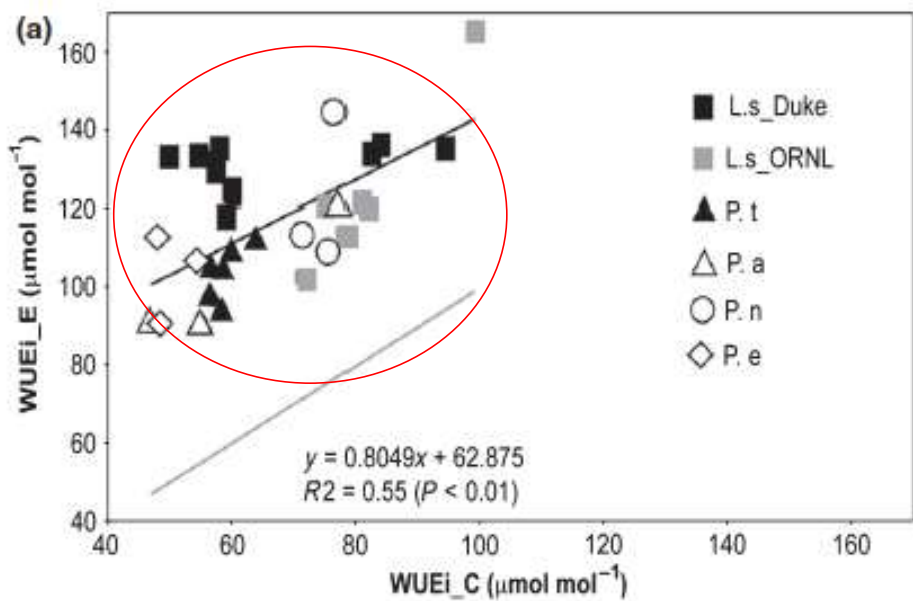
# The dual isotope approach

- Described in: **Scheidegger *et al.*** (2000) Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: a conceptual model. *Oecologia* 125: 350-357
- The model attempts at distinguishing whether differences in intercellular CO<sub>2</sub> concentration are caused by a response of *g<sub>s</sub>* or *A*.

Changes between two treatments are shown by arrows



Regression is significantly different from 1:1 line



Response of  $WUE_i$  to elevated  $\text{CO}_2$  is consistent across a wide range of species

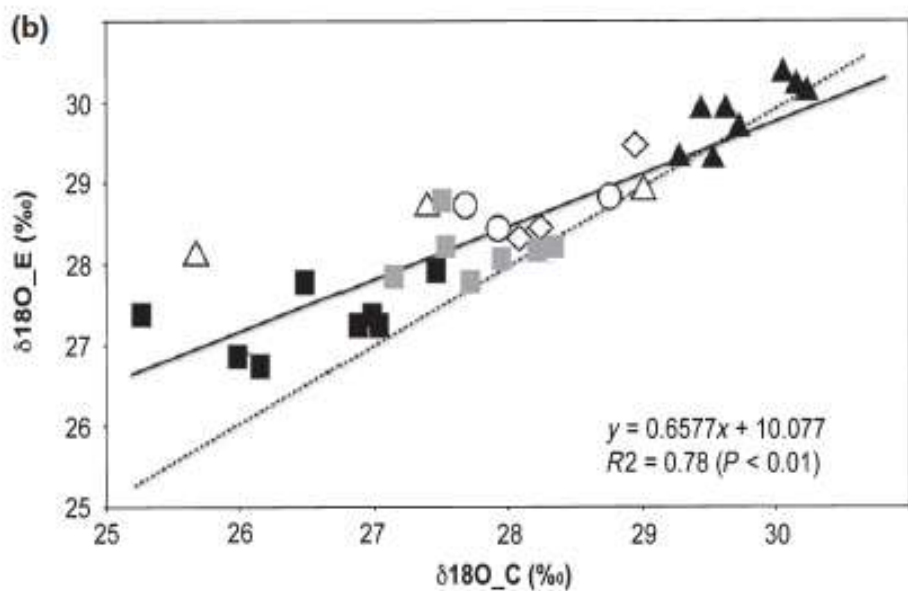


WUE<sub>i</sub> increases in all species

Variations of  $\delta^{18}\text{O}$  are different in the different species



Variation in stomatal conductance ( $g_s$ )



Parameter	Site	Species	$\delta^{18}\text{O}$		BAI	
			$r^2$	$b$	$r^2$	$b$
WUE <sub>i</sub>	ORNL	L. s	0.65*	0.59	0.003	0.50
WUE <sub>i</sub>	Duke	L. s	0.68*	0.48	0.10	0.26
WUE <sub>i</sub>	Duke	P. t	0.12	0.08	0.30*	0.80
WUE <sub>i</sub>	POPEUROFACE	P. a	0.45*	0.53	0.38*	0.55
WUE <sub>i</sub>	POPEUROFACE	P. n	0.48*	0.35	0.74**	0.27
WUE <sub>i</sub>	POPEUROFACE	P. e	0.38*	0.51	0.91***	0.70

**L.s** → gs ↓ → WUE<sub>i</sub> ↑ → TRW =

**P.t** → gs = → WUE<sub>i</sub> ↑ → TRW ↑

**Pop.** → gs ↓ → WUE<sub>i</sub> ↑ → TRW ↑

Battipaglia et al\_2013\_NPH

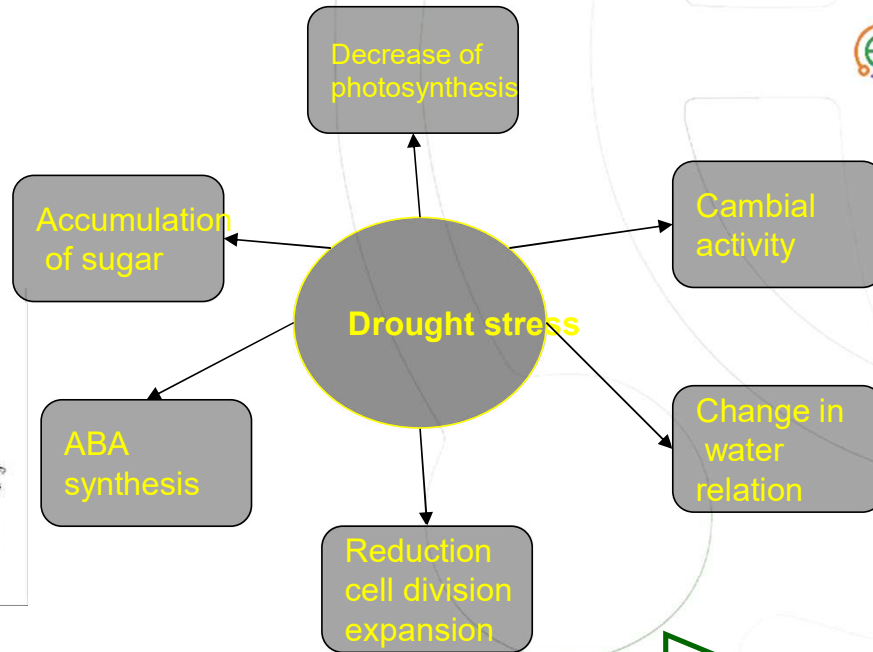
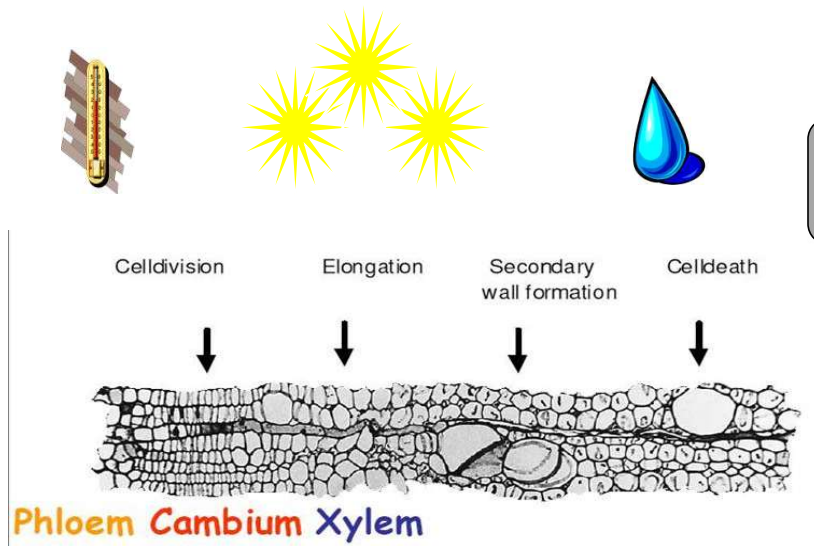
Across all the sites, elevated CO<sub>2</sub> increased <sup>13</sup>C-derived water-use efficiency on average by **73%** for *Liquidambar styraciflua*, **77%** for *Pinus taeda* and **75%** for *Populus* sp., but through different ecophysiological mechanisms:

- In *L. styraciflua*, warmer conditions seemed to be coupled with a **reduction of stomatal conductance (gs)**, increasing **WUE<sub>i</sub>** but without a parallel stimulation of **tree growth**.
- A reduction of **gs** was also observed in the fast-growing *Populus* sp., accompanied by **positive tree growth** responses, and was partially limited by high temperature during 2003 and 2004.
- Finally the rise of **WUE<sub>i</sub>** in *P. taeda* was mainly related to soil moisture increases under elevated CO<sub>2</sub> and opens new questions about the ability of this isohydric species (with tight stomatal control) to withstand the expected reduction in soil water in combination with an increase in drought.

