Life+ Environment Policy and Governance
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AN INTEGRATED SYSTEM APPROACH TO IMPROVE PLANT NUTRITION BY COMBINING RECYCLED COMPOSTED BIOMASSES WITH MICROBIAL CONSORTIUM

HIARHI MONDA – Ph.D.

BIO FECTOR

EUTRIPHICATION

SOIL FERTILITY

BIODIVERISTY

COMPOST
BIO-EFFECTORS
N
P

- Improve soil structure
- Reduce soil erosion
- Increase soil fertility
- Furnish 30% to 70% of soil's CEC
- Promote water retention
- C food supply for beneficial microbes

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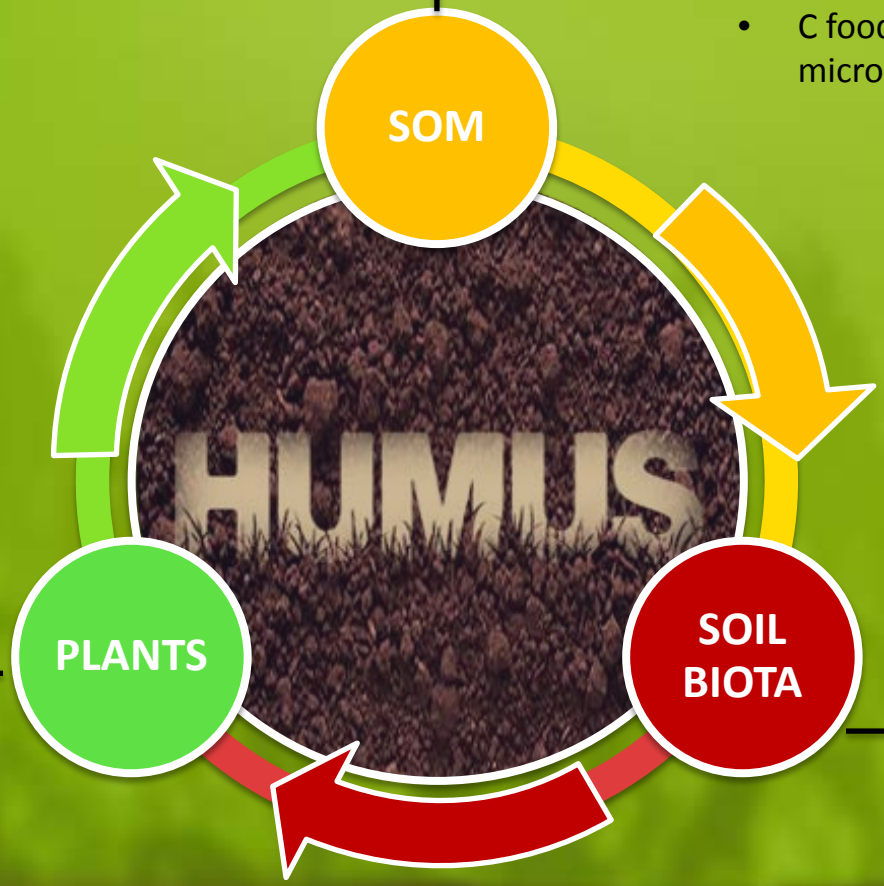
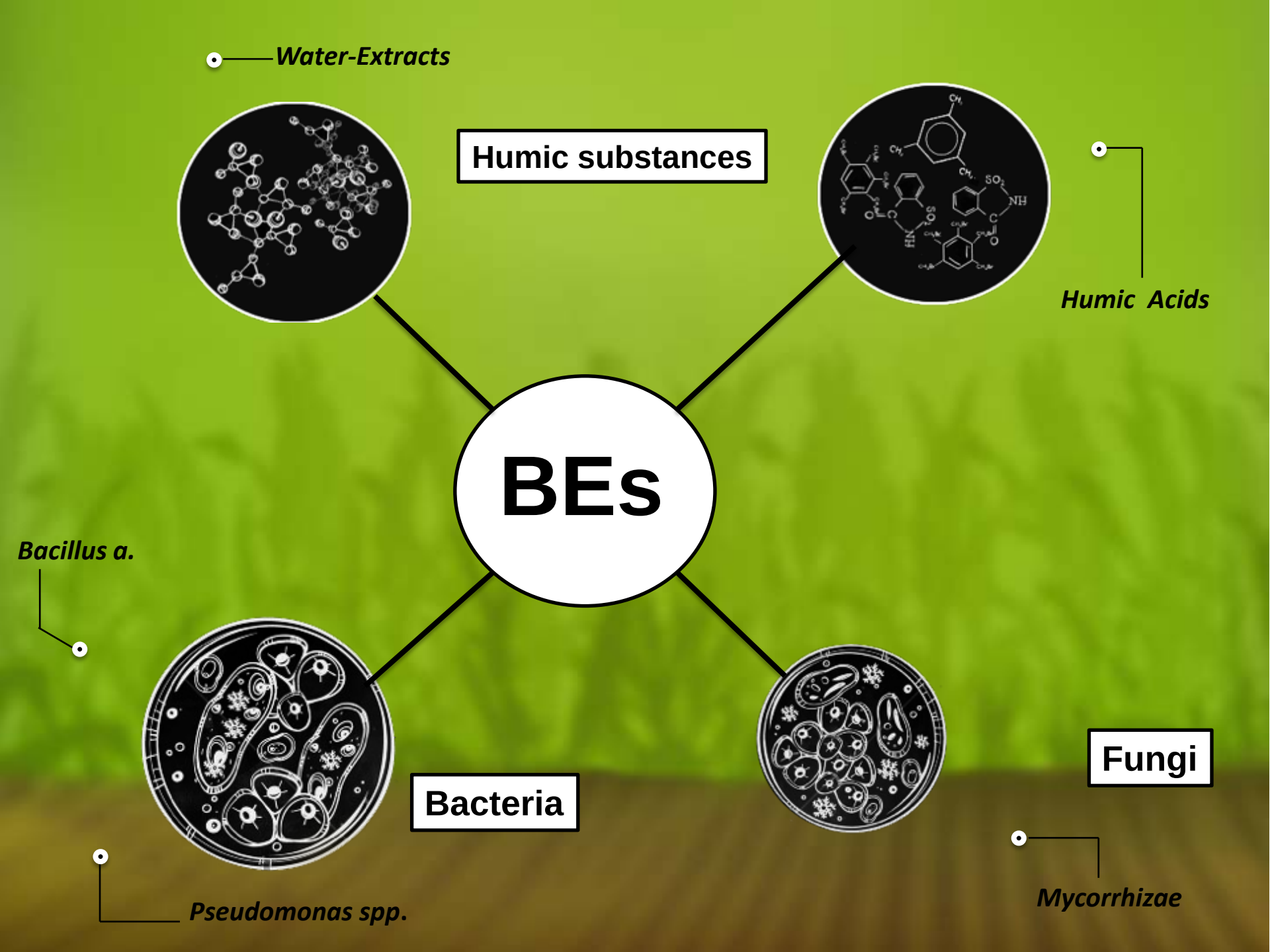
- Compelx interaction with microbes
- Selective pressure on microbial community
- Root exudates dramatically affect the chemistry of rhyzosphere
- Keep soil moisture



- Soil engineers
- Nutrient cycling
- Promote mineralization of C
- Promote sequestration of C
- Breaking down OM into humus
- Enhance soil mosture and porosity
- Improve crop productivity

COMPOST → **ALTERNATIVE RECYCLED SOURCE OF ORGANIC MATTER AND NUTRIENTS**


Water-Extracts
Humic substances
Humic Acids
BEs
Bacillus a.
Bacteria
Fungi
Pseudomonas spp.
Mycorrhizae

- Development and characterization of new biostimulants from recycled organic biomassess

- Eco-friendly approach to improve nutrients uptake by use of PGPM in combination with biostimulants

1

Molecular characteristics of water-extractable organic matter from different composted biomasses as related to effects on seed germination and early growth of maize

C-CYN

8.0% artichoke, 20% woodchips and 2% compost as starter

C-CYNF

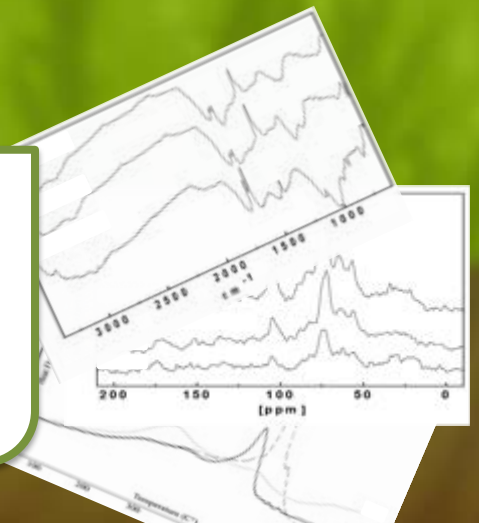
43% artichoke, 23% fennel, 11% escarole residues, 20% woodchips 2% compost as starter

C-TOM

50.0% tomato residues, 48% woodchips 2% compost as starter

C-MSW

comercial urban waste compost (FORSU)



CHARACTERIZATION

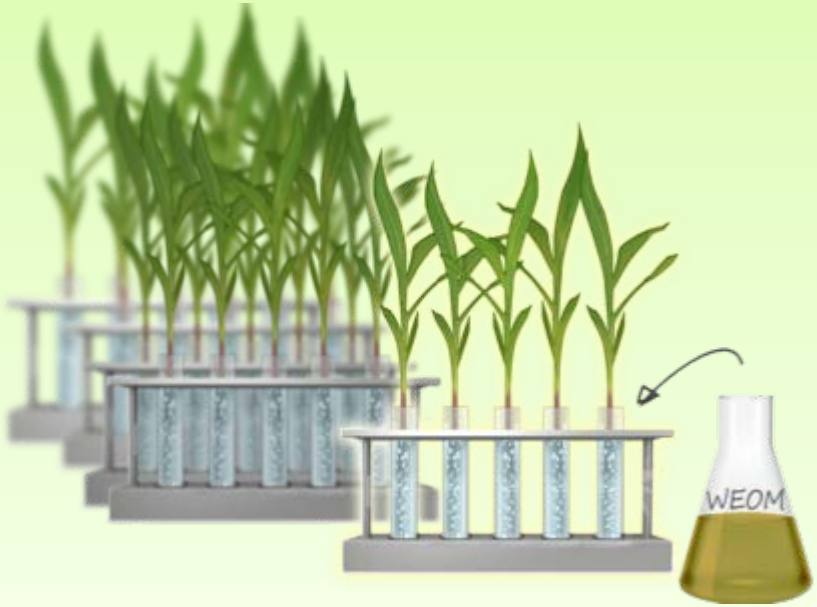
Elemental analysis
13C-CPMAS Spectroscopy
1H Spectroscopy
IR-ATR Spectroscopy
Thermal analysis (TGA, DSC)

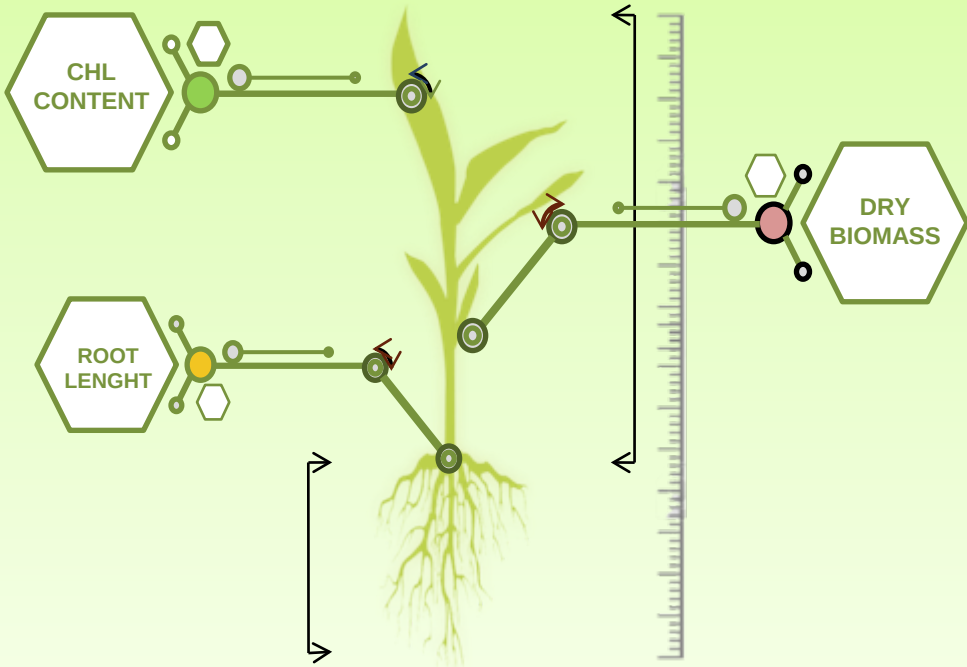
Experimental Setup

GERMINATION TEST: 5 days at 25°C in the dark 85% RH
15 seeds x petri dish (x5 replicates)
10 ml WEOM extract (50 ppm CL-1) or water (control)

ANALYSES
length of coleoptile
relative seed germination (RSG),
relative primary root growth (1RRG),
relative secondary root growth (2RRG)
germination index (GI)

