Life+ Environment Policy and Governance
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Humeomics:

A molecular zoom into Soil Organic Matter

Marios Drosos

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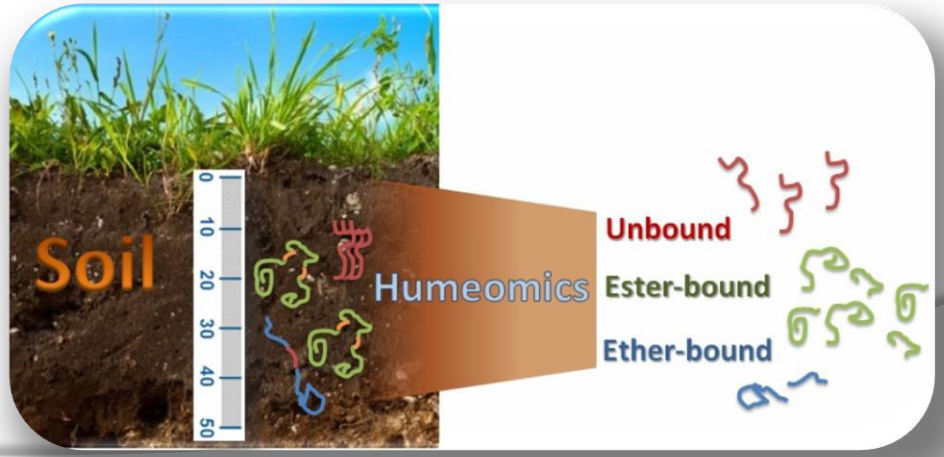
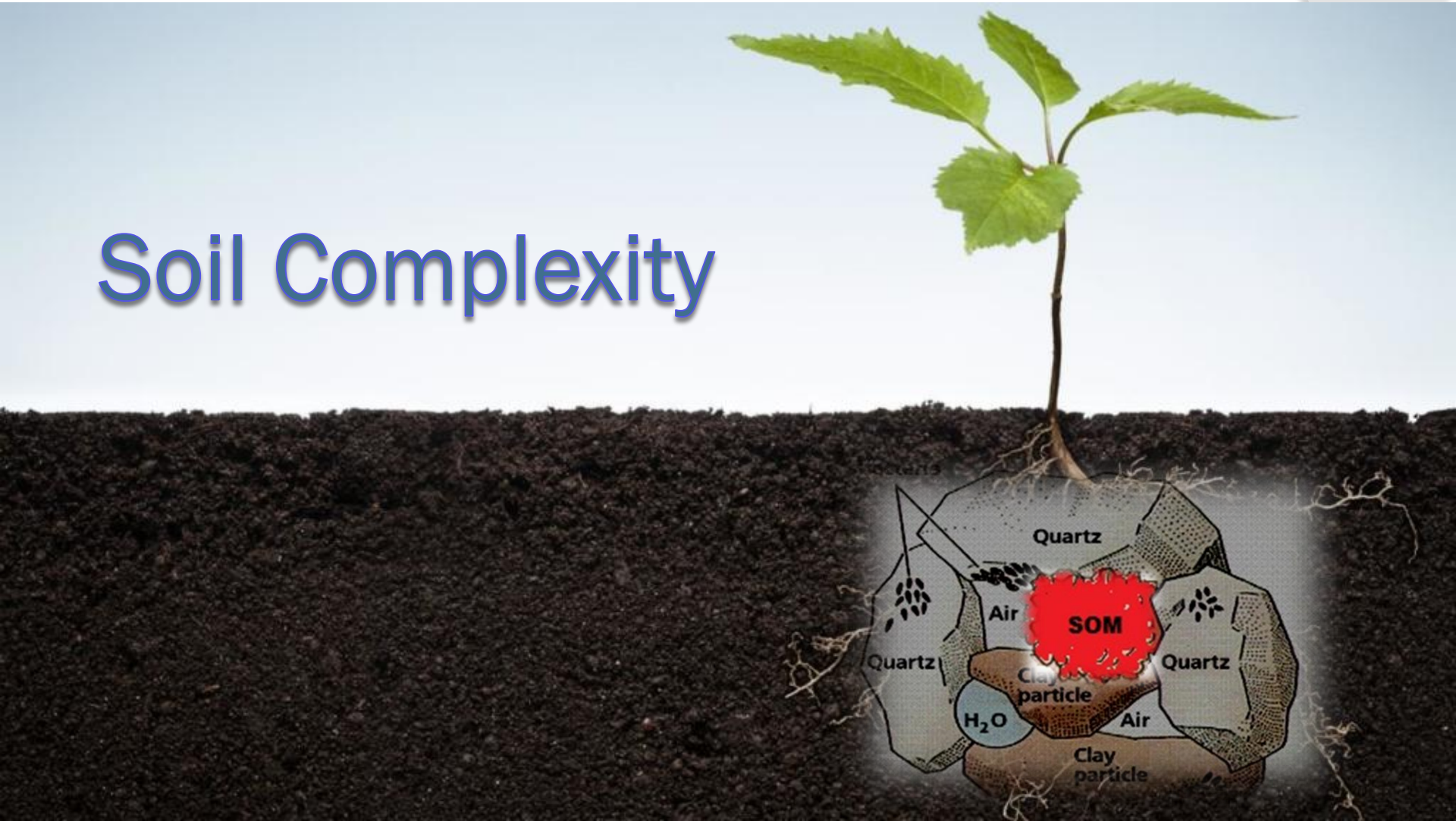
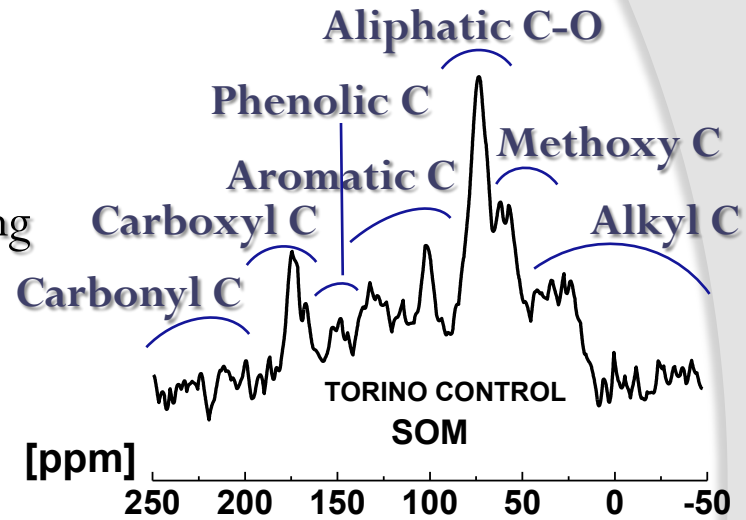
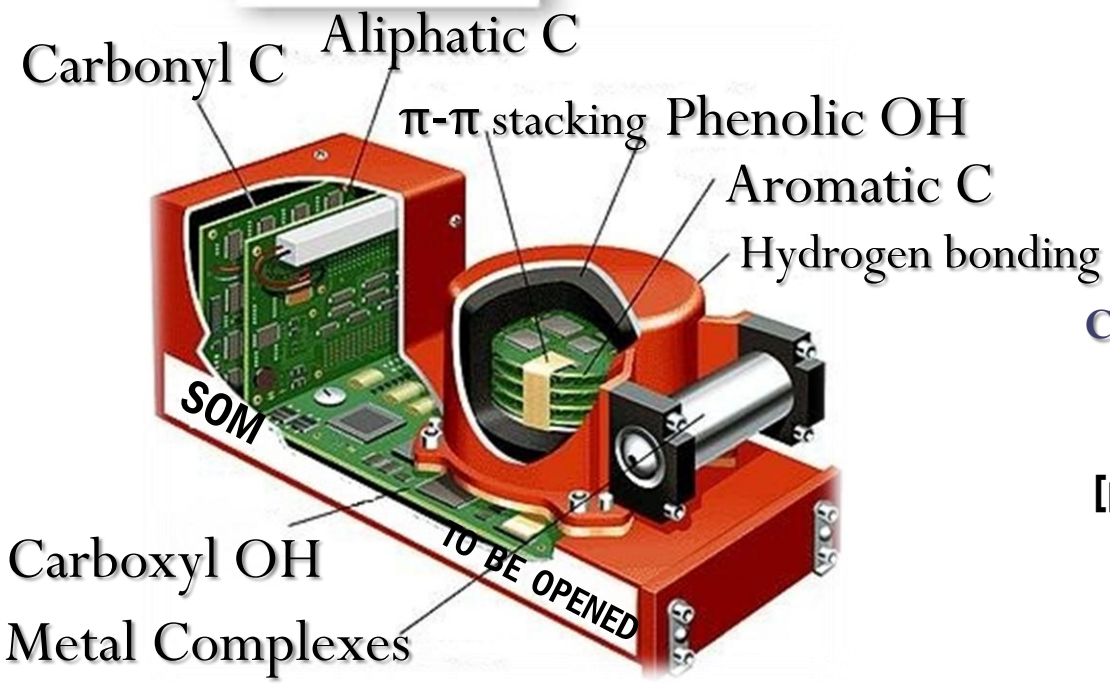


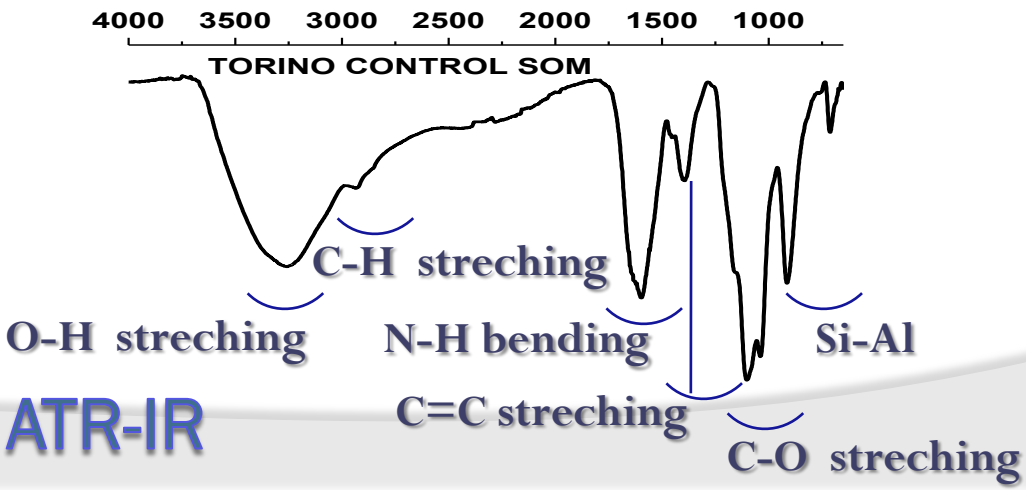
Soil Complexity



^{13}C-CPMAS-NMR



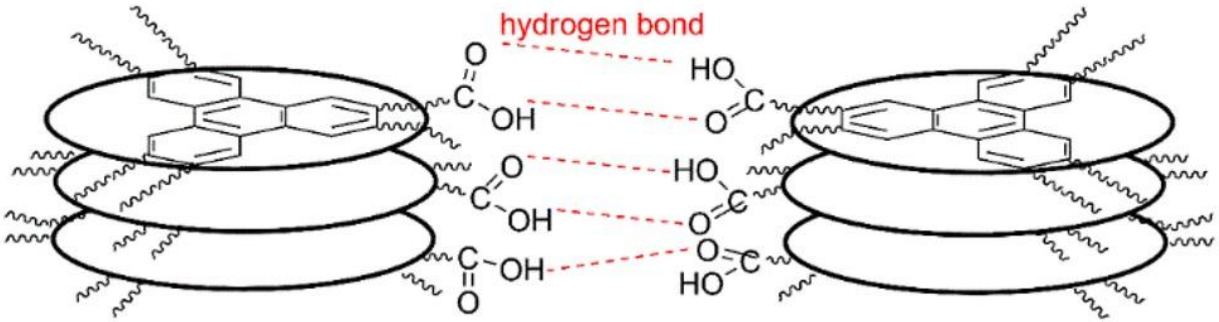




ATR-IR

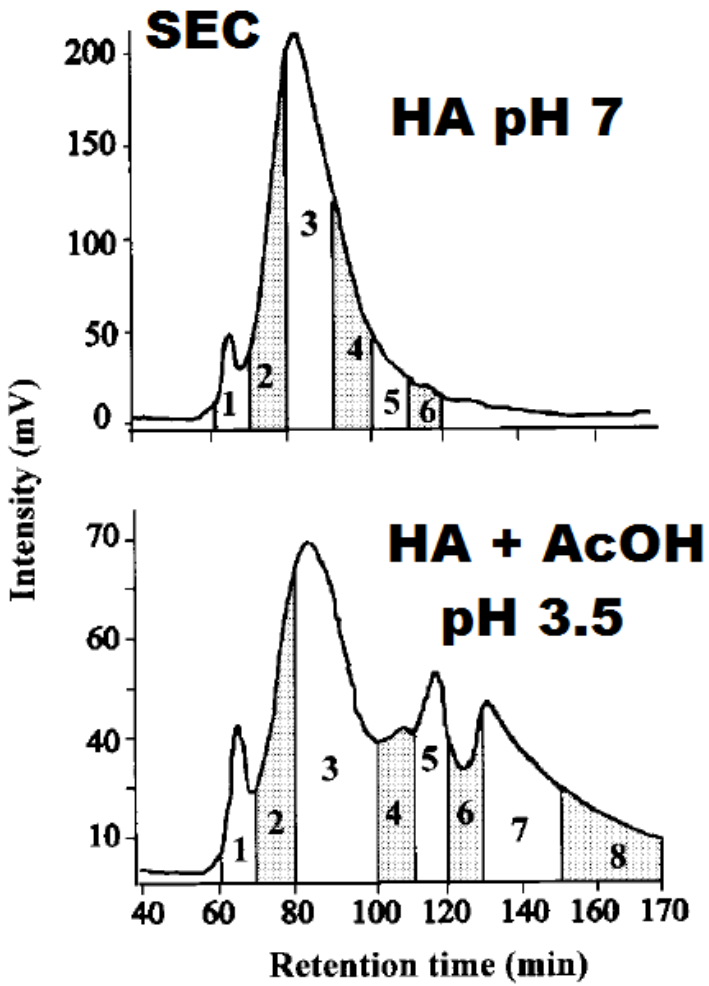
Supramolecular Structure

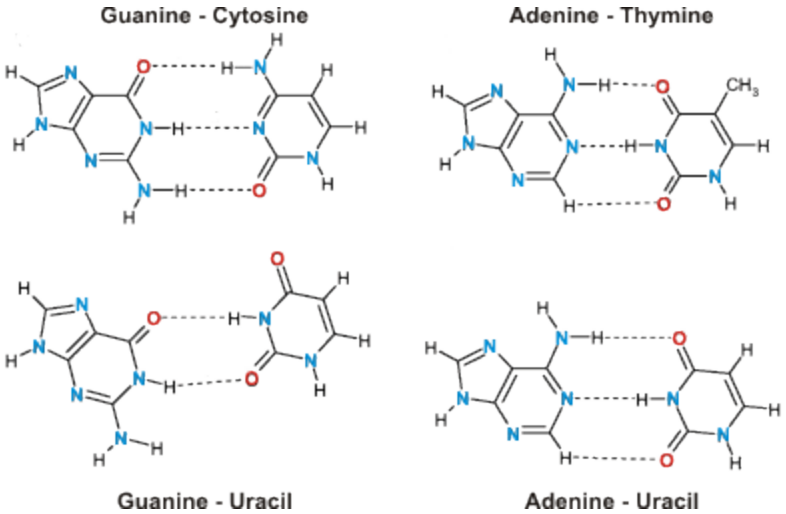
- Small size organic molecules linked together by metal, hydrogen and π-π bonds.

