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1º CONGRESO SOBRE FERTILIZACIÓN
26 al 27 Septiembre 2017
Madrid - España

Los ácidos húmicos en la rizosfera: vectores principales del crecimiento y nutrición de las plantas

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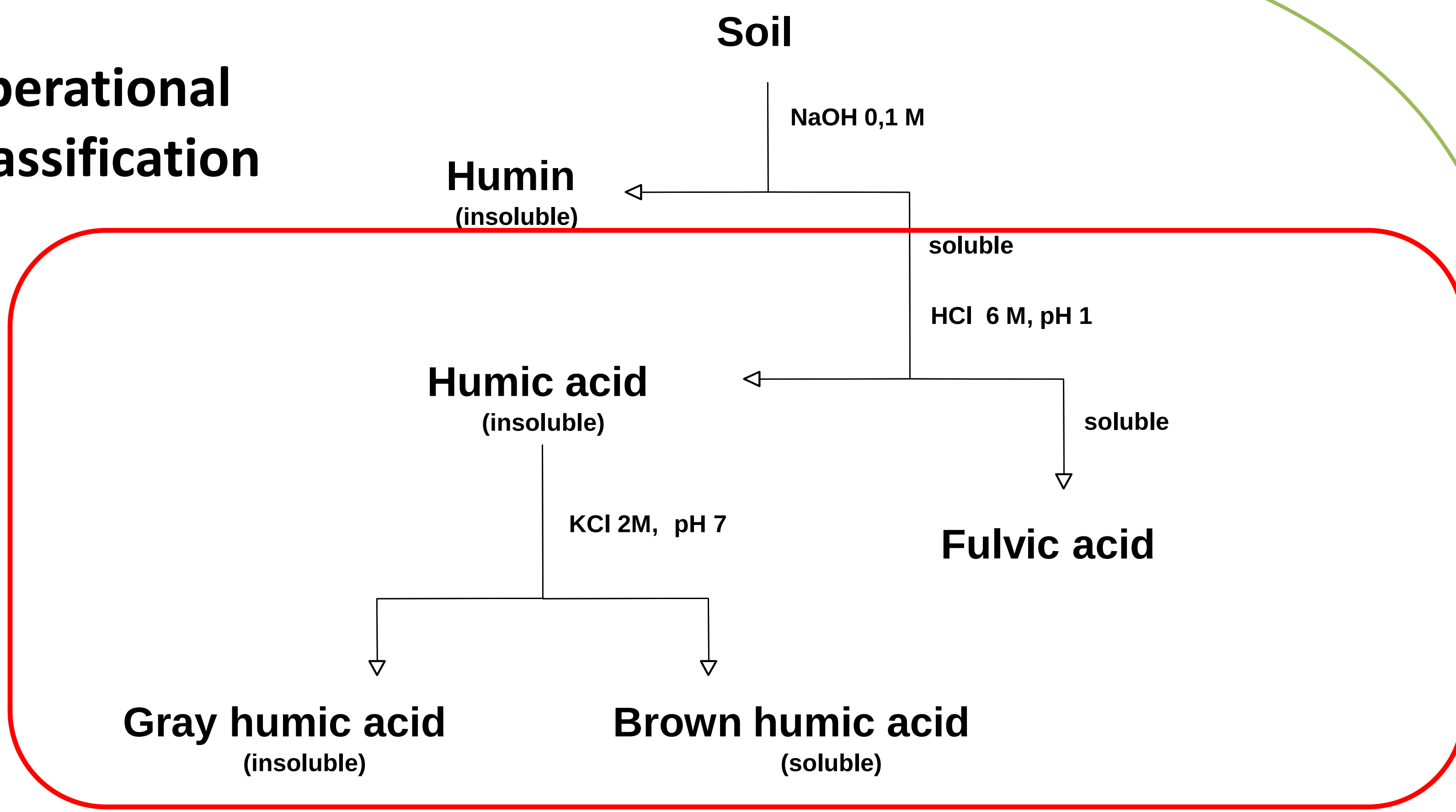
3 Centre Mondial d'Innovation (CMI), Grupo Roullier, Saint Maló, Francia

Operational Classification

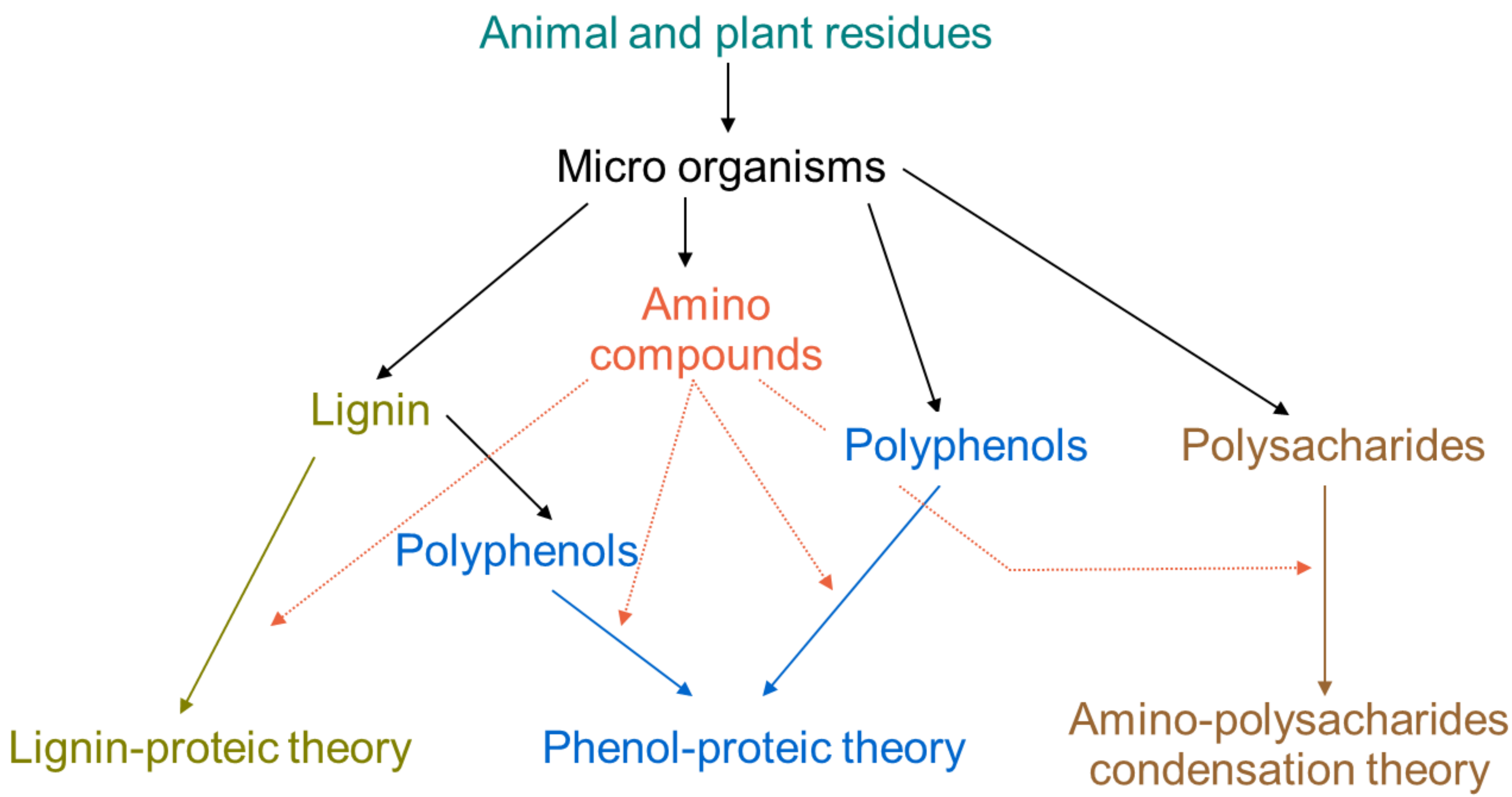


Stevenson, 1995

Alternativas a la clasificación operacional IHSS?

Artificial	Fresh	Compost	Sedimentary
(Char-Biochar, Chemical Oxidation, Anaerobic digestion…..)	(fresh organic matter)		(soil, aquatic organic matter)
Artificial HS (AHS)	Fresh HS (FHS)	Compost HS (CHS)	Sedimentary HS (SHS)
AHA	FHA	CHA	SHA
AFA	FFA	CFA	SFA

Mora et al., 2014 (In M. N. Khan et al. (eds.) Nitric Oxide in Plants: Metabolism and Role in Stress Physiology, Springer., 2014)


Animal and plant residues
Micro organisms
Amino compounds
Lignin
Polyphenols
Polysacharides
Polyphenols
Lignin-proteic theory
Phenol-proteic theory
Amino-polysacharides condensation theory

Controversia

PERSPECTIVE

doi:10.1038/nature10386

Persistence of soil organic matter as an ecosystem property

Michael W. I. Schmidt[1]*, Margaret S. Torn[2,3]*, Samuel Abiven[1], Thorsten Dittmar[4,5], Georg Guggenberger[6], Ivan A. Janssens[7], Markus Kleber[8], Ingrid Kögel-Knabner[9], Johannes Lehmann[10], David A. C. Manning[11], Paolo Nannipieri[12], Daniel P. Rasse[13], Steve Weiner[14] & Susan E. Trumbore[15]

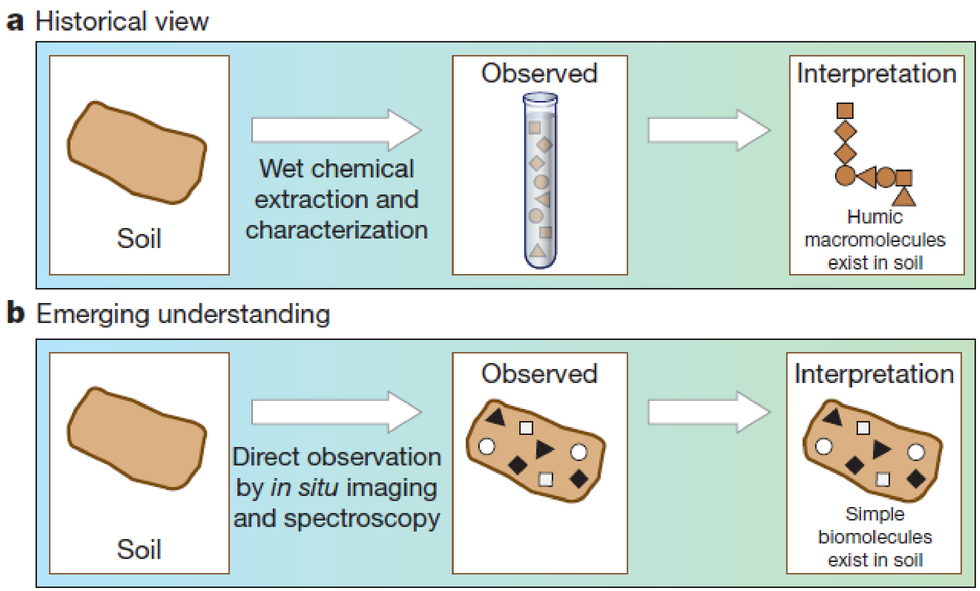


Figure 2 | In soil, the existence of humic substances has not been verified by direct measurements. **a**, Based on chemical analysis of the extracted materials (Observed), the *de novo* formation of humic polymers (Interpretation) was postulated to be an important source of recalcitrant SOM. **b**, Direct high-resolution *in situ* observations with non-destructive techniques (Observations) have been able to explain the functional group chemistry of the extracted humic substances as relatively simple biomolecules (Interpretation), without the need to invoke the presence of unexplainable macromolecules[100].

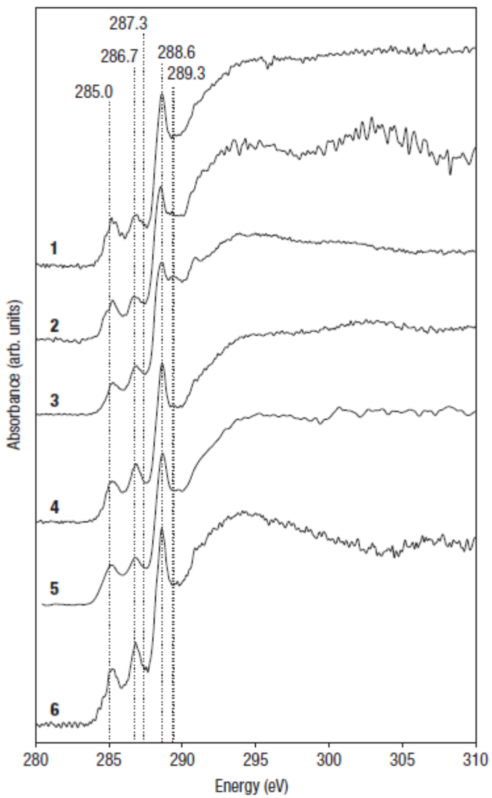


Figure 1 Molecular characterization of total soil carbon by C-1s NEXAFS spectroscopy using transmission spectroscopy of soil extracts obtained from different forest sites. 1, Arnot Forest on schist parent material in New York State, USA. 2, McGowen Forest on glacial till in New York State, USA. 3, Barro Colorado Island Forest on volcanic facies in Panama. 4, Embrapa Forest on Tertiary sediments in central Amazonia, Brazil. 5, Nandi Forest on biotite-gneiss in western Kenya. 6, Franz Josef Forest on schist outwash terraces in New Zealand.

