Department of Environmental Chemistry

-Faculty of Organic Agricultural Sciences-

University of Kassel

Application of biogas slurries from energy crops to arable soils and their impact on carbon and nitrogen dynamics

Dissertation

submitted to the Faculty of Organic Agricultural Sciences of the

University of Kassel

to fulfill the requirements for the degree Doktorin der Naturwissenschaften

(Dr. rer. nat.)

by Dipl. Geoecologist

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Witzenhausen, January 2012

This work has been accepted by the Faculty of Organic Agricultural Sciences of the University of Kassel as a thesis for acquiring the academic degree of Doctor of Science (Dr. rer. nat.).

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Defence Day: 31st May 2012

Eidesstattliche Erklärung

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(Anja Sänger)

Acknowledgments

First I would like to sincerely thank Prof. Dr. Bernard Ludwig, my supervisor and the spokesman of the DFG Research Training Group for giving me the opportunity to prepare this dissertation. Thanks to his continuous support and motivation this work could be completed within the target three years.

Furthermore, I want to thank Prof. Dr. Rainer Georg Jörgensen for co-supervising this work.

I am grateful to Dr. Daniel Geisseler for fruitful discussions and the scientific guidance as well as for his support with the experimental design of the studies.

I thank Dr. Mirjam Helfrich, Dr. Kerstin Michel, my former colleagues of the first cohort of the Research Training Group and my current colleagues for welcoming me into the Department of Environmental Chemistry, for many helpful advices concerning being a doctoral candidate as well as for their help during the last years.

I wish to thank Anja Sawallisch for the continuous support in the laboratory and in the climate chambers as well as for enjoyable work breaks. I also want to thank Margit Rode for her technical assistance. I thank the Department of Soil Biology and Plant Nutrition and the Department of Organic Farming and Cropping Systems for the opportunity to use their equipment, sometimes even at short notice and for a longer period of time.

For proof-reading and helpful comments I would like to thank Christoph Stang, Rouven Andruschkewitsch, Deborah Linsler and Juliane Wetzel.

I want to thank my mother for any kind of support not only during the time of writing my dissertation.

I am very grateful to Christoph Stang for his patience and for encouraging me whenever I needed it, even when hundreds of kilometers away. Hopefully this is the final step for a common future, wherever this may be.

For enabling this dissertation I wish to thank the *Deutsche Forschungsgemeinschaft* which is funding the Research Trainings Group 1397 "Regulation of soil organic matter and nutrient turnover in organic agriculture".

Preface

This thesis was prepared within the DFG Research Training Group 1397 “Regulation of soil organic matter and nutrient turnover in organic agriculture” and is submitted to the Faculty of Organic Agricultural Sciences to fulfill the requirements for the academic degree "Doktorin der Naturwissenschaften" (Dr. rer. nat.).

This cumulative dissertation consists of three papers as first author. Two of them are published in international refereed journals whereas the third one is submitted. The papers are included in chapters 4, 5 and 6, respectively.

Chapter 1 gives a general introduction of biogas slurries and the impact of organic fertilizers on C and N mineralization whereas chapters 2 and 3 include the objectives of the present thesis and additional information concerning the methodology. Following the papers a general conclusion covering the entire thesis is given in chapter 7. Finally, the utilized references of chapter 1 are listed in chapter 8.

The following papers are incorporated:

Chapter 4:

Sänger A, Geisseler D, Ludwig B (2010): Effects of rainfall pattern on carbon and nitrogen dynamics in soil amended with biogas slurry and composted cattle manure. Journal of Plant Nutrition and Soil Science 173: 692-698

Chapter 5:

Sänger A, Geisseler D, Ludwig B (2011): Effects of moisture and temperature on greenhouse gas emissions and C and N leaching losses in soil treated with biogas slurry. Biology and Fertility of Soils 47: 249-259 (DOI: 10.1007/s00374-010-0528-y)

Chapter 6:

Sänger A, Geisseler D, Ludwig B: C and N dynamics of a range of biogas slurries as a function of application rate and soil texture: a laboratory experiment. Journal of Plant Nutrition and Soil Science (submitted)

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List of abbreviations

ANOVA	Analysis of variance
BS	Biogas slurry
C	Carbon
C_t	Total carbon
$CaCl_2$	Calcium chloride
CH_4	Methane
CM	Composted cattle manure
CO_2	Carbon dioxide
K_2SO_4	Potassium sulfate
N	Nitrogen
N_2	Molecular nitrogen
NH_3	Ammonia
NH_4^+	Ammonium
N_{min}	Mineral nitrogen
N_2O	Nitrous oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
N_t	Total nitrogen
O_2	Oxygen
Q_{10}	Temperature coefficient
TDN	Total dissolved nitrogen
TOC	Total organic carbon
WHC	Water holding capacity
WFPS	Water filled pore space

Summary

The use of renewable primary products as co-substrate or single substrate for biogas production has increased consistently over the last few years. Maize silage is the preferential energy crop used for fermentation due to its high methane (CH_4) yield per hectare. Equally, the by-product, namely biogas slurry (BS), is used with increasing frequency as organic fertilizer to return nutrients to the soil and to maintain or increase the organic matter stocks and soil fertility. Studies concerning the application of energy crop-derived BS on the carbon (C) and nitrogen (N) mineralization dynamics are scarce. Thus, this thesis focused on the following objectives: I) The determination of the effects caused by rainfall patterns on the C and N dynamics from two contrasting organic fertilizers, namely BS from maize silage and composted cattle manure (CM), by monitoring emissions of nitrous oxide (N_2O), carbon dioxide (CO_2) and CH_4 as well as leaching losses of C and N. II) The investigation of the impact of differences in soil moisture content after the application of BS and temperature on gaseous emissions (CO_2, N_2O and CH_4) and leaching of C and N compounds. III) A comparison of BS properties obtained from biogas plants with different substrate inputs and operating parameters and their effect on C and N dynamics after application to differently textured soils with varying application rates and water contents.

For the objectives I) and II) two experiments (experiment I and II) using undisturbed soil cores of a Haplic Luvisol were carried out. Objective III) was studied on a third experiment (experiment III) with disturbed soil samples. During experiment I three rainfall patterns were implemented including constant irrigation, continuous irrigation with periodic heavy rainfall events, and partial drying with rewetting periods. Biogas slurry and CM were applied at a rate of 100 kg N ha^{-1}. During experiment II constant irrigation and an irrigation pattern with partial drying with rewetting periods were carried out at 13.5°C and 23.5°C. The application of BS took place either directly before a rewetting period or one week after the rewetting period stopped. Experiment III included two soils of different texture which were mixed with ten BS's originating from ten different biogas plants. Treatments included low, medium and high BS-N application rates and water contents ranging from 50% to 100% of water holding capacity (WHC).

Experiment I and II showed that after the application of BS cumulative N_2O emissions were 4 times (162 mg N_2O-N m^{-2}) higher compared to the application of CM caused by a higher content of mineral N (N_{min}) in the form of ammonium (NH_4^+) in the BS. The cumulative emissions of CO_2, however, were on the same level for both fertilizers indicating similar amounts of readily available C after composting and fermentation of organic material. Leaching losses occurred predominantly in the mineral form of nitrate (NO_3^-) and were higher in BS amended soils (9 mg NO_3^--N m^{-2}) compared to CM amended soils (5 mg NO_3^--N m^{-2}). The rainfall pattern in experiment I and II merely affected the temporal production of C and N emissions resulting in reduced CO_2 and enhanced N_2O emissions during stronger irrigation events, but showed no effect on the cumulative emissions. Overall, a significant increase of CH_4 consumption under inconstant irrigation was found. The time of fertilization had no effect on the overall C and N dynamics. Increasing temperature from 13.5°C to 23.5°C enhanced the CO_2 and N_2O emissions by a factor of 1.7 and 3.7, respectively. Due to the increased microbial activity with increasing temperature soil respiration was enhanced. This led to decreasing oxygen (O_2) contents which in turn promoted denitrification in soil due to the extension of anaerobic microsites. Leaching losses of NO_3^- were also significantly affected by increasing temperature whereas the consumption of CH_4 was not affected. The third experiment showed that the input materials of biogas plants affected the properties of the resulting BS. In particular the contents of DM and NH_4^+ were determined by the amount of added plant biomass and excrement-based biomass, respectively. Correlations between BS properties and CO_2 or N_2O emissions were not detected. Solely the ammonia (NH_3) emissions showed a positive correlation with NH_4^+ content in BS as well as a negative correlation with the total C (C_t) content. The BS-N application rates affected the relative CO_2 emissions (%

of C supplied with BS) when applied to silty soil as well as the relative N_2O emissions (% of N supplied with BS) when applied to sandy soil. The impacts on the C and N dynamics induced by BS application were exceeded by the differences induced by soil texture. Presumably, due to the higher clay content in silty soils, organic matter was stabilized by organo-mineral interactions and NH_4^+ was adsorbed at the cation exchange sites. Different water contents induced highest CO_2 emissions and therefore optimal conditions for microbial activity at 75% of WHC in both soils. Cumulative nitrification was also highest at 75% and 50% of WHC whereas the relative N_2O emissions increased with water content and showed higher N_2O losses in sandy soils.

