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Review

The impact of anaerobic digestate on soil life: A review

Christina van Midden[a,*], Jim Harris[a], Liz Shaw[b], Tom Sizmur[b], Mark Pawlett[a]

[a] School of Water, Energy, and Environment, Cranfield University, Bedford, MK43 0AL, UK
[b] Department of Geography and Environmental Science, University of Reading, Reading, RG6 6AB, UK

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ABSTRACT

Using organic amendments to fertilise crops is a crucial part in the sustainability of agricultural systems. The residual slurry remaining after biogas production (anaerobic digestate) contains a rich source of plant nutrients that provides an alternative to mineral fertilisers. The delivery of many nutrients to plants is facilitated by a healthy soil biota: free-living and symbiotic microflora (e.g. archaea, bacteria and fungi) mineralize, solubilize and facilitate plant uptake of nutrients and the soil fauna (e.g. protozoa, microarthopods and earthworms) influence nutrient cycling processes as higher-level consumers and litter transformers. The delivery of nutrients to plants via the activity of this soil food web is influenced by fertiliser inputs. Here we review the impact of anaerobic digestate on soil biota. The quantity and composition of the carbon in digestate has a large influence on soil heterotrophic microbial dynamics and their subsequent influence on nutrient bioavailability. The main points are (1) digestate low in carbon has little effect on soil microorganisms, whereas digestate higher in carbon increases soil microbial abundance and diversity; (2) labile carbon stimulates fast-growing bacteria, whereas recalcitrant carbon shifts the microbial community in favour of slower-growing fungi and Gram-positive bacteria; and (3) earthworms, springtails and nematodes dwelling in the soil surface layer can be negatively affected by digestate application due to toxicity when compounds such as ammonia are present in high concentrations. Generalized understanding of the effect by digestates on soil biota is made difficult by differences in digestate properties caused by varying feedstock and production methods and the inherent heterogeneity of soil. There is a lack of research investigating the impact of repeated digestate application on soil biota and subsequently soil health. This information would give end users more confidence to substitute mineral fertilisers with digestate.

1. Introduction

Anaerobic digestion transforms organic matter into energy in a well-developed industrial process that generates biogas. During anaerobic digestion organic matter is broken down in oxygen-free conditions, producing CH_4 and CO_2 that are used to generate electricity and heat (Al Seadi et al., 2008; Fig. 1). In 2009 the European Union set a mandatory target that, by 2020, 20 % of all energy consumption should come from renewable sources (European Parliament and Council of European Union, 2009). This target resulted in numerous EU governments subsiding biogas plants installations (Edwards et al., 2015), with over 18,000 biogas plants being registered by end of 2018, an increase of 192 % from 2009 (EBA, 2020). Anaerobic digestion to produce renewable energy has several advantages; biogas can be produced when needed, the produced biogas can supply the current natural gas grid, and energy is produced from organic wastes such as household, food and drink processing, agriculture, and sewage works.

After biogas production the resulting slurry, known as anaerobic digestate, requires removal from the biogas plant. Originating from organic matter feedstock, and with only carbon and hydrogen removed as biogas (Möller, 2015), digestate contains the remaining nutrients from the digested feedstock (Fig. 1). Digestate can be used as a fertiliser in agriculture and has been shown to support crop yields equivalent to mineral fertilisers (Šimon et al., 2015; Riva et al., 2016; Ehmann et al., 2018; Walsh et al., 2018; Barzee et al., 2019; Zicker et al., 2020). However, digestate has a low nutrient to volume content when compared to mineral fertilisers (Table 1), therefore the cost of transporting it from biogas plants to farms increases with distance and becomes uneconomical (Möller et al., 2010). To address this limitation, digestate is often separated into a "liquid" and a more fibrous "solid" fraction to reduce the volume and therefore the cost of transporting (Al Seadi et al., 2012).

* Corresponding author.
E-mail addresses: christina.van-midden@cranfield.ac.uk (C. van Midden), j.a.harris@cranfield.ac.uk (J. Harris), e.j.shaw@reading.ac.uk (L. Shaw), t.sizmur@reading.ac.uk (T. Sizmur), m.pawlett@cranfield.ac.uk (M. Pawlett).

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The separation of digestate causes uneven nutrient distribution; between 65–75 % of the total nitrogen and 70–80 % of the potassium remains in the liquid fraction, while 55-65 % of the phosphorous and 60-70 % of the carbon remains in the solid fraction (Fuchs and Drosg, 2013). As liquid digestate receives the majority of the nitrogen, of which typically over 70 % is in the readily available form of ammonium (Drosg et al., 2015) it has good potential as a fertiliser. The solid fraction contains a greater amount of phosphorous, but also carbon as organic matter (Table 2) and is considered both a source of nutrients (Al Seadi et al., 2012) and a soil conditioner to build soil organic matter (SOM)) (Logan and Visvanathan, 2019). Little work has been done to understand the influence of solid or liquid fractions of digestate on SOM, particularly the living component of SOM. Therefore, this review aims to address this knowledge gap by providing a greater understanding of the impact of digestate application on soil biota.

Table 1
Comparison of nutrient content between a 30:11:24 NPK compound fertiliser and the average values from four digestates used by Abubaker et al. (2012).

	Digestate kg t^{-1}	NPK kg t^{-1}
Total nitrogen	5.4	303
Ammonia nitrogen	3.6	148
Phosphorous	0.6	114
Potassium	2.3	245

Table 2
Nutrient content per weight of the whole, solid and liquid fractions of digestate. Data from screw extractor and rotary screen separator experiments by Bauer et al. (2009).

	Liquid phase kg t^{-1}	Whole phase kg t^{-1}	Solid phase kg t^{-1}
Dry matter	45.0	73.1	193.1
Volatile solids	31.3	53.8	165.4
Total nitrogen	4.0	4.2	4.6
Ammonia nitrogen	2.6	2.7	3.0
Phosphorous	0.9	1.2	2.5
Potassium	3.5	3.6	3.4

2. The influence of anaerobic digestate on soil microorganisms

A significant proportion of SOM consists of living and dead microorganisms (Liang and Balser, 2011). Soil microorganisms consist of archaea, bacteria, fungi, and protozoa, though the majority of studies investigating the impact of digestate on soil microorganisms have focused on bacteria and fungi as dominant groups in terms of abundance and biomass. The focus on these two microbial groups is largely because they are considered the largest functional groups responsible for nutrient cycling in soil (Buerkert et al., 2012).

