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REVIEW

Impact of biogas digestates on soil microbiota in agriculture: a review

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Abstract

The global production of biogas has increased threefold during the last decade to partly replace fossil fuels, yet biogas production by anaerobic digestion generates substantial amounts of by-products named digestates. These biogas digestates can be recycled in soils to fertilize crops and to sequester carbon. Nonetheless, the impact of digestates on the soil biological is actually poorly known. Here, we reviewed the impact of digestates published in 56 articles reporting 23 microbial parameters. Half of the articles show neutral effects of biogas digestates and 7% showed negative effects. 25% of the articles show more stimulation of the soil microbial quality by biogas digestates, whereas 17% of the articles show less stimulation, compared to other organic fertilizers.

Keywords Anaerobic digestate · Biogas · Soil · Microorganisms · Agriculture · Meta-analysis

Introduction

The biogas sector has been developing sharply round the world since the 2000’s, with a dual goal: producing a renewable energy such as electricity/heat or biomethane, and managing organic waste (Rawoof et al. 2021). In a context of climate change and ecological transition, this can represent an opportunity to decrease greenhouse gas emissions and enhance carbon sequestration in the soil by returning biogas residues to it. Three main regions are implied in biogas production around the world: the largest producer is Europe with more than 18 million tons of oil equivalent (Mtoe) in 2018, followed by China and the USA with about 7 Mtoe and 4 Mtoe, respectively (IEA 2018). Two-thirds of European biogas plants are located in Germany, which is by far the largest market among European countries. For ten years, other countries such as the UK, France, Switzerland, Denmark and The Netherlands have stepped up the development of their biogas industry (International Energy Agency (IEA) 2019).

Biogas is produced by anaerobic digestion of organic matter in an oxygen-free environment. A wide variety of feedstocks can be used, classified into four categories: crop residues, animal manure, the organic fraction of municipal and industrial solid waste, and wastewater sludge (IEA 2020). The process yields biogas and a by-product composed of liquid and solid residues called “digestate”. These residues are the major final product due to the important biomass and volume they represent (Fig. 1).

In Europe, the digestates were classified as waste but the recent regulation of the European Commission authorized that a fertilizer was composed of digestates (European Commission 2019). This new regulation facilitates the use of digestates on crop lands, which is the common way to manage the huge amounts of anaerobic digestion residues

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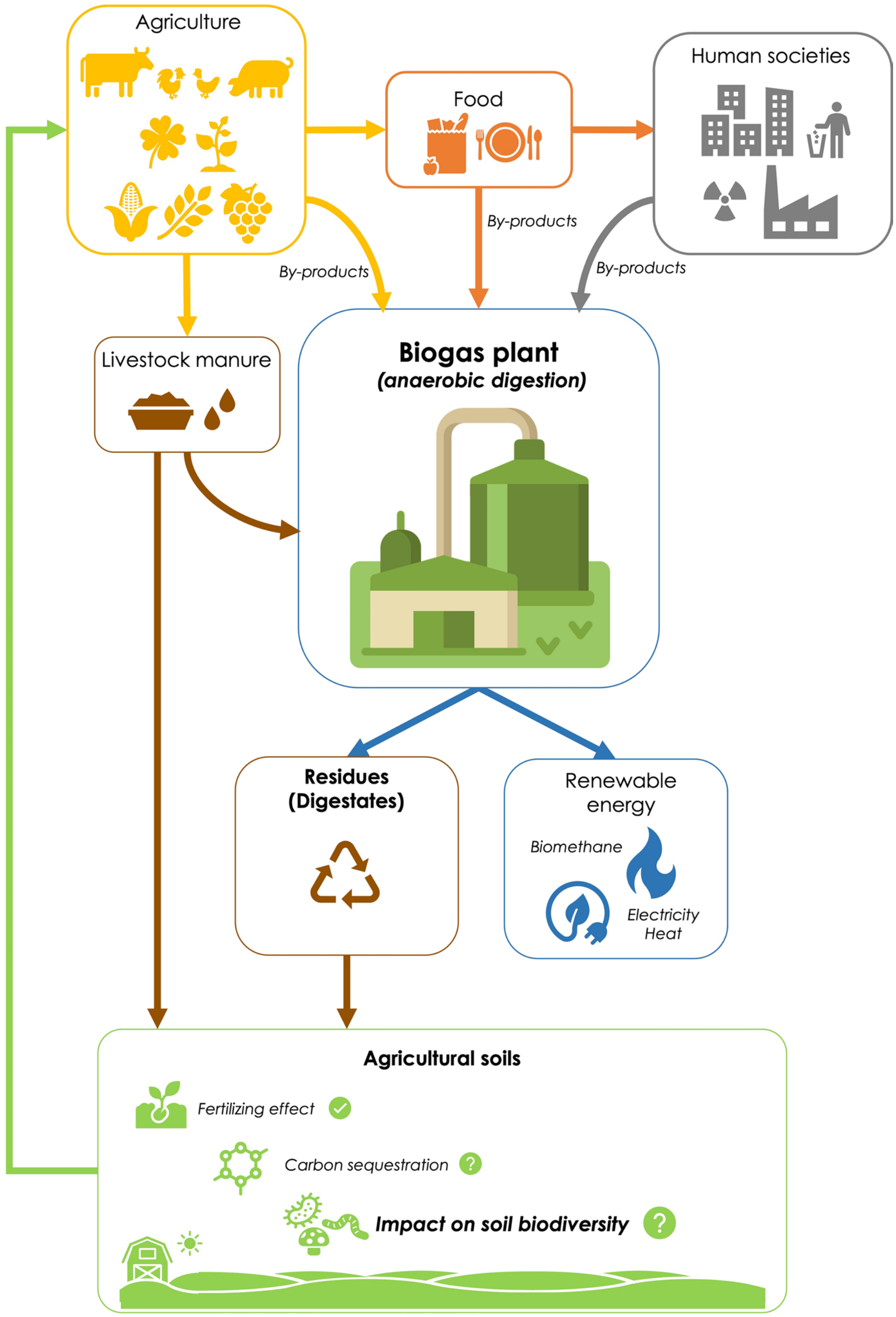
Fig. 1 Relationships between agriculture, biogas industry and soils. The feedstock of biogas plant come from agriculture, i.e., crop residues and livestock, and from food and human societies waste. The anaerobic digestion of these feedstocks in the biogas plant produces renewable energy such as electricity, heat and/or biomethane, and digestates. The digestates are spread on the agricultural soil for their fertilizing effect. They could also help to carbon sequestration. However, the impact of digestate on the soil biodiversity remain unclear and it urge to be answered with the acceleration of the biogas industry development



produced by the biogas industry. In the current context aimed at an agro-ecological transition and at reducing the environmental impact of agriculture, this practice could represent an alternative to mineral fertilizers and a solution for organic matter recycling by farmers. However, like for the other organic products, the potential deleterious impacts of digestates on the environment–or even beneficial ones–must be characterized in order to drive their sustainable use in favor of environmental and soil protection (Thangarajan et al. 2013; Urra et al. 2019).

Since the last decade, numerous studies have described that the application of a range of organic fertilizers could increase the soil organic carbon and improve the soil biological communities (Sabir et al. 2021). Concerning digestates, the review by Nkoa (2014) highlighted environmental risks such as potentially higher NH_3 emission than undigested organic matter or the concentration of trace elements (Cu, Zn, Mn) from pig and cattle slurry feedstock that could induce toxic side effects in agricultural soils (Nkoa 2014). More globally, an essential environmental issue needs to be answered, namely the impact of digestates on the whole soil biological quality.

The soil biological quality is defined as the capacity of a soil to host a large quantity and diversity of living organisms involved in its functioning and in the supply of ecosystem services (Karimi et al. 2020). A suitable soil biodiversity can supply numerous benefits for agricultural production, such as promoting organic matter degradation (Baumann et al. 2012), creating a barrier effect to pathogen populations (Vivant et al. 2013), maintaining the soil structure (Le Guillou et al. 2012), reducing plant sensitivity to drought (Prudent et al. 2020), reducing atmospheric pollution (Abis et al. 2020), and more globally maintaining soil functioning stability (Tardy et al. 2014; Maron et al. 2018).

Although digestates have often been proved efficient fertilizers or amendments, their effects on the soil biodiversity are still unclear, and their harmlessness for soils as biodiversity reservoirs remains to be demonstrated. Shedding light on this issue is awkward due to the diversity of soil organisms, the wide range of biological parameters that can be assessed (Karimi et al. 2020) and the variety of digestates (Guilayn et al. 2019). Digestates vary in terms of type, composition and quality, according to the feedstock quality and the technology used for biogas production, which themselves depend on the localization and size of the biogas plant and the availability of the different feedstocks (Guilayn et al. 2019). To date, few syntheses dealing with a comprehensive impact of digestates on soil biological quality are available, which makes it difficult to conclude robustly on this point.

In this context, a summary of all available scientific knowledge is needed to objectively assess the implications of digestate applications for the agricultural soil biodiversity. We carried out a meta-analysis to elucidate the impact of digestates on the biological quality of agricultural soils by systematically inventorying the international academic literature issued in the last 20 years. Surprisingly, the soil biodiversity has been essentially investigated from the angle of soil microorganisms to date, with only rare studies on nematodes and the soil fauna (according to a search on Web of Science in March 2021). Thus, our meta-analysis was mainly focused on the soil microbiological quality.

In the present study, we first analyzed the evolution of the number of studies over time and localized the geographical origin of the studies. Then, we produced a scientific summary of the questions investigated in the literature and of the experimental approaches set up to answer them. To evaluate the global ecological impact of digestates, we quantified the proportion of studies reporting deleterious, neutral and beneficial effects following digestate application compared to a fertilizer-free control, a mineral fertilizer, and any other organic fertilizer. Finally, we summarized the results concerning the most relevant questions addressed in the literature for the 23 most measured parameters related to microbial abundance, diversity, and activity. As a result of this review, we identified orphan lines of research that need to be investigated to provide most accurate and operational recommendations for stakeholders and environmental policies at the European and national scales.

Bibliometric analysis

A global search with the words [*Digestate* AND soil** AND (**diversity OR microb* OR faun* OR nematode* OR earthworms*)] identified 222 articles dealing with digestates and soil biology. Among them, 200 addressed microorganisms, 4 addressed nematodes, and 17 addressed the soil fauna. The 4 articles about nematodes addressed the suppression of phytoparasitic nematodes only, without investigating the whole ecological community. Concerning the soil fauna, two provided data on collembola and 7 on earthworms. These 9 articles investigated various questions related to digestates. The low number of scientific articles and data on nematodes and the soil macrofauna did not provide a robust synthetic analysis allowing us to conclude objectively on the impact of digestates on these biological groups. Consequently, our study was focused on the soil microbial community, which has been studied more intensively by the international scientific community.

The first filters checked the adequacy of the themes in the title, keywords and abstract with the scope of the meta-analysis. They were applied to the 200 articles dealing with microorganisms and resulted in 66 articles providing data on the impact of digestates. The bibliometric analysis – consisting in a study of the metadata of the articles–was conducted on this pool of articles. We first mapped the geographical origin of the studies to identify the main scientific teams interested in this research topic in the world. Then, an analysis of the issuance date of the articles showed how the interest of scientific research for the topic evolved.

