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Review paper

Manure-based biogas fermentation residues – Friend or foe of soil fertility?

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ABSTRACT

Anaerobic digestion of organic residues has the potential to significantly contribute to a shift from fossil to renewable energy. The by-product, anaerobic slurry, does have properties that are different from the undigested material. There are concerns of soil organic matter depletion in soils, enhanced greenhouse gas and odour emissions, and pathogen spread upon production and use of biogas slurries as fertilizer. However, considering the pros and cons, anaerobic digestion of residues does have positive effects for the climate, the environment and for the farmer, compared to the use of undigested matter.

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1. Introduction

Public concern about use of resources, and political directives in many regions of the world, are promoting the production and subsequent use of biogas (biomethane) as a renewable energy resource. The generation of biomethane from manures is a cost-effective option, because not only does it solve an environmental problem of excess raw manure but it also allows the livestock farms to become self-sustaining in electrical and/or heat energy supply (Borges de Oliveira et al., 2011; Insam et al., 2014). Last but not least, it is also one of the bioenergy options with the smallest ecological footprint (German National Academy of Sciences – Leopoldina, 2012).

Anaerobic digestion (AD) is the degradation of organic substrates to biogas (~70% CH_4 and 30% CO_2) by a microbial consortium of four functional groups that act successively during the process: hydrolytic bacteria, acidogenic bacteria, acetogenic bacteria, and methanogenic archaea. However, the sustainability of the production of biogas depends on the suitable recycling of the digested material, which must be treated, disposed or reused properly, avoiding any negative environmental impacts. The use of the digested material as an organic fertilizer seems to be the best option, since it contains significant amounts of residual organic C and nutrients for plants (Alburquerque et al., 2012a,b). The application of digestate resulting from the AD of different types of waste is a common practice in several countries and it has been shown to improve the properties of soils (Petersen et al., 2003; Odlare et al., 2008; Frąc et al., 2012; Fernández-Delgado Juárez et al., 2013). The use of digestate as a fertilizer is considered beneficial since it provides nutrients (N, P, K) and improves the structure of the soil with the addition of organic matter (Nkoa, 2014). The magnitude of nutrient accumulation and its distribution in the soil profile are directly influenced by the soil type, the climate, the frequency of application and the properties of the digestate (Stumborg et al., 2007).

The substrates range from liquid manure to energy crops, as well as from sewage sludge to domestic organic wastes. Residues remaining after AD of all these substrates are called digestates. The benefits and disadvantages of the use of digestates of various origins have been addressed in numerous papers (Guanaseelan, 1997; Sahlström, 2003; Yadvika et al., 2004; Arthurson, 2009; Demirel and Scherer, 2011; Makádi et al., 2012; Zieminski and Frąc, 2012), and some of them with a special emphasis on plant growth (Gurung, 1997) or pathogens (Mawdsley et al., 1995). There is abundant literature on the use of digestates from wastewater treatment plants (WWTP) (anaerobically treated *Biosolids*), with

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very controversial discussions concerning their benefit or risk (e.g. Insam and Palojärvi, 1995; Insam and Merschak, 1997; Rigby and Smith, 2013; Lucid et al., 2014). While by imission control heavy metal loads in biosolids have decreased over the years, an ever increasing and steady changing load with xenobiotics is posing new questions. In their study on Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol), contained in many healthcare products, Horswell et al. (2014) concluded that the presence of co-contaminants in biosolids may produce synergistic or additive ecotoxicological impact upon soil function.

For a long time, it was particularly the contents of heavy metals that led to strong objections against the use of anaerobic sludges from WWTP for agricultural, horticultural or silvicultural purposes. In many places, such sludges are only allowed for reclamation purposes of waste dumps. Very often, sludges from WWTP are either deposited in waste dumps, or are incinerated. Sludges from WWTP are not the focus of this review. However, most recently, in many places operators of WWTP realised that the capacities in their anaerobic fermenters are high enough to allow for co-substrates from other sources than from the wastewater stream. Residues from the food industry or organic household wastes, after proper pre-treatment, are co-digested and are able to greatly increase the biogas yield and thus the process economy of WWTP. Together with other measures, WWTP are thus converted from energy consumers to energy producers. Interestingly, the addition of certain easily degradable co-substrates decreases the amount of residual digestate, an effect that has not been fully understood yet but that might work in analogy to the well-known priming effect with soil organic matter. In any case, this reduces the costs for the further treatment or disposal of the digestate, and thus WWTP may, in the future, curb this way of treatment. The result is, however, a product that comes from the (organic) waste stream and the wastewater stream with special requirements concerning hygiene, heavy metals and organic pollutants.

Concerning the contents of toxic materials, residues from the digestion of organic household wastes are of little concern, in particular since in several countries there are existing end-of-waste regulations after proper treatment. Such treatment may be, in analogy to composting or other thermal treatment, also anaerobic digestion, or, in some cases, a combination thereof. Some concerns are existing with regard to the spread of both plant and human pathogens, but it is well acknowledged that anaerobic digestion is able to decrease pathogen numbers, even at the common digestion temperature of 39 °C, and even more so if the thermophilic digestion temperature of 55 °C is chosen (Franke-Whittle and Insam, 2013).

After the recent wave of newly installed anaerobic digestion plants using energy crops (canola, maize, elephant grass etc.) huge amounts of digestates are becoming available. Concerning the use of these digestates for fertilizer purposes, a major problem is the considerable distance from the crop production to the large-scale digesters. Just too often, plant operators are tempted to avoid long transportation distances and overcharge nearby fields with digestates. Excessive nitrogen loads may not only put the groundwater at risk, but it also may have negative effects on soil properties, such as air porosity and aggregate stability. Biogas from energy crops is, from the viewpoint of the environmental footprint, very much disputed, and due to the competition with land for food production, it is also disputed from an ethical viewpoint.

Recent environmental assessments have shown that biomethanation does only make sense from an environmental viewpoint if agricultural or other wastes are used as a substrate (Moreira Vilar, 2014). The use of liquid and solid manure for biogas production is becoming increasingly popular in many countries, despite the relatively low energy content. This is compensated by high feed-in tariffs for the produced electricity, e.g. up to 0.28 per kWh in Germany and Italy. Along with the use of manure, farmers are tempted to add some energy-rich substrates (grass clippings, domestic organic wastes, feed wastes etc.) to make their reactors more efficient. To some extent, such extra input may be allowed (e.g. up to 20% in Germany) and thus increase plant efficiency, or may compromise the feed-in tariff (e.g. in Italy). While the production of biomethane from farm wastes undoubtedly reduces the greenhouse gas (GHG) production compared to conventional manure treatment (Amon et al., 2006; Insam and Wett, 2008), the effects of AD on the soil and its microbiota are not undisputed which will be discussed below.

This review is destined to give an overview over current knowledge and opinion on the use of digestates in general, but with a particular emphasis on digestion of (liquid) manures. Thus, as a first overview for the reader, a comparison of the physico-chemical properties of the substrates and the resulting digestates is given (Table 1).

2. General effects on the soil biota

The application of organic fertilizers may significantly change the structure and diversity of soil microbial communities, resulting in improvements of the soil quality (Sullivan et al., 2006). Due to the fact that changes in microbial communities can occur more quickly than changes in other soil characteristics, the study of microbial parameters is deemed a sensitive indicator when evaluating the soil disturbance and impact assessment after application of (organic) fertilizers (Odlare et al., 2008, 2011; Abubaker et al., 2013a).