2.1. Effect of digestate on soil microorganism activity and abundance

The application of the liquid fraction and non-separated whole digestate to soils rapidly stimulates microbial activity (Risberg et al., 2017; Iocoli et al., 2019; Meng et al., 2022). Similar increases in the soil microbial biomass have been observed within hours after digestate application (Johansen et al., 2013; Monard et al., 2020), but both changes in activity, abundance and biomass are temporary and often subside within days of application (Alburquerque et al., 2012a; Galvez et al., 2012; Iocoli et al., 2019; Barduca et al., 2021) and are not detectable after a few weeks (Walsh et al., 2012, 2018; de la Fuente et al., 2013; Gómez-Brandón et al., 2016; Viaene et al., 2017; Mórtola et al., 2019; Gebremikael et al., 2020; Ren et al., 2020; Różyło and Bohacz, 2020; Valentinuzzi et al., 2020).

The majority of soil microorganisms are heterotrophic and use organic carbon as their energy source. As the anaerobic microbes in the biogas tank have already converted much of the readily available carbon in the feedstock into CH_4 and CO_2 (Thomsen et al., 2013) there is less readily available carbon present for soil microbes to utilise, compared to undigested feedstock materials (Chen et al., 2012). In pot studies that added a high amount (10-50 % w/w) of whole or liquid digestate to soil (García-Sánchez et al., 2015; Muscolo et al., 2017; Panuccio et al., 2021) microbial biomass increased. Manfredini et al. (2021) altered the concentration of dissolved organic carbon in the digestate and observed that higher levels of dissolved organic carbon resulted in increased microbial biomass by the end of the study. These studies show that it is when carbon concentrations are increased beyond standard field application rates, that microbial activity and abundance increase for more than a few weeks. This indicates that typical liquid or whole digestate application rates do not supply enough available carbon for soil microorganisms to support sustained growth.

The application of solid digestate led to sustained increases in microbial biomass and activity (de la Fuente et al., 2013; Badagliacca et al., 2020; Cattin et al., 2021) indicating that the solid fraction did not result in the carbon-limited microbial growth observed for whole or liquid digestate. Furthermore, de la Fuente et al. (2013) observed that solid digestate increased microbial biomass to a greater extent than any other form of digestate and reported a concurrent increase in nitrogen within

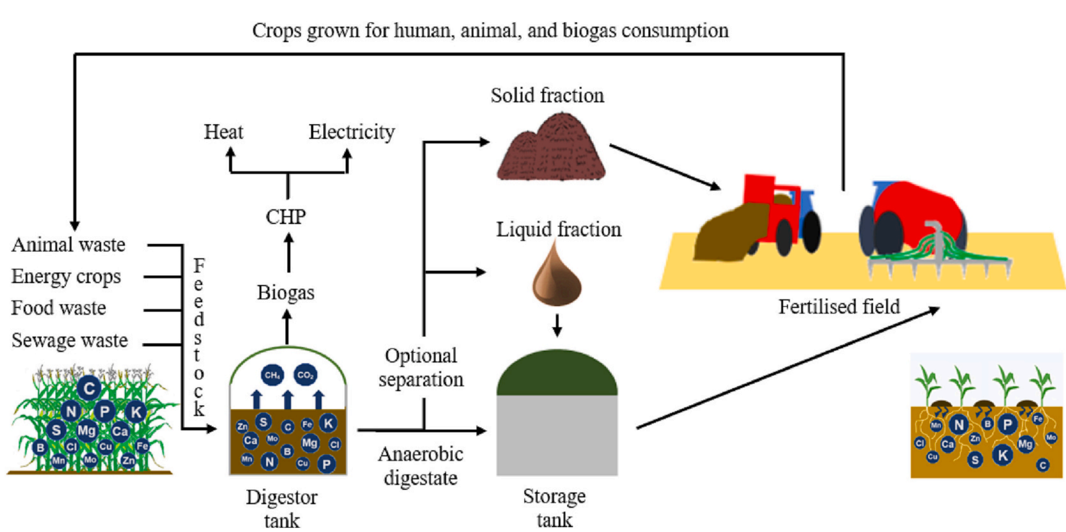

Fig. 1. The process of anaerobic digestion and end-use of anaerobic digestate as a fertiliser.

the microbial biomass in solid digestate treated soils that was absent in others. Therefore, the authors reasoned that the high immobilisation of nitrogen in the microbial biomass receiving this treatment had an important influence over the growth and activity of the soil microorganisms. Although the digestate separation process removes most of the nitrogen in the liquid fraction (Tambone et al., 2017), it is apparent that the solid fraction still contains sufficient nitrogen to support microbial growth.

The characteristics of the feedstock can influence the impact that digestate has on soil microorganisms. The rate of liquid and whole digestate applied to land is routinely based on its nitrogen content; subsequently digestate with a higher C:N ratio delivers more carbon to the soil, which influences its impact on soil microbial activity (Abubaker et al., 2013; Iocoli et al., 2019). Muscolo et al. (2017) observed that the biochemical nature of the carbon is important, since, when both liquid and solid digestate with a lower percentage of carbon as recalcitrant plant material was applied, a greater positive effect on microbial biomass growth was observed. Alburquerque et al. (2012a) and Risberg et al. (2017) both reported significant differences in the effects of digestate on microbial activity due to the digestate feedstock type; digestates containing a greater amount of readily available carbon resulted in increased levels of microbial activity.

2.2. Effect of digestate on soil microbial community

Hupfauf et al. (2016) showed that microbial community level physiological profiles (using principal components analysis of MicroResp™) for soils receiving applications of solid digestate were distinct from those receiving liquid digestate, whilst whole digestate resulted in a community profile that lay between the two. The physiochemical characteristics of the digestate influence microbial community composition. The high ammonium and water content of whole and liquid digestate create favourable conditions for bacterial groups associated with the nitrogen transformation, with increases in the abundance of bacterial nitrifiers and denitrifiers being reported (Sawada and Toyota, 2015; Brenzinger et al., 2018; Ogbonna et al., 2018).

