The interaction of MOLTRO, a new product based on *Bacillus atrophaeus* and plant-derived organic compounds, with the microbiota of a natural soil in presence of tomato plants

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INTRODUCTION

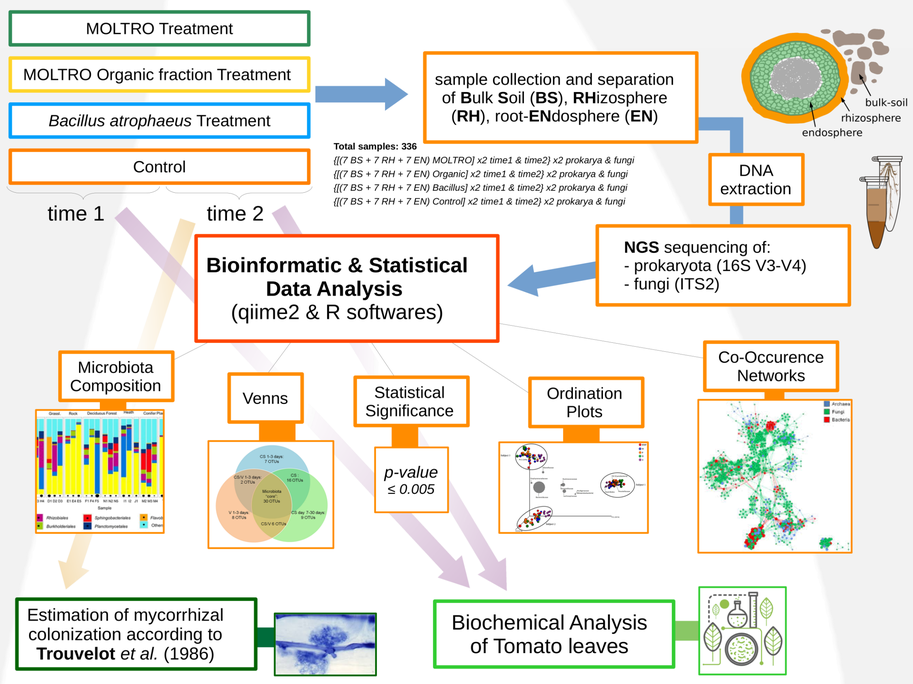
MOLTRO is a liquid product based on the acknowledged plant growth-promoting bacterium *Bacillus atrophaeus* ($3*10^8$ CFU) and plant-derived organic compounds such as phenols, fatty acids, glicynbetaine and peptides. MOLTRO has been formulated to maintain the fertility of soil, positively influencing the plant nutrients availability and the efficiency of their use. Its application on different horticultural crops showed beneficial effects on plant growth, increased chlorophyll content, nutrient use efficiency and yield.

AIM

For an effective and sustainable usage of MOLTRO, we investigated whether and to which extent its application can influence the structure of the microbial communities that thrive in soils, and in particular those living in strict association with plant roots (i.e. the rhizosphere).
We focused on:

A. characterizing the impact of MOLTRO both in complete formulation (MO) and separated in its organic (OR) and microbial components (BA), on the microbial diversity of an agrarian soil through molecular metabarcoding techniques
B. evaluating the effect of MOLTRO on the arbuscular mycorrhizal fungi (AMF) naturally present in an agrarian soil, or added as an AMF commercial inoculum
C. verifying the molecular traceability of *Bacillus atrophaeus* RIZ901 (microbial component of MOLTRO) in soil and roots of the treated plants, using quantitative Real Time PCR (qPCR)

METHODS



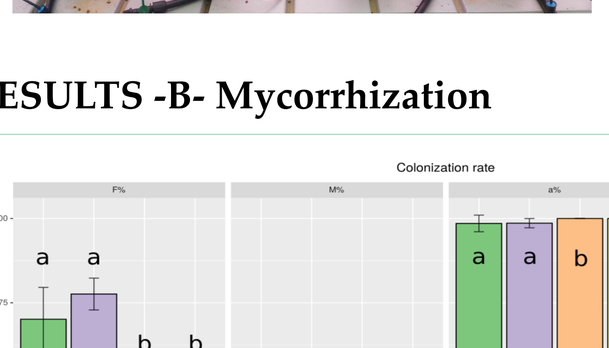
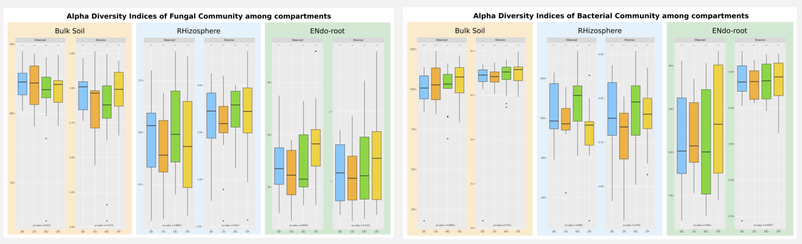


Fig.1 - trial carried out in the Green Has Italia greenhouse. The plants were grown in pots containing agrarian soil for the microbiota study. Whereas for the influence of MOLTRO on mycorrhization evaluation, plants grew on pots containing sand.