# Mediterranean species and Drought effect

*Plant strategies to cope with drought..*

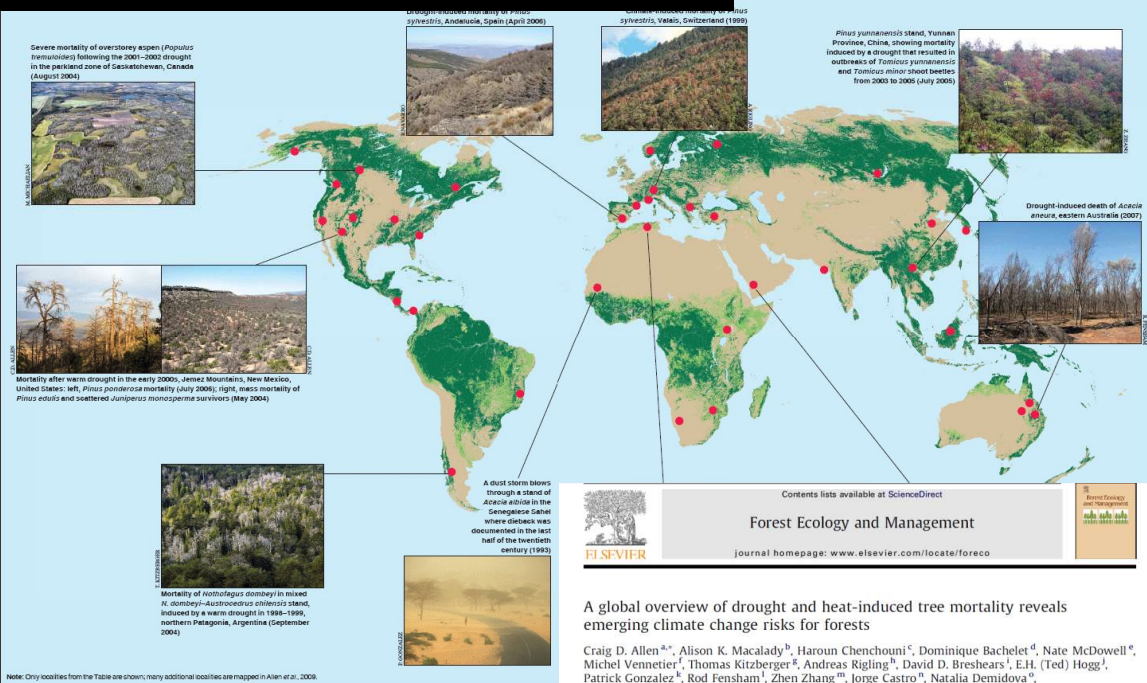
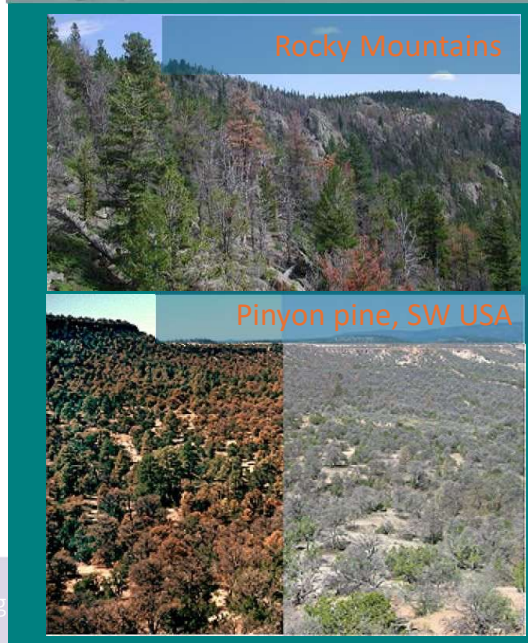
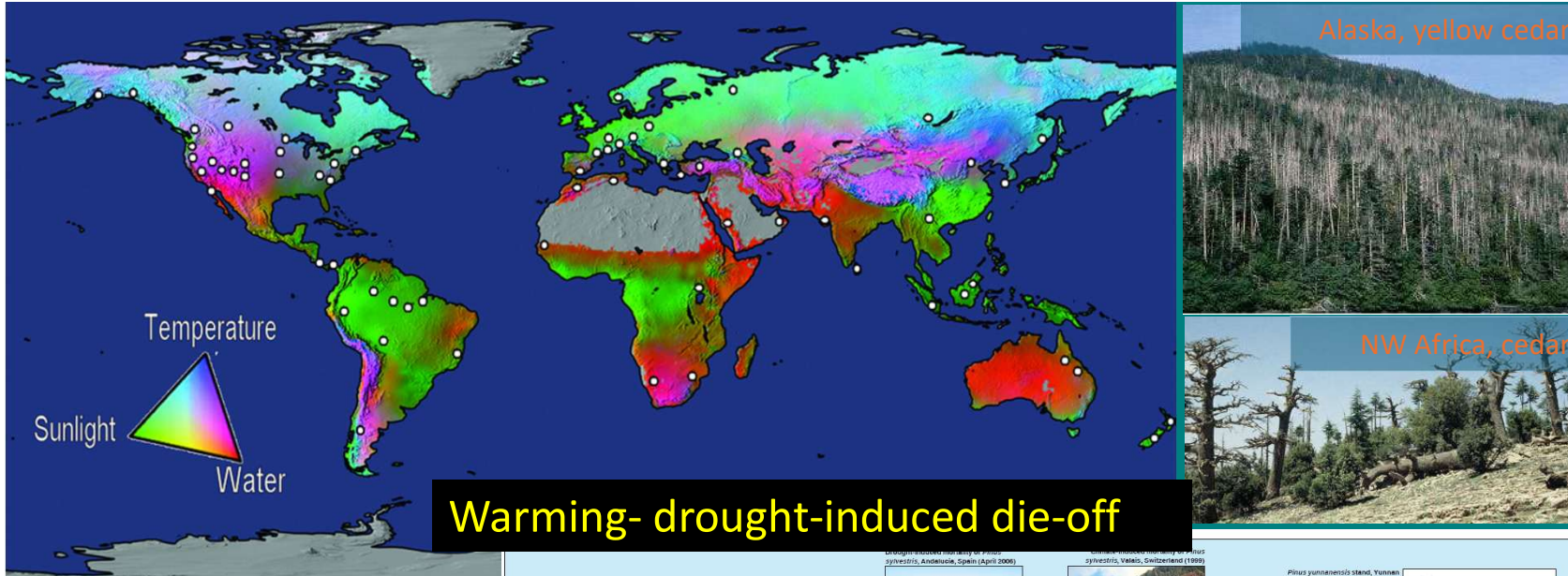
# Wood formation



Retrospective analysis



Forecasting analysis



Note: Dry oases from the Table are shown; many additional oases are mapped in Allen et al., 2009.

A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests

Craig D. Allen<sup>a,\*</sup>, Alison K. Macalady<sup>b</sup>, Haroun Chenchouni<sup>c</sup>, Dominique Bachelet<sup>d</sup>, Nate McDowell<sup>e</sup>, Michel Vennetier<sup>f</sup>, Thomas Kitzberger<sup>g</sup>, Andreas Rigling<sup>h</sup>, David D. Breshears<sup>i</sup>, E.H. (Ted) Hogg<sup>j</sup>, Patrick Gonzalez<sup>k</sup>, Rod Fensham<sup>l</sup>, Zhen Zhang<sup>m</sup>, Jorge Castro<sup>n</sup>, Natalia Demidova<sup>o</sup>

## Which physiological mechanisms drive tree mortality under drought?

Three possible, not mutually exclusive, mechanisms:

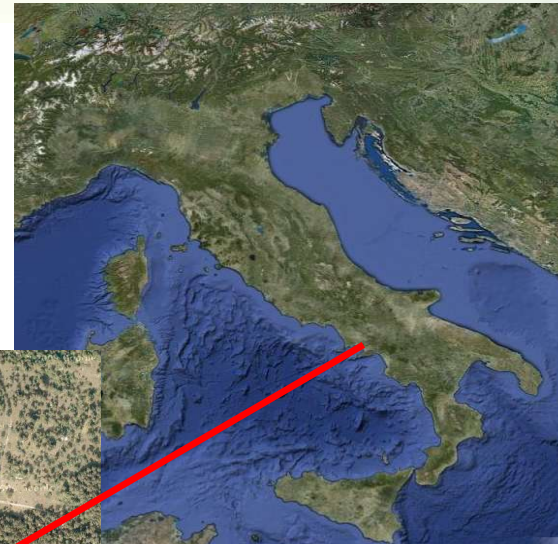
1. Hydraulic failure
2. Carbon starvation
3. Biotic attacks

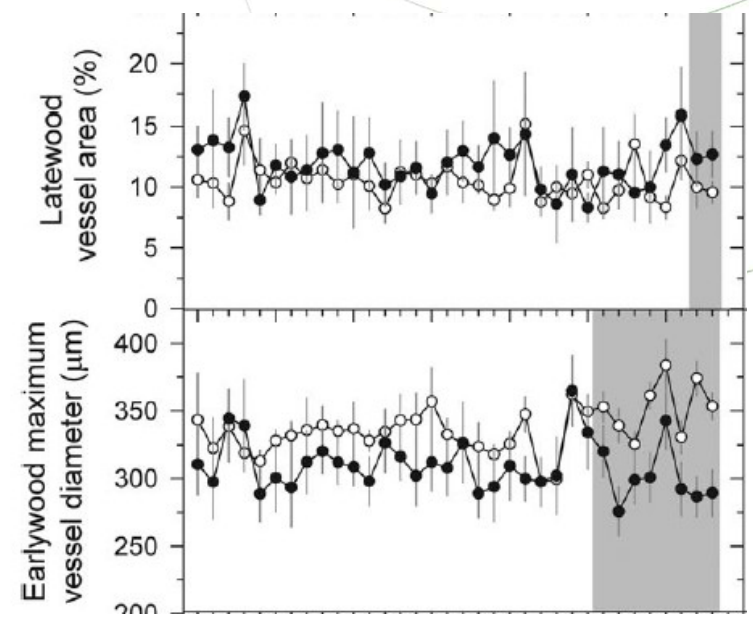
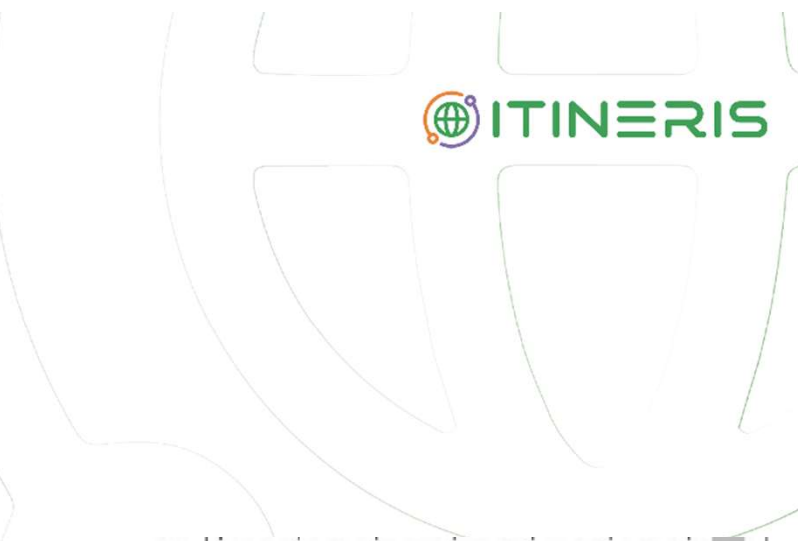
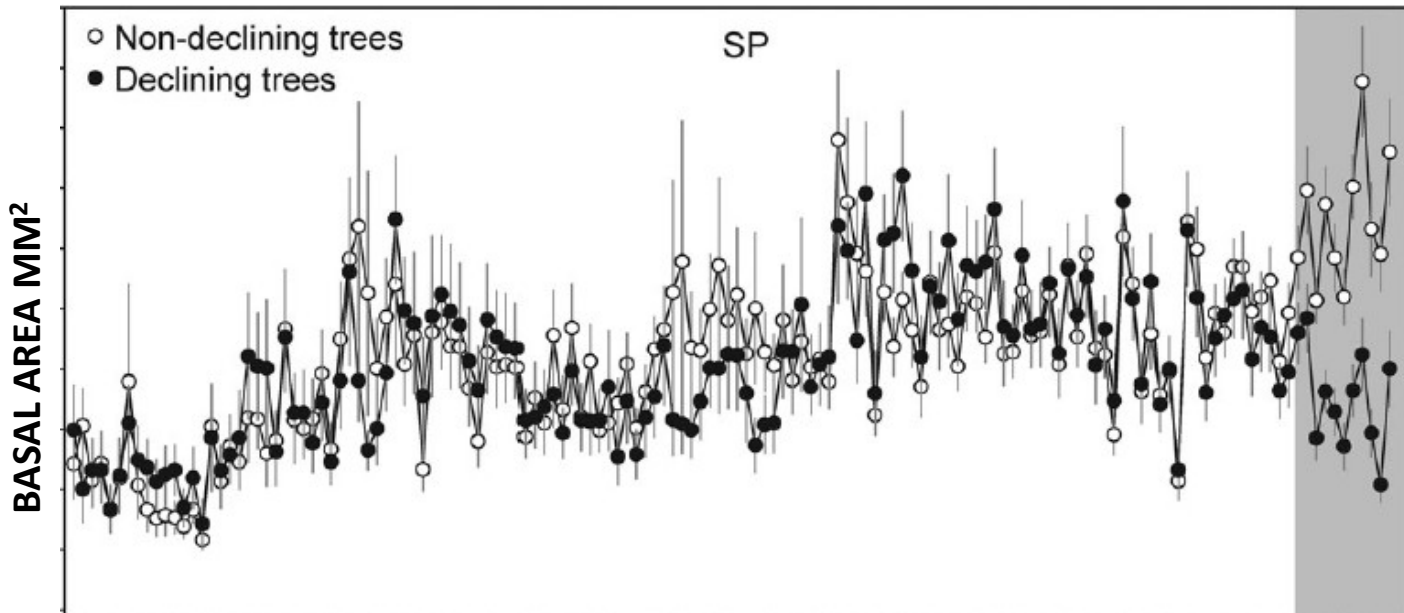
## A multi-proxy assessment of dieback causes in a Mediterranean oak species

