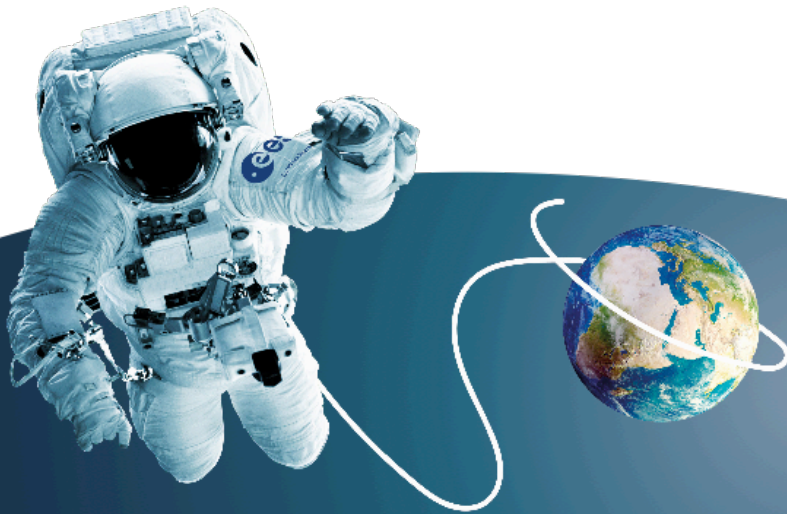




# Effectiveness of Bacterial Amendments on Lettuce Performance Inside a Plant Factory with Artificial Lighting

Thijs Van Gerrewey, Maarten Vandecruys, Nele Ameloot, Maaïke Perneel, Marie-Christine Van Labeke, Nico Boon, and Danny Geelen

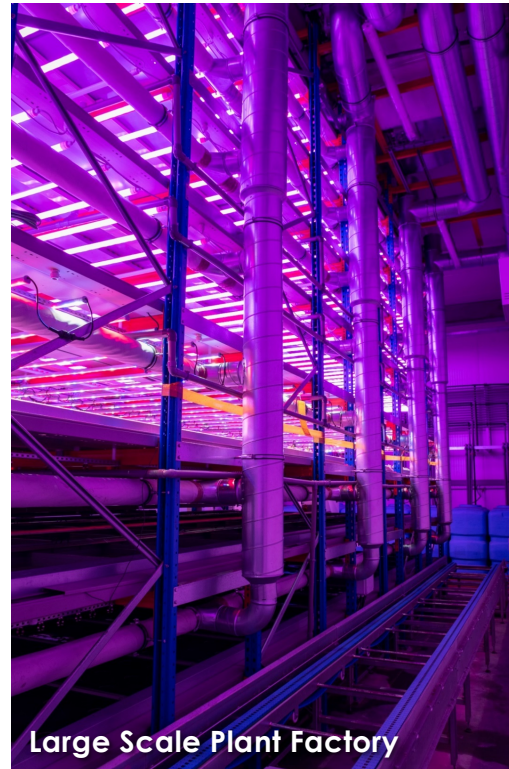
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# Plant Factory with Artificial Lighting (PFAL)

- PFALs maximize plant growth in a resource use efficient way.
- Obstacles:
  1. Pests and diseases.
    - Improve PFAL system robustness.
  2. Expensive.
    - PFALs only for niche markets (geographical niches and value chain models).
- Niches need to be expanded:
  1. Engineering improvement.
    - Reaching its limits.
  2. Plant biology (e.g. breeding).
    - Microbe-assisted cultivation.





# Plant Growing Media

- PFALs utilize soilless culture methods which typically require a plant growing medium (e.g. peat plugs).
- Peat:
  - Widely used.
  - Sustainability and environmental concerns.
  - Expanding world population → increase in demand for plant growing media.
- Development of sustainable peat alternatives (e.g. coir pith, wood fiber, composts, biochar).
- Peat will remain essential.

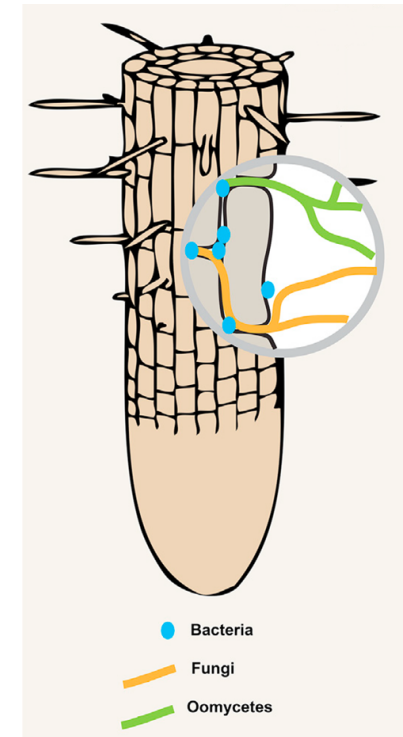






# Microbe-Assisted Cultivation

- Little attention given to the microbial properties when selecting new plant growing media.
- Microbial inoculation can enhance plant performance.
- Plant growing medium composition may be a determining factor in the successful amendment of bacteria.
- Use of plant growing media as a substrate for bacterial amendments.