More than 80% of the studies were conducted in European countries, mostly in Germany and Italy with 15% of the studies each. Most of the other studies were carried out in Asian country such as China and Japan. Africa and America were poorly represented (Fig. 2a). The first articles were published in 2008. Since then, 1 to 6 articles *per* year were issued, except in 2015 and 2020. Thus, 83% of the articles were published between 2015 and 2021 (Fig. 2b).

Finally, the journals that published the articles and their scope were analyzed to better understand the context in which the scientific community was interested in the question. The 66 articles were issued in 40 different journals related to 6 main scopes: Microbiology, Pollution and waste, Energy, Food, Soil and agriculture, and Ecology and environment. As showed in Fig. 2c, the diversity of journals and their scope increased with the number of

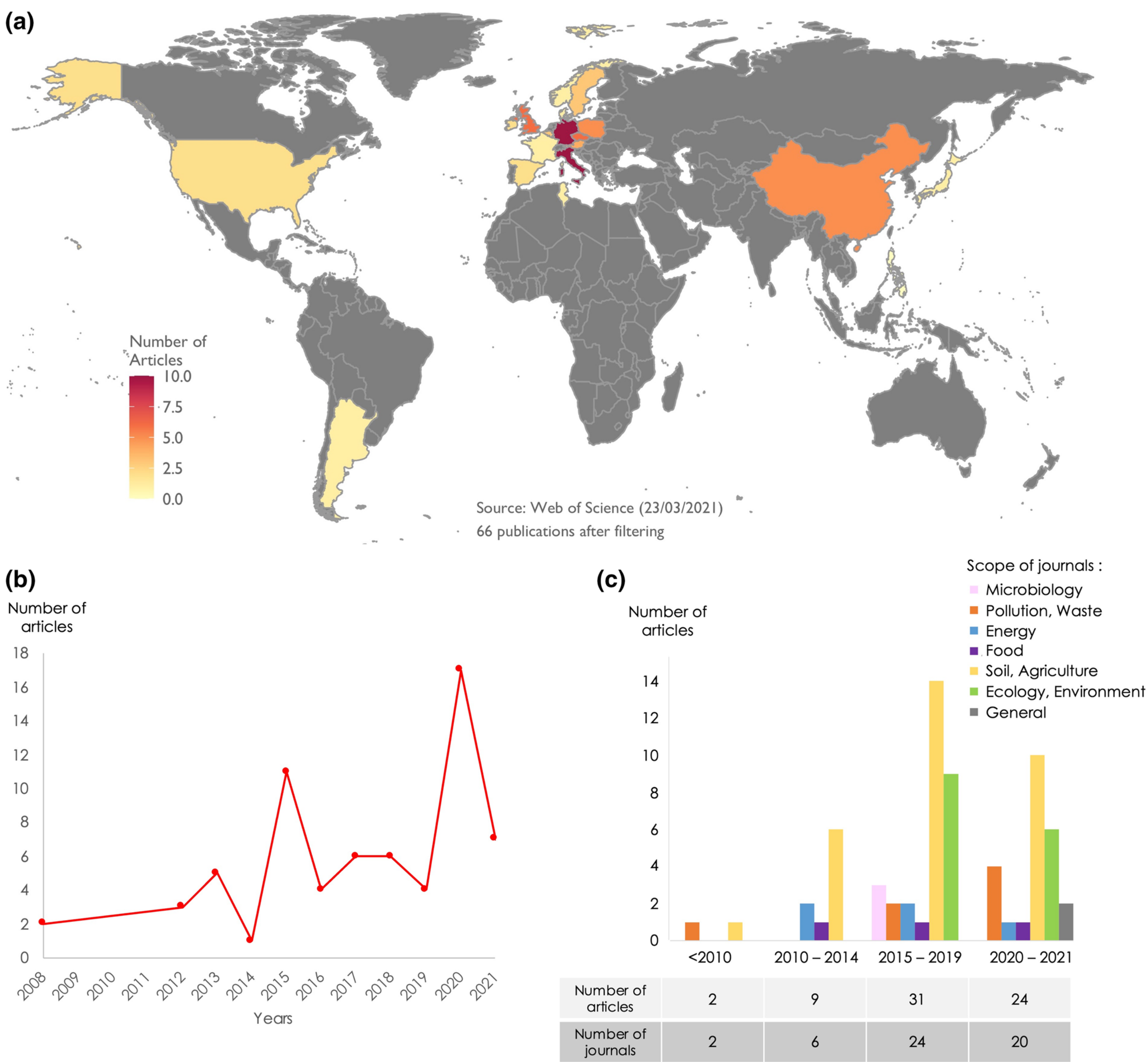


Fig. 2 Bibliometric analysis of the impact of digestates on the soil microbiological quality: **a** Mapping of the geographical origin of the studies. **b** Temporal dynamics of the number of articles for 15 years. **c** Evolution of the scopes of the journals publishing the studies. Large part of studies was conducted in European countries, mostly in Germany and Italy, and in China. The first articles were published in 2008. but 83% of the articles were issued between 2015 and 2021 in journals with two main scopes: Soil and agriculture, and Ecology and Environment. From 2020, generalist journals became interested in the publication of research articles about the impact of digestates on soil biodiversity

published articles. Interestingly, most of the articles were related to "Soil and Agriculture" or "Ecology and Environment", while "Energy", "Pollution and Waste" and "Food" were present since 2010 but little represented. From 2020, generalist journals also became interested in the publication of research articles about the impact of digestates on soil biodiversity. They target a larger readership and propose articles with scientific and societal issues.

Analysis of experimental strategies

The analysis and summary of the characteristics of the experimental strategies used in the studies concerned: (i) the type of approach: microcosms, greenhouse, or field experiments; (ii) the time scale of the experiment; (iii) the soil type; (iv) the digestate type and fraction; (v) the measured microbiological parameters. Reviewing these characteristics provided an overview of the diversity of results but also pointed out the strengths and the weaknesses of the state of current scientific knowledge.

Types of experiment

The analysis of experimental strategies showed that two main approaches were used by the experimenters: the laboratory approach or the *in situ* approach. The laboratory approach consisted of microcosm or mesocosm experiments set up in laboratory under controlled conditions. The different treatments were mostly applied and tested on one or two different soils, and the experiment took place over the short term, *i.e.,* from a few days to a few months. The *in situ* approach consisted of field experiments set up in real agronomical and pedological conditions. In this approach, the treatments were applied on three or four blocks on a same plot of one or two farms. This type of approach usually took place on the mid or long term, *i.e.,* from several months to a few years.

A major part of the studies–more than 70%–used the laboratory approach, compared to 25% based on an *in situ* approach (Fig. 3a). One study used both approaches. Interestingly, no study provided data from a network of farms: each farm applied different digestates in its own agronomical and pedological conditions (Fig. 4).

Time scale

The experiments were carried out on a large range of time scales. The shortest time was a few days or one week between digestate spreading and the measurement of biological parameters. The longest time was two years between the first digestate application and the measurements. A study in which the biological parameters were measured 6–24 months after digestate application was considered as an evaluation of their mid-term impact. When the delay was greater than 2 years, the study was considered to be evaluating the long-term impact of digestates. As presented in Fig. 3b 60% of experiments lasted 1 week to 6 months, which is considered as an evaluation of the short-term impact of digestates. Besides, 88% of these short-term experiments involved microcosm or mesocosm approaches. The studies that lasted more than 6 months represented 40% of the experiments, and 75% of them were *in situ* approaches.

Types of soils

Seventy-four per cent of the studies relied on one soil type only, while 20% were based on two soil types. A few studies included a diversity of soils – the maximum was 8 soil types. The soils were 57% agricultural soils, covered by grass, crops, orchards or fallow, but bare soil was used in 24% of the cases. This datum was not available in 18% of the studies. Although all studies were focused on the topsoil, the sampling depth varied. Twenty-three *per* cent of the studies targeted the first 10 cm of soil, 42% targeted the first 20 cm,

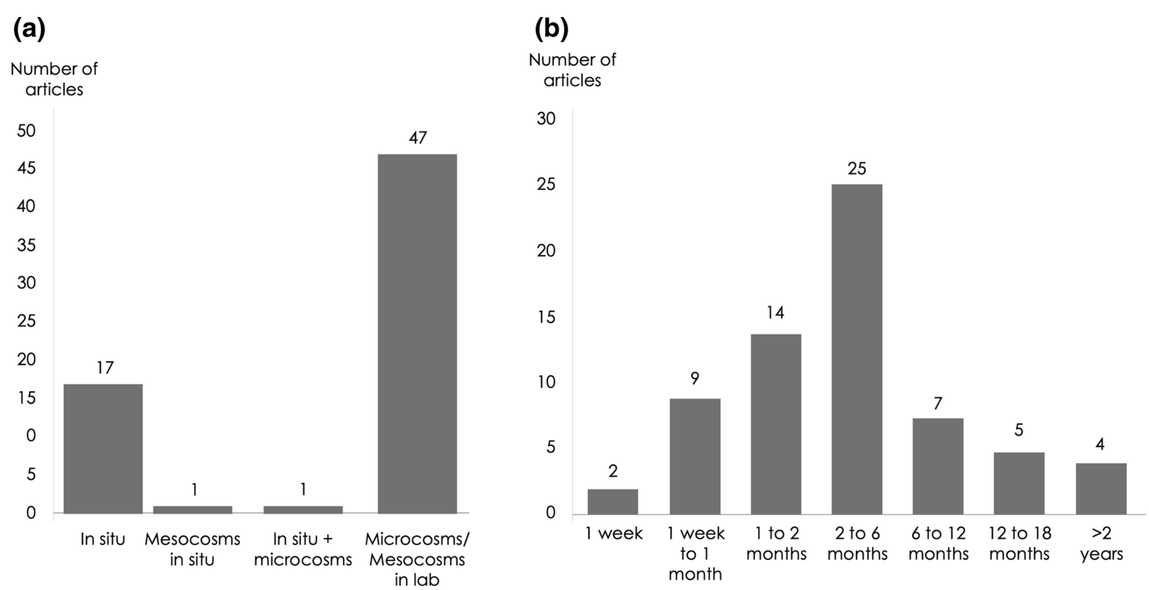


Fig. 3 Number of scientific articles on the impact of digestates on the soil microbiological quality according to **a** the type of experiment (experimental strategy developed) and **b** the time scale of the studies. Two third of studies set up in laboratory and most of them are short-term experiments. The long-term experiments generally take place on the field, in realistic pedoclimatic conditions