Another explanation for the differences in the physiological profiles of the microbial community may be due to changes in fungi:bacteria (F:B) ratios, as the two groups occupy different functional niches in the soil. Walsh et al. (2012) observed an increase in bacterial growth six months after applying liquid digestate, which reduced the F:B ratio. Similarly, Pezzolla et al. (2015) applied a digestate with a dry matter equivalent to liquid digestate, and observed an increase in gram-negative bacteria, causing a decrease in the F:B ratio. These quick growing bacteria are better able than fungi to take advantage of the labile carbon supplied in the liquid digestate, whilst very little complex carbon is added that fungi can use. This may explain the negative effect of liquid digestate on fungi that Wentzel and Joergensen (2016), Elbashier et al. (2018) and Barduca et al. (2021) found. However, Coelho et al. (2019) and Gryń et al. (2020) observed negligible changes to both groups.

The application of whole digestate resulted in transient (Ren et al., 2020; Różyło and Bohacz, 2020) or insignificant changes (Makádi et al., 2016; Brenzinger et al., 2018) to bacterial or fungal abundance and no changes to the F:B ratio were observed (Gebremikael et al., 2020). In contrast, Chen et al. (2012) observed a shift in microbial community, as inferred from growth kinetic parameters, to one dominated by slower growing organisms under the application of whole digestate made from maize. This response was interpreted to be due to the presence of the recalcitrant plant fibres that the microbes in the anaerobic digestor did not break down, which support the relatively slower growing microbes, such as fungi and Gram-positive bacteria (Meidute et al., 2008; Bastian et al., 2009). Chen et al. (2012) used a digestate made only from maize, and therefore a comparatively larger proportion of its organic carbon would be in a recalcitrant form compared to the digestates used in the other studies.

When digestate with a higher ratio of carbon to nitrogen is applied, an increase in fungal content (García-Sánchez et al., 2015; Barduca et al., 2021; Panuccio et al., 2021) and F:B ratio (Cattin et al., 2021) were observed. The solid fraction of digestate contains a greater availability and variability of organic carbon, including a high quantity of recalcitrant organic matter that saprophytic fungi utilise (Meidute et al., 2008). Furthermore, Tambone et al. (2017) demonstrated that the nitrogen content in the solid fraction is high enough to consider it an organic fertiliser, consequently reducing direct competition between fungi and bacteria for nitrogen and thereby relieving the nitrogen limitation on fungal growth (Rousk and Bååth, 2007).

Not all fungi are decomposers and an important fungal group, the arbuscular mycorrhizal fungi (AMF), gain their carbon from a symbiotic relationship with plants. Despite having their carbon needs met by the plants, AMF are affected by digestate application and the fraction of digestate applied determines the direction of the effect. Solid digestate application has a positive effect on AMF colonisation (Caruso et al., 2018). This effect may be due to the slow release of phosphorus from both the decay of its fibrous material (Gosling et al., 2006) and the struvite minerals that precipitate during the anaerobic process (Marti et al., 2008), This makes it beneficial for the host plant to maintain the symbiosis through supply of photosynthate for the purposes of improved phosphorus acquisition.

Unlike phosphorus, nitrogen addition has been shown to have positive effects on AMF stimulation (Nouri et al., 2014; Johnson et al., 2015) through increasing phosphorus demand by alleviation of nitrogen as the nutrient most limiting to plant growth. Although liquid and whole digestate are rich in nitrogen, positive effects on AMF colonisation were not seen (Wentzel and Joergensen, 2016; Caruso et al., 2018; Dahlqvist, 2018; Ren et al., 2020), though Ren et al. (2020) did measure an increase in hyphal length. Ren et al. (2020) observed a slight but significant decrease of 0.18 in soil pH as they increased digestate dosage rates. Since they applied digestate in its whole form, it will have contained a high concentration of ammonium N. Although ammonium N initially increases soil pH due to its alkaline nature, it reduces soil pH as it undergoes nitrification. Furthermore, as plants take up ammonium ions they release acidic hydrogen ions into the soil around the roots to balance their internal pH (Smith and Read, 2008). These factors result in soil acidification, which has been shown by Pan et al. (2020) as a cause for suppressing AMF colonisation.

The physiochemical properties of digestate (such as carbon content and type, nutrients, and water volume) influence the physiological profile of the microbial community. However not all the aforementioned studies observed the same result for the same form of digestate. These differences are due to variability in digestate characteristics caused by different feedstock sources (Tables 3–4). Other factors contributing to different patterns observed in these studies include different soil properties, dose rates and analytical methodologies adopted by the researchers (Tables 3–4). These differences between disparate studies makes understanding the effects of digestate application on microbial community structure difficult to quantify. Currently the number of studies investigating the impact of digestate application on distinct groups of soil microorganisms are too low to generate consensus by reviewing only those using similar measurements and current trends identified should be taken with caution.

2.3. Effect of repeated digestate application on soil microbial community

The changes in microbial community previously discussed were observed in experiments that ran for a short time (< 1 year) and under controlled laboratory conditions. A two-year field experiment run by Coelho et al. (2020), showed no significant changes in soil bacteria and fungi abundance and diversity through repeated liquid digestate applications. Similarly, Makádi et al. (2016) saw no significant change in the microbial groups they studied over two years. Furthermore, no significant increases in microbial biomass were observed after three years of repeated liquid or whole digestate application (Johansen et al., 2015;

Table 3
The effects of the three forms of digestate on soil microbial biomass as measured by chloroform fumigation extraction method. In all experiments the comparison of effect by digestate is against a non-fertilised control. Only studies longer than 30 days were selected. In multi-year trials, only data from first year was considered.