In summary it can be stated that the findings of the present thesis confirmed the high fertilizer value of BS's, caused by high concentrations of NH_4^+ and labile organic compounds such as readily available carbon. These attributes of BS's are to a great extent independent of the input materials of biogas plants. However, considerably gaseous and leaching losses of N may occur especially at high moisture contents. The emissions of N_2O after field application corresponded with those of animal slurries.

Zusammenfassung

Die Erzeugung von Biogas aus nachwachsenden Rohstoffen als Kosubstrat bzw. Monosubstrat hat in den letzten Jahren stark zugenommen. Vor allem Mais wird aufgrund seines hohen Methanertrags für die Energiegewinnung genutzt. Zugleich nimmt auch die Nutzung des Nebenprodukts Biogasgülle als organischer Dünger zu, um dem Boden die Nährstoffe zurückzuführen und zur Aufrechterhaltung der organischen Substanz und Bodenfruchtbarkeit beizutragen. Bisher haben sich erst wenige Studien dem Thema Biogasgülle-Düngung und deren Einfluss auf die Kohlenstoff (C)- und Stickstoff (N)-Dynamik gewidmet. Aus diesem Grund beinhaltet die vorliegende Arbeit folgende Zielsetzungen:

I) Die Untersuchung des Einflusses unterschiedlicher Beregnungsmuster auf die C- und N-Dynamik nach einer Biogasgülledüngung (die Biogasgülle stammt ausschließlich aus der Fermentation von Maissilage) im Vergleich zu Rottemistdüngung. Verglichen werden die Emissionen von Distickstoffmonoxid (N_2O), Kohlendioxid (CO_2), Methan (CH_4) sowie C- und N-Sickerwasserausträge.

II) Die Quantifizierung des Einflusses verschiedener Bodenfeuchten nach einer Biogasgülledüngung sowie die Untersuchung des Einflusses der Temperatur auf die Emissionen (CO_2, N_2O und CH_4) und C- und N-Austräge mit dem Sickerwasser.

III) Vergleich der chemischen Eigenschaften verschiedener Biogasgüllen, die aus unterschiedlichen Biogasanlagen mit unterschiedlichem Substrateinsatz und Betriebsparametern stammen. Die Bestimmung des Einflusses dieser Biogasgüllen nach Einarbeitung in Böden auf die C- und N-Dynamik in Abhängigkeit der Textur, verschiedener Biogasgülle-N-Raten und unterschiedlicher Wassergehalte.

Für die Ziele I) und II) wurden zwei Versuche (Experiment I und II) mit ungestörten Bodensäulen zweier Parabraunerden durchgeführt. Für Ziel III) wurde ein weiterer Versuch (Experiment III) mit gestörten Bodenproben angelegt. In Experiment I wurden drei Beregnungsmuster eingesetzt. Dazu zählten konstante Beregnung, kontinuierliche Beregnung mit regelmäßigen Starkniederschlägen sowie Trockenperioden mit

regelmäßiger Wiederbefeuchtung. Die Biogasgülle und der Rottemist wurden mit einer Düngerrate von 100 kg N ha^{-1} ausgebracht. In Experiment II erfolgte die Beregnung konstant oder unterbrochen durch mehrwöchige Trockenperioden bei 13.5°C und 23.5°C. Die Biogasgülle wurde mit einer Düngerrate von 33 kg N ha^{-1} entweder direkt vor einem Niederschlagsereignis ausgebracht oder eine Woche nach Aussetzen der Beregnung. In Experiment III wurden zwei Böden mit unterschiedlicher Textur jeweils mit den zehn Biogasgüllen vermischt. Zudem wurden verschiedene Biogasgülle-N-Raten aufgebracht sowie verschiedene Wassergehalte eingestellt.

Experimente I und II zeigten, dass nach der Ausbringung von Biogasgülle die kumulierten N_2O-Emissionen 4-mal höher (162 mg N_2O-N m^{-2}) waren als nach Ausbringung von Rottemist. Dies wurde bedingt durch den hohen Gehalt an mineralischem N, in Form von Ammonium (NH_4^+), in der Biogasgülle. Die kumulierten CO_2-Emissionen waren jedoch bei beiden Düngern etwa gleich hoch, was auf einen ähnlichen Anteil an leicht verfügbaren C nach Kompostierung sowie Fermentation hindeutet. Austräge mit dem Sickerwasser traten überwiegend als Nitrat (NO_3^-) auf und waren nach Biogasgülledüngung höher (9 mg NO_3^--N m^{-2}) als nach Rottemistdüngung (5 mg NO_3^--N m^{-2}). Die Beregnungsmuster der Experimente I und II übten lediglich einen temporären Effekt aus, welcher in verminderte CO_2- und erhöhte N_2O-Emissionen während einer Starkregenphase resultierte. Die kumulierten Emissionen blieben jedoch unbeeinflusst, während eine signifikante Erhöhung des CH_4-Verbrauchs bei nicht-konstanter Beregnung festgestellt werden konnte. Der Düngezeitpunkt hatte keinen Einfluss auf die Gesamtemissionen von C und N. Eine Temperaturerhöhung um 10°C steigerte die CO_2-Emissionen um den Faktor 1,7 und die N_2O-Emissionen um den Faktor 3,7. Aufgrund zunehmender mikrobieller Aktivität mit steigender Temperatur, erhöhte sich auch die Respiration im Boden. Hierdurch nahm der Sauerstoffgehalt im Boden ab und die damit einhergehende Ausdehnung anaerober Bereiche begünstigte wiederum die Denitrifikation im Boden. Sickerwasseraustäge in Form von NO_3^- wurden ebenfalls signifikant erhöht, während der CH_4-Verbrauch unbeeinflusst blieb. Das dritte Experiment zeigte, dass die Art des Ausgangsmaterials die chemischen Eigenschaften der entstehenden Biogasgüllen beeinflusste. Insbesondere der Trockensubstanzgehalt wurde durch den Anteil an zugefügter pflanzlicher Biomasse bestimmt und der NH_4^+-Gehalt durch den Anteil an zugefügten tierischen Exkrementen. Zwischen der chemischen Zusammensetzung der Biogasgülle und den CO_2- und N_2O-Emissionen konnten keine Korrelationen festgestellt werden. Einzig die Ammoniakemissionen zeigten einen positiven Zusammenhang mit dem NH_4^+-Gehalt sowie einen negativen Zusammenhang mit dem Gesamt-Kohlenstoffgehalt. Die Biogasgülle-N-Raten beeinflussten die relativen CO_2-Emissionen (% des zugefügten C mit der Biogasgülle) im schluffigen Boden sowie die relativen N_2O-Emissionen (% des zugefügten N mit der Biogasgülle) im sandigen Boden. Dies verdeutlicht den starken und teilweise überdeckenden Einfluss der Bodentextur, vermutlich aufgrund des höheren Tongehalts im schluffigen Boden im Vergleich zum sandigen Boden. Hierbei wird die organische Substanz durch organisch-mineralische Verbindungen stabilisiert und NH_4^+-Ionen werden an den Kationenaustauschern adsorbiert. Die Anwendung unterschiedlicher Wassergehalte verdeutlichte bei 75% der Wasserhaltekapazität die höchsten CO_2-Emissionen und damit optimale Bedingungen für mikrobielle Aktivität in beiden Böden. Die kumulierte Nitrifikation erreichte ebenfalls Maxima bei 50% und 75% der Wasserhaltekapazität während die relativen N_2O-Emissionen mit dem zunehmendem Wassergehalt anstiegen und zwar im sandigen Boden stärker als im schluffigen Boden.

Zusammenfassend kann gesagt werden, dass die Ergebnisse dieser Arbeit den hohen Düngewert der Biogasgülle unterstreichen. Dieser Düngewert basiert auf einem hohen Anteil an schnell wirksamen N in Form von NH_4^+ sowie dem Vorliegen leicht verfügbaren Kohlenstoffs. Diese Eigenschaften von Biogasgüllen sind weitestgehend unabhängig vom Ausgangsmaterial der Biogasanlagen. Dies bedingt jedoch auch ein großes Potential für gasförmige N-Verluste oder N-Verluste mit dem Sickerwasser, besonders bei hohen Wassergehalten. Zudem wurde festgestellt, dass die Emissionen nach Biogasgülledüngung denen nach Gülledüngung entsprechen.