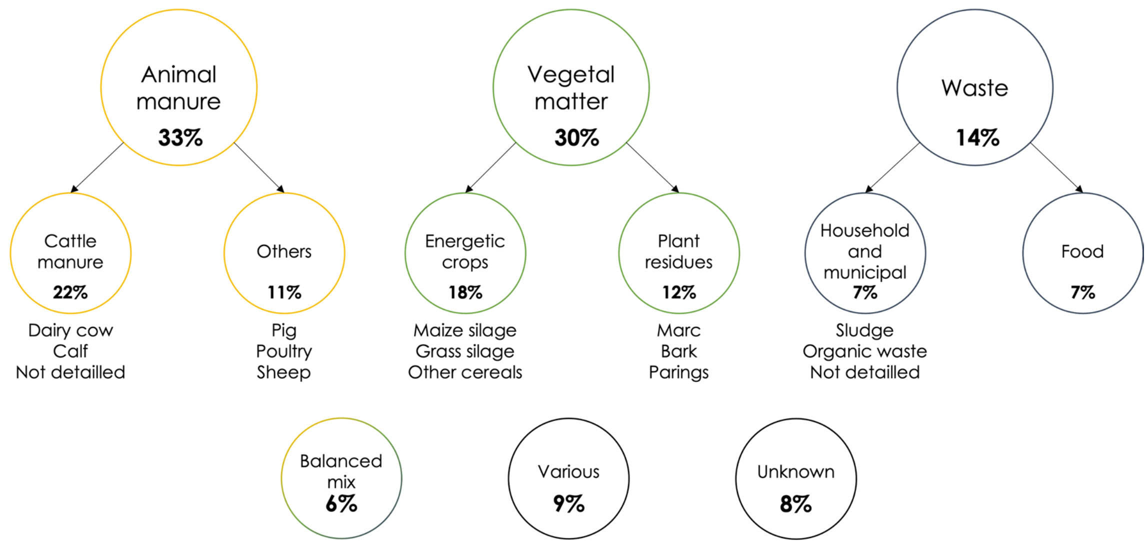


Fig. 4 Analysis of the types of feedstocks used for anaerobic digestion in the studies on the impact of digestates on the soil microbiological quality. Generally, in the studies, applied digestate are composed of animal or vegetal matter. Digestate based on waste are less frequently tested

and 17% targeted the first 30 cm. This datum was not available in 18% of the studies.

Types of digestates

The feedstocks used for anaerobic digestion were inventoried across the studies. They were classified in 3 groups according to the main component of the feedstock (Fig. 5):

1. feedstock of animal origin called "animal manure",
2. feedstock of vegetal origin called "vegetal matter",
3. feedstock from various waste products called "waste".

Animal manure digestates were applied in one third of the studies, and most of it was cattle manure. Manure from other animals like pigs, poultry or sheep was also used. Plant matter digestates were applied in 30% of the studies. Plant matter came from energetic crops such as maize or grass, or plant residues such as marc, bark or parings. Waste were used in only 14% of the studies, and were food waste in more than half of the cases. Digestates based on a balanced mix of different types of feedstocks were applied in 6% of the studies, and a various range of digestates was tested in 9% of them.

In 60% of the studies, the process used for anaerobic digestion was not reported. Among the remaining 40%, the authors mentioned mesophilic digestion in 36% of the cases. However, behind the same nomenclature, the temperature varied from 35 to 48 °C depending on the studies. The last 4% of studies mentioned thermophilic digestion.

Despite an attempt to review the fraction of digestates used in all studies, this datum was complex to inventory in a significant part of the articles. Several words were used to identify the digestates: "Slurry", "Manure", "Digestate", "Residues", but a same word referred to various products depending on the studies. For example, "slurry" can be used for raw digestate, for mainly liquid digestate or for the liquid fraction after separation. In some studies, the word "manure" referred to the liquid fraction, but it was essentially used as the solid fraction or raw solid digestate. These observations highlight the need to clarify terms at the international scale and establish a common nomenclature based on the characteristics of the product such as production process or proportion of dry matter of the final product.

Soil microbiological parameters

As previously defined in Karimi et al. (2020), the soil biological quality is the capacity of a soil to host a large quantity and diversity of living organisms involved in its functioning and in the provision of ecosystem services. By translation, the soil microbiological quality refers to the huge quantity and diversity of microorganisms living and interacting in the soil and involved in its ecological functioning. Therefore, all the microbiological parameters–more than 55 in total – studied in the pool of 66 articles were inventoried and reviewed, and classified according to the type of information provided on the soil microbiological quality (Table 1): (i) – biomass or abundance of microbial communities, (ii) microbial taxonomic diversity, (iii) microbial metabolic activity, (iv) abundance of functional genes or groups, v- microbial functional diversity, (vi) the microbial network, and vii- the sanitary state of the soil.

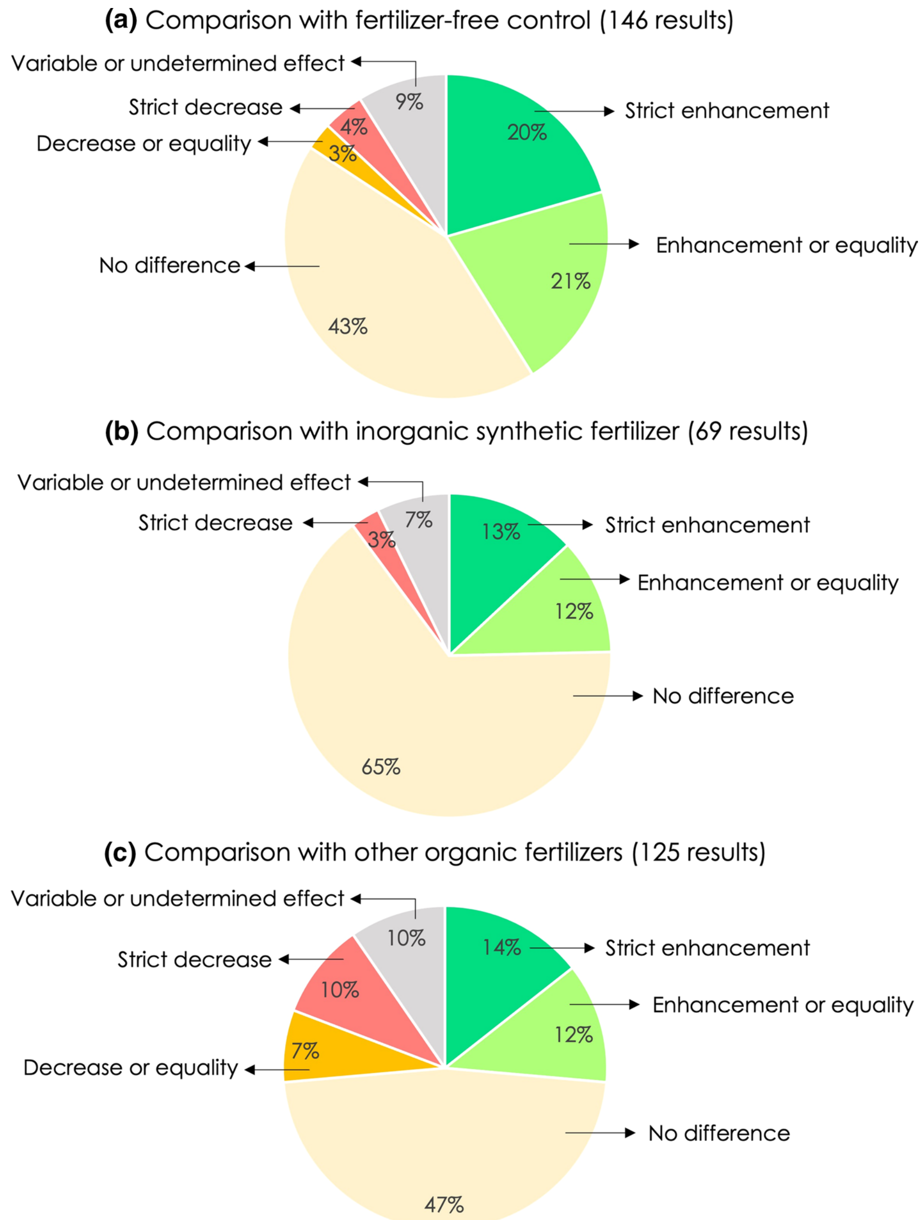


Fig. 5 Pie-charts summarizing the global ecological impact of digestates application on the soil microbiological quality in comparison with: **a** fertilizer-free control; **b** with a classical inorganic mineral fertilizer; **c** other classical organic fertilizers. The results from 56 studies measuring 23 microbial parameters are summarized. The number of results indicated in parentheses correspond to the number of comparisons of any soil microbial parameters between the digestates and the controls. Digestate present globally neutral or positive effects compared to the 3 different controls. However, negative effects have been recorded for 7% of results compared to fertilizer-free control and for 17% of results compared to other organic fertilizers

Among these 55 parameters, some were rarely evaluated, while 23 were more commonly measured and more easily interpretable in terms of soil microbiological quality. These parameters concerned the whole microbial community, the bacterial community, the fungal community, or the archaeal community. They quantified their biomass and abundance, their taxonomic diversity in term of richness or community structure, and their metabolic activity measured by enzymatic activities, metabolic quotient and metabolic diversity. The present study relies on these 23 microbiological parameters to review the ecological impacts of digestates and to offer a robust and generic conclusion on the implication of digestates in the soil microbiological quality.

Within these 23 parameters, some were more assessed than others. Far ahead, microbial biomass, which gives the quantity of microorganisms in the soil, was measured in 32 out of 66 studies. Dehydrogenase activity was measured in 18 studies and was the second most measured parameter. Dehydrogenase is an oxidoreductase, and its measurement evaluates the global activity of organic matter decomposition by the microbial communities. Four parameters were assessed in about 10 studies: bacterial and fungal abundance, bacterial richness, and alkaline phosphatase activity which is a microbial activity depending on the PO_4^- ions available in the soil. All other parameters were assessed in less than 10 studies, which could be explained by the newness of some methods such as molecular approaches to characterize

Table 1 Microbiological parameters monitored in the 66 studies analyzed in this review, classified according to the type of information they provide. For each parameter, the table indicates the number of articles provided data and if it was included in this review. Parameters with too few studies providing data were discarded

Type	Microbiological parameters	Number of articles	Reviewed in this study
Biomass—Abundance	Microbial biomass	32	X
	Microbial abundance	3	X
	Bacterial abundance	11	X
	Fungal abundance	12	X
	Archael abundance	4	X
	Fungi:bacteria ratio	3	
Taxonomic diversity	Bacterial diversity (richness)	10	X
	Fungal diversity (richness)	5	X
	Archaeal diversity (richness)	2	X
	Microbial community structure	5	X
	Bacterial community structure	8	X
	Fungal community structure	4	X
	Archaeal community structure	2	X
Metabolic activity	Metabolic quotient (qCO2)	9	X
	Fluorescein Diacetate Hydrolytic activity	4	X
	Dehydrogenase activity	18	X
	Alkalin phosphatase activity	12	X
	Acid phosphatase activity	6	X
	Betaglucosidase activity	8	X
	Protease activity	5	X
	Urease activity	3	X
	Arylsulfatase activity	3	X
	Catalase activity	2	X
	Metabolic diversity (richness, shannon index, structure)	7	X
	Betaglucosaminidase activity	1	
	O-diphenol oxydase activity	1	
	Cellobiohydrolase activity	1	
	Xylanase activity	1	
	Chitinase activity	1	
	Leucine amino-peptidase activity	1	
	Bacterial growth	1	
	Fungal growth	1	
Abundance of functional genes or groups	amoA-AOA abundance	4	
	amoA-AOB abundance	4	
	nifH abundance	2	
	nrfA abundance	1	
	nirK abundance	2	
	nirS abundance	2	
	nosZ abundance	3	
	mcrA abundance	1	
	pmoA abundance	1	
	Ammonifying bacteriaabundance	1	
	Copiotrophic bacteria abundance	1	
	Oligotrophic bacteria abundance	1	
	Arbuscular Mycorhizal Fungi abundance (AMF)	1	
	Abundance of proteolytic microorganisms	1	

Table 1 (continued)

Type	Microbiological parameters	Number of articles	Reviewed in this study
Functional diversity	Rhizospheric bacterial richness	1	
	Denitrifying bacterial richness	1	
	Rhizospheric bacterial structure of community	2	
	AOA community structure	1	
	Denitrifying bacterial structure of community	1	
Microbial network	Bacterial network	1	
	Fungal network	1	
Sanitary state	R. solani infection index	1	
	Antibiotic resistance genes	1	

microbial communities, or the lack of references which complexifies the interpretation of the results.