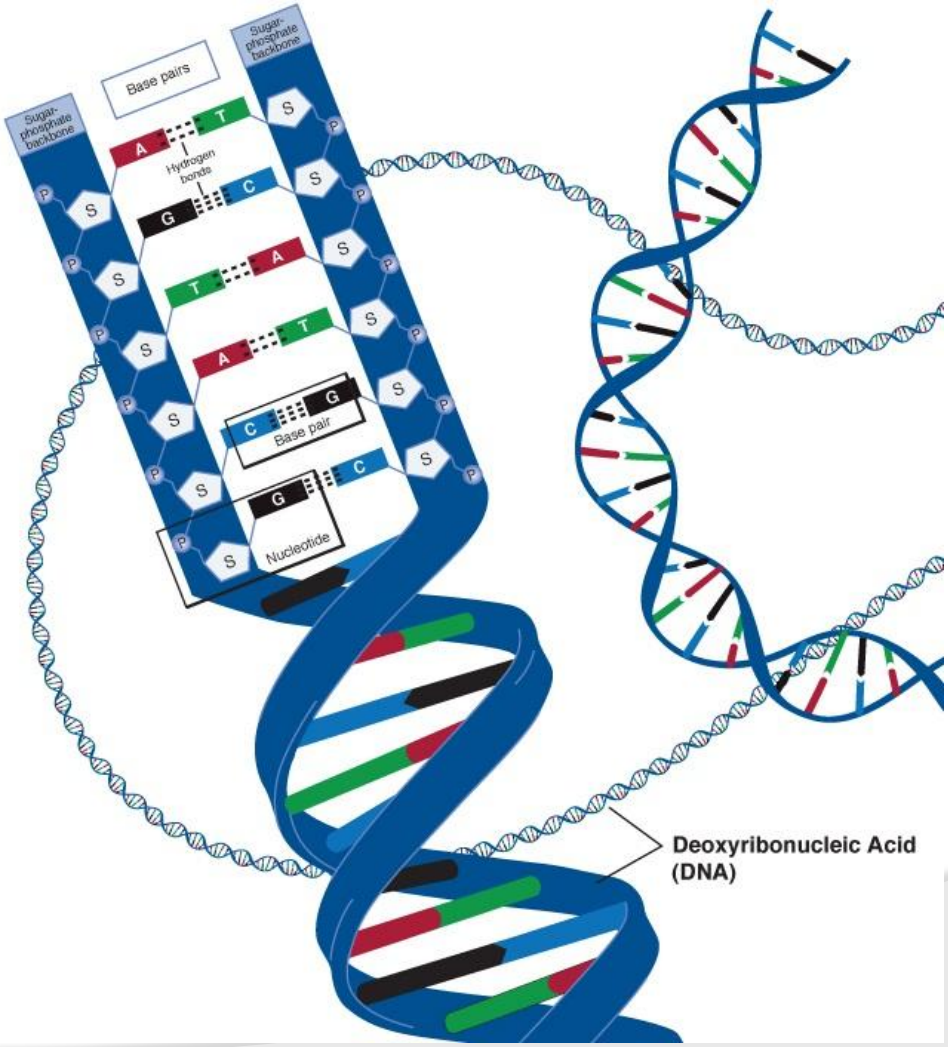


Alessandro Piccolo (2001) The Supramolecular structure of humic substances. Soil Science, Vol. 166, pp. 810-832.

Environmental Science & Technology, Vol. 36 (2002) pp.76-84.

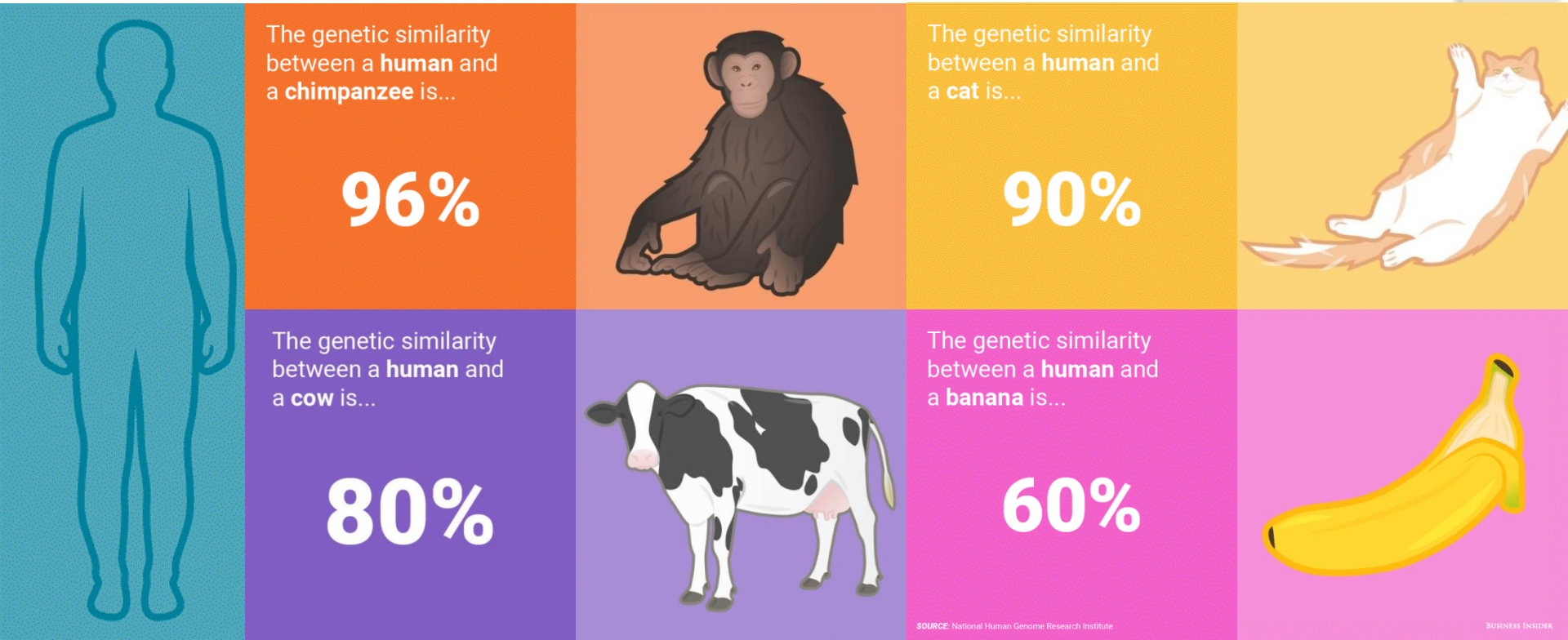





Sugar-phosphate backbone
Base pairs
Sugar-phosphate backbone
Hydrogen bonds
Base pair
Nucleotide
Deoxyribonucleic Acid (DNA)

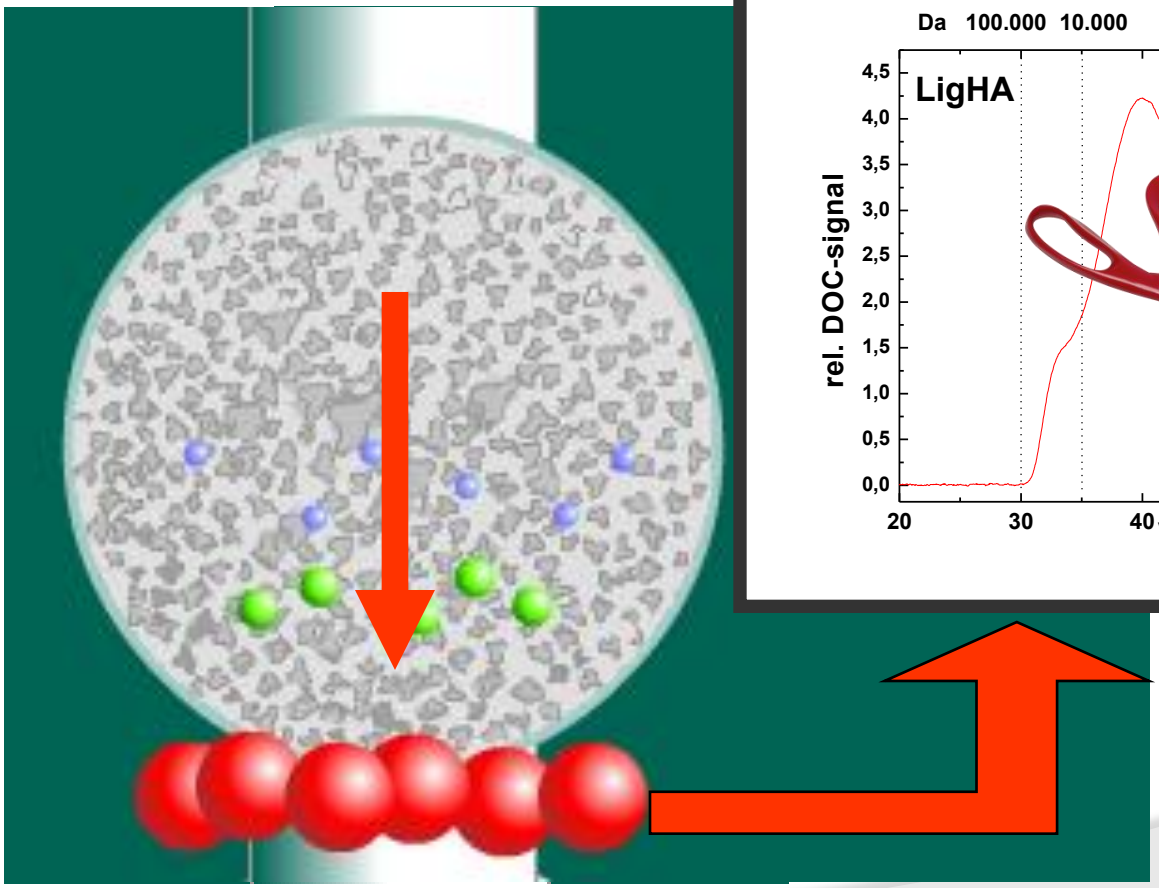
DNA sequence (Biology)

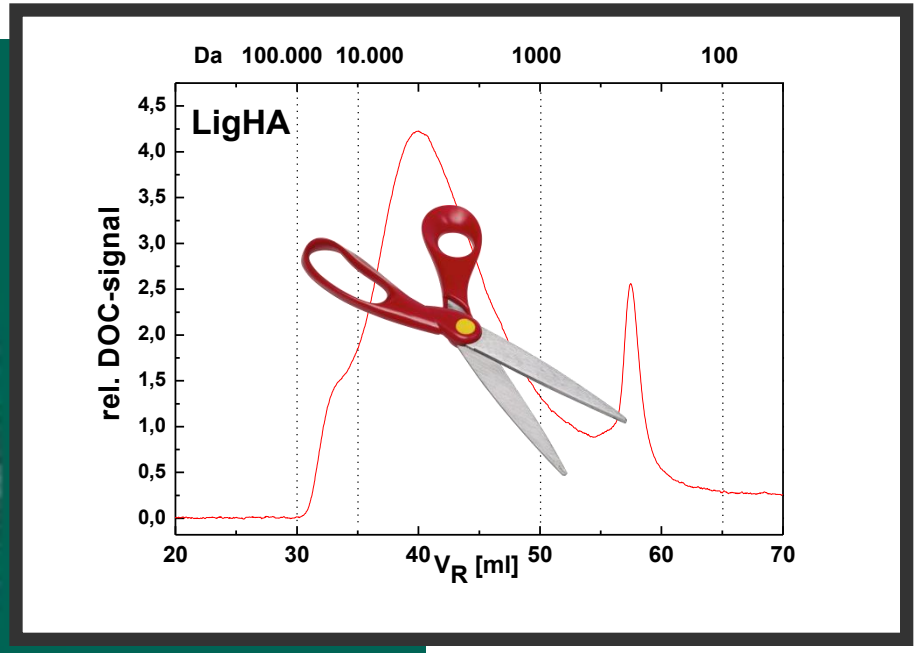
Supramolecular assembly of the building blocks (Chemistry)



