Quality and function of anaerobic digestion residues

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Cover: Digestate spread to growing crop
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Abstract

The growing number of biogas plants in Europe has resulted in increased production of nutrient-rich digestate, which has potential as fertiliser on arable land. Many different organic materials can be degraded in the anaerobic digestion process, with most macronutrients and micronutrients retained in the digestate. Depending on the ingoing organic substrate and management of the biogas process, the nutrient content of digestate varies widely. It can also contain compounds such as heavy metals and organic pollutants that are potentially toxic to soil microorganisms. Previous studies on the effect of digestate on soil microorganisms and crop yield have yielded contradictory results, so further investigations are needed to determine its true fertiliser value. In this thesis, the fertiliser effect of different types of digestate originating from biogas plants operating with various substrates and operating parameters was determined by measuring: 1) general and specific soil microbial activity, 2) bacterial and archaeal community composition, 3) crop growth and 4) chemical and physical composition of different digestates. Soil respiration generally displayed a positive response to digestate addition, but soil respiration curves revealed differences in quality and quantity of organic carbon between digestate, pig slurry and cow manure. However, the total utilisation rate of the organic carbon in digestate, pig slurry and cow manure did not differ. Moreover, digestate showed both stimulatory and inhibitory effects on potential ammonia oxidation, while pig slurry and cow manure had a more consistent inhibitory effect. Addition of digestate to soil resulted in increased wheat yield compared with control soil and mineral fertiliser, but final yield was not as high as that from pig slurry. Digestate was also generally characterised by a higher content of ammonium and lower content of organic carbon than pig slurry and cow manure. Addition of digestate to soil resulted in changes in the microbial community structure, with less pronounced effect in sandy soils, but no change in diversity was detected.

It can be concluded that the digestate from biogas plants has great potential as a fertiliser in crop production and does not seem to pose a greater risk of disturbing soil microorganisms than pig slurry and cow manure when spread on arable land.

Keywords: digestate, animal manure, biogas, microbial activity, microbial community structure, crop yield, soil respiration, potential ammonia oxidation

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List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Abubaker, J., Risberg, K. & Pell, M. (2010). Biogas residues as fertilisers – Effects on wheat growth and soil microbial activities. *Applied Energy* 99, 126-134.

II Abubaker, J., Risberg, K., Jönsson, E., Dahlin, S., Cederlund, H. & Pell, M. (2015). Short-term effects of biogas digestates and pig slurry application on soil microbial activity. *Applied and Environmental Soil Science* 2015, Article ID 658542.

III Risberg, K., Sun, L., Levén, L., Horn, S.J. & Schnürer, A. (2013). Biogas production from wheat straw and manure – Impact of pretreatment and process operating parameters. *Bioresource and Technology* 149, 232-237.

IV Risberg, K., Cederlund, H., Pell, M., Arthurson, V. & Schnürer, A. Biogas residues as agricultural crop fertilizers – comparative characterization with pig slurry and cow manure (submitted).

V Risberg, K., Sun, L., Arthurson, V., Pell, M. & Schnürer, A. Characterization of bacterial and archaeal communities in soil after amendment with anaerobic digestate as fertilising agent (manuscript in preparation).

The contribution of Kajsa Risberg to the papers included in this thesis was as follows:

I Participated in planning the work and performed most of pot-experiment. Performed some of the laboratory work and data evaluation, apart from the statistical analyses, and helped writing the manuscript.

II Participated in planning the work and measuring the potential ammonia oxidation. Had a minor contribution to writing the manuscript.

III Participated in planning the project and performed most of the laboratory work and data evaluation, apart from the statistical analyses. Main writer of the manuscript.

IV Participated in planning the project and performed most of the laboratory work and data evaluation, apart from the statistical analyses. Main writer of the manuscript.

V Participated in planning the project. Performed the incubation experiment and most of the molecular work and some data evaluation. Wrote most of the manuscript.

Abbreviations

AD	anaerobic digestion
CSTR	completely stirred tank reactor
VFA	volatile fatty acids
WWTP	waste water treatment plant
D	digestate
WHC	water-holding capacity
CEC	cation exchange capacity
OM	organic matter
MDS	minimum dataset
NGS	next-generation sequencing
AO	ammonia-oxidation
SIR	substrate-induced respiration
AOB	ammonia-oxidising bacteria
AOA	ammonia-oxidising archaea
VS	volatile solids
C/N	carbon to nitrogen ration
GHG	greenhouse gas
HRT	hydraulic retention time
NMC	nitrogen mineralisation capacity
OLR	organic loading rate
PAO	potential ammonium oxidation
PDA	potential denitrification activity
TS	total solid

1 Introduction

In order to reduce greenhouse gas (GHG) emissions and the stress they impose on the global climate, the European Commission (EC) has set the goal that 20% of all energy consumed in member states by the year 2020 should originate from renewable energy sources (EREC, 2008). As a result, interest in anaerobic digestion (AD) technology has increased in recent years. Within the AD process, a wide range of different types of organic materials can be degraded by microorganisms, resulting in biogas containing the energy-rich compound methane. This biogas can be used for producing heat, electric power and vehicle fuel. Most of the plant nutrients in the digested raw material are retained in the anaerobic digestion residue (digestate) and it can be used as a fertiliser on arable land. This enables the recycling of plant nutrients, thus potentially reducing the need for fossil fuel-dependent mineral fertiliser (Holm-Nielsen *et al.*, 2009).

The substrates commonly used in AD include manure, agricultural waste, energy crops, waste from food-processing industries, sewage sludge and organic municipal waste (Appels *et al.*, 2011). The type of substrate used not only determines the amount of biogas produced, but also the amount of digestate and its content of plant nutrients. The management regime used for the AD process also affects the resulting nutrient characteristics of the digestate (Zirkler *et al.*, 2014; Möller & Müller, 2012). Among different positive effects, spreading digestate on soil can increase the soil organic matter content, which is very important for maintaining soil fertility (Masciandaro & Ceccanti, 1999). However, in addition to the beneficial effects, spreading digestate on soil can carry the risk of adding potentially toxic compounds occasionally found in digestate. These compounds may originate from the substrate used as feedstock or may be formed as intermediates in the AD process or from the operating conditions (Kupper *et al.*, 2014; Limam *et al.*, 2013; Hellstrom *et al.*, 2011; Odlare *et al.*, 2008; Leven *et al.*, 2006; Engwall & Schnurer, 2002; Angelidaki *et al.*, 2000; Kirchmann & Witter, 1992). These compounds might affect the

soil microbial ecosystem negatively. The microbial ecosystem is an integral and crucial component of soil quality affecting plant growth. Microorganisms in a soil amended with organic residues of various origins can be studied in many different ways. One way is by evaluating the activity of the total community or specific groups (Stenberg, 1999). Changes in soil microbial activity have frequently been proposed to occur faster than changes in soil chemical and physical properties and are therefore considered ideal early indicators of changes in soil quality (Stenberg, 1999). Changes in the community structure of soil microorganisms can also be investigated after addition of different fertilisers, to reveal information about the effects of the fertiliser on soil quality. A decline in microbial diversity can result in *e.g*. less efficient suppression of soil diseases (Garbeva *et al.*, 2004). Another way of evaluating the fertiliser effect of organic residues is to study the plant-soil system, *i.e*. how the growing crop responds to different organic residues. The plant can be viewed as the ultimate integrator of changes in the soil ecosystem.

Digestate is a relatively new product and the results from previous studies on the effect of digestate on soil microorganisms and crop yield are often contradictory. Therefore further investigations are needed to draw conclusions on the fertiliser value of digestate.

1.1 Aims

The overall aim of this thesis was to evaluate different aspects related to the use of digestate as a fertilising agent. Specific objectives were to analyse the impact of feedstock source and various operating parameters in the anaerobic digestion (AD) process on the quality of the digestate produced, by measuring:

- General and specific soil microbial activity
- Bacteria and archaea community composition
- Crop growth
- Chemical and physical composition

To evaluate and compare different digestates, samples were collected from biogas processes in both large-scale biogas plants (**I**, **II**, **IV**) and small-scale (laboratory) plants (**III**, **V**) operating with a wide variety of substrate mixtures and under different process parameters, *e.g*. temperature, hydraulic retention time (HRT) and organic loading rate (OLR). The response in terms of soil microbial activity after addition of digestate was studied in both a short-term (direct response) and long-term (three-month) perspective (**I**, **II**, **IV**). The effect of digestate on crop growth was evaluated and compared with the effect

of pig slurry and mineral fertiliser (NPK) (**I**). The response in microbial activity in soil amended with digestate was also compared with that in soil amended with a large set of pig slurries and cattle manures (**IV**).

