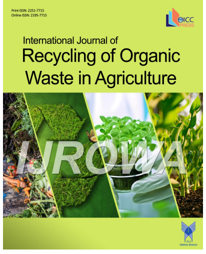

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Digestate biofertilization: a sustainable pathway to increase global soil C content

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Abstract:

Purpose: This paper is aimed at the determination of digestate potential in a long-term carbon accumulation after its application as a biofertilizer.
Method: Literature survey of $>$ 1000 papers was conducted and resulted in a selection of 21 papers that involved data of soil C accumulation after digestate addition for at least a period of 12 months. Meta-analysis was used for data analysis and interpretation of a large database. The results of incorporation of total organic carbon in the soil after digestate biofertilization were measured by one-way analysis of variance (ANOVA).
Results: A comprehensive literature review showed trends for carbon increase in the soil for different experiment periods up to 84 months and initial content of carbon in the soil. It was demonstrated that application of digestate, a byproduct of anaerobic digestion, to agricultural soils resulted in an increase of soil carbon content for a period of up to 8 years. Specifically, digestate derived from cow and pig manure had the highest potential to enhance soil carbon accumulation compared to digestate from other organic residues including food waste and sewage sludge, highlighting the need for a proper choice of the waste substrate used in anaerobic digestion. Soil carbon accumulation is notably more pronounced when digestate is applied to soils with low organic matter content, particularly sandy and loam soils.
Conclusion: While digestate application to soils is typically used to substitute mineral fertilizers, it also leads to an overall increase in soil carbon content.

Keywords: Biofertilizer; Climate impact; Food security; Organic matter; Soil carbon; Soil restoration

1. Introduction

Soil degradation due to the intensive agriculture and subsequent global loss of agriculture soil carbon (C) stocks have been reported as a worldwide problem (Kopittke et al., 2019; Amelung et al., 2020). Agricultural use of soil leads not only to soil degradation, but also to loss of organic matter and fertility, increased production costs, maintaining of soil productivity, and contributes to CO_2 emissions (Pezzolla et al., 2012). Moreover, the engineered farming system based on the application of chemical fertilizers and pesticides doesn't fit sustainability to principles according to EN Agenda 2030, and therefore should be replaced by organic fertilizer or biofertilizer. Organic farming concept assumes an increase of the percentage of organically farmed land up to 25% in the EU till 2030 according to the European Green Deal initiatives (Kowalska and Bieniek, 2022). Angouria-Tsorochidou et al. (2023) identified anaerobic digestate that is a leftover from biogas reactors to be a perspective candidate among possible biofertilizers. Full or partial substitution of mineral NPK fertilizer by biofertilizer from digestate has a real potential for the improvement of chemical properties of soil, growth characteristics of plants and crop yield (Mahmoud et al., 2023; Kumar et al., 2019). Sustainable approaches, for instance, in agricultural production, could increase the resilience of soil quality in the face of climate change.

On the other hand, Lynch et al. (2021) has reported that agriculture is an economic sector with a great potential to mitigate the atmospheric concentrations of greenhouse gases (GHG) that is in accordance with the Intergovernmental Panel on Climate Change (IPCC) focus. Soils play an important role in mitigating and adapting to climate change (Tóth et al., 2018). Carbon accumulation can be achieved through soil carbon sequestration during photosynthesis, and the predominance of the humification process over the mineralization process of soil organic matter (SOM) resulted in the increasing of soil organic carbon (SOC) levels over time (Don et al., 2023). The fluxes of GHG can be reduced by more efficient management of carbon and nitrogen (N) flows in agricultural ecosystems (Foereid et al., 2021; Reuland et al., 2022). Significant amounts of soil carbon could be stored using appropriate sustainable agricultural techniques such as digestate biofertilization instead of mineral fertilizers use and crop rotation system leading to prevention of soil depletion, improvement of soil quality parameters, increasing of crop yield and accumulation of carbon in the soil due to the photosynthesis intensification (García-López et al., 2023). Additionally, agricultural residues were effectively used as a substrate for bio-fuels production, avoiding GHG emissions from its combustion (Chubur et al., 2022; Aduba et al., 2023). Moreover, anaerobic digestion (AD) has been reported as promising technology for waste management by generating green energy and allowing the recovery of elements (Holm-Nielsen et al., 2009). This technology has several advantages contributing to the achievement of some of the 17 Sustainable Development Goals (SDGs) (Ablieieva et al., 2022a; Obaideen et al., 2022).

Thus, an appropriate application and valorization of anaerobic digestate in agricultural systems is a significant approach in terms of reducing the use of mineral fertilizers, which leads to positive results, namely decrease of energy costs and consumption of mineral resources for its production, preventing of GHG emissions and carbon storage in the soil with resulting climate change mitigation, as well as maintaining of soil quality.

The topic of digestate application as biofertilizer and carbon accumulation in the soil was found with a huge importance among the researchers all over the world during the period of 2009 – 2023 based on the literature survey performed using Web of Science Core Collection database. The number of papers has been raised from 11 in 2010 to 125 in 2023, at the same time, the publications were covered by 5 main fields of knowledge according to the Web of Science Categories, among them category Environmental Sciences prevailed (47% of total publications). Such categories as Engineering Environmental, Energy Fuels, Agronomy and Soil Science involved from 15% to 13% of publications, consequently, for the whole period of the research search

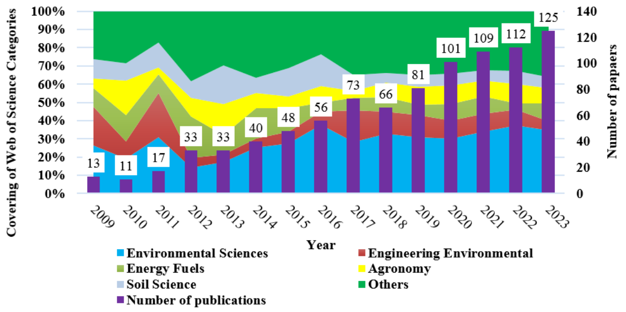

Figure 1. Dynamics of publications activities and distribution of identified Web of Science Categories referred to the topic “Digestate application as biofertilizer and carbon accumulation in the soil” for the period 2009 – 2023.
(based on Web of Science Core Collection database searching tools).

