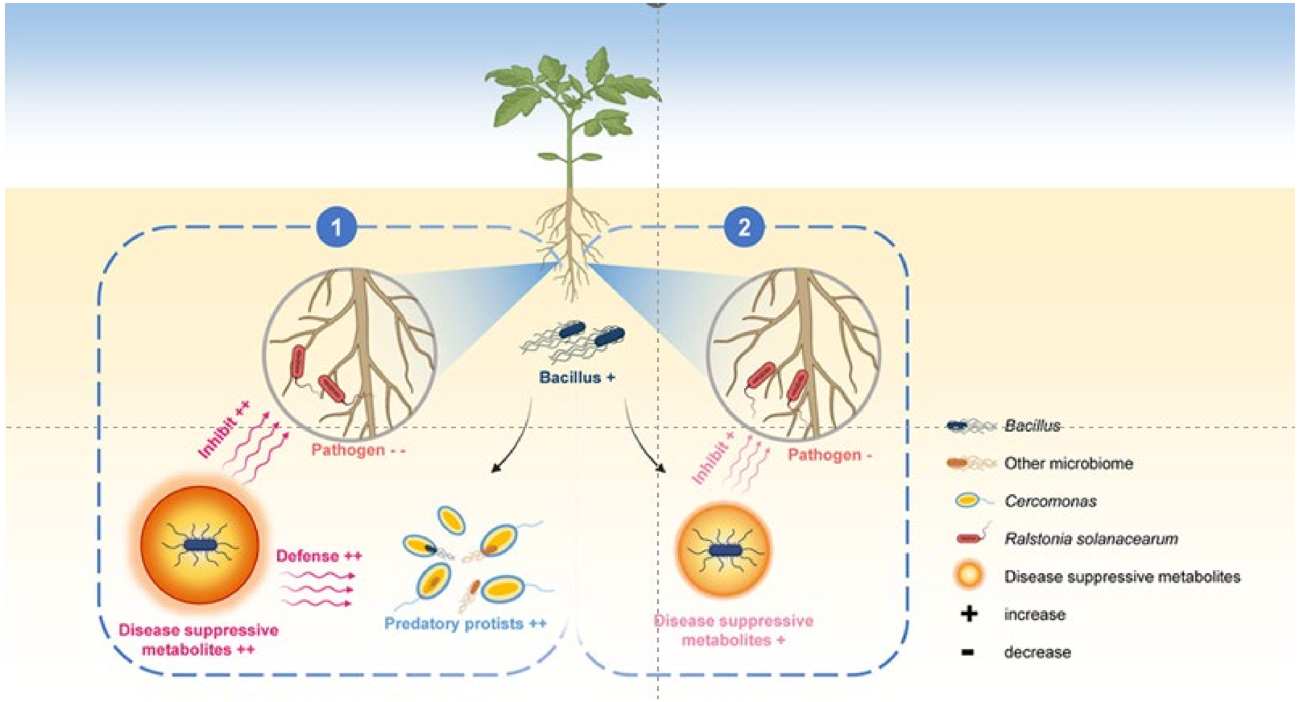
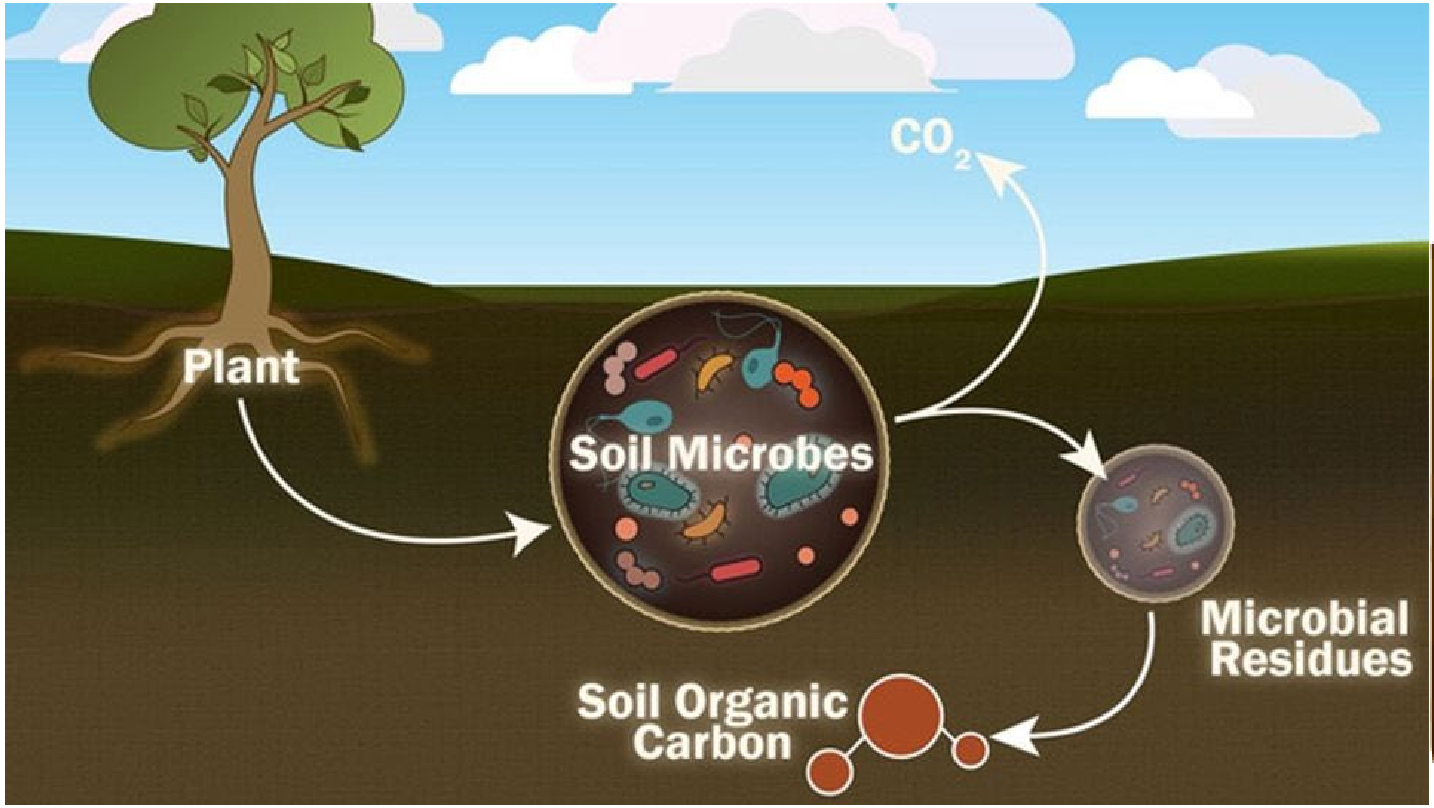
Soil resilience:

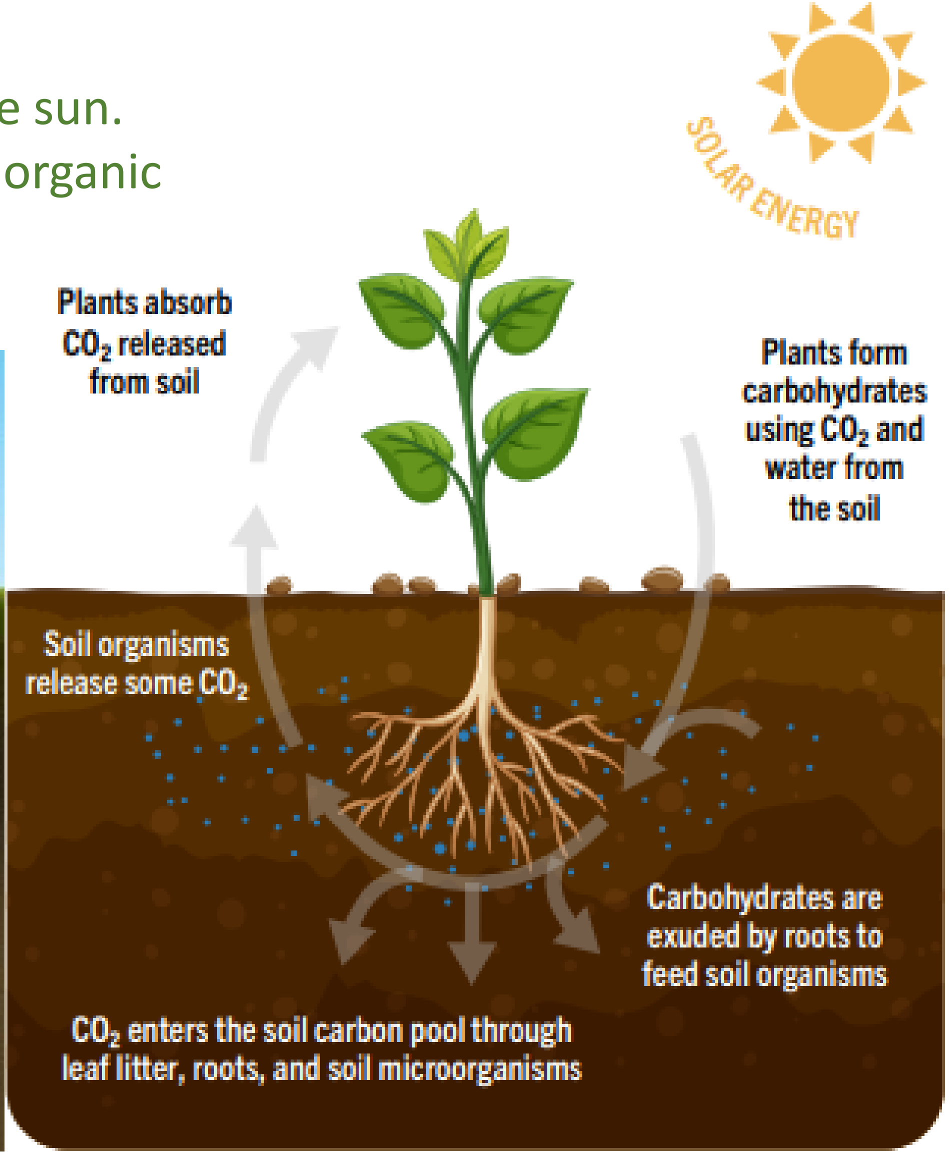
Amazing effect(ive)



Pier Oosterkamp
Managing director Ingredients at Eurostyle / ECOstyle

Introduction:
Plants are the intermediate between the soil and energy from the sun. Partly direct through sugars around the roots and partly through organic material like fallen leaves and wood that is stored in the soil.





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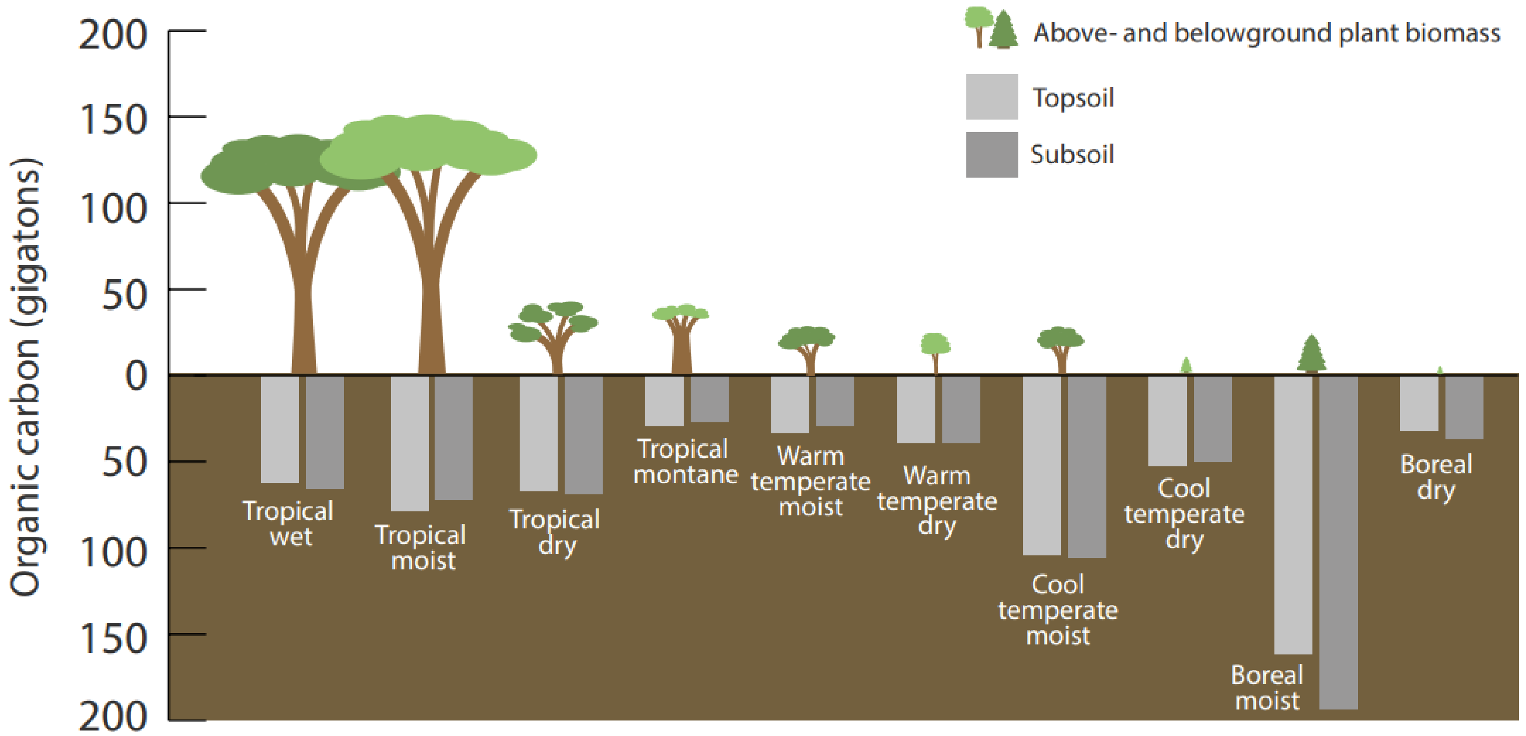
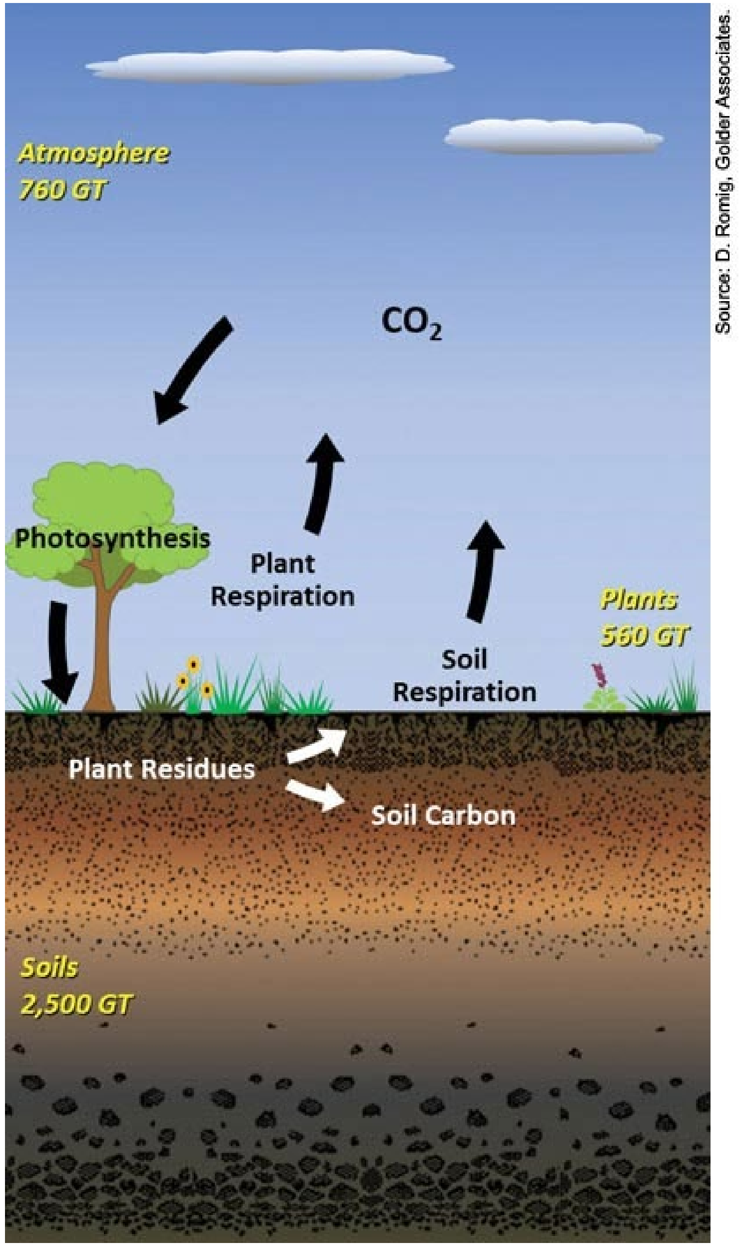
In soil there is more than twice as much carbon stored in biological vorm (organic carbon) than in the whole atmosphere and all life on the ground put together..
There also is MUCH more life in the soil than above ground.

