



## Review paper

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

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## ARTICLE INFO

## Article history:

Received 23 September 2014

Received in revised form

9 February 2015

Accepted 10 February 2015

Available online 21 February 2015

## Keywords:

Anaerobic digestion

Biogas residues

Anaerobic slurry

Digestate

Soil

## 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<sub>4</sub> and 30% CO<sub>2</sub>) 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; Frac 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 Frac, 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 Palojarvi, 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 ground-water 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](#); [Fr  c 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 <sub>tot</sub> [kg/m <sup>3</sup> ]   | 4.1    | 5.0       | ↑     |
| NH <sub>4</sub> –N [kg/m <sup>3</sup> ] | 1.8    | 2.8       | ↑     |
| K [kg/m <sup>3</sup> ]                  | 4.2    | 4.9       | →     |
| MgO [kg/m <sup>3</sup> ]                | 0.85   | 0.95      | →     |
| CaO [kg/m <sup>3</sup> ]                | 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  $\text{NH}_4\text{-N}$ , and not the available organic C, was responsible for the higher  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$ . The soil water content and the temperature, along with the ammonium concentration in the manure, were also found to have a significant impact on  $\text{N}_2\text{O}$  emission potential (Clemens and Huschka, 2001; Wulf et al., 2002; Heller et al., 2010; Sängler et al., 2011).

The increased  $\text{NH}_4\text{-N}$  and pH-value as a result of AD give rise to evaporative  $\text{NH}_3$  loss (Loria et al., 2007). According to IPCC guidelines (2007), around 1% of  $\text{NH}_3$  deposited to soils is transformed to  $\text{N}_2\text{O}$  via microbial processes, which could notably offset GHG savings of biogas energy (Köster et al., 2014). Emissions of  $\text{NH}_3$  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  $\text{NH}_3$  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  $\text{NH}_3$  emissions at low temperatures to the fact that the frozen soil could have restricted the infiltration of most of the applied digestate, thereby  $\text{NH}_3$  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  $\text{NO}_3^-$ , which could be drained to surface waters, leached to ground waters or denitrified into gaseous forms (Bardgett and Wardle, 2010). The movement of  $\text{NO}_3^-$  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  $\beta$ -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  $30 \text{ m}^3 \text{ ha}^{-1} \text{ a}^{-1}$ . 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  $\text{CO}_2$  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. Hupfaut 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  $\text{K}^+$  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 ( $\text{S}^{2-}$ ), carbonate ( $\text{CO}_3^{2-}$ ) and phosphate ( $\text{PO}_4^{3-}$ ) (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 bimethanisation 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 streptomycin (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 (Udikov-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<sup>-1</sup> to  $10^4$  ml<sup>-1</sup>. 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

**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<br>(70 °C) | Anaerobic digestion |          | Composting |
|                                     |                           | At 37 °C            | At 55 °C |            |
| <i>Escherichia coli</i>             | ++                        | +                   | +        | +          |
| <i>Salmonella</i>                   | ++                        | +                   | ++       | ++         |
| <i>Clostridium</i>                  | –                         | –                   | –        | –          |
| <i>Bacillus anthracis</i>           | –                         | –                   | –        | –          |
| <i>Mycobacterium bovis</i>          | ++                        | +                   | ++       | Not known  |
| <i>Erysipelothrix rhusiopathiae</i> | ++                        | +                   | ++       | ++         |
| BSE prion                           | –                         | –                   | –        | –          |
| <i>Cysticercus bovis</i>            | ++                        | ++                  | ++       | ++         |

++ Total inactivation; + inactivation; – survival.

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.

### 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.

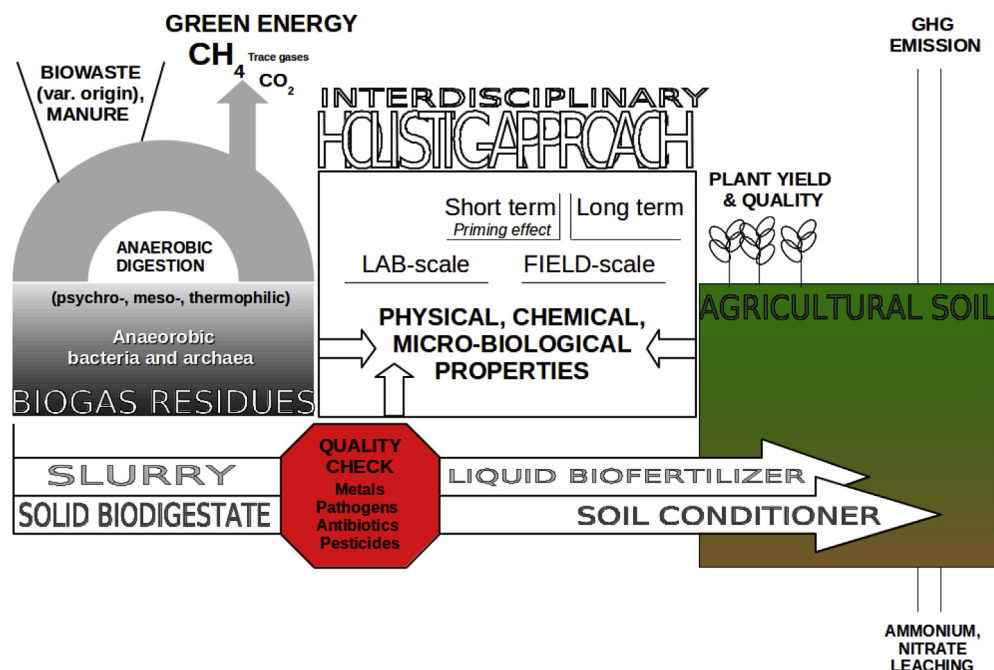
Any accurate monitoring of the ecological impact of BRs used as fertilizers has to take into account also the quantity of GHGs released upon their application to soil ([Möller et al., 2008](#); [Odlare et al., 2012](#); [Singla et al., 2013](#); [Singla and Inubushi, 2014](#)). The methods are indicated 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 meta-genomic 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 bio-wastes) 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, sul-phamethazine, lincosamin, 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 phytotoxicity 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. *hlyA* gene; genes encoding the virulence factor listeriolysin O, which is essential for the virulence of *Listeria monocytogenes*; and *invA* 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).

