Smart use of microbial-rich vermicomposting to enhance tripartite plant-microbe-soil interactions

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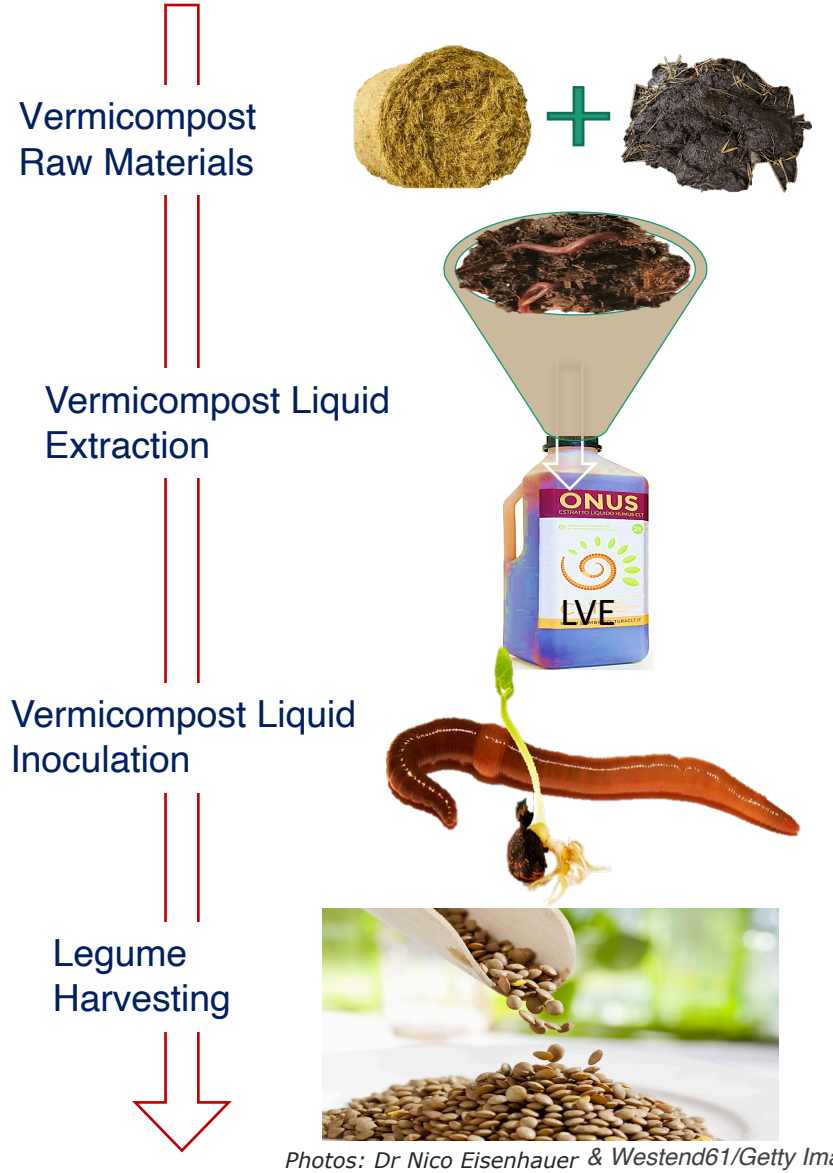
Introduction
Vermicomposting: A key product to legume cultivation

- **Vermicomposting** is a process that utilizes **earthworms** and the associated **microbiome** to transform organic wastes into **biofertilizers**.
- **Legumes**' **interaction capabilities** with diverse **native soil** microorganisms and introduced microbial-rich **inoculants** could be exploited to enhance associated agroecosystem services.
- The **dynamics of native arbuscular mycorrhizal fungi (AMF)** within the legume root rhizosphere as influenced by the **liquid vermicompost extract (LVE)** inoculation is largely **unexplored**.