In addition, controlled laboratory-scale biogas reactors were operated with manure and straw to evaluate: a) the impact of pre-treatment, operating temperature and C/N ratio on process performance, gas yield and digestate composition (**III**); and b) the impact of addition of digestate on soil microbial community composition in three different soils after 12 weeks of incubation (**V**).

2 Biogas production

Formation of biogas is a natural process that requires the activity of a multitude of different microorganisms which are dependent on each other and form a complex ecosystem. The biogas process occurs spontaneously in *e.g*. wetlands and other oxygen-limited places in the environment, but also in the guts of ruminants and termites. By utilising these specialist microorganisms under controlled conditions, the biogas process can be used for treating a variety of organic materials in a biogas reactor (also called an anaerobic digester). Anaerobic degradation of the organic material results in formation of a gas mixture mainly composed of methane (CH_4) and carbon dioxide (CO_2), but also low levels of hydrogen gas (H_2), hydrogen sulphide (H_2S), nitrogen gas (N_2) and ammonia (NH_3) (Angelidaki *et al.*, 2011). Most of the energy within the organic material digested can be found in the methane produced (Angelidaki *et al.*, 2011). The energy-rich methane can be used for production of heat, electric power and vehicle fuel (Weiland, 2010). In addition to the biogas, a nutrient-rich slurry, so-called biogas digestate, is also produced. Digestate contains more plant-available forms of the inorganic nutrients than the ingoing substrate (Arthurson, 2009) and is therefore a valuable fertilising agent.

Many different types of organic materials can be treated by AD. Some of these substrates, such as agricultural wastes, manure, sewage sludge and source-separated organic household waste, are more interesting than various energy crops since they do not compete with food production as regards of land use. The nutrient composition of the ingoing substrate affects the biogas composition and specific methane yield, as well as the composition of the digestate (Weiland, 2010).

Anaerobic digestion processes can be divided into wet and dry fermentation (Weiland, 2010). Wet fermentation is typically used for materials with total solids (TS) content below 10%, allowing stirring and pumping of substrate and digested material. Organic substrate with higher TS content has to be diluted

with some kind of liquid (water, liquid manure or recycled process water) to enable its use in wet fermentation. The most common configuration for wet fermentation processes is a completely stirred tank reactor (CSTR), which is the type of process studied in this thesis. This type of biogas plant is continuously fed with organic material, while at the same time an equal amount of digested residue is removed. Wet fermentation is the most commonly used process in the agricultural sector in Europe (Weiland, 2010). Dry fermentation is used for substrates with TS content between 15-35% and can either be a continuous or a batch process (Weiland, 2010). The digestate from the dry fermentation process generally has a higher TS content than the digestate originating from wet fermentation processes.

Figure 1. Source-separated organic household waste, commonly used as feedstock in Sweden.

2.1 Biogas in Europe and Sweden

In 2013, there were 264 biogas plants in Sweden and the production of biogas corresponded to 1686 GWh per year (Swedish Energy Agency, 2014) (Table 1). In the same year, there were more than 14 500 biogas plants within the European Union (EU), with total annual production of 52.3 TWh biogas (EurObservÉR, 2014). Total biogas production in terms of GWh per year in Sweden has increased by 30% since 2005 and co-digestion plants (treating many different types of substrates simultaneously) have been responsible for

the largest GWh increase (356%), while biogas production from landfill has decreased, by 53% from 2005 to 2013.

Table 1. *Number and type of biogas production plants in Sweden in 2014 and their contribution to total biogas production in Sweden (Swedish Energy Agency, 2014)*

Type of biogas plant	Number of plants	% of total biogas production (GWh)
Sewage treatment	137	40
Co-digestion	23	34
Farm-scale	39	5
Industrial	5	7
Landfill	60	14
Total	**264**	**100**

In Sweden today, the most common substrate used in AD is sewage sludge from municipal wastewater treatment plants (Swedish Energy Agency, 2014) (Table 2). The most widely used process type is CSTR (Swedish Energy Agency, 2014).

Table 2. *Substrate used for biogas production (ton wet weight) in different types of anaerobic digestion process in Sweden in 2013 (Swedish Energy Agency, 2014)*

Substrate	WWTP	Co-digestion	Farm-scale	**Total**
Source-separated household waste	79 316	225 035	2 400	**306 751**
Sewage sludge	5 923 163	0	0	**5 923 163**
Manure	0	225 473	347 867	**573 340**
Food processing waste	582 617	231 028	3 258	**816 903**
Slaughterhouse waste	0	108 239	9 800	**118 039**
Energy crops	0	13 087	16 651	**29 738**
Others	149 206	142 469	5 293	**296 969**

In 2014, a new subsidy to support biogas production from manure was introduced in Sweden to stimulate the development of agricultural biogas production (Swedish Energy Agency, 2014). However, even before the subsidy, biogas production from manure had increased over the previous years, with 204 365 tons of manure being digested in 2009 and 573 340 tons in 2013 (Swedish Energy Agency, 2014). Treating manure under controlled conditions in anaerobic digesters also has the positive effect of potentially reducing

uncontrolled emissions of CH_4 from the methanogenesis process naturally occurring in manure during storage (Appels *et al.*, 2011). In Europe, two of the main biogas producers, Germany with 10 000 plants (2014) and Italy with 750 plants (2009) (IEA Bioenergy, 2014; ENEA, 2011), have in recent years faced new energy laws and biogas policy, leading to less profitable biogas production from energy crops and lower subsidies for building new biogas plants. The substrate used should instead be based on by-products and organic waste. In Italy, a decrease in newly installed biogas capacity was seen in 2013 and a decline in new biogas installation is also expected in Germany from 2015 onwards (EurObservÉR, 2014).

2.2 Microbiology in an anaerobic digester

Anaerobic digestion of organic material is a multi-step process performed by diverse groups of microorganisms that are closely dependent on each other (Angelidaki *et al.*, 2011). The four main steps are: hydrolysis, fermentation, acetogenesis and methanogenesis.

In the hydrolysis stage, complex organic compounds such as carbohydrates, proteins and fats are hydrolysed by extracellular enzymes secreted by hydrolytic bacteria, to form monomers such as sugars, amino acids and fatty acids. In the second step, fermentation, the fermentative bacteria continue to degrade the monomers produced into short-chain volatile fatty acids (VFA), alcohols, lactate, succinate, hydrogen gas, carbon dioxide and ammonia. In the third step, acetogenesis, the VFA, alcohols and sugars are further degraded by syntrophic bacteria into hydrogen and acetate. The final step, methanogenesis, is performed by methanogens belonging to the domain Archaea. This last step involves two main types of methanogens, hydrogenotrophic methanogens, which form methane mainly from hydrogen gas and carbon dioxide, and acetotrophic methanogens, which form methane mainly from acetate.

The activity and structure of the microbial community found in AD processes are related to the original inoculum, the feedstock and the operating parameters (Demirel & Scherer, 2008). Analyses of bacterial communities have revealed that one of the most commonly found phyla in digesters, are Firmicutes, where the classes Clostridia and Bacilli dominate, and Bacteroidetes, which is typically represented by the class Bacteroidia (**V**; St-Pierre & Wright, 2014; Sundberg *et al*., 2013). Moreover, representatives from other phyla have also been detected at lower abundances in various digesters operating on different substrates, *e.g*. phyla Spirochaetes, Actinobacteria and Proteobacteria (Li *et al.*, 2014b; Sundberg *et al*., 2013). Among archaeal communities the dominant phylum is Euryarchaeota, with the highest sequence

abundances belonging to the classes Methanobacteria and Methanomicrobia (**V**; Sundberg *et al*., 2013; Zakrzewski *et al.*, 2012). Among operational parameters, temperature has shown to have a strong impact on the microbial community. Typically the abundance of the class Clostridia increases with increasing temperature and a decrease in diversity has also been reported (**V**; Sun *et al*., 2014; Leven *et al*., 2007).

2.3 Factors of importance for the biogas process

Many factors such as plant design, type of substrate and operating parameters affect the biogas production process in terms of both the amount and quality of the gas and the digestate composition.

