The effect of digestate on indigenous soil microbial communities and mesofauna diversity and activity

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Biogas production plants produce a vast amount of residue by-product through the anaerobic digestion process called digestate. This residue is a digested material with no market that needs to be properly managed or disposed in order to prevent any limitations on the advancement of anaerobic digestion systems. The addition of digestate to the soil, which has advantages for agricultural and ecological development, is seen as an acceptable choice since legislative trends in the field of waste management are focused on integrated management, adding value to these by-products (Directive, 2008/98/EC). Furthermore, the recycling of digestate has favorable impacts on mineral resources conservation, mitigating climate change and maintaining soil health. In turn, soil quality is linked with the soil microbial communities since they actively participate in nutrient cycling, plant development and disease prevention. However, conventional chemical fertilizers are believed to have a significant negative impact on the intrinsic soil microbial abundance and composition and have been linked to a decrease in soil organic matter (Kibblewhite et al., 2008). The enzyme activity associated with the soil microbial communities is also one of the key elements that needs to be addressed when evaluating soil quality (Leno et al., 2021). Although digestates have frequently been proven to be effective fertilizers or amendments, their effects on the biodiversity and microbial activity of the soil are still unknown, thus, it is still uncertain if their applications are exclusively beneficial. In order to holistically evaluate the ecological status of the soil, the determination of some microbiological and biochemical indices is necessary. To date, few studies have utilized molecular and biochemical approaches to compare changes in soil microbial populations after applying digestate and conventional fertilizer. Notably, even less research has been conducted involving nematodes and the soil fauna (Karimi et al., 2022). Regarding diversity and richness, nematodes are among the most prevalent metazoan in the soil biota (van den Hoogen et al., 2019). They are widely used as biological markers of both soil health and quality because their abundance and diversity reflect the soil's physical and chemical conditions as well as the microorganisms that inhabit it. In addition, predator nematodes which are responsive to environmental changes can be used to assess the effects of soil fertilizers (Lu et al., 2020). In this study, we aim to evaluate in depth the effect of digestate application on the field in comparison with a conventional fertilizer.

For this purpose, field plots with a surface of $10m^2$ per treatment will be installed following a randomized design. Three different treatments -with three replicates each- will be examined and compared in total: fertilization with conventional fertilizer, fertilization with digestate, and without fertilization. Soil samples will be collected before the application of fertilizer and a physicochemical analysis will be performed. A fertilizer recommendation will be generated based on the results and the restrictions permitted by European and National legislation,. The digestate will be collected from a local full-scale biogas plant (Biogas Lagada S.A) operating in mesophilic conditions. Both fertilizing materials will be manually distributed to the plots and quickly integrated into the soil with a rotavator to achieve homogeneous dispersion and prevent nitrogen volatilization. After the application, all plots will be drip irrigated using the same amount of water for 2-3 weeks. This stabilization period is required for soil organisms to assimilate nutrients and will allow changes of microbial and nematode community composition and activity to occur. Soil samples will be collected (0-20 cm depth) in triplicates from each plot and will be analyzed further. In order to determine physicochemical properties, the mechanical composition, pH, electrical conductivity, organic carbon concentration, macronutrients and trace elements will be measured. Furthermore, since the proposed soil fertilizer is produced through a biological process, it is essential to investigate its effect on indigenous microbiota. Therefore, high throughput 16S rRNA and ITS amplicon sequencing will be performed to map the soil's bacterial and fungal profile using the Illumina MiSeq platform. Enzyme activity of acid phosphatase, β-glycosidase and urease -representative for P, C and N cycle, respectively- will also be measured using a colorimetric assay. Soil nematodes will be extracted from each sample and their abundance will be determined using a stereoscope. After identification down to the genus level using light microscopy, nematode genera will be categorized into trophic groups and allocated along the colonization–persistence gradient (c–p values).

Upcoming results will shed light on this topic and help to deeply investigate the effect of digestate application on the soil, not from a fertilization perspective, but in terms of microbial diversity and activity. Furthermore, results from soil nematodes composition – a neglected soil taxonomic group- will aid in drawing conclusions regarding the ecological impact of biogas digestates on these soil biodiversity niches.

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