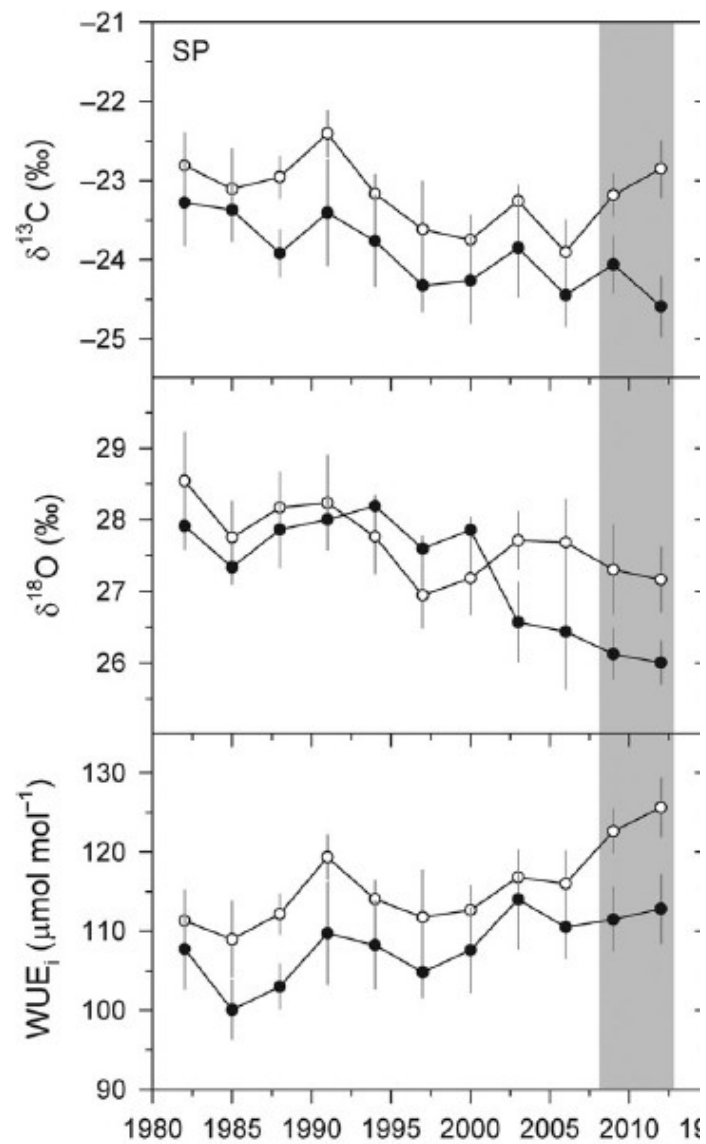
Michele Colangelo<sup>1</sup>, J. Julio Camarero<sup>2</sup>, Giovanna Battipaglia<sup>3,4</sup>, Marco Borghetti<sup>1</sup>, Veronica De Micco<sup>5</sup>, Tiziana Gentilesca<sup>1</sup> and Francesco Ripullone<sup>1,6</sup>



*Q. cerris*

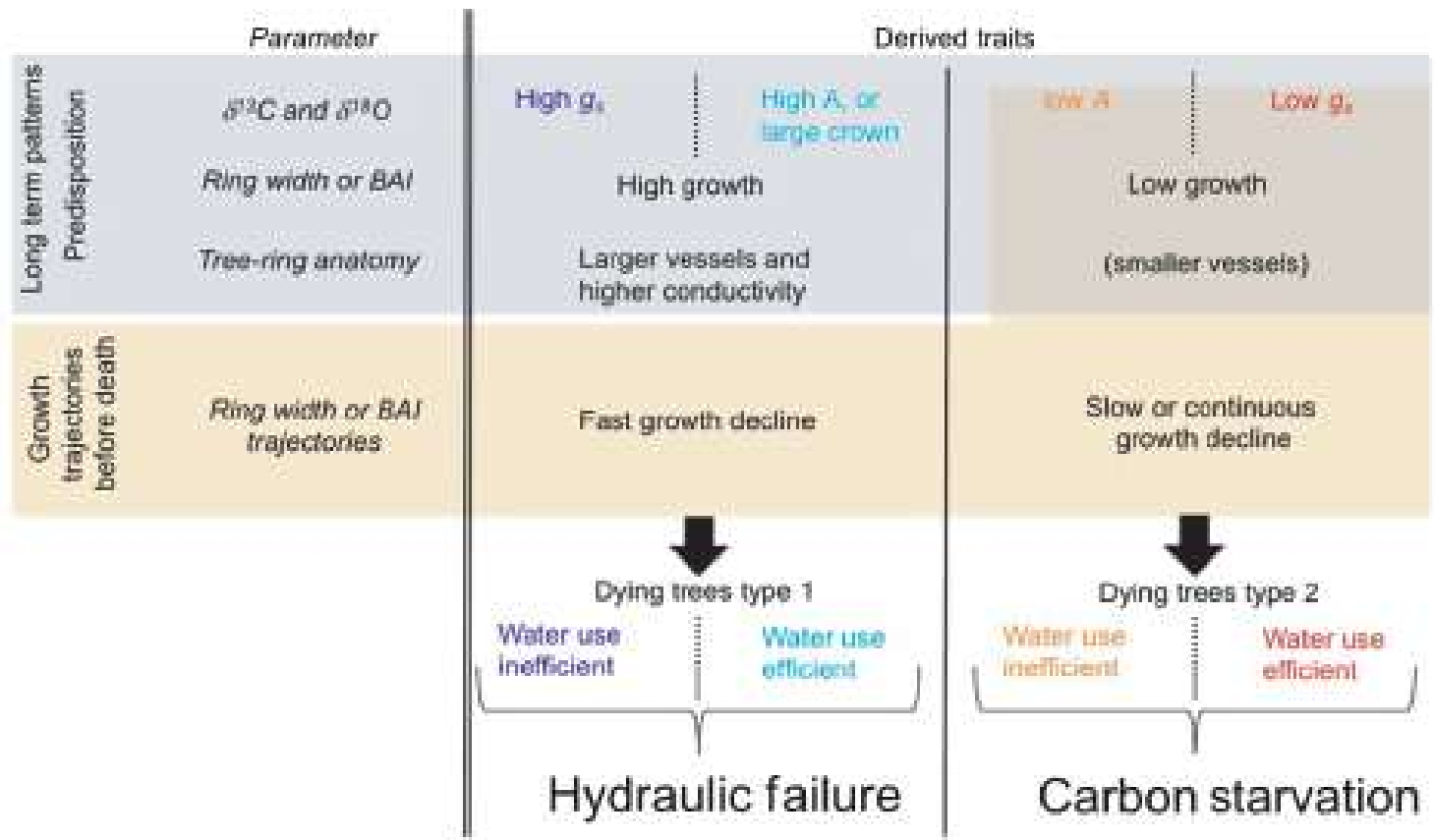






○ Non-declining trees  
● Declining trees





most vulnerable to drought are characterized by:

- lower wood density
- Low WUEi

Gessler et al. 2018, *New Phytologist* 219:485-490

# Fire Effect on plants

# Study sites and PB conditions

*P. halepensis*- National Park of Cilento (NPC) PB May 2009



*P. pinea*- National Reserve of Castelvolturno (NRC) PB March 2014

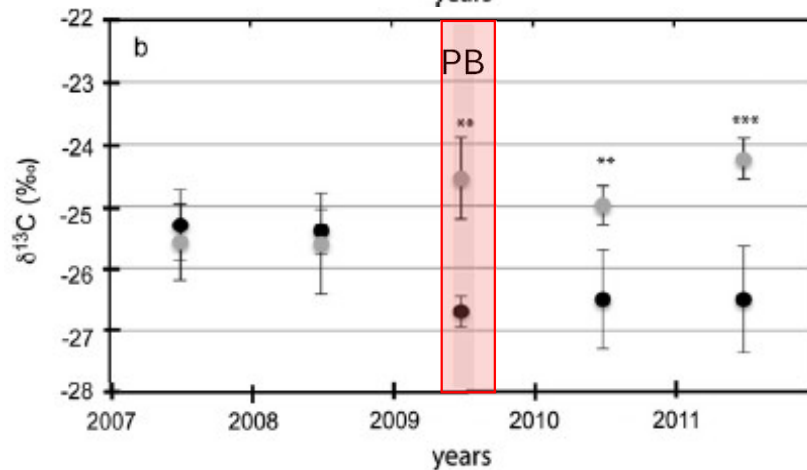
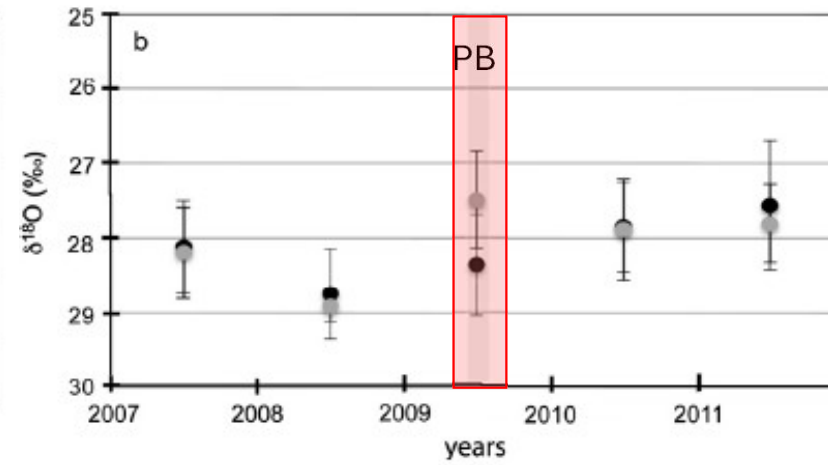
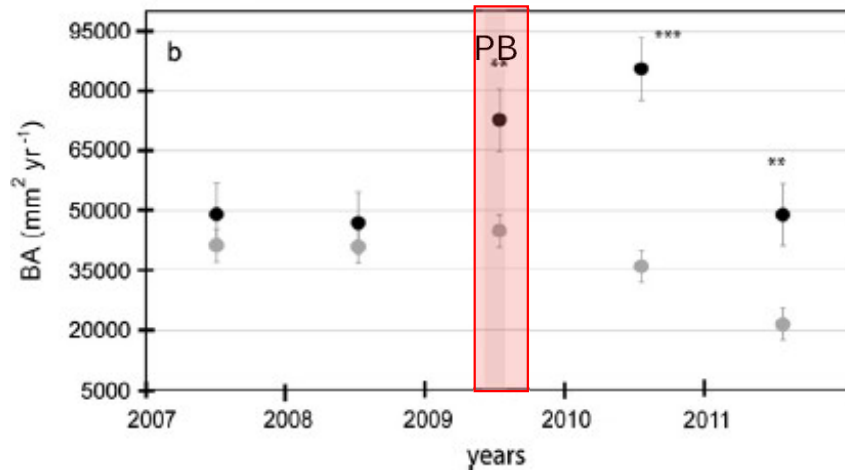


## PREScribing BURNING CONDITIONS

	NPC	NRC
AIR TEMPERATURE (°C)	23	18
RELATIVE AIR HUMIDITY (%)	53	54
WIND SPEED (km/h)	4	1-3 Km/h
FLAME HEIGHT (m)	<1	<1
SURFACE TMAX (°C)	737	851
FIRE INTENSITY (kW/m)	62	<200

## Old stand

- burned
- control

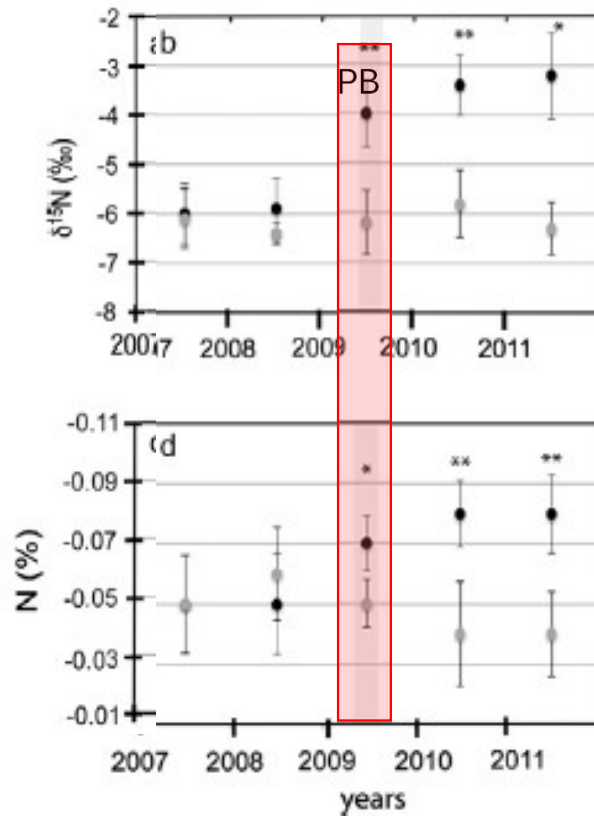


- Increase in BA in the short and mid term
- Decrease in  $\delta^{13}\text{C}$  in short and mid term
- No change in  $\delta^{18}\text{O}$  in short and mid term



Variation in productivity and  $^{13}\text{C}$  derived  $\text{WUE}_i$  related to an increase of photosynthetic activity