2.3.1 Process parameters

The biogas process is usually run at temperatures around 35-42 °C (mesophilic) or 45-60 °C (thermophilic) (Weiland, 2010). Mesophilic temperatures are reported to give a relatively stable process that is also typically less sensitive to high levels of ammonium (NH_4^+) (Kim *et al.*, 2002). Anaerobic digesters operating at thermophilic temperatures have been shown to have some potential advantages, such as increased degradation rate of the organic material, higher kill-off of pathogens and potentially higher methane yield (Bagge *et al.*, 2005; Sahlstrom, 2003; Buhr & Andrews, 1977). However, disadvantages with higher temperatures include lower microbial diversity (**V**; Leven *et al*., 2007), with an accompanying risk of a less stable process and less efficient degradation of certain chemical compounds, such as phenols (Leven *et al.*, 2012; Weiland, 2010). Moreover, a higher process temperature needs a higher energy input in the form of heating. However, equal performance and biogas yield from some substrates can be observed at temperatures between 37 and 52 °C (**III**).

The average process time of the substrate in the biogas plant is called hydraulic retention time (HRT) (Angelidaki *et al.*, 2011). The HRT lies between 15-30 days for mesophilic temperatures and 10-20 days for thermophilic temperatures (Angelidaki *et al.*, 2011). However, some materials might need longer times to be sufficiently degraded and thus HRT may be as long as 60-80 days. The HRT affects the degree of degradation, *i.e*. the percentage of the organic material that is converted to biogas. A longer HRT often results in better degradation of the organic material in the reactor, thus leading to less carbon ending up in the digestate (Bauer *et al.*, 2009). The amount of organic material loaded to the AD per unit of time and volume is called organic loading rate (OLR). If the OLR is low the biogas process might

be inefficient and not fully exploit the reactor volume, resulting in low biogas yield and a digestate with low dry matter content and low concentrations of plant nutrients. On the other hand, a high OLR may result in overloading of the process, which can lead to VFA accumulation, followed by a drop in pH and process failure (Rincon *et al.*, 2008).

2.3.2 Substrate

The nutrient composition of the ingoing material is of great importance for creating a stable and efficient biogas process. The ratio of carbon to nitrogen, *i.e.* the C/N ratio of the feedstock, influences the growth of microorganisms. A high C/N ratio carries a risk of nitrogen limitation for the growth of microorganisms, which can result in low efficiency of the biogas process (Igoni *et al.*, 2008). A low C/N ratio, on the other hand, may lead to an increase in ammonia concentration, which may inhibit the biogas process (Rajagopal *et al.*, 2013). To obtain maximum growth in biogas reactors, the C/N ratio of the organic substrate should be about 15-30:1 (Weiland, 2010; Igoni *et al.*, 2008). However, stable operation of biogas processes at both lower and higher C/N ratio is possible (**III**; Moestedt *et al.*, 2015; Yan *et al.*, 2015), illustrating that other factors such as macronutrients and micronutrients, pH, alkalinity, toxic and inhibitory compounds, dry matter and biodegradability of substrate are also relevant (Mata-Alvarez *et al.*, 2014).

Trace elements are very important for an efficient biogas process, since many active sites on enzymes contain metals, which hence determine the enzymatic activity (Banks *et al.*, 2012; Demirel & Scherer, 2011; Schattauer *et al.*, 2011). Deficiency of trace elements can be one reason for low efficiency of the process when no other clear reasons can be found. This limitation can be overcome by addition of extra trace elements (Demirel & Scherer, 2011). Addition of nickel (Ni), iron (Fe) and cobalt (Co) to biogas processes operating on different materials can improve the efficiency of the process and allow an increase in OLR, without risking instability (Moestedt *et al.*, 2015; Pobeheim *et al.*, 2011; Jarvis *et al.*, 1997). Another way of handling the problem of imbalanced nutrient composition is to co-digest various substrates, *i.e.* digestion of two or more substrates at the same time, which can result in a more efficient process (Mata-Alvarez *et al.*, 2014; Westerholm *et al.*, 2012; Wu *et al.*, 2010). Co-digestion can also enable digestion of materials that are difficult to degrade as single substrates due to *e.g.* a high lipid fraction or high ammonia concentration (Appels *et al.*, 2011).

Pretreatment (chemical, biological, thermal or mechanical) of lignocellulosic material can increase the biodegradability of the substrate and result in higher biodigestion efficiency and methane production (Appels *et al.*,

2011; Chandra *et al.*, 2007). Pretreatment can be of a chemical, biological or thermal nature and the aim is to solubilise hemicellulose and lignin, granting the enzyme greater access to the cellulose (Chandra *et al.*, 2007). Some pretreatments can result in solubilisation of lignin, *e.g*. steam explosion at temperatures above 160 °C following an acid pretreatment (Hendriks & Zeeman, 2009). This pretreatment process can result in production of furans and phenolic compounds, which can exert an inhibitory effect on the following biogas process, leading to a decrease in gas production (Hendriks & Zeeman, 2009). Moreover, the phenolic compounds and furans can end up in the digestate, from where they can exert an inhibitory effect on soil microorganisms if applied to soil (Leven *et al.*, 2012; Nyberg *et al.*, 2006).

3 Biogas digestate

In Sweden, approximately 1 360 000 tons of digestate were produced in 2013 (not including digestate from AD treating wastewater). The composition of the digestate in terms of plant macronutrients, micronutrients and organic components and its biological features (content of microorganisms) depend on the origin of the ingoing raw organic substrate and on the management of the anaerobic digestion process.

3.1 Chemical composition

When organic material is anaerobically digested, most of the nutrients in the feed material are retained in the process and hence end up in the digestate (Debosz *et al.*, 2002). The digestate contains most of the macronutrients (N, P, K, Ca, S and Mg) and micronutrients (B, Cl, Mn, Fe, Zn, Cu, Mo and Ni) needed by plants. Since biogas reactors can operate on many types of organic raw materials and sometimes also receive process additives such as trace metals, the resulting digestate can display a wide range of nutrient composition (**I**, **II**, **IV**; Möller & Müller, 2012; Moller *et al.*, 2008; Odlare *et al.*, 2008; Kirchmann & Witter, 1992) (Table 3). When co-digesting different organic materials, temporal variations in substrate delivery to the biogas plant may lead to high variation in nutrients in the final digestate. Such variation is difficult to predict compared with when a single feed with constant composition is used. The chemical composition is important for the digestate quality, as the content of organic matter (Doran, 2002) and the nutrients should sustain the soil microbial ecosystem and fulfil crop requirements in arable systems when the digestate is used as fertiliser (Möller & Müller, 2012).

The TS content of the organic material digested and plant design, *i.e.* wet or dry fermentation, also affect the TS content of the digestate. Digestate with higher TS will contain more organic matter and will possibly have a positive effect on soil quality.

The content of carbon in digestate is generally lower than in the substrate used as feedstock to the biogas process. This lower carbon concentration in digestate can be explained by the release of mineralised CO_2 and formation of CH_4, both originating from the degraded carbon present. In studies comparing fresh manure with digested manure, carbon losses of up to 25-53% have been reported (**IV**; Möller & Stinner, 2009; Kirchmann & Witter, 1992).

The nitrogen (N) content in digestate is correlated to the nitrogen concentration in the feed, meaning that substrates rich in proteins such as slaughterhouse waste, food waste and manure will result in an N-rich digestate (**IV**; Möller & Müller, 2012). The total nitrogen (Tot-N) content in the digestate has been reported to remain at its initial level during the biogas process, *i.e*. the same level as that in the feed. High Tot-N in organic fertilisers is desirable due to its importance as a plant nutrient. However, the proportion of mineral nitrogen in Tot-N is also important, since mineral nitrogen is the plant-available form of nitrogen. The proportion of Tot-N:NH_4-N is increased during AD, since organic nitrogen is mineralised (**IV**; Möller & Stinner, 2009; Kirchmann & Witter, 1992). It should be borne in mind, however, that high ammonium nitrogen level poses a risk to the stability of the anaerobic digestion process due to inhibition of the methanogens (Rajagopal *et al.*, 2013). As a consequence of this inhibition, high ammonium processes are typically associated with accumulation of VFA (Weiland, 2010).

A small amount (<10%) of phosphorus (P) is reported to be lost during the anaerobic digestion process (Möller & Müller, 2012; Masse *et al.*, 2007), while the content of potassium (K) is not changed (Masse *et al.*, 2007; Field *et al.*, 1984). Moreover, the process does not affect the plant availability of P or K (Möller & Müller, 2012; Field *et al.*, 1984). However, Masse *et al.* (2007) reported a decrease in the content of calcium (Ca) and magnesium (Mg) during the anaerobic digestion process compared with that in the feedstock used in the process.