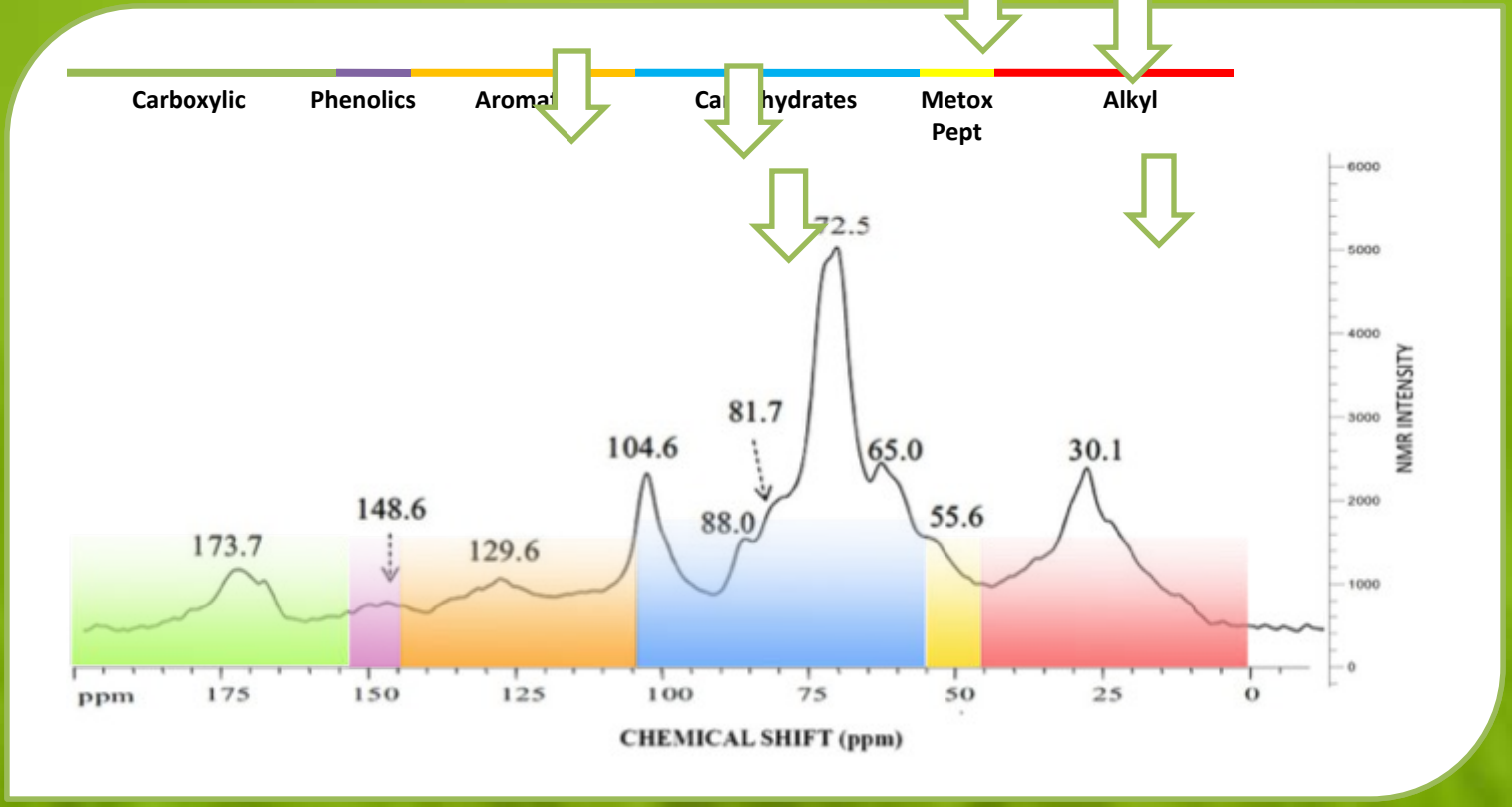
BIOACTIVITY ASSAY: 15 days, 75% RH, 20-27°C T°, photoperiod 8h dark/16h light





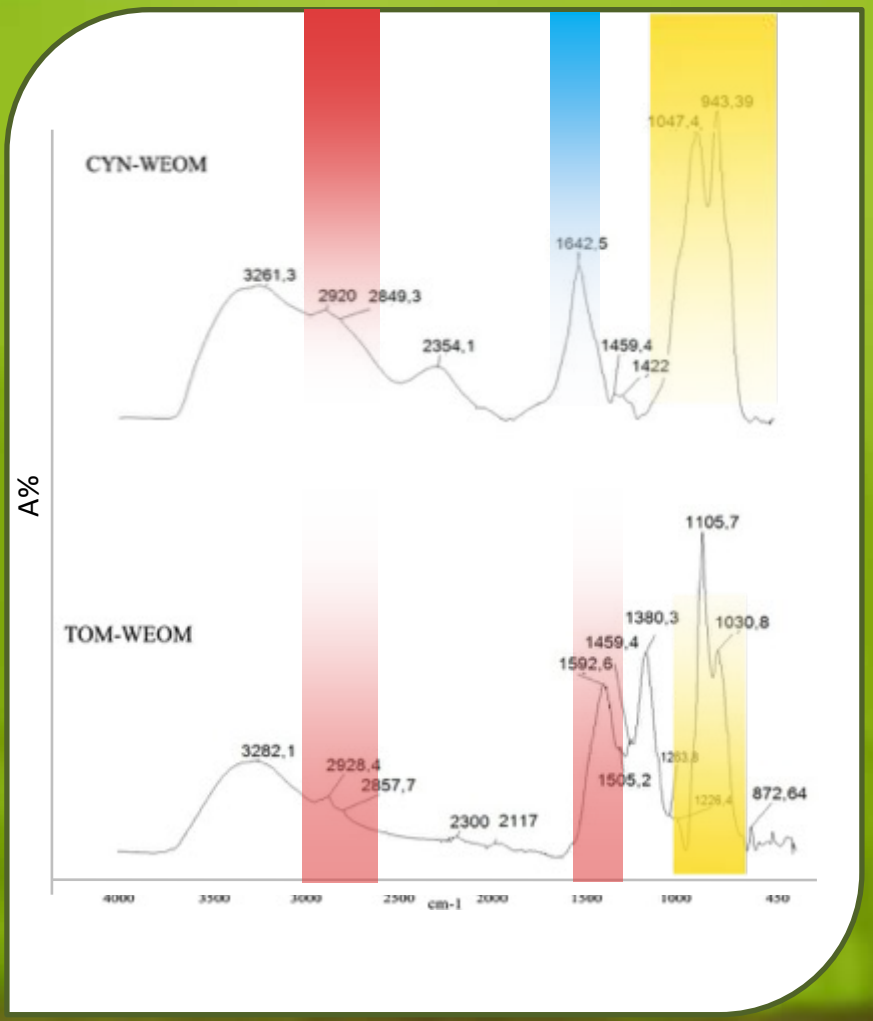
^{13}C CPMAS NMR

RESULTS - CHARACTERIZATION



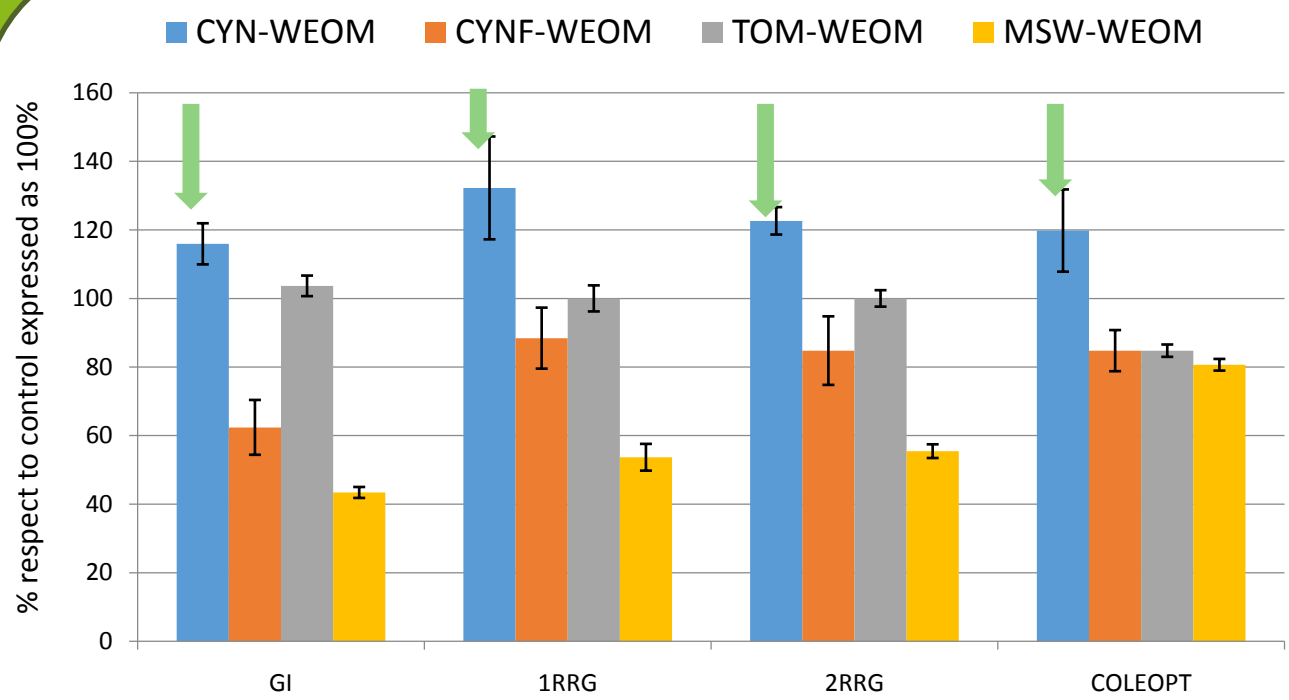
FT- IR ATR WEOMs

RESULTS - CHARACTERIZATION



GERMINATION

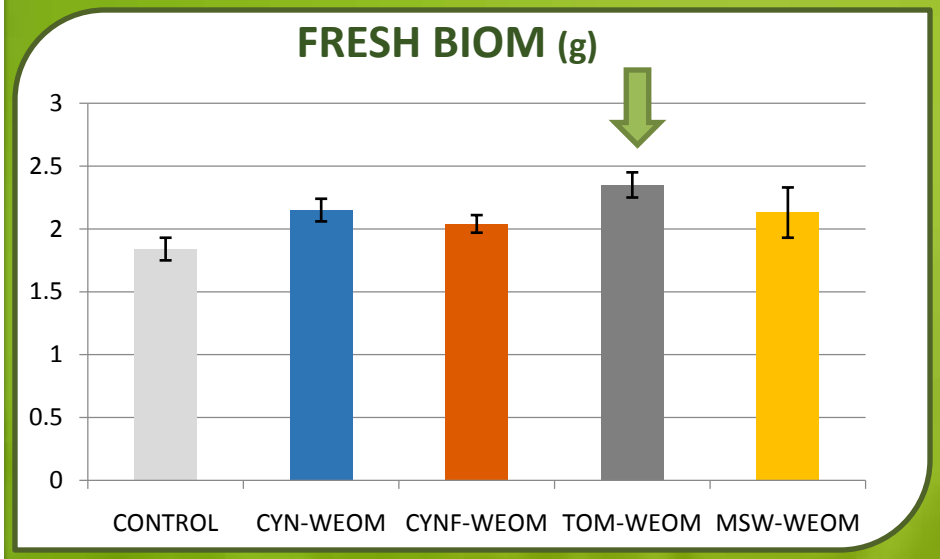
RESULTS – BIOACTIVITY ON PLANT

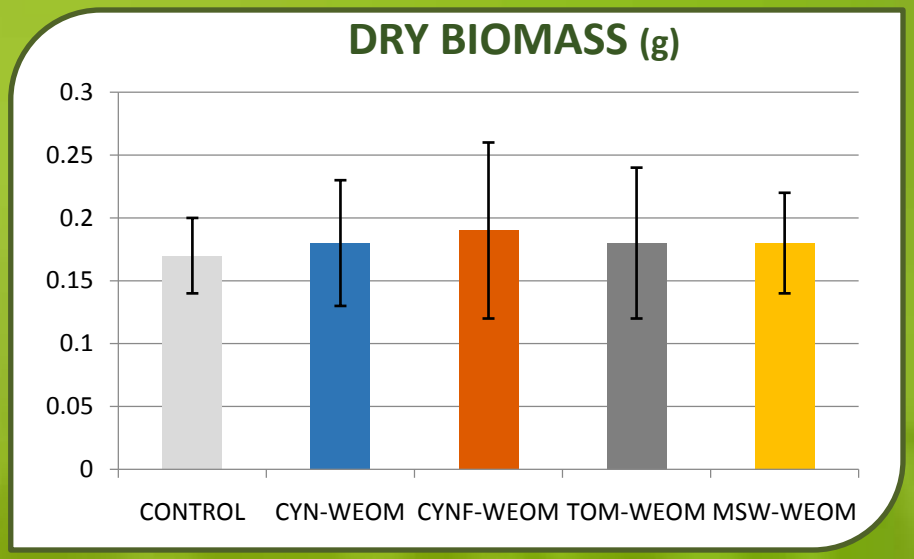


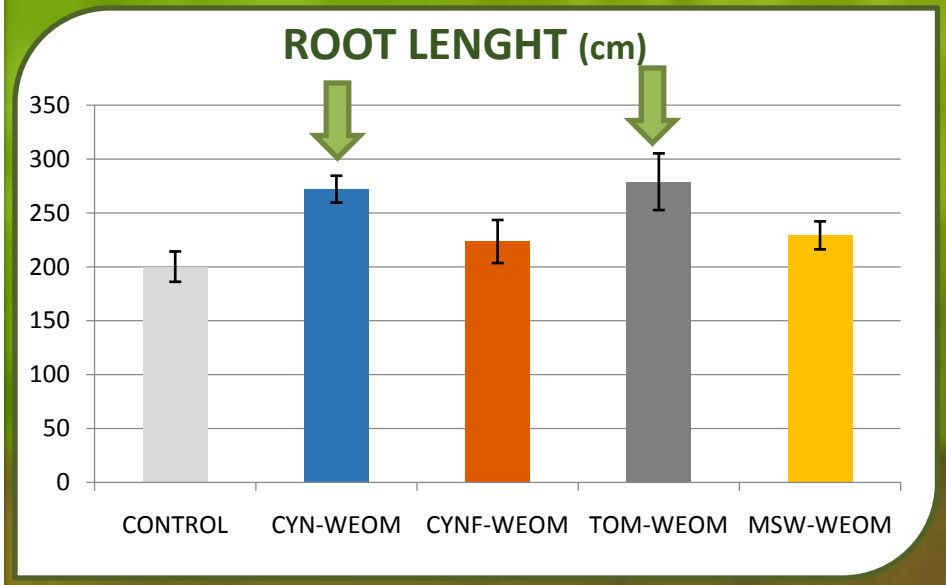
GI = Germination index
1RRG = Relative primary roots
2RRG = Relative secondary roots
Coletopt = Coleopltile

