Soil microbiohumeome: feed the microbes for restoring soils, increasing resource-efficiency and stress resistance of agroecosystems

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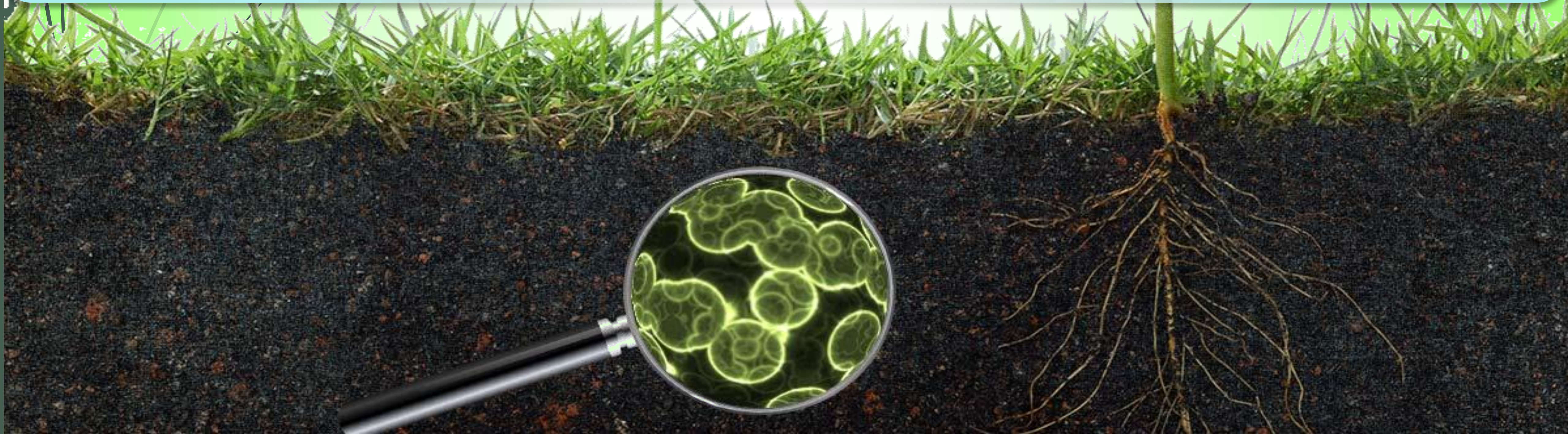


Issues and challenges…

Issues..

One-third of all soils and more than half of agricultural soils are moderately or highly degraded. **Erosion**, **loss** of **organic carbon**, **compaction salinization** and **pollution** reduce soil's fertility and ability to **hold moisture**.

Tractors in Pantanal, Brazil, erode soils and kill marshland.

Challenges: more resilient systems

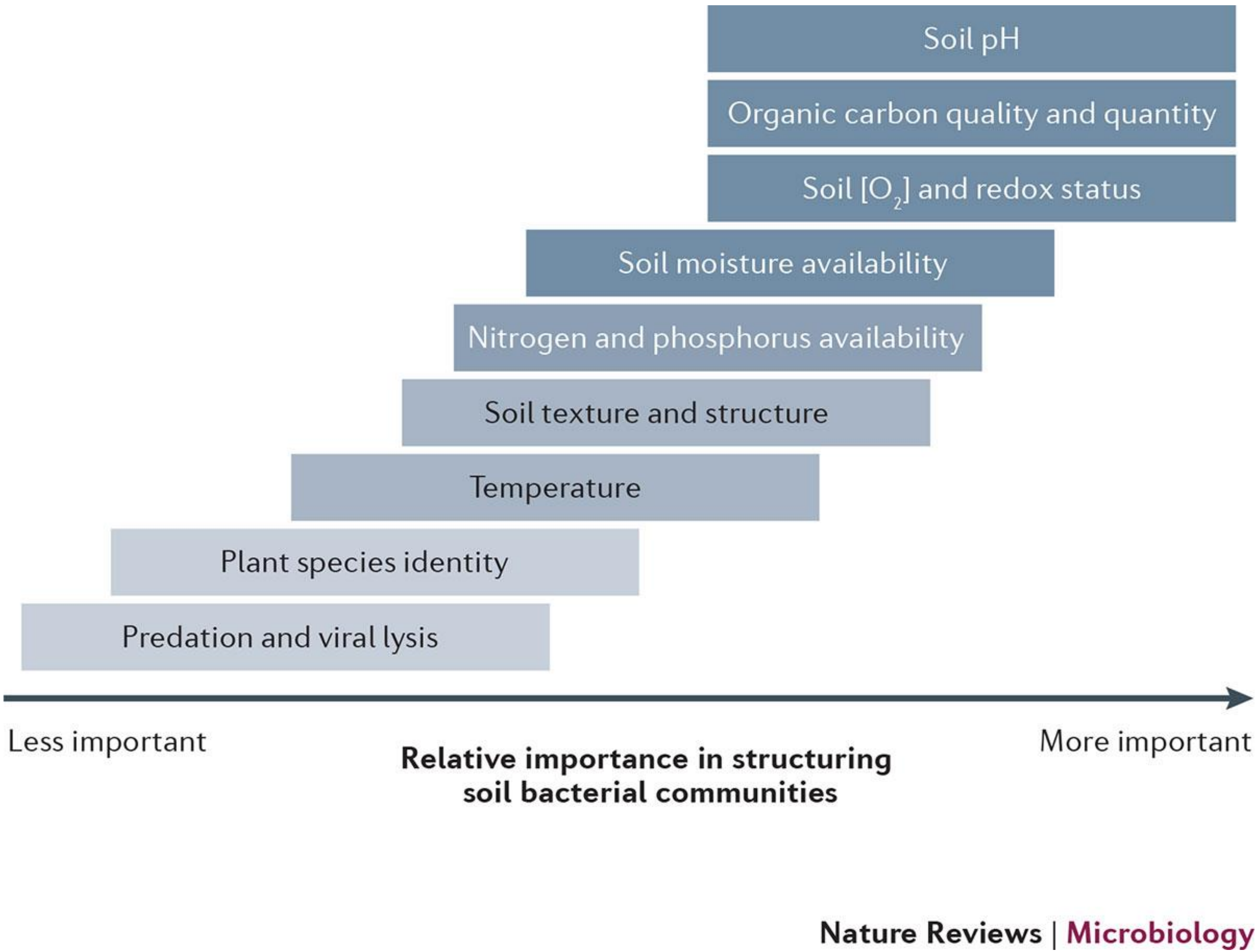
- WE NEED TO WORK WITH THE NATURAL SYSTEM INSTEAD OF TRYING TO FIGHT AGAINST IT.’

Agricultural soils can be chilly environment for native microbes

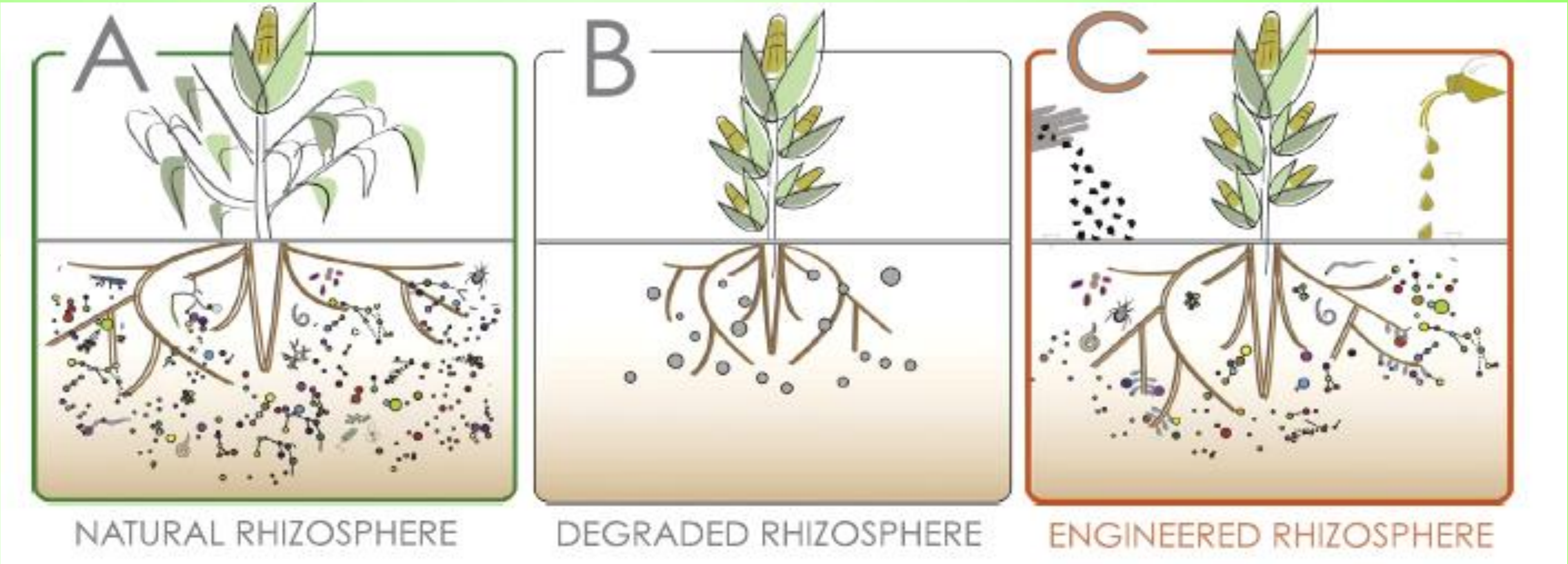
- ❖ Not enough carbon inputs: removal of large portion of plant biomass (not returning
- ❖ stubble) or simply not enough plant biomass
- ❖ Physical disturbance from tillage (disturbs habitat and disrupts hyphal networks) and
- ❖ compaction from machinery
- ❖ Bare soils during fallow periods—no C, no protection from heat, no water
- ❖ Agrichemicals decrease some groups –fungi, micro/macrofauna– and select others—
- ❖ e.g., some bacteria that degrade chemical or "bloom" after application. Selection of copiotrophs vs oligotrophs
- ❖ Fertilizer concentrations too high for symbiotic organisms w/plants.

Many recommended agricultural practices are:

- based on rapid test
- targeting single issues rather than systems oriented: address symptoms not underlying causes
- Have only short term perspective (that season)



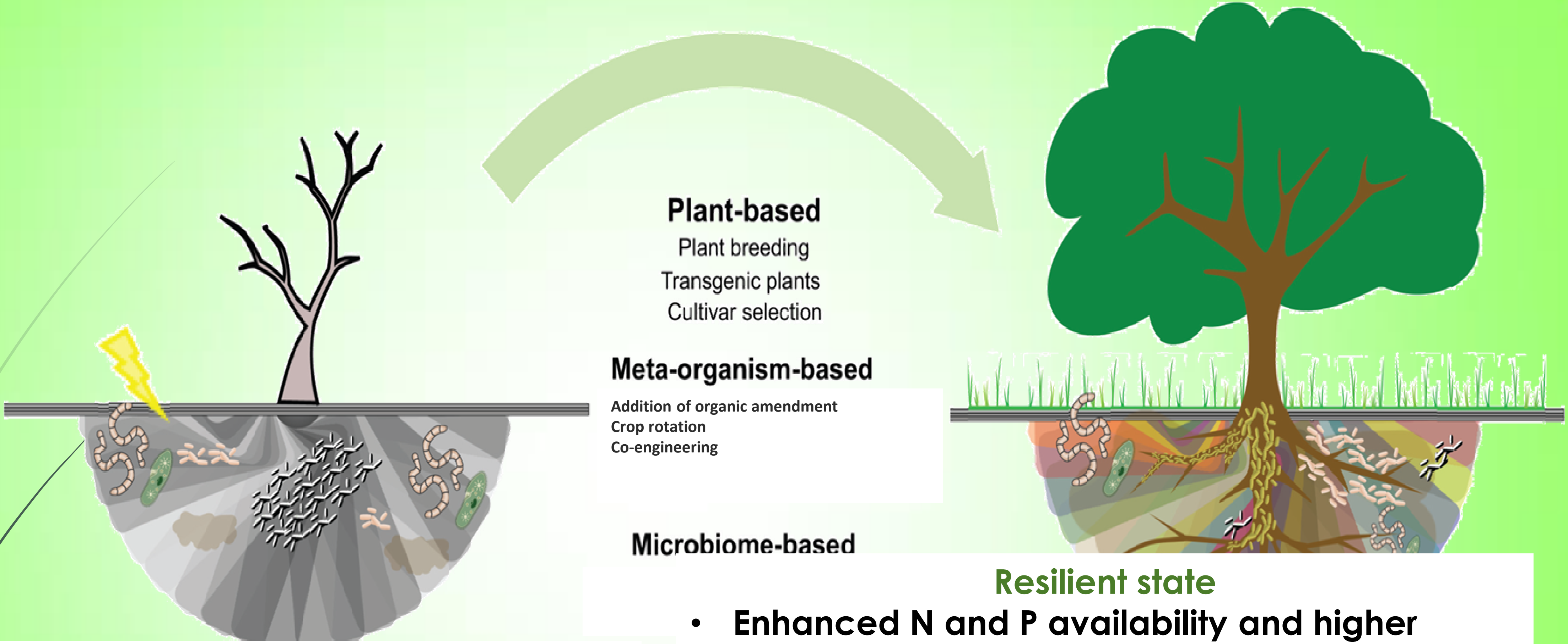
Soil pH
Organic carbon quality and quantity
Soil [O2] and redox status
Soil moisture availability
Nitrogen and phosphorus availability
Soil texture and structure
Temperature
Plant species identity
Predation and viral lysis
Less important
More important
Relative importance in structuring soil bacterial communities
Nature Reviews | Microbiology



A. Natural ecosystems.They are characterized by highly structured and interactive microbiomes and food webs because plants co-evolved with their microbiomes and natural organic molecules

B. Degraded systems such as those under intensive management and high fertilization. The connections decreased.

C. Engineered systems”. Engineered systems through inoculants that form connections with the native microbiome or soil amendments that stimulate microbial activity. Restore beneficial microorganisms activity.

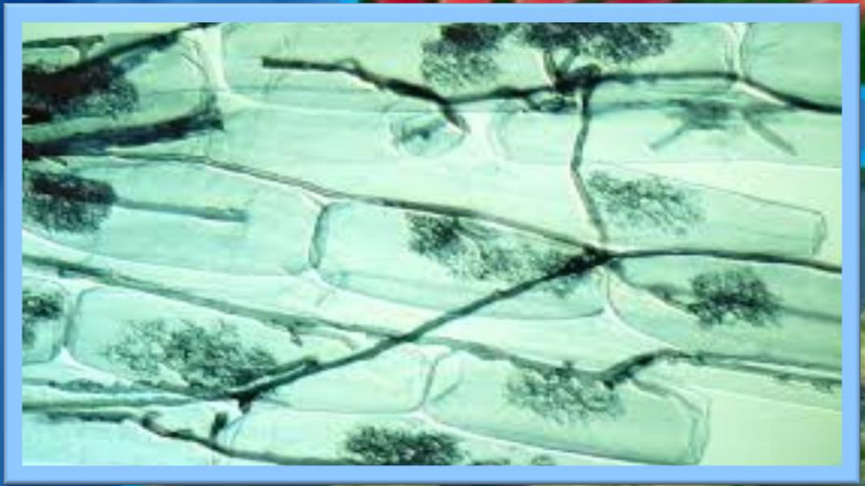


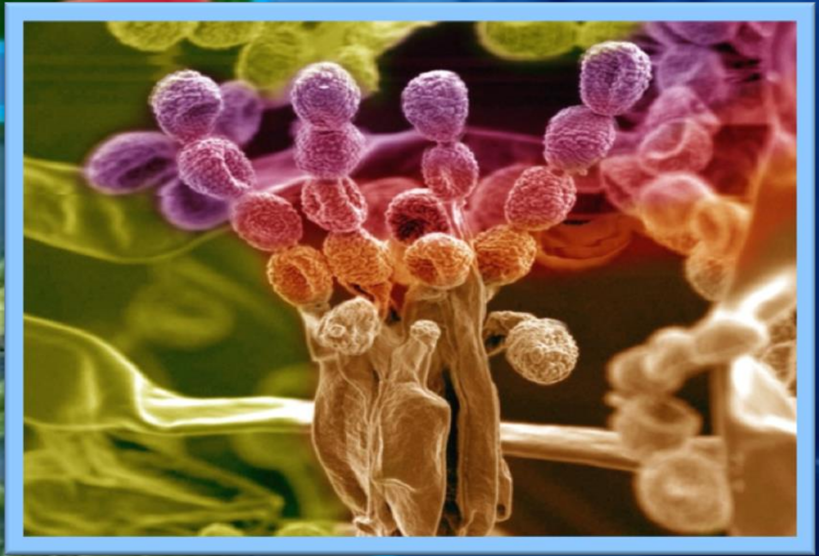
Vulnerable state

- Low levels of nutrient cycling
- Reduced growth
- Low productivity
- Low diversity
- Lower resistance to abiotic stress

Resilient state

- Enhanced N and P availability and higher levels of nutrient cycling
- Improved growth
- Enhanced disease supressiveness
- Functional redundancy (niche saturation)
- Higher resistance to abiotic stress
- Enhanced restoration capacity of degraded soils