The nutrient requirement can vary between different soils depending on soil characteristics and on the agricultural practices applied. In addition, different crops have different demands. Therefore the variation in nutrient composition between different digestates might allow customised and optimised fertilisation.

Table 3. *Chemical and physical composition of 20 digestates from different biogas production plants operating with different substrates and under different process conditions* ***(IV)***

Digestate	DM	Tot-N	Org-N	NH_4-N	Tot-C
	(%)		(kg ton^{-1} fw^{-1})		
1	3.7	6.4	2.2	4.1	9.0
2	6.1	7.6	7.6	5.0	19.0
3	1.7	3.4	0.6	2.8	7.0
4	6.6	5.6	2.2	3.1	28.0
5	6.0	4.3	1.2	3.1	27.0
6	3.1	4.5	0.8	3.7	11.0
7	7.4	3.4	1.2	2.2	34.0
8	3.9	4.8	1.2	3.6	15.0
9	6.5	5.4	2.1	3.3	27.0
10	5.2	5.7	1.7	4.1	21.0
11	6.1	4.1	1.3	2.8	26.0
12	3.3	6.6	1.3	5.3	15.2
13	2.2	4.6	1.0	3.6	8.0
14	4.2	5.1	1.7	3.4	14.6
15	1.1	2.6	0.5	2.1	4.3
16	5.9	5.5	2.0	3.5	24.0
17	1.4	2.4	0.5	1.9	6.3
18	4.3	3.5	1.1	2.4	16.9
19	4.8	3.8	1.4	2.4	20.0
20	4.1	5.7	1.5	4.2	16.0

fw=fresh weight

Besides plant macronutrients and micronutrients, the digestate may also contain various heavy metals (Kupper *et al.*, 2014; Govasmark *et al.*, 2011; Odlare *et al.*, 2008; Kirchmann & Witter, 1992), organic pollutants (Limam *et al.*, 2013; Govasmark *et al.*, 2011; Hellstrom *et al.*, 2011; Leven *et al.*, 2006; Engwall & Schnurer, 2002; Angelidaki *et al.*, 2000) and antibiotics (Spielmeyer *et al.*, 2014; Martinez-Carballo *et al.*, 2007; Hamscher *et al.*, 2005). A survey of eight commercial anaerobic digestion plants (not including wastewater treatment plants) and 43 commercial composting facilities in Switzerland revealed that the concentrations of cadmium (Cd), zinc (Zn), cobalt (Co) and lead (Pb) were lower in digestate than in compost (Kupper *et al.*, 2014). In the same survey, the concentrations of chromium (Cr), copper (Cu) and nickel (Ni) were found to be similar in both residue types. Govasmark *et al.* (2011) found that the concentrations of Zn, Cu, Cd, Ni, Cr, Pb, and mercury (Hg) in digestate were lower than those reported for poultry litter,

composted swine manure, organic food waste and municipal sewage sludge. However, little is known about the plant bioavailability of heavy metals in digestate, although many of them are thought to have the same bioavailability as *e.g*. heavy metals in compost (Govasmark *et al.*, 2011).

Organic pollutants and pesticides have been found in digestate from anaerobic digesters treating industrial food waste and source-separated house hold waste (Govasmark *et al.*, 2011; Nilsson *et al.*, 2000). In such cases, they may enter the digestate as contaminants in the organic substrate treated in the anaerobic digester or may be formed as intermediates during anaerobic digestion of these substrates, but may also be naturally occurring compounds such as aromatic polymers and aromatic amino acids (Angelidaki *et al.*, 2000; van Schie & Young, 1998). Phenols are one type of organic compound found in digestate (Leven *et al.*, 2006; Nyberg *et al.*, 2006). These phenols may originate from pig manure, as they are formed during bacterial degradation of tryptophan and tyrosine in the gut system or during storage of the slurry (Wu *et al.*, 1999), but may also originate from various pesticides present in some AD substrates (Limam *et al.*, 2013; Rosenkranz *et al.*, 2013). Some pretreatment methods used on the substrate can also result in formation of phenolic compounds that end up in the digestate (Hendriks & Zeeman, 2009). Moreover, thermophilic process temperatures in the anaerobic digester have been shown to result in less efficient phenol degradation than mesophilic temperatures (Leven *et al.*, 2012).

3.2 Microbial composition

Digestate is a living material and contains a wide variety of different microorganisms. For example, microorganisms from the preceding AD can be found in the digestate. These microorganisms are still active during storage of the digestate, as shown by post-production of biogas. Moreover, there is a risk of organisms not needed for the biogas process *per se* being present, among which are potentially pathogenic bacteria and fungi (Sahlstrom *et al.*, 2008; Schnurer & Schnurer, 2006; Bagge *et al.*, 2005; Sahlstrom, 2003). Different types of these organisms can pose a risk not only to the humans handling the digestate, but also to the soil and plant system and to animals grazing on digestate-fertilised fields.

Pathogenic bacteria such as *Listeria, Salmonella, Escherichia coli*, *Mycobacterium*, *Clostridium*, *Campylobacter* and *Yersinia* have been found in different substrates such as farm and slaughterhouse wastes and wastes from food processing industries (Sahlstrom, 2003). Spore-forming *Clostridia* and

fungal spores have also been detected in digestate (Schnurer & Schnurer, 2006; Bagge *et al.*, 2005).

To reduce the load of pathogens and minimise their presence in the digestate, pasteurisation of the raw substrate can be performed at 70 °C for 1 h (Sahlstrom *et al.*, 2008). The origin of the substrate determines the need for pasteurisation. For example, animal by-products can be grouped into three different categories (European Parliament and Council, 2002). Substrate belonging to category one, *i.e*. high risk material such as bone marrow and substrate from sick animals among others, should be incinerated and not used at all for biological treatment. Substrate in category two should be pasteurised at 70 °C for 1 h before further digestion. Slaughterhouse waste and manure are examples of category two substrates. Manure however, may be digested without heat-treatment if it contains no or low concentrations of *salmonella*, *enterococaceae* or *Escherichia coli* according to Commission of the European Communities (2006). Category three covers substrates such as different organic household wastes, waste from food processing industries and slaughterhouse waste not included in category two. The category three substrates do not need to be heat-treated before digestion (European Parliament and Council, 2002).

Bagge *et al.* (2005) reported a reduction in most bacterial pathogens studied after pasteurisation for 1 h at 70 °C of raw substrate prior to anaerobic digestion. The AD process itself also causes a reduction in bacterial pathogens for which both temperature and HRT are of importance, with more efficient inactivation at higher process temperature and longer HRT (Sahlstrom *et al.*, 2008). The ammonia level has also been shown to be important, with improved pathogen reduction with increasing ammonia levels (Ottoson *et al.*, 2008). However, spore-forming bacteria have been shown to survive both anaerobic digestion and pasteurisation (Bagge *et al.*, 2005). Regrowth of pathogens in digestate storage tanks at farm sites has also been reported (Bagge *et al.*, 2005). Another source of contamination and regrowth of pathogens can be the transportation vehicle used for digestate, if not cleaned properly between runs (Bagge *et al.*, 2005). Moreover, Schnurer and Schnurer (2006) reported that pasteurisation at 70 °C for 1 h did not inactivate fungal spores detected in source-separated household waste intended for anaerobic digestion. When the heat-treated substrate was digested the number of fungal spores was lowered, but some thermotolerant spores survived with the risk of ending up in the digestate and posing a potential hazard for the plant-soil system and for humans and animals (Schnurer and Schnurer, 2006). Moreover plant pathogenic fungi have been shown to survive anaerobic digestion (Bandte *et al*., 2013).

Figure 2. Storage tanks for digestate at Lövsta biogas plant in Uppsala, Sweden.

3.3 Certification of biogas digestate

Digestate is a relatively new type of fertiliser that, as mentioned above, risks containing heavy metals, organic pollutants and microbial contaminants. To create confidence in digestate as a fertilising agent, in 1999 Swedish Waste Management launched a certification system called SPCR 120. The system is voluntary and was developed in cooperation between producers of compost and biogas digestates, the food industry, soil producers, authorities, researchers and Swedish Waste Management. The certification rules are based on the EU health rules regarding animal by-products and derived products not intended for consumption (European Parliament and Council, 2002). Within the EU, different regulations regarding disposal of digestates can be found in a report from Biogasmax, (2010). Prior to receiving certification, a biogas plant has to pass a qualification year verifying that the product meets the requirements contained in the standards. The certification rules include raw material, supplier, collection and transportation, reception, treatment process, end product, declaration of contents and advice and instructions for use (SPCR 120, 2010). The declaration of content currently does not cover organic contaminants. Examples of raw materials permitted as substrate for treatment in a certified biogas process are park and garden waste, and manure and slurry

from pigs, cattle, sheep, horses, poultry and other animals. Furthermore, digestate from biogas plants operating with waste from the food industry containing approved additives for food production can be certified. Biogas digestate produced from sewage sludge is not covered by the SPCR 120 certification system. If a digestate is certified, the producer may label the product as ‘Certified Recycling' (*Certifierad återvinning*). By 2014, 17 biogas plants in Sweden were certified according to SPCR 120.