1 General introduction

Organic agriculture names an economic system producing crops and animals without the use of artificial fertilizers, pesticides and genetically modified organisms. One of the main tasks of organic agriculture is to maintain and increase the organic matter content in soil as well as its fertility to support arable crops. More precisely, soil fertility names the ability of natural and agricultural soil to serve as habitat for plants (Blume et al. 2010). Therefore, the supply of nutrients via legume cultivation and organic fertilizers such as crop residues, farmyard manure, slurry and green manure is of major importance and should be provided by closed nutrient cycles. The above-mentioned organic fertilizers and their impact on carbon (C) and nitrogen (N) mineralization dynamics are already well studied. However, one organic fertilizer gained increasingly importance during the last few years, namely biogas slurry (BS), the by-product of biogas production. Studies concerning BS application are scarce but of great significance caused by the chemical composition and high potential for considerable N losses by leaching or gaseous emissions.

This thesis focuses on the investigation of the influence of BS application to soil on C and N mineralization compared to composted cattle manure (CM) and on factors influencing C and N dynamics in soil, such as chemical properties of BS, temperature, soil moisture and texture. Whereas chapters 4.2, 5.2 and 6.2 will give specific introductions the following general introduction presents the corresponding background information.

1.1 Biogas production

The production of biogas from sewage sludge and dung has a long history. Since the 1980's biogas was mainly obtained from the fermentation of animal slurry. The biogas yield could be enhanced by adding co-substrates such as plant material, industrial and household wastes. In recent years the production of biogas as a renewable energy source has gained increasing importance by the effort to enhance the proportion of renewably energies in electricity generation to 30% until 2020 (§1 II German Renewable Energy Sources Act (EEG) 2009). In organic agriculture, plant material, animal manure and slurry originated from organic farms can be fermented. The resulting BS can be applied to arable land at a rate of up to 170 kg N ha^{-1} relating to the animal based portion (Council Regulation (EC) No 834/2007) whereas there is no upper limit for the N application of fermented plant biomass (Reinhold et al. 2011). If in-house fertilizers are insufficient to maintain or increase the soil fertility and microbial activity the use of BS including the following material are permitted according to the Council Regulation (EC) 837/2007 on Organic Agriculture Annex I: Plant and animal based household wastes, animal slurry as well as renewable raw materials as long as none of them contain genetically modified organisms. Furthermore, it must be ensured that animal slurry is not originated from industrial animal husbandry. Some associations of organic agriculture such as *Bioland*, *Demeter* and *Naturland* underlie more strict guidelines for the use of biogas slurries, for instance no use of conventional animal slurry or generally farmyard manure (*Demeter*) and a limitation of the total amount of N application to 112 kg N ha^{-1} (Bioland e.V. 2011; Demeter e.V. 2010, Naturland 2011). With increasing amounts of biogas plants for energy production the use of the by-product BS as organic fertilizer also increased.

1.1.1 Fermentation process

Type and amount of input substrates for biogas plants vary widely but mainly animal slurry and energy crops are used. Besides differences in feeding material, biogas plants may differ in type of digestion (wet or dry), numbers of digesters and post-digesters, dwell time, process temperature (mesophilic, thermophilic) and other operating parameters. These factors may have an impact on the degree of fermentation (Sensel and Wragge 2008) and hence on greenhouse gas emissions during storage and after field application.

The fermentation process is subdivided into 4 steps: hydrolysis, acidogenesis, acetogenesis and methanation (Weiland 2010). During hydrolysis long chain organic compounds namely carbohydrates, proteins and lipids are decomposed to monosaccharides, amino acids, fatty acids and glycerine, respectively. These products are converted to short chain acids (acidogenesis), hydrogen and CO_2. The involved microorganisms of the first two steps of biogas production are hydrolytic bacteria and anaerobic acidifiers which require pH values of 4.5 to 6.3. In the process of acetogenesis conversion to acetic acid, hydrogen and carbon dioxide (CO_2) takes place followed by the production of methane (CH_4) and CO_2 (methanation). Optimal pH values for these two processes are between 6.8 and 7.5. More acidic or alkaline conditions lead to an inhibition of the acetogenesis and methanation. Total amounts of nutrients persist unchanged within the fermentation except for the amounts of C, hydrogen and oxygen (Asmus et al. 1988; Kirchmann and Witter 1992; Möller et al. 2008; Möller et al. 2010). Due to the reduction of fresh matter volume and mass caused by organic biomass degradation and biogas emission (Möller et al. 2010) the ratio of nutrients to fresh matter is higher in biogas slurries compared to feed material. Especially, the increased availability of N of BS indicates a valuable fertilizer (Gutser et al. 2005; Weiland 2010).

1.2 Aerobic and anaerobic manure treatment

Organic fertilizers differ in their fertilizer effect, which is strongly influenced by manure treatment and storage (anaerobic, aerobic). During aerobic manure treatment (composting) large amounts of C and N may be lost due to microbial conversion of organic material to CO_2 and ammonia (NH_3). Overall, composting results in a fertilizer with maximum C stabilization efficiency indicating the potential for C sequestration as well as for preserving and increasing the humus content in soil (Kirchmann and Bernal 1997, Kirchmann and Lundvall 1998, Thomson 2000). The amount of mineral N (N_{min}) in composted manure is less than 10% of total N (N_t) which is similar to the N_{min}

content in untreated manure. In contrast, anaerobically treated manure contains a higher amount of volatile fatty acids compared to aerobically stored manure and about 50% of N_t as N_{min}. Similar N_{min} contents were found in cattle slurry whereas the N_{min} content increases for pig and chicken slurry (60-70% of N_t) and liquid manure (which contains almost exclusively urine and faeces, 90% of N_t) (Knittel and Albert 2003, Thomson 2000). Nitrogen uptake of plants occurs in the mineral forms of ammonium (NH_4^+) and nitrate (NO_3^-), therefore, anaerobically treated material and untreated slurries are more suitable to be used as fertilizer because of their N composition (Kirchmann and Bernal 1997). However, highest microbial activity and CO_2 emissions are found after application of anaerobically treated organic waste to soil due to the higher amount of volatile fatty acids and thereby the higher N immobilization compared to soil amended with aerobically treated manure which partially compensate the potentially better fertilizer value of anaerobically treated manure (Kirchmann and Bernal 1997, Thomson 2000). Furthermore, when the application of anaerobically treated fertilizer does not match the crop demand high gaseous and leaching N losses may occur. In all cases, NH_3 volatilization is considerably higher because of higher contents of NH_4^+ (Kirchmann and Lundvall 1998) compared to the application of composted material. Due to surface incorporation or injection of anaerobically treated materials into soil NH_3 emissions may be reduced by 35-95% and 80-100%, respectively (Bussink and Oenema 1998; Huijsmans et al. 2003). Ammonia is considered as an indirect greenhouse gas and could be transformed after deposition to nitrous oxide N_2O, nitrate (NO_3^-) and other N-forms inducing gaseous and leaching losses.

1.3 C and N mineralization

The mineralization of organic substances results in the production of compounds such as CO_2, NO_3^- and NH_4^+. This process depends on the type of organic material, temperature and soil conditions (soil moisture, texture) (Blume et al. 2010, Kás 2011) and under specific circumstances gaseous emissions such as N_2O and CH_4, classified as greenhouse gases, are formed. Agriculture contributes about 60% and 50% of the global anthropogenic N_2O and CH_4 emissions, respectively. The net flux of CO_2 related to agriculture is estimated to be approximately balanced (Smith et al. 2007). The global warming potential of N_2O and CH_4 is 298 and 25 times higher compared to CO_2, respectively. The most important processes producing N_2O are nitrification and denitrification. Nitrification is the biological oxidation of (1) NH_4^+ to nitrite (NO_2^-) and (2) NO_2^- to NO_3^-. The first step is predominantly performed by chemoautotrophic *Nitrosomonas*. One by-product could be nitroxyl (NOH) which may further be converted to N_2O. The second step is carried out by *Nitrobacter* (Gisi et al. 1997; Subbarao et al. 2006). Factors influencing the nitrification process are oxygen (O_2) and CO_2 levels, soil moisture content, temperature, soil texture, especially the clay content as well as chemical factors such as pH value, C to N ratio, cation exchange capacity, organic matter etc. (Gisi et al. 1990; Subbarao et al. 2006). Optimal O_2 and CO_2 levels for nitrification are >20% and between 1 and 5%, respectively (Subbarao et al. 2006). The soil moisture content is correlated with the aeration in soil and highest nitrification rates are expected at field capacity. During the denitrification process NO_3^- is reduced to NO_2^-, nitric oxide (NO), N_2O and molecular nitrogen (N_2) under anaerobic conditions by heterotrophic bacteria such as *Bacillus subtilis, Escherichia coli, Achrobacter aerogenes, Aspergillus flavus, Pseudomonas* spec., *Micrococcus* spec. and *Pencillium atrovenetum* (Mosier et al. 1996). Main regulating factors for denitrification are soil oxygen, NO_3^- and C. The oxygen content in turn is affected by the water content and soil texture and the lower the oxygen content, the higher the denitrification rates (Barton et al. 1999). Nitrate serves as an electron acceptor, C as an electron donor for denitrifiers and additionally influences the consumption of oxygen in soil due to the heterotrophic respiration under aerobic conditions which in turn accelerates the development of anaerobic microsites (Barton et al. 1999). Emissions of N_2O could arise within the nitrification process when water filled pore space (WFPS) is below 50% as well as within the denitrification process when WFPS is above 75% (Mosier et al. 1996).