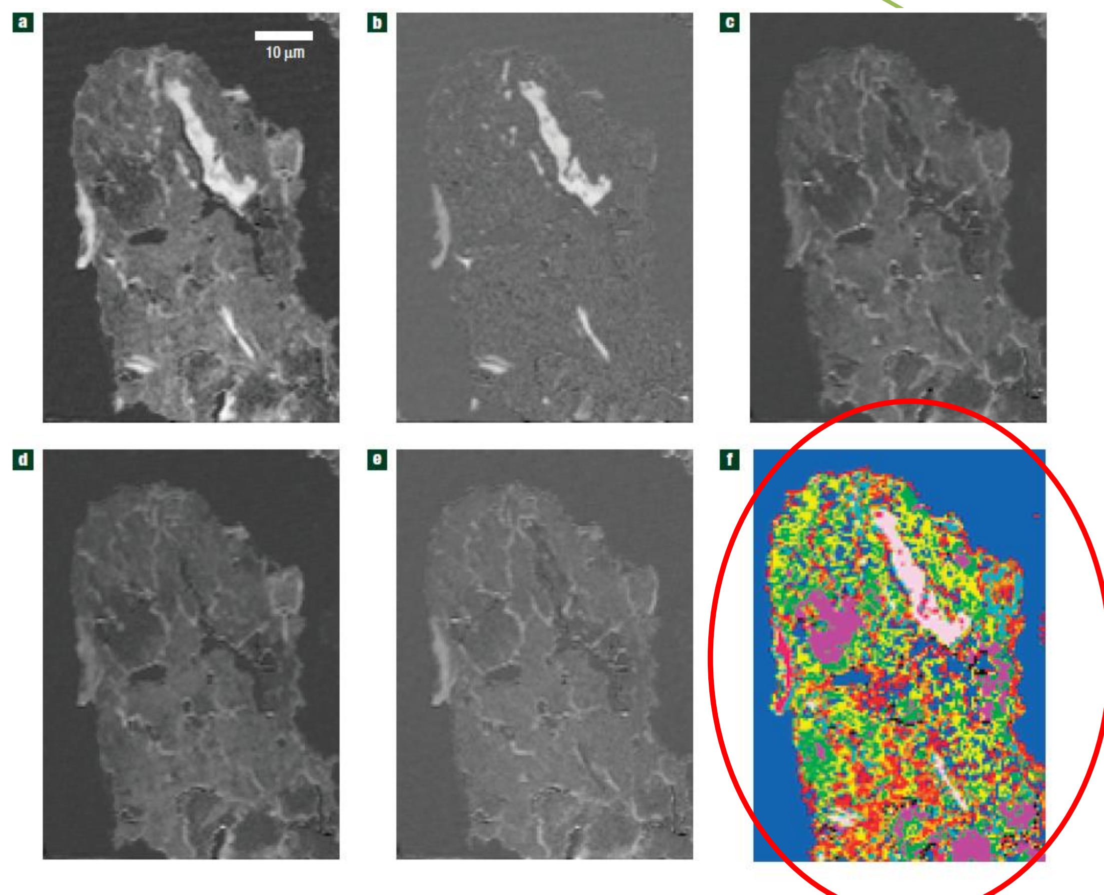
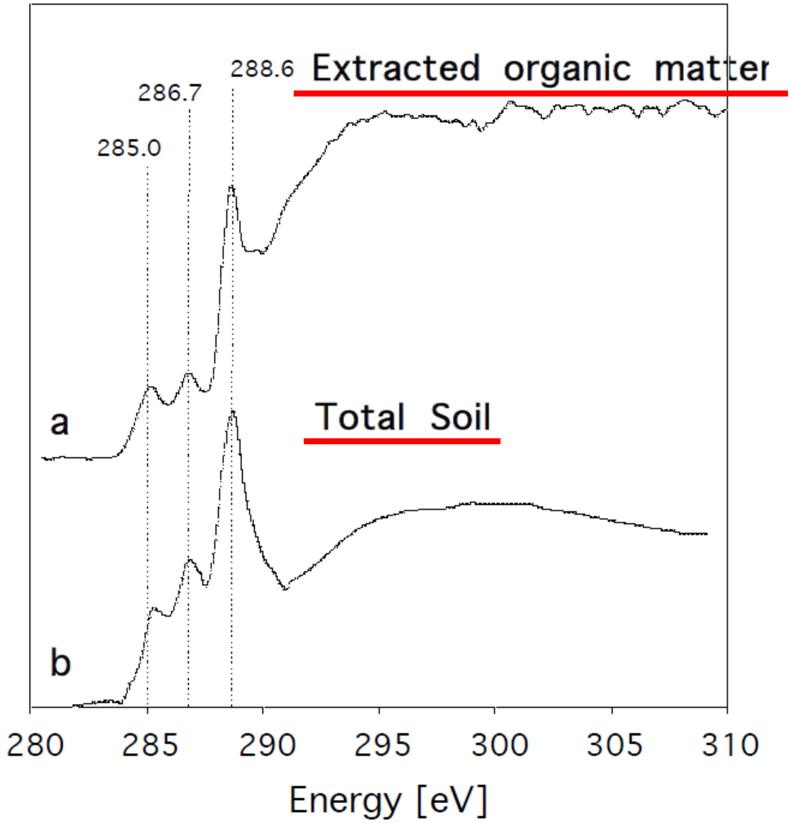


Figure 2 Distribution of carbon contents and molecular forms in a soil microassemblage from Nandi Forest (Kenya) determined by NEXAFS in combination with STXM. a, Total carbon (subtraction of energy region at 280–282 eV from 290–292 eV). **b,** Aromatic carbon (subtraction of energy region at 280–282 eV from 284–286 eV). **c,** Aliphatic carbon (subtraction of energy region at 284–286 eV from 287.3–287.8 eV). **d,** Carboxyl carbon (subtraction of energy region at 284–286 eV from 288–289 eV). **e,** Phenolic carbon (subtraction of energy region at 284–286 eV from 286.4–287.4 eV). **f,** Cluster map of carbon forms (clusters are shown separately with their spectra in Fig. 3).



Supplementary Figure 6. Molecular characterization of total carbon in soil (Nandi Forest soil from Kenya). C (1s) NEXAFS spectra of **a**, extracts of organic matter obtained by transmission (STXM) in comparison to **b,** total soil organic matter determined by total fluorescence yield (SGM).

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Fernan ... -NMR)

AQUATIC HUMIC SUBSTANCES

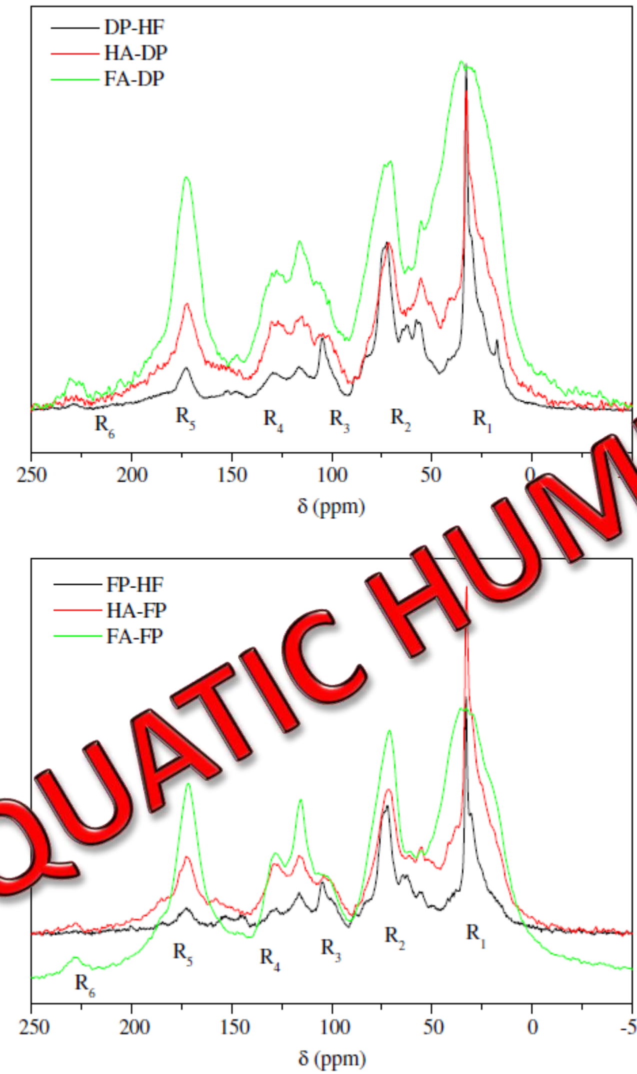

Fig. 3. ^{13}C CP/MAS-NMR spectra of the peat samples DP and FP and their respective FA and HA.

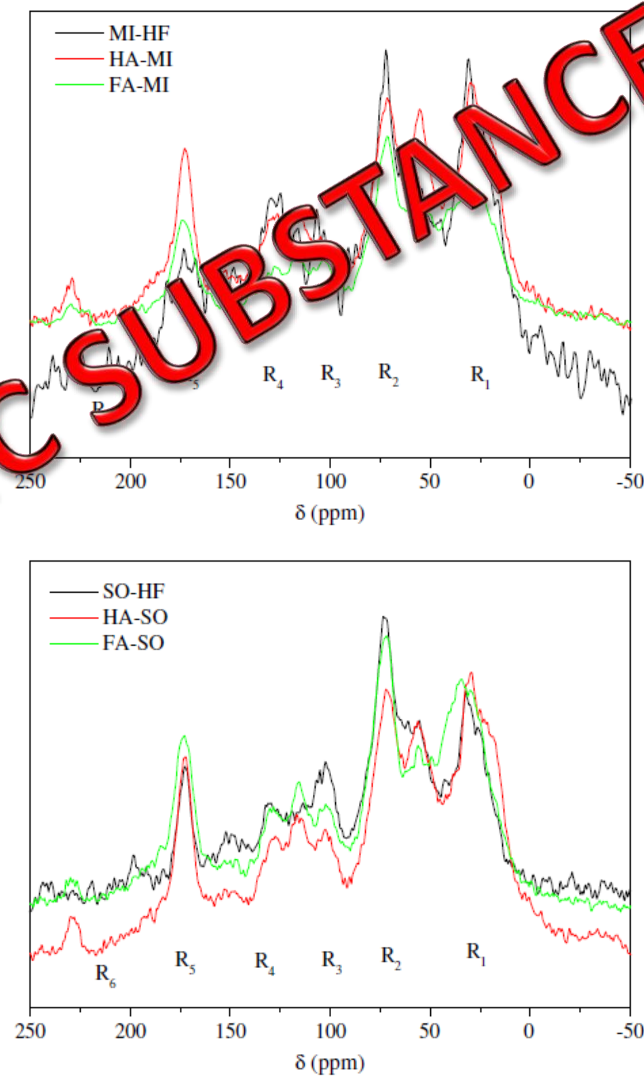

Fig. 4. ^{13}C CP/MAS-NMR spectra of the soil samples MI and SO and their respective FA and HA.

Soil

Distribution in organomineral complexes

- **Partially transformed : aa, sugars, lignin ????**
- **New molecules from humification ???**
- **Mixed-systems**

↓ Rainfall; Soil water infiltration

Aquatic – Soil Solution ======= Biological-Chemical properties

- **Reorganization of SOM in DOM**
- **Fractionation ===== Deep knowledge on chemical humic nature**

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About the chemical nature of HS

Soil Science 164 (11) 1999
Soil Science 166 (11) 2001

Open Debate

Macromolecules
Polyelectrolites
Ramdom coil

Swift R.

Aggregates
Micelle-like

Wershaw R.

Supramolecules

Piccolo A.

Self-associating aggregate

Artifacts ??

Burdon J.