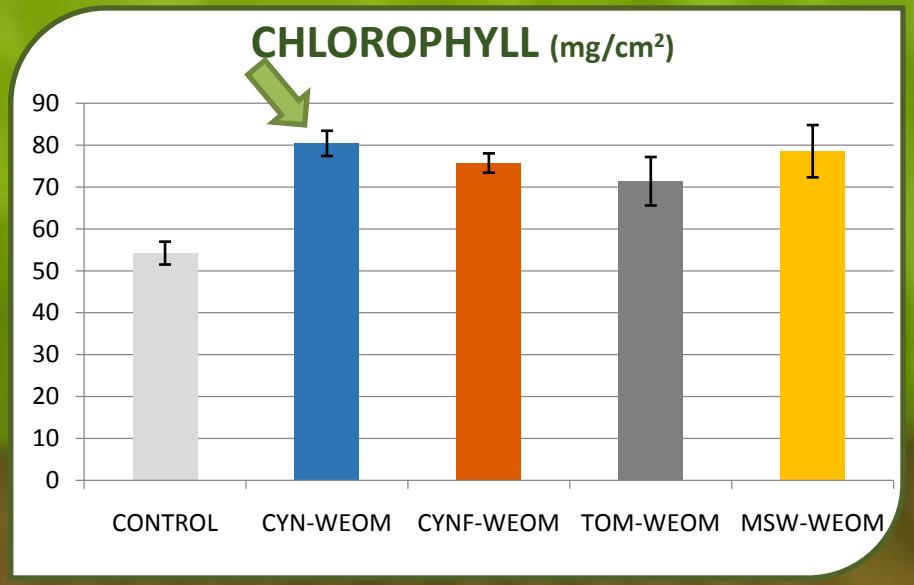
EARLY GROWTH

RESULTS – BIOACTIVITY ON PLANT



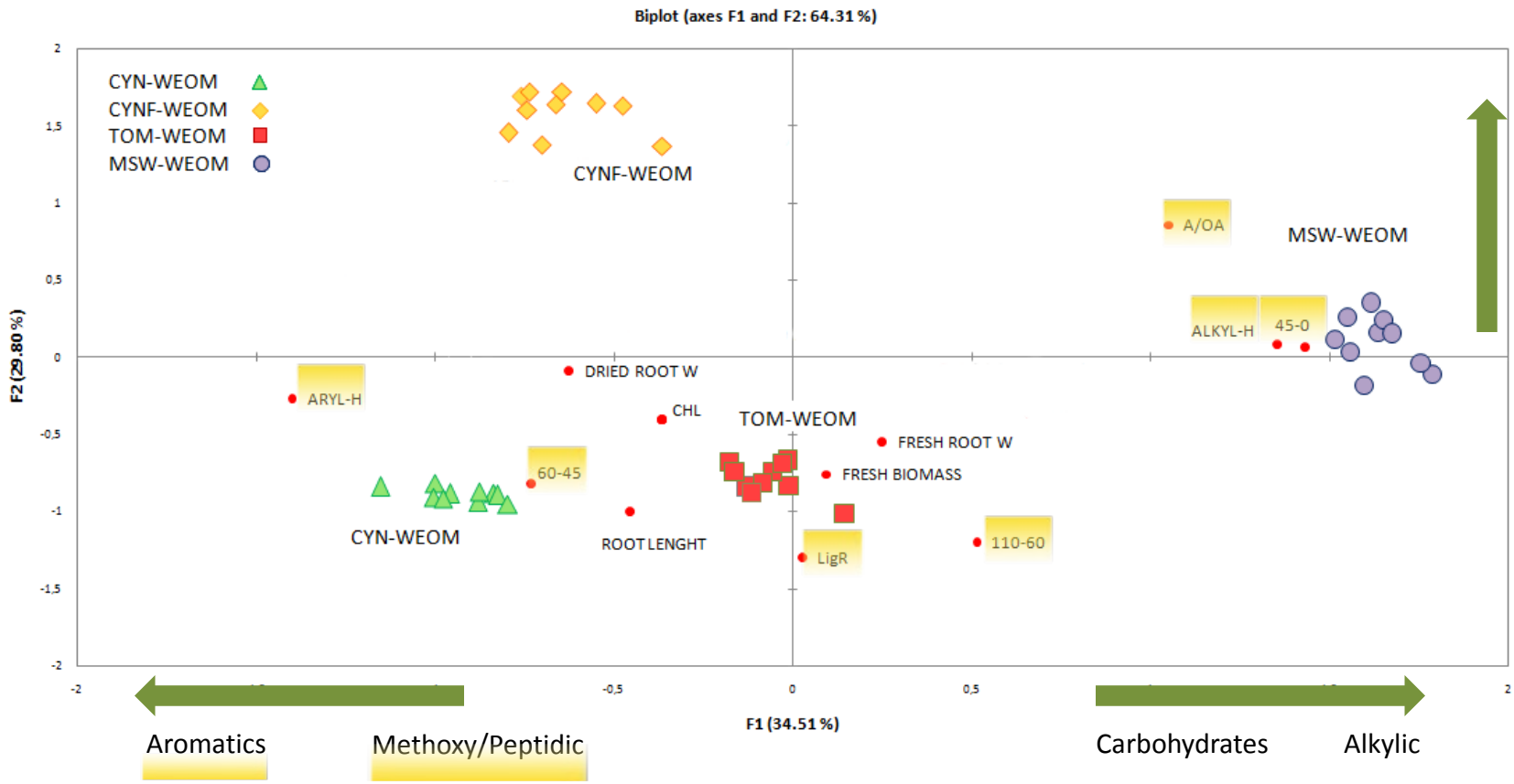






PRINCIPAL COMPONENT ANALYSIS

RESULTS – CORRELATION STRUCTURE-ACTIVITY



CONCLUSION

- Water Extractable organic matter (WEOM) stimulates plant growth
- CYN-WEOM and TOM-WEOM application showed the best general performances on plant growth
- Bioactivity is mainly correlated to aromatic features of WEOMs
- The combination of aromatic and carbohydrate structures observed mainly in CYN-WEOM and TOM-WEOM may confer to conformational structure of these WEOMs the flexibility to allow bioactive molecules to exert their biological activity on plant growth

2

Biostimulation of humic acids isolated from different on-farm composts: Relationship between chemical structure and biological effects on maize early growth

C-CYN

8.0% artichoke, 20% woodchips and 2% compost as starter

C-CYNF

43% artichoke, 23% fennel, 11% escarole residues, 20% woodchips 2% compost as starter

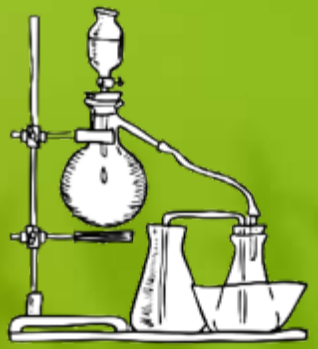
C-TOM

50.0% tomato residues, 48% woodchips 2% compost as starter

C-CAV

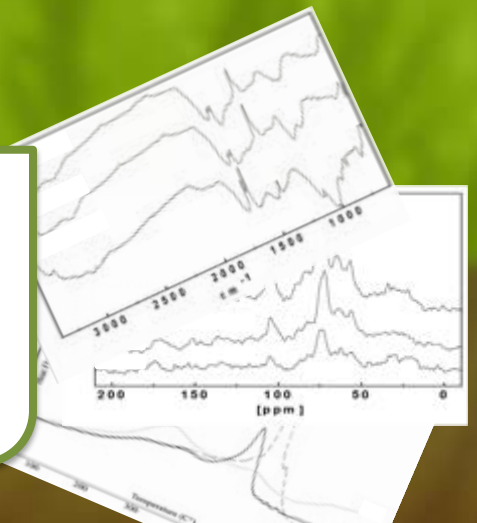
53% cauliflower residues, 45% woodchips and 2% mature compost as starter

EXTRACTION



CHARACTERIZATION

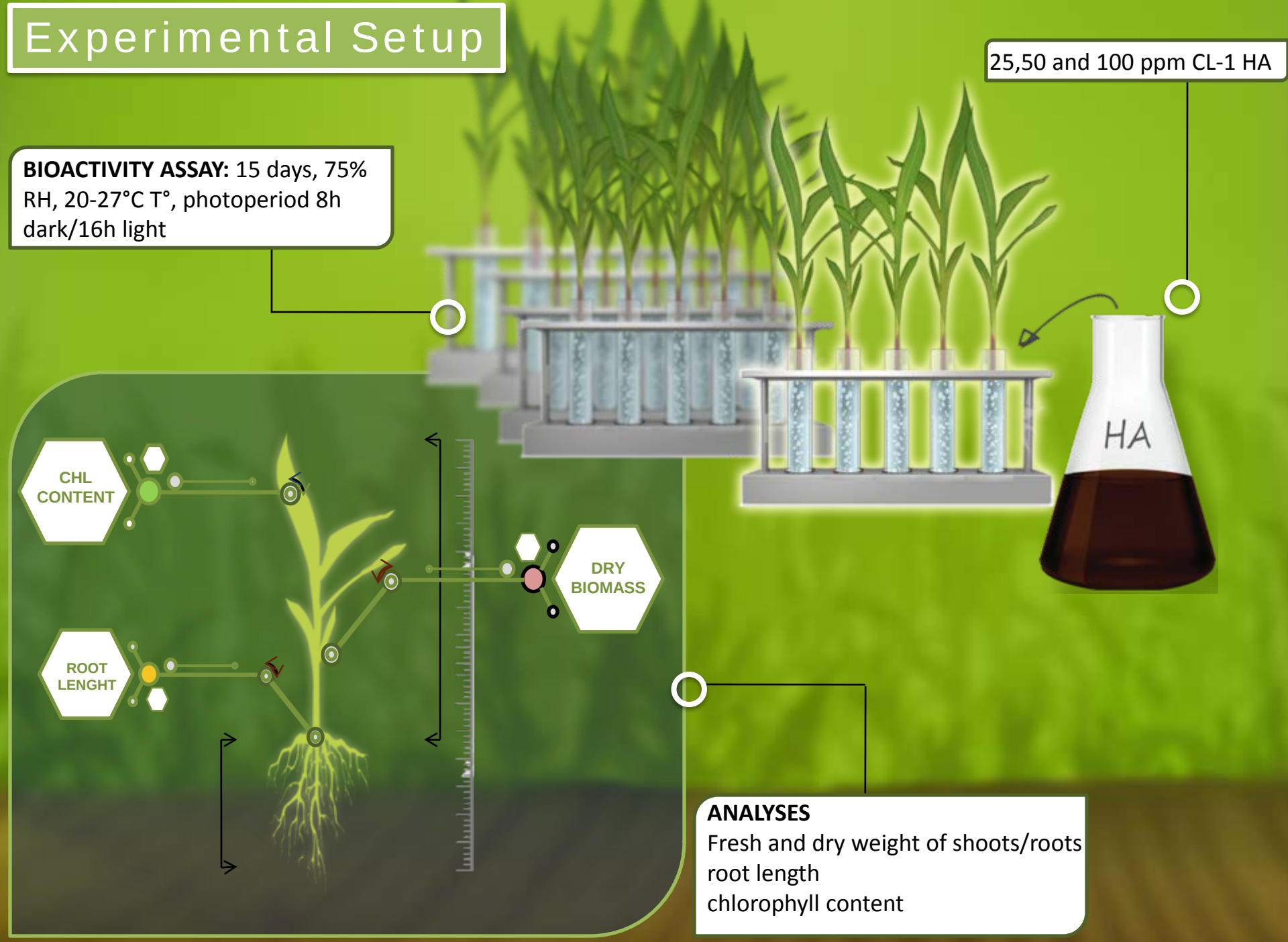
13C-CPMAS Spectroscopy
TMAH-Pyrolisis
IR-ATR Spectroscopy
Thermal analysis (TGA, DSC)