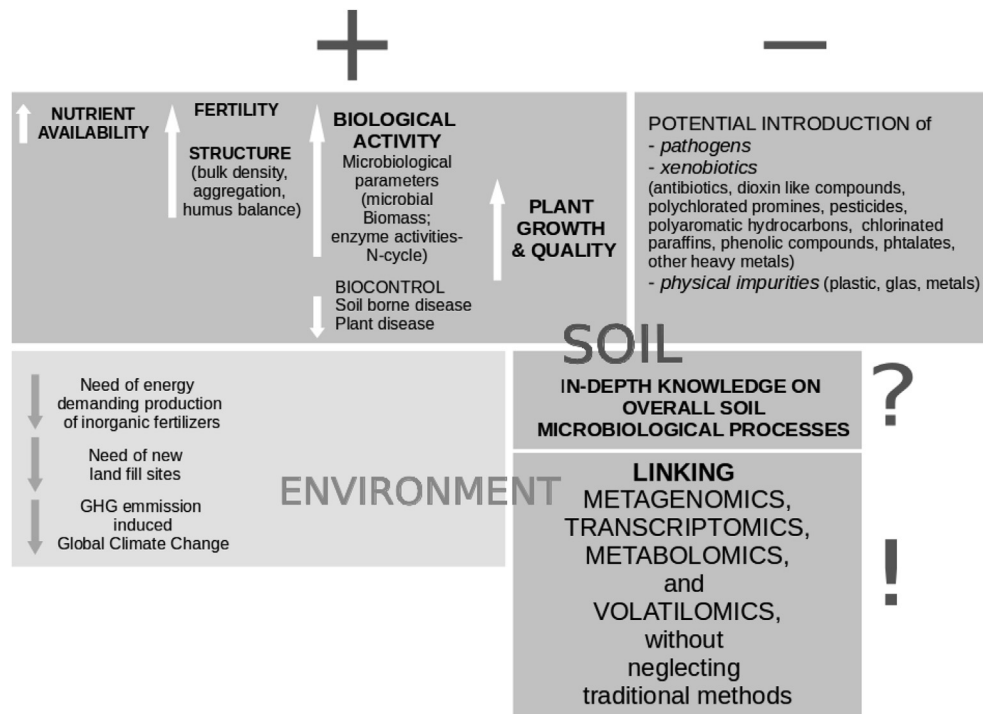
### 11.5. Antibiotics

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 ( $\mu_{SIR}$ ) 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 ( $\mu_{PDA}$ ) (Odlare et al., 2008); v) potential ammonia oxidising rate (PAO) (Odlare et al., 2008; Abubaker et al., 2012); vi) microbial metabolic quotient ( $q_{CO_2}$ ) (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<sup>®</sup> (Johansen et al., 2009, 2013) and community level physiological profiling by Biolog<sup>®</sup> (Garland and Mills, 1991; Insam and Ranggner, 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   |
|---|---|--|
| <b>Soil nutrients</b>                                   |   |  |
| 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)<br>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 analyser (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 <sub>4</sub> <sup>+</sup> –N and NO <sub>3</sub> <sup>–</sup> –N) | NH <sub>4</sub> –N by the method ST9002–NH <sub>4</sub> D and NO <sub>3</sub> –N by the method ST9002–NO <sub>3</sub> D with dialysis on an AutoAnalyzer TRAACS 800 (Kontram, Stockholm, Sweden) (Odlare et al., 2008)<br>The soil mineral N (SMN) content in the filtrates (100 g of fresh samples mixed with 200 ml CaCl <sub>2</sub> 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 analyser (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 <sub>3</sub> ) (Abubaker et al., 2012)  |
|   | Available P   | Olsen et al. (1954) and Bachmann et al. (2011); fractionated analysis (Demetz and Insam, 1999)   |
| Potassium, K  | Available K   | Ammonium lactate (AL) method according to Egnér et al. (1960) and del Pino et al. (2014)<br>Ammonium lactate (AL) method according to Egnér et al. (1960) and del Pino et al. (2014)<br>Available K extracted with 0.1 N NH <sub>4</sub> OAC buffered at pH 7.0 (K–HN <sub>4</sub> OAC, Al-Juhaimi et al., 2014)   |
| <b>Metals/heavy metals</b>                              |   |  |
| 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 <sub>2</sub> /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)   |
| <b>Exchangeable cations (EC):</b>                       |   |  |
| 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)  |
| <b>Electrical conductivity (EC, mS cm<sup>–1</sup>)</b> |   | Electrical conductivity in a soil:water suspension (1:2.5) (Insam et al., 2009);<br>Determination by saturated soil paste extraction (Al-Juhaimi et al., 2014)   |
| <b>pH</b>   |   | Determined in a soil:0.01 M CaCl <sub>2</sub> suspension (1:2.5) with a pH meter (Insam et al., 2009);<br>Assessed in 0.01 M CaCl <sub>2</sub> with the vol/vol ratio 1:2 (pH CaCl <sub>2</sub> ) (Odlare et al., 2008)  |
| <b>Bulk density</b>                                     |   | Core method according to Blake (1965)  |
| <b>Particle size distribution</b>                       |   | According to Jung (1987) and Odlare et al. (2008)  |
| <b>Soil texture</b>                                     |   | Hydrometer method (Hossain et al., 2014)   |
| <b>Soil structure (aggregate stability)</b>             |   | Texture estimation according to Blake (1965) and Al-Juhaimi et al. (2014)<br>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<sup>–1</sup> dw m<sup>2</sup>; 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<sup>®</sup> 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          | $\beta$ -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 <sub>2</sub> 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)    |
|                       | Acid phosphatase                 | Organic P  | Elfstrand et al. (2007) and Stumpe et al. (2012) |
| Phosphorus-cycle      | Arylsulfatase                    | Hydrolysis of sulfates                                 | Elfstrand et al. (2007)                          |
| Sulphur-cycle         | Catalase                         | Decomposition of hydrogen peroxide to water and oxygen | Chen et al. (2012) and Fan et al. (2012)         |

- To avoid ammonia volatilisation and N<sub>2</sub>O 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<sub>4</sub>.
- A low remaining biodegradation potential will lead to reduced N<sub>2</sub>O 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.

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 macro-fauna ([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

**Table 5**

Post-treatment options for biogas slurries.

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

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.