Fractionation

Preparative Size Exclusion Chromatography



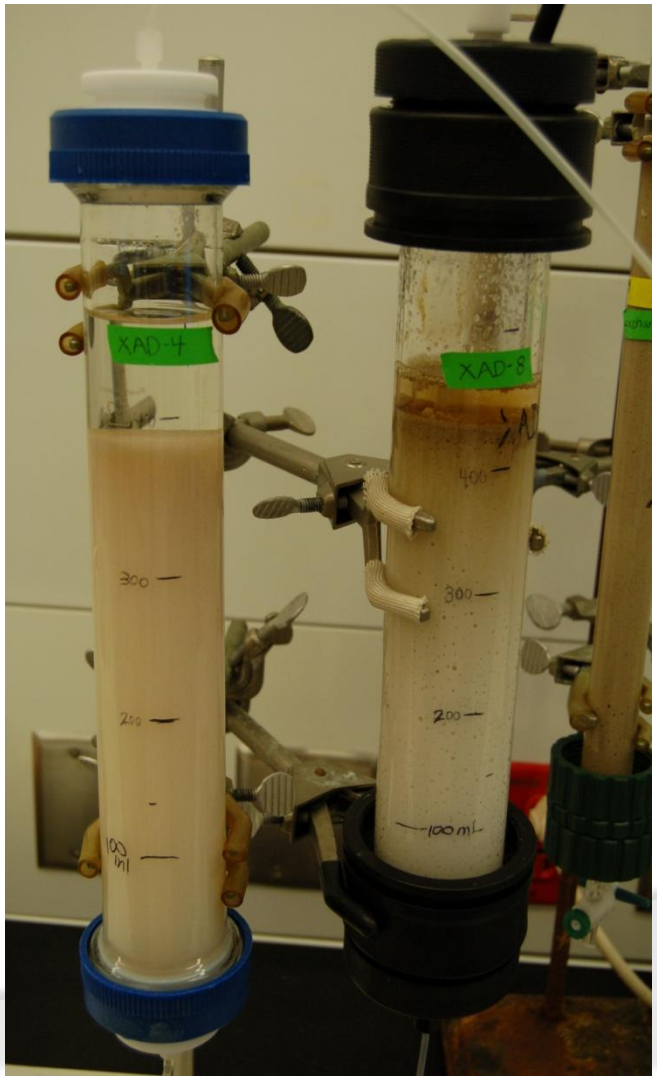


Fractionation

Polarity

Adsorption in XAD resin

pH



release

Limitations

- Only for hydrosoluble organic matter!

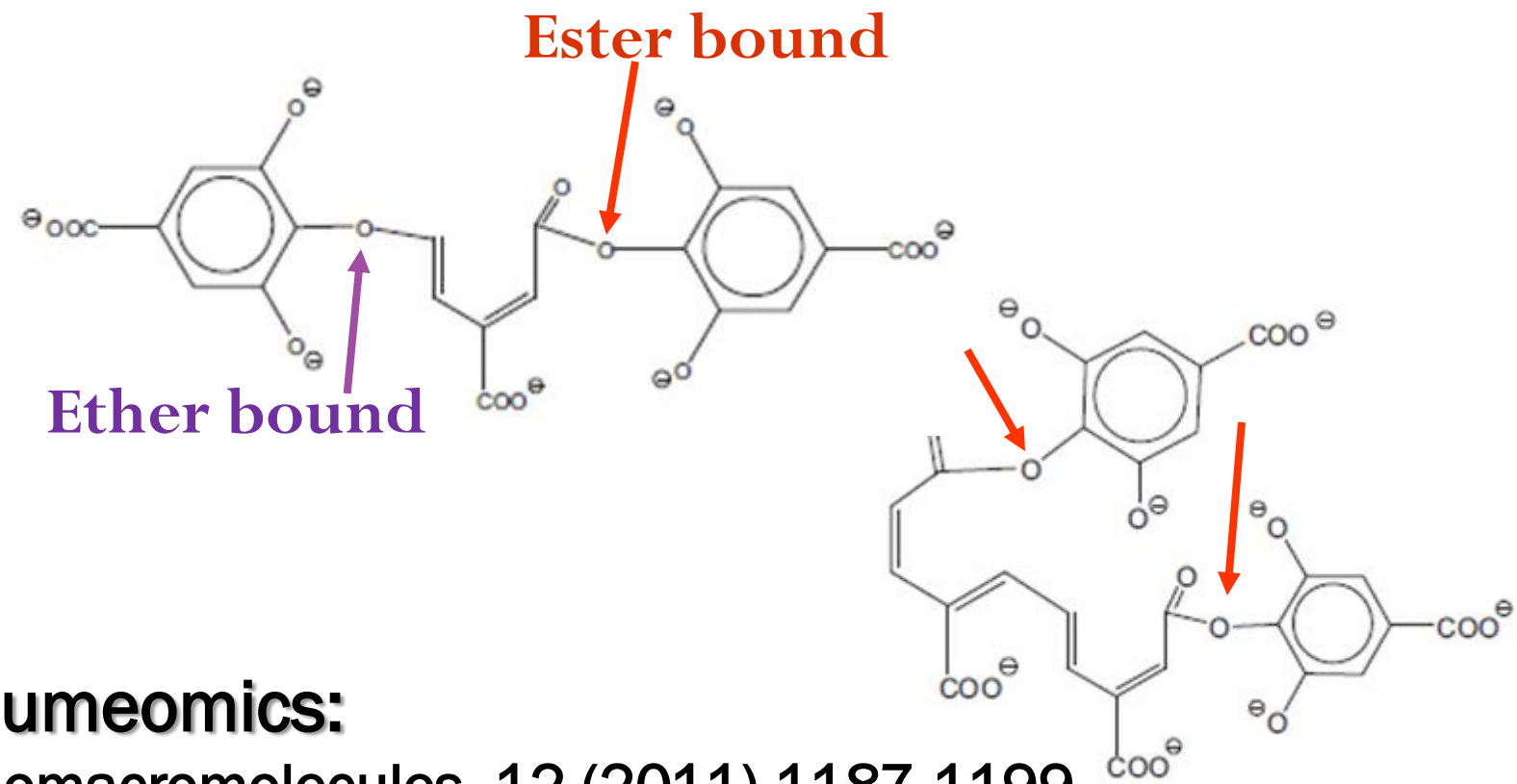
  FA, DOM, HA at high pH

- Not for **SOM!**

A humic-acid-like polycondensate produced with no use of catalyst

J. Colloid Interface Sci. 336 (2009) 59–66.

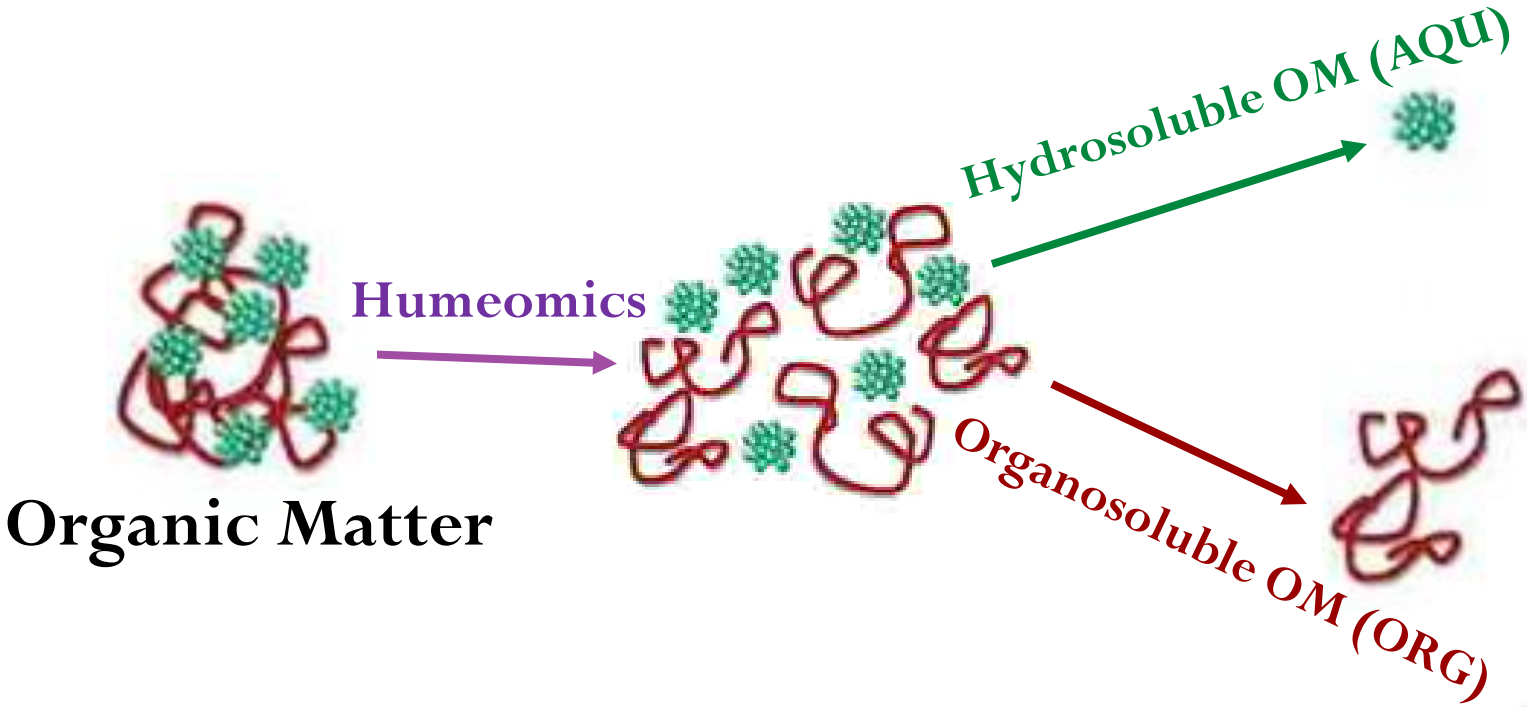
Col. & Surf. A: Physic. & Engineer. Aspects 389 (2011) 254-265.



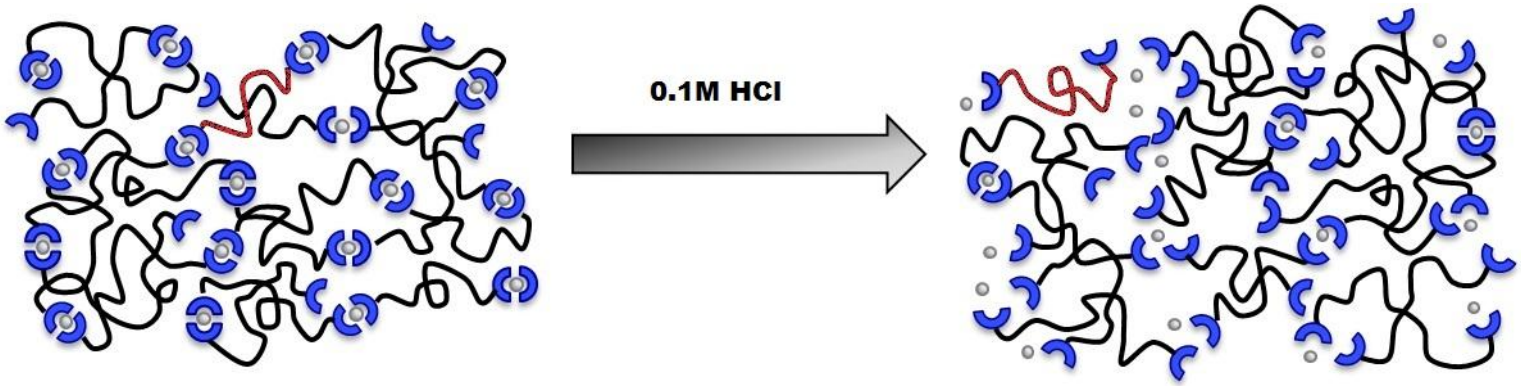
Humeomics:

Biomacromolecules 12 (2011) 1187-1199.