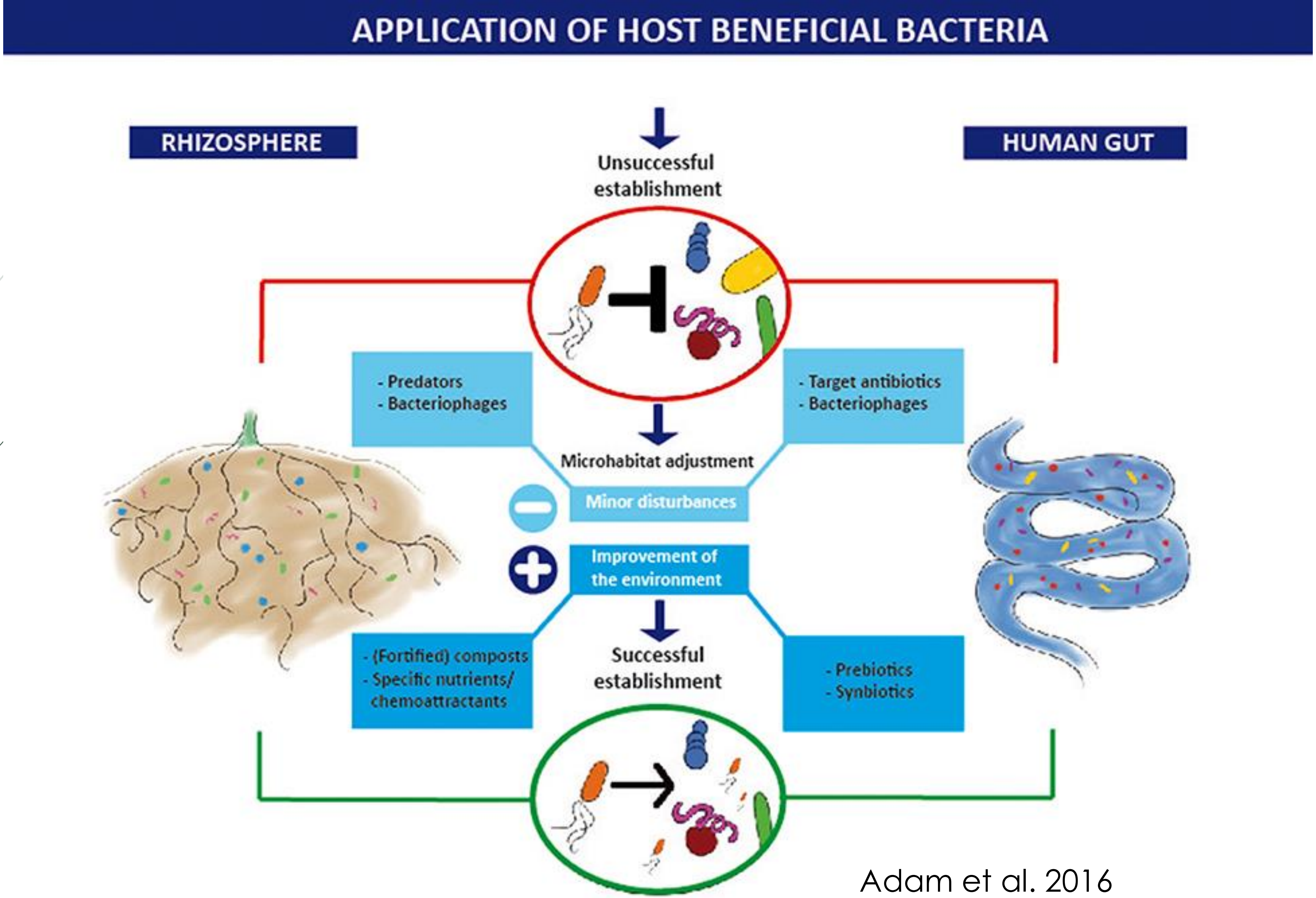


Improvement of the Environment—The Human Gut as a Paragon for Concepts in Healthy Soils

Just as human microbiome research is increasingly focused on manipulating our gut microbiomes to improve human health, soil microbial research is increasingly focused on leveraging our increasing understanding of the soil microbiome to improve the management of agricultural soils.

Human microbiome, to alleviate competition and increase the chance of establishment of host beneficial microbes in an environment that harbors a highly diverse microbial community utilizing all available resources can be enabled by adding specific energy resources, for example prebiotics.

Parallels with prebiotics can be seen the application of compost or green manure to the soils that can favor the development or the establishment of beneficial microbes.



Adam et al. 2016

There is a tremendous opportunity to process this organic waste and return it back to the farm. If these resource pulses coincide with beneficial inoculants, the temporary decrease in competition by native microbes could enhance their success.

Currently, our ability to manage and manipulate the rhizosphere microbiome is limited.

Inoculation with commercial inocula of bacteria and fungi.

Most of them were isolated under traditional culturing conditions under controlled lab and greenhouse conditions that are not reproducible under natural field conditions, so there is a limited evidence that these inoculated microbes establish, compete and function in agricultural soils.

Not only soil parameter as pH, texture, nutrient stoichiometry, but they also must integrate themselves not only with native microbiome, but with the food web.

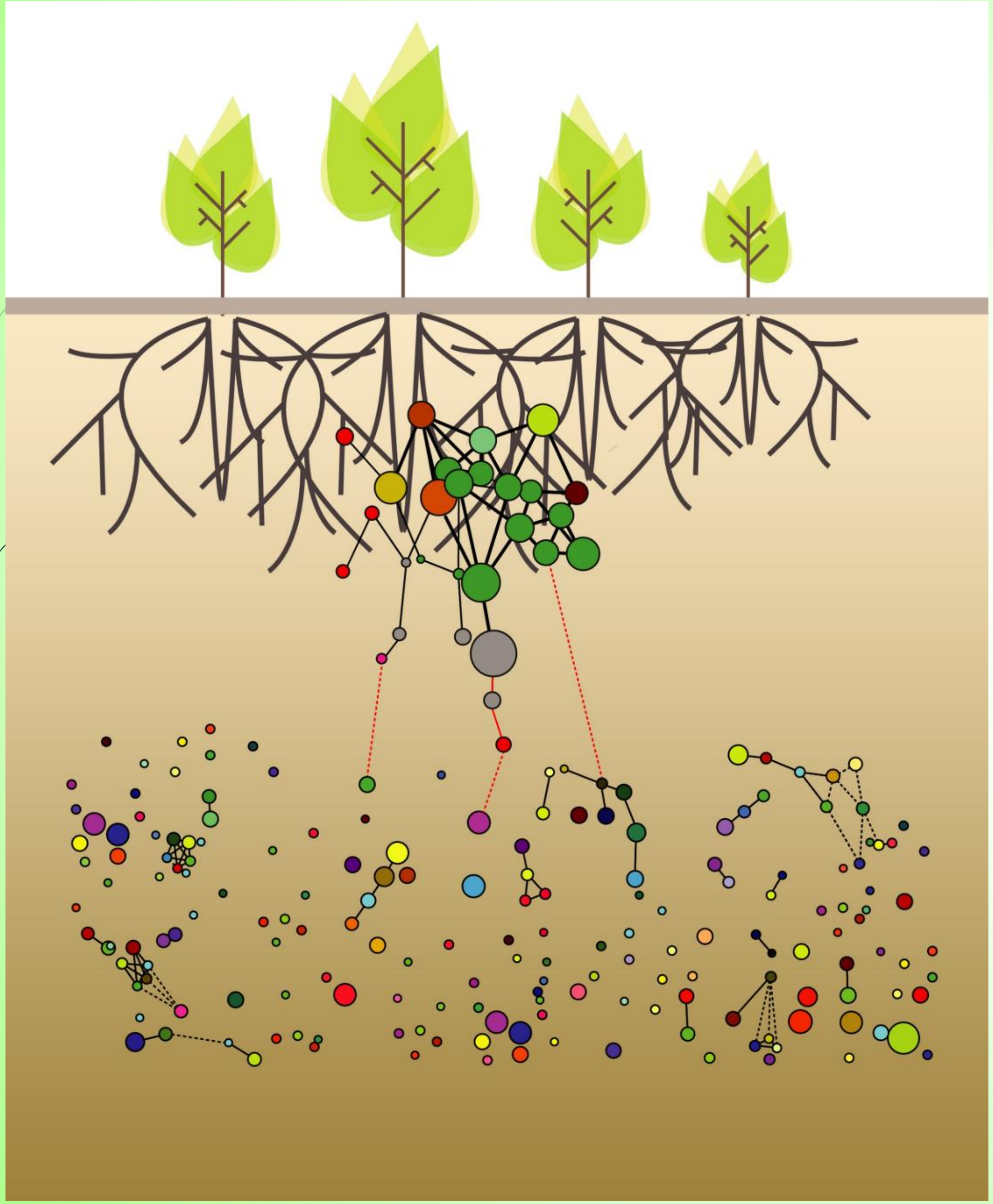
Compost quality is variable, which results in inconsistent colonization for soil beneficial microorganisms.

Many inoculants may fail under field conditions simply because they are quickly consumed by predators or outcompeted for resources by native microbes.

In nature, healthy plants recruit microbes from highly diverse, but weakly connected bulk soils, and favor rhizosphere microbiomes that are less diverse but highly structured into modules of highly interactive microbes and soil fauna.

Effective inoculants must form associations with the rest of the microbiome, emulating the strongly structured networks in native rhizosphere soils

RHIZOSPHERE INTERACTIONS FOR SUSTAINABLE AGRICULTURE MODELS

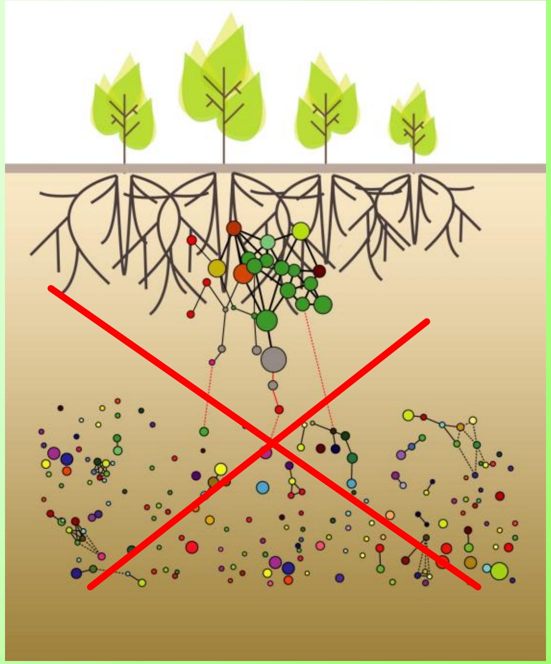


Understanding and manipulating network structure of both rhizosphere and bulk soil networks in agricultural soils, and the connections between them, is a promising avenue for optimising healthy soils and the benefits they provide for sustainable food production.
(De Vries and Wallenstein 2017 J. Ecology).

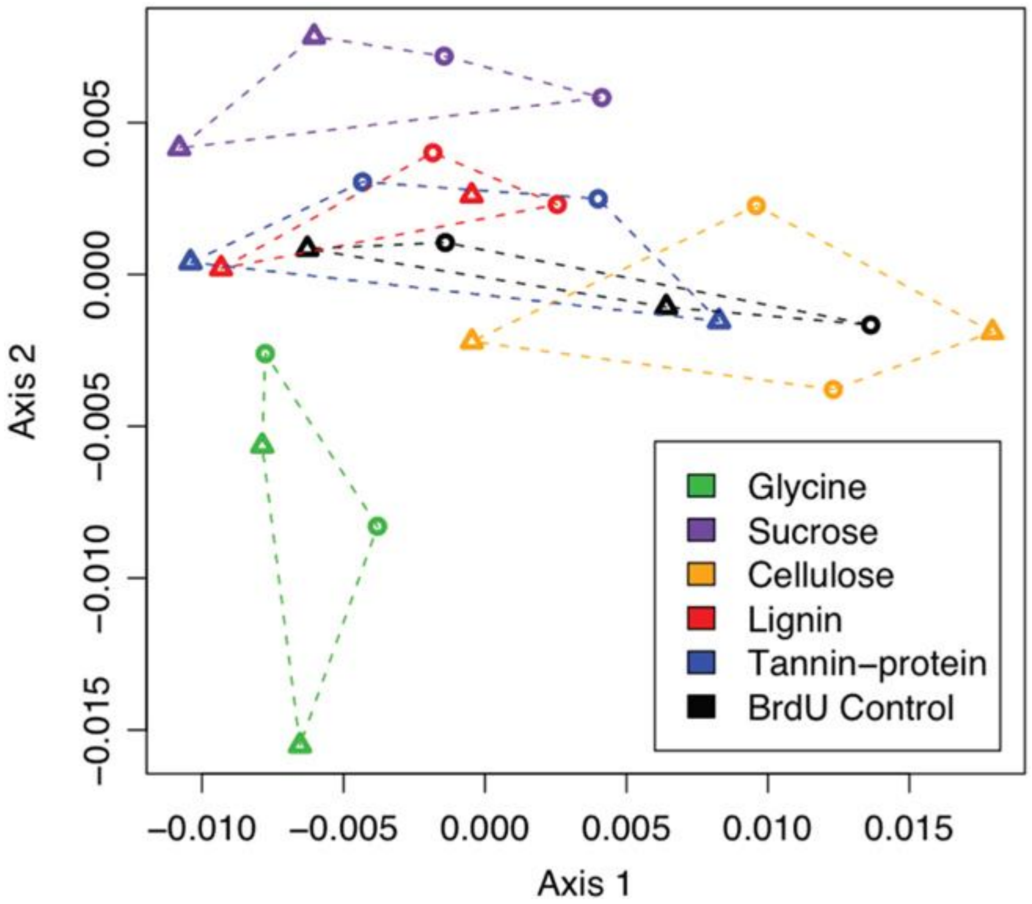
The bulk soil network provides the ‘seed bank’ from which rhizosphere networks are recruited, and crops will be able to recruit a functioning rhizosphere network as long as this seed bank is intact.

The composition and structure of the recruited rhizosphere network depends on the traits, and in particular root traits, of the crop grown, as well as on the abundance and composition of the bulk soil community.

Conventional agricultural management reduces the ability of the rhizosphere to recruit from the bulk soil.



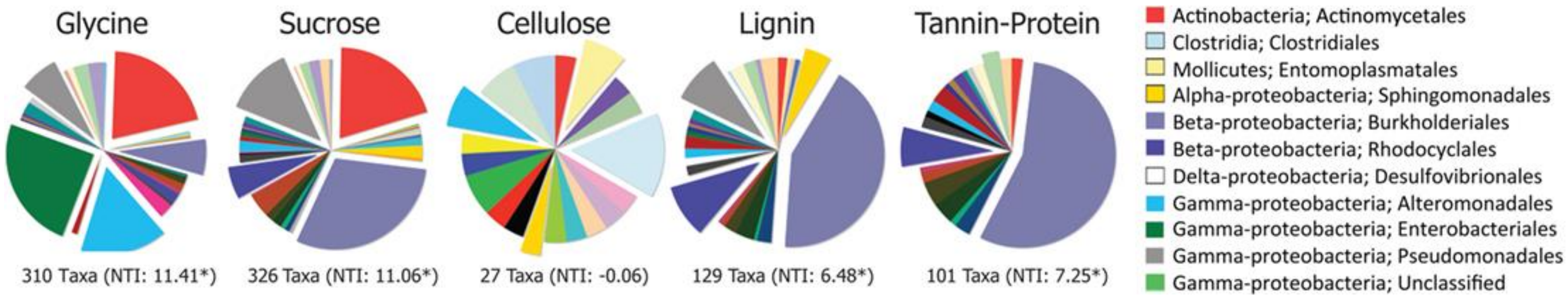
Differential growth responses of soil bacterial taxa to carbon substrates of varying chemical recalcitrance



Non-metric Multidimensional Scaling of inter-sample Bray– Curtis distances, based on PhyloChip probe set intensities. Addition of labile C (glycine or sucrose) resulted in a greater divergence of bacterial communities from controls than did chemically recalcitrant C

Goldfarb et al. 2011 Front. Microb.