Battipaglia et al 2014 For Ecol Manage



- Increase in  $\delta^{15}\text{N}$  in the short and mid term
- Increase in N% in the short and mid term

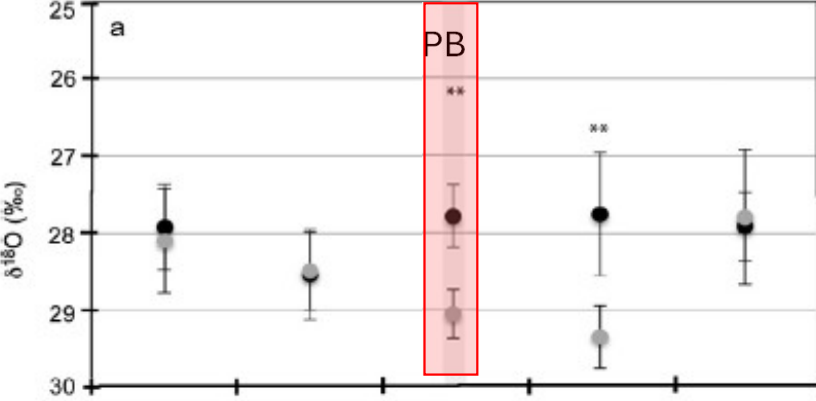
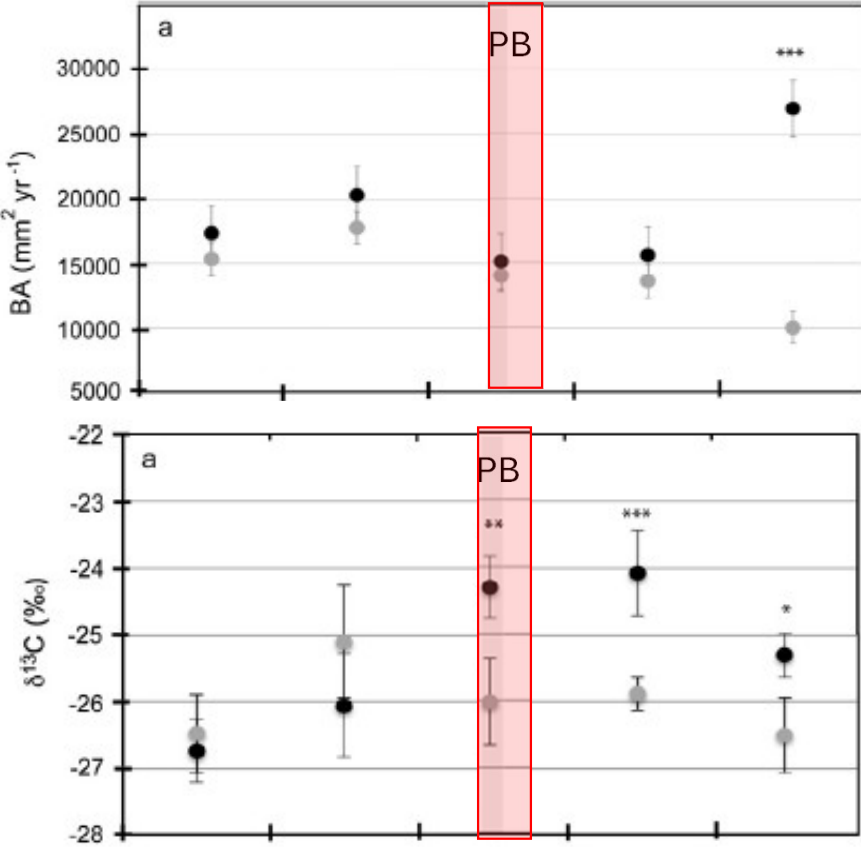


Prescribed fire has a positive effect on old individuals of *P. halepensis* for reduction of competition and the fertilizer effect of nitrogen and water

Battipaglia et al 2014 For Ecol Manage

# Young stand

- burned
- control

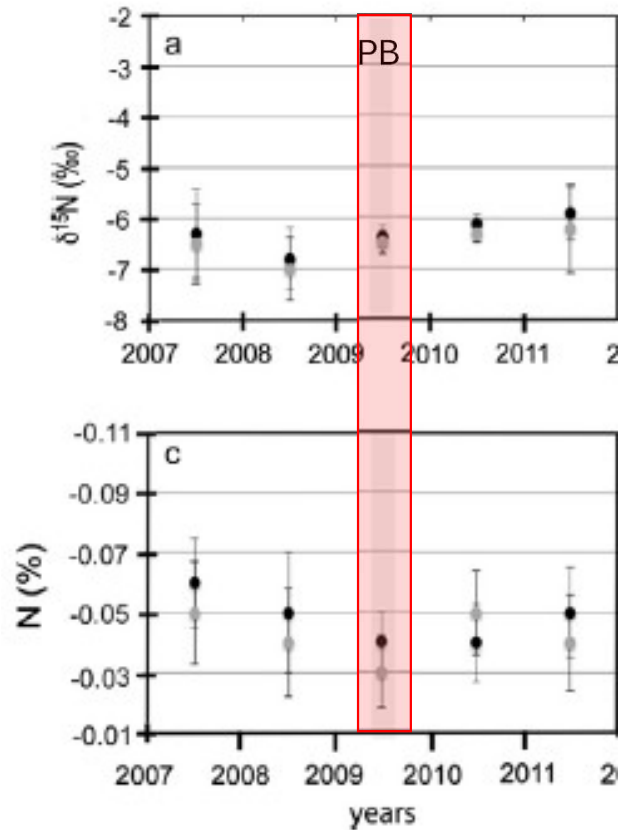


- No change BA in the short term
- Increase in  $\delta^{13}\text{C}$  in short and mid term
- Increase in  $\delta^{18}\text{O}$  in short term



Stomatal conductance is the dominant limitation to photosynthesis

Battipaglia et al 2014 For Ecol Manage



- No change in  $\delta^{15}\text{N}$  in the short and mid term
- No change in N% in the short and mid term



Young trees do not benefit from prescribed burning, but they suffer from an increase of competition with the older individuals.

*Battipaglia et al 2014 For Ecol Manage*

## Take home message

- Prescribed fire effects depend on tree's age
- Need to extend the study to PB with different burning conditions and different species

Tree Physiology 36, 1019–1031  
doi:10.1093/treephys/tpw034

Research paper

**Effects of prescribed burning on ecophysiological, anatomical and stem hydraulic properties in *Pinus pinea* L.**

Giovanna Battipaglia<sup>1,2,3,8</sup>, Tadeja Savi<sup>4</sup>, Davide Ascoli<sup>5</sup>, Daniele Castagneri<sup>6</sup>, Assunta Esposito<sup>1</sup>, Stefan Mayr<sup>7</sup> and Andrea Nardini<sup>4</sup>

- Two areas: burned area and control area.
- litter fuels added around the trunk of ten trees to produce a gradient of heat treatment
- Thermocouples placed at the stem base, as a proxy of fire heating.
- 20 trees sampled in each area



*Burned area*



*Trunk sampled*



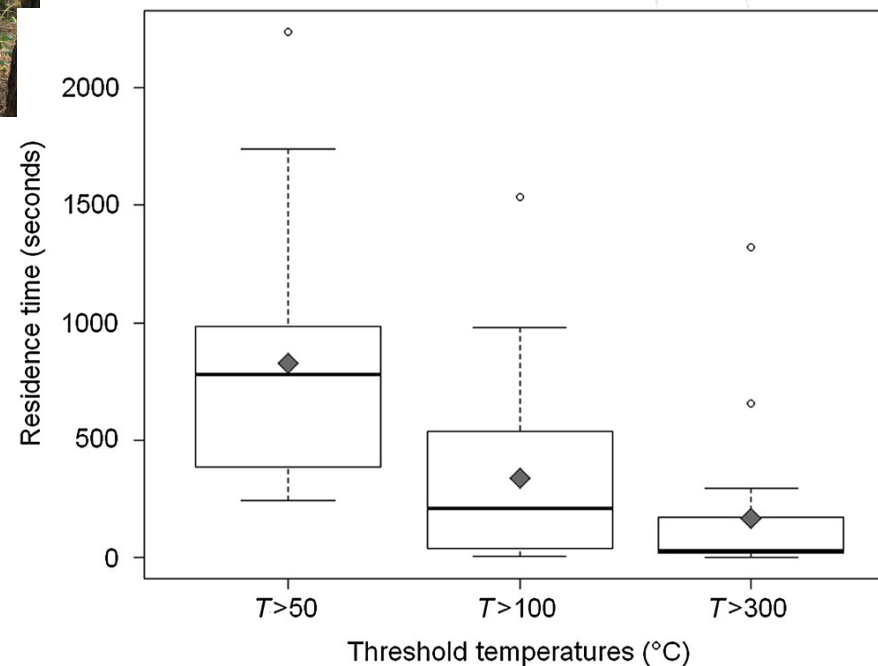
## *Pinus pinea* stand



- Three cores were collected with a 5 mm diameter borer (Suunto, Finland) per each trees: two for dendro-isotopic measurements and one for anatomical analyses
- Trunk sampled for hydraulic measurements, two weeks after PB



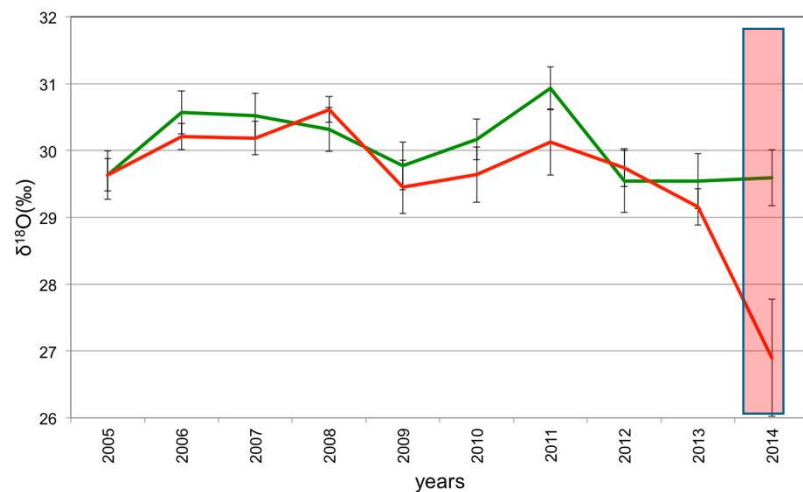
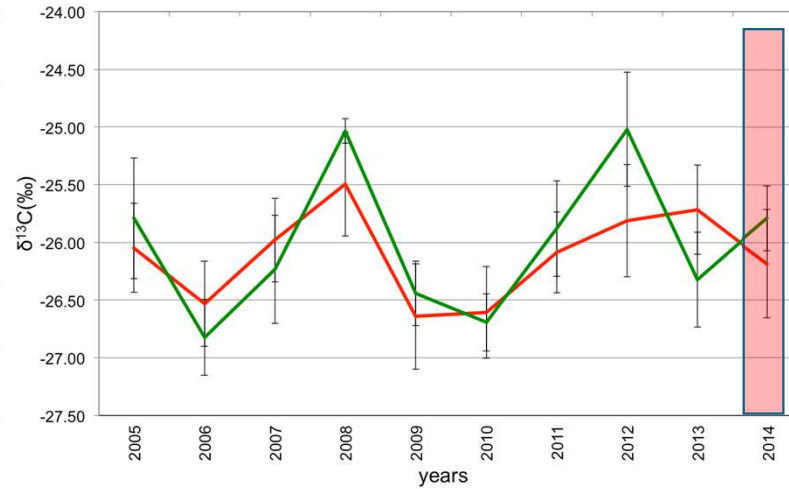
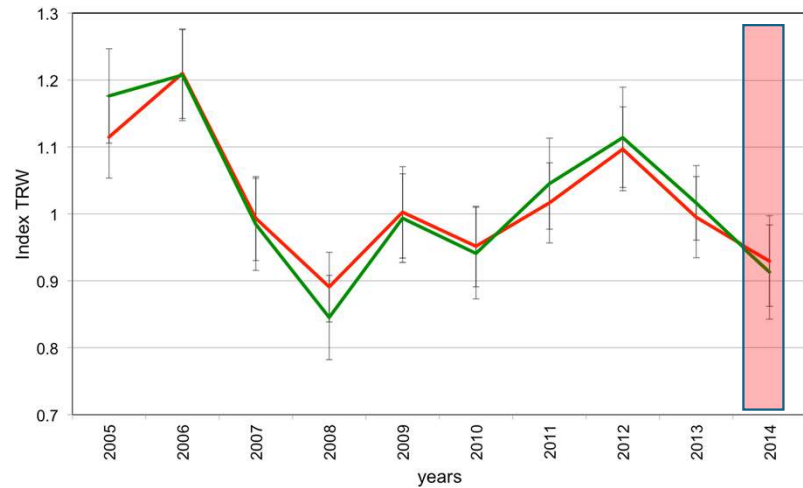
## Temperature distribution



The time–temperature integration above 50°C ranged between 27,825 and 595,948 s °C. The maximum temperature range was 361–851 °C, but peaks were reached only for few seconds. The residence time of temperatures above 50 and 300 °C ranged between 242–2239 and 0–1323 s, and on average was 826 and 165 s, respectively.

