PhD School on Agriculture, Environment and Bioenergy

(http://sites.unimi.it/dottorato_aab/)

(XL cycle, 2024-27)

Project draft

1.Field of interest

*Indicare **il/i** settore/i scientifico disciplinari:* AGRI-06/B - Chimica agraria

2.Project title

Microbial consortia and bio-based fertilizers: A synergy towards circular economy

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4.Relevance of the topic and state of the art:

Bio-based fertilizers (BBFs) recovered from animal manure are promising products to optimize resources recovery and generate high agricultural yields. However, their fertilization value may be limited and it is necessary to enrich BBFs with microbial consortia to enhance their fertilization.

Different soils have different levels of efficiency in carrying out different soil functions, and certain farming practices should be targeted where more soil functions can thrive (Schulte et al., 2014). Synthetic fertilizers are commonly used to sustain the always increasing food demand, however they are produced at high energy costs and greenhouse gases emissions. An important alternative to synthetic fertilizers is found in "RENURE" (REcovered Nitrogen from manURE) products that have the advantages of being produced from manure which becomes, through anaerobic digestion followed by nutrient recovery techniques a source of renewable energy while reducing emissions and producing fertilisers in the form of nutrient-rich digestate (Huygens et al., 2020; Vaneeckhaute et al., 2017).

Overall, biological activation has been shown to enhance the fertilization performance of BBFs, increasing yields and improving the nutritional values of the crops (Clagnan et al., 2023; Reuland et al., 2022). In the near future, biologically activated BBFs could completely replace synthetic fertilizers. However, full-scale application of biological activated BBFs (to meet Circular Economy Strategy objectives) and the development of new techniques to use microbial activation on-field, especially in combination with BBFs application, represents the main objective of future studies. Additionally, plant-growth promoting (PGP) microorganisms-based

fertilizers are thought to be of high importance as key mechanism of N acquisition and through their expected long-lasting effects on soil fertility.

Within this project, custom organic-based fertilisers enhanced with plant-growth promoting (PGP) microorganisms will be tested and soil-plant microbiome characterised with particular focus on the N cycle, therefore moving forward the development of high-quality organic fertilisers and their validation oriented toward optimal and sub-optimal environmental ranges.

5.Layout of the project (draft)

5.1. Materials & Methods:

The project will follow this experimental design:
1)   <u>Fertilizers and bio-stimulants selection</u>
Biobased fertilizers from multiple sources (e.g. various animal slurries and manures) and treated through on site technologies will be selected and collected. PGP microorganisms (e.g. *Azospirillum*, *Sinorhizobium*, *Trichoderma*) will be further chosen to use in combination with the organic fertilizers. Different combination of microorganisms and biobased fertilizers will be set up.
2)   <u>Controlled conditions experiments</u>
The resulting biofertilizers will be chemically characterised then pot-tests will be carried out on multiple agriculturally relevant plants (e.g. tomatoes, strawberries, lettuce) under a greenhouse to assess the efficiency of these fertilizers in promoting plant growth and nutrient uptake: difference in plant growth, fruit production and differences in organic compounds (e.g. polyphenols).
3)   <u>Microbial characterisation</u>*
Microbial communities will be assessed in depth for soil, rhizosphere and roots:
a.   qPCR will be used to quantify the PGP microorganisms within soil, roots and rhizosphere in order to understand if these bio-stimulants are present and thriving therefore possibly pushing plant growth.
b.   Next generation sequencing analyses will be used to understand variations in the microbial communities possibly led by the PGP microorganisms.
c.   qPCR will be again used to quantify N-cycle related genes and identify the main roads through which N is used within the system (e.g. bacterial denitrification genes (*nirS*, *nirK*, *nosZ1* and *nosZ2*), nitrification (*amoA*), anammox (*hzo* cluster 1) dissimilatory nitrate reduction to ammonium (*nrfA*) and fixation (*nifH*)).
4)   <u>Field validation under real conditions</u>
When the outcome from the previous three points is positive and a biofertilizer is deemed effective in enhancing plant growth, the testing on of the biofertilizer could be reproduced under real field conditions.

* The techniques that have been selected for the microbiome characterisation within this project are 16S rRNA (for bacteria) and ITS (for fungi) rRNA genes next generation sequencing (NGS) together with quantitative real time polymerase chain reaction (qPCR) of the N-cycle genes.
The rRNA genes NGS provides an efficient way to analyse microbial community structure (Edgar et al., 2020) as it enables to identify and characterise the communities in terms of most abundant genera/species (taxa summaries), diversity measures, networks and potential functions. Whole

genome analysis could be further added as a technique for a selected set of samples, as this provides a direct link between microbial identity (as determined by analysis of 16S rRNA gene sequences) with potential function (as determined from the presence of genes encoding specific enzymes), however this approach is generally impractical for soils given their great microbial diversity (10^6 different 16S rRNA for soil gram) (Gans et al., 2005; Quince et al., 2008).

Additionally, a subset of genes will be used as suitable proxies to quantify the variation in the potential dominant pathways of the N-cycle (using 16S rRNA gene abundance as measure of total microbial abundance) despite the problem of primer bias being exacerbated with functional genes (Osborn and Smith, 2005).

5.2. Schedule and major steps (3 years):

Year 1 - Development of the microbial consortia and review and characterisation of the BBFs.

Year 2 - Green house pot experiments and characterization of the effects of the microbiologically activated BBFs (applied in different formulations) on 1. plant (yield and fruit quality), 2. rizospheric community, 3. nitrogen cycle within the soil.

Year 3. – On field trials.

6. Available funds (to support research): 100,000 Euro – Adani

7. Co-Financing (to support the bourse):

8. Literature:

1.   Clagnan E., Cucina M., De Nisi P., Dell'Orto M., D'Imporzano G., Kron-Morelli R., Llenas-Argelaguet L., Adani F., 2023. Effects of the application of microbiologically activated bio-based fertilizers derived from manures on tomato plants and their rhizospheric communities. Sci Rep. 18, 13(1), 22478

2.   Huygens, D., Orveillon, G., Lugato, E., Tavazzi, S., 2020. Technical Proposals for the Safe Use of Processed Manure above the Threshold Established for Nitrate Vulnerable Zones by the Nitrates Directive (91/676/EEC). JRC, Ispra, Italy.

3.   Reuland, G., Sigurnjak, I., Dekker, H., Sleutel, S., Meers, E., 2022. Assessment of the Carbon and Nitrogen Mineralisation of Digestates Elaborated from Distinct Feedstock Profiles. Agron. 12, 456.

4.   Schulte, R.P.O., Creamer, R.E., Donnellan, T., Farrelly, N., Fealy, R., O'Donoghue, C., O'hUallachain, D., 2014. Functional land management: A framework for managing soil-based ecosystem services for the sustainable intensification of agriculture. Environ. Sci. Policy 38, 45-58.

5.   Vaneeckhaute, C., Lebuf, V., Michels, E., Belia, E., Vanrolleghem, P.A., Tack, F.M.G., Meers, E., 2017. Nutrient Recovery from Digestate: Systematic Technology Review and Product Classification. Waste Biomass Valoriz. 8, 21–40.