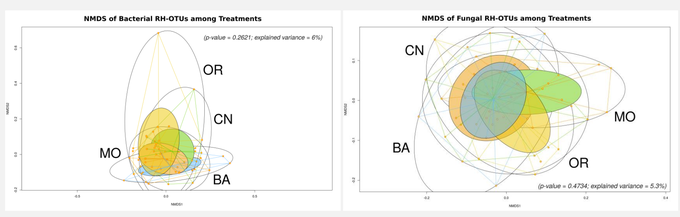
RESULTS -A- Microbial diversity

α-diversity index



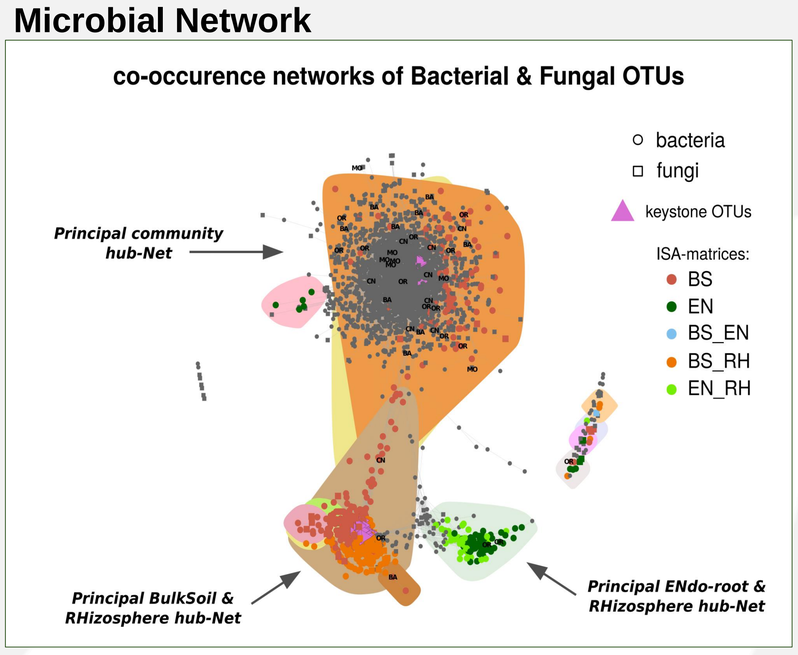
Barplots of α-diversity indexes (Observed and Shannon) illustrate the composition of bacterial and fungal communities for each compartment (BS, RH, EN). Tukey's HSD (honestly significant difference) test were conducted among the 4 conditions tested, and no significant differences were observed (p-value > 0.05), except for the biologically not meaningful case of fungal "Observed" richness in the EN compartment

ß-diversity index



NDMS ordination (β-diversity) confirmed the absence of significant differences in bacterial and fungal communities for each matrix (BS, RH, EN) among the 4 conditions (BA, CN, MO, OR).
The above plots present NMDS for RH, where most plant-microbe interactions occur. PERMANOVA analyses corroborate the NMDS ordinations with no significance (p-value > 0.05)