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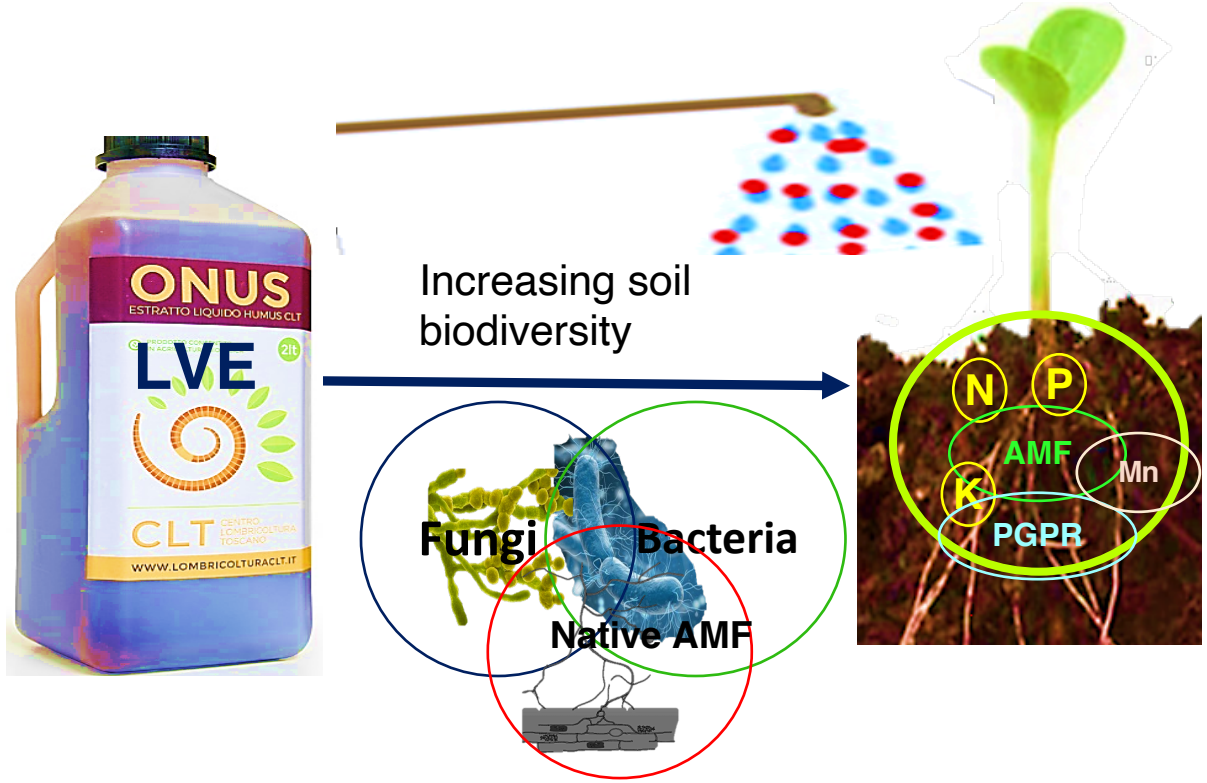
Research question

- **LVE** inoculation could augment soil microbial interactions (**AMF & PGPRs), improve soil quality** and provide soil related **agroecosystem services**.
- Does the **LVE inoculant** contain a **diverse composition** of microbial (bacteria and fungi) communities known for **plant-growth promoting traits**?
- How will the **vermicompost-legume interaction** affect the soil **AMF** dynamics in a low-input management system?
- Test effects on:
  a) the changes in **soil mycorrhizal inoculation potential (MIP)**
  b) the changes in **AMF root colonization**



Agroecosystem services

- N- fixation
- Biocontrol
- Siderophore production
- Nutrient cycling (N, P, K)

NB: LVE, traded as ONUS® was sourced from Centro Lombricoltura Toscano, Pisa, Italy.