(Fig. 1).

The distribution of paper numbers among different countries showed that Peoples Republic of China and Germany are leaders in the research of this topic (Fig. 2). Italy, Poland, and Sweden were following by them, but number of papers were less by 2 – 3 times. At the same time, research findings showed that number of papers within Research Area Environmental Sciences Ecology has been increasing since 2020, and Agriculture Research Area covering was still constant. This tendency highlighted the importance of the topic related to digestate biofertilization and its potential for carbon storage in the soil in terms of environmental and agricultural issues.

A bibliometric network and keywords maps were performed using the software tool VOSviewer (version 1.6.20). A cluster visualization of the digestate application as biofertilizer and its effect on the carbon accumulation in the soil was generated (Fig. 3).

Due to this analysis (Fig. 3) five clusters of research directions were defined as follows:

1. Green cluster involved research related to the process of anaerobic digestion with digestate production as biogas residue, quality of digestate as fertilizer, impact of digestate application on soil fertility, plants growth, nutrient availability (nitrogen and phosphorus), crop yield and microbial activity.

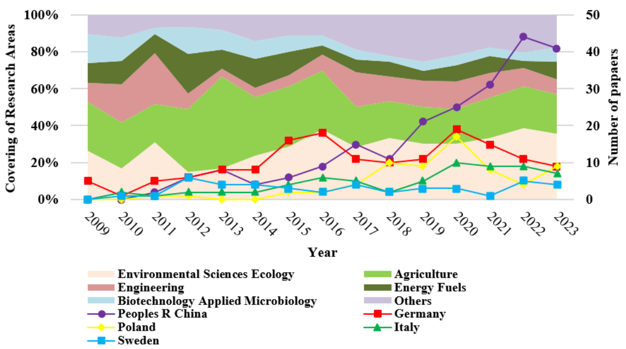

Figure 2. Dynamics of publications activities within different countries and distribution of Research Areas referred to the topic “Digestate application as biofertilizer and carbon accumulation in soil” for the period 2009 – 2023.
(based on Web of Science Core Collection database searching tools).

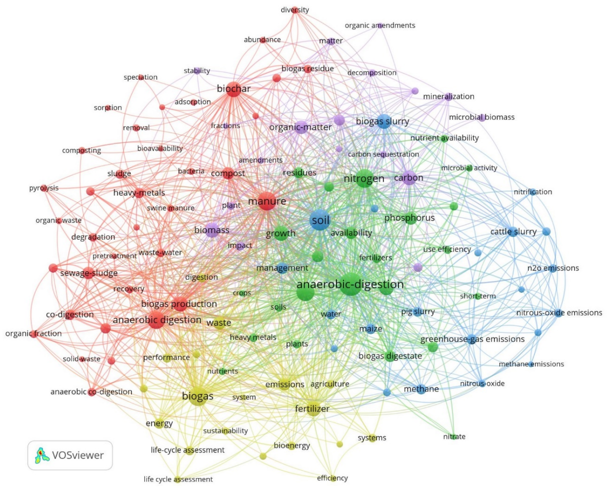

Figure 3. Cluster visualization of the most used keyword network (frequency > 15) related to the topic "Digestate application as biofertilizer and carbon accumulation in the soil" for the period 2009 – 2023.

2. Red cluster referred to the research of biogas production process, main types of organic waste (manure, food waste, sewage sludge, wastewater) that are used as substrates and co-substrates for anaerobic digestion, possible pre-treatment techniques and composting as frequent approach for digestate stabilization. Biochar production is investigated as common post-treatment pyrolysis technology allowing to influence the biogas residue quality as an amendment that is applied for soils contaminated with heavy metals.
3. Yellow cluster involved research related to the performance of biogas and biofertilizer production through the approach of life cycle assessment of bioenergy technologies in the sustainability context as well as effect of digestate fertilizer application on decrease of the emissions from the agriculture.
4. Blue cluster referred to the research of biogas slurry (digestate) application as fertilizer and the effect on soil properties as well as its contribution to greenhouse gas emissions mitigation. The special research focus is on how nitrous oxide emissions decrease through nitrification and comparison of methane emissions in case of the application of raw manure and digested manure as organic fertilizers.
5. Purple cluster research was focusing on the effect of digestate organic matter on carbon sequestration and carbon storage in the soil through its decomposition and mineralization by microbial biomass and plants.

The current study was conducted mainly according to the research directions of green, red and purple clusters, however, research covered by yellow and blue clusters was supplemented for discussion.

Thus, digestate produced as the residual material from AD has been successfully applied as a biofertilizer replacing the use of mineral NPK fertilizer meaning saving of fossil fuels and natural resources (Sogn et al., 2018) and as a biostimulant for bioremediation of soils contaminated with heavy metals (Chernysh et al., 2021). Organic wastes such as manure, agricultural residues and crops, food waste, and sewage sludge were commonly used as feedstock for AD, either alone or in combination (Reuland et al., 2022). However, the addition of digestate from different feedstocks to different types of soil has yielded contrasting results in the literature, where digestate addition can lead to a large increase in soil C accumulation in some studies while having no effect in others.