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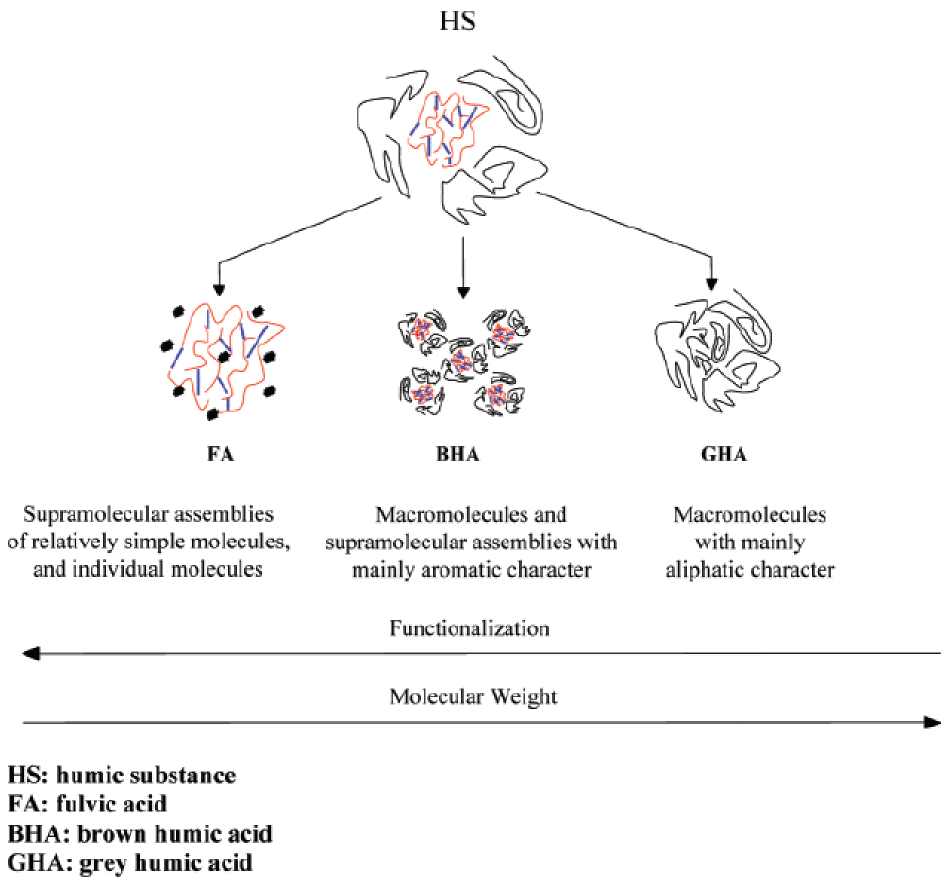
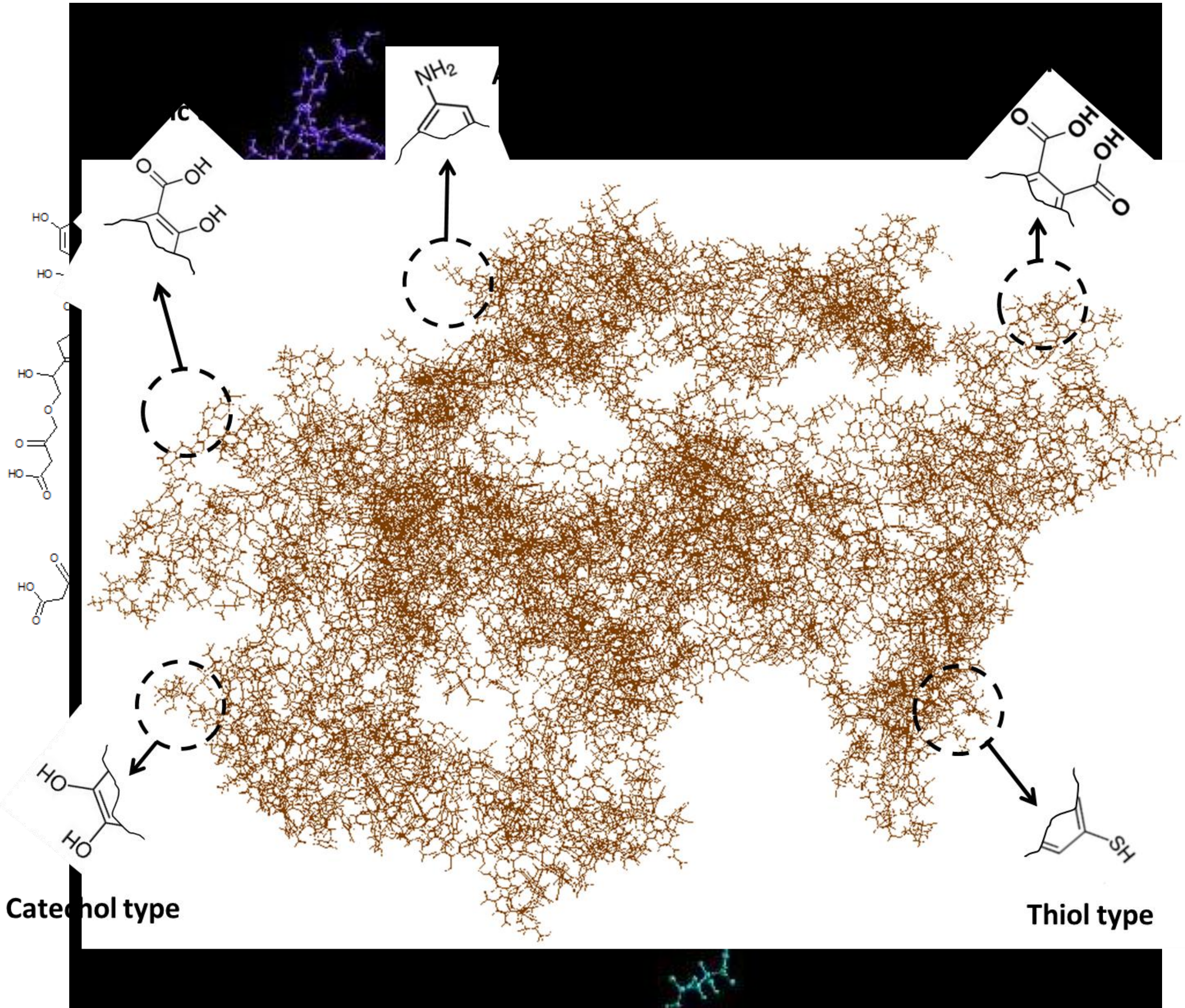


Figure 4. Main molecular patterns associated with the principal fractions of the studied humic substances in solution.

Baigorri et al., J. Phys Chem B., 2007


NH2
OH
OH
O
OH
OH
O
HO
HO
SH
Cate[redacted]hol type
Thiol type

Soil Conditioner

Indirect Effects

Physical features , porosity, aggregation,
Nutrient bioavailability ,
microbiota increase

Biostimulant

Direct Effects

Phytoregulator pathways

Nutrient root uptake regulation

HS Action on plants

Mechanisms ??

Intrinsic Factors

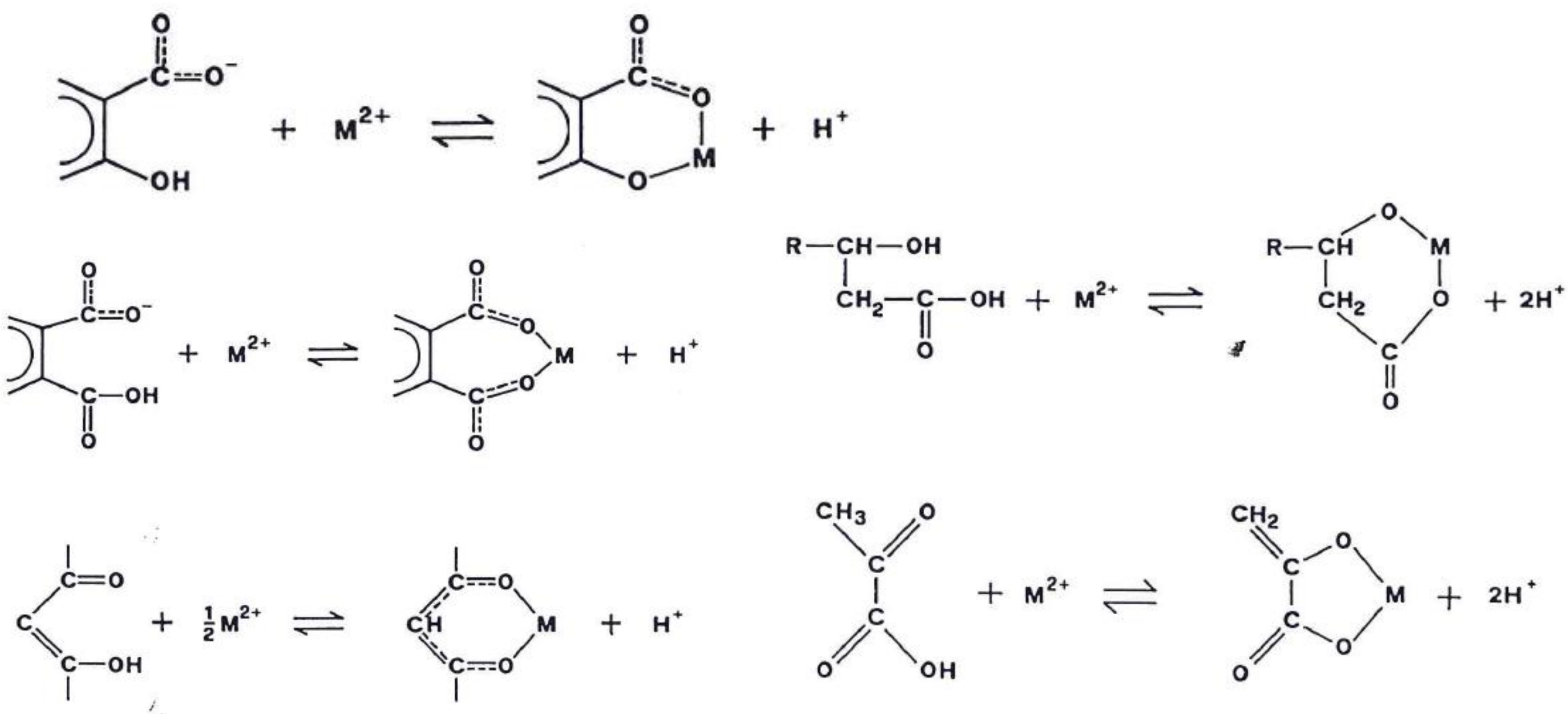
1ª - 3ª Structural features

Extrinsic Factors

Substrate characteristics,
Crop management,
Environmental conditions,
Plant species,
Application type and moment

Indirect Effects

Capacidad de quelatación de metales



TDD

Table 1. Stability (Log K_0) and solubility (IS) of different

Table 2. Effect of metal-humic complexes (Cu–SHE, Zn–SHE and Fe–SHE) on the concentration of soil available Zn, Cu and Fe, and on shoot dry weight, root dry weight and shoot Zn-, Cu- and Fe-content of wheat plants cultivated in Velate soil (humic rate in SHE and metal-SHE treatments was 53 mg kg^{-1})

Velate soil	Shoot dry weight ($g\ pot^{-1}$)	Root dry weight ($g\ pot^{-1}$)	Zn content ($mg\ kg^{-1}$)		Cu content ($mg\ kg^{-1}$)		Fe content ($mg\ kg^{-1}$)	
Treatments			Shoot	Soil#	Shoot	Soil#	Shoot	Soil#
Control	2.31a†	1.41ab	21.89a	2.22a	2.87a	0.28a	66.35bc	268a
SHE	1.87a	1.48ab	25.76a	2.46a	2.83a	0.72a	61.87b	269a
Cu – SHE (metal rate)								
Dose 1 (2.50 mg kg^{-1})	3.90c	2.62c	23.14a	2.46a	4.52b	1.99b	41.42a	297a
Dose 2 (5.00 mg kg^{-1})	3.23b	2.67c	28.57a	2.73a	6.76c	3.19c	82.02c	284a
Zn – SHE (metal rate)								
Dose 1 (2.50 mg kg^{-1})	1.80a	0.89a	34.58b	3.55b	2.61a	0.46a	55.53ab	271a
Dose 2 (5.00 mg kg^{-1})	2.40a	0.98a	39.29b	4.21b	2.37a	0.36a	84.37c	267a
SHE – Fe (metal rate)								
Dose 1 (2.50 mg kg^{-1})	2.31a	1.56ab	25.17a	2.65a	3.88ab	0.48a	82.79c	279a
Dose 2 (5.00 mg kg^{-1})	2.46a	1.70b	23.17a	2.61a	2.76a	0.45a	69.61bc	285a

† IS is defined as the SHE:metal ratio in the reaction corresponding to the precipitation of SHE over 50%.

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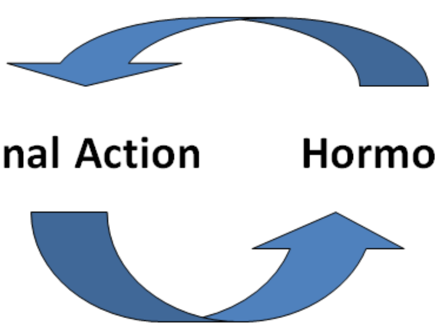
Direct Effects

Nutritional hypothesis

Pseudo-hormonal Hypothesis

Important relationships between both Hypothesis



Nutritional Action **Hormonal Action**

Table 1. Reports of humic substances effects on plant growth regulation via hormone-like activity and proton pump activation