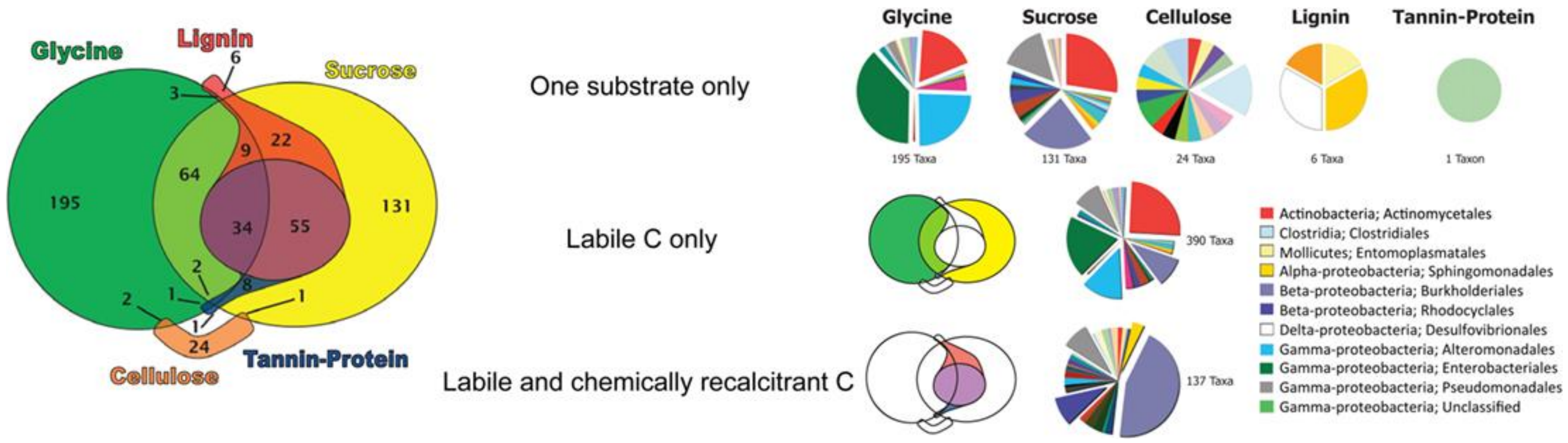
Differential growth responses of soil bacterial taxa to carbon substrates of varying chemical recalcitrance



Many more bacterial taxa (>300) were significantly enriched by the addition of a labile substrate, whereas the addition of more chemically recalcitrant substrates stimulated 27 to 129 taxa

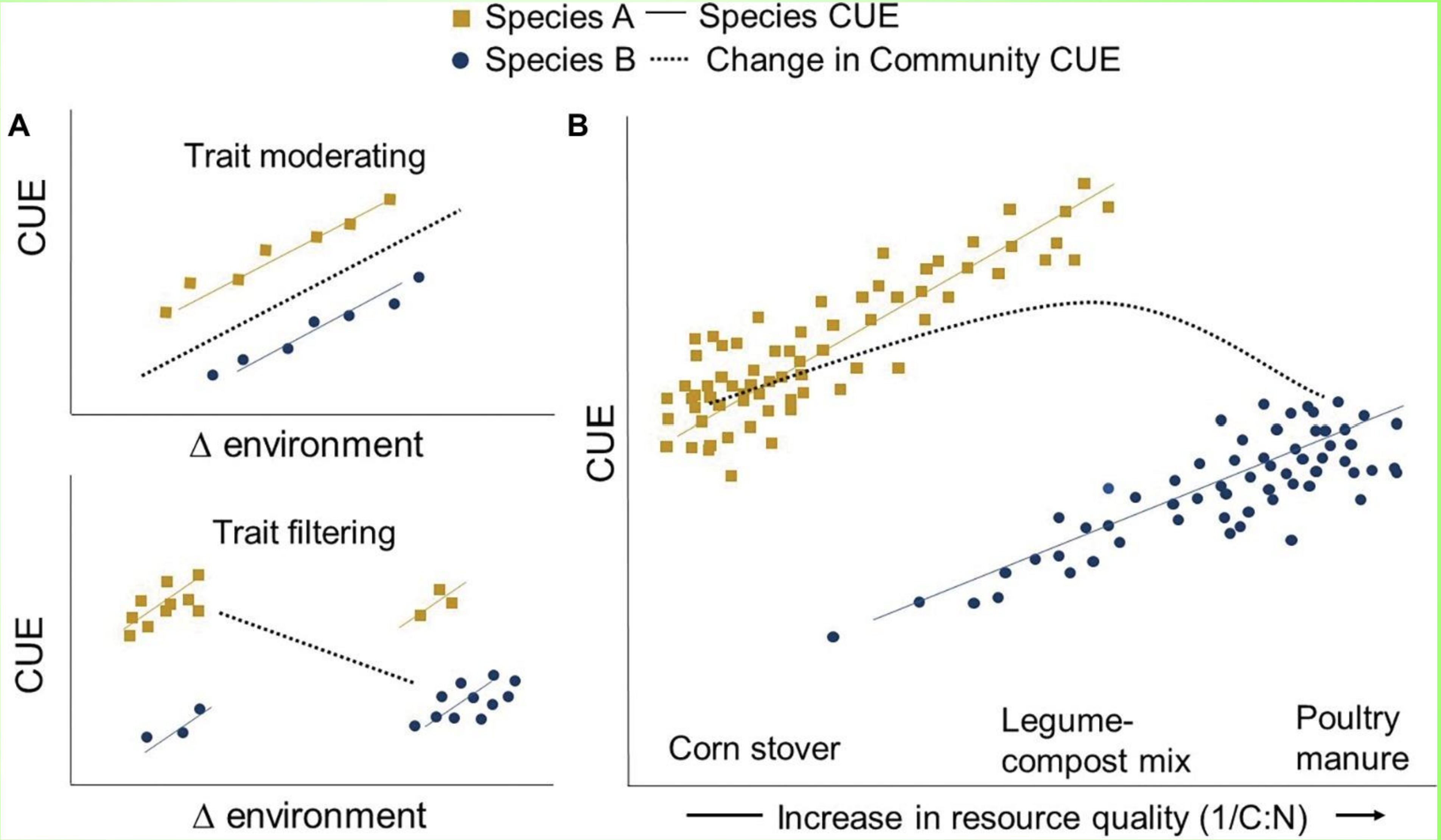
Goldfarb et al. 2011 Front. Microb.

Differential growth responses of soil bacterial taxa to carbon substrates of varying chemical recalcitrance



Increases in the availability of specific substrates can stimulate the growth of the taxa that can best compete for those resources, which may quickly lead to changes in microbial community composition.

On the other hand, the addition of substrates that are resistant to degradation (e.g., lignin and cellulose) did not change the overall composition of the active microbial community, although a small number of specialist taxa did show a growth response.



Kallebach et al. Front. Microb. 2019

A diversity of inputs representing a wide range of C and nutrient availability and chemistry might facilitate a balance between individual and community-level carbon use efficiency (CUE) optimization

Practices such as diversifying crop rotations or mixing legume cover crop biomass with corn or wheat residues could provide resources that promote species with different life histories to coexist.

Thus, community CUE might be maximized just before a threshold in community shift occurs, where a diversity of inputs provides resources for each member to realize their optimum CUE without shifting toward an overabundance of inefficient microbes.

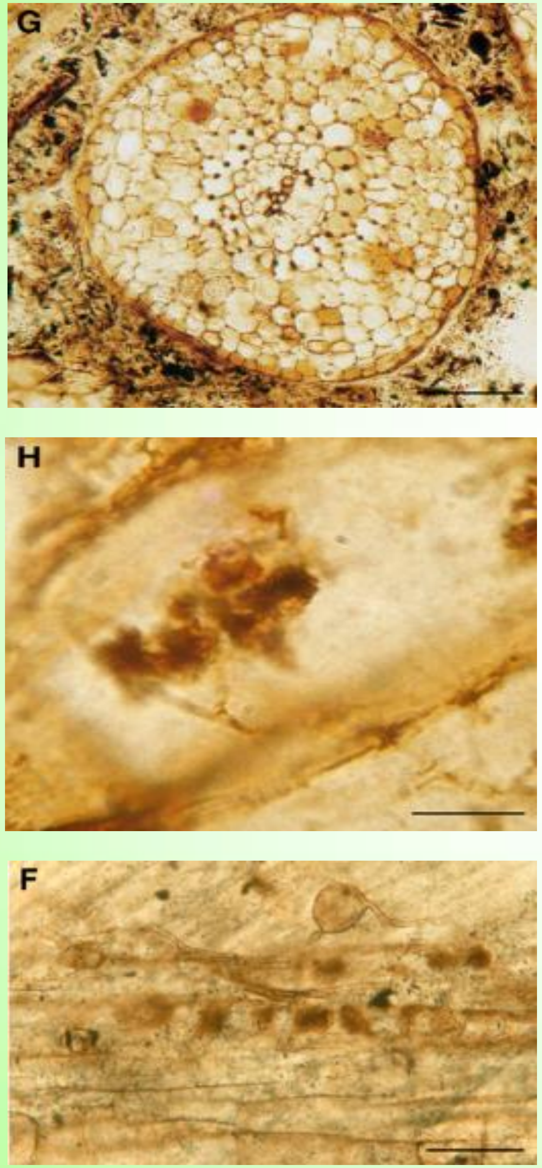
More structured heterogenous environments also theoretically favor k-strategists outcompeting R-strategists, characterized by a relatively lower CUE

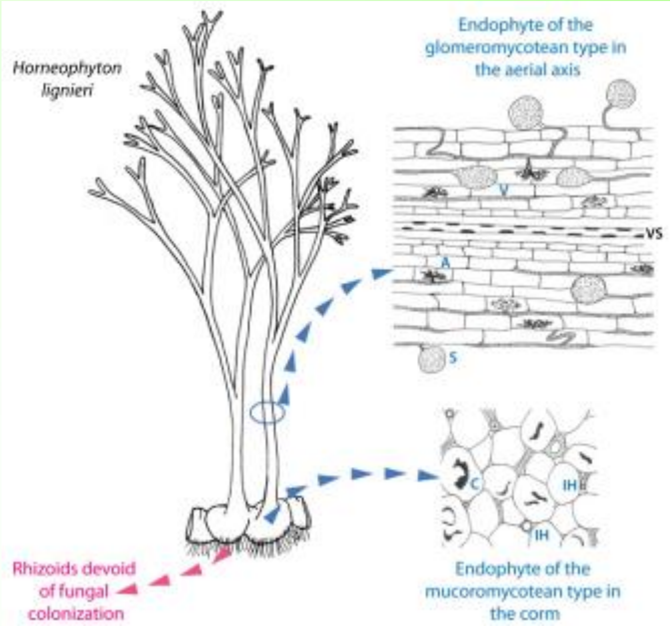
ARBUSCULAR MYCORRHIZAL FUNGI (AMF)

AM symbiosis is the default situation for most crop plants (80%) in the field. With the exception ……

…of the species belonging to the families of Brassicaceae (broccoli, cauliflowers etc.) and Chenopodiaceae (beets, spinach etc.)

Fungal partnership in Devonian plants

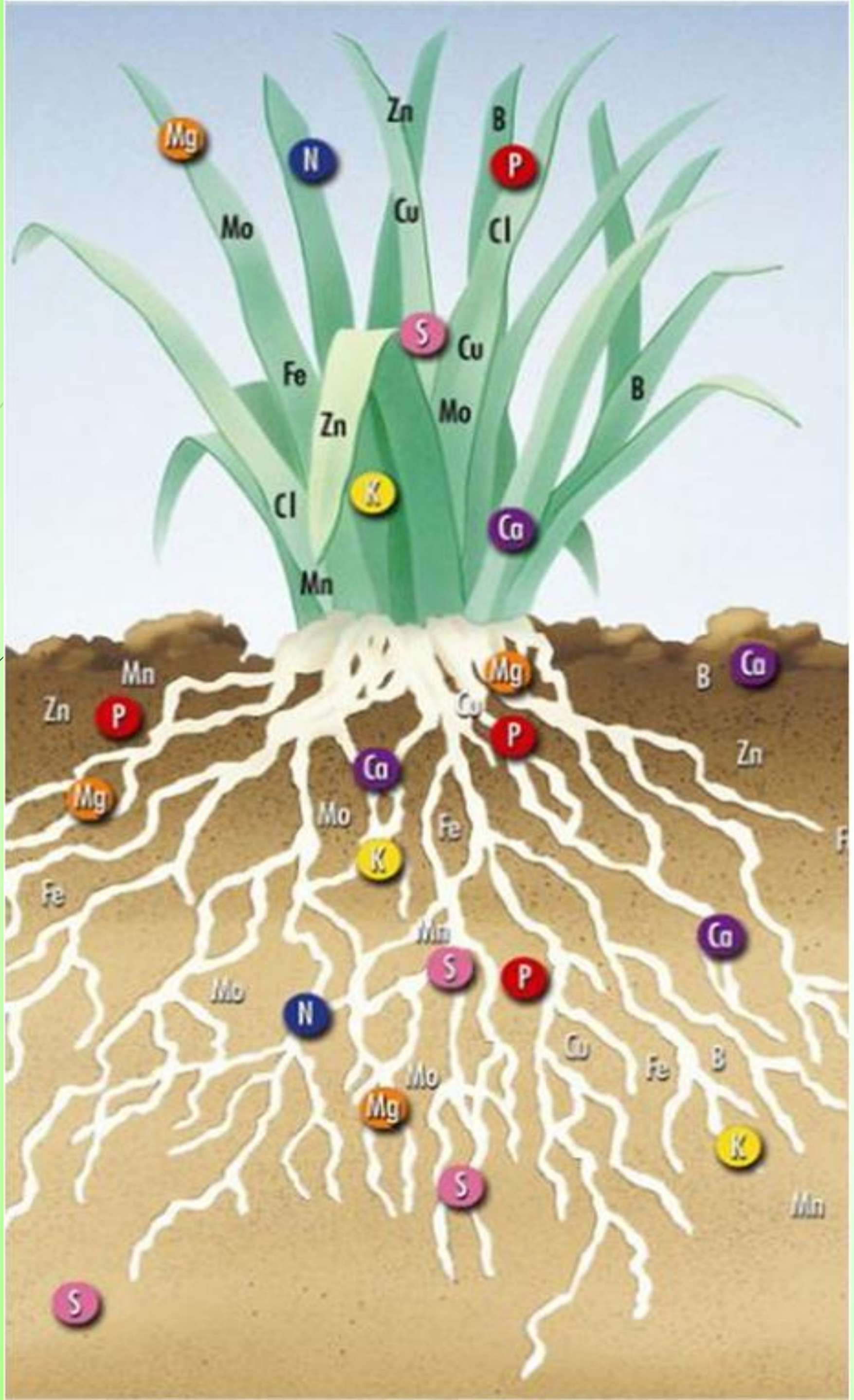


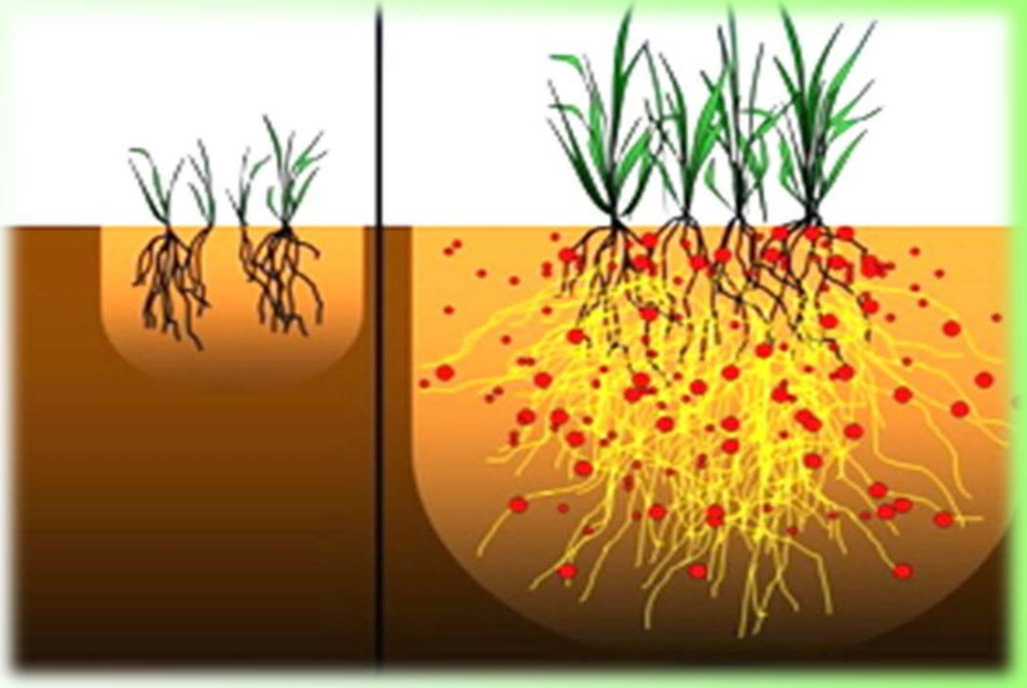


Exceptional sites of fossil preservation (*Horneophyton lignieri*)such as the 407-million-year-old Rhynie Chert provide direct evidence on the nature and function of roots and rhizoid in early terrestrial ecosystems and on their interactions with fungi. Rhizoid predate the evolution of roots, and they were widespread in both the sporophyte and gametophyte generations of early vascular plants.