Following the analysis of questions and experimental strategies, the pool of articles was reduced from 66 to 56. The 10 removed articles provided data on questions not treated in this review, such as the impact of biochar produced from digestates, or on microbial parameters not reviewed here (Table1).

Global ecological impact of digestates on the soil microbiological quality

To evaluate the global impact of digestates on the soil microbial biodiversity–that we called the "global ecological impact" –, we inventoried the positive, neutral and negative effects reported in the 56 articles finally selected based on the 23 main microbiological parameters measured in the studies. To be in accordance with both the ecological and the agronomical points of view, we recorded the effects in comparison to 3 different references: (i) the fertilizer-free control; (ii) an inorganic synthetic fertilizer at a similar dose of nitrogen; and (iii) any other organic fertilizer or amendment. In each case, the proportion of strictly positive, positive or neutral, strictly neutral, negative or neutral and negative effects reported in the articles were calculated. An effect was considered as significant when the statistical test reported a p value lower than 0.05. Some study provided two different results: for example, a positive effect and no effect according on the studied soil. The global result was then counted in "Enhancement or equality" or in "Decrease or equality" categories. These counts were reported on pie charts summarizing the global ecological impact of digestates (Fig. 5).

Interestingly, the articles reported twice as much data when the control was fertilizer-free than when the control was an inorganic fertilizer. This observation can be partly explained by the choice of laboratory experiments, which are often poorly linked to real agronomical conditions and to farmers' concerns. These data had the advantage of measuring the net effect of digestates on soil microorganisms, which corresponds to the criteria of an ecological evaluation.

Whatever the control, the results showed that a large part of studies – 43 to 65% – concluded to no effect of digestates compared to the reference (Fig. 5). Positive effects of digestates were found in 25–41% of the results, while negative effects were found in 3 to 17% of the results depending on the level of reference.

Compared to the fertilizer-free control (Fig. 5), the microbial parameters responded negatively to digestate application in 7% of the cases whatever the experimental conditions, suggesting a slightly deleterious global ecological impact. Digestates had a net stimulating effect on the soil microbial communities in several cases. When the control was an inorganic fertilizer, digestates often had the same effect as the inorganic fertilizer, or a more stimulating effect in 25% of the cases. Microbial parameters decreased compared to the inorganic fertilizer in only two cases. In parallel, 17% of the results showed that digestates stimulated the microbial communities less than other organic fertilizers did, and particularly concerned microbial biomass and activity.

Overall, these results indicate that digestates were neutral for the soil microbiological quality in half of the situations. However, negative effects were detected in 7% of the cases, so that we cannot conclude to the absence of any ecological risk of these products on soils. Moreover, the comparison with other organic fertilizers highlighted that digestates seemed less beneficial for the soil microbial communities in 17% of the cases.

Impact of digestate characteristics on the soil microbiological quality

Characteristics investigated in the literature

As regards frequency criteria, we identified and ranked all the questions related to the impact of digestates on the soil microbial biodiversity investigated in the scientific literature to date, and used them to detect the well-informed questions and the questions that need further investigations. Among the core reviewed articles, 16 questions were inventoried, characterized by different levels of investigation (Fig. 10). Six major questions were identified for which substantial scientific literature was found:

1. The comparison of digested and undigested organic matters (18 articles)
2. The comparison of the effect of digestates with the effect of other organic products (15 articles)
3. The effect of the feedstock type (14 articles)
4. The effect of digestate spreading whatever its form: raw, liquid, or solid (11 articles)
5. The effect of digestate doses (11 articles)
6. The differential effects of the liquid and solid digestate fractions (9 articles).

The effects of the digestion process, fraction separation or stabilization on the soil microbiological quality have been poorly studied so far or not studied at all, and so have the effects of the soil type, of the historical record of fertilization and of the interaction with other agricultural practices.

All the results of each of the 6 main questions were reviewed and summarized, and are presented on one radar chart *per* question. As the most frequent reference was the fertilizer-free control, it was used as a reference on the charts. To contextualize and appreciate the genericity of the results, different data were summarized in addition to the effects, *i.e.*, (i) the number of articles about the same combination [condition x microbiological parameter]; (ii) the temporal scale of the study: less than 6 months was considered as short-term, more than two years was considered as long-term, and 6 to 24 months was considered as mid-term; (iii) the type of digestate concerned by the results: vegetal feedstock, animal feedstock, a mix of vegetal and animal feedstocks, and waste; (iv) the digestate fraction: raw residues, liquid fraction, solid fraction; and (v) the type of organic materials when the digestates where compared with other fertilizers or amendments. All these data were used to plot the generic character of each result on one synthetic figure and return to the specificities of the observed effect if necessary.

Effect of digestate application

Nine articles reported the simple effect of digestate application, without dealing with another question (Table 2). The results are summarized in Fig. 6. As far as their global ecological impact was concerned, most data showed positive or null effects of digestates on the soil microbial parameters compared to the fertilizer-free control, whatever the parameters and the digestate fraction. The digestates used in these studies were mainly based on vegetal or animal feedstocks, and the experiments were set up on a short time scale. As depicted in Fig. 6, only one result indicated that the application of liquid digestates of animal origin induced dehydrogenase activity lower than or equal to the control (Fernández-Delgado Juarez et al. 2015). This result, reported by one article only, was not generic.

The overall input of digestates mainly had a positive effect on the soil microbiological quality, whatever the fraction and the feedstock type. Most of these results were obtained for one or a few soil types and need to be validated across a larger diversity of pedoclimatic conditions and by long-term survey.

Effect of the digestate fraction

The effect of the digestate fraction was investigated in 9 articles that provided results about a reduced set of microbial parameters. The studies generally included solid and liquid fractions, but the raw materials were poorly compared with these fractions. Most of the experiments were conducted on a short time scale, *i.e.,* 6 months or less. Most of the studies showed positive or null effects on the microbial abundance and activity parameters compared to the fertilizer-free control. Significant differences were rarely observed when raw digestates, the solid fraction and the liquid fraction were compared, whatever the microbial parameter and the feedstock type. As for this latter point, no datum was available about the response of microbial diversity parameters (Fig. 7).

As illustrated in Fig. 7, fungal abundance and dehydrogenase activity results were not clear. The impact of the digestate fraction on fungal abundance was investigated in two studies (Barduca et al. 2021; Panuccio et al. 2021) that provided opposite results. The first one showed that the liquid fraction and raw digestates decreased fungal abundance compared to the solid fraction and the fertilizer-free control (Barduca et al. 2021). The second study highlighted that fungal abundance was higher after applying the liquid fraction than after applying the solid fraction, and both were stimulating compared to the control (Panuccio et al. 2021). The digestates used in both studies were obtained from a mix of animal manure and plant material such as ensiled crops or grass.

Table 2 Topics of publications on the effect of digestate application

Questions	References
Effect of digestate application	
Raw	Sapp et al. (2015), García-Sánchez et al. (2016), Różyło and Bohacz (2020)
Solid	García-Sánchez et al. (2016), Száková et al. (2016), Badagliacca et al. (2020)
Liquid	Fernández-Delgado Juarez et al. (2015), Zhao et al. (2017), Tang et al. (2021)
Effect of the digestate fraction	de la Fuente et al. (2013), Hupfauf et al. (2016), Muscolo et al. (2017), Ibeto et al. (2020), Nielsen et al. (2020), Valentinuzzi et al. (2020), Barduca et al. (2021), Cattin et al. (2021), Panuccio et al. (2021)
Effect of the feedstock type	Johansen et al. (2013); Martin et al. (2014,Barra Caracciolo et al. (2015, Sawada and Toyota (2015, Wentzel et al. (2015, Wentzel & Joergensen (2016, Hupfauf et al. (2016, Viaene et al. (2017, Muscolo et al. (2017, Coelho et al. (2019, 2020), Nielsen et al. (2020), Pagliaccia et al. (2020), Manfredini et al. (2021)
Effect of the digestate dose	
Raw	Brenzinger et al. (2018), Cardelli et al. (2018), Gryta et al. (2020), Różyło and Bohacz (2020), Pastorelli et al. (2021
Solid	Barra Caracciolo et al. (2015), Muscolo et al. (2017), Telesiński et al. (2017), Valentinuzzi et al. (2020)
Liquid	Johansen et al. (2015),Muscolo et al. (2017), Mortola et al. (2019), Gryń et al. (2020), Valentinuzzi et al. (2020)
Effect of the anaerobic digestion of organic matter	Chen et al. (2012), Pezzolla et al. (2013), de la Fuente et al. (2013), Fernández-Delgado Juárez et al. (2013), Johansen et al. (2013), 2015; Bachmann et al. 2014, Wentzel and Joergensen (2016, Hupfauf et al. (2016, Viaene et al. (2017, Wolters et al. (2018, Podmirseg et al. (2019, Muscolo et al. (2019, Zicker et al. (2020, Monard et al. (2020, Nielsen et al. (2020

Table 2 (continued)

Questions	References
Comparison of digestates with other types of organic fertilizer	Odlare et al. (2008), Ernst et al. (2008, Alburquerque et al. (2012, Walsh et al. (2012, Martin et al. (2014, Ramezanian et al. (2015, Šimon et al. (2015, Bhogal et al. (2018, Siebielec et al. (2018, Brenzinger et al. (2018, Cardelli et al. (2018, Coelho et al. (2019, 2020, Valentinuzzi et al. (2020), Gebremikael et al. (2020), Gryta et al. (2020), Manasa et al. (2020a)

Regarding dehydrogenase activity, the liquid fraction had a more stimulating effect than the solid fraction in 2 studies where the digestates were produced from animal feedstock (Nielsen et al. 2020; Panuccio et al. 2021). An opposite result was observed in 2 cases where the feedstocks were animal manure or a mix of animal and vegetal matter, respectively (Muscolo et al. 2017; Nielsen et al. 2020). Moreover, 2 studies showed no difference in the effects induced by each fraction; they were carried out on digestates produced from olive and animal manure, respectively (Muscolo et al. 2017; Valentinuzzi et al. 2020). The various results on fungal abundance and dehydrogenase activity highlighted that no general trend can be drawn about the effect of the digestate fraction on these two microbial parameters.

