Standardized metabarcoding pipeline for soil microbiome analysis on MinION platform

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The comprehensive characterization of soil microbiomes is pivotal for understanding ecosystem functions and evaluate soil health practices. In the frame of WP1 and WP4, the GAIA project aims to develop a workflow, from samples stabilization with specific solutions to a metabarcoding pipeline utilizing universal genetic markers, such as the 16S rRNA for bacteria and 18S rRNA or ITS for fungi, with the **possibility to zoom in specific taxa by adding more resolutive markers** (e.g., H.P.M.E markers – patent EP3488015B1) targeting specific taxa (e.g., *Bacillus* genus) or any critical metabolic function. Libraries sequencing is conducted on short reads platform (Illumina) and on MinION platform (long reads), **enabling easy and fast data acquisition and analysis** on the developed pipelines.



Results

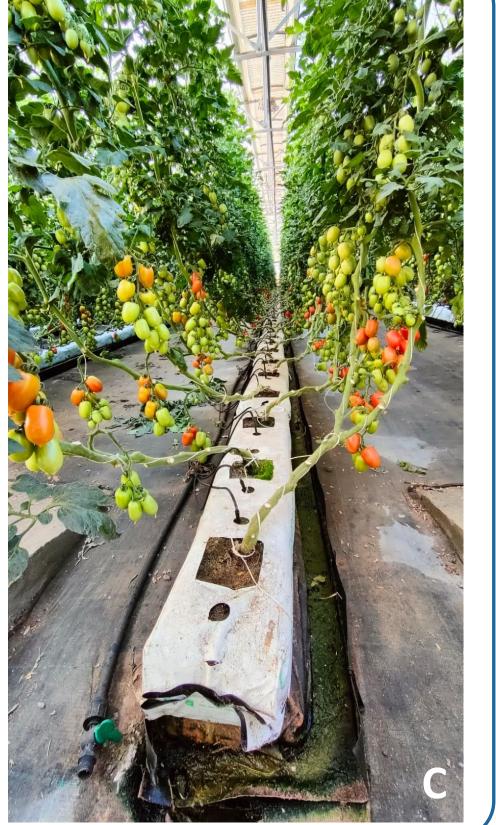
The research incorporates two distinct series of soil samples: one from wheat fields (144 samples) and another from greenhouse-grown tomatoes (45 samples), both cultivated in Sicily. Moreover, a third series of soil samples (36) treated with *Bacillus* containing biostimulant will be characterized. These diverse agricultural settings provide a broad spectrum of microbial communities, to test the robustness and applicability of the metabarcoding pipeline.

Conclusions and perspectives



Pictures showing the on-field trial of wheat intercropping and greenhouse trial with tomatoes in Sicily: A) intercropping wheat/forage legume field trial set up 12 December 2024 (minimum tillage vs conventional tillage); B) Intercropping wheat/*Medicago polimorpha* in minimum tillage; C) greenhouse trial evaluates the effect of different citrus sewage sludge doses and water content concentrations on tomato growth.





The establishment of a standardized metabarcoding workflow and a qualified database of high-resolution markers on the MinION platform, represents a significant advancement in soil microbiology. This framework not only enhances our understanding of microbial diversity and function but also offers the possibility to apply qualified data for AI training to further improve soil health on a global scale.

By applying machine learning techniques to metabarcoding data, this approach promises to uncover novel correlations between microbial communities and soil conditions, potentially leading to innovative strategies for soil management and rehabilitation.



"GAIA" PROJECT

AIM: To study the impact of regenerative agriculture techniques, to increase sustainable productivity and promote resilience to climate change; to increase the availability of natural resources and reduce waste; to improve soil fertility and biodiversity in the agroecosystem, including microorganisms.

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