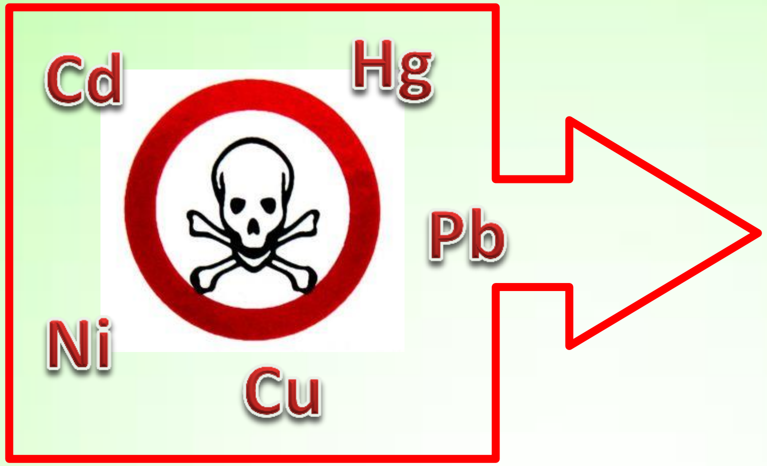
Strullu-Derren et al, 2014

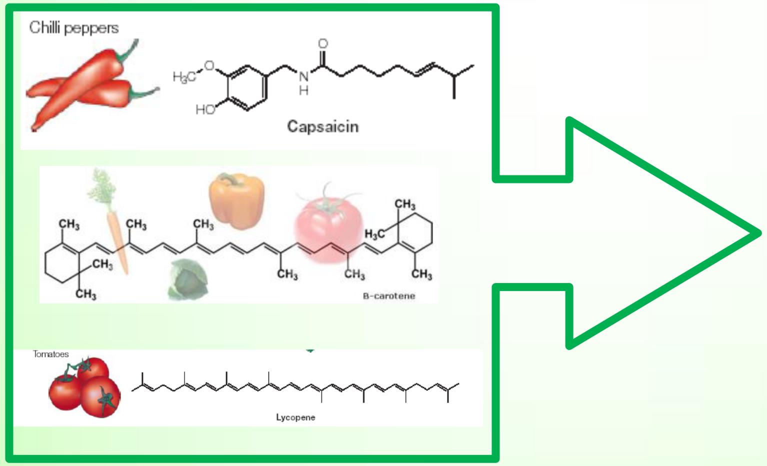
Increase soil nutrient uptake





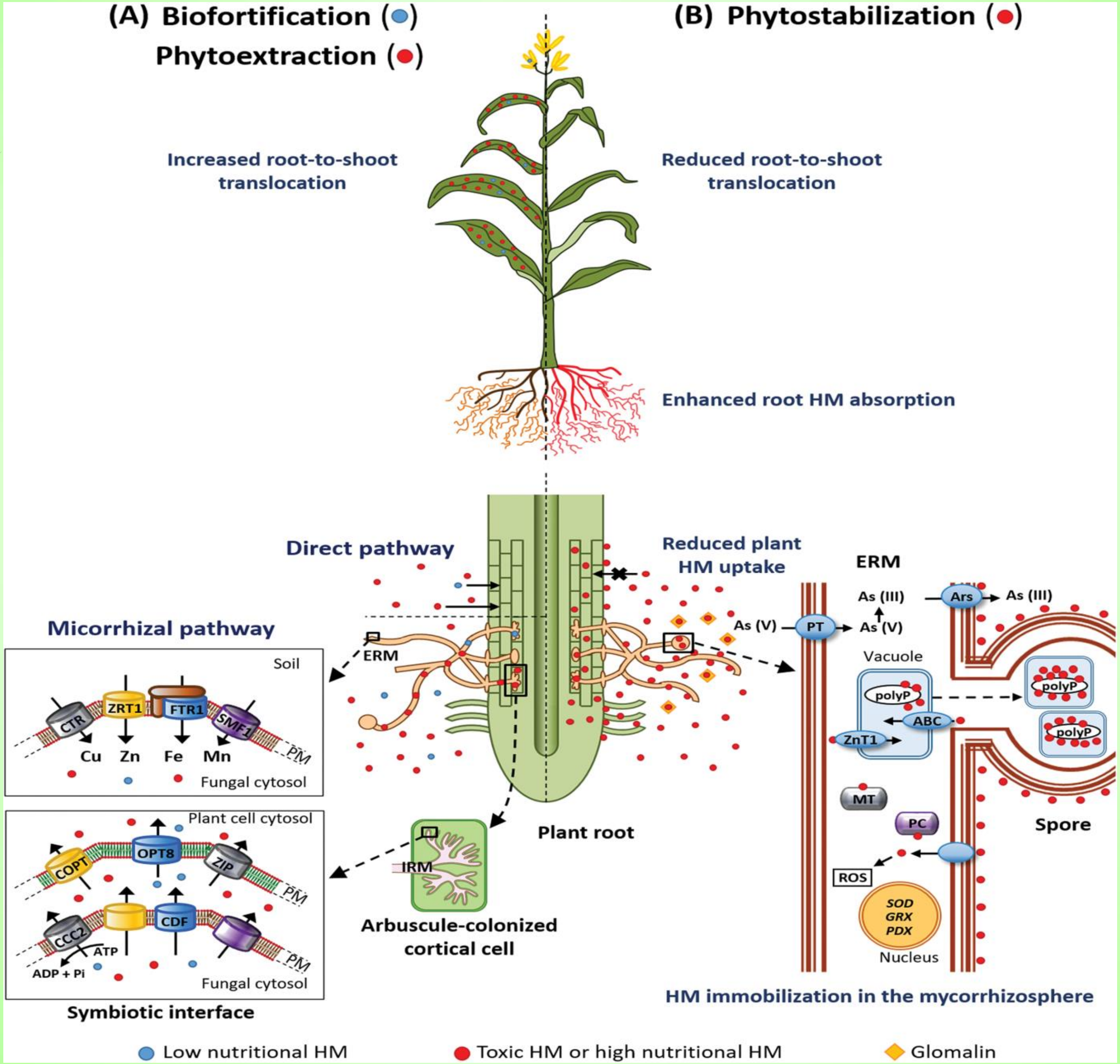
P, N, S, B, Cu, K, Zn, Ca, Mg, Na, Mn, Fe, Al, and Si.

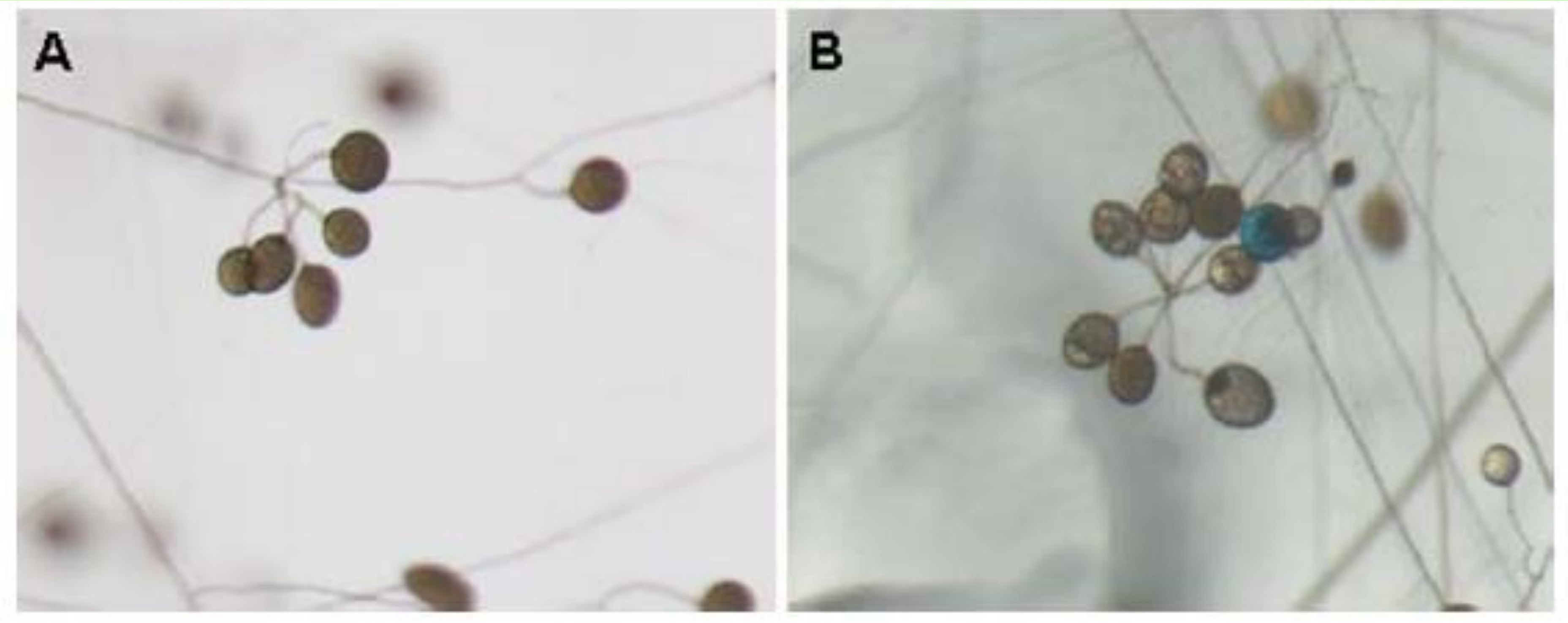




Decrease heavy metal uptake
Increase nutraceutical compunds

AM contribution to plant heavy metals acquisition and distribution.

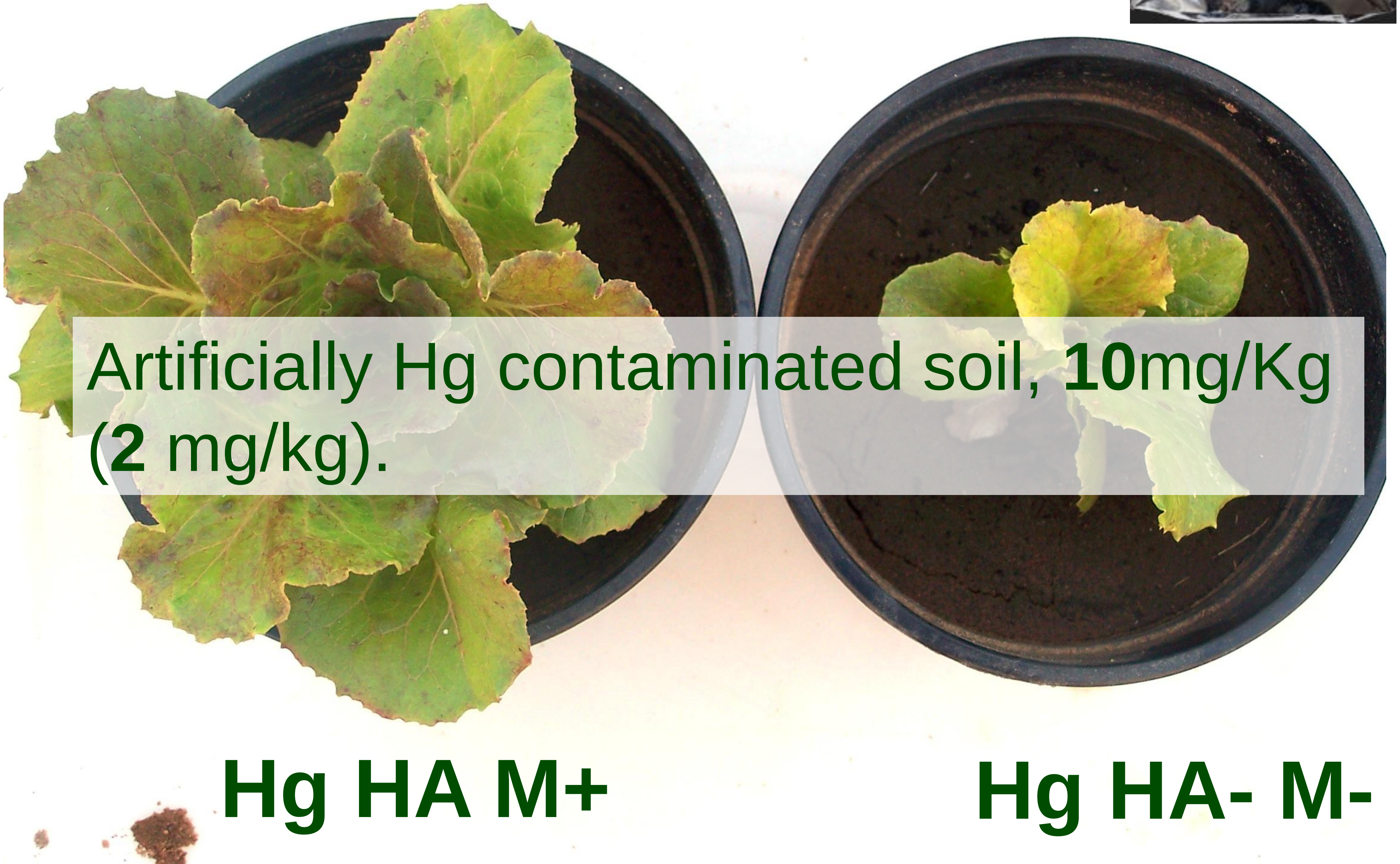




Fungal mycelium culture containing 0.5µM Cu (A) or 50 µM Cu (B) per 48h (Ferrol et al., 2009 Phytochem Rev.) .

In presence of high concentrations of toxic elements AMF can survive concentrating the elements in some spores, protecting in this way the rest of fungal colony

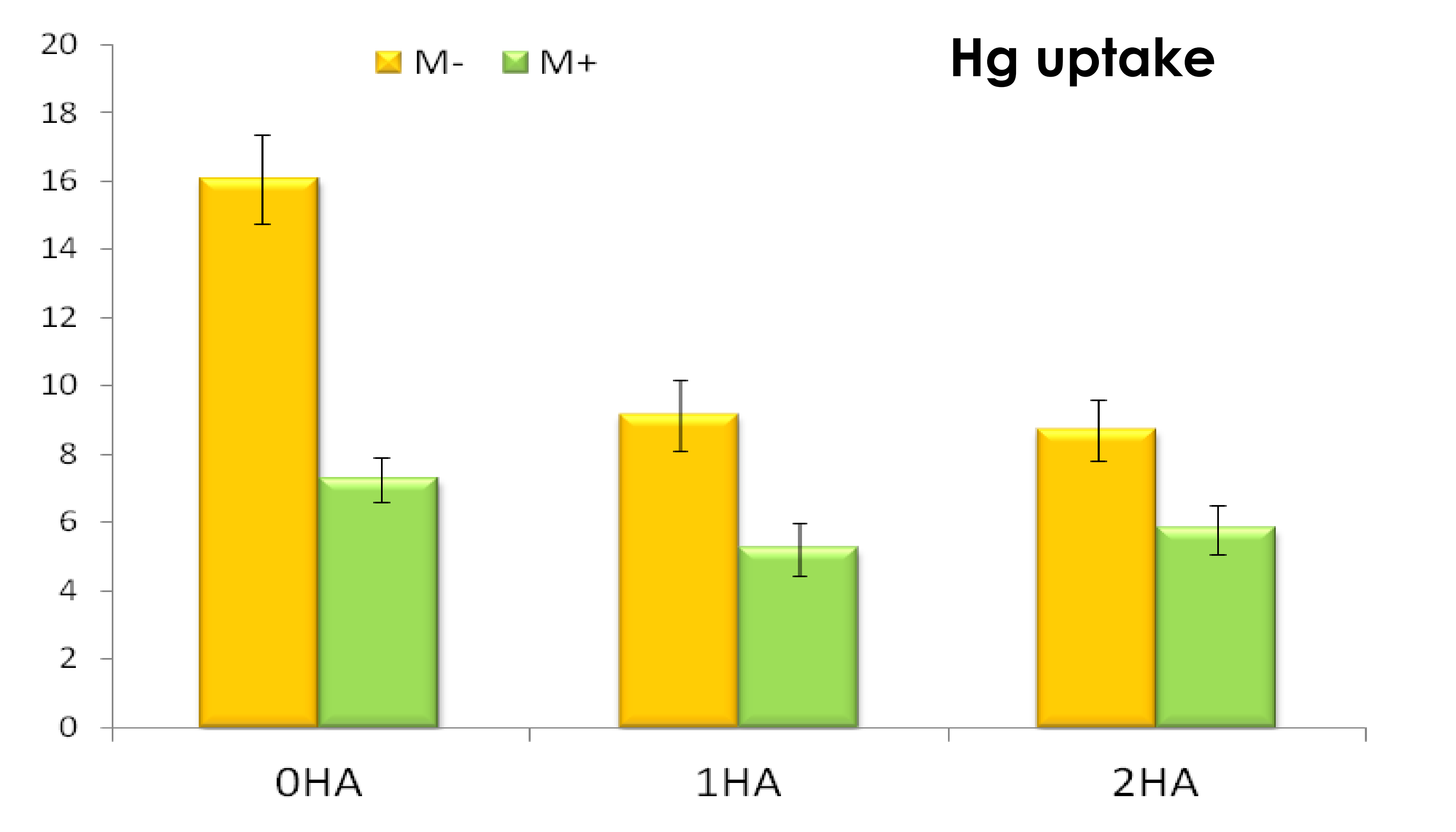
Mercury contamination



Artificially Hg contaminated soil, **10**mg/Kg (**2** mg/kg).