Humeomics: Opening the SOM Black Box



Metals release



Unbound organosoluble material (ORG1) release

1. Soil Preparation to remove Metals/ break humic aggregates in soil (*HCl 0.1M*)

2. Mild extraction to obtain the Unbound fraction ORG1 (*DCM/MeOH 2:1*)

3. Extraction out of Clay Minerals to obtain the Weakly bound ester fraction ORG2 + AQU2 (*BF_3/MeOH 12%*)

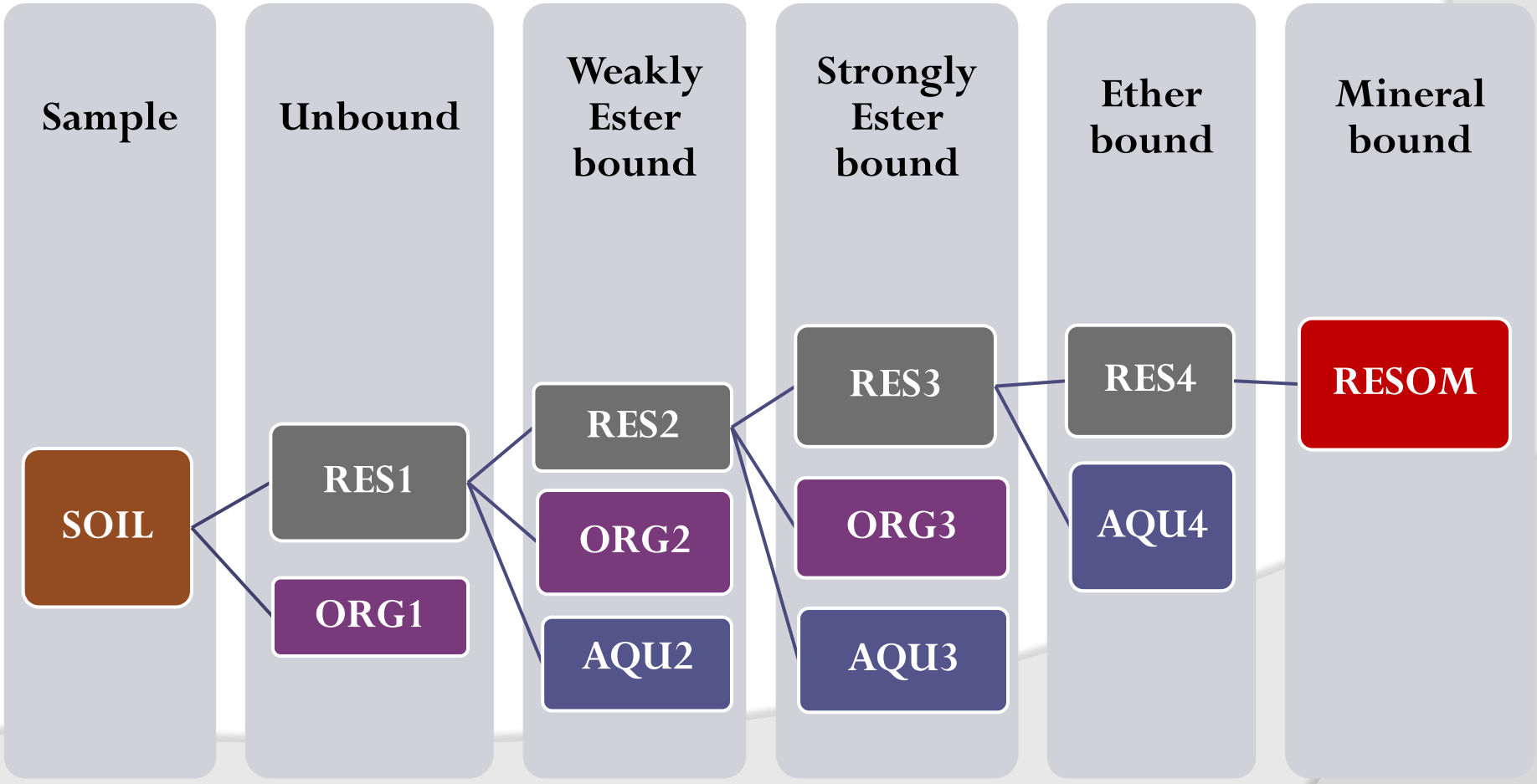
4. Heavy Extraction to obtain the Strongly bound ester fraction ORG3 + AQU3 (*KOH/MeOH 1M*)

5. Residual Extraction to obtain the Ether bound fraction AQU4 (*HI/H_2O 47%*)

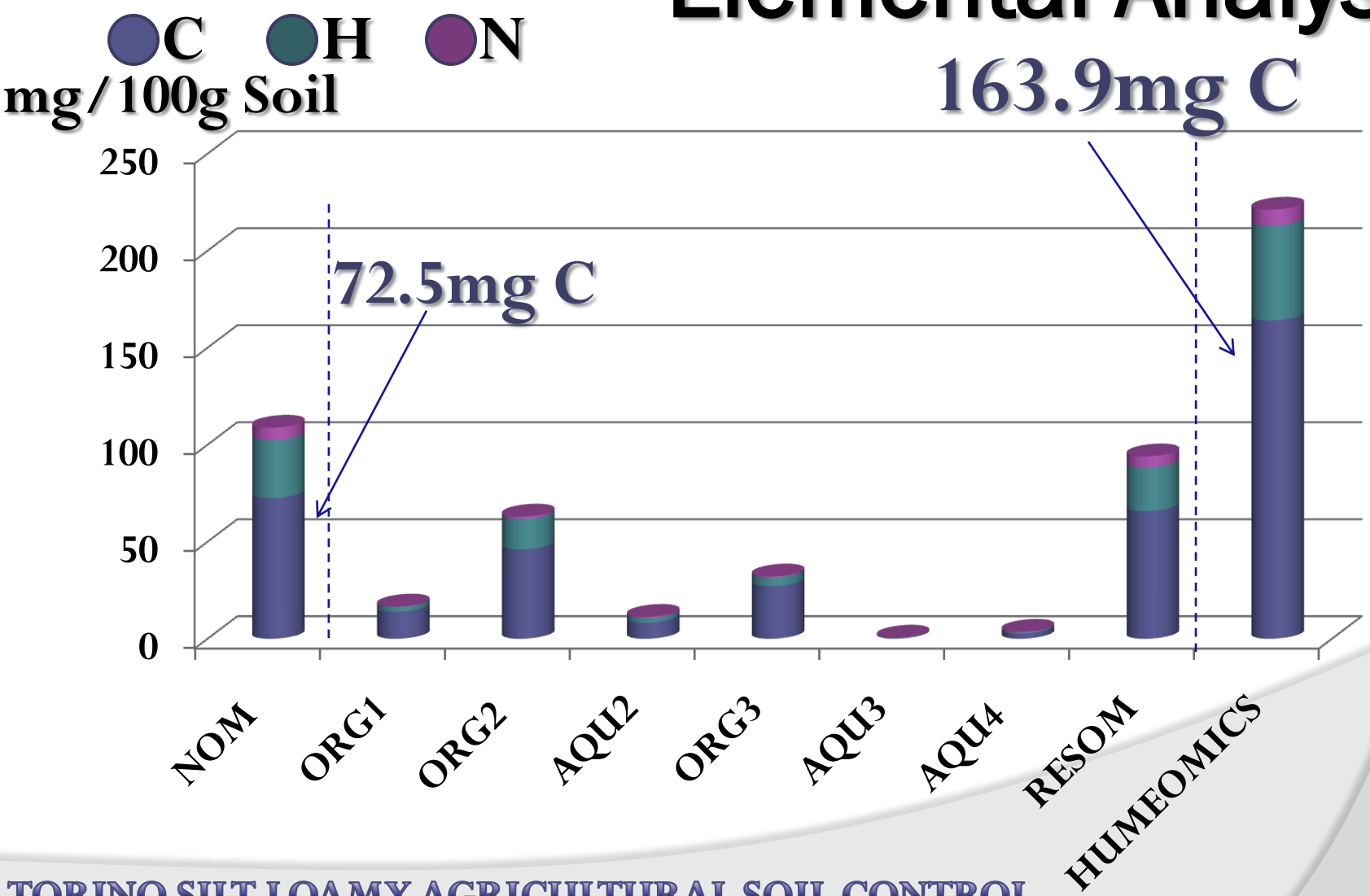
6. Final Extraction to obtain Residual SOM (*NaOH 0.5M/$Na_4P_2O_7$ 0.1M*)

SOIL → eSOM

TORINO SILT-LOAMY AGRICULTURAL SOIL



Elemental Analysis



TORINO SILT-LOAMY AGRICULTURAL SOIL CONTROL

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A molecular zoom into soil Humeome by a direct sequential chemical fractionation of soil

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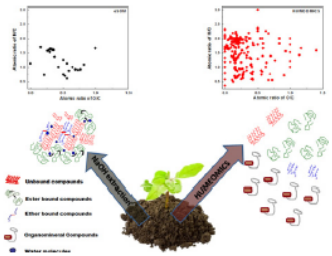
HIGHLIGHTS

- Soil Humeome was for the first time directly fractionated from soil.
- 235% more carbon extracted by Humeomics than traditional alkaline extraction.
- Separation of soil Humeome unraveled soil organominerals arrangement.
- HR-ESI-Orbitrap-MS revealed humic molecules covalently bound to Fe and AlSi.
- 66% of unextractable carbon by alkaline solution was solubilised by Humeomics.

GRAPHICAL ABSTRACT



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Carbon stabilization
Organomineral complexes

ABSTRACT

A Humeomics sequential chemical fractionation coupled to advanced analytical identification was applied directly to soil for the first time. Humeomics extracted ~235% more soil organic carbon (SOC) than by the total alkaline extraction traditionally employed to solubilise soil humic molecules (soil Humeome). Seven fractions of either hydro- or organo-soluble components and a final unextractable humic residue were separated from soil. These materials enabled an unprecedented structural identification of solubilised heterogeneous humic molecules by combining NMR, GC–MS, and ESI-Orbitrap-MS. Identified molecules and their relative abundance were used to build up structure-based van Krevelen plots to show the specific contribution of each fraction to SOC. The stepwise isolation of mostly hydrophobic and unsaturated molecules of progressive structural complexity suggests that humic suprastructures in soil are arranged in multi-molecular layers. These comprised molecules either hydrophobically adsorbed on soil aluminosilicate surfaces in less stable fractions, or covalently bound in amorphous organo-iron complexes in more recalcitrant fractions. Moreover, most lipid molecules of the soil Humeome appeared to derive from plant polyesters rather than bacterial metabolism. An advanced understanding of soil humic molecular composition by Humeomics may enable control of the bio-organic dynamics and reactivity in soil.

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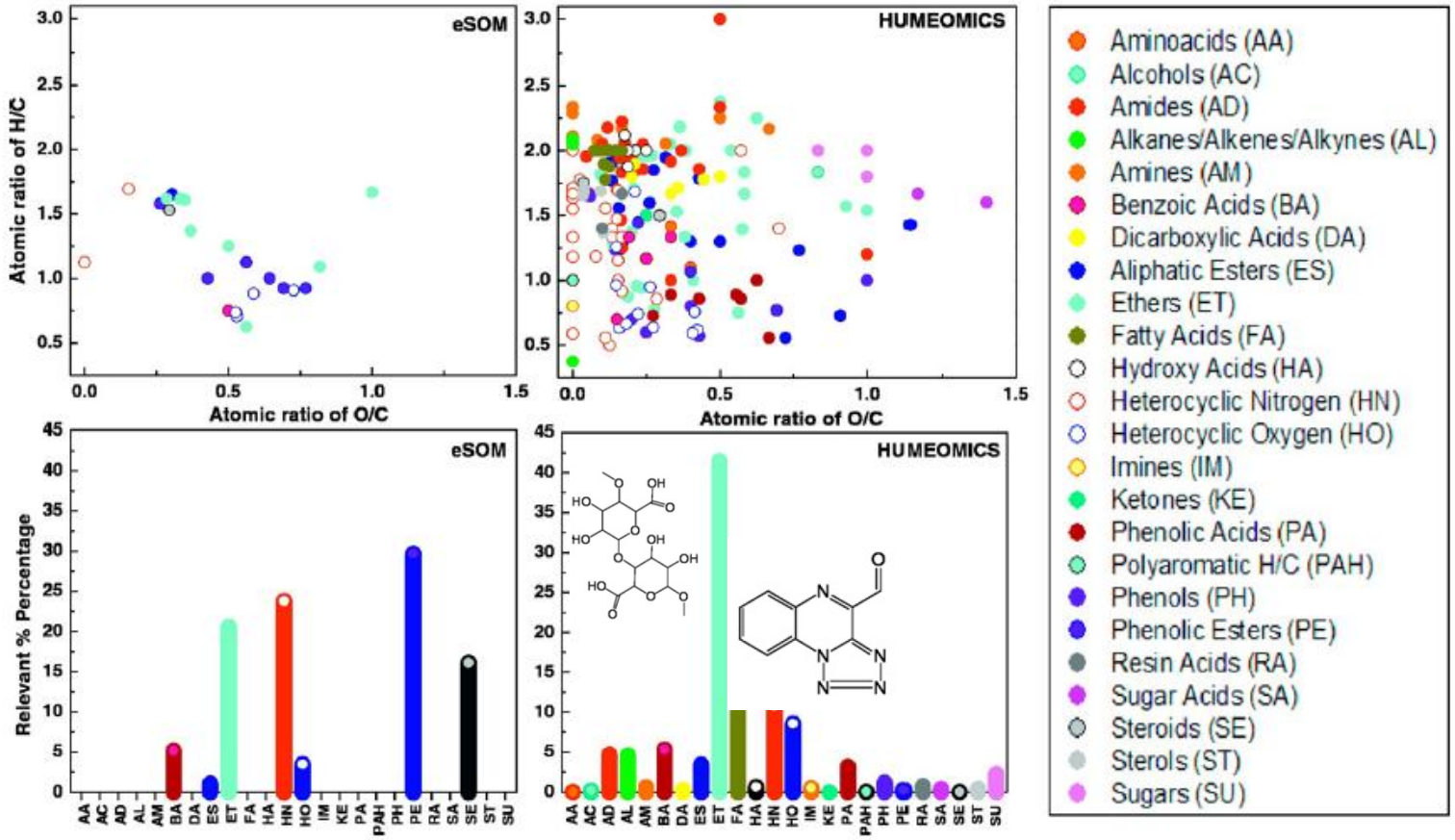
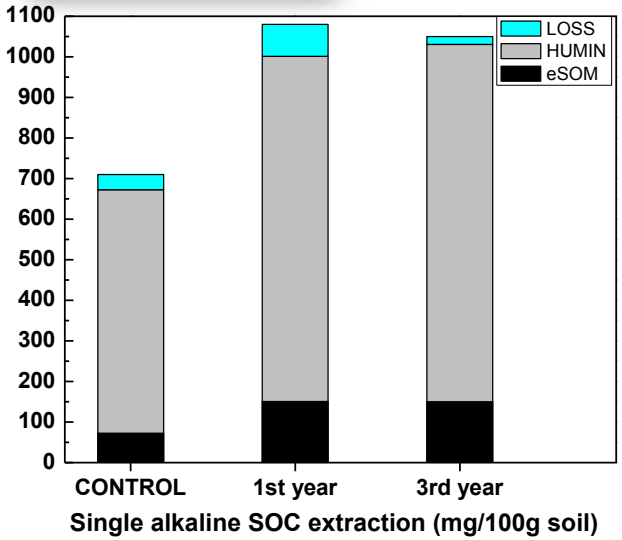
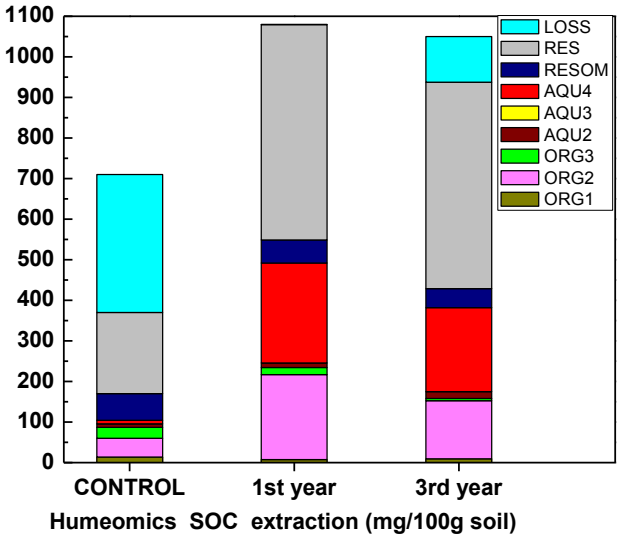

eSOM
HUMEOMICS
Atomic ratio of H/C
Atomic ratio of O/C
Relevant % Percentage
AA AC AD AL AM BA DA ES ET FA HA HN HO IM KE PA PAH PH PE RA SA SE ST SU
Aminoacids (AA)
Alcohols (AC)
Amides (AD)
Alkanes/Alkenes/Alkynes (AL)
Amines (AM)
Benzoic Acids (BA)
Dicarboxylic Acids (DA)
Aliphatic Esters (ES)
Ethers (ET)
Fatty Acids (FA)
Hydroxy Acids (HA)
Heterocyclic Nitrogen (HN)
Heterocyclic Oxygen (HO)
Imines (IM)
Ketones (KE)
Phenolic Acids (PA)
Polyaromatic H/C (PAH)
Phenols (PH)
Phenolic Esters (PE)
Resin Acids (RA)
Sugar Acids (SA)
Steroids (SE)
Sterols (ST)
Sugars (SU)