Humic substances			Treatment	Plant material	Pump activity (%)			Effects		Reference
Source	Size	Dose						Nutrient & others	Hormonal	
Podzol Soil	Unfractionated	25 mg C L^{-1}	20 min, *in vitro*	*A. sativa*	-41	N/V	N/V	K^+ and SO_4 uptake	N/V	Maggioni et al. 1987
	>100 kDa				-34	N/V	N/V	K^+ and SO_4 uptake	N/V	
	<9 kDa				-27	N/V	N/V	K^+ and SO_4 uptake	N/V	
Vermicompost	Unfractionated		20 min, *in vitro*	*A. sativa*	-27	N/V	N/V	K^+ and SO_4 uptake	N/V	
	>100 kDa				-24	N/V	N/V	K^+ and SO_4 uptake	N/V	
	<9 kDa				-27	N/V	N/V	K^+ and SO_4 uptake	N/V	
Vermicompost	>3.5 kDa	1.1−10 mg C L^{-1}	5 h, *in vivo*	*A. sativa*	N/V	N/V	N/V	NO_3 uptake	IAA, GA and CK	Dell'Agnola and Nardi, 1987
	<3.5 kDa	1.1−10 mg C L^{-1}	5 h, *in vivo*	*A. sativa*	N/V	N/V	N/V	NO_3 uptake	IAA, GA and CK	
Sphagnum peat	<5 kDa	0.5 mg L^{-1}	5 min, *in vitro*	*A. sativa*	35	35	N/V	H^+ extrusion	N/V	Pinton et al. 1992
Lythic rendoll soil	<5 kDa	0.075 mg C L^{-1}	20 min, *in vivo*	*A. sativa*	435	N/V	N/V	NO_3 uptake	N/V	Varanini et al. 1993
	>5 kDa	0.5 mg C L^{-1}	20 min, *in vitro*	*A. sativa*	30	N/V	N/V	NO_3 uptake	N/V	
Vermicompost	<3.5 kDa				N/V	N/V	N/V	N/V	IAA	Muscolo et al. 1998
Sphagnum peat	WEHS	5 mg C L^{-1}	4 h, *in vivo*	*Zea mays*	28	N/V	N/V	NO_3 and NH_4^+	N/V	Pinton et al. 1999
Cambisol soil	LMW	0.1 mg L^{-1}	16 h, *in vivo*	*Z. mays*	89	N/V	N/V	NO_3 uptake	GA	Nardi et al. 2000
Vermicompost	HMW	20 mg C L^{-1}	7 d, *in vivo*	*Z. mays*	66	N/V	N/V	H^+ extrusion	IAA	Canellas et al. 2002
Vermicompost	<3.5 kDa	0.75 mg C L^{-1}	48h, *in vivo*	*Z. mays*	N/V	N/V	N/V	NO_3 uptake	IAA	Quaggiotti et al. 2004
Vermicompost	HMW	20 mg C L^{-1}	5 d, *in vivo*	*Z. mays*	89	166	23	H^+ extrusion	IAA	Zandonadi et al. 2007
Sewage sludge	HMW	20 mg C L^{-1}	5 d, *in vivo*	*Z. mays*	191	113	46	H^+ extrusion	IAA	
Ultisol soil	HMW	20 mg C L^{-1}	5 d, *in vivo*	*Z. mays*	75	173	146	H^+ extrusion	IAA	
Incept Soil	HMW	20 mg C L^{-1}	5 d, *in vivo*	*Z. mays*	196	60	123	H^+ extrusion	IAA	
Sphagnum peat	WEHS	50 mg L^{-1}	8h, *in vivo*	*Arabidopsis thaliana*	N/V	N/V	N/V	N/V	No auxin activity	Schmidt et al. 2007
Oxisol soils	HMW	3 – 300 mg L^{-1}	2 w, *in vivo*	*A. Thaliana*	N/V	N/V	N/V	N/V	IAA	Dobbss et al. 2007
		40 mg L^{-1}	10 d, *in vivo*	*Solanum lycopersicum*	N/V	N/V	N/V	N/V	IAA	
	LMW	3 – 300 mg L^{-1}	2 w, *in vivo*	*A. Thaliana*	N/V	N/V	N/V	N/V	IAA	
Vermicompost	Unfractionated <14 kDa	1 mg C L^{-1}	16 h, *in vivo*	*Z. mays*	N/V	N/V	N/V	Several proteins expression, including those related to auxin pathway, proton pumps and nutrient metabolism and uptake.	IAA	Carletti et al. 2008
Leonardite	HMW	5 mg L^{-1}	48 h, *in vivo*	*Cucumis sativus*	130	N/V	N/V	NO_3 uptake and enzymes of N metabolim	N/V	Elena et al. 2009
Leonardite	HMW	5 mg L^{-1}	48h, *in vivo*	*C. sativus*	135	N/V	N/V	Nutrients analysis, NO_3 uptake and enzymes of N metabolim	CK, PA and ABA	Mora et al. 2010
Vermicompost	HMW	20 mg C L^{-1}	4 d, *in vivo*	*Z. mays*	50	N/V	N/V	H^+ extrusion	IAA and NO	Zandonadi et al. 2010
Vermicompost	>3.5 kDa	0.5-2.0 mg C L^{-1}	48 h, *in vivo*	*Z. mays*	N/V	N/V	N/V	N metabolism, secondary metabolism	IAA	Schiavon et al. 2010
Vermicompost	Unfractionated <14 kDa	1 mg C L^{-1}	30, 120 min, *in vivo*	*Z. mays*	N/V	N/V	N/V	HS function by auxin dependent and independent, nutrients uptake, vesicle trafficking, transport mechanism, gene transcription and protein regulation	IAA-independent and dependent effects	Trevisan et al. 2011
Leonardite	HMW	100 mg L^{-1}	72 h, *in vivo*	*C. sativus*	N/V	N/V	N/V	N/V	IAA, ET and NO	Mora et al. 2012

N/V: not verified; IAA: indole acetic acid; ET: ethylene; GA: gibberellic acid; CK: citokynin; PA: polyamines; ABA: abscisic acid; WEHS: water extractable humic substances; HMW: high molecular weight; LMW: low molecular weight.

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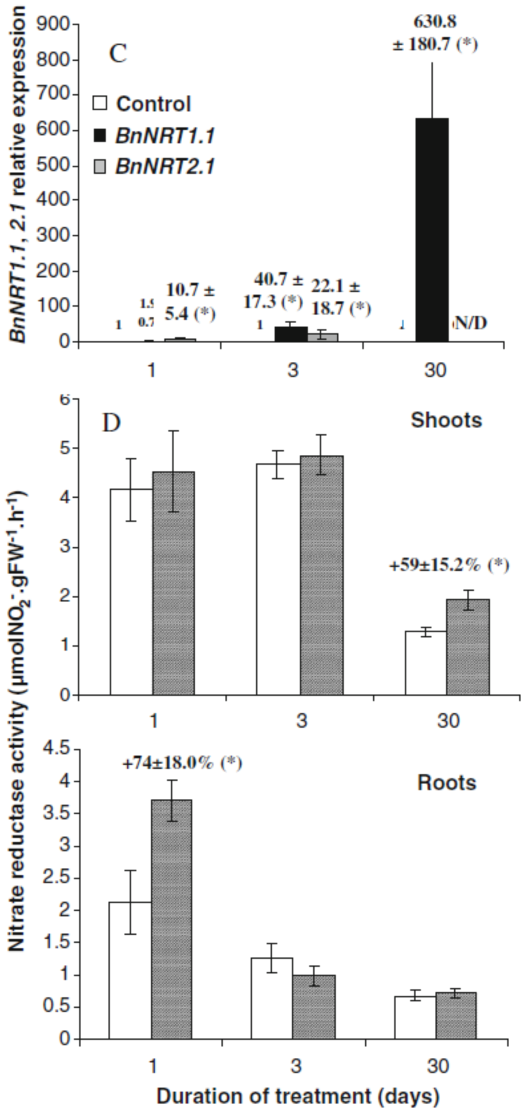

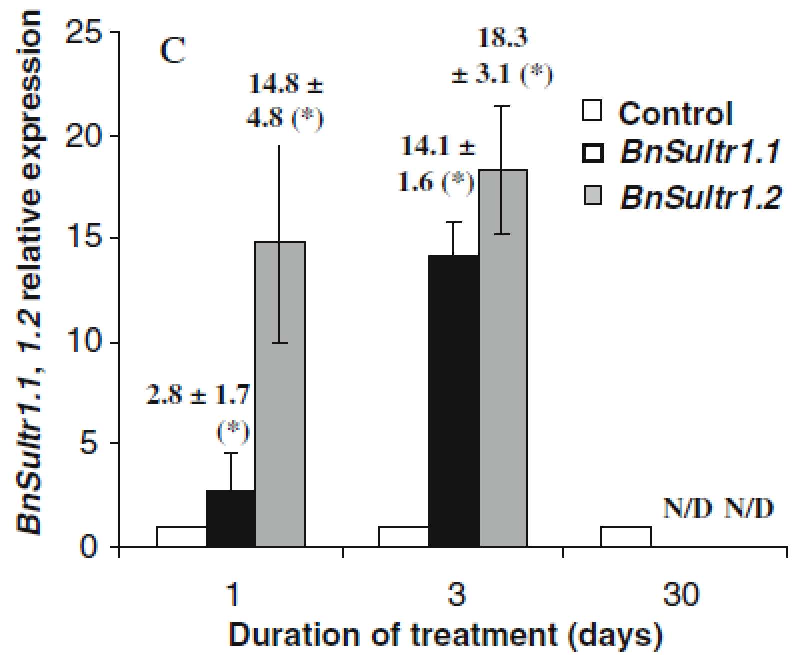

NO_3^-, SO_4^{2-}

Jannin et al., PLSO 2012

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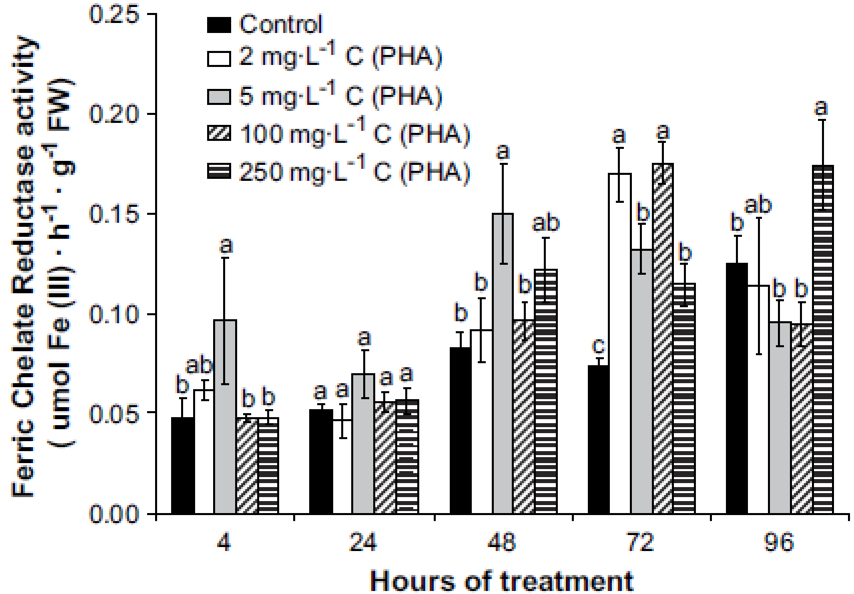
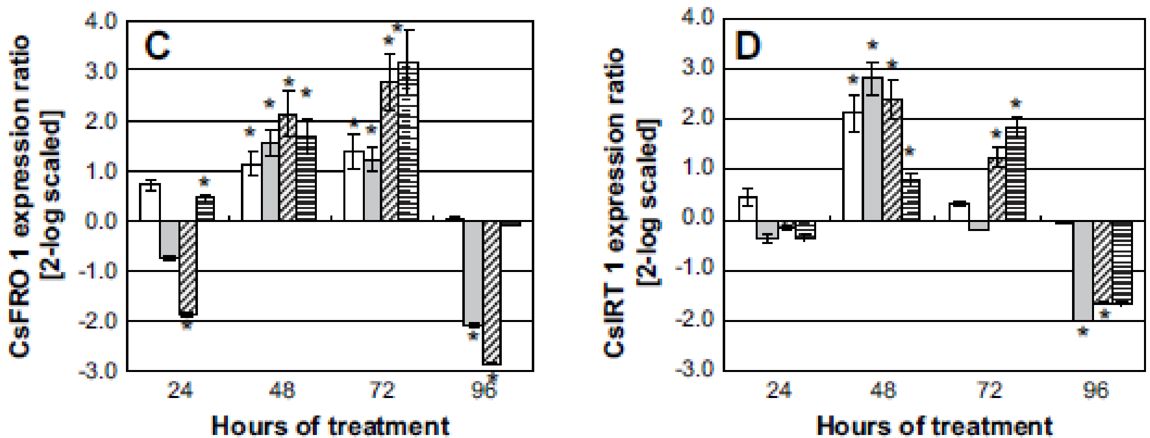
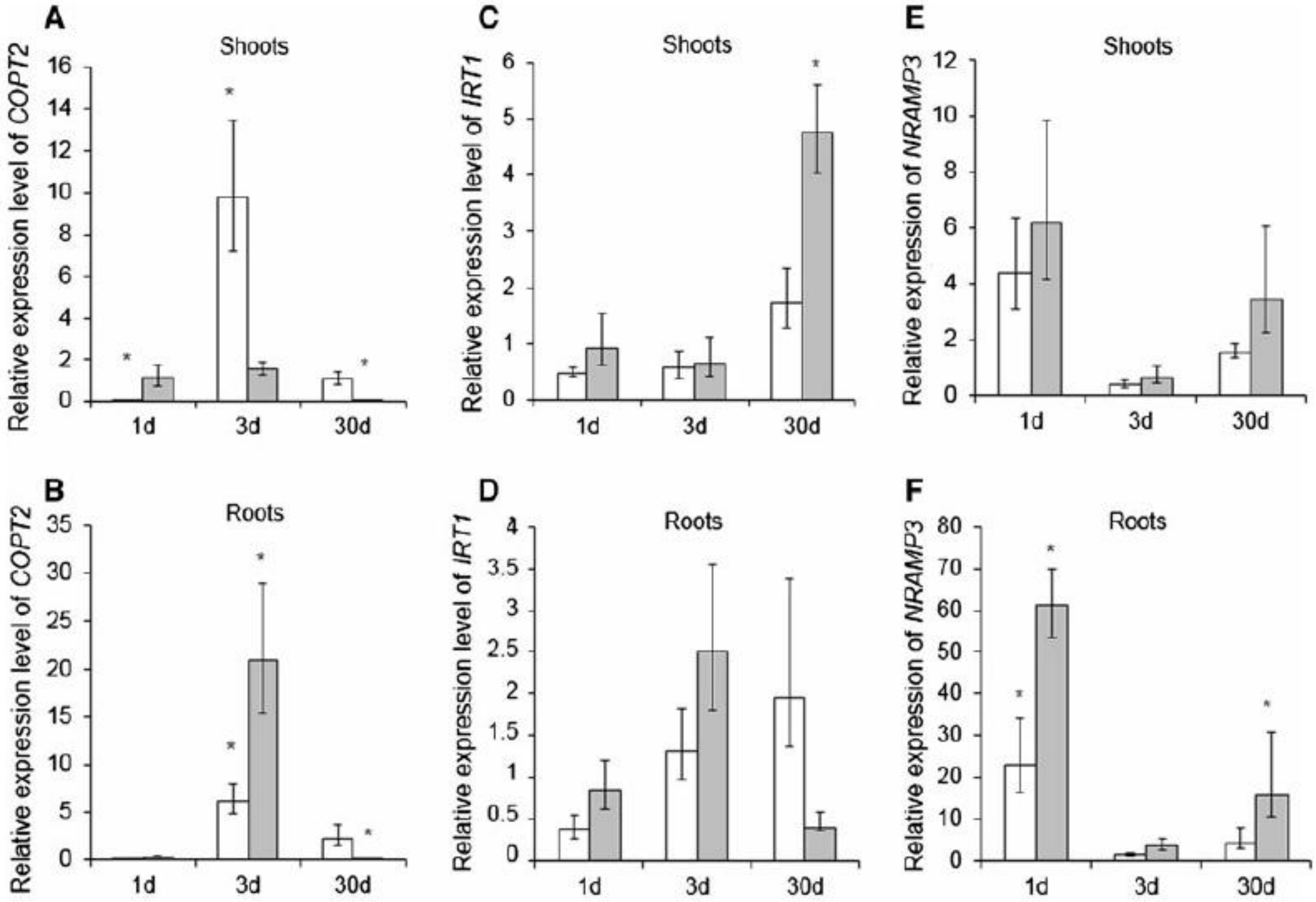
Fe