Durán et al., 2018





# Materials and Methods

## Collection of Root-Associated Bacterial Communities

**Table 1.** Overview of Rhizosphere Sampling Locations.

Sample	Collection Date	Location	Crop	Cultivation Method	Plant Growing Medium
S1	03-Oct-2017	Wachtebeke, Belgium	Lactuca sativa var. crispa (oakleaf)	Organic open field	Sand
S2	17-Oct-2017	Moerbeke-Waas, Belgium	Lactuca sativa var. crispa (oakleaf)	Organic open field	Loamy sand
S3	21-Nov-2017	Onze-Lieve-Vrouw-Waver, Belgium	Lactuca sativa var. crispa (lollo bionda)	Soilless	Black peat
S4	12-Dec-2017	Ardooie, Belgium	Lactuca sativa var. capitata (butterhead)	Soilless	Black peat
S5	05-Jun-2018	Lochristi, Belgium	Lactuca sativa var. crispa (lollo bionda)	Organic open field	Sand



Positive control: *Bacillus* sp.





# Materials and Methods

## Plant Growing Media Composition

**Table 2.** Composition of Plant Growing Media. Each plant growing medium consists of 4 raw material groups at different volume per volume (% v/v).

Plant Growing Medium	Peat (60% v/v)	Other Organics (20% v/v)	Composted Materials (10% v/v)	Inorganic Materials (10% v/v)
M1	white peat	coir pith	bark	perlite
M2	white peat	wood fiber	bark	perlite
M3	black peat	coir pith	bark	sand
M4	white peat	coir pith	green waste	perlite
M5	white peat	wood fiber	bark	sand
M6	white peat	coir pith	bark	sand
M7	black peat	wood fiber	bark	perlite
M8	black peat	coir pith	green waste	sand
M9	white peat	wood fiber	green waste	sand
M10	black peat	wood fiber	green waste	perlite

Positive control: commercial plant growing medium (75% peat and 25% coco coir).







# Materials and Methods

## Plant Growth and Inoculation

- Batavia lettuce was sown in each plant growing medium (M1-10).
- Two weeks after sowing:
  - Inoculation with the bacterial community inocula (BCI S1-5) and positive control (PGPR) at a dose of  $3.2 \times 10^9$  CFU/L.
  - Non-inoculated negative control (C).
  - Transfer to PFAL.
- Harvest three weeks after inoculation.







# Materials and Methods

## Plant Sample Analysis

- Yield and quality analysis:
  - Shoot fresh weight (FW)
  - Lettuce head area (LHA)
  - Root fresh weight (RW)
  - Shoot dry weight (DW)
  - Total phenolic content (TPC)
  - NO<sub>3</sub>-content
  - Leaf pigments
- Fractional factorial analysis (DOE)
- Principal component analysis (PCA)



