Agro-ecological aspects when applying the remaining products from agricultural biogas processes as fertilizer in crop production

Dissertation

Zur Erlangung des akademischen Grades

doctor rerum agriculturarum
(Dr. rer. agr.)

eingereicht an der
Landwirtschaftlich- Gärtnerische Fakultät
der Humboldt-Universität zu Berlin

von

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Tag der mündlichen Prüfung: 11.06.2012

Abstract

With the increase of biogas production in recent years, the amount of digestates or the remaining residues increased accordingly. Every year in Germany more than 50 million tons of digestates are produced, which are used as fertilizer. Thus nutrients return into the circulation of agricultural ecosystems. However, the agro-ecological effects have not been deeply researched until now. For this reason, the following parameters were quantified: the influence of dry and liquid fermentation products on the yield of three selected crops in comparison to or in combination with mineral-N-fertilizers in on-farm experiments; the growth, development and yield of two selected crops in comparison to mineral-N-fertilizer, liquid manure and farmyard manure in a randomized complete block design; selected soil organisms as compared to mineral-N-fertilizer, liquid manure and farmyard manure in a randomized complete block design. In addition, the mineralization of dry and wet digestates in comparison with liquid manure and farmyard manure was investigated in order to evaluate the effects of different fertilizers on the humus formation under controlled conditions.

The 2-year results of on-farm experiments showed that for a sandy soil, the combination of digestates in autumn and mineral-N-fertilizer in spring for winter crops (wheat, rye and rape) brought the highest yields. The wet digestate achieved the highest dry-matter yield as the only fertilizer for maize in spring. In a clayey soil, the use of 150 kg ha^{-1} N mineral-N-fertilizer brought the highest grain yield. These results were similar to the ones obtained by the application of dry digestates, if they were applied in two doses. Maize showed no significant differences between the dry-matter yields of the different treatments.

The results in the field experiments from 2009 to 2011 showed that the effect of digestates on the yield of winter wheat and *Sorghum sudanense* was up to 15 % lower than the effect of the mineral-N-fertilizer.

There were no negative effects from both digestates on the population of earthworms one month after the application. The enchytraeid populations were negatively influenced by wet digestate, liquid manure and mineral-N-fertilizer on the short term. This effect was reversed seven months after application.

Under controlled conditions, the results from 2010 and 2011 showed that due to the CO_2 exhalation curve and the lignin content, the liquid manure and wet digestate seem to be the most stable fertilizers with regards to the humus reproduction in the soil.

However, further studies should be done to quantify the effect of digestates under an agro-ecological more reliable view.

Zusammenfassung

Durch die Zunahme der Biogasproduktion in den letzten Jahren hat sich auch die Menge von Gärprodukten, die verbleibenden Rückstände, entsprechend erhöht. In Deutschland fallen jährlich mehr als 50 Millionen Tonnen Gärprodukte an, die als Dünger verwendet werden. Damit werden Nährstoffe in den Kreislauf landwirtschaftlicher Ökosysteme zurückgeführt. Allerdings sind die agroökologischen Wirkungen noch nicht hinreichend erforscht. Aus diesem Grund wurden die folgenden Parameter untersucht: Einfluss von trockenen und flüssigen Gärprodukten auf dem Ertrag von drei ausgewählten Fruchtarten im Vergleich zu oder in Kombination mit Mineraldünger in On-Farm Versuchen; Wachstum, Entwicklung und Ertrag von zwei ausgewählten Fruchtarten im Vergleich zu Mineraldünger, Gülle und Stallmist in einer vollständig randomisierten Blockanlage; Einfluss auf ausgewählte Bodenlebewesen im Vergleich zu Mineraldünger, Gülle und Stallmist in einer vollständig randomisierten Blockanlage. Zusätzlich wurde die Mineralisierung von flüssigen und festen Gärprodukten im Vergleich zu Gülle und Stallmist untersucht, um Effekte der verschiedenen Düngestoffe auf die Humusbildung unter kontrollierten Bedingungen zu bestimmen.

Die Ergebnisse der 2-jährigen On-Farm-Versuche zeigten, dass auf sandigen Böden die Kombination von Gärprodukten im Herbst und Mineraldünger im Frühjahr für die Fruchtarten der Winterung die besten Ernteresultate erzielte (Weizen, Roggen und Raps). Das flüssige Gärprodukt als alleiniger Dünger für Mais im Frühjahr erbrachte vergleichbare Ergebnisse wie Mineraldünger. Auf einen tonigen Boden erbrachte die Anwendung von 150 kg ha^{-1} N Mineraldünger den höchsten Winterweizen-Kornertrag. Vergleichbare Ergebnisse wurden bei der Anwendung von trockenen Gärprodukten erzielt, wenn sie ebenfalls in zwei Gaben ausgebracht wurden. Silomais zeigte keine deutlichen Unterschiede zwischen dem Trockenmasseertrag der verschiedenen Prüfglieder.

Im Feldversuch zeigten die Ergebnisse von 2009 bis 2011, dass die Wirkung der Gärprodukte auf den Ertrag von *Sorghum sudanense* und Winterweizen in der Regel bis zu 15 % niedriger war, als die Wirkung des Mineraldüngers.

Die Gärprodukte hatten keine negativen Auswirkungen auf die Populationen von Regenwürmern einen Monat nach der Ausbringung. Die enchytraeidenpopulationen wurden durch flüssige Gärprodukte, Gülle und Mineral-N-Dünger kurzfristig negativ beeinflusst. Dieser Effekt kehrte sich sieben Monate nach der Anwendung wieder um.

Unter kontrollierten Bedingungen zeigten die Ergebnisse von 2010 und 2011, dass aufgrund der CO_2-Exhalationskurve und der Lignin-Gehalte Stallmist und flüssiges Gärprodukt die stabilsten Düngestoffe mit Blick auf die Humusreproduktion im Boden zu sein scheinen.

Allerdings müssen weitere Studien gemacht werden, um die Wirkung der Gärprodukte unter agroökologischer Sicht sicherer zu quantifizieren.

Key Words: Biogas, Digestates, Agro-ecosystem, Crop production, Soil biota

Schlagwörter: Biogas, Gärprodukte, Agrarökosystem, Pflanzenbau, Bodenlebewesen

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Literature Review

1 Literature Review

1.1 Biogas market development in recent years and outlook

A new era is upon us for energy and the environment in the European Union. Strategies like the reduction of the dependency on imported energy, the improvement of the security of its supply, the reduction of greenhouse-gas emissions, the increase of renewable energy usage and the improvement of energy efficiency, are on the forward-looking political agenda by 2020 (BOSCH *et al.*, 2009). Renewable energy is already seen as one of the short-term and medium-term options for mitigating greenhouse-gas (GHG) emissions and replacing fossil fuels. This is certainly evident in Europe, where a variety of activities and programs for developing and encouraging renewable energies have been implemented, both at European (European Commission – EC) and national level. Basically, every country in Europe has included renewable energy in its energy and climate policies (FAAIJ, 2006). In Germany, the main sources of renewable energy in 2010 were wind, which produced 36.5 billion kilowatts hour (KWh); hydropower, which produced 19.7 billion KWh; biomass, which produced 33.5 billion KWh; photovoltaic, which produced 12.2 billion KWh; and geothermal, which produced < 0.1 billion KWh. Biogas, as part of biomass energy, produced 12.8 billion KWh (BÖHME *et al.*, 2010).

In Europe, biogas production comes from three main sources: landfills, sewage sludge and others such as decentralized agricultural plants. However, Germany has opted to develop agricultural anaerobic digestion plants by encouraging the planting of energy crops. As a result of this strategy, Germany is the leading European biogas producer, alone accounting for half of Europe's primary energy output (50.5 % in 2009) and half of its biogas-sourced electricity output (49.9 % in 2009) (Eurobserver, 2010). According to the German Agency for Renewable Resources (Fachagentur für Nachwachsende Rohstoffe), in Germany over 5,900 biogas plants are currently operating with a total electrical capacity of approximately 2,300 megawatts (MW) (Figure 1). The steady increase in crop digesters in Germany can be directly attributed to a favorable supportive national legal framework coupled with the tariffs paid for renewable energy. Staggered feed-in tariffs (which depend on the electrical power capacity of the biogas plants) are guaranteed for the entire depreciation period of the investment (Murphy et al., 2011). The electricity generation from biogas in 2010 was about 12.8 billion kWh, equivalent to about 2.1 % of the total electricity consumption in Germany, or about 12.6 % of the electricity supply from renewable energies.

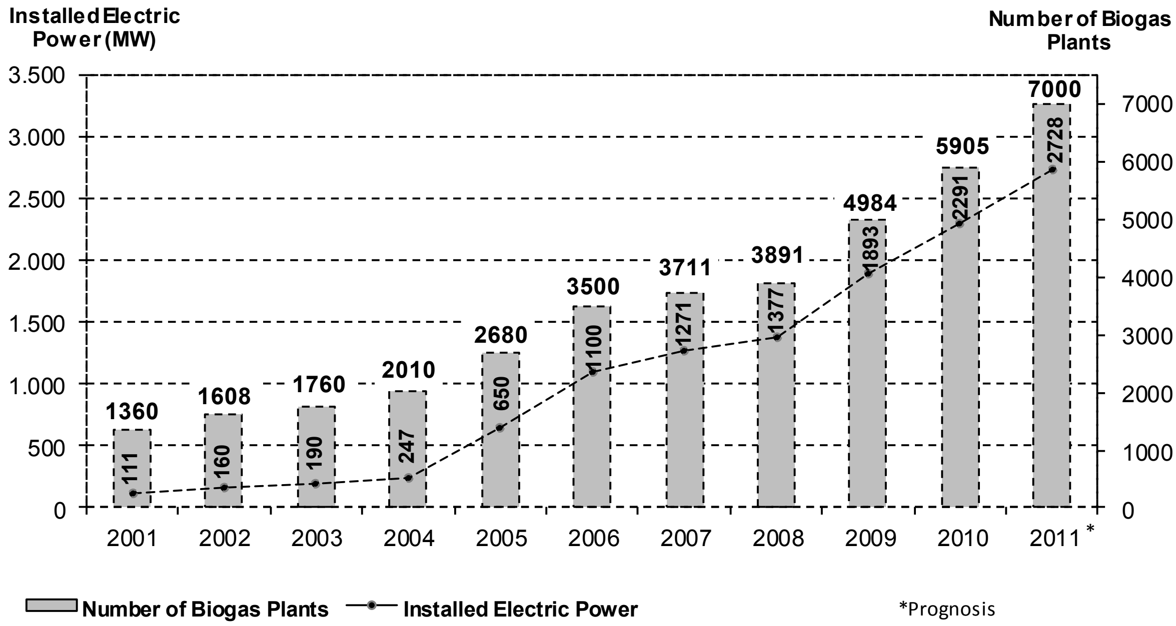


Figure 1: Development in the number and electrical installed capacity of biogas plants in Germany (FNR, 2011).

Biogas can not only be used for power generation, but also for admixing products (in blends with natural gas) or for transport application as biofuel (HERR *et al.*, 2010). The concept of biogas production by digestion and its subsequent conversion into electricity in combined heat and power (CHP) plants or feeding as biogas into the gas networks is an essential contribution to utilizing biowaste from households, communities and agriculture (GRAAF & FENDLER, 2010). The German market for the feed-in of biogas into the grid is still young. The first two biogas plants were put into operation at the end of 2006. In 2007, five more plants were opened. Around 30 plants had been connected to the network by the end of 2009. By the end of 2010, more than 70 plants were expected to be feeding into the German gas grid with a total hourly feed-in capacity of 54,000 cubic meters of biogas. With the plants that were presumably installed by the end of 2010, almost 4.2 billion kilowatt hours of biogas could be generated and fed-in. This amount suffices to cover the final energy demand for heating and hot water of 215,000 four-person households with a yearly consumption of 20,000 kilowatt hours of natural gas each. Biogas can also be used as a substitute for natural gas for natural-gas-powered vehicles. By means of gas-grid feed-in, the gas can be distributed around the German gas-station network and be a substitute for fossil fuels. (HERR *et al.*, 2010).

1.2 Anaerobic digestion processes and biogas production

Numerous plant species and plant residues have been tested for their methane potential. In principal, many varieties of grass, clover, cereals and maize, including whole plants, as well as rape and sunflower have proved feasible for methane production. Hemp, flax, nettles, potatoes, beets, kale, turnip, rhubarb and artichokes have all been tested successfully. These crop materials can be used directly as input material for the anaerobic digestion process after the harvest, or can be stored in silage clamps. There are two types of anaerobic digestion processes depending on the type of processed material. The most common is the co-digestion process in which crops are combined with manure or other liquid substances to promote homogeneous or stable conditions. The second and less common way to produce biogas is mono-digestion, in which only crops are used as input material for biogas production. In this type of digestion process, the recirculation of the digestate is required in order to maintain homogeneous as well as buffered digester conditions. In 2004 and 2009, an evaluation of the German biogas plants was carried out. It was observed that most plants used manure-based substrate mixtures, with a range of crops including maize, grass and cereals. Food and vegetable wastes, potato-processing residues, whey and fat-trap contents were also used as co-substrates with liquid manure. In the 2004 study, manure was the dominant substrate (75-100 %) in nearly 50 % of the plants considered. About 83 % of these German agricultural biogas plants operated with a mixture of crops and manure; 15 % used crops only and just 2 % were operated with manure only. Nearly 90 % operated with wet-digestion technology while the remainder used dry digestion (MURPHY *et al.*, 2011). Anaerobic digestion processes can be also termed "wet" or "dry" depending on the total solids concentration of the substrate. If the concentration is less than 15 % the anaerobic digestion is defined as wet, and if the concentration reaches 20-40 % it is defined as dry (NAYONO, 2010). Depending on the temperature there are two main digestion processes: thermophilic which ranges from 35 to 45 ° C and mesophilic which ranges from 45 to 65 ° C (HAKE, 2004). In the thermophilic range, decomposition and biogas production occur more rapidly than in the mesophilic range. However, the process is highly sensitive to disturbances, such as changes in feed materials or temperature. While all anaerobic digesters reduce the viability of weed seeds and disease-producing (pathogenic) organisms, the higher temperatures of thermophilic digestion result in more complete destruction (TILAK & DEY, 2010). Digesters can be designed for batch feeding or continuous feeding. In batch digesters, a full charge of raw material is placed into the digester which is then sealed off and left to ferment as long as gas is produced. When gas production has ceased, the digester is emptied and refilled with a new batch of raw material (TILAK & DEY, 2010). With continuous-load digesters the raw material enters the digester continuosly and an equal amount of digested material is removed (NAYONO, 2010). Digestion systems can also be configured with different levels of complexity depending on the number of stages of fermentation. In a single-stage process the digestion occurs simultaneously in a single reactor, whereas in a two- or multi-stage reactor the reaction takes places sequentially in at least two reactors. Both, the two- and multi-stage system allow the intermediate steps of the anaerobic digestion to be controlled and investigated. Otherwise, the one-step system due to its simple design suffers less frequent technical failures and has lower investment costs (MATA-ALVAREZ, 2003). The residence time of the feed material in the digester depends on the amount and type of the input material, the configuration of the digestion system and whether it has one or two stages (MANDAN & MANDAN, 2009). Normally the time of residence in a mesophilic digestion is between 15 and 30 days. The thermophilic digestion needs a residence time of 12 to 14 days (ARVANITOYANNIS, 2008).

Anaerobic digestion (AD) is a biological process that happens naturally when bacteria breaks down organic matter in the absence of oxygen. The main products of the process are carbon dioxide and methane, but minor quantities of nitrogen, hydrogen, ammonia and hydrogen sulphide are also generated. As a result of the removal of carbon, organic bound minerals and salts are released in their soluble inorganic form (AHRING, 2003). The main steps in the anaerobic digestion process are:

– *Hidrolysis*, in which complex insoluble organic polymers such as carbohydrates, cellulose, proteins and fats are broken down and liquified by eytracelullar enzymes produced by hydrolytic bacterias. Proteins are converted into amino acids, fats into long-chain fatty acids and carbohydrates into simple sugars (EVANS, 2001).

– *Acidogenesis*, in which facultative anaerobic and hydrogen-producing acidogenic bacterias convert the simple organic material via oxidation/reduction reactions into intermediate products, generally acetic acid and a small proportion of organic volatile fatty acids (porpionic, butiric and valeric acids) hydrogen, carbon dioxide (MARKANDEY & RAJVAIDYA, 2004), alcohol and acetic acid (WATTER, 2009).

– *Acetogenesis* in which the fatty acids are converted into acetate, hydrogen and carbon dioxide via acetogenic dehydrogenation by obligate hydrogen-producing bacteria and,

– *Methanogenesis* in which the acetate and hydrogen plus the carbon dioxide are converted by methane-producing bacteria into methane, carbon dioxide, water and remaining products or by-products (MARKANDEY & RAJVAIDYA, 2004) (Figure 2).

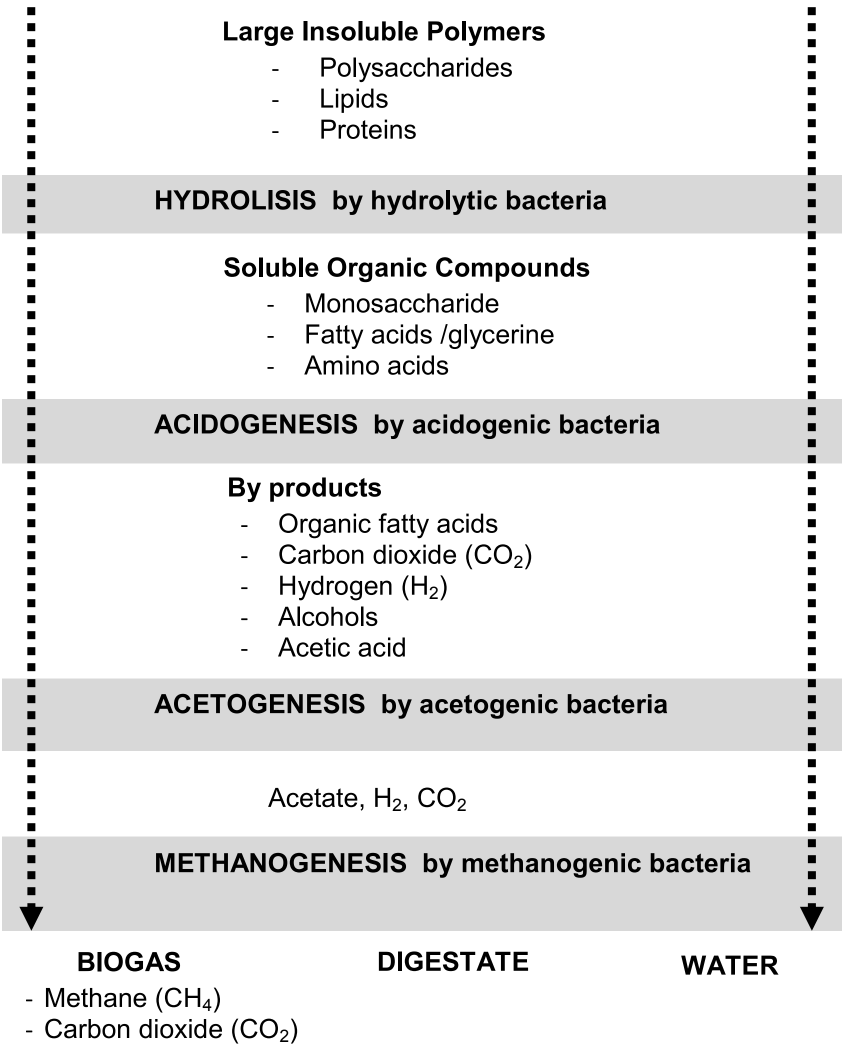


Figure 2: Phases of the anaerobic digestion process (own illustration).

Besides the variety of possible applications of biogas like heat and electricity generation, fuel for cars and feeding into natural grids, the produced biogas can be stored for later use according to fluctuation demands (KARAGIANNIDIS, 2012).

1.3 Digestates: remaining products from agricultural biogas production

The by-product of the anaerobic digestion is termed “digestate” and consists of raw organic material that cannot be used by microorganisms and water. It also consists of the mineralised remains of the dead bacteria from within the digesters (MANDAN & MANDAN, 2009). More than 90 % of nutrients entering anaerobic digesters are retained within the digestate, which can therefore be used as soil conditioner (OECD, 2010). Digestate typically contains elements such as lignin that cannot be broken down by the anaerobic microorganisms (MANDAN & MANDAN, 2009). Due to the reduction of organic matter also a part of the organically bound nitrogen is mineralized. This results in a higher amount of ammonium than in other conventional organic fertilizers like cattle manure (ROSCHKE & PLÖCHL, 2006). During the fermentation process, the organic dry matter is decomposed into methane (CH_4) and carbon dioxide (CO_2); the carbon content in the digestate is reduced and this consequently results in a narrowing of the C/N ratio (LEITHOLD, 2010). The reduction of the organic dry matter depends on the duration and intensity of the fermentation and also on the composition of the starting material (BRENNER, 2008). A typical organic dry-matter content of 50-60 % is often seen in manure-based systems. In the case of digestates, the organic dry-matter content is normally 40-50 % primarily in the form of fibers (AHRING, 2003). The anaerobic digestion results in an increase in pH in the digestate in comparison to the original material (BRENNER, 2008; CLEMENS *et al.*, 2001). This is presumaby due to the production of amonium (SCOTTISH EXECUTIVE ENVIRONMENT AND RURAL AFFAIRS, 2007). The pH value of the digestates is higher than that of liquid manure and farmyard manure. A comparative measurement over a twenty-week period of one digester biogas plant in Germany yielded an average of 0.45 pH units higher pH of the digestate in comparison to fresh manure (BERENDONK, 2011). Phosphorus, potassium and magnesium do not undergo extreme change during the fermentation process. As with nitrogen, some of the phosphorus is also converted into an inorganic form or a readily available form for the plants. Potassium and magnesium contents do not suffer significant changes during the fermentation process (FNR, 2004).

1.4 Agronomic aspects when applying digestates as fertilizers

The digestates produced by most operational plants are destined for use as soil conditioner or fertilizer. They demonstrate a useable nutrient level, both in terms of concentration and in a form which makes them available for plant uptake. Consequently, the use of digestates results in a reduced requirement for mineral fertilizers (EVANS, 2001) and therefore lower fertilizer investment costs. However, digestates have characteristics that are specific to each digester tank. These characteristics can vary between batches from the same digester and even within the same batch of digestate, following storage (LUKEHURST *et al.*, 2010), so a continuous monitorization of digestates before the application is required (MÖLLER, *et al.*, 2009).

According to the German Fertilizer Ordinance (DüMG 1977, § 3 No. 1 and 2), organic fertilizers can be authorized in the market only if they are used properly and they do not cause harm to the soil fertility, the health of humans, domestic animals and crops and do not compromise the balance in the ecosystem. According to the German Fertilizer

Ordinance, organic fertilizers are "…liquid manure, farmyard manure, straw and similar by-products from agricultural production, and further treated, which promote growth, increase the yield and improve the quality of plants". Most digestates in Germany are a typical form of further by-products from agricultural production, so they are included in the fertilizer legislation (DITTRICH, 2011). According to the German Fertilizer Regulation (DüV) before applying any fertilizer the analysis of available nutrients in the soil and in the applied fertilizer is recommended. The soil conditions at the time of application should be taken into account. The application of fertilizer when the soil is flooded, saturated, frozen or has more than a 5 cm layer of snow is forbidden. The maximum amount of applied organic fertilizer is 170 kg N ha^{-1} per year, with a maximum permissible excess of 60 kg N ha^{-1} per year and 20 kg ha^{-1} P_2O_5 per year (only if the P content in the soil is <20 mg P_2O_5 100 g^{-1} soil (CAL method) or <3.6 mg P_2O_5 100 g^{-1} soil (FUE method)). After harvest of winter crops or catch crops an application of no more than 80 kg ha^{-1} of total nitrogen is recommended (SCHNEIDER & MASTEL, 2008). Plant security, safety against diseases and unwanted substances like heavy metals should be an important aspect for consideration. By using cattle or pig manure as input material for the production of biogas the levels of certain heavy metals (for example copper and zinc) should be taken into account since values over the limits have already been documented. This aspect is also managed through the German Waste Regulations (BioABFV) to prevent damage to the health of humans and animals through the release of pathogens and to prevent possible damage to the agricultural ecosystem from the spread of harmful organisms or substances (DITTRICH, 2011). In relation to hygenisation, the recommendations given by the German Waste Regulations (BioAFV) are scarce, giving only the analysis of salmonella from digestates. The use of digestate is also regulated by the German Animal By-Product Regulations (TierNebV) which recommend further analysis of the *Escherichia coli* (TIERISCHE NEBENPRODUKTE-BESEITIGUNGSVERORDNUNG, 2006). In addition, Commission Regulation No. 1774/2002 also recommends the analysis of *Enterococaceae*.

Digestate is produced throughout the year and must therefore be stored until its use (LUKEHURST *et al.*, 2010). The concentration of ammonia in the biogas inside the reactor is controlled by the pH and temperature-dependent NH_4/NH_3 equilibrium in the liquid phase. When the gas is transported from the reactor to, e.g. a storage tank or gas engine, the gas temperature drops and water vapour condenses, thereby trapping water-soluble compounds such as ammonia. As a consequence, the handling of the condensate from gas cooling can be of some environmental concern (CHRISTENSEN, 2011). These emissions can be reduced if the surface of the digestate is covered by a protective layer such as tight membranes or flexible storage bags (LUKEHURST *et al.*, 2010). Wet digestates are applied with the same equipment used for applying raw slurry. Similarly, the equipment used for spreading farmyard manure can also be used to spread dry digestate. At the moment of the application of the digestate it is advisable to avoid windy and warm days due to higher losses of NH_3. The application should be as close as possible to the soil surface and the soil should be directly plowed after the fertilization (MÖLLER *et al.*, 2009).

With the information found so far in the literature it can be firstly summarized that the digestates are used by many farmers as fertilizer; secondly that a few effects on some agricultural aspects are already known; and thirdly the existence of laws which regulate their application. However, there are still many aspects that can be investigated in depth.

2 Aim of the Study

The aim of this study is to describe the potential effects of wet and dry digestates from the biogas production on agro-ecosystems in comparison to farmyard and liquid manure, and mineral-N-fertilizers. The following questions should be answered:

1. How is the biomass and crop yield of five selected plants affected by the use of dry and wet digestates as fertilizer in comparison to and in combination with mineral-N-fertilizer in on-farm experiments?
2. How are growth, development and crop yield of two selected plants influenced by application of dry and wet digestates in comparison to farmyard and liquid manures and mineral-N-fertilizer in a randomized complete block design?
3. What is the impact of the application of dry and wet digestates on the soil biota in comparison to farmyard, liquid manure and mineral-N-fertilizer in a randomized complete block design?
4. What is the mineralization rate of wet and dry digestates in comparision to farmyard and liquid manures and which is the most stable for promoting humus formation under environmental-controlled conditions?

Chapter 1

Digestates from the agricultural anaerobic digestion process as fertilizers on selected plants in on-farm experiments

3 Chapter 1: Digestates from the agricultural anaerobic digestion process as fertilizers on selected plants in on-farm experiments

3.1 Introduction

The Agency of Renewable Resources (Fachagentur für Nachwachsende Rohstoffe) has been documenting the production of biogas in Germany since 1999. In this time, a growing number of farmers have become involved in the production of biogas, using agricultural residues as input material. The remaining products of biogas production, the so-called digestates, have been used as fertilizer in their fields. However, it has not been corroborated since when the farmers have been using these digestates. The need to know about their effects on agricultural production has increased over the past few years. A few studies were carried out in the early years of biogas production, but from 2005 German scientists started focusing on the effects of the digestates on the dry-matter yield and grain yield of different crops in different locations. STINNER & MÖLLER (2005) observed that the grain yield (dt ha^{-1}) obtained from spring and winter wheat fertilized with wet digestate tended to be higher than that from the unfertilized control. A significantly higher straw and grain yield of barley and winter wheat was also reported by CLEMENS *et al.*, 2005 in comparison to the unfertilized control. SCHNEIDER-GÖTZ *et al.,* 2007 observed that the fertilization with wet digestate achieved high yields of winter wheat and maize. These were quite comparable to the results of using mineral fertilizers or to the utilization of a combination of liquid manure and mineral fertilizer. The results of KAUTZ *et al.,* 2007 showed that the digestate from biogas production, in which organic material from agricultural production was used as input material, had a positive but not significant effect on the maize dry-matter yield (dt ha^{-1}) compared to the digestates coming from pig manure as input material or an unfertilized control. According to the research studies of PACHOLSKI *et al.*, 2010, the dry-matter yield of maize by using digestates as fertilizer was quite comparable, but not significantly different to that of mineral fertilizer. However, the mineral fertilizer had a significant positive effect on winter wheat in comparison to the use of digestate.

Overall, from the literature it can be observed that the effect of digestates as fertilizers in crop production is quite comparable to the effect of mineral fertilizer, and that their effects depend on the crop. However, most of the research is focused on the influence of the digestates on dry-matter yield and grain yield. There is a lack of information on the yield formation, crop quality and local conditions. Specifically, the effects of the soil type on the fertilizer effectiveness have not yet been studied in detail.

The main focus of this chapter is to describe the effect of dry and wet digestates on dry-matter yield, grain yield, yield formation and yield quality of winter wheat, winter rye, winter oilseed rape and silage maize in two different locations with different soil types.