Aguirre et al., PPB 2009

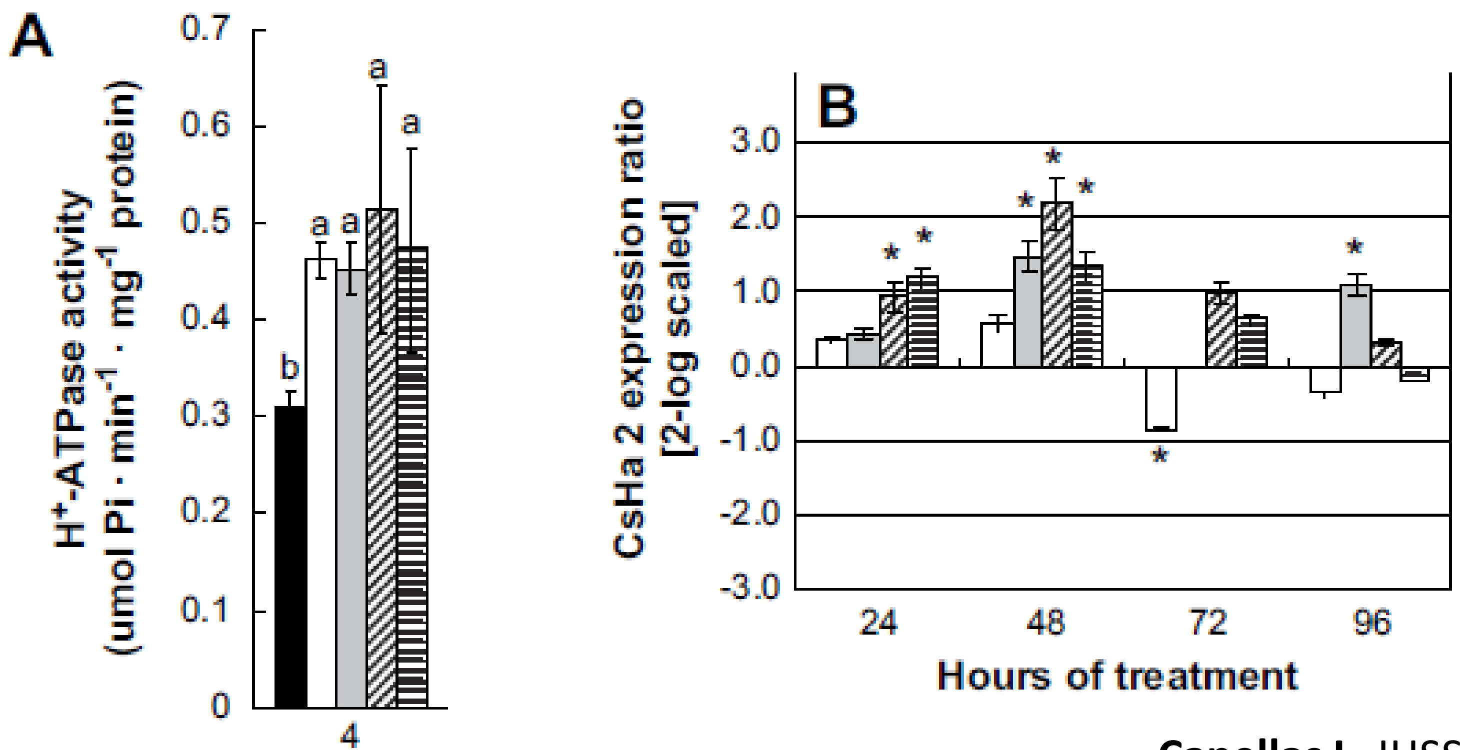




Fe, Cu, Mn, Zn

Billard et al., JPGR, 2013

ATPasa



Canellas L, IHSS 17
Nardi's Group
Pinton's Group

Aguirre et al., PPB, 2009; **Mora et al.**, JPP 2010

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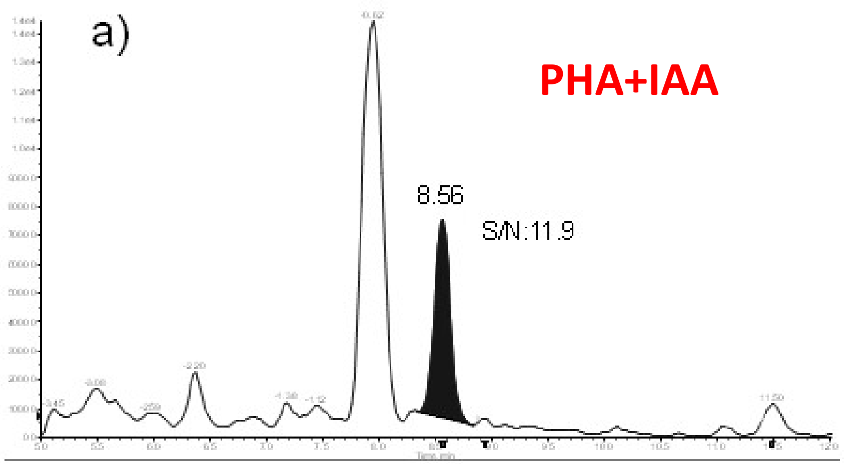
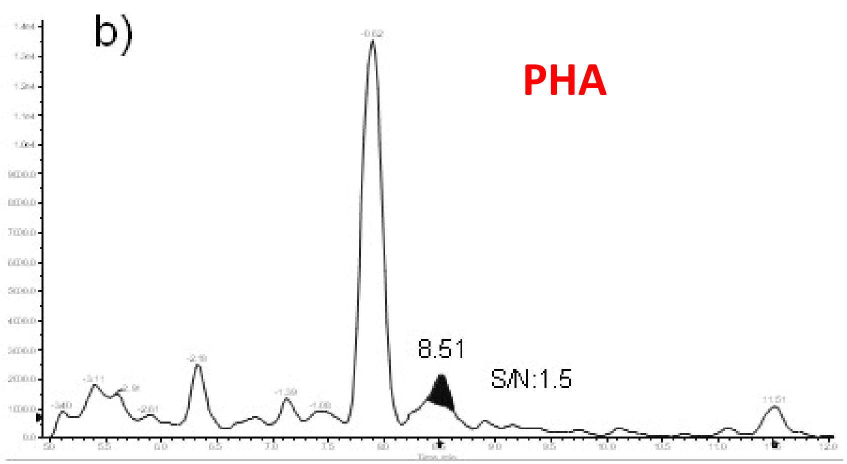
Leonardite Humic acid (PHA)

Without the presence of the Main phytoregulators in its Composition





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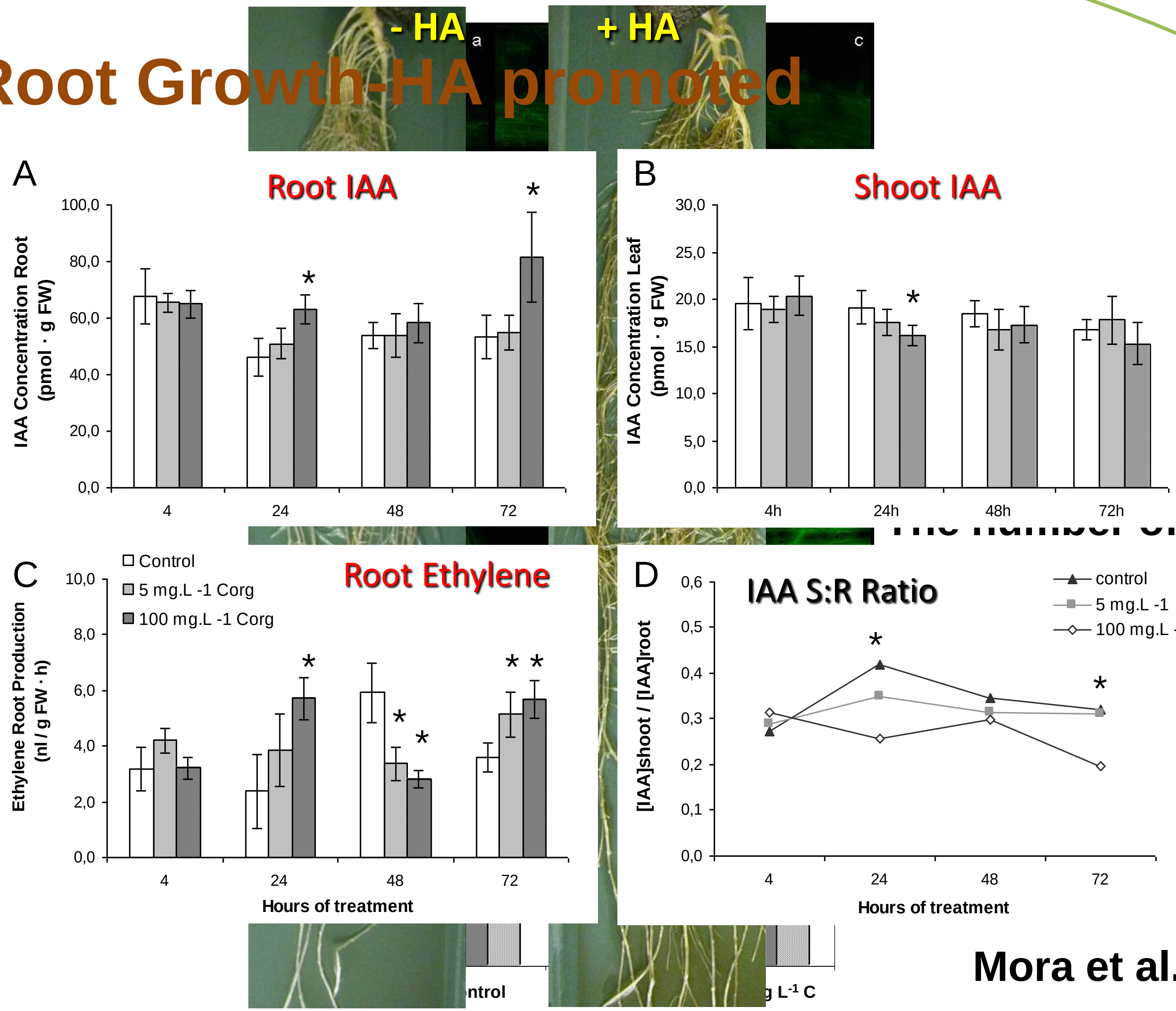
Root Growth-HA promoted



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secondary

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Mora et al., EEB 2012

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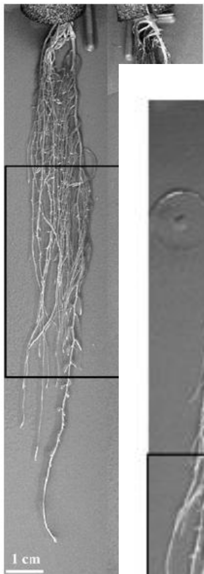
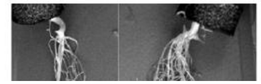




PCIB - HA PCIB + HA

Co+2 - HA Co+2 + HA

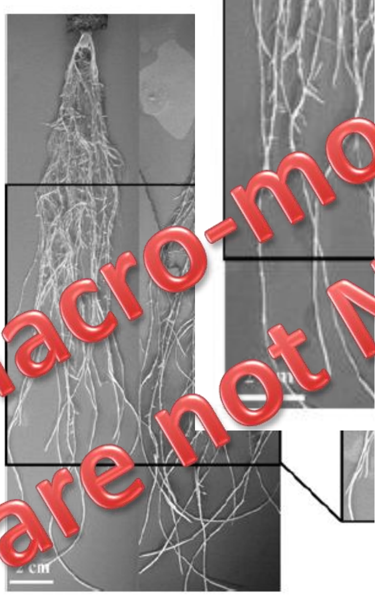


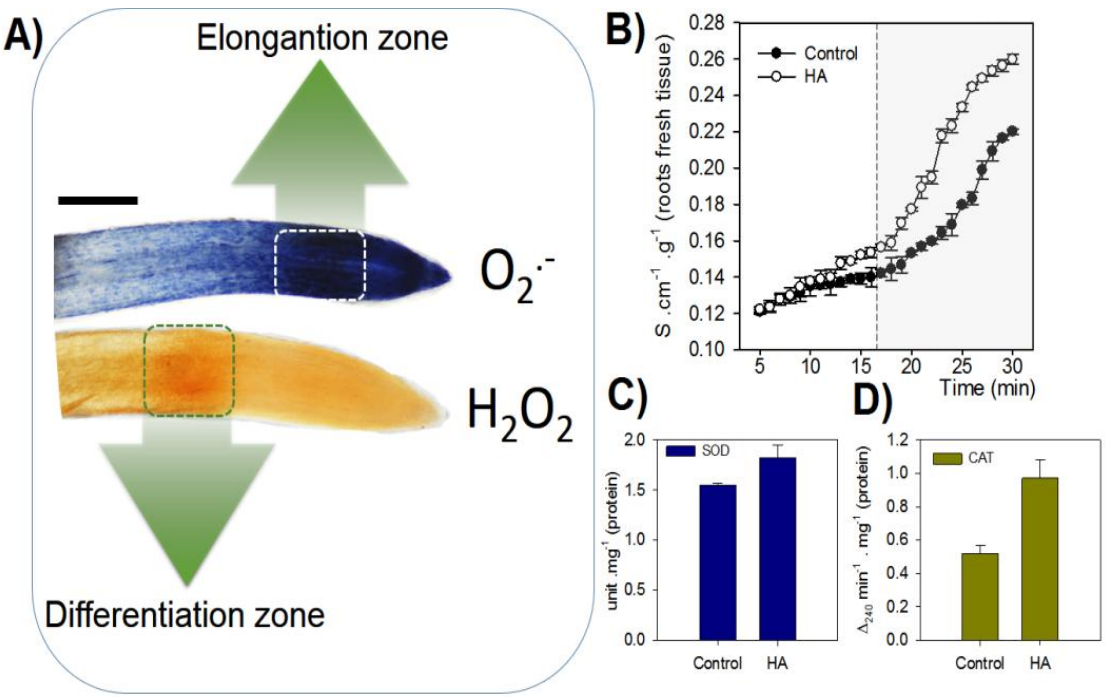
Table 3
Effect of 100 mg L−1 C purified humic acid (PHA), 50 µM PCIB, 50 µM TIBA, 200 µM PTIO, 10 µM cobalt and 200 µM STS on root development: root fresh weight (g), % of secondary roots and thickness root. Data are the mean ± SE ($n = 5$).