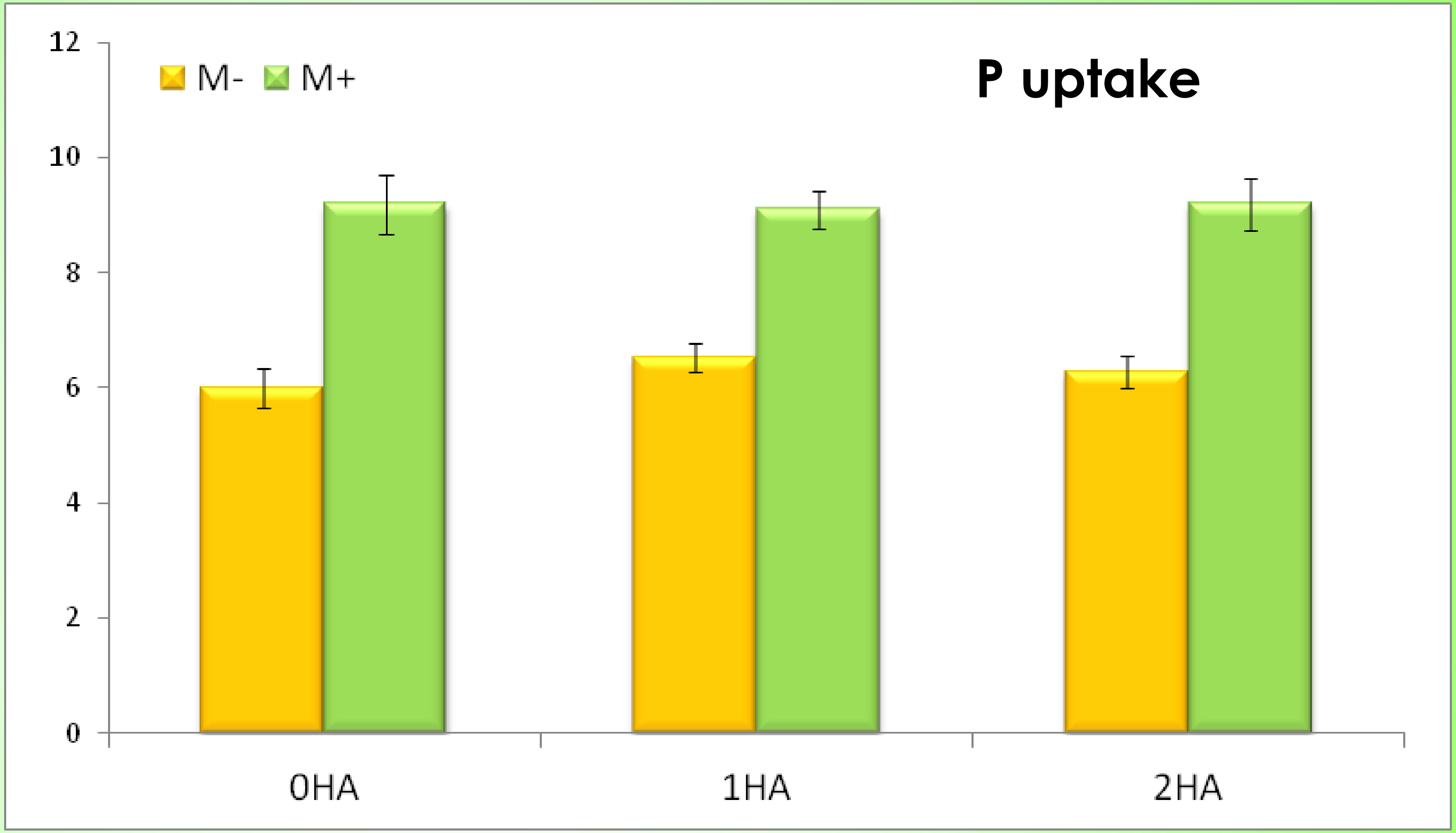
Hg HA M+

Hg HA- M-



Total Hg uptake per root dry weight (µg g^{-1} root DW) in lettuce plants inoculated (M+) and uninoculated (M-), with and without amendment with humic acids (HA).

Cozzolino et al. 2016 Environ Sci Pollut Res



Total P uptake per root dry weight (mg g^{-1} root DW) in lettuce plants inoculated (M+) and uninoculated (M-), with (HA) and without amendment (0HA) with humic acids.

Cozzolino et al. 2016 Environ Sci Pollut Res

AMF Agro-ecosystem service

Overview of the main roles that AM symbiosis can play as an agroecosystem service	
AM function	Ecosystem services provided
Root morphology modification and development of a ramifying mycelial network in soil	Increase plant/soil adherence and soil stability(binding action and improvement of soil structure
Increasing mineral nutrient and water uptake by plants	Promote plant growth while reducing fertilizer requirement
Buffering effect against abiotic stresses	Increased plant resistance to drought, salinity, heavy metals pollution and mineral nutrient depletion
Secretion of “glomalin” into the soil	Increased stability and water retention
Protecting against root pathogens	Increased plant resistance against biotic stresses while reducing phytochemical input
Modification of plant metabolism and physiology	Bioregulation of plant development and increase in plant quality for human health

Gianninazzi et al., 2010

How different compost typologies can affect arbuscular mycorrhizal fungi growth and crop growth?

Experimental Setup

Treatments

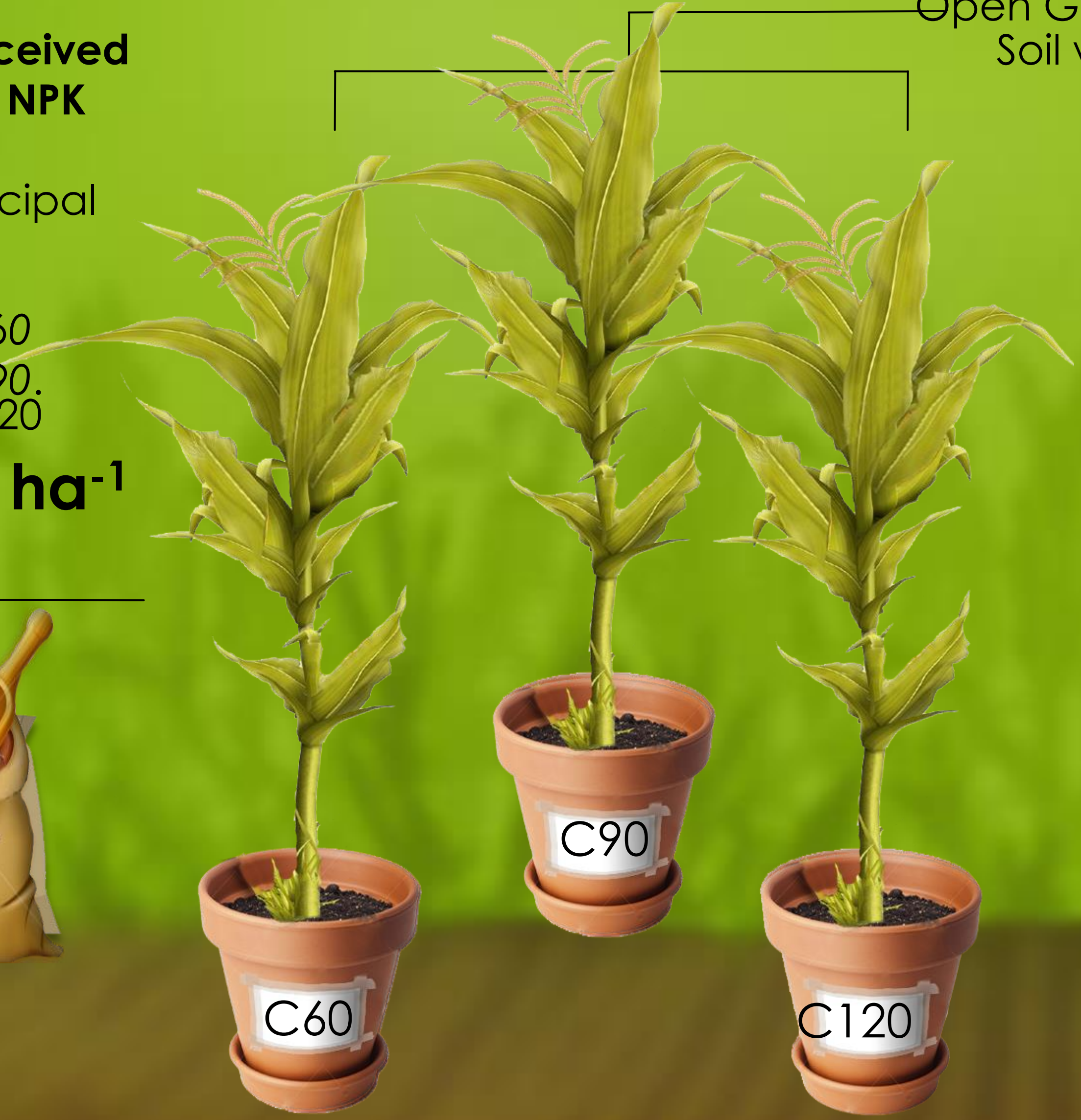
All treatment received
Mineral fertilizer NPK

Compost= Municipal organic wastes
CT= control,
C60= *compost 60*
C90= *compost 90*.
C120compost 120

15ton ha^{-1}

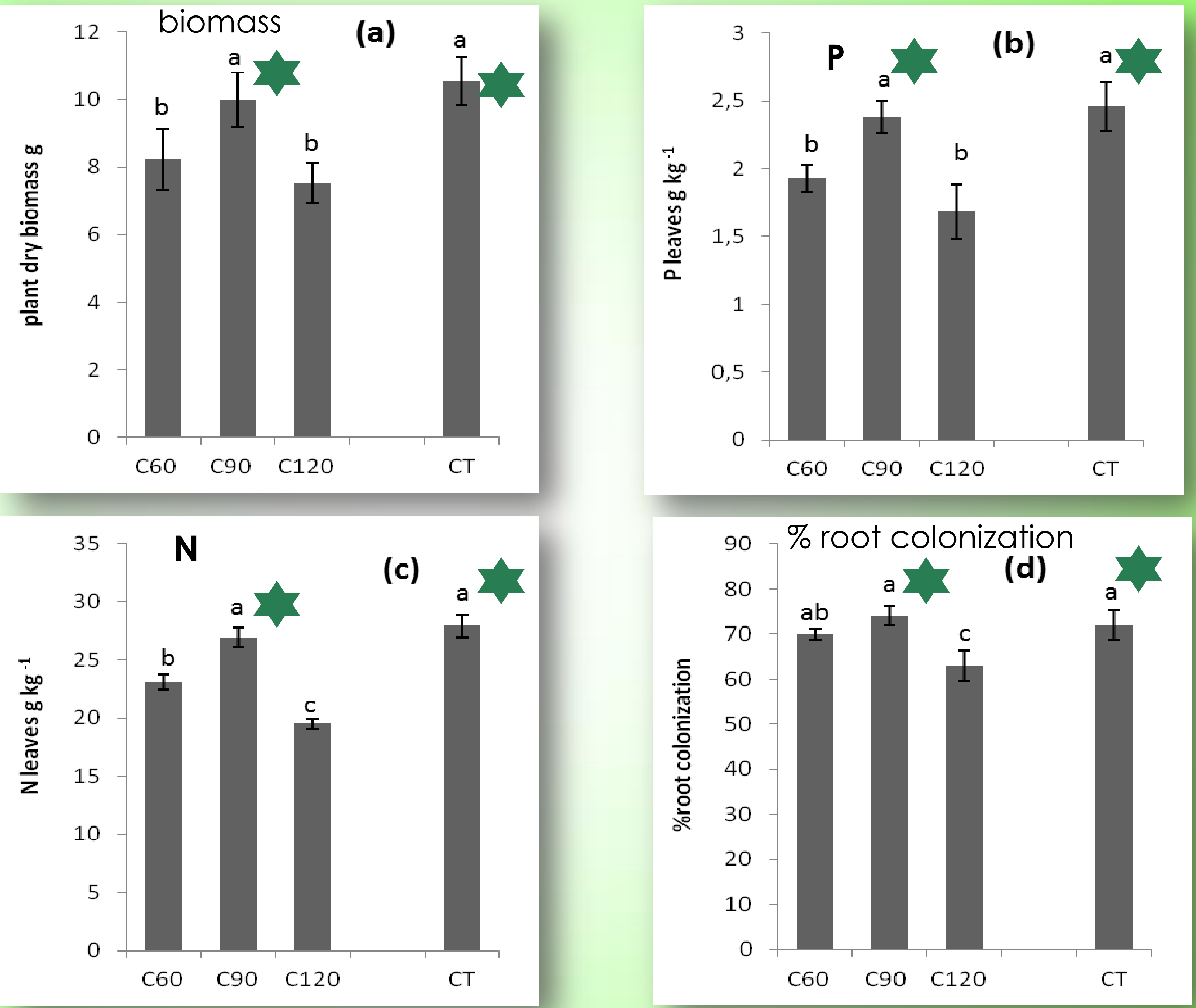
Conditions

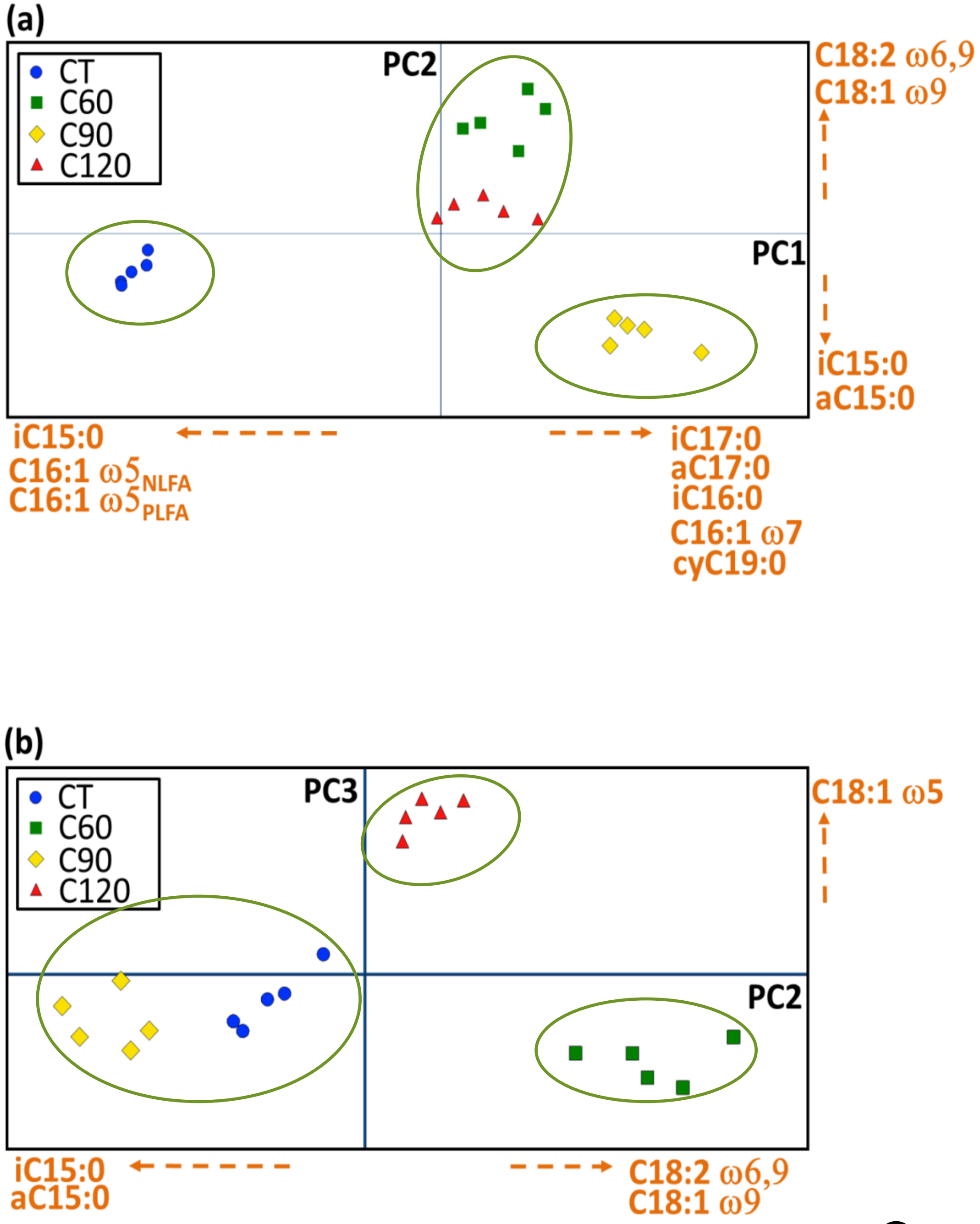
8 weeks growt
Open Greenhouse 5 replicates
Soil water content kept at