## Dendro-isotopic analyses

- burned
- control



- No differences in TRW
- No differences in  $\delta^{13}\text{C}$
- Decrease in  $\delta^{18}\text{O}$  in 2014



Increase in photosynthetic activity and stomatal conductance. But too dense stand for an increase in productivity in the short term

## Take home message

- PB have minor impacts on the ecophysiology of *P. pinea*
- No reduction in radial growth
- No alteration of xylem characteristics
- PB doesn't have major effect on hydraulic functionality and safety

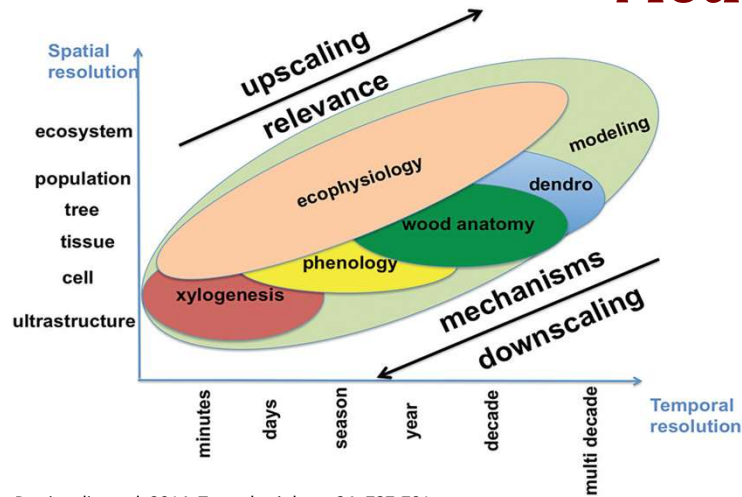
PB effects on plants are different from wildfire effects

Ecophysiological effects of PB depend on species

**July 2017**  
**Southern Italy struck by wildfire!**

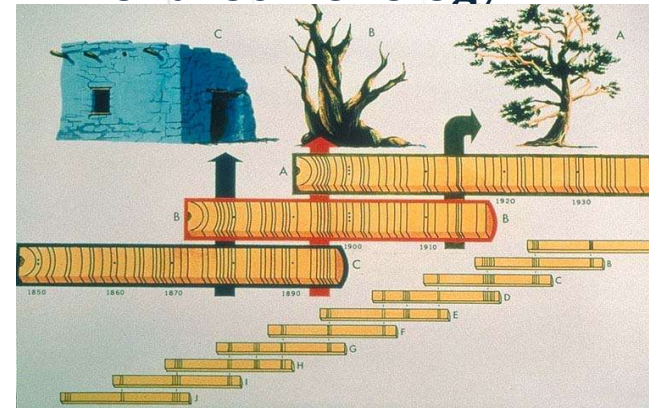


# Methods



Battipaglia et al. 2014, Tree physiology, 34: 787-791.

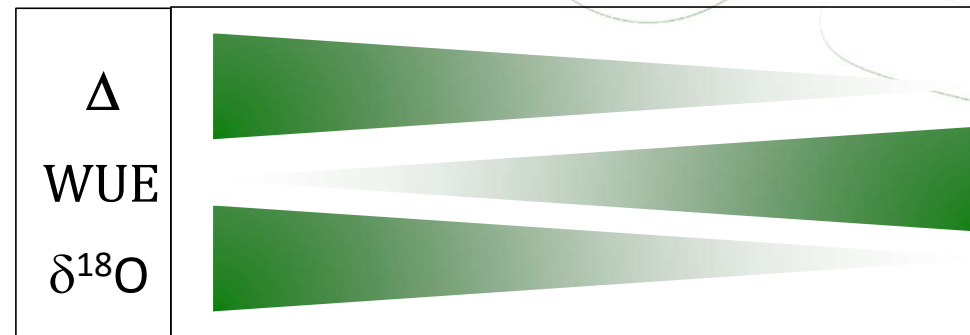
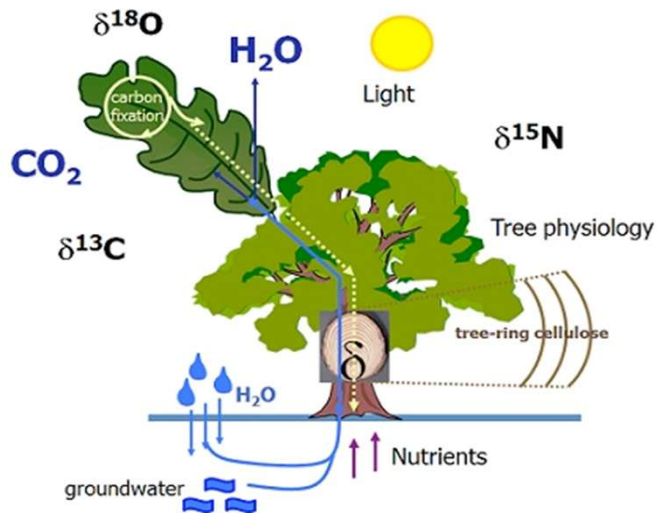
## Dendrochronology



<http://www.ncdc.noaa.gov/paleo/treering.html>

**TIME** →

## Stable isotopes



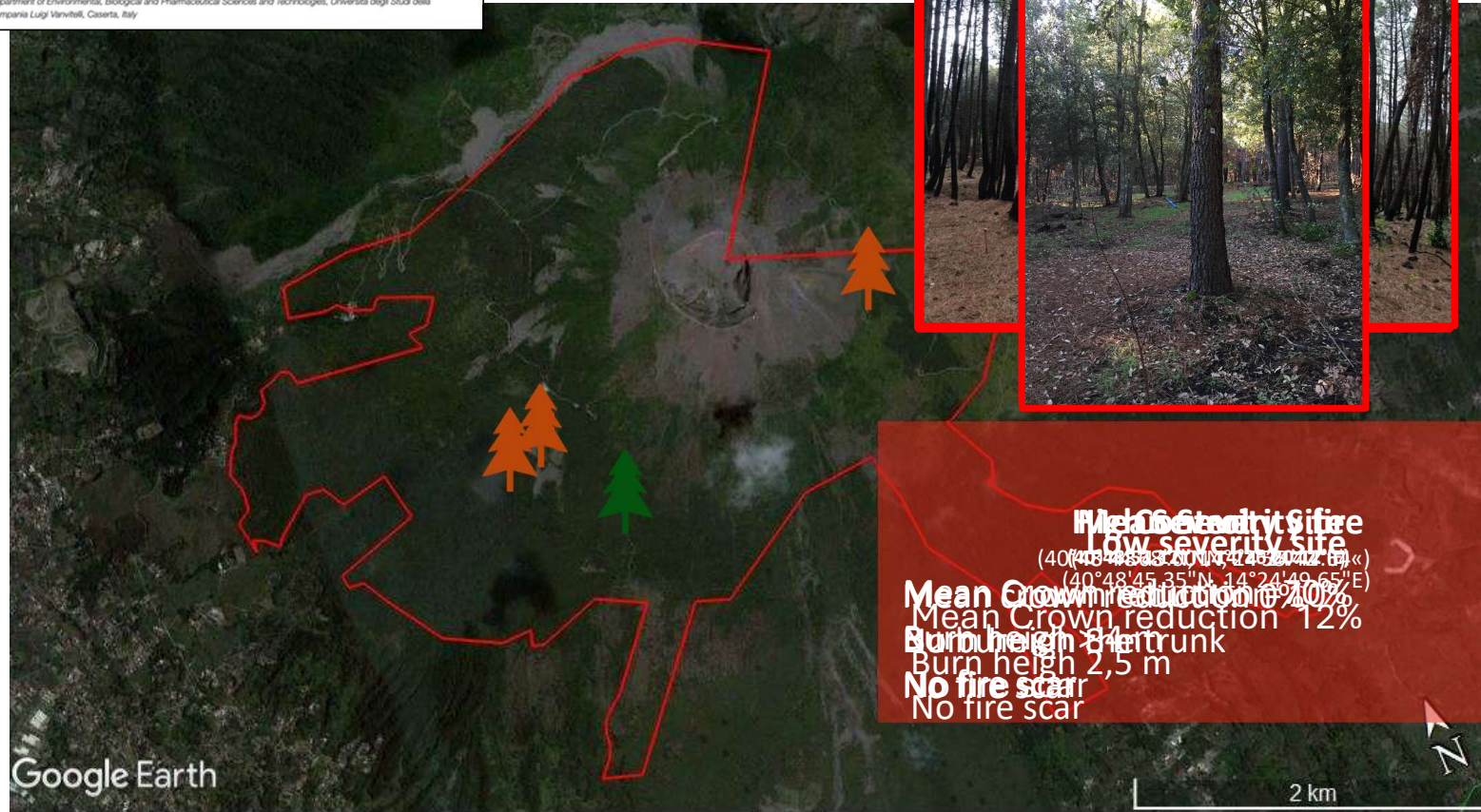
$\Delta^{13}C$  as proxy of Water use efficiency  
 $\delta^{18}O$  as proxy of stomatal conductance



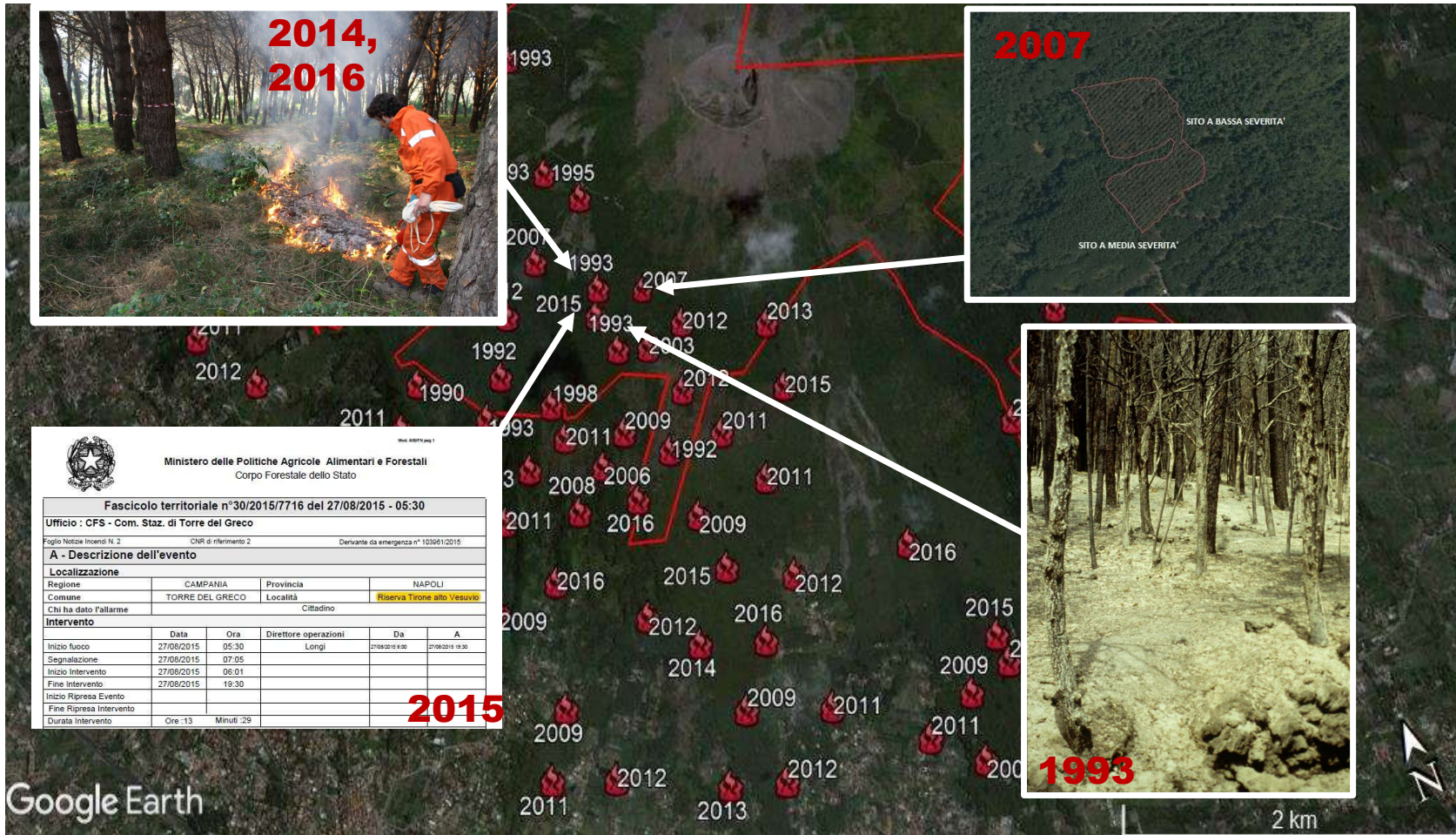
## Fire Severity Influences Ecophysiological Responses of *Pinus pinaster* Ait