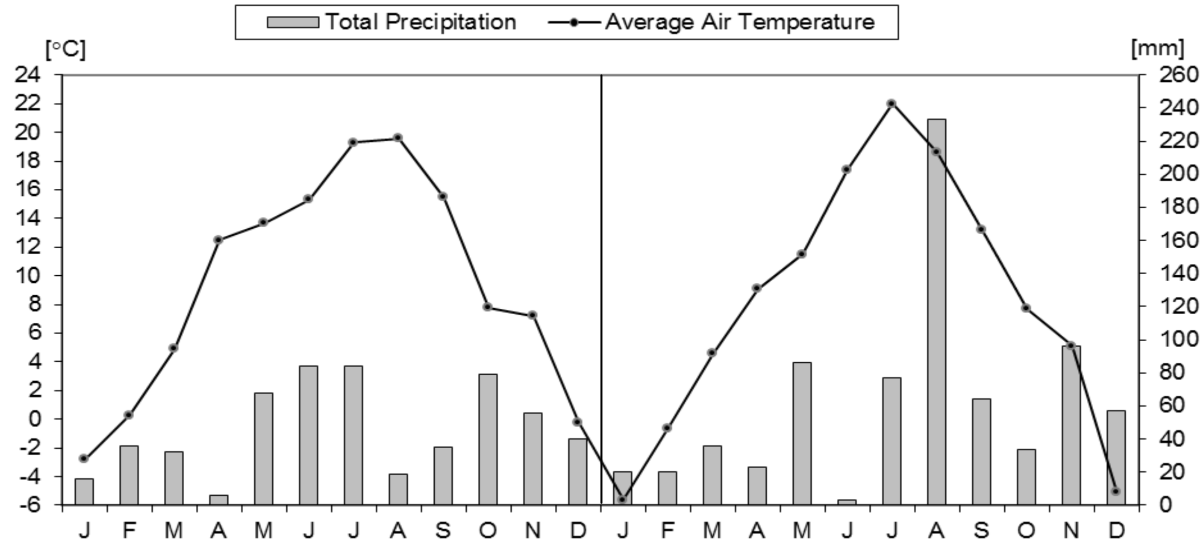
To assess the effects of dry and wet digestates on crop production, since 2009 the Department of Agronomy and Crop Science from Humboldt University of Berlin has been working with two agricultural companies which produce biogas and currently use the digestates as fertilizers on their fields. The company Friedersdorfer Ackerbau GbR produces biogas from maize through a dry mono-digestion process under mesophilic conditions (38 ° C), where the batch-feeding material is percolated with water and stored under anaerobic conditions for 32 days in a single reactor. The remaining product of the fermented silage maize is a digestate which contains up to 17 % of water. The company Agrargenossenschaft Trebbin produces biogas through a wet co-fermentation from liquid manure, silage maize, silage grass, and millet with a duration of 42.5 days without a disposal facility and 69.7 days including it. The digestion process is continuous and mesophilic (38 ° C). The remaining product, also called wet digestate, which is a pumpable, homogeneous, and semi-liquid residue, is obtained. In both companies, the biogas is stored until it is converted into electrical energy by subsequent combustion. The company Friedersdorfer Ackerbau GbR produces 9,200 MW per day (d^{-1}) and the company Agrargenossenschaft Trebbin 24,000 MW d^{-1}.

3.2 Material and Methods

From 2009 until 2010, field experiments were carried out with different crops in fields of the above-mentioned agricultural companies.

3.2.1 Field site description

The company Friedersdorfer Ackerbau GbR in Märkisch-Oderland, is situated 70 km east of Berlin. The geographical coordinates of its location are 52 °31 'N, 14 °24' W and its height above sea level is 46 m. Data from 2009 and 2010 was obtained from a meteorological station in Manschnow (Oderbruch), situated 10 km from the experimental field (DEUTSCHER WETTERDIENST, 2010). In 2009 the average annual temperature was 9.4 ° C. 2009 and 2010 were marked by a cold period from December until February. The coldest average temperature was reached in January 2010 with a minimum of -6 ° C. The warmest month in the two years was August 2010, reaching a maximum average air temperature of 22 ° C. January and April were the months with the least total precipitation during 2009; June 2010 had only 5mm. In 2009 higher precipitation rates were recorded in June, July and October, reaching a maximum rainfall of 80 mm in June and July. In 2010 the month with the highest precipitation was August, which reached 233 mm. The total annual precipitation was 555 mm in 2009 and 749 mm in 2010 (Figure 3).



	2009	2010	1981-2010
Total annual air Temperature (° C)	9.4	8.2	-
Total annual precipitation (mm)	555	749	-

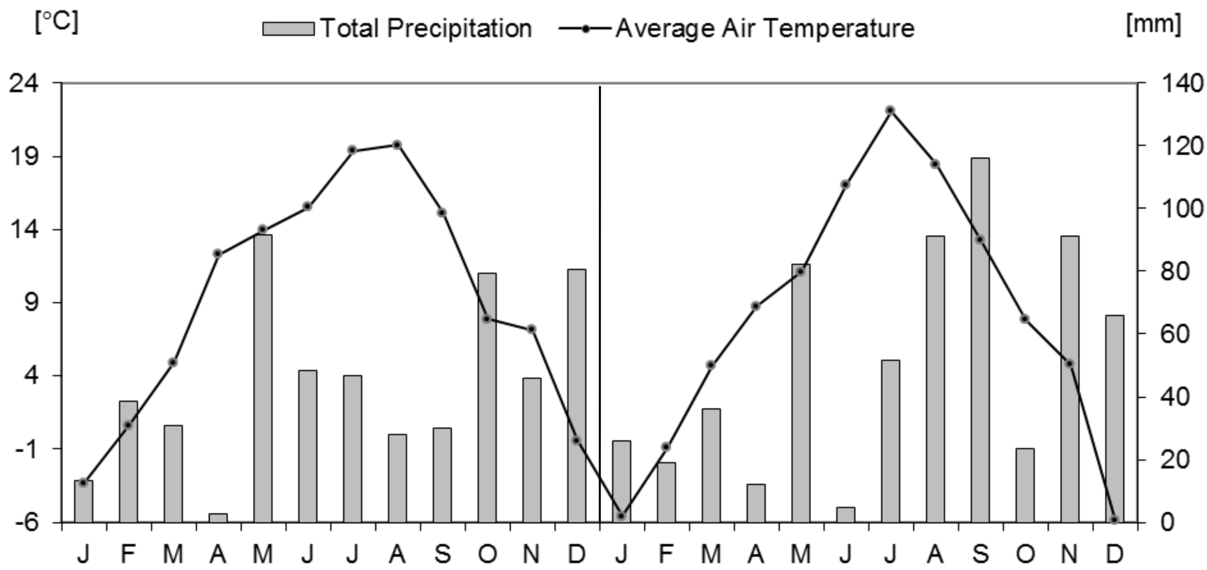
Figure 3: Total precipitation and average air temperature from 2009-2010 at the Friedersdorf location. Data from Manschnow (Oderbruch).

The company Agrargenossenschaft Trebbin is located in the district Teltow-Fläming, 36 km from Berlin, at 39 m above sea level with geographical coordinates 52° 13' 0" N 13° 11' 59" E. Meteorological data was collected using an automatic weather station of the Department of Agronomy and Crop Science in an experimental station of Humboldt University at the Thyrow location. The distance from the cultivated areas was about 10 km.

The total annual air temperature in 2009 was 9.4 ° C, which was 1.5 ° C warmer than 2010. The coldest periods were from December to February, with a minimum average air temperature reached in January of -5 ° C. December 2010 was 5 ° C colder than December 2009. The highest average air temperatures were recorded in the months July and August, with a maximum of 23 ° C in July 2010 (Figure 4).

The total annual precipitation in 2010 reached 619 mm, which was 80 mm higher than in 2009. In 2009 the highest precipitations were observed in the months of October and December, reaching 120 mm in October. The driest months were March, April and September.

2010 was characterized by regular precipitation, reaching a maximum precipitation rate in September of 120 mm and a minimum of 5 mm in July (Figure 4).



	2009	2010	1981-2010
Total annual air Temperature (° C)	9.4	7.8	9.2
Total annual precipitation (mm)	535.4	619.0	509.8

Figure 4: Total precipitation and average air temperature from 2009-2010 in comparison to the mean over the last 30 years at the Thyrow experimental station.

3.2.2 Management practices

The following management practices were carried out at both locations:

3.2.2.1 Friedersdorf location

<u>Soil</u>

The soil type at this location was characterized with the help of the soil texture feel test or "Fingerprobe" method (BODENKUNDE UND STANDORTLEHRE, HUMBOLDT UNIVERSITÄT ZU BERLIN, 2011). In 2009 the soil of the winter wheat experimental field was found to be composed of clay down to a depth of 40 cm and was ploughed down to 30 cm. From 40 to 90 cm sandy clay loam was found. From 90 cm there was a pure sand horizon with the groundwater depth greater than 100 cm. The soil of the silage maize experimental field in 2009 and 2010 up to the exploratory limit of 100 cm was clay and from 50 to 100 cm signals of oxidation were observed. Table 1 below, shows the relative values of total nitrogen (TN), total carbon (TC), total organic carbon (TOC), phosphor (P), potassium (K) and magnesium (Mg) of the soil samples from the experimental fields before they were fertilized.

Table 1: Chemical parameters from soil samples from the Friedersdorf location collected before the fertilization of the field. TC: total carbon, TOC: total organic carbon. TN: total nitrogen, P_{DL} and K_{DL}: phosphorous and potassium in Doppel-Lactat.

	TC (%)	TOC (%)	TN (%)	P_{DL} (mg 100 g^{-1})	K_{DL} (mg 100 g^{-1})
Winter wheat 2009	1.50 ± 0.09	1.40 ± 0.07	0.10 ± 0.01	2.20 ± 0.41	7.35 ± 0.59
Silage maize 2009	2.38 ± 0.11	2.06 ± 0.10	0.18 ± 0.01	6.90 ± 1.93	12.85 ± 4.41
Silage maize 2010	2.77 ± 0.33	2.61 ± 0.32	0.25 ± 0.03	19.93 ± 3.14	17.27 ± 2.89

The determination of total carbon (TC) and total nitrogen (TN) was carried out by means of elemental analysis. The prepared samples were measured in an elemental analyzer (CNS VarioMAX, from Elemental Analysis GmbH, Hanau). The total organic carbon (TOC) was analyzed with the VarioMAX TIC/TOC from Elemental Analysis GmbH, Hanau. The soluble phosphorus and potassium were determined after their extraction from the soil samples with a calcium solution (VDLUFA I, A 6.2.1.2, 1997). The phosphorus was measured spectrophotometrically from the filtrated aliquot using Continuous Flow Analysis (CFA) and the potassium was measured by flame photometry with Atomic Absorption Spectroscopy (AAS).

Crops

In the fields of the company Friedesdorfer Ackerbau GbR investigations were conducted on two types of crop: winter wheat and silage maize.

Winter Wheat

In 2009, the selected area for the experiment with winter wheat had a size of 13,200 m^2, divided into seven strips of 2,400 m^2 each. These strips were fertilized with mineral-N-fertilizer (calcium ammonium nitrate) and dry digestate, either combined or applied separately. Fertilization was split into two application dates (Table 2). The application rate was based on the analytically assessed total nitrogen contents of the dry digestate and was estimated based on the quantities of fertilizer used by the agricultural company.

To determine the yield and the yield structure, the plant material was harvested manually from five plots of 1 m^2 (1 x 1 m) on each experimental strip. These plots were located in the same position for all stripes (Figure 5). Due to extreme moist soil conditions during the sowing time in 2010, it was only possible to carry out the experiment with winter wheat in 2009.

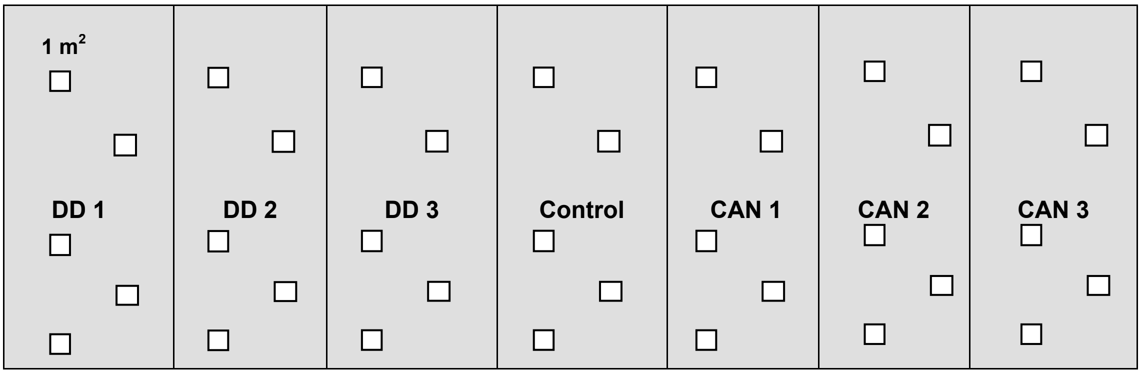


Figure 5: Winter wheat experimental field plots in Friedersdorf. DD: dry digestate, CAN: calcium ammonium nitrate.

Silage Maize

The experimental area used for the silage maize had a size of 12,000 m^2, divided into five strips of 2,400 m^2 each. These plots were fertilized with dry digestate and mineral-N-fertilizer in different application rates and at a single application date in spring (Table 2).

Table 2: Experimental fields in Friedersdorf. Different combination of dry digestate (DD) and calcium ammonium nitrate (CAN) applied to winter wheat and silage maize.

	Treatment	Autumn (kg ha^{-1} N)	Fertilizer	Spring (kg ha^{-1} N)	Fertilizer	Total N (kg ha^{-1})	Total P (kg ha^{-1})	Total K (kg ha^{-1})
Winter wheat	Control	0	--	0	--	0	0	0
	DD 1	75	DD	25	CAN	100	5.2	34.3
	DD 2	100	DD	50	CAN	150	6.9	45.8
	DD 3	150	DD	0	CAN	150	10.4	68.7
	CAN 1	0	--	100	CAN	100	0	0
	CAN 2	50	CAN	100	CAN	150	0	0
	CAN 3	0	--	150	CAN	150	0	0
Silage Maize	Control	0	--	0	--	0	0	0
	DD 1	--	--	100	DD	100	6.9	45.8
	DD 2	--	--	150	DD	150	10.4	68.7
	CAN 1	--	--	100	CAN	100	0	0
	CAN 2	--	--	150	CAN	150	0	0

To determine the dry-matter yield on each experimental strip, the plant material was harvested by hand from five plots of 2 m² (1.5 x 1.33 m) at each experimental strip. These plots were marked in each stripe at the same position.

3.2.2.2 Trebbin location

Soil

The soil was characterized for every experimental field with the help of the soil texture feel test method (BODENKUNDE UND STANDORTLEHRE, HUMBOLDT UNIVERSITÄT ZU BERLIN, 2011). In 2009, in the winter oilseed rape experimental field, the soil down to 70 cm consisted of loamy sand of which the first 30 cm were handled as plough horizons. Up to a lower limit of 100 cm there was sandy clay. In 2010 this plot experiment was carried out in a different field of the company Agrargenossenschaft Trebbin eG. In this field, loamy sand was only found down to 35 cm, followed up to 100 cm by sand.

In 2009 the soil of the winter rye experimental field down to 100 cm consisted of sand combined down to 40 cm with a colluvium horizon, ploughed down to a depth of 25 cm. In 2010 the plot experiments were also conducted in another field of the company. In this field, the soil down to 90 cm consisted of loamy sand of which the first 30 cm were handled as plough horizons. Down to a lower limit of 100 cm there was sandy clay loam.

In 2009 and 2010 the soil of the silage maize experimental field down to 75 cm consisted of loamy sand, of which the first 35 cm were handled as a plough horizon. Sand was present down to an assessment depth of 100 cm. The relative values of the total nitrogen (TN), total carbon (TOC), total organic carbon (TOC), phosphor (P) and potassium (K) of the soil samples from the experimental fields before being fertilized are represented in Table 3.

Table 3: Chemical parameters of soil samples from the Trebbin location collected before the fertilization of the fields. W.: winter, TC: total carbon, TOC: total organic carbon. TN: total nitrogen, P_{DL} and K_{DL}: phosphorous and potassium in Doppel-Lactat.

	TC (%)	TOC (%)	TN (%)	P_{DL} (mg 100 g^{-1})	K_{DL} (mg 100 g^{-1})
Winter rye 2009	0.93 ± 0.02	0.92 ± 0.05	0.06 ± 0.00	3.74 ± 0.37	13.28 ± 2.36
Winter rye 2010	1.64 ± 0.11	1.54 ± 0.17	0.18 ± 0.02	11.13 ± 1.84	16.76 ± 4.78
W. oilseed rape 2009	0.76 ± 0.02	0.71 ± 0.03	0.05 ± 0.00	6.17 ± 1.56	12.63 ± 0.64
W. oilseed rape 2010	4.33 ± 0.89	3.88 ± 0.64	0.36 ± 0.05	9.06 ± 4.10	25.10 ± 3.94
Silage maize 2009	0.90 ± 0.08	0,89 ± 0.08	0.05 ± 0.00	4.28 ± 0.30	16.06 ± 3.51
Silage maize 2010	1.75 ± 0.11	1.69 ± 0.11	0.17 ± 0.00	6.58 ± 0.41	12.63 ± 0.24

Crops

In the fields of the company Agrargenossenschaft Trebbin investigations were carried out on three types of crop: winter oilseed rape, winter rye and silage maize.

Winter oilseed rape

The experimental field used for winter oilseed rape had a dimension of 12,600 m^2 and was divided into 7 strips of 1,800 m^2 each. These experimental strips were fertilized with mineral-N-fertilizer and wet digestate in various application rates and at two different dates (Table 4).

Table 4: Experimental fields in Trebbin. Different combinations of wet digestate (WD) and calcium ammonium nitrate (CAN) by winter wheat, applied at two different dates.

	Treatment	Autumn (kg ha^{-1} N)	Fertilizer	Spring (kg ha^{-1} N)	Fertilizer	Total N (kg ha^{-1})	Total P (kg ha^{-1})	Total K (kg ha^{-1})
Winter oilseed rape	Control	0	--	0	--	0	0	0
	WD 1	40	WD	80	CAN	120	3.3	18.8
	WD 2	80	WD	80	CAN	160	6.7	37.7
	CAN 1	40	CAN	80	CAN	120	0	0
	CAN 2	80	CAN	80	CAN	160	0	0
	CAN 3	--	--	120	CAN	120	0	0
	CAN 4	--	--	160	CAN	160	0	0
Winter rye	Control	0	--	0	--	0	0	0
	WD 1	40	WD	80	CAN	120	3.3	18.8
	WD 2	80	WD	0	--	80	6.7	37.7
	WD 3	80	WD	40	CAN	120	6.7	37.7
	WD 4	0	--	80	WD	80	6.7	37.7
	WD 5	0	--	80/40	WD/CAN	120	6.7	37.7
	CAN 1	0	--	80	CAN	80	0	0
	CAN 2	0	--	120	CAN	120	0	0
	CAN 3	40	CAN	40	CAN	80	0	0
	CAN 4	80	CAN	40	CAN	120	0	0
Silage maize	Control	--	--	0	--	0	0	0
	WD 1	--	--	80	WD	80	6.7	37.7
	WD 2	--	--	120	WD	120	10	56.7
	WD 3	--	--	160	WD	160	13.3	75.5
	CAN 1	--	--	80	CAN	80	0	0
	CAN 2	--	--	120	CAN	120	0	0
	CAN 3	--	--	160	CAN	160	0	0

The application rate for both on-farm experiments was based on the analytically total assessed nitrogen contents of the wet digestate and was estimated regarding the quantities of fertilizer used by the agricultural company. Due to technical reasons, it was not possible to trace the selected parcels in order to collect the plant material like in the other plots. In order to obtain a precise yield sampling, Agrargenossenschaft Trebbin allowed 6 harvest plots of 27 m^2 each to stand during the harvest in each strip. From these harvest plots the yield and yield structure were estimated.

Winter rye

The experimental field of winter rye had a size of 18,000 m^2 and was organized into 10 strips of 1,800 m^2 each. Every strip was fertilized in various application rates and at different times with mineral-N-fertilizer and wet digestate (Table 4). To determine the yield and yield structure, the plant material was harvested manually from five plots of 1 m^2 each on every experimental strip. These plots were marked in each stripe at the same position.

Silage maize

The experimental field with silage maize had a size of 9,000 m^2, divided into 5 test strips with a size of 1,800 m^2 each. The strips were fertilized with mineral-N-fertilizer and wet digestate in different application rates and on one application date (Table 4). To determine the dry-matter yield, the plant material was also harvested by hand from five plots of 2 m^2 on each experimental strip. These plots were marked in each stripe at the same position.

3.2.3 Dry-matter yield, grain yield, yield formation and crop quality

From every experimental field the dry matter of the straw from each crop was determined. Also, the dry-matter was determined from the whole silage maize plant material. The grain yield of winter wheat, winter rye and winter oilseed rape were also calculated. The grain yield of winter wheat and winter rye were calculated based on 86 % dry matter (DM) and the oilseed rape on 91 % DM.

The grains of winter wheat, winter rye and winter rape were used for the analysis of the yield formation under the principle of HEUSER (1954). Through this analysis, yield components such as stand density (spikes per square meter), corn number per spike and the thousand kernel weight was determined. As external quality parameter the hectoliter weight (EGGER & MOREL, 1989) was determined. As an internal quality parameter the protein content of winter wheat by near-infrared spectroscopy (NIRS) reflectance was estimated.

3.2.4 Statistics

The data of the dry-matter yield, grain yield, yield formation and crop quality of the harvested plots was calculated as arithmetic means (± standard error of mean). No other statistical analyses were carried out because the basic principles of establishing an exact field experiment were not met. The specific functions of this experiment were testing, demonstration and transfer of information to the farmers.

3.3 Results

3.3.1 Friedersdorf location

3.3.1.1 Winter wheat 2009

In 2009 it was observed that in a clay soil the highest grain yield was obtained with the application of 150 kg ha^{-1} N mineral fertilizer in two doses, one of 50 kg ha^{-1} N in autumn and one of 100 kg ha^{-1} N in spring. With these application rates the highest grain yield, one of the highest population densities and one of the highest protein contents were achieved. The application of 150 kg ha^{-1} N mineral fertilizer in one dose in spring led to the highest stand density and the highest protein content (Table 5).

Table 5: Yield Parameters of winter wheat at the Friedersdorf location. DD: Dry digestate. CAN: calcium ammonium nitrate. Mean values and standard deviations.

	2009					
	Straw yield (dt ha^{-1} DM)	**Grain yield (dt ha^{-1} 86 % DM)**	**Population density (Spikes m^{-2})**	**Corn number per Spike**	**Thousand kernel weight (g)**	**Protein content (%)**
Control	40.0 ± 15.5	37.6 ± 9.6	295 ± 44.5	28.4 ± 6.7	44.9 ± 2.0	14.0 ± 0.8
DD 1	67.9 ± 16.6	70.1 ± 16.1	428 ± 92.9	35.6 ± 2.7	46.1 ± 2.0	15.0 ± 3.3
DD 2	60.6 ± 24.8	73.9 ± 19.6	435 ± 77.9	36.8 ± 4.6	45.8 ± 3.1	17.0 ± 3.1
DD 3	53.5 ± 20.5	53.0 ± 17.3	360 ± 95.8	32.0 ± 4.7	45.5 ± 0.9	14.9 ± 1.6
CAN 1	52.9 ± 6.9	67.0 ± 6.7	403 ± 24.8	39.3 ± 4.4	42.4 ± 1.8	16.9 ± 0.7
CAN 2	63.6 ± 17.1	76.3 ± 16.2	430±104.1	40.2 ± 4.1	44.6 ± 1.2	19.1 ± 1.1
CAN 3	60.6 ± 14.8	72.7 ± 7.8	500 ± 80.8	35.1 ± 4.1	42.0 ± 1.4	21.2 ± 0.9

The application of dry digestate had a positive effect in comparison to the unfertilized control. The use of 150 kg ha^{-1} N dry digestate divided into a dose of 100 kg ha^{-1} N in autumn and a second dose of 50 kg ha^{-1} N in spring, led to results which were quite comparable to the use of 150 kg ha^{-1} N mineral fertilizer in one or two doses. The highest straw yield was obtained after the application of 100 kg ha^{-1} N dry digestate, divided into two doses, 75 kg ha^{-1} N in autumn and 25 kg ha^{-1} N in spring, respectively. The highest dry-matter yields were observed in two consecutive years (2009 and 2010) in the unfertilized strips. In 2009 the use of 150 kg ha^{-1} N dry digestate had a positive effect of 6 % compared to the application of 150 kg ha^{-1} N mineral fertilizer and 24 % compared to the fertilization with 100 kg ha^{-1} N mineral fertilizer (Table 6).

Table 6: Dry-matter yield (dt ha^{-1} DM) of silage maize at the Friedersdorf location. DD: Dry digestate. CAN: calcium ammonium nitrate. Mean values and standard deviations.

Dry-matter yield (dt ha^{-1} DM)	Year	Treatment				
		Control	DD 1	DD 2	CAN 1	CAN 2
	2009	222.6 ± 79.8	155.6 ± 46.8	179.3 ± 44.2	137.7 ± 35.7	168.2 ± 44.0
	2010	160.3 ± 28.2	147.5 ± 18.2	122.9 ± 53.4	154.7 ± 8.2	136.1 ± 23.1

In 2010, the application of 100 kg ha^{-1} N mineral fertilizer led to the highest dry-matter yield, being 4.5 % higher than the treatment fertilized with 100 kg ha^{-1} N dry digestate and 20 % higher than achieved by the application of 150 kg ha^{-1} N dry digestate (Table 6).

3.3.2 Trebbin location

3.3.2.1 Winter rye

In 2009 on a sandy soil, it was observed that the highest grain yield and population density were obtained after the use of 120 kg ha^{-1} N mineral fertilizer in one application in the spring. However, the highest straw yield was reached with 80 kg ha^{-1} N mineral fertilizer in one dose also in spring. The combination of 80 kg ha^{-1} N wet digestate in autumn and 40 kg ha^{-1} N mineral fertilizer in spring led to a straw and grain yield, a population density, a number of grains per spike and thousand kernel weight comparable to the use of 120 kg ha^{-1} N mineral fertilizer. The use of a single application of digestate of 80 kg ha^{-1} N in autumn resulted in a higher protein content (Table 7).

In 2010 the results varied in relation to 2009. In a loamy sand soil, followed down to 90 cm by sandy clay loam, the best results were obtained by using 40 kg ha^{-1} N mineral fertilizer in autumn and 40 kg ha^{-1} N mineral fertilizer in spring, reaching the highest straw and grain yield, the highest population density and highest thousand kernel weight. Comparable results were observed in parameters like the straw and grain yield and grains per spike after the use of 40 kg ha^{-1} N wet digestate in autumn and 80 kg ha^{-1} N mineral fertilizer in spring (Table 7).

Table 7: Yield parameters of winter rye at the Trebbin location. WD: wet digestate. CAN: calcium ammonium nitrate. Mean values and standard deviations.

	Straw yield (dt ha^{-1} DM)	Grain yield (dt ha^{-1} 86 % DM)	Stand density (Spikes m^{-2})	Corn number per Spike	Thousand kernel weight (g)	Protein content (%)
			2009			
Control	20.4 ± 4.1	19.0 ± 3.9	171 ± 15.3	33.1 ± 3.0	33.3 ± 1.0	9.4 ± 0.7
WD 1	28.3 ± 2.0	33.1 ± 3.2	198 ± 1.0	48.7 ± 2.2	34.3 ± 1.1	8.5 ± 0.3
WD 2	29.5 ± 2.1	19.0 ± 4.6	144 ± 26.8	38.6 ± 8.1	34.6 ± 3.0	9.9 ± 0.8
WD 3	32.0 ± 6.1	35.3 ± 6.2	215 ± 16.2	46.7 ± 3.6	35.0 ± 2.4	8.4 ± 1.0
WD 4	24.4 ± 4.5	23.4 ± 3.8	171 ± 36.5	40.2 ± 5.0	34.4 ± 1.8	9.0 ± 0.5
WD 5	27.4 ± 6.9	31.6 ± 4.9	169 ± 97.0	45.1 ± 3.6	34.2 ± 1.2	8.0 ± 0.5
CAN 1	32.4 ± 4.8	37.3 ± 4.4	227 ± 24.7	46.8 ± 3.0	35.1 ± 1.2	8.5 ± 0.5
CAN 2	32.1 ± 5.2	40.2 ± 5.3	242 ± 32.7	46.6 ± 2.8	35.8 ± 0.6	9.8 ± 0.6
CAN 3	29.8 ± 1.8	33.4 ± 1.7	208 ± 4.4	46.7 ± 2.2	34.4 ± 0.4	8.1 ± 0.3
CAN 4	26.2 ± 6.3	33.2 ± 6.2	206 ± 31.6	46.5 ± 1.4	34.6 ± 0.8	7.9 ± 0.5
			2010			
Control	43.4 ± 12.9	33.9 ± 8.8	272 ± 15.3	39.7 ± 8.0	29.8 ± 4.0	n.a. ± n.a.
WD 1	69.5 ± 5.5	32.2 ± 5.5	350 ± 18.0	52.0 ± 3.8	17.7 ± 2.3	n.a. ± n.a.
WD 2	53.2 ± 4.4	37.9 ± 4.4	305 ± 26.8	50.4 ± 6.6	24.9 ± 2.7	n.a. ± n.a.
WD 3	68.1 ± 10.8	37.3 ±10.8	359 ± 16.2	49.9 ± 4.8	20.6 ± 4.4	n.a. ± n.a.
WD 4	64.0 ± 9.8	38.4 ± 9.8	344 ± 36.5	50.4 ± 3.7	21.5 ± 1.8	n.a. ± n.a.
WD 5	67.4 ± 11.2	32.3 ±11.2	367 ± 97.0	54.6 ± 6.4	17.6 ± 4.0	n.a. ± n.a.
CAN 1	58.5 ± 9.8	44.4 ± 9.8	299 ± 24.7	62.0 ± 10.1	25.3 ± 5.2	n.a. ± n.a.
CAN 2	66.4 ± 9.4	49.9 ± 9.4	353 ± 32.7	58.3 ± 1.4	24.2 ± 0.9	n.a. ± n.a.
CAN 3	72.7 ± 3.3	56.9 ± 3.3	362 ± 4.4	58.1 ± 2.2	27.1 ± 1.0	n.a. ± n.a.
CAN 4	55.9 ± 4.7	52.9 ± 4.7	327 ± 31.6	58.4 ± 1.8	27.8 ± 1.9	n.a. ± n.a.

The application of wet digestate in one dose in autumn had a positive effect on the thousand kernel weight (Table 7).

3.3.2.2 Winter oilseed rape

Similar results were obtained in 2009 and 2010. The soil in 2009 corresponded to a loamy sand soil down to a depth of 70 cm, followed by a sandy clay soil. In 2010 this type of soil was only found down to 35 cm, followed by a sand soil. The highest grain yield was observed after using 80 kg ha^{-1} N wet digestate in autumn and 80 kg ha^{-1} N mineral fertilizer in spring (WD 2). Comparable results were observed after the use of 160 kg ha^{-1} N mineral fertilizer in two doses (CAN 2). The development of the thousand-grain weight was aided by the use of 120-160 kg ha^{-1} N mineral fertilizer at one dose in the spring (Table 8).