Experimental Setup

25,50 and 100 ppm CL-1 HA

BIOACTIVITY ASSAY: 15 days, 75% RH, 20-27°C T°, photoperiod 8h dark/16h light



ANALYSES

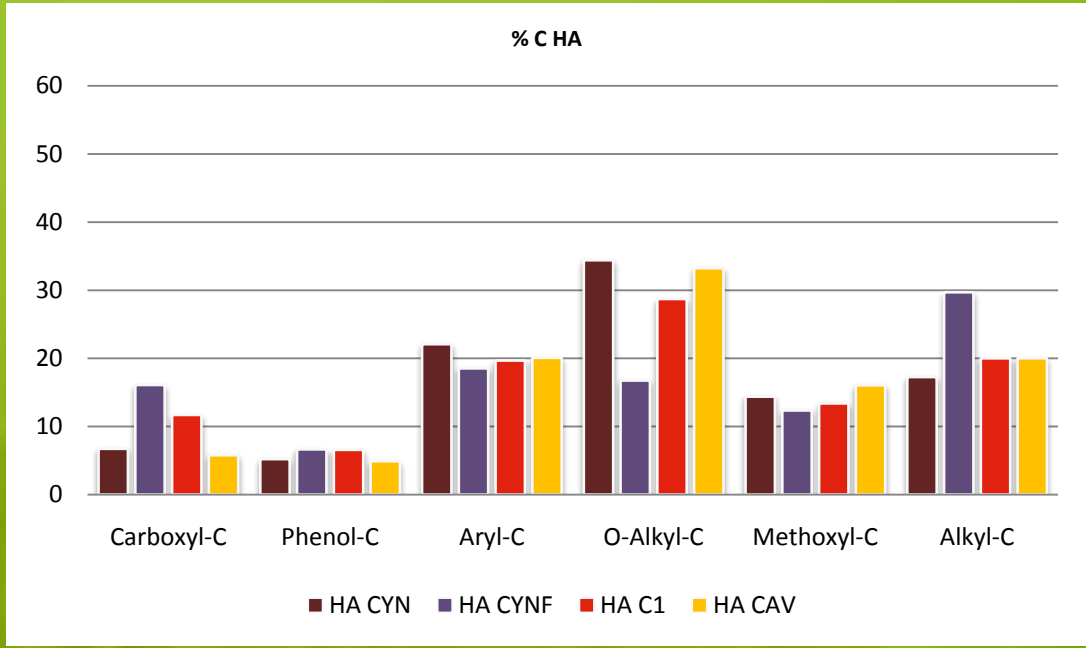
Fresh and dry weight of shoots/roots

root length

chlorophyll content

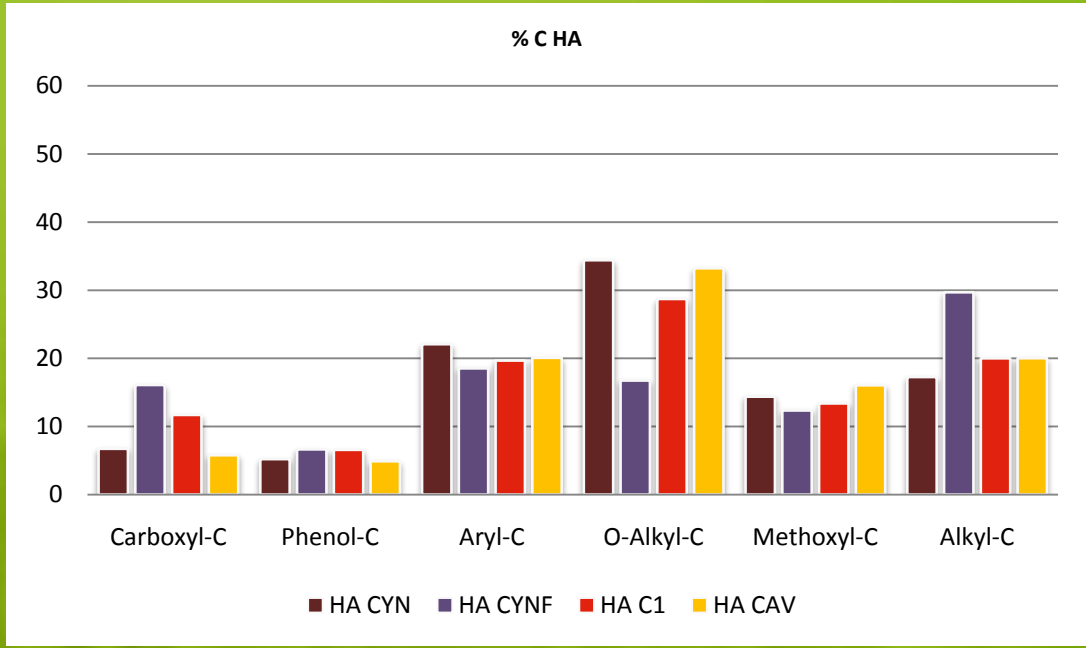
^{13}C CPMAS NMR

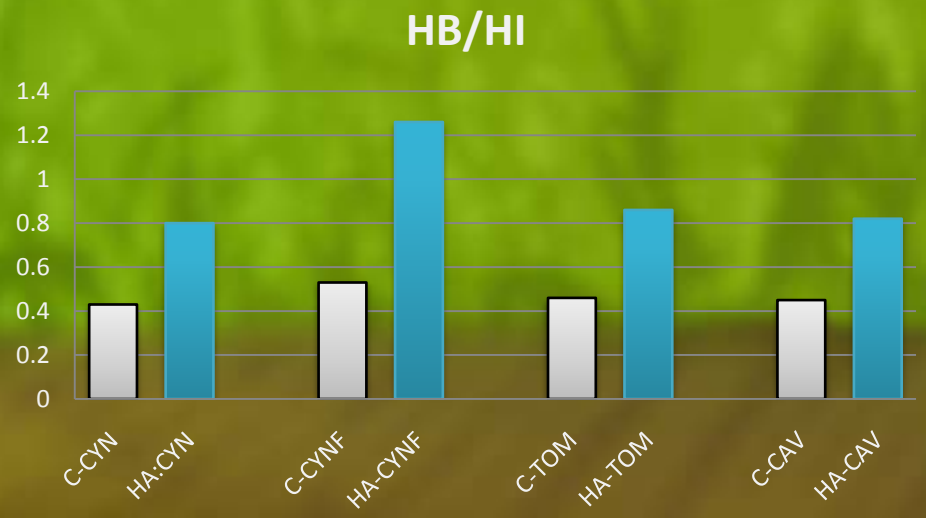
RESULTS - CHARACTERIZATION

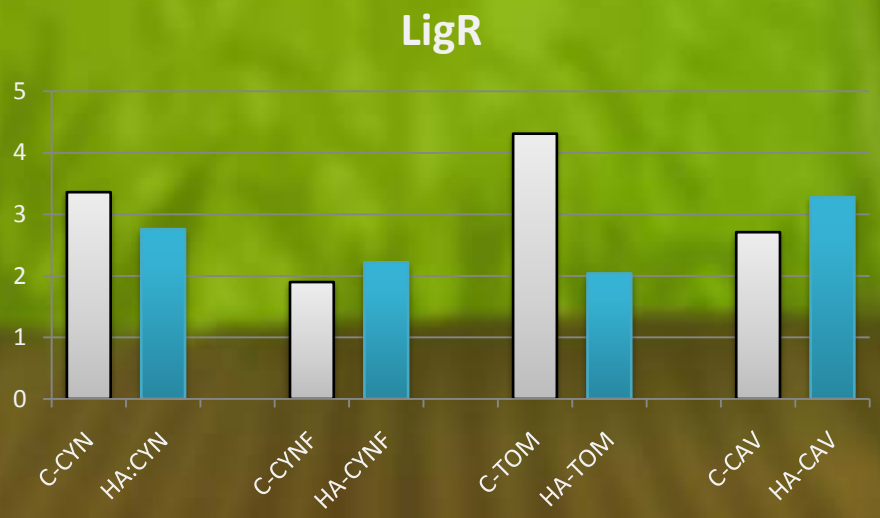


^{13}C CPMAS NMR

RESULTS - CHARACTERIZATION







TMAH-PYROLISIS GC-MS

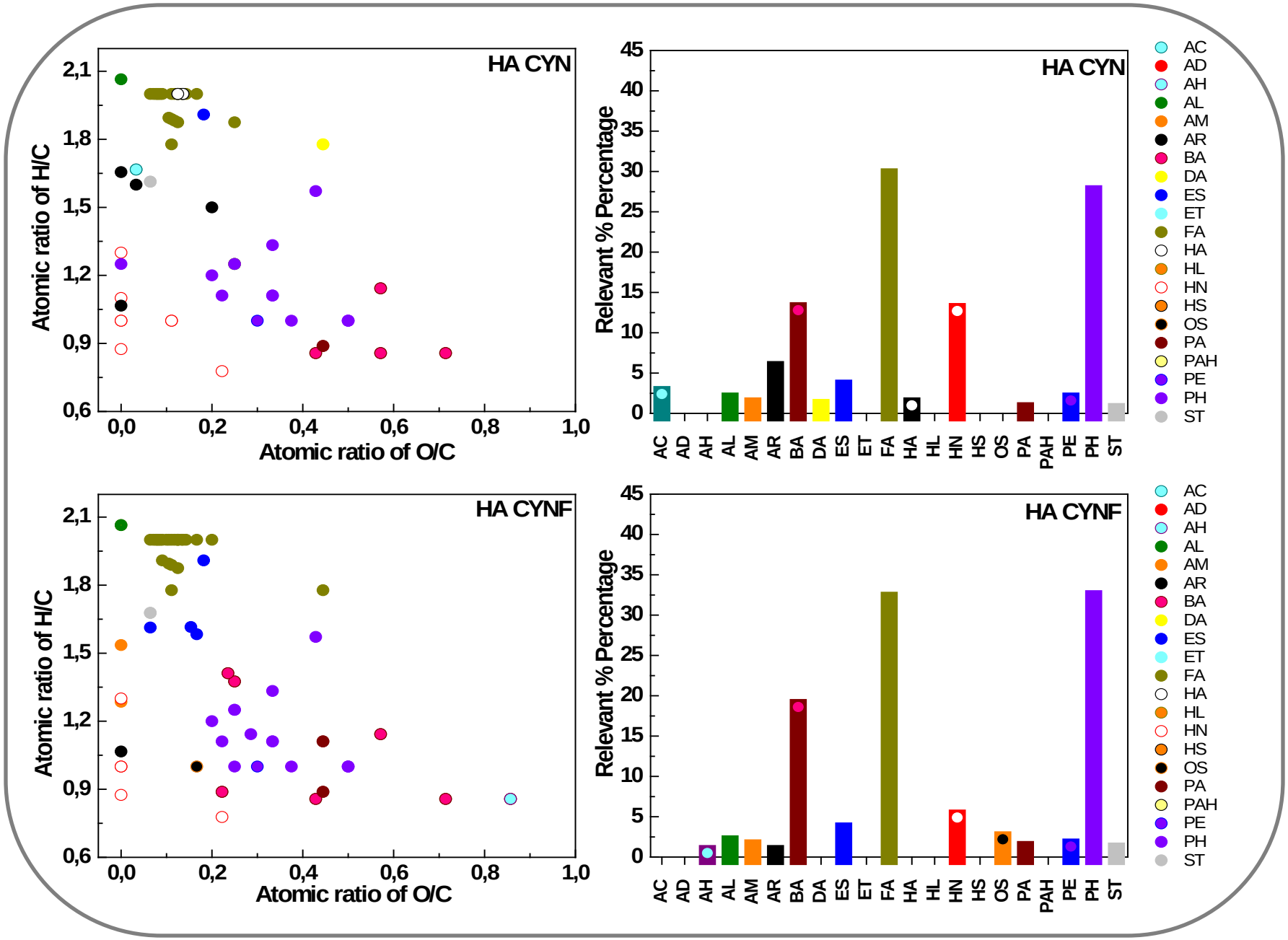
RESULTS - CHARACTERIZATION

	C-CYN	C-CYNF	C-TOM	C-CAV
Lignin	9738	10557	15736	9459
FAME	2857	8174	1824	1390
Mic	3322	1903	3439	tr.
alka/alke	574	591	5250	863.
AD/AL_G	1,78	4,55	0,91	1,89
AD/AL_S	2,48	8,83	2,67	2,92

Yields (µg g−1 dry weight) and composition of main thermochemolysis products released from the four composts. FAME fatty acid methyl ester, Mic microbial origin fatty acids, Alka/Alke alkane and alkene, AD/ALG G6/G4, AD/ALS S6/S4.

TMAH-PYROLISIS GC-MS

RESULTS - CHARACTERIZATION



TMAH-PYROLISIS GC-MS

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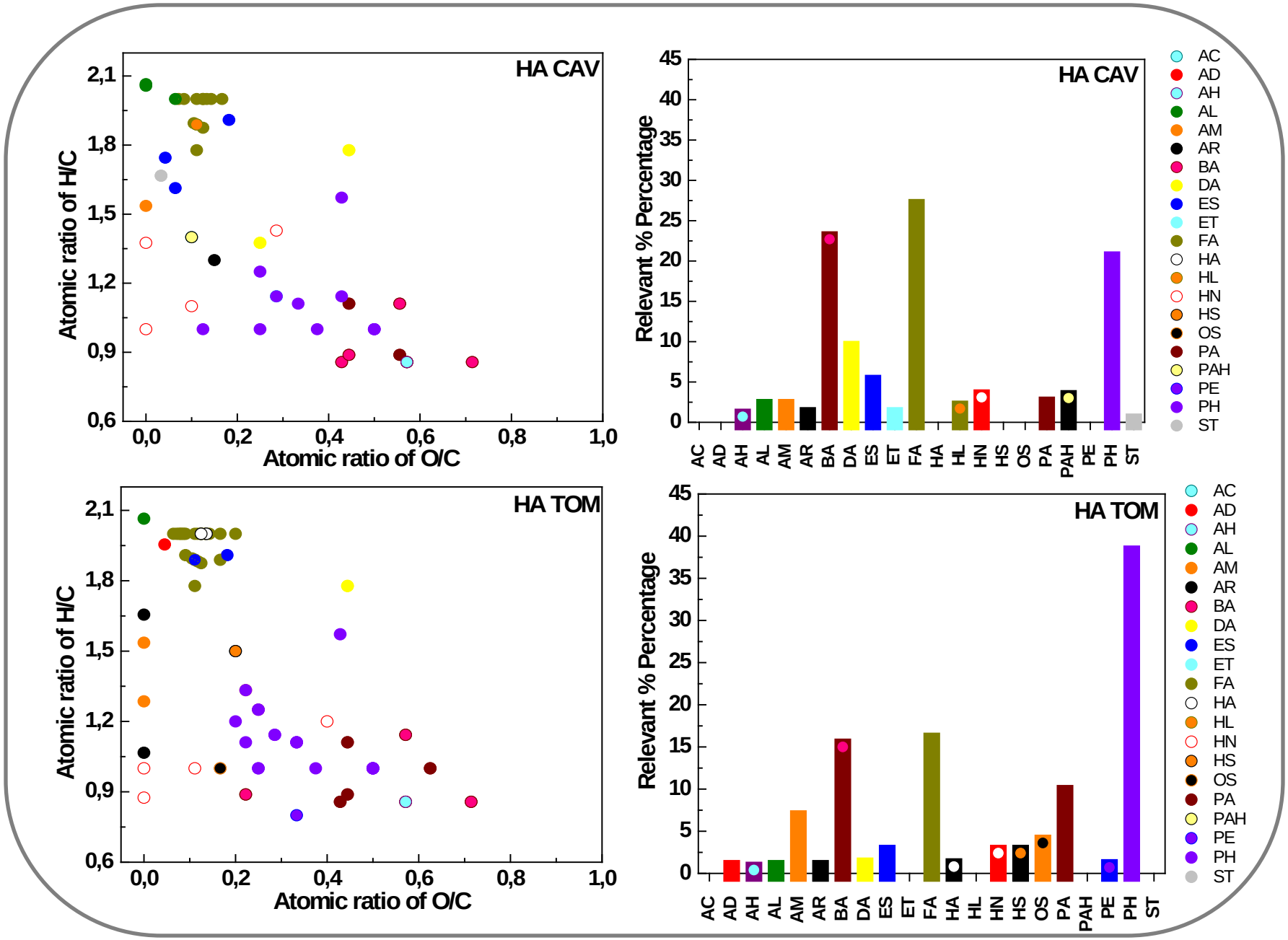
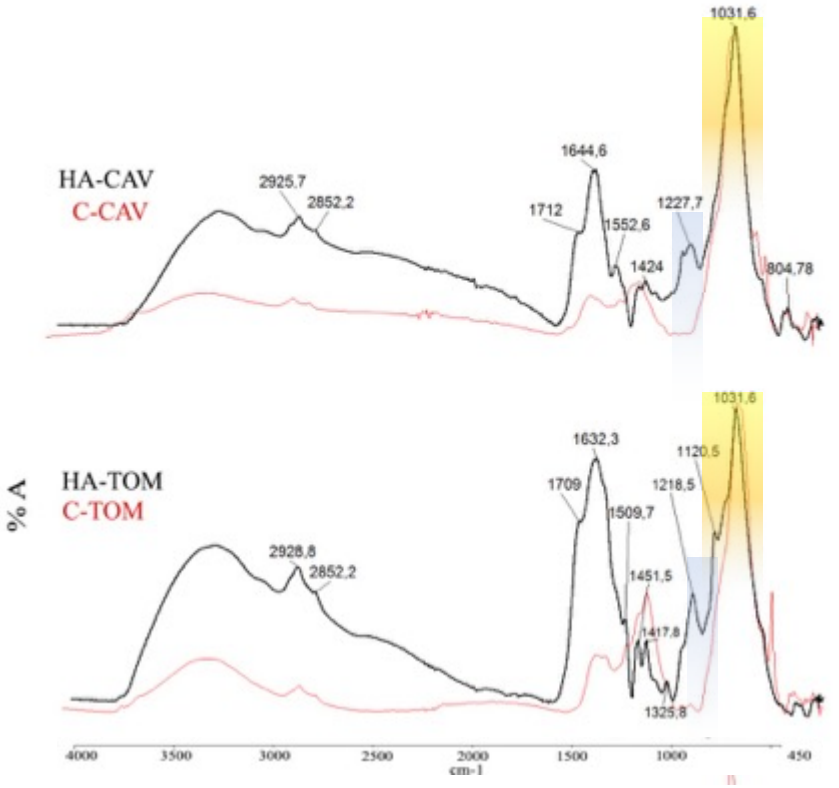
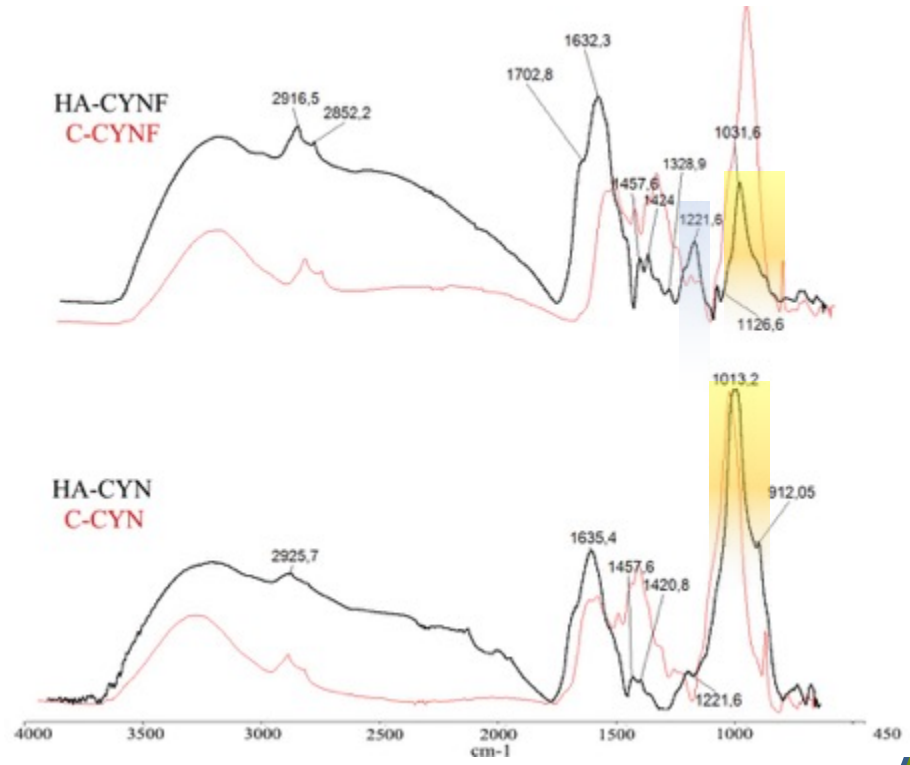
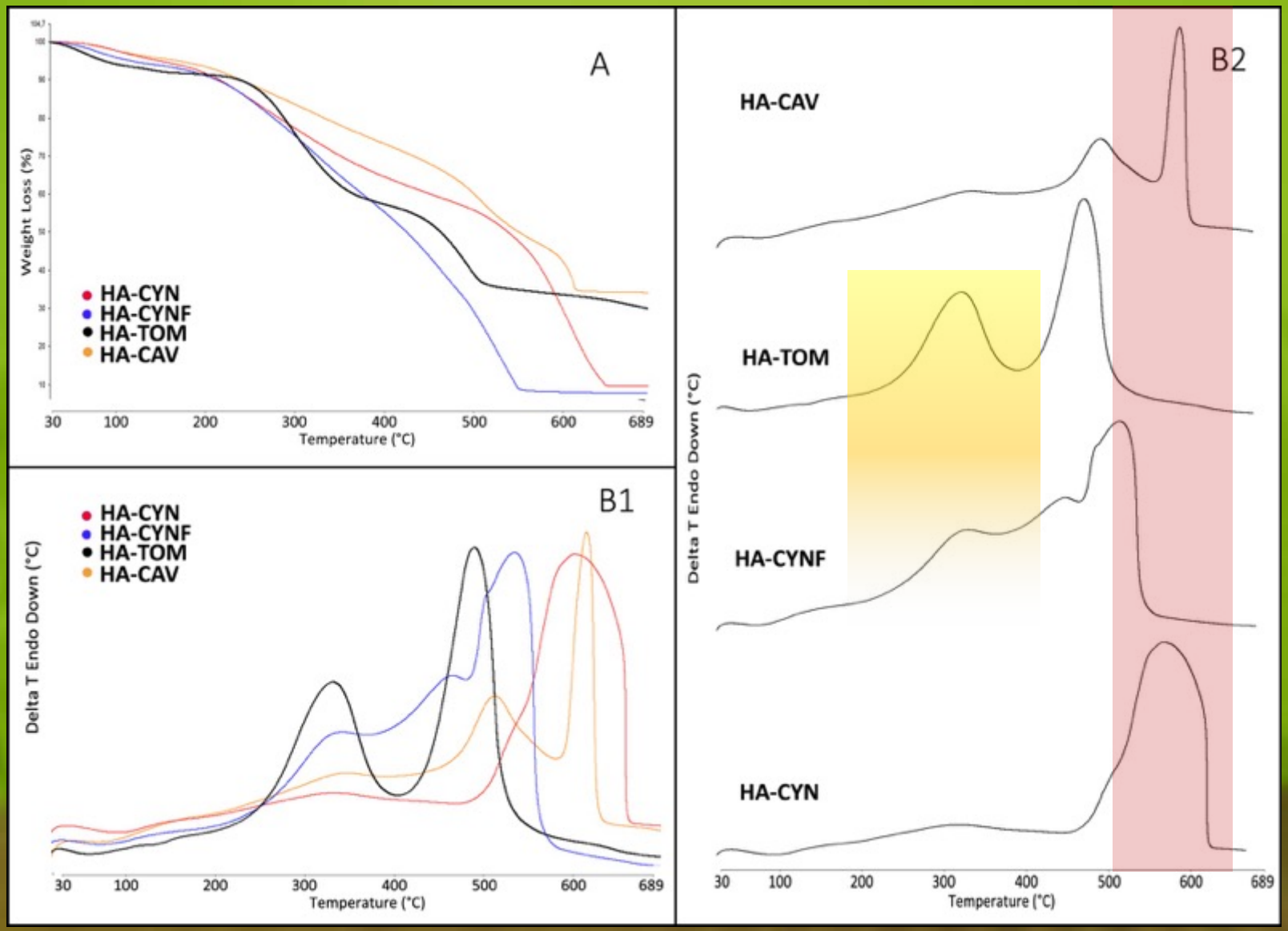