Francesco Niccoli, Assunta Esposito, Simona Altieri and Giovanna Battipaglia\*

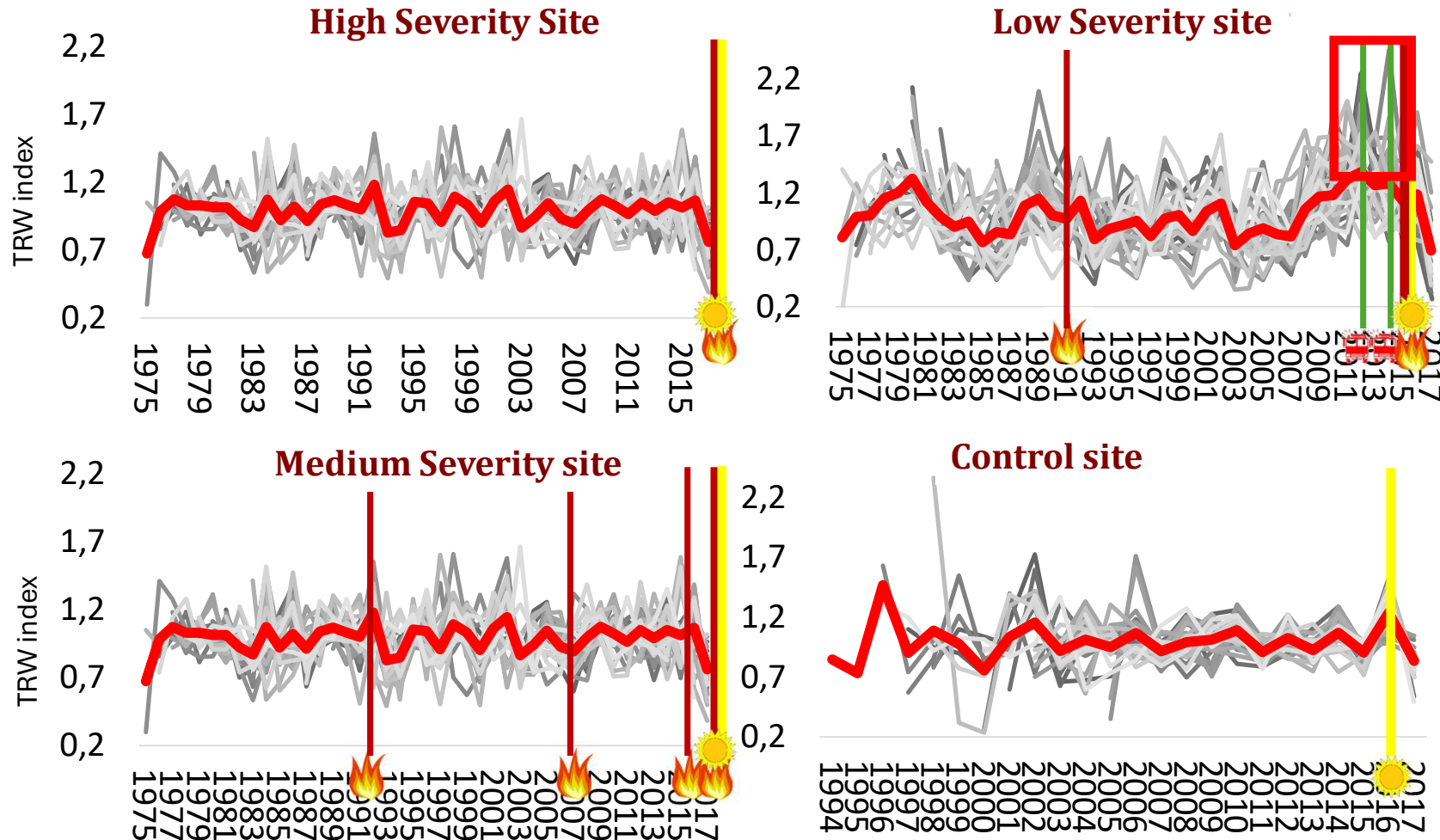
Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, Università degli Studi della Campania Luigi Vanvitelli, Caserta, Italy



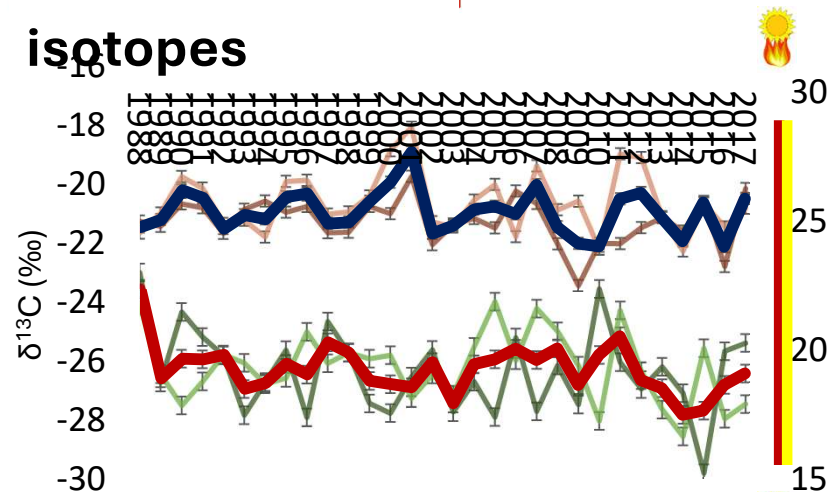
# HISTORICAL RECONSTRUCTION OF PAST WILDFIRES