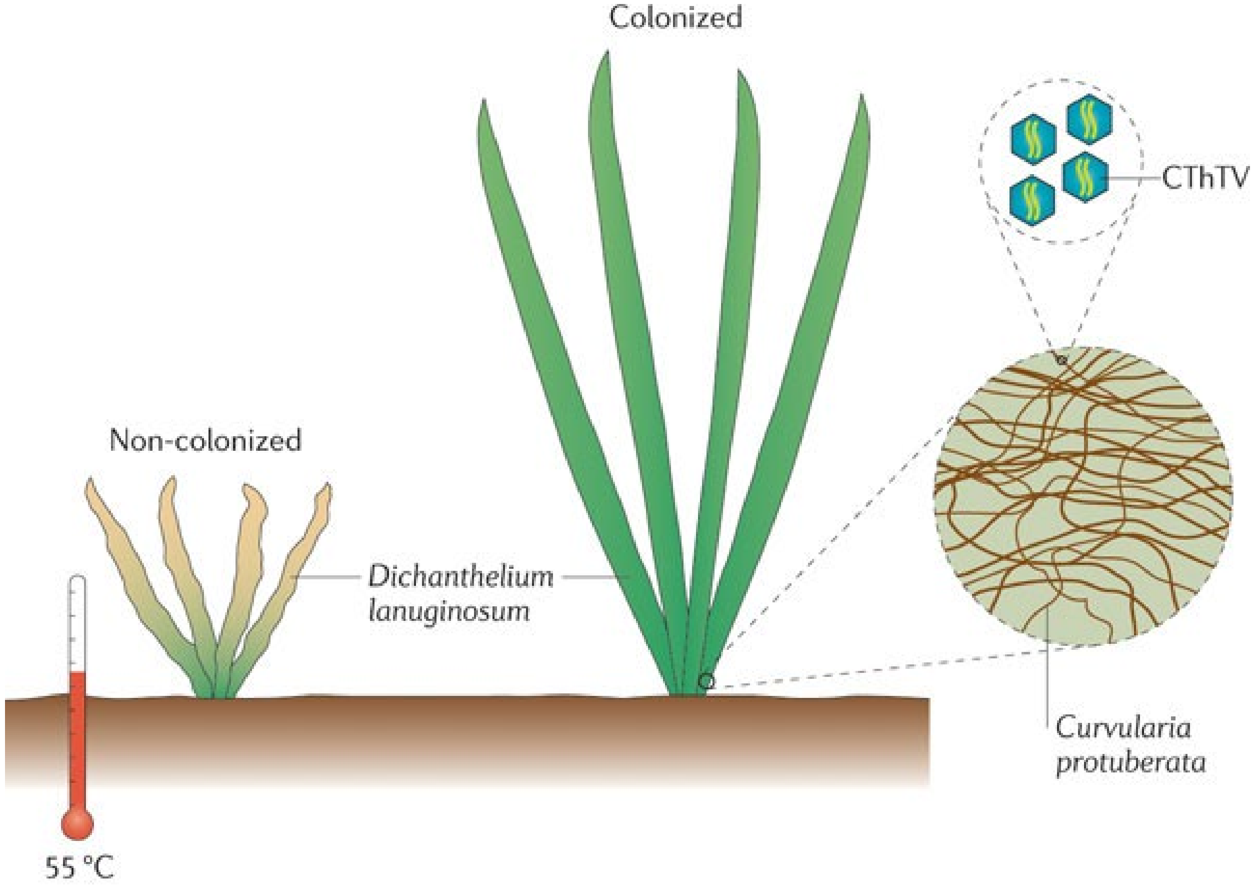




ECOstyle

Except plant nutrition, soil life also profides resistence to A-biotic stress.

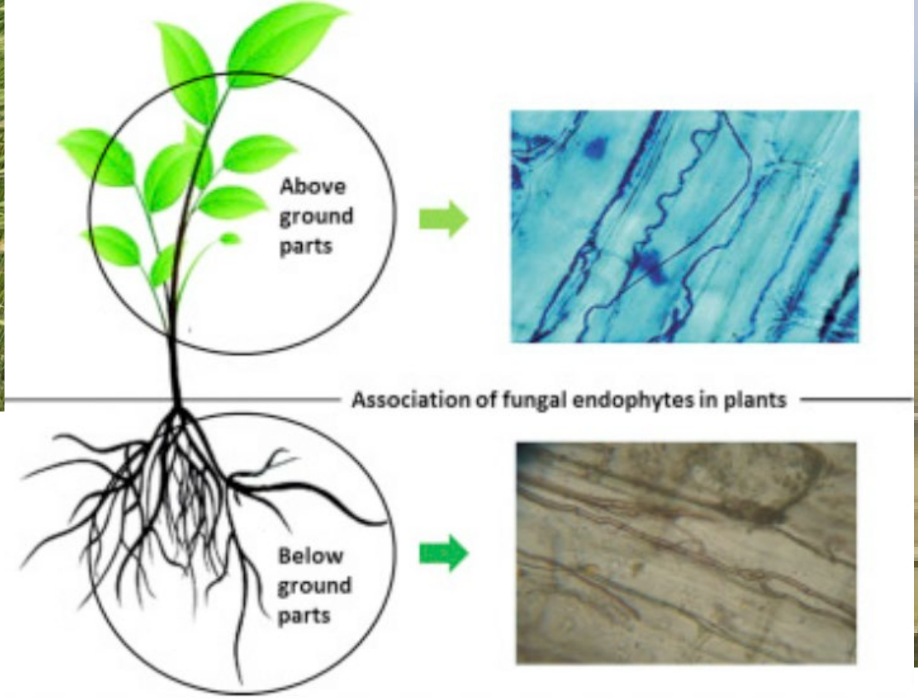
Aloe vera plant can only survive the desert heat thanks to a fungus AND a virus inside its cells.



Nature Reviews | Microbiology

Or, closer to home: (RUG Groningen research)
Both sides of the “wadden”dike the plant Festuca rubra can be found flourishing..
This is remarkeble since the soil on the left hand side is very salt (wadden sea) and the right side (arable seed potato land) is in sweet water land..

The plants proved to be identical the only difference is a fungus that lives in the plants in the salt region...



- I will dive deeper in the soil life bye highlighting 3 researches:

  - 1) Testing difference between good and bad arable soil in Friesland.

  - 2) Research
    - potatoMETAbiome: Plant –soil life synergy

Harnessing microbiome-plant synergies: Microbiome-interactive traits enhance plant growth and support sustainable agriculture

Tianci Zhao, Xiu Jia, Xipeng Liu, Jyotsna Nepal, Rémy Guyoneaud, Krzysztof Treder, Anna Pawłowska, Dorota Michałowska, Gabriele Berg, Franz Stocker, Tomislav Cernava, J. Theo M. Elzenga, Eléonore Attard, Joana Falcão Salles*

*Corresponding author for this work

  - 3) Research
    - Indirect effect on plant health through soil life interactions

Predatory protists impact plant performance by promoting plant growth-promoting rhizobacterial consortia

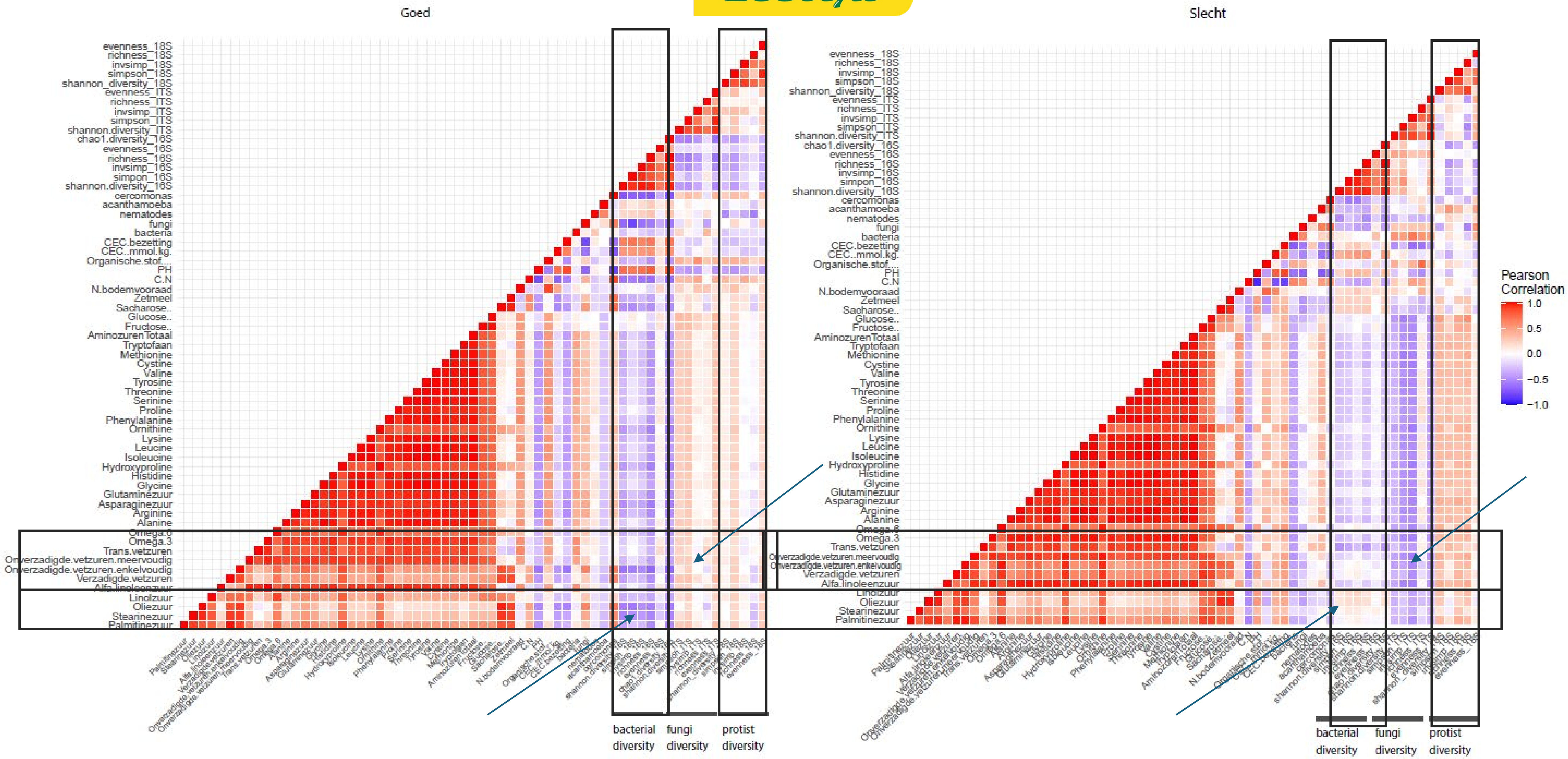
Sai Guo, Stefan Geisen, Yani Mo, Xinyue Yan, Ruoling Huang, Hongjun Liu, Zhilei Gao, Chengyuan Tao, Xuhui Deng, Wu Xiong, Qirong Shen, George A Kowalchuk, Rong Li

  - 4) Market approach: effectiveness / claims

- 1) Good and bad soil: 3 tests at two locations:
  - 1) Standard Eurofins soil analyses:
    - N-Soil
    - C/N ratio
    - pH
    - Organic matter
    - CEC of the soil and the
    - CEC saturation

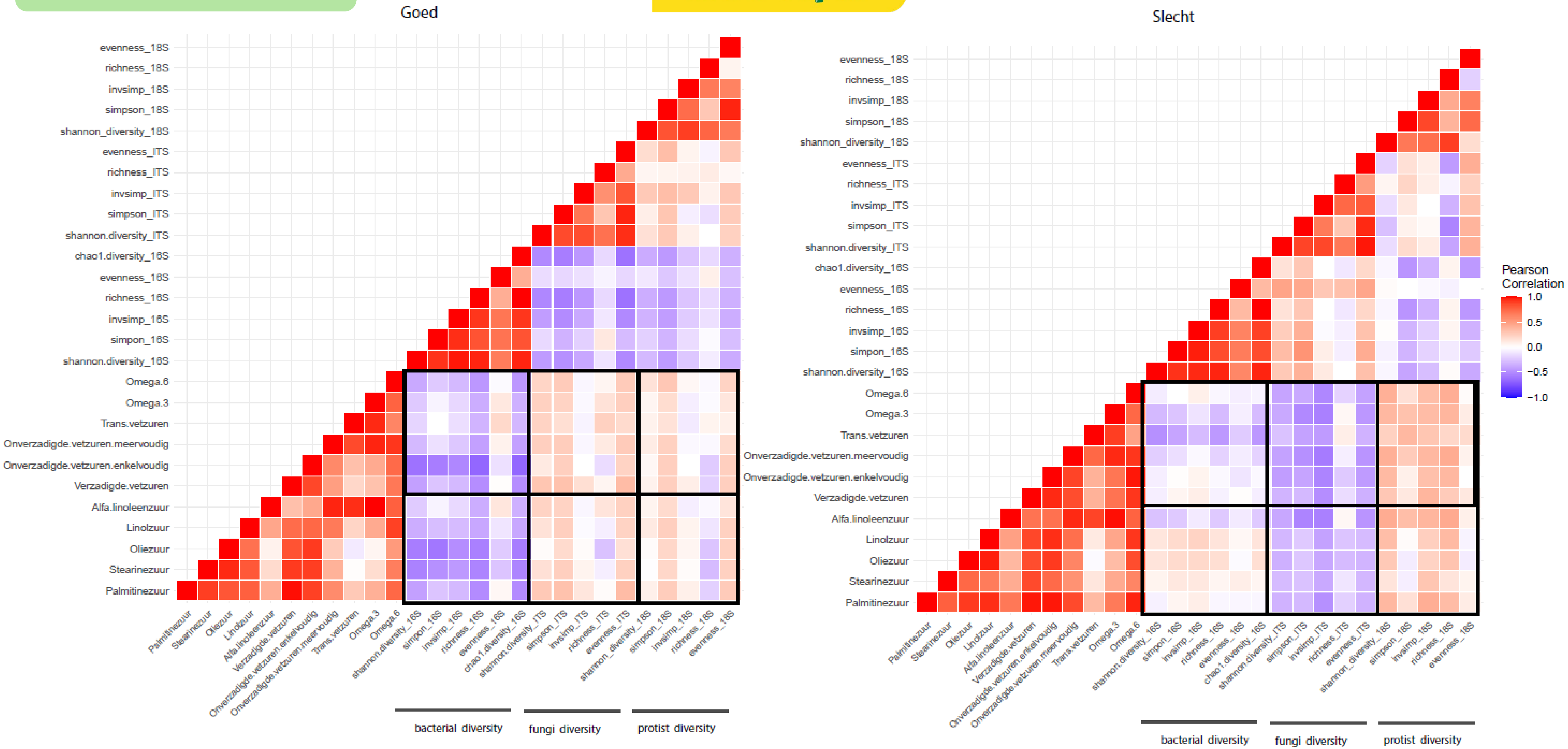
  - 2) Soil microbiology:
    - Number of bacteria and the diversity
    - Number of fungi and the diversity
    - Number of protozoa and the diversity

  - 3) Later in the season : plant analyses (organic componants, biological building blocks.)
    - Fatty acids
    - Sugars / starch
    - Amino acids



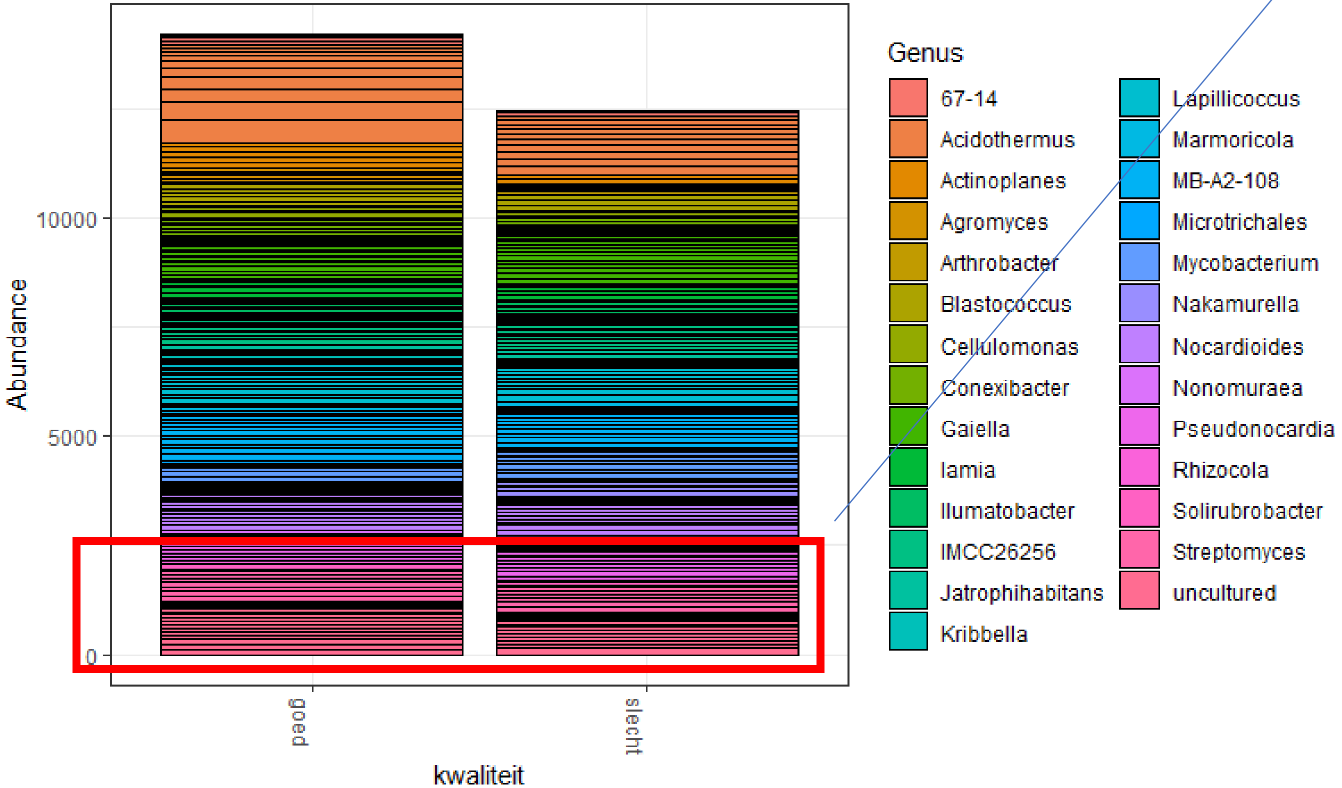
One of the results: The correlation between the parameters.

