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Potential of using digestate to regenerate soil and stimulate its biological life

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Abstract. The future of humans and our planet, and food security, require innovative insights across many sectors of the economy (industry, agriculture, forestry, science and technology development). Reducing the use of chemicals, recycling carbon and recovering nutrients, caring for soil health, producing healthy food and adapting to climate change are the main challenges facing modern agriculture. The high proportion of soils low in organic matter, combined with manure shortages in some regions of Poland, poses a serious problem for maintaining the soil's ability to perform productive and environmental functions. The use of selectively collected biodegradable waste, which contains significant amounts of organic matter, can be a key strategy for supplementing soil organic matter deficits. Green waste, kitchen waste, plant biomass produced in agriculture are valuable materials that, when processed through energy production,should become biofertilisers in line with the circular economy. Soil micro-organisms play an important role in the decomposition of organic matter and participate in the circulation and provision of nutrients to plants. Their role also includes fixing atmospheric nitrogen, stabilising soil aggregates, participating in the formation of soil humus and detoxifying soil from harmful substances present in the soil environment. Research to date confirms that biogas plant digestate can be a valuable fertiliser and has the potential to restore soil biological quality. There are virtually no reports indicating a negative effect of the digestate on the biological quality of the soil, especially when using digestate from agricultural substrates. This fact indicates that the potential of digestate in soil regeneration is significant, given its effects on soil biology, soil carbon and nutrient provision, and soil structure. It is more difficult to assess the impact of the digestate on soil biodiversity, especially the structure of the microbial population, which is strongly dependent on a number of soil, climatic and crop influences. An important aspect this review is the presentation of research needs for the potential of using digestate to regenerate soil and stimulate its biological life.

Keywords: digestate, soil, regeneration, biofertiliser, biodiversity, agriculture, organic matter.

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INTRODUCTION

Climate change, soil degradation and depletion of natural resources are becoming key challenges for crop productivity as well as environmental sustainability in modern agriculture (Tanveer et al., 2017). The answer to the challenges mentioned is the concept of sustainable agriculture. Sustainable management of the natural environment is based on understanding the protection of the natural environment as a condition for ensuring human health and the health of ecosystems, which are inextricably linked (Delany-Crowe et al., 2019). It is estimated that between one and six billion hectares (up to 30%) of land worldwide has been degraded (Nilsson et al., 2016). Land degradation is a significant problem for food security, ecosystem services and biodiversity. The productivity of agricultural land depends on both natural and anthropogenic factors. Soil contains nutrients, organic matter and microorganisms in a natural dynamic equilibrium. Disruption of this equilibrium due to human pressure and improper land use leads to increasing soil degradation (AbdelRahman, 2023) (Fig. 1).

One of the most important elements of sustainable soil use is the maintenance of adequate levels of soil organic matter (SOM). Organic matter has a key role in maintaining a soil's ability to perform its productive, regulatory, and environmental functions. It determines properties such as the sorption and buffering capacity of the soil and the biological activity associated with the soil microbiome and mycobiome. It stabilises soil structure and increases resistance to soil compaction, which can reduce the risk of degradation by water and wind erosion (Johannes et al., 2017). The preservation of soil carbon resources is also important for the role of soils in fixing carbon dioxide from the atmosphere. In addition, organic matter plays an important role in water and nutrient cycling, and stimulating soil and the landscape biodiversity (Craswell, Lefroy, 2001). Increasing SOM content minimises soil erosion and reduces soil desertification processes (Lal, 2004; Reeves,

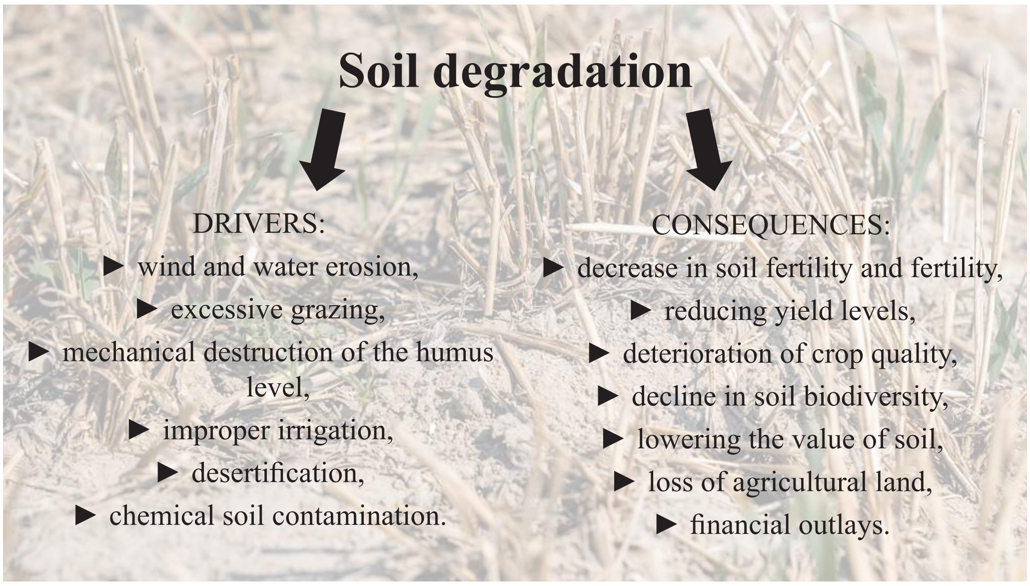

Figure 1. Factors influencing the progressive degradation of soil (own scheme).