4 Soil quality and organic residues

Soil quality has been defined as: "the capacity of a soil to sustain plant and animal productivity, maintain water and air quality and promote plant and animal health" (Doran, 2002). However, the meaning of soil quality can vary from soil to soil and also depends on the usage of the soil. One important feature of soil quality frequently pointed out is the content of soil organic matter (Doran, 2002), as this is associated with high soil nitrogen and phosphorus content (Jakobsen, 1995), improves the water-holding capacity (WHC) and structure of the soil and reduces the risk of plant diseases (Hoitink & Boehm, 1999).

In general, the interactions between chemical, physical and microbial soil components greatly influence the soil (Kennedy & Papendick, 1995). If changes occur in one of these components, the other two will also be affected (Doran, 2002; Stenberg, 1999). Chemical properties of a soil include pH, cation exchange capacity (CEC), organic matter (OM) content and C/N ratio (Darilek *et al.*, 2009). Physical properties include soil texture, porosity, bulk density, structure and WHC. Biological properties of the soil include indigenous microorganisms which perform many important functions, for example mineralisation of organic material, by which they enhance cycling of plant nutrients and increase nutrient availability to the roots (Kennedy & Papendick, 1995). Soil microorganisms can also help aggregate the soil, which reduces the risk of erosion, allows good water infiltration and maintains good aeration of the soil (Bronick & Lal, 2005). Changes in soil microbiology have been shown to appear faster than changes in physical and chemical properties, since microbial communities constantly adapt to their environment. Soil microorganisms are therefore considered to be a good indicator of early changes in soil quality (Odlare *et al.*, 2008; Kennedy & Papendick, 1995). Thus, studying the soil microbial response to addition of organic and mineral fertilisers should provide early information on perturbations in the soil environment (Stenberg, 1999).

Adding organic residues produced in different processes (anaerobically digested, composted, pig slurry and cow manure, among others) may have both positive and negative effects on soil quality. For example, organic residues can improve soil phosphorus and nitrogen content (Jakobsen, 1995), improve WHC and soil structure (Joshua *et al.*, 1998) and conserve soil organic matter (Doran, 2002). Moreover, adding organic residues to soil can have both positive and negative effects on soil microbial functions (**I**, **II**, **IV**; Sanger *et al.*, 2014; Odlare *et al.*, 2011; Liu *et al.*, 2009; Leven *et al.*, 2006; Nyberg *et al.*, 2004; Svensson *et al.*, 2004; Kirchmann & Lundvall, 1993). Mineral fertilisers can also have positive and negative effects on soil microbial communities and their activity (**I**; Sapp *et al.*, 2015; Geisseler & Scow, 2014). Even soil organic carbon has been shown to increase after long-term use of mineral fertilisers (Geisseler & Scow, 2014). This is most likely a result of the increased crop yield obtained in fields treated with mineral fertilisers. However, Hati *et al.* (2006) observed no difference in organic carbon content between unfertilised soil and soil treated with mineral fertiliser in a three year field trial.

4.1 Soil microorganisms

The surface of the soil contains a large share of the earth's living biomass. The four main microbial taxa are bacteria, archaea, fungi and viruses (Fierer *et al.*, 2007), with bacteria being the most frequently found organism in soil. However, fungi are the most dominant soil microorganism in terms of biomass (Metting, 1992). Soil microorganisms can be evaluated in different ways, for example by studying the metabolic activity of the total community, by focusing on the activity of specific microbial groups, or by investigating changes in soil microbial community structure. A minimum dataset (MDS) covering chemical, physical and biological properties have been suggested when analysing and interpreting soil quality (Stenberg, 1999; Kennedy & Papendick, 1995). Suggested analyses for indicators of microbial activity are soil respiration, microbial biomass, nitrogen mineralisation capacity (NMC), potential denitrification activity (PDA) and potential ammonia oxidation (PAO), among others. However, with the development of new molecular technologies, the possibilities to analyse soil microbial communities and to link groups of soil microorganisms to soil quality are steadily increasing.

4.1.1 Microbial community structure

The soil microbial community has been described as: "multi-species assemblages, in which organisms live together in a continuous environment and interact with each other" (Konopka, 2009). Knowledge of the soil

microbial community is important for understanding soil fertility and also fundamental ecological processes occurring in the ecosystem. Microbial diversity has been shown to have an effect on *e.g*. plant growth, as soils with greater microbial diversity often have fewer problems with plant diseases (Garbeva *et al.*, 2004). Soil microorganisms are constantly adapting to their environment and changes in microbial communities as a response to fertilisation have been investigated in a number of recent studies (**V**; Sapp *et al.*, 2015; Su *et al.*, 2015; Bissett *et al.*, 2014; Geisseler & Scow, 2014; Li *et al.*, 2014a). It is important to bear in mind in this regard that the entire microbial community is not always active at the same time. However, by detecting and separating actively growing soil microbial communities from those not metabolically active, specific microorganisms with functionally important traits can be identified (Nannipieri *et al.*, 2003).

Different approaches can be adopted when studying soil microbial community structure in soil. One such approach is based on analysing the diversity in 16S rRNA sequences, by which the total microbial community or specific microbial groups or species can be targeted. Another way of studying soil microorganisms is by targeting specific functional genes. This allows the microorganisms responsible for a specific function in the environment, such as cycling of nutrients, to be scrutinised (He *et al.*, 2012). Some techniques that have been used to investigate soil microbial communities and response to fertilisation include different fingerprinting methods such as terminal-restriction fragment length polymorphism (T-RFLP) (Cederlund *et al.*, 2014; Aiken, 2011; Odlare *et al.*, 2011), temperature and denaturing gradient gel electrophoresis (TGGE and DGGE) (Enwall *et al.*, 2007; Muyzer, 1999), quantitative real-time polymerase chain reaction (qPCR) (Cederlund *et al.*, 2014; Zhang & Fang, 2006) and clone libraries (Odlare *et al.*, 2011; Daniel, 2005). However, it should be borne in mind when investigating microbial communities that analyses based on DNA will only reveal information on the microbial community present and not specifically the active organisms. To analyse active microorganisms, RNA can instead be targeted (Ding *et al.*, 2015; Wang *et al.*, 2012). Moreover, the metabolically active fraction of the community can be investigated with methods such as bromodeoxyuridine (BrdU) immunocapture (Nyberg *et al.*, 2012; Borneman, 1999) or stable isotope probing (SIP) (Ding *et al.*, 2015; Kreuzer-Martin, 2007).

In the last couple of years, next-generation sequencing (NGS) technologies have revolutionised biological science. NGS techniques, in particular Illumina-based strategies, allow detection and analysis of subdominant microbial species and groups at deeper levels than previously achievable using molecular techniques (Shokralla *et al.*, 2012). Illumina provides paired reads of the same

DNA fragment, offer multiplexing capability and the amount of sequence data generated is large compared with *e.g.* 454 pyrosequencing (Shokralla *et al.*, 2012). Among the Illumina platforms currently available, the MiSeq sequencer offers most potential in 16S rRNA sequence studies, since it is relatively cheap to use and generates longer sequence reads (Kozich *et al.*, 2013).

The dominant phyla of bacteria found in most soils are the Proteobacteria, Acidobacteria, Actinobacteria, Verrucomicrobia, Bacteriodetes, Cloroflexi, Planctomycetes, Gemmatimonadetes and Firmicutes (**V**; Sapp *et al.*, 2015; Janssen, 2006). The most abundant archaea reported in soil samples belong to the phylum Chrenarchaeota (**V**; Bates *et al.*, 2011).