Both CO_2 and N_2O emissions increase with increasing water content and the C and N turnover rates in soil are in particular affected by dry and rewetting phases. Rewetting of dried soil induces a stimulation of C and N mineralization due to metabolization of dead microbial biomass and degradation of previously unavailable and physically released easily decomposable organic material (Wu and Brookes 2005). However, this boost, a priming effect termed as “Birch effect” (Jarvis et al. 2007) is temporary and cumulative C and N mineralization after repeated dry and rewetting phases are smaller than the corresponding soil adjusted to optimal moisture level (Borken 2009).

2 Objectives

As described above the effect of soil moisture on the C and N mineralization dynamics of organic matter is well studied. However, little is known about the influence of different rainfall patterns having the same total amount of irrigation without complete dry out on C and N dynamics. Furthermore the effect of BS obtained by biogas plants fed with varying substrate input (including energy crops) applied to soil on C and N turnover is less studied compared to other organic fertilizers. Thus this thesis focuses on the investigation of BS originated from energy crops on C and N mineralization dynamics.

Specific objectives include:

i) Comparison of the effect of the application of BS obtained by maize silage and CM to soil on C and N dynamics (chapter 4).

ii) Determination of the effect of different rainfall patterns possessing the same total amount of irrigation and the time of BS application (dry period or wet period) (chapter 4) on gaseous emissions (CO_2, CH_4 and N_2O) and on leaching losses such as NO_3^- and total organic carbon (TOC) (chapters 4 and 5).

iii) Quantification of the temperature and soil moisture effect on CO_2, N_2O and CH_4 emissions as well as on C and N leaching losses after the application of BS obtained by maize silage (chapter 5).

iv) Comparison of BS properties obtained from biogas plants with different substrate inputs and operating parameters and their effect on C and N dynamics after application to soil (chapter 6).

v) Effect of BS-N rate and water content expressed as WHC in differently textured soils (chapter 6).

3 Methodology

All investigations during this thesis were carried out in incubation experiments in a climate chamber. Advantages of incubation experiments over field studies are the controlled experimental conditions concerning constant temperature, equal soil moisture and irrigation events for all samples. Additionally, more frequent or even continuous and accurate measurements can be performed more easily within incubations. However, findings of incubation experiments are not directly transferable to the field.

The first two experiments (chapters 4 and 5) were conducted with undisturbed soil cores taken directly from the field using plexiglass cylinders. These soil cores possessed their natural structure, stratification and aggregation which regulate the transport of water and therefore the supply of nutrient and oxygen. The organic fertilizers were incorporated in the upper 4-6 cm of top soil to avoid direct emission in the form of NH_3 from the soil surface.

For the third experiment (chapter 6) air dried and sieved soils were used to receive a large number of homogeneous samples for the determination of differences in C and N mineralization processes depending on the composition of biogas slurries, BS-N rates and water contents.

Ammonia volatilization was determined with H_2SO_4 traps and subsequently analyzed according to DIN 38406/5 1983 (chapter 6). To validate the extent of captured volatilized NH_3, ammonium chloride (NH_4Cl) and sodium hydroxide (NaOH) were mixed and the formed and emitted NH_3 trapped in H_2SO_4 where it was transformed to NH_4^+. Acid traps were sampled after 3, 7, 24 and 48 hours and analyzed for NH_4^+.

4 Effects of rainfall pattern on carbon and nitrogen dynamics in soil amended with biogas slurry and composted cattle manure

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4.1 Abstract

Soil moisture affects the degradation of organic fertilizers in soils considerably, but less is known about the importance of rainfall pattern on the turnover of carbon (C) and nitrogen (N). The objective of this study was to determine the effects of different rainfall patterns on C and N dynamics in soil amended with either biogas slurry (BS) or composted cattle manure (CM). Undisturbed soil cores without (control) or with BS or CM, which were incorporated at a rate of 100 kg N ha^{-1}, were incubated for 140 days at 13.5 °C. Irrigation treatments were (i) continuous irrigation (cont_irr; 3 mm day^{-1}); (ii) partial drying and stronger irrigation (part_dry; no irrigation for 3 weeks, one week with 13.5 mm day^{-1}); and (iii) periodic heavy rainfall (hvy_rain; 24 mm day^{-1} every 3 weeks for 1 day and 2 mm day^{-1} for the other days). The average irrigation was 3 mm day^{-1} in each treatment. Cumulative emissions of carbon dioxide (CO_2) and nitrous oxide (N_2O) from soils amended with BS were 92.8 g CO_2-C m^{-2} and 162.4 mg N_2O-N m^{-2}, respectively, whereas emissions from soils amended with CM were 87.8 g CO_2-C m^{-2} and only 38.9 mg N_2O-N m^{-2}. While both organic fertilizers significantly increased CO_2 production compared to the control, N_2O emissions were only significantly increased in the BS-amended soil. Under the conditions of the experiment, the rainfall pattern affected the temporal production of CO_2 and N_2O, but not the cumulative emissions. Cumulative nitrate (NO_3^-) leaching was highest in the BS-amended soils (9.2 g NO_3^--N

m^{-2}) followed by the CM-amended soil (6.1 g NO_3^--N m^{-2}) and lowest in the control (4.7 g NO_3^--N m^{-2}). Nitrate leaching was also independent of the rainfall pattern. Our study shows that rainfall pattern may not affect CO_2 and N_2O emissions and NO_3^- leaching markedly provided that the soil does not completely dry out.

4.2 Introduction

Soil moisture has a considerable impact on C and N turnover of organic material in soil. In general, CO_2 production and net N mineralization increase with increasing soil moisture (*Stanford* and *Epstein*, 1974; *Thomsen* et al., 1999). At high soil moisture, denitrification results in the production of nitrous oxide (N_2O), an important greenhouse gas (*Robertson* and *Groffman*, 2007). For instance, *Clemens* and *Huschka* (2001) found that N_2O emissions in soils amended with slurry increased with increasing water-filled pore space from 35 to 71%. In addition, drying/rewetting phases have marked effects on CO_2 and N_2O emissions (*Jørgensen* et al., 1998, *Muhr* et al., 2008). However, less information is available on the effect of different rainfall patterns in cases where the soil does not dry out completely. Under rainfed crops, even with identical sums of precipitation, differences in the distribution of rainfall during the season result in fluctuating soil moisture contents, which may potentially affect C and N dynamics. For example, the rainfall pattern not only affects the number of days when the soil moisture content is high enough for denitrification to occur, but also the products of denitrification. When the water-filled pore space is above 70%, denitrification results predominantly in the release of N_2, whereas below 70%, the N_2O to N_2 ratio increases (*Masscheleyn* et al., 1993; (Bareth 2000).

The effects of organic fertilizers on the C and N turnover in soils has been studied intensively because of their importance for soil fertility and nutrient supply to plants, and because of potential negative environmental effects such as nitrate (NO_3^-) leaching and N_2O emissions. The chemical composition of organic fertilizers has been found to significantly affect C and N dynamics in soil (*Pansu* and *Thuries*, 2003). In contrast to most other organic fertilizers, the use of biogas slurry (BS) from energy crops as a fertilizer has not received much attention yet. However, biogas production from energy crops is of growing importance. As maize has one of the highest methane (CH_4) yields per hectare, its use for biogas production is likely to increase in the future (*Amon* et al., 2007) and therefore the availability of BS as an organic fertilizer. *Möller* and *Stinnner* (2009) compared the N_2O emissions of clover/grass-ley when mulched and incorporated as green manure with the N_2O emissions resulting from the field application of BS made from the same clover/grass-ley. The field applications of BS resulted in a strong increase in N_2O emissions. However, compared with the N_2O emissions that resulted when the same plant material was incorporated, digestion decreased N_2O emissions by 38%.

In general, anaerobic fermentation increases the ammonium (NH_4^+) content in the substrate as well as the stability of organic matter, but decreases the C to N ratio remarkably, resulting in a product with a high content of directly available N. During anaerobic digestion for CH_4 production, volatile fatty acids and other labile organic compounds are formed as intermediates (*Cysneiros* et al., 2008; *Jacobi* et al., 2009). These compounds, when still present in the BS when applied to the field, are readily available C sources for soil microorganisms. Aerobic decomposition also increases the stability of the organic matter, but reduces the mineral N content (*Thomsen* and *Olesen*, 2000; *Gutser* et al., 2005). These changes also take place when manure is composted (*Eghball* et al., 1997). In addition, N mineralization from composted manure has been found to be lowered, which may be attributed to the loss of easily convertible C and N compounds during composting, resulting in a relative accumulation of more stable C and N forms (*Eghball* et al., 2002 *Lynch* et al., 2004).