ANALYSIS PLANNED/PERFORMED

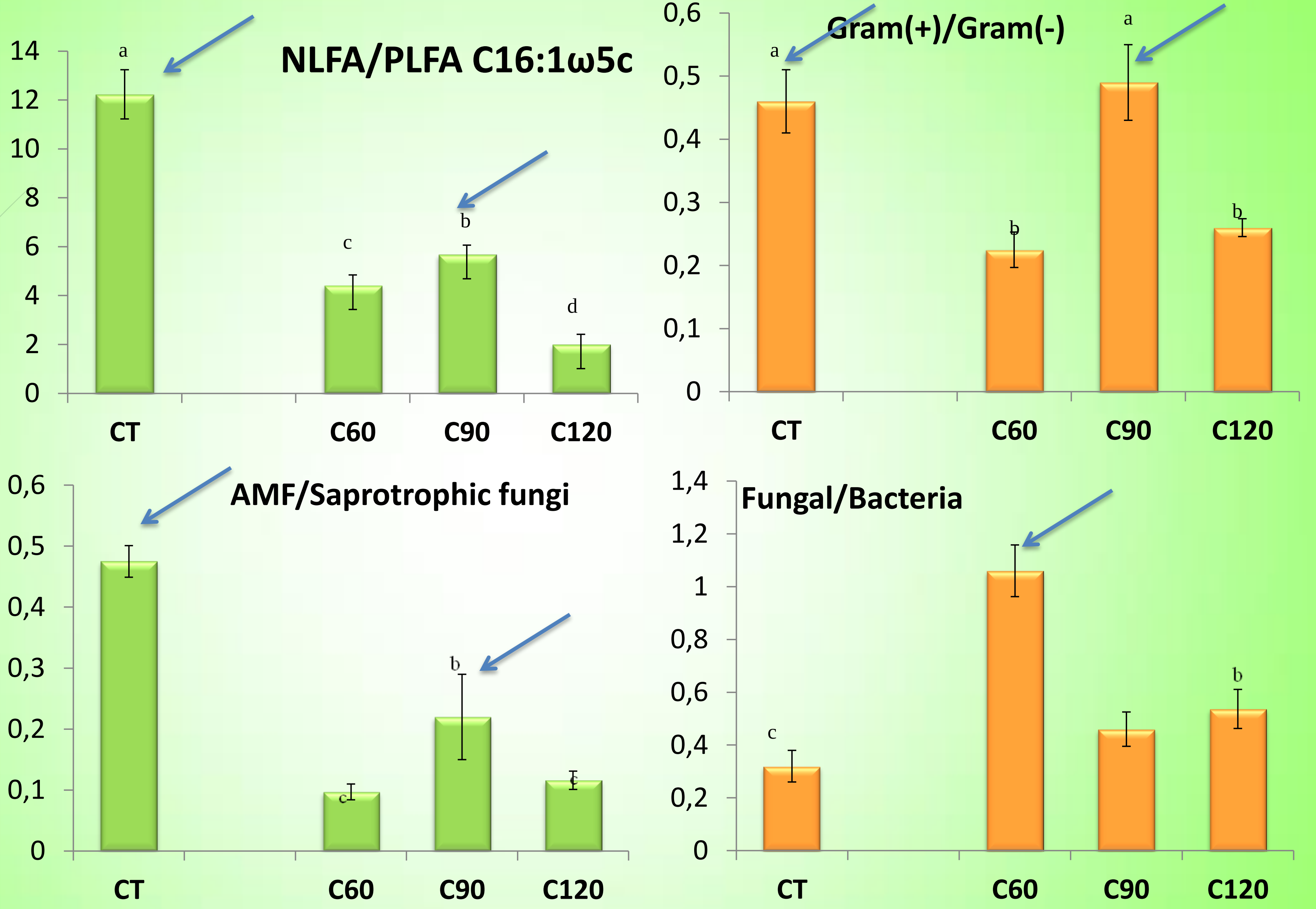
Cozzolino et al., 2016 Biol. Fert Soil


biomass
(a)
plant dry biomass g
12
10
8
6
4
2
0
b
a
b
a
C60
C90
C120
CT
P
(b)
P leaves g kg -1
3
2,5
2
1,5
1
0,5
0
b
a
b
a
C60
C90
C120
CT
N
(c)
N leaves g kg -1
35
30
25
20
15
10
5
0
b
a
c
a
C60
C90
C120
CT
% root colonization
(d)
%root colonization
90
80
70
60
50
40
30
20
10
0
ab
a
c
a
C60
C90
C120
CT



PCA score-plots of samples treated without or with compost amendments resulting from **60, 90 and 120** (**C60, C90, C120**) days of maturation. The combination of PC1 (47.3% explained variance) with PC2 (23.6% explained variance) as well as that of PC2 with PC3 (16.2% explained variance) are shown in Figure 2A and 2B, respectively

Cozzolino et al., 2016 Biol. Fert Soil



Cozzolino et al., 2016 Biol. Fert Soil

Compost	190-160 Carboxyl-C	160-145 O-aryl-C	145-110 Aryl-C	110-60 O-Alkyl-C	60-45 CH_3O/CN	45-0 Alkyl-C	HB/HI[a]	A/OA[b]
C60	5.3a	2.6a	9.9a	52.3a	8.9a	21.1a	0.58a	0.40a
C90	5.3a	2.7a	8.2ab	48.0b	8.6a	27.2b	0.73b	0.57b
C120	5.2a	2.9a	9.0a	54.3a	8.3a	20.4a	0.54a	0.38a

[a] HB/HI = Hydrophobicity index = (Aryl-C + Phenol-C+ Alkyl-C)/(Carboxyl-C + O-Alkyl-C);
[b] A/OA = Alkyl ratio = (Alkyl-C)/(O-Alkyl-C)

Cozzolino et al., 2016 Biol. Fert Soil

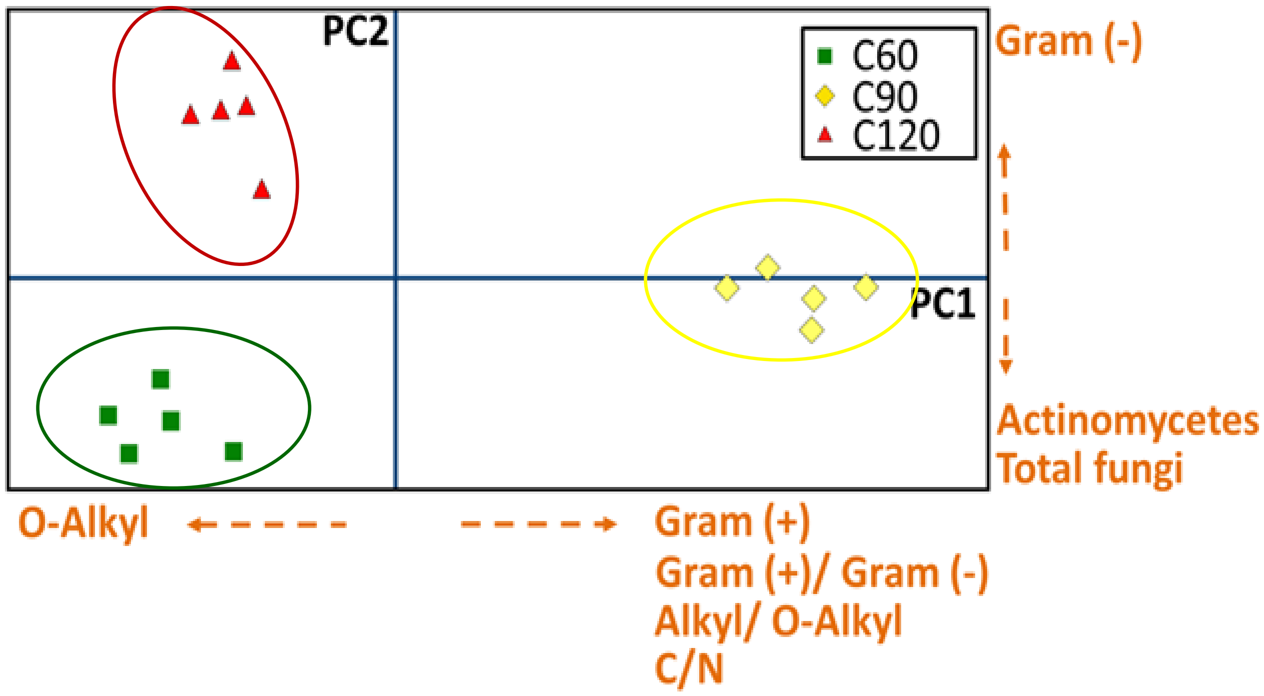


Table 5 Correlation coefficients (R) for statistical relationship of compost molecular properties, as estimated by NMR parameters and microbial markers in soil

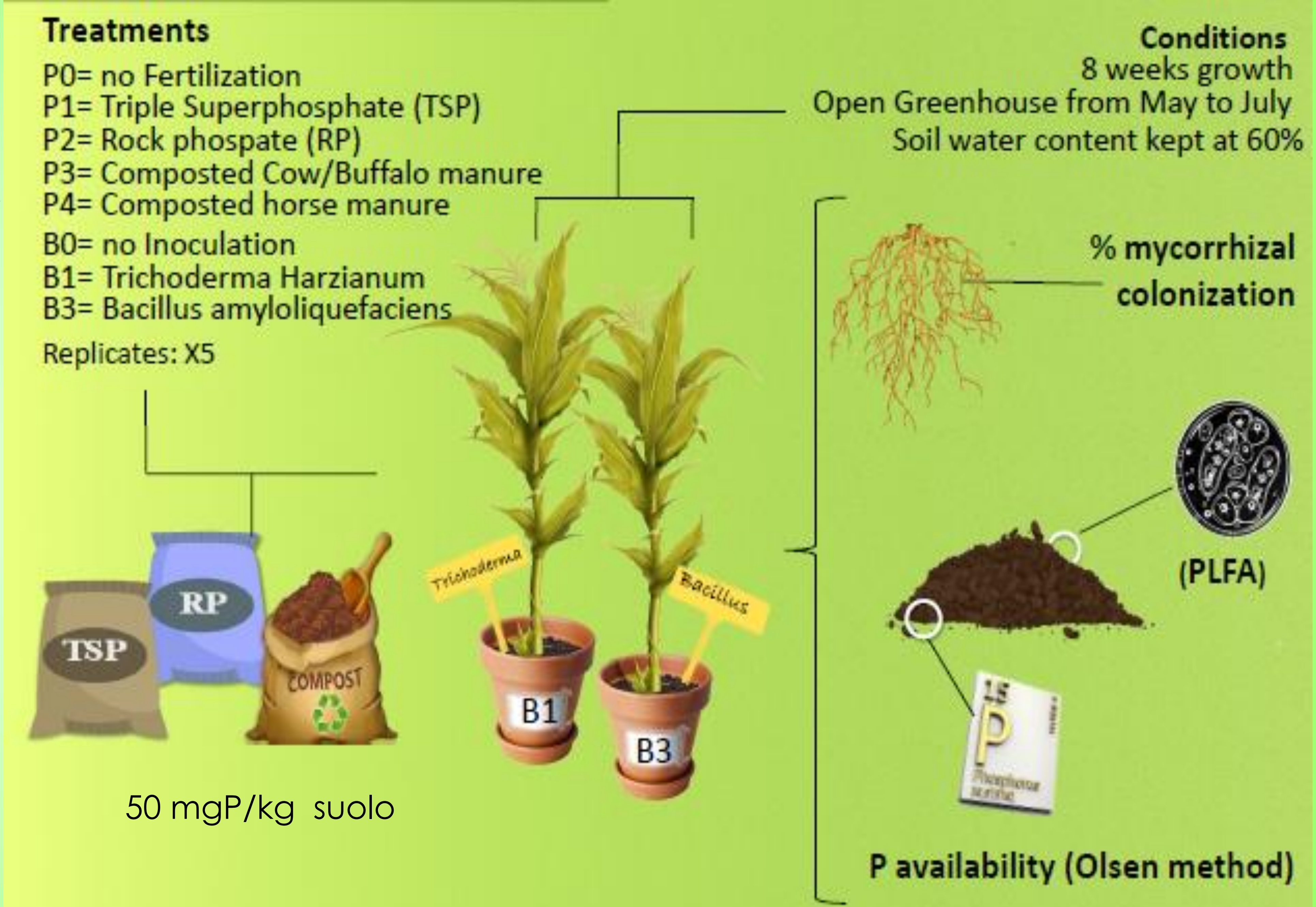
	Alkyl-C[a]	O-alkyl-C[a]	HB/HI[b]	A/OA[c]	C/N
NLFA 16:1ω5	*R*=0.6224 *P*=0.013	*R*=−0.8012 *P*=0.0003	*R*=0.6323 *P*=0.0114	*R*=0.6858 *P*=0.0048	*R*=0.5490 *P*=0.011
Gram(+)/Gram(−)	*R*=0.7129 *P*=0.0029	*R*=−0.7932 *P*=0.0004	*R*=0.7895 *P*=0.0005	*R*=0.7865 *P*=0.0005	*R*=0.9435 *P*=0.0003
AMF/saprotrophic fungi	*R*=0.6198 *P*=0.01	*R*=−0.7967 *P*=0.0004	*R*=0.7325 *P*=0.0019	*R*=0.7325 *P*=0.0019	*R*=0.7936 *P*=0.0009
Total fungi	*R*=−0.5647 *P*=0.012	*R*=0.4506 *P*=0.09	*R*=−0.5857 *P*=0.032	*R*=−0.4416 *P*=0.09	*R*=−0.7712 *P*=0.01

[a] See Table 4 for chemical shift range

[b] HB/HI=hydrophobicity index=(aryl-C+phenol-C+alkyl-C)/(carboxyl-C+O-alkyl-C)

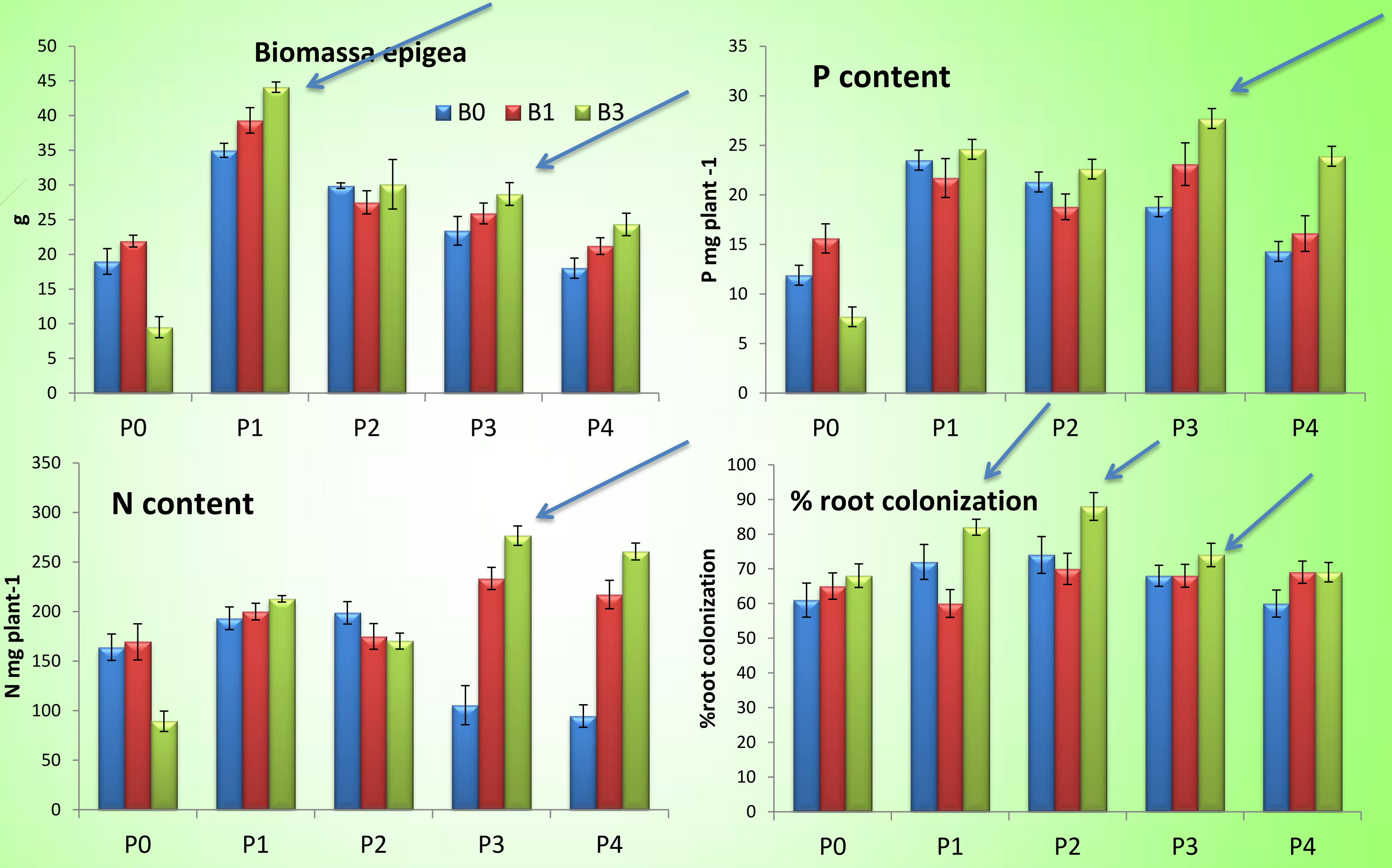
[c] A/OA=alkyl ratio=(alkyl-C)/(O-alkyl-C)