1997). Therefore, maintaining or increasing SOM content is key to sustaining agroecosystem productivity and ensuring food security (Schmidt et al., 2011; Stavi et al., 2016).

One way to increase SOM and provide organic matter and nutrients to the soil is to use organic materials, such as digestate (Barłóg et al., 2020; Baştabak et al., 2020). Given the plans to develop the biogas production sector, an increase in waste production in the form of digestate is expected (Ministerstwo Aktywów Państwowych, 2019). Increasing the natural use of biodegradable organic waste can regenerate soils while addressing landfill and gas emissions from biodegradable waste. Increased use of biofertilisers in agriculture based on organic materials, such as digestate, will promote a positive balance of organic matter in soils (Jandl et al., 2023). The effect of this can be to reduce soil degradation by increasing soil carbon stock, maintaining proper soil structure, reusing nutrients from waste, increasing soil biodiversity and reducing erosion.

The presumed agricultural benefits of using digestate to fertilise soils are an incentive for its wider use in agriculture, in line with the circular economy principles. The justification for the use of digestate as soil amendment is certainly the possibility of nature-based management of the by-product of biogas production. The widespread use of digestate can only be proposed if there are no negative effects on soil health and environmental quality. Therefore, there is a need to systematise existing knowledge and the need for further research towards the effects of using digestate in soil regeneration.

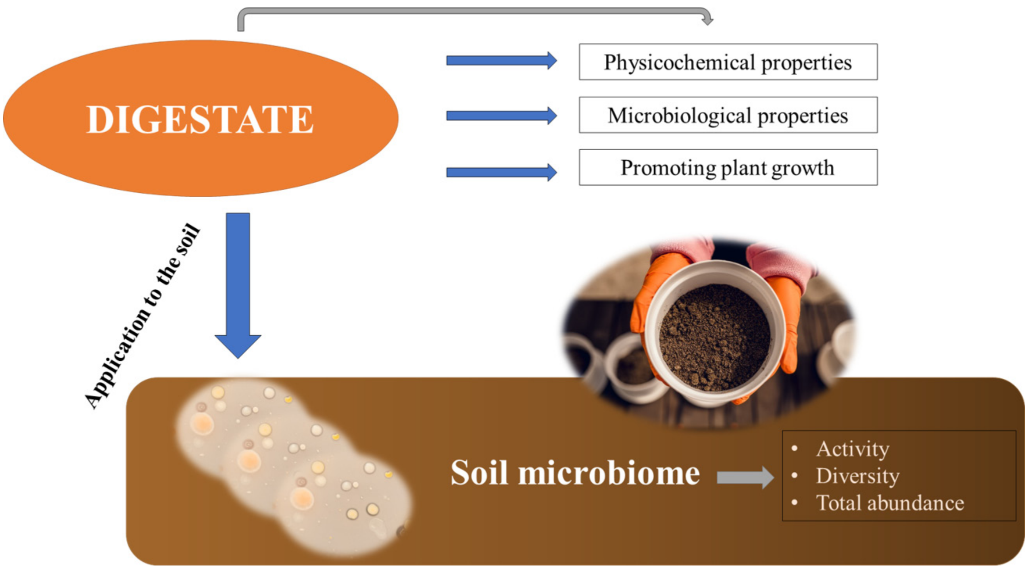

Figure 2. Paths of influence of digestate on soil properties and plant yield (own scheme).

Research into the use of digestate as a fertiliser is relatively new and has focused on the agricultural benefits that digestate can bring, on the one hand, and the associated environmental risks, on the other. Relatively little is known about the impact of digestate on biological properties (e.g. bacterial and fungal communities), which are key factors in soil functioning. There are still knowledge gaps in this area and the field of research is complex, as it has to take into account different types of substrates, crops, soils and climatic conditions (Pastorelli et al., 2021). Available literature data generally report on the level of enzymatic activity of soils after the application of digestate (Nielsen et al., 2020; Siebielec et al., 2018). However, there is a lack of scientific reports on modern methods, including genetic analyses, that enable evaluation of the effects of digestate on microbial populations (Siebielec et al., 2018). The impact of digestate on soil biology is therefore not fully understood (Fig. 2).

The aim of the following literature review was therefore to summarise existing information on the effects of digestate on soil properties, particularly soil biological activity.

DIGESTATE – SIGNIFICANCE FOR THE SOIL ENVIRONMENT

Operating a biogas plant requires efficient and rational management of the by-product remaining after anaerobic digestion. The digestate pulp, is the mass remaining after fermentation which contains organic matter and plant-essential minerals. The digestate is the result of biogas production from various organic raw materials and wastes, among which we can list: plant biomass (e.g. maize silage); waste from the agri-food industry (fermentation, fruit and vegetable residues, beet pulp, out-of-date food); natural fertilisers (slurry, manure), etc. (Czekała et al., 2012; Kowalczyk-Juśko, Szymańska, 2015).

Global biogas production has tripled over the past decade to partially replace fossil fuels, consequently generating significant amounts of digestate (Banaszuk et al., 2015). This by-product of biogas production recycled into the soil for crop fertilisation and carbon sequestration could be an important component of future scenarios of the agriculture.