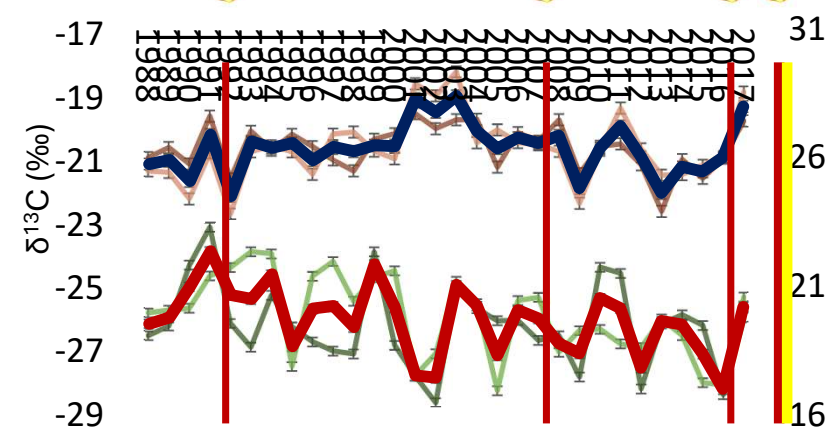
# TREE GROWTH



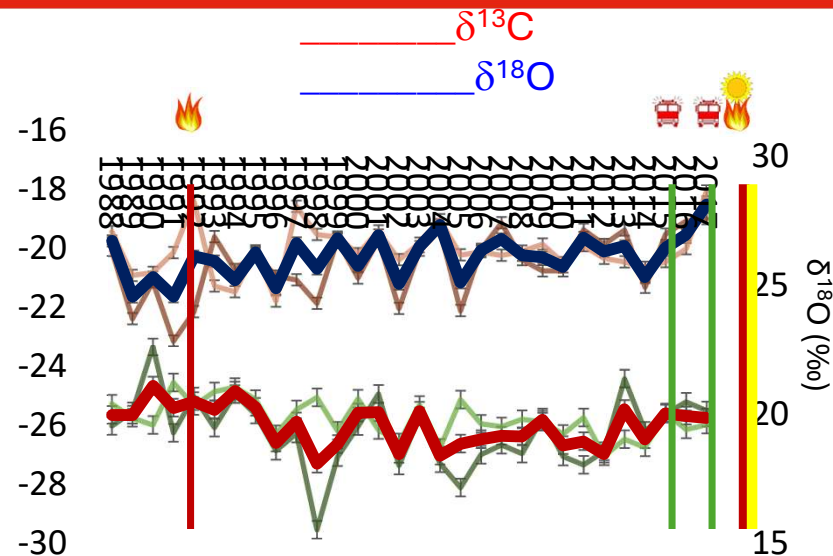
# C and O isotopes



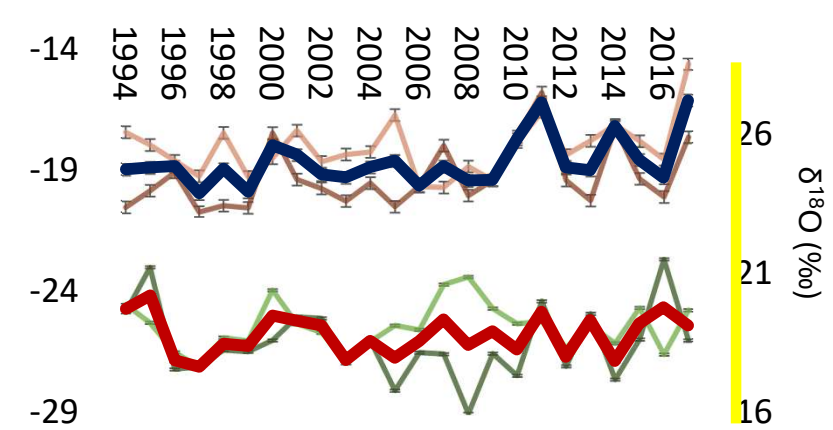
High Severity



Medium severity

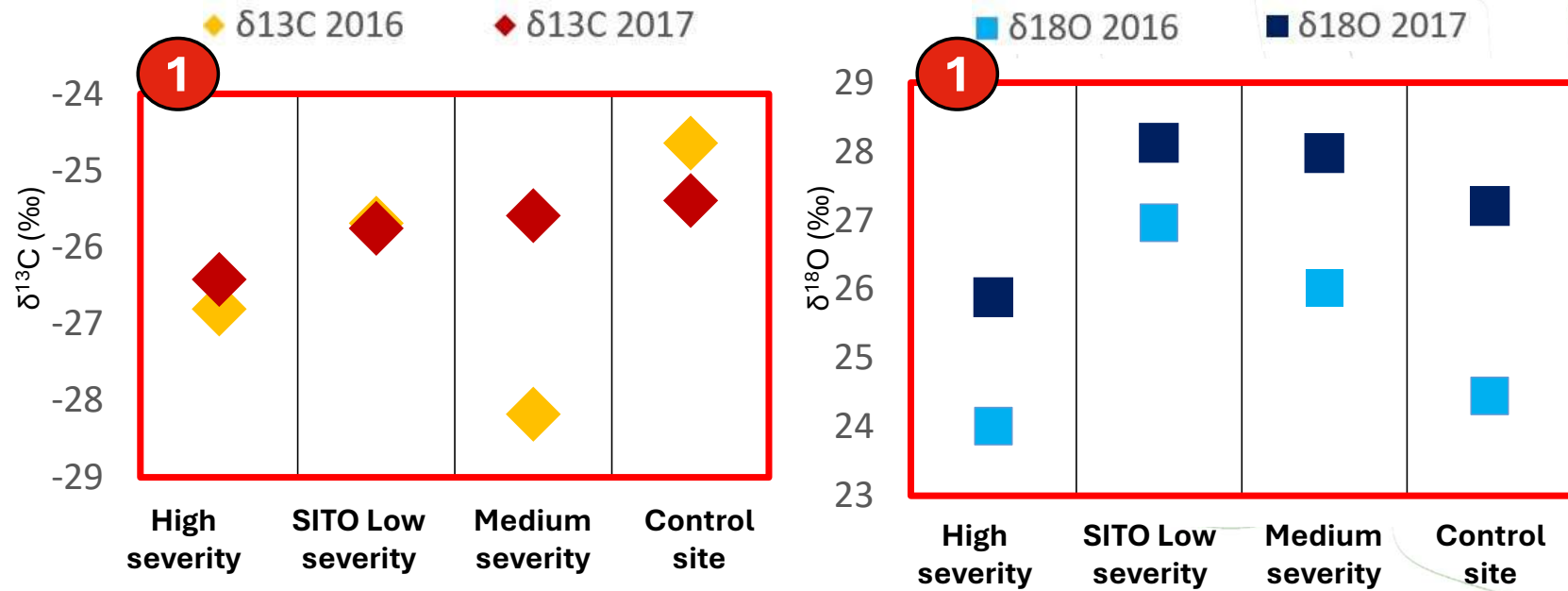


Low severity



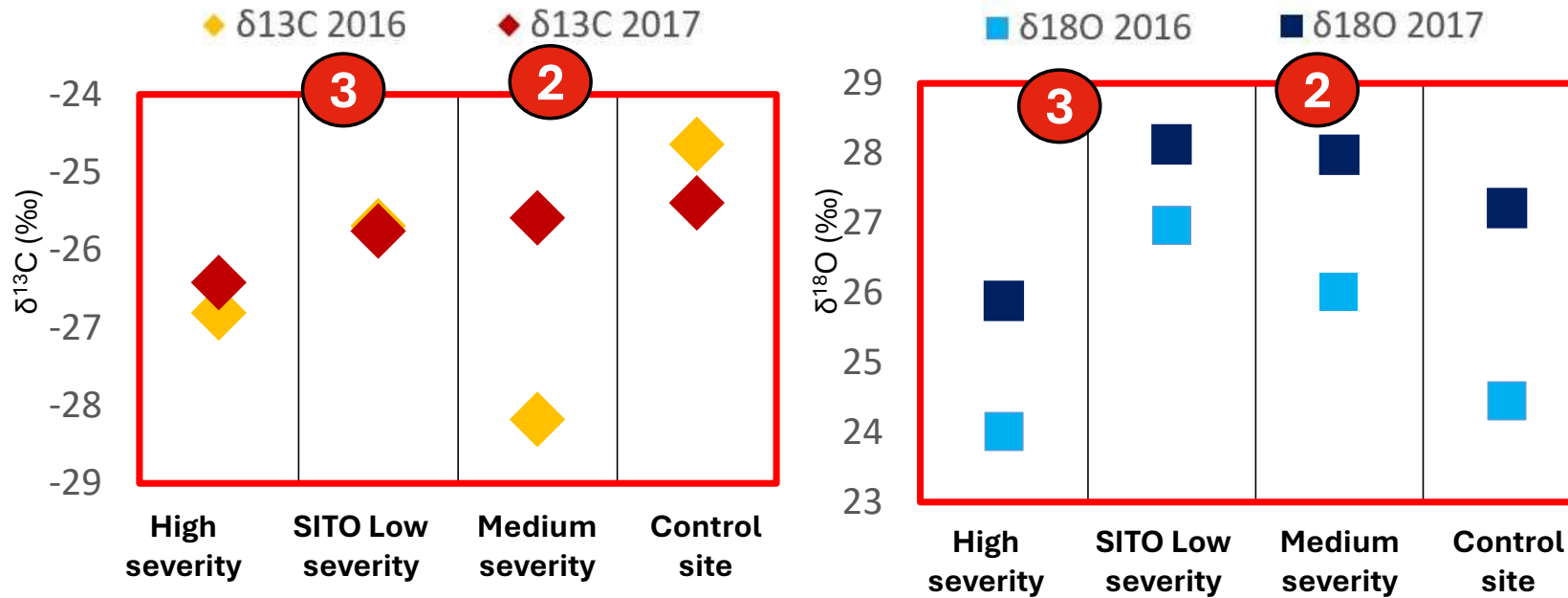
Control Site

# What the isotopes tell us?



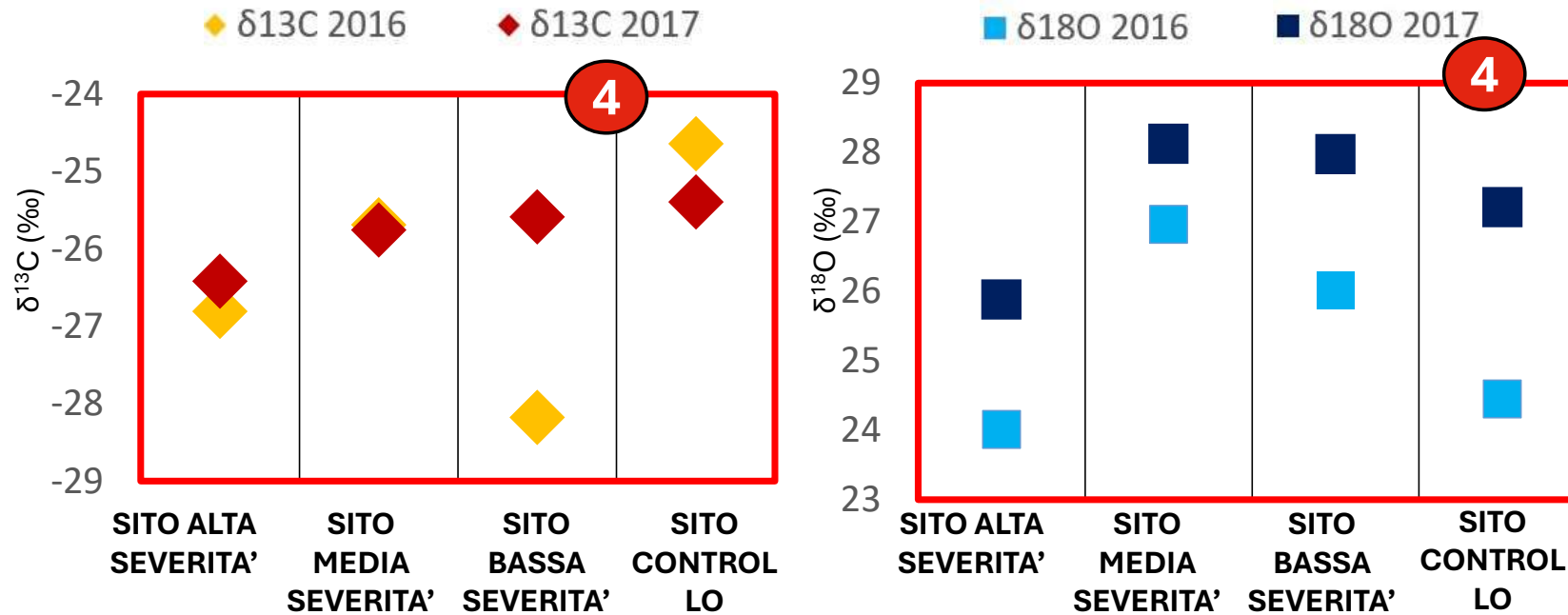
**1 High severity site  $\uparrow\delta^{13}\text{C}$   $\uparrow\delta^{18}\text{O}$**   
we could hypothesize that the plants to protect themselves from strong stress condition, due to fire, and as a consequences of the serious observed crown damage, closed their stomata and lowered their photosynthetic activity. These conditions explain the observed reduction in growth of 2017 tree ring.

# What the isotopes tell us?



**3** Medium severity site  $\uparrow\delta^{13}\uparrow\delta^{18}\text{O}$ ; Low severity  $\approx\delta^{13}\uparrow\delta^{18}\text{O}$   
Slow Stomatal conductance and photosynthetic activity

# What the isotopes tell us?



4

## Control site $\downarrow\delta^{13}\text{C}$ $\uparrow\delta^{18}\text{O}$

This variation, compared to the previous year, can indicate a lower photosynthetic activity and an unchanged stomatal conductance (Scheidegger et al., 2000) probably related to the extreme hot conditions experienced in that summer by all the plants.

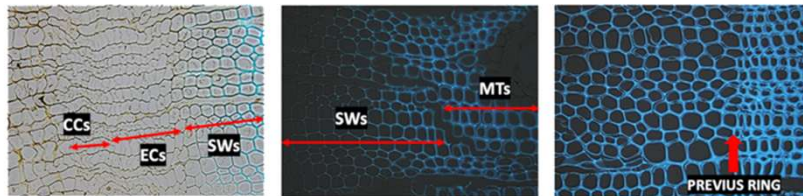
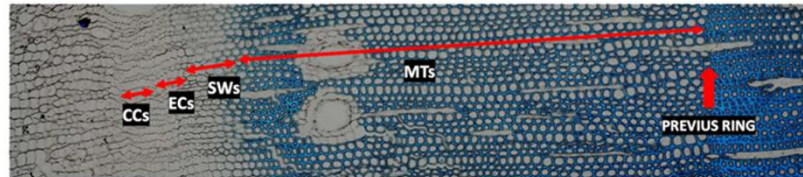
All the sampled trees in the burned sites showed a decrease in tree growth in 2017, in particular in the latewood at high-severity site. In all the sites the high temperatures and the time of exposure to the flames were not sufficient to determine the death of the cambium and all the trees, even if reduced their stomatal conductance and photosynthetic activity, were able to complete the 2017 seasonal wood formation.

Our findings demonstrated that *P. pinaster* growth reduction is strictly linked to the percentage of crown scorch and that even trees with high level of crown scorched could survive.

Further, the dendrochronology analyses showed that several individuals experienced and endured higher fire severity than the 2017 wildfire.

## Medium long-term study

From 2019 to 2020



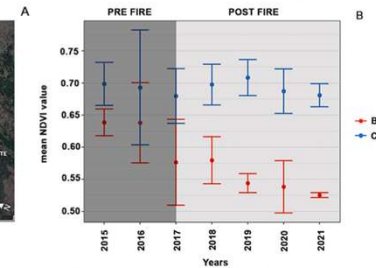
### Xylogensis monitoring



Fire affects wood formation dynamics and ecophysiology of *Pinus pinaster* Aiton growing in a dry Mediterranean area

F. Niccoli<sup>a,b</sup>, A. Pacheco-Solana<sup>a,b</sup>, V. De Micco<sup>c</sup>, G. Battipaglia<sup>a</sup>

<sup>a</sup> Department of Environmental, Biological, Pharmaceutical Sciences and Technologies, University of Campania "Luigi Vanvitelli", Via Vivanti 43, 81100 Caserta, Italy  
<sup>b</sup> The Earth Institute, Tree-ring Laboratory, Lamont-Doherty Earth Observatory of Columbia University, New York 10964, United States of America  
<sup>c</sup> Department of Agricultural Sciences, University of Naples Federico II, via Università 100, 80055, Portici, Italy