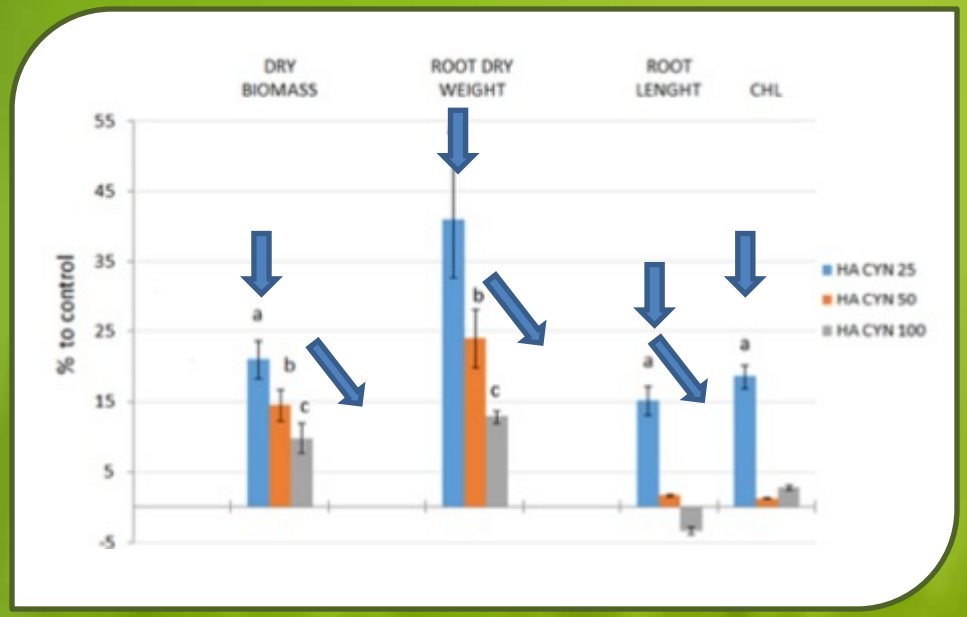
FT-IR ATR OF HAs

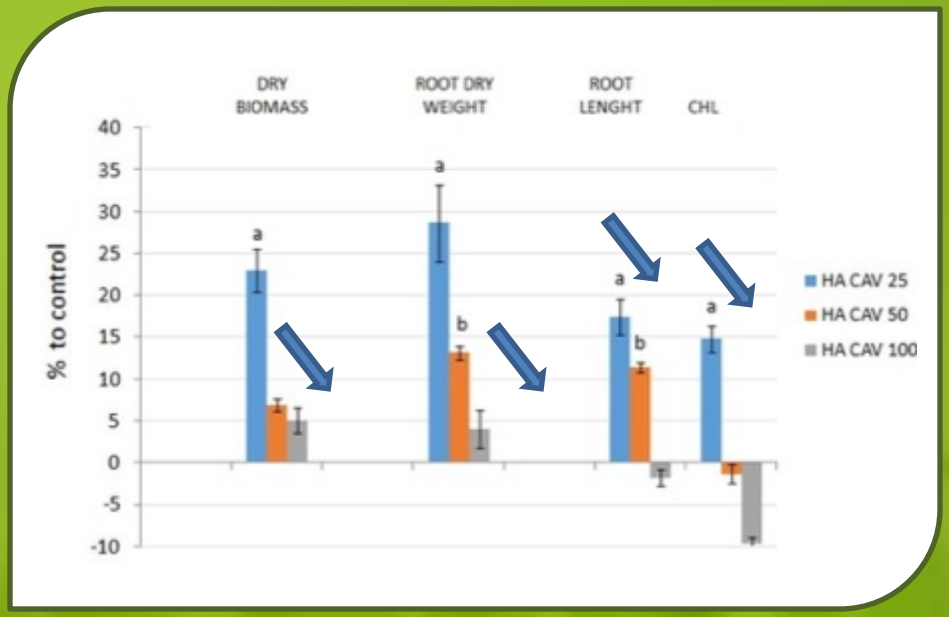


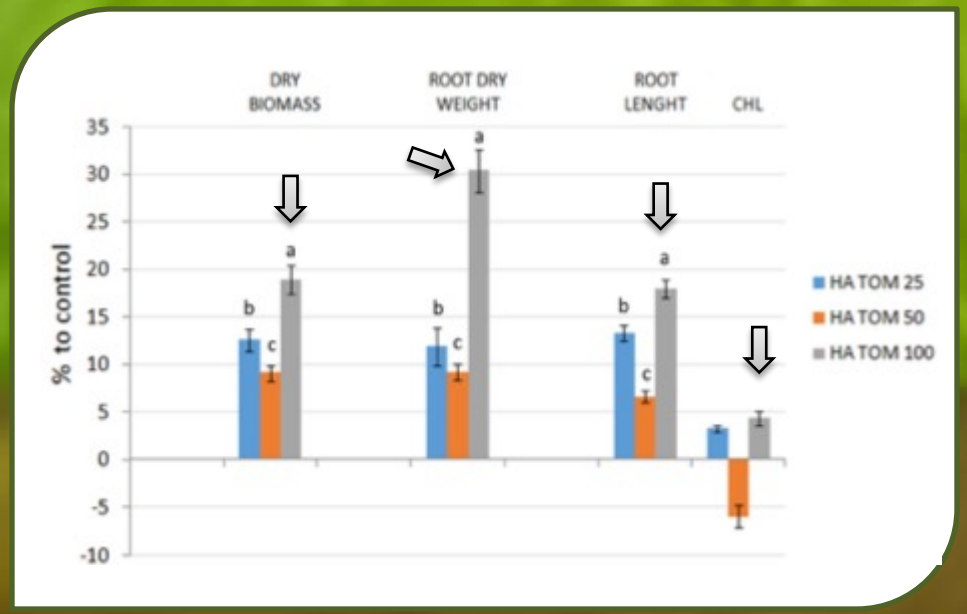


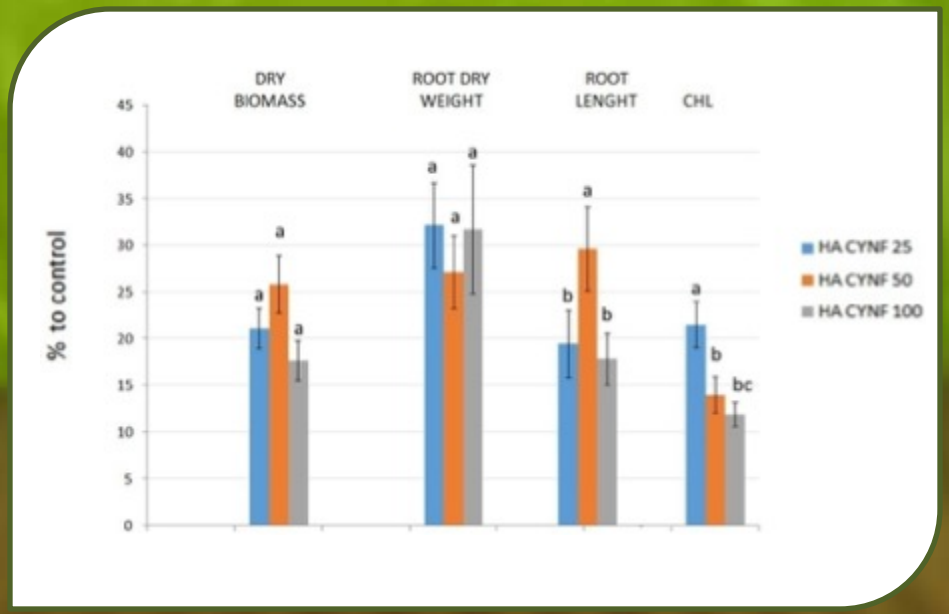

A
Weight Loss (%)
HA-CYN
HA-CYNF
HA-TOM
HA-CAV
30
100
200
300
400
500
600
689
Temperature (°C)
B1
Delta T Endo Down (°C)
HA-CYN
HA-CYNF
HA-TOM
HA-CAV
30
100
200
300
400
500
600
689
Temperature (°C)
B2
Delta T Endo Down (°C)
HA-CAV
HA-TOM
HA-CYNF
HA-CYN
30
100
200
300
400
500
600
689
Temperature (°C)