The benefits of using digestate as a soil additive include the introduction of organic matter into the soil, activation of soil biological life, reuse of nutrients, improvement of soil structure, improvement of soil resistance to erosion, improvement of plant health through stimulation of microorganisms and provision of humic substances (Barłóg et al., 2020; Baştabak et al., 2020; Garg et al., 2005; Siebielec et al., 2018; Siebielec et al., 2020; Simon et al., 2015). Enrichment of the soil with organic matter and an increase in the abundance and activity of microorganisms are among the main advantages of digestate application to soil (Baryga et al., 2021). The digestate is an important source of readily available nitrogen, as a significant part of the nitrogen occurs in the digestate in mineral forms (Kowalczyk-Juśko, Szymańska, 2015). Interest in the agricultural use of digestate is growing, especially in Europe (Scarlat et al., 2018). In 2020, European Commission put forward a new plan for a circular economy (GOZ), which has become a key element of the so-called ‚green transition'. The aim is to take steps to reprocess waste (European Commission, 2020). The recycling of organic matter and nutrients from digestate through its incorporation into the soil is considered the most sustainable use of digestate (Al Seadi et al., 2013; Barłóg et al., 2020; Czekała et al., 2022; Tampio et al., 2016a). The direct application of digestate to the soil is furthermore considered an inexpensive way to utilise by-product and recover nutrients in agricultural systems (Alburquerquea et al., 2012).

The digestate can be derived from a wide range of bio-waste from a variety of sources, it can also be in different forms (solid, liquid (Fig. 3), semi-liquid) and can be subjected to various chemical or physical treatments before being applied to the soil. There are many methods of processing raw digestate, such as dehydration, solid-liquid separation, drying, evaporation, biological processes (e.g. composting, anaerobic digestion) and thermal processes (e.g. direct combustion, pyrolysis or gasification) (Czekała et al., 2022; Logan, Visvanathan, 2019). Depending on the aforementioned factors, the digestate can have variable physico-chemical properties, which, when introduced into the soil, can affect soil biological life, which is crucial for ensuring soil function and soil resistance to degradation (Siebielec et al., 2018).

The digestate is usually dominated by persistent organic compounds such as cellulose, lignin and lipids, which are precursors of humic substances. Organic compounds containing nitrogen and phosphorus are easily broken down and are a source of plant-available nitrogen and phosphate ions (Lorenz et al., 2007). Therefore, digestate can be used as a relatively fast-acting soil additive, more akin to mineral fertilisers than organic additives (Siebielec et al., 2018). The digestate, especially in solid form, also contains significant amounts of organic matter that can increase the soil organic carbon pool, which in turn has a positive effect on microbial activity and improves the enzymatic properties of the soil (Odlare et al., 2011).

Scientific data indicate that digestate is generally a very good fertiliser, providing large amounts of plant-available elements such as nitrogen and phosphorus in a short time, improving the biological properties of the soil and providing high crop yields compared to mineral fertilisers. The average dry matter content of the digestate is usually very low, ranging from 1.5% (liquid digestate) to 46% (solid digestate) (Nkoa, 2014). Previous studies have indicated that the digestate can have a positive effect on both the biological (Simon et al., 2015) and the physical properties of the soil (Garg et al., 2005). It is also noteworthy that the application of digestate has a positive effect on soil struc-

Figure 3. Liquid digestate from an agricultural biogas plant (own photo).

ture and increases the soil water retention capacity (Nabel et al., 2017) (Fig. 2).

The use of digestate as a fertiliser presents a wide range of logistical challenges especially due to its lower nutrient content compared to synthetic fertilisers, especially if the digestate is to be used in conventional agriculture. Urban and peri-urban facilities face challenges in transporting digestate to agricultural regions due to high costs caused by the low nutrient concentration in liquid digestate and the large volumes of digestate required to feed crops (Tampio et al., 2016b). Other constraints on digestate as a source of nutrients include waste and environmental regulations and social, i.e. odour controversies. In addition, digestate is susceptible to significant N losses depending on storage conditions, method of application and environmental conditions during soil application (Nkoa, 2014).

CHARACTERISTICS AND FUNCTIONS OF THE SOIL MICROBIOME

Investigating the effects of the digestate on soil microorganisms is key to assessing the effects of its use, which relates to the vitally important functions of the soil microbiome. Much scientific work in the field of soil microbial ecology in recent years has focused on cataloguing the diversity of soil bacteria and documenting how bacterial communities are affected by ongoing environmental changes or disturbances. As a result, we know that soil bacterial diversity is enormous and that it can be influenced by many biotic and abiotic factors. Given the wide range of mechanisms by which soil microbes can support plants (alleviating stress, providing nutrients through nitrogen fixation and phosphorus solubilisation, facilitating micronutrient uptake) and the remarkable diversity of microorganisms, it is also important to observe their activity following the application of exogenous organic matter to soil in the form of digestate (Siebielec et al., 2018, 2020).

It should be noted that soil organic matter levels and soil biodiversity are closely linked. Progress in understanding the expected level of soil biodiversity depends on our ability to assess changes in biodiversity over time and the influence of different factors (Chase et al., 2018; Estes et al., 2018). Experimental data also suggests that there are strong links between biodiversity, plant health and soil health (Delgado-Baquerizo et al., 2020).