## References

- Abaye, D.A., Brookes, P.C., 2006. Relative importance of substrate type and previous soil management in synthesis of microbial biomass and substrate mineralization. *European Journal of Soil Science* 57, 179–189.
- Abubaker, J., Risberg, K., Pell, M., 2012. Biogas residues as fertilizers – effects on wheat growth and soil microbial activities. *Applied Energy* 99, 126–134.
- Abubaker, J., Cederlund, H., Arthurson, V., Pell, M., 2013a. Bacterial community structure and microbial activity in different soils amended with biogas residues and cattle slurry. *Applied Soil Ecology* 72, 171–180.
- Abubaker, J., Odlare, M., Pell, M., 2013b. Nitrous oxide production from soils amended with biogas residues and cattle slurry. *Journal of Environmental Quality* 42, 1046–1058.
- Aitken, M.D., Walters, G.W., Crunk, P.L., Willis, J.L., Farrell, J.B., Schafer, P.L., Arnett, C., Turner, B.G., 2005. Laboratory evaluation of thermophilic-anaerobic digestion to produce class A biosolids. Stabilization performance of a continuous-flow reactor at low residence time. *Water Environment Research* 77, 3019–3027.
- Alburquerque, J.A., de la Fuente, C., Bernal, M.P., 2012a. Chemical properties of anaerobic digestates affecting C and N dynamics in amended soils. *Agriculture, Ecosystems & Environment* 160, 15–22.
- Alburquerque, J.A., de la Fuente, C., Ferrer-Costa, A., Carrasco, L., Cegarra, J., Abad, M., Bernal, M.P., 2012b. Assessment of the fertilizer potential of digestates from farm and agroindustrial residues. *Biomass and Bioenergy* 40, 181–189.
- Alburquerque, J.A., de la Fuente, C., Campoy, M., Carrasco, L., Nájera, I., Baixauli, C., Caravaca, F., Roldán, A., Cegarra, J., Bernal, M.P., 2012c. Agricultural use of digestate for horticultural crop production and improvement of soil properties. *European Journal of Agronomy* 43, 119–128.
- Al-Juhami, F.Y., Hamad, S.H., Al-Ahaidib, I.S., Al-Otaibi, M.M., El-Garawany, M.M., 2014. Effects of fertilization with liquid extracts of biogas residues on the growth and forage yield of alfalfa (*Medicago sativa* L.) under arid zone conditions. *Pakistan Journal of Botany* 46, 471–475.
- Alt, D., Peters, I., Gaus, S., 1994. Die CaCl<sub>2</sub>-Methode zur Untersuchung gärtnerischer Erden auf Mengen und Spurenelemente. *Agrobiological Research* 47, 75–83.
- Alvarez, J.A., Otero, L., Lema, J.M., Omil, F., 2010. The effect and fate of antibiotics during the anaerobic digestion of pig manure. *Bioresource Technology* 101, 8581–8586.
- Amon, B., Kryvoruchko, V., Amon, T., Zechmeister-Boltenstern, S., 2006. Methane, nitrous oxide and ammonia emissions during storage and after application of dairy cattle slurry and influence of slurry management. *Agriculture, Ecosystems and Environment* 112, 153–162.
- Anderson, J.P.E., Domsch, K.H., 1978. A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biology and Biochemistry* 10, 215–221.
- Appels, L., Baeyens, J., Degreëve, J., Dewil, R., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. *Progress in Energy and Combustion Science* 34, 755–781.
- Arthurson, V., 2009. Closing the global energy and nutrient cycles through application of biogas residue to agricultural land – potential benefits and drawbacks. *Energies* 2, 226–242.
- Ascher, J., Ceccherini, M.T., Pantani, O.L., Agnelli, A., Borgogni, F., Guerri, G., Nannipieri, P., Pietramellara, G., 2009. Sequential extraction and genetic fingerprinting of a forest soil metagenome. *Applied Soil Ecology* 42, 176–181.
- Bachmann, S., Wentzel, S., Eichler-Löbermann, B., 2011. Codigested dairy slurry as a phosphorus and nitrogen source for *Zea mays* L. and *Amaranthus cruentus* L. *Journal of Plant Nutrition and Soil Science* 174, 908–915.
- Bachmann, S., Gropp, M., Eichler-Löbermann, B., 2014. Phosphorus availability and soil microbial activity in a 3 year field experiment amended with digested dairy slurry. *Biomass and Bioenergy* 70, 429–439.
- Bagge, E., Sahlström, L., Albiñ, A., 2005. The effect of hygienic treatment on the microbial flora of biowaste at biogas plants. *Water Research* 39, 4879–4886.
- Bagge, E., 2009. Hygiene Aspects of the Biogas Process with Emphasis on Spore-forming Bacteria. PhD thesis, Uppsala.
- Bardgett, R.D., Wardle, D.A., 2010. Aboveground–Belowground Linkages: Biotic Interactions, Ecosystems Processes, and Global Change. Oxford University Press, Oxford, UK.
- Barret, M., Delgadillo-Mirquez, L., Trably, E., Delgenes, N., Braun, F., Cea-Barcia, G., Steyer, J.P., Patureau, D., 2012. Anaerobic removal of trace organic contaminants in sewage sludge: 15 years of experience. *Pedosphere* 22, 508–517.
- Bassil, R.J., Bashour, I.I., Sleiman, F.T., Abou-Jawdeh, Y.A., 2013. Antibiotic uptake by plants from manure-amended soils. *Journal of Environmental Science and Health B* 48, 570–574.
- Bertin, L., Capodicasa, S., Occulti, F., Girotti, S., Marchetti, L., Fava, F., 2007. Microbial processes associated to the decontamination and detoxification of a polluted activated sludge during its anaerobic stabilization. *Water Research* 41, 2407–2416.
- Bertora, C., Alluvione, F., Zavattaro, L., van Groenigen, J.W., Velthof, G., Grignani, C., 2008. Pig slurry treatment modifies slurry composition, N<sub>2</sub>O, and CO<sub>2</sub> emissions after soil incorporation. *Soil Biology & Biochemistry* 40, 1999–2006.
- Blagodatsky, S.A., Heinemeyer, O., Richter, J., 2000. Estimating the active and total soil microbial biomass by kinetic respiration analysis. *Biology and Fertility of Soils* 32, 73–81.
- Blake, G.R., 1965. Bulk density: core method. In: Black, C.A. (Ed.), *Methods of Soil Analysis, Part I, Agronomy*, vol. 9. American Society of Agronomy, Madison WI, pp. 375–377.
- Borges de Oliveira, S.V., Leoneti, A.B., Magrini Caldo, G.M., Borges de Oliveira, M.M., 2011. Generation of bioenergy and biofertilizer on a sustainable rural property. *Biomass and Bioenergy* 35, 2608–2618.
- Bougnom, B.P., Niederkofler, C., Knapp, B.A., Stimpfl, E., Insam, H., 2012. Residues from renewable energy production: their value for fertilizing pastures. *Biomass and Bioenergy* 39, 290–295.
- Boxall, A.B.A., Sinclair, C.J., Fenner, K., Kolpin, D., Maund, S.J., 2004. When synthetic chemicals degrade in the environment. *Environmental Science and Technology* 38, 368–375.
- Brändli, R.C., Bucheli, T.D., Kupper, T., Mayer, J., Stadelmann, F.X., Tarradellas, J., 2007. Fate of PCBs, PAHs and their source characteristic ratios during composting and digestion of source-separated organic waste in full-scale plants. *Environmental Pollution* 148, 520–528.
- Bremner, J.M., Mulvaney, C.S., 1982. Nitrogen-total. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), *Methods of Soil Analysis Part 2*. American Society of Agronomy, Madison, pp. 595–624.
- Bujoczek, G., Oleszkiewicz, J., Sparling, R., Cenkowski, S., 2000. High solid anaerobic digestion of chicken manure. *Journal of Agricultural Engineering Research* 76, 51–60.
- Cayuela, M.L., Sinicco, T., Mondini, C., 2009. Mineralization dynamics and biochemical properties during initial decomposition of plant and animal residues in soil. *Applied Soil Ecology* 41, 118–127.
- Cayuela, M.L., Oenema, O., Kuikman, P.J., Bakker, R.R., van Groenigen, J.W., 2010. Bioenergy by-products as soil amendments? Implications for carbon sequestration and greenhouse gas emissions. *GCB Bioenergy* 2, 201–213.
- Cesaro, A., Velten, S., Belgioirio, V., Kuchta, K., 2014. Enhanced anaerobic digestion by ultrasonic pretreatment of organic residues for energy production. *Journal of Cleaner Production* 74, 119–124.
- Chadwick, D., 2007. Building the market for digestate as a fertilizer. In: Session 4 of Biogas Stakeholders Workshop. Exeter University, UK. DEFRA (with contributions from Ken Smith, ADAS).
- Chadwick, D., Sommer, S., Thornman, R., Fanguiero, D., Cardenas, L., Amon, B., Misselbrook, T., 2011. Manure management: implications for greenhouse gas emissions. *Animal Feed Science and Technology* 166–167, 514–531.
- Chae, K.J., Jang, A., Yim, S.K., Kim, I.S., 2008. The effects of digestion temperature and temperature shock on the biogas yields from the mesophilic anaerobic digestion of swine manure. *Bioresource Technology* 99, 1–6.
- Chantigny, M.H., Angers, D.A., Rochette, P., Bélanger, G., Massé, D., Côté, D., 2007. Gaseous nitrogen emissions and forage nitrogen uptake on soils fertilized with raw and treated swine manure. *Journal of Environmental Quality* 36, 1864–1872.
- Chauret, C., Springthorpe, S., Sattar, S., 1999. Fate of *Cryptosporidium* oocysts, *Giardia* cysts, and microbial indicators during waste- water treatment and anaerobic sludge digestion. *Canadian Journal of Microbiology* 45, 257–262.
- Chen, Y., Cheng, J.J., Creamer, K.S., 2008. Inhibition of anaerobic digestion process: a review. *Bioresource Technology* 99, 4044–4064.
- Chen, R., Blagodatskaya, E., Senbayram, M., Blagodatsky, S., Myachina, O., Dittert, K., Kuzyakov, K., 2012. Decomposition of biogas residues in soil and their effects on microbial growth kinetics and enzyme activities. *Biomass and Bioenergy* 45, 221–229.
- Clemens, J., Huschka, A., 2001. The effect of biological oxygen demand of cattle slurry and soil moisture on nitrous oxide emissions. *Nutrient Cycling in Agroecosystems* 59, 193–198.
- Crane, S.R., Moore, J.A., 1986. Modelling enteric bacterial die-off—a review. *Water, Air and Soil Pollution* 27, 411–439.
- Czerniawska-Kusza, I., Ciesielczuk, T., Kusza, G., Cichoń, A., 2006. Comparison of the phytotoxicity microbioassay and chemical variables for toxicity evaluation of sediments. *Environmental Toxicology* 21, 367–372.
- Dawson, C.J., Hilton, J., 2011. Fertilizer availability in a resource limited world: production and recycling of nitrogen and phosphorus. *Food Policy* 36, 14–22.
- Debosz, K., Petersen, S.O., Kure, L.K., Ambus, P., 2002. Evaluating effects of sewage sludge and household compost on soil physical, chemical and microbiological properties. *Applied Soil Ecology* 19, 237–248.
- de la Fuente, C., Alburquerque, J.A., Clemente, R., Bernal, M.P., 2013. Soil C and N mineralisation and agricultural value of the products of an anaerobic digestion system. *Biology and Fertility of Soils* 49, 313–322.
- del Pino, A., Casanova, O., Barbazán, M., Mancassola, V., Arló, L., Borzacconi, L., Passaggi, M., 2014. Agronomic use of slurry from anaerobic digestion of agro-industrial residues: effects on crop and soil. *Journal of Sustainable Bioenergy Systems* 4, 87–96.
- Demetz, M., Insam, H., 1999. Phosphorus availability in a forest soil determined with a respiratory assay compared to chemical methods. *Geoderma* 89, 259–271.
- Demirel, B., Scherer, P., 2011. Trace element requirements of agricultural biogas digesters during biological conversion of renewable biomass to methane. *Biomass and Bioenergy* 35, 992–998.