AD form / fraction	Digestate feedstock[a]	Application rate	Plant present	Sampling time post application	Effect	Authors
Liquid	M/OFMIW/EC	10-50 % soil w/w	No	6 months	increase in biomass	Panuccio et al., 2021
Solid	M/OFMIW/EC	25-75 % soil w/w	No	6 months	increase in biomass	Panuccio et al., 2021
Whole	S/EC/OFMIW	25-35 t FW / ha	No	112 days	no change in biomass	Gebremikael et al., 2020
Liquid	S	4.25 L/m2	No	60 days	no change in biomass	Monard et al., 2020
Liquid	M	180 kg N/ha	No	7 weeks	no change in biomass	Valentinuzzi et al., 2020
Whole	M	70 and 210 kg N/ha	Lettuce	34 days	no change in biomass	Mórtola et al., 2019
Liquid	M/OFMIW/EC	10-30 % soil	No	3 months	increase in biomass	Muscolo et al., 2017
Liquid	OFMIW/M/EC	10-30 % soil	No	3 months	no change in biomass	Muscolo et al., 2017
Solid	M/OFMIW/EC	20-75 %	No	3 months	increase in biomass	Muscolo et al., 2017
Solid	OFMIW/M/EC	20-75 %	No	3 months	increase in biomass	Muscolo et al., 2017
Whole	M	80 kg N/ha	No	60 days	no change in biomass	Gómez-Brandón et al., 2016
Liquid	S/EC	120 kg N/ha	Ryegrass	70 days	no change in biomass	Wentzel and Joergensen, 2016
Whole	S	1.4 g/kg soil	No	90 days	no change in biomass	Pezzolla et al., 2015
Liquid	S/M/EC	$96m^3$/ha	No	56 days	no change in biomass	de la Fuente et al., 2013
Solid	S/M/EC	48 Mg/ha	No	56 days	increase in biomass	de la Fuente et al., 2013
Whole	S/M/EC	$96m^3$/ha	No	56 days	increase biomass	de la Fuente et al., 2013
Liquid	S/OFMIW/SS	$64m^3$/ha	Watermelon	152 days	increase in biomass	Alburquerque et al., 2012b
Whole	S	20 t/ha	No	30 days	increase in biomass	Galvez et al., 2012
Whole	S/EC	120 kg NH_4-N/ha	No	6 weeks	increase in biomass	Ernst et al., 2008

[a] S: animal slurry, M: animal manure, OFMIW: organic fraction of municipal and industrial waste, EC: energy crops, SS: sewage sludge.

Table 4
the effects of the three main forms of digestate on the soil bacterial, fungal and mycorrhizal fungal (MF) abundance. CFU = colony forming units. GCN = gene copy numbers. PLFA = phospholipid fatty acids. For digestate effects on MF, only colonisation measurement was accepted for comparison. For the effects of digestate on fungi and bacteria, different measurement techniques had to be accepted for enough studies to be selected to provide a pattern.

AD form / fraction	Digestate Feedstock[a]	Application rate	Plant present	Sampling point after application	Effect	Authors
Bacteria						
Liquid	S/C/OFMIW	170kgN/ha	No	150 days	No change in CFU	Gryń et al., 2020
Whole	C/OFMIW/M	3.4 t DW/ha	No	6 months	No change in CFU	Różyło and Bohacz, 2020
Liquid	OFMIW; SS	33 m3 FW/ha	Grass-sward mix	6 months	No change in GCN	Coelho et al., 2019
Liquid	M	1100 L/ha	Melon	<1 yr	No change in CFU	Elbashier et al., 2018
Solid	OFMIW/C	10 g/100 g soil	Wheat	60 days	Increase in PLFAs	García-Sánchez et al., 2015
Whole	S	340 kg ha	No	90 days	Increase in PLFAs	Pezzolla et al., 2015
Liquid	S	150kgN/ha	Grass mix	16 weeks	Increase in growth (leucine)	Walsh et al., 2012
Fungi						
Liquid	S/C/OFMIW	170kgN/ha	No	150 days	No change in CFU	Gryń et al., 2020
Whole	C/OFMIW/M	3.4 t DW/ha	No	6 months	No change in CFU	Różyło and Bohacz, 2020
Liquid	OFMIW; SS	33 m3 FW/ha	Grass mix	6 months	No change in GCN	Coelho et al., 2019
Liquid	M	1100 L/ha	Melon	<1 year	Decrease in CFU	Elbashier et al., 2018
Liquid	M/C/OFMIW	170kgN/ha	Sweetcorn	1st year	No change in CFU	Makádi et al., 2016
Liquid	S/C	120kgN/ha	Ryegrass	70 days	Decrease in ergosterol	Wentzel and Joergensen, 2016
Solid	OFMIW/C	10 g/100 g soil	Wheat	60 days	Increase in PLFAs	García-Sánchez et al., 2015
Whole	S	340 kg ha	No	90 days	no change in PLFAs	Pezzolla et al., 2015
Liquid	S	150 kgN/ha	Grass mix	16 weeks	No change in growth (ergosterol)	Walsh et al., 2012
Mycorrhiza fungi						
Whole	OFMIW	25 and 50kgN/ha	Ryegrass	75 days	No effect on colonisation	Ren et al., 2020
Liquid	–	140kgN/ha	Triticale	223 days	Non-sig. decrease in colonisation	Caruso et al., 2018
Solid	–	140kgN/ha	Triticale	223 days	Increased colonisation	Caruso et al., 2018
Whole	OFMIW	100kgN/ha	Spring wheat	10 weeks	No effect on colonisation	Dahlqvist, 2018
Liquid	S/C	120kgN/ha	Ryegrass	70 days	Decreased colonisation	Wentzel and Joergensen, 2016

[a] S: animal slurry, M: animal manure, OFMIW: organic fraction of municipal and industrial waste, EC: energy crops, SS: sewage sludge.

Šimon et al., 2015; Bhogal et al., 2016; Pastorelli et al., 2021), although Alburquerque et al. (2012b) did observe a positive effect after two years. This positive effect may have been due to the application of digestate twice per year in the rotation, whereas the others only applied once per year. Similarly, Odlare et al. (2008) observed a significant increase in microbial biomass after four annual digestate applications. It could be that fluctuating environmental conditions in the field masked the impact of the digestate influence on soil microbes. Indeed Pastorelli et al. (2021) observed that the season when soil was sampled had a greater influence on microbial community composition than the digestate treatment. Therefore, for sustained changes to be detected, more than three digestate applications are recommended.