Table 8: Grain yield (dt ha^{-1} 91 % DM) and thousand kernel weight (g) of winter oilseed rape at the Trebbin location. WD: wet digestate. CAN: calcium ammonium nitrate. Mean values and standard deviations.

	2009		2010	
	Grain yield (dt ha^{-1} 91 % DM)	Thousand-kernel weight (g)	Grain yield (dt ha^{-1} 91 % DM)	Thousand kernel weight (g)
Control	18.7 ± 3.4	4.6 ± 0.1	31.6 ± 2.3	n.a. ± n.a.
WD 1	36.4 ± 3.5	4.6 ± 0.2	39.6 ± 2.0	n.a. ± n.a.
WD 2	38.9 ± 5.2	4.8 ± 0.2	43.1 ± 1.6	n.a. ± n.a.
CAN 1	24.4 ± 11.9	4.8 ± 0.5	38.0 ± 2.9	n.a. ± n.a.
CAN 2	32.3 ± 4.8	5.0 ± 0.2	38.0 ± 0.5	n.a. ± n.a.
CAN 3	24.3 ± 5.7	5.1 ± 0.5	42.6 ± 2.5	n.a. ± n.a.
CAN 4	26.8 ± 6.9	5.1 ± 0.4	40.6 ± 0.6	n.a. ± n.a.

3.3.2.3 Silage maize

In a loamy sand soil, the use of wet digestate in one dose of 120 kg ha^{-1} N in 2009 and 160 kg ha^{-1} N in 2010 led to the highest dry-matter yield. Comparable results were obtained after the use of 160 kg ha^{-1} N mineral fertilizer (Table 9).

Table 9: Dry-matter yield (dt ha^{-1} DM) of silage maize at the Trebbin location. WD: Wet digestate. CAN: calcium ammonium nitrate. Mean values and standard deviations.

Dry-matter yield (dt ha^{-1} DM)	Year	Treatment						
		Control	WD 1	WD 2	WD 3	CAN 1	CAN 2	CAN 3
	2009	110.2±11.4	119.9±18.4	163.7±27.1	160.7±13.5	136.8±14.3	136.2± 8.3	146.9±37.3
	2010	148.7±21.1	165.3±25.6	171.0±15.7	192.3±14.2	179.5±24.4	180.3±25.7	187.0±31.7

3.4 Discussion

Winter wheat

The highest crop yield was obtained after application of 150 kg ha^{-1} N mineral fertilizer split into two doses with 50 kg ha^{-1} N in autumn and 100 kg ha^{-1} N in spring (CAN 2). The second dose corresponded to 60 % of the total fertilizer, which was the amount of fertilizer applied at the phenological development stage 32. This application rate contributed to an increased yield, presumably due to a high supply of nitrogen readily available during the stem extension until the heading. This dose also resulted in a higher number of grains per spike, and one of the highest protein contents, thousand kernel weights and population densities.

Results comparable to those observed after the use of mineral-N-fertilizer applied in two doses (CAN 2) were also observed after the combination 100 kg ha^{-1} N of dry digestate and 50 kg ha^{-1} N mineral fertilizer in spring (DD 2). This application led to a crop yield 4 % lower than the one obtained after the application of 150 kg ha^{-1} N mineral fertilizer in two doses (CAN 2). The spring dose corresponding to 50 kg ha^{-1} N mineral fertilizer at the phenological development stage 32 contributed to a higher protein and starch content in the grain, higher grains per spike and thousand kernel weight compared to a lower application of dry digestate combined with mineral-N-fertilizer (DD 1) or one application of 150 kg ha^{-1} N of dry digestate in autumn (DD 3). This fact is due to the direct availability of the nitrogen in the form of mineral fertilizer during the grain formation.

The highest population density was observed in the treatment fertilized with 150 kg N ha^{-1} mineral fertilizer in spring (CAN 3). This led to a reduced production of grains per spike and a lower thousand kernel weight. This is probably due to competition between plants for soil-available resources from previous treatments. This treatment also resulted in a 5 %-lower yield than the one obtained with CAN 2, but with a 10 % higher protein content. Furthermore, the protein content was higher than expected (14-15 %). The wheat grain N content and the yield components are largely influenced by the amount of N absorbed after the anthesis and by the amount of remobilized N originating from pre-anthesis uptake since these two sources of N are used for the storage protein synthesis. After the anthesis, leaves become a source of N because the N in the leaves is hydrolyzed and exported in the form of amino acids to the grains. 60-95 % of the N in the grains comes from the remobilization of N stored in roots and shoots before the anthesis and a less important fraction of seed N comes from post-flowering uptake and N translocation to the grain (KICHEY *et al.*, 2007). As soon as the content of available N from previous crops was high enough for the development of the plants, probably the high second application in spring contributed to a direct movement of the nitrogen to the grains.

The dry digestate applied in this experiment had a C/N ratio of 12. JARVIS *et al.* (1996) suggested that C/N characteristics of organic materials provide an indication of whether net mineralization or immobilization will occur when organic materials are added to soil. The mineralization is the conversion of organic forms of N into mineral forms, which are readily available for the plants. The process is quite complex since it takes place under the influence of the physicochemical properties of the added organic material; abiotic factors such as pH, temperature, water and clay content of the soil; and the characteristics of the soil organisms involved in the decomposition process (NETT *et al.,* 2010). The C/N ratio of dry digestates is not as high as that of farmyard manure (20), but also the N does not show as being readily available as by mineral fertilizer. Presumably the dry digestate needs time to be mineralized before it can be compared with the effect of the mineral-N-fertilizer and it also seems to be easily retained due to physicochemical properties of this type of soil.

Silage maize

The soil nitrogen supply depends on the reserves of nitrogen in the soil at the beginning of spring, after the leaching period and before the beginning of mineralization, the effect of the previous crop and the mineralization of the soil (ELIZALDE, 1987).

The results of the crop yield from 2009 and 2010 showed that the supply of nitrogen in the soil in the unfertilized control was high enough to achieve the highest harvest. This fact is reflected in the soil analysis carried out before the fertilization of the experimental field. This indicates that the unfertilized control stripe was probably fertilized in previous years with N not readily available. A slower process of mineralization of the dry digestate compared with the one of mineral-N-fertilizer and the kind of soil at this location might be that the conditions for the N remained in the soil in 2009 at an optimal dose for cultivation. It can also be observed that in 2010 the supply of nitrogen in the unfertilized control was also optimal for maize. This confirms the previously exposed. The nitrogen that has been slowly mineralized was not so easily washed out and resulted in a progressively use of N. In addition, part of the nitrogen probably also comes from the rest of the previous crops.

From the emergence of the plants until they produce five leaves (30-40 days after emergence) the growth of maize is slow. The highest absorption rate of mineral elements begins after this stage in a fast growth phase, with an elongation of internodes, leaf elongation and appearance of new leaves increasing the surface exposed to sunlight and increasing the rate of accumulation of dry matter. Nitrogen uptake reaches its greatest intensity when ears are formed and the hairs appear. Then, during the ripening of grain, nitrogen absorption decreases and at the time of harvest 65 % of the nitrogen will be concentrated in the grains (ELIZALDE, 1987).

In 2009 the application of 150 kg ha^{-1} N dry digestate (DD 2) led to the highest yield of all fertilized plots. This might be because the plant may have used the readily available nitrogen from previous crops in the elongation phase and after mineralization of dry digestate the plants have used the N available for the formation of spikes. The application of 150 kg ha^{-1} N mineral fertilizer (CAN 2) delivers comparable results to the application of digestates. Based on the data for the control, the amount of available N from the mineralization of the digestate from previous years resulted in a 20 %-lower harvest in 2009. The effectiveness of mineral-N-fertilizers at a rate of 100 kg ha^{-1} N (CAN 1) is comparable to that of 100 kg ha^{-1} N dry digestate (DD 1) and exceeds yields obtained by the application of 150 kg ha^{-1} N of both fertilizers (CAN 2 / DD 2). This could be due to a cumulative effect of N in the soil. According to HAY & PORTER, 2006 the biomass of the crop tends to increase linearly with N application up to a limit in which the yield is maintained or reduced. As a result, it could be possible that the application of 150 kg ha^{-1} N of both fertilizers has exceeded the physiological needs of the plant resulting in the re-mineralization of the remaining material and long-term assimilation by soil microorganisms. Inevitably a significant part remains in the soil as relatively stable organic N (JINGGUO & BAKKEN, 1997).

Winter rye

In spring, at the time of intense growth, the nutritional need of the rye is extremely large because the nutrient uptake for the formation of dry matter hurries ahead (REINER, ET AL., 1979). In 2009, in a purely sandy soil, the crop physiological needs were met with a single dose of 120 kg ha^{-1} N mineral fertilizer in spring (CAN 2). The application of mineral-N-fertilizer in winter probably would have led to losses of N through leaching. The application of 80 kg ha^{-1} N wet digestate in autumn and 40 kg ha^{-1} N mineral fertilizer in spring (WD 3) led to comparable results. The C/N ratio of the wet digestate was 10. Fertilizers with a relatively low C/N as in the case of the wet digestate, have fast nitrogen availability. However, part of the N is still linked to an organic form and needs to be mineralized so the nitrogen becomes available to the plant. It can be observed that the plant took the nutrients that were probably released gradually through the process of mineralization, necessary for the growing process. The dose of mineral-N-fertilizer in spring favored the development in later stages contributing to a high plant density, corn number per spike, thousand kernel weight and protein content.

In 2010, the best results were obtained combining a dose of 80 kg ha^{-1} N wet digestate in autumn and 40 kg ha^{-1} N mineral fertilizer in spring (WD 3). These results were probably achieved due to the soil type. A soil with relatively high silt content like the one in this experimental field has a lower infiltration rate and a medium level of nutrient storage as compared to a sandy soil (PORTA, *et al.*, 1999). Moreover, it is known that the combination of organic and mineral fertilization leads to higher crop yields compared to the use of only inorganic or organic fertilizers (KELLER *et al.*, 1997). The organic dose applied in autumn was not only optimal for the crop in this kind of soil, but also the grain yield results were superior to those of 2009. Presumably, the plant obtained the necessary nutrients throughout all of its growth stages and the second dose was crucial in the formation of structural organs.

Comparable results were observed after the application of wet digestate in autumn in a dose of 80 kg ha^{-1} N (WD 2) and also after application of 80 kg ha^{-1} N in a dose in the spring (WD 4). This indicates that the ability of N losses by leaching in these soils is low, and the time of application of the wet digestate seems to be indifferent to the plant.

Winter oilseed rape

The highest grain yield in a loamy sand soil in two consecutive years (2009 and 2010) was obtained after the fertilization with 160 kg ha^{-1} N divided into two doses of 80 kg ha^{-1} N wet digestate in autumn and 80 kg ha^{-1} N mineral fertilizer in spring (WD 2). For a normal autumn development oilseed rape takes about 50-80 kg ha^{-1} N. Under normal growth conditions, N-fertilizer is not necessary before the sowing of oilseed rape because the mineralization in autumn usually provides enough nitrogen for the development of the plant in winter. In spring, the nitrogen plays a crucial role for the yield formation. It induces the mass growth, the silique formation and thus the number of seeds per unit area (CHRISTEN & FRIEDT, 2007). In spring, the necessities of the plants were also met due to the availability of N after the mineralization process of the wet digestate applied in autumn and the second doses of direct available N in spring. The application of 50 % less digestate in autumn (WD 1) delivered comparable results in 2009. However, it can be observed that the best results in 2010 were obtained after the application in two consecutive years of 80 kg ha^{-1} N wet digestate in autumn (WD 2), probably due to the progressive release of N after the mineralization process. The best results for the mineral fertilizer were obtained with the application of 160 kg ha^{-1} N divided into two doses of 80 kg ha^{-1} N in spring and in autumn (CAN 2). In 2010 the application of 120 kg ha^{-1} N in one dose in spring (CAN 3) led to better results. This fact shows that the loss of N in these soils was not high. The thousand kernel weight was positively influenced by an application in spring of 120-160 kg ha^{-1} N in a directly available form (CAN 3 - CAN 4).

Silage maize

Due to its slow early growth, maize has late requirements of nutrients. Maize in relation with other crop species is more capable to use more efficiently the released nitrogen from organic compounds (ANONYM, 2011). That could explain why the best results were obtained after the application of wet digestate in a dose of 120-160 kg ha^{-1} N (WD 2 - WD 3) in 2009 and 2010. The risk of transfer of nitrogen in the form of nitrate in the deeper soil layers during the early growth phase from maize through rainfall is high (ANONYM, 2011). Therefore the application of wet digestate is preferable in order to avoid leaching losses. However, the results of 2010 were not as expected. The results after using mineral-N-fertilizers were quite comparable to the use of 160 kg ha^{-1} N digestate. This could indicate that the plant nutritional needs were met after the mineralization of the previous crop and the directly available dose of nitrogen was applied in spring.

3.5 Conclusions

The results of this study are consistent with those found in the literature so far. Firstly, there has been a positive effect of the digestate in most cultivars compared with the unfertilized control. Secondly, it has been observed that the effect depends on the nutritional needs of the plant. Therefore, the application of the digestates is more effective in those plants that require a late fertilization. It should also be taken into account that in a sandy soil the digestates are not washed from the soil profile as easily as the mineral-N-fertilizers and, as long as they remain in the profile, the organic material is mineralized and the nutrients, which are attached in an organic form, are released gradually and the effect of fertilization remains for longer. In a clayey soil, a tendency of accumulation of nutrients has been documented. If the nutrient content exceeds the nutritional needs of the plant, the remaining nutrients could be re-mineralized and in the long-term can be assimilated by soil microorganisms leading to a less effective nutrient utilization by the plant. An important fact to consider is the combination of the digestate with mineral-N-fertilizers, especially for those crops that have high nitrogen requirements in spring.

In this chapter the effects of two types of digestate on different crops and soil conditions have been studied based on the needs of the farmer. However, there are other aspects that need to be investigated in more depth. In the next chapter both digestates will be compared with mineral-N-fertilizers, as well as with other organic fertilizers with similar properties in an exact field experiment. Two crops will be monitored throughout the period of growth and development.

Chapter 2

Effects of digestates and other fertilizers on selected plants in a randomized complete block design

4 Chapter 2: Effects of digestates and other fertilizers on selected plants in a randomized complete block design

4.1 Introduction

In this study two crops, which are of great interest to the farmer, were chosen. *Sorghum bicolor* var. *sudanense* is a C-4 plant similar to maize, from the northeastern region of equatorial Africa. Because of its origin, this plant is drought-tolerant and at the same time has high biomass with a relatively low demand for nutrients and soil quality (ROLLER *et al.*, 2009). In comparison to maize, sorghum has a higher tolerance to drought because of its extensive root system and needs 30 % less water to produce a similar biomass (ROUX, 2010). It is also an interesting candidate as a co-substrate for the production of biogas because of its content in high dry matter, its silage capacity and high production of methane (FNR, 2010). In relation to its spread and persistence strategy, it is not considered an invasive plant species, although so far there have been no long-term studies on the effect of sorghum in agricultural ecosystems (ROLLER *et al.*, 2009). However, the potential of *Sorghum sudanense* as co-substrate for the production of biogas is not only of great interest in this study; wheat has also been chosen because it is one of the main sources of vegetable protein in human nutrition. The winter wheat variety "Discus" has good properties like a high resistance to cold temperatures and it easily adapts to many different environmental conditions. Besides, it is a quality A grain, which means that it has a high and stable yield, high protein content, high falling number and a stable hectoliter weight (DEUTSCHE SAATVEREDELUNG AG., 2010).

Most of the literature is focused on the effect of wet digestate as fertilizer on wheat, maize, rapeseed and rye, but not on *Sorghum sudanense*. In this study, literature references of studies describing the effect of the digestates as fertilizer on maize have been taken into consideration, since sorghum has similar physiological characteristics as maize. According to research conducted by KAUTZ & RAUBER (2006), the use of digestate from biogas production using, on the one hand, manure as input material for the biogas production and, on the other hand, renewable resources, results in a maize yield which tends to be higher than the unfertilized control. SCHNEIDER-GÖTZ & MASTEL (2007) found that the application of digestates as fertilizer led to comparable results to the use of mineral fertilizer or the combination of liquid manure and mineral fertilizers. WENDLAND *et al.*, 2006 compared the effect of digestates with other organic fertilizers. They found that the digestate had a positive effect on maize yields in comparison to the unfertilized control and a comparable effect on the use of liquid manure.

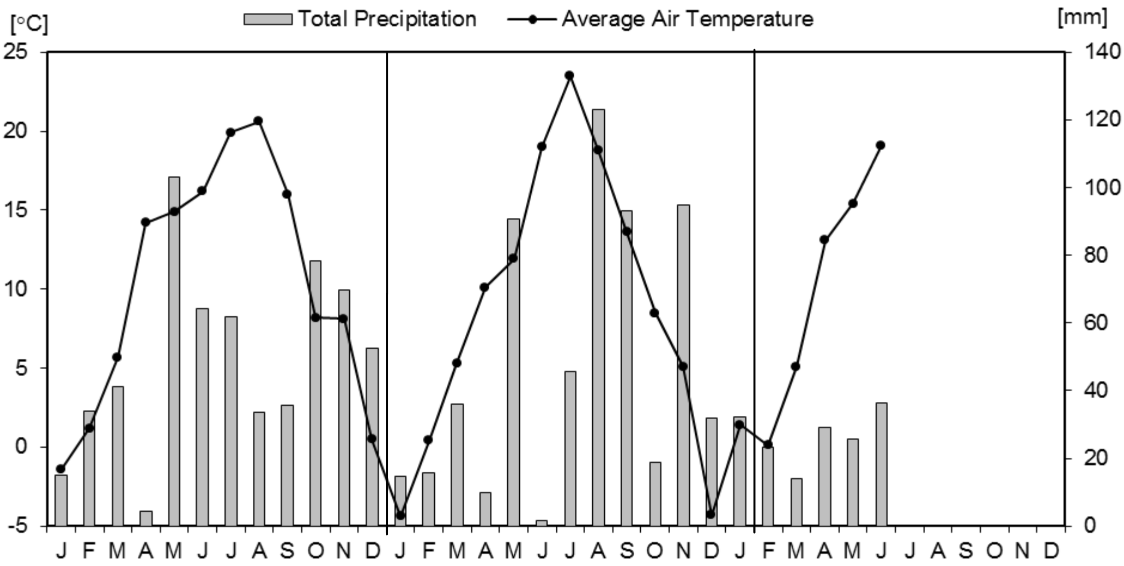
Some studies have focused not only on the grain yield of wheat, but also on some aspects of internal and external quality. A study by BRENNER & CLEMENS (2005) described the effect of wet digestate on the grain yield, the straw yield and the thousand kernel weight of winter wheat in comparison to liquid fertilizer and the unfertilized control. These authors observed no significant differences between the effects of wet digestate and liquid manure on grain yield of wheat. But they did find significant differences when comparing to the unfertilized control. STINNER & MÖLLER (2005) compared the effect of a treatment with dry digestate applied before the sowing and wet digestate applied as topdressing on the grain yield and grain protein content and compared them to an unfertilized control. They found that the grain yield fertilized with both digestates tends to be higher than the unfertilized control. However, the treatment fertilized with both digestates showed significantly higher protein content than the unfertilized control.

This chapter is focused not only on the effect of wet and dry digestate on the dry-matter yield and grain yield of both crops, but also describes their process of growth and development. Because winter wheat is an important plant for human consumption, the effects of digestate fertilization on the internal and external quality of the grain are also taken into account. In addition, the effects of dry and wet digestates will be compared with the effect of organic and mineral fertilizers.

4.2 Material and Methods

4.2.1 Field site description

From 2009 until 2011 field experiments were carried out on the experimental station of the Humboldt University of Berlin in Dahlem. This is located at 52° 28' north latitude and 13 ° 18' east longitude on 51 m above sea level.
The climatic data from the meteorological station of Berlin-Dahlem was collected from 2009 until 2011. 2009 was characterized by a minimal precipitation of 4.2 mm in April and a maximum of 103.1 mm in May. The average temperatures increased gradually from January with -1.4 ° C until August with 20.6 ° C. From August, the average temperatures decreased progressively and reached a minimum of -4.4 ° C in January 2010. The same pattern was observed in 2010, with maximum temperatures reaching about 23.5 ° C in July and the minimum was reached with -4.3 ° C in December. Precipitation varied in comparison to 2009, reaching a minimum of 1.6 mm in June and a maximum of 122.9 mm in August. 2011 was characterized by regular and scant precipitations in May in comparison to the previous two years and temperatures tended to be higher (Figure 6).



	2009	2010	2011	1981-2000
Average annual Air Temperature (° C)	10.3	9.0	-	9.9
Total annual Precipitation (mm)	592.7	575.8	-	561.9

Figure 6: Monthly values, total precipitation and average air temperature from 2009-2011 at the experimental station in Berlin-Dahlem.

The soil type in Berlin-Dahlem is an Albic Luvisol (FAO, 2006) which consist, from 0 to 30 cm, of a plowed weakly to moderately humic horizon followed from 30 to 50 cm by a brownish upper horizon produced by leaching. From 50 to 70 cm the soil consists of a horizon with an irregular or broken upper boundary resulting from the tonguing of bleached soil material into an illuviation horizon (BERGER, ET AL., 2010). From 70 to 90 cm there is a subsoil horizon enriched with clay. The effective rooting depth reaches down till 10 dm, the usable field capacity in the effective root zone is medium (180 mm) and the cation exchange capacity in the root zone is low (4.8 cmolc kg^{-1}). Soil texture is composed from 0 to 30 cm soil depth of loamy sand followed by a sandy loam to a depth of 80 cm (Table 10) (LEHR- UND FORSCHUNGSSTATION HUMBODLT UNIVERISTÄT ZU BERLIN, 2010).

Table 10: Soil Texture at the experimental field in Berlin Dahlem.

Layer	**Particle size fractions (%)**		
(cm)	Sand	Silt	Clay
0 - 30	72.1	25.0	2.9
30 - 50	69.8	25.5	4.7
60 - 80	65.3	23.4	11.3

4.2.2 Experimental design and assessment parameters

To investigate the effect of wet and dry digestates in direct comparison to conventional fertilizers such as mineral-N-fertilizer (calcium ammonium nitrate), liquid manure, and farmyard manure, a field experiment was carried out from 2009 until 2011 within a randomized complete block design of 40 x 28.5 m. An untreated control was used as a reference. The randomized complete block design consisted of six treatments with four replications (Figure 7).

4. Rep.	**2**	**6**	**4**	**5**	**1**	**3**
3. Rep.	**6**	**5**	**2**	**3**	**4**	**1**
2. Rep.	**4**	**3**	**1**	**6**	**2**	**5**
1. Rep	**1**	**2**	**3**	**4**	**5**	**6**

Treatments

1 Control
2 CAN
3 Wet digestate
4 Dry digestate
5 Liquid manure
6 Farmyard manure

Figure 7: Randomized complete block design on the experimental fields in Berlin-Dahlem. Rep.: replication. CAN: calcium ammonium nitrate.

Every plot had an extension of 9 m in length and 4.5 m in width. To prevent edge effects, a core of 8 m in length and 1.5 m in width was harvested. Each field was surrounded by an edge of 1.5 m in width.

Sorghum bicolor var. *Sudanense* was sown as preceding experimental crop in spring 2009. Winter wheat variety "Discus" was sown in autumn 2009 as successive crop. The intermediate crop previous to sorghum was potato (*Solanum tuberosum*). The same experiment was conducted in 2010-2011 in an adjacent plot, in which *Phacelia tanacetifolia* was sowed as preceding intermediary crop (Table 11).

Table 11: Crop sequences corresponding to the experiment 1 and 2.

2008-2009	**2009**	**2009-2010**	**2009-2010**	**2010**	**2010-2011**
Solanum tuberosum	*Sorghum sudanense*	Winter wheat	*Phacelia tanacetifolia*	*Sorghum sudanense*	Winter wheat
Experiment 1			**Experiment 2**		

Soil chemical analyses were carried out before and after the experiments. The objective was to obtain information about the effect of the digestates and the organic and mineral conventional fertilizers on the soil chemical status after two vegetative periods (Table 12).

Table 12: Soil chemical properties 1) before and 2) after the winter wheat vegetative period corresponding to the experiment 1 and 2. TN: total nitrogen, TC: total carbon, TOC: total organic carbon, P_{DL}/K_{DL}: phosphorous and potassium in Doppel-Lactat.

	Year	TN (%)	TC (%)	TOC (%)	P_{DL} mg 100 g^{-1}	K_{DL} mg 100 g^{-1}
Experiment 1	Spring 2009	0.06 [1)]	0.94	0.89	25.85	24.13
	Autumn 2010	0.05 [2)]	0.87	0.68	26.75	20.44
	Difference	**-0.01**	**-0.07**	**-0.21**	**0.90**	**-3.69**
Experiment 2	Spring 2010	0.07	1.05	0.72	33.17	24.53
	Autumn 2011	0.07	1.06	0.71	34.58	16.56
	Difference	**0.00**	**0.01**	**-0.01**	**1.41**	**-7.97**

In order to acquire more detailed information, soil analysis for each treatment were also performed one month after fertilization and after the growing season for winter wheat corresponding to experiment 2 (Table 13).

Table 13: Soil chemical properties 1) one month after the fertilization of the experiment 2 and 2) after vegetative period corresponding to winter wheat. TN: total nitrogen, TC: total carbon, TOC: total organic carbon, P_{DL}/K_{DL}: phosphorous and potassium in Doppel-Lactat.

	Treatment	TN (%)	TC (%)	TOC (%)	P_{DL} mg 100 g^{-1}	K_{DL} mg 100 g^{-1}
Experiment 2	**Control**	0.069 [1)]	1.105	0.748	33.24	18.75
		0.070 [2)]	1.064	0.715	34.58	16.56
	Difference	**0.002**	**-0.041**	**-0.033**	**1.66**	**-2.19**
	Mineral	-	-	-	-	-
		0.073	1.073	0.725	33.81	16.81
	Difference	-	-	-	-	-
	Wet digestate	0.076	1.113	0.797	36.96	20.75
		0.074	1.062	0.765	29.80	17.64
	Difference	**-0.002**	**-0.051**	**-0.032**	**-7.16**	**-3.11**
	Dry digestate	0.073	1.150	0.792	35.36	20.84
		0.076	1.066	0.735	34.12	17.85
	Difference	**0.003**	**-0.084**	**-0.057**	**-1.24**	**-2.99**
	Liquid manure	0.075	1.078	0.788	37.64	21.14
		0.074	0.991	0.757	34.90	17.50
	Difference	**-0.001**	**-0.087**	**-0.031**	**-2.74**	**-3.64**
	Farmyard manure	0.075	1.142	0.821	35.00	22.18
		0.072	1.057	0.752	32.44	19.97
	Difference	**-0.003**	**-0.085**	**-0.069**	**-2.56**	**-2.21**

Before the application of the different fertilizers, content of total nitrogen (TN), ammonia (NH_4^+-N), total carbon (TC), total organic carbon (TOC), phosphorous (P_{DL}) and potassium (K_{DL}) of each fertilizer were assessed (Table 14).

Table 14: Chemical and physical parameters of the applied fertilizers. CAN: calcium ammonium nitrate. n.a.: not available. FW: fresh weight. TN: total nitrogen. DM: dry matter. P_{DL}/K_{DL}: phosphorous and potassium in Doppel-Lactat. Mean values from original data from IASP.

Year	Fertilizer						
	Parameter	Control	CAN	Wet digestate	Dry digestate	Liquid manure	Farmyard manure
2009	TN (% FW)	0	27	0.45	0.54	0.36	0.61
	NH_4^+-N (% FW)	0	13.5	0.23	0.21	0.20	0.03
	TC (% DM)	0	0	3.22	6.42	4.93	8.01
	TOC (% DM)	0	0	2.85	6.41	3.39	6.75
	DM (%)	0	100	8.11	14.60	8.75	22.68
	P_{DL} (mg $100g^{-1}$)	0	0	59.64	59.28	38.21	66.69
	K_{DL} (mg $100g^{-1}$)	0	0	373.6	354.9	277.1	378.1
	pH	-	-	7.93	8.15	7.51	7.62
2010	TN (% FW)	0	27	0.49	0.52	0.37	0.53
	NH_4^+-N (% FW)	0	13.5	0.25	0.16	0.17	0.07
	TC (% DM)	0	0	2.80	6.77	3.40	15.44
	TOC (% DM)	0	0	2.71	6.51	3.30	15.23
	DM (%)	0	100	7.18	15.12	7.90	35.51
	P_{DL} (mg $100g^{-1}$)	0	0	n.a.	n.a.	n.a.	n.a.
	K_{DL} (mg $100g^{-1}$)	0	0	n.a.	n.a.	n.a.	n.a.
	pH	-	-	7.93	7.99	6.95	9.05
2011	TN (% FW)	0	27	0.39	0.58	0.34	0.94
	NH_4^+-N (% FW)	0	13.5	0.22	0.19	0.17	0.03
	TC (% DM)	0	0	3.26	7.13	3.34	6.41
	TOC (% DM)	0	0	2.39	6.99	3.30	6.12
	DM (%)	0	100	7.5	16.2	7.69	29.15
	P_{DL} (mg $100g^{-1}$)	0	0	n.a.	n.a.	n.a.	n.a.
	K_{DL} (mg $100g^{-1}$)	0	0	n.a.	n.a.	n.a.	n.a.
	pH	-	-	7.66	8.44	6.90	7.97

The determination of total carbon and total nitrogen was carried out by means of elemental analysis. The prepared samples were measured in an elemental analyzer (CNS VarioMAX, from Elemental Analysis GmbH, Hanau). The total organic carbon (TOC) was analyzed with the VarioMAX TIC/TOC from Elemental Analysis GmbH, Hanau. The soluble phosphorus and potassium were determined after their extraction from the soil samples with a calcium solution (VDLUFA I, A 6.2.1.2, 1997). The phosphorus was measured spectrophotometrically in a filtrated aliquot with the Continuous Flow Analysis (CFA) and the potassium was measured by flame photometry with the Atomic Absorption Spectroscopy (AAS).

The parameters TN, TC, TOC, K_{DL} and P_{DL} from the fertilizer samples were analyzed in the same way as those for the soil samples. NH_4^+-N was determined from the fresh material by the Kjeldahl method followed by the conversion of the total nitrogen organically bound into an ammonium form, the alkalization, distilling and capturing of ammonium content and finally, titration (BKG, 1998). To estimate the pH of the soil and fertilizers, 10 gram of each were mixed with 25 ml of a 0.01 M $CaCl_2$ solution during 10 minutes. After one hour, the pH was measured with a pH-electrode (VDLUFA).