Soil type has been suggested to be the dominant factor determining variations in soil microbial community composition (Larkin *et al.*, 2006; Girvan *et al.*, 2003), displayed as variations in abundance and composition of the major phyla between different types of soil (**V**; Janssen, 2006; Larkin *et al.*, 2006; Girvan *et al.*, 2003). For example, the relative abundance of the three most dominant phyla, Proteobacteria, Acidobacteria and Actinobacteria, found in three different soils varied between 17-23%, 18-24% and 13-21% respectively (**V**). The biological, physical and chemical factors in different soils that may have an impact on the variation in abundance are not completely clear (Janssen, 2006). Factors shown to impact on the soil microbial community structure, besides soil type, include organic matter content in soil, nutrient addition (nitrogen and phosphorus), pH and plant growth (Sapp *et al.*, 2015; Geisseler & Scow, 2014; Enwall *et al.*, 2007). For example, on adding different organic residues to soils of varying origin, the microbial communities are reported to stay clustered according to soil and not according to residue treatment (**V**; Abubaker *et al.*, 2013). Still, the community structure in different soils have been shown to change as a response to organic residue addition, where *e.g.* Abubaker *et al.*, (2013) identified a more pronounced change for sandy soils than soils with a high clay content. However, in this thesis contradictory results were obtained, i.e. a larger change in the community after fertilization with different digestates as well as with manure was seen for a soil with high content of clay compared to a sandy soil (**V**). Microbial communities in sandy soils, with low natural biomass content, have also been suggested to respond more to organic input than soils with a higher content of organic biomass (Abubaker *et al.*, 2013). Moreover, some fertilisers may change soil pH after repeated addition and this pH change can in turn result in a shift in microbial community structure (Enwall *et al.*, 2007).

Even though changes in the whole microbial community structure are not always seen, there may still be variations within specific groups of soil microorganisms responsible for important microbial ecosystem services. For

example, the ammonia-oxidising (AO) communities have been shown to change in response to soil fertilisation even when the whole soil community structure does not show any variation (Bissett *et al.*, 2014; Enwall *et al.*, 2007). When connecting the community structure of specific groups to soil microbial activity, different results have been reported. Bissett *et al.* (2014) found that even though a community structure change was evident for a specific group (AO community), a change in microbial activity could not be detected. In contrast, Enwall *et al.* (2007) found a connection between changes in AO community and microbial activity patterns.

Changes in soil microbial community structure after addition of different organic residues can for some phyla be a result of addition of microorganisms originating from the residue. As shown in this thesis (**V**), the relative abundance of Firmicutes increased in three different soils after addition of organic residues. This increase was represented by an increase in the abundance of the class Clostridia (**V**). Clostrida are usually harmless but might impose risks as pathogenic Clostridia have been found to be present in digestate from various biogas plants (Neuhause *et al*. 2015; Bagge *et al.* 2005). However, after 12 weeks of incubation with the residues the increases in relative abundance of Firmicutes had in most cases decreased again, but were for two of the soils still higher than incubated control soil (**V**). In general it is important to consider that some microorganisms can have a large impact even when present in only small amounts. For example, *Campylobacter* originally present in untreated manure can enter soil when the manure is spread as a fertiliser and can ultimately end up in the food chain, causing illness in humans consuming the contaminated food (Jaderlund *et al.*, 2011).

Addition of different fertilisers (both organic and inorganic) has been reported to result in a decrease in the diversity and richness of the microbial community (Sapp *et al.*, 2015). However, this decline in diversity was not seen in a study included in this thesis evaluating the effect of five different digestates on three different soils (**V**). The microbial diversity in the study by Sapp *et al.* (2015) was shown to be slightly higher for soils treated with digestate compared with soils treated with mineral fertiliser.

Some of the dominant phyla in soil (Acidobacteria, Verrucomicrobia and Gemmatimonadetes) have been shown to decrease in relative abundance after addition of digestate (**V**; Sapp *et al.*, 2015), and after addition of mineral fertiliser (Sapp *et al.*, 2015), most likely as a response to nitrogen addition (Cederlund *et al.*, 2014). The relative abundance of the phylum Planctomycetes, which is involved in the nitrogen cycle, has also been reported to decline after addition of organic residues or mineral fertiliser (Sapp *et al.*, 2015). It is clear that the soil microbial community structure, and possibly also

the activity of the community, is affected by application of different fertilisers. However, it is unclear exactly what the change occurring in response to fertilisation actually means, as many different factors can affect the community structure, for example different soils and types of fertiliser.

4.1.2 Soil respiration

Soil respiration is a general process performed by most microorganisms and methods for measuring this activity are probably the most common tool for investigating soil microbial activity (Stenstrom *et al.*, 2001; Stenberg, 1999). Several methods exist for determination of soil respiration based on either oxygen consumption or release of carbon dioxide. The background respiration activity of a soil microbial community, also called basal respiration, can simply be measured as CO_2 produced without any addition of substrate. Measuring basal respiration gives an estimate of the overall availability of carbon in the soil. Substrate-induced respiration (SIR), a measure of the response of the total soil microbial biomass in terms of CO_2, is determined after addition of an optimal amount of a carbon source, typically glucose. The SIR response to glucose is frequently used as a biomass index, as most microorganisms are believed to respond to this universal molecule. The effect of different carbon sources on soil respiration can also be used to assess changes in microbial communities. MicroResp^{TM} is one such method where the response in respiration to a multitude of simple organic molecules can be measured after addition of one carbon source at a time to small soil samples, so-called multi-SIR (Chapman *et al.*, 2007). Instead of adding glucose or a set of carbon sources, the respiratory response of the active microbial biomass can also be measured after addition of different organic fertilisers (**I**, **II**, **IV**; Alburquerque *et al.*, 2012; Odlare *et al.*, 2008). This assesses the capacity of the soil community to utilise a complex mixture of organic substances under more natural conditions where the microorganisms in the soil sample have to compete for the substrates. Adding organic residues to soil generally increases soil respiration, since carbon serves as an energy source for most soil microorganisms (Ryan & Law, 2005). Mineral fertiliser, on the other hand, contains no carbon and should therefore not affect respiration in any pronounced way directly after addition. However, the carbon content in soil has been shown to increase after long-term use of mineral fertiliser (Geisseler & Scow, 2014), which may therefore increase respiration. However, in a long-term field trial comparing mineral fertilisation to fertilisation with digestate and compost, the response in SIR was found to be significantly lower for the mineral fertiliser (Odlare *et al.*, 2011). The origin of organic residues (digestate, animal manure, compost) also causes different responses in soil

respiration (**II**, **IV**; de la Fuente *et al.*, 2012; Odlare *et al.*, 2008; De Neve *et al.*, 2003; Kirchmann & Lundvall, 1993). The factor suggested to have the greatest impact on main peak time ($t_{peakmax}$), *i.e.* the time when the maximum respiration activity takes place, and main peak height ($h_{peakmax}$), *i.e.* the maximum respiration activity, is the quality of the carbon, *i.e.* how easily degradable the carbon source is to the soil microorganisms (**II**; de la Fuente *et al.*, 2013; Alburquerque *et al.*, 2012; Kirchmann & Lundvall, 1993). On amending soil with digestate, an instant flush of high CO_2 (response in respiration) production has been reported, unlike with other organic residues (**II**, **IV**; Alburquerque *et al.*, 2012; Odlare *et al.*, 2011). This instant response in respiration is most likely an effect of a comparatively higher fraction of easily degradable carbon in the digestate becoming immediately accessible to the soil microorganisms (**II**, **IV**; Odlare *et al.*, 2011), compared with *e.g.* non-digested animal manure (Ernst *et al.*, 2008) and compost (Odlare *et al.*, 2011). The amount and origin of such easily available carbon in digestate has been discussed and organic loading rate (OLR) and hydraulic retention time (HRT) are two operating factors suggested to have an impact on the level (Bauer *et al.*, 2009). Longer HRT has been suggested to result in more thorough degradation of the organic matter, with less easily available carbon ending up in the digestate. Moreover, the organic materials used as substrate in an anaerobic digester, together with HRT, are suggested to affect the biodegradability of the digestate (**II**; Bernal *et al.*, 1998). For example, Mata-Alvarez *et al.* (2014) discuss the "risk" of co-digesting different substrates, as this might result in an unstable digestate, *i.e.* a digestate with a high concentration of easily-degradable carbon. A fast response in soil respiration after addition of organic residues can possibly have a negative effect on nitrogen availability in soil. Unstable organic matter, occasionally found in digestate, can stimulate soil microbial cell synthesis and growth, resulting in immobilisation of dissolved inorganic nitrogen (Alburquerque *et al.*, 2012; Kirchmann & Lundvall, 1993). However, the immobilisation of inorganic nitrogen is reported to be temporary, as the nitrogen is later re-mineralised (Alburquerque *et al.*, 2012).