Cozzolino et al., 2016 Biol. Fert Soil



50 mgP/kg suolo

Vinci et al. 2018 Plant Soil



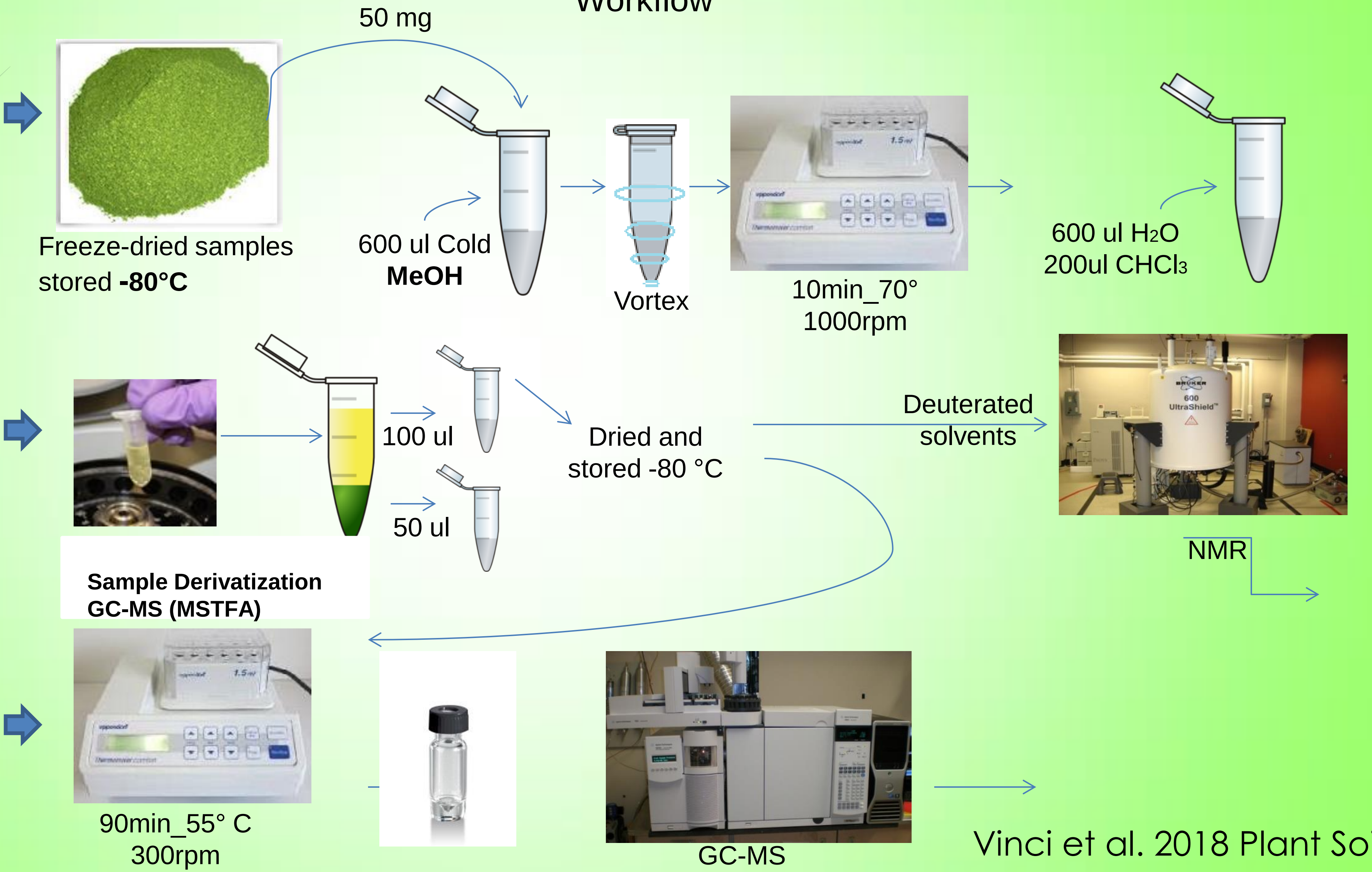
B0= not inoculated; B1=T. harzianum; B3=B. amyloliquefaciens

P0= no P; P1=TSP; P2=RP; P3= Composted cow manure; P4=Composted horse manure

Vinci et al. 2018 Plant Soil

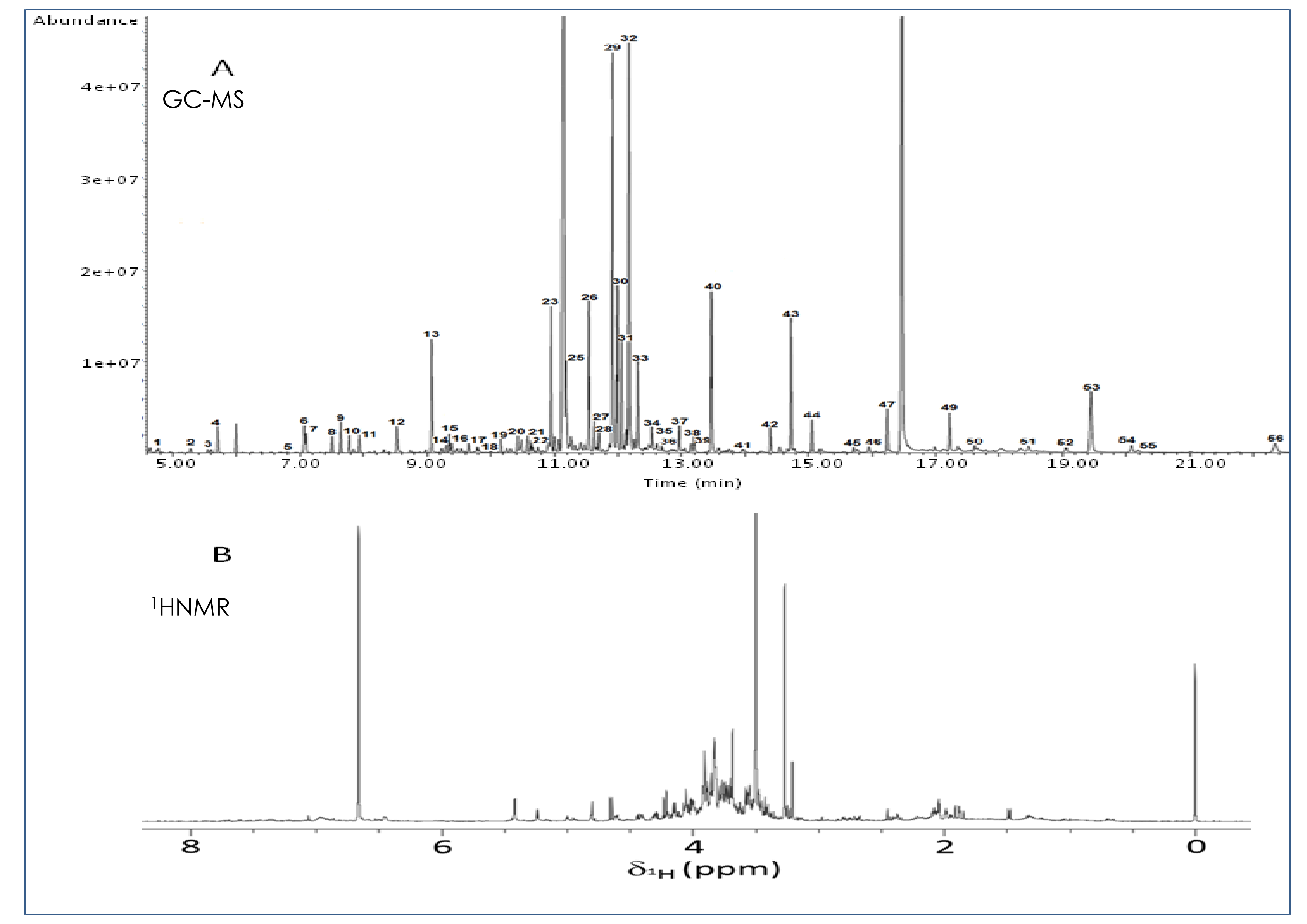
Extraction and Analysis of polar metabolites

Workflow

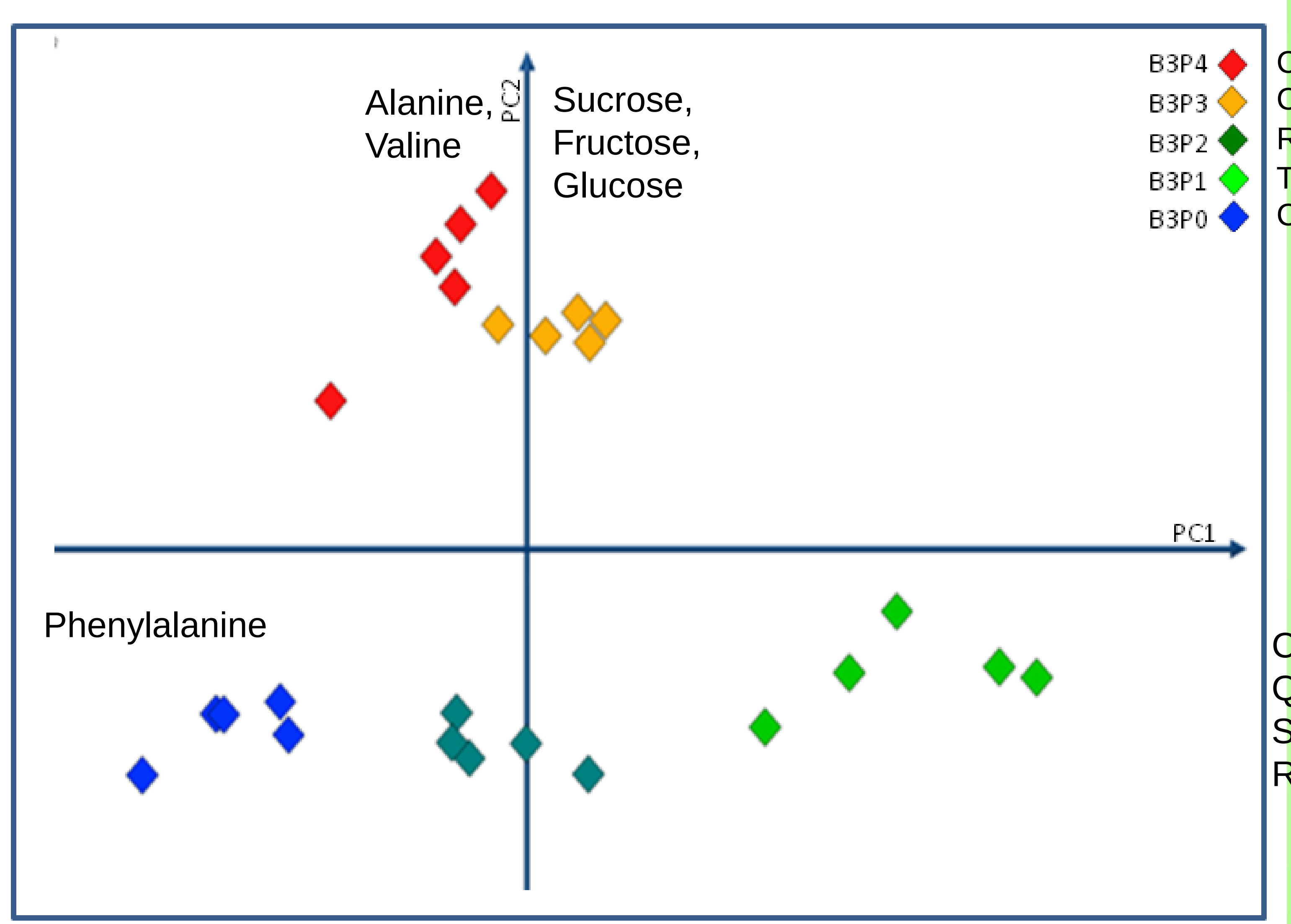


Vinci et al. 2018 Plant Soil

Total Ion Chromatogram (TIC) **(A)**, 1HNMR spectrum **(B)** of metabolites extracted from *Zea mays* leaves inoculated with *B. amyloliquefaciens*



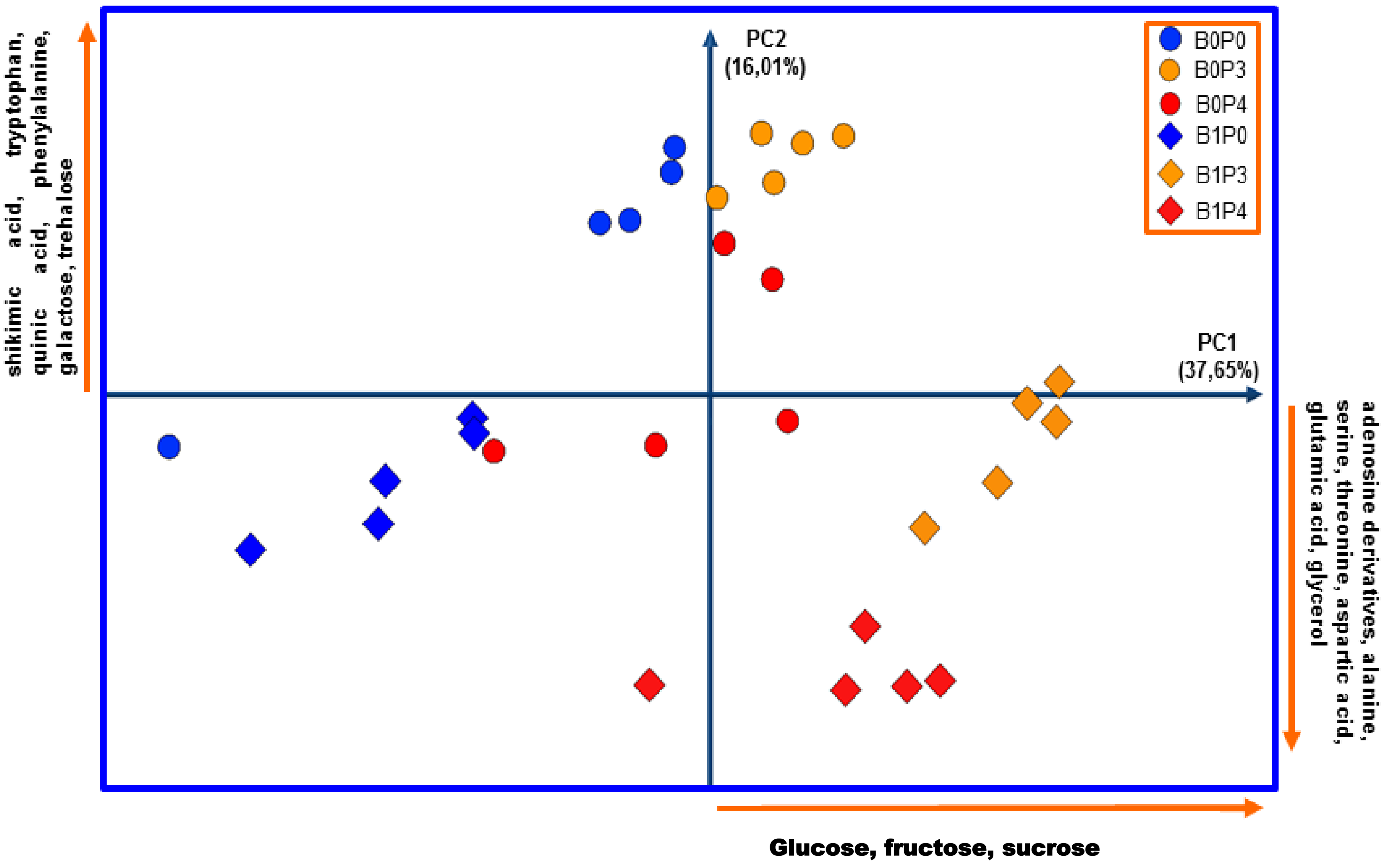
P fertilizers combined with *Bacillus amyloliquefaciens*



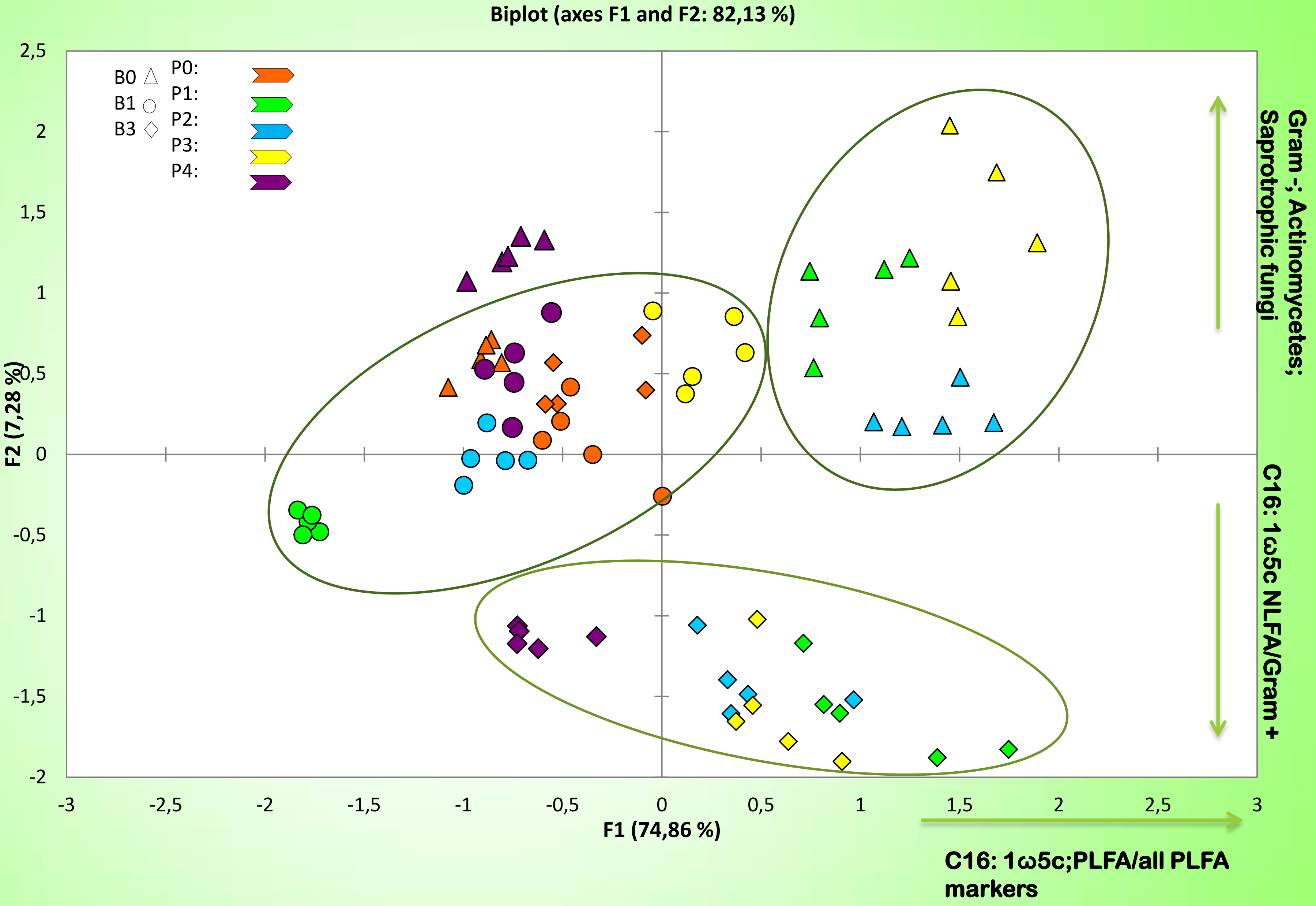
Compost H.
Compost B.
RP
TSP
Control

Chlorogenic acid,
Quinic acid,
Shikimic acid,
Raffinose

PCA score plot obtained by processing the GC-MS and NMR metabolic data
PC1;PC2 (68.44%)