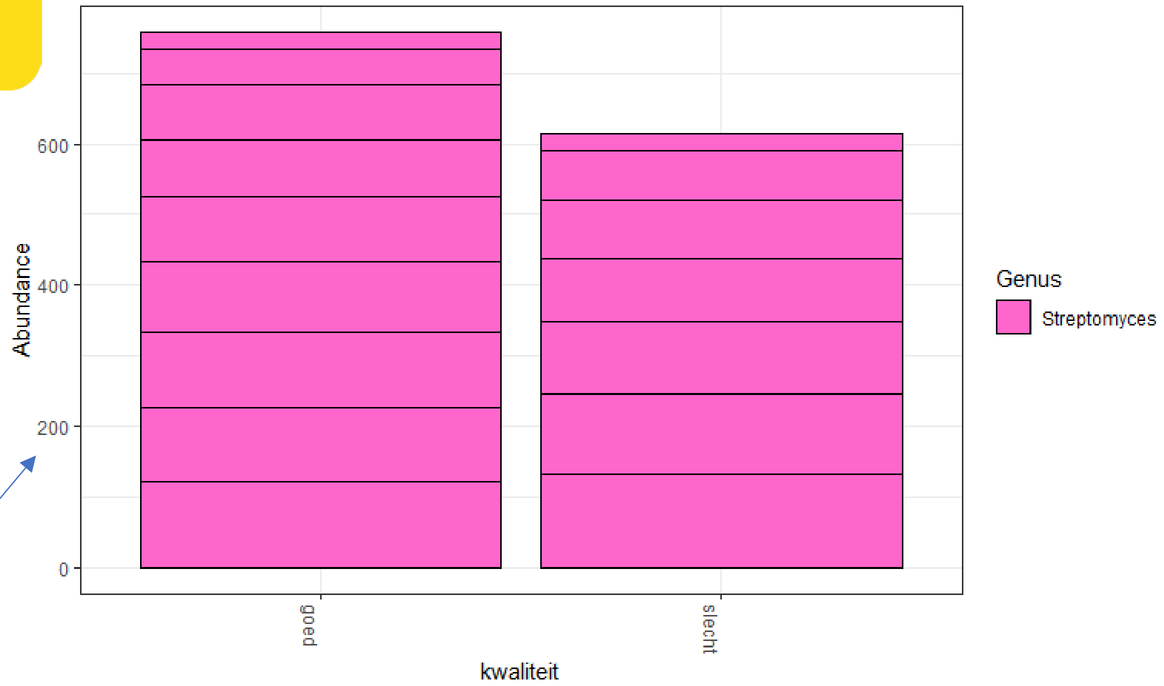
Correlation_fatty acids



Other result:
Marker species?

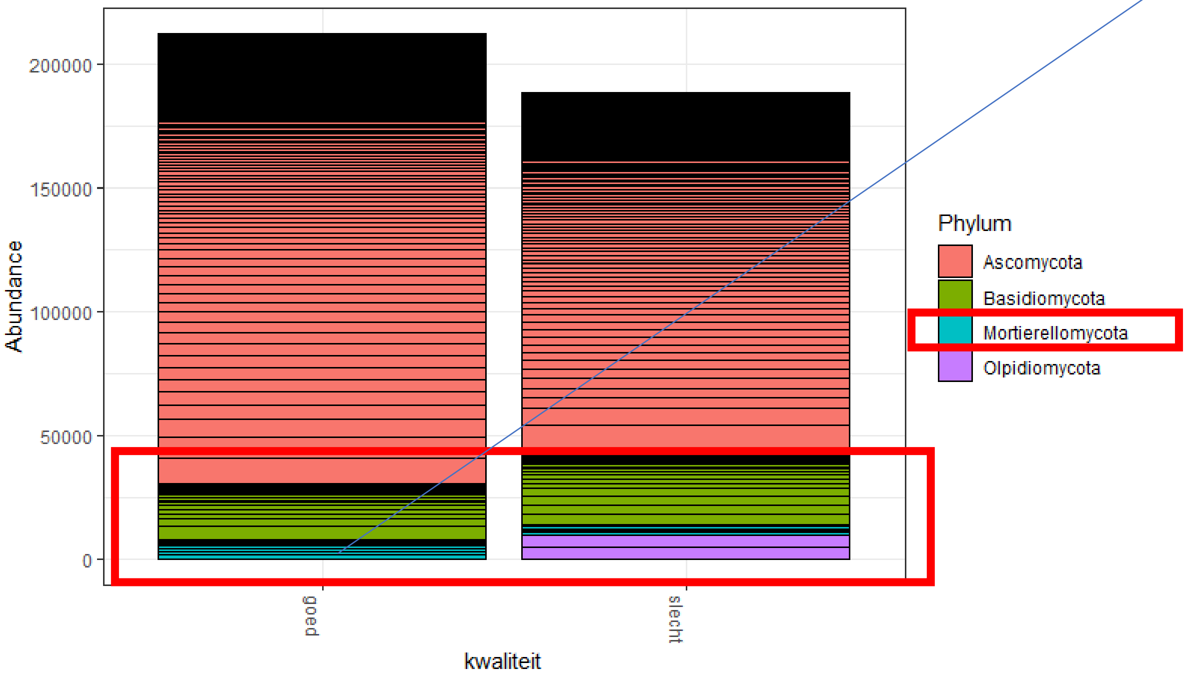
Bacterie genus
Streptomyces

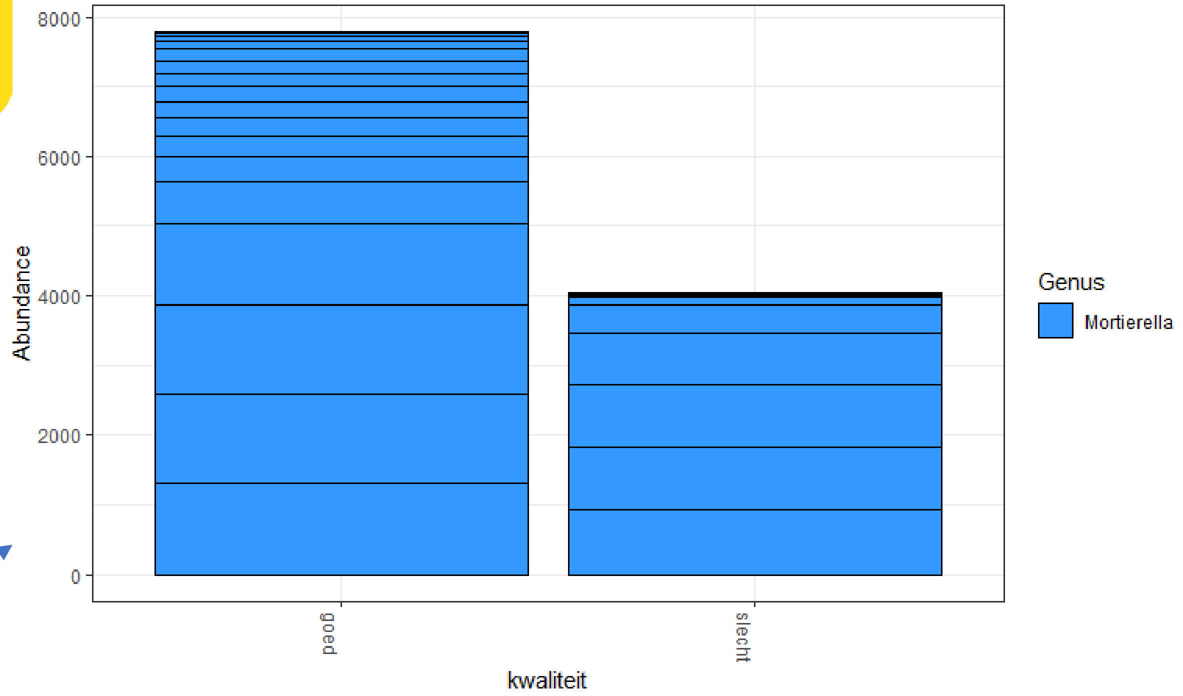




- Streptomyces are more abundant in the good soil. In all tested years! (2020-2023) .
- They are bacteria that feed predominantly on dead plant material (smell of wet forest soil is caused by Streptomyces.) They are known for the production of antibiotics (*streptomycene).* Thus for soil resiliance. And in addition to that for releasing minerals from the soilcomplex and making this available to plants..

Fungus genus Mortierella





- In all years (2020 – 2023) a clear difference between good and bad soil. Good soil had more Mortierella fungi. WIKIPEDIA:
- The species *Mortierella alpina* is used for the production of multi-unsaturared fatty acid through fermentation. The oil produced contains over 40% arachidonacid (= Omega 6 fatty acid). This oil is suitable for human consumption and is used as food additive in farmaceutica..

Streptomyces showed high heavy metal resistance and mineralization properties to form crystallized P–containing switzerite ($Mn_3(PO_4)_2•7H_2O$) and hydrated nickel hydrogen phosphate [125]. Therefore, PSM-induced Pi mineral dis solution can both supply bioavailable orthophosphate to plants and accelerate metal precipitation to form secondary P-containing minerals in soil

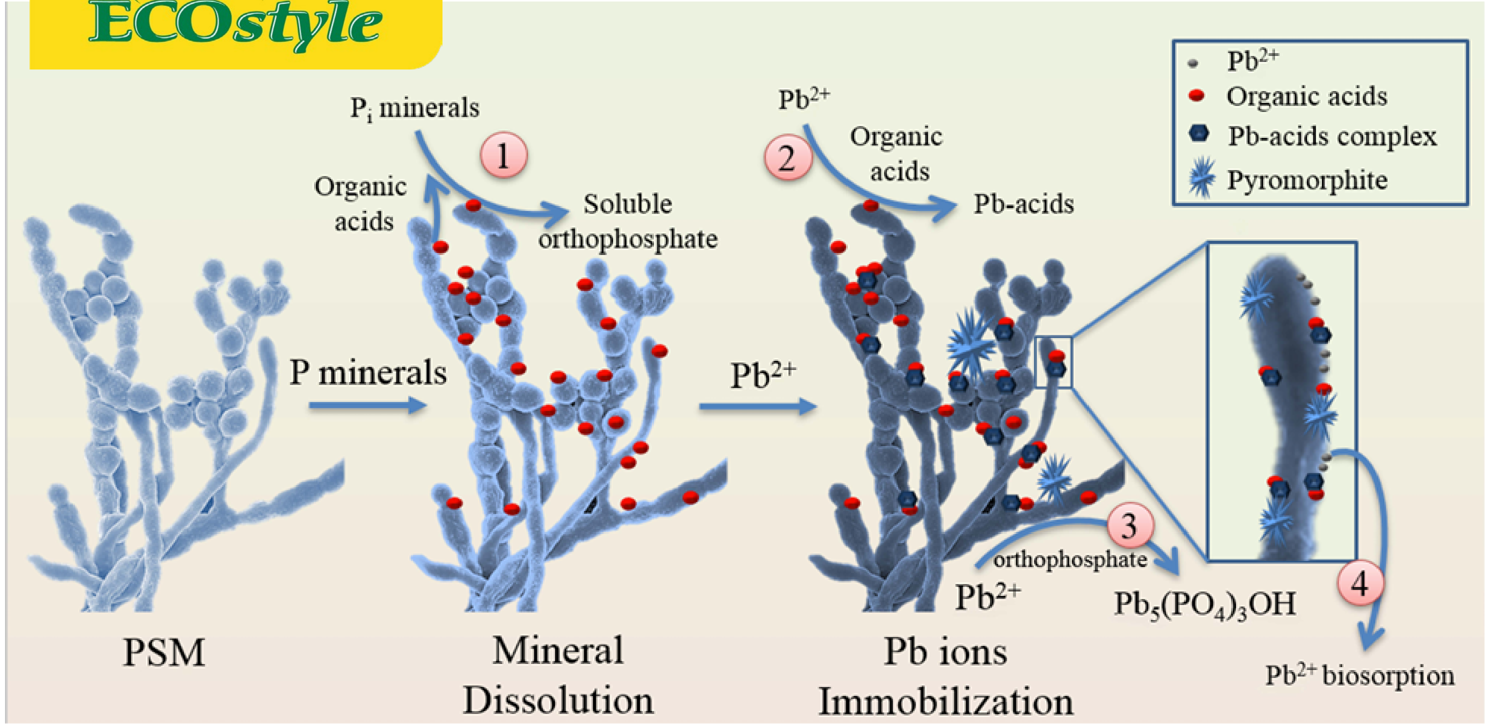


Figure 3. Simplified representation of phosphate solubilizing microorganisms (PSM)-induced dissolution to accelerate lead (Pb) precipitation to form secondary inorganic P (P_i) minerals. ① PSM can dissolve Pi minerals to soluble orthophosphate by releasing organic acids. ② Pb ions in the solution are precipitated to Pb–acid complexes by reacting with organic acids that are released by PSM. ③ In the presence of orthophosphate ($H_2PO_4^-$, HPO_4^{2-}), Pb ions are precipitated to the relatively stable pyromorphite. ④ Pb ions in the solution are biosorbed to PSM surface due to the negative charges of the surface functional groups.

As the capacity of PSM to desorb P depends on the Pi adsorptioncapacity, surface areas of clayminerals, and the saturation of Pi-absorbing sites,the effectiveness of Mortiella sp. To desorb P is ranked as montmorillonite >kaolinite>goethite>allophane[90]. Furthermore, inoculation with Mortiella sp. can significantly increase in situ soil Pi solution through desorption.

Streptomyces sp. and Mortierella sp. combined

- Tian et al. 2021 "*Roles of Phosphate Solubilizing Microorganisms from Managing Soil Phosphorus Deficiency to Mediating Biogeochemical P Cycle*"
- From this article: de Streptomyces sp. en Mortierella sp. Both are PSM, *phosphate solubilizing microorganisms*. They free phosphates from organic material (manure, plant rest material etc.) with the help of other acids and enzymes they convert this to a form that can be taken up by the plants.
- Streptomyces sp. is apart from that also capable of getting heavy metals from the soil and linking these to soil particles while freeing the phosphates in the same process.
- Mortierella sp. Can free phosphates from clay particles and make them available to the crop.
- **Streptomyces and Mortierella are very valuable soil organisms.**

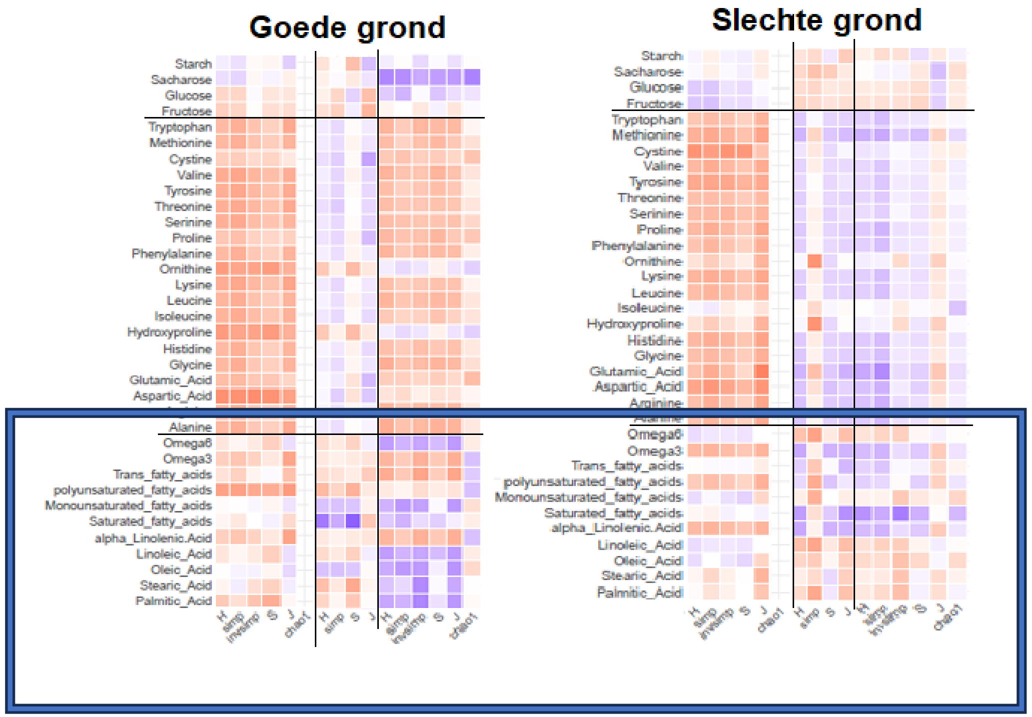
ECOstyle

Fatty acids and plant health..

Dale Walters, Lynda Raynor, Anne Mitchell, Robin Walker & Kerr Walker

Abstract

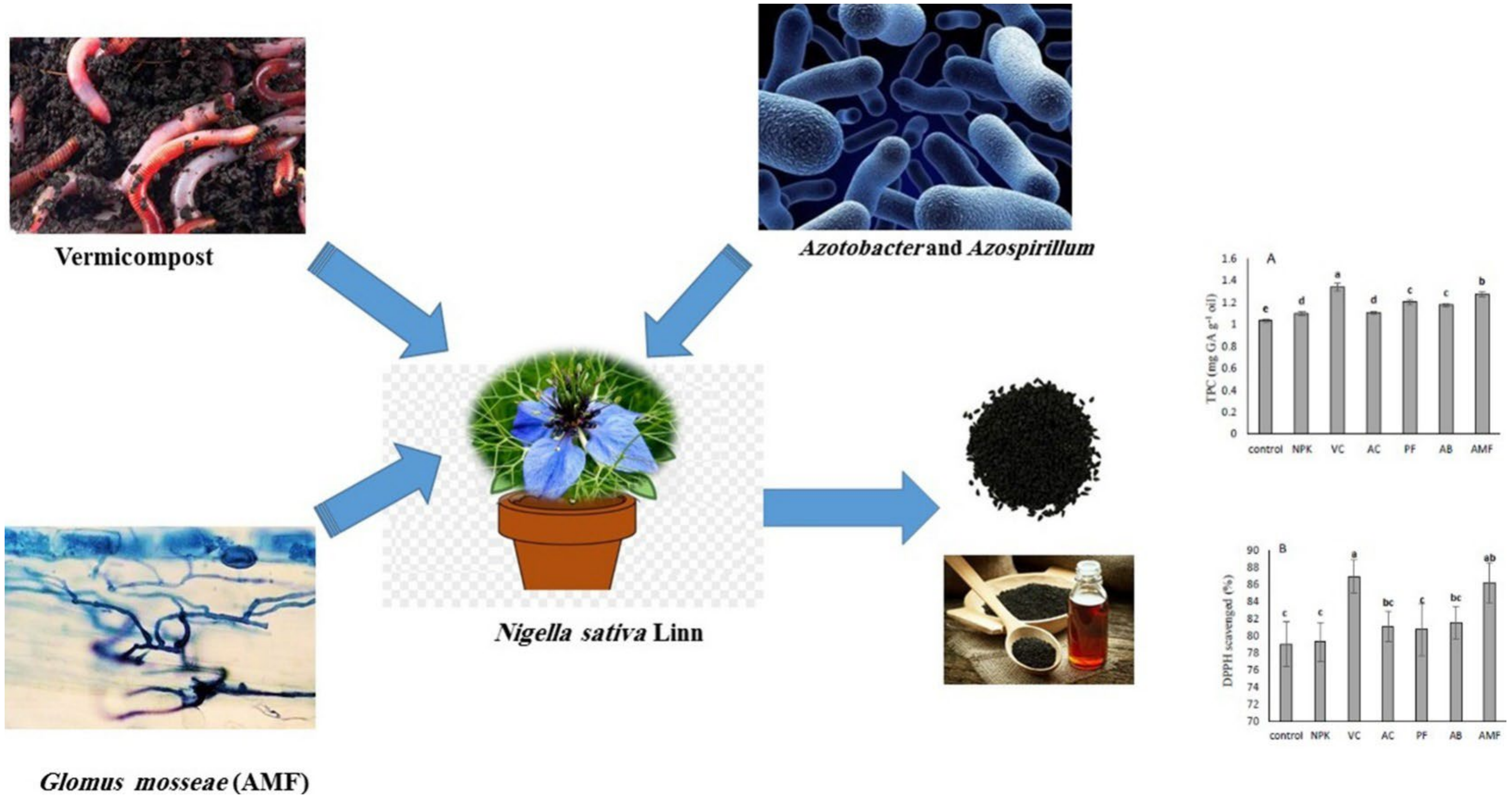
The effect of the fatty acids linolenic acid, linoleic acid, erucic acid and oleic acid on the growth of the plant pathogenic fungi *Rhizoctonia solani*, *Pythium ultimum*, *Pyrenophora avenae* and *Crinipellis perniciosa* were examined in *in vitro* studies. Linolenic and linoleic acids exhibited activity against all of the fungi. However, whereas linolenic acid reduced mycelial growth of *R. solani* and *C. perniciosa* at 100μM, the concentration had to be increased to 1000 μM before any effect on mycelial growth of *P. ultimum* and *P. avenae* was observed. Linoleic acid only reduced mycelial growth of *R. solani*, *P. ultimum* and *P. avenae* at 1000 μM, but led to a significant reduction in growth of *C. perniciosa* at 100 μM. In contrast, oleic acid had no significant effect on growth of *R. solani* or *P. avenae*, but gave significant reductions in mycelial growth of *P. ultimum* at 100μM and reduced growth of *C. perniciosa* significantly at 1000 μM. All of the fatty acids reduced biomass production by all of the fungi significantly in liquid culture when added to the media at 100 μM. Erucic acid had no effect on fungal growth at any concentration examined. The antifungal activities exhibited by linolenic, linoleic and oleic acids may be useful in the search for alternative approaches to controlling important plant pathogens, such as those examined in this study.