- De Neve, S., Sleutel, S., Hofman, G., 2003. Carbon mineralization from composts and food industry wastes added to soil. *Nutrient Cycling in Agroecosystems* 67, 13–20.
- Di, H.J., Cameron, K.C., Shen, J.-P., Winefield, C.S., O'Callaghan, M., Bowatte, S., He, J.-Z., 2010. Ammonia-oxidizing bacteria and archaea grow under contrasting soil nitrogen conditions. *FEMS Microbiology Ecology* 72, 386–394.
- Dick, R.P., 1992. A review: long-term effects of agricultural systems on soil biochemical and microbial parameters. *Agriculture, Ecosystems and Environment* 40, 25–36.
- Dijkstra, F.A., West, J.B., Hobbie, S.E., Reich, P.B., Trost, J., 2007. Plant diversity, CO<sub>2</sub>, and N influence inorganic and organic N leaching in grasslands. *Ecology* 88, 490–500.
- Di Salvatore, M., Carafa, A.M., Carratù, G., 2008. Assessment of heavy metals phytotoxicity using seed germination and root elongation tests: a comparison of two growth substrates. *Chemosphere* 73, 1461–1464.
- Dohrmann, A.J., Baumert, S., Klingebiel, L., Weiland, P., Tebbe, C.C., 2011. Bacterial community structure in experimental methanogenic bioreactors and search for pathogenic clostridia as community members. *Applied Microbiology Biotechnology* 89, 1991–2004.
- Doi, T., Matsumoto, H., Abe, J., Morita, S., 2010. Application of rice rhizosphere microflora for hydrogen production from apple pomace. *International Journal of Hydrogen Energy* 35, 7369–7376.
- Domene, X., Alcaniz, J.M., Andrés, P., 2007. Ecotoxicological assessment of organic wastes using the soil collembolan *Folsomia candida*. *Applied Soil Ecology* 35, 461–472.
- Domene, X., Ramírez, W., Mattana, S., Alcaniz, J.M., Andrés, P., 2008. Ecological risk assessment of organic waste amendments using the species sensitivity distribution from a soil organisms test battery. *Environmental Pollution* 155, 227–236.
- Dosch, P., Gutscher, R., 1996. Reducing N losses (NH<sub>3</sub>, N<sub>2</sub>O, N<sub>2</sub>) and immobilization from slurry through optimized application techniques. *Fertilizer Research* 43, 165–171.
- Drury, C.F., Oloya, T.O., McKenney, D.J., Gregorich, E.G., Tan, C.S., van Luyk, C.L., 1998. Long-term effects of fertilization and rotation on denitrification and soil carbon. *Soil Science Society of America Journal* 62, 1572–1579.
- Egnér, H., Riehm, H., Domingo, W.R., 1960. Untersuchungen über die chemische Bodenanalyse als Grundlage der Beurteilung des Nährstoffzustandes der Böden. II. Chemische Extraktionsmethoden zur Phosphor- und Kaliumbestimmung. Statens Jordbruksforsk, Särtryck och Småskrifter nr. 133, Uppsala.
- Elfstrand, S., Båth, B., Mårtensson, A., 2007. Influence of various forms of green manure amendment on soil microbial community composition, enzyme activity and nutrient levels in leek. *Applied Soil Ecology* 36, 70–82.
- Elliott, E.T., 1986. Aggregate structure and carbon, nitrogen and phosphorus in native and cultivated soils. *Soil Science Society American Journal* 50, 627–633.
- Engeli, H., Edelmann, W.F.J., Rottermann, K., 1993. Survival of plant – pathogens and weed seeds during anaerobic-digestion. *Water Science and Technology* 27, 69–76.
- Engwall, M., Schnürer, A., 2002. Fate of Ah-receptor agonists in organic household waste during anaerobic degradation—estimation of levels using EROD induction in organ cultures of chick embryo livers. *Science of the Total Environment* 297, 105–108.
- Ernst, G., Müller, A., Göhler, H., Emmerling, C., 2008. C and N turnover of fermented residues from biogas plants in soil in the presence of three different earthworm species (*Lumbricus terrestris*, *Aporrectodea longa*, *Aporrectodea caliginosa*). *Soil Biology & Biochemistry* 40, 1413–1420.
- EU, 2002. European Regulation (EC) No 1774/2002. Laying Down Health Rules Concerning Animal By-products Not Intended for Human Consumption. European Parliament and Council, Brussels, Belgium.
- EU, 2009. European Regulation (EC) No 1069/2009. Laying Down Health Rules as Regards Animal By-products and Derived Products Not Intended for Human Consumption and Repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation). European Parliament and Council, Brussels, Belgium.
- Fan, W.H., Liu, J.F., Wang, Z.W., Shan, J.Y., 2012. Effects of biogas slurry fertilizer on soil microorganisms and enzyme activity in greenhouse. In: Proceedings of the 12th Conference of China Soil Science Society of China, Sichuan, China, pp. 768–774.
- Fernández-Delgado Juárez, M., Waldhuber, S., Knapp, A., Partl, C., Gómez-Brandón, M., Insam, H., 2013. Wood ash effects on chemical and microbiological properties of digestate- and manure-amended soils. *Biology and Fertility of Soils* 49, 575–585.
- Ferrer, I., Ponsa, S., Vazquez, F., Font, X., 2008. Increasing biogas production by thermal (70 °C) sludge pre-treatment prior to thermophilic anaerobic digestion. *Biochemical Engineering Journal* 42, 186–192.
- Fornasier, F., Ascher, J., Ceccherini, M.T., Tomat, E., Pietramellara, G., 2014. A simplified, low-cost and versatile DNA-based assessment of soil microbial biomass. *Ecological Indicators* 45, 75–82.
- Frąc, M., Oszust, K., Lipiec, J., 2012. Community level physiological profiles (CLPP), characterization and microbial activity of soil amended with dairy sewage sludge. *Sensors* 12, 3253–3268.
- Franke-Whittle, I.H., Goberna, M., Pfister, V., Insam, H., 2009. Design and development of the ANAEROCHIP microarray for investigation of methanogenic communities. *Journal of Microbiological Methods* 79, 279–288.