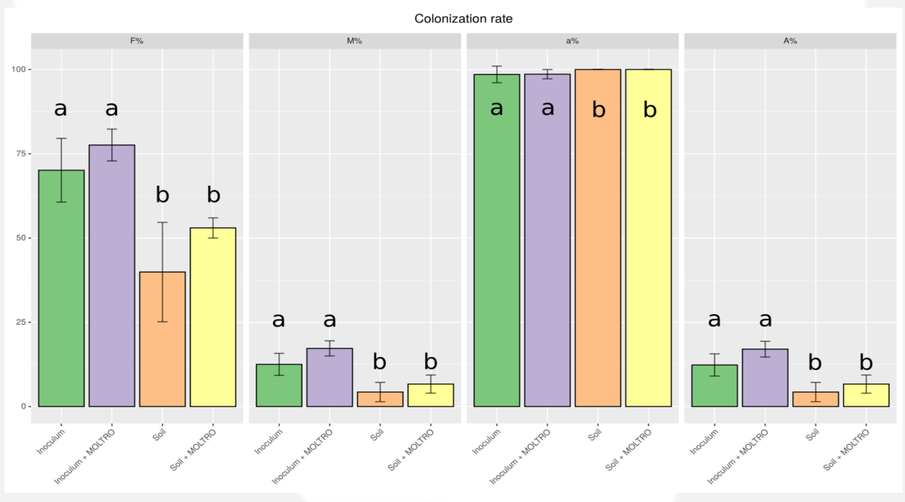
Microbial Network

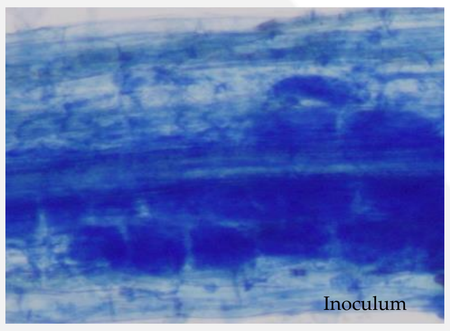


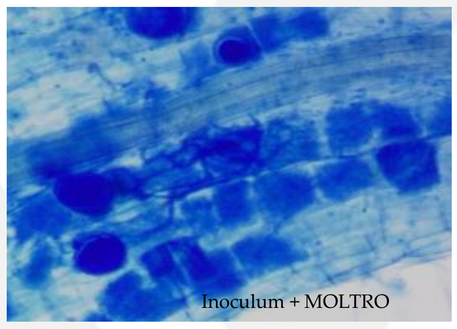
The co-occurence network between bacterial and fungal communities, shows three main hub-nets.
Principal Community hub-net of the whole bacterial and fungal co-occurence, revealed no significant compartment- or thesis-related sub-nets.
Principal Bulk-Soil-Rhizosphere and Principal Rhizosphere-Endoroot hub-nets, by contrast, revealed relations between compartments, as highlighted by Indicator Species Analysis (ISA, colored dots). Thesis-related ISA are indicated with acronyms on the plot.
In more detail, OR-ISA bacterial taxa belong to EN and BS_RH matrices, indicating that the organic component of MOLTRO could positively influence on the bacterial composition of both compartments.
BA-ISA taxa belong to the BS matrix, but within a sub-net module, suggesting a positive interaction between *Bacillus atrophaeus* inoculation in BS and Soil-Rhizosphere community enhancement.

Keystone species (pink triangles), that frequently co-occur with many others and may therefore play an ecologically important role in microbiome dynamics and functioning, were identified in the "Principal Community" and "Principal Bulk-Soil-Rhizosphere".

RESULTS -B- Mycorrhization

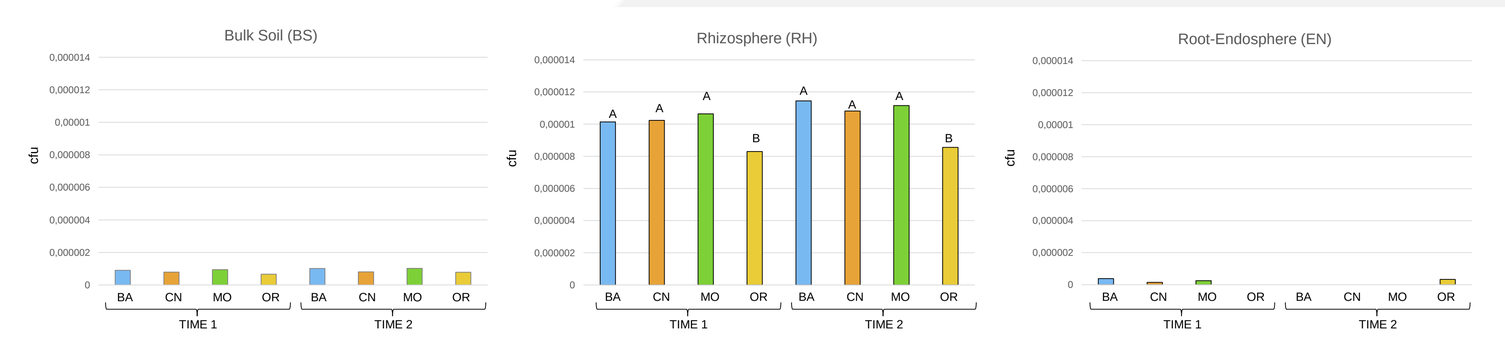






MOLTRO has no negative effect on the extension of tomato root colonization by native or inoculated AM fungi.
On the contrary, the highest mycorrhization intensity (albeit statistically not significant) **are recorded under MOLTRO treatment.**

RESULTS -C- Traceability *Bacillus atrophaeus*



Bacillus atrophaeus was abundant in Bulk Soil and Rhizosphere but not significantly detected in root-Endosphere
Bacillus atrophaeus is much more abundant in Rhizosphere than Bulk Soil (letters indicate statistically significant differences).
Bacillus atrophaeus population in Bulk Soil and Rhizosphere does not increase upon inoculation either as pure culture or as MOLTRO at any time point.

CONCLUSIONS

Overall, our results indicate that repeated MOLTRO treatments - while improving plant growth and yield - have no negative effect on the composition or functionality of soil microbial communities at any level, from bulk soil to rhizosphere and endosphere, including AM symbiosis. As expected, the rhizosphere (root-soil interface) was confirmed to be the niche where most of the microbes thrive and interact in agrarian soils, also under our pot-based experimental system. The low impact on soil microbiota is further confirmed by *B. atrophaeus* short permanence in soil, as revealed by our molecular tracing analyses.
In conclusion, MOLTRO emerges as an environmentally-friendly product that is able to preserve the natural microbiota diversity in the soil and therefore its fertility, inducing a positive effect on yield and on the efficiency of nutrient use in shortage conditions - for more information visit the poster "The efficacy of MOLTRO, a new product based on *Bacillus atrophaeus* and plant-derived organic compounds on the mitigation of nutrient shortage effects".