It should be emphasised that microbial diversity is essential in nitrogen and carbon cycles, as it ensures the decomposition of organic matter and the mineralisation of organic forms of these elements, and thus the sustainable functioning of the soil (Maron et al., 2018).

The tasks of soil microorganisms also include fixing atmospheric nitrogen, stabilising soil aggregates, participating in the formation of soil humus and detoxifying harmful substances present in the soil environment. Soil microorganisms are extremely abundant and characterised by high taxonomic and functional diversity (Bertrand et al., 2011). Five overarching communities are responsible for the biodiversity of the soil environment. These include: microbes and microfauna, which are less than 100 μm in size; mesofauna, which range in size from 100 μm to 2 mm; and macrofauna and megafauna, which are greater than 2 mm (Tibbett et al., 2020). These organisms differ in their ap-

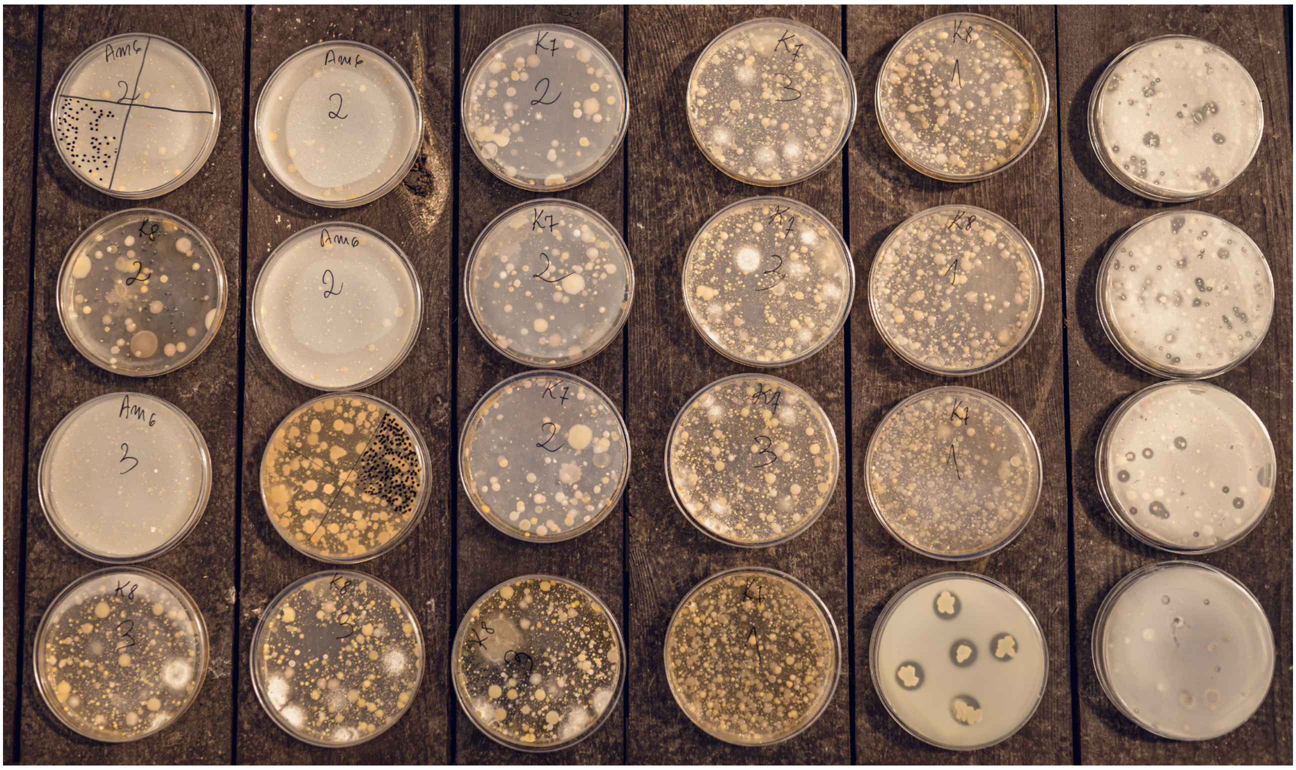

Figure 4. Petri dishes with solid medium showing the diversity and number of bacteria (own photo).

pearance, ability to carry out biochemical transformations, ability to grow in different environments and interactions with other organisms (Six et al., 2004). Figure 4 shows bacteria isolated from the soil environment cultured under laboratory conditions on solid agar medium, includes copiotrophic (Fig. 5) and oligotrophic bacteria and bacteria with the ability to solubilise phosphates (Fig. 6). From literature data, we know that copiotrophic bacteria show high growth rates under conditions of high nutrient availability, while oligotrophs are able to function under nutrient-deficient conditions (Koch, 2001). Ammonification bacteria, on the other hand, play an important role in soil nitrogen metabolism (Paśmionka, 2017). The process of ammonification is crucial for reincorporating nitrogen from plant residues into the soil cycle and converting N from biomass into forms readily available to plants (Strock, 2008). Another important group are bacteria of the genus *Azotobacter*. These bacteria have the ability to fix atmospheric nitrogen (Martyniuk, 2008), and the maximum amount of nitrogen fixed annually by these bacteria is generally several kg ha^{-1} (Nannipieri et al., 2007). These bacteria are quite sensitive to soil properties, especially soil pH. They usually prefer alkaline or neutral soil, and are rarely found in soils with a pH below 6.0 (Martyniuk, Martyniuk, 2003). Although these free-living diazotrophs introduce fairly small amounts of assimilable nitrogen into the soil environment from the point of view of nitrogen levels used in agricultural production, even such amounts of assimilated nitrogen are extremely important in remediation systems where regular nitrogen fertilisation is not applied and soils poor in fertiliser elements. The growth site

Figure 5. Copiotrophic soil bacteria growing on a solid medium with the addition of agar (own photo).