Organic amendments are known to have positive effects on soil structure, water-holding capacity and microbial activity, and so do digestates (Debosz et al., 2002; Arthurson, 2009; Goberna et al., 2011). Generally, the soil microbial biomass and metabolic activities are stimulated after the application of digestate, which is attributed to increased C and nutrient availability (Odlare et al., 2008; Frąc et al., 2012). However, these increases may conceal adverse effects on more specialized microbial groups.

Nyberg et al. (2004) found that depending on the dose digestates may contain substances that inhibit the activity of ammonium-oxidizing bacteria (AOB) in agricultural soils. The

Table 1
Typical properties of liquid manure and their respective biogas slurries (Mokry et al., 2008) and generalizable trends (see this paper) indicated by the arrows (increase, decrease and slight increase).

	Manure	Digestate	Trend
% DM	9.1	7.7	⇩
C/N	10.7	7.0	⇩
pH	7.4	8.2	⇧
N_{tot} [kg/m^3]	4.1	5.0	⇧
NH_4–N [kg/m^3]	1.8	2.8	⇧
K [kg/m^3]	4.2	4.9	⇨
MgO [kg/m^3]	0.85	0.95	⇨
CaO [kg/m^3]	2.1	2.2	⇨

populations of bacterial and archaeal nitrifiers, as well as denitrifying and N_2-fixing populations will respond rapidly to the addition of ammonium (Di et al., 2010; Long et al., 2013). There are indications that the archaeal community in the soil will be enhanced when the soil is treated with digestate compared to manure, while the eubacterial community will be less affected (Johansen et al., 2013; Abubaker et al., 2013a). The abundance of methanogens and acetogenic bacteria will show a higher increase in soil treated with digestate than in soil treated with manure. This is also supported by Walsh et al. (2012) who found that the liquid digestate compared to mineral fertilizer enhanced, while undigested slurry reduced bacterial growth. Chen et al. (2012) observed that the application of biogas residues (BRs) to soil resulted in a shift of active microbial community to slower-growing microorganisms, and simultaneously to a moderate increase in microbial biomass.

Concluding, digestate is likely to enhance microbial activity and biomass, not only compared to mineral fertilization but in most cases also compared to undigested manure. The effects, however, may vary among the different functional or phylogenetic groups of soil microorganisms.

3. Carbon cycle

Anaerobic digestion converts organic matter to methane, which escapes the on-farm carbon cycle. Thus, researchers and farmers are often concerned that compared to undigested manure, biogas slurries would on the long-term impair the soil organic C status (Løes et al., 2010; Johansen et al., 2013). Organic farmers argue that fertilizing with the digestates may impair the soil microbiota and fertility because they contain more mineral N and less organic C than undigested manure. Senbayram et al. (2009) discussed that the elevated N concentration in digested effluents indicates their potential as a readily available N source, but it may also enhance carbon mineralization (priming effect). Direct comparisons with the effect of undigested manure/slurries show clearly that in most cases the above concerns can be discarded. De Neve et al. (2003) reported that the organic carbon in biogas slurry is more stable compared to other organic wastes. Stumpe et al. (2012) compared organic carbon dynamics in agricultural soils and found that respiration rates were higher after liquid manure than digestate application. Similar results were also found by Løes et al. (2010). Overall, the effect on soil organic matter dynamics was limited.

Johansen et al. (2013) performed an incubation study where different combinations of raw cattle slurry or grass-clover mixtures, as well as anaerobically digested products thereof were applied to soil. Grass-clover contributed with four times more readily degradable organic C than the other materials, causing an increased microbial biomass which depleted the soil for mineral N and caused a 10-fold increase in emissions of CO_2 compared to any of the other treatments during the nine days of incubation. There were no indications of an enhanced C loss upon fertilization with digestate. Investigating African soils, Smith et al. (2014) stated that AD has a great potential to increase soil C sequestration, finding a similar stabilization of organic matter by anaerobic digestion and composting. Based on simulation with the RothC model, they concluded that digestates would be superior to untreated slurry. This corroborates the work of Marcato et al. (2009) who reported a stabilization of organic matter by AD through degradation of the most labile fractions.

Even though AD does not appear to negatively affect soil organic C status, some authors suggest post-treatment of the digestates. de la Fuente et al. (2013) found no differences in soil C sequestration between slurries and digestates, however, they emphasized that a post-processing by composting could considerably help to retain the organic matter in the soil. Abubaker et al. (2012) demonstrated that the use of biogas residues as organic amendments including digestate showed positive effects on wheat crop yields and soil microbial functions. The authors suggest devoting more effort to developing biogas processes that not only produce biogas but also custom-tailed high-yielding fertilizers that are also suited for maintaining soil organic carbon. Numerous papers are comparing digestates with organic amendments other than manures or slurries (Bertora et al., 2008; Ernst et al., 2008; Stinner et al., 2008; Möller and Stinner, 2009; Cayuela et al., 2010; Abubaker et al., 2013a,b), and also from these findings we do not anticipate long-term negative effects on the soil organic carbon status if manures are anaerobically digested. Post-treatment, like liquid–solid separation, or composting may, however, in many cases improve the C sequestration potential of digestates.

4. Nitrogen cycle

Nitrogen is a critical nutrient in all plant-based systems and, upon entering the soil, may follow a number of pathways including mineralisation, immobilisation, nitrification and denitrification, as well as leaching and ammonia volatilisation, mainly depending on the form of deposited N, the soil chemistry, precipitation rates and the land-use strategy (Bardgett and Wardle, 2010; Manning, 2013). Concerning the introduction of N into the soil via digestate amendment, its liquid (LS) and solid fraction (SS) was shown to either promote or suppress N mineralization and N apparent recovery fraction (ARF) (Grigatti et al., 2011). However, the use of the composted SS resulted in the mitigation of N immobilization, even though a relevant loss of C to the atmosphere was recorded. Johansen et al. (2013) found that digestates increased the concentration of NO_3^- by approx. 30–40% compared to undigested matter. Likewise, Friedel et al. (1996) recorded a 37% increase in inorganic N during a 60-day incubation period of farmyard manure-derived biogas slurry in soil at 15 °C, while they have found no evidence of net N mineralization under field conditions on fallow plots. On the other hand, Goberna et al. (2011) observed that amending soils with digestate enhanced nitrate losses during the first 30 d of a 100-day incubation period. Around 23% and 45% of the total N contained in the soil (natural + added) was lost from soils amended with cattle manure and digestate, respectively. In their pot study, however, the soil was not cropped with plants. Evidence suggests that the extent of nitrate leaching may be modified by plant community composition, as shown by Dijkstra et al. (2007), who found that N losses were lower in species rich grasslands, probably due to a more complete resource use.

A reduction of N_2O emissions after the soil application of digestates compared to undigested manure is likely (Amon et al., 2006; Vallejo et al., 2006; Chantigny et al., 2007; Möller and Stinner, 2009; Joo et al., 2013), probably as a consequence of the digestion of the easily mineralizable N from the manure during the AD process (Massé et al., 2011). Indeed, the N_2O model by Sommer et al. (2004) predicted that AD could reduce N emissions by more than 50%. Using a modelling approach (CAPRI) for all countries in the EU, Leip et al. (2010) found that N_2O emission following field would be up to 40% lower. Nonetheless, Bertora et al. (2008) observed that the N_2O emission pattern derived from the anaerobically digested liquid fraction of pig slurry was similar to that observed for the original slurry after soil application during a 58-day mesocosm study. These different patterns in N_2O emissions could be due to various factors; the input of easily available organic C (measured as biological oxygen demand, BOD) seems to be the dominant factor driving the amount of N_2O emitted after digestate application (Clemens and Huschka, 2001). The higher the BOD, the more N_2O was emitted from the soil (Dosch and Gutser, 1996; Drury et al., 1998). Contrarily, Möller and Stinner (2009) emphasized that the higher supply of readily available NH_4—N, and not the available organic C, was responsible for the higher N_2O emissions after the injection of digested slurry, which was characterised by a low content in C and organic dry matter, into soil in closed slots. In this latter case, an enhanced denitrification was the main driving force for the course and amount of emitted N_2O. The soil water content and the temperature, along with the ammonium concentration in the manure, were also found to have a significant impact on N_2O emission potential (Clemens and Huschka, 2001; Wulf et al., 2002; Heller et al., 2010; Sänger et al., 2011).