ECOstyle

Physiological and biochemical responses of black cumin to vermicompost and plant biostimulants: Arbuscular mycorrhizal and plant growth-promoting rhizobacteria

Seyyed Ali Sadegh Sadat Darakeh [a], Weria Weisany [a], Nawroz Abdul-Razzak Tahir [b], Peer M. Schenk [c]



This research shows that the use of different fertilizers creates a difference in fatty acid content in this plant oil..!
In short:

Fertilized with articicial fertilizer the plant produces more saturated fatty acids.
If the plant is fertilized with Mycorrhiza fungi the plant produces more (mono) unsaturated fatty acids.

And if the plant is fertilized with compost the oil had the highest amount of multi-unsaturated fatty acids.

Appropriate soil conditions as a cultural practice are the basis of medicinal plant production. The experiment was carried out to investigate the effect of NPK, vermicompost (VC), *Pseudomonas fluorescens* (PF), *Azotobacter chroococcum* (AC), *Azospirillum brasilense* (AB), *Glomus mosseae* (AMF) on plant weight, antioxidant capacity and fatty acid (FA) profile of seed oil, and leaf nutrients of black cumin (*Nigella sativa* Linn) in 2018 and 2019. The results showed that dry weight in plants treated with VC (24.9 g and 28.8 g) or in AMF-inoculated plants (23.4 g and 27.8 g) was significantly higher compared with other treatments in 2018 and 2019, respectively. The highest (1.34 mg gallic acid (GA) g^{-1} oil) total phenolic content (TPC) and DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging activity (86.8 %) was observed in plants treated with VC, and followed by AMF. The main fatty acid component was linoleic acid (51.92–55.46 %), followed by oleic acid (22.6–24.5 %) and palmitic acid (13.24–16.27 %). The saturated fatty acid (SFA) under NPK application (23.39 % and 24.21 % in 2018 and 2019) was higher than other experimental treatments. Monounsaturated fatty acid (MUFA) concentration in plants inoculated with AMF (25.15 % and 25.41 % in 2018 and 2019, respectively) or supplied with AB and PF was greater. The seed oil of black cumin contained the significant level of polyunsaturated fatty acid (PUFA), which its highest content was obtained at in plants treated with VC as 55.74 % and 55.79 % in 2018 and 2019, respectively. Sustainable agricultural practices are required to increase ecofriendly performance, by using soil micro-organisms, conserving the natural resource base for improved medicinal plant cultivation.

Microorganisms selected for function

(S18 analysis = eukaryots in the soil.) (predominantly fungi and protozoa) (results are significant).

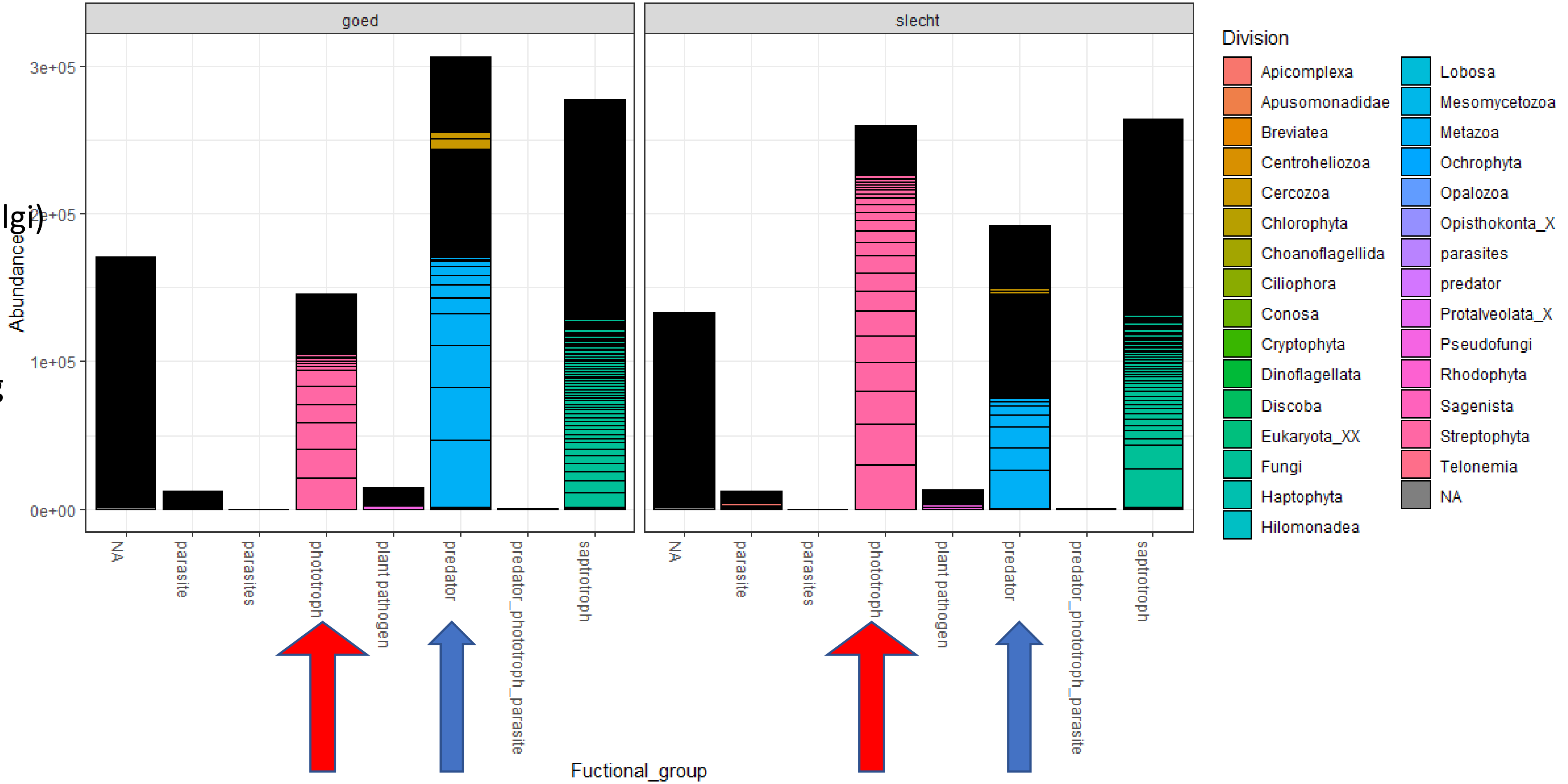
Parasites

Phototroph = Fotosynthesis (algi)

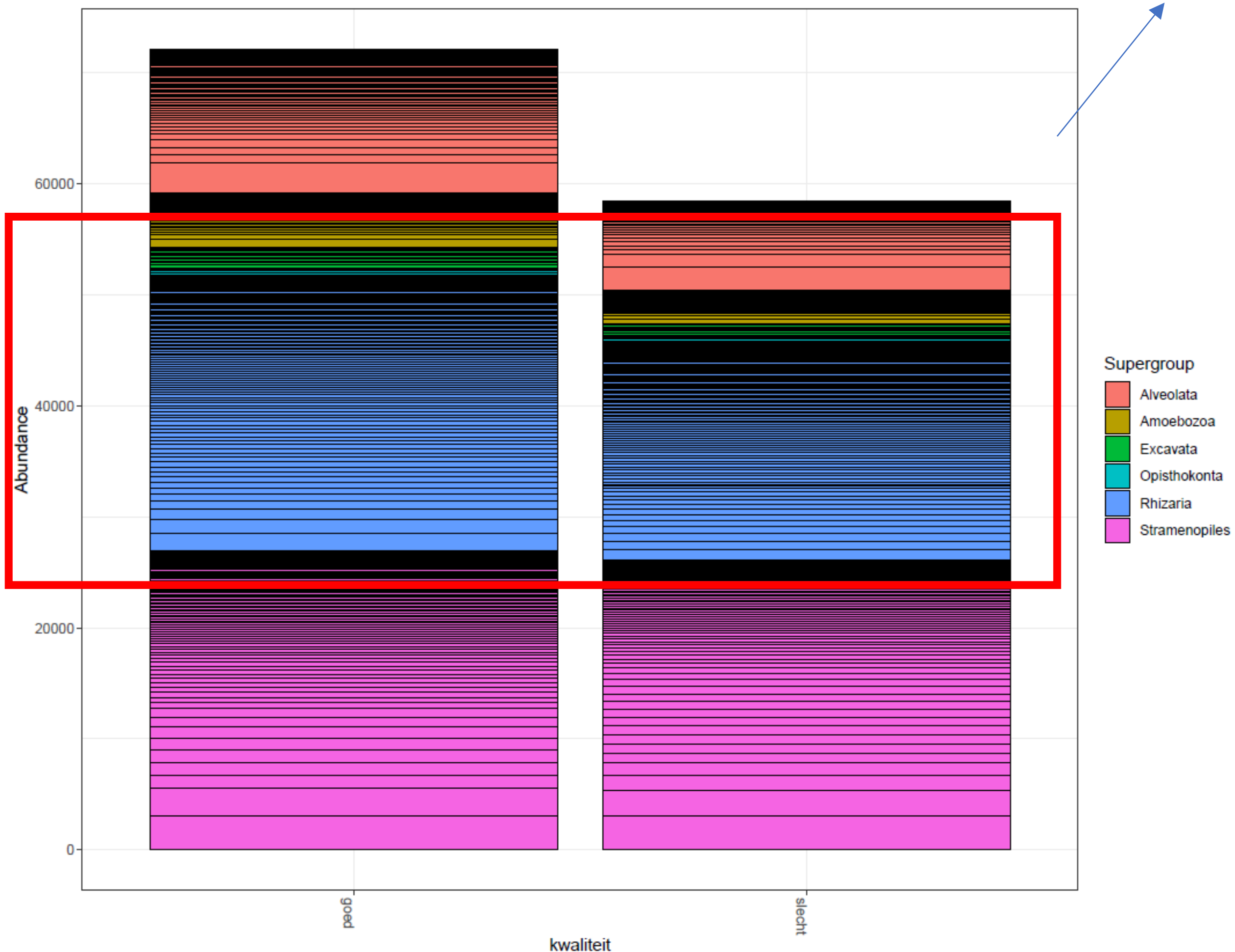
Plantdisease

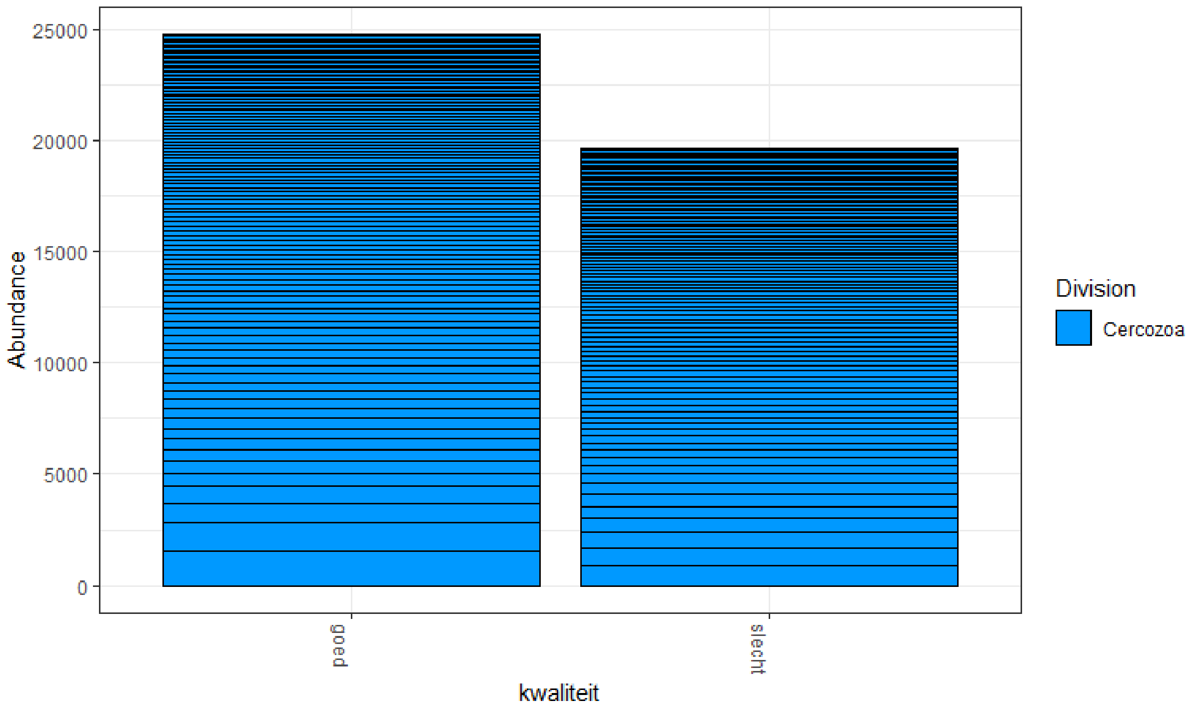
Predator = Phagotroph eating other microbers.

Saprotroph = eten houtstof



Protozoa divisie
Cercozoa

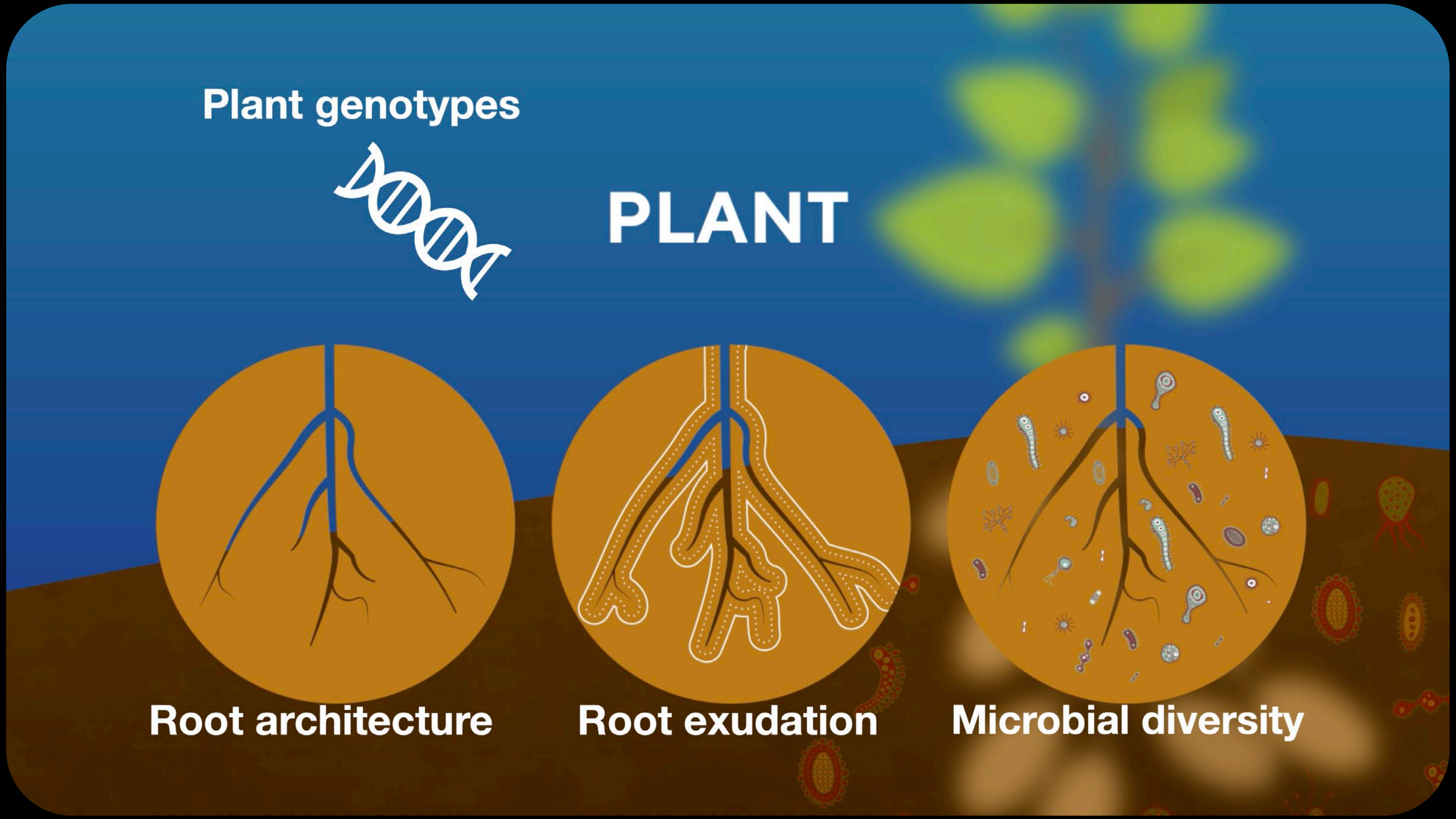




- In all years (2020-2023) there are considerable more Cercozoa in good soil.
- Protozoa are less studied compared to bacteria and fungi.....
- But more and more the role of the phagotrophe species seems to be quite interesting...