In this experiment an amount of fertilizer corresponding to 120 kg N ha^{-1} for each treatment was applied, therefore the amounts of the organic and mineral-N-fertilizers varied according to their nitrogen content in fresh weight (FW) (Table 15).

Table 15: Application rates (kg ha^{-1} FW) of the different fertilizers depending on their amount of nitrogen. CAN: calcium ammonium nitrate.

Year	Control	CAN	Wet Digestate	Dry Digestate	Liquid Manure	Farmyard Manure
2009	0	444	25806	22430	27714	16644
2009-2010	0	444	24440	21164	35294	16461
2010	0	444	24440	22945	32787	22472
2010-2011	0	444	25696	25478	48980	28169

4.2.3 Plant Growth and Development

Sorghum and wheat were monitored during the growth and development period until harvest. As indicators of growth and development, plant length in cm (HEADY, 1957), the Leaf Area Index (LAI) (m^2/m^2) and the green color of the leaves were measured. The LAI is geometrically defined as the total one-sided area of photosynthetic tissue per unit ground surface area (BARKER SCHAAF *et al.*, 2008). The LAI of sorghum was measured with the Plant Canopy Analyzer LAI 2000, which determines the attenuation of diffuse sky radiation at five zenith angles simultaneously (LI-COR, Inc., 1992). The stand reflectance of wheat was measured with the HandySpec Field (Tec5 AG). The measuring head of this device consists of two optical receive channels, of which the upper one quantifies the incoming light as reference and the lower one records the reflectance by vegetation and ground (MÜLLER *et al.*, 2008). The green color of the youngest leaves is an indicator of the chlorophyll content and it provides indirect information on the plant nitrogen supply. This was measured with the Yara-N-Tester in which internal radiation source emits light and the transmission through a leaf is measured in the red (650 nm) as well as near-infrared (920 nm) spectral regions (SCHMIDHALTER *et al.*, 2008).

In 2010, the monitoring of sorghum could not be carried out after calendar week 32. Due to an intense precipitation on the 12.08.2010 which reached 21 mm (Figure 8), the plants bent irregularly to the ground. From calendar week 30 until the 32 of that year, also the Leaf Area Index could not be measured due to technical problems with the Plant Canopy Analyzer LAI 2000.

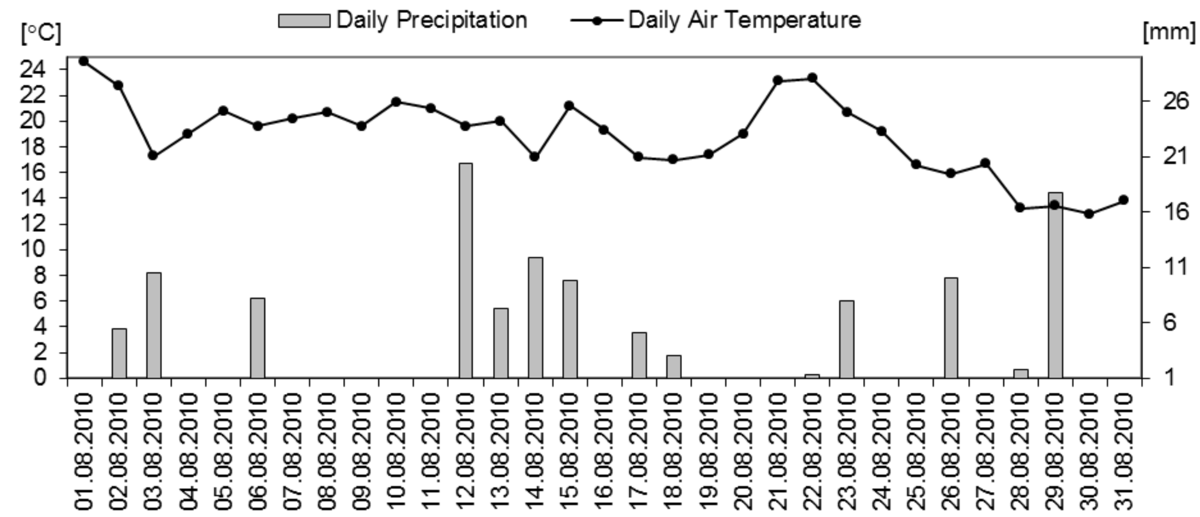


Figure 8: Daily precipitation and air temperature from August in 2010 at the experimental fields in Berlin-Dahlem.

The following Table (16) represents the mean values of the parameters soil temperature up to 20 cm, air temperature, solar radiation and precipitation from the BBCH stage 1 to 89. Since the sowing of sorghum in 2010 (CW 19) was carried out one week earlier than in 2009 (CW 20), calendar week corresponding to the different phenological stages from both years are also represented. The sowing of wheat was carried out in 2009 one week earlier than in 2010.

Table 16: Soil-air temperature, precipitation and solar radiation during the germination, growth and development of *Sorghum sudanense* and winter wheat. MV: mean value.

			Sorghum sudanense		Winter wheat		
			2009	**2010**	**2009**	**2010**	**2011**
BBCH 1- 9	Calendar week		**20-21**	**19-20**	**39-41**	**40-42**	**-**
	Soil temperature (° C)	MV	16.9	15.6	14.1	9.0	-
	Precipitation (mm)		17.2	30.1	60.2	18.8	-
	Solar radiation (MJ m^{-2})		20.3	17.8	8.6	6.3.	-
BBCH 9 - 13	Calendar week		**21-24**	**20-23**	**-**	**-18**	**-19**
	Air temperature (° C)	MV	15.4	13.8	-	4.2	4.0
	Precipitation (mm)		90.8	61.5	-	299.7	248.9
	Solar radiation (MJ m^{-2})		19.2	14.8	-	5.8	6.6
BBCH 13 - 89	Calendar week		**24-38**	**23-38**	**-**	**18-29**	**19-29**
	Air temperature (° C)		18.9	19.2	-	18.0	17.7
	Precipitation (mm)	MV	177.7	256.7	-	90.5	136.7
	Solar radiation (MJ m^{-2})		17.85	17.52	-	19.3	20.7

It was only possible to monitor the growth and development parameters of winter wheat, from the experiment 2 in 2011, until calendar week 22. Because the plants were in the BBCH phenological growth stage 69/71, end of the flowering and beginning of the development of the fruit, the risk of being eaten by birds was high. Therefore, the plants were covered with a net. This net was also used in 2010, but three weeks later because the plants reached the phenological stadium 69/71 just in the 25 calendar week.

4.2.4 Dry-matter yield, grain yield, yield formation and crop quality

The dry matter of the whole sorghum plant material was determined. The grain yield of winter wheat was calculated based on 86% dry matter (DM). The grains of winter wheat were used for the analysis of the yield formation under the principle of HEUSER, 1954. As external quality parameter the hectoliter weight (EGGER & MOREL, 1989) was determined. Also, internal quality parameters like the protein content according to the Dumas method, the sedimentation value according to Zeleny and the Falling Number (HAGBERG, 1960) were assessed. For the determination of the protein content according to Dumas, the samples were combusted in an oxygen-rich environment, at about 1000 ° C. Other products resulting from the combustion phase were removed by selective absorption and the nitrogen gas was measured with a thermal conductivity detector (INTERNATIONAL ASSOCIATION FOR CEREAL SCIENCE AND TECHNOLOGY (ICC), 2000). The sedimentation value according to Zeleny is the degree of sedimentation of flour suspended in a lactic acid solution during a standard time interval. This value is taken as a measure for the baking quality (ICC, 1972). The Falling Number according to Hagberg is defined as the time in seconds required to stir and to allow a viscometer stirrer to fall a measured distance through a hot aqueous meal, flour or starch gel undergoing liquefaction due to alpha-amylase activity (ICC, 1968).

4.2.5 Statistic

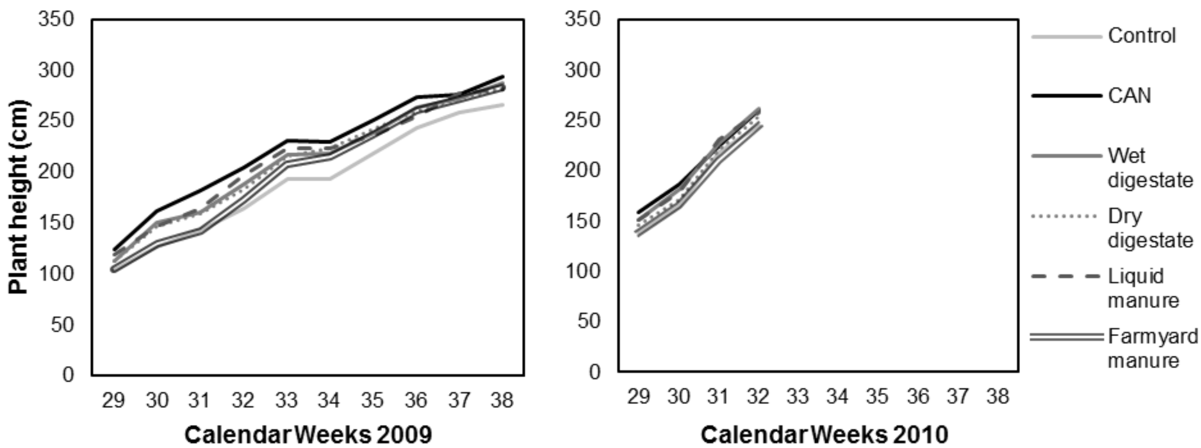
All parameters were due to their normal distribution and homogeneity of variance, statistically analyzed with one-way ANOVA followed by the Tukey-Test to analyze differences between treatments (SPSS Statistics Desktop 17.0 for Windows). Data was calculated as arithmetic means ± standard deviation of 4 replicates.

4.3 Results

4.3.1 *Sorghum bicolor* var. *sudanense*

Plant height

The results for 2009 showed a progressive increase in the plant height from calendar week 29 to 33. Until calendar week 33, the plants fertilized with mineral-N-fertilizers and liquid manure had a height significantly greater than the unfertilized control. Plants fertilized with dry and wet digestates were placed in an intermediate position (Figure 9).



C	a	a	a	a	a	a	a	a	a	a	C	a	a	a	a		-	-	-	-	-
CAN	b	b	c	c	b	a	a	a	a	a	CAN	a	a	a	a	-	-	-	-	-	-
WD	ab	a	a	abc	ab	a	a	a	a	a	WD	a	a	a	a	-	-	-	-	-	-
DD	ab	a	ab	abc	ab	a	a	a	a	a	DD	a	a	a	a	-	-	-	-	-	-
LM	b	ab	bc	bc	b	a	a	a	a	a	LM	a	a	a	a	-	-	-	-	-	-
FM	a	a	a	ab	ab	a	a	a	a	a	FM	a	a	a	a	-	-	-	-	-	-

Figure 9: Plant height development of Sorghum sudanense during 2009 and 2010. Treatments compared by assessment date. C: control, CAN: calcium ammonium nitrate, WD: wet diges-

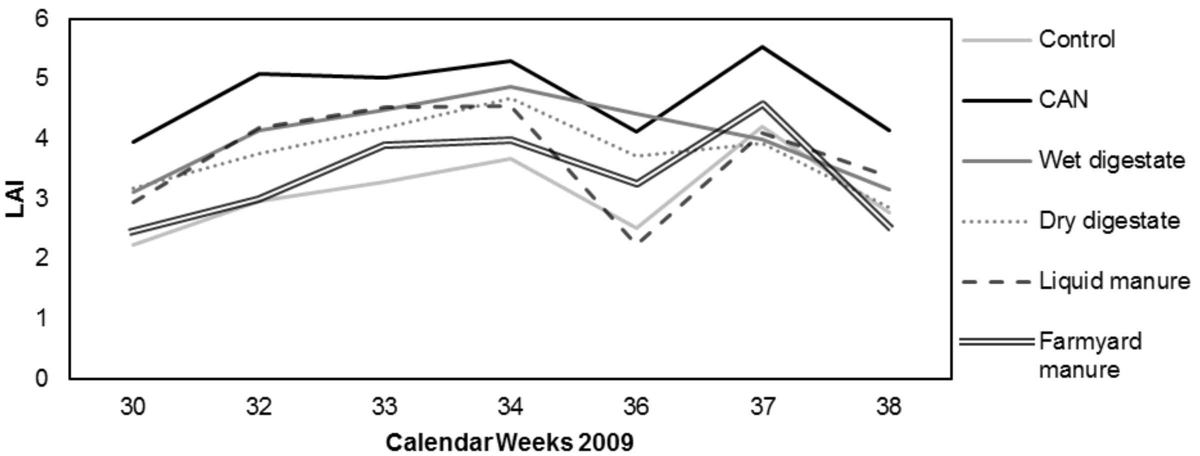
tate, DD: dry digestate, LM: liquid manure, FM: farmyard manure. Mean values and Tukey-Test (p≤ 0.05). Means from the same homogeneous group are followed by the same letter.

The plants fertilized with farmyard manure had a height comparable to the unfertilized control, with the exception of calendar weeks 33 and 34 where the plant height was in an intermediate position between those fertilized with mineral-N-fertilizer and the unfertilized control. After 33 weeks, no significant differences between the treatments were observed (Figure 9).

In the experiment 2, conducted in 2011 on an adjacent field, no significant differences were observed between treatments. Until calendar week 32, the highest plants were those fertilized with the mineral-N-fertilizer, followed by the ones fertilized with the wet digestate, liquid manure, dry digestate, farmyard manure and finally the unfertilized control (Figure 9).

Leave Area Index (LAI)

The results for the Leaf Area Index referring to 2009 showed a gradual increase until calendar week 34 followed by a decline until calendar week 38. A decreasing peak in all the treatments can be also observed in calendar week 36 (Figure 10).



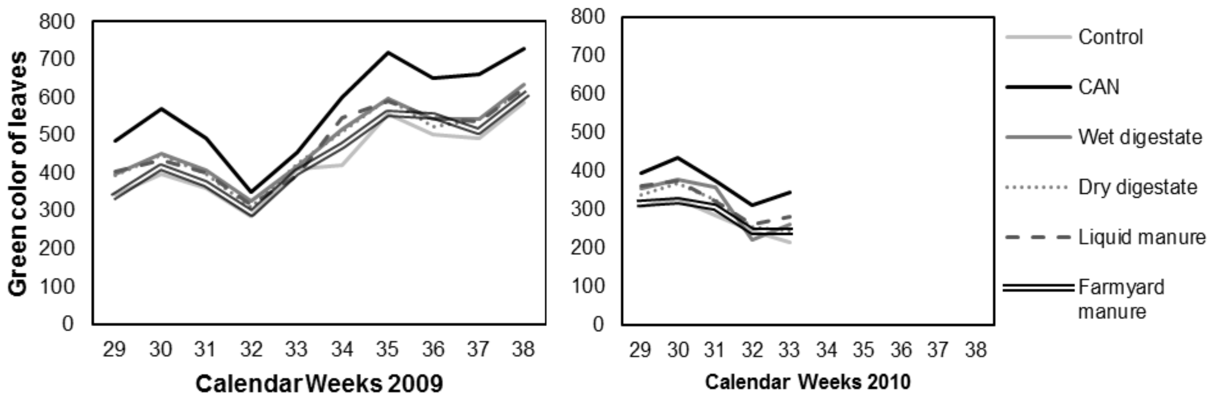
C	a	a	a	a	a	a	a
CAN	b	b	c	b	a	a	a
WD	ab	ab	bc	ab	a	a	a
DD	ab	ab	abc	ab	a	a	a
LM	ab	ab	bc	ab	a	a	a
FM	a	a	ab	ab	a	a	a

Figure 10: Leave Area Index (LAI) development by *Sorghum sudanense* during 2009. Treatments compared by assessment date C: control, CAN: calcium ammonium nitrate, WD: wet digestate, DD: dry digestate, LM: liquid manure, FM: farmyard manure. Mean values and Tukey-Test (p≤ 0.05). Means from the same homogeneous group are followed by the same letter.

From the 30th until the 34th calendar week, significant differences in the Leaf Area Index of those plants fertilized with mineral-N-fertilizers in relation to the unfertilized control were observed. The plants fertilized with liquid manure and dry and wet digestates were in an intermediate position between the unfertilized control and the mineral-N-fertilizer. The Leaf Area Index of plants fertilized with farmyard manure was comparable to the unfertilized control until calendar week 32. From calendar week 33 to 34 the effect was comparable to the other treatments. After the 36 calendar week, there were no significant differences (Figure 10).

Green color of the youngest leaves

The green color of the youngest leaves was measured from week 29 to 38. In 2010, a progressive increase in their intensity was observed in all treatments with the exception of week 31 and 32, in which a decreasing peak was observed. The plants fertilized with mineral-N-fertilizer had an intensity of green color significantly higher than the unfertilized control in most of the weeks. In calendar week 30, 33 and 38 both digestates and the liquid manure acquired an intermediate position between the mineral-N-fertilizer and unfertilized control. The farmyard manure did not differ significantly from the unfertilized control, with the exception of calendar week 30 (Figure 11).



C	a	a	a	a	ab	a	a	a	a	a	**C**	a	a	a	a	a	-	-	-	-	-
CAN	b	b	b	a	b	a	b	a	a	b	**CAN**	d	c	c	a	c	-	-	-	-	-
WD	a	ab	a	a	ab	a	a	a	a	ab	**WD**	bcd	b	bc	a	ab	-	-	-	-	-
DD	a	ab	a	a	ab	a	a	a	a	ab	**DD**	abc	ab	ab	a	ab	-	-	-	-	-
LM	a	ab	a	a	a	a	a	a	a	ab	**LM**	cd	b	ab	a	bc	-	-	-	-	-
FM	a	ab	a	a	a	a	a	a	a	a	**FM**	ab	a	a	a	ab	-	-	-	-	-

Figure 11: Development of the green color of the youngest leaf by *Sorghum sudanense* during 2009 and 2010. Treatments compared by assessment date C: control, CAN: calcium ammonium nitrate, WD: wet digestate, DD: dry digestate, LM: liquid manure, FM: farmyard manure. Mean values and Tukey-Test (p≤ 0.05). Means from the same homogeneous group are followed by the same letter.

In 2011, also significant differences between the plants fertilized with mineral-N-fertilizers and unfertilized control were observed. The effect of farmyard manure was comparable to the control with the exception of weeks 27 and 31. All other variants with organic fertilizer had an intermediate position between the mineral-N-fertilizer and unfertilized control (Figure 11).

Dry-matter yield

In 2009, the highest dry-matter yield was obtained after the fertilization with mineral-N-fertilizer followed by the liquid manure, the wet digestate, the dry digestate, the farmyard manure and finally the unfertilized control.

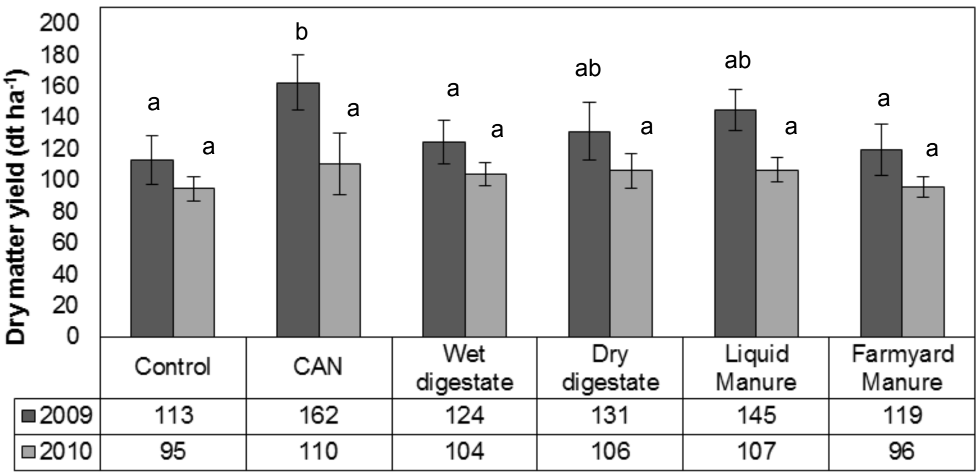


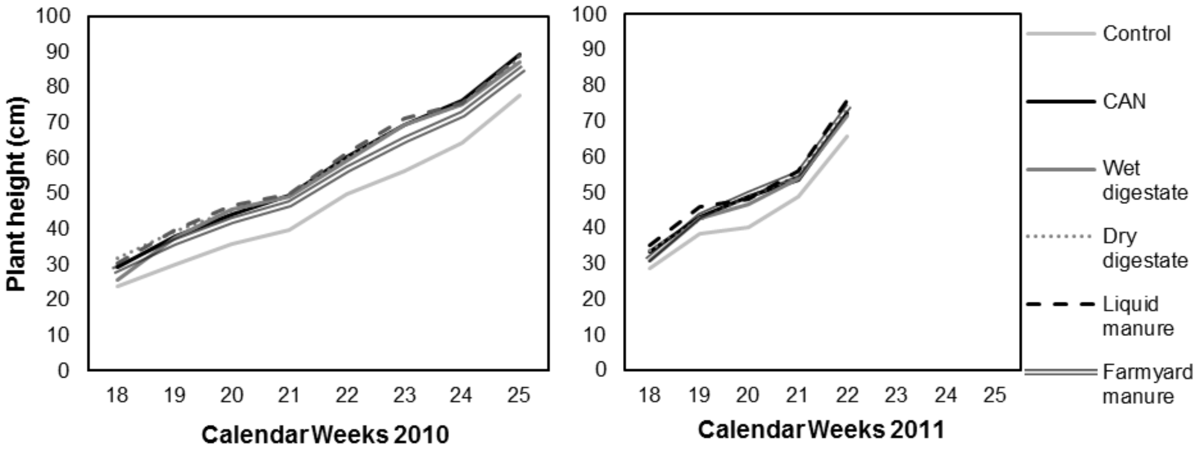
Figure 12: Dry-matter yield (dt ha^{-1}) of *Sorghum sudanense* (2009-2010). CAN: calcium ammonium nitrate. Mean values, standard deviations and Tukey-Test (p≤ 0.05). Means from the same homogeneous group are followed by the same letter.

Significant differences were found between dry-matter yields in the variant fertilized with mineral-N-fertilizer as compared to the unfertilized control, the wet digestate and farmyard manure. The results of 2010 followed the same pattern as the ones of 2009 but due to the bent of the plant in calendar week 32, there were no significant differences between the treatments (Figure 12).

4.3.2 Winter wheat

Plant height

The different treatments had a significant positive effect on the plant height in comparison to the unfertilized control. In calendar weeks 22 and 23 the plants, which were fertilized with liquid manure, were significantly higher than the other treatments. In these two weeks the effect of the mineral-N-fertilizer, the wet digestate and the dry digestate were between the liquid fertilizer and the farmyard manure. Their effect was significantly greater than the un-fertilized control (Figure 13).



C	a	a	a	a	a	a	a	a	**C**	a	a	a	a	a	-	-	-
CAN	ab	b	b	b	bc	bc	b	b	**CAN**	b	b	b	ab	ab	-	-	-
WD	b	b	b	b	bc	bc	b	b	**WD**	a	b	b	ab	a	-	-	-
DD	b	b	b	b	bc	bc	b	b	**DD**	b	ab	b	b	b	-	-	-
LM	b	b	b	b	c	c	b	b	**LM**	c	b	b	b	b	-	-	-
FM	b	b	b	b	b	b	b	b	**FM**	b	b	b	b	b	-	-	-

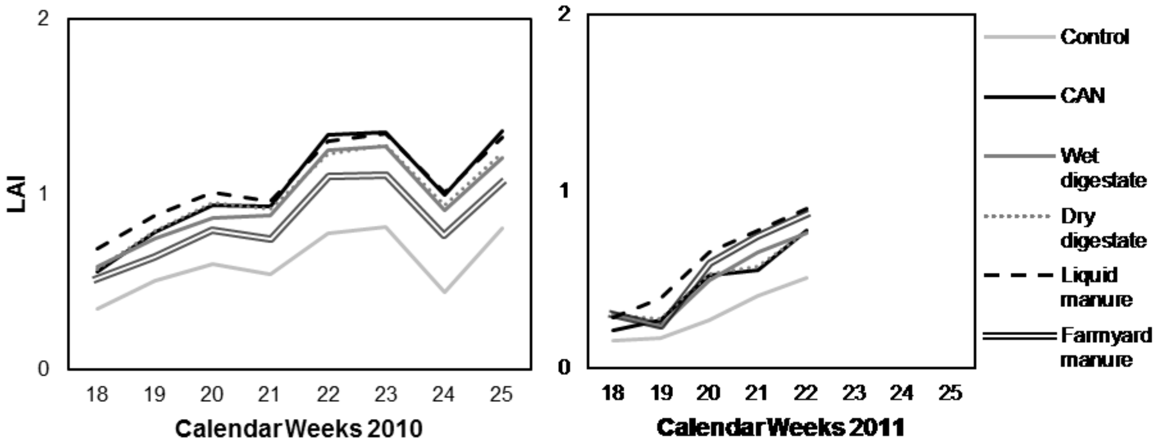
Figure 13: Plant height development by winter wheat during 2010 and 2011. Treatments compared by assessment date C: control, CAN: calcium ammonium nitrate, WD: wet digestate, DD: dry digestate, LM: liquid manure, FM: farmyard manure. Mean values and Tukey-Test ($p \leq 0.05$). Means from the same homogeneous group are followed by the same letter.

In 2010, the effect of all treatments on the plant height was significantly higher in comparison to the unfertilized control. The effect of the liquid fertilizer in calendar week 18 was significantly higher than the other treatments. The effects of the mineral-N-fertilizer, the dry digestate and the farmyard manure were placed between the control and the liquid fertilizer. In calendar week 19, 21 and 22, the effects of the dry digestate, the wet digestate and the mineral-N-fertilizer were in an intermediate position between the control and the rest of treatments (Figure 13).

Leave Area Index

The Leaf Area Index of winter wheat in 2009 was significantly higher in all treatments in relation to the unfertilized control. There were no significant differences between treatments with the exception of calendar weeks 19 and 23, in which the plants fertilized with liquid manure had a greater leaf area than other treatments.

In 2011, from calendar week 19 there were significant differences between the treatments fertilized with liquid manure and farmyard manure in comparison to the unfertilized control. The effects of the mineral-N-fertilizer, the dry and wet digestates were in an intermediate position (Figure 14).



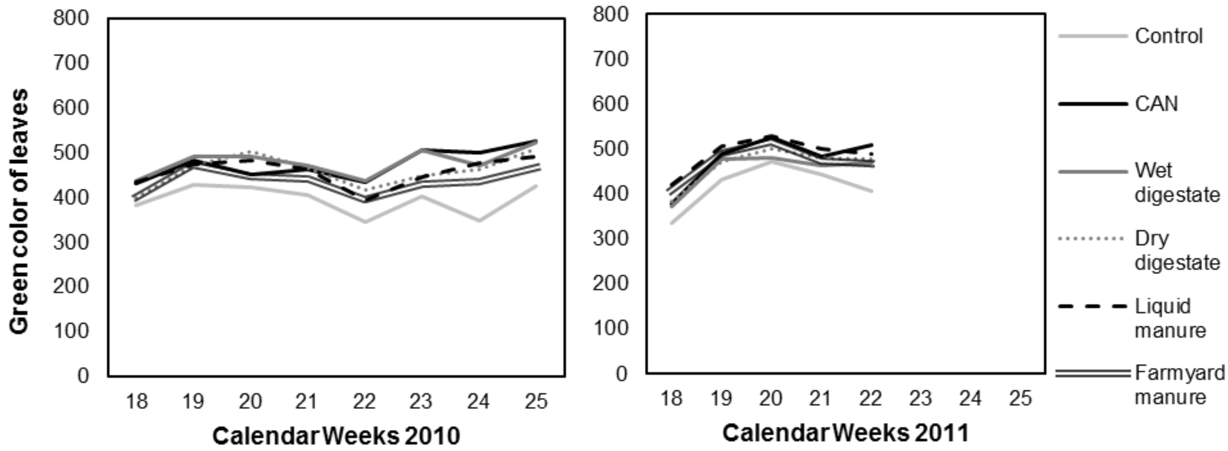
C	a	a	a	a	a	a	a	a	**C**	a	a	a	a	a	-	-	-
CAN	b	bc	b	b	b	bc	b	bc	**CAN**	a	ab	ab	ab	ab	-	-	-
WD	b	bc	b	b	b	bc	b	bc	**WD**	a	ab	ab	ab	ab	-	-	-
DD	b	bc	b	b	b	bc	b	bc	**DD**	a	ab	ab	ab	ab	-	-	-
LM	b	c	b	b	b	c	b	c	**LM**	a	b	b	b	b	-	-	-
FM	a	ab	a	ab	b	b	b	a	**FM**	a	ab	b	b	b	-	-	-

Figure 14: Leave Area Index development by winter wheat during 2010 and 2011. Treatments compared by assessment date C: control, CAN: calcium ammonium nitrate, WD: wet digestate, DD: dry digestate, LM: liquid manure, FM: farmyard manure. Mean values and Tukey-Test (p≤ 0.05). Means from the same homogeneous group are followed by the same letter.

Green color of the youngest leaves

There were no significant differences in the intensity of the color of the leaves among treatments during the first three weeks and calendar weeks 22 and 23. In calendar week 21 the intensity of the green color of the leaves was significantly higher in those plants fertilized with the digestates in comparison to the unfertilized control. The plants in the other fertilizer variants had an intermediate intensity in green leaf color between the unfertilized control and both digestates. In calendar week 24, the fertilized plants had a higher intensity of green color of leaves than the unfertilized control. In calendar week 25, significant differences were observed between the plants fertilized with mineral-N-fertilizer and the unfertilized control. The other treatments acquired an intermediate position.

In 2011, significant differences were observed in calendar weeks 19 and 22. In calendar week 19 the intensity of green color of leaves was higher in those plants fertilized with liquid manure and farmyard manure, compared with the unfertilized control. In calendar week 22, significant differences were observed among those plants fertilized with mineral-N-fertilizer in comparison to the unfertilized control (Figure 15).