- Franke-Whittle, I.H., Insam, H., 2013. Treatment alternatives of slaughterhouse wastes, and their effect on the inactivation of different pathogens: a review. *Critical Reviews in Microbiology* 39, 139–151.
- Franke-Whittle, I.H., Walter, A., Ebner, C., Insam, H., 2014. Investigation into the effect of high concentrations of volatile fatty acids in anaerobic digestion on methanogenic communities. *Waste Management* 34, 2080–2089.
- Friedel, J.K., Bezler, A., Fischer, W.R., 1996. Stickstoffmineralisierung von verflüssigtem, anaerob fermentiertem Rindermist im Boden. *Agrobiological Research* 49, 1–9.
- Fründ, H.C., Butt, K., Capowiez, Y., Eisenhauer, N., Emmerling, C., Ernst, G., Potthoff, M., Schädler, M., Schrader, S., 2010. Using earthworms as model organisms in the laboratory: recommendations for experimental implementations. *Pedobiologia* 53, 119–125.
- Fuchs, J.G., Berner, A., Mayer, J., Smidt, E., Schleiss, K., 2008. Influence of compost and digestates on plant growth and health: potentials and limits. In: *Compost and Digestate: Sustainability, Benefits, Impacts for the Environment and for Plant Production*, Solothurn, Switzerland.
- Garland, J.L., Mills, A.L., 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole carbon source utilization. *Applied and Environmental Microbiology* 57, 2351–2359.
- Gasser, M.O., Chantigny, M.H., Angers, D.A., Bittman, S., Buckley, K.E., Rochette, P., Massé, D., 2012. Plant-available and water-soluble phosphorus in soils amended with separated manure solids. *Journal of Environmental Quality* 41, 1290–1300.
- Ge, G., Li, Z., Fan, F., Chu, G., Hou, Z., Liang, Y., 2010. Soil biological activity and their seasonal variations in response to long-term application of organic and inorganic fertilizers. *Plant and Soil* 326, 31–44.
- Gell, K., van Groenigen, J., Cayuela, M.L., 2011. Residues of bioenergy production chains as soil amendments: immediate and temporal phytotoxicity. *Journal of Hazardous Materials* 186, 2017–2025.
- German National Academy of Sciences Leopoldina, 2012. Bioenergy – Chances and Limits. Halle (Saale).
- Gianfreda, L., Rao, A.A., 2014. *Enzymes in Agricultural Sciences*. Published by OMICS Group eBooks, Gull Ave, Foster City, CA 94404, USA.
- Goberna, M., Insam, H., Franke-Whittle, I.H., 2009. Effect of biowaste sludge maturation on the diversity of thermophilic bacteria and archaea in an anaerobic reactor. *Applied and Environmental Microbiology* 75, 2566–2572.
- Goberna, M., Gadermaier, M., García, C., Wett, B., Insam, H., 2010. Adaptation of methanogenic communities to the cofermentation of cattle excreta and olive mill wastes at 37 °C and 55 °C. *Applied and Environmental Microbiology* 76, 6564–6571.
- Goberna, M., Podmirseg, S.M., Waldhuber, S., Knapp, B.A., García, C., Insam, H., 2011. Pathogenic bacteria and mineral N in soils following the land spreading of biogas digestates and fresh manure. *Applied Soil Ecology* 49, 18–25.
- González-Fernández, C., García-Encina, P.A., 2009. Impact of substrate to inoculum ratio anaerobic digestion of swine slurry. *Biomass and Bioenergy* 33, 1065–1069.
- Grigatti, M., Di Girolamo, G., Chincari, R., Ciavatta, C., Barbanti, L., 2011. Potential nitrogen mineralization, plant utilization efficiency and soil CO<sub>2</sub> emissions following the addition of anaerobic digested slurries. *Biomass and Bioenergy* 35, 4619–4629.
- Guanaseelan, V.N., 1997. Anaerobic digestion of biomass for methane production: a review. *Biomass and Bioenergy* 13, 83–114.
- Gurung, J.B., June 1997. Review of Literature on Effect of Slurry Use on Crop Production. In: *Biogas Support Program*. Nepal.
- Hansen, K.H., Angelidaki, I., Ahring, B.K., 1997. Anaerobic digestion of swine manure: inhibition by ammonia. *Water Research* 32, 5–12.
- Hansen, K.H., Angelidaki, I., Ahring, B.K., 1999. Improving thermophilic anaerobic digestion of swine manure. *Water Research* 33, 1805–1810.
- Hartmann, H., Ahring, B.K., 2003. Phthalic acid esters found in municipal organic waste: enhanced anaerobic degradation under hyper-thermophilic conditions. *Water Science and Technology* 48, 175–183.
- Hass, D., Défago, G., 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature Reviews Microbiology* 3, 307–319.
- He, Z., Gentry, T.J., Schadt, C.W., Wu, L., Liebich, J., Chong, S.C., Huang, Z., Wu, W., Gu, B., Jardine, P., Criddle, C., Zhou, J., 2007. GeoChip: a comprehensive microarray for investigating biogeochemical, ecological and environmental processes. *The ISME Journal* 1, 67–77.
- Heller, H., Bar-Tal, A., Tamir, G., Bloom, P., Venterea, R.T., Chen, D., Zhang, Y., Clapp, C.E., Fine, P., 2010. Effects of manure and cultivation on carbon dioxide and nitrous oxide emissions from a corn field under mediterranean conditions. *Journal of Environmental Quality* 39, 437–448.
- Hernandez, D.L., Hobbie, S.E., 2010. The effects of substrate composition, quantity, and diversity on microbial activity. *Plant and Soil* 335, 397–411.
- Horswell, J., Prosser, J.A., Siggins, A., van Schaik, A., Ying, L., Ross, C., McGill, A., Northcott, G., 2014. Assessing the impacts of chemical cocktails on the soil ecosystem. *Soil Biology & Biochemistry* 75, 64–72.
- Hossain, N., Islam, M., Alamgir, M., Kibria, M.G., 2014. Growth response of Indian spinach to biogas plant residues. *IOSR Journal of Pharmacy and Biological Sciences* 9, 1–6.
- Hupfaut, S., Bachmann, S., Fernández-Delgado Juárez, M., Insam, H., Eichler-Löbermann, B., 2015. Digestate application strategies affect soil P availability and microbial community composition. *Science of the Total Environment* (submitted in publication).