Figure 6. Soil bacteria with the ability to solubilize phosphates (transparent shell around the bacteria, the so-called „halo zone") (own photo).

of these bacteria is the rhizosphere of plants, from which these plants can draw assimilated nitrogen (Martyniuk, 2008). Moreover, these bacteria secrete active substances (auxins, gibberellins, cytokinins, amino acids, vitamins) into the soil environment, which have beneficial effects on plant development (Gonzales-Lopez et al., 1991; Kozieł, 2023). Another important group are phosphate solubilising bacteria, which can convert insoluble phosphorus into plant-available forms (Zhang et al., 2019). Therefore, their presence plays a key role in the transformation and biogeochemical cycling of phosphorus in the soil environment (Yu et al., 2019). In addition to phosphorus, these bacteria also introduce many substances into the soil that stimulate plant growth and resistance, i.e.: siderophores, auxins, cytokinins or vitamins. Their ability to mineralise organic phosphorus and solubilise inorganic and insoluble phosphates varies between bacterial strains and also depends on the chemical properties of the substrate (Parastesh et al., 2019).

Only soils with adequate chemical and physical properties and characterised by high microbial activity can guarantee the right conditions for plant growth and development (Jha et al., 1992; Widiwati et al., 2005). Therefore, proper land use involves the conservation and maintenance of soil biodiversity at the correct level. This is a prerequisite for the stability and resilience of the soil to external disturbances and for providing soil ecosystem services (Thiele-Bruhn et al., 2012).

Microbial parameters are often used as early indicators of changes in soil quality, susceptible to changes in the soil environment and the influence of agricultural practices. Research confirms that soil type and fertilisation practices are two important factors influencing soil microbial activity (Li et al., 2018; Chen et al., 2020).

High soil biological quality, meaning a diverse and abundant microbial community and activity, is a prerequisite for plant growth and, consequently, crop production (Paz-Ferreiro, Fu, 2016). In particular, soil microbial biomass, activity and diversity are indicators of soil health (Singh, Gupta, 2018) (Fig. 2).

For this reason, they are used as indicators of soil quality and health (Doran, Zeiss, 2000). Soil quality is defined as the ability of a particular soil to sustain plant and animal productivity, maintain or improve water and air quality, and support human health and habitat (Karlen et al., 1997). Microbial populations vary according to various factors such as soil type, presence/absence of plants and climate; their responses to fertiliser treatments may therefore vary according to the abiotic factors mentioned above. During the long-term evolutionary process, soil, plants and microorganisms have co-evolved to form relatively stable relationships within a given ecosystem (Mercado-Blanco et al., 2018). Therefore, changes in soil microbial communities induced by environmental changes and the introduction of biofertilisers can undoubtedly affect soil community structure and microbial-plant relationships. Therefore, studying the effects of biofertilisers on the microbial community is crucial.

Given the importance of microbial functions to the soil, understanding how these functions change is important for assessing the impact of applied agricultural practices on the soil ecosystem (Decaëns et al., 2006; Hermans et al., 2023; Thiele-Bruhn et al., 2012). Wider insights into the role of microbial communities using modern research methods will allow the development of important monitoring tools to support land managers in addressing key environmental issues related to agriculture.

IMPACT OF THE DIGESTATE ON THE SOIL AND ITS BIOLOGICAL ACTIVITY

Impact on chemical properties

Digestate is a relatively new type of waste that can be used for soil fertilisation. The most common use of digestate is for fertilising field crops and pastures. However, despite the potential benefits of agricultural use of digestate, there is a need for a comprehensive assessment of its impact on soils and the nutrient use efficiency (Burg et al., 2023).

The use of digestate as exogenous organic matter has many benefits, such as the delivery of waste-borne nutrient replacing mineral fertilisers. In addition, soils increase their carbon stocks, which are important not only for maintaining the productive functions of soils, but also for the soil role in sequestering carbon from the atmosphere and shaping the soil retention properties (Westphal et al., 2016). Organic fertilisers are therefore an important factor in maintaining soil fertility (Möller et al., 2015).

Soil organic carbon (SOC) deficiency can be supplemented from exogenous sources such as digestate, especially in solid form. The organic matter of the digestate then undergoes processes of decomposition and transformation. In the soil, it is decomposed by microorganisms and then transformed to form humic compounds. The carbon introduced into the soil with the digestate represents an additional supply of carbon, which is partly permanently incorporated into the soil organic matter. As reported by Cavalli et al. (2017), the mineralisation (loss) of C from digested manure during the growing season was between 32–34% of the C added to the soil. This was significantly lower than for raw manure (51% of manure C after 181 days). After one season, this pool of C remained in the soil, so it can be assumed that this fraction will be included in the SOC cycle, assuming soil conditions do not change drastically. Similarly, the study by Beghin-Tanneau et al. (2019) shows that no more than 40% of the C introduced into the soil is released as CO_2 during the growing season. Greenberg et al. (2019), on the other hand, showed that the liquid fraction of digestate derived from maize silage and applied to a sandy soil under temperate conditions increases SOC content in soil aggregates compared to the application of mineral fertilisers.