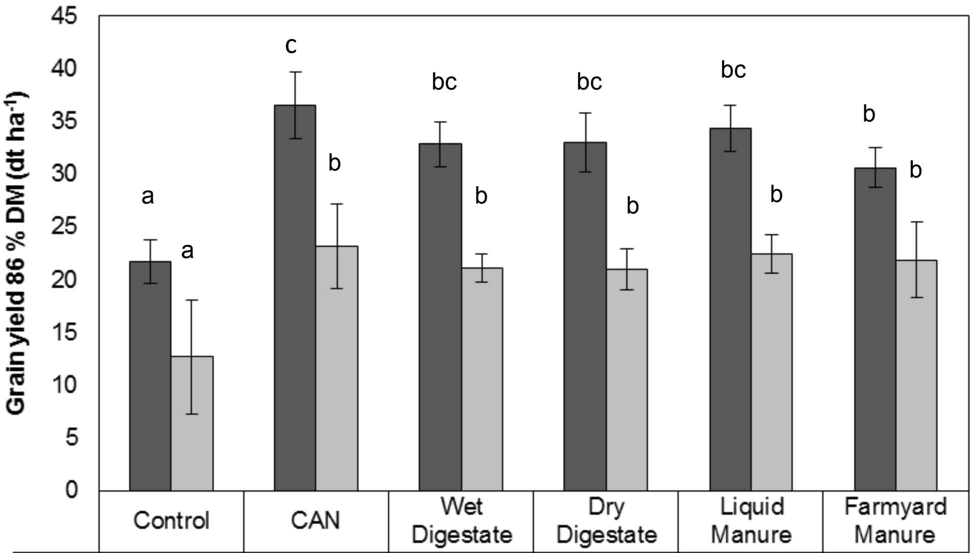
C	a	a	a	a	a	a	a	a	**C**	a	a	a	a	a	-	-	-
CAN	a	a	a	ab	a	a	b	c	**CAN**	a	ab	a	a	b	-	-	-
WD	a	a	a	b	a	a	b	bc	**WD**	a	ab	a	a	ab	-	-	-
DD	a	a	a	b	a	a	b	bc	**DD**	a	ab	a	a	ab	-	-	-
LM	a	a	a	ab	a	a	b	bc	**LM**	a	b	a	a	b	-	-	-
FM	a	a	a	ab	a	a	b	ab	**FM**	a	b	a	a	ab	-	-	-

Figure 15: Development of the green color of the youngest leaf by winter wheat during 2010 and 2011. Treatments compared by assessment date. C: control, CAN: calcium ammonium nitrate, WD: wet digestate, DD: dry digestate, LM: liquid manure, FM: farmyard manure. Mean values and Tukey-Test ($p \leq 0.05$). Means from the same homogeneous group are followed by the same letter.

Grain yield

The use of mineral-N-fertilizer resulted in the highest grain yield of winter wheat in 2010. These results were significant compared to the unfertilized control and the farmyard manure. The effect of the digestate and liquid manure on the grain yield of winter wheat had an intermediate position between the mineral-N-fertilizer and farmyard manure. The farmyard manure was less effective than other fertilizers, but significantly higher than the unfertilized control.

In 2011 there were no significant differences between the effect of different fertilizers on the grain yield of winter wheat; however, all treatments had a significantly greater effect compared to the unfertilized control (Figure 16).



	Control	CAN	Wet Digestate	Dry Digestate	Liquid Manure	Farmyard Manure
2010	21.73	36.55	32.87	33.02	34.36	30.65
2011	12.70	23.11	21.08	21.02	22.45	21.88

Figure 16: Grain yield (dt ha^{-1}) of winter wheat (2010-2011). CAN: calcium ammonium nitrate. Mean values, standard deviations and Tukey-Test (p≤ 0.05). Means from the same homogeneous group are followed by the same letter.

External and internal quality of the grains

In 2010 the use of mineral-N-fertilizers and liquid manure showed a tendency toward greater population density with lower number of grains per spike. Also, these fertilizers led to a significantly increased thousand kernel weight and showed a tendency toward greater hectolitre weight in comparison to other treatments. The results closest to achieving optimal crude protein content for quality wheat (12.5 %) were obtained after the fertilization with liquid manure (11 %) and wet digestate (10.6 %). In relation to the Falling Number, the results closest to achieving an optimal backing quality (250 to 300 seconds) were achieved with the unfertilized control and the use of mineral-N-fertilizer. The sedimentation values closest to quality wheat (> 35 ml) were tendentially obtained with the fertilization with liquid manure (12.4 %) and the dry digestate (12.4 %) (Table 17).

In 2011, the use of dry digestate and liquid manure resulted in a greater population density with a lower number of grains. The thousand kernel weight and the hectolitre weight were higher in those plants which were fertilized with dry digestate and mineral-N-fertilizer. The best results in relation to the crude protein content in the grains were obtained after fertilization with both digestates. The best Falling Number was obtained in those plots fertilized with liquid manure and mineral-N-fertilizer. Regarding the sedimentation value, the best results were obtained using wet digestate and farmyard manure as fertilizers. None of the differences assessed in this year were significantly different (Table 17).

Table 17: External and internal quality parameters of winter wheat. CAN: calcium ammonium nitrate, TKW: thousand kernel weight. Mean values, Standard Errors and Tukey-Test (p≤ 0.05). Means from the same homogeneous group are followed by the same letter.

	Control	CAN	Wet digestate	Dry digestate	Liquid manure	Farmyard manure
			2010			
Spikes m^{-2}	243 ± 19.42 [a]	273 ± 34.04 [a]	264 ± 33.04 [a]	268 ± 26.73 [a]	275 ± 0.25 [a]	231 ± 16.92 [a]
Corn number per $spike^{-1}$	23 ± 1.45 [a]	29 ± 4.96 [ab]	34 ± 4.16 [b]	30 ± 2.49 [b]	30 ± 0.19 [b]	33 ± 1.92 [b]
TKW (g)	39.0 ± 0.85 [a]	42.2 ± 1.34 [b]	41.5 ± 1.28 [b]	41.3 ± 0.46 [b]	41.8 ± 1.27 [b]	40.6 ± 0.61 [ab]
Hectolitre weight (kg)	79.8 ± 0.25 [a]	80.8 ± 0.64 [a]	80.5 ± 0.30 [a]	80.6 ± 0.34 [a]	80.5 ± 0.74 [a]	80.5 ± 0.43 [a]
Protein content (% DM)	9.7 ± 0.21 [a]	10.1 ± 0.43 [a]	10.6 ± 0.24 [a]	10.4 ± 0.34 [a]	11.0 ± 1.14 [a]	10.4 ± 0.83 [a]
Falling number (sec)	310.7 ± 11.5 [a]	322.5 ±17.6 [a]	323.5 ±19.4 [a]	327.0 ± 7.3 [a]	336.2 ±26.9 [a]	323.0 ±24.6 [a]
Sedimentation value (ml)	11.7 ± 0.29 [a]	12.0 ± 0.24 [a]	11.2 ± 0.52 [a]	12.4 ± 0.44 [a]	12.4 ± 0.89 [a]	12.0 ± 0.67 [a]
			2011			
Spikes m^{-2}	256 ± 33.5 [a]	265 ± 19.08 [a]	263 ± 23.80 [a]	286 ± 18.06 [a]	280 ± 14.82 [a]	271 ± 20.84 [a]
Corn number per $spike^{-1}$	12 ± 5.07 [a]	20 ± 3.32 [ab]	18 ± 1.65 [b]	17 ± 1.25 [b]	18 ± 2.16 [b]	19 ± 3.43 [b]
TKW (g)	41.5 ± 0.92 [a]	44.3 ± 1.07 [b]	43.8 ± 0.67 [b]	44.0 ± 0.75 [b]	43.9 ± 0.62 [b]	43.5 ± 1.14 [ab]
Hectolitre weight (kg)	80.4 ± 0.40 [a]	81.2 ± 0.82 [a]	80.0 ± 0.39 [a]	80.4 ± .21 [a]	79.9 ± 0.28 [a]	80.0 ± 0.63 [a]
Protein content (% DM)	10.2 ± 0.3 [a]	10.3 ± 0.2 [a]	12.0 ± 1.3 [a]	10.5 ± 0.4 [a]	10.4 ± 0.2 [a]	10.2 ± 0.2 [a]
Falling number (sec)	280.9 ± 5.1 [a]	276.7 ± 4.0 [a]	279.5 ± 5.3 [a]	282.2 ± 5.7 [a]	267.7 ± 4.8 [a]	285.6 ± 7.5 [a]
Sedimentation value (ml)	12.37 ± 0.5 [a]	13.25 ± 0.3 [a]	13.43 ± 0.3 [a]	13.00 ± 0.6 [a]	13.19 ± 0.4 [a]	13.31 ± 0.9 [a]

4.4 Discussion

Sorghum bicolor var. sudanense

Mineral-N-fertilizer (CAN) had a significantly positive effect on plant height, green color of the leaves and the Leaf Area Index of *Sorghum sudanense*. This is probably due to the availability of nitrogen, in the form of ammonium nitrate (NH_4NO_3), a chemical compound which is highly hygroscopic and soluble in water (TORRES DUGAN, 2007). In many soils nitrate (NO_3^--N) is usually movable and easily available to plants. The ammonium ion (NH_4^+-N) interacts ionically with clays and colloids from the soil organic matter. It is usually present in an interchangeable form and is converted to nitrate by Nitrosomonas and Nitrobacter through a nitrification process, becoming readily available for plants (KRISHNA, 2002). After the application of mineral-N-fertilizer, short term nitrogen losses in the form of NH_3, N_2O_2 and NO_3^--N and long-term nitrogen losses in the form of NO_3^--N and N_2O are usually observed. It is considered that the plant absorbs 50 to 80 % of the applied nitrogen (GUTSER *et al.*, 2005). Sorghum has a high nutritional demand in the vegetative growth stage (QUINTERO & CASANOVA, 2000) a stage in which, in comparison to other fertilizers, the nutritional requirements were met by the use of mineral-N-fertilizer. It must be taken into account that the supply of phosphorus (P) and potassium (K) in this locality is not necessary due to high levels of these two macronutrients in the soil (Table 12). The phosphorus in the soil can be found dissolved in small quantities in the soil solution or associated with soil minerals (inorganic-P) or organic matter (organic-P). The relative amount of different forms of P varies depending on the soil and its release depends on several factors such as soil moisture, the composition of organic matter and pH (ESPINOSA *et al., n.d.*).

There were no significant differences in the effect of liquid manure on plant height, the green color of leaves and Leaf Area Index of *Sorghum sudanense* compared with both digestates. Besides the fact that the effects of these organic fertilizers were tendentially comparable with the effect of mineral-N-fertilizer (CAN). The nutrients found in the liquid manure and in both digestates are partly in a form in which they are not directly available to plants because part of the nitrogen is attached to the organic matter. The nitrogen is gradually released after the mineralization process carried out by the soil fauna (SCHILLING, 2000). Because of this, the liquid manure and both digestates probably had an effect which was 5 % lower on the plant height, 15-20 % lower on the Leaf Area Index, 15 % lower on the green of the leaves and 10-20 % lower on the dry-matter yield compared to the effect of the mineral-N-fertilizer. At the same time there were no significant differences between liquid manure and both digestates on the parameters listed above.

The P from organic fertilizers is bound in an organic form. It is estimated that 60 % of P is assimilated by the first crop and 40 % by the next crop (SCHILLING, 2000). K is normally directly assimilated, but under specific conditions can also be percolated. The K available from the organic fertilizer becomes part of the soil solution. Part is absorbed by the plant and part remains in the colloidal complex (clay, organic matter, hydroxides). The remaining P can be in an exchangeable form which constitutes a backup system for plants (FASSBENDER, 1975). K also can be accumulated to form part of the structures and crystal lattices of primary minerals such as feldspars and micas (NAVARRO, 2003). The analysis of soil taken before fertilizing and after two growing seasons showed, that the amount of P in the soil increased by 0.90 mg 100 g^{-1} in the experiment 1 and by 1.41 mg 100 g^{-1} in the experiment 2. This indicates that P is organically bound in this agro-ecosystem. On the other hand, K did not remain in the system (Table 13). The corresponding analyzes from the soil samples taken one month after fertilization and after the wheat growing season, showed higher losses of P and K from the organic fertilizers with lower DM content % (wet digestate and liquid manure) than from organic fertilizers with higher DM content % (dry digestate and farmyard manure). The fertilizers with lower C/N ratio, as in the case of the wet digestate (8-9) and liquid manure (11), have a higher availability of nutrients than those with a higher C/N ratio (dry digestate: 13-14, farmyard manure: 16) (GUTSER *et al.*, 2005).

Winter Wheat

For winter wheat the variation of the effects of the tested fertilizers on the parameters of growth and development named above was not as prominent as in the case of sorghum. However, there were significant differences in their effects in relation to the unfertilized control. In the early stages of development, the wheat seedlings used the existing nutrient reserves in the endosperm. The nutritional requirements before the beginning of tillering (early spring in the case of winter wheat) are low and usually fulfilled by the available minerals in the soil (KOHLI & MARTINO, 1997). Normally the first application in the form of mineral-N-fertilizer takes place by or just after the start of early seedling development. This application is usually 25 % of the total N application amount (DENNERT, 2007) due to the possible loss of nutrients by leaching during the winter. The second application is usually 35 % of the total N application amount in the growth stage 31-32 with the aim to provide the nutrients necessary for the formation of the main stems and the third application is usually 40 % of the total application of fertilizer on the growth stage 37-49 with the aim of achieving a high level of quality. This last application is destined exclusively to grain filling (CHRISTEN, 2009). Depending on the nitrogen in the soil before planting winter wheat, the amount of fertilizer to be applied varies (SCHNEIDER & MASTEL, 2008). The amount of N to be applied is also dependent on the desired type of grain yield, and is regulated as a function of the quantity or quality. If the objective is to obtain a higher quantity, the nutritional needs are lower than those for a high quality (DENNERT, 2007).

A single application of 120 kg ha^{-1} N in autumn, probably led to leaching losses of nitrogen during the winter which resulted in non-significant differences in the growth and development between the different treatments. However, some significant trends in the yield structure were observed. In 2010 the numbers of spikes m^{-2} were higher in the treatment fertilized with liquid manure and mineral-N-fertilizer. This led to a reduction of the number of grains per spike and to a higher thousand kernel weight. The direct availability of N in both fertilizers and consequently the possible loss of this nutrient by leaching during the winter probably led to the reduction of the number of grains once the plant had produced the spikes. This is probably due to a compensation mechanism of the plant (CHRISTEN, 2009). This reduction in turn led to increased grain weight. However, the results were not significant. In 2011, the results were not confirmed.

The hectoliter weight is a measure which indicates the status of homogeneity of the wheat grain. A low hectoliter weight (<70-80 kg l^{-1}) is indicative for impurities, damages or immature kernels. Grain damages can be produced by negative weather influences, such as a severe drought at the time of grain filling, and due to diseases (ENGELBRECHT, 2008). In 2010 and 2011 there were no significant differences between treatments and therefore there is no clear influence of the type of fertilizer on the hectoliter weight. The values were also suitable for wheat, indicating that there were no apparent grain damages.

In the years 2010 and 2011 there were no significant differences in Falling Number between treatments. The values for 2010 were 10 % higher than the estimated values for quality wheat (250 and 320 seconds). This is probably due to the low activity of the alpha amylase enzyme. This enzyme is naturally present in the grain of wheat and is responsible for the transformation of starch into sugars during germination (DENDY & DOBRASZYK, 2001). Low amylase activity is indicative for drought periods (WASSERMANN, n.d.). So probably the activity of this enzyme was affected by the availability of water in the stage of fruit formation. In 2010 the precipitations during the period of formation of the fruit were scarce (0.1 mm). In 2011 the Falling Number values were within the range expected for this parameter of quality; however, no significant differences between treatments were observed. The precipitations during the fruit formation in 2011 were 2 mm, a fact that probably had a positive effect on the activity of alpha amylase. The results obtained in both years lead to the conclusion that a single application of fertilizer in a quantity of 120 kg ha^{-1} N, regardless of the type of fertilizer, has no visible effect on the Falling Number. However, there seems to be a closer relationship between the precipitation during the fruit formation until the harvest and the amylase activity.

A parameter associated with the protein quality of wheat is the sedimentation value, which determines the ability of hydration and expansion of the gluten protein in a slight acid (MELLADO, 2001). Gluten is responsible for forming a gas-tight structure which increases the power and extensibility of flour (FIGONI, 2011). The corresponding values of sedimentation in both years were 50 % lower than the ones estimated for wheat quality. The protein content of the different treatments was also 12-15 % lower than expected. In addition, there were no significant differences between treatments in any of the parameters listed above. This indicates that the fertilization with 120 kg ha^{-1} N in autumn, as expected, was not sufficient to meet the quality parameters of wheat grain. It also indicates that the mineralization of the organic fertilizer used, probably occurred before the stage of formations of the fruits.

4.5 Conclusions

Comparing the results of this study with those found in the literature, it should be noted that the effect of the digestates on the chosen indicators of growth and development and on the yield of both plants is comparable with that of mineral-N-fertilizer and liquid manure. As the effect in this study is usually up to 15 % lower than the effect of mineral-N-fertilizer, it would be recommended to combine the application of digestate with mineral-N-fertilizers to obtain an increased yield.

In case of winter wheat, due to its nutritional needs, it would be recommended to apply 25 % of the fertilizer in the form of digestate (preferably dry) before sowing, a second application with 35 % of the fertilizer in the form of wet digestate and due to the rapid availability required in the phase of fruit filling, a third application of fertilizer in the form of calcium ammonium nitrate. STINNER AND MÖLLER (2005) corroborated this fact, observing that a second application with wet digestate increased significantly the protein content in the grain in comparison to an unfertilized control.

However, in this experiment a second or third application of fertilizer on wheat was not considered because another important objective of this study was the monitoring of the soil biological activity. Because the organisms to be investigated were extracted from the field where the wheat was monitored, it was considered that both applications would interfere with the observation of the development and behavior of soil organisms in this agro-ecosystem.

Chapter 3

Impact of digestates and other fertilizers on the soil biota in a randomized complete block design

5 Chapter 3: Impact of digestates and other fertilizers on the soil biota in a randomized complete block design

5.1 Introduction

This chapter comprehends the effect of the digestates from biogas production on soil quality as a fundamental component of the agro-ecosystem. The concept of soil quality has been suggested by many authors as a tool to assess the long-term sustainability of agricultural practices on local, national and international levels (KARLEN, *et al.*, 1994; BAUTISTA *et al.,* 2004). However, the assessment of the soil quality is difficult because despite its importance there are no clearly defined parameters to determine what is meant as soil quality (BAUTISTA CRUZ *et al.*, 2004). According to the Soil Science Society of America, 1997, the soil quality is the ability of soil to function within the limits of an ecosystem, sustain biological productivity, maintain environmental quality and promote the health of plants and animals (KNOEPP *et al.*, 2000). In order to operationalize this concept it is necessary to have parameters that can be measured to evaluate it. These parameters are known as indicators, as they give information about the improvement, maintenance or degradation of soil quality (LARSON & PIERCE, 1994; KNOEPP *et al.*, 2000).

To describe the effect of digestates on soil quality, soil organisms were used as biological indicators because they play an important role in the agro-ecosystem. They are in contact with stress factors via soil solution, solid phase and gas phase and, therefore, are sensitive to changes in the soil system. The abundance and biomass of earthworms and enchytraeids were considered as bioindicators in the presented field experiments. Besides, avoidance-response tests (STEPHENSON *et al.*, 1998) were conducted in the laboratory to assess the effect of different concentrations of the digestates on the behavior of earthworms and enchytraeids.

Earthworms belong to the phylum Annelida, the class Clitellata and the family Lumbricidae. They are the most important soil invertebrates in most soils worldwide, in terms of both biomass and activity. Earthworms are also known to influence soil structure, soil chemistry and in particular processes like the organic matter decomposition (RÖMBKE *et al.*, 2005). In agro-ecosystems, earthworm numbers can reach up to 328 individuals m^{-2} in non-tilled systems and 344 individuals m^{-2} in tilled organic systems (SMITH *et al.*, 2008)). They can be divided into three ecological groups (RÖMBKE *et al.*, 2005):

– Epigeic species: they live above the mineral soil surface and feed on litter and/or the attached microorganism and ingest little or no soil.

– Anecic species: they live in permanent vertical burrows in mineral soil layers (up to 3 m deep) and feed at the soil surface by dragging leaves and other organic materials into the soil. They also ingest some soil.
– Endogeic species: they inhabit mineral soils, making horizontal non-permanent burrows, mainly in the uppermost 10-15 cm of the soil. They feed on soil more or less enriched with organic matter and probably the most important part of their diet is the attached microorganisms.
Enchytraeids belong to the family Enchytraeidae, class Clitellata and phylum Annelida and are the closest relatives to earthworms (JÄNSCH *et al.*, 2005). They play a major role in soil functions like the decomposition of organic matter. The enchytraeids belong to the saprophagous mesofauna of the litter layer and the upper mineral soil. They usually feed on slightly to strongly decomposed remains of plants and microorganisms (bacteria and fungi), 80 % of their diet consist of microorganisms and 20 % of dead organic matter (JÄNSCH *et al.*, 2005). In agricultural systems, enchytraeid abundances range from 4,650 to 30,000 per m^2 (VAN VLIET *et al.*, 1997).
Few studies have described the effect of digestates on the soil biota. ELSTE *et al.*, 2010 documented the effect of a type of digestate (classic, not processed) on the abundance and biomass of earthworms in comparison to liquid manure and mineral-N-fertilizer. They observed that in a loam soil the abundance and biomass of earthworms was higher in those treatments which were fertilized with liquid manure, followed by the use of digestate and mineral-N-fertilizer. In a silt loam soil, the abundance of earthworms tended to be higher in the unfertilized control, followed by the use of digestate, liquid manure and mineral fertilizer. A study of BRAUCKMANN *et al.* (2009) showed the effect of a digestate with a dry-matter content of 1.2 % on earthworms in a controlled environment (Terrestrial Ecosystem Model; VGL). They observed that under these conditions the digestate had a tendency to increase earthworm populations more than the liquid manure (mixture of cow and pig manure) and significantly increase abundance as compared to unfertilized control. SENSEL & WRAGGE (2008) carried out an avoidance response test to investigate the effect of a type of digestate with 6.19 % dry-matter content on a determined population of *Eisenia fetida* in comparison to an unfertilized control. In this study, the reaction of the earthworms depended on the amount of digestate applied to the system. With the application of 6.2 g of digestate per 500 g of soil compared with unfertilized soil, 64 % of the earthworms tended to move to the soil mixed with digestate. At an application rate of 30.6 g of digestate per 500 g of soil, 98 % of the earthworms moved to the unfertilized control. They also observed in field experiments that the abundance of earthworms decreased as soon as the nitrogen application

rate of one type of wet digestate increased. This digestate had 6.19 % dry matter, the pH-value was 7.96, the total amount of nitrogen was 0.50 %, the ammonium content was 0.33 % and the C/N ratio was 8.8.

Studies related to the effect of digestates on the population of enchytraeids are not available. However, there are some investigations regarding the effect of farmyard manure, liquid manure and mineral fertilizers on their abundance. GRAENITZ & BAUER (2000) found that the application of farmyard manure increased the abundance of enchytraeids by 80 % in spring compared with the applications of mineral fertilizer and by 20 % compared with the unfertilized control. This effect was also observed in autumn, with increases of 50 % and 30 % respectively. A research conducted by DE GOEDE *et al.* (2003) showed that after the application of 76 kg ha^{-1} N of liquid manure, the enchytraeid abundance in autumn was 10-20 % higher than after the application of mineral fertilizer in two different locations.

Based on this information, the aim of this chapter is to describe the effect of the application of both digestates compared with farmyard manure, liquid manure and mineral fertilizers on earthworm and enchytraeid populations as bioindicators of the soil quality in a randomized complete block design and under laboratory conditions.

5.2 Material and Methods

5.2.1 Experimental design and soil parameters

The experiments with soil organisms were carried out from 2009 until 2011 in the experimental fields 1 and 2. All information concerning the fertilization, soil analysis and the design of the field experiment can be found in the previous chapter. Each plot was divided into three sections: one section of 1.5 x 8 m^2 to prevent edge effects, a second section of the same dimension which was harvested, and a third section of the same dimension in which assessments of soil organisms and monitoring of the plants were conducted (Figure 17).

5.2.2 Determination of the soil biota

Field experiment

Earthworms were assessed in calendar week 42 of 2009 on field 1 and in calendar week 43 of 2010 on field 2. They were conducted one month after the fertilization and during the BBCH stage 9 of winter wheat. To obtain information about the population distribution of earthworms in the different fertilizer treatments, eight samples of 0.125 m^2 and 20 cm depth from each variant were collected. Because in this volume of soil the probability of finding only epigeic and endogeic species was high, an extraction with a 0.2 % formalin solution from deeper soil layers in the holes was added, allowing also the anecic species to be sampled (Figure 17).

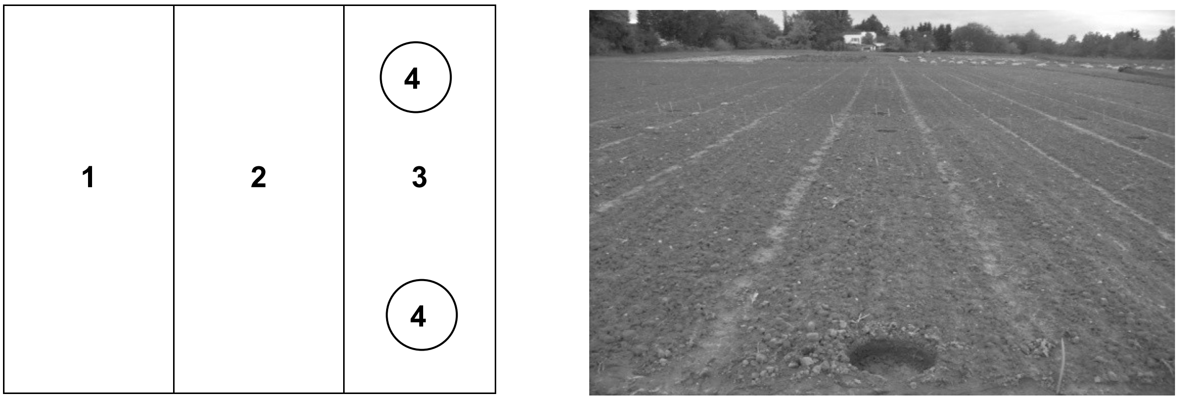


Figure 17: Example of division of one of the treatments on the experimental field in Berlin Dahlem. 1: plot of prevention of edge effect. 2: harvested plot. 3: plot for monitoring of plants and soil organisms. 4: soil samples of 0.125 m^2 and 20 cm deep.

The samples were taken at two points at the plot ends, always in the same position, to avoid damages of the winter wheat crop. The earthworms were removed by hand from the soil samples, counted, weighed and finally the age and species were determined (ISO 23611-1:2006).

The assessments of enchytraeids were conducted on the experimental field 2. The first sampling took place in calendar week 21 of 2010 during BBCH stage 9 of sorghum. The second sampling took place in calendar week 44 of 2010 during BBCH stage 9 of winter wheat. The third sampling took place in calendar week 19 of 2011 at the BBCH stage 13 of winter wheat.

To obtain information about the distribution of the population of enchytraeids in the different fertilizer treatments, 16 samples of 12.6 cm^2 and 10 cm depth for each treatment were collected. The enchytraeids were extracted from the soil samples by the O'Connor method (ISO 23611-3:2007). The soil samples were placed in containers whose bottoms had a 0.5 mm mesh opening. The container was placed in a funnel. The funnel was fixed with a flexible rubber hose to a cylindrical glass container. The funnel and the glass container were filled with water, so that the container with soil samples stayed immersed in water. These three components were immersed in a water bath at a temperature of 12 ± 2 ° C. The samples were heated with an infrared lamp over 2 hours, gradually increasing the temperature until it reached 40 ° C. This temperature was then held for 2 hours. The content of the cylindrical glass containers (water and enchytraeids) was transferred into a petri dish and the animals were counted with a binocular (Vision Engineering Cobra Stereoscope) (Figure 18). The enchytraeids were collected in glass tubes with water and were stored at 5 ° C while they were counted.

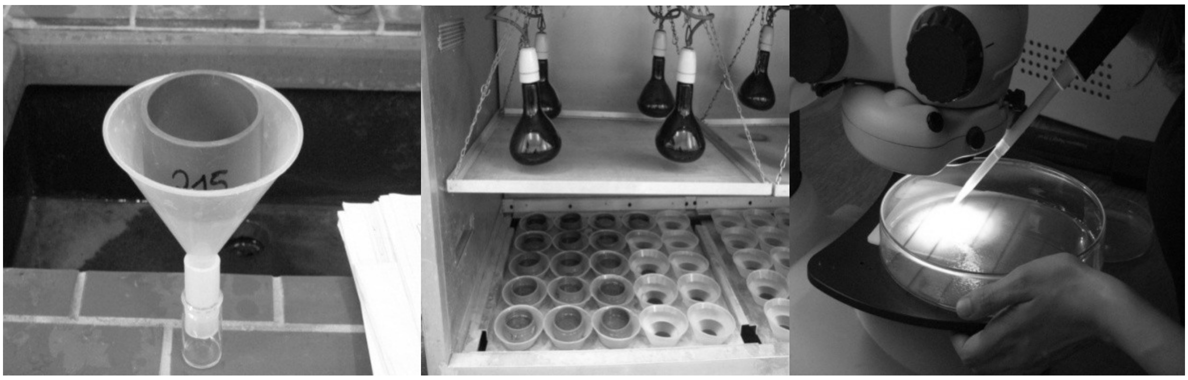


Figure 18: O'Connor method: left photo: container with mesh base, funnel and glass recipient. Central photo: samples submerged in water bath and infrared lights. Right photo: counting of echytraeids under the binocular.

Experiments in the laboratory

As laboratory experiment the avoidance response test was carried out (STEPHENSON *et al.*, 1998) to obtain predicting information about the response of earthworms to the different fertilizers. The first test consisted of a black vessel of 23 cm in diameter and 8 cm depth, divided into six chambers through the use of a star-shaped acrylic glass device. The center of the device was a cylindrical chamber of 6.5 cm in diameter which could be accessed through holes in the base from the adjacent six chambers. The six chambers were also connected through these holes in the bottom of the Plexiglas separation. Every chamber could store about 500 g of soil (Figure 19).