The aim of this paper was to determine the digestate potential in a long-term carbon accumulation in the soil after its application as a biofertilizer. To obtain this aim research tasks were set as follows:
1) to assess the impact of initial content of carbon in the soil before digestate application as biofertilizer;
2) to evaluate the effect of substrate type for biogas production on carbon accumulation in the soil in the long-term perspective;
3) to analyze the influence of soil type and soil properties on the long-term carbon accumulation in the soil;
4) to define the effect of crop management and crop type on the processes in soils leading to carbon accumulation;
5) to compare advantages and disadvantages of digestate application as biofertilizer in terms of sustainable agriculture management.

2. Materials and method

To rigorously evaluate the effect of digestate on carbon concentration and storage in the soil, a large literature survey of over 1000 papers was conducted using the Web of Science Core Collection database. For this purpose, a special code for topic category "(biogas OR biogas manure OR biogas digestate) AND (amendments OR compost OR soil improver OR fertilizer) AND (soil OR soil organic matter OR soil organic carbon OR SOM OR SOC)" was used resulted in finding of 918 papers after categories, year, language, and document type clarification. Only studies that assessed the effect of digestate on the carbon content in the soil for at least 12 months were considered. The methodological basis of this study was a meta-analysis of initial 168 articles and final 21 articles in which the effect of digestate addition on the soil carbon accumulation was evaluated (Fig. 4).

Meta-analysis table included four main categories: (i) physical and chemical parameters of digestate; (ii) physical and chemical parameters of soil before digestate biofertilization; (iii) physical and chemical parameters of soil after digestate biofertilization including plant type; (iiii) climate and growing conditions (Table 1).

Changes (increase or decrease) of carbon in the soil after biofertilization was calculated according to the formula (1):

$$\%C = \frac{TOC_f - TOC_c}{TOC_i} \times 10\% \qquad (1)$$

where %C is changes of carbon content in the soil after digestate biofertilization, %; TOC_f is a final content of total organic carbon in the soil after digestate addition in the end of experiment, g/kg; TOC_c is content of total organic carbon in the soil without digestate addition (control) in the end of experiment, g/kg; TOC_i is an initial content of total organic carbon in the soil before digestate addition at the beginning of experiment, g/kg.

Statistical analysis of data was performed using Stata IC 14.2 Software, MS Excel software. The results of incorporation of total organic carbon (TOC) in the soil were

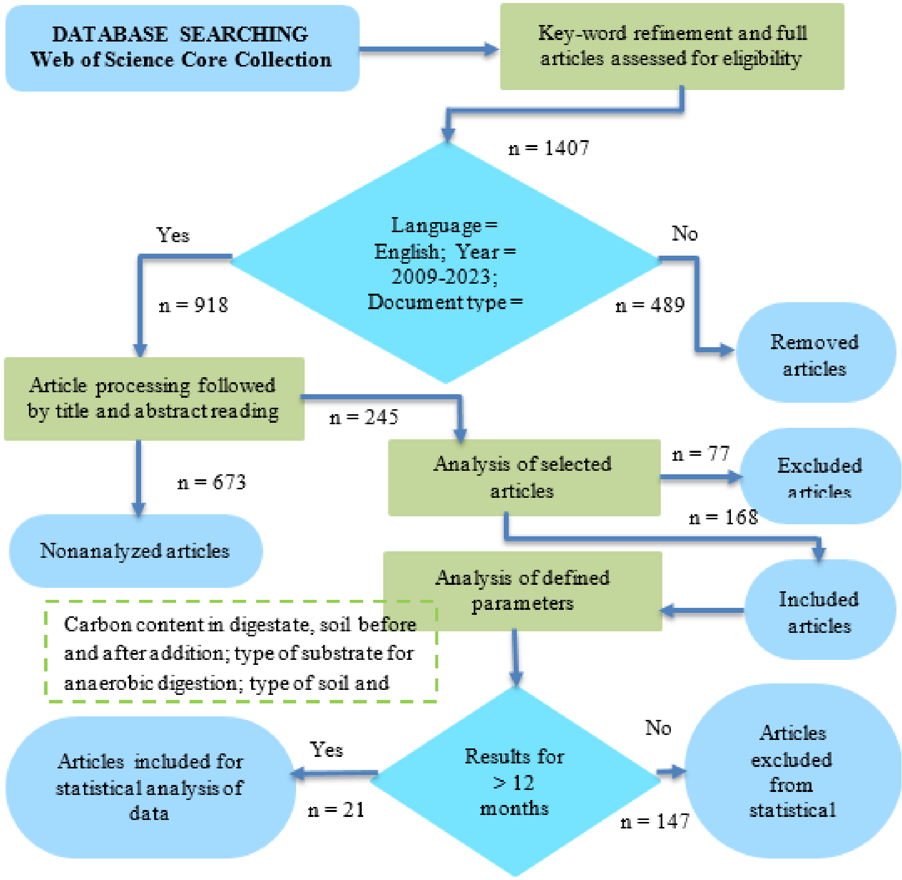

Figure 4. Overview formalization of the methodological basis of the study including literature survey in Web of Science Core Collection database and meta-analysis approach.

measured by one-way analysis of variance (ANOVA) consider as significant *p-value* 0.05 using GraphPad PRISM 7. During ANOVA analysis TOC level was considered as a dependent variable and such parameters as initial content of carbon in the soil, type of substrate for AD, type of soil and type of plant were used as independent factors. At the same time, differences between data from different categories were evaluated. For post-hoc analysis, the Tukey HSD (honestly significant difference) test was used to establish a statistically significant difference between the data

Table 1. Description of the analyzed categories and parameters used for meta-analysis table.