ECOstyle
Cercomonas lenta ECO P-01
00:00,00

2) Research PotatoMETAbiome: Consortium led by Prof. Joana Falcao Salles (RUG)



Identification of **plant genetic markers** associated with **high microbial interactive traits (MIT)**

↓

Future breeding strategies

Field trial set up

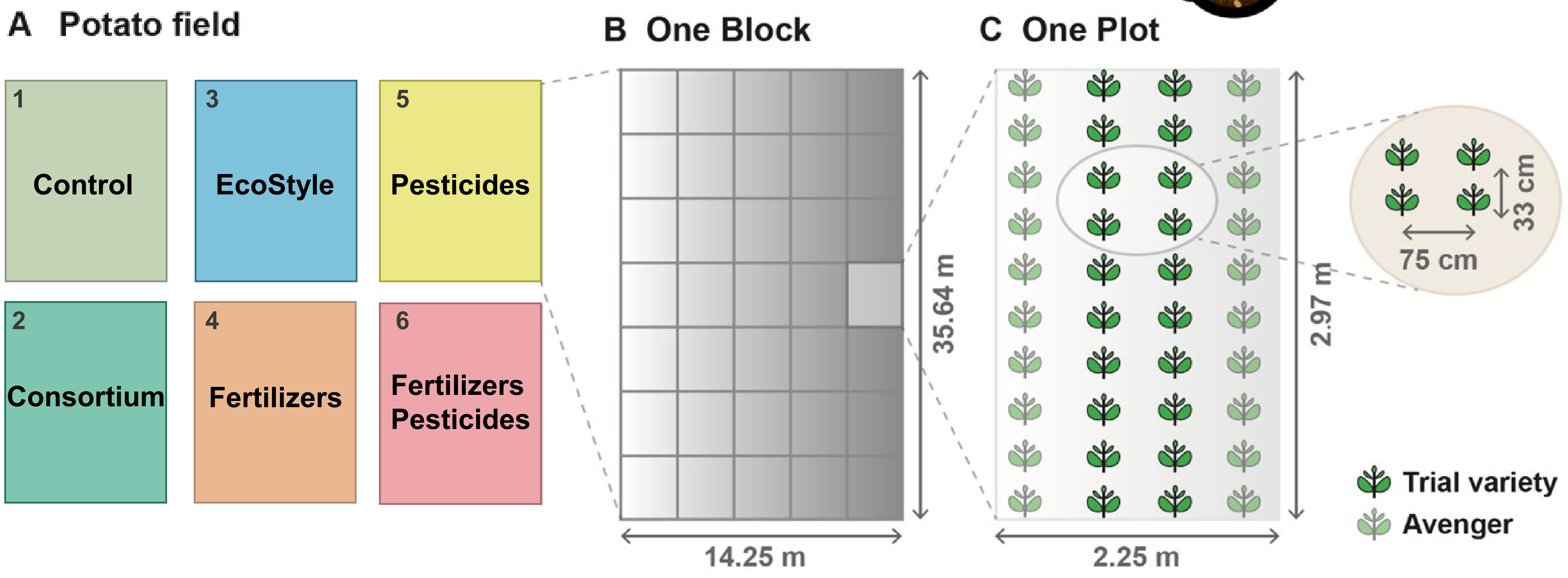


Fig.1 Schematic plot represented the field trial setup. (A) The distribution of six treatments in the field trial. (B) In each treatment/block, 3 replicates/plots of 11 potato varieties are distributed in 35 plots (5 × 7). Potato variety Avenger is used to fill up blocks in which the plots were not distributed the trial varieties. (C) In each plot, 40 plant individuals (10 × 4) are planted, in which 20 individuals in the middle two columns will be the trial varieties.

potatoMETAbiome

Rhizosphere soil microbial community composition

5th week

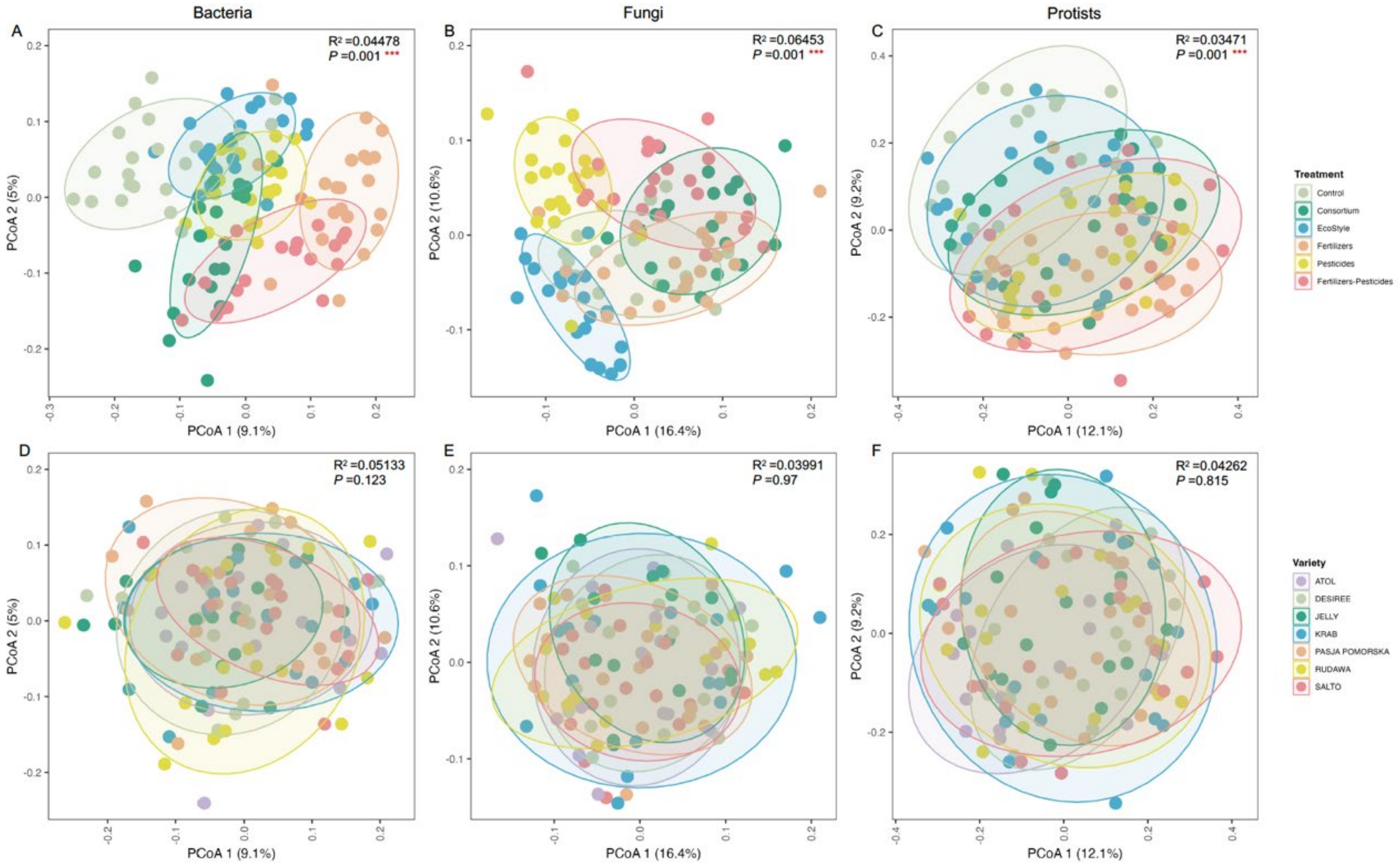
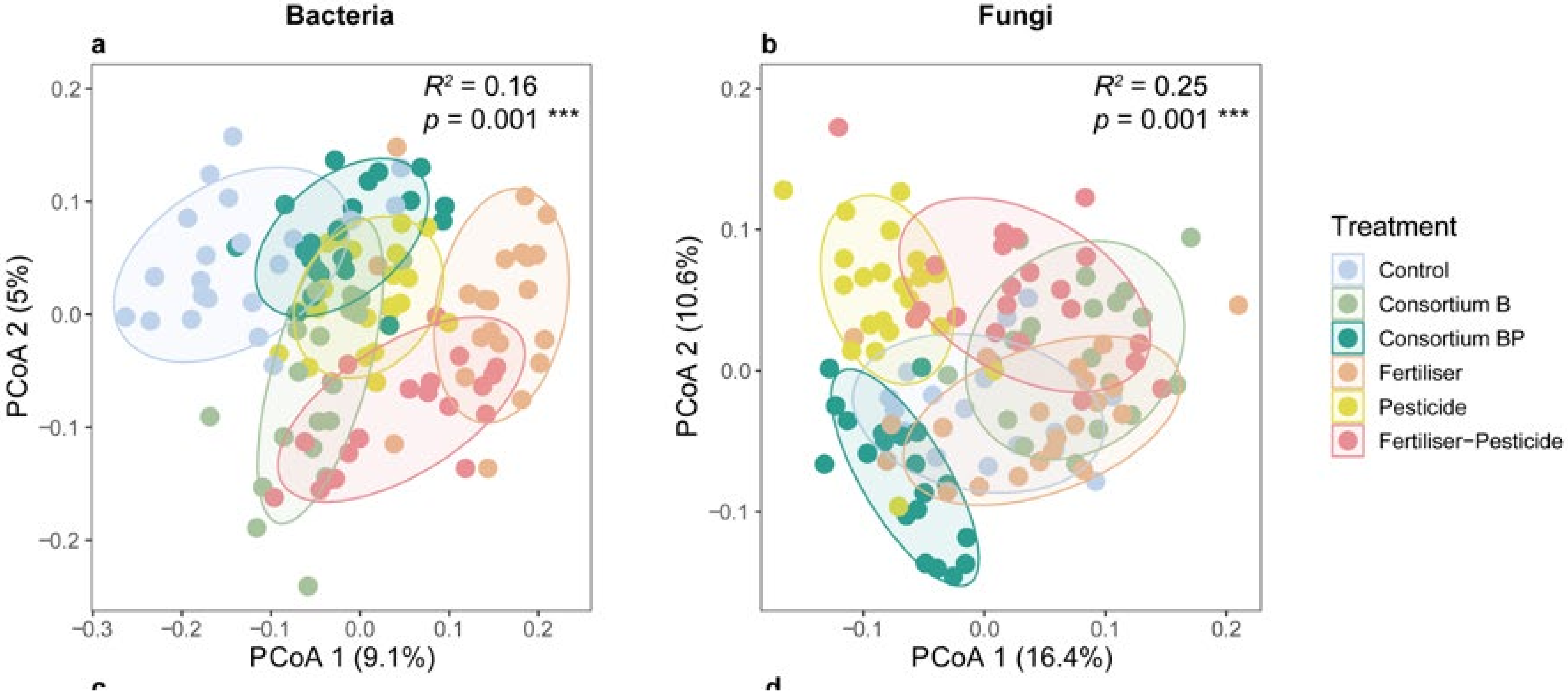


Fig.3 The effect of treatment and variety on the composition of rhizosphere soil microbial communities, displayed for bacteria, fungi and protists .

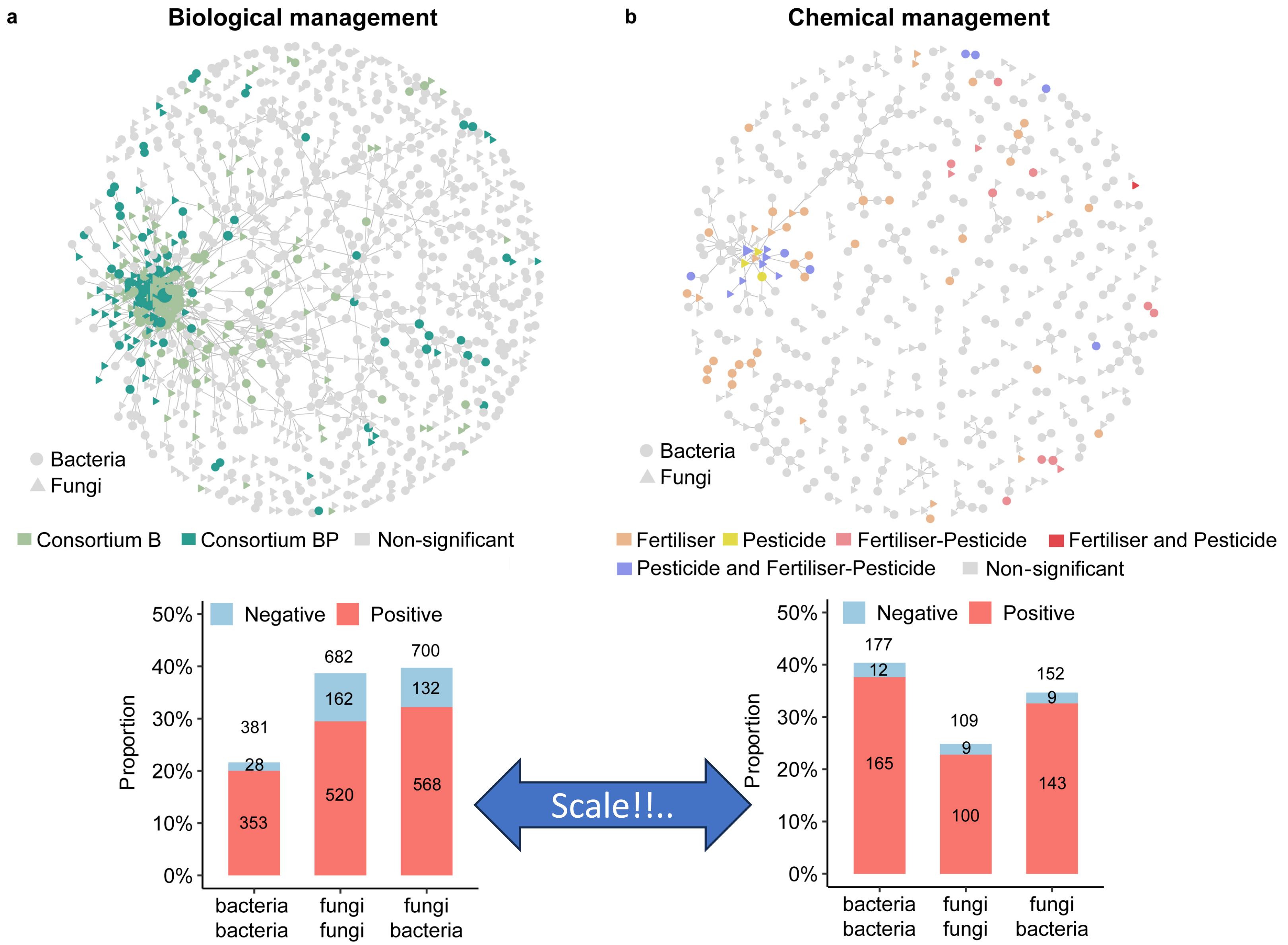
ECOstyle has big influence on the soil microbial composition

Harnessing Microbiome-Plant Synergies:
Microbiome-Interactive Traits Enhance Plant Growth and Support Sustainable Production



[a]Genomics Research in Ecology and Evolution in Nature (Green), Groningen Institute for Evolutionary Life Sciences (GELIFES), University of Groningen, Groningen, 9747AG, the Netherlands

Figure 4. Co-occurrence networks of rhizosphere microbial communities, displayed for biological (a) and chemical (b) management regimes. The upper panels visualise the significant correlation (coefficient > 0.6, *p* < 0.01) between bacterial and fungal species in rhizosphere soil communities. Circles and triangles refer to bacteria and fungi, respectively. ASVs/OTUs are colour-coded based on their association with different treatments, with each colour indicating a significantly higher ASVs/OTUs abundance in the corresponding treatment, that is treatment-responsive ASVs/OTnUs. Grey ASVs/OTUs are unresponsive to treatments in management. The size of nodes indicates the degree of edges connected to other ASVs/OTUs. The lower panels show the proportion of positive and negative correlations in biological and chemical management. The numbers on each bar, from bottom to top, represent the count of positive, negative, and total links, respectively.