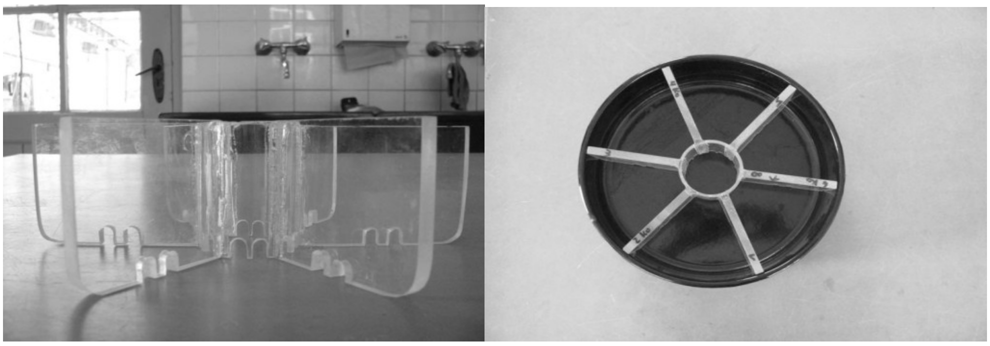
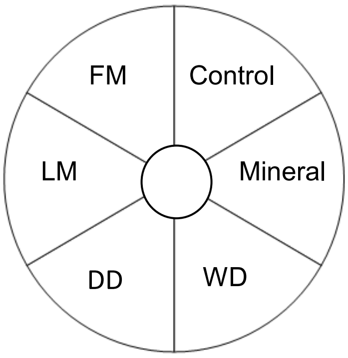


Figure 19: Avoidance test. Chamber divided into 6 chambers by a star-shaped acrylic glass device.

Once the fertilized soil samples to be tested (Tables 18 and 19) were introduced in the chambers, 12 adult earthworms were weighed and placed in the middle of the cylinder. *Aporrectodea caliginosa* was chosen as the experimental earthworm because it is the representative specie of the region of Brandenburg. Due to the phototactic response of earthworms to light, they moved into the chambers filled with the soil to be tested. The vessel was covered to prevent earthworms from escaping during the experiment, and they were kept at about 10-15 ° C. After 72 hours, the earthworms from each chamber were counted and weighed. The chambers were filled with different fertilizers mixed into 500 g of soil and one chamber with unfertilized soil was used as a control (Figure 20).



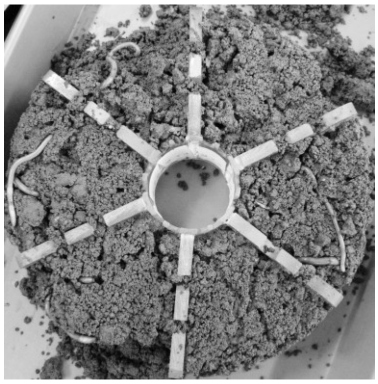


Figure 20: Example of a vessel divided into 6 chambers. Each chamber shows a treatment to be tested. WD: wet digestate, DD: dry digestate, LM: liquid manure, FM: farmyard manure.

Two types of experiments were conducted to obtain information about the preference for a certain kind of fertilizer and avoidance to increased concentrations of fertilizers:

1 - The chambers of the first vessel were filled with unfertilized soil, and then for each treatment 20 g of fertilizer mixed into 500 g of soil. In a second vessel the amounts of fertilizer for each substance was increased to 30 g of fertilizer per 500 g of soil, in a third vessel to 60 g of fertilizer and in a fourth vessel to 80 g of fertilizer mixed into 500 g of soil. The maximum water-holding capacity was calculated and water was added until 60 % of the maximum water-holding capacity was reached. For each of the described concentrations two vessels were prepared as experimental replicates, and vessels were set up in a randomized design. The following table shows the amount of fertilizer used in every vessel and equivalent quantity to be applied in the field.

Table 18: Experiment 1: Quantity of fertilizer per vessel and chamber and equivalent quantity to be applied in the field (kg ha^{-1} N). WD: wet digestate, DD: dry digestate, LM: liquid manure, FM: farmyard manure.

	Chamber		Equivalent in the field (kg ha^{-1} N)					
	Fertilizer (g)	Soil (g)	Control	Mineral	WD	DD	LM	FM
Vessel 1	20	500	0	120	81.58	105.1	65.3	103.2
Vessel 2	30	500	0	178.6	122.4	157.7	97.9	155.0
Vessel 3	60	500	0	357.3	244.7	315.4	195.8	310.0
Vessel 4	80	500	0	477.2	326.3	420.6	261.1	413.3

2 - In each vessel only one substance, but in varying amounts, was tested in order to observe the quantity of fertilizer which is most preferable to earthworms. The order of the concentrations in the sections of each vessel was randomly selected and changed in each vessel. All five substances (mineral-N-fertilizer, wet digestate, dry digestate, liquid manure and farmyard manure) were tested, treatment rates were replicated twice. Soil was wetted to achieve a moisture content of 60 % of the maximum water-holding capacity. The following table shows the amount of fertilizer and soil applied in every chamber, and the equivalent amount of fertilizer to be applied in the field (kg ha^{-1} N).

Table 19: Experiment 2: Quantity of fertilizer per chamber and equivalent amount of fertilizer to be applied in the field (kg ha^{-1} N). WD: wet digestate, DD: dry digestate, LM: liquid manure, FM: farmyard manure.

Fertilizer per chamber (g)	Soil per chamber (g)	Equivalent in the field (kg ha^{-1} N)				
		Vessel 1 Mineral	Vessel 2 WD	Vessel 3 DD	Vessel 4 LM	Vessel 5 FM
Control	500	0	0	0	0	0
10	500	58.7	40.8	52.6	32.6	51.7
15	500	89.9	61.2	78.8	48.9	77.5
30	500	178.6	122.4	157.7	97.9	155.0
40	500	239.8	163.1	210.3	130.5	206.6
50	500	299.8	203.9	262.8	163.1	258.3

The second avoidance response test was carried out with enchytraeids. A vessel of 10 x 8 cm and 5 cm depth was divided into two chambers through a plastic sheet. The chambers were filled with 20 g of soil from the experimental field. Soil was taken from each treatment directly after fertilizer application to the field experiment in autumn 2010. The plastic sheet was removed and 12 enchytraeids were placed at the center of the chamber. The chamber was covered during 72 hours and stored at 10-15 ° C (Figure 21). The maximum water-holding capacity was calculated and water was added until reaching 60 % of the maximum water-holding capacity.

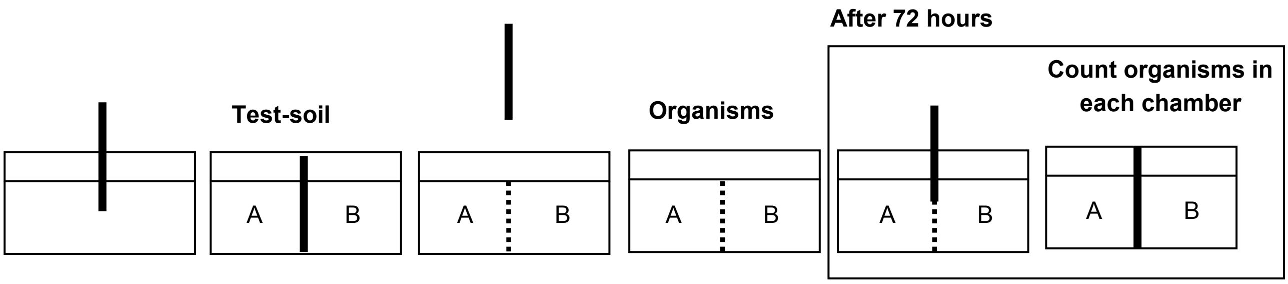


Figure 21. Scheme of avoidance response test (LOUREIRO *et al.,* 2005).

The next table shows the different combination of tested treatments. The experiment was repeated twice (Table 20).

Table 20: Enchytraeid experiment. C: control; M: mineral; WD: wet digestate; DD: dry digestate; LM: liquid manure; FM: farmyard manure.

Organism	Date	Vessel 1		Vessel 2		Vessel 3		Vessel 4		Vessel 5	
Enchytraeids	Autumn 2010	WD	LM	DD	FM	WD	DD	LM	FM	C	M

The selected enchytraeid specie *Echytraeidus albidus* was chosen for their facility to be obtained and for its relatively large size and easy visibility. In this type of test, the "habitat function" can be assessed which proposes that the habitat function of soils is considered to be limited if less than 20 % of the test organisms (on average) are found in the test soil, which indicates an impact on behavior (LOUREIRO *et al*., 2005).

5.2.3 Statistics

All parameters were statistically analyzed based on their normal distribution and homogeneity in variance with one-way ANOVA followed by the Tukey-Test to analyze differences between treatments (SPSS Statistics Desktop 17.0 for Windows). Data was calculated as arithmetic means ± standard deviation. To obtain information about the relationship among the parameters, linear Pearson correlations were estimated at significant level $P < 0.01$ and $P < 0.05$.

5.3 Results

5.3.1 Field experiments with earthworms

In 2009 the highest abundance of earthworms was observed in the farmyard manure followed by liquid manure, dry digestate, wet digestate and the unfertilized control. The total number of endogeic earthworms (*Aporrectodea caliginosa, Aporrectodea icterica*, and *Aporrectodea rosea*) and the juveniles followed the same order of distribution as the total abundance. A higher number of anecic species (*Aporrectodea longa* and *Lumbricus terrestris*) were found in the wet digestate in comparison to other fertilizers. The number of adults was significantly higher in the treatment fertilized with wet digestate in relation to the dry digestate.

The highest biomass was observed in the treatment fertilized with liquid manure followed by the farmyard manure, dry digestate, wet digestate and control. The biomass of the endogeic species followed the same order as the total biomass. The highest biomass of anecic species was found in the wet digestate and the liquid manure. The lowest biomass was observed in the dry digestate and farmyard manure. The juveniles found in the farmyard manure had the highest biomass followed by liquid manure, dry digestate, the wet digestate and the control. The biomass of adults was greater in the liquid manure and farmyard manure (Table 21).

Table 21: Experimental field 1, 2009. Abundance (Individuals m^{-2}) and Biomass (g m^{-2}) of earthworms one month after application of 120 kg ha^{-1} N in various organic fertilizer treatments and an untreated control. Mean values and Tukey-Test (p≤ 0.05). Means from the same homogeneous group are followed by the same letter.

	Control	Wet Digestate	Dry Digestate	Liquid Manure	Farmyard Manure
Abundance (Individuals m^{-2})					
Total Number	61 ^{a}	93 ^{a}	101 ^{a}	124 ^{a}	139 ^{a}
Total Endogeic	58 ^{a}	89 ^{a}	100 ^{a}	120 ^{a}	136 ^{a}
Total Anecic	1 ^{a}	4 ^{a}	1 ^{a}	2 ^{a}	2 ^{a}
Total Juvenils	54 ^{a}	86 ^{a}	96 ^{a}	106 ^{a}	127 ^{a}
Total Adults	5 ^{ab}	6 ^{ab}	4 ^{a}	14 ^{b}	10 ^{ab}
Biomass (g m^{-2})					
Total Biomass	8.57 ^{a}	11.40 ^{a}	13.38 ^{a}	23.06 ^{a}	16.16 ^{a}
Total Endogeic	8.03 ^{a}	10.74 ^{ab}	13.32 ^{ab}	22.22 ^{b}	18.85 ^{ab}
Total Anecic	0.37 ^{a}	0.66 ^{a}	0.05 ^{a}	0.65 ^{a}	0.12 ^{a}
Total Juvenils	4.26 ^{a}	7.44 ^{a}	8.90 ^{a}	9.67 ^{a}	9.95 ^{a}
Total Adults	4.14 ^{a}	3.67 ^{a}	4.15 ^{a}	12.91 ^{a}	8.86 ^{a}

In 2010 the highest abundance of earthworms was observed in the treatment fertilized with wet and dry digestate. In the treatment fertilized with liquid manure and farmyard manure, unlike the previous year, fewer earthworms were found (Table 22).

Table 22: Experimental field 2, 2010. Abundance (Individuals m^{-2}) and Biomass (g m^{-2}) of earthworms 1 month after application of 120 kg ha^{-1} N in various organic fertilizer treatments and an untreated control. Mean values and Tukey-Test (p≤ 0.05). No significance.

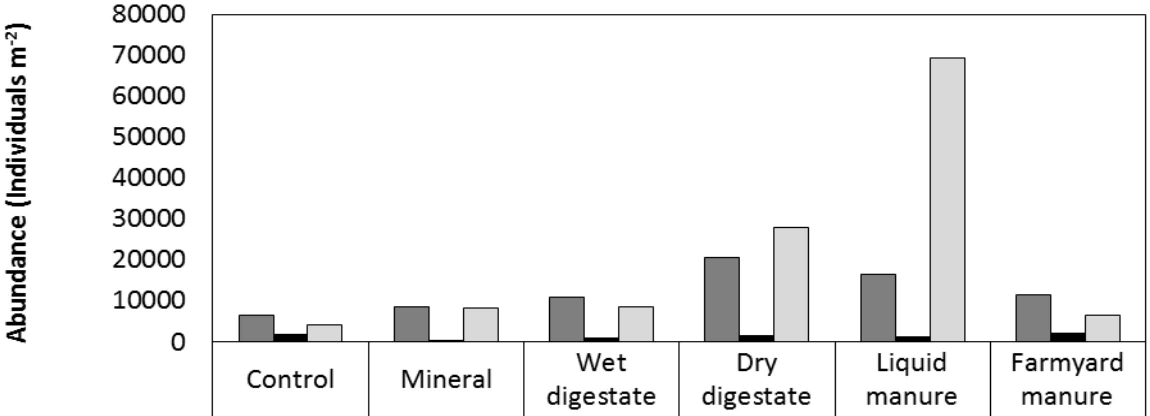
	Control	Wet Digestate	Dry Digestate	Liquid Manure	Farmyard Manure
Abundance (Individuals m^{-2})					
Total Number	71	89	74	68	68
Total Endogeic	67	86	73	65	65
Total Epigeic	3	1	0	2	0
Total Anecic	1	1	1	0	1
Total Juvenils	48	63	48	49	52
Total Adults	21	22	24	17	13
Biomass (g m^{-2})					
Total Biomass	25.75	23.35	29.04	23.97	19.05
Total Endogeic	25.34	25.98	28.82	22.70	18.51
Total Epigeic	0.18	0.08	0.00	0.70	0.00
Total Anecic	0.23	0.07	0.21	0.00	0.09
Total Juvenils	4.50	7.43	5.61	6.08	7.21
Total Adults	20.67	18.27	23.04	17.26	11.14

The abundance of endogeic species and adults followed the same distribution as the total abundance. The anecic species were distributed equally between treatments, with the exception of liquid manure. In this experimental field one epigeic specie was found (*Eisenia fetida*) in the unfertilized control, in the liquid manure and wet digestate (Table 22).

The highest total biomass was found in the variant fertilized with dry digestate, followed by the unfertilized control and liquid manure. The biomass of the endogeic species found in the digestate was higher than in other treatments. Anecic species had greater biomass in the unfertilized control and the dry digestate. The biomass of juveniles, adults and endogeic species is consistent with the number of individuals found (Table 22).

5.3.2 Field experiments with enchytraeids

Figure (22) below illustrates the abundance of individuals of enchytraeids per square meter of spring and autumn 2010 in relation to spring 2011.



	Control	Mineral	Wet digestate	Dry digestate	Liquid manure	Farmyard manure
Spring 2010	6647 a	8581 ab	10962 ab	20486 c	16468 bc	11657 ab
Autumn 2010	1885 a	546 a	1141 a	1786 a	1488 a	2331 a
Spring 2011	4067 a	8234 a	8581 a	27877 ab	69395 b	6498 a

Figure 22: Development of the abundance of enchytraeids during 2010-2011. Mean values and Tukey-Test ($p \leq 0.05$).

It was observed that the abundance of individuals was higher in spring than in autumn. In the spring of 2010 and 2011 the results were comparable in their distribution pattern. The lowest abundance of individuals was found in the unfertilized control and it was 39 % less than in 2011. In 2010, the abundance of enchytraeids was by 20 % higher in the mineral-N-fertilizer, 40 % in the wet digestate, 43 % in the farmyard manure, 60 % in the liquid manure and 68 % in the dry digestate in relation to the unfertilized control. In spring 2011, the difference was 37 % higher for farmyard manure, 51 % for mineral-N-fertilizer, 53 % for wet digestate, 85 % for dry digestate and 94 % for liquid manure in relation to the unfertilized control. In spring 2011, a high percentage of enchytraeids was found in liquid manure and dry digestate. In autumn 2010 the distribution of individuals differed. The lowest number of enchytraeids was found in liquid fertilizers (wet digestate and liquid manure) and the greatest abundance was found in dry fertilizers (dry digestate and farmyard manure) and the unfertilized control.

In order to obtain more detailed information from the experiment 2, a Pearson correlation between the abundance of earthworms, the analyzed soil chemical parameters at the time of sampling and abundance of enchytraeids was carried out. A significantly opposite distribution of both groups (earthworms and enchytraeids) was observed. A significant effect of the TN and TC of the organic fertilizers on the enchytraeid population was also observed (Table 23).

Table 23: Pearson correlation coefficient (r) between soil chemical parameters and the abundance of echytraeids and earthworms in the experimental field 2. TN: total nitrogen, TC: total carbon, TOC: total organic carbon, DM: dry matter, P_{DL}/K_{DL}: phosphorous and potassium in Doppel-Lactat, C/N: carbon-nitrogen ratio.

	Abundance earthworms	**Abundance enchytraeids**
TN (%)	0.23	-0.35 *
TC (%)	0.25	-0.45 **
TOC (%)	0.15	-0.06
DM (%)	-0.13	0.22
P_{DL} (mg 100 g^{-1})	0.20	-0.22
K_{DL} (mg 100 g^{-1})	0.26	-0.20
C/N	0.26	0.08
Abundance earthworms	-	-0.35 *
Abundance enchytraeids	-0.35 *	-

* significance at 0.01, ** significance at 0.05

5.3.3 Experiments in the laboratory

The results for the first experiment, in which different fertilizers were tested per chamber, showed that when the amount of fertilizer per chamber was 20 g per 500 g of soil, 42 % of the earthworms tended to move to the unfertilized control and farmyard manure and 8 % to the mineral-N-fertilizer and dry digestate. If the amount of fertilizer in the system increases to 30 g per 500 g of soil, 25 % of earthworms tend to move to the unfertilized control, 4 % to the mineral-N-fertilizer, 12.5 % to the dry digestate, 17 % to the liquid manure and 42 % into the farmyard manure. Testing a quantity of 60 g of fertilizer per 500 g of soil, 21 % of the earthworms tend to move to the control, 29 % to the dry digestate, 33 % to the liquid manure and 12.5 % to the farmyard manure. After the application of 80 g of fertilizer per 500 g of soil, 62.5 % of earthworms tend to move to the control, 4 % to the mineral-N-fertilizer, 12.5 % to the dry digestate and 21 % to the liquid manure (Table 24).

Table 24: Abundance of earthworms after the avoidance test. Mean values and standard deviations.

Treatment	20 g **500g soil^{-1}**	30 g **500g soil^{-1}**	60 g **500g soil^{-1}**	80 g **500g soil^{-1}**
	Earthworms found in fertilized soil (%)			
Mineral	8.3 ± 11.7	4.2 ± 5.9	0.0 ± 0.0	4.2 ± 5.9
Wet digestate	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Dry digestate	8.3 ±11.7	12.5 ± 17.7	29.3 ± 41.2	12.5± 5.9
Liquid manure	0.0 ± 0.0	16.7 ± 11.8	33.4 ± 0.0	20.8 ± 5.9
Farmyard manure	41.7 ± 0.0	41.7 ± 23.6	12.5 ± 17.7	0.0 ± 0.0
	Earthworms found in the unfertilized control (%)			
Control	41.7 ± 0.0	25.0 ± 11.7	20.8 ± 29.5	62.5 ± 17.7

In the second experiment the response of earthworms to the progressive increase in the amount of one type of fertilizer per vessel was tested. By increasing the amount from 0 to 50 g mineral-N-fertilizer per 500 g of soil it could be observed that 96 % of individuals tend to move to the unfertilized chamber and 4 % to the chambers fertilized with 40 g of mineral-N-fertilizer per 500 g of soil. In the chamber where the wet digestate was tested, 79 % of the earthworms moved into the unfertilized chambers and 21 % to the chambers with 10 g per 500 g of soil (Figure 23).

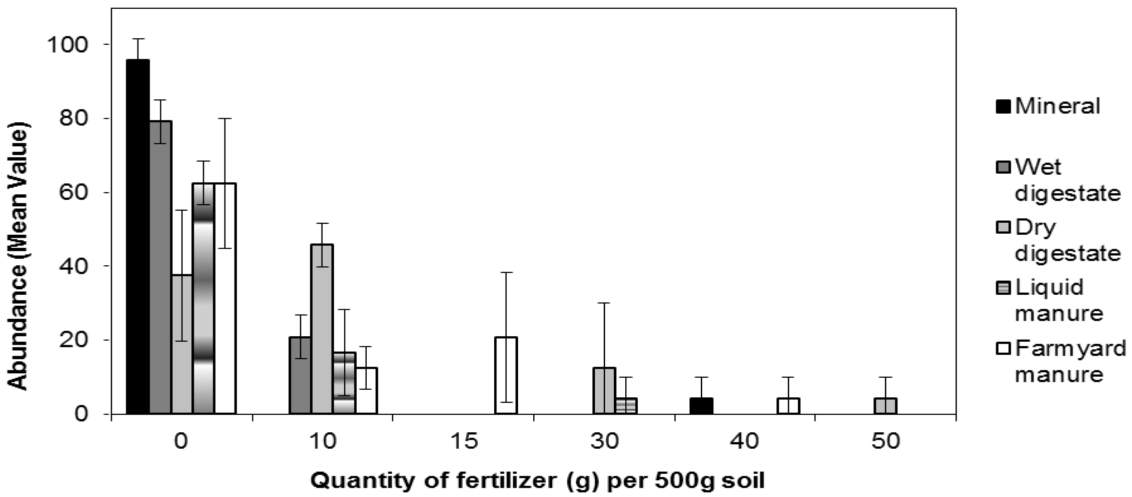


Figure 23: Abundance of earthworms after the avoidance test 2. Mean values and standard deviations.

In the chamber with dry digestate, 37 % of the earthworms were found in the unfertilized chambers, 46 % in the chambers fertilized with 10 g per 500 g of soil, 13 % in the chambers with 30 g per 500 of soil and 4 % in the chambers fertilized with 50 g per 500 g of soil. In the chamber in which the liquid manure was tested, 62 % of earthworms were found in the unfertilized chamber, 17 % in the chambers fertilized with 10 g per 500 g of soil and 4 % in the chambers fertilized with 30 g per 500 g of soil. The distribution of the earthworms in the chamber fertilized with farmyard manure was 62 % for the unfertilized chamber, 12 % for the chambers with 10 g per 500 g of soil, 21 % for the chambers with 15 g per 500 g of soil and 4 % in the chamber with 40 g per 500 g of soil.

Echytraeids 2 chambers

The results in the avoidance test with enchytraeids show that 100 % of individuals tended to move towards the liquid manure when compared with the wet digestate (Figure 24).

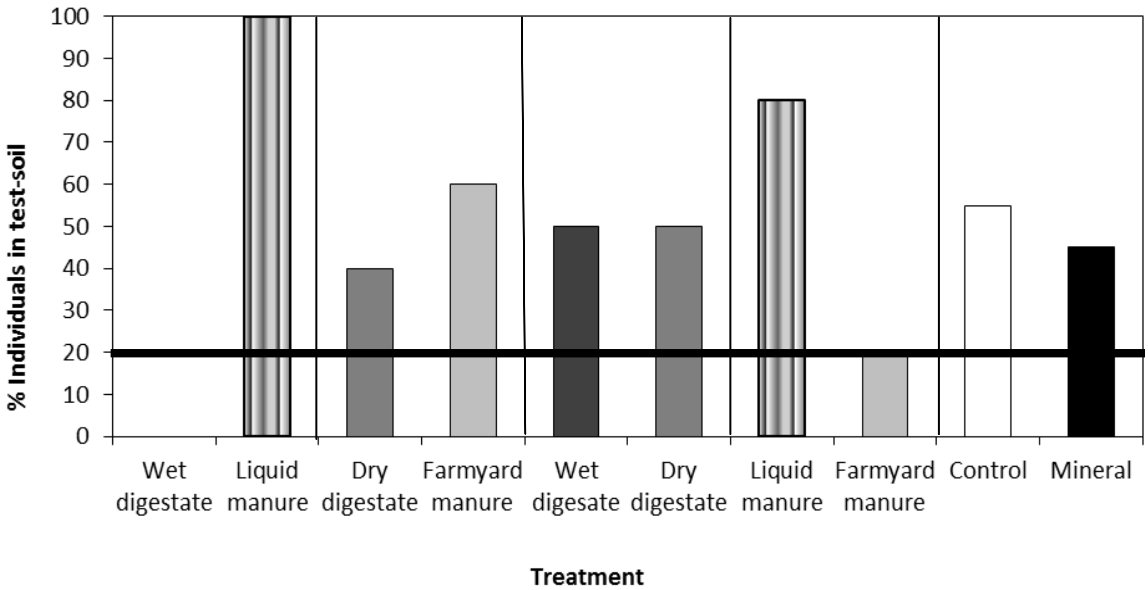


Figure 24: Abundance of enchytraeids after the avoidance test with 2 chambers.

Comparing the dry fertilizers, it was observed that 60 % of individuals moved to the farmyard manure and 40 % to the dry digestate. There was no preference for the type of digestate for enchytraeids. Comparing liquid manure with farmyard manure, 80 % more individuals were found in the liquid manure. The enchytraeids tended to move to the unfertilized control, when compared with mineral-N-fertilizer.

5.4 Discussion

The results of the years 2009 and 2010 showed that the digestates did not negatively affect the population of earthworms. In autumn 2009, one month after the application of the different fertilizers, the highest abundance of earthworms was found in the treatment fertilized

with farmyard manure followed by the treatment fertilized with liquid manure. It is known that organic manures are one of the most important factors in increasing the abundance of earthworms in agricultural soils due to the increased food supply (EDWARDS & LOFTY, 1982). In addition, the C/N ratio of the farmyard manure was the highest. AIRA *et al.,* 2006 have observed that fertilizers with high C/N ratios have a strong positive influence on earthworm populations. However, even though the organic matter content and C/N ratio in the dry digestate was also high, the abundance of earthworms in this treatment was lower than for liquid manure. Earthworms can consume very large amounts of litter and the amount they ingest seems to depend more on the total amount of suitable organic matter available than other factors (EDWARDS & BOHLEN, 1996). The carbon soluble fraction constitutes the available form of the soil organic carbon quickly utilized as a source of nutrition and energy for microorganisms. In 2009, most of the species found were endogeic ones. This group of earthworms feed on soil more or less enriched with organic matter and probably the most important part of their diet is the attached microorganisms (RÖMBKE *et al.*, 2005). This suggests that one month after fertilization, the earthworms were found not only where there was a higher content of organic matter available but where there was higher microbial activity. The average of two analyses of the amount of available carbon of the different fertilizers in 2009 (January and March) showed a greater availability of carbon in the farmyard manure and liquid manure (Chapter 2). This could be the reason for the higher number of earthworms in these two treatments; however, this fact could not be ascertained since only the amount of nitrogen was analyzed in order to calculate the fertilizer application rate.
In autumn 2010, in an adjacent field the results did not confirm the distribution of 2009. The effect of both digestates was 10-20 % higher than the organic conventional fertilizers. Through a Pearson correlation an opposite distribution of the population of earthworms and enchytraeids was observed. This negative interaction was also documented by other authors (TOPOLIANZ *et al.*, 2000). There seems to be a competitive relationship between the groups for the resources to survive in this agro-ecosystem. HAUKKA (1987) observed that one species of enchytraeid (*Enchytraeus albidus*) negatively influenced the abundance of earthworms (*Eisenia fetida*). He proposed that this may be due to metabolic substances which are produced by enchytraeids affecting the food intake of the earthworms. Also, the enchytraeid population seems to be more affected by the chemical parameters of the different fertilizers than earthworms (Table 24). The impact of wet digestate was negative for enchytraeids compared to the unfertilized control. This was probably caused by the higher amount of NH_4^+-N of the wet digestate (PRENDERGAST-MILLER *et al.*, 2009). Due to the aggregated distribution of animals in the soil and the resulting dispersion of data, the results

were not statistically significant. On the other hand, the results corresponding to the avoidance test with soil samples obtained directly after fertilization of the soil in autumn 2010 confirmed the distribution of enchytraeids in the field experiment. The enchytraeids tended to move toward the treatment fertilized with liquid manure and farmyard manure when compared with both digestates. In addition, according to the "habitat function" (LOUREIRO *et al.*, 2005), no negative impact of the wet digestate on the behavior of enchytraeids was observed.

However, after monitoring the behavior of the population of enchytraeids during a year, it is notable that in spring 2010 and 2011 they were distributed mostly in the treatment fertilized with liquid manure and dry digestate. The enchytraeids usually ingest both mineral and organic particles in soil, though generally in a smaller range than that ingested by earthworms. Because enchytraeids lack the necessary enzymes in the intestine to digest recalcitrant organic matter in soil, they feed mostly on tissues of the macrofauna and macroflora responsible for organic matter break down (COLEMAN *et al.*, 2004) and organic material decomposed by the mentioned organisms (CURRY, 1994). The sampling carried out in both years in spring, delivered indirect information on the precise moment of the mineralization process. At this time, more than seven months had passed in which the organic material of different fertilizers had been progressively degraded. The fact that a higher population of enchytraeids was found in wet manure and in dry digestate, suggests that the organic matter in these two fertilizers was sufficiently decomposed to be a suitable habitat for enchytraeids. However, it had been also expected to find a higher population of enchytraeids in the treatment fertilized with wet digestate, which was not the case. This is probably due to the negative effect of ammonia on the population of enchytraeids already mentioned above.

What is clear so far is that there is an antagonistic relationship of both groups. At the time of the extraction of the organisms and the soil sampling the fertilizers appeared to have a greater effect on the enchytraeid population than on the earthworm population and the same effect could be observed in the enchytraeid avoidance test with soil samples taken directly after fertilization. What still remains to be comprehended, is the effect of different fertilizers on earthworms at the time of the application. However, this was also not possible to investigate in the experimental field, because at the time of application only the TN (%) content of each fertilizer was analyzed in order to calculate the amount of fertilizer to be applied in each treatment.