Analyzed category	Parameters within category
Digestate properties description and added rate	Type of substrate or co-substrate Type of fraction or whole digestate Physical and chemical parameters (pH, N, P, K, TS, VS, TOC, OM, C/N) Amount of digestate and amount of added N
Soil properties before biofertilization	Class and type of soil Phase ratio Depth of sampling Physical and chemical parameters (pH, bulk density, N, P, K, C/N, TOC, SOM)
Soil and plant properties after biofertilization	Depth of sampling Physical and chemical parameters (pH, bulk density, N, P, K, C/N, TOC, SOM) Type of grown plant and crops yield
Climate and growing conditions	Country and region Temperature and precipitation Vegetation period, research duration Start and end months of the fieldwork experiment

Notes: N - nitrogen content; P - phosphorus content; K - potassium content; TS - total solids; VS - volatile solids; TOC - total organic carbon; OM - organic matter; C/N - ratio of carbon to nitrogen; SOM - soil organic matter.

($p < 0.05$). Regression models for time-related changes of carbon content in the soil were build using trendline tool and checked through a statistical measure of the coefficient of determination (R-Squared or R^2) that determined the proportion of variance in carbon content (TOC) that can be explained by the time or initial value of TOC in the soil. Calculated regression model equations were used to define the type of regression.

Based on this meta-analysis research studies outline of the general tendency were defined such as absolute changes in TOC after digestate addition of 15.2 and 21 gC/kg dry soil in the case of cow manure and agricultural wastes as feedstock for anaerobic digestion, respectively. In the first study winter wheat and summer corn were grown on an unknown type of soil, and in the second one loam soil was used for *Sida hermaphrodita* vegetation.

Results of relative changes in TOC (% for the whole period of the experiment and %/month) of soil after digestate addition showed outline dates of 162.5 and 152.9% (6.77 and 6.37%/month respectively) for sand soil. At the beginning of this experiment, the amount of TOC in the soil was 0 g/kg. In that case poultry manure was used as feedstock for anaerobic digestion. Results of outline relative changes in TOC after digestate addition of 210% and 300% (30%/month and 100%/month, respectively) and 153.42% (4.26%/month) were observed in the case of loam and sandy soils and agricultural wastes and manure as feedstocks for anaerobic digestion, respectively.

3. Result and discussion

Trends for a long-term carbon accumulation in the soil: relationship between initial and final carbon content after digestate biofertilization

The treatment with the digestate application leads to more increase in soil C content in contrast to mineral fertilizer application treatment (Table 1 of Appendix). Analysis showed that the addition of digestate increased soil C content, but the efficiency of the C incorporation depended on the type of substrate used for anaerobic digestion and type of soil that digestate was being added.

The addition of digestate increased the soil C content in 17 of the studied cases and a decrease or no effect was observed in 4 of the studies. In some cases, a great increase was reported even after 8 years of digestate addition. Fig. 5 shows through changes in absolute and relative measurements that the highest accumulation rate was up to 36 months, but accumulation was still also after 84 months of experiment. Initial C content also affected the efficiency of carbon storage in the soil (Fig. 6). This was an intuitive result and could represent those soils with lower C content the addition of soil may contribute to the overall C content. Soils with higher C content may already have a well-established microbial community that was efficiently recycling organic matter and the extra organic matter that was added, may simply be metabolized, and be incorporated into the existing biota or being emitted to the atmosphere (Insam et al., 2015).

This may indicate that digestate can be very successfully used by helping reforestation efforts, as they usually have

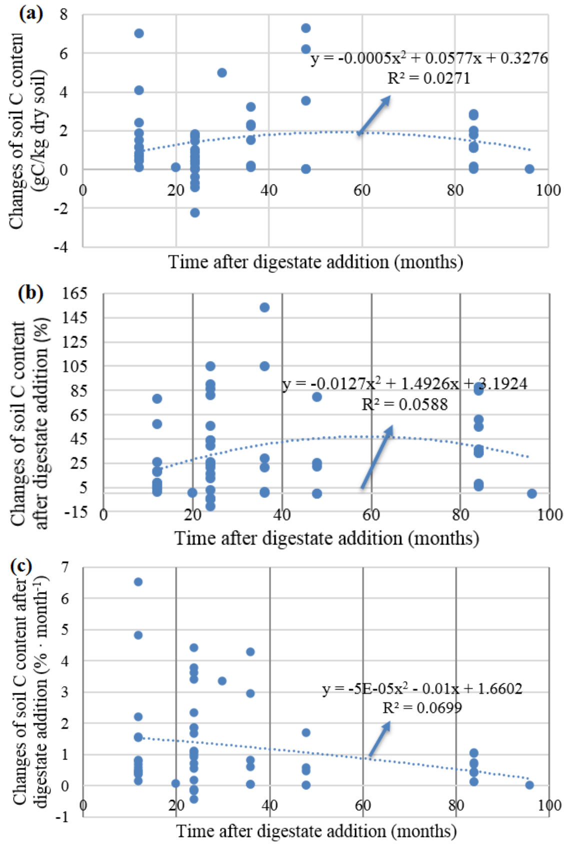

Figure 5. Accumulation of the total organic carbon (TOC) in the soil after more than 12 months of digestate addition through absolute changes of TOC content in the soil after digestate addition (a) and relative changes of TOC content in the soil after digestate addition for whole time of experiment, % (b) and calculated for 1 month, %/month (c).

lower soil C content than natural areas. Moreover, rehabilitation of the degraded land to pastoral land use had a potential for the soil organic carbon (SOC) sequestration (De Rose, 2013; Basher et al., 2011). Mineralization of soil organic carbon is managed by different processes; among them biochemical processes are the most important. Microbial growth efficiencies, along with mineral-matrix interactions with specific carbon compounds, were thought to be dominant forces controlling soil carbon stabilization (Bradford et al., 2016). Meanwhile, soil microbes and their indicators (activity, abundance, and composition) had a strong predom-

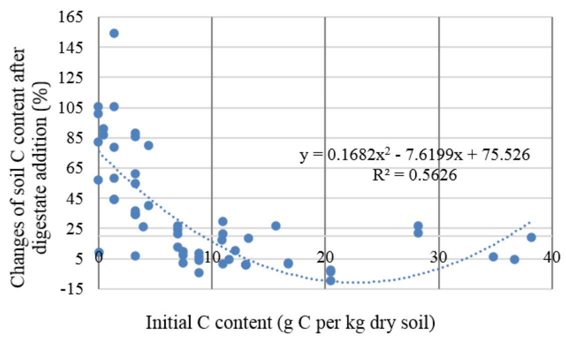

Figure 6. Relationship between soil total organic carbon (TOC) accumulation through the changes of carbon content in the soil after digestate addition and initial carbon content in the soil.

inant effect on carbon accumulation in the soil (Creamer et al., 2015).