The increased NH_4—N and pH-value as a result of AD give rise to evaporative NH_3 loss (Loria et al., 2007). According to IPCC guidelines (2007), around 1% of NH_3 deposited to soils is transformed to N_2O via microbial processes, which could notably offset GHG savings of biogas energy (Köster et al., 2014). Emissions of NH_3 also entail an environmental hazard in terms of soil acidification through nitrification to nitric acid (Quakernack et al., 2012). For instance, Möller and Stinner (2009) found that ammonia losses could take place when the incorporation of digestate into soil occurs more than 12 h after field spreading. This suggests that digestates can provide particularly positive agronomic effects in terms of their N content if they stay on the soil surface for only a short time. Furthermore, weather conditions have been shown to notably affect NH_3 emissions when digestates are applied (Quakernack et al., 2012; Köster et al., 2014). These authors found that the major portion of ammonia was released during the first two days after land spreading, and they attributed these higher NH_3 emissions at low temperatures to the fact that the frozen soil could have restricted the infiltration of most of the applied digestate, thereby NH_3 remaining on the soil surface. In conclusion, the application of biogas digestates to soil should accurately follow precise agricultural practice bearing in mind the ready availability of nitrogen and the crop N demand, so to avoid N loss as NO_3^-, which could be drained to surface waters, leached to ground waters or denitrified into gaseous forms (Bardgett and Wardle, 2010). The movement of NO_3^- can also carry cations along, potentially depleting potassium, calcium and magnesium in the soil and causing the leaching of aluminium, which may contaminate drinking water supplies (Manning, 2013).

The characterization of digestates in terms of form and content of N is critical so as to evaluate their leaching, volatilization and phytotoxic potential. This way the proper rating and timing of application is possible, and additional costs of post-digestion conditioning may be avoided (Alburquerque et al., 2012b).

5. Phosphorus and potassium

To date, little is known about the P-fertilizer effect of biogas residues in soil compared to undigested slurry. It is therefore of particular importance to delve deeper into this issue, as natural P sources are expected to be exhausted in the short to medium term (Dawson and Hilton, 2011).

In a field experiment by Alburquerque et al. (2012c) the usefulness of a digestate as fertilizer was evaluated with two horticultural crops (watermelon and cauliflower). The digestate application to soil led to yields comparable to mineral fertilisation for the summer watermelon crop. However, for the winter cauliflower crop, only plots treated with the mineral fertilizer showed good yields. The authors stated that N from the digestate is rapidly plant available but also it can be easily lost. A slow rate of microbial processes due to low temperatures as in the winter application could reduce the fertilizer potential of cattle manure and digestate. Of great interest is that the digestate led to an increased amount of available P in the soil. Overall, digestate application may affect the P availability in the soil and plant P nutrition either directly by adding inorganic and organic P compounds, or indirectly by influencing the activity of soil microorganisms as a consequence of the supply of organic matter. This is supported in that study by positive digestate effects on soil biological properties such as microbial biomass C and several enzyme activities (i.e., dehydrogenase, alkaline phosphatase and β-glucosidase activities).

Bachmann et al. (2014) evaluated the P-fertilizer value of a biogas residue, which was obtained from AD of dairy slurry, maize silage and wheat corn, in a 3-year field experiment with maize. The impact of the digestate on the biomass yield and P and N uptake, as well as on P cycle-related enzymes, was evaluated in comparison with its ingestate and a mineral fertilizer treatment (NK without P) that was used as a control. The organic treatments were applied at a rate of 30 m^3 ha^{-1} a^{-1}. After three years the plant-available P, as well as the uptake of P and N was higher in the digestate treated than in the control soils, and no differences were found relative to the ingestate. This emphasizes the potential of biogas residues as valuable and readily available P sources for crops. However, lower dehydrogenase and alkaline phosphatase activities with digestate compared to its ingestate, even though these differences were not reflected in the overall soil CO_2 flux. In an earlier study, consisting of an 8-week pot experiment on a sandy and a loamy soil using two crop species (*Zea mays* L., *Amaranthus cruentus* L.), Bachmann et al. (2011) observed that the effect of co-digested slurry on P uptake was comparable to that of the dairy slurry (ingestate) and mineral P fertilizer. Accordingly, Gasser et al. (2012) recorded comparable crop yields to those obtained with mineral P fertilizer in a pot experiment with oat (*Avena sativa* L.) grown in four soils with varied P-fixing capacities and P saturation levels. Hupfauf et al. (submitted for publication) found that the metabolic quotient increased upon fertilization with digestate compared to undigested slurry. This was attributed to the formation of recalcitrant P minerals like struvite and hydroxylapatite which in the short term reduced P availability, affording a higher respiratory turnover.

Summarized, total P contents will not change upon AD, however, the short-term availability may be reduced. AD seems not to alter substantially the fertilizer value of slurries as P sources for crops. Separation of liquid and solid fraction may shift P contents to the solid part, thus care has to be given to the potential effects of post-treatments.

Due to C loss, the K contents increase during AD, but total amounts in ingestate and digestate remain constant. Digestates may, however, contain large fractions of free K^+ ions (Unterfrauner et al., 2010), which in the case of light, poorly buffered soils may overload the sorption complex and destroy aggregates. In such cases, post-treatment like solid—liquid fractionation, or the use of clay minerals as amendment may be a remedy.

6. Heavy metals

To avoid risks upon the production of food and feed, it is important that the digestate used for fertilization contains adequate nutrient and micronutrient levels, however, at levels below toxicity. Solubility and availability of heavy metals are usually decreasing during AD, mainly due to precipitation processes with sulfide (S^{2-}), carbonate (CO_3^{2-}) and phosphate (PO_4^{3-}) (Möller and Müller, 2012). Decrease of Cd, Zn and Mg has been attributed to sulfide and struvite production (Zirkler et al., 2014). The formation of precipitates, however, does not mean that the total heavy metal contents decrease, if not a solid/liquid fractionation is performed and thus heavy metal contents are enriched in the solid phase. Massé et al. (2007) found that 18.4% of Zn and 41.4% of Cu have remained in swine manure biodigesters. While a decrease in heavy metal availability has been shown in pot experiments, no such evidence has been found in field experiments (Möller and Müller, 2012) and thus long-term heavy metal immobilization seems unlikely.