Recent investigations of large sets of digestates, included in this thesis, did not show any parallels between operating parameters of the digester or substrate type fed to the process and soil respiration (**IV**; Sanger *et al.*, 2014), suggesting that factors other than those studied affect the bioavailability of carbon and the response in soil respiration.

The utilisation rate, *i.e.* the amount of organic carbon added divided by the accumulated CO_2-C, has been reported to be lower for digestate than for animal manure (**II**; de la Fuente *et al.*, 2012; De Neve *et al.*, 2003; Sorensen,

1998; Kirchmann & Lundvall, 1993). However, studies performed as part of this thesis showed that the utilisation rate of digestate, pig slurry and cow manure did not differ (**IV**), indicating that the total carbon mineralised is more dependent on the concentration than the biodegradability of the carbon source added. The explanation for these contradictory results could be large variations in the content of nutrients and easily degradable carbon in different animal manures (Yang & Ha, 2013; Moreno-Caselles *et al.*, 2002).

4.1.3 Potential ammonia oxidation

The first step of nitrification is ammonia oxidation, where NH_3 is oxidised to NO_2^- by the ammonia-oxidising bacteria (AOB) (Ernst *et al.*, 2008; Kowalchuk & Stephen, 2001). The AOB are highly specialised and possess complex cell machinery as a result of their dual chemolithotrophic and autotrophic life, making them a very sensitive group of microorganisms (vanBeelen & Doelman, 1997) that can respond quickly to perturbations in the soil system. Until quite recently, the AOB were thought to be the only ammonia-oxidising organisms. However, during the past decade ammonia-oxidising archaea (AOA) have also been discovered and have been shown to be present at high abundance in soil (Leininger *et al.*, 2006). Their contribution to overall ammonia oxidisation is speculated to be larger than initially thought (Kelly *et al.*, 2011). However, the AOB are more commonly found in agricultural soils, *i.e.* soils with high disturbance through *e.g.* tillage. Moreover, nitrogen fertilisation has been shown to stimulate AOB more than AOA (Bissett *et al.*, 2014). Potential ammonia oxidation (PAO) is defined as the increase in NO_2^- concentration per unit of time and under non-limited substrate concentration and optimal pH (Pell *et al.*, 1998). It can be used to estimate the overall activity of the ammonia-oxidising community. In the PAO assay performed under optimal conditions for AOB, chlorate is added to inhibit further transformation of nitrite to nitrate, which makes the method simple and rapid as nitrite can easily be analysed using automated colorimetric method. Analysing PAO can give an early warning of the presence of possible toxic contaminants such as organic pollutants, pesticides and heavy metals in the environment (Odlare & Pell, 2009; Pell *et al.*, 1998).

When organic residues (digestate, pig slurry and cow manure) have been added to soil, both positive and negative effects on PAO have been reported (**I**, **II**, **IV**; Odlare *et al.*, 2008; Leven *et al.*, 2006; Nyberg *et al.*, 2006). In comparison, mineral fertilisers have been reported to have only stimulating effects on PAO compared with control soil (with no fertiliser) (Odlare *et al.*, 2011). However, in these studies various organic residues had an even more stimulating effect than the mineral fertiliser (**I**; Odlare *et al.*, 2011). The

lithotrophic ammonia-oxidising community is not dependent on organic matter as its energy source, and is instead stimulated by ammonia (vanBeelen & Doelman, 1997). Therefore, when fertilising soil with organic residues, the content of ammonium has a large impact on the activity of AOB. Negative effects of anaerobic digestate on PAO activity were demonstrated in this thesis, at fertilisation rates considered realistic field rates or lower (**II**, **IV**). One reason for the inhibitory effect on PAO can be the content of xenobiotic compounds and heavy metals negatively affecting the ammonia-oxidising community. Digestate from biogas reactors treating source-separated household waste and industrial food waste has been reported to contain *e.g.* different organic pollutants (PCB 6, PBDE, DEHP and PAH 16) and pesticides (imazalil, thiabendazole, fludioxynil and 2-phenylphenol) (Govasmark *et al.*, 2011; Nilsson *et al.*, 2000). Phenols are one organic compound proven to affect PAO negatively and are present in digestate (Leven *et al.*, 2006; Nyberg *et al.*, 2006). Digestate originating from the same type of substrate, household or organic waste, but from different locations, was shown in this thesis to have both stimulatory and inhibitory effects on PAO (**IV**). Therefore even if different digestates originate from the same type of waste, for example household waste, it is obvious that their quality can vary from location to location and also between different batches. Moreover, the temperature in the AD process affects the degradation of organic compounds, and thus the quality of the digestate, with *e.g.* mesophilic temperatures resulting in relatively more efficient degradation of phenols than thermophilic temperatures (Leven *et al.*, 2012). However, when a large set of digestates was studied regarding their effect on PAO in this thesis, no clear parallels to the origin of the substrate was seen (**IV**).

The varying response in PAO observed after addition of digestate to soil in different studies could also be a result of differences in the set-up of the experiments. In some studies PAO is measured directly after addition of digestate to soil (**II**, **IV**; Leven *et al.*, 2006) and in others at the end of an incubation trial (**I**; Odlare *et al.*, 2008; Nyberg *et al.*, 2006). The inhibitory effect of PAO can also be temporary, as the same digestate was shown in this thesis to cause immediate inhibitory effects, but after a certain period of incubation to stimulate PAO (**I**, **II**). This effect might have been caused by microbial adaptation to the inhibitors or decomposition of the inhibiting compounds (Nyberg *et al.*, 2012). It should also be noted that in some studies extracts of digestate, and not the whole digestate, have been used to test effects on PAO (Leven *et al.*, 2006; Nyberg *et al.*, 2006). Furthermore, the variation in results between different studies may be related to the soil type, as soils

possess different physical and chemical characteristics and also display differences in soil microbial population (**V**; Nyberg *et al.*, 2012).

The inhibition of PAO observed in soil after addition of organic residues should probably be taken as an early warning of disturbance, as organic fertiliser can affect soil fertility by reducing the turnover of ammonia to nitrite. However, inhibition of PAO might also have an indirect positive effect by lowering the risk of the soil system emitting the greenhouse gas nitrous oxide (N_2O) and leaching nitrate, the product of full nitrification.

4.1.4 Nitrogen mineralisation capacity

Nitrogen mineralisation is the biological transformation of organic N to NH_4^+ (Nahm, 2005). Mineralisation can take place under aerobic and anaerobic conditions and involves enzymatic degradation of organic nitrogen such as that in animal manure and crop residues. The enzymes involved in mineralisation are produced by most organisms. Mineralisation of organic nitrogen in soil is very important to soil fertility, as it supplies the plants and soil microorganisms with mineral N (Ros *et al.*, 2011). In addition, nitrogen mineralisation has an indirect effect on the environment, as it contributes to the risk of nitrate leaching and losses of N_2O (Ros *et al.*, 2011; Akkal-Corfini *et al.*, 2010). Nitrogen mineralisation capacity (NMC) refers to the amount of mineral N released in a soil during a certain time (Ros *et al.*, 2011). When organic fertiliser is added to soil, NMC has been shown to increase compared with non-amended control soil (**I**, **II**; Odlare *et al.*, 2008) and soil amended with mineral fertiliser (**I**, **II**). This effect of the organic residue is most likely a result of the input of organic material, with the fertiliser serving as an energy and nitrogen source for many soil microorganisms. When the microorganisms utilise the energy source, ammonium is released and may be emitted to the environment or immobilised, depending on the C/N ratio of the soil and fertiliser. The importance of organic N was demonstrated in part of the work reported in this thesis, where the digestate containing the highest amount of added organic N stimulated NMC the most (**I**). Another factor reported to affect nitrogen mineralisation is the size of the water-soluble fraction in the fertiliser, *i.e*. the water-soluble organic nitrogen content (Qafoku *et al.*, 2001; DeNeve & Hofman, 1996). Furthermore, the fertiliser dose has been shown to influence whether net mineralisation or assimilation of N occurs (**II**; Azam *et al.*, 1993). In Paper **II**, high doses of organic residues resulted in high nitrogen addition to the soil, which seemed to result in a deficiency of easily available carbon in the residue and soil. This low content of easily available carbon resulted in a limitation for the microorganisms, which were unable to

assimilate the NH_4^+ or NO_3^- released, and thus net mineralisation took place (**II**).