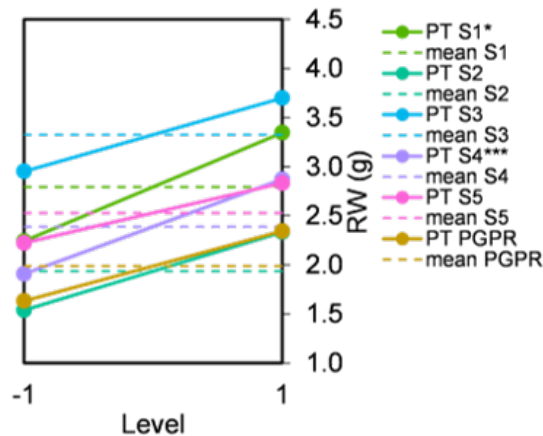


# Results and Discussion

## Plant Growing Medium Constituents Have Differing Effects on Lettuce Performance

### 1. Changing black peat to white peat increased RW:

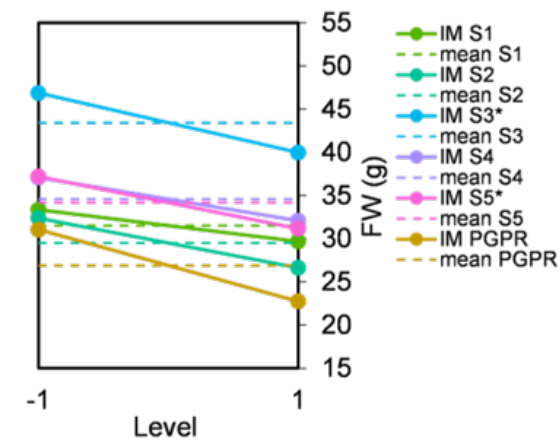
- Higher air volume in white peat mixtures improved the rooting of lettuce.



**Figure 1.** Main effect of peat on root fresh weight (RW; g) under different bacterial treatments (S1-5 and positive control PGPR). Peat (PT; -1 = black peat and 1 = white peat). Dashed lines indicate mean levels of RW for each bacterial treatment. Asterisks indicate level of significance:  $P < 0.05$  (\*),  $P < 0.01$  (\*\*) and  $P < 0.001$  (\*\*).

### 2. Perlite increased FW compared to sand (and LHA, RW):

- Higher air volume and water capacity in plant growing media amended with perlite.



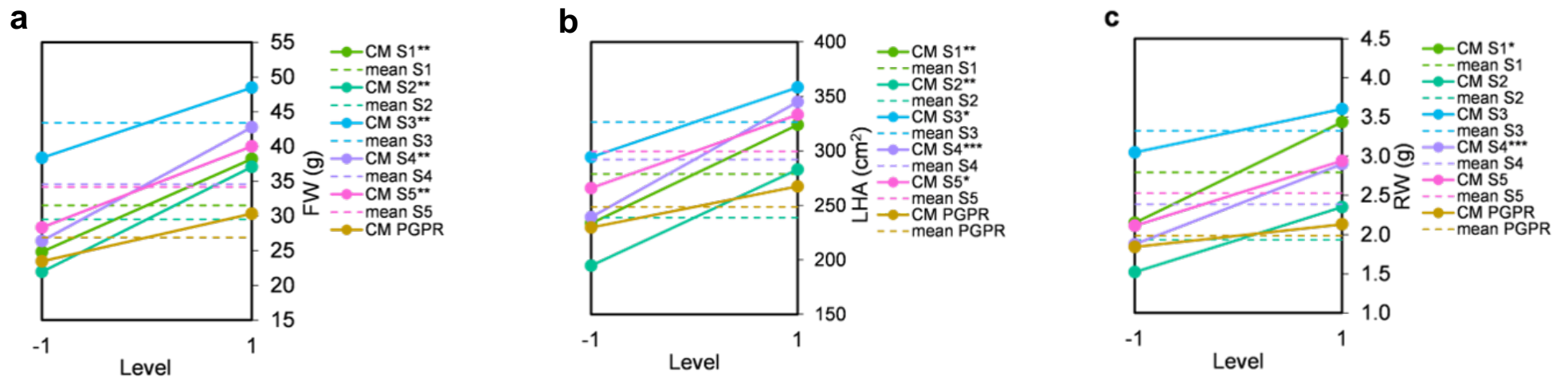
**Figure 2.** Main effects of inorganic materials on shoot fresh weight (FW; g) under different bacterial community inoculum treatments (S1-5 and positive control PGPR). Inorganic materials (IM; -1 = perlite and 1 = sand). Dashed lines indicate mean levels of FW for each bacterial treatment. Asterisks indicate level of significance:  $P < 0.05$  (\*),  $P < 0.01$  (\*\*) and  $P < 0.001$  (\*\*).



# Results and Discussion

## Plant Growing Medium Constituents Have Differing Effects on Lettuce Performance

3. Green waste compost significantly increased lettuce growth (FW, LHA, RW):
- The increased availability of salts, and especially  $K^+$ , was advantageous for lettuce growth.



**Figure 3.** Main effects of composted materials on (a) shoot fresh weight (FW; g), (b) lettuce head area (LHA; cm<sup>2</sup>), and (c) root fresh weight (RW; g) under different bacterial community inoculum treatments (S1–5 and positive control PGPR). Composted materials (CM; -1 = composted bark and 1 = green waste compost). Dashed lines indicate mean levels of FW for each bacterial treatment. Asterisks indicate level of significance:  $P < 0.05$  (\*),  $P < 0.01$  (\*\*) and  $P < 0.001$  (\*\*\*).