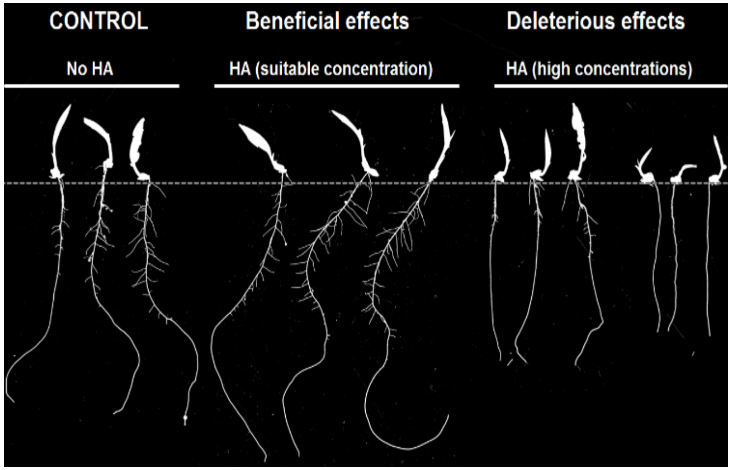
	Root fresh weight (g)	% Secondary roots[a]	Thickness root
[illegible] – PHA	[illegible] ± [illegible]	100	–
Control + PHA	[illegible].01 ± 0.71	136*	+
PCIB	2.20 ± 0.18	100	–
PCIB + PHA	2.80 ± 0.21	145*	+
TIBA	1.39 ± 0.21	100	–
TIBA + PHA	1.24 ± 0.18	103	–
PTIO	1.89 ± 0.14	100	–
PTIO + PHA	2.59 ± 0.47	136*	+
cobalt	4.41 ± 0.32	100	+
cobalt + PHA	5.12 ± 0.53	145*	++
STS	4.23 ± 0.29	100	–
STS + PHA	4.80 ± 0.37	163*	+

[a] Secondary roots density in the medium region of the root, considering the control as 100%.
* $p \leq 0.05$ t-test.

Macro-morphological changes in roots are not NO, IAA Ethylene – Dependent

Reactive Oxygen Species (ROS)???





Berbara's Group
García's Group

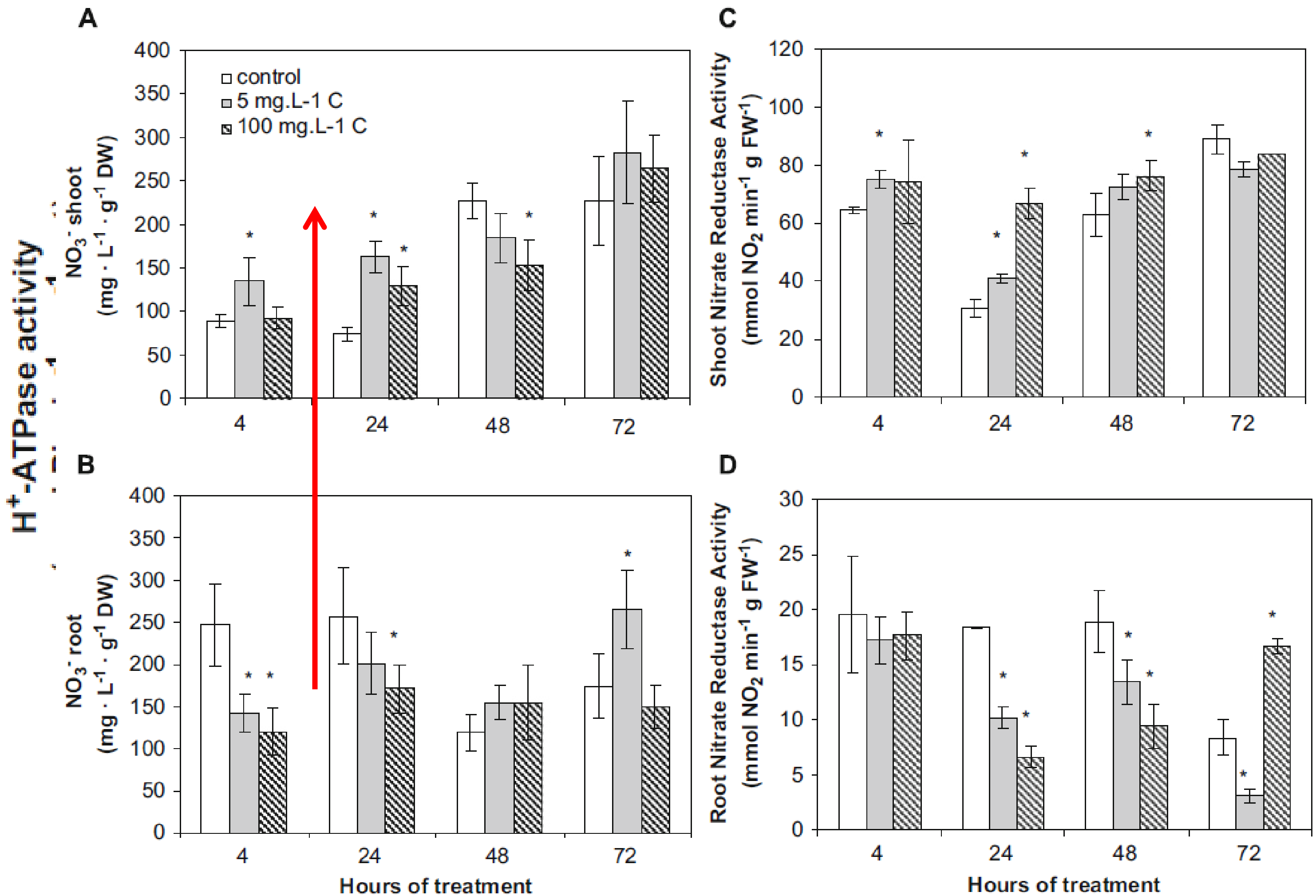
Shoot Growth-HA promoted

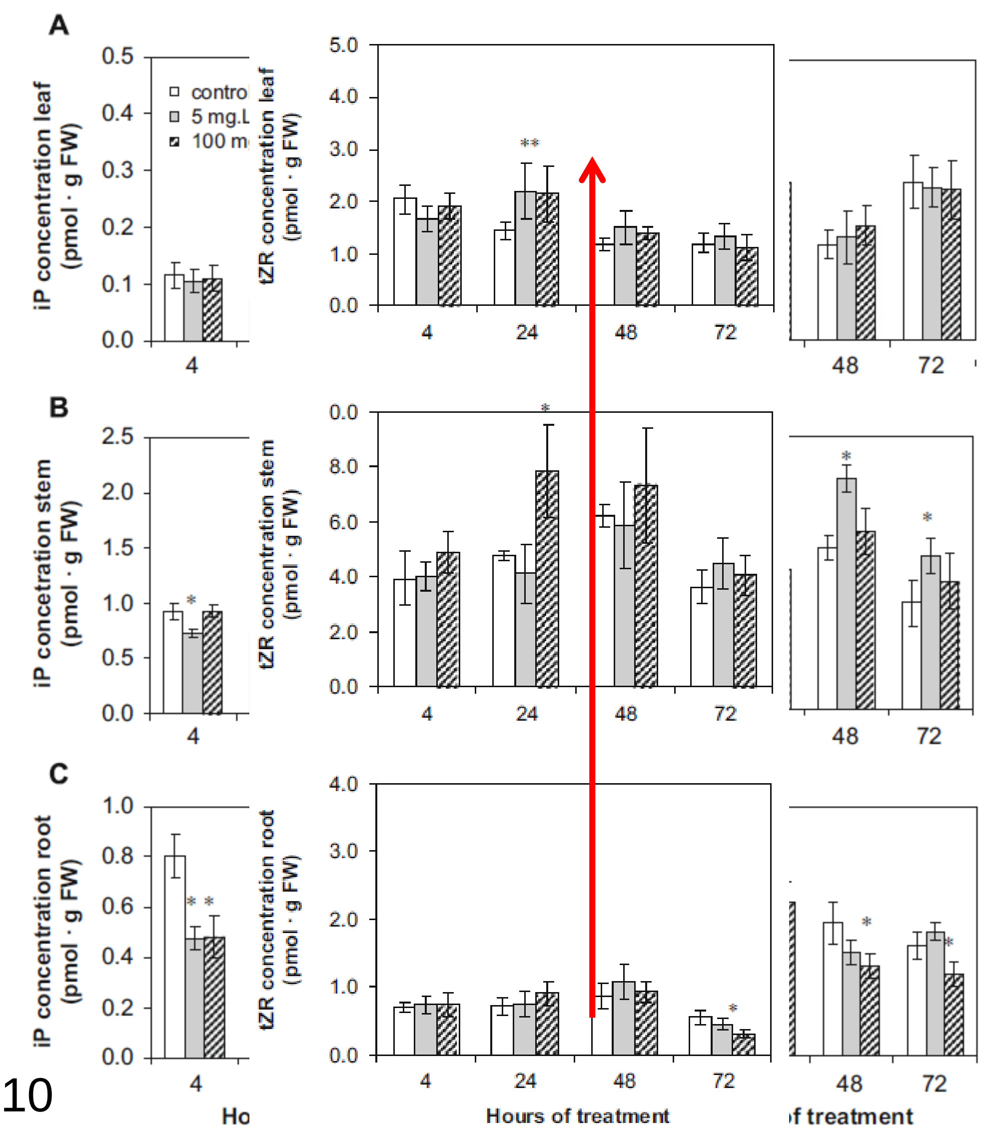
Working Hypothesis

HA-ATPase-Nitrate (Canellas's, Pinton's & Nardi's group)

Signal effect of Nitrate – increase in CKs translocation
(Sakakibara's & Von Wiren's group; Garnica et al JPP (2010))

HA – Nitrate – CKs – Shoot growth; Nutrient translocation


A
B
C
D
H+-ATPase activity
control
5 mg.L-1 C
100 mg.L-1 C
NO3- shoot (mg · L-1 · g-1 DW)
NO3- root (mg · L-1 · g-1 DW)
Shoot Nitrate Reductase Activity (mmol NO2 min-1 g FW-1)
Root Nitrate Reductase Activity (mmol NO2 min-1 g FW-1)
Hours of treatment
Hours of treatment
4
24
48
72



Mora et al., JPP 2010

Shoot Dry Weigh (g)				
Treatments	48 h		72 h	
control	0.588 ± 0.155	b	0.575 ± 0.053	c
5 mg L^{-1} C	0.714 ± 0.085	ab	0.743 ± 0.065	b
100 mg L^{-1} C	0.837 ± 0.100	a	0.904 ± 0.130	a

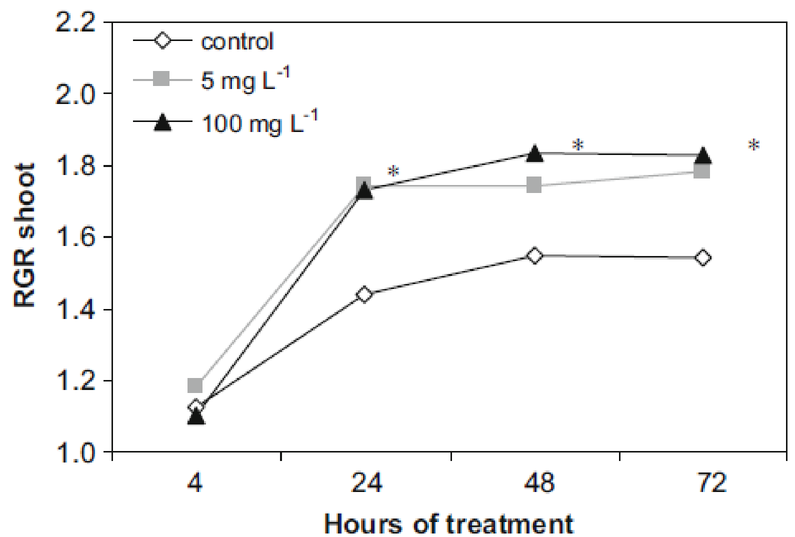


Fig. 1. Effect of a low (5 mg L^{-1} C) and a high (100 mg L^{-1} C) concentration of HA on shoot Relative Growth Rate (RGR). RGR is calculated as RGR=(ln W_2–ln W_1)/(t_2-t_1), where W_1 and W_2 are plant dry weights at times t_1 and t_2. The results are the mean ± SE (n=5). Differences between mean values indicated as * are significant ($p<0.05$) according *Tukey-b* test.