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Materials and Methods

Illumina Miseq Sequencing

LVE inoculation at a rate = 25.6 litres/ha

- Lentil
- Chickpea
- Berseem clover
- Soybean
- Sunflower

Liquid Vermicompost (LVE)

At seeding stage

At stem-elongation stage

Soil sampling before planting (**MIP_0**)

Root sampling at flowering (**AMF%**)

Crop Harvesting (July-Sept. 2019)

Soil sampling after harvesting (**MIP_1**)

NB: Illumina Miseq Sequencing of LVE was done by BMR Genomics (Italy) using primers from Takahashi et al.2014 (bacteria), White et al., 1990 (fungi) and Qiagen (DNA extraction, amplification & sequencing) protocol. Analyses was performed on QIIME2 pipeline and taxonomic annotation was done on GreenGene and UNITE databases.

- A field trial was carried out at the 'Centro di Ricerche Agro-Ambientali' (CiRAA), San Piero a Grado, Pisa, Italy between March and September 2019.
- A Split plot layout design, with 5 replicates was used.
- **Test factors included:**
  - **a)** **Crop Species** (4 **Legumes,** 1 **non-legume control**, 1 empty control plot).
  - **b)** **Liquid Vermicompost Extract** (LVE) **inoculation** with 2 levels i.e. with or without.

Results 1 – Vermicompost alpha diversity

- Our LVE inoculant was **rich** in bacteria and fungi with a **diverse** community composition.

Bacteria and fungal diversity

LVE Sample	Simpson Index	Shannon (*H'*)	Observed OTUs (Richness)
Fungi (ITS2 region)			
FB3	0.76 ± 0.02	0.93 ± 0.06	50.37 ± 0.70
Bacteria (16s rRNA V3-V4 region)			
FB3	0.89 ± 0.01	4.17 ± 0.12	159 ± 22.27

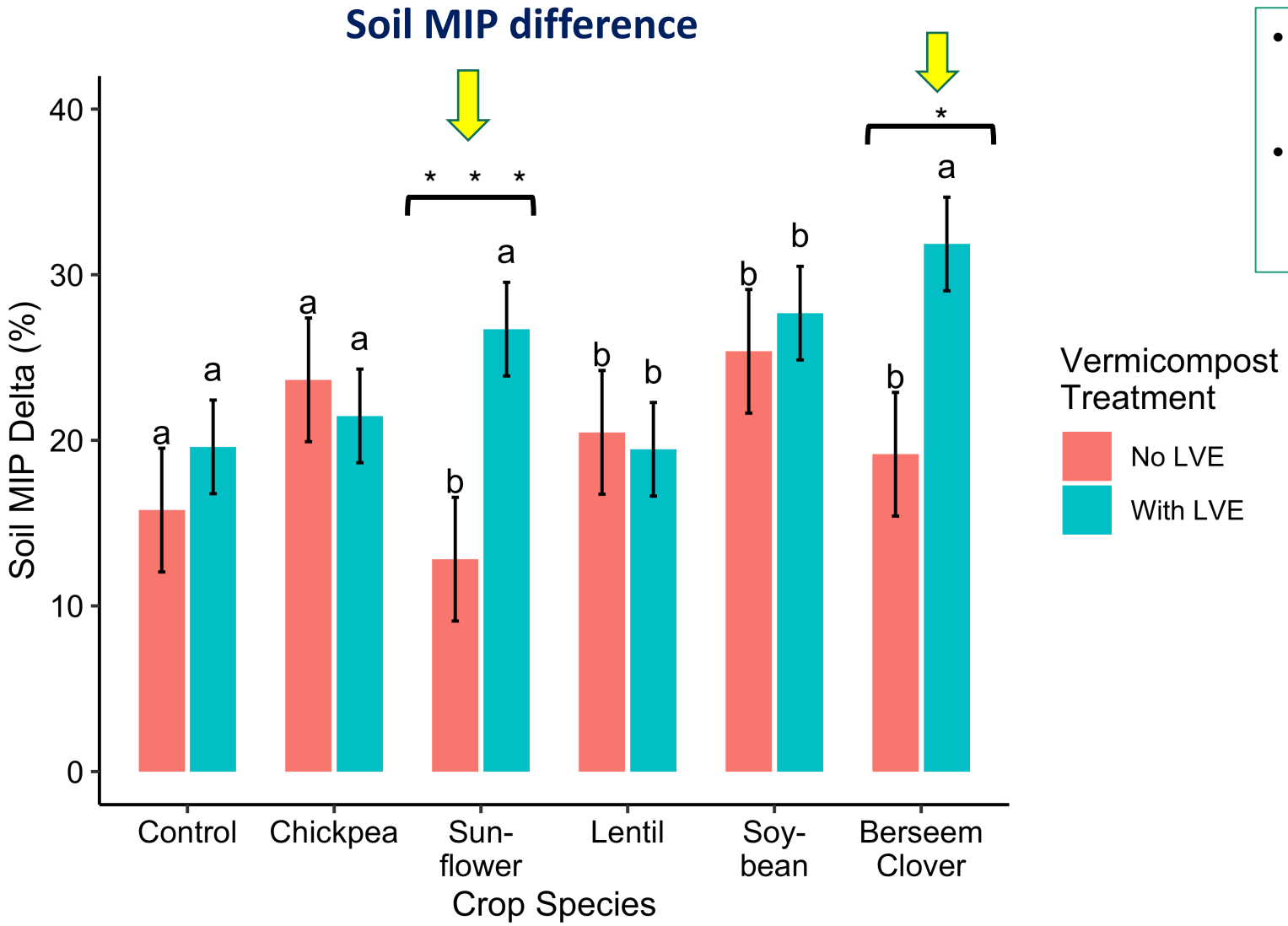
NB: Mean ± SE of 3 sample replicates from the same LVE batch. Universal primers and protocols used - Takahashi et al., 2014 & White et al., 1990.