According to Guo et al. (2019) understanding the mechanism of microbial regulation of soil carbon pool mineralization is of great significance for the accumulation and sustainable development of farmland soil carbon pool. Organic fertilizer treatment can significantly increase organic carbon mineralization. Microbial abundance of several non-dominant bacterial species contributed more to the differences in C mineralization than the overall microbial community distribution. Thus, soil microbial communities may be functionally redundant with respect to native organic C mineralization (Guo et al., 2019).

According to Basile-Doelsch et al. (2020) long-term stabilization and short-term degradation of organic matter are regulated by different forces. Mineralization–stabilization balance is controlled by biotic factors (plant, fauna, microbial activity, and biodiversity) as well as abiotic factors (mineralogy, temperature, water content, pO_2, soil solution chemistry and pH, N and P availability).

In this relation long-term effect of the digestate addition to the soil is more visible for C accumulation based on the organic matter transformation in soil. However, short term studies in general showed an increase in soil TOC as the result of determining the effect within one growing season, which reflected the increase in carbon due to carbon digestate, rather than biological conversion. The main processes that regulate organic matter (OM) dynamics in soils were summarized in Fig. 7.

Regarding the cultivated land, the soil carbon input flux is the net primary production of the ecosystem minus the exported crop production, losses from herbivory (production and respiration), and dissolved and particulate organic matter outputs.

The effect of substrate type for anaerobic digestion on carbon accumulation rates in the soil

In this study several types of substrates for anaerobic digestion were identified and analyzed. It should be noted that agricultural residues, food waste, manure and combination of manure and organic waste were used for production of digestate applied for experiment period more than 12 months after digestion addition. Results obtained during meta-analysis showed some trends and relationship between rates of carbon accumulation in the soil after digestate biofertilization and type of substrate for anaerobic digestion. Thus, the digestate from the reactors where manure was the main substrate showed statistically significant higher results in terms of soil C accumulation compared to digestate from biogas reactors fed with food waste and manure mixed with sewage sludge and agricultural residues (Fig. 8 (a)). Agricultural wastes as feedstock for biogas reactors were used primarily in short-term experimental studies and only one research was related to the long-term period. The last experiment showed a rather high increase in the soil TOC but initial carbon content in sand soil was 0 g/kg of dry

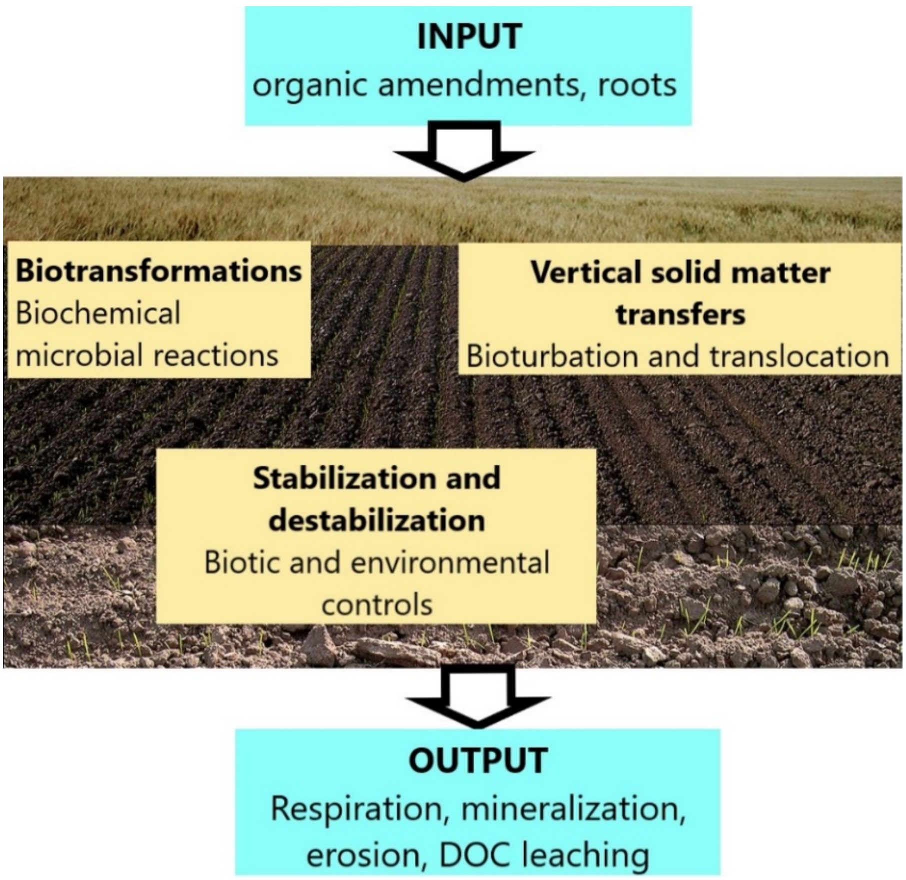

Figure 7. Interactions between inputs and outputs as well as biotic and abiotic processes controlling carbon fate in the soil including transformation pathways for carbon storage in soils. DOC – dissolved organic carbon (based on Basile-Doelsch et al. (2020).