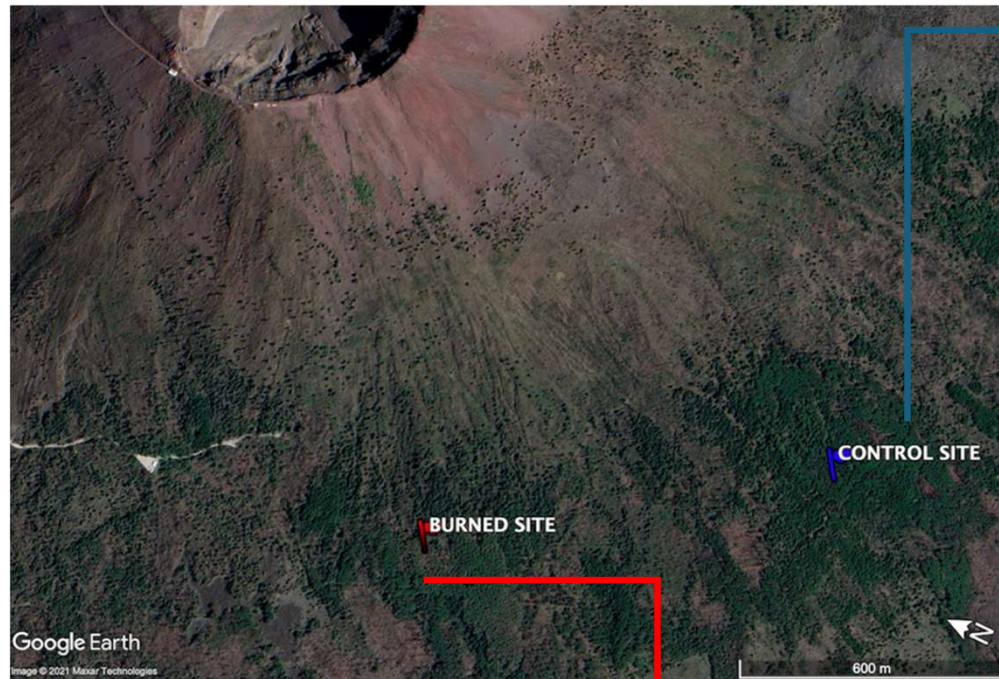


Effects of wildfire on growth, transpiration and hydraulic properties of *Pinus pinaster* Aiton forest

Francesco Niccoli<sup>a,\*</sup>, Arturo Pacheco-Solana<sup>a,b</sup>, Sylvain Delzon<sup>c</sup>, Jerzy Piotr Kabala<sup>a</sup>, Shahla Asgharina<sup>d</sup>, Simona Castaldi<sup>d</sup>, Riccardo Valentini<sup>d</sup>, Giovanna Battipaglia<sup>a</sup>

Study case of ecophysiology with the TreeTalker system

Study area



Unburned trees not defoliated



Burned trees with crown damage (50% of defoliation)

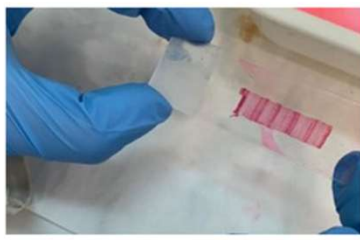
Study case of ecophysiology with the TreeTalker system Approach used

CONTROL TREES vs BURNED TREES

Cavitation test

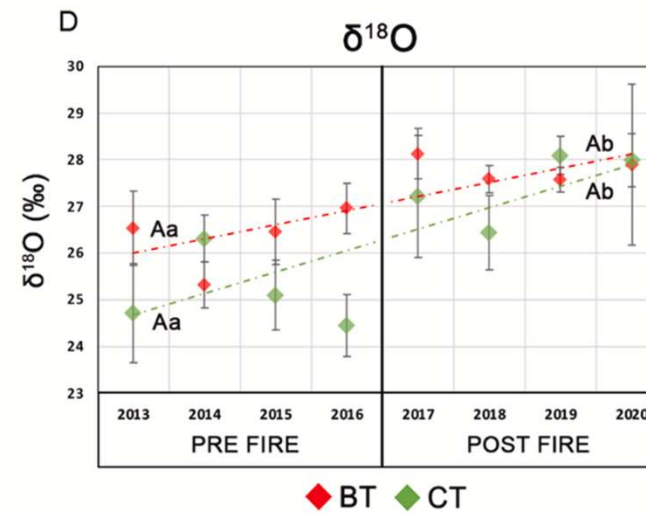
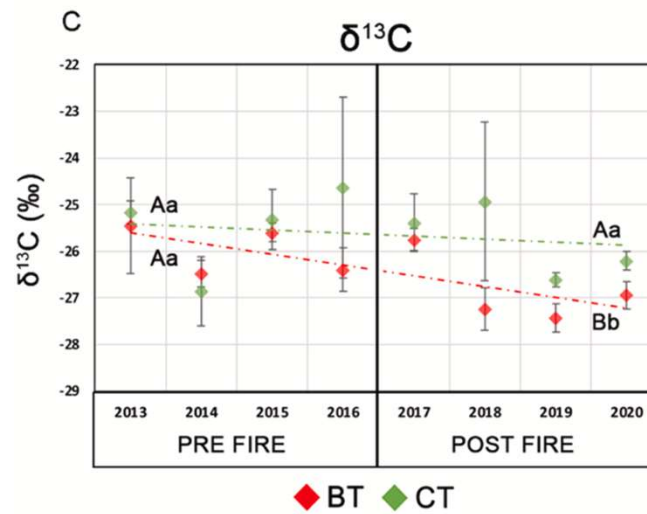
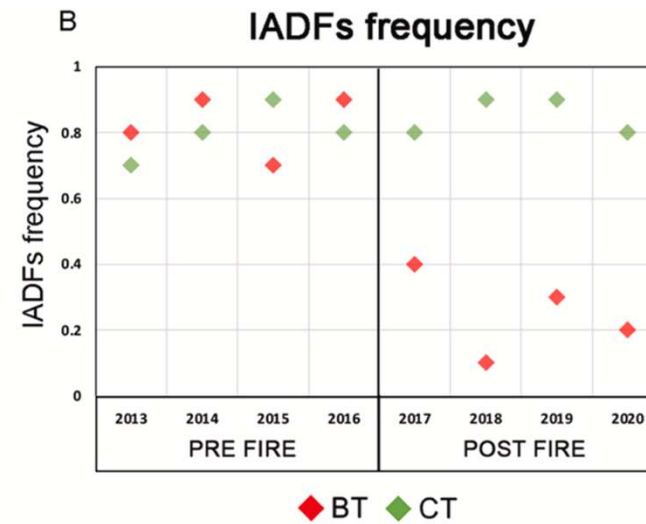
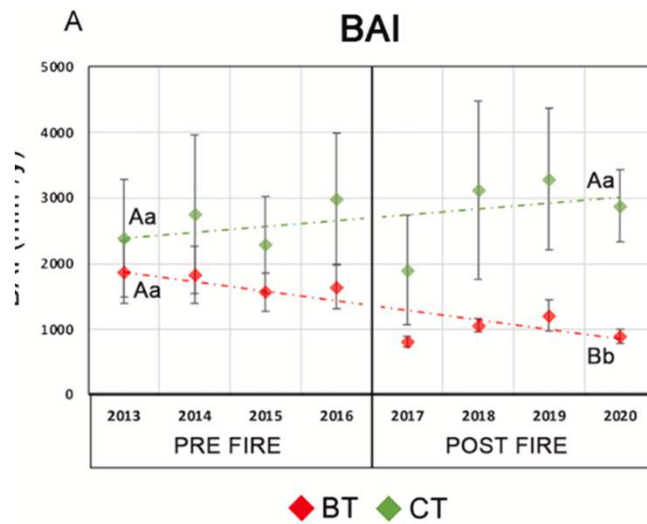


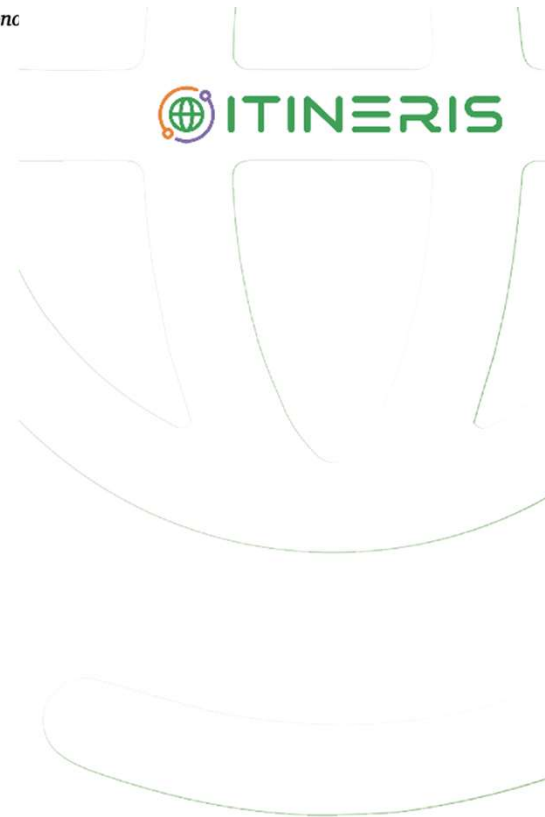
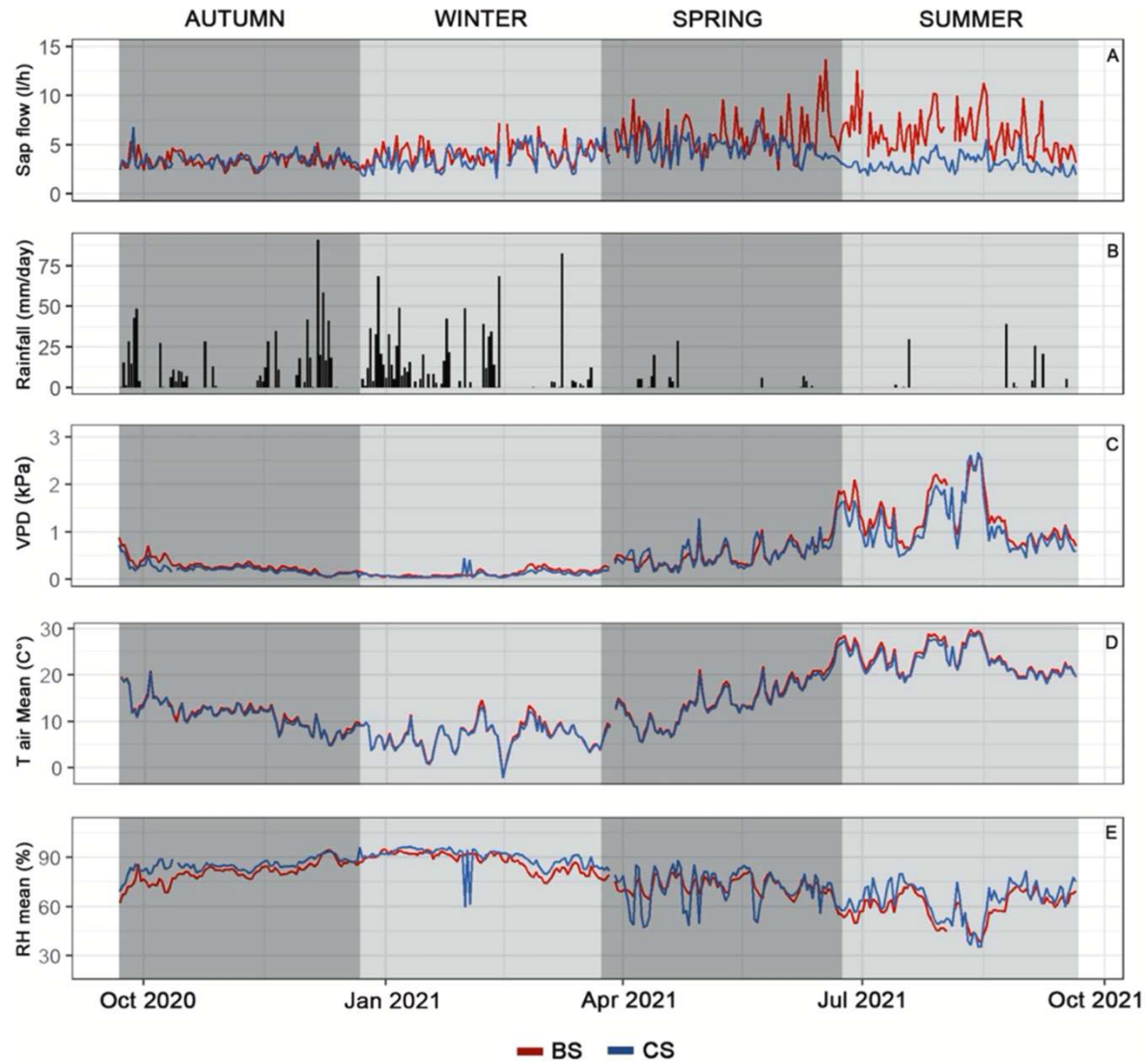
Dendro-anatomy analysis



TreeTalker monitoring







Tree Physiology

Rolf T. W. Siegwolf  
J. Renée Brooks  
John Roden  
Matthias Saurer *Editors*

# Stable Isotopes in Tree Rings

Inferring Physiological, Climatic and  
Environmental Responses

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THANKS!

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3.1: "Fund for the realisation of an integrated system of research and innovation infrastructures"