Overall, no strong and generic difference was observed in the soil microbiological quality between the solid and liquid fractions of the digestates. More data, particularly about microbial diversity, are needed to establish an objective and robust conclusion.

Effect of the feedstock type

Results about the effect of the feedstock type were provided in 14 articles that measured a large range of microbial parameters on the short or mid term, *i.e.,* 6 months or less and up to 18 months respectively. Figure 8 shows that apart from fungal abundance and archaeal abundance, the other microbial abundance parameters were not affected by the type of feedstock used to produce the digestates. Among the microbial diversity parameters, only bacterial diversity and community structure were sensitive to the type of feedstock. Microbial activities were more largely concerned, with 7 parameters sensitive to the type of feedstock: the metabolic quotient, fluorescein diacetate hydrolytic activity, acid phosphatase activity, beta-glucosidase activity, urease activity, catalase activity, and metabolic diversity.

The analysis of all these results did not highlight any one type of feedstock proved to be systematically deleterious or favorable to all microbial parameters. Animal feedstocks produced digestates that stimulated fluorescein diacetate hydrolytic activity, urease activity and bacterial diversity, but had a negative impact on fungal abundance compared to mixed or waste feedstocks (Barra Caracciolo et al. 2015; Wentzel & Joergensen 2016; Muscolo et al. 2017; Coelho et al. 2019, 2020; Pagliaccia et al. 2020). The digestates from vegetal feedstocks showed lower fluorescein diacetate hydrolytic activity, acid phosphatase, beta-glucosidase and urease activities than the digestates from animal feedstocks (Muscolo et al. 2017). Conversely, they seemed to stimulate catalase activity more strongly than the digestates based on animal feedstocks did (Muscolo et al. 2017). Concerning the digestates based on waste feedstocks, different digestates provided similar results for most microbial parameters, except the metabolic quotient on which the effects were highly variable according to the waste (Manfredini et al. 2021).

Overall, it was difficult to highlight a strong and robust effect of the feedstock type on the soil microbiological quality due to the lack of genericity of the results on some microbial parameters. Available knowledge suggests that the diverse compositions of digestates can induce diverse soil microbiological responses, but no trend can be drawn as to specific effects of the types of feedstocks.

Effect of the digestate dose

The dose effect of digestates was studied in 11 articles, and the effects were measured on almost all microbial parameters except archaeal communities and aryl-sulfatase activity on the short or mid-term, *i.e.,* up to 18 months. The results highlighted that the dose effect was more frequently studied on the solid fraction than on the liquid fraction or raw digestates. When a dose effect was observed, higher doses generally stimulated the microbial parameters: all the parameters of microbial abundance, fluorescein diacetate hydrolytic activity, dehydrogenase activity, alkaline phosphatase activity. In some cases, the highest dose was less stimulating

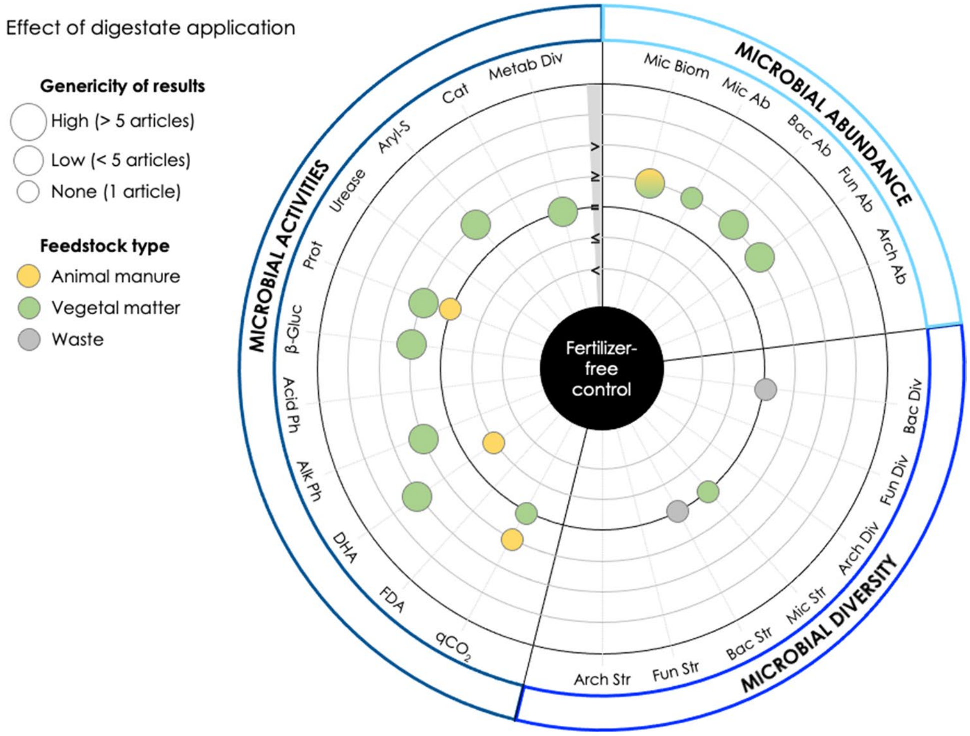


Fig. 6 Effect of digestates application on the microbiological parameters of abundance, diversity and activity in soils: Mic Biom = Microbial biomass, Mic Ab = Microbial abundance, Bac Ab = Bacterial abundance, Fun Ab = Fungal abundance, Arch Ab = Archaeal abundance, Bac Div = Bacterial diversity, Fun Div = Fungal diversity, Arch Div = Archaeal diversity, Mic Str = Microbial community structure, Bac Str = Bacterial community structure, Fun Str = Fungal community structure, Arch Str = Archaeal community structure, qCO_2 = Metabolic quotient, FDA = Fluorescein Diacetate hydrolytic Activity, DHA = Dehydrogenase activity, Alk Ph = Alkaline phosphatase activity, Acid Ph = Acid phosphatase activity, β-Gluc = Beta-glucosidase activity, Prot = Protease activity, Urease = Urease activity, Cat = Catalase activity, Metab Div = Metabolic diversity). The reference is the fertilizer-free control. The results of the studies showing similar effects between digestate and control are indicated by circles on the median bold line (= sign). Circles placed above (or below) the median bold line indicate the results of the studies showing a positive (or negative) effect of digestate compared with the control. The closer the circle is to the center of the diagram, more negative the effect of the digestate is compared to the control. The closer the circle is to the periphery of the diagram, more positive the effect of the digestate is compared to the control. The circle size is related to the genericity of the results: greater the circle, more generic the result. The color indicates the feedstock type used for digestate production: yellow for animal manure, green for vegetal matter and grey for waste. Synthesis from 9 references shows that digestates tends to have a positive or neutral effect on microbial abundance, diversity and activity

than the lower doses, suggesting the existence of an optimal dose, at least for bacterial diversity, acid phosphatase activity, beta-glucosidase activity, urease activity and catalase activity (Barra Caracciolo et al. 2015; Muscolo et al. 2017; Telesiński et al. 2017; Różyło and Bohacz 2020). Finally, only two studies showed a negative effect of the digestate dose. Acid phosphatase activity decreased by increasing the dose of a digestate mixing animal and vegetal feedstocks, 56 days after application (Telesinski et al. 2017). A similar effect was observed on urease and betaglucosidase activities 3 months after application of an olive-based digestate (Muscolo et al. 2017).

Dose effects can be strictly positive or with an optimum, possibly explained by the variability of the doses tested in the studies. Depending on the study, the choice of the doses was based on either the equivalent in mineral nitrogen content, or the carbon content, or the mass quantity, or the volume of the digestates. This made it difficult to compare the impact of the range of doses tested in the studies.

An overall positive or neutral effect of the digestate dose was frequently found on the soil microbiological quality, whatever the digestate type. However, the effect on some microbial parameters was less beneficial at too high doses than at lower doses, evidencing an optimum. This result raises the question of the long-term effects induced by the repeated accumulation of digestates in the soil, and the potential long-term risks for microbial communities.

Comparison of the digestate with undigested organic matter

Figure 9 compares the application of digested organic matter with the application of the same organic matter in

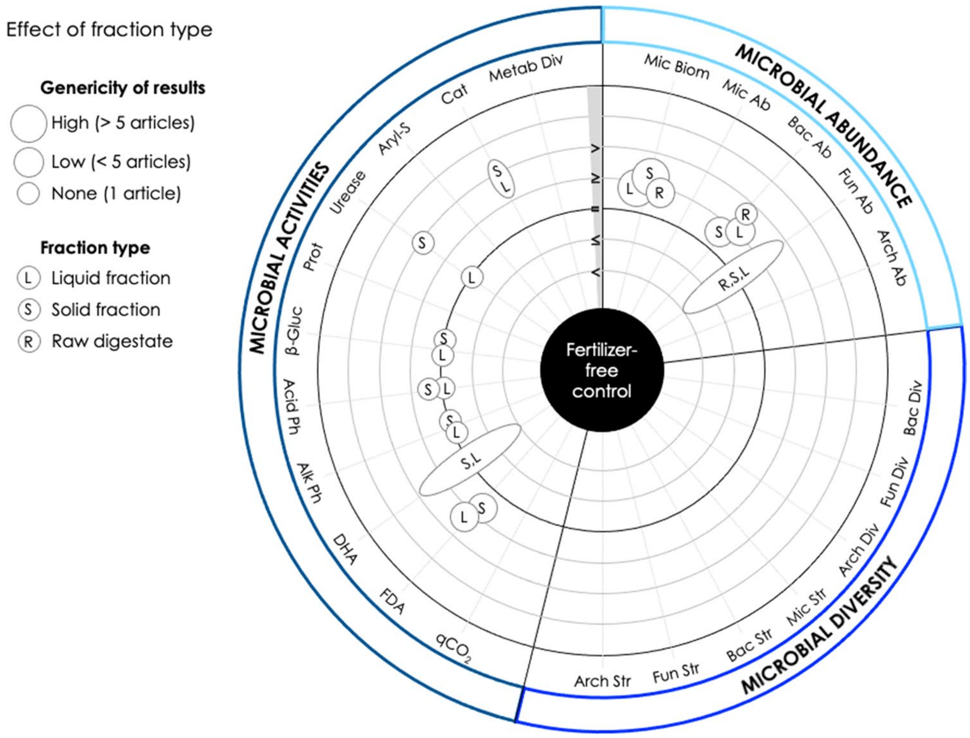


Fig. 7 Effect of the digestate fraction applied on the microbiological parameters of abundance, diversity and activity in soils: Mic Biom = Microbial biomass, Mic Ab = Microbial abundance, Bac Ab = Bacterial abundance, Fun Ab = Fungal abundance, Arch Ab = Archaeal abundance, Bac Div = Bacterial diversity, Fun Div = Fungal diversity, Arch Div = Archaeal diversity, Mic Str = Microbial community structure, Bac Str = Bacterial community structure, Fun Str = Fungal community structure, Arch Str = Archaeal community structure, qCO_2 = Metabolic quotient, FDA = Fluorescein Diacetate hydrolytic Activity, DHA = Dehydrogenase activity, Alk Ph = Alkaline phosphatase activity, Acid Ph = Acid phosphatase activity, β-Gluc = Beta-glucosidase activity, Prot = Protease activity, Urease = Urease activity, Cat = Catalase activity, Metab Div = Metabolic diversity). The reference is the fertilizer-free control. The results of the studies showing similar effects between digestate and control are indicated by circles on the median bold line (= sign). Circles placed above (or below) the median bold line indicate the results of the studies showing a positive (or negative) effect of digestate compared with the control. The closer the circle is to the center of the diagram, more negative the effect of the digestate is compared to the control. The closer the circle is to the periphery of the diagram, more positive the effect of the digestate is compared to the control. The circle size is related to the genericity of the results: greater the circle, more generic the result. The letter in the circle indicates the fraction of digestate applied on the soil: L for liquid fraction, S for solid fraction, R for the raw digestate. Synthesis from 9 references did not evidence a significant difference between the impact of solid, liquid and raw digestates on microbial abundance and activity. No data was found about the microbial diversity