The indirect effects of repeated digestate application on microorganisms due to changes in soil physiochemical properties are currently unknown. The most important soil property for determining microbial biomass and community diversity is soil pH (Fierer and Jackson, 2006; Hermans et al., 2017; Ma et al., 2019). Variability in soil pH influences many other soil properties, including the solubility of inorganic and organic compounds such as nutrients and metals (Veroney and Heck, 2015). A significant reduction or increase in pH leads to microbial community changes (Lauber et al., 2009; Rousk et al., 2009). pH may also have a direct effect on microorganisms, many of which have intracellular pH levels close to neutral and a significant alteration in soil pH may exert a physiological stress that tolerant or extremophile taxa can grow better in (Hozzein et al., 2013; Quatrini and Johnson, 2018). Digestates tend to be slightly alkaline (Abubaker et al., 2012; Wentzel and Joergensen, 2016; Prays et al., 2018; Coelho et al., 2019; Iocoli et al., 2019). However digestate application can cause soil acidification depending on ammonium load that gets transformed into nitrate (Ren et al., 2020) or the content of volatile fatty acids (Risberg et al., 2017). Multi-year trials running between 2 and 4 years found no change in soil pH (Odlare et al., 2008; Alburquerque et al., 2012b; Bhogal et al., 2018; Elbashier et al., 2018; Barłóg et al., 2020) but after six years Zicker et al. (2020) observed a significant decrease in soil pH from digestate application, indicating that the effects of digestate application take time before they are noticeable. However, these effects can be remediated by liming, a common practice in agriculture.

Soil organic carbon (SOC) is a major determinant of soil microbial biomass (Hu et al., 2014) and community composition (Drenovsky et al., 2004) with low SOC concentrations favouring oligotrophic microbes (Semenov, 1991). Multi-year field trials have reported no change in SOC (Odlare et al., 2008; Šimon et al., 2015; Barłóg et al., 2020; Pastorelli et al., 2021) or SOM (Bhogal et al., 2018), of which SOC is a major component. It may be that these trials were too short in duration for changes to be seen, as Smith (2004) showed that it takes between 6 and 10 years for changes in SOC to be detected under various rates of carbon inputs, land uses and soil types.

There is some concern that digestate application may lead to the accumulation of heavy metals in soils, particularly as some studies have shown that digestate sourced from sewage, industrial, and urban waste contain levels of copper, cadmium, nickel, lead and zinc above those set by the relevant governing bodies as acceptable for land application (Govasmark et al., 2011; Bonetta et al., 2014; Coelho et al., 2018). A high concentration of heavy metals in the soil can reduce enzyme function, inhibit respiration, and shift the composition of the microbial community to favour organisms that can tolerate the contamination (Giller et al., 2009; Chu, 2018). Multiple studies have analysed digestates made from a range and mix of organic materials for their heavy metal content and found them to be below the advised threshold levels set by their nation or federation (Kuusik et al., 2017; Coelho et al., 2018; Mórtola et al., 2019; Panuccio et al., 2021) and so are considered safe to apply. However, the long-term cumulative effect of repeated digestate applications on heavy metal concentration in soils is unexplored, either due to direct accumulation in the soil or indirectly due to changes in metal solubility through an alteration of pH.

There are concerns about the presence of hazardous compounds in digestate based on animal, industrial or household waste, such as antibiotics (Widyasari-Mehta et al., 2016), hormones (Withey et al., 2016; Congilosi and Aga, 2021), pesticides (Govasmark et al., 2011), pharmaceuticals and personal care products (Narumiya et al., 2013; Samaras et al., 2014; Malmborg and Magnér, 2015), phenols (Levén et al., 2012; Limam et al., 2013), salinity (Pawlett and Tibbett, 2015), microplastics (Weithmann et al., 2018), and persistent organic compounds including PAHs, phthalates, and dioxin-like compounds (Govasmark et al., 2011; Bhogal et al., 2016). The presence of these compounds can have negative effects on microorganisms (Levén et al., 2006; Chen et al., 2013; Lipińska et al., 2014; Molaei et al., 2017; Al-Ani et al., 2019; Mahfouz et al., 2020), but their influence on the soil microbiota due to digestate application is underexplored. Some studies have shown that pesticides and phthalates can stimulate microbial growth as the compounds provide an energy source to species able to utilise them (Iocoli et al., 2019; Osadebe et al., 2020; Zhang et al., 2020a). However, they can also inhibit the activity of other microorganisms (Baćmaga et al., 2018; Gao et al., 2020) and therefore will alter the microbial community structure.

Digestate contains a consortium of microorganisms that are introduced to the soil when applied, which can be negative in the case of pathogens and altering the native microbial community composition. Pathogen transfer is a particular concern for biogas facilities that supply digestate to multiple farms. To do so, they must meet quality assurance schemes set by governmental legislation, such as the EU's ECN-QAS or the UK's BSI PAS110. The thermophilic conditions of the anaerobic digestion process reduce pathogen load (Jiang et al., 2020; Nag et al., 2019) compared to original feedstock and pre-or post- pasteurization further sanitize the digestate (Thwaites et al., 2013; Nag et al., 2019). Regarding digestate sourced microorganisms altering the soil microbial community, Coelho et al. (2020) observed that these microorganisms did not replace the native microbial populations and attributed this to two factors. Firstly, most digestate sourced microorganisms are obligate or facultative anaerobes and therefore cannot survive the aerobic conditions in the soil surface and secondly that digestors operate at higher temperatures than those found in soil, which impacts growth and activity. Fernández-Bayo et al. (2017) and Podmirseg et al. (2019) tested the establishment of digestate sourced microorganisms that can survive in the soil by applying digestate to sterilised and non-sterilised soil. They discovered that only in sterilised soils could the digestate-sourced microorganisms establish.

3. The influence of anaerobic digestate on soil meso-organisms

Very few studies have looked at how anaerobic digestate impacts soil meso-organisms. Meso-organisms contribute to the carbon and nitrogen cycles via herbivory on belowground plant and fungal structures (Zhao and Neher, 2014), predation (Murray et al., 2009) and fragmentation of plant litter (Song et al., 2020), such as recalcitrant plant fibres remining in digestate. These actions free up the carbon locked in complex plant, fungal and faunal bodies into smaller particles and compounds which microorganisms can utilise. Meso-organisms include a diverse faunal range including nematodes and small arthropods such as springtails and mites. However, of the studies found looking at meso-organisms in relation of digestate application to soil, only springtails, mites, and plant parasitic nematodes were investigated.