potatoMETAbiome

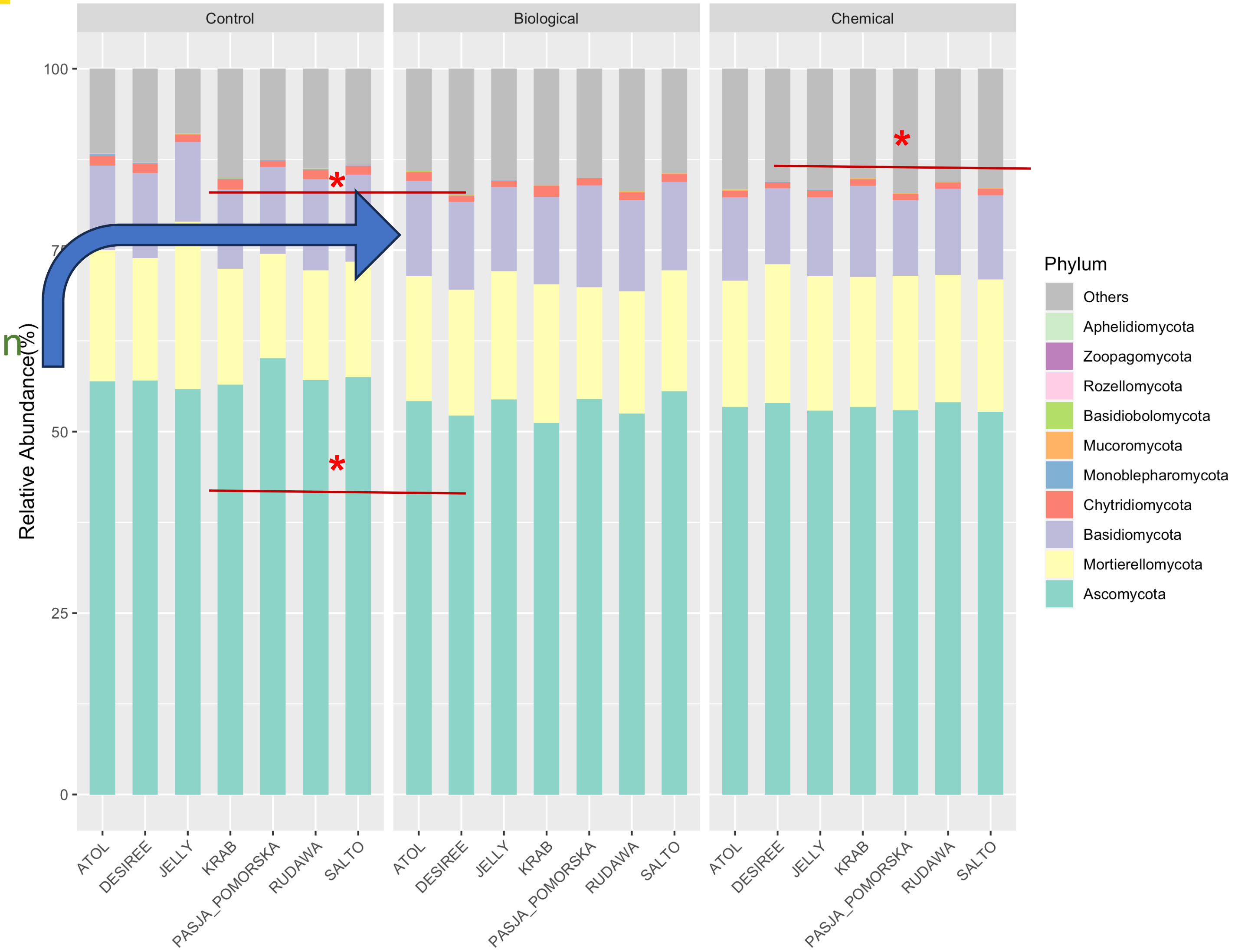
ECOstyle professional

Fungal community composition

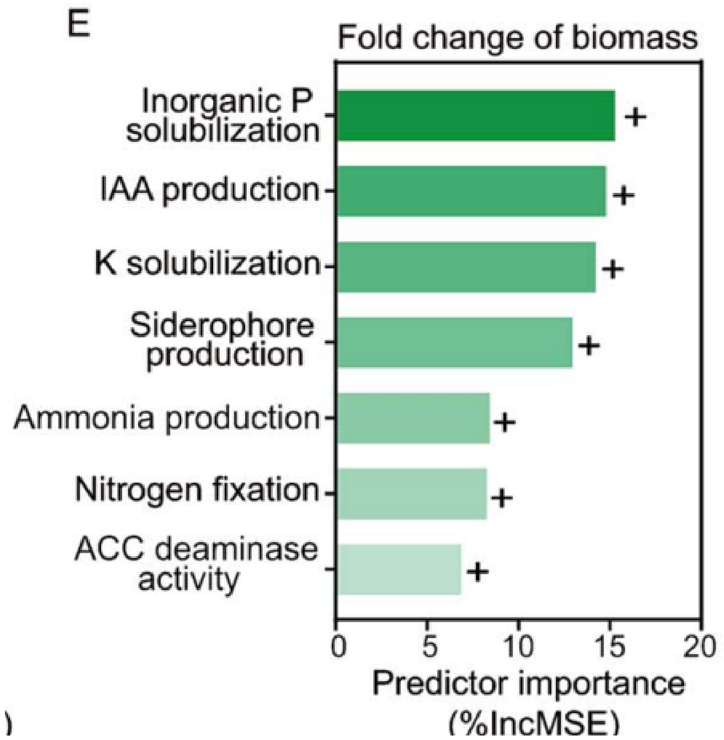
Control has more Ascomycota, Monoblepharomycota, and Zoopagomycota than others.

Biological (ECOstyle) has more Basidiomycota than Chemical. (and slightly less Ascomycota)

Chemical has less Chytridiomycota and Rozellomycota than control and biological.



Biofilm formation incraese with protozoa ECOP01



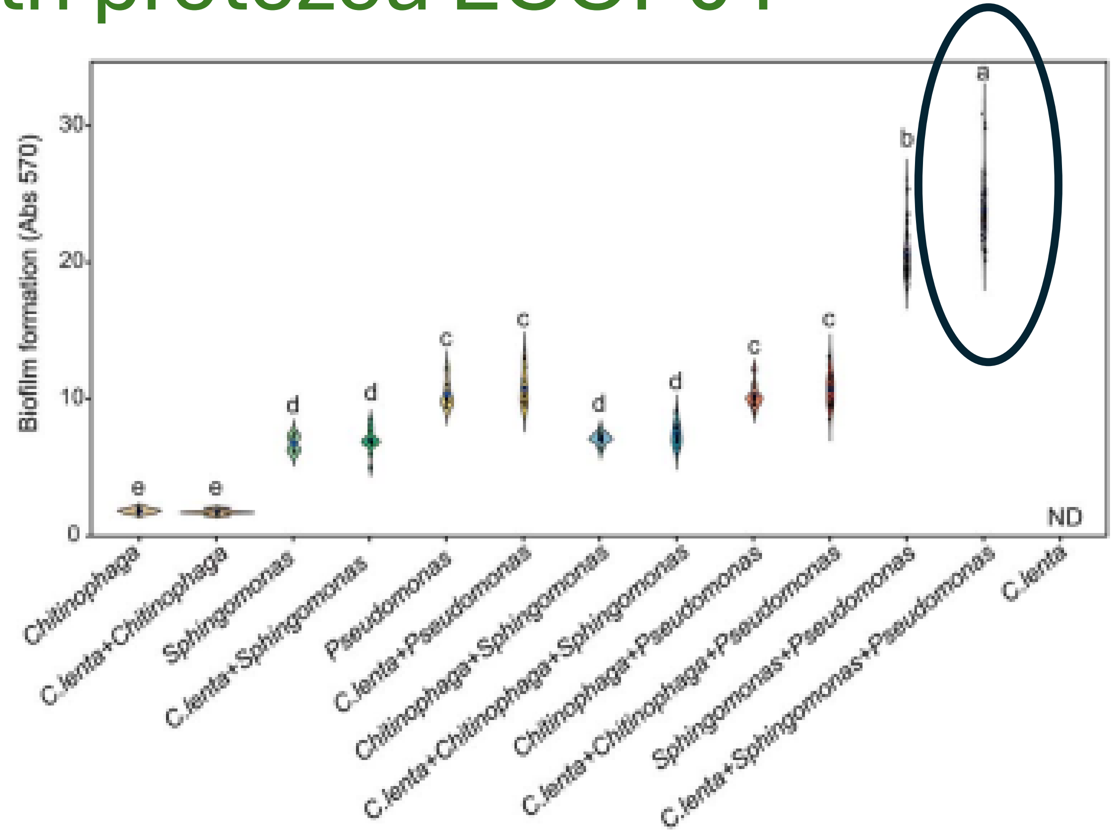


Figure 4. Biofilm formation of selected bacterial strains and the predatory protist *C. lenta* in coculture and monoculture systems. Different lowercases indicate significant differences ($P < 0.05$) between different treatments (Welch one-way test with pairwise t-test was used to calculate statistical significance). ND = no value was detected.

ISME Commun. 2022 Sep 5;2:82. doi: 10.1038/s43705-022-00166-9

The impact of protozoa addition on the survivability of *Bacillus* inoculants and soil microbiome dynamics

Panji Cahya Mawarda [1,2], Xavier Le Roux [3], Melissa Uribe Acosta [4,5], Jan Dirk van Elsas [1], Joana Falcão Salles [1]

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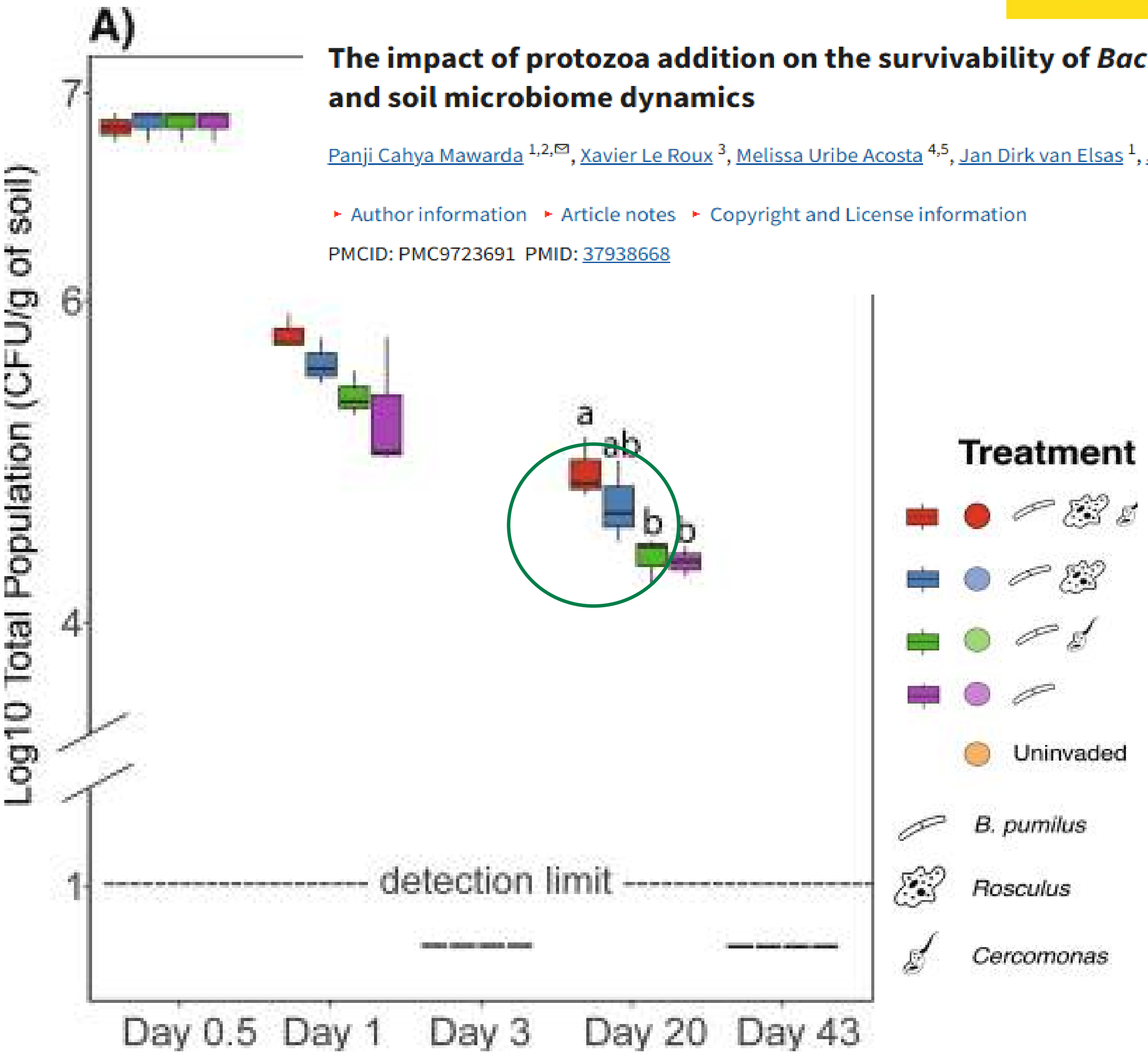
PMCID: PMC9723691 PMID: 37938668



It has been Scientifically proven that the two protozoa strains *Rosculus terrestris* ECO P002 and Cercomonas lenta ECO P001 increase the quantaty of *Bacillus pumilus* in the soil

+

3)

Indirect effect on plant health through soil life interactions

Predatory protists impact plant performance by promoting plant growth-promoting rhizobacterial consortia

Sai Guo, Stefan Geisen, Yani Mo, Xinyue Yan, Ruoling Huang, Hongjun Liu, Zhilei Gao, Chengyuan Tao, Xuhui Deng, Wu Xiong, Qirong Shen, George A Kowalchuk, Rong Li

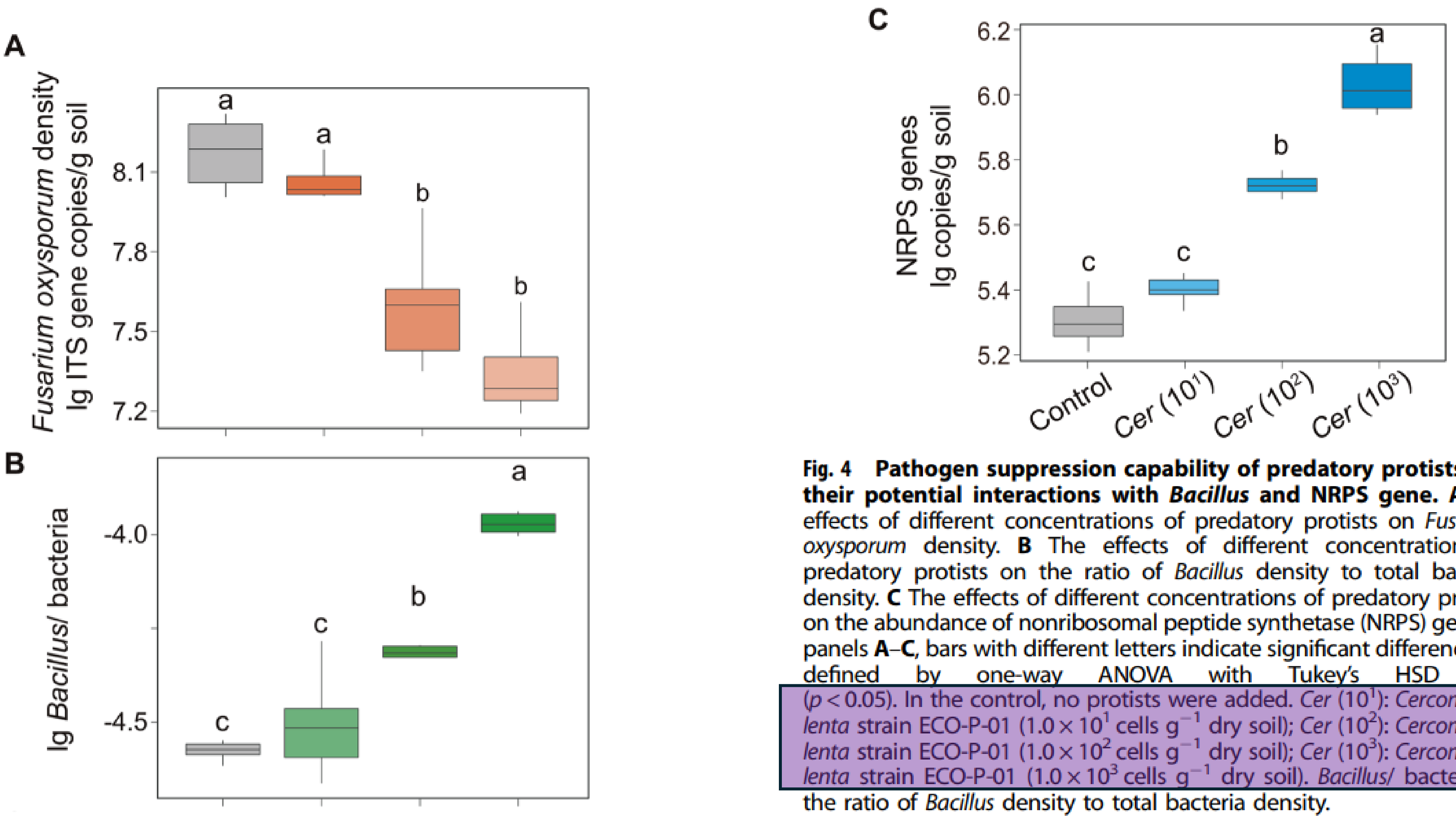
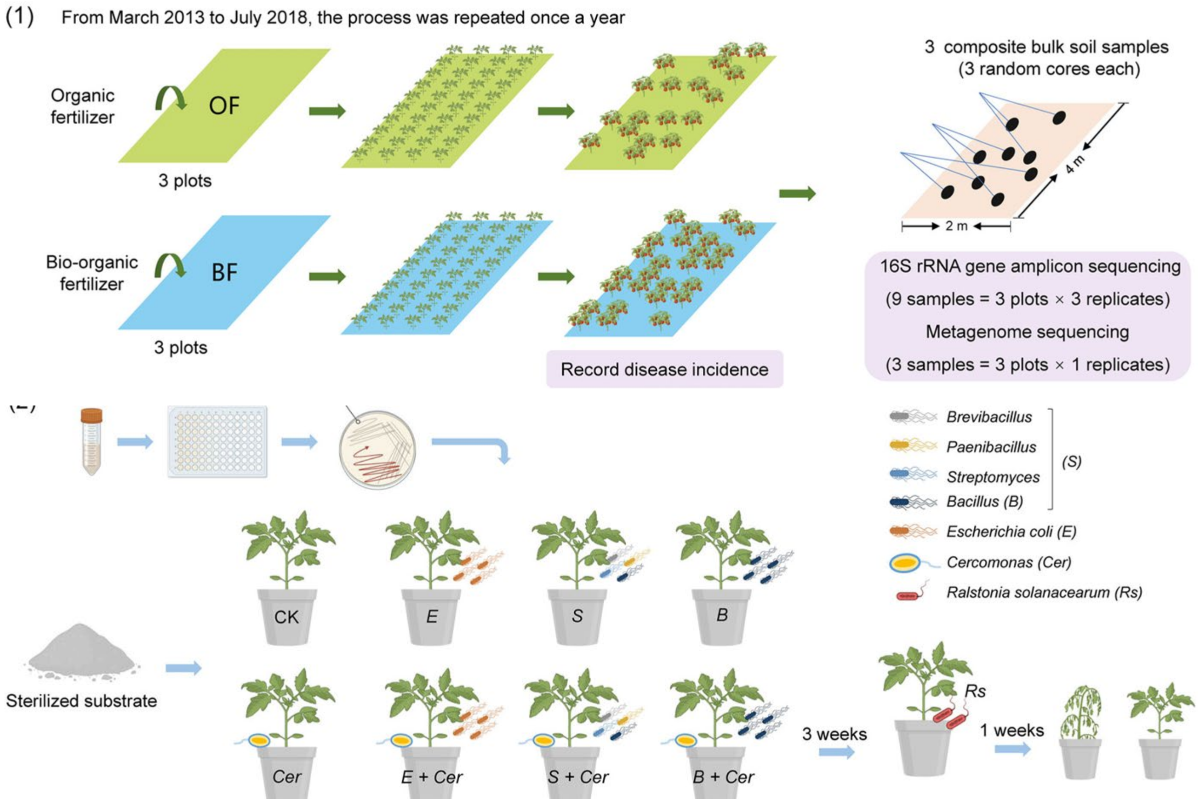


Fig. 4 Pathogen suppression capability of predatory protists and their potential interactions with *Bacillus* and NRPS gene. A The effects of different concentrations of predatory protists on *Fusarium oxysporum* density. **B** The effects of different concentrations of predatory protists on the ratio of *Bacillus* density to total bacteria density. **C** The effects of different concentrations of predatory protists on the abundance of nonribosomal peptide synthetase (NRPS) gene. In panels **A**–**C**, bars with different letters indicate significant differences as defined by one-way ANOVA with Tukey's HSD test ($p < 0.05$). In the control, no protists were added. *Cer* (10^1): *Cercomonas lenta* strain ECO-P-01 (1.0×10^1 cells g^{-1} dry soil); *Cer* (10^2): *Cercomonas lenta* strain ECO-P-01 (1.0×10^2 cells g^{-1} dry soil); *Cer* (10^3): *Cercomonas lenta* strain ECO-P-01 (1.0×10^3 cells g^{-1} dry soil). *Bacillus/* bacteria = the ratio of *Bacillus* density to total bacteria density.