another form, such as raw farm manure, green manure or plant residues, composts, and biochar. This question was investigated in 18 articles, and the effects were mainly measured on microbial biomass and microbial diversity parameters on a short time scale. Digestates decreased the soil fungal abundance and fluorescein diacetate hydrolytic activity compared to unfertilized soil and other types of materials (Wentzel and Joergensen 2016; Muscolo et al. 2019). Digestates also stimulated betaglucosidase activity less than raw plant residues did, but without having a deleterious impact (Chen et al. 2012). In contrast, digestates had effects close to those of other types of materials for many microbial parameters: microbial biomass, microbial abundance, bacterial, fungal et archaeal diversity, the fungal and archaeal community structures, dehydrogenase activity, and metabolic diversity (de la Fuente et al. 2013; Fernández-Delgado Juárez et al. 2013; Johansen et al. 2013; Pezzolla et al. 2013; Wentzel & Joergensen 2016; Viaene et al. 2017; Wolters et al. 2018; Muscolo et al. 2019; Podmirseg et al. 2019; Monard et al. 2020; Nielsen et al. 2020). No study showed a higher stimulating effect of digestates than of the other types of organic matter on any parameters of soil microorganisms.

Overall, anaerobically digested organic matter had a similar effect as that of undigested organic matter on the soil microbiological quality. However, several deleterious effects were also observed on a few essential microbial parameters which regulate soil biological functioning, *i.e.,* the fungal abundance and global microbial activity.

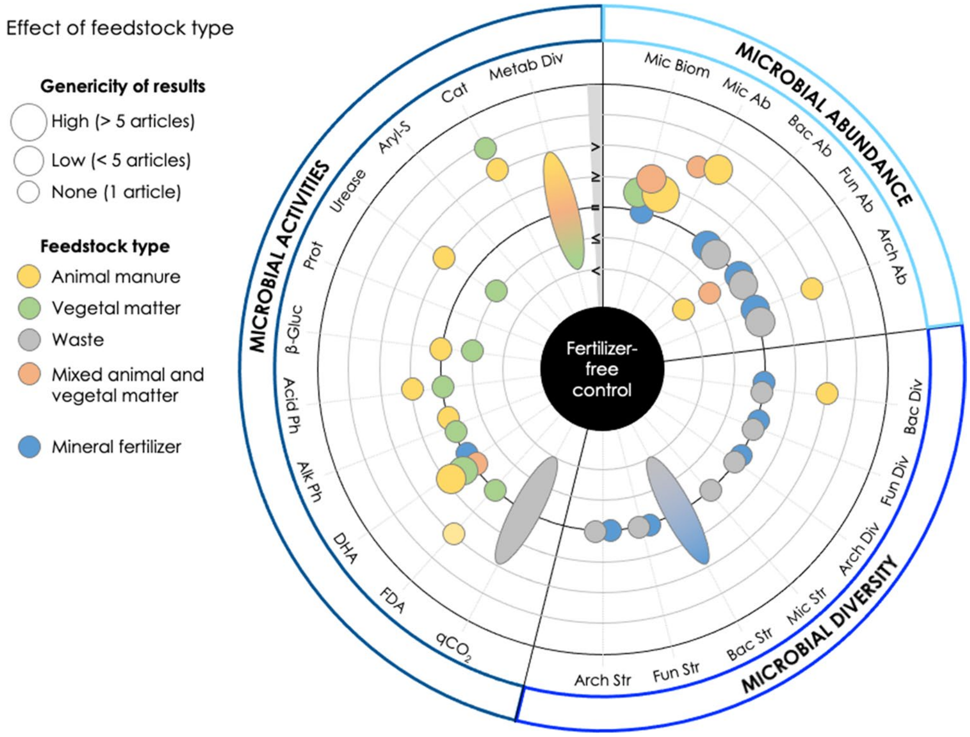


Fig. 8 Effect of the feedstock type on the microbiological parameters of abundance, diversity and activity in soils: Mic Biom = Microbial biomass, Mic Ab = Microbial abundance, Bac Ab = Bacterial abundance, Fun Ab = Fungal abundance, Arch Ab = Archaeal abundance, Bac Div = Bacterial diversity, Fun Div = Fungal diversity, Arch Div = Archaeal diversity, Mic Str = Microbial community structure, Bac Str = Bacterial community structure, Fun Str = Fungal community structure, Arch Str = Archaeal community structure, qCO_2 = Metabolic quotient, FDA = Fluorescein Diacetate hydrolytic Activity, DHA = Dehydrogenase activity, Alk Ph = Alkaline phosphatase activity, Acid Ph = Acid phosphatase activity, β-Gluc = Beta-glucosidase activity, Prot = Protease activity, Urease = Urease activity, Cat = Catalase activity, Metab Div = Metabolic diversity). The reference is the fertilizer-free control. The results of the studies showing similar effects between digestate and control are indicated by circles on the median bold line (= sign). Circles placed above (or below) the median bold line indicate the results of the studies showing a positive (or negative) effect of digestate compared with the control. The closer the circle is to the center of the diagram, more negative the effect of the digestate is compared to the control. The closer the circle is to the periphery of the diagram, more positive the effect of the digestate is compared to the control. The circle size is related to the genericity of the results: greater the circle, more generic the result. The circle color indicates the feedstock type used for digestate production: yellow for animal manure, green for vegetal matter, grey for waste and orange for mix between animal and vegetal matter. The blue color indicates a mineral fertilizer control. Synthesis from 14 references does not evidence a systematic difference in the impact of feedstock type on the microbiological parameters. The impacts, which are different from one parameter to another, were generally reported by only one reference

Comparison of digestates with other organic fertilizers

In most farms, digestates were usually used to replace other types of fertilizers made from another source of materials. Thus, the comparison between of digestates and other types of organic fertilizers or mineral fertilizers was needed to evaluate the impact of fertilization change on the soil microbiological quality. Fifteen articles reported results on the comparison of digestates with farm manure, green manure made from plant residues, composts, biochar, and mineral fertilizers. Most microbial parameters were investigated, except microbial abundance, acid phosphatase activity, and arylsulfatase activity. Measurements were performed on the short-, mid-, and long-term in laboratory microcosm experiments as well as field experiments.

Figure 10 highlights a few significant deleterious effects of digestates compared to the unfertilized control. The parameters of microbial abundance were more improved by digestates than by mineral fertilizers, and the effects of different types of organic fertilizers were similar. More precisely, digestates had a similar positive effect than other organic fertilizers on microbial biomass, except biochar which had no effect (Ernst et al. 2008; Odlare et al. 2008; Alburquerque et al. 2012; Šimon et al. 2015; Bhogal et al. 2018; Cardelli et al. 2018; Gebremikael et al. 2020; Manasa et al. 2020a; Valentinuzzi et al. 2020). Digestates had a higher or neutral effect on bacterial, fungal, and archaeal abundances compared to farm manure and compost (Walsh

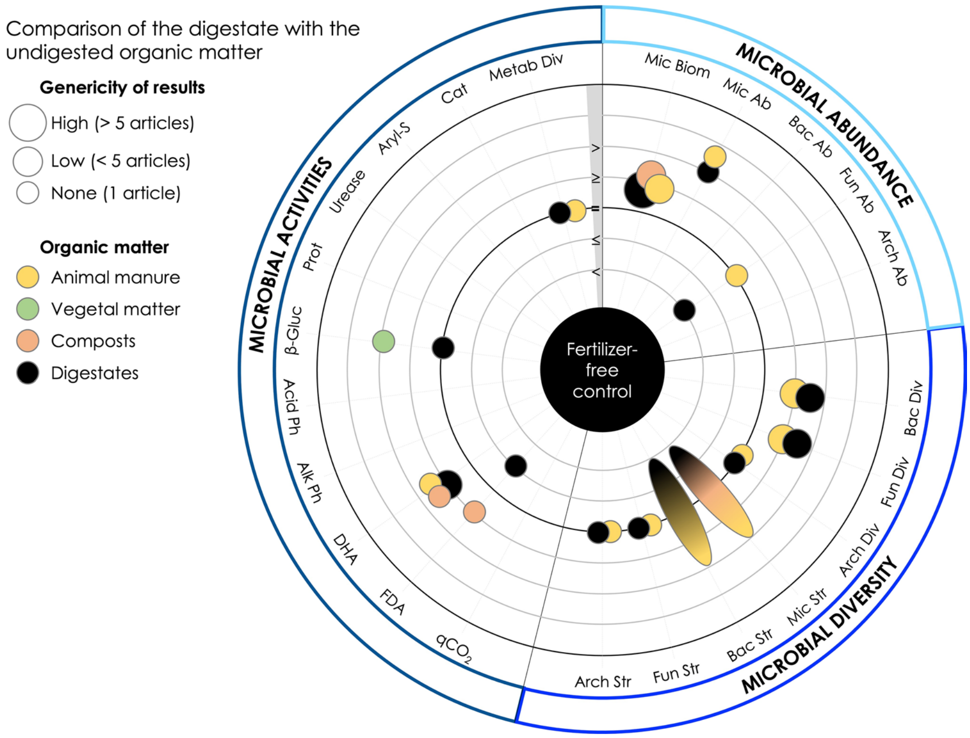


Fig. 9 Effects of digested and undigested organic matters on the microbiological parameters of abundance, diversity and activity in soils: Mic Biom = Microbial biomass, Mic Ab = Microbial abundance, Bac Ab = Bacterial abundance, Fun Ab = Fungal abundance, Arch Ab = Archaeal abundance, Bac Div = Bacterial diversity, Fun Div = Fungal diversity, Arch Div = Archaeal diversity, Mic Str = Microbial community structure, Bac Str = Bacterial community structure, Fun Str = Fungal community structure, Arch Str = Archaeal community structure, qCO_2 = Metabolic quotient, FDA = Fluorescein Diacetate hydrolytic Activity, DHA = Dehydrogenase activity, Alk Ph = Alkaline phosphatase activity, Acid Ph = Acid phosphatase activity, β-Gluc = Beta-glucosidase activity, Prot = Protease activity, Urease = Urease activity, Cat = Catalase activity, Metab Div = Metabolic diversity). The reference is the fertilizer-free control. The results of the studies showing similar effects between digestate and control are indicated by circles on the median bold line (= sign). Circles placed above (or below) the median bold line indicate the results of the studies showing a positive (or negative) effect of digestate compared with the control. The closer the circle is to the center of the diagram, more negative the effect of the digestate is compared to the control. The closer the circle is to the periphery of the diagram, more positive the effect of the digestate is compared to the control. The circle size is related to the genericity of the results: greater the circle, more generic the result. The circle color indicates the organic matter type applied: yellow for animal manure, green for vegetal matter, orange for compost and black for digestates. The digestate is systematically composed of the same feedstock than the undigested organic matter applied. Synthesis from 16 references shows that digestate has negative effect, or lower positive effect, compared with the undigested organic matter on fungal abundance, fluorescein diacetate hydrolytic activity and beta-glucosidase activity. Other parameters are less impacted by the form of the organic matter applied on soils

et al. 2012; Brenzinger et al. 2018; Siebielec et al. 2018; Coelho et al. 2019, 2020). However, raw vegetal organic matters increased microbial biomass, bacterial abundance and fungal abundance more than digestates did (Brenzinger et al. 2018; Gebremikael et al. 2020).