7. Organic pollutants

Most substrates used for biomethanisation will have levels of organic contaminants that are low enough for not inhibiting the process. However, if not sufficiently degraded in the anaerobic digestion process, they can render the digestate unsuited as a fertilizer and impair soil health. Such compounds may be dioxin-like (Engwall and Schnürer, 2002; Olsman et al., 2002, 2007; Brändli et al., 2007), polyaromatic hydrocarbons (PAH; Brändli et al., 2007), polychlorinated biphenyls (PCBs) and pesticides (Brändli et al., 2007), chlorinated paraffins (Nilsson et al., 2001; Brändli et al., 2007), phthalates (Nilsson, 2000; Hartmann and Ahring, 2003; Brändli et al., 2007) and phenolic compounds (Levén and Schnürer, 2005; Levén et al., 2006). Levén et al. (2012) presented an overview on phenols in context with digestates. The résumé of their study was that, firstly, phenols may be produced from other xenobiotic precursor substances during anaerobic digestion; secondly, phenols are more likely to be degraded under mesophilic than thermophilic conditions due to a higher metabolic potential of mesophilic microorganisms; and thirdly that remaining phenols in the digestate may inhibit AOBs in the soil. Wastewater sludges usually carry particularly high loads of organic pollutants, for these, Bertin et al. (2007) found evidence for the degradation of several pollutants, including poly-chlorinated biphenyls and polycyclic aromatic hydrocarbons in particular at mesophilic reactor conditions. Barret et al. (2012) studied four families of compounds, polycyclic aromatic hydrocarbons (PAHs), polychlorobiphenyls (PCBs), the phthalic acid esters (PAEs), and nonylphenol ethoxylates (NPEs) during anaerobic digestion in WWTP. They emphasized that the overall biodegradation was dependent on the interactions between the organic matter matrix and the microbiome, and in particular on the physico-chemical sorption properties of the compounds. It was shown that microbial co-metabolism was essential and efficient for removal of xenobiotics. In general it may be concluded that anaerobic digestion is a valid process for degradation of xenobiotics and usually the concentrations of organic pollutants are lower after the process than before, albeit not all of the compounds can be entirely degraded.

8. Antibiotics

Antibiotics are extensively used in livestock farming to promote growth and reduce diseases. Therefore, animal manure often contains antibiotics of various kinds (Massé et al., 2014). Once manure is applied to agricultural land to improve soil productivity, crops would be exposed to antibiotics which may persist in soils from a few to several hundred days.

Numerous studies are available on the effects of antibiotics used in livestock production on soils and plants, and it is known that antibiotics may directly be taken up by plants and stored in their leaves (e.g. Bassil et al., 2013). Generally the levels of antibiotics in plant tissue increased with increasing antibiotic concentration in the manure, where gentamicin (small molecule) was taken up in higher amounts than streptomycine (large molecule). Besides their negative effects in terms of ecological impact, antibiotics, e.g. those commonly used in pig treatment, are undesired due to their inhibitory effect on the AD process (Lallai et al., 2002). For example, Alvarez et al. (2010) reported a reduction in methane production by >50% induced by oxytetracycline and chlortetracycline; in line, Massé et al. (2000) reported that penicillin and tetracycline, added to the pig diet at their maximum prescribed level, showed to reduce methane production by 35% and 25%, respectively, while the other antibiotics (carbadox, tylosin, sulphamethazine, lyncomycin) showed no significant inhibitory effect. Thus, farmers using AD will take care to reduce the amount of antibiotics they apply to their animals. Interesting would further be the effect of antibiotics on the survival of pathogens during AD which relates to chapter 9.

The half-life of antibiotics during manure storage ranges from <2 d (e.g. impersistent macrolides) to 100 d as for very persistent tetracyclines (Boxall et al., 2004). As a comparison, the observed biodegradative reduction of chlortetracycline during 21 d of AD was found to be 7, 80 and 98% at 22 °C, 38 °C and 55 °C, respectively (Varel et al., 2012). Massé et al. (2014) conclude that anaerobic digestion is a quite efficient means of reducing antibiotic loads to soils, in particular at higher than ambient temperatures. However, it has been shown that antibiotic resistance genes proliferate in manure-fertilized soil even when the cattle had not been fed with antibiotics (Udikovic-Kolic et al., 2014). If this is the case also with AD treated manures needs to be investigated.

9. Pathogens

Pathogens may survive the AD process and persist in the digestate (Sahlström et al., 2004; Bagge et al., 2005), in particular spore-forming bacteria (Dohrmann et al., 2011; Saunders et al., 2012). Hygienisation of the end-product depends on the quality of the substrates, and on the reactor performance such as digestion temperature, slurry retention time, pH and ammonium concentration, and also any pre- or post-treatment like pasteurisation or composting (Sahlström, 2003; Ottoson et al., 2008). Goberna et al. (2011) found that AD eliminated cultivable *Escherichia coli* and *Salmonella*, and that *Listeria* was reduced from 10^5 ml^{-1} to 10^4 ml^{-1}. One month after application of manure and digestate to the soils, neither *E. coli* nor *Salmonella* could be detected any more (unless the manure was applied to sterilized soil). *Listeria* numbers in soil were reduced by one order of magnitude within three months. Specifically, the survival period of *E. coli* in soil is usually not longer than some days or weeks; although there exist previous studies in which survival periods of up to 40–68 d in soil have been shown for these pathogenic bacteria following the application of pig manure (Unc and Goss, 2004). Several environmental factors including temperature, soil moisture and pH may have a large influence on the survival time of faecal bacteria in soil (Pourcher et al., 2007). The manure application method as well as the microbial competition with the native soil microbiota also can be determinants in governing the survival of potential pathogens in amended soils (Unc and Goss, 2004; Goberna et al., 2011).

The numbers of spore-formers, which are commonly found in animal wastes (Snell-Castro et al., 2005), are not reduced (Olsen and Larsen, 1987; Sahlström et al., 2004; Bagge et al., 2005; Goberna et al., 2009). Accordingly, Olsen and Larsen (1987) observed that the spores of *Clostridium perfringens* were not inactivated, neither by mesophilic nor by thermophilic digestion. Similar results were observed by other authors (Chauret et al., 1999; Aitken et al., 2005) in a reactor operating under mesophilic and thermophilic conditions, respectively. Pepper et al. (2006) emphasized the potential for pathogen re-growth during storage or transport, which may be attributed to non-hygienic conditions of the tanks (Bagge et al., 2005) or to re-inoculation by animal vectors (Zaleski et al., 2005). Crane and Moore (1986) emphasized that raw and treated manures can involve a risk for the environment, even when they are applied at low pathogen concentration, due to the fact that some pathogens can exhibit a regrowth after deposition to soil.

In a review on the effects of treatment options on the pathogen survival in slaughterhouse wastes, Franke-Whittle and Insam (2013) came to the conclusion that an AD process with either a pre- or post-pasteurisation step would most likely inactivate the majority of microorganisms, excluding, however, prions and spore-forming bacteria. Importantly, any form of anaerobic treatment decreased pathogen loads compared to undigested slurry, and this was particularly so if the digestion plants were run under thermophilic conditions (Table 2). Furthermore, there is evidence of considerable sanitation potential of biowaste treatment involving high concentrations of ammonia, since the associated high pH may inhibit the growth of bacterial pathogens (Bujoczek et al., 2000; Ottosson et al., 2008; Bagge, 2009). However, elevated ammonia concentrations are also known to exert inhibitory effects on the AD process (Hansen et al., 1997) and increase the risk of ammonia volatilisation upon spreading the residues as fertilizer (Bagge, 2009).

Despite known risks of both untreated and anaerobically digested manures, AD would in no case increase the risk of pathogen spread, but usually decrease the risk. European Regulations (EC) 1774/2002 and 1069/2009 allow the use of manure and digested residues, respectively, as organic fertilizers and soil improvers (EU, 2002, 2009) and set the limits of the pathogenic loads admitted in both types of amendments (EU, 2002).