The avoidance test with 6 chambers provided additional information. Despite the nitrogen quantity in the liquid manure, wet digestate and mineral-N-fertilizer was lower than in other fertilizers:

- If the earthworms could choose between different fertilizers, they tended to choose those with lower ammonium content like the unfertilized control and the farmyard manure. In addition, there were no earthworms in any chamber fertilized with wet digestate. There is no clear trend in the behavior of earthworms up to 60 g of fertilizer per 500 g of soil – a fact that can be verified by the high standard deviation values. Up to 80 g per 500 g of soil, most of the earthworms were found in the unfertilized control.
- If the amount of fertilizer was increased in the same vessel, the earthworms tended to move toward the unfertilized control and the remaining number of earthworms tended to decrease as soon as the amount of fertilizer increased. The earthworms were no longer present up to 10 g wet digestate per 500 g of soil, if they had the chance to move to a chamber with a lower quantity of fertilizer.

5.5 Conclusion

With the information obtained from the field and laboratory experiments it could be concluded:

Despite changes in the population distribution of earthworms in the two field experiments (autumn 2009-2010), no negative effect of both digestate on the population of earthworms was observed one month after the application of fertilizers. This fact can be confirmed in the literature (ELSTE *et al.*, 2010; KNACKER *et al.*, 2009). ELSTE *et al.* (2010) also documented a negative effect of one type of digestate on the earthworm population in a silty loam soil, but these results were not significant. Even though there was insufficient scientific basis to clarify the different distributions of earthworms, the idea that the distribution is dependent on the amount of available carbon in the fertilizer was not discarded. Endogeic species not only feed on soil, but also of the attached microorganisms and the abundance of microorganisms is closely related to the amount of nutrients readily available.

One month after fertilization in both the field and the laboratory experiments, the echytraeids seemed to have a greater tendency for conventional organic fertilizers than for the digestates. Also, in the field the mineral-N-fertilizer and wet digestate seemed to have a tendentially negative effect on the enchytraeids population one month after the application. Additions of the different N forms had an effect on soil pH and it seems likely that the enchytraeid response to the different N additions reflects these pH changes (PRENDERGAST-MILLER *et al.*, 2009). Seven months after fertilization of the field, the enchytraeids seemed to have a higher tendency for liquid manure and dry digestate. This fact led to the idea that

these two fertilizers were at a higher decomposition level than the rest of the fertilizers, because due to the lack of enzymes responsible for digesting organic recalcitrant material, enchytraeids feed on decomposed material; moreover, in agreement with the literature, the effect of conventional organic fertilizers on enchytraeid populations was positive in comparison to the unfertilized control and was higher than the effect of the mineral-N-fertilizer.
There was a relationship of competition between the two groups for the existing resources within this agro-ecosystem. This has been documented by several authors. However, it could not be figured out which group was more affected directly after fertilization in autumn 2010.
As soon as the amount of fertilizer under controlled environmental conditions in the laboratory was increased and the earthworms had the option of choosing between various fertilizers, the trend was directed toward those fertilizers with lower ammonium content. The dose from which the earthworms start to display avoidance behavior of other fertilizers or other doses was up to 30 g of fertilizer per 500 g of soil. This means 178 kg N of mineral-N-fertilizer, 122 kg N of wet digestate, 157 kg N of dry digestate, 97 kg N of liquid manure and 155 kg N of farmyard manure ha^{-1} (Table 18 and 19). This fact was also documented by SENSEL & WRAGGE, 2008. They found an avoidance behavior of 98 % of the earthworms to the unfertilized control in a two-chamber system.

Chapter 4

Influence of digestates from agricultural biogas production and organic fertilizers on the humus formation

6 Chapter 4: Influence of digestates from agricultural biogas production and organic fertilizers on the humus formation

6.1 Introduction

The primary aim of this chapter is to investigate the process of mineralization or degradation of digestates compared to conventional organic fertilizers. For this investigation, the respiration of the soil was measured as this makes it possible to measure the activity of the microorganism and predict the process of mineralization and the stability of the carbon in the soil (ZAGAL, *et al.*, 2002). The mineralization is a process in which the edaphic organisms digest and reduce the organic material into inorganic components. The inorganic material is released and constitutes essential nutrients for the plants. The soil moisture, the temperature and the aeration regulate the activity of the microorganisms in the soil, which are the main factors regulating the mineralization rate of the organic fertilizers (MURPHY, 2001). The organic material of the soil consists of several functional pools which are stabilized by specific mechanisms and have different decomposition rates. The decomposition rates represent the time that the carbon remains in a certain pool. The active pool is composed of microbial biomass and components which are easily degradable, such as, for example, roots exudates and plant litter with a relatively short time of decomposition (weeks to years). The passive pool consists of organic matter in a stable form (highly humidified organic compounds) and persists in the soil for thousands of years. There is also an intermediary pool, which is also called a slow pool, in which the organic material persists in a range of years to centuries (TRUMBORE, 1997) (Figure 25).

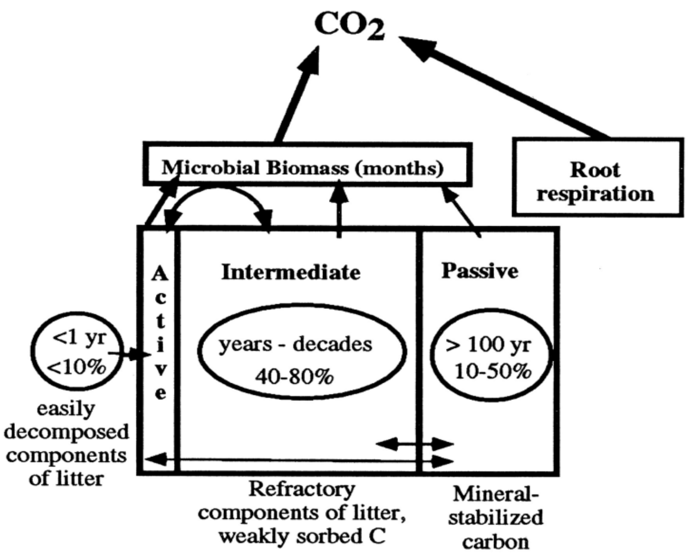


Figure 25: Conceptual model of soil organic matter (TRUMBORE, 1997).

Soil respiration (CO_2) is a product of the metabolic respiration of the roots and the decomposition of the active pools of the organic material in soils by soil organisms (TRUMBORE, 1997). By quantifying the quantity of CO_2, it is possible to obtain information about the decomposition rate of the organic fertilizers. The decomposition rate of the organic fertilizers can provide information about their effect on the formation and stability of the existing humus in a soil. A positive effect in the formation and stability of the humus is based on the presence of stable organic substances with a high C/N ratio (EBERTSEDER, 2007). Enriching the surface of a soil with organic material improves the quality of the soil in relation to the infiltration, aeration, germination of seeds and plant nutrition and by this, also the quality of the soil (HILLEL AND ROSENZWEIG, 2011).

Assessments of the decomposition of organic material and CO_2 flow in the field are usually complicated since they are affected by seasonal changes and it is also necessary to use correction factors by taking into consideration the respiration of the roots. Because of this, the objective of this study was the observation of the CO_2 in the decomposition process in a controlled environment and in the absence of roots.

6.2 Material and Methods

6.2.1 Experimental Greenhouse design

The soil used for this experiment was composed of 83 % sand, 14.2 % silt and 2.7 % clay. In the following table some physical-chemical parameters of the soil are represented before being mixed with the corresponding fertilizers (Table 25).

Table 25: Soil chemical parameters corresponding to 2010 and 2011. TC: total carbon, TN: total nitrogen, TOC: total organic carbon, DM: dry matter, WHC_{max}: maximum water-holding capacity.

	TC (% DM)	TN (% DM)	TOC (% DM)	DM (%)	WHC_{max} (%)
Soil 2010	0.30	0.01	0.22	93.51	25.16
Soil 2011	0.38	0.05	0.33	99.71	24.30

A total of 105 “Mitscherlich-Gefäße” (Mitscherlich vessels) of 23 cm in diameter and 26 cm in height were used for the experiment. In each of these vessels, 5 kg of soil sifted through sieves with a mesh width of 2 mm were mixed with wet digestate, dry digestate, liquid manure or farmyard manure. The applied organic fertilizers presented the following physical-chemical characteristics (Table 26).

Table 26: Chemical parameters of the organic fertilizers corresponding to 2011. TC: total carbon, TN: total nitrogen, TOC: total organic carbon, DM: dry matter.

	TC (% DM)	TN (% DM)	TOC (% DM)	DM (%)
Wet Digestate	2.49	0.30	2.39	5.73
Dry digestate	7.98	0.35	6.99	18.13
Liquid manure	3.34	0.29	3.30	7.69
Farmyard manure	6.41	0.53	6.12	29.14

Because the digestates and organic fertilizers presented different percentages in dry matter, these were dried at 35 ° C for 14 days. With this drying temperature possible damage to the existing microorganisms was avoided. Once the fertilizers were dried, they were sifted to 2 mm as well. According to the quantity of organic carbon in the various fertilizers, different quantities of fertilizers were applied in each vessel in order to achieve the required organic carbon content in the soil. The organic carbon in each vessel reached 0.76 % DM. Taking into account the maximum water retention capacity in the soil, water was added until reaching 60 % of the maximum water-holding capacity.

The experiment was carried out in a greenhouse under controlled environmental conditions. The temperature was maintained during the incubation period between 20 and 22 ° C. The vessels were randomly distributed on two tables of 1 m height each. The treatments were separated in two blocks:

- **Block A**: the following 5 treatments, wet digestate, dry digestate, farmyard manure liquid manure, and an unfertilized control were repeated 7 times and the 35 vessels were randomly distributed. The CO_2 emissions were measured regularly (every two days) during 100 days (description 6.2.2). Once this period finished, the samples were analyzed in the laboratory.
- **Block B**: The above mentioned 5 treatments were repeated 14 times in order to analyze in the laboratory two repetitions per treatment on 7 sampling times. The samples were analyzed in the laboratory after 2, 7, 14, 30, 45, 60 and 100 days from the beginning of the experiment. In these vessels the concentration of CO_2 was not measured.

6.2.2 Measurements of CO_2 efflux from soil surface

The emission of CO_2 from the surface of the soil was measured by the use of a portable infrared analyzer (EGM4 – ENVIRONMENTAL GAS MONITOR PP SYSTEMS, UK) which measures the concentration of CO_2 in a chamber with surface area 78.5 cm^2 and height 10 cm. This chamber was placed on the soil surface and measures the CO_2 flow in a certain time period. To avoid errors in the CO_2 measurement, due to the rupture of the structure of the soil surface, a ring with the same diameter as the chamber was inserted into the soil in every vessel, protruding 1 cm above the soil surface. This made it possible to measure the same soil area on each measuring date (Figure 26). The estimated period of time for the measurement of the CO_2 was 200 seconds.

Figure 26: Environmental Gas Monitor (EGM-4).

The portable infrared analyzer was connected to a thermometer which was inserted into the soil close to the respiration measurement point (approximately 10 cm deep) to monitor the temperature at the moment when the respiration measurement was being carried out. Also, the water content in the soil was measured by using a TDR sensor (TIME DOMAIN REFLECTROMETRY) in order to control that every vessel had 60 % of maximum water holding capacity during the whole experiment. The vessels were covered with a lid to avoid water losses from evaporation as well as possible external contamination.

6.2.3 Analysis of the soil and organic fertilizers

Analysis of the total nitrogen, total carbon, total organic carbon and pH of the soil samples were carried out in the laboratory. Information about the methodology used for this analysis can be found in Chapter 1. For the investigation of the hot-water-soluble carbon (HWSC), each sample was boiled with distilled water for a duration of one hour at reflux. The content of HWSC was determined from the centrifuged extracts with the LiquiTOC (VDLUFA-SCHULTZ, 2010). Soluble soil nitrogen (N_{min}) was extracted with $CaCl_2$ (VDLUFA, 1991). For the determination of the structural components (hemicellulose, cellulose and lignin) of the fertilizers the analysis of neutral detergent fiber (NDF) was carried out. For this, 1 g of soil sample was mixed with a detergent solution (90.35 g EDTA, 34.05 g disodium tetraborate x 10 H_2O in 2 l distilled hot water, 150 g sodium borate, decahydrate, 22.8 g sodium phosphate, dibasic, pH 6.9-7.1), boiled for 1 hour, washed with hot water and acetone and dried at 100 ° C. Finally the samples were incinerated at 500 ° C for 4 hours. The neutral detergent fiber was calculated by using the following formula:

Formula 1: Calculation of the Neutral Detergent Fiber (NDF) percentage

$$NDF\ (\%) = (DW\ (g) - IW\ (g))\ /\ iW\ (g) \times 100$$

DW: dry weight
IW: incinerated weight
iW: initial weight

For the determination of cellulose and lignin the analysis of acid detergent fiber (ADF) was carried out. The same procedure as for the analysis of neutral detergent fiber but with a different detergent solution (267 ml concentrated H_2SO_4, 5 l distilled water, 20 g cetyltrimethylammonium bromide) was performed. The acid detergent fiber was calculated using the following formula:

Formula 2: Calculation of the Acid Detergent Fiber (ADF) percentage

$$ADF\ (\%) = (DW\ (g) - IW\ (g))\ /\ iW\ (g) \times 100$$

DW: dry weight
IW: incinerated weight
iW: initial weight

To determine the lignin content the analysis of acid detergent lignin (ADL) was carried out. Also, the same procedure as for the analysis of acid detergent fiber but with 72 % sulfuric acid was performed. Sulfuric acid was stirred at constant coverage for half an hour and filtered under vacuum for 3 hours. Finally the samples were washed with hot water, dried at 105 ° C and incinerated at 500 ° C for 4 hours. Acid detergent lignin was calculated by the following formula:

Formula 3: Calculation of the percent Acid Detergent Lignin (ADL)

$$ADL\ (\%) = (DW\ (g) - IW\ (g))\ /\ iW\ (g) \times 100$$

DW: dry weight
IW: incinerated weight

iW: initial weight

NDF, ADF and ADL from the samples were analyzed with the Fibertec System (Fa.Tecator, heute Fa. Foss).

Formula 4: Calculation of the different fractions

NDF: Celullose + Hemicellulose + Lignin
ADF: Celullose + Lignin
ADL: Lignin

6.2.4 Soil biological activity

In the vessels belonging to Block B (in which the CO_2 was not measured), a bait lamina test (terra protecta GmbH, 1999) was carried out in order to measure the microbiological activity. For this, plastic strips or bait laminas of length 16 cm and width 1 cm were inserted into the soil (Figure 27).

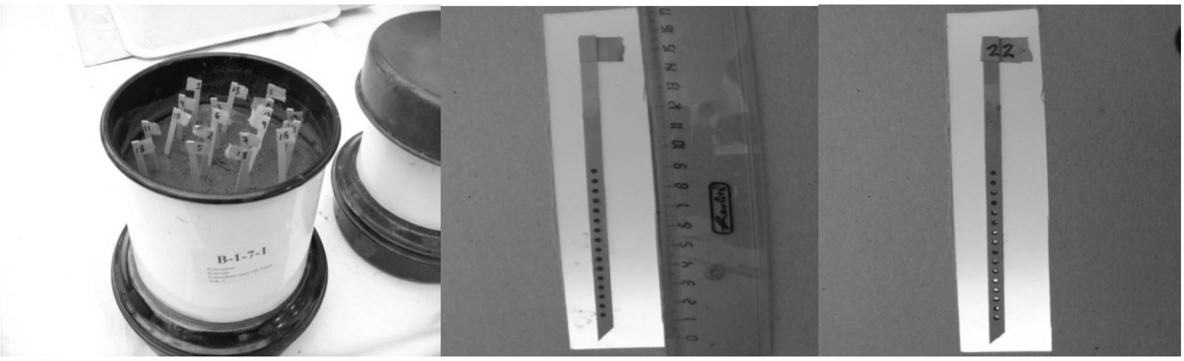


Figure 27: Bait laminas used as bioindicators for the biological activity.

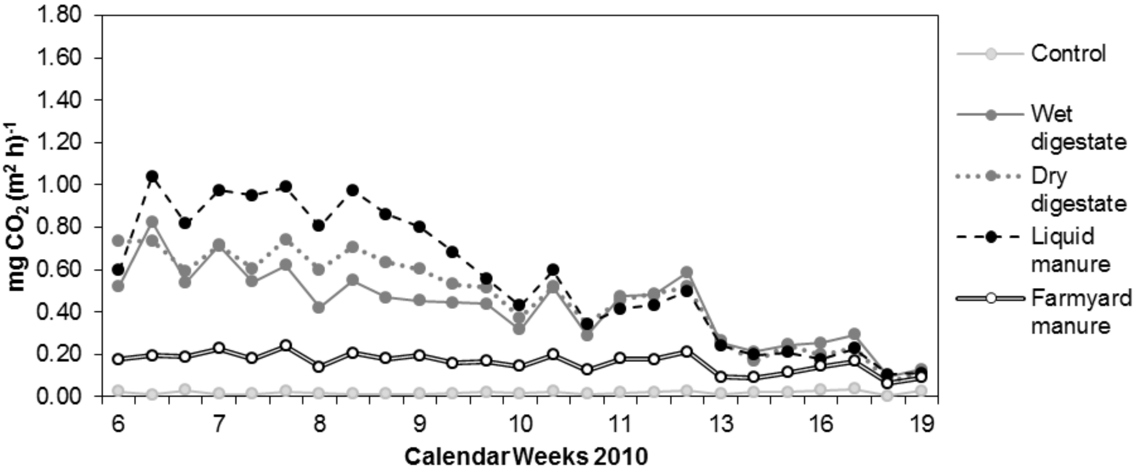
Every lamina contained 16 baits of approximately 3.14 cm^2 which were filled with active coal and cellulose. 20 bait laminas were inserted into the soil of each treatment in calendar weeks 10, 13 and 20. After three days the strips were removed and the percentage of empty baits was counted.

6.2.5 Statistics

All parameters were, based on their normal distribution and homogeneity of variance, statistically analyzed with one-way ANOVA followed by the Tukey-Test to analyze differences between treatments (SPSS Statistics Desktop 17.0 for Windows). Data was calculated as arithmetic means ± standard deviation. To obtain information about the relationship between the parameters, linear Pearson correlations were estimated at significant levels P <0.01 and P< 0.05.

6.3 Results

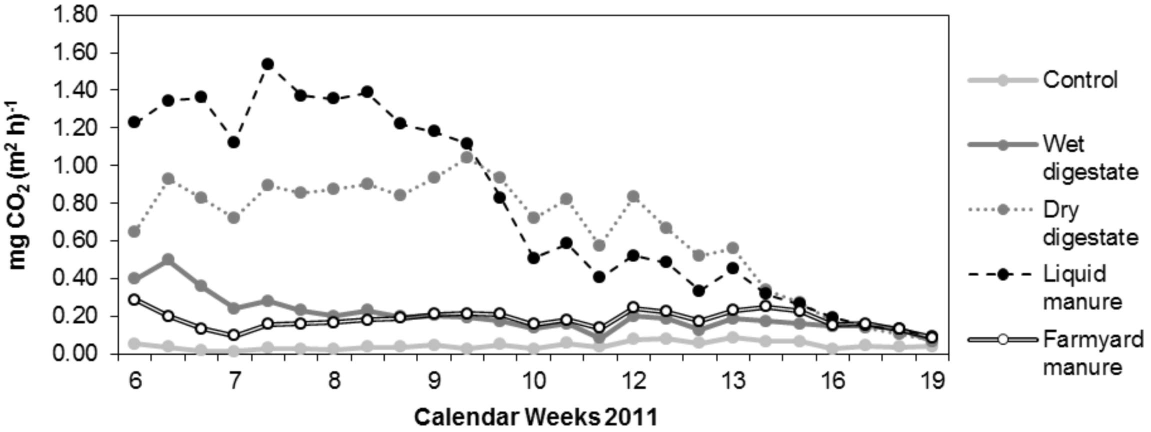
In 2010 over a period of 9-10 weeks differences in the respiration were observed between the organic fertilizers. The liquid manure exhaled 80 % more CO_2 than the farmyard manure and the digestates exhaled 70-75 % more than the farmyard manure. As the weeks elapsed the release of CO_2 decreased, but the above-mentioned order of CO_2 release still remained. From calendar week 10 onwards no significant differences between liquid manure and the digestates were observed. However, these differed significantly from the farmyard manure and the unfertilized control until calendar week 16. From calendar week 16 onwards, there were significant differences between the different treatments (Figure 28).



C	a	a	a	a	a	a	a	a	a
WD	c	bc	bc	bc	b	b	b	b	b
DD	bc	bc	cd	cd	b	b	b	b	b
LM	bc	c	d	d	b	b	b	b	b
FM	ab	ab	ab	ab	a	a	a	b	b

Figure 28: 100 days of decomposition process during 2010. C: control, WD: wet digestate, DD: dry digestate, LM: liquid manure, FM: farmyard manure.

In 2011 the decomposition pattern of the organic matter was similar to the pattern observed in 2010. However, there were three notable differences: Firstly, in 2011 the amount of CO_2 exhaled from the liquid manure and dry digestate was higher than in the previous year. Secondly, the amount of CO_2 exhaled from the wet digestate was 40 % lower than the year before. Thirdly, the exhalation of CO_2 of the liquid manure between calendar weeks 9 and 10 decreased by 60 %. Also, there was a progressive and lower decrease of the CO_2 exhalation in the dry digestate in comparison to the liquid manure (Figure 29).



C	a	a	a	a	a	a	a	a	a
WD	b	b	b	a	a	a	a	b	a
DD	c	c	c	b	c	c	b	b	a
LM	d	d	d	c	b	b	b	b	a
FM	b	a	ab	a	a	a	a	b	a

Figure 29: 100 days of decomposition process during 2011. C: control, WD: wet digestate, DD: dry digestate, LM: liquid manure, FM: farmyard manure.

In order to observe the process of decomposition of organic matter in the different treatments, polysaccharides (hemicellulose and cellulose) and organic polymers (lignin) present in the fertilizers were analyzed. The highest content of hemicellulose and cellulose was observed in the dry digestate and the liquid manure. The lowest percentage of hemicellulose was observed in the farmyard manure and, in the case of cellulose, in wet digestate. The highest percentages of lignin were observed in the wet digestate and farmyard manure followed by the dry digestate and the liquid manure. In 2011 the lignin content in the wet digestate was 45 % higher and the quantity of hemicelullose was 80 and 90 % lower than in previous years (Table 27).

Table 27: Hemicelullose, celullose and lignin content of the different organic fertilizers in 2011. MV: mean value 2009-2010: Data von IASP.

Treatment	**Hemicellulose (% DM)**		**Cellulose (% DM)**		**Lignin (% DM)**	
	2011	*MV 2009/2010*	2011	*MV 2009/2010*	2011	*MV 2009/2010*
Wet digestate	1.78	*8.95*	10.59	*8.28*	25.19	*11.81*
Dry digestate	20.28	*20.34*	25.30	*20.52*	10.23	*8.83*
Liquid manure	17.12	*17.15*	20.24	*16.93*	10.15	*6.15*
Farmyard manure	2.18	*22.26*	13.32	*10.34*	18.20	*23.06*

The following figure (30) represents the amount of ammonia (NH_4^+-N) in the different treatments. It should be observed that the treatment with liquid manure and wet digestate contained 70-80 % more ammonia than the other treatments. The ammonia content in the different fertilizers was reduced progressively until calendar week 10; from then on no differences between the treatments were observed.

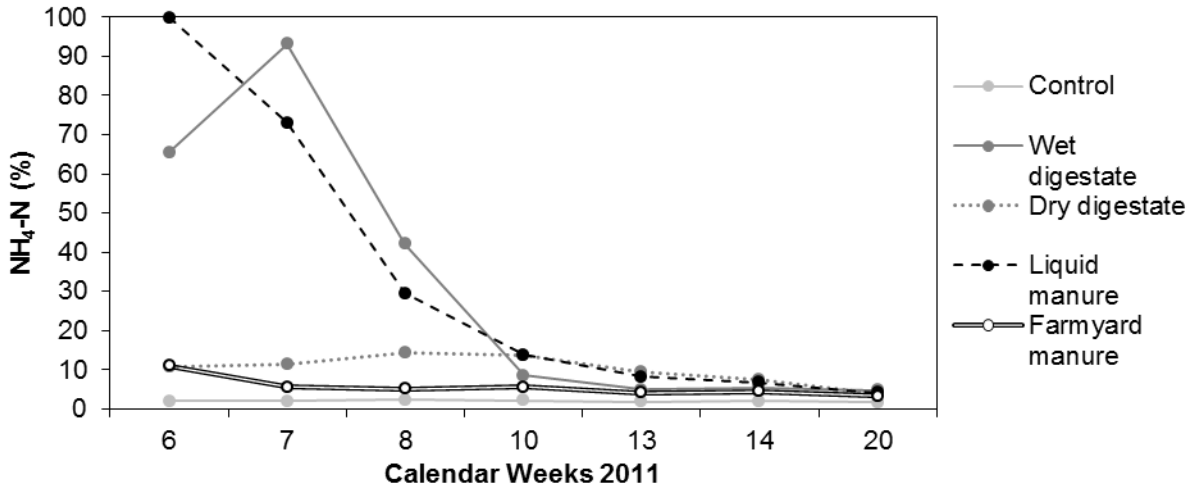


Figure 30: Ammonia content in the different treatments during the decomposition process in 2011.

As soon as the process of decomposition of the organic matter advanced, the amount of nitrate in each treatment increased. The following figure (31) shows that until calendar week 10 the amount of nitrate released from the liquid manure and wet digestate was higher than from the other treatments.

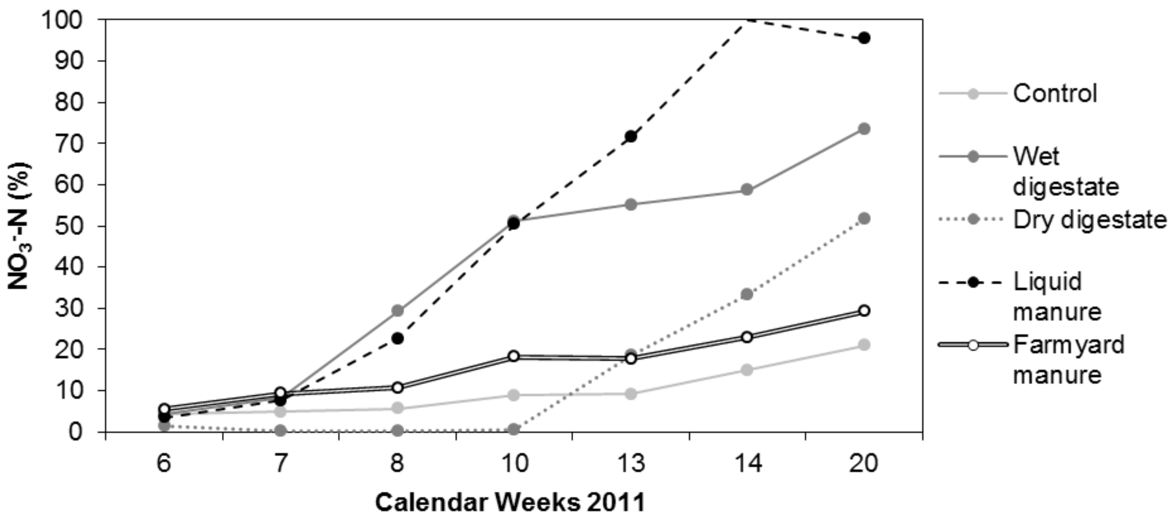


Figure 31: Nitrate content in the different treatments during the decomposition process in 2011.

From this point, the amount of nitrate found in the treatment fertilized with liquid manure increased exponentially until calendar week 14. The amount of nitrate in the treatment fertilized with wet digestate also increased progressively and in calendar week 20 achieved 25 % less nitrate than the liquid fertilizers. The amount of nitrate in the treatment fertilized with dry digestate was below the unfertilized control until calendar week 10. At this point, it started to increase until it reached 50 % less nitrate than the liquid manure. The amount of nitrate in farmyard manure increased progressively until it reached 75 % less than in the liquid manure (Figure 31).

The following figure (32) represents the C/N ratio from the different organic fertilizers. In both years similar patterns were observed. In 2010 the C/N ranged between 20 and 30 at the beginning of the experiment, falling to 10 and 15 by the end. In 2011, the C/N ratio varied from 10 to 20 at the beginning of the experiment and from 10 to 15 at the end. Although the development of the C/N ratio in both years was irregular, it can be observed that the farmyard manure and dry digestate in general have a higher C/N ratio than the liquid manure and wet digestate.

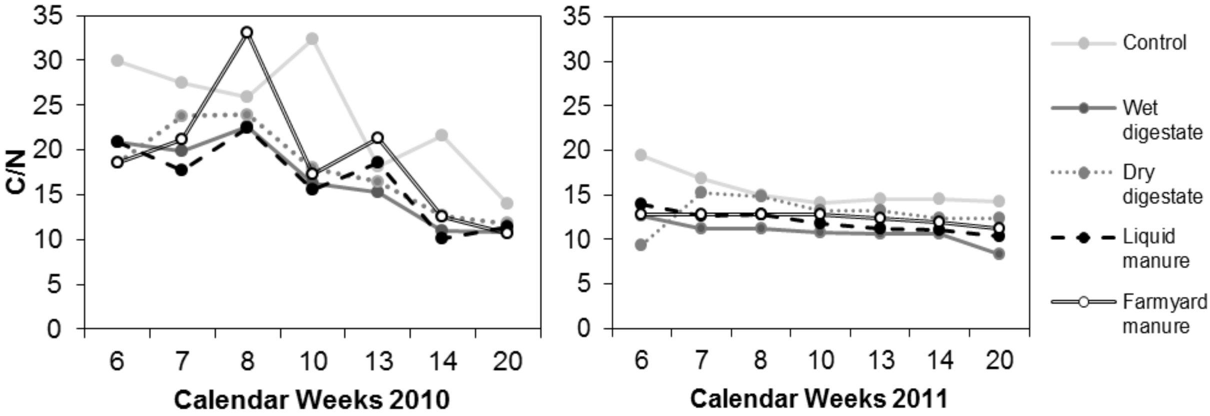


Figure 32: C/N ratio of the different treatments during the decomposition period of the organic matter in 2010 and 2011.

In the following figure (33) the percentages of the mineralized or available carbon of the different organic fertilizers are represented. It should be observed that the wet digestate and farmyard manure in both years had a lower percentage of mineralized organic carbon than the dry digestate and liquid manure.