The objective of this study was to determine the effects of rainfall pattern on the C and N dynamics from two contrasting organic fertilizers, namely BS from corn and composted cattle manure (CM), by monitoring gas emissions (N_2O, CO_2) and leaching losses of C and N from undisturbed soil cores during a 140-day incubation.

4.3 Material and methods

4.3.1 Soil and substrates

Soil samples were taken at the long-term experimental site Garte Süd near Göttingen. The mean annual precipitation at the site is 645 mm and the mean annual temperature 8.7 °C (30-year average; *Deutscher Wetterdienst*, 2009). The soil type is a Haplic Luvisol (*FAO*, 1998) derived from loess (*Ehlers* et al., 2000) with sand, silt, and clay contents of 120, 730, and 150 g kg^{-1}, respectively. The samples were taken from a field under minimum tillage since 1971. At the time of soil sampling the field lay fallow. The bulk density of the sampled cores was 1.4 g cm^{-3}, pH was 6.4 and the contents of total C and N were 15.1 and 1.6 g kg^{-1}, respectively.

Thirty undisturbed soil cores of 15 cm diameter and 30 cm length were sampled by driving plexiglass cylinders with a sharpened edge into the soil. The cylinders containing the soil cores were then dug out and capped at the bottom for transportation. The soil cores remained in the cylinders for the duration of the incubation. The cores were kept in the climate chambers for three months before the start of the experiment under conditions identical to the cont_irr treatment (see below).

Composted cattle manure consisted of cow dung mixed with straw which had been stored under aerobic conditions for five months. It was obtained from the Institute for Biodynamic Research in Darmstadt. Total C and Kjeldahl-N contents were 370 and 30 g kg^{-1} dry matter, respectively. The NH_4^+ content was very low (Table 1). Biogas slurry was obtained from the Institute for Anaerobic Technique from the University of Applied Sciences of Giessen. The slurry is a by-product of the anaerobic digestion of corn silage for CH_4 production (4 months at 55 °C) in a laboratory scale reactor. The slurry had total C, Kjeldahl-N, and NH_4^+-N contents of 419, 59, and 29 g kg^{-1} dry matter, respectively (Table1).

Therefore, BS contained about twice as much Kjeldahl-N compared to CM, which resulted in a lower C to N ratio. In addition, a much larger proportion of the N in BS was in the form of NH_4^+.

Table 1: Characterization of the organic fertilizers used in this study.

	Organic fertilizer	
	Composted cattle manure	Biogas slurry
Dry matter (%)	22.0	8.4
Total C (g kg^{-1} dry matter)	370	419
Total Kjeldahl-N (g kg^{-1} dry matter)	30	59
C to N ratio	12.3	7.1
NH_4^+-N (g kg^{-1} dry matter)	3	29
pH	8.7	8.5

4.3.2 Incubation

At the beginning of the incubation, the organic fertilizers were incorporated into the upper 6 cm of the soil at a rate of 100 kg N ha^{-1}. This equated to a CM and BS application of 1.52 and 2.03 kg fresh matter m^{-2}, respectively. In order to obtain a uniform application, substrates were suspended with 50 ml of 0.01 M calcium chloride ($CaCl_2$) solution. For the control, 50 ml of 0.01 M $CaCl_2$ solution were applied. After a conditioning phase of one to two weeks with an irrigation rate of 3 mm day^{-1} (Figure 1), three irrigation treatments were carried out. The treatments differed in the rainfall pattern but had the same average irrigation of 3 mm day^{-1} over the entire incubation, including the conditioning phase. The treatments were continuous irrigation (cont_irr; 3 mm day^{-1}); partial drying and stronger irrigation (part_dry; 3 out of 4 weeks without irrigation, 1 out of 4 weeks with 13.5 mm day^{-1}); and periodic heavy rainfall (hvy_rain; 24 mm day^{-1} every 3 weeks for one day and 2 mm day^{-1} for the other days). The soil cores were irrigated with a 0.01 M $CaCl_2$ solution to simulate the ionic strength of soil solution. The number of replicates for each irrigation treatment was 4 for the control soils without organic fertilizer and 3 for the soils amended with CM or BS.

The incubation was carried out for 140 days at 13.5 °C using an automated microcosm system (Hantschel et al. 1994). The 30 soil cores were placed on a ceramic plate with a 1 µm pore diameter to which a constant suction of 100 hPa was applied. Leachates were collected weekly, quantified, and analyzed for concentrations of NO_3^-, total organic C (TOC) and total dissolved N (TDN). After the application of the organic fertilizers, the soil cores were hermetically sealed. Fresh air was purged through the headspace at a rate of 25 ml min^{-1}. Every 3.7 h, a gas sample was analyzed for CO_2, and N_2O. Several times during the incubation, subsamples of the leachate (n = 64) were filtered through a 0.45 µm polyamide filter before analysis to determine dissolved organic carbon (DOC), which accounted for at least 77 % of the TOC (data not shown).

4.3.3 Chemical analyses

Dried soil and organic fertilizer samples were used to determine total C by dry combustion on a Vario Max C elemental analyzer (Elementar Analysesysteme, Hanau, Germany). The same instrument was used to determine total N in the soil samples. Fresh manure and slurry samples were used to determine total Kjeldahl-N and NH_4^+-N by steam distillation on a Büchi 323 (Büchi Labortechnik, Essen, Germany). The pH was measured in a 0.01 M $CaCl_2$ solution (2.5 ml solution per ml fresh organic fertilizer or per g dry soil). Dry matter content was determined by drying the samples for 24 h at 105 °C.

Gaseous emissions of CO_2 and N_2O were measured by a gas chromatograph equipped with a flame ionization detector (KNK 3000 HRGC, Konik Instruments, Barcelona, Spain) and an electron-capture detector (ECD control, Model 400, Carlo Erba Strumentazione, Milan, Italy), respectively. Nitrate concentrations in the leachates were determined on a continuous flow analyzer (Evolution II auto-analyzer, Alliance

Instruments, Salzburg, Austria). TDN and TOC in the leachate were determined by thermal oxidation using a DIMATOC® 2000 (Dimatec Analysentechnik, Essen, Germany).

To determine the gravimetric moisture content, the cores were kept under the same irrigation regimes for two more months after the 140-day incubation. Periodically, soil samples from the top 6 cm of the cores were taken and dried for 24 h at 105 °C. Water filled pare space (WFPS) was then calculated using the bulk density (1.4 g cm^{-3}) and a particle density of 2.65 g cm^{-3}.

4.3.4 Statistical analyses

Statistical analyses were performed using SPSS 14.0.1 (*SPSS*, 2005). Means and standard deviations were calculated for cumulative production of CO_2, N_2O, NO_3^-, TDN and TOC. The datasets were analyzed as a two-way analysis of variance (ANOVA) with organic fertilizer treatment and rainfall pattern as the two factors. Mean comparisons were performed using the Scheffé test. Effects were considered significant for $p<0.05$.

4.4 Results and discussion

4.4.1 Carbon dynamics

In the unfertilized control soil, the cumulative CO_2 production across all irrigation treatments averaged 54.7 g CO_2-C m^{-2}. The addition of manure and slurry markedly increased CO_2 emissions throughout the 140 days of incubation with largest emissions during the first 10 days (Fig. 1). The cumulative CO_2 emissions for the soils amended with BS and CM were 92.8 and 87.8 g CO_2-C m^{-2}, respectively. The difference between the amended soils and the control accounted for 54 and 27% of the C applied with BS and CM, respectively. While there were no significant differences in the 140-day cumulative CO_2 emissions between the soils amended with organic fertilizers (Table 2), the BS-amended soils were characterized by very high CO_2 emissions during the first day after application. This may be due to the presence of labile organic compounds in BS, such as volatile fatty acids, which form as intermediates during anaerobic digestion (*Cysneiros* et al., 2008; *Jacobi* et al., 2009).

The rainfall pattern artificially created in our study affected the temporal pattern of CO_2 release. CO_2 emissions were negatively correlated with WFPS, most likely due to the fact that an increased WFPS limits oxygen diffusion through the pore space, which in turn lowers microbial activity and CO_2 production (Murwira et al. 1990)*Hillel*, 1998; (Davidson et al. 2000). During periods of increased irrigation, the WFPS reached 83 and 80% in the part_dry and hvy_rain treatments, respectively, and the CO_2 emissions were low (Figure 1). While in the hvy_rain treatment the WFPS did not drop below 76% during the periods of reduced water application, it decreased to 71% in the part_dry treatment. This decrease in WFPS was associated with an increased CO_2 release from the unamended soil, but not from the amended cores. The temporal fluctuations in CO_2 release and WFPS (ranging from 75 to 77%) were small in the cont_irr treatment.