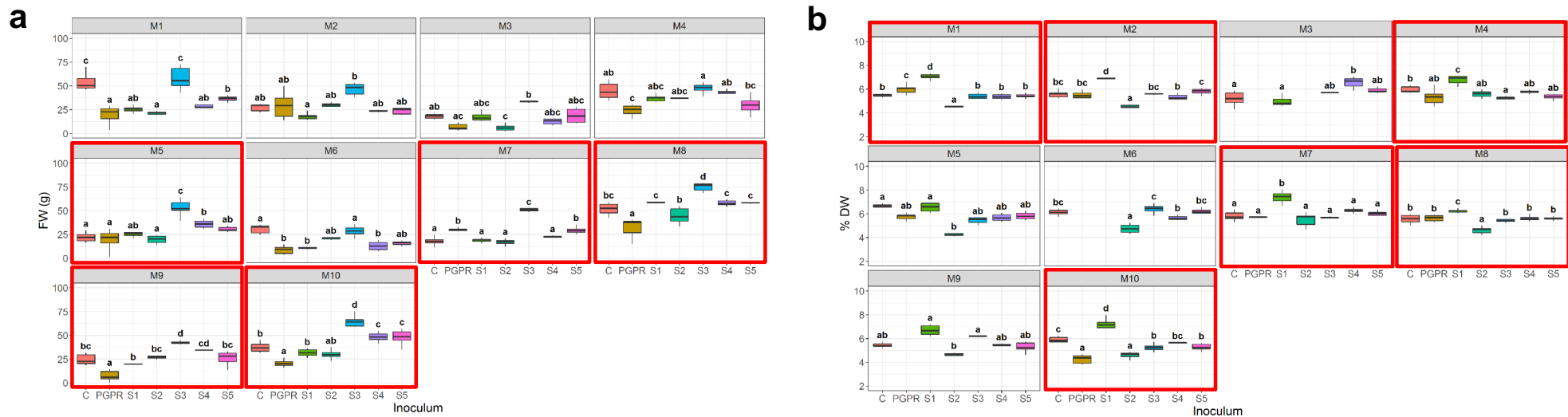




# Results and Discussion

## Microbe-Plant Growing Medium Interactions Determine Plant Performance

- The interaction between BCI and plant growing medium affected all plant performance parameters.
- BCI S1 and S3 positively affected plant performance depending on plant growing medium composition.



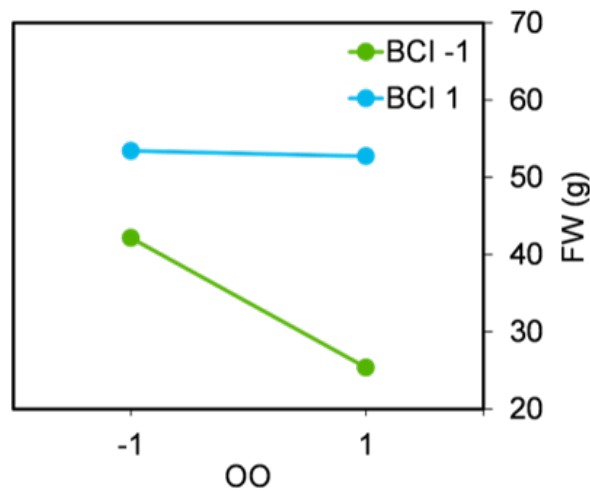
**Figure 4.** Boxplot of (a) shoot fresh weight (FW; g) and (b) shoot dry weight (%DW) grouped per plant growing medium. Letters show comparison of BCI means per plant growing medium at the 95% confidence level. S indicates the bacterial community inoculum, M indicates the plant growing medium, C indicates the negative control treatment without addition of inoculum, and PGPR indicates the positive control treatment with a *Bacillus* sp. inoculum. Number of plants  $\geq 3$ .



# Results and Discussion

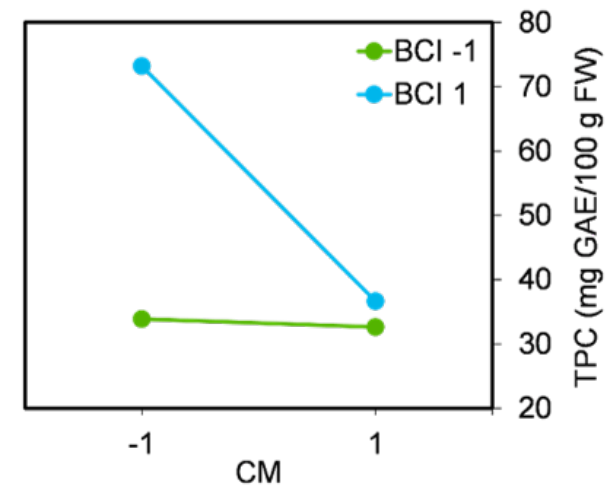
## Microbe-Plant Growing Medium Interactions Determine Plant Performance

1. BCI S3 and other organics interaction effect on FW:



**Figure 5.** Interaction effect between other organics (OO; -1 = coir pith and 1 = wood fiber) and BCI S3 (-1 = C and 1 = S3) on shoot fresh weight (FW; g) ( $P = 0.024$ ).