BIOACTIVITY – EARLY GROWTH



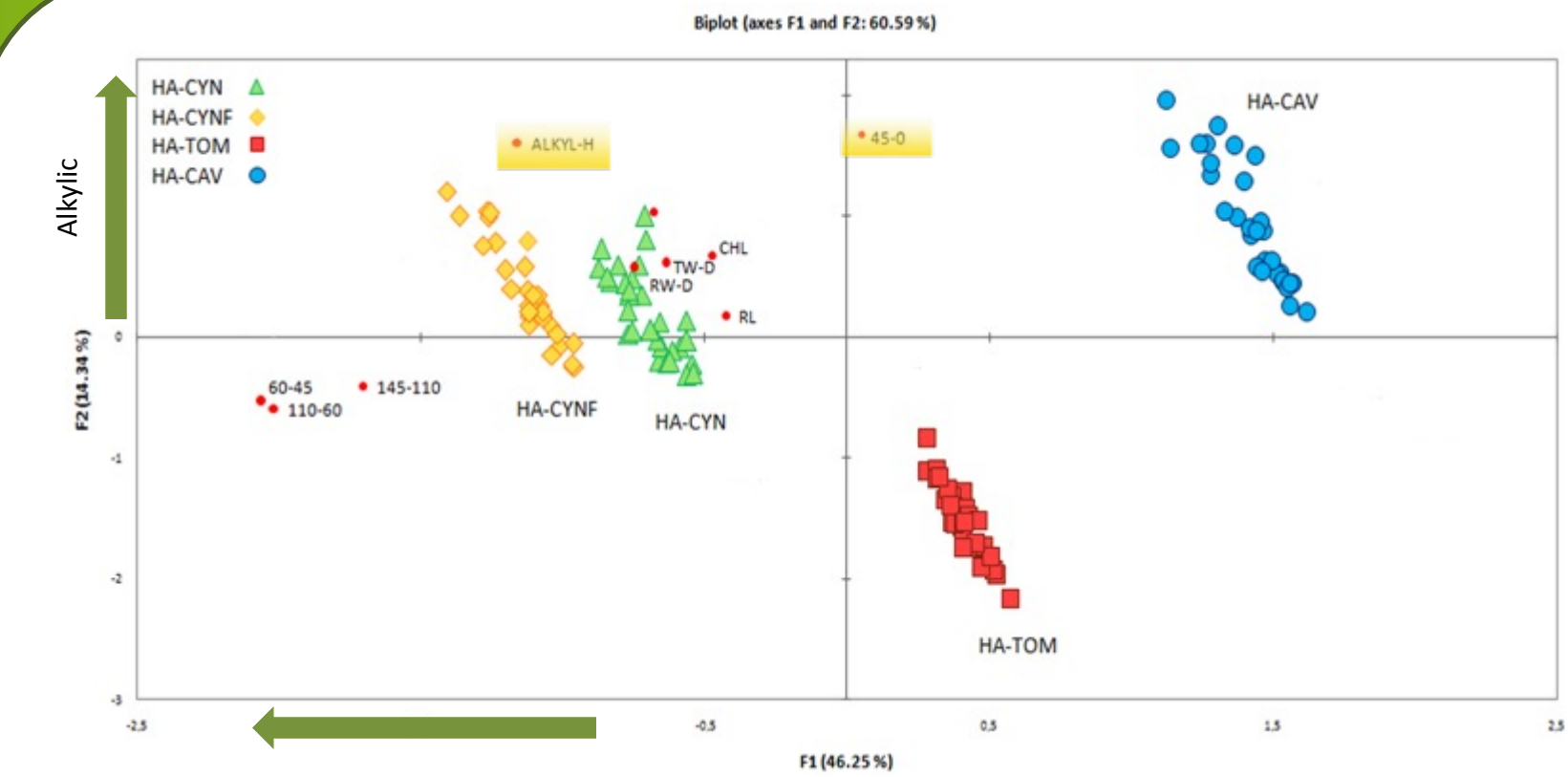






PRINCIPAL COMPONENT ANALYSIS

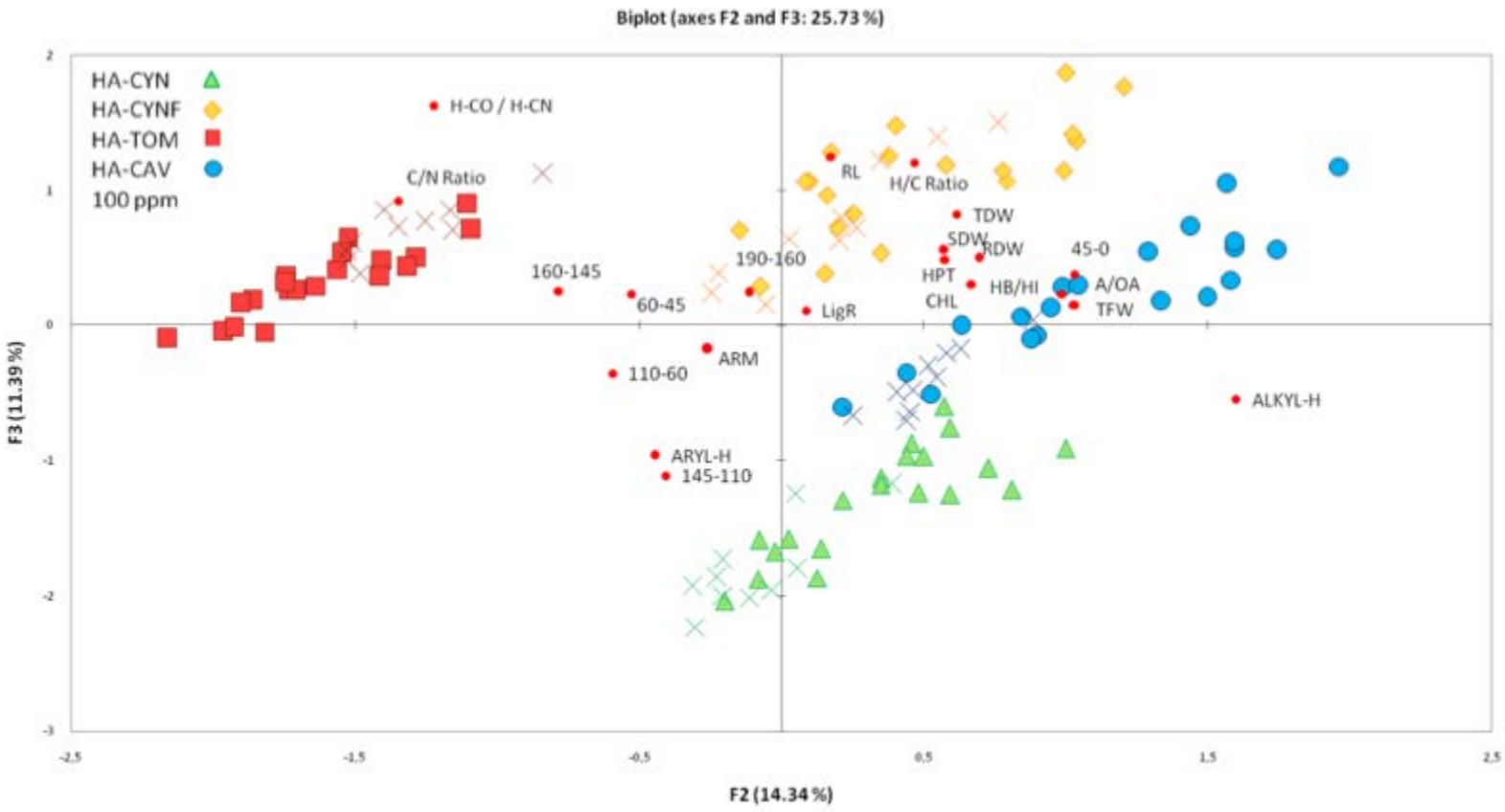
RESULTS – CORRELATION STRUCTURE-ACTIVITY



Lignin residues ← Carbohydrates ← Aromatics

PRINCIPAL COMPONENT ANALYSIS

RESULTS – CORRELATION STRUCTURE-ACTIVITY



- HA-TOM: MAJOR CONTENT OF UNALTERED LIGNIN COMPONENTS → LESS REFRACTORY HUMIFIED MATERIAL
- HA-CYN/HA CYNF: PROTEINACEOUS BIOLABILE MOIETIES → THEIR PERSISTANCE SUGGESTS THEIR STABLE INCORPORATION INTO THE PROTECTIVE HYDROPHOBIC DOMAINS
- HA-CYN : LOWER YIELD OF LIGNIN COMPONENTS AND EXCLUSIVE PRESENCE OF OXIDIZED MOLECULES → COMPLETE DECOMPOSITION OF COMPOST LIGNIN MOIETIES → INCORPORATION IN HA OF PRODUCTS THAT REACHED EXTENSIVE DEGRADATION

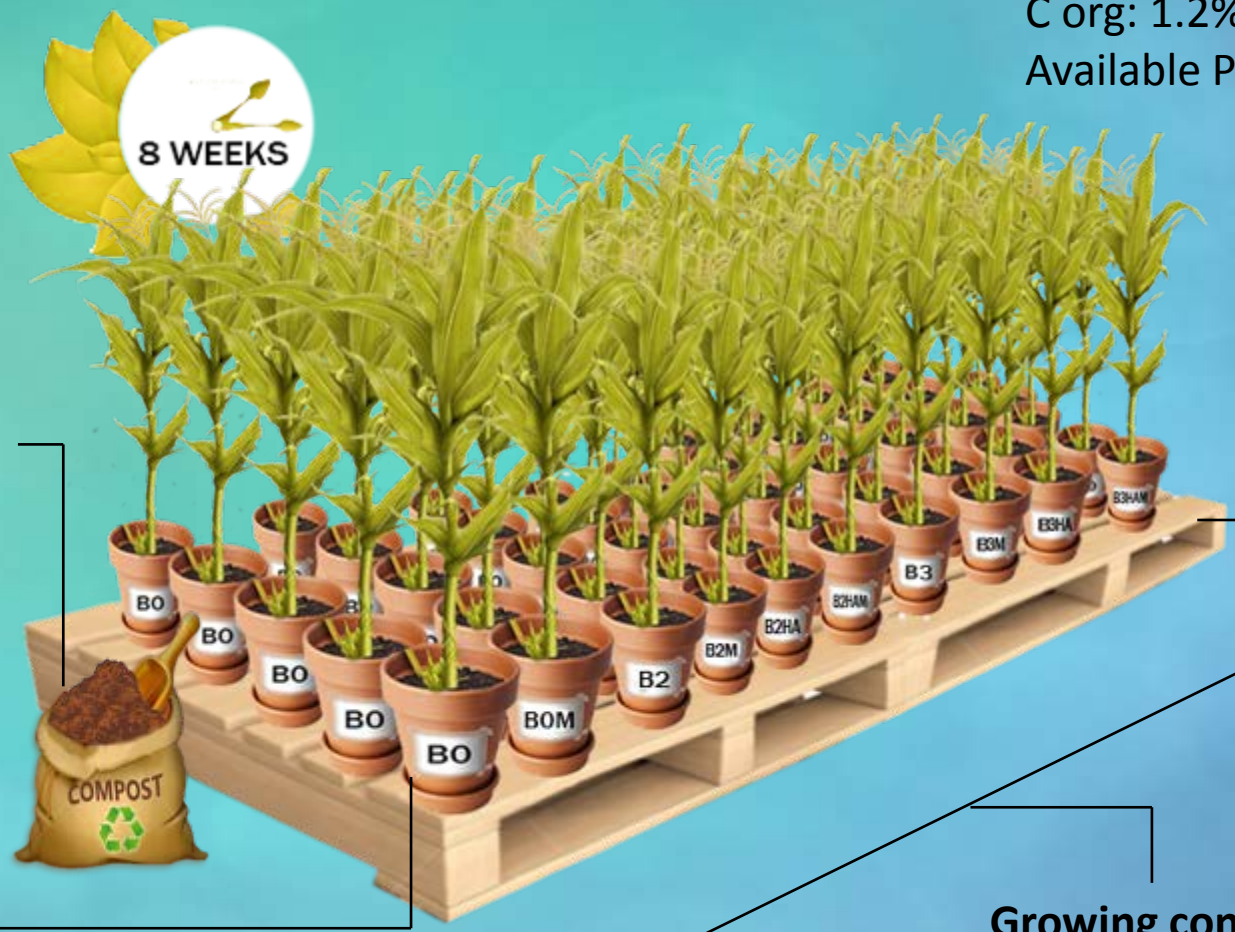
- ALL HA PROMOTE PLANT GROWTH WITH BEST RESULTS AT LOW APPLICATION RATES
- RATIO BETWEEN HYDROPHOBIC/HYDROPHILIC COMPOUNDS IS A KEY FACTOR IN DETERMINING BIOACTIVITY
- THE CONCENTRATION COULD ACT MODIFYING THE CONFORMATIONAL STRUCTURE OF HUMIC SUBSTANCES ALLOWING SOLUBILITY OF BIOACTIVE MOLECULES CAPABLE TO INTERACT WITH PLANT PHISIOLOGY

Soil characteristics
Texture: clay-loam
pH: 8.5 (H2O)
C org: 1.2%
Available P: 18mg Kg-1

Colture
Maize (Aapotheoz)

P fertilization treatments
Composted cow/buffalo manure (50 mg P/kg)

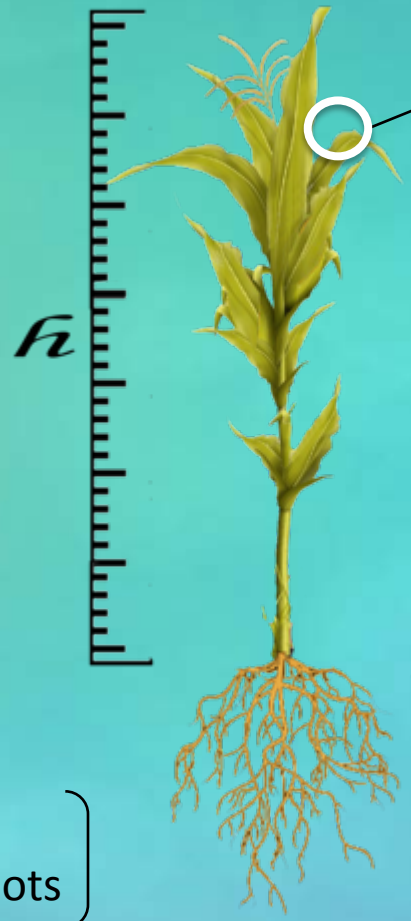
Replicates: X5

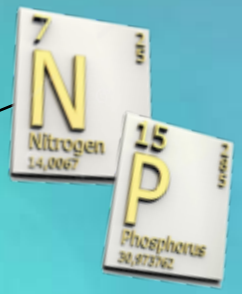


Biofectors
B2: Proradix (Pseudomonas spp.)
B3: Rhizovital (Bacillus amyloliquefaciens)
MYC:Aegis (Glomus mosseae–Rhizophagus irregularis)
HA: Humic acids extracted from artichoke compost

Growing conditions
- Greenhouse
- 5 L pots (1 plant/pot)
- Duration: 8 weeks

ANALYSES PERFORMED





Nutrient content N/P

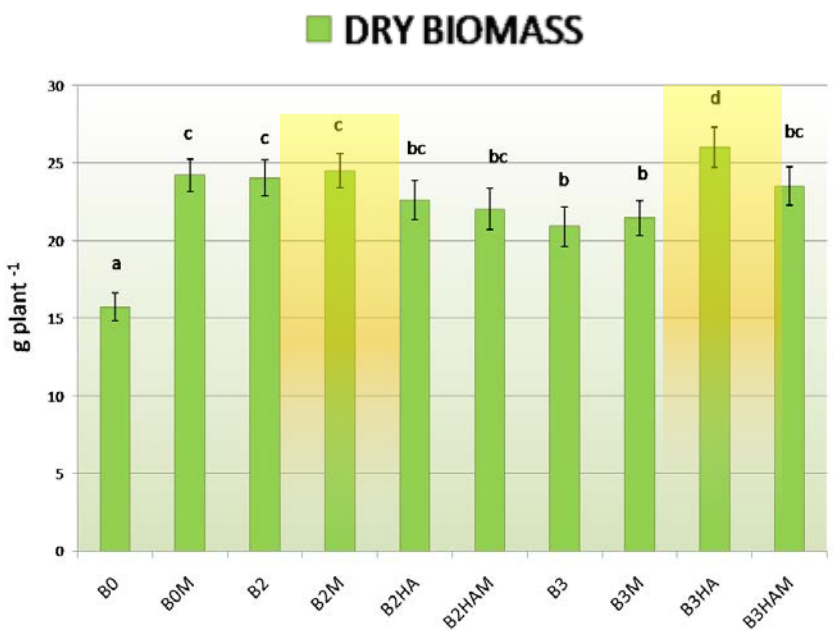
Weight
Fresh/dry for leaves/trunks/roots