The changes in soil chemical properties resulting from the introduction of the digestate into the soil also have the effect of altering microbiological properties. The interaction between changes in the chemical state of the soil, its water retention properties, and its biological state is very often observed after the supply of digestate to the soil. For this reason, in most cases, the effects of the digestate application should be considered with regard to the interaction between the chemical, water retention and microbiological properties of the soil, the consequence of which is also the response of the crop plants.

Impact on microbiological properties

Climate change and increasingly frequent extreme weather events have a significant impact on crop growth and yield. While prolonged drought causes pronounced yield losses in susceptible crops, it can also have a significant impact on the activity and structure of soil microbial populations and therefore also on the rate of carbon and nutrient cycling processes and greenhouse gas emissions from the soil. It is also reasonable to assume that sudden and significant changes in soil moisture, e.g. heavy rain following a prolonged drought, can significantly affect microbial functionality and microbe-driven processes, including processes that support crop resistance to abiotic stresses. Literature data indicate that periods of drought and associated water stress, followed by rapid rewetting of the soil environment, can have a significant impact on soil biological activity (Report, 2012; Young, Ritz, 2000). It can be assumed that increasing the amount of organic matter through the application of digestate improves the level of water retention in the soil, reducing the negative effects of drought on soil biological activity. Organic fertilisation undoubtedly contributes to increasing the availability of nutrients to plants (Chu et al., 2007), influences the activity of soil microorganisms (Makdi et al., 2012; Siebielec et al., 2018), which in turn improves crop yields (Simon et al., 2015).

Active soil regeneration is an important strategy for restoring soil microflora activity, which is a guarantee of soil fertility and productivity. One possibility is the use of digestate to fertilise soils, which enables the recovery of macro- and micro-nutrients by plants and represents an opportunity to reduce the use of mineral fertilisers produced from non-renewable raw materials. While the scientific literature is quite rich in reporting the effects of digestate on the chemical characteristics of the soil environment, the effects of digestate on soil biology are poorly understood. Karimi et al. (2022), in a review article, assessed the effects of digestate on soil microorganisms based on the number of positive, negative and neutral effects found in 56 scientific articles. The results showed that a large proportion of the studies – 43 to 65% – concluded that there was no significant effect of the digestate on soil microorganisms compared to the control. A positive effect of the digestate was found in 25–41% of the results obtained, while a negative effect was found in 3–17%. At the same time, 17% of the results showed that digestate products stimulated microbial communities to a lesser extent than other organic fertilisers, which was particularly evident for microbial biomass and activity. In summary, these results indicate that digestates were neutral for soil microbial quality in up to half of the cases. However, the authors emphasise that these conclusions should be treated with caution due to the specificity of the soil biological activity studies and the varying conditions of the studies conducted. Instead, these data provide a sound basis for further key studies to assess the benefits of using digestate for soil fertilisation (Karimi et al., 2022).

Cardelli et al. (2018), showed that the use of digestate in agriculture has a positive effect not only on crop production but also on microbial activity in the soil. According to the authors, the introduction of digestate into the soil can change not only the chemical and physical characteristics, but can also affect the biological life of the soil. The ecotoxicological tests carried out suggested that the application of the digestate has a positive or no significant effect on the test organisms (*Microbacterium* spp., *Staphylococcus warneri* or *Pichia anomala*). Therefore, digestate can be an effective material for improving soil biological properties (Stefaniuk et al., 2015). Other studies show that soil microbial biomass and metabolic activity are stimulated by the application of digestate, which is attributed to increased carbon and nutrient availability. In addition, the study concludes that changes in soil microbial properties occur more rapidly than for most chemical properties. This suggests that soil microbial processes may function as more sensitive indicators of short-term changes in soil properties as a result of organic additives introduced into the soil (Odlare et al., 2008). According to other researchers, digestate enhances microbial activity and biomass, not only compared to mineral fertilisation, but in most cases also compared to manure (Insam et al., 2015).

In a three-year rotation of maize and triticale, the short-term effect of the digestate on the physical, chemical and microbiological properties of the soil was studied and its effectiveness in supplementing mineral fertilisers was evaluated. The digestate increased total soil organic C and total N and K contents (Pastorelli et al., 2021). Sampling time, in turn, was the main factor influencing the diversity of soil microbial communities. Therefore, the study showed that the digestate had a transient effect on microbial community structure. Soil microbial communities developed a new equilibrium over time following digestate treatments, thus strengthening the hypothesis that microbial communities are resilient to anthropogenic changes. Agronomic recycling of digestate ensured adequate crop yields and soil quality. It was confirmed that the digestate can be an important element of sustainable soil fertility management in energy crops. The authors point out that further research is extremely important in order to improve the knowledge of the optimal dose of digestate to apply depending on soil and crop specificities and the best application method to minimise the potential negative effects of digestate (Pastorelli et al., 2021).