2. BCI S1 and composted materials interaction effect on TPC:



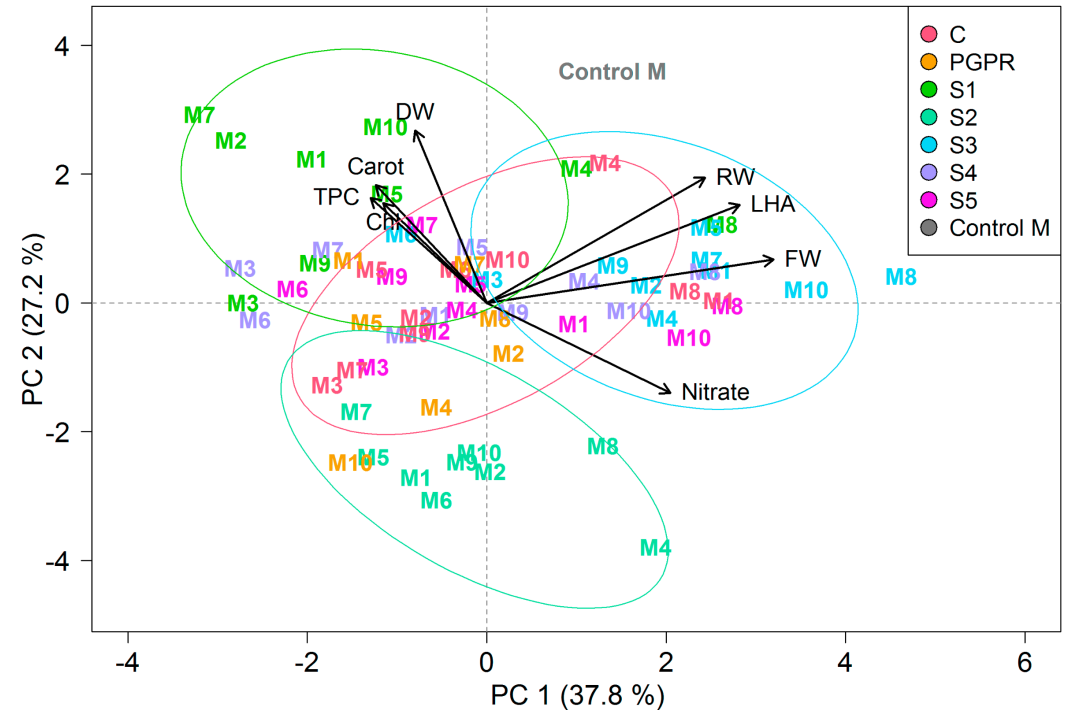
**Figure 6.** Interaction effect between composted materials (CM; -1 = bark compost and 1 = green waste compost) and BCI S1 (-1 = C and 1 = S1) ( $P = 0.001$ ) on total phenolic content (TPC; mg GAE/100 g FW) under BCI S1 treatment.



# Results and Discussion

## The Bacterial Source Determines Plant Performance

- Bacterial amendment resulted in different effects on plant performance depending on the bacterial source.
- PCA analysis showed a grouping of BCI-plant growing medium combinations.
- The BCIs were collected at separate locations with different cultivation method, fertilizer management, soil type, crop species, etc. which shaped the community.
- A complex bacterial community is a driver for successful bacterial amendment.



**Figure 7.** PCA biplot of the lettuce yield and quality variables under different BCI-plant growing medium treatments.





## Conclusions

- Plant growing medium composition determines plant performance.
- Bacterial amendment is a key driver affecting plant performance.
- The effectiveness of bacterial amendment depends on the bacterial source and on its interaction with the plant growing medium.
- Potential in using bacterially enhanced plant growing media to modulate plant performance and improve PFAL and MELISSA system robustness.
- Further research will focus on the rhizosphere bacterial community structure.





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## Microbe-Plant Growing Media Interactions Modulate the Effectiveness of Bacterial Amendments on Lettuce Performance Inside a Plant Factory with Artificial Lighting

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