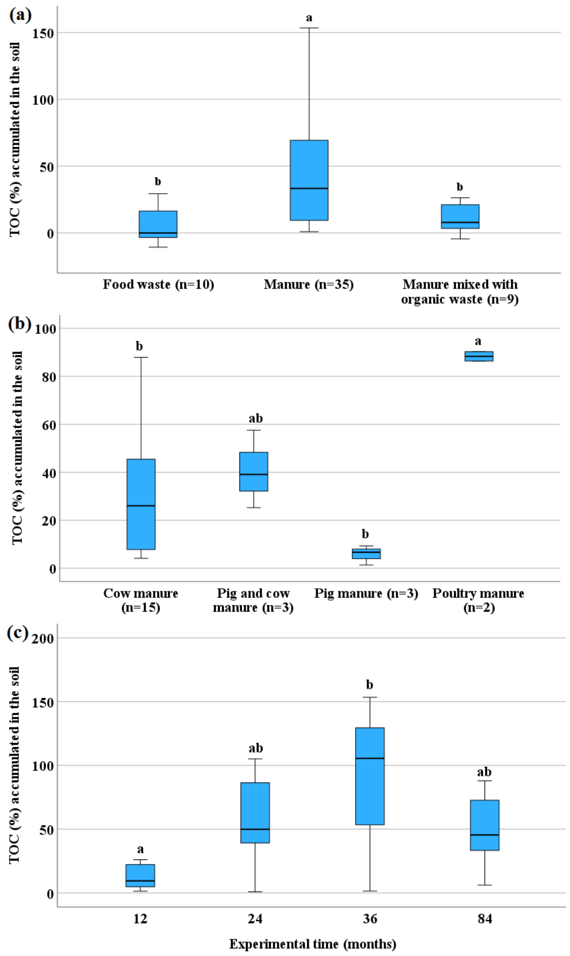

Figure 8. TOC accumulation in the soil after more than 12 months of digestate biofertilization as a response from the primary substrate added to biogas reactor: (a) efficiency of soil TOC incorporation of the digestate originated from different substrate sources; (b) efficiency of soil TOC incorporation of the digestate originated from different types of manure added to biogas reactor; (c) distribution of TOC accumulation in the soil after digestate biofertilization depending on the experimental period. Values mean ± SD, 95% confidence interval (CI); significantly different rates ($p < 0.05$) are indicated by the lowercase letters.

soil that can be controversial and must be further discussed. The same situation was observed in the case of different types of manure, where the poultry manure had statistically significantly higher results than pig manure and cow manure in terms of soil C increase (Fig. 8 (b)). Nevertheless, experiments with the using of poultry manure as feedstock were only two that cannot be representative and statistically approved. Based on the mentioned among different types of manure, the mix of cow and pig manure showed the highest results in the relation of long term carbon accumulation in the soil after digestate biofertilization. However, this result was not approved as statistically significant.

In the case of cow manure feedstock for anaerobic digestion, soil C increase reached more than 40% per month (Fig. 8 (b)). This can be easily explained as manure was very rich in lignocellulose that was very difficult to degrade during anaerobic digestion. It has been recognized that biogas production from cow manure can be very challenging given the nature of the substrate. This was different with pig and poultry manure that had less lignocellulosic material (Yan et al., 2018). Moreover, digestate based on the mix of cow and pig manure had higher results in terms of carbon increase in the soil compared to single cow manure or pig manure as substrate for anaerobic digestion. This observation was in line with the finding of the recent study (Pinto et al., 2023) which resulted in the best proportion of co-digestion of bovine and swine manure for biogas reactors as 4:1.

Results of the distribution of soil TOC accumulation depending on the experimental period shown on the graph (Fig. 8 (c)) could be analyzed from different points of view because different types of feedstocks, soil and crops were applied for these experiments. It was seen a general tendency of lower increase for the period of 12 months and further increasing with the statistically significant highest rate for the period of 36 months.

The biogas sector had pointed out manure and more specifically, cow manure as major substrates to increase the biogas production in that sector (Aso, 2020; Walsh et al., 2018). Obtained results added another value to this decision, because the digestate from manure can more easily be used in agriculture, which would not only add nutrients to the soil but also increase C content in the soil. The use of digestate from cow manure in agriculture would also be more economically feasible, as the costs for digestate transport is likely to be decreased if it is applied in the agricultural areas. This is a different situation from the digestate produced from food waste or from sewage sludge, that are located in large cities, likely to be more distant from the producing areas.

The comparison of the effect of different sources of substrates on the increase of soil TOC content by ANOVA (p-$value < 0.05$), indicated a significant difference in the efficiency of the incorporation of TOC between manure and food waste as different sources for digestate.

Climate change is a major driver of terrestrial ecosystem degradation, particularly desertification and biodiversity loss and vice versa. Forests and soils are major carbon sinks and can be used as powerful tools for climate change mitigation (SDG 13) and integrated land-use activities to foster land degradation neutrality (Zucca et al., 2024). Soil organic carbon was considered as a main compartment of the global C cycle, thus providing opportunities for climate change mitigation (Basile-Doelsch et al., 2020).

In 2015, France released a global soil initiative called, '4 per 1000 Initiative', which aimed to increase the carbon in soils by 0.4% annually to stabilize the climate and to ensure food security (Minasny et al., 2017). The initiative allows different actors to contribute what they feel is within their means to prevent soil degradation. SOC sequestration may be an effective solution to mitigate climate change, to take atmospheric CO_2 and convert it into soil carbon which is long-lived (Minasny et al., 2017).

The digestate used as fertilizer had the capability to increase yields of crops by C accumulation in the soil. It seemed like a promising solution for the problem of poverty reduction which met SDG 2, by demonstrating the sustainability of an integrated and self-sufficient renewable energy access model (Piadeh et al., 2024).