Another study aimed to evaluate the use of digestate to optimise bioremediation of soils contaminated with petroleum hydrocarbons. The study was conducted on two soils: a clay soil and a sandy soil. After the application of digestate, microbial respiration was enhanced in the sandy soil and inhibited in the more compact soil due to aggregate formation. In addition, the application of the digestate to the soil resulted in the development of distinct microbial groups. The overall conclusion of the study indicated the potential of the digestate as a source of nutrients and bacteria for soil bioremediation (Gielnik et al., 2019).

The aim of the work by other researchers was to compare the effects of the addition of food waste digestate and urea-ammonium nitrate liquid solution on plant growth, rhizosphere bacterial community composition and diversity, and mycorrhizal fungal (AM) counts. Both types of fertilisers significantly increased plant growth. In addition, the digestate significantly increased the density of AM fungal hyphae. The application of both fertilisation variants significantly changed the composition of the bacterial population. The application of digestate had less effect on soil microflora diversity and function than liquid urea-ammonium fertiliser, the community composition and functional genes involved in N and C metabolism changed over time. This study showed that the use of digestate as a soil additive can provide a balanced nutrient supply, and increase soil organic C content (Ren et al., 2020).

The possibility of combining digestate and biochar in organic fertilisation of soils and their interactions is of interest to researchers (Fig. 7). In a study by Bede et al. (2022) such a mixture of exogenous matter was used in a pot experiment. Tomato (*Solanum lycopersicum* L.)

Figure 7. Biochar (own photo).

plants were grown for 45 days in a medium enriched with five doses of food waste digest with and without biochar. At harvest, plant growth parameters and soil characteristics from the pot experiment were measured. The bacterial community of the rhizosphere was profiled by amplicon sequencing followed by analysis of putative functional genes. The amount of biomass and N content of the shoots increased with the addition of digestate proportionally to the dose of digest. Biomass increases were slower when the digestate was combined with biocarbon, but both treatments produced similar final shoot yields. The use of biochar reduced the mineral nitrogen content of the soil, which may reduce the risk of nitrogen leaching from the soil when using digestate. The rhizosphere bacterial population structure differed significantly for the variants with and without biochar (Bede et al., 2022).

In contrast, another study assessed the effect of biochar encapsulated in digestate and other additives in the form of exogenous organic matter based on plant growth, the characteristics of a given soil after additive application, nutrient leaching and the soil microbiome. The results showed that the tested fertilisers and soil additives, i.e. digestate, compost, commercial fertiliser, biochar encapsulated in digestate, had a significantly positive effect on the test plant. In particular, this effect was found after the application of biochar encapsulated in digestate. It had the best efficiency, as evidenced by a 9–25% increase in chlorophyll index, fresh weight, leaf area and flowering frequency. Regarding the effect of fertilisers or soil additives on soil properties and nutrient retention, biocarbon encapsulated in the digestate leached the least nitrogen (<8%), while compost, digestate and mineral fertiliser leached up to 25% nitrogen. Based on the study, the authors concluded that, according to the microbiological analysis, biochar encapsulated in the digestate has a comparable role with compost in improving soil resistance to pathogen infection. The combination of metagenomic studies, i.e. metagenomics with qPCR analysis, allowed the conclusion that this biochar – digestate combination accelerates the nitrification process and inhibits the denitrification process (Yan et al., 2023).

A study by Doyeni et al. (2023) compared the role of different digestate products (chicken manure digestate, cow manure digestate and pig manure digestate) on spring wheat productivity, soil microbial activity and greenhouse gas emissions in clay and sandy loam soils under controlled conditions. The addition of the digestate had a positive effect on plant yields, especially in the sandy loam soil, due to the increased availability of nutrients to plants in the early phase of crop growth and development. A greater effect on soil microbial activity was also found in the sandy loam soil compared to the clay soil. The highest value of microbial parameters, such as dehydrogenase activity and