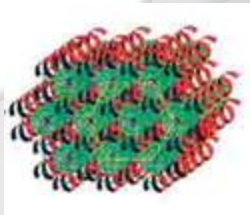


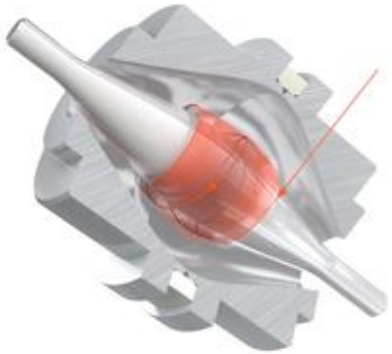
Extractable OM Chemical Protection Ratio (CPR): $\frac{\sum ORG_i\, OC_{mg}}{RESOM\, OC_{mg} + \sum AQU_i OC_{mg}}$

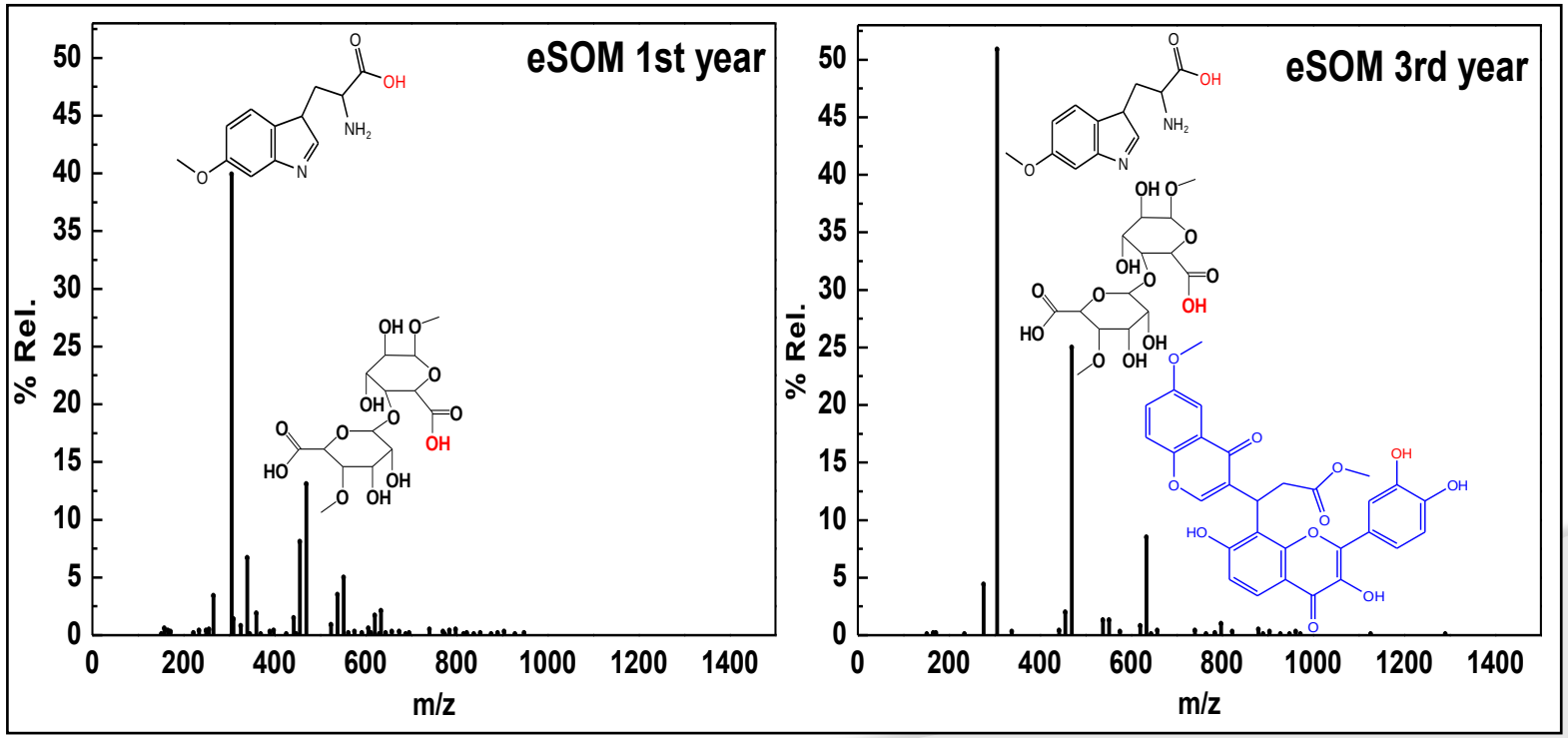
CPR TO CONTROL **= 87.1/83.2 = 1.05**

CPR TO 1st year Tillage **= 234.3/304.4 = 0.77**

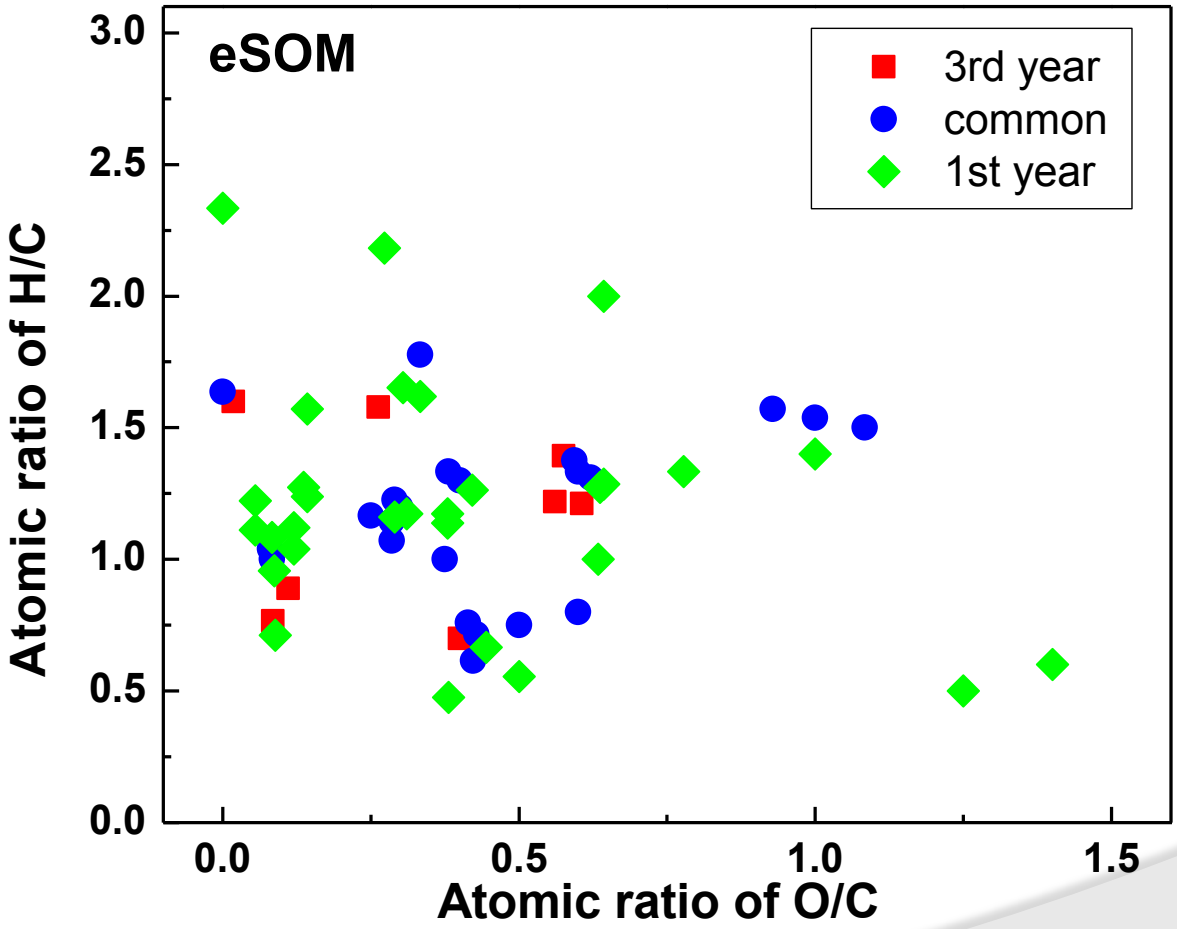
CPR TO 3rd year Tillage **= 158.4/270.3 = 0.59**