10. Phytotoxicity and plant disease suppression

In the end, not the effects on the environment and not the effects on soil but the effects on plants are concerning the farmers. In particular, the enhanced levels of ammonia in the digestate may increase its phytotoxicity, thereby affecting seed germination and plant growth (Engeli et al., 1993). Various volatile organic compounds may act in a phytotoxic way (Lee et al., 2014), but this will more likely be the case in undigested slurries due to the higher biological activity. Comparing phytotoxic effects of various by-products of bioenergy production, Gell et al. (2011) did not find any phytotoxic effects of residues from anaerobic digestion, be the substrate cow manure, pig slurry or human excreta. Phytotoxic effects may also be exerted by elevated heavy metal contents, or elevated contents of certain nutrients, such as P which may immobilize trace elements or render iron inaccessible; these effects are discussed in the respective sections.

There are reports that confirm a pesticidal effect of digestate on nematodes in tomatoes (Jothi et al., 2003). Kupper et al. (2006) reported the successful control of citrus black spot disease caused by *Phyllosticta citricarpa*. There are recent evidences of pathogen suppression by soil microbes through the production of antibiotics, the deprivation of nutrients mediated by chelators and the interference with pathogenicity factors (Haas and Défago, 2005; Goberna et al., 2011). In a study with 100 composts in Switzerland it was found that composts that had undergone some sort of anaerobic phase, or that contained AD slurries as a substrate component exerted a higher level of plant disease suppression than other composts (Fuchs et al., 2008).

Concluding, AD-induced increases of phytotoxic substances like ammonia, volatile organic compounds, or nutrient imbalances may be counteracted by agronomic measures and do usually not offset the beneficial effects of digestates. Rather, improved plant growth and disease suppression may be expected.

Table 2
Inactivation of pathogens by AD at 37 °C and 55 °C compared to pasteurization and thermophilic composting (modified from Franke-Whittle and Insam, 2013).

Pathogen	Inactivation by			
	Pasteurisation (70 °C)	Anaerobic digestion		Composting
		At 37 °C	At 55 °C	
Escherichia coli	++	+	+	+
Salmonella	++	+	++	++
Clostridium	−	−	−	−
Bacillus anthracis	−	−	−	−
Mycobacterium bovis	++	+	++	Not known
Erysipelothrix rhusiopathiae	++	+	++	++
BSE prion	−	−	−	−
Cysticercus bovis	++	++	++	++

++ Total inactivation; + inactivation; − survival.

11. Monitoring methods for biogas residue quality, soils and plant growth

Considering the potential benefits and risks of biogas residues (BRs) used as biofertilizers, it is of paramount importance to ensure the quality of digestates prior to their application to agricultural soil, so to prevent any negative environmental impact (Fig. 1).

This chapter aims to give an overview of soil biological methods adequate to judge the effects of digestates as organic fertilizers. Next to a brief listing of standard methods (routinely performed to assess the major soil characteristics), emphasis is given on recommended inter-disciplinary/multidisciplinary approaches, to be performed both at laboratory and field scale, both in short-term (initial stages; *priming effect*) and long-term experiments (Fig. 1). To gain an in-depth knowledge on the overall AD process, there is the need to analyse and control not only the quality of BRs and their effects on soil properties and consequently the impact on plant growth and GHG emission, but also the raw substrates (feedstocks), the used inocula, and the digester conditions.

Any accurate monitoring of the ecological impact of BRs used as fertilizers has to take into account also the quantity of GHGs released upon their application to soil (Möller et al., 2008; Odlare et al., 2012; Singla et al., 2013; Singla and Inubushi, 2014). The methods are indicated in the following sections.

11.1. Pre-treatment of substrates and inoculum

There are several pre-treatment options for substrates fed to the anaerobic digester. They usually aim at accelerating the hydrolysis of macromolecules, predominantly cellulose, and are based on thermal, mechanical, chemical, ultrasound or bacterial/enzymatic action (e.g. Appels et al., 2008; Ferrer et al., 2008; Cesaro et al., 2014). The methane fermentation process is possible under psychrophilic, mesophilic, and/or thermophilic conditions (Hansen et al., 1999; Ziemiński and Frąc, 2012; Zhu and Jha, 2013) that need to be controlled throughout the process (Turoviskiy and Mathai, 2006; Appels et al., 2008).

Any deviation of the theoretical potential would be an indicator of process failure, and thus a warning signal for an inappropriate and still reactive end product. During the AD process microbial culturing methods and/or genetic fingerprinting techniques (e.g. DGGE; Rincón et al., 2008; Wagner et al., 2009; Doi et al., 2010; Zhu and Jha, 2013), as well as microarrays like the ANAEROCHIP (Franke-Whittle et al., 2009, 2014; Goberna et al., 2010; Walter et al., 2012), together with metagenomic approaches may be used to assess and monitor microbial communities and population dynamics.

Among the principal inhibitors of the methanation process there are: i) high concentrations of ammonia (Hansen et al., 1999); ii) sulfide; iii) suboptimal availability of light metal ions (Na, K, Mg, Ca, Al); iv) heavy metals; and v) organic compounds (e.g. alkyl benzenes, phenols, alkanes, alcohols), as reviewed by Chen et al. (2008). In order to avoid inhibition of the AD process, parameters such as soluble chemical oxygen demand, volatile

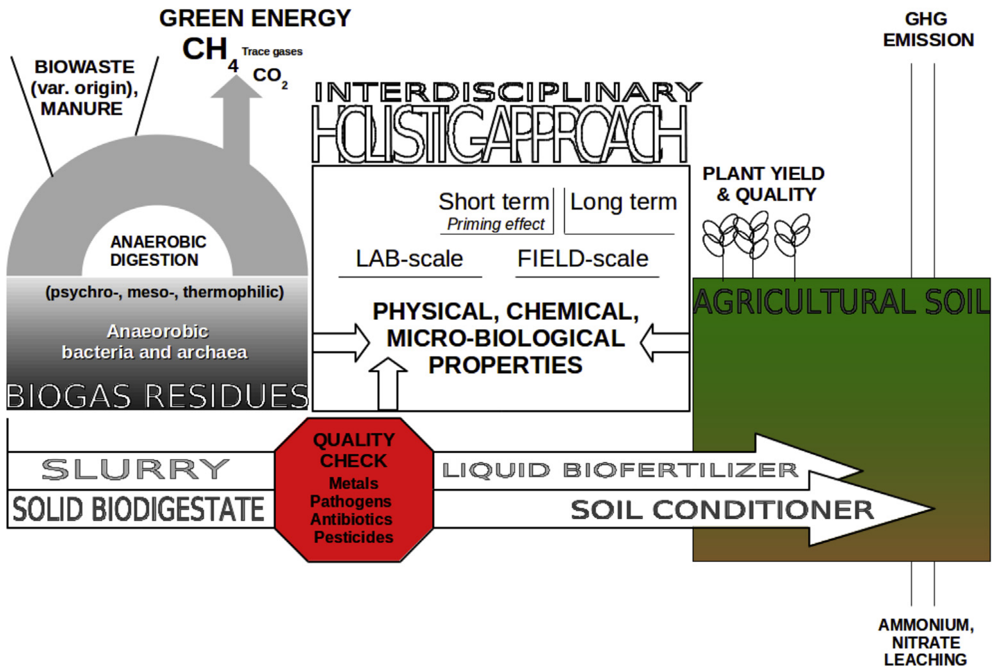

Fig. 1. Schematic overview of anaerobic digestion (methane fermentation) of organic wastes and required analyses to assess and monitor the impact of its nutrient rich by-products, liquid and solid biogas residues, on overall soil properties.

fatty acids (VFAs) concentration and pH have to be regularly measured during the AD process (Rincón et al., 2008; González-Fernández and García-Encina, 2009; Doi et al., 2010; Scherr et al., 2012).