Response of soil type and growing plant type to the carbon accumulation in the soil

The carbon increase was much larger, up to one order of magnitude, when the digestate was added to sand or loam than to silt or clay soils (Fig. 9). Obtained results for the period more than 12 months showed the highest soil C increase (more than 2% per month) for sand (Fig. 9). The incorporation of TOC in the soil from the addition of digestate did not have a performance significantly delayed by ANOVA (*p-value* < 0.05) in different types of soils.
However, interactions between soil type and biofertilization were not significant as presented in the study (Xiong and Kätterer, 2010). Moreover, Ostrowska et al. (2010) found that SOC accumulation is to a greater extent affected by the site type and the age of pine stands than by the soil type.
In case of alkaline pH of the digestate and calcium-rich soil humus will be formed with a predominance of humic acids, which are higher molecular weight, contain more C and less O than fulvic acids. Humic acids are not well dissolved in water, and therefore are poorly released from the soil, that's why humus and carbon must gradually accumulate.
The addition of a slightly alkaline digestate will neutralize the soil pH and change it closer to neutral, which, firstly, helps to consolidate and accumulate humus (carbon will accumulate), increase fertility (cultivated plants prefer a neutral medium), and secondly, prevents the migration of heavy metals, in particular cadmium, which is in the soil as a natural background or has been migrated from phosphorus fertilizers as it was shown in the study (He et al., 2021).
Dependence of the soil TOC increase from different types of grown crops are shown on Fig. 10. Wheat and maize were the best crops after maize in terms of the potential for TOC accumulation in the soil.
According to the obtained results some plants such as eggplant, lettuce and melon were used only for one experimental setup, therefore, they were not statistically representative and were excluded from the statistical analysis. The comparison of the effect of different cultivated plants on the increase of the soil TOC content by ANOVA (*p-value* $<$ 0.05), indicated significant difference in the efficiency of the TOC incorporation for maize with the highest results compared to barley, peanut, rice, clover, wheat with maize. Additionally, the effect on carbon increase in soils cultivated

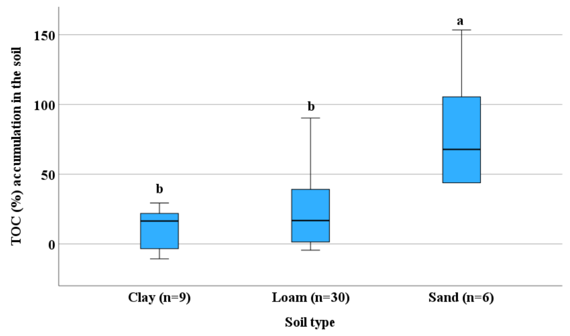

Figure 9. The effect of the type of soil treated with anaerobic digestate on the carbon accumulation in the soil for an experimental period of more than 12 months. Values mean ± SD, 95% confidence interval (CI); significantly different rates ($p < 0.05$) are indicated by the lowercase letters.

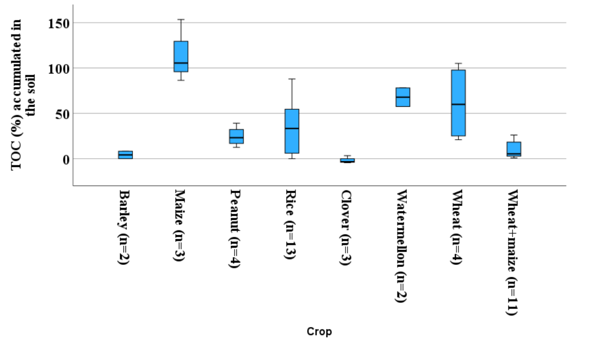

Figure 10. Relative changes in TOC accumulation in the soil after digestate addition for different types of cultivated crops. Values mean ± SD, 95% confidence interval (CI); significantly different rates ($p < 0.05$) are indicated by the lowercase letters and star.

with wheat was statistically significantly higher than with clover and wheat with maize. Such differences in results can be explained by different types of soils and used substrates for digestate production in case of wheat and maize cultivation separately and together.
Cultivated plants play a significant role in the process of organic carbon transformation in the soil. Soil microorganisms interact with plant roots providing carbon mineralization with further humification. Moreover, plants returned organic carbon to the soil after harvesting, and therefore anaerobic digestate had a positive effect on the crop yield and biomass growth. Thus, different plants were indicated with different potential for contribution to the carbon storage in the soil. Additionally, crop rotation systems are another aspect for further investigation and discussion as well as crop cultivation management.

Additional advantages of digestate biofertilization related to environmental and agricultural issues

Addition of the digestate to soils kept the nutrients N, P and K longer in the soil compared to mineral fertilizers (Sogn et al., 2018). The addition of digestate also added several micronutrients to the soil (Głowacka et al., 2020).
Organic fertilizers are one of the greatest sources for remediation of soil but there are not enough available ones to meet agricultural demand worldwide. The agricultural sector is one of the most vulnerable to climate change, and its negative effects (such as extreme weather events, increasing temperatures, declining availability of water and other resources). So, finding new sources of organic matter for degraded land is relevant. The use of digestate as a biological fertilizer will increase the fertility of the land during periods of drought and keep the crop yields stable. It can increase the carbon sequestration potential of agricultural soils by adding appropriate doses of digestate for biomass production.
Digestate as soil improver must be suitable for organic matter content according to the EU standards on fertilizer properties. Among the most significant and necessary organic materiasl carbon and nitrogen play key roles in microbiological processes in the soil as well as carbon is the most widely used energy source.
The conversion of organic matter in the soil depended on

microbial and enzymatic activity, which determined the release and availability of nutrients in the soil (Daunoras et al., 2024). Therefore, changes in enzyme activity (urease, protease-dietary supplement, alkaline phosphatase and -glucosidase) were clear indicators of changes in soil fertility, as they were associated with the process of mineralization and nutrient supply.
Different types of pre-treatments are used to decrease the number of fungi, bacteria, other pathogens, or substances that could cause problems by the use of digestate as biofertilizer. Sanitation (thermal pre-treatment), electrokinetic disintegration, ultrasound treatment and solid waste detoxification (fungal pre-treatment) are the most common techniques. Additionally, pasteurization may be applied for pre-treatment as well as post-treatment that showed better results of pathogens indicators (Nolan et al., 2018).
Finally, digestate application into soil as fertilizer has higher ecological safety indicators in contrast with untreated animal wastes such as manure or slurry. This can be explained by biochemical processes led by anaerobic bacteria during anaerobic digestion with the following transformation of organic substances including pollutants and pathogens.