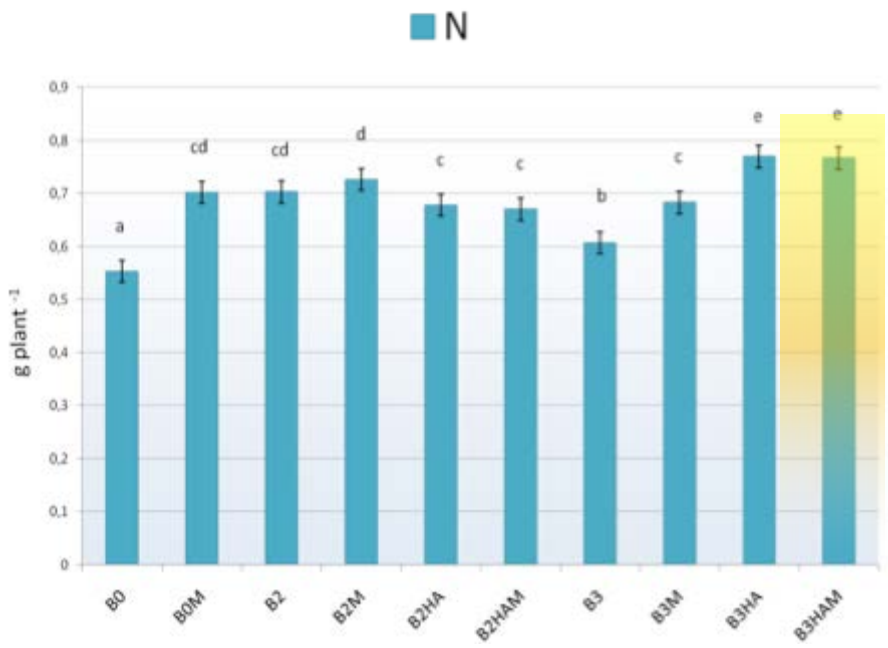
DGGE Fingerprint

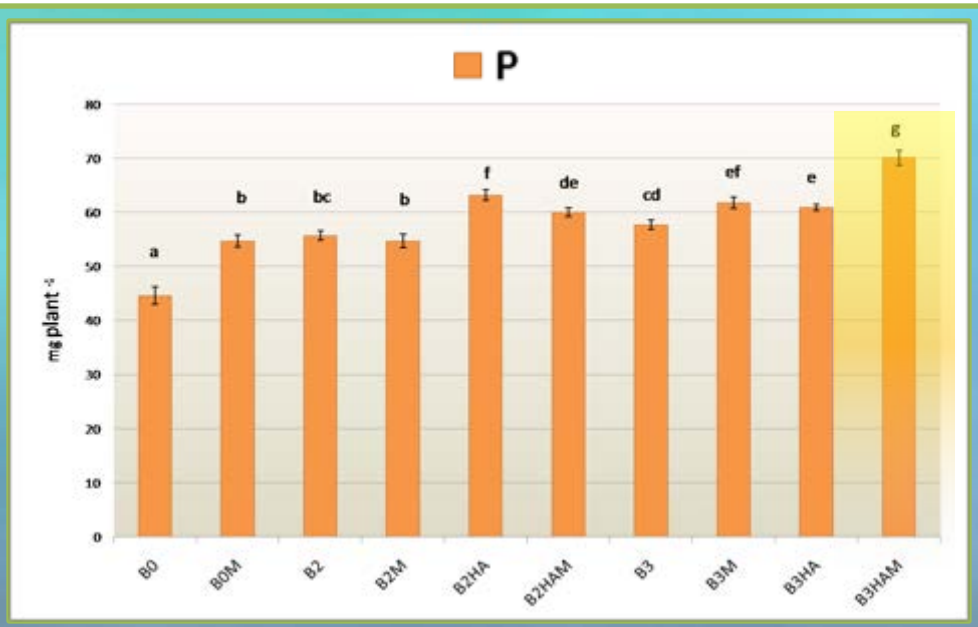
PLFA

BIOMASS – N/P

RESULTS – YIELD/NUTRIENTS

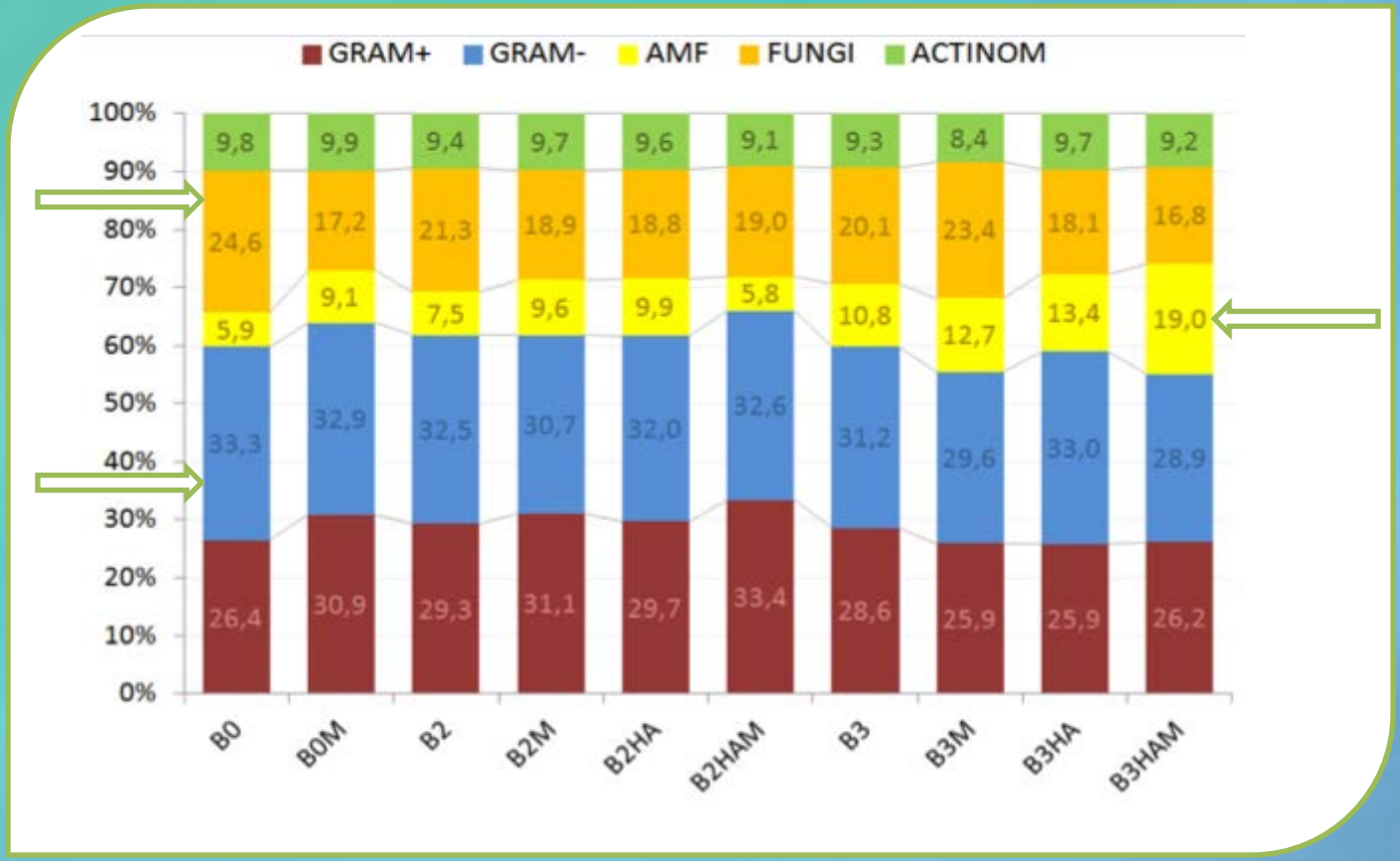






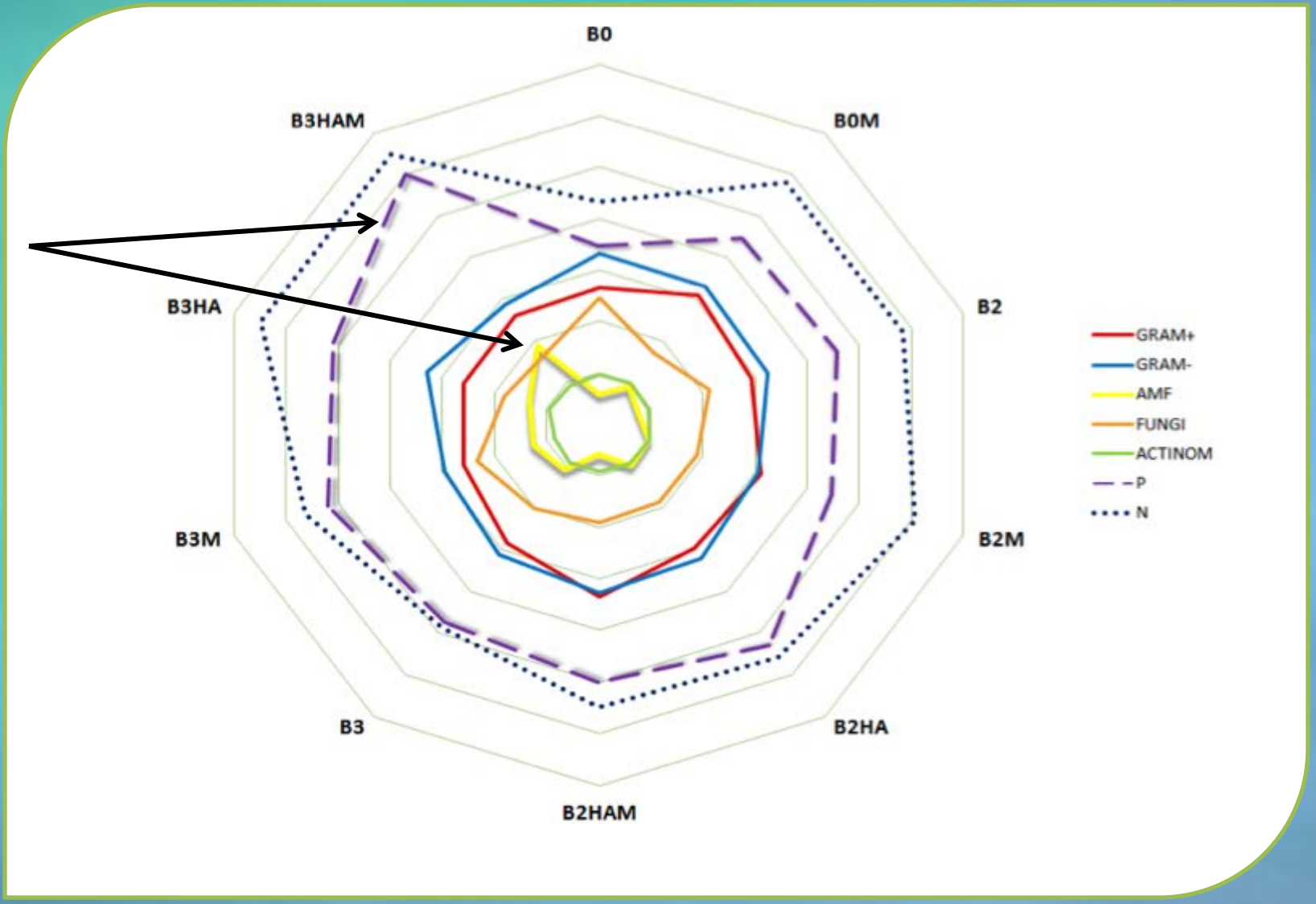
PLFA/NLFA

RESULTS – MICROBIAL COMMUNITY



PLFA/NLFA

RESULTS – MICROBIAL COMMUNITY



16S rRNA Bacterial community

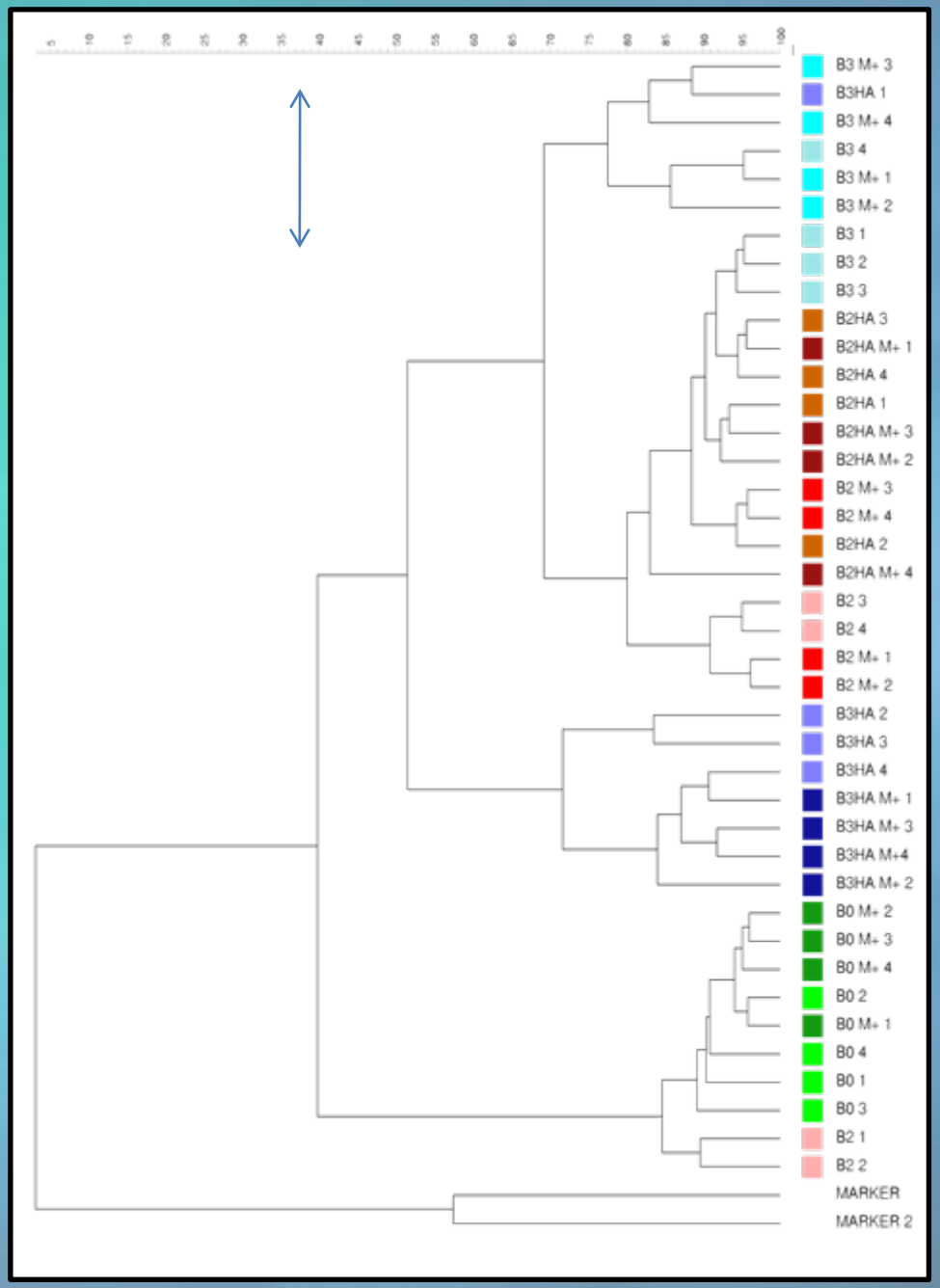
UPGMA Cluster analysis based on Pearson correlation