11.2. Characteristics of BRs

Physico-chemical characteristics of feedstocks (and biowastes) of different origins, as well as of BRs can be assessed by standard methods applied in soil ecology (see Section 11.6). Among the routinely assessed characteristics of BRs there are the total nutrient pools (N, C, P) and other essential plant macro-microelements; inorganic/mineral nitrogen (N_{inorg}, N_{min}), NH_4^+–N and NO_3^-–N, pH, electrical conductivity (EC), the C/N ratio, cation exchange capacity (CEC) and dry mass (total solids).

11.3. Phytotoxicity

The assessment of slurry characteristics, raw *vs.* digested slurry, is essential to evaluate phytotoxic action, also by assessing the predicted no-effect concentration (PNEC) (Domene et al., 2008). Among the potential phytotoxic contaminants contained in BRs there are: i) heavy metals (e.g. Cu, Zn, Cr, Cd, Pb, As, Ni, Mn and Se); ii) antibiotics (e.g. penicillin, tetracycline, carbadox, tylosin, sulphamethazine, lyncomycin, oxytetracycline and chlortetracycline (Massè et al., 2000; Alvarez et al., 2010); iii) pathogens; iv) xenobiotics and v) a multitude of other, often unknown, effectors. Basically, three types of phytotoxixity tests are used, including the seed germination test, and the determination of shoot and root elongation (Zucconi et al., 1981). Various plants, like cress, ryegrass or vegetables like radish or lettuce may be used, depending on the exact purpose. The fastest approach may be considered the seed germination test (e.g., Di Salvatore et al., 2008). Test kits like the Phytotoxkit are also commercially available (Czerniawska-Kusza et al., 2006).

11.4. Hygiene

Hygiene assessment in terms of pathogen abundance can be performed by culture dependent methods (plate counts; e.g. cultivable *E. coli*, *Salmonella* and *Listeria*) and culture independent, molecular detection of pathogenicity genes, e.g. *hly*A gene; genes encoding the virulence factor listeriolysin O, which is essential for the virulence of *Listeria monocytogenes*; and *inv*A gene coding for invasine, a protein largely determining the invasive abilities of several pathogens, including *Salmonella* sp. (Mawdsley et al., 1995; Sahlström, 2003; Bagge et al., 2005; Goberna et al., 2011). However, there is still a lack of idoneous indicators of hygiene that can be applied in every case (Sahlström, 2003; Chae et al., 2008; Zongqiang et al., 2011).

11.5. Antibiotics

The effect of antibiotics on the AD process has been discussed above. Specific PCR-based monitoring of antibiotic resistance genes might be a potential tool to assess and monitor the soil quality before and after the application of BRs to soil (Udikovic-Kolic et al., 2014; Yang et al., 2014).

11.6. Effects of BRs on soil properties

To evaluate the impact of BRs on overall soil properties, the assessment and monitoring of physical, chemical, and microbiological soil properties before and after its application to soil is crucial and it has to be performed in short-term (initial stages) *vs.* long-term survey studies, at both the laboratory and the field scale (Fig. 2).

An adequate experimental design, based on a valid soil sampling strategy (independent replicates avoiding pseudo-replicates) is of paramount importance in order to obtain reliable and inter-laboratory comparable data; therefore, efforts toward standardised basic methods (including soil conservation; soil treatment

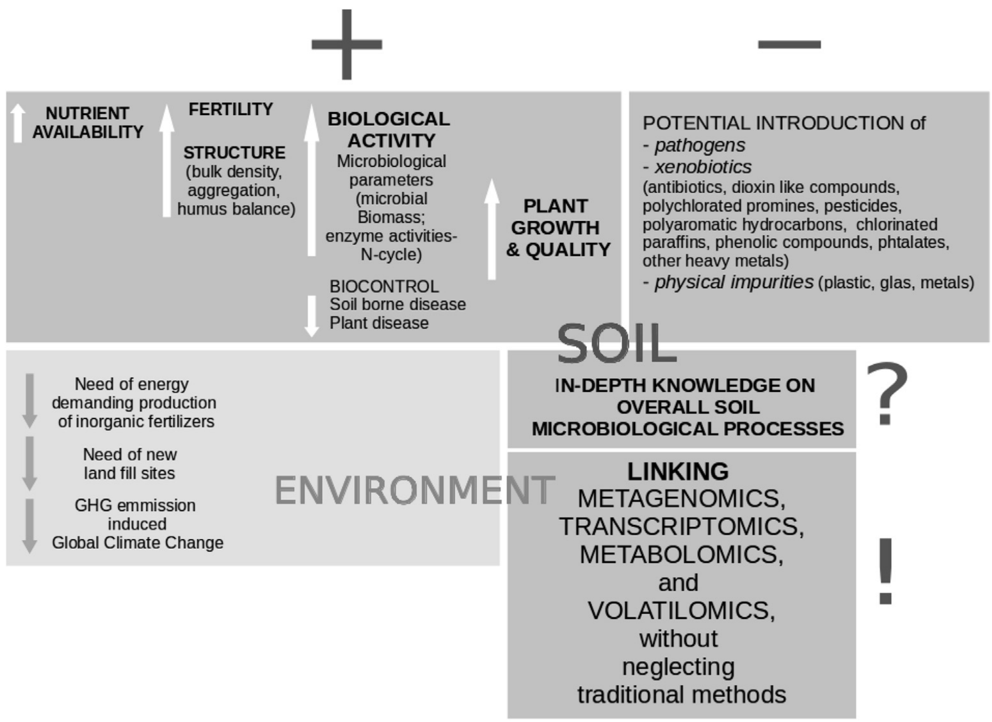

Fig. 2. Positive environmental effects of methane fermentation and potential positive (+), negative (−), and still unknown (?) effects of the application of its by-products, biogas residues (BRs), as fertilizer in agriculture; long-term field survey studies performing a holistic OMICs-approach might fill this gap (!).

such as sieving, etc.) are (still) of crucial concern. In order to accurately assess the impacts of BRs on soil properties, also in terms of soil fertility and plant growth promotion (PGP) potential, a comparative monitoring of rhizosphere *vs.* bulk soil has been recommended (e.g. Singla et al., 2013).

The most commonly used methods to assess and monitor changes in soil physical and chemical properties induced by BRs amendment are summarised in Table 3. Regarding the microbiological properties, soil microbial biomass has been recognised as the driving force for residues mineralization in soils, although it usually comprises only about 1–3% of total soil organic carbon (Abaye and Brookes, 2006; Chen et al., 2012). There are several standard methods to assess the soil microbial biomass, such as i) the substrate-induced respiration (SIR) as a measure of microbial C, e.g. according to Anderson and Domsch (1978) (Odlare et al., 2008; Insam et al., 2009; Bougnom et al., 2012; Chen et al., 2012); ii) fumigation–extraction method (Jenkinson and Powlson, 1976); iii) substrate-induced growth response (SIGR) in the soil, using a model presented by Panikov and Sizova (1996), to estimate the kinetic parameters of soil microbial growth in response to substrate amendments (Blagodatsky et al., 2000; Chen et al., 2012) and therefore to quantify the active fraction of the microbial biomass. Other standard methods like the quantification of total phospholipid fatty acids (PLFAs) or double-strand DNA (dsDNA) (Fornasier et al., 2014) may also be recommended.