Despite different emission patterns (Figure 1), cumulative CO_2 emissions did not differ significantly between the irrigation treatments (Table 2). Thus, the lower emissions during the phases of stronger irrigation were balanced by higher emissions in the drier phases compared to the treatments with continuous irrigation (Figure 1).

The high initial CO_2 emissions in the soils amended with BS and CM suggest the presence of labile C. However, the cumulative amounts of TOC leached from the columns amended with organic fertilizers were lower than in the unamended control (Table 2).

Highest concentrations of TOC in the leachate were observed during and after rewetting periods. However, the increase in TOC concentration during and after rewetting periods did not compensate for the very low concentrations during dryer periods. On the contrary, significantly less TOC leached from the soils subjected to partial drying and heavy rainfall than from the soils under continuous irrigation.

Table 2: Cumulative C and N outputs from soils amended with either biogas slurry (BS) or composted cattle manure (CM) or without amendment (control). The undisturbed soil cores were incubated for 140 days and subjected to different rainfall patterns. Values shown are means (n=3 for BS and CM; n=4 for control) and standard deviations. Values followed by the same letter are not significantly different ($p<0.05$). Capital letters refer to the comparison of rainfall patterns, while lower case letters refer to manure treatments.

	Continuous irrigation	Partial drying and stronger irrigation	Heavy rainfall
CO_2-C [g m^{-2}]			
BS	95.2 (20.5) Aa	92.5 (3.7) Aa	90.8 (8.1) Aa
CM	83.4 (7.2) Aa	93.7 (16.2) Aa	86.3 (28.4) Aa
Control	45.6 (10.7) Ab	65.2 (14.1) Ab	53.3 (4.5) Ab
N_2O-N [mg m^{-2}]			
BS	170.9 (37.3) Aa	162.4 (25.5) Aa	153.9 (28.9) Aa
CM	29.8 (3.2) Ab	42.5 (6.7) Ab	44.4 (19.7) Ab
Control	34.6 (8.0) Ab	54.4 (20.1) Ab	48.3 (40.7) Ab
NO_3^--N [g m^{-2}]			
BS	9.9 (1.7) Aa	8.9 (0.4) Aa	8.7 (0.4) Aa
CM	6.2 (0.6) Ab	5.9 (0.7) Ab	6.2 (1.5) Ab
Control	5.2 (1.3) Ac	5.0 (0.9) Ac	4.0 (0.3) Ac
TDN [g m^{-2}]			
BS	11.0 (2.5) Aa	9.7 (0.9) Aa	9.5 (0.7) Aa
CM	7.4 (0.9) Ab	6.7 (1.0) Ab	7.2 (1.8) Ab
Control	5.9 (1.4) Ac	5.3 (0.8) Ac	4.8 (0.2) Ac
TOC [g m^{-2}]			
BS	2.9 (0.7) Ab	2.5 (0.5) Bb	3.6 (0.1) Bb
CM	3.6 (1.1) Aab	2.5 (0.4) Bab	2.8 (0.8) Bab
Control	4.6 (0.6) Aa	2.9 (0.5) Ba	3.1 (0.3) Ba

4.4.2 Nitrogen dynamics

The amount of NO_3^- leached during the incubation was highest in the soils amended with BS, followed by the CM-amended soils and lowest in the control (Table 2). The difference between the amended soils and the control accounted for 44 and 14% of the N applied with BS and CM, respectively. The rainfall pattern had little effect on NO_3^-

leaching and no significant differences were observed between the NO_3^- leached in the different irrigation treatments (Table 2). Leaching of organic N was small. In all treatments, at least 80% of the N leached was in the form of NO_3^-. A noteworthy observation is that, independent of the rainfall pattern, all BS-amended soils showed a NO_3^- leaching peak after about five weeks, (Figure 2). This indicates that N mineralization in the BS amended soil during the initial phase of the incubation largely exceeded N demand by the microbial community, which is mainly due to the narrow C to N ratio of the slurry (Table 1).

Nitrous oxide emissions in the BS-amended soil were extremely high during the first two weeks of incubation, reaching rates of up to 880 µg N_2O-N m^{-2} h^{-1}. In contrast, the maximum N_2O emissions in the CM-amended soil and the control did not exceed 120 and 20 µg N_2O-N m^{-2} h^{-1}, respectively. Nitrous oxide may be produced by denitrification or nitrification. At the WFPS observed, denitrification was likely the major source of N_2O. In addition, high initial rates of CO_2 emissions in the BS-amended soils indicate that labile C was available and the oxygen (O_2) demand was high. In the BS-amended soil, and to a lesser degree in the CM-amended soil, the high O_2 demand by microorganisms may have exceeded the supply by diffusion, resulting in O_2 shortage, which is conductive to denitrification. Therefore, during the initial 14 days of the experiments, the conditions were favorable for denitrification to occur. Later, the C supply seemed to be limiting, as evidenced by the high leaching losses of NO_3^- and the low losses of TOC. After day 50, TOC leaching losses increased, while NO_3^- losses decreased, suggesting that the availability of N was limiting denitrification during periods with high moisture content. These observations were most pronounced in the soil amended with BS and smallest in the control.

Cumulative N_2O emissions in the soils amended with BS were increased by a factor of ≈ 4 compared to the other treatments, while the cumulative N_2O emissions from the CM-amended soil tended to be slightly lower than in the unamended control (Table 2).

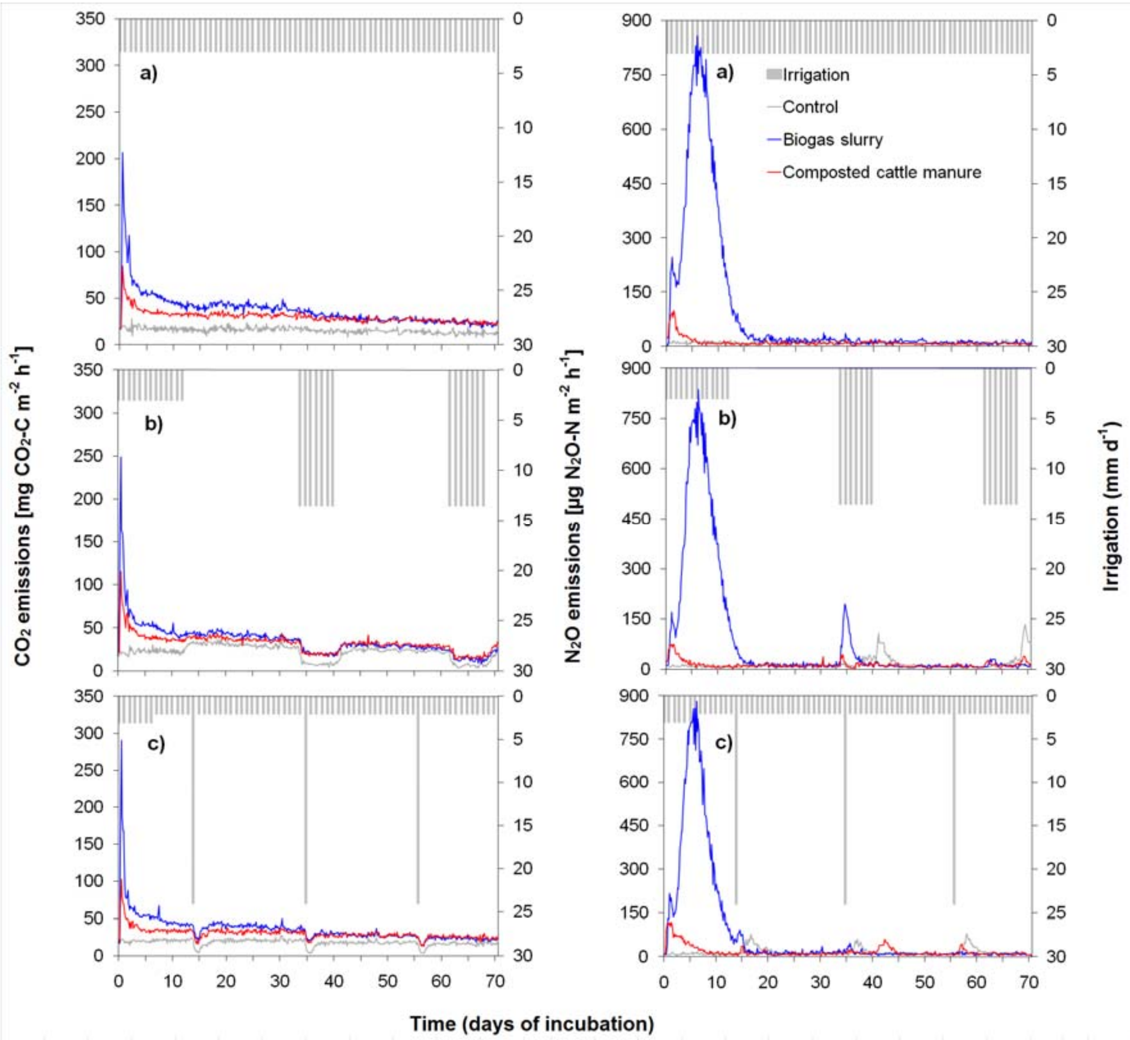

Figure 1: Average N_2O and CO_2 emissions from soils amended with composted cattle manure or biogas slurry, and unfertilized; a) continuous irrigation, b) partial drying and stronger irrigation, c) heavy rainfall; results are shown only for the first 70 days of the incubation, since no marked changes occurred later on.