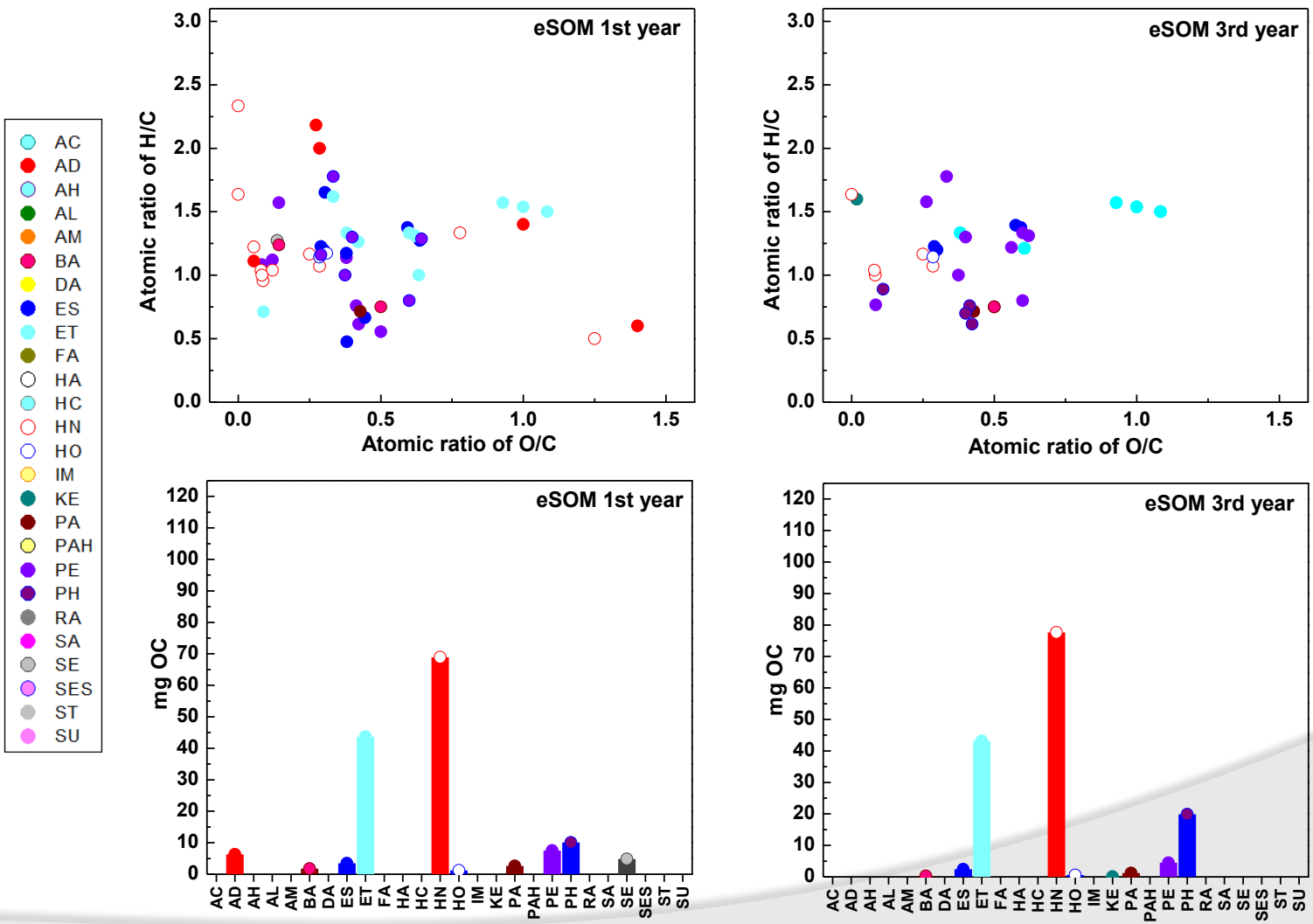




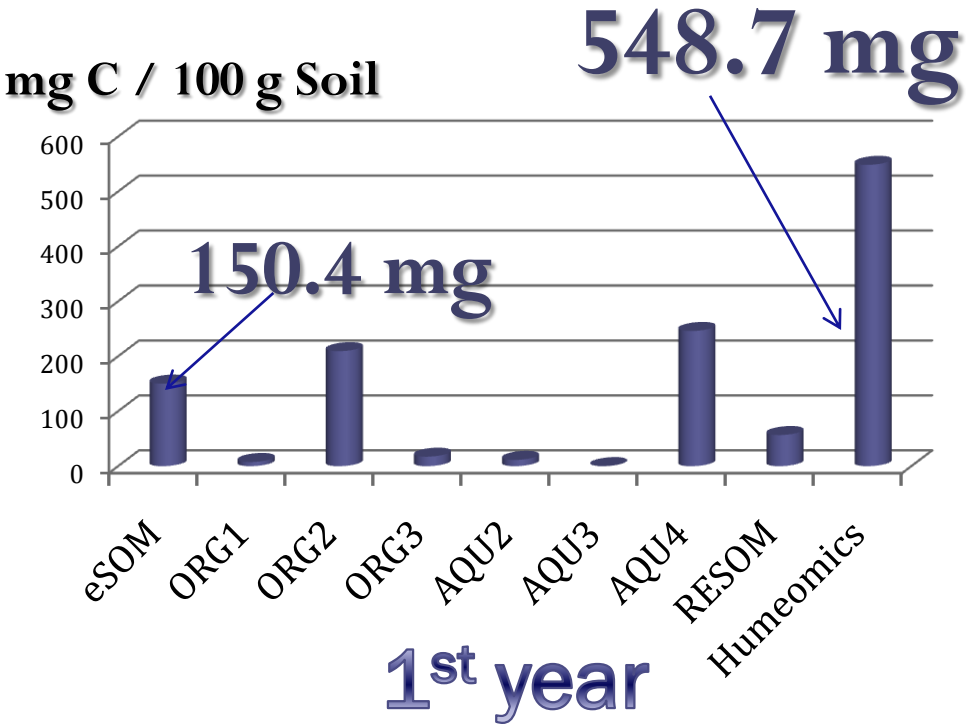


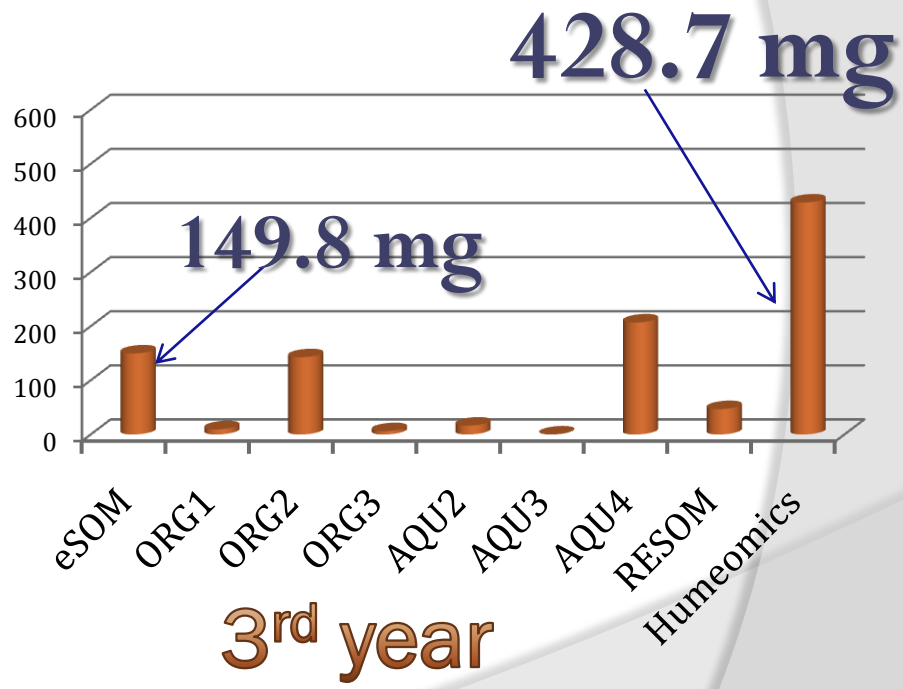
TRADITIONAL TILLAGE (maize crop)

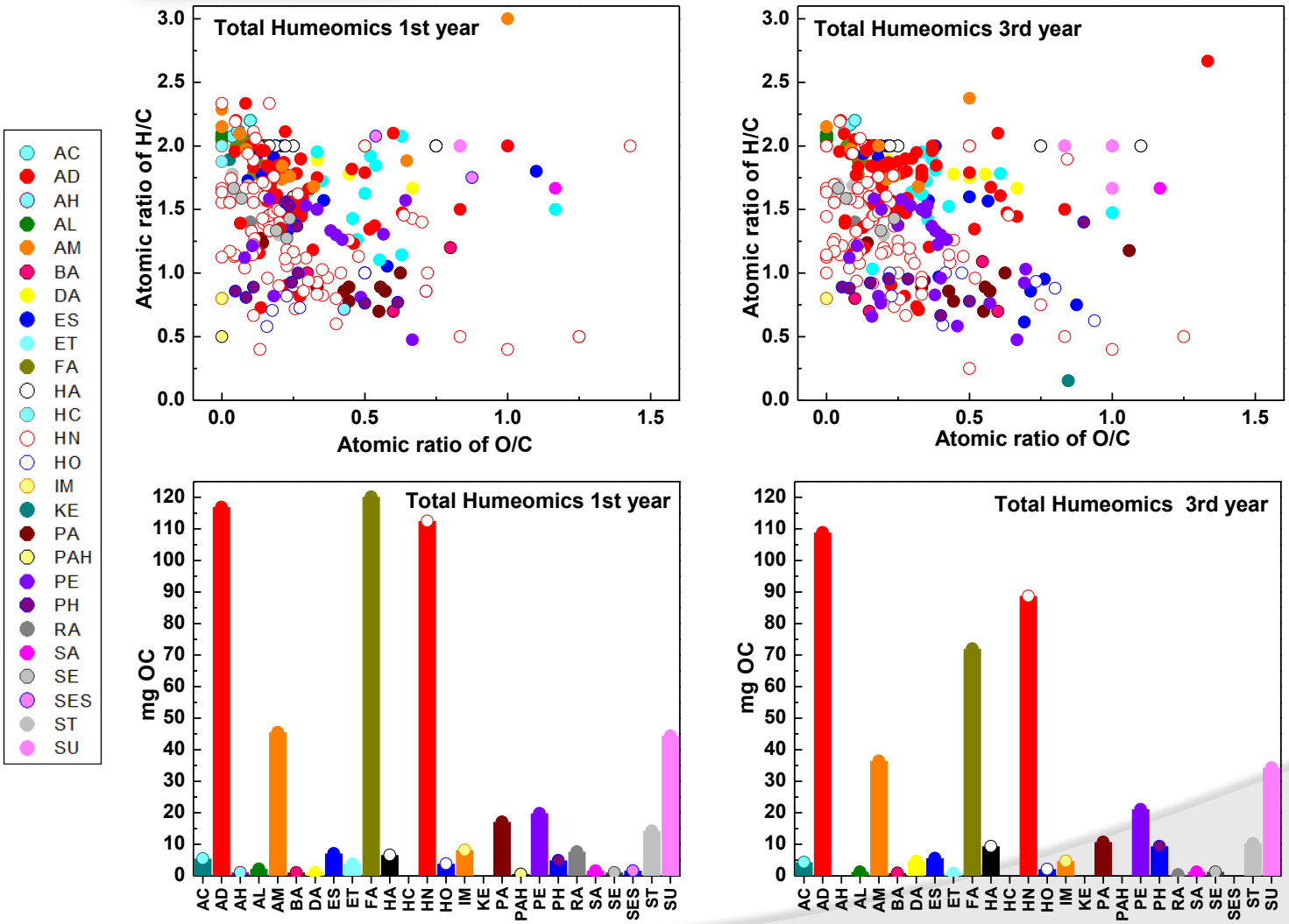

eSOM
3rd year
common
1st year
Atomic ratio of H/C
Atomic ratio of O/C
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0.0
0.5
1.0
1.5


AC
AD
AH
AL
AM
BA
DA
ES
ET
FA
HA
HC
HN
HO
IM
KE
PA
PAH
PE
PH
RA
SA
SE
SES
ST
SU
eSOM 1st year
eSOM 3rd year
Atomic ratio of H/C
Atomic ratio of O/C
mg OC

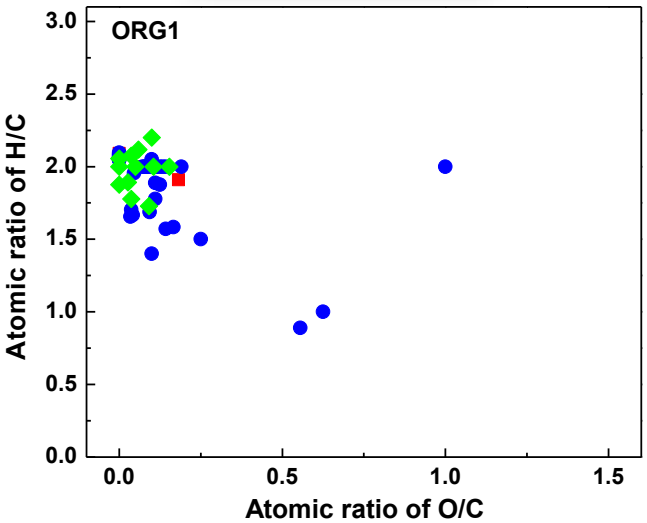
TRADITIONAL TILLAGE (maize crop)

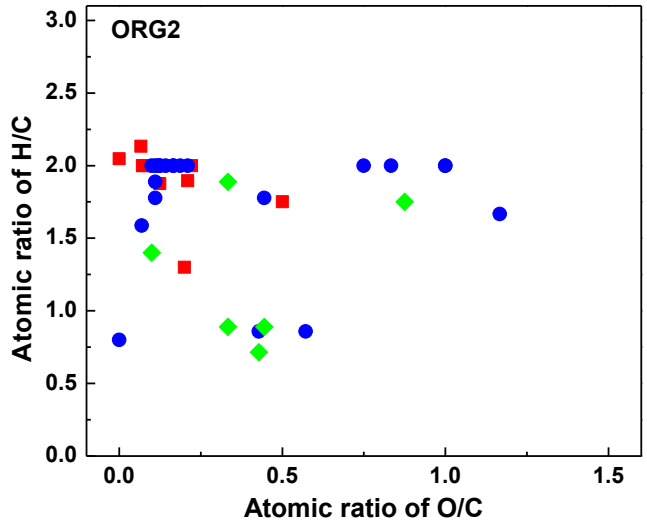





Total Humeomics 1st year
Atomic ratio of H/C
Atomic ratio of O/C
Total Humeomics 3rd year
Atomic ratio of H/C
Atomic ratio of O/C
Total Humeomics 1st year
mg OC
Total Humeomics 3rd year
mg OC
AC
AD
AH
AL
AM
BA
DA
ES
ET
FA
HA
HC
HN
HO
IM
KE
PA
PAH
PE
PH
RA
SA
SE
SES
ST
SU

In ORG1 most of the lipids are degraded after 2 years.