The application of digestate had either no effect on springtails (Alves, 2016; Pommeresche et al., 2017) or a positive effect on both springtails and mites (Platen and Glemnitz, 2016) over the course of multiple applications. Platen and Glemnitz (2016) observed a positive correlation between soil moisture and springtail abundance, with the liquid digestate providing more water to the soil than a mineral nitrogen control. Yet, Pommeresche et al. (2017) observed a reduction in surface dwelling springtails shortly after liquid digestate application. This reduction may be due to elements or compounds in the digestate being toxic, as Renaud et al. (2017) observed depressive effects on springtail reproduction caused by cadmium and zinc. Digestate also contains a high concentration of ammonium (Möller and Müller, 2012) which Domene et al. (2010) showed was the main reason for springtail mortality after sewage sludge application. This mortality may be due to an increase in soil pH beyond levels that springtails could tolerate, as observed by Maccari et al. (2020) under high doses of ammonium rich poultry litter application. However, springtails produce multiple generations within a year (Badejo and Van Straalen, 1993), indicating that populations may well recover a few months after application. This ability to recover could explain why Platen and Glemnitz (2016) observed a positive effect on springtails, as the temporary negative effects may have been negated by more permanent beneficial changes in soil properties from digestate application.

Several studies have investigated the use of digestate on suppressing plant parasitic nematodes, as these cause considerable damage to important crops. Laboratory studies demonstrated reductions in the number of root knot nematodes (Jothi et al., 2003; Westphal et al., 2016; Wang et al., 2019; Das et al., 2022), and eggs produced by soybean cyst nematodes (Xiao et al., 2007) between digestate and non-digestate treated soils. Mechanisms proposed for the suppressive effects of digestate include: promoting populations of nematode suppressing bacteria (Westphal et al., 2016), nematicidal compounds from plants in digestate mixtures (Wang et al., 2019) or elevated ammonium and organic acids content produced from the digestion process (Min et al., 2007). Xiao et al. (2007) compared ammonium enriched digestate against volatile fatty acid enriched digestate and observed the latter being more effective at reducing egg counts. However, the suppressive effects declined over time, and after 2 (Xiao et al., 2007; Wang, 2019) and 6 months (Westphal et al., 2016) from application no differences between treatments were found. This indicates that these nematodes are likely to produce multiple generations during their host plants' growing season, enabling their population to recover. Indeed, in an experiment growing mangolds, Westphal et al. (2016) observed an increase in nematode egg and cyst numbers in digestate treated soils compared to soils receiving no digestate after 5 months, despite a reduction early in the growth of the mangold.

There is great difficulty in directly attributing the effects of digestate applications to changes in meso-organism abundances due to too few studies having been conducted (Table 5). Whilst the research here indicates that meso-organisms living close to the soil surface are negatively impacted, they can recover due to quick generation times and even be positively impacted in the longer term due to changes in soil properties caused by the digestate. However, both the number of studies involving meso-organisms and the number of meso-organism groups studied, are too small to make a scientifically robust generalisation. Much more work in this area is needed to properly understand the effects of digestate application on meso-organisms. This is a challenge due to the immense diversity of meso-organisms, but necessary to do as they are a key link in carbon and nutrient cycling.

4. The influence of anaerobic digestate on soil macro-organisms

Earthworms are the most studied soil macro-fauna in relation to impacts of anaerobic digestate application. Earthworms are considered ecological engineers (Lavelle et al., 1997); mixing of organic matter through the soil profile, aerating and improving soil fertility, increasing soil porosity, and breaking down organic matter into segments that other decomposers can utilise (Blouin et al., 2013). As such they are candidate indicators of soil health (Fusaro et al., 2018) and the reasons for selecting this group to determine the effect of digestate application on macro-organisms are logical. The majority of arable field experiments showed no significant change in earthworm abundance after whole digestate application (Bermejo et al., 2010; Clements, 2013; Frøseth et al., 2014; Johansen et al., 2015; Koblenz et al., 2015; Rollett et al., 2020; Moinard et al., 2021). The overall lack of an effect may be due to the inherently low numbers of earthworms found in arable fields (Stroud, 2019) as Rollett et al. (2020) observed a decrease in earthworms abundance in a densely populated perennial ley field after digestate application. To understand the reason for this decrease, looking at how digestate influences individual ecological groups of earthworms is necessary.

Earthworms can be broadly defined into three ecological groups: epigeic, endogeic and anecic (Bouché, 1977), though species are found corresponding to multiple categories (Bottinelli et al., 2020). Epigeic (litter dwelling) earthworms actively avoided digestate amended soils where possible (Clements, 2013; Ross et al., 2017). Whilst endogeic (topsoil dwelling) did not express such clear avoidance behaviour (Ross et al., 2017), their biomass decreased after digestate application (Ernst et al., 2008; Bhogal et al., 2016). In contrast epi-anecic (subsoil dwelling who collect food from soil surface) earthworms responded positively to digestate application (Ernst et al., 2008). Digestate is commonly applied either to the top of the soil surface or shallowly injected, and the negative effects caused by digestate indicate the presence of potentially toxic constituents, such as high ammonium and salt contents, which were both found to contribute to greater earthworm mortality (Bhogal et al., 2016; Natalio et al., 2021). Epi-anecic earthworms can avoid these toxic effects due to their deep burrowing nature, although a small number were found dead shortly after digestate application as a result of being present in the surface soil immediately after application (Moinard et al., 2021). Overall, very few ecotoxicological tests have been done to understand the impact of digestate application on earthworms and there may be other factors involved.

Digestate is applied to a rate of total nitrogen per hectare, to match the nutrient requirements of the crop, which can require high volumes of digestate to be applied. At a volume of 50/ha to supply 170kgN/ha, more dead earthworms were found compared to a lower volume of 25 t/ha (Johansen et al., 2015). As such a method to mitigate earthworm mortality would be to reduce the application rate, which can be done by using a split application method where the crop is fertilised at two or more time periods during its growth. Another option is to alter the method by which digestate is applied, which is either broadcast,

Table 5
the effects of whole digestate application on soil mesofauna (springtails and nematodes) and macrofauna (earthworms).