Results 2 - Vermicompost composition

- We identified genera of bacteria and fungi and in some cases, species with **plant growth promoting traits**, **siderophore production**, **N-fixation** and **disease suppression**, which supports the importance of vermicompost use in crop production.

Taxonomic genera:	Species	Ecological importance
Bacteria:		
▪ Citrobacter (36%)	- *C. freundii & C. werkmanii*	- Play a key role in soil N cycle (*Line, 1990*).
▪ Arcobacter (30%)	- *A. aquimarinus & A. ellisii*	- Associated to human & animal effluents (*Van Driessche et al. 2004*).
▪ Pseudomonas (10%)	- *P. viridiflava*	- Produce peptide antimycotics ecomycin B & C (*Miller et al., 1998*).
▪ Flavobacterium (4%).	- *F. succinicans*	- Possess PGPR traits: P-solubilization, IAA & ACC deaminase production (*Soltani et al., 2010*).
▪ Clostridium (2%)	- *C. pasteurianum*	- Fermenter & an anaerobic N fixer under gnotobiotic conditions (*Meurial and Kumar, 2018*).
Fungi:		
▪ Mucor (92%)	- *M. circinelloides*	- Lignocellulolytic degrader (*Chen et al., 2015*).
▪ Cyberlindnera (1%)	- *Cyberlindnera* sp.	- IAA producing soil-borne yeast (*Nassar et al., 2005*).
▪ Dipodascus (1%)	- *D. geotrichum*	- Decomposition & breaking down of tough debris (*Bonito et al., 2010*).
▪ Candida (1%)	- *C. boidinii*	- Siderophore production & reduce post- emergence damping-off of seedlings (*El-Tarabily, 2004*).