- Insam, H., Palojärvi, A., 1995. Effects of forest fertilization on nitrogen leaching and soil microbial properties in the Northern Calcareous Alps of Austria. *Plant and Soil* 168–169, 75–81.
- Insam, H., Rangger, A., 1997. *Microbial Communities*. Springer, Berlin, 261 pp.
- Insam, H., Merschak, P., 1997. Nitrogen leaching from forest soil cores after amending organic recycling products and fertilizers. *Waste Management and Research* 15, 277–291.
- Insam, H., Wett, B., 2008. Control of GHG emission at the microbial community level. *Waste Management* 28, 699–706.
- Insam, H., Franke-Whittle, I.H., Knapp, B.A., Plank, R., 2009. Use of wood ash and anaerobic sludge for grassland fertilization: effects on plants and microbes. *Die Bodenkultur* 60, 39–51.
- Insam, H., Seewald, M., 2010. Volatile organic compounds (VOCs) in soil. *Biology and Fertility of Soils* 46, 199–213.
- Insam, H., Franke-Whittle, I.H., Podmirseg, S.M., 2014. Agricultural waste management in Europe, with an emphasis on anaerobic digestion. *Journal of Integrated Field Science* 11, 13–17.
- IPCC, 2007. In: Metz, B., Davidson, O.R., Bosch, P.R., Dave, R., Meyer, L.A. (Eds.), *Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Jenkinson, D.S., Powelson, D.S., 1976. The effects of biocidal treatments on metabolism in soil – V. A method for measuring soil biomass. *Soil Biology & Biochemistry* 8, 209–213.
- Jieqiong, M., Hongguang, Z., Min, F., 2013. Distribution of heavy metals in pig farm biogas residues and the safety and feasibility assessment of biogas fertilizer. *International Journal of Agricultural and Biological Engineering* 6, 35–43.
- Johansen, A., Hauggaard-Nielsen, H., Ambus, P., Carter, M.S., August 2009. Recycling of Bioenergy Waste-stream Materials to Soil in Organic Farming Systems, pp. 25–27. Poster at: NJF Seminar 422, Tartu in Estonia. <http://orgprints.org/16281/1/16281.pdf>.
- Johansen, A., Carter, M.S., Jensen, E.S., Hauggaard-Nielsen, H., Ambus, P., 2013. Effects of digestate from anaerobically digested cattle slurry and plant materials on soil microbial community and emission of CO<sub>2</sub> and N<sub>2</sub>O. *Applied Soil Ecology* 63, 36–44.
- Joo, H.S., Ndegwa, P.M., Harrison, J.H., Whitefield, E., Fei, S., Wang, X., Neerackal, G., Heber, A.J., Ni, J.Q., 2013. Potential air quality impacts of anaerobic digestion of dairy manure. In: *From Waste to Worth Conference*, Denver, Colorado.
- Jothi, G., Pugalendhi, S., Poornima, K., Rajendran, G., 2003. Management of root-knot nematode in tomato *Lycopersicon esculentum*, Mill., with biogas slurry. *Bioresource Technology* 89, 169–170.
- Jung, G., 1987. Mechanical analysis – a description of a rational method for determination of soil type, Report 87:2. The Section for Hydrotechnique, Department of Soil Science, The Swedish University for Agricultural Sciences, Uppsala.
- Köster, J.R., Dittert, K., Mühling, K.-H., Kage, H., Pacholski, A., 2014. Cold season ammonia emissions from land spreading with anaerobic digestates from biogas production. *Atmospheric Environment* 84, 35–38.
- Kupper, K.C., Bettiol, W., de Goes, A., de Souza, P.S., Bellotte, J.A.M., 2006. Bio-fertilizer for control of *Guignardia citricarpa*, the causal agent of citrus black spot. *Crop Protection* 25, 569–573.
- Lallai, A., Mura, G., Omnis, N., 2002. The effects of certain antibiotics on biogas production in the anaerobic digestion of pig waste slurry. *Bioresource Technology* 82, 205–208.
- Larsen, T., Luxhøi, J., Magid, J., Jensen, L.S., Krogh, P.H., 2007. Properties of anaerobically digested and composted municipal solid waste assessed by linking soil mesofauna dynamics and nitrogen modelling. *Biology and Fertility of Soils* 44, 59–68.
- Lee, S., Hung, R., Schink, A., Mauro, J., Bennett, J.W., 2014. *Arabidopsis thaliana* for testing the phytotoxicity of volatile organic compounds. *Plant Growth Regulation* 74, 177–186.
- Leip, A., Weiss, F., Wassenaar, T., Perez, I., Fellmann, T., Loudjani, P., Tubiello, F., Grandgirard, D., Monni, S., Biala, K., 2010. Evaluation of the Livestock Sector's Contribution to the EU Greenhouse Gas Emissions (GGELS) – Final Report. European Commission, Joint Research Centre.
- Levén, L., Schnürer, A., 2005. Effects of temperature on biological degradation of phenols, benzoates and phthalates under methanogenic conditions. *International Biodeterioration and Biodegradation* 55, 153–160.
- Levén, L., Nyberg, K., Korkea-Aho, L., Schnürer, A., 2006. Phenols in anaerobic digestion processes and inhibition of ammonia oxidising bacteria (AOB) in soil. *Science of the Total Environment* 364, 229–238.
- Levén, L., Nyberg, K., Schnürer, A., 2012. Conversion of phenols during anaerobic digestion of organic solid waste – a review of important microorganisms and impact of temperature. *Journal of Environmental Management* 95, 99–103.
- Loes, A.-K., Johansen, A., Pommeresche, R., Riley, H., 2010. Anaerobic Digestion of Manure – Consequences for Plant Production. <http://orgprints.org/24552/2/24552p.pdf> (accessed 29.12.14.).
- Long, A., Heitman, J., Tobias, C., Phillips, R., Song, B., 2013. Co-occurring anammox, denitrification, and codenitrification in agricultural soils. *Applied and Environmental Microbiology* 79, 168–176.
- Long, P., Siu, P., Gao, W.-S., Wang, B.-B., Huang, J.-X., Yan, P., Zou, J.-X., Yan, L.-L., Chen, Y.-Q., 2014. Aggregate stability and associated C and N in a silty loam soil as affected by organic material inputs. *Journal of Integrative Agriculture Advance Online Publication*. [http://dx.doi.org/10.1016/S2095-3119\(14\)60796-6](http://dx.doi.org/10.1016/S2095-3119(14)60796-6).