AD form / fraction	Application rate	Field site	Sampling time post application	Effect	Authors
Springtails					
Whole	147 kg N/ha	Grassland	1.5 months	No change in abundance	Pommeresche et al., 2017
Whole	–	Arable	4 months	No change in abundance	Alves, 2016
Whole	196 kg N/ha	Arable	1-6 months	Increase in abundance	Platen and Glemnitz, 2016
Nematodes		Pot and plant			
Whole	300 kg NH_4-N/ha	Pot – None	3 months	No change in abundance	Wang, 2019
Whole	120 kg N/ha	Pot - Sugarbeet	6 months	No change in egg / cyst count	Westphal et al., 2016
Whole	120 kg N/ha	Pot - Mangold	5 months	Increase in egg / cyst count	Westphal et al., 2016
Whole NH_4^+ enriched	23.4-187.2 m^3/ha	Pot – Soybean	2 months	No change in egg count	Xiao et al., 2007
Whole VFA enriched	23.4-187.2 m^3/ha	Pot – Soybean	2 months	No change in egg count	Xiao et al., 2007
Earthworms					
Whole	140-167 kg N/ha	Arable	2 years	Non-significant increase in abundance	Moinard et al., 2021
Whole	120-250 kg N/ha	Arable	3.5 years	No change in abundance	Rollett et al., 2020
Whole	120-250 kg N/ha	Grassland	3.5 years	Decrease in abundance	Rollett et al., 2020
Whole	160 kg N/ha	Arable	4 weeks	Non-significant increase in abundance	Koblenz et al., 2015
Whole	71.9 kg N/ha	Arable	6 weeks	Non-significant increase in abundance	Clements, 2013
Whole	120 kg N/ha	Arable	1 month	Non-significant increase in abundance	Bermejo et al., 2010

bandspread or injected. Investigation into the effects of these application methods on earthworm mortality has not been undertaken. Thirdly, it would be worth investigating whether transforming the physiochemical properties of digestate would influence its toxicity.

5. Transforming anaerobic digestate

5.1. Composting

The high ammonium nitrogen content of digestate that makes it a good fertiliser has negative effects on micro- to macro-organisms as discussed previously, and on the environment through leaching and volatilisation (Nkoa, 2014). Transforming it through composting reduces these problems, with additional benefits such as reduction in pathogen contamination (Bustamante et al., 2012; Tambone et al., 2015; Subirats et al., 2022) and odours (Rincón et al., 2019). In order to be composted effectively, additional materials such as woodchips, corn stalks, or oyster shells as bulking agents, and, in the case of organic materials, to increase the C:N ratio are required (Zeng et al., 2016; Li et al., 2020; Lu et al., 2020). Usually, only the solid fraction is composted, but the liquid fraction can be used to water compost piles (Bustamante et al., 2013; Vu et al., 2015).

Applying composted digestate to soil had a positive and lasting effect on microbial abundance (de la Fuente et al., 2013) but it reduced the peak of microbial activity, compared to when solid digestate was applied, and lowered the amount of carbon that was mineralised. This dampening of respiration is because compost contains a higher amount of carbon that is resistant to decomposition, as the readily and semi degradable carbon has already been decomposed during the anaerobic digestion and aerobic composting stages. Maynaud et al. (2017) demonstrated that the solid fraction of digestate still contained a substantial amount of the easily accessible carbon of the digestate. As a result, the microbial biomass did not increase as much under composted digestate application compared to the application of the solid digestate, yet was still higher than the biomass in whole digestate or liquid digestate treated soils. Adding composted digestate to degraded agricultural land had a positive influence on the soil microbial diversity (Caracciolo et al., 2015; Manasa et al., 2020).

5.2. Additives

Attention is being given to studying the effects of adding biochar into digestate to reduce environmental pollution risks from its application. Biochar is a material derived from the thermal decomposition of organic material in the absence of oxygen (pyrolysis), often using feedstock materials that are otherwise considered a waste product. Biochar is a high carbon and highly porous material and has been found to reduce N_2O emissions (Dicke et al., 2015; Martin et al., 2015) and nitrate leaching (Plaimart et al., 2021) when applied with digestate.

Multi-year field trials running between 1.5 and 4 years showed that the co-application of digestate with biochar had a positive effect on soil microbial biomass compared to soil receiving digestate only (Hewage, 2016; Greenberg et al., 2019). This increase could be due to a variety of reasons. The biochar provides a surface for bacteria to adhere to (Hill et al., 2019), preventing them being leached by the liquid in the digestate. Similarly, nutrients may sorb to the surface of biochar due to its high cation exchange capacity, which steadies the supply of nutrients delivered from the digestate and thereby increases the availability of nutrients to microbes over time (Zhu et al., 2017). The highly porous nature of biochar can increase the water holding capacity of sandy soils (Glaser et al., 2002), the soil texture used in both aforementioned studies, retaining moisture from sources such as the digestate and ensuring microorganisms have access to water during drier periods. The pH of biochar should also be considered. An alkaline biochar may offset soil acidification by digestate, thereby creating a more favourable environment for microorganisms, as Hewage (2016) observed that soils applied with digestate and a biochar of pH 8 had a higher soil pH than digestate treated soils by the end of their experiment.

5.3. Nutrient recovery

The recovery of nutrients from anaerobic digestate is of interest to the biogas industry as it reduces problems of storage and cost of transporting the bulky liquid material. Techniques are being investigated to remove nutrients which can then be applied to soils as a fertiliser. Methods can be physical, such as drying or filtering the digestate to concentrate the nutrients and clean the water for safe disposal or reuse (Knoop et al., 2018; Chiumenti et al., 2013). Chemical methods include ammonia stripping to recover nitrogen (Liu et al., 2015; Zarebska et al., 2015), the formation of struvite crystals to capture phosphorous and nitrogen (Zhang et al., 2020b; Muhmood et al., 2019), and the use of materials such as biochar or zeolites that have a high cation exchange capacity to absorb nutrients (Kocatürk-Schumacher et al., 2017; Shepherd et al., 2016). Biological methods include reed beds and algae runways (Nielsen and Stefanakis, 2020; Díez-Montero et al., 2020).