Biofertilizer induces soil disease suppression by activating pathogen suppressive protist taxa



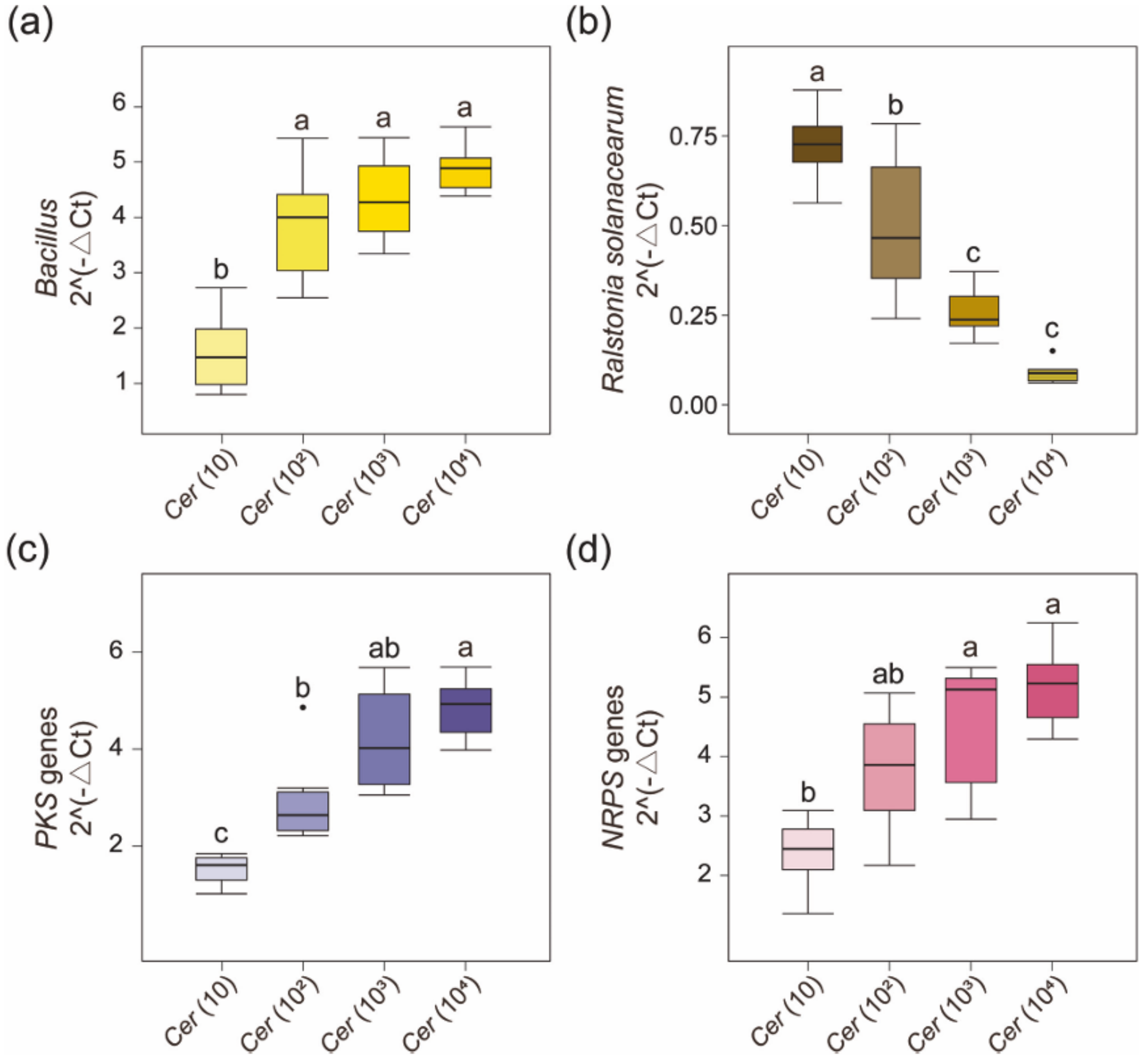


Fig. 5 | Potential interactions between *Cercomonas* and *Bacillus*, *R. solanacearum*, *PKS* genes, and *NRPS* genes. a Effects of different concentrations of *Cercomonas* on *Bacillus* density. **b** Effects of different concentrations of *Cercomonas* on *R. solanacearum* density. **c** Effects of different concentrations of *Cercomonas* on the abundance of the polyketide synthase (*PKS*) genes. **d** Effects of different concentrations of *Cercomonas* on the abundance of the nonribosomal peptide synthetase (*NRPS*) gene. In **a**–**d**, different letters represent significant differences, as determined by one-way ANOVA followed by Tukey's HSD test ($p < 0.05$). *Cer* (10): 10 cells/g dry soil *Cercomonas*; *Cer* (10^2): 10^2 cells/g dry soil *Cercomonas*; *Cer* (10^3): 10^3 cells/g dry soil *Cercomonas*; *Cer* (10^4): 10^4 cells/g dry soil *Cercomonas*. $\Delta Ct = Ct$ (treatment) − Ct (control). *Cercomonas* was not added to the control group.

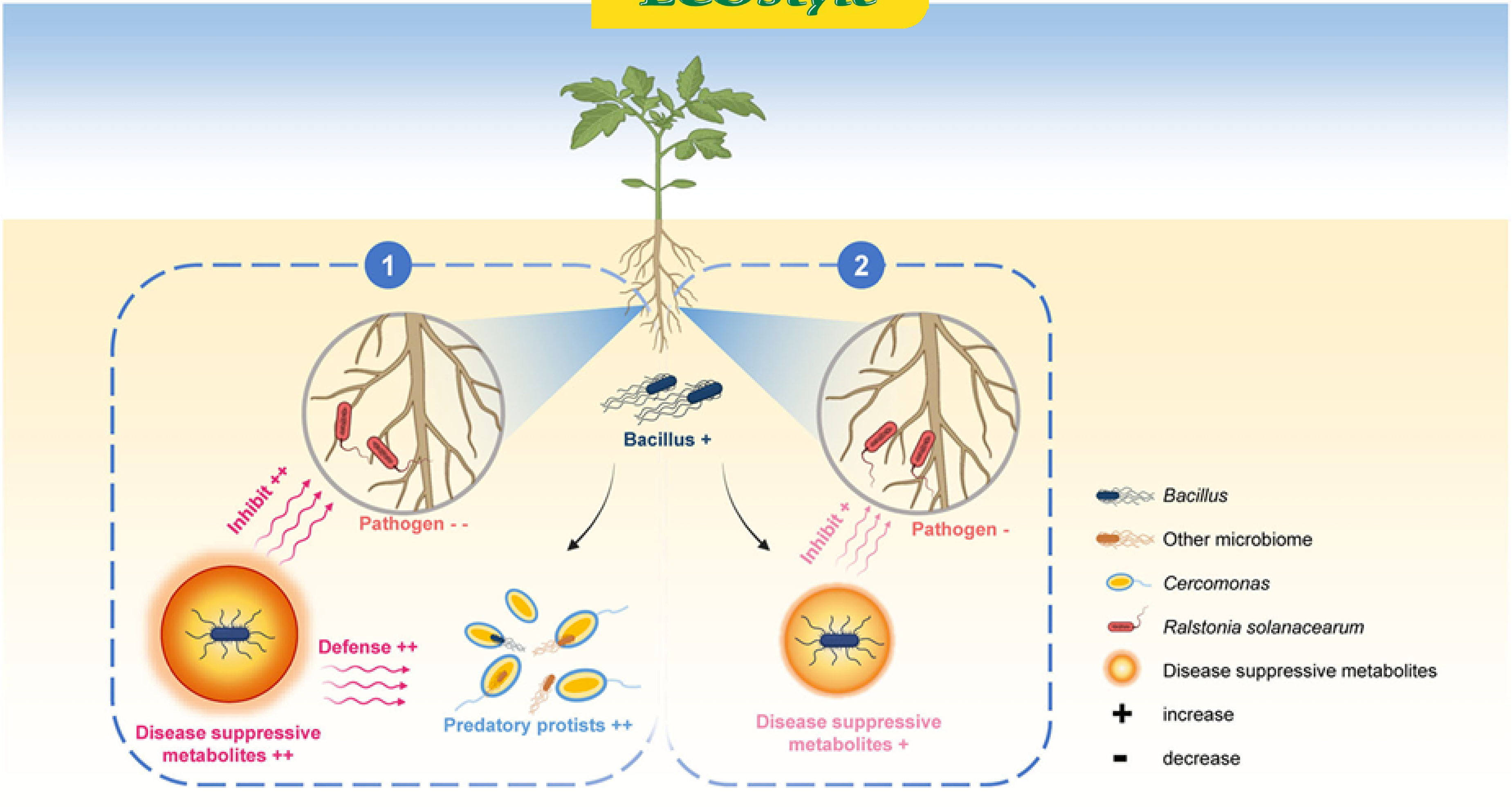
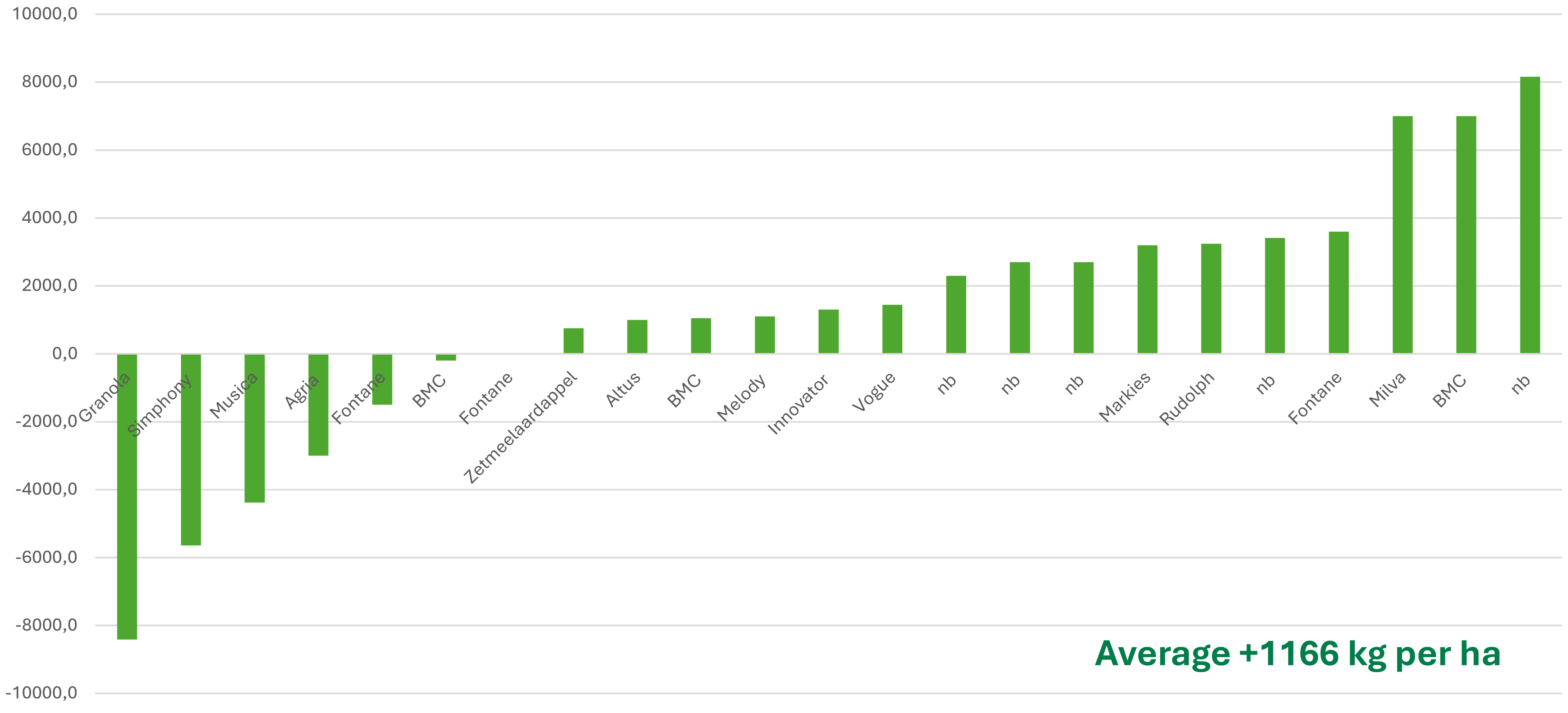


Fig. 6 | Conceptual model. This conceptual model illustrates how applied biocontrol bacteria enhance pathogen inhibition by stimulating predatory protists, which subsequently increases the proportion of antibacterial genes. Pathway 1: Biocontrol bacteria stimulate the proliferation of predatory protists (*Cercomonas*). Under predation pressure, biocontrol bacteria (e.g., *Bacillus*) upregulate the production of disease-suppressive metabolites, which not only mediates their defense against protist grazing but also enhances the inhibition of pathogens (*R. solanacearum*). Pathway 2: Biocontrol bacteria directly secrete disease-suppressive metabolites to inhibit pathogens. The number of '+' and '-' signs indicates the significance of given effect.

4) How to approach the market

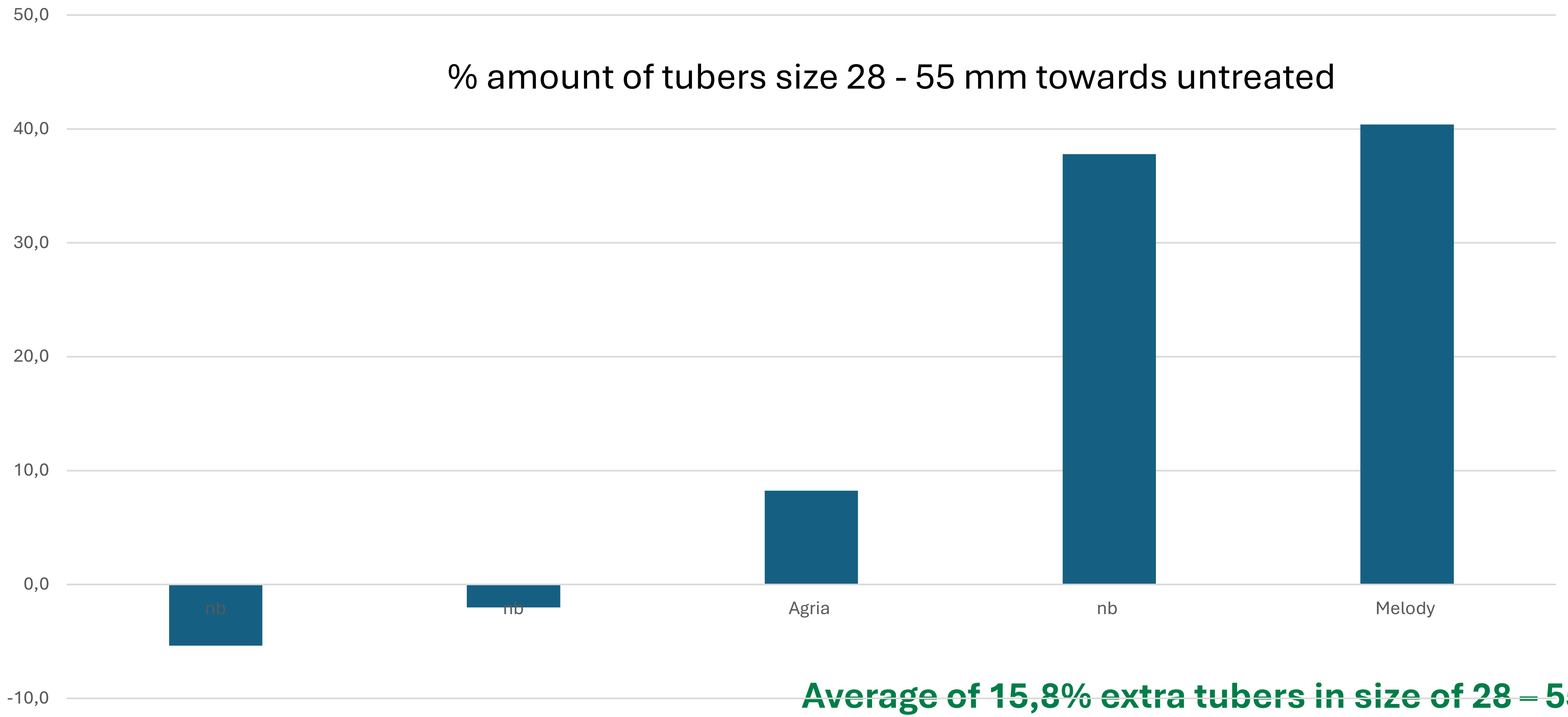
- Legal aspects
- Selection of crop
- Understand your product
- Uptimize production for a stable and affordable (farmers) costing price
- Cooperate with the known organisations in the market.
- Trials trials and trials.
- Proven effect / claims backed up with test results.