Stronger irrigation after partial drying (part_dry treatment) increased N_2O emissions from the control soil in all cycles (Figure 1) and from the BS-amended soil during the first cycle, whereas the CM-amended soil responded only slightly to stronger irrigation phases. Heavy rainfall affected the temporal N_2O emissions even less (treatment hvy_rain). The temporal differences did not result in significant differences in the cumulative emissions between the irrigation treatments (Table 2).

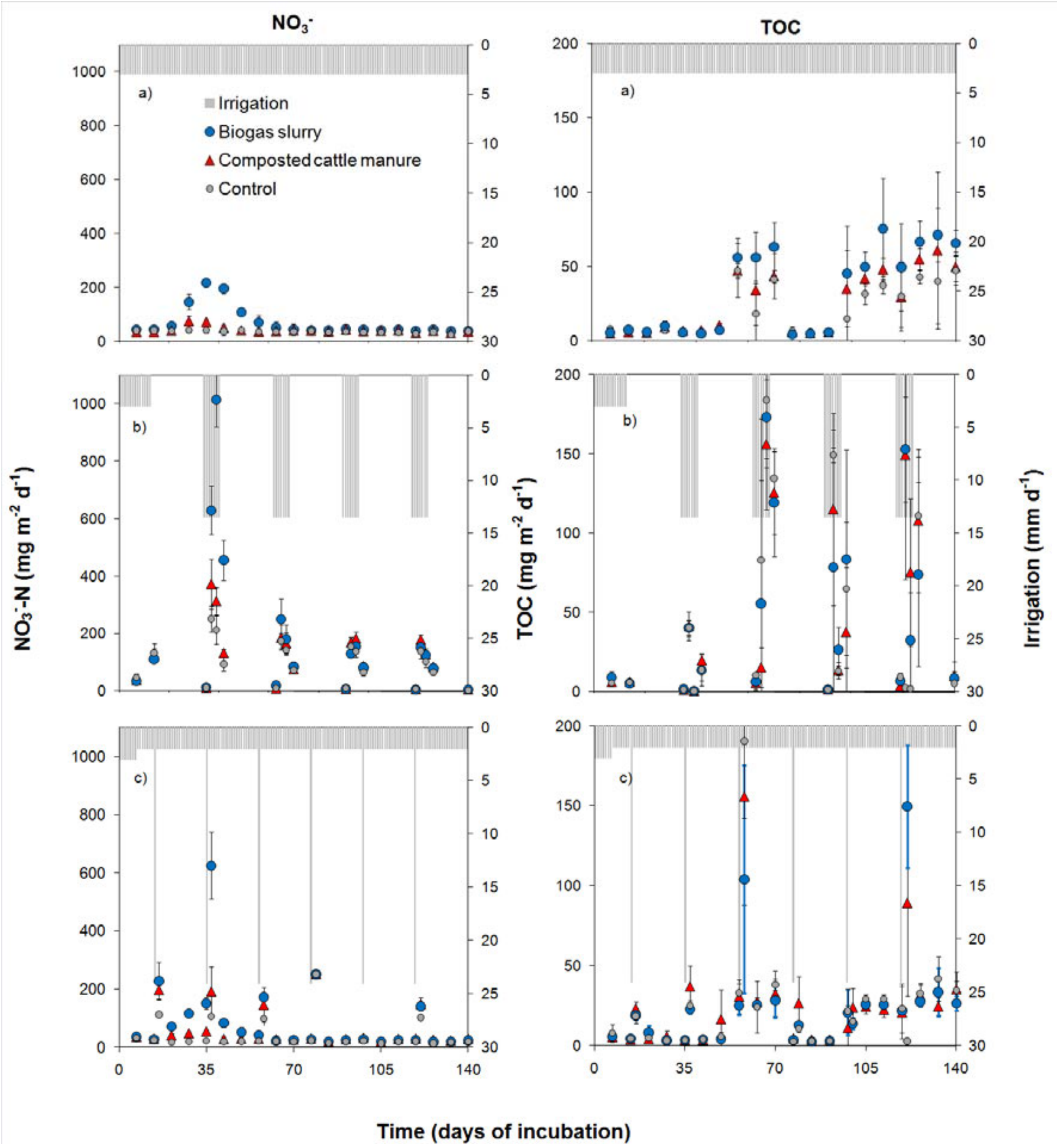

Figure 2: Leaching losses of NO_3^- and total organic carbon (TOC) from the soil columns amended with composted cattle manure or biogas slurry, and an unamended control; a) continuous irrigation, b) partial drying and stronger irrigation, c) heavy rainfall. Data shown are means (n=3 for soils amended with biogas slurry and cattle manure; n=4 for control) and standard deviations.

The rainfall pattern had little effect on cumulative N_2O emissions, which may be partly due to the fact that most of the N_2O was emitted during the initial two weeks of the incubation when the differences in soil moisture were still small.

The alkaline pH of the organic fertilizers may have resulted in N losses due to ammonia (NH_3) volatilization, especially in the samples amended with BS, which had a high NH_4^+ content. However, the organic fertilizers were diluted and incorporated into the moist soil, which had a pH of 6.4. In addition, the temperature was relatively low (13.5 °C). These factors have been found to considerably reduce NH_3 volatilization (*Huijsmans* et al., 2003; *Søgaard* et al., 2002).

4.5 Conclusions

Biogas slurry application to soils resulted in considerable N_2O emissions and NO_3^- leaching during the initial weeks of the incubation, indicating that a large proportion of the N was readily available. While this may be an attractive property for an organic fertilizer, it highlights the need to carefully choose the amount and timing of application to match plant uptake in order to minimize N losses.

Under the conditions of only partial drying of the soils as in this study, rainfall pattern slightly affected the temporal emissions of CO_2 and N_2O, and the leaching losses of NO_3^- and TOC, but had little effect on the overall C and N dynamics in the soils.

4.6 Acknowledgements

We would like to thank Anja Sawallisch and Margit Rode for technical assistance and Mirjam Helfrich for her help with the design of the experiment and four anonymous reviewers for their valuable comments on the manuscript. We also thank the Institute for Biodynamic Research in Darmstadt and the Institute for Anaerobic Technique from the University of Applied Sciences of Giessen for the fertilizer samples. This project is financed by the Deutsche Forschungsgemeinschaft (DFG-Research Training Group 1397 ‘‘Regulation of soil organic matter and nutrient turnover in organic agriculture’’).

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5 Effects of moisture and temperature on greenhouse gas emissions and C and N leaching losses in soil treated with biogas slurry

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5.1 Abstract

The objective of this study was to examine the effects of soil moisture, irrigation pattern and temperature on gaseous and leaching losses of carbon (C) and nitrogen (N) from soils amended with biogas slurry (BS). Undisturbed soil cores were amended with BS (33 kg N ha^{-1}) and incubated at 13.5 °C and 23.5 °C under continuous irrigation (2 mm day^{-1}) or cycles of strong irrigation and partial drying (every 6 weeks 1 week with 12 mm day^{-1}). During the 6 weeks after BS application on average 30% and 3.8% of the C and N applied with BS were emitted as carbon dioxide (CO_2) and nitrous oxide (N_2O), respectively. Across all treatments, a temperature increase of 10 °C increased N_2O and CO_2 emissions by a factor of 3.7 and 1.7, respectively. The irrigation pattern strongly affected the temporal production of CO_2 and N_2O, but had no significant effect on the cumulative production. Nitrogen was predominantly lost in the form of nitrate (NO_3^-). On average, 16% of the N applied was lost as NO_3^-. Nitrate leaching was significantly increased at the higher temperature ($p<0.01$), while the irrigation pattern had no effect ($p=0.63$). Our results show that the C and N turnover was strongly affected by BS application and soil temperature whereas irrigation pattern had only minor effects. A considerable proportion of the C and N in BS were readily available for soil microorganisms.