Mora et al., JPP 2010

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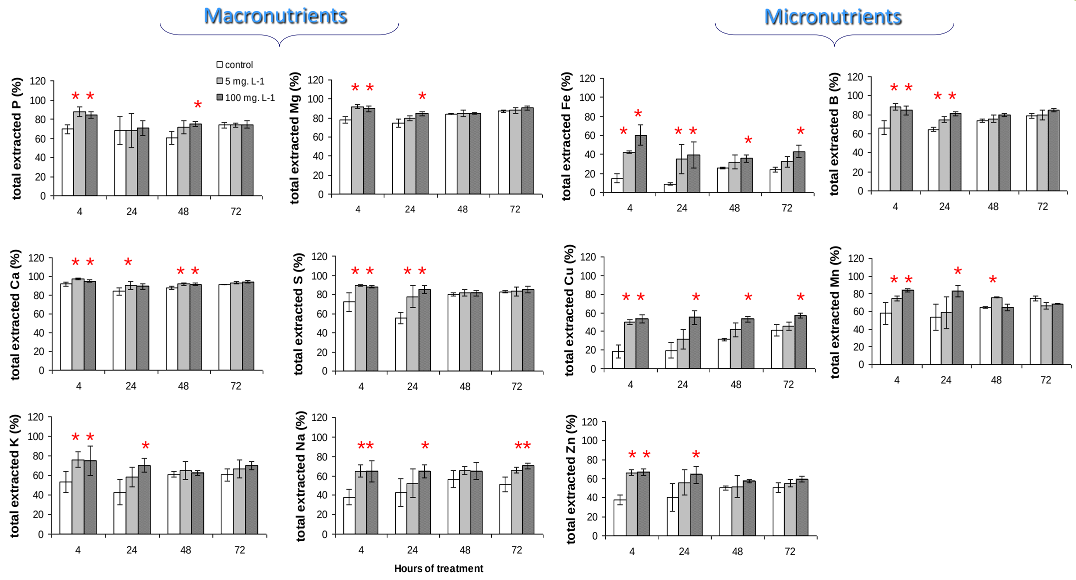
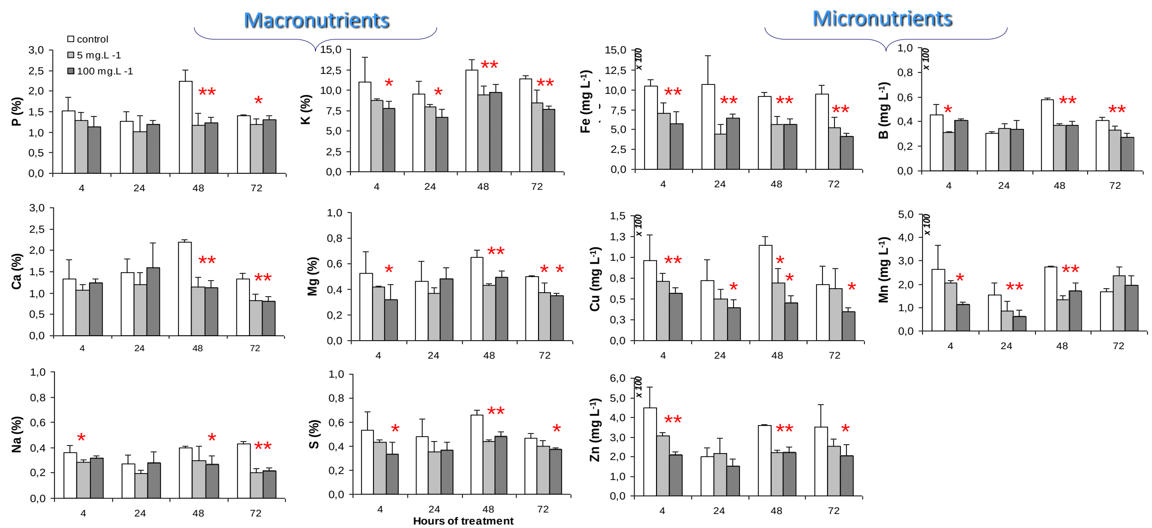
Shoot



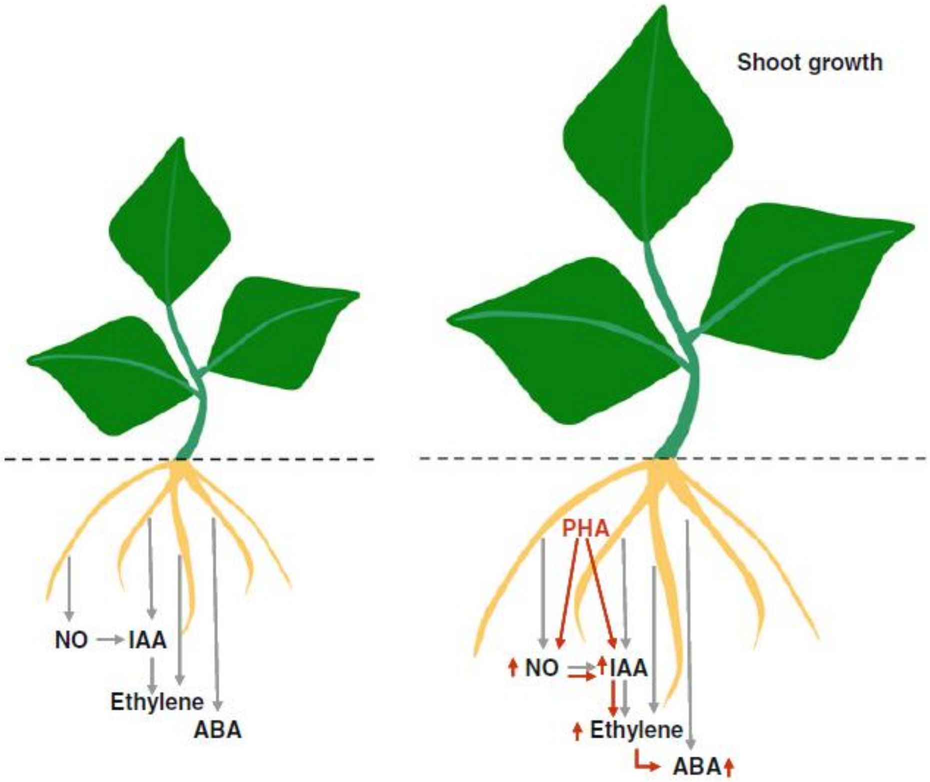
Root



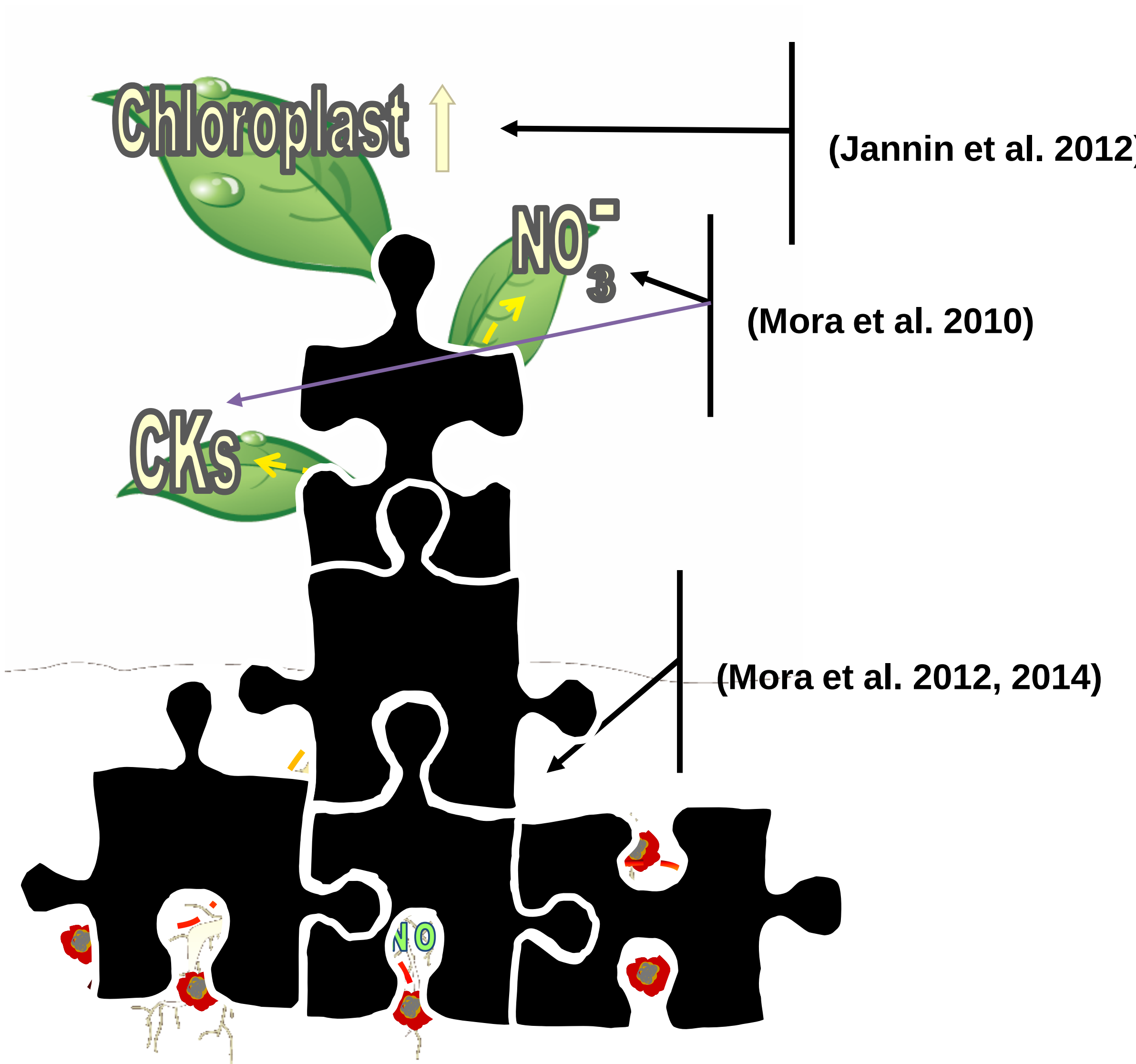
Root-shoot signaling crosstalk

Regarding de relationships between the PHA effects on IAA, Ethylene, NO in root and PHA-induced shoot growth

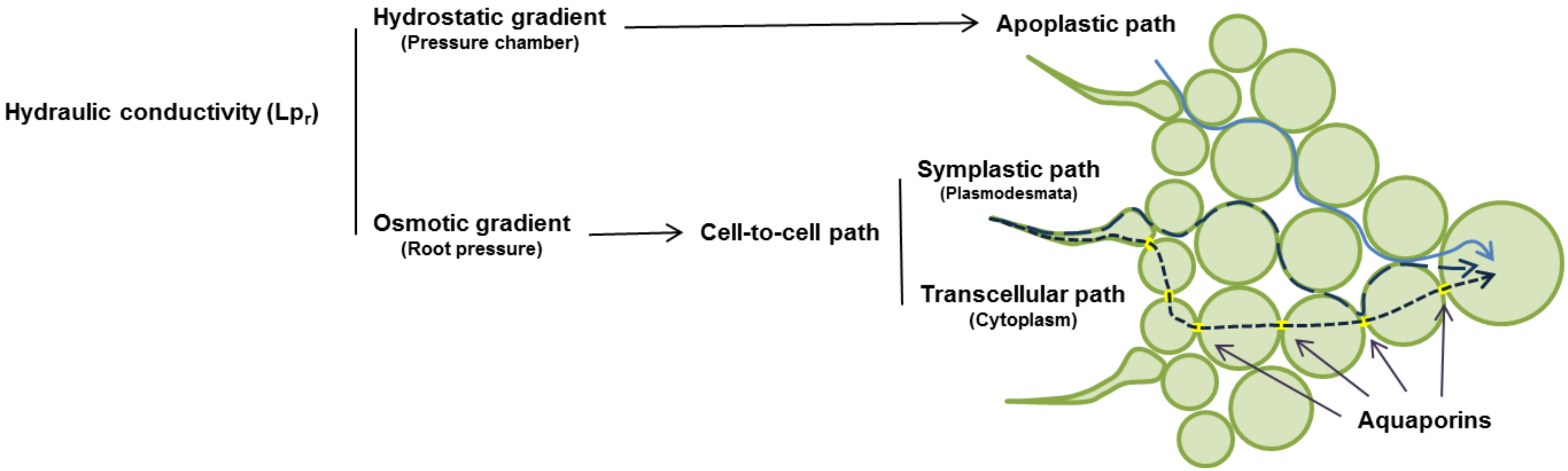
	Shoot Fresh Weight (g)
Control – PHA	4.71 ± 0.87
Control+ PHA	6.81 ± 0.88
PCIB	3.83± 0.0.51
PCIB + PHA	4.62 ± 0.29
PTIO	3.83 ± 0.40
PTIO + PHA	4.50 ± 0.35
Cobalt	5.90 ± 0.52
Cobalt + PHA	6.33 ± 0.47
STS	5.36 ± 0.31
STS + PHA	6.32 ± 0.77



Mora et al., JPGR 2014


Chloroplast
NO_3^-
CKs
NO
(Jannin et al. 2012)
(Mora et al. 2010)
(Mora et al. 2012, 2014)

- Some authors have described the ABA action on water-plant relationships (Hose et al., 2000; Thompson et al. 2007) through aquaporins
- When aquaporins channels are opened, cell to cell water pathway increases and root hydraulic conductivity (Lp_r) is enhanced