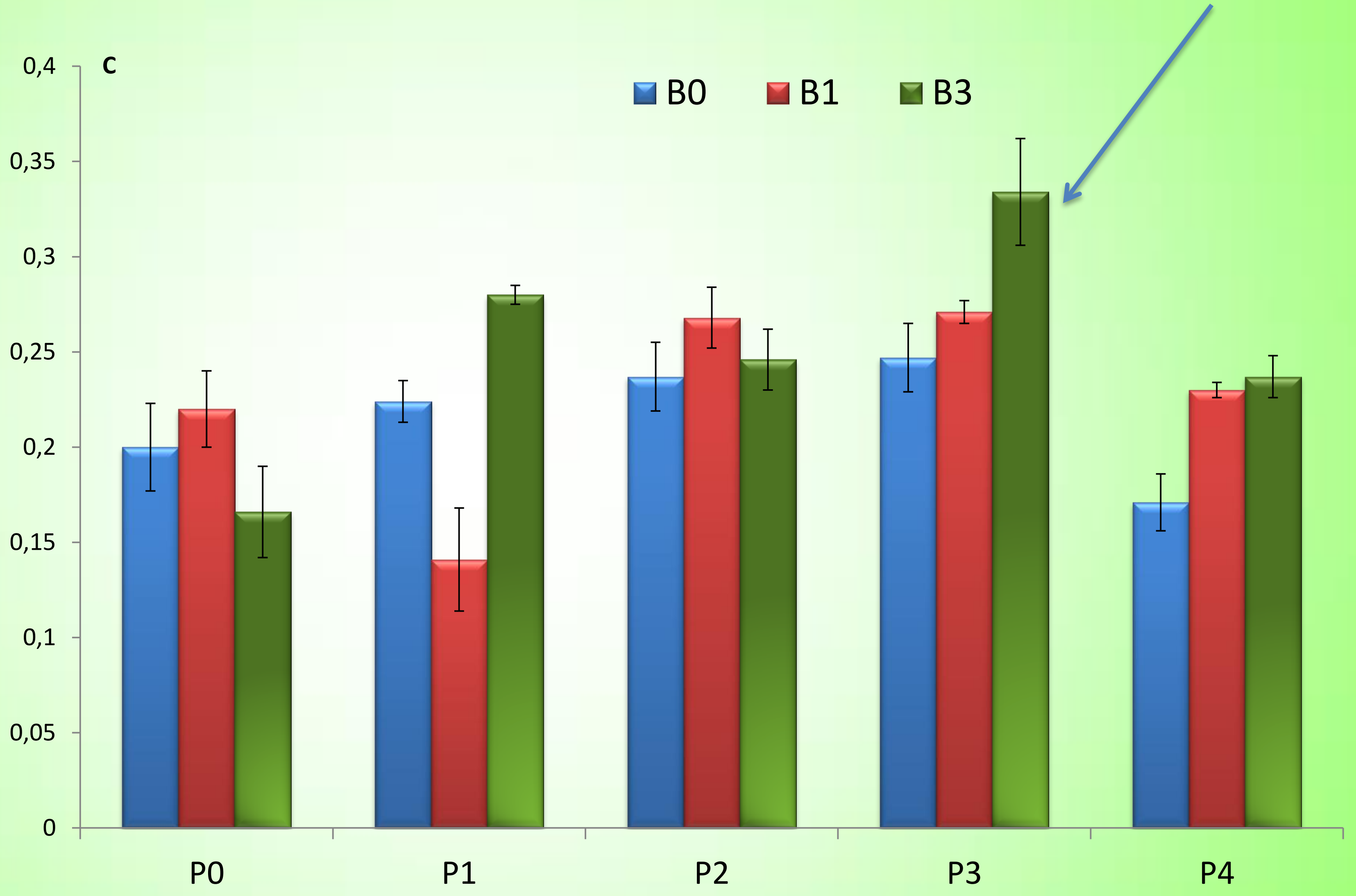

PC2
(16,01%)
PC1
(37,65%)
B0P0
B0P3
B0P4
B1P0
B1P3
B1P4
shikimic acid, tryptophan, quinic acid, phenylalanine, galactose, trehalose
adenosine derivatives, alanine, serine, threonine, aspartic acid, glutamic acid, glycerol
Glucose, fructose, sucrose

A

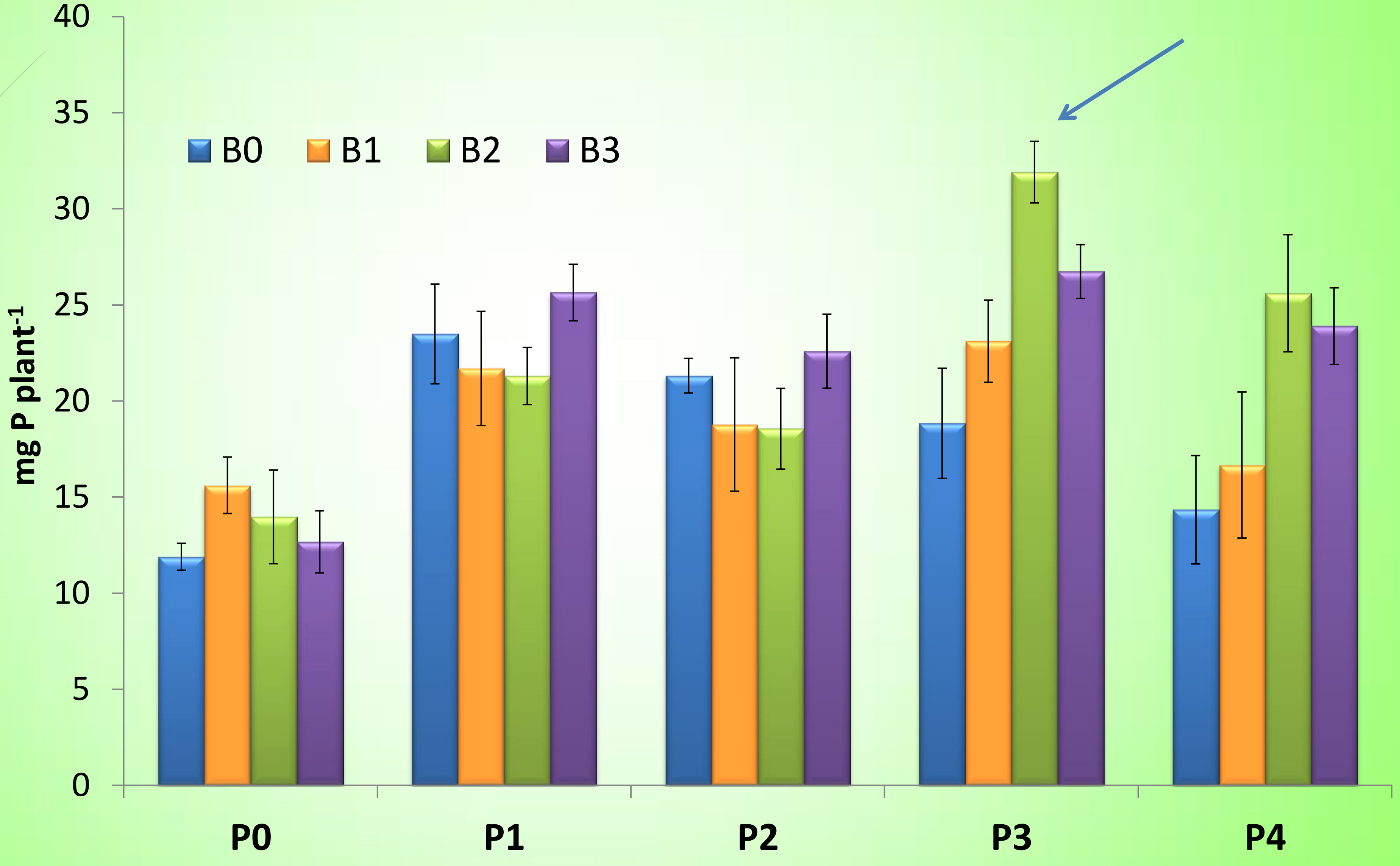


B0: not inoculatedB1: T.harzianum; B3: B. amyloliquefaciens

AMF/saprotrophic fungi .



P content in shoots



The composition of the endogenous or exogenous organic substance selects the microbial communities composition.
They, in turn, influence the cycling of nutrients in the soil and plants, affecting their productivity.
The quality and bioaccessibility of humic molecules present in the soil or in the exogenous organic substance could select the activity of the microorganisms in using the metabolic C required for their growth

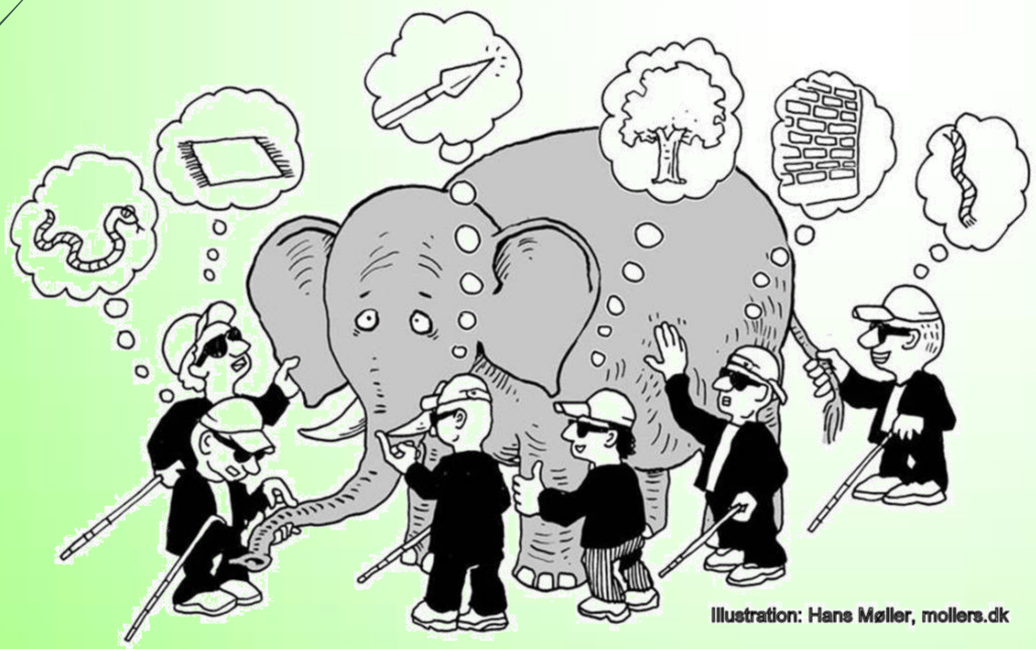
Another approach is needed

Yesterday

Reductionist approach to agricultural sciences

Understanding parts individually

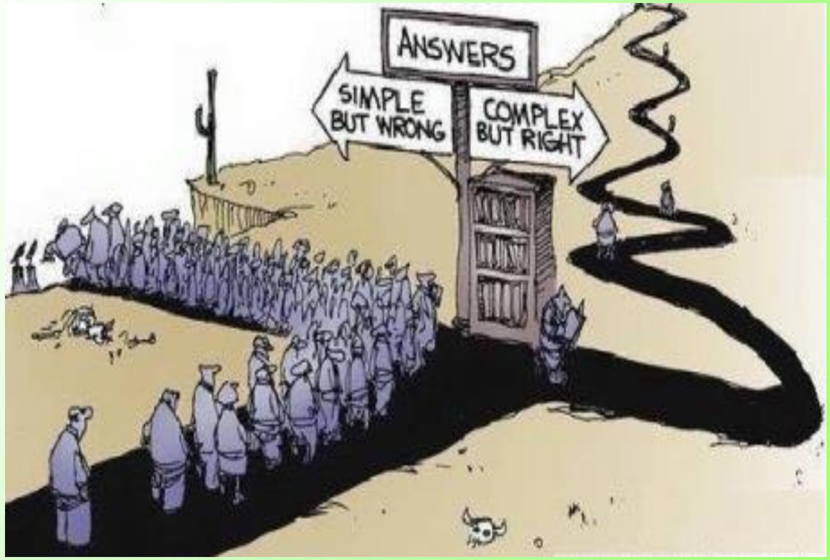
Reliance on partial knowledge-genetics or environmental factor, soil or plant, plant or microbe, microbe or organic matter



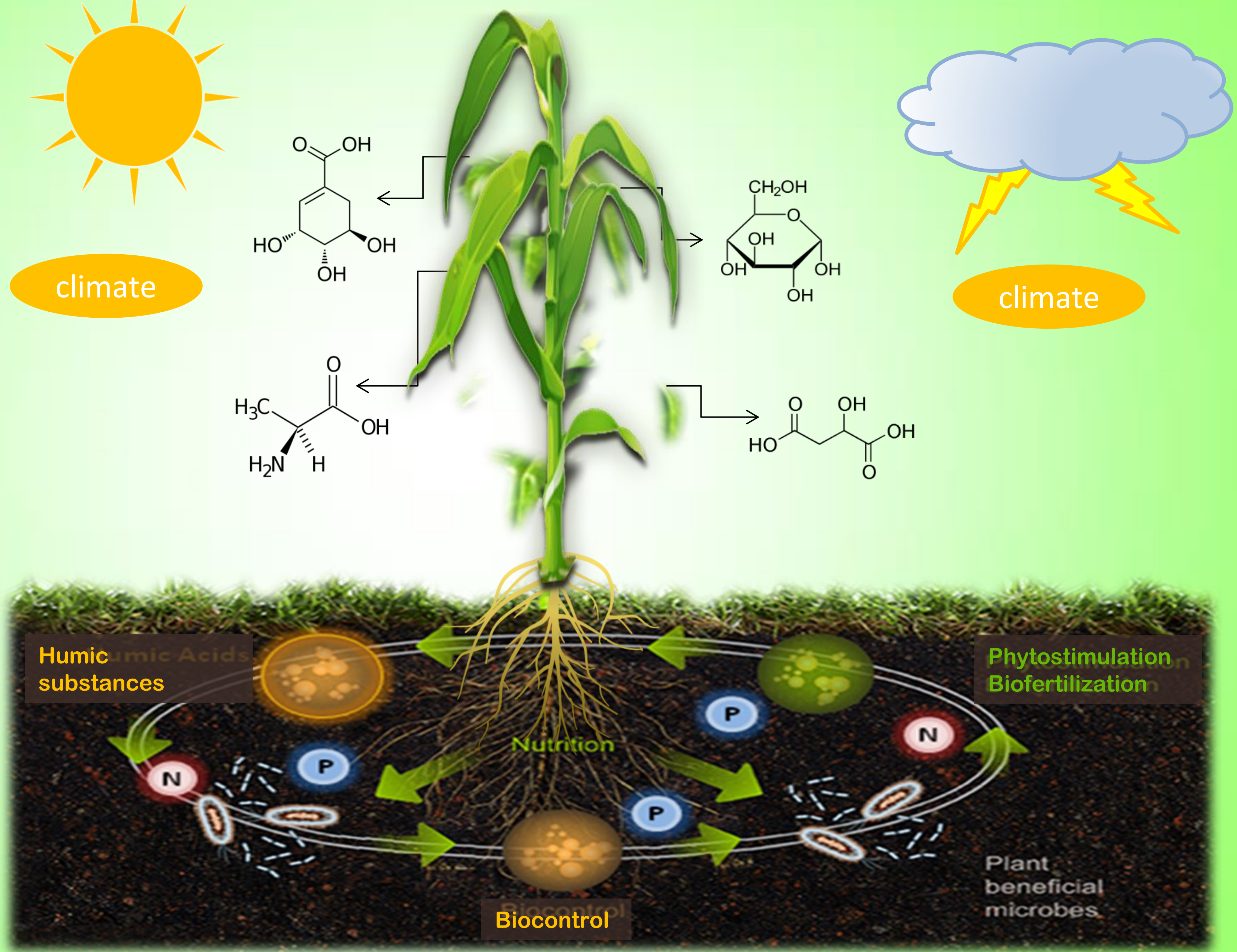
Today

Complex, non linear organization

Microbiohumeome



Micro-bio-umeoma



Challenges and expected outcomes

Expected outcomes….

- Achieve a sustainable crop productivity through a system level understanding of diverse interacting components,looking for benefits in the long term and not only during the current season
- Develop predictive indicators of soil and crop healthy
- Manage or enginereed“micro-bio-humeome that promote effective rehabilitation of degraded and depleted land worldwide

Expected outcomes….

- Increased resilience of our cropping systems to pest, pathogens, pollution, water and nutrient limitation

- A better understanding of the effects of the different management practices (fertilization, application of organic amendements, soil processing, type of crops, rotations) on the quality and quantity of soil organic matter


Thank you
For your attention