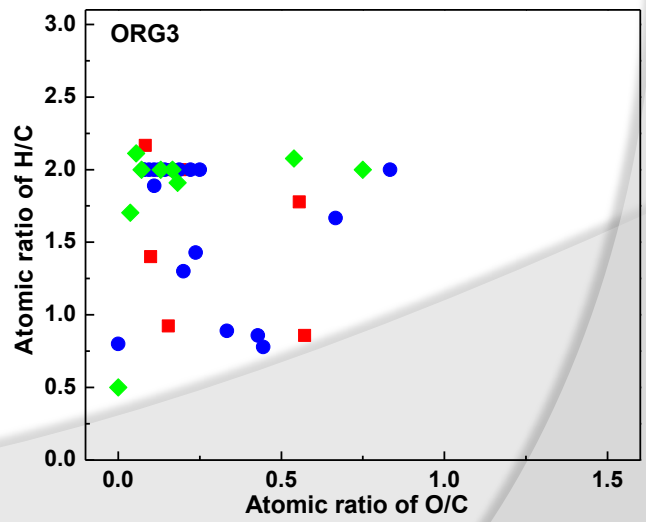


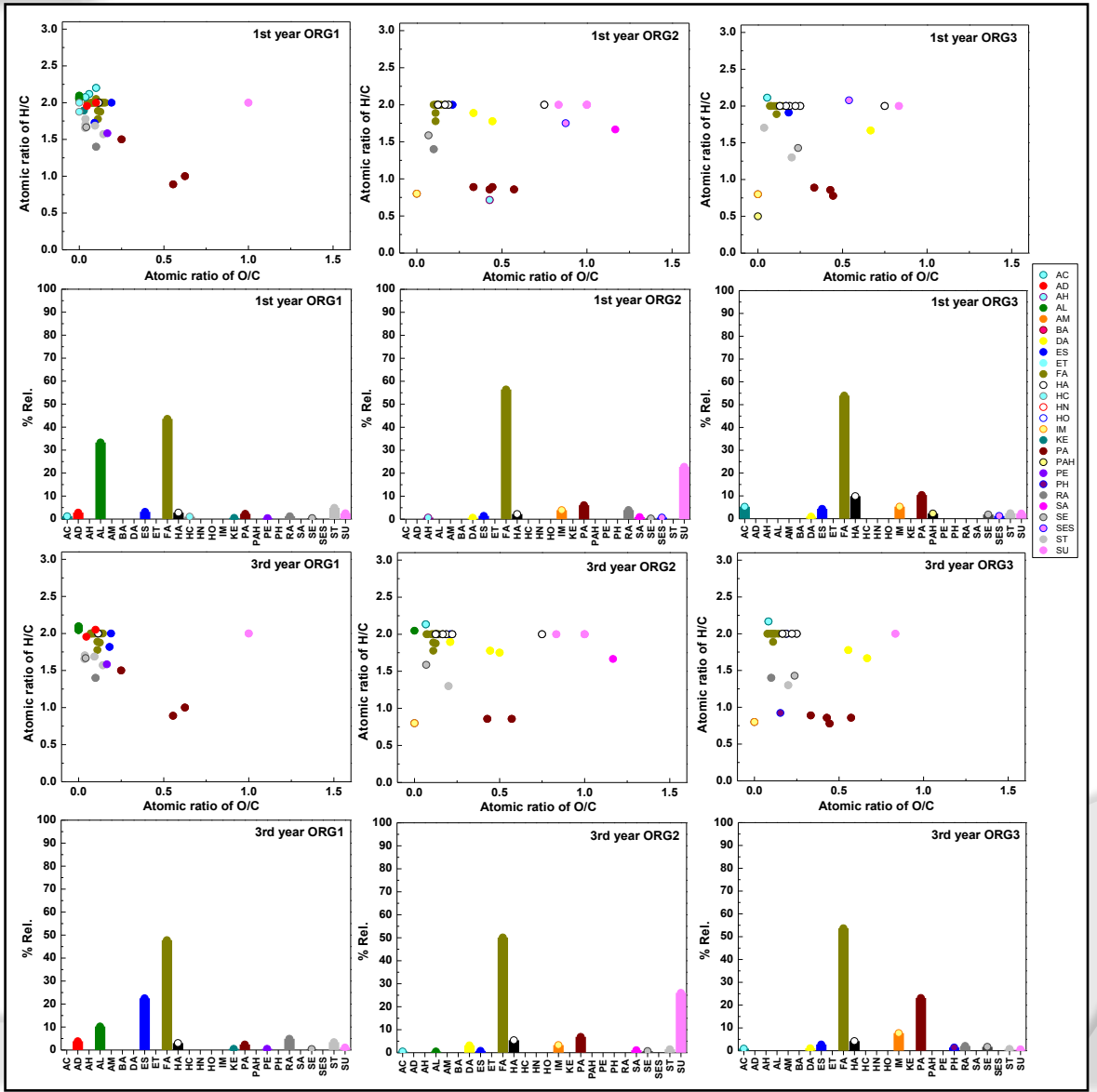


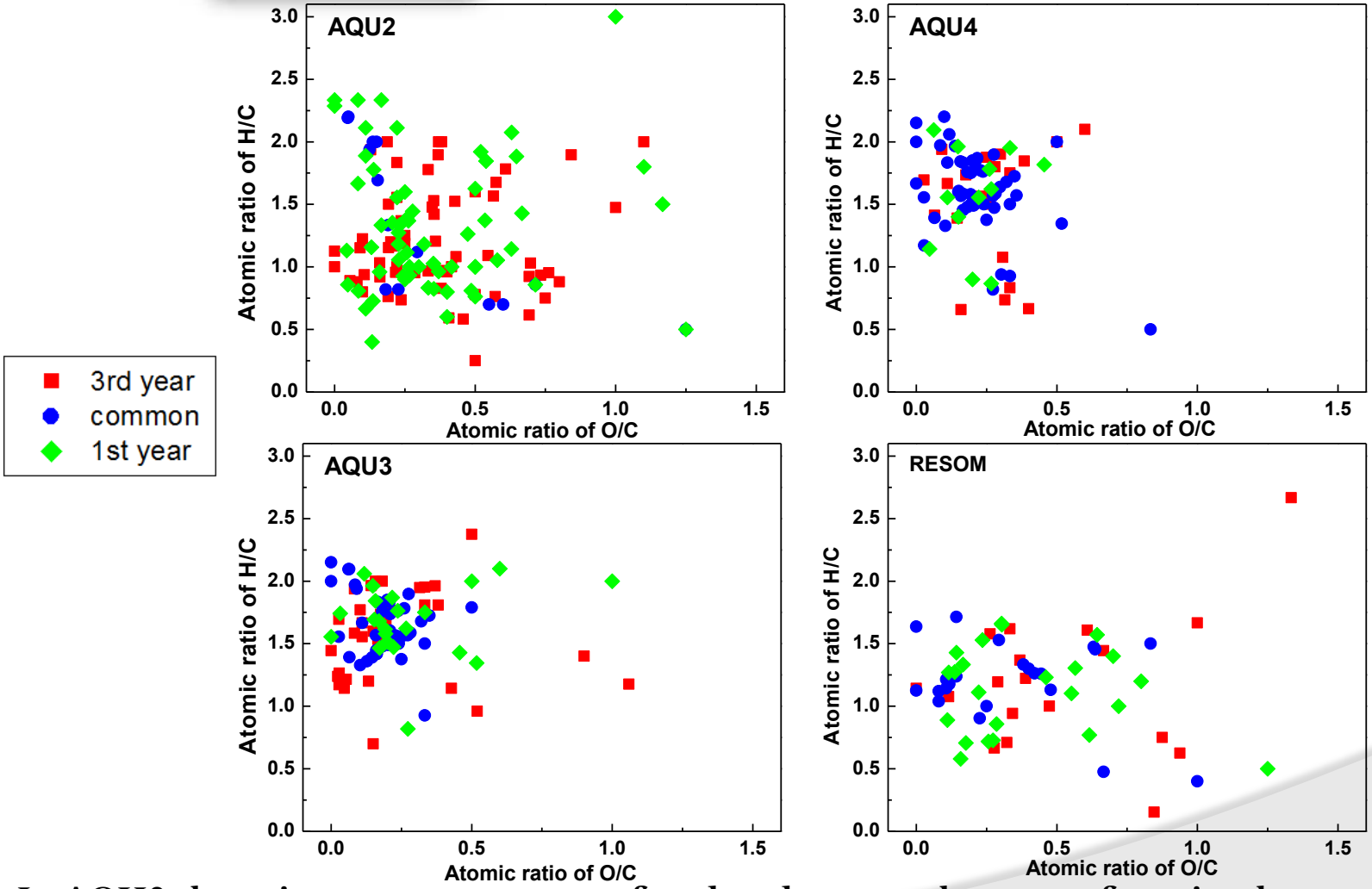
3rd year
common
1st year

ORG2 loses aromatic compounds in favor of lipids.

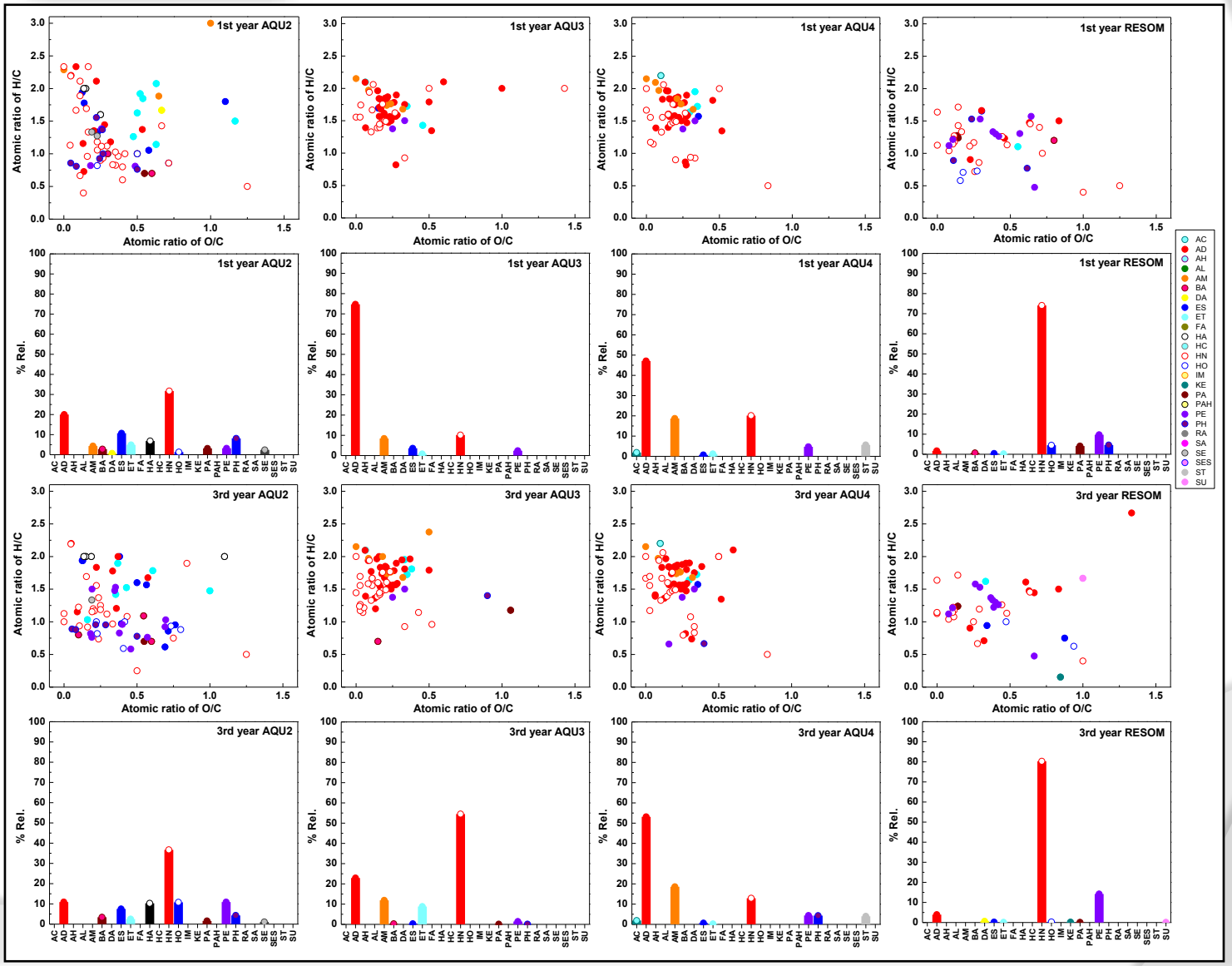
ORG3 gets more oxidized.

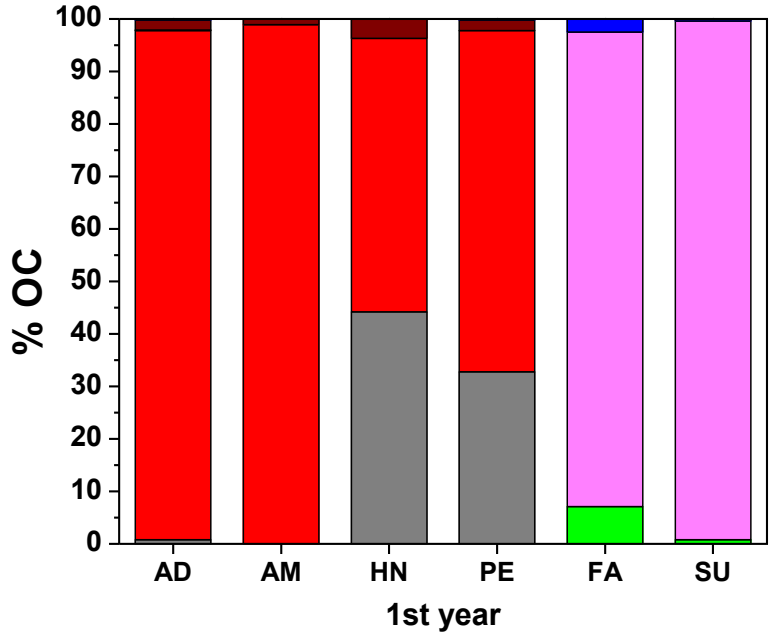


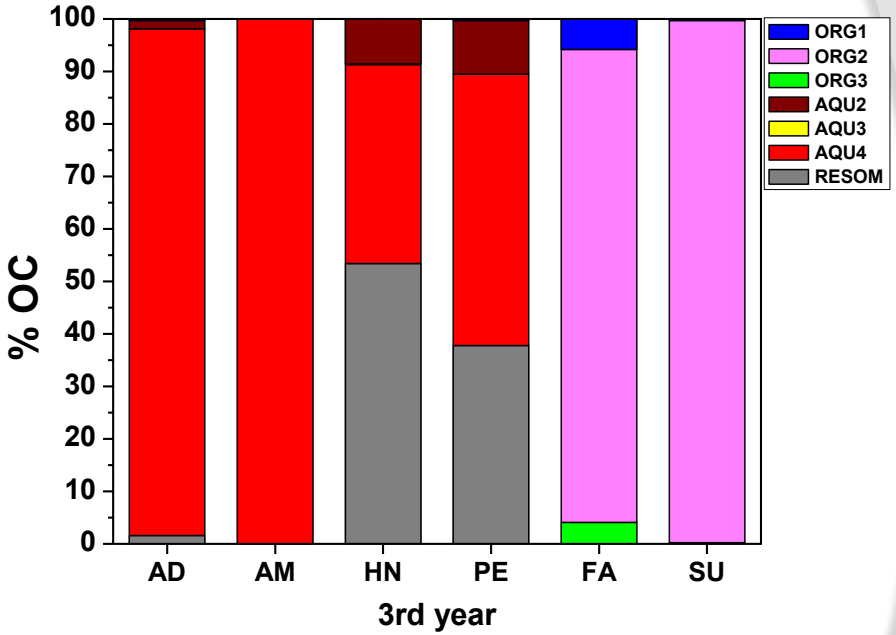

1st year ORG1
1st year ORG2
1st year ORG3
3rd year ORG1
3rd year ORG2
3rd year ORG3
Atomic ratio of H/C
Atomic ratio of O/C
% Rel.
AC
AD
AH
AL
AM
BA
DA
ES
ET
FA
HA
HC
HN
HO
IM
KE
PA
PAH
PE
PH
RA
SA
SE
SES
ST
SU



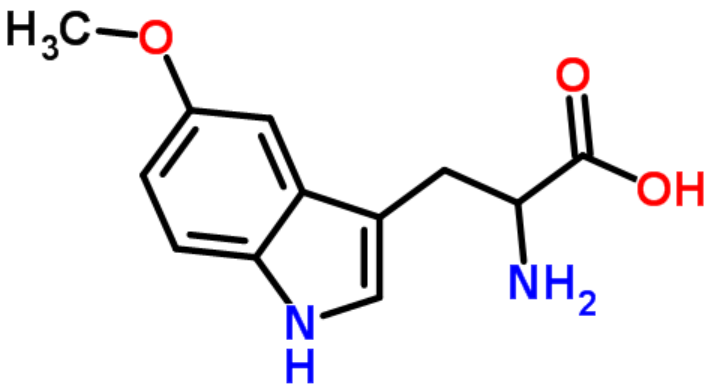
In AQU2 there is a great turnover of molecules over the years, favoring homogeneity.
AQU3 becomes more heterogeneous and oxidized.
AQU4 and RESOM get more oxidized.