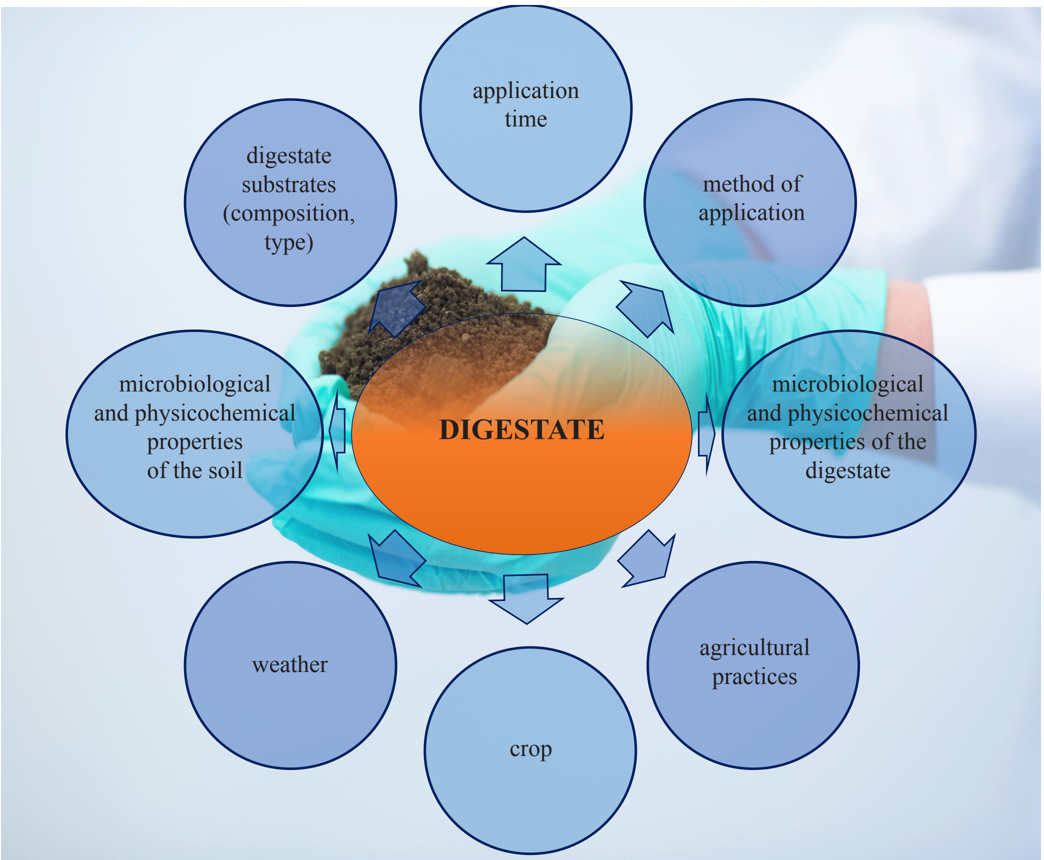

Figure 8. Factors modifying the impact of digestate on the soil microbiome (own scheme).

soil microbial biomass, was found after the application of chicken manure digestate. Overall, the results showed that the two soil types responded differently to the addition of the digestate, and the benefits depended on the soil properties and the type of digestate applied (Doyeni et al., 2023).

A study by other researchers aimed to assess the effect of manure-based digestate application on soil diversity in the cultivation of three different cereals (spring wheat, triticale and barley). Three different types of digestate (based on substrates: pig manure, chicken manure and cow manure) were applied. The soil microbial community was characterised using Illumina MiSeq next-generation sequencing (NGS) technology. This study showed that the most prevalent were the Actinobacteria, Acidobacteria and Proteobacteria, which accounted for more than 55% of the total prokaryotic community. Other phylogenetic groups, such as *Verrucomicrobia* and *Bacteroidetes*, were also identified as an important part of the soil microflora (Suproniene et al., 2022). There was no statistically significant relationship between the type of digest and the diversity of microorganisms, which were instead characterised by high seasonal variability and dependence on soil pH and plant species.

The effect of using digestate as a fertiliser can be characterised by large temporal dynamics. For example, Möller (2015) reported that digestate with high degradability of organic matter has a strong but short-lived effect on soil microbial activity. Similarly, Nielsen et al. (2020) reported in their review article that a greater effect of digestate application on some soil microorganisms (parameters such as metabolic activity and respiration) is observed in the short term. In contrast, Luo et al. (2009) reported changes in microbial community structure in a long-term study, which was also confirmed in other literature reports (Chu et al., 2007; Guo et al., 2019). Changes in the soil microbial community were dependent on climatic conditions. Soil microbial populations are constantly subject to change and need to adapt to environmental conditions, e.g. moisture, nutrient availability and temperature (Bardgett, Caruso, 2020).

The soil microbial community can be significantly altered in response to the exogenous organic matter, but also depending on the timing of soil sampling and the length of the experiment. Therefore, it is important to study in detail and understand the main factors in shaping the physicochemical properties and soil community in the results of digestate application (Fig. 8). Soil biodiversity is therefore a complex concept that is difficult to encapsulate within the strict framework of a single research factor. In turn, a realistic assessment of the role of soil biodiversity in regulating soil multifunctionality is urgently needed to better understand the potential consequences or benefits of changes in soil biodiversity, including as a result of waste organic matter application (Delgado-Baquerizo et al., 2020).

SUMMARY

Studies available in the scientific literature confirm that digestate can be a valuable fertiliser, with significant potential to partially replace mineral nitrogen fertilisation. In an era of environmental care and a closed-loop economy, its use in agriculture can be part of efficient waste management and regeneration of soil health. There are practically no reports indicating a negative impact of digestate on the biological quality of soil, especially when using digestate from agricultural substrates. Research results reported in the literature indicate a positive or neutral effect of the digestate on a wide range of soil microbial activity parameters and diversity. This fact indicates that the potential of digestate in soil regeneration is significant, especially that, in addition to its effect on soil biology, a beneficial effect of this fertiliser on soil carbon and nutrient availability can be assumed. In general, the digestate stimulates the enzymatic activity of soils or the biomass of microorganisms. It is much more difficult to assess the effect of the digestate on soil biodiversity and especially the structure of the soil microbiome. This is largely due to the limited capacity, as yet, of interpreting changes in population structure as a result of biofertiliser application and valuing the benefits of observed changes in soil biodiversity. An additional difficulty is the simultaneous interaction of many other factors shaping microbial diversity, such as soil chemical properties, soil texture, soil moisture, plant influence and temporal dynamics of soil diversity. Further detailed studies involving different types of digestate and diverse soil types are needed to draw complete conclusions on the impact of digestate properties on soil biodiversity (Karimi et al., 2022).

Research needs for the potential of digestate to regenerate the biological quality of soils therefore include the use of methods to assess the genetic or functional diversity of soils in conjunction with changes in other soil parameters and soil ecosystem services, potentially enabling a more holistic valuation of the impact of digestate on changes in soil biological traits.

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