Permutation test

16s - 30,15% inoculation effect
p-value and dissimilarity value for main comparisons

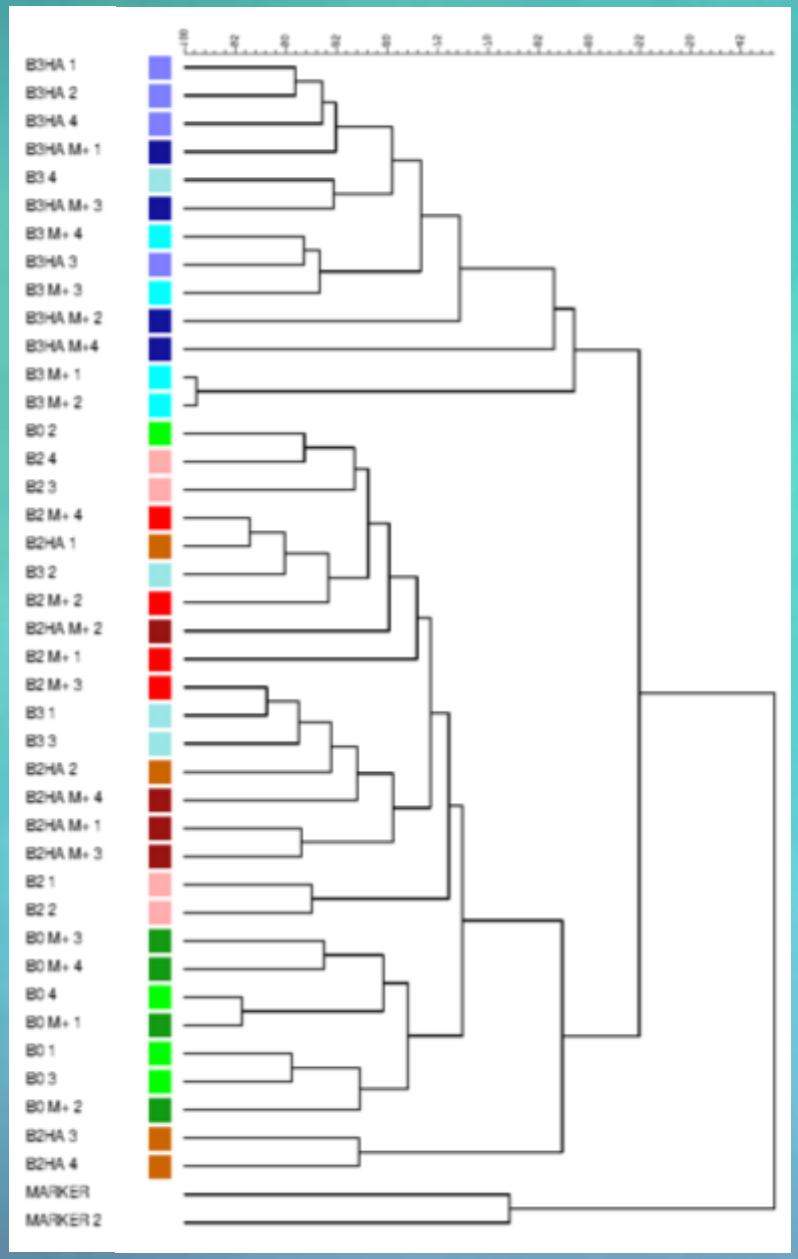
			d	p
B2	vs	B0	9,34	0,032
B2M	vs	B0	46,19	0,032
B2HA	vs	B0	58,71	0,032
B2HAM	vs	B0	54,21	0,032
B3	vs	B0	50,36	0,03
B3M	vs	B0	47,125	0,03
B3HA	vs	B0	67,51	0,03
B3HAM	**vs**	**B0**	**82,95**	**0,03**
B0M	vs	B0	0,74	0,17

Significant difference (P ≤ 0.05) 0

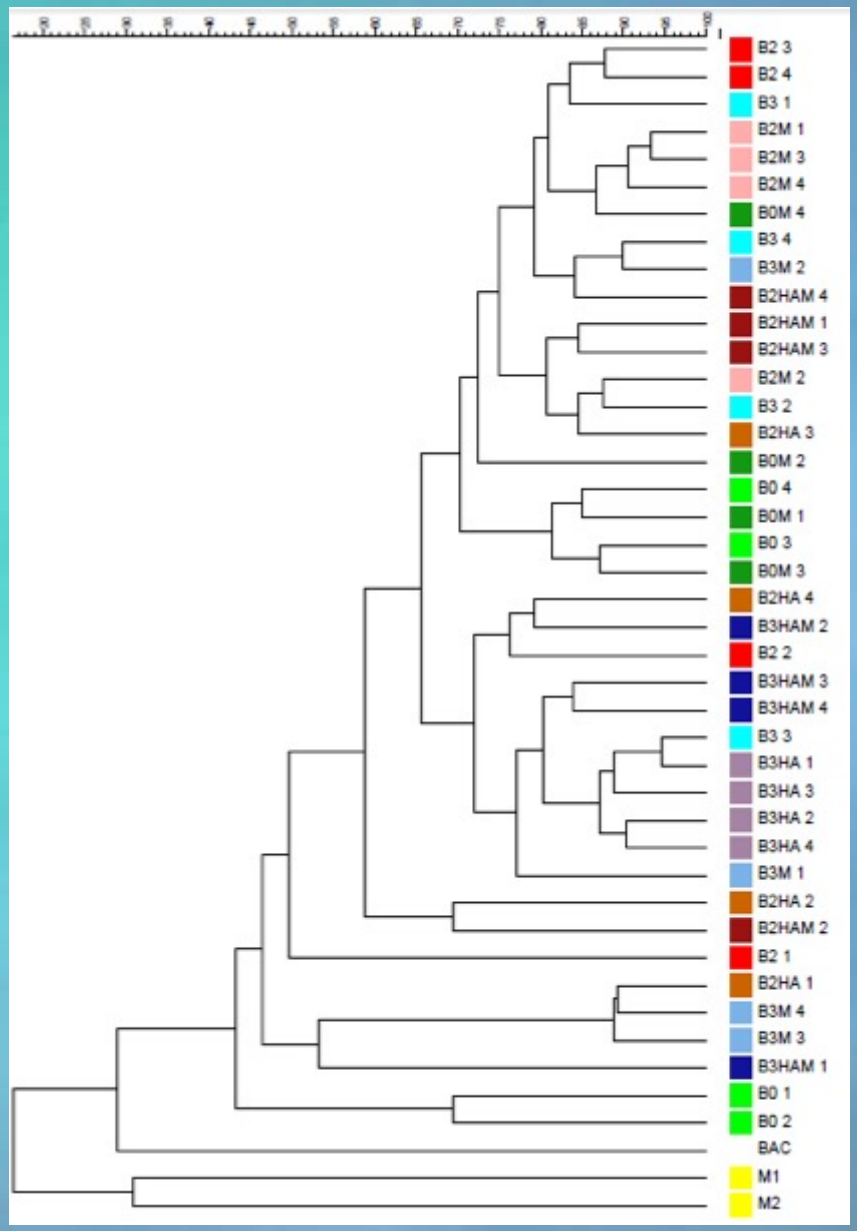


DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE)

RESULTS – MICROBIAL COMMUNITY CHANGES



PSEUDOMONAS



BACILLUS

DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE)

Permutation test

Pseudomonas – 5,44% inoculation effect
p-value and dissimilarity-value for main comparisons

			d	p
B2	vs	B0	0,89	0,33
B2M	vs	B0	5,98	0,027
B2HA	vs	B0	6,41	0,11
B2HAM	**vs**	**B0**	**14,16**	**0,031**
B3	vs	B0	6,97	0,026
B3M	vs	B0	9,94	0,061
B3HA	vs	B0	4,8	0,03
B3HAM	vs	B0	2,27	0,26
B0M	vs	B0	3,23	0,08

Significant difference (P ≤ 0.05)

Bacillus– 9,87% inoculation effect
p-value and dissimilarity-value for main comparisons

			d	p
B2	vs	B0	4,88	0,26
B2M	vs	B0	22,28	0,03
B2HA	vs	B0	3,07	0,26
B2HAM	vs	B0	7,94	0,16
B3	vs	B0	7,66	0,19
B3M	vs	B0	15,05	0,116
B3HA	vs	B0	14,48	0,05
B3HAM	vs	B0	9,98	0,17
B0M	vs	B0	7,94	0,16

Significant difference (P ≤ 0.05)

DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE)

RESULTS – MICROBIAL COMMUNITY CHANGES

Fungal community

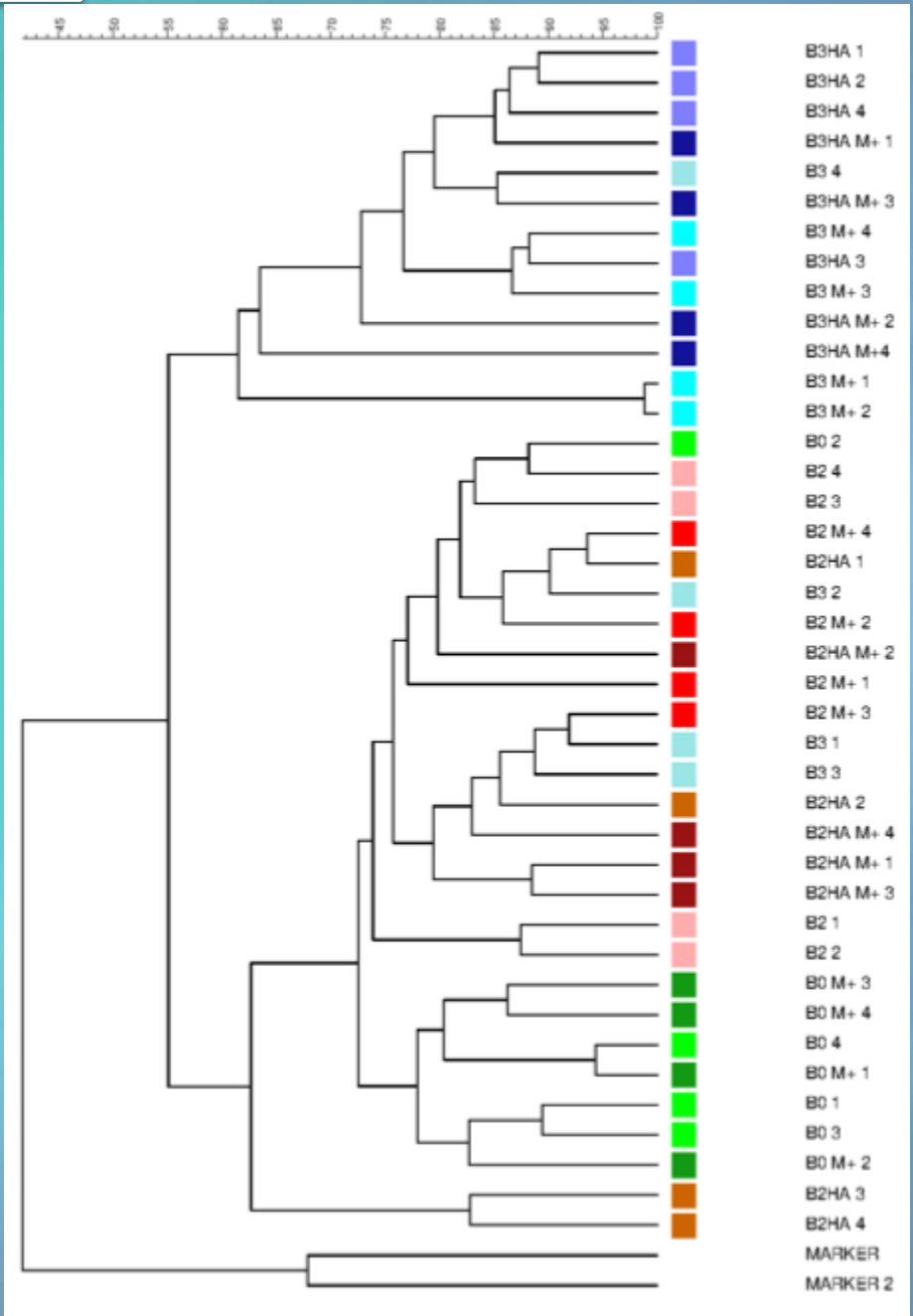
UPGMA Cluster analysis based on Pearson correlation

Permutation test

ITS – 11,72% inoculation effect
p-value and dissimilarity value for main comparisons

			d	p
B2	vs	B0	8,56	0,026
B2M	vs	B0	8,56	0,03
B2HA	vs	B0	18,73	0,026
B2HAM	vs	B0	10,14	0,026
B3	vs	B0	7,81	0,028
B3M	vs	B0	22,65	0,032
B3HA	vs	B0	18,72	0,03
B3HAM	vs	B0	22,88	0,03
B0M	vs	B0	3,23	0,08

Significant difference ($P \leq 0.05$)



CONCLUSION

- Both microorganisms improved plant growth and nutrient uptake
- Best performances were obtained by combining bacteria/HA/mycorrhizae
- Combination of Bacillus a. HA and mycorrhizae showed sinergistic effect
- humic substances could have promoted plant roots development allowing mycorrhizal colonization on root surface and at the same time been used as C source by microbial population thus triggering:

  - Fast solubilization of phosphorus allowed by bacterial community
  - Better traslocation of nutrients allowed by mychorrizal fungal population

FINAL REMARKS

- **Water Extractable organic matter (WEOM) as well as humic acids (HA) stimulate plant growth**
- **Chemical characteristics are strictly correlated to the bioactivity**
- **Hydrophobic /hydrophilic domains determine different conformational flexibility of HS resulting in:**
  - **Protection of bioactive molecules with hormone-like activity**
  - **release of molecules in solution determining biostimulation effect on plant growth**

- **The combination of humic substances with P-solubilizing microorganisms boost plant response stimulating nutrients uptake and determining a shift in the microbial community that favor beneficial association**
- **Mineral fertilization is still the best option in terms of yield, however, organic fertilization allows a better nutrient efficiency use**
- **Optimization and improvements in the use of bioeffector as well as organic recycled biomassess as fertilizer are required but promising**

Thank you

For your attention