1st year AQU2
1st year AQU3
1st year AQU4
1st year RESOM
3rd year AQU2
3rd year AQU3
3rd year AQU4
3rd year RESOM
Atomic ratio of H/C
Atomic ratio of O/C
% Rel.
AC
AD
AH
AL
AM
BA
DA
ES
ET
FA
HA
HC
HN
HO
IM
KE
PA
PAH
PE
PH
RA
SA
SE
SES
ST
SU


% OC
100
90
80
70
60
50
40
30
20
10
0
AD
AM
HN
PE
FA
SU
1st year


% OC
100
90
80
70
60
50
40
30
20
10
0
AD
AM
HN
PE
FA
SU
3rd year
ORG1
ORG2
ORG3
AQU2
AQU3
AQU4
RESOM

5-methoxytryptophan



+ FeO

Orbitrap mass (in negative mode): **305.022**

Orbitrap mass +H mass -FeO mass

305.022 + 1.008 -71.930 = **Monoisotopic mass**: **234.100**

Average mass: 234.251

Environmental Science & Technology

Article

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DOI: 10.1021/acs.est.5b03608
Environ. Sci. Technol. 2015, 49, 14257−14266

olicy and Governance
Farm
NV/IT/000719

Revealing Brown Carbon Chromophores Produced in Reactions of Methylglyoxal with Ammonium Sulfate

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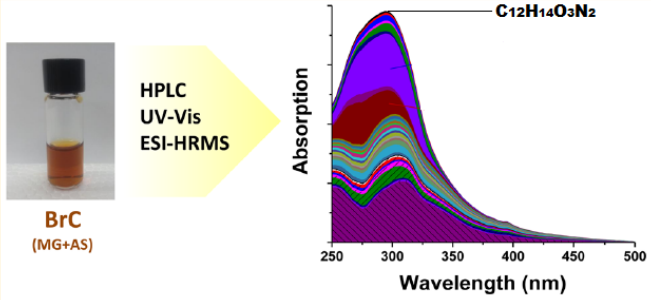
[‡]Physical Sciences Division, Pacific Northwest National Laboratory, Richland, Washington 99354, United States
[§]Department of Chemistry, University of California, Irvine, California 92697, United States

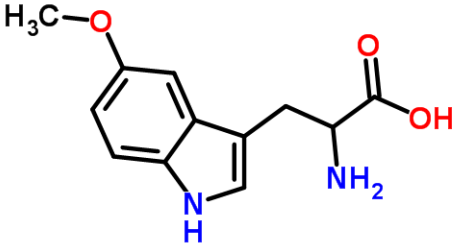
Supporting Information

ABSTRACT: Atmospheric brown carbon (BrC) is an important contributor to light absorption and climate forcing by aerosols. Reactions between small water-soluble carbonyls and ammonia or amines have been identified as one of the potential pathways of BrC formation. However, detailed chemical characterization of BrC chromophores has been challenging and their formation mechanisms are still poorly understood. Understanding BrC formation is impeded by the lack of suitable methods which can unravel the variability and complexity of BrC mixtures. This study applies high performance liquid chromatography (HPLC) coupled to photodiode



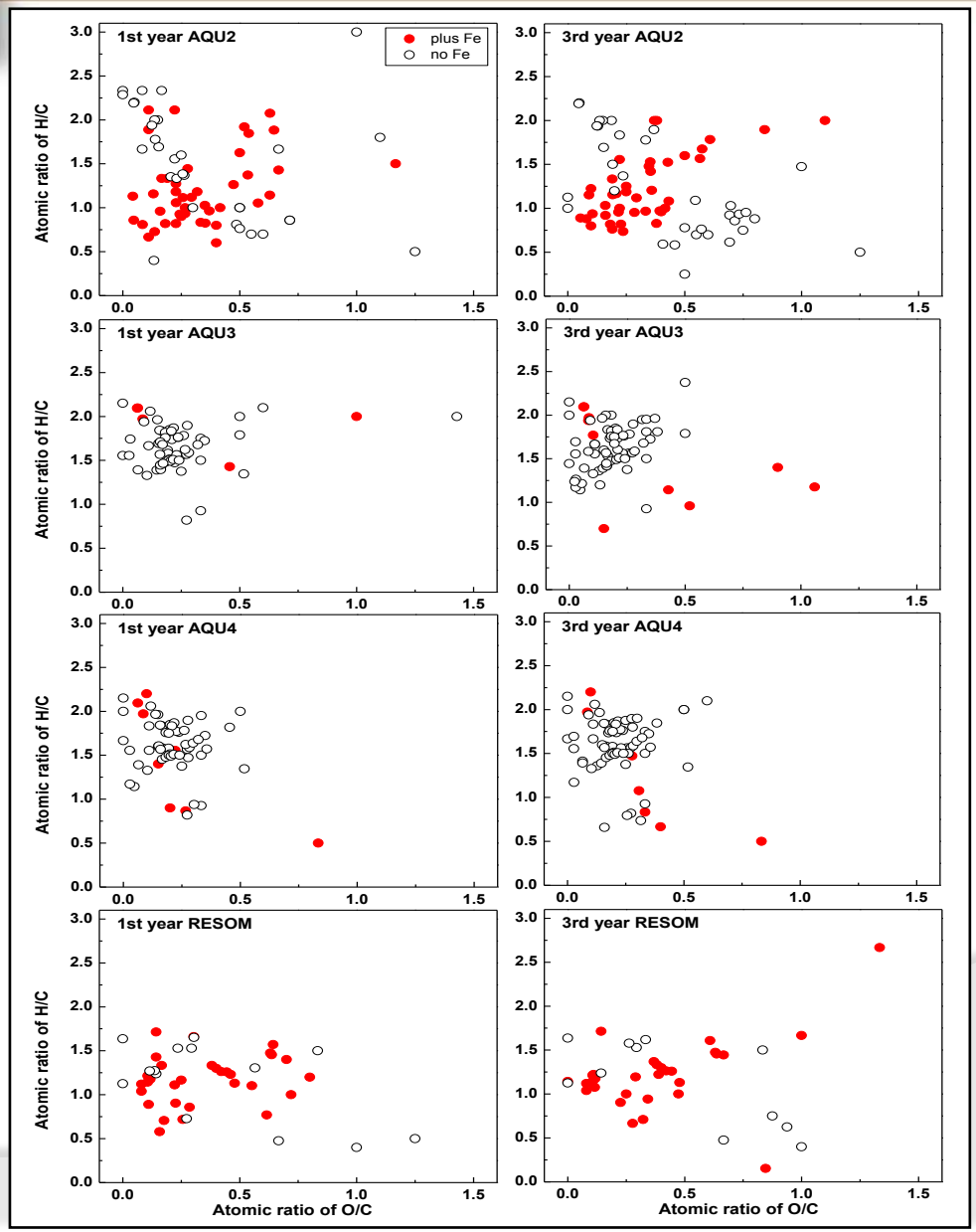
5-methoxytryptophan

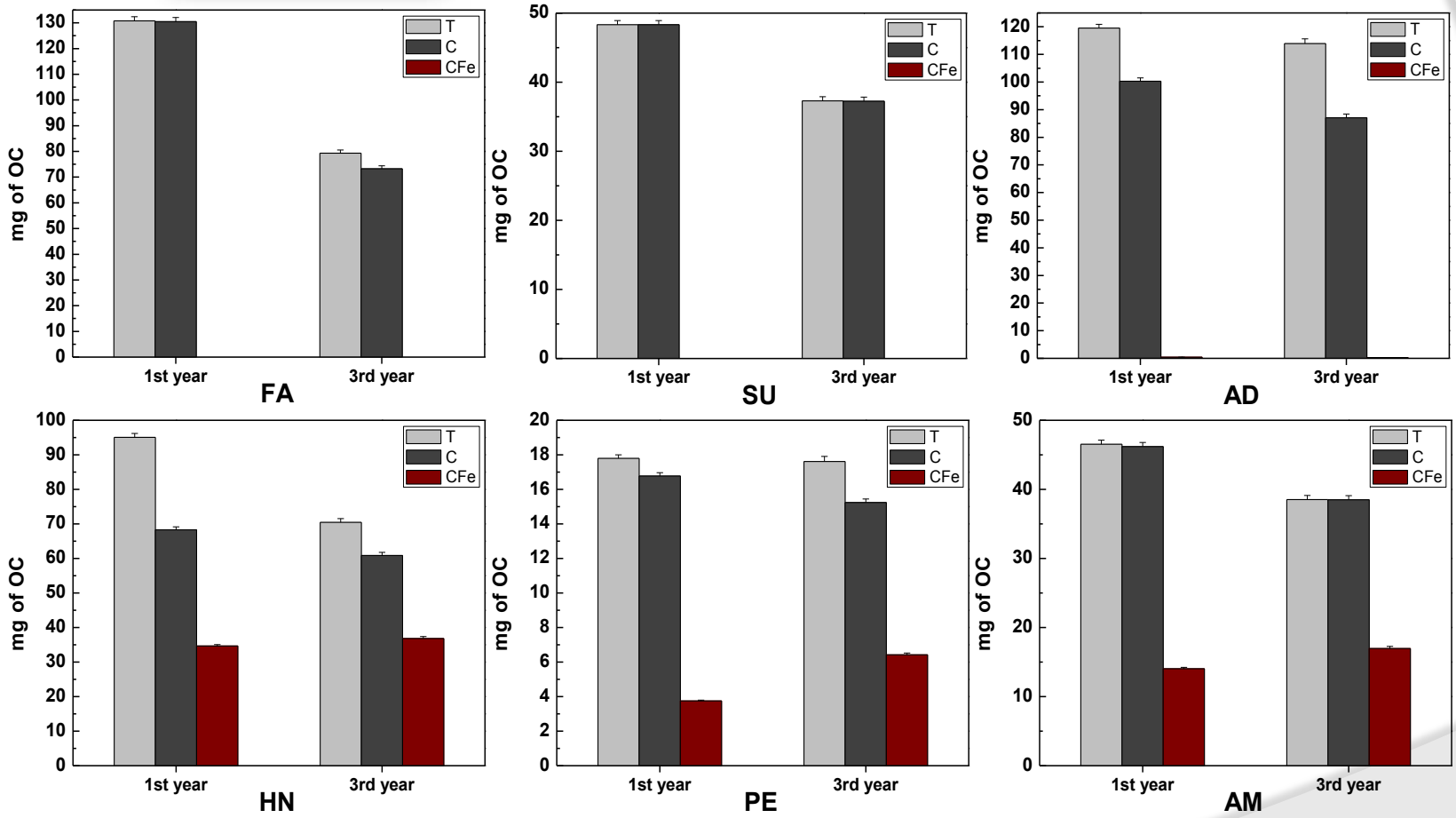
$C_{12}H_{14}O_3N_2$



There is a visual trend of Fe being detached from lipids (AQU2) and get attached to more oxidized material such as polysaccharides and lignin derivatives (AQU2, AQU3, and RESOM).

More oxidized and less condensed Fe-bound moleclules in 3rd year AQU2 and RESOM fractions.





Changes in the carbon yield, specifically in those molecules which remain stable in time, reveal a chemical affinity to iron.

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Synthetic iron complexes as models for natural iron-humic compounds: Synthesis, characterization and algal growth experiments

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Higher affinity of AM to Fe than HN

Perspectives

- More research is needed in revealing Fe affinity to certain groups of molecules.
- **Iron complexation to OM seems to be group-specific and could eventually lead to an *in-situ* stabilization technology of soil organic carbon.**
- More soil humeomics research is needed to reveal humification/carbon sequestration mechanisms.

Extractable OM Chemical Protection Ratio (CPR): $\frac{\sum ORG_i\ OC_{mg}}{RESOM\ OC_{mg} + \sum AQU_i OC_{mg}}$

TORINO CONTROL

CPR = 87.1/83.2 = 1.05

TORINO Maize 1st year

CPR = 234.3/304.4 = 0.77

TORINO Wheat 1st year

CPR = 122.5/249.8 = 0.49

TORINO Wheat 1st year + Iron porphyrin

CPR = 107.6/70.0 = 1.54

TORINO Compost 1st year

CPR = 150.5/83.2 = 1.81

Brazilian Terra Preta Soil

CPR = 454.3/504.0 = 0.90

Brazilian Oxisol Control Soil

CPR = 363.0/253.2 = 1.43

Iron Stabilization mechanism

Wheat Biochar (WB)

CPR = 379.9/158.0 = 2.40

Chinese Control Rice Paddy Soil

CPR = 190.6/408.1 = 0.47

Chinese Rice Paddy Soil + 20 tn/ha WB

CPR = 153.0/285.3 = 0.54

Chinese Rice Paddy Soil + 40 tn/ha WB

CPR = 274.8/163.5 = 1.68

NMR CERMANU

Life+ Environme
LIFE Carb
progetto: LIFE

Università degli Studi di Napoli Federico II

Thank you for your attention!