Disadvantages and challenges on the way of replacing mineral fertilizers by digestate biofertilizer

The most significant ecological negative backside was possible chemical and biological contamination. Moreover, the content of different chemicals depended mostly on the feedstock type and digestion regime. For example, digestate produced from green waste matter, maize silage, beet pulp, stillage, wheat under case-study (Koszel and Lorencowicz, 2015) did not contain any heavy metals.
Digestate and digestate mixed with the compost meet the requirements for the quality and chemical and biological safety of fertilizers by the number of heavy metals such as Cu, Pb, Cd, Ni, Hg and Cr, toxicological properties according to the EU Regulation on fertilizers (Fertilizer Regulation, 2019/1009). Some of the proposed strategies are to combine digestates from different substrates to dilute the relative levels of heavy metals. This allowed them to mitigate the environmental risk posed by each starting material and to valorize their nutrient content (Pecorini et al., 2020).
In general, digestate may be polluted by heavy metals and metalloids, persistent organic pollutants (POPs), pesticides, pharmaceuticals, hormones, microorganisms, and viruses depending on the used feedstock type (manure, food or agriculture wastes, sewage sludge etc.). That's why before soil addition the digestate effluent from a recirculating anaerobic digestion system must be analyzed for heavy metals (Ni et al., 2017).
The high ammonium concentrations in the digestate (Alburquerque et al., 2012) can also be a source of concern. Ammonia (NH_3) is a volatile molecule and depending on soil pH, it would be released to the atmosphere. NH_3/NH_4^+ also had a high potential to be transported to the groundwater through leaching processes (Malovanyy et al., 2014; Malovanyy et al., 2021). Ammonia stripping from the liquid fraction (air, steam) is usually used to reduce the nitrogen content in the digestate in the case of soil application or return to the process of anaerobic fermentation. Under such conditions, it is possible to produce ammonium sulfate (ammonia water) as the derivative product that is a valuable commercial grade fertilizer (Drosg et al., 2015).
Separation of digestate into solid and liquid phases is the first stage of the post-treatment approaches. There are different solid–liquid separation technologies such as decanter centrifuges, screw press separators, bow sieves, double circle bow sieves, sieve belt presses, and sieve drum presses (Ablieieva et al., 2022c). To increase solid-liquid separation, flocculation or precipitation agents were commonly applied (Logan and Visvanathan, 2019).
The processes of adsorption and granulation allowed to obtain a granular organic slow-release fertilizer as a concentrated product from the liquid fraction (Ablieieva et al., 2022b). That's why the quality of digestate as a biofertilizer and related potential for carbon accumulation in the soil depends on different parameters. Based on provided meta-analysis, the type of substrate for anaerobic digestion, type of soil and cultivated plant were identified as the most significant. Nevertheless, such factors as treatment techniques (pre-treatment of substrate and post-treatment of raw digestate) as well as technological approaches for fertilizer production are of great importance in terms of patterns for carbon storage in the soil and climate change mitigation and must become the subject of further research.

4. Conclusion

Based on meta-analysis results, initial lower carbon content in the soil responded to the higher accumulation rate of carbon in the soil after digestate biofertilization in case of long-term perspectives meaning more than 12 months. Digestate can be very successfully used by helping reforestation efforts, as they usually have lower soil carbon content than natural areas. Digestate originated from manure as the main substrate for anaerobic digestion showed significantly higher potential for carbon content increase in the soil compared to digestate from food waste or combination of manure and other organic waste. Among different types of manure cow and pig manure showed the highest results in terms of long term ($>$ 12 months) carbon accumulation in the soil after biofertilization. General tendency of lower total organic carbon increase for the period of 12 months and further increase at 36 months was found. Obtained results for the period of more than 12 months showed the highest soil carbon increase (more than 2% per month) for sand. Wheat and maize were found to be the best crops in terms of the potential of total organic carbon accumulation.
The biogas production contributes to the global efforts in reduction of fossil fuel consumption, societal decarbonization and in case of the digestate application as biofertilizer global soil carbon content can be increased. The potential negative aspects of digestate biofertilizer addition such as possible high levels of chemical and biological contamination can be eliminated through feasible technical and operational solutions.
Further research will be related to the experimental setup

of the long-term carbon storage in two types of soil (sand and silty clay) after application of the digestate produced from the four types of local feedstock (food waste, sewage sludge, manure, and crops) and treated of three different post-treatment types (ammonia stripping, calcium hydroxide addition and zero valent iron addition). Besides this, the investigation of organic matter of different above mentioned types of digestate is in progress that will define the main patterns for the process of organic matter mineralization and humification in the soil.

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Authors contributions
The authors confirm the study conception and design: A. Enrich-Prast, I. Ablieieva; data collection: I. Ablieieva, O. Burla; analysis and interpretation of results: T.M. Anacleto, I. Ablieieva, I. Sipko; draft manuscript preparation: A. Enrich-Prast, I. Ablieieva. The results were evaluated by all authors, and the final version of the manuscript was approved.

Availability of data and materials
The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Conflict of interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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