07 Results 3 - Soil mycorrhizal inoculation potential (MIP) delta

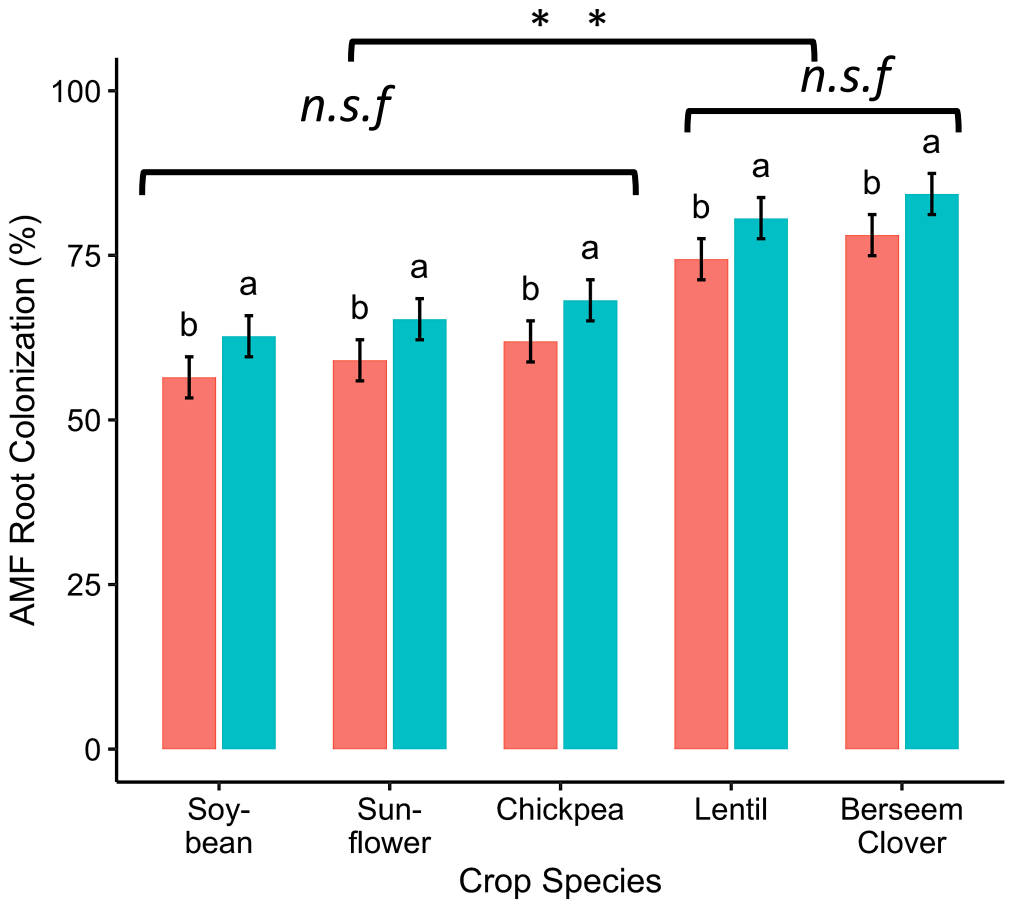


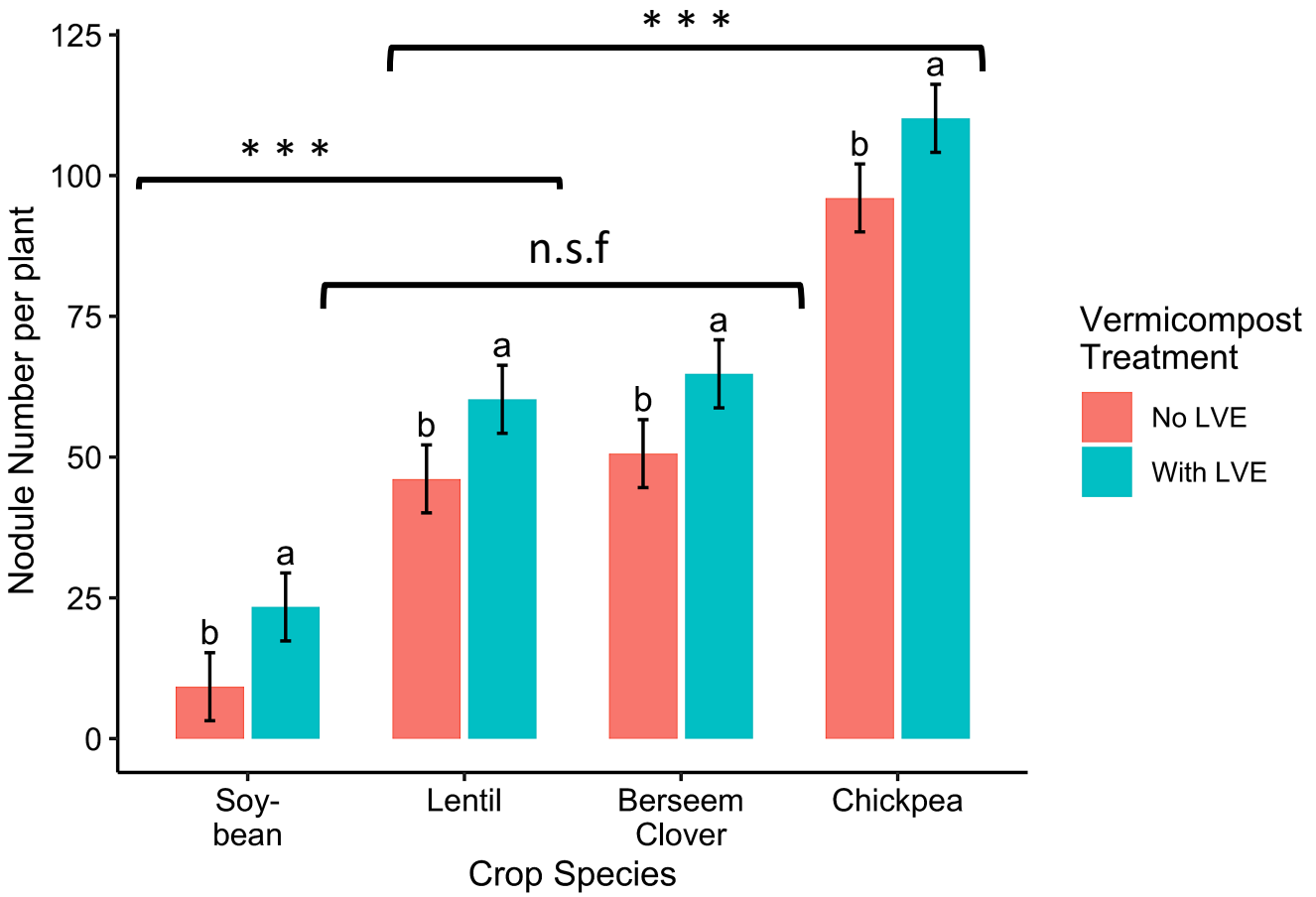
- **There is a general positive increase in soil MIP.**
- **Higher MIP increase in LVE inoculated plots with Sunflower & Berseem Clovers.**

*Significant P- values: * < 0.05, ** < 0.01, *** < 0.001*

08 Results 4 - AMF root colonization and nodulation at flowering stage

- LVE inoculation in **overall increased** AMF root colonization & nodulation; means of **+ 6.24%** AMF & **+ 14.2** nodules, respectively.





*Significant P- values: * < 0.05, ** < 0.01, *** < 0.001, n.s.f not significant at 0.05*

Conclusions

- ✓ The liquid vermicompost extract (LVE) had a high bacterial and fungal diversity.
  - The genera/species identified have been found in vermicompost and agricultural soils by other researchers and are known to induce plant growth promoting traits and disease suppression.
- ✓ LVE inoculation significantly increased soil MIP, legume AMF root colonization and nodulation.
  - Plant-vermicompost-soil interactions could have played a key role in promoting root colonization of indigenous AMF and rhizobia communities.

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