- Loria, E.R., Sawyer, J.E., Barker, D.W., Lundvall, J.P., Lorimor, J.C., 2007. Use of anaerobically digested swine manure as a nitrogen source in corn production. *Agronomy Journal* 99, 1119–1129.
- Lucid, J., Fenton, O., Grant, J., Healy, M., 2014. Effect of rainfall time interval on runoff losses of biosolids and meat and bone meal when applied to a grassland soil. *Water, Air and Soil Pollution* 225, 1–11.
- Makádi, M., Tomócsik, A., Orosz, V., 2012. Digestate: a new nutrient source – review. In: Kumar, S. (Ed.), *Biogas*. Intech, pp. 295–309.
- Manning, P., 2013. The impact of nitrogen environment on ecosystems and their services. In: Wall, D.H. (Ed.), *Soil Ecology and Ecosystem Services*. Oxford University Press, Oxford, UK, pp. 256–269.
- Marcato, C.E., Mohtar, R., Revel, J.-C., Pouech, P., Hafidi, M., Guirese, M., 2009. Impact of anaerobic digestion on organic matter quality in pig slurry. *International Biodeterioration and Biodegradation* 63, 260–266.
- Massé, D.I., Lu, D., Masse, L., Droste, R.L., 2000. Effect of antibiotics on psychrophilic anaerobic digestion of swine manure slurry in sequencing batch reactors. *Bioresource Technology* 75, 205–211.
- Massé, D.I., Croteau, F., Masse, L., 2007. The fate of crop nutrients during digestion of swine manure in psychrophilic anaerobic sequencing batch reactors. *Bioresource Technology* 98, 2819–2823.
- Massé, D.I., Talbot, G., Gilbert, Y., 2011. On farm biogas production: a method to reduce GHG emissions and develop more sustainable livestock operations. *Animal Feed Science and Technology* 166–167, 436–445.
- Massé, D.I., Saady, N.M.C., Gilbert, Y., 2014. Potential of biological processes to eliminate antibiotics in livestock manure: an overview. *Animals* 4, 146–163.
- Mawdsley, J.L., Bardgett, R.D., Merry, R.J., Pain, B.F., Theodorou, M.K., 1995. Pathogens in livestock waste, their potential for movement through soil and environmental pollution. *Applied Soil Ecology* 2, 1–15.
- Mokry, M., Bolduan, R., Michels, K., Wagner, W., 2008. Fertilization of Arable Crops with Digestates of Agricultural Biogas Plants. Poster by staff at Landwirtschaftliches Technologiezentrum, Karlsruhe. [www.ltz-augustenbergl.de](http://www.ltz-augustenbergl.de).
- Möller, K., Stinner, W., Deuker, A., Leithold, G., 2008. Effects of different manuring systems with and without biogas digestion on nitrogen cycle and crop yield in mixed organic dairy farming systems. *Nutrient Cycling in Agroecosystems* 82, 209–232.
- Möller, K., Stinner, W., 2009. Effects of different manuring systems with and without biogas digestion on soil mineral nitrogen content and on gaseous nitrogen losses (ammonia, nitrous oxides). *European Journal of Agronomy* 30, 1–16.
- Möller, K., Müller, T., 2012. Effects of anaerobic digestion on digestate nutrient availability and crop growth: a review. *Engineering in Life Sciences* 12, 242–257.
- Moreira Vilar, M.T., 2014. Environmental impacts of biogas production systems. In: Gübitz, G., Gronauer, A., Bauer, A., Bochmann, G., Scheidl, S., Weiß, S. (Eds.), *Conference Proceedings of Biogas Science 2014*, Vienna, p. 82.
- Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. *European Journal of Soil Science* 54, 655–670.
- Nannipieri, P., Pietramellara, G., Renella, G., 2014. In: Nannipieri, P., Pietramellara, G., Renella, G. (Eds.), *Omics in Soil Science*. Caister Academic Press, ISBN-13: 978-1908230324. ISBN-10: 1908230320.
- Nilsson, M.-L., 2000. Occurrence and Fate of Organic Contaminants in Wastes. PhD thesis, Uppsala.
- Nilsson, M.-L., Waldebeck, M., Liljegen, G., Kylan, H., Markides, K.E., 2001. Pressurized-fluid extraction (PFE) of chlorinated paraffins from the biodegradable fraction of source-separated household waste. *Fresenius Journal of Analytical Chemistry* 370, 913–918.
- Nkoa, R., 2014. Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review. *Agronomy for Sustainable Development* 34, 473–492.
- Nömmik, H., 1974. Ammonium chloride-imidazole extraction procedure for determining titratable acidity, exchangeable base cations, and cation exchange capacity in soils. *Soil Science* 118, 254–262.
- Nyberg, K., Sundh, I., Johansson, M., Schnürer, A., 2004. Presence of potential ammonia oxidation (PAO) inhibiting substances in anaerobic digestion residues. *Applied Soil Ecology* 26, 107–112.
- Odlare, M., Pell, M., Svensson, K., 2008. Changes in soil chemical and microbiological properties during 4 years of application of various organic residues. *Waste Management* 28, 1246–1253.
- Odlare, M., Arthursen, V., Pell, M., Svensson, K., Nehrenheim, E., Abubaker, J., 2011. Land application of organic waste-Effects on the soil ecosystem. *Applied Energy* 88, 2210–2218.
- Odlare, M., Abubaker, J., Lindmark, J., Pell, M., Thorin, E., Nehrenheim, E., 2012. Emissions of N<sub>2</sub>O and CH<sub>4</sub> from agricultural soils amended with two types of biogas residues. *Biomass and Bioenergy* 44, 112–116.
- Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *USDA Circular* 939.
- Olsen, J.E., Larsen, H.E., 1987. Bacterial decomposition times in anaerobic digestions of animal slurries. *Biological Wastes* 21, 153–160.
- Olsman, H., Björnfoth, H., van Bavel, B., Lindström, G., Schnürer, A., Engwall, M., 2002. Characterisation of dioxin-like compounds in anaerobically digested organic material by bioassay-directed fractionation. *Organohalogen Compounds* 58, 345–348.
- Olsman, H., Engwall, M., Kammann, U., Klempt, M., Otte, J., van Bavel, B., Hollert, H., 2007. Relative differences in aryl hydrocarbon receptor-mediated response for 18 polybrominated and mixed halogenated dibenzo-*p*-dioxins and -furans in