Microbial diversity parameters were little influenced by the type of fertilizer (one mineral fertilizer and different organic fertilizers were tested) (Coelho et al. 2020; Gebremikael et al. 2020). Only the bacterial community structure changed between unfertilized soil, soil fertilized with the digestates, and soil fertilized with pot ale from whisky distilleries (Ramezanian et al. 2015). Finally, the microbial activity parameters also showed similar results whatever the fertilizer, except metabolic diversity (Ernst et al. 2008; Alburquerque et al. 2012; Ramezanian et al. 2015; Cardelli et al. 2018; Siebielec et al. 2018; Gebremikael et al. 2020; Gryta et al. 2020; Manasa et al. 2020b; Valentinuzzi et al. 2020).

Overall, compared to other fertilizers, digestate application induced various effects according to the soil microbial parameters. The stimulating effect of digestates was either higher or lower than that of other organic fertilizers on microbial biomass and abundance. These conclusions should be taken with caution because the genericity of these results is weak, implying that a low diversity of soil types was studied. Previous studies on the impact of various organic fertilizers on a range of soil types evidenced that

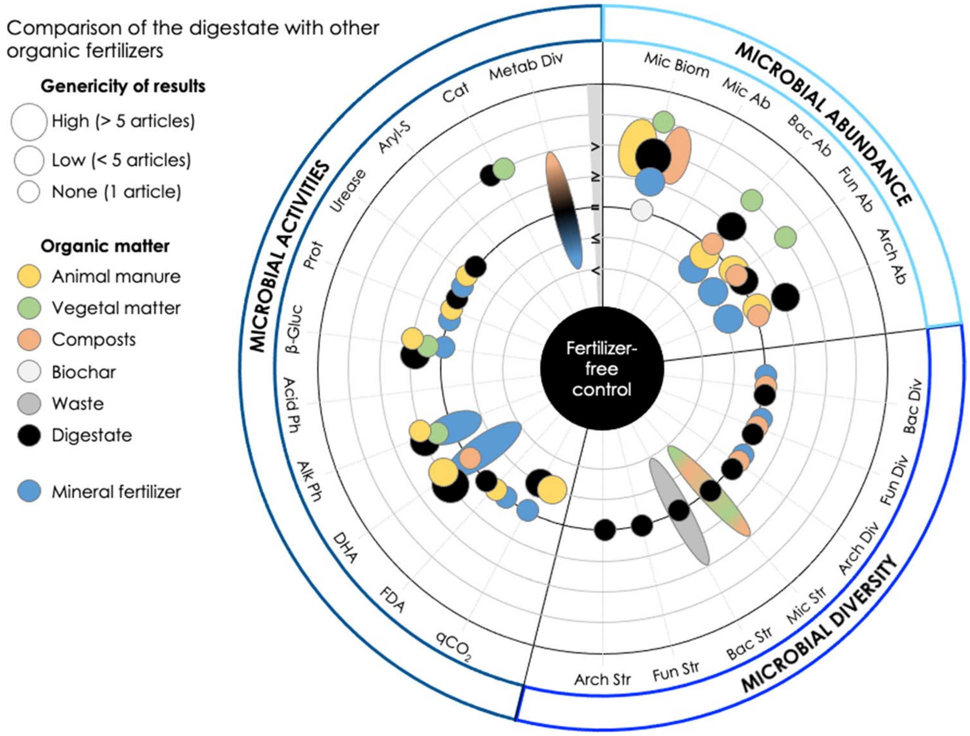


Fig. 10 Effects of digestate with other organic fertilizers on the microbiological parameters of abundance, diversity and activity in soils: Mic Biom = Microbial biomass, Mic Ab = Microbial abundance, Bac Ab = Bacterial abundance, Fun Ab = Fungal abundance, Arch Ab = Archaeal abundance, Bac Div = Bacterial diversity, Fun Div = Fungal diversity, Arch Div = Archaeal diversity, Mic Str = Microbial community structure, Bac Str = Bacterial community structure, Fun Str = Fungal community structure, Arch Str = Archaeal community structure, qCO_2 = Metabolic quotient, FDA = Fluorescein Diacetate hydrolytic Activity, DHA = Dehydrogenase activity, Alk Ph = Alkaline phosphatase activity, Acid Ph = Acid phosphatase activity, β-Gluc = Beta-glucosidase activity, Prot = Protease activity, Urease = Urease activity, Cat = Catalase activity, Metab Div = Metabolic diversity). The reference is the fertilizer-free control. The results of the studies showing similar effects between digestate and control are indicated by circles on the median bold line (= sign). Circles placed above (or below) the median bold line indicate the results of the studies showing a positive (or negative) effect of digestate compared with the control. The closer the circle is to the center of the diagram, more negative the effect of the digestate is compared to the control. The closer the circle is to the periphery of the diagram, more positive the effect of the digestate is compared to the control. The circle size is related to the genericity of the results: greater the circle, more generic the result. The circle color indicates the organic fertilizer type: yellow for animal manure, green for vegetal matter, orange for compost, light grey for biochar, dark grey for waste, black for digestates. The mineral fertilizer control is in blue. The digestate is composed of different feedstock than the undigested organic matter applied. Synthesis from 17 references shows that the digestates have currently a similar effect on the microbial abundance, diversity and activity than other organic fertilizers

similar products can have different effects according to the soil (Sadet-Bourgeteau et al. 2019).

Summary of the impact of digestates on the soil microbiological quality

We evaluated the global ecological impact of biogas digestates on the soil microbiological quality by referencing the number of positive, negative, and neutral effects found in 56 articles. Based on 146 experimental comparisons of soils with digestate application on unfertilized soils and 23 microbial parameters, this meta-analysis evidences low cases of global ecological impact on the soil microorganisms. Most studies showed neutral or positive effects (Fig. 5a), while 7% of the results corresponded to altered microbial parameters. This points a low but significant risk of digestate application for soil microbial communities that needs further studies to better understand the soil, climate and digestate application conditions in which the risk occurs.

To complete this evaluation, we needed to determine if anaerobically digested feedstocks had a similar effect as the same undigested feedstocks had on the soil microbial quality. Our meta-analysis evidenced that the application of anaerobically digested organic matters had the same impact on several microbial community parameters as the application of the same organic matters in the absence of anaerobic digestion. However, fungal abundance, global microbial activity measured by fluorescein diacetate hydrolytic activity and beta-glucosidase activity were improved to a lesser extent or even decreased when the matter was digested. This suggests that the process of anaerobic digestion provides less beneficial organic matter for the soil microbial communities and the regulation of soil biological functioning. This conclusion needs to be verified by measuring supplementary environmental indicators such as the nematodes or the soil fauna, the soil carbon storage, the soil nitrogen rate, or the contaminants concentrated during the digestion process.

For farmers, applying biogas digestates from a local biogas plant can represent an alternative to the use of other commercial fertilizers, whether mineral or organic ones. In that case, not only is the digestion of the feedstock changed, but so are the type and origin of organic matter (animal, plant, waste, mix and others). The meta-analysis showed no significant difference in the soil microbiological quality between the application of digestates and the application of other fertilizers in half of the cases. When the digestates were compared with mineral fertilizers, only 28% of the effects were different, and almost all led to a higher stimulation of microorganisms. When the digestates were compared with other organic fertilizers, 26% of the effects were a higher stimulation of microbial communities, and 17% were a lesser stimulation.

These effects were essentially observed on microbial abundance parameters, *i.e.,* microbial biomass, bacterial abundance, fungal abundance, archaeal abundance, and few studies provided results on microbial diversity and activity. Overall, this corroborates that the application of digestates instead of mineral fertilizers is rather beneficial for the soil microbiological quality (Sabir et al. 2021). Concerning the replacement of other organic fertilizers by digestates, further case-by-case studies according to the soil type and the type of available feedstock are needed to identify the most beneficial products for soil microorganisms in terms of abundance, diversity and activity.

Other questions identified the specific conditions in which the use of digestates is more deleterious or beneficial. The scientific literature mainly provided data on 3 questions: the type of feedstock, the fraction, and the dose. This meta-analysis highlights that increasing doses to some extent has a positive impact on the soil microbiological quality, but too high doses can be less beneficial for the soil microbiological quality. In parallel, the available data did not allow concluding to an effect of the feedstock type or of the digestate fraction applied on the soils. Although all these results are based on more than 50 articles, each of them is characterized by low or no genericity and should be taken with caution. In numerous cases, the effects reported here on each microbial parameter were provided by only one study – five studies at most. Moreover, in each article, the experiment was frequently carried out on only one soil, so that extrapolation to other soil types was limited. As previously demonstrated for other types of organic matter, the impact of an organic product on microbial communities can differ among soil types (Ho et al. 2017; Sadet-Bourgeteau et al. 2019).

To conclude, the scientific knowledge available to date reveals that:

- Biogas digestates are neutral for the soil microbiological quality in half of the situations
- Due to the observation of negative effects in 7% of the experimental assessments, it is impossible to conclude to the absence of any ecological risks of the digestates on soils
- Digestates were less beneficial than other organic fertilizers for the soil microbial communities in 17% of the cases, particularly when comparing the same digested and undigested feedstock
- The dose effect was determined by an optimal dose that seemed to vary according to the feedstock type, the fraction, and the soil type; this reveals a possible ecological risk of recurrent digestate application on the soils on the mid or long term
- No strong and generic difference was observed in the soil microbiological quality following the application of the solid or liquid fraction of the digestates
- The diverse compositions of the digestates could induce a diversity of the soil microbiological response, but no trend can be drawn as to specific effects of the feedstock type for now.

Conclusion

Several perspectives can be drawn from the limitations of our review. First, field experiments–which are closest to realistic agro-pedo-climatic conditions–were under-represented in our meta-analysis, with only 25% of the studies. In field conditions, the effect of digestates is modulated by the weather conditions at the time of spreading and after spreading, the soil is not entirely bare and the application of digestates is repeated over time at a frequency adapted to the needs of the crops and soils. Moreover, these experiments are the most appropriate approach to monitor the impact of biogas digestates on short-, mid- and long-term scales, and thus smooth out the transient effect.