Summary overall yield in kg potato per hectare:
All tests with 4 replicates (not diversivicated)



Synthesis of 23 trials

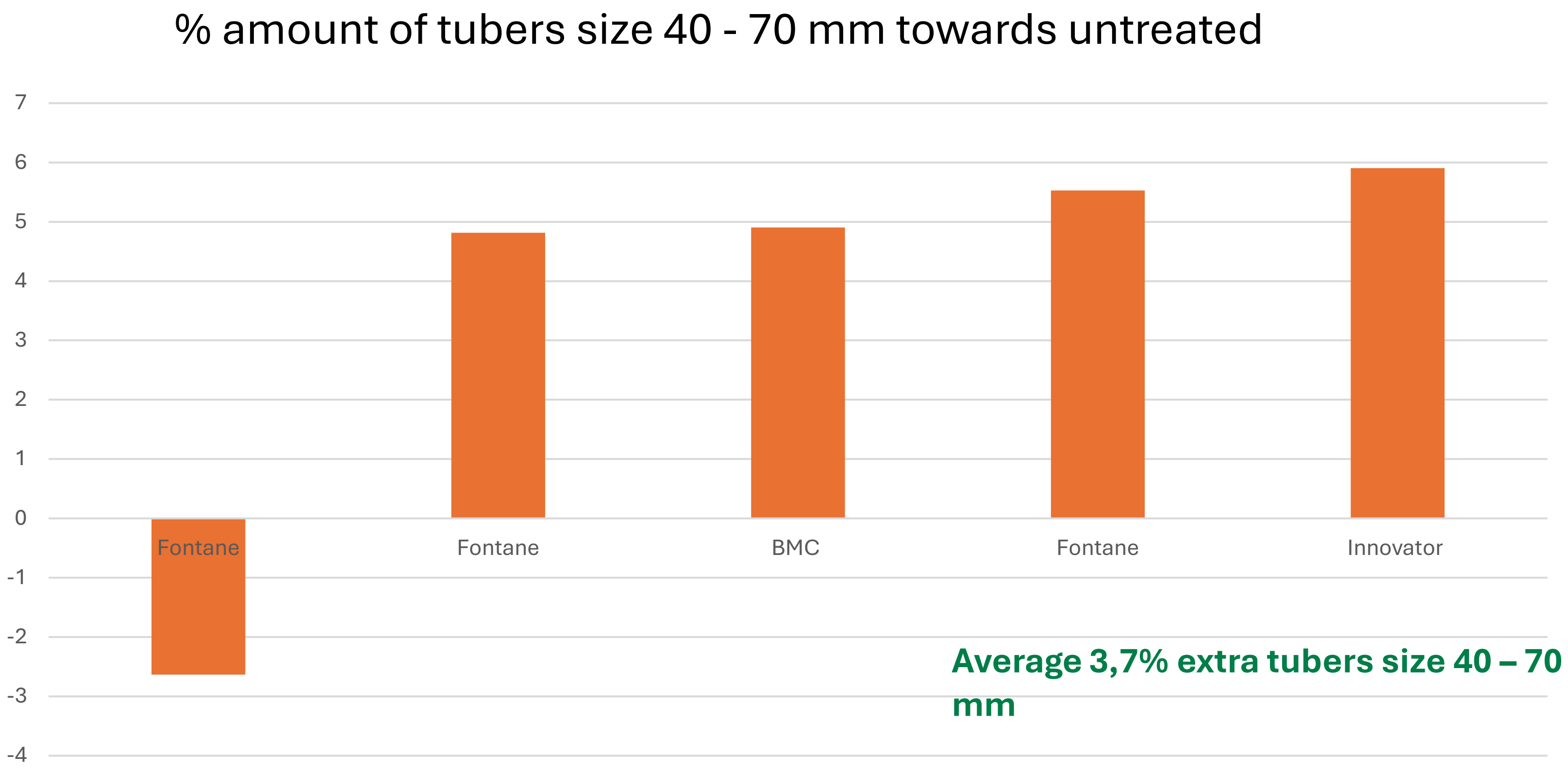
Amount of tubers size 28 – 55 mm seed potatoes

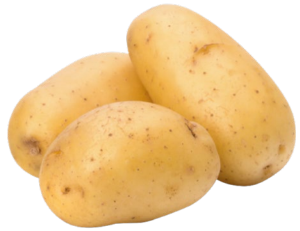


Average of 15,8% extra tubers in size of 28 – 55 mm

Synthesis of 5 trials

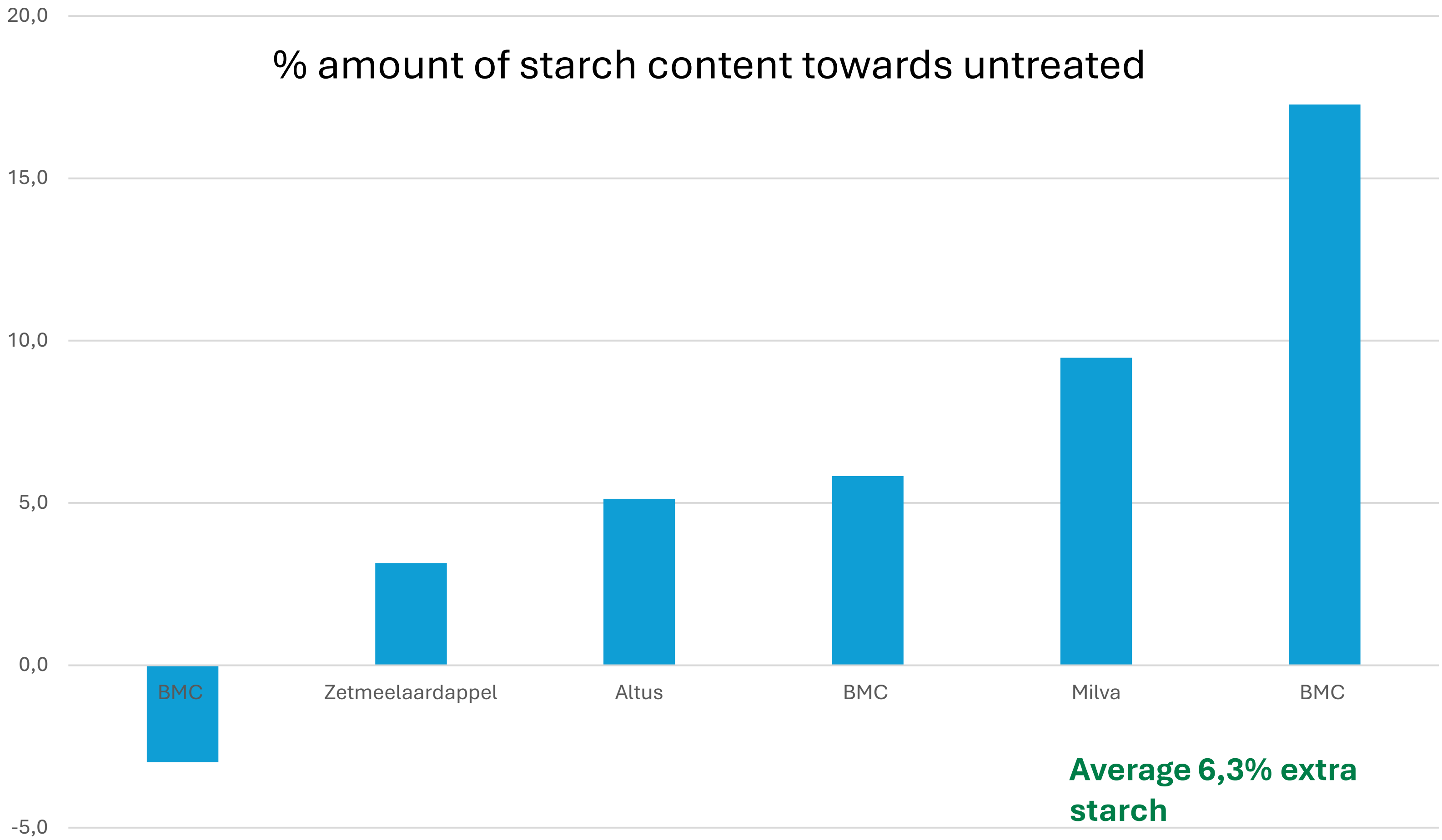
Amount of tubers size 40 – 70 mm table potatoes





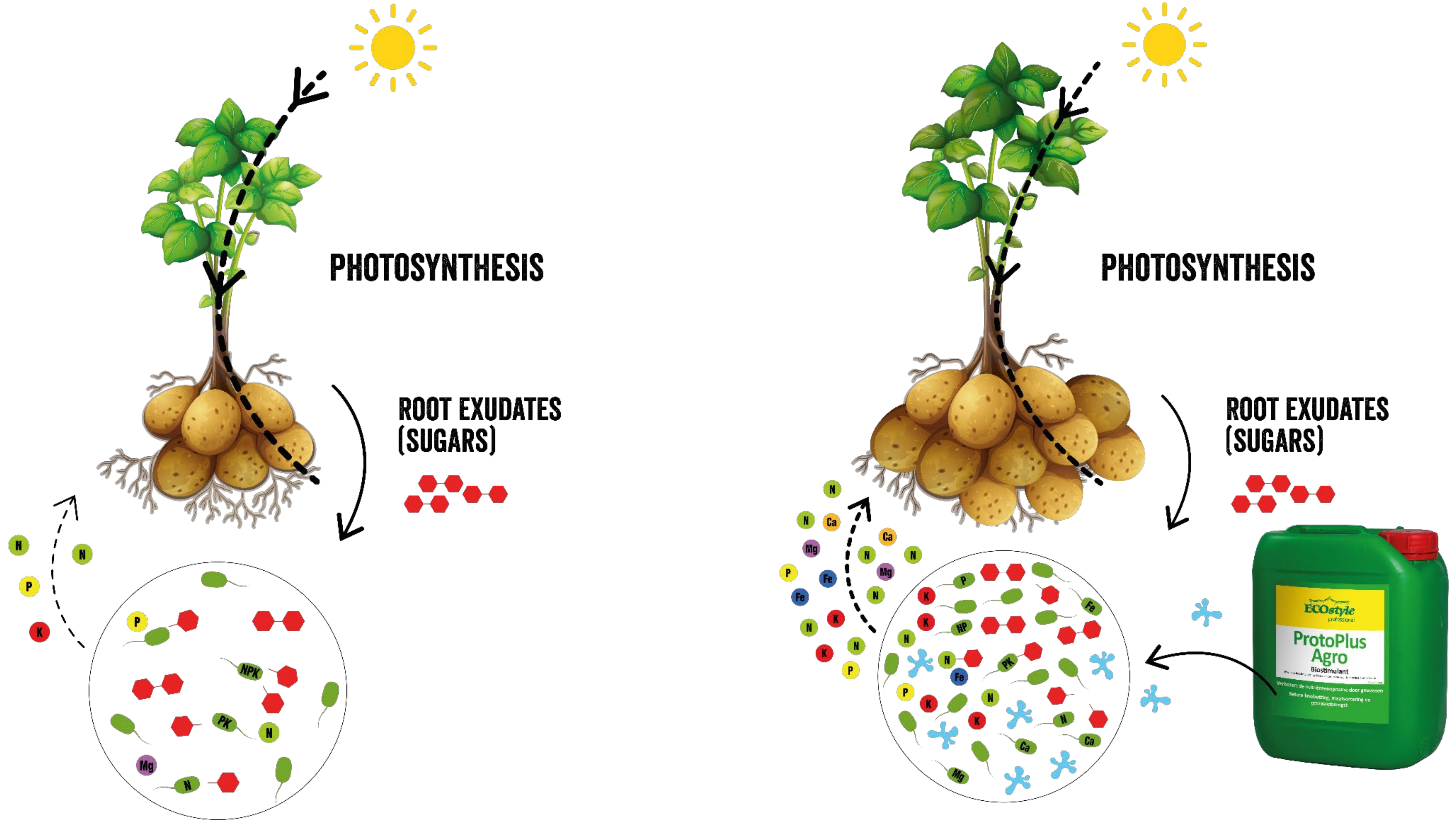
Synthesis of 5 trials

Amount of starch content



Synthesis of 6 trials

Mode of action ProtoPlus Potato trials



Amazing: Effect (ive)

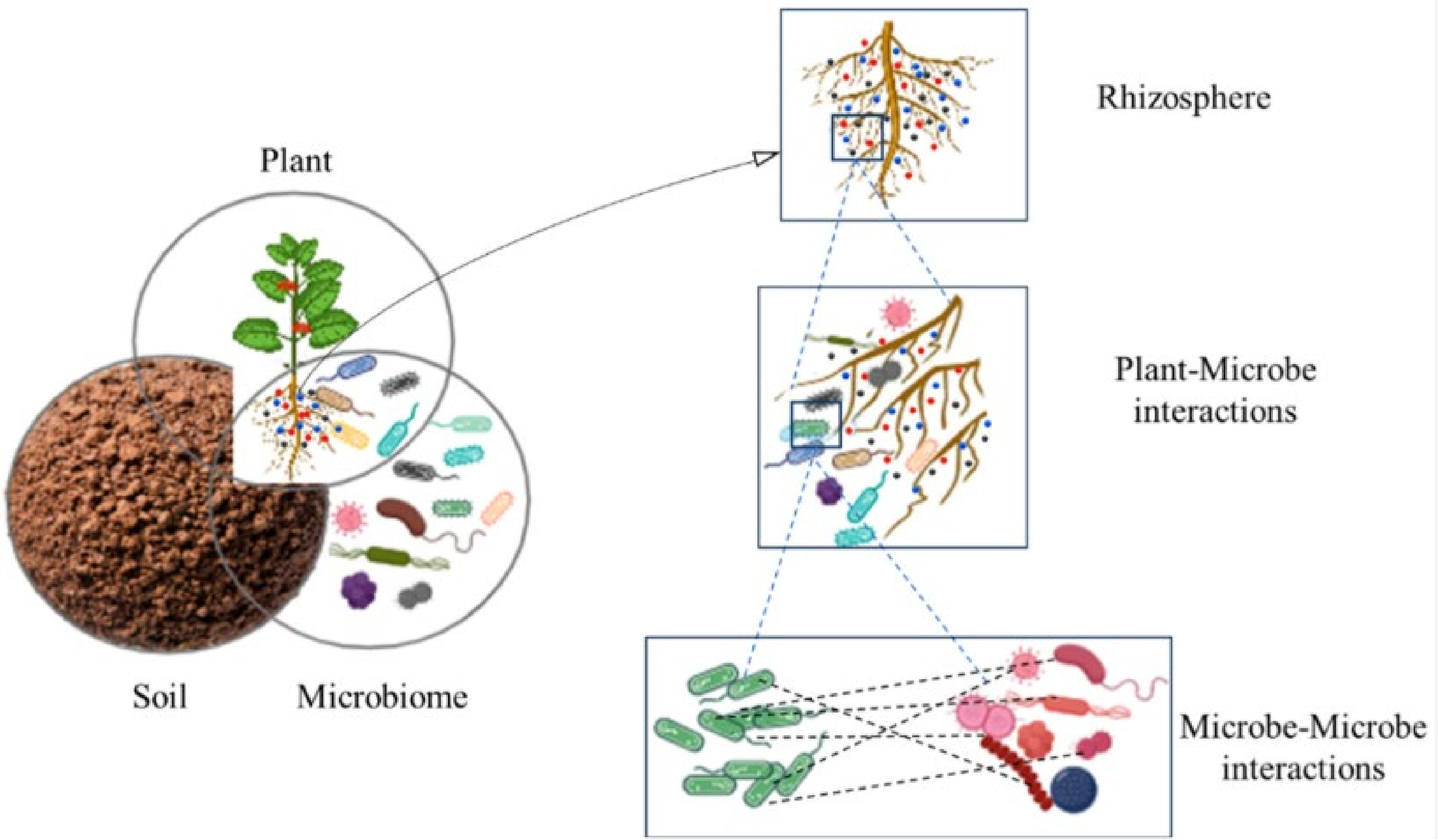
- We add (only) 2 species of protozoa
  - *Cercomonas lenta* *ECO P 01* 50.000.000 cysts
  - *Rosculus terrestris* *ECO P 02* 50.000.000 cysts

  (both together: 1 x 10 high 8)

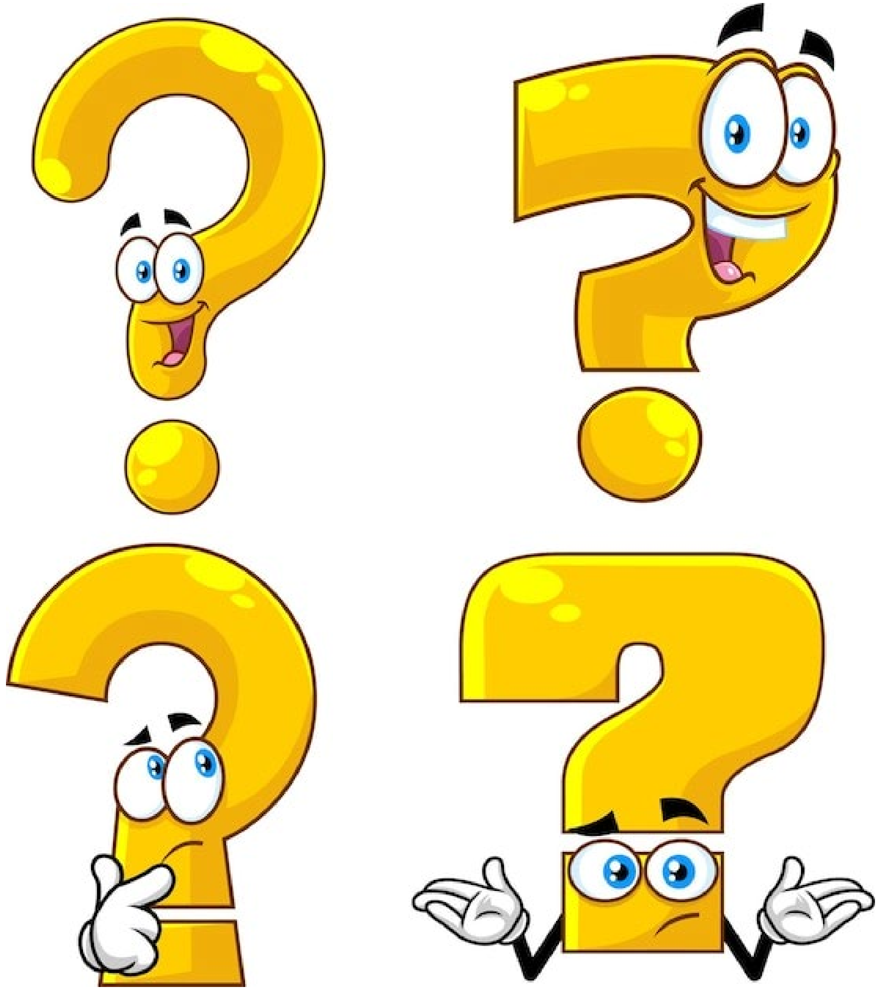
- To 1 hectare of soil.
- During plantation / seeding, only 1 treatment.
- We measure the effect at harvest.

Amazing effect(ive)
The power behind this effect ?

- We work with living organisms in their natural habitat.
- NOT disturbing or destroying organisms.
- But stimulating and enhancing the rhizosphere microbiome in the direction of increasing plant development. Both in growth and in health.



Questions ?



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