- cell lines from four different species. *Environmental Toxicology and Chemistry* 26, 2448–2454.
- Ottosson, J.R., Schnürer, A., Vinnerås, B., 2008. In situ ammonia production as a sanitation agent during anaerobic digestion at mesophilic temperature. *Letters in Applied Microbiology* 46, 325–330.
- Panikov, N.S., Sizova, M.V., 1996. A kinetic method for estimating the biomass of microbial functional groups in soil. *Journal of Microbiological Methods* 24, 219–230.
- Pepper, I.L., Brooks, J.P., Gerba, C.P., 2006. Pathogens in biosolids. *Advances in Agronomy* 90, 1–41.
- Petersen, S.O., Henriksen, K., Mortensen, G.K., Krogh, P.H., Brandt, K.K., Sørensen, J., Madsen, T., Petersen, J., Gron, C., 2003. Recycling of sewage sludge and household compost to arable land: fate and effects of organic contaminants, and impact on soil fertility. *Soil and Tillage Research* 72, 139–152.
- Podmirseg, S.M., Schoen, M.A., Murthy, S., Insam, H., Wett, B., 2010. Quantitative and qualitative effects of bioaugmentation on ammonia oxidizers at a two-step WWTP. *Water Science and Technology* 61, 1003–1009.
- Pourcher, A.M., Françoise, P.B., Virginie, F., Agnieszka, G., Vasilica, S., Gérard, M., 2007. Survival of faecal indicators and enteroviruses in soil after land-spreading of municipal sewage sludge. *Applied Soil Ecology* 35, 473–479.
- Quakernack, R., Pacholski, A., Techow, A., Herrmann, A., Taube, F., Kage, H., 2012. Ammonia volatilization and yield response of energy crops after fertilization with biogas residues in a coastal marsh of Northern Germany. *Agriculture, Ecosystems and Environment* 160, 66–74.
- Rigby, H., Smith, S.R., 2013. Nitrogen availability and indirect measurements of greenhouse gas emissions from aerobic and anaerobic biowaste digestates applied to agricultural soils. *Waste Management* 33, 2641–2652.
- Rincón, B., Borja, R., González, J.M., Portillo, M.C., Sáiz-Jiménez, C., 2008. Influence of organic loading rate and hydraulic retention time on the performance, stability and microbial communities of one-stage anaerobic digestion of two-phase olive mill solid residue. *Biochemical Engineering Journal* 40, 253–261.
- Ros, M., Goberna, M., Pascual, J.A., Klammer, S., Insam, H., 2008. 16S rDNA analysis reveals low diversity in microorganisms discriminating community level physiological profiles. *Journal of Microbiological Methods* 72, 221–226.
- Rousk, J., Bååth, E., 2011. Growth of saprotrophic fungi and bacteria in soil. *FEMS Microbiology Ecology* 78, 17–30.
- Sahlström, L., 2003. A review of survival of pathogenic bacteria in organic waste used in biogas plants. *Bioresource Technology* 87, 161–166.
- Sahlström, L., Aspán, A., Bagge, E., Danielsson-Tham, M.-L., Albiñ, A., 2004. Sewage sludge hygiene in Swedish sewage treatment plants. *Water Research* 38, 1989–1994.
- Sänger, A., Geisseler, D., Ludwig, B., 2011. Effects of moisture and temperature on greenhouse gas emissions and C and N leaching losses in soil treated with biogas slurry. *Biology and Fertility of Soils* 47, 249–259.
- Saunders, O., Harrison, J., Fortuna, A.M., Whitefield, E., Bary, A., 2012. Effect of anaerobic digestion and application method on the presence and survivability of *E. coli* and fecal coliforms in dairy waste applied to soil. *Water, Air and Soil Pollution* 223, 1055–1063.
- Scherr, K.E., Lunda, T., Klose, V., Bochmann, G., Loibner, A.P., 2012. Changes in bacterial communities from anaerobic digesters during petroleum hydrocarbon degradation. *Journal of Biotechnology* 157, 472–564.
- Senbayram, M., Chen, R.R., Muhling, K.H., Dittert, K., 2009. Contribution of nitrification and denitrification to nitrous oxide emissions from soils after application of biogas waste and other fertilizers. *Rapid Communication of Mass Spectrometry* 23, 2489–2498.
- Singla, A., Dubey, S.K., Iwasa, H., Inubushi, K., 2013. Nitrous oxide flux from *Komatsuna (Brassica rapa)* vegetated soil: a comparison between biogas digested liquid and chemical fertilizer. *Biology and Fertility of Soils* 49, 971–976.
- Singla, A., Inubushi, K., 2014. Effect of biogas digested liquid on CH<sub>4</sub> and N<sub>2</sub>O flux in paddy ecosystem. *Journal of Integrative Agriculture* 13, 635–640.
- Smith, J., Abegaz, A., Matthews, R.B., Subedi, M., Orskov, E.R., Tumwesige, V., Smith, P., 2014. What is the potential for biogas digesters to improve soil carbon sequestration in Sub-Saharan Africa? Comparison with other uses of organic residues. *Biomass and Bioenergy* 70, 73–86.
- Snell-Castro, R., Godon, J.-J., Delgenès, J.-P., Dabert, P., 2005. Characterisation of the microbial diversity in a pig manure storage pit using small subunit rDNA sequence analysis. *FEMS Microbiology Ecology* 52, 229–242.
- Sommer, S.G., Petersen, S.O., Møller, H.B., 2004. Algorithms for calculating methane and nitrous oxide emissions from manure management. *Nutrient Cycling in Agroecosystems* 69, 143–154.
- Steinfeld, H., Gerber, P., Wasenauer, T., Castel, V., Rosales, M., de Haan, C., 2006. *Livestock's Long Shadow. Environmental Issues and Options. Environmental Issues and Options. Food and Agriculture Organisation (FAO) of United Nations.*
- Stinner, W., Möller, K., Leithold, G., 2008. Effects of biogas digestion of clover/grass-leys, cover crops and crop residues on nitrogen cycle and crop yield in organic stockless farming systems. *European Journal of Agronomy* 29, 125–134.
- Stumborg, C., Schoenau, J.J., Malhi, S.S., 2007. Nitrogen balance and accumulation pattern in three contrasting prairie soils receiving repeated applications of liquid swine and solid cattle manure. *Nutrient Cycling in Agroecosystems* 78, 15–25.
- Stumpe, B., Werner, S., Jung, R., Heinze, S., Jüschke, S., Strippel, C., Marschner, B., 2012. Organic carbon dynamics and enzyme activities in agricultural soils amended with biogas slurry, liquid manure and sewage sludge. *Agricultural Sciences* 3, 104–113.
- Sullivan, T., Stromberger, M., Paschke, M., Ippolito, J., 2006. Long-term impacts of infrequent biosolids applications on chemical and microbial properties of a semi-arid rangeland soil. *Biology and Fertility of Soils* 42, 258–266.
- Sundberg, C., Yu, D., Franke-Whittle, I., Kauppi, S., Smårs, S., Insam, H., Romantschuk, M., Jönsson, H., 2013. Effects of pH and microbial composition on odour in food waste composting. *Waste Management* 33, 204–211.
- Taberlet, P., Prud'Homme, S.M., Campione, E., Roy, J., Miquel, C., Shehzad, W., Gielly, L., Rioux, D., Choler, P., Clément, J.C., Melodelima, C., Pompanon, F., Coissac, E., 2012. Soil sampling and isolation of extracellular DNA from large amount of starting material suitable for metabarcoding studies. *Molecular Ecology* 21, 1816–1820.
- Tao, G., Wei, F., HuaCen, W., YongHua, W., Yunji, Z., TianCai, G., 2010. Effect of biogas slurry top-dressing on soil microorganisms and enzyme activity in the rhizosphere of winter wheat field. *Journal of Triticeae Crops* 30, 721–726.
- Turoviskiy, I.S., Mathai, P.K., 2006. *Wastewater Sludge Processing*. New York, Wiley.
- Udikovic-Kolic, N., Wichmann, F., Broderick, N.A., Handelsman, J., 2014. Bloom of resident antibiotic-resistant bacteria in soil following manure fertilization. *Proceedings of the National Academy of Sciences of the United States of America* 111, 15202–15207.
- Unc, A., Goss, M.J., 2004. Transport of bacteria from manure and protection of water resources. *Applied Soil Ecology* 25, 1–18.
- Unterfrauner, H., Somitsch, W., Peticzka, R., Brauneis, S., Schlaipfer, M., 2010. Auswirkung von Biogasgülle auf Bodenparameter. 2. In: *Umweltökologisches Symposium Gumpenstein*, pp. 59–64.
- Vallejo, A., Skiba, U.M., García-Torres, L., Arce, A., López-Fernández, S., Sánchez-Martín, L., 2006. Nitrogen oxides emission from soils bearing a potato crop as influenced by fertilization with treated pig slurries and composts. *Soil Biology & Biochemistry* 38, 2782–2793.
- Van der Meer, H., 2007. Optimising manure management for GHG outcomes. In: *Green House Gas and Animal Agriculture Conference (GGAA)*, Christchurch, New Zealand.
- Varel, V.H., Wells, J.E., Shelver, W.L., Rice, C.P., Armstrong, D.L., Parker, D.B., 2012. Effect of anaerobic digestion temperature on odour, coliforms and chlortetracycline in swine manure or monensin in cattle manure. *Journal of Applied Microbiology* 112, 1365–2672.
- Wagner, A.O., Malin, C., Illmer, P., 2009. Application of denaturing high-performance liquid chromatography in microbial ecology: fermentor sludge, compost, and soil community profiling. *Applied and Environmental Microbiology* 75, 956–964.
- Walkley, A., Black, L.A., 1934. An examination of the method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science* 37, 29–38.
- Walsh, J.J., Rousk, J., Edward-Jones, G., Jones, D.L., Williams, A.P., 2012. Fungal and bacterial growth following the application of slurry and anaerobic digestate of livestock manure to temperate pasture soils. *Biology and Fertility Soils* 48, 889–897.
- Walter, A., Knapp, B.A., Farbmacher, T., Ebner, C., Insam, H., Franke-Whittle, I.H., 2012. Searching for links in the biotic characteristics and abiotic parameters of nine different biogas plants. *Microbial Biotechnology* 5, 717–730.
- Wulf, S., Maeting, M., Clemens, J., 2002. Application technique and slurry co-fermentation effects on ammonia, nitrous oxide, and methane emissions after spreading: II. Greenhouse gas emissions. *Journal of Environmental Quality* 31, 1795–1801.
- Yadvika, S., Sreekrishnan, T.R., Kohli, S., Rana, V., 2004. Enhancement of biogas production from solid substrates using different techniques-a review. *Bioresource Technology* 95, 1–10.
- Yang, Y., Li, B., Zou, S., Fang, H.H.P., Zhang, T., 2014. Fate of antibiotic resistance genes in sewage treatment plant revealed by metagenomic approach. *Water Research* 62, 97–106.
- Zaleski, K.J., Josephson, K.L., Gerba, C.P., Pepper, I.L., 2005. Survival, growth, and regrowth of enteric indicator and pathogenic bacteria in biosolids, compost, soil, and land applied biosolids. *Journal of Residuals Science and Technology* 2, 49–63.
- Zhu, G., Jha, A.K., 2013. Psychrophilic dry anaerobic digestion of cow dung for methane production: effect of inoculum. *Science Asia* 39, 500–510.
- Ziemiński, K., Frac, M., 2012. Methane fermentation process as anaerobic digestion of biomass: transformations, stages and microorganisms. *African Journal of Biotechnology* 11, 4127–4139.
- Zirkler, D., Peters, A., Kaupenjohann, M., 2014. Elemental composition of biogas residues: variability and alteration during anaerobic digestion. *Biomass and Bioenergy* 67, 89–98.
- Zongqiang, Z., Guanwen, C., Yinian, Z., Honghu, Z., Rongrong, W., Caichun, W., 2011. The effects of different anaerobic fermentation temperature on biogas fermentation of swine manure. In: *International Conference on Computer Distributed Control and Intelligent Environmental Monitoring*, pp. 1410–1413.
- Zucconi, F., Forte, M., Monaco, A., De Bertoldi, M., 1981. Biological evaluation of compost maturity. *Biocycle* 22, 27–29.