Field experiments including several digestate applications should be set up and monitored for at least two years. Another approach–the farm network–can provide interesting results by measuring the impact of digestates on a large diversity of soils and includes more diverse and complex farming systems. This approach has not been found in the literature to date. However, it is a powerful way of drawing generic conclusions. With the increasing number of biogas plants and farmers interested in the use of digestates at the European scale, building such a network should be affordable to robustly assess the impact of different types of digestates.

Second, most bioindicators are related to microbial abundance parameters, more particularly to microbial biomass. The studies measuring microbial diversity and activity parameters were less frequent and provided results with low genericity or no genericity at all. Nevertheless, microbial diversity is essential in the nitrogen and carbon cycles because it ensures organic matter decomposition and carbon mineralization, and in turn sustainable soil functioning (Maron et al. 2018). Thus, the evaluation of changes in bacterial and fungal diversity and community structures should provide data on the sustainability of digestate application on agricultural soils.

Apart from microorganisms, other soil taxonomic groups remain poorly studied. This review points the lack of data about the soil macro- and mesofauna, including nematodes, collembola, earthworms and all macro-arthropods. With less than 10 articles available, it was impossible to draw robust conclusions about the ecological impact of biogas digestates on these compartments of soil biodiversity. Obviously enough, in addition to the response of microbial communities, the response of the soil macro- and mesofauna to digestates should be investigated by using microcosm/mesocosm and field experiments on the short and long terms, as proposed by the latest article published about this topic (Moinard et al. 2021).

Finally, the research perspectives include the questions that need to be deeply investigated and the orphan topics. The effects of the feedstock type and of the digestate fraction are not clearly answered in this meta-analysis due to the lack of studies. More studies and results on different digestate types and soil types are required to conclude objectively and robustly about the impact of the digestate characteristics on the soil biodiversity.

Questions still little or not addressed in the academic literature are the effect of the different processes used to transform raw organic matter into digestate, *i.e.*, the digestion process, mainly characterized by the temperature of incubation and the retention time, the fraction separation process, and the stabilization process (Fig. 11). All these processes determine the organic and sanitary quality of the outgoing digestates, and can change the ecological impact of digestates on the soil biodiversity.

Other orphan questions are the interactions between digestate application and the agro-pedological context. This includes the soil type but also the historical record of fertilization and the interactions with other agricultural practices such as additive fertilization, tillage and the plant cover (type of crop or cover crop, rotation or plant diversity). Understanding all these interactions will lead to the digestate application conditions most favorable to the soil biodiversity and rule out risky and unsustainable agricultural practices.

Methods

The methodological process of this review is presented in Fig. 12. The combination of key-words used for the search was the following one:

Digestate AND soil** AND (**diversity OR microb* OR faun* OR nematode* OR earthworms*).

where "AND" indicates that the words had to occur simultaneously in the search results, "OR" indicates that at least one of the terms had to occur in the results, and * indicates that the search targeted all the words containing the given letter sequence associated to a prefix if * came before the letter sequence, and/or associated to a suffix if * came after the letter sequence (e.g., **diversity* included *biodiversity*, microb* included *microbial* and *microbiological*). The search was carried out in the Web of Science database in March 2021, and no restriction was applied as to the date or the geographical origin of the articles.

Scientific articles that referred to the topic in a relevant way were identified through several filtering steps: (i) Were the themes of the title, keywords and abstract in adequacy with the theme of the meta-analysis? (ii) Did the article contain original data about digestates and the

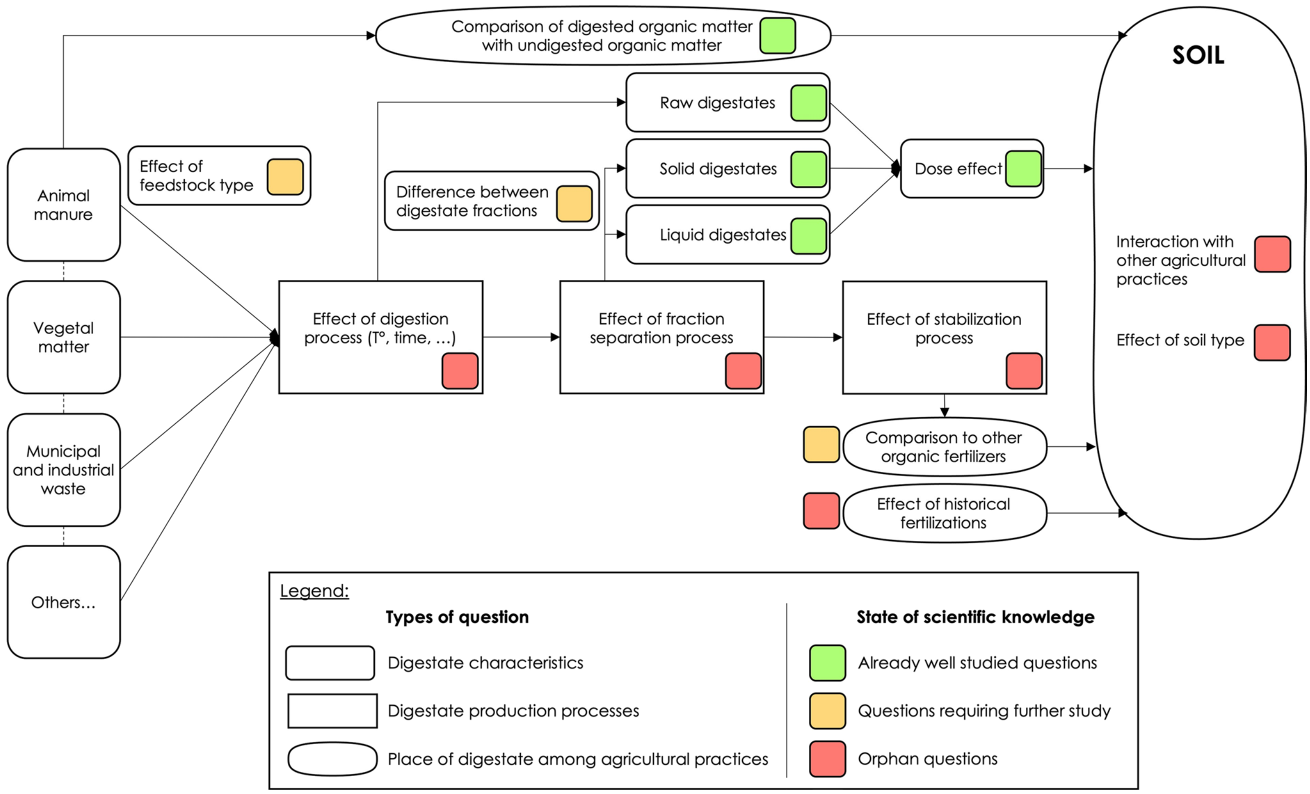


Fig. 11 Questions related to the impact of digestates on the biological quality of soils. The form type indicates the type of question investigated by the scientists, relying on the characteristics of digestate, the production processes of digestate or on the relation between digestate and other agricultural practices. Green, amber, and red boxes inform on the state of current knowledge for each question

soil biodiversity? (iii) Were the experimental design or the tested modalities suitable to answer the question?

The final core of selected articles was analyzed finely to constitute 3 different data sets composed of the metadata of articles, the contextual data of studies and the measured effects, respectively. These databases were the support of the bibliometric analysis, of the analysis of experimental strategies and of the analysis of impacts.

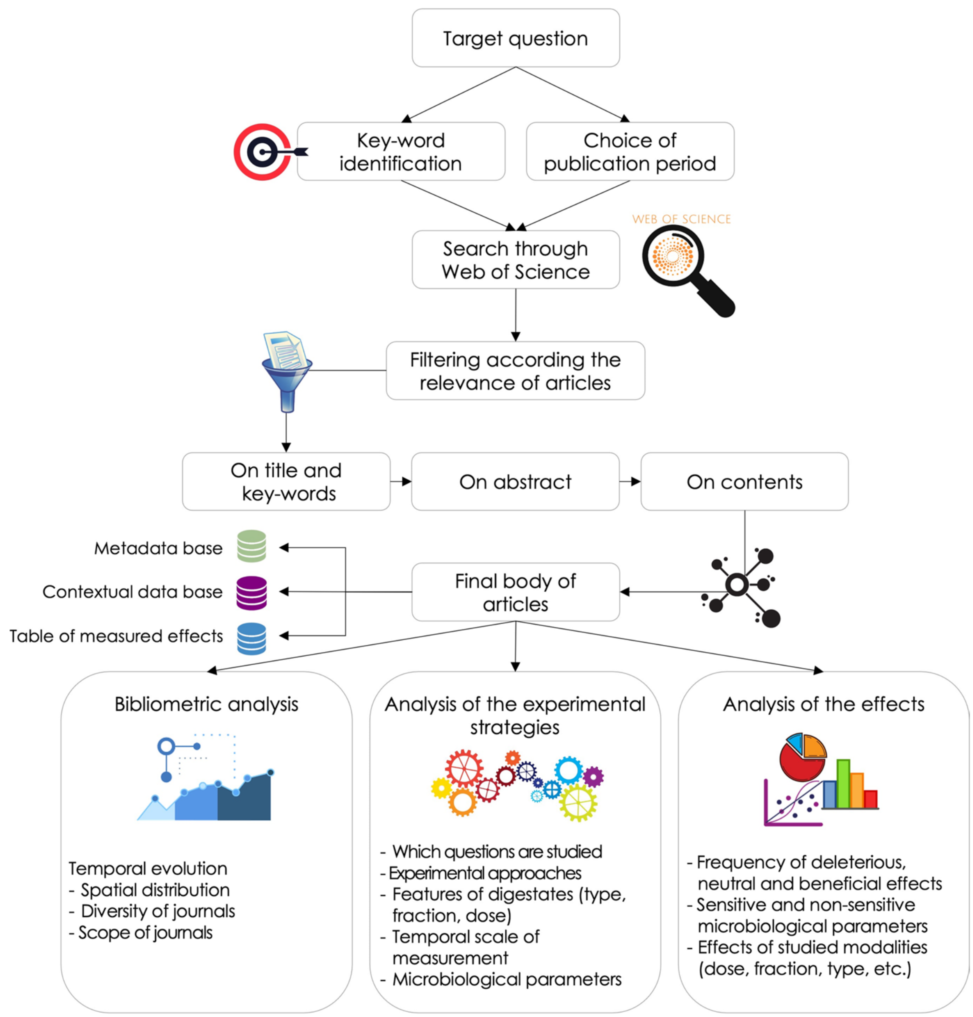


Fig. 12 Methodological process implemented for this systematic review. The final box details all the information recorded from the articles.

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Author contribution BK did the review and meta-analysis work. All authors contributed to the technical and scientific discussion on the topic. BK and LR established the structure of the manuscript. BK wrote the draft of the manuscript with the help of LR. All the authors commented and validated the manuscript.

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Availability of data and material Not applicable.

Code availability Not applicable.

Declarations

Conflicts of interest The authors declare that they have no conflict of interest.

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