These techniques are mostly in the early stages of development (Khoshnevisan et al., 2021; Shi et al., 2018; Logan and Visvanathan, 2019) and their effects on soil biota is not a primary research concern. However, a similarity with all these products is the zero to very low carbon content, or in the case of biochar highly recalcitrant carbon. Therefore, it can be conjectured that the application of these products will have indirect benefits to soil microbial community should they stimulate crop yield, with bigger crops equalling more roots for decomposition as well as triggering nutrient mining by plants through increased exudates. Sorbent materials such as zeolites and biochar positively influence microorganisms involved in nitrogen cycling (Costamagna et al., 2020; Karličić et al., 2017). Yet these benefits may be outweighed by any significant changes in the soil physiochemical status, particularly pH. Nitrogen based fertiliser has been shown to acidify soils (Pan et al., 2020). P-struvite crystals may increase levels of magnesium to above optimum, turning this essential metal toxic (Gell et al., 2011). Being of organic material origin there are also potential toxic elements in biochar based on its feedstock and pyrolysis process that can have subsequent negative effects on the soil biology (Godlewska et al., 2021).

Unlike chemical and physical nutrient recovery techniques, biological nutrient recovery methods may be most promising for benefiting soil microbes. Algae grown in a digestate substrate can be processed and used as a biofertiliser (Hussain et al., 2021; Solovchenko et al., 2016). The application of algae as a fertiliser has been shown to have positive effects on the microbial biomass in the soil (Marks et al., 2019). When applied as necromass, the algae cells decompose and release nutrients and carbon into the soil, providing resources to support microbial growth. Living algae are also applied and can contribute to microbial biomass growth in multiple ways, which include the following. Firstly, some algae such as cyanobacteria can grow in the soil and directly add to the abundance (Perin et al., 2019). Secondly, algae produce extracellular polysaccharides, which provide a carbon source to other microbes (Marks et al., 2019). Thirdly they may be able to ameliorate soil pollution (Subashchandrabose et al., 2011) and improve conditions for soil microorganisms. The application of algae as a nutrient recovery technology is facing challenges for implementation, such as digestate turbidity and algae biomass processing (Xia and Murphy, 2016).

6. Conclusion and future research requirements

The addition of anaerobic digestate to soil has variable effects on the soil biota (Fig. 2) and long-term research is needed to understand the cumulative effects of repeated digestate application on soil organisms. Digestate can be altered by physical separation to liquid and solid fractions. Evidence from the reviewed literature suggests that the solid fraction of digestate has positive effects on all groups of soil microorganisms. The liquid fraction only slightly benefits bacteria and

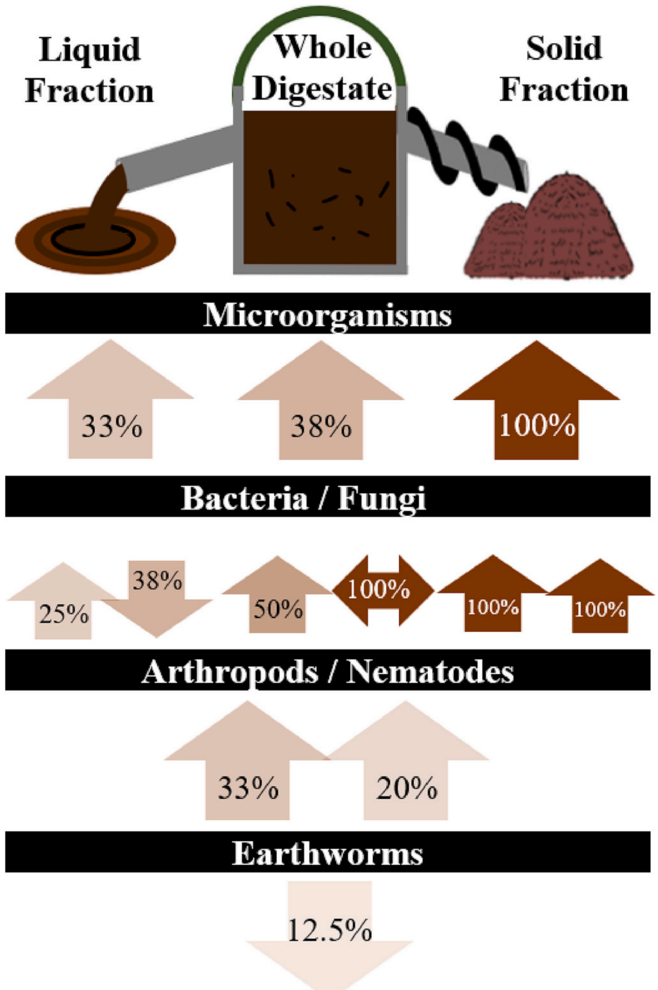

Fig. 2. Effect of anaerobic digestate and its fractions on the abundance of soil biota groups, based on the percentage of studies indicating the effect out of all the studies that included this measurement. Studies used to generate this figure are in Tables 3-5.

negatively affects mycorrhizal and saprophytic fungi. Digestate in its whole form negatively affects litter surface dwelling springtails, nematodes and earthworms, though these effects are reduced for organisms that inhabit deeper layers of soil. The negative effects of digestate on soil organisms are due to a combination of factors including, but not limited to; (i) lack of carbon supplied to support growth, (ii) toxicity due to ammonia and contaminant content, and (iii) changes to habitat conditions caused by shifting soil pH.

The focus of biogas production should include optimisation of digestate quality for fertiliser use, without detrimentally effecting biogas production. Plant operators can separate the digestate for fertiliser use to reduce handling costs or add materials such as biochar to the digestate to improve the retention of nutrients in the soil. Digestate can be stabilised by composting, reducing its toxicity and the negative environmental impacts such as nutrient losses at application, and positively benefiting soil microorganisms. In all cases research needs to be done to understand the long-term effects of these digestate products on soil organic matter, including the life within, which underpins all soil processes necessary for productive crop growing. By ensuring that anaerobic digestate promotes the development of soil organic matter and functioning of soil biota, biogas facilities can provide farmers with a sustainable alternative to mineral fertilisers.

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CRediT authorship contribution statement

Christina van Midden: Conceptualisation; writing - original draft; writing-review & editing. Mark Pawlett: Conceptualisation; supervision; writing-review & editing. Jim Harris: Conceptualisation; supervision; writing-review & editing. Liz Shaw: supervision; writing-review & editing. Tom Sizmur: supervision; writing-review & editing.

Declaration of competing interest

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.

Data availability

No data was used for the research described in the article.

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