4.1.5 Potential denitrification activity

Denitrification is a respiration process where NO_3^- and NO_2^- are stepwise reduced to NO, N_2O and N_2 in an oxygen-limited environment. Denitrification is performed by a wide variety of heterotrophic and chemoorganotrophic bacteria (Philippot *et al.*, 2007). Potential denitrification activity (PDA), *i.e.* the denitrification activity expressed under optimal conditions, reflects the amount of denitrifying enzymes in the system under study. As denitrification is a process sensitive to changes, PDA assays can be used to detect and evaluate disturbances in the soil system after addition of *e.g.* organic residues and pesticides (Odlare & Pell, 2009; Pell *et al.*, 1998). When soil was fertilised with digestate in one of the studies in this thesis, inhibition of PDA was observed (**II**). This inhibition could be an effect of *e.g.* heavy metals such as Cu, Ca and Zn, all three of which are frequently found in digestate in concentrations previously reported to have negative effects on denitrification (Holtan-Hartwig *et al.*, 2002). Moreover, inhibitory effects on denitrification have been reported from the presence of silver (Ag) in concentrations detected in sewage sludge (Johansson *et al.*, 1998). Besides heavy metals, some organic pollutants such as polyaromatic hydrocarbons and pesticides, which can be found in different digestates (Angelidaki *et al.*, 2000; van Schie & Young, 1998) have been shown to inhibit denitrification (Philippot *et al.*, 2007; Pell *et al.*, 1998).

4.2 Effect of organic fertilisers on crop yield

When investigating digestate and its potential as a fertiliser, one of the most obvious ways is to study the effect on plant growth and to compare crop yield with the fertilising effect of other well-studied fertilisers, such as animal manure and mineral fertilisers.

Many studies have been performed to evaluate the effects of digestate on crop yield. The results can be categorised into four groups: 1) Performance equal or better than mineral fertiliser (**I**; Ahmad & Jabeen, 2009; Chantigny *et al.*, 2008); 2) performance equal or lower than mineral fertiliser (Bougnom *et al.*, 2012; Odlare *et al.*, 2011; Svensson *et al.*, 2004); 3) performance equal or better than the raw substrate (Chantigny *et al.*, 2008; Moller *et al.*, 2008; Loria *et al.*, 2007); and 4) performance similar to an unfertilised control (Svensson *et al.*, 2004). Due to the wide variation in organic substrate treated in AD plants, the plant nutrient profile of the digestate varies (**I**, **II**, **IV**) and therefore the

fertilising effect of different digestates can also be expected to vary. For example, four different digestates evaluated in a well-controlled pot experiment in this thesis all resulted in higher wheat biomass yields (root, straw and ear) than mineral fertiliser (**I**) when dosed at rates corresponding to 35, 70 and 140 kg N ha^{-1}. The lower yield from mineral fertiliser was possibly an effect of the poor nutrient status of the sandy soil used in the study, which had a low ability to provide the crop with macronutrients and micronutrients (**I**). The digestate studied might contain a range of micronutrients not present in the mineral fertiliser. However, in a long-term fertilisation trial on clay soil, mineral fertiliser gave higher yields than digestate (Odlare *et al.*, 2011).

Lower yield of wheat and barley after addition of digestate compared with pig slurry or cow manure has been reported (**I**; Odlare *et al.*, 2008). The content of phosphorus (P) found in pig slurry is usually higher than that in digestate, which could be one explanation for the lower yield with the latter. Phosphorus is a plant macronutrient and digestate is known to contain low concentrations (Svensson *et al.*, 2004). To avoid P deficiency symptoms in crops when fertilising with digestate, complementary fertilisation with P has been suggested (Svensson *et al.*, 2004).

Most studies investigating crop yield and fertilisation with digestate only report the effect of a single digestate (Bougnom *et al.*, 2012; Odlare *et al.*, 2011; Ahmad & Jabeen, 2009; Chantigny *et al.*, 2008; Moller *et al.*, 2008; Svensson *et al.*, 2004), which makes it difficult to draw any general conclusions regarding the fertiliser effect of digestate.

5 Conclusions

In this thesis, the effect of application of a wide variety of anaerobic digestate to soil as a fertilising agent was investigated by studying its effect on soil microbial activity, soil microbial community composition and crop growth. Moreover, the chemical and physical composition of different digestates was characterised. The effect of the digestates was compared with those of pig slurry, cow manure and mineral fertilizer. The results, taken together, provide a comprehensive and informative overview of digestate as a fertilising agent.

Different digestates showed large variation in chemical composition due to differences in origin of the raw substrate treated, but probably also due to process parameters such as operating temperature and hydraulic retention time. Compared with pig slurry and cow manure, digestates were characterised by a high content of ammonium, the result of anaerobic degradation of the raw substrate treated, and a lower content of organic carbon, as some of the carbon in the raw substrate is transformed to methane and carbon dioxide during anaerobic degradation.

Soil fertilisation with digestate increased crop yield compared with control soil and mineral fertiliser, but not compared with pig slurry. The lower yield from mineral fertiliser compared with digestate and pig slurry was most likely a result of the nutrient-poor sandy soil used in the study, which had limited ability to provide the crop with micronutrients and macronutrients beyond those added with the fertiliser.

Addition of digestate to soil had both stimulatory and inhibitory effects on soil microbial activity. Different responses in the soil respiration curves revealed differences in the quality and quantity of organic carbon in digestate, pig slurry and cow manure. As pig slurry and cow manure generally contain more organic carbon than digestate, on addition to soil they resulted in a higher response in respiration activity. Digestate on the other hand, displayed

immediate response demonstrating presence of a more easily available fraction of carbon compared with pig slurry and cow manure. Despite these different respiration patterns, however, total utilisation rate of the organic carbon in digestate, pig slurry and cow manure did not differ significantly after 12 weeks of incubation. While the response by soil respiration was positive, addition of digestate at field fertilisation rates had both stimulatory and inhibitory effects on potential ammonia oxidation (PAO) and potential denitrification activity (PDA). The inhibitory effect observed should be taken as an early warning sign of the presence of potentially hazardous compounds in these digestates. However, if PAO and PDA are inhibited, there may be a reduced risk of nitrate leaching and gaseous emissions from the soil. It is difficult to identify where the potentially inhibitory compounds come from, since the response proved difficult to correlate to origin of raw substrate and process parameters, *e.g*. temperature of the biogas reactor. Inhibitory effects on PAO were also seen after addition of pig slurry and cow manure and thus in this regard digestate does not seem to pose a greater risk of disturbing soil microorganisms than pig slurry and cow manure when spread on arable land.

The soil microbial community structure and also the activity of the community was clearly affected by addition of different fertilisers. However, it is unclear exactly what the response to fertilisation actually entails, as there are so many different factors affecting the community structure. For example, soil type is believed to be the dominant factor for changes in community composition, while pH, nutrient addition and crop growth are also believed to have an impact.

Overall, the results of this thesis reveal that digestate from biogas plants has great potential as a fertiliser in crop production. However, to fully exploit its potential it should preferably be used on heavy soils, while pig slurry and cow manure may be more suitable for lighter soils.

6 Perspectives

Overall, the results in this thesis show that digestate has great potential as a fertilising agent on arable soil and that it compares well to other organic residues such as pig slurry and cow manure, but also to mineral fertilisers.

There are many interesting questions to be taken into consideration when thinking about future research in this area. One question left unanswered in this thesis concerned the inhibition of some soil microbial activities observed after addition of digestate. Further investigations regarding the content of possible toxic compounds and where these originate from are needed to minimise a potential risks of spreading digestate on the soil. This can be done by further investigating connections between chemical content, origin of raw substrate treated in the process and process parameters such as temperature and hydraulic retention time and the responses in terms of microbial activity and microbial community structure in soil. Moreover, linking responses in microbial activity in soil to the metabolically active fraction of the soil microbial community after addition of digestate would not only increase knowledge, but would also link soil microbial communities to important ecosystem services.

Furthermore, increased knowledge on the effects of digestate on crop yield is needed. To my knowledge, most results reported today only refer to a few digestates and one soil type at a time, making it difficult to draw any general conclusions about the effect of digestate on crop yield. This reveals the need of long-term field studies where different soils and large set of digestates are investigated.

Another area of interest not investigated in this thesis is the profitability of digestate recycling for large-scale biogas plants. Most biogas plants make little or no money from their digestate, even though it has been shown to function well as a fertiliser on arable land, especially compared to pig slurry and cow manure but also mineral fertiliser. If more long-term studies are performed on

soil microorganisms and on crop growth, further supporting the positive effects of digestate reported in this thesis, the interest among farmers in using digestate as fertiliser will hopefully increase.

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