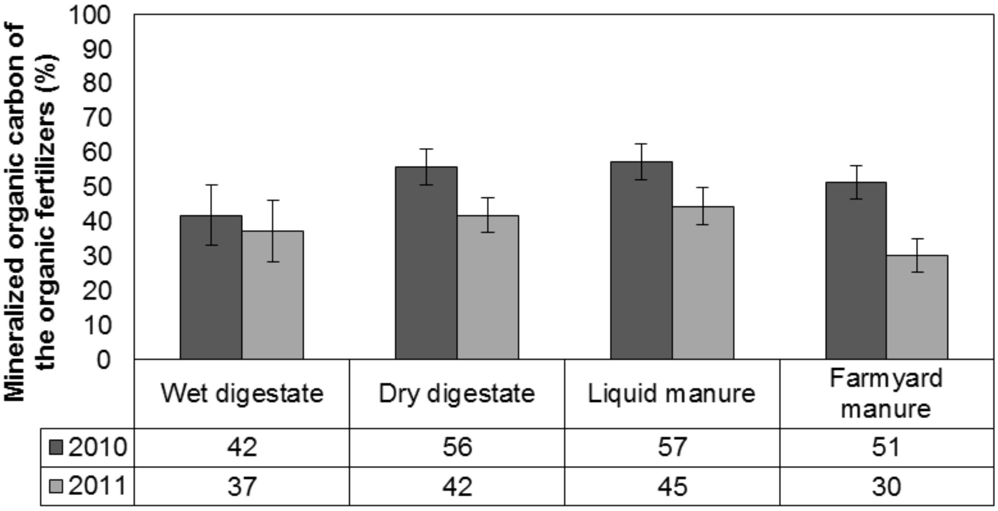


Figure 33: Percentage of mineralized carbon of the organic fertilizers (%).

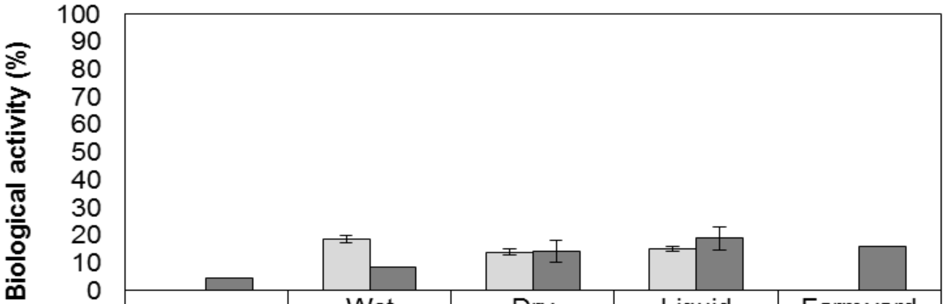
Pearson correlations were performed to observe the relationship between the amounts of CO_2 exhaled and the chemical analyzed parameters, in addition to the pH values. The following table shows different levels of correlation between the investigated parameters (Table 28).

Table 28: Pearson correlation coefficient (r) between the exhaled CO_2, total organic carbon (TOC), hot water soluble carbon (HWSC), ph, nitrate (NO_3^--N), ammonia (NH_4^+-N) and C/N ratio.

				2010			
	CO_2	**TOC**	**HWSH**	**pH**	**NH_4^+-N**	**NO_3^--N**	**C/N**
CO_2	-	0.55**	0.73**	0.65**	n.a.	n.a.	0.03
TOC	0.55**	-	0.66**	0.71**	n.a.	n.a.	0.40
HWSH	0.73**	0.66**	-	0.80**	n.a.	n.a.	-0.05
pH	0.65**	0.71**	0.70**	-	n.a.	n.a.	-0.23
NH_4^+-N	n.a	n.a	n.a	n.a	-	n.a.	n.a
NO_3^--N	n.a	n.a	n.a	n.a	n.a.	-	n.a
C/N	0.28	-0.40	-0.05	-0.23	n.a.	n.a.	-
				2011			
CO_2	-	0.33	0.75**	0.72**	0.53**	-0.24	-0.04
TOC	0.33	-	0.61**	0.66**	0.25	-0.31	-0.50**
HWSH	0.75**	0.61**	-	0.87**	0.72**	-0.14	-0.23
pH	0.72**	0.66**	0.87**	-	0.69**	0.11	-0.46*
NH_4^+-N	0.53**	-0.078	0.72**	0.69**	-	-0.26	-0.08
NO_3^--N	-0.24	-0.031	-0.14	0.11	-0.26	-	-0.60**
C/N	-0.43	-0.50**	-0.23	-0.46*	-0.08	-0.60**	-

The Pearson correlation analysis showed a significant positive and negative linear relationship between most of the parameters. In 2010, the highest significant correlations (0.70 to 0.80) were found in the pH, HWSH and the CO_2 exhalation. In 2011, this pattern was repeated with the addition of a high significant correlation between the NH_4^+-N and the HWSH (Table 28).

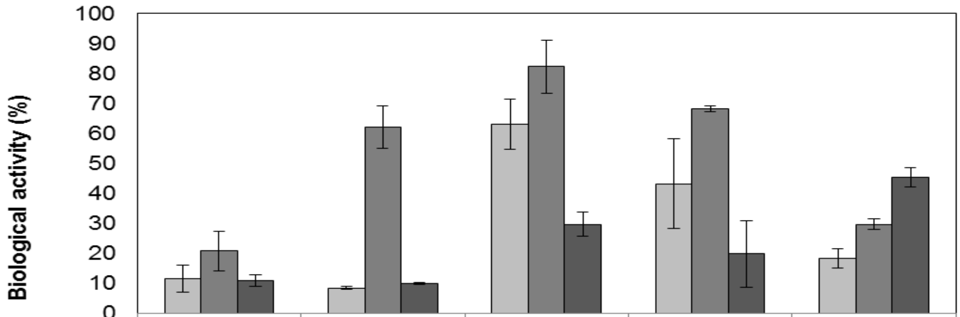
Additional data was obtained after the observation of the biological activity in the different treatments. In 2010, the highest biological activity was observed at calendar week 10 in the liquid digestate, liquid manure and dry digestate. In calendar week 18, the activity changed, and a higher activity in the treatment fertilized with liquid manure followed by the farmyard manure and dry the digestate was observed (Figure 34).



	Control	Wet digestate	Dry digestate	Liquid manure	Farmyard manure
□ Calendar Week 10	0	19	14	15	0
■ Calender week 18	5	8	14	19	16

Figure 34: Percentage of biological activity in the different treatments in 2010.

At calendar week 10 of 2011, the biological activity followed a similar pattern to the one in calendar week 10 of 2010, with the exception of the treatment fertilized with wet digestate. In this treatment the percentage of biological activity was 10 % lower. In 2011 the biological activity was also measured in calendar week 13. The results showed a higher biological activity in the treatment fertilized with dry digestate followed by liquid manure and wet digestate. In calendar week 20 the highest biological activity was observed in farmyard manure and dry digestate (Figure 35).



	Control	Wet digestate	Dry digestate	Liquid manure	Farmyard manure
□ Calendar Week 10	11	8	63	43	18
■ Calendar Week 13	21	62	82	68	30
■ Calendar Week 20	11	10	30	20	45

Figure 35: Percentage of biological activity in the different treatments in 2011.

6.4 Discussion

Soil organic matter is thermodynamically unstable under oxidative conditions. It is oxidized into water and carbon dioxide. This process is influenced by heterotrophic organisms (VAN BREEMEN & BUURMAN, 2002). The first stages of decomposition of organic matter include the decrease in size of the organic particles and the consumption and rapid degradation of the labile fraction by the fauna and microorganisms. This process results in the release of CO_2 and other inorganic elements such as ammonium, phosphate and sulfate. In addition, recalcitrant forms of organic matter such as lignin and alkyl structures accumulate in the soil (SENESI *et al.*, 2009).

Observations of different organic fertilizers under controlled environmental conditions showed that the exhalation of CO_2 followed a clear pattern of decomposition for the different fertilizers. It was observed that there is an increased exhalation in the liquid manure and a low release of CO_2 in the farmyard manure. Both digestates occupied an intermediate position. In both years, a significant linear relationship between the exhaled CO_2 and labile carbon (HWSC) was observed. This indicates that during decomposition the readily available carbon was consumed by microorganisms. As the relationship is positively linear, the CO_2 exhalation decreases as soon as the amount of available carbon decreases in the system. This fact is also reflected in the % of biological activity measured with the bait laminas.

The mineralization process of N consists mainly of an ammonification process that gives rise to the release N from the organic matter. The results corresponding to the analysis of ammonium and nitrate from 2011 showed that as the mineralization process progresses, the ammonium content decreased and the amount of nitrate increased in the system. In addition, it should be observed that the amount of ammonium in the soil analyzed was 70 % higher in the liquid manure and 80 % higher in the wet digestate than in the other organic fertilizers. This suggests that the state of decomposition of these organic fertilizers is more advanced than the dry digestate and farmyard manure. The second step in the mineralization process is the transformation of NH_4^+-N into NO_3^--N through the process of nitrification. This occurs through the activity of two groups of autotrophic and aerobic bacteria, the Nitrosomonas which transform NH_4^+-N into NO_2^- and Nitrobacter which transform NO_2^--N to NO_3^--N (BLOEM *et al.*, 2006). During the process of nitrification there was a greater release of nitrate in the liquid manure and wet digestate than in the rest of the fertilizers. The amount of nitrate produced was lower in the wet digestate than in the liquid manure despite having 10 % more ammonia. This fact suggests that the NH_4^+-N may have subsequently followed one of two paths: The ammonia was released as ammoniac into the atmosphere, or was used by heterotrophic microorganisms to decompose organic residues. In the second case the NH_4^+-N is immobilized constituting part of the microbial tissue (PERDOMO & BARBAZÁN, n.d.).

The C/N ratio has been frequently used as a predictor for the decomposition rate (CHAPIN *et al.*, 2002). If the C/N ratio of fresh organic matter amounts to 30, the immobilization of nitrogen takes place. A C/N ratio between 20 and 30 may indicate both immobilization and mineralization. Values below 20 indicate the release of nitrogen through the mineralization process (GOWARIKER *et al.*, 2009). The correlation between the C/N ratio of different fertilizers and the CO_2 exhalation was low and not significant. In addition, there was a negative correlation with low significance between the C/N and NO_3^--N. This correlation was also observed by other authors (SAGUER & GISPERT, 1996; HEIJ, 1991). This means that as soon as the C/N ratio decreases, the amount of nitrate increases in the system and this fact is indicative of the mineralization process.

The CO_2 exhalation in both years was similar. In 2011 the exhalation of CO_2 in the wet digestate was lower than in 2010; nevertheless the mineralization process continued (a fact which can be observed in the release of NH_4^+-N and its transformation into NO_3^--N). This fact leads to the assumption that the liquid digestate contained less readily usable labile carbon. The order of breakdown of organic matter begins with the consumption of labile carbon by microorganisms followed by hemicellulose, cellulose and finally lignin (CHEN & AVNIMELECH, 1986). Further, it should be noted that the amount of hemicellulose, cellulose and lignin of the wet digestate is comparable to that of farmyard manure. In both fertilizers, there was a high state of decomposition of cellulose and hemicellulose and high lignin content. These results can be also confirmed by the fact that these fertilizers also had the lowest percentage of mineralized organic matter (Figure 33). The results for the analysis showed that the lignin content in 2011 was 53 % higher than the mean value of 2009 and 2010 and the hemicellulose content was 80 % lower than those of 2009-2010 (Table 27). The decomposition of these three components is slower in anaerobic conditions than under aerobic conditions. It was estimated that during the early stages of aerobic decomposition, microorganisms decompose 40-60 % of the original organic matter. Under anaerobic conditions, this percentage drops to 5 % (CHEN & AVNIMELECH, 1986). This indicates that this batch of wet digestate was probably stored in aerobic conditions for the time necessary for the microorganisms to consume the hemicellulose and cellulose. The high lignin content could be explained due to a higher input of raw material in the fermenter or by the mixture of wet digestate with raw material.

One aspect that should not be forgotten in this study is the potential of the fertilizer to contribute positively to the formation of humus in the soil. According to STEVENSON (1999), the humus is a matrix of interlocking and complex humic and non-humic substances adsorbed to mineral components and contains complexes of metal ions. The humic substances are composed of carbohydrates, proteins, lipids, organic acids and other low-molecular-weight compounds from plant and animal debris (CRUZ-GUZMAN ALCALÁ, 2007). Humic substances are composed of pigmented polymers such as fulvic acid and humic acids. For many years it was considered that humic substances came from lignin, incompletely utilized by microorganisms (Waksman theory). According to STEVENSON (1999), there are three other possible routes of formation of humic substances: In the first route the lignin still plays an important role, but it is transformed into polyphenol which is further transformed into quinones via enzymatic process. The quinones polymerize in the presence of amino compounds to form humic macromolecules. In the second route, the polyphenols are synthesized by microorganisms through non-lignin sources, such as cellulose. The third route proposes that the humus is produced through the decomposition of macromolecules, including lignin monomers. The metabolism of these monomers results in an increase in microbial mass which is followed by repeated recycling of C and N in the biomass and the synthesis of new cells. Finally these monomers are polymerized into molecules with high molecular weight. According to the literature, the humic substances may be derived from several passages; however, it seems that in most soils the most important way is that involving condensation reactions from polyphenols and quinones (Porta et al., 2003). This fact can be used to predict the potential of an organic fertilizer to contribute to the formation of humus in soils.

According to VDLUFA (2004), the ability of an organic fertilizer to form humus in the soil depends on its composition and its degree of decomposition. A farmyard manure with a DM of 25 % will produce 40 kg humus-c per ton of substrate, a cattle manure with a DM of 4 - 10 % will produce 6-12 kg humus-c per ton of substrate, a dry digestate with 25 -35 % DM will produce 36-50 kg per ton humus-c substrate and finally a wet digestate with a DM of 4-10 % will produce 6-12 kg per ton humus-c of substrate. This means that the formation of humus is higher for farmyard manure and dry digestate followed by liquid manure and wet digestate. This does not follow the CO_2 exhalation pattern in both years; however, it coincides with the curves obtained with the values of NH_4^+-N, NO_3^--N and C/N. Also, the amount of lignin and cellulose in the different fertilizers before starting the experiment followed the same order of CO_2 release up to calendar week 10, in which the release of CO_2 begins to stabilize. This could indicate that once the CO_2 is exhaled from the labile fraction, the stable fraction begins to become visible. And this stable fraction coincides with the amount of lignin and celullose of each fertilizer, a fact which suggests that these fractions contribute to the formation of humus.

6.5 Conclusion

The results obtained in this study might lead to the conclusion that the most stable fertilizer, as a function of the CO_2 exhalation and the lignin and cellulose content in the fertilizer would be the farmyard manure and the least stable would be the liquid manure. The digestates occupy an intermediate position; the dry digestate being less stable than the wet digestate. Because the formation of humus is a complex issue, it would be recommended to obtain detailed information of the fertilizer applied before and during the mineralization process. The parameters HWSC, NO_3^--N, NH_4^+-N, pH and C/N have contributed to understanding the process of mineralization of the applied organic fertilizers. The CO_2 exhalation and the lignin and celulose content of the different fertilizers have helped to elucidate which fertilizer has the highest potential to contribute in the formation of humus. However, these results should be repeated and also compared with long-term field experiments in order to obtain more reliable information.

General discussion

7 General discussion

After answering the hypotheses set forth earlier in this study, another aspect to take into consideration is the relationship between the various points investigated in each chapter.

The first aspect to consider is the impact of the digestates on the soil fauna directly after their application. In the field, there seemed to be no negative effect of digestates on the population of earthworms one month after the application. However, under laboratory conditions a dose of 30 g of fertilizer per 500 g of soil induced the earthworms to move either to other fertilizers or to a lower dose of the same fertilizer. In the field, the equivalent doses for mineral-N-fertilizer would have been 178 kg per hectare, 122.4 kg for wet digestate, 157 kg for dry digestate, 97 kg for liquid manure and 155 kg for farmyard manure. It was observed that due to the low dry-matter content of the wet digestate and liquid manure the applied quantities of nitrogen and carbon were also lower than for other fertilizers. This led to the assumption that the intolerance of the earthworms to these two fertilizers was high. This was probably due to the higher ammonium content which had a negative effect on the earthworm population immediately after the application of the fertilizer. Another reason could be the lower food income in the fertilizers with lower dry-matter content. One month after the application of wet digestate the enchytraeid population also appeared to be affected. However, the decrease of the enchytraeid population in this treatment could also have been induced by competition for the resources with the earthworm population (Figure 36-A).

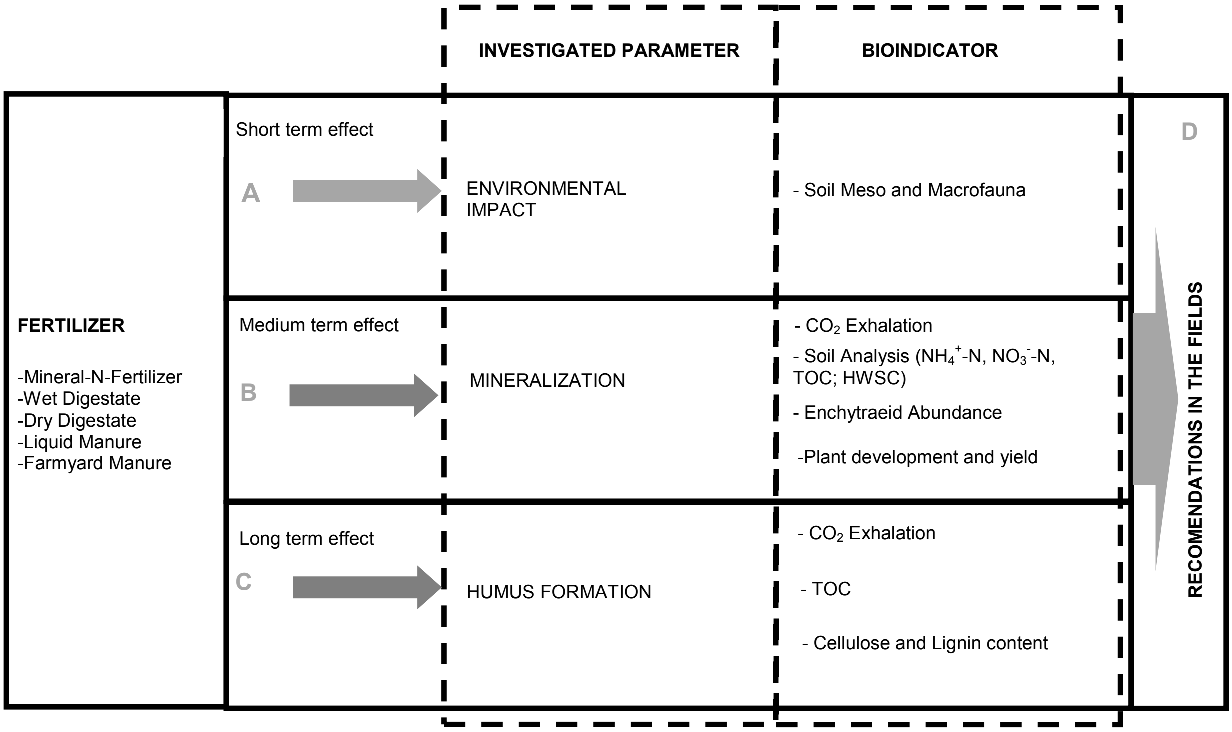


Figure 36: Summary of the aspects investigated in this study (own illustration).

The experiment with vessels under controlled environmental conditions demonstrated that the decomposition rate of digestates was slower than that of liquid manure, but faster than that of farmyard manure. The dry-matter yield of sorghum in the field experiments followed the same pattern. This fact ratifies that the availability of nutrients is dependent on their chemical form in each fertilizer and its influence in the develpment of the plants. Wheat presented a similar pattern, but due to their nutritional needs the difference between various fertilizers was not significant. During the period of growth and development of both cultivars, the effect of different fertilizers roughly confirmed the pattern previously pointed out, but the differences between treatments were not significant. By monitoring enchytraeids it was also possible to investigate one specific moment in the process of mineralization. Because of the saprophytic habits of these animals the greatest abundance of individuals seven months after fertilization was found in the treatment fertilized with liquid manure. As in the CO_2 exhalation experiment and the development and yield of sorghum and wheat, the effect of liquid manure was followed by that of dry digestate, wet digestate and farmyard manure. The lack of enzymes responsible for the digestion of organic matter in the gut of these animals was an indicator to found a higher population in those treatments in which organic matter was in a higher degree of decomposition (Figure 36-B).

According to the VDLUFA (2004) farmyard manure has due to its higher dry-matter content the greatest potential for formation of humus followed by the dry digestate. However, the results obtained in this study suggest that those fertilizers with the greatest potential for the formation of humus are those that exhaled less CO_2 during the decomposition process, had the lowest losses in the balance of organic matter and a higher lignin and cellulose content. Liquid manure and wet digestate were the ones which met these requirements. The results for the exhalation of CO_2 obtained in this study are comparable to those found by the IASP in Berlin where the CO_2 exhalation was measured with a respirometer (Respicond) in an incubation experiment under anaerobic conditions. Also, the percentage of mineralized organic matter coincided (NIELSEN *et al.*, 2011). Because the lignin and cellulose content pointed out by several authors as a precursor to be taken into account for the formation of humus; these should be considered when evaluating the potential of a fertilizer for the formation of humus (Figure 36-C).

Due to the similar physiological characteristics of sorghum and maize (TECHNOLOGIE-UND FÖRDERZENTRUM, 2009) and the comparable type of soil in Dahlem and Trebbin, the dry-matter yield of sorghum was compared with the dry-matter yield of maize. By fertilizing with 120 kg ha^{-1} N mineral-N-fertilizer 23 % (2009) and 5 % (2010) higher dry-matter yield of sorghum in comparison to the use of wet digestate was attained. This effect was also observed in the dry-matter yield of maize in Trebbin (+5 %) but only in the second year. In 2009 the effect of wet digestate on the dry-matter yield of maize in Trebbin was 17 % higher than the one of mineral-N-fertilizer, but the differences between the replications in this treatment were high. SENSEL & WRAGGE (2008) observed that by applying 100 kg ha^{-1} N of wet digestate (with similar chemical characteristics as the wet digestate use in this study) at the location of Dahlem, the dry-matter yield of maize was 14 % (2006) and 32 % (2007) higher in the treatment fertilized with mineral-N-fertilizer in comparison to the one fertilized with wet digestate. The same fact occurred by applying 150 kg ha^{-1} N. The dry-matter yield was 7 % (2006) and 30 % (2007) higher in those treatment fertilized with mineral-N-fertilizer in comparison to the one fertilized with wet digestate. In general, there seems to be a higher effect of the mineral-N-fertilizer on the dry-matter yield of sorghum and maize in comparison to wet digestate. In the Trebbin location, even the application of 160 kg ha^{-1} N wet digestate (WD3) led to the highest dry-matter yield of maize. This application can also be recommended for sorghum as it is already accepted by the Germany's fertilizer regulation or "Düngerverdordnung" (SCHNEIDER & MASTEL, 2008) (Figure 36-D).

The application of dry digestate at the Dahlem location led to 4-19 %-lower dry-matter yield of sorghum than the application of mineral-N-fertilizers. Unfortunately, this data could not be directly compared to those for a clay soil (Friedersdorf) because the amount of fertilizer applied at this location was not the same. In addition, the soil of the Friedersdorf location had a 60 to 70 % higher carbon and nitrogen content than the one of the Dahlem location. This led to increased crop yield in the unfertilized control. In clay soils minerals tend to be not so easily washed from the profile and therefore become a reservoir for the microorganisms with the establishment of a competition with plants (JINGGUO & BAKKEN, 1997; KAYE & HART, 1997). As soon as the minerals are easily available for microorganisms, N immobilization can occur (MARY *et al.*, 1996) and lead to competition between the plant and microorganisms for minerals uptake. Therefore, due to the specific characteristics of this type of soils, soil analysis before the application of fertilizers would be recommended. This analysis should inform about the availability of nutrients at the time of application. If the content of minerals available to the plant is sufficient to meet its needs in the early stages of development, it would be recommendable to apply dry digestate so that the plant will progressively acquire the necessary nutrients. If soil tests show that the nutrient content is not sufficient for the plant, the application of mineral-N-fertilizer is more advisable, because in dry digestate nutrients are bound organically and their release depends on the factor time (Figure 36-D).

As already pointed out in Chapter 2, the application of wet digestate in a dose of 120 kg ha^{-1} N in a sandy soil as the one in the Dahlem location, led to a lower winter wheat grain yield than usual in this location (EREKUL *et al.*, 2005). Therefore, it is advisable to apply 25 % of the dose in the form of digestate (preferably dry one), a second dose of 35 % as wet digestate and finally 40 % of the dose in the form of mineral-N-fertilizer at the time of fruit filling. In the Trebbin location no experiments were carried out with winter wheat, but with winter rye. However, being of the same family (Poaceae) than wheat and having similarities (CAVARACA *et al.*, 2003), the grain yield of winter rye in Trebbin was taken as reference for the doses to be applied. Therefore, the recommended dose for higher crop yields is 80 hg ha^{-1} N wet digestate in autumn combined with 40 kg ha^{-1} mineral-N-fertilizer in spring. For a higher-quality yield 80 kg ha^{-1} N wet digestate in autumn combined with 80 kg ha^{-1} N wet digestate in spring is recommended. The dose in autumn corresponds to the maximum accepted by the German regulation or "Düngeverordnung" (SCHNEIDER & MASTEL, 2008). The lowest crop yield was obtained after the application of 80 kg ha^{-1} N wet digestate in autumn. These results were comparable to the grain yield of winter wheat in Dahlem (19-32 dt ha^{-1}) after applying in autumn one dose of 120 kg ha^{-1} N. In order to obtain a grain yield and grain quality comparable to that of mineral-N-fertilizer in clay soils like the one at the Friedersdorf location, the application of 100 kg ha^{-1} N dry digestate in autumn combined with 50 kg ha^{-1} mineral-N-fertilizer in spring is recommended. This dose is also permitted by the German regulations or "Düngeverordnung" (SCHNEIDER & MASTEL, 2008) (Figure 36-D).

In the case of rapeseed, for a sandy soil as the one of the Trebbin location a dose of 80 kg ha^{-1} N wet digestate in autumn and 80 kg ha^{-1} N wet digestate spring is recommended. The last dose in the development of the seed as pointed out in Chapter 1, plays an important role for growth, the formation of the silique and the number of seeds per area cultivated (CHRISTEN & FRIEDT, 2007) (Figure 36-D).

8 Conclusions

Based on the information found in the literature so far and the results obtained in this study it could be concluded that the digestates from agricultural production do not appear to have a negative effect on the studied agro-ecosystems. However, the mixed view in the literature and the two years of research are not considered sufficient to consider these conclusions as statistically relevant. There are several aspects that have still to be studied in depth like the short-term effect of digestates on the population of enchytraeids and other members of the soil fauna. Also, in the literature appear to exist some doubts on the phytotoxicity, the presence of certain heavy metals (e.g., zinc and copper) stemming from the utilization of liquid manure as input material in biogas production and the agricultural contamination by weed seeds.

Apart from these aspects additional scientific focus needs to be placed on the balance of nutrients in the agricultural ecosystem. The amount of digestates applied is normally calculated as a function of the amount of nitrogen content of the same. The content of other nutrients in the digestate is not taken into consideration in many cases which leaves aside the possible negative effects on the soil nutrients and therefore on the plants. Therefore, it is highly recommended to carry out an analysis of a variety of nutrients in the digestates and the soil before the application. This would avoid problems of accumulation of nutrients easily adsorbed by the organic matter such as P, Zn and Cu and thus avoid a possible contamination of aquatic ecosystems caused by contact of the topsoil surface through water erosion. It can also avoid problems associated with the high concentration of K in soil, such as Mg and Ca deficiency in plants.

One possible solution to this problem has been documented by several research groups (Bustamante et al., 2010, Smidt et al., 2011) where digestates are composted before being applied in the field. According to these authors, digestates can be stabilized by composting by reducing the volume and mass (which would also save transportation costs), and at the same time eliminating phytotoxic substances as well as unwanted seeds. Also, through water percolation and the addition of low bulking agents, the amount of phosphorus and potassium in the digestates can be minimized. However, other aspects must also be taken into account. During composting, nitrogen losses occur in the form of NH_4^+-N (Epstein, E., 2011) and consequently leading to an under supply of nitrogen from the composted digestate required in the fast phase of growth of those crops with high nutrient demands. Also, the cost of composting of the digestates needs be added to the assessment of the use of composted digestates.

In order to avoid the problems described above when applying digestates the implementation of a fertilization plan according to the type of soil and the plant needs is necessary.

The ultimate goal would be efficient fertilization based on the proper diagnosis of the agricultural ecosystem as a way to achieve economically viable agriculture, environmental protection and a sustainable agriculture by using energy from renewable sources like the production of biogas.

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Acknowledgements

I want to thank all those who contributed in some way or another in the last three and a half years of investigation.

First of all I would like to thank Prof. Ellmer for trusting me anytime, for his support on the taken decisions and for his patient guidance and supervision.

Many thanks also to Prof. Rueß for her continued interest in this topic and for her advices in the area of soil biology. Also I would like to thank Dr. Seiml-Buchinger, for helping me in the practical work related to enchytraeids.

Special thanks to Dr. Krück, for guiding me not only in theoretical and practical aspects of soil biology, but to have a more global vision of the research work. Also, for reviewing earlier versions of this manuscript and for suggesting helpful improvements.

Thanks to all who took time to listen, answer my questions and comment constructively: Prof. Engels, Prof. Achazi, Prof. Graefe, PD.Dr. Schenk, Dr. Hübner, Dr. Götz, Dr. Schweitzer, Mr. Baumecker, Dr. Einhorn, Dr. Weiß, Dr. Kroschewski, Dr. Köhler, Mr. Schmelz, Dr. Römbke and all doctoral students who have participated in the seminars organized in our faculty.

Many thanks to those who contributed to make the practical work more bearable and effective: Mr. Schmidt and his team of the experimental station, Dr. Weiß and her team of the laboratory, Mr. Vogel, Jorita Krieger, Thomas Gäbert, Janna Sayer, Markus Theiss, Marcel Budras, the tutors, student assistants and all those exchange students who not only gave me their help but from whom I've learned a lot.

Thanks for the close collaboration and exchange of data with the researchers involved in the Project: Karen Sensel, Verena Wragge and Kerstin Nielsen.

I dedicate this work to the people who have been always by my side anytime and anywhere: my husband, my family and my friends.