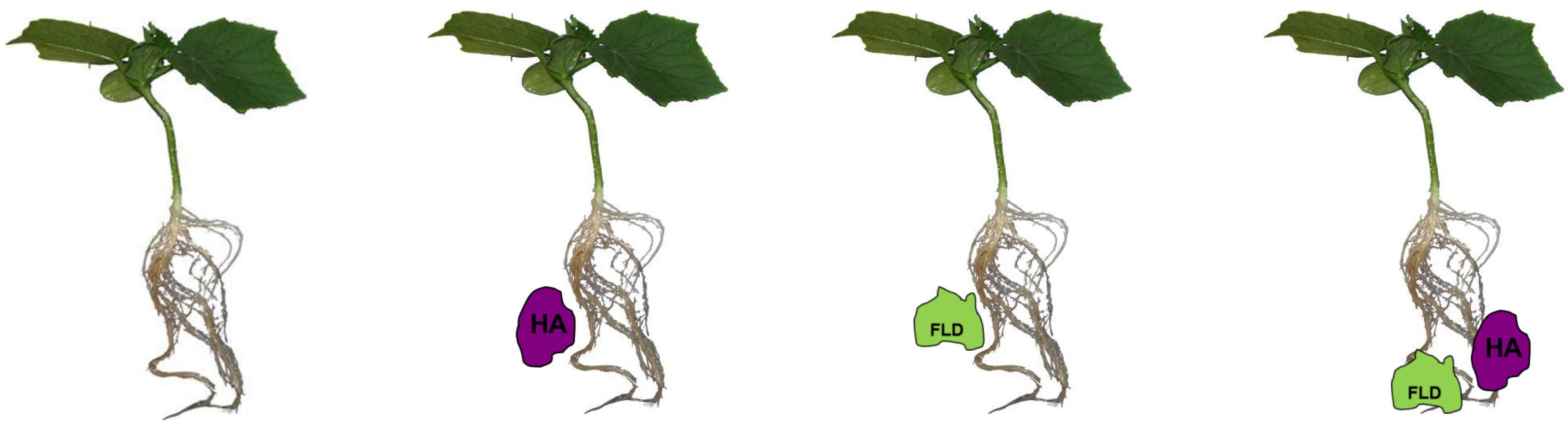


- As consequence a higher movement of water and nutrients is observed from root to shoot

Experiment with HA measuring Lp_r, ABA and aquoporins was carried out in order to explore the hypothetical HA action through ABA.

This experiment was completed using Fluridone (Fld) a ABA synthesis inhibitor

Control | **HA** | **Fld** | **Fld + HA**



Nutrient solution

Nutrient solution + 100 ppm Leonardite HA

Nutrient solution + 10 µM Fld

Nutrient solution + 10 µM Fld + 100 ppm Leonardite HA

Olaetxea et al., PP 2015

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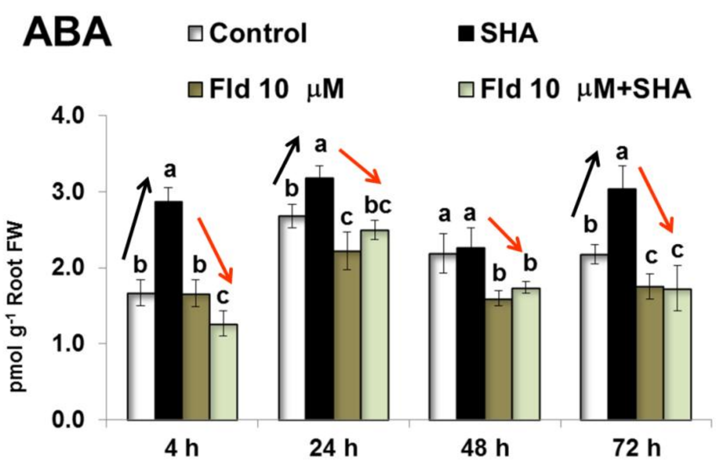

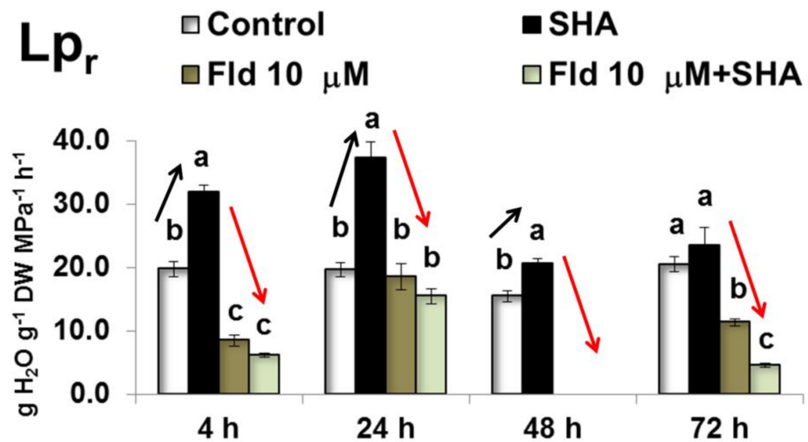

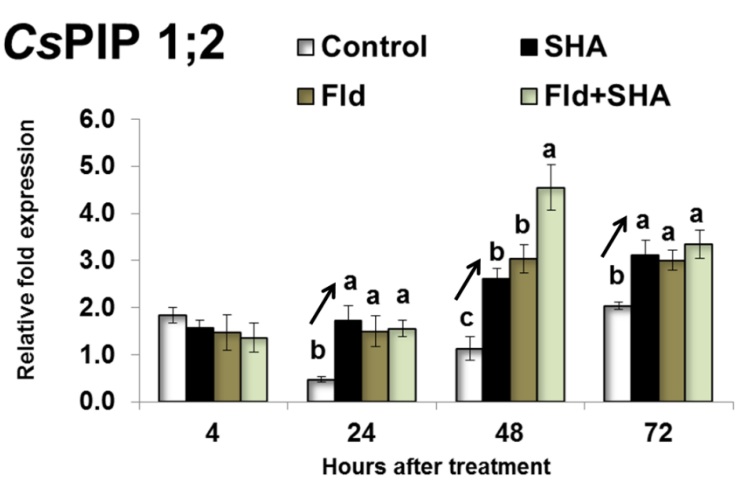

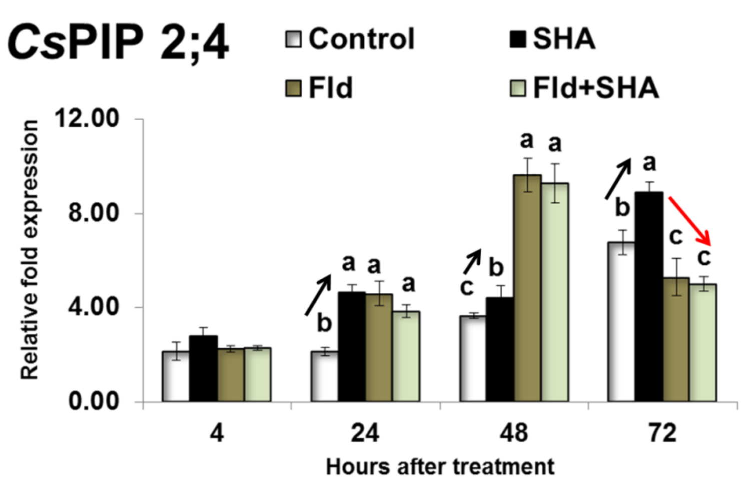

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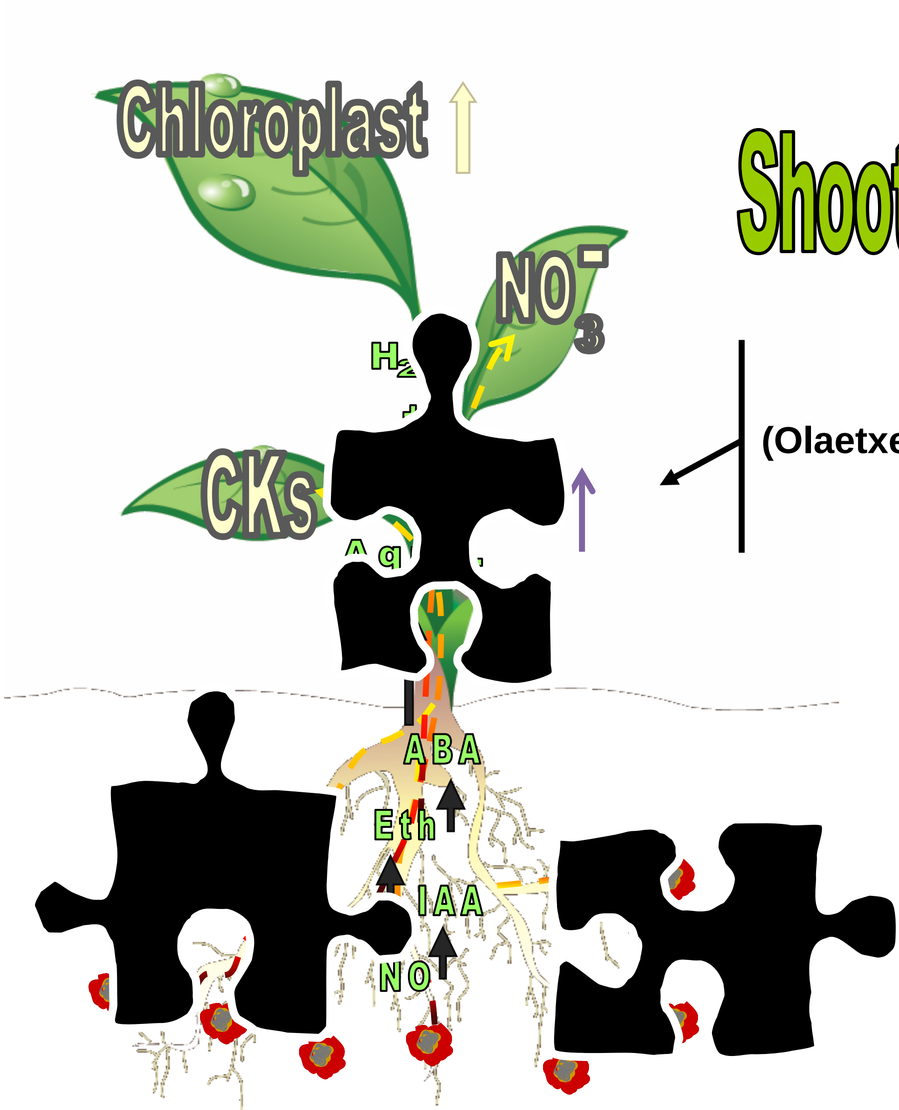

Shoot Growth ↑

(Olaetxea et al. 2015)

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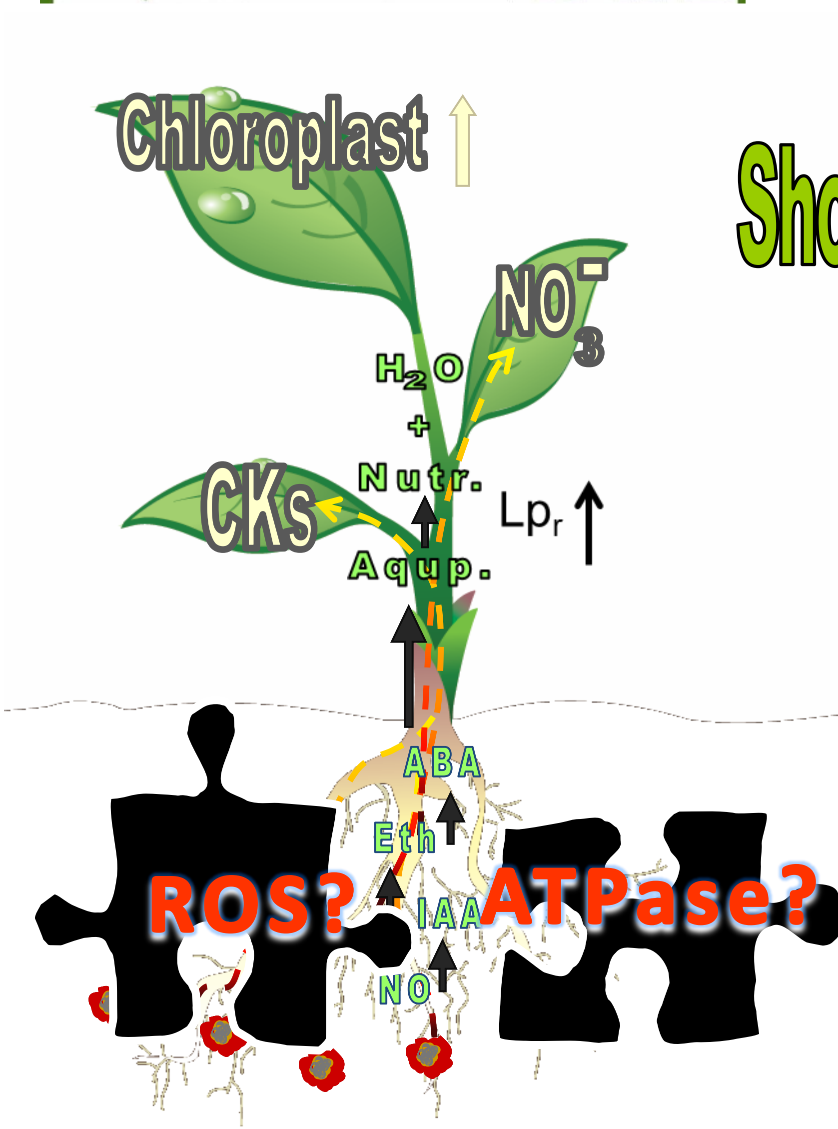

Shoot Growth

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¡Gracias por su atención!