Parameters like basal respiration (R_{mic}) as a measure of biological activity, and assessed as CO_2 evolution or O_2 consumption (Odlare et al., 2008; Bougnom et al., 2012); and/or the determination of the specific growth rate (μSIR) of active and dormant microbes (Odlare et al., 2008) have been used as indicators of microbial activity. In addition, the organic carbon mineralization can be determined by measuring the evolved total CO_2 (e.g. trapped in 10 ml of 0.1 M KOH solution placed inside the incubation vessels in the respirometer) via the changes in EC of the KOH solution (Stumpe et al., 2012). Other estimators of soil microbial activity can be obtained by measuring the i) nitrogen mineralization capacity (NMC) (Odlare et al., 2008; Abubaker et al., 2012); ii) Alk-P (Odlare et al., 2008); iii) potential nitrification activity (PDA) (Odlare et al., 2008); iv) specific growth rate of denitrifiers (μPDA) (Odlare et al., 2008); v) potential ammonia oxidising rate (PAO) (Odlare et al., 2008; Abubaker et al., 2012); vi) microbial metabolic quotient (qCO_2) (respiration-to-biomass ratio) (Insam et al., 2009); and vii) bacterial and fungal growth rates (reviewed by Rousk and Bååth, 2011), among others.

Soil microorganisms degrade SOM by excreting enzymes; extracellular enzymes (EE) are biological catalysts of specific reactions, and as such they play a key role in the decomposition of native and exogenous OM in soils (Cayuela et al., 2009; Chen et al., 2012; Stumpe et al., 2012), regulating its turnover and overall carbon dynamics. Since soil enzymes respond promptly to environmental changes, such as changes in agricultural practices (Ge et al., 2010), they are considered as sensitive indicators of soil biological processes and soil fertility (Dick, 1992; Nannipieri et al., 2003; Gianfreda and Rao, 2014) (Table 4).

The microbial community structure in terms of diversity (composition, evenness, richness) can be assessed and monitored by fingerprinting techniques such as: i) metabolic fingerprinting, e.g. MicroResp® (Johansen et al., 2009, 2013) and community level physiological profiling by Biolog® (Garland and Mills, 1991; Insam and Rangger, 1997). The latter, frequently used method, however, is clearly not recommended since the growth conditions in the Biolog wells allow only a small fraction of the soil microbiota to grow (Ros et al., 2008); ii) biochemical fingerprinting, e.g. phospholipid fatty acid (PLFA) analysis, (Elfstrand et al., 2007; Johansen et al., 2009, 2013); and iii) genetic fingerprinting, e.g terminal restriction fragment length polymorphism (TRFLPs) (Abubaker et al., 2013a), denaturing gradient gel electrophoresis (DGGE) (Insam et al., 2009), and denaturing high-performance liquid chromatography (dHPLC) (Wagner et al., 2009), or iv) microarrays like the GeoChip (He et al., 2007).

Table 3
Overview of routine analyses used to monitor the impact of biogas residues amendment on physical and chemical soil properties.

Parameters assessed		Method/reference
Soil nutrients		
Carbon, C	Total C	Combustion at 1250 °C and determination using a C–N–S analyser (CNS-2000, LECO Equipment Corp., St. Joseph, MI, USA) (Odlare et al., 2008)
	Organic C	Tot-C was corrected for carbonate to give organic C (Odlare et al., 2008)
		TOC on air-dried and sieved (0.25-mm) soil samples using an exogenous thermal process with potassium dichromate according to Walkley and Black (1934) and Long et al. (2014)
Nitrogen, N	Total N	Combustion at 1250 °C and determination using a C–N–S analyzer (CNS-2000, LECO Equipment Corp., St. Joseph, MI, USA) (Odlare et al., 2008)
	Organic N	Kjeldahl method according to Bremner and Mulvaney (1982)
	Mineral-inorganic N (NH_4^+–N and NO_3^-–N)	NH_4–N by the method ST9002-NH_4D and NO_3–N by the method ST9002-NO_3D with dialysis on an AutoAnalyzer TRAACS 800 (Kontram, Stockholm, Sweden) (Odlare et al., 2008)
		The soil mineral N (SMN) content in the filtrates (100 g of fresh samples mixed with 200 ml $CaCl_2$ diluted in a.d. and shaking for 60 min) by colorimetry (Skalar Analytical) (Möller et al., 2008)
Sulphur, S	Total S	Combustion at 1250 °C and determination using a C–N–S analyzer (CNS-2000, LECO Equipment Corp., St. Joseph, MI, USA) (Odlare et al., 2008)
Phosphorus, P	Total P	Contents of total phosphorus were measured in soil extracts (7 M HNO_3) (Abubaker et al., 2012)
	Available P	Olsen et al. (1954) and Bachmann et al. (2011); fractionated analysis (Demetz and Insam, 1999)
		Ammonium lactate (AL) method according to Egnér et al. (1960) and del Pino et al. (2014)
Potassium, K	Available K	Ammonium lactate (AL) method according to Egnér et al. (1960) and del Pino et al. (2014)
		Available K extracted with 0.1 N NH_4OAC buffered at pH 7.0 (K–HN_4OAC, Al-Juhaimi et al., 2014)
Metals/heavy metals		
Cu, Zn, Cr, Cd, Pb, As, Ni, Mn and Se		Determination via ICP-AES plasma analyser (Perkin–Elmer ICP Optima 3000) according to SS02 83 11 (Odlare et al., 2008)
Available Fe, Mn, Cu, Zn		$CaCl_2$/DTPA extraction method coupled with ICP-AES analyses according to Alt et al. (1994) and Bougnom et al. (2012)
Hg		Direct combustion after freeze-drying, using an Advanced Mercury Analyser (Bougnom et al., 2012)
Exchangeable cations (EC):		
Total cation-exchange capacity (CEC)		According to Nömmik (1974) and Hossain et al. (2014)
Total exchangeable base cations (TEB)		According to Nömmik (1974) and del Pino et al. (2014)
Electrical conductivity (EC, mS cm^{-1})		Electrical conductivity in a soil:water suspension (1:2.5) (Insam et al., 2009); Determination by saturated soil paste extraction (Al-Juhaimi et al., 2014)
pH		Determined in a soil:0.01 M $CaCl_2$ suspension (1:2.5) with a pH meter (Insam et al., 2009); Assessed in 0.01 M $CaCl_2$ with the vol/vol ratio 1:2 (pH $CaCl_2$) (Odlare et al., 2008)
Bulk density		Core method according to Blake (1965)
Particle size distribution		According to Jung (1987) and Odlare et al. (2008)
Soil texture		Hydrometer method (Hossain et al., 2014)
		Texture estimation according to Blake (1965) and Al-Juhaimi et al. (2014)
Soil structure (aggregate stability)		Separation of soil samples (50 g) into four fractions (aggregate sizes) by wet sieving according to Elliott (1986)

In-depth knowledge on microbial community structures and population dynamics can be gained by i) metagenetic approaches, e.g. sequencing of PCR amplified and cloned 16S rRNA gene fragments (Sundberg et al., 2013); and ii) metagenomic approaches using high throughput sequencing techniques.

11.7. Plant growth promoting potential of BRS

The assessment of biomass and quality of plants provides information about the plant growth promoting (PGP) potential of BRs, as reviewed by Gurung (1997). Some commonly assessed parameters as descriptors of the PGP potential are: i) plant height, number of leaves, shoot and root dry weight (Möller et al., 2008; Singla et al., 2013; Hossain et al., 2014); and ii) total biomass yield (g^{-1} dw m^2; Singla et al., 2013) and relative yields of ear, straw and root fractions (Abubaker et al., 2012). In addition, the nitrogen agronomic efficiency can be calculated as the ratio of the yield with the applied N minus the yield of the control to the total amount of the applied N (Singla et al., 2013).

12. Post-treatment and application issues

Above, without considering the options of post-treatment of biogas slurries we have focused on general effects. Any of these effects may, however, be enhanced or mitigated by several post-treatment options (Table 5). Each of the post-treatment options has its advantages and disadvantages. A fast and low cost treatment of biogas slurry prior to its application to agricultural soils is that of centrifuging the biodigestate; C-rich compounds and also heavy metals (Jieqiong et al., 2013) remain in the solid phase, while much of the nitrogen stays in the liquid phase. In case of excess nitrogen in the liquid phase, ordinary wastewater treatment techniques may be applied, or advanced methods like treatment with the DEMON® anaerobic ammonia oxidation process (Podmirseg et al., 2010). Drying yields a nutrient-rich organic fertilizer that may also be transported over large distances, and by pelletizing the material custom-designed products with different nutrient release dynamics may be produced. Composting is able to stabilise most of the nutrients to obtain a slow-release product. Both drying and composting have a positive effect on hygienisation (Franke-Whittle and Insam, 2013).

The loss of C during digestion reduces viscosity and thus facilitates the handling and further management. Digestate is a more uniform product allowing for better prediction of nutrient use; and it is known for reduced odour emissions and less “burn” after topdressing (Chadwick, 2007; Chadwick et al., 2011; Van der Meer, 2007). The benefits of a higher ammonia fraction in digestate (or the liquid fraction thereof) require, however, careful management when it is field applied:

Table 4
Overview of enzyme activities assessed as indices for soil quality in response to BRs amendment as biofertilizer.

Biogeochemical cycles	Enzyme	Catalysed reaction	Reference
Carbon-cycle	β-glucosidase	Cellulose decomposition	Chen et al. (2012) and Stumpe et al. (2012)
	Cellobiohydrolase	Cellulose decomposition	Chen et al. (2012) and Stumpe et al. (2012)
	Xylanase	Hemicellulose decomposition	Chen et al. (2012)
	Invertase	Hydrolysis of sucrose to glucose and fructose	Chen et al. (2012)
Nitrogen-cycle	Chitinase	Break down of glycoside bonds	Chen et al. (2012)
	Leucine amino peptidase	Hydrolysis of L-peptide bonds	Chen et al. (2012)
	L-leucine	Organic N	Stumpe et al. (2012)
	Tyrosine	Organic N	Stumpe et al. (2012)
	Arginine amino- peptidase	Organic N	Stumpe et al. (2012)
	Dehydrogenase	Oxidoreductase	Bachmann et al. (2011) and Chen et al. (2012)
	Urease	Hydrolysis of urea in CO_2 and ammonia	Tao et al. (2010) and Fan et al. (2012)
	Protease (peptidase, proteinase)	Proteolysis, the hydrolysis of peptide bonds	Elfstrand et al. (2007) and Tao et al. (2010)
Phosphorus-cycle	Acid phosphatase	Organic P	Elfstrand et al. (2007) and Stumpe et al. (2012)
Sulphur-cycle	Arylsulfatase	Hydrolysis of sulfates	Elfstrand et al. (2007)
Other oxidoreductases	Catalase	Decomposition of hydrogen peroxide to water and oxygen	Chen et al. (2012) and Fan et al. (2012)

- To avoid ammonia volatilisation and N_2O emissions its application is recommended late in the day, just before or during light rainfall. To avoid odour, only mature digestate with a low remaining potential of methane and volatile organic compounds (VOCs; Insam and Seewald, 2010) emission should be used.
- Application by trench-hose: the remaining VOCs with their methane potential are degraded before entering the atmosphere (Van der Meer, personal communication) and methane oxidisers consume remaining CH_4.
- A low remaining biodegradation potential will lead to reduced N_2O emissions from anaerobically digested manures (Steinfeld et al., 2006).

13. Research outlook

To gain in-depth knowledge on the ecological impact of BRs applied to soil, further research on overall soil processes is needed, e.g. by performing inter-disciplinary approaches on soil physical, chemical, and (micro)biological properties (Fig. 2), including 'omics' approaches (Nannipieri et al., 2014). To cover all the aspects related to BRs, there is also the need of i) idoneous indicators capable to evaluate and monitor the fate of pathogens and antibiotics during the AD process; and ii) monitoring the effects of antibiotics (digestates *vs.* untreated manures) on the autochthonous and allochthonous soil microbiota. A potential tool could be a PCR-based screening and quantification (qPCR) of antibiotic resistance genes (BRs *vs.* soil after BRs application). Furthermore, the sequential extraction of the extracellular (eDNA) and intracellular (iDNA) fraction of the total soil DNA pool is not only capable to markedly improve the overall DNA extraction efficiency (Ascher et al., 2009); but enables also to specifically analyse the soil eDNA, which might contain genetic information about the fate of pathogens and antibiotics, potentially introduced into the soil via the application of BRs. In line with this, the recent approach of the barcoding technique based on extracellular soil DNA proposed by Taberlet et al. (2012) could constitute a powerful tool to assess the eukaryotic biodiversity at a large scale; the principal challenge is that of extrapolating the collected data from the regional to a global scale.

Table 5
Post-treatment options for biogas slurries.

Post-treatment		Product
Liquid/solid separation	Liquid fraction	Liquid fertilizer
(settlement or centrifugation)	Solid fraction → drying	Solid matter, pellets
Drying		Solid matter, pellets
Composting		Compost
Pyrolysis		Biochar

The recently proposed method by Fornasier et al. (2014), consisting in the direct extraction and PicoGreen based fluorimetric measurement of double stranded DNA (dsDNA), is believed to provide a reliable estimator of soil microbial biomass; it relies on the fact that the unavoidable loss of DNA during purification procedures, required for downstream analysis, and thus an underestimation of soil microbial biomass is omitted. This simple, fast and low cost method is suggested for monitoring changes in microbial biomass in BRs amended soils.

Most studies dealing with the impact of biogas residues (in comparison to other organic waste fertilizers), focussing on C and N dynamics, are so far conducted at the short-term (*priming effect*) and at the laboratory scale (e.g. Möller et al., 2008; Chen et al., 2012; Stumpe et al., 2012; Hossain et al., 2014). However, for an overall agronomic evaluation, long-term field trials to validate the results from pot experiments are required.

Multiple-enzyme assays performed on key enzymes involved in the biogeochemical N-cycle, which are assumed to be promoted by N-enriched organic components accumulated in BRs during the biogas fermentation process; so far there are only few reports, e.g. on chitinase and LAP activities (Hernandez and Hobby, 2010; Chen et al., 2012), but also enzymes involved in P and S cycle need to be addressed in order to gain a more complete picture of principal biogeochemical nutrient cycles in response to BR amendment.

Furthermore, investigations on the effects of BRs on the soil ecosystem should be also extended to the soil meso- and macrofauna (Domene et al., 2007; Larsen et al., 2007; Fründ et al., 2010) and correlated to the responses of the microbiome.

14. Conclusions

From an environmental perspective, manure management by biogas production is believed to reduce potential GHG production. From an agricultural perspective, AD residues are believed to act as potent fertilizers. When applied correctly, nutrient (e.g. gaseous nitrogen) losses are lower than with undigested matter, and evidence suggests that also soil organic C pools presumably do not suffer from reduced C input, at least when compared to mineral fertilization.

Overall, if looking at all the pros and cons, AD of agricultural residues may be considered ecologically sound. Care, however, has to be taken if the centralization of AD plants causes a local oversupply of AD residues and, as a consequence, nutrients. This will in particular be the case upon use of energy crops, or when residues of larger operations like slaughterhouses or WWTP are being taken care of. At the farm level, we consider AD superior to any other manure treatment option. A supplementation by a post-treatment, however, may be advised in certain cases.

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