5.2 Introduction

Agriculture accounted for 10-12% of total global anthropogenic emissions of greenhouse gases in 2005 (Smith et al. 2007). The most important greenhouse gases released from agricultural land are methane (CH_4), nitrous oxide (N_2O) and carbon dioxide (CO_2) which contribute 6.5%, 5.5% and 0.1% to total global anthropogenic greenhouse gas emissions, respectively. The net flux of CO_2 related to agriculture is estimated to be approximately balanced whereas agriculture contributes about 60% and 50% of the global anthropogenic N_2O and CH_4 emissions, respectively (Smith et al. 1998, 2007). Due to concerns about the greenhouse effect, renewable energy sources, such as biogas production from energy crops, are becoming increasingly important. As maize has one of the highest CH_4 yield per hectare, its use for biogas production is likely to increase in the future (Amon et al. 2007) and therefore the availability of maize-derived biogas slurry (BS) as an organic fertilizer. However, the use of BS from energy crops as fertilizer and the effect of field applications on greenhouse gas emissions have not received much attention yet. Moeller and Stinner (2009) compared the N_2O emissions of clover/grass-ley when mulched and incorporated as green manure with the N_2O emissions resulting from the field application of BS made from the same clover/grass-ley. The field applications of BS resulted in a large increase in N_2O emissions, but it was 38% less than that from the mulched and incorporated clover/grass-ley. Senbayram et al. (2009) reported that soils treated with BS derived from maize emitted more N_2O than soils treated with mineral nitrogen (N) fertilizer. In a laboratory study they found that 2.6% of the applied BS-N was emitted as N_2O.

In soil, N_2O is produced through denitrification and nitrification, both of which are mediated by microorganisms. While nitrification takes place under aerobic conditions, denitrification predominantly occurs under anaerobic conditions (Dittert and Mühling 2009). Nitrification and denitrification rates have been found to be closely related to the water filled pore space (WFPS) of a soil. Nitrification rates are generally highest when soil moisture is below field capacity and nearly stops in saturated soils due to lack of oxygen (O_2), whereas denitrification rates generally increase with increasing soil moisture content (Davidson 1992, Maag and Vinther 1996). Bateman and Baggs (2005) found that all of the N_2O emitted from a silt loam at 70% WFPS was produced during denitrification, while at 35%–60% WFPS nitrification was the main process producing N_2O. Besides soil moisture content and temperature, the availability of substrates, ammonium (NH_4^+) for nitrification and nitrate (NO_3^-) as well as available carbon (C) for denitrification, affects the rates of N_2O emissions (Smith et al. 1998). In general, anaerobic fermentation increases the NH_4^+ content in the substrate as well as the stability of organic matter, but decreases the C to N ratio considerably (Gutser et al. 2005), resulting in a product with a high content of directly available N. However, during anaerobic digestion for CH_4 production, volatile fatty acids and other labile organic compounds are formed as intermediates (Cysneiros et al. 2008; Jacobi et al. 2009). These compounds, when still present in the BS, may be readily available C sources for soil microorganisms.

The aim of this study was to investigate the effects of soil moisture, irrigation pattern and temperature on gaseous emissions (CO_2, N_2O and CH_4) and leaching losses of C and N compounds from undisturbed soil cores in an incubation experiment.

5.3 Materials and methods

5.3.1 Soil and biogas slurry

Soil was sampled in March 2009 at the long-term experimental site "Hohes Feld" near Göttingen in Lower Saxony, Germany. The preceding crop was winter wheat, harvested in July 2008. In August 2008, the field was tilled with a rotary harrow. During winter, the field lay fallow. The mean annual precipitation at the site is 645 mm and the mean annual temperature 8.7 °C (Deutscher Wetterdienst 2009). The soil type is a Haplic Luvisol (FAO 1998) derived from loess (Ehlers et al. 2000). The soil sampled had a sand, silt and clay content of 15%, 67% and 18%, respectively, a bulk density of 1.42 g cm^{-3} and a pH of 6.9. The total carbon (C_t) content gradually decreased with depth in the plow layer (26 cm) from 13.3 in the top 9 cm to 8.2 g kg^{-1} dry soil in the lowest 9 cm of the profile. The C to N ratio ranged from 9.5 to 10.0 and was not affected by depth. When the incubation started (see below), the microbial biomass C was 328 mg kg^{-1} in the top 9 cm of the profile and 125 mg kg^{-1} in the lower part of the plow layer (the methods used are described below).

Undisturbed soil cores were sampled using plexiglass cylinders with an inner diameter of 15 cm. The cylinders were inserted into the soil to a depth of 26 cm and dug out with a spade. The soil cores remained in the cylinders for the duration of the incubation.

Biogas slurry was obtained from the Institute for Anaerobic Technology at the University of Applied Science of Giessen. The BS is a by-product of the anaerobic digestion (4 months at 55 °C) of corn silage for CH_4 production in a laboratory scale reactor. The BS had a dry matter content of 8.4% and a pH of 8.9. The C and N contents in the dry matter were 41% and 5.1%, respectively, resulting in a C to N ratio of 8. About 24% of the N was in the form of NH_4^+, while no NO_3^- was detectable.

5.3.2 Incubation

The study was carried out in a climate chamber equipped with an automated microcosm system (Hantschel et al. 1994). The cores were placed on a ceramic plate with a 1 µm pore diameter to which a constant suction of 100 hPa was applied. Prior to the incubation, the cores were conditioned at 13.5 °C for 6 months and irrigated with 2 mm day^{-1} of a 0.01 M $CaCl_2$ solution adjusted to a pH of 5.5. After the conditioning phase, the cores were randomly assigned to one of the treatments (see below). All cores were incubated for 30 weeks. During the first 16 weeks of the experiment the temperature was maintained at 13.5 °C, while it was increased to 23.5 °C for weeks 17 to 30. Three factors were investigated, namely (1) BS application (cores amended with BS and unamended controls), (2) temperature (13.5 °C and 23.5 °C), and (3) irrigation treatment. Three irrigation treatments were included:

1. Continuous irrigation (2 mm day^{-1}),
2. Cycles of strong irrigation and partial drying (1 week with an irrigation rate of 12 mm day^{-1} was followed by 5 weeks without irrigation) with BS application immediately before a period of strong irrigation, and
3. Cycles of strong irrigation and partial drying with BS application 1 week after the end of a period of strong irrigation.

In all treatments, the cumulative irrigation over any 6-week period was 84 mm. Separate cores were used for BS applications at the two temperatures. To the cores fertilized at 13.5 °C, BS was applied during week 5 when applied before the period of strong irrigation or week 8 when applied after the period of strong irrigation. To the cores fertilized after the temperature rise, BS was applied during week 17 or 20. Four cores per treatment were incubated. Biogas slurry was applied at a rate of 33 kg total N per hectare. For this, a 3-4 cm thick layer of the topsoil was removed, the slurry, suspended in 50 mL of 0.01 M $CaCl_2$ solution, added to the cores and the topsoil placed back on top. The control cores were treated identically, except that 50 mL of 0.01 M $CaCl_2$ solution without BS were added. The irrigation was reduced by the amount of liquid added in the fertilized cores. During the incubation, the soil cores were hermetically sealed and fresh air flow through the headspace was 20 ml min^{-1}.

Leachates were collected weekly, weighed, and analyzed for concentrations of NO_3^-, NH_4^+, total organic C (TOC) and total dissolved N (TDN). Every 3.7 h a gas sample was analyzed for CO_2, CH_4 and N_2O. Soil moisture content was monitored in two cores per treatment with soil moisture probes (ECH2O EC-5; Decagon Devises, Inc., Pullman, WA, USA). Measurements were taken every 30 min. The soil moisture probes were placed at a depth of 5-6 cm. During week 18 and at the end of the incubation, soil moisture content in the topsoil of all cores was determined using a ThetaProbe Soil Moisture Sensor - ML2x (Delta-T Devices Ltd., Cambridge, UK). To calibrate the soil moisture probes, gravimetric water content was determined in the top 5 cm of each core at the end of the experiment by drying a sample at 105 °C for 24 h.

5.3.3 Chemical analysis

Two soil cores were used for determination of soil characteristics after the conditioning phase. Soil moisture content was determined by drying at 105 °C for 24 h. The pH was measured in a 0.01 M $CaCl_2$ solution (2.5 ml solution per g fresh soil) and particle size distribution with the pipet method according to DIN ISO 11277 (2002). Soil microbial biomass was determined with the chloroform fumigation extraction method (Vance et al. 1987). Total contents of C and N were determined by dry combustion on a CN Elemental Analyzer (Elementar Vario El, Heraeus, Hanau, Germany). The same methods were used for BS analyses, except that total N was analyzed by the Kjeldahl method on a Büchi 323 (Büchi Labortechnik, Essen, Germany). Ammonium and NO_3^- were extracted from the BS with 0.5 M K_2SO_4 (20 ml per g fresh BS) and determined on a continuous flow analyzer (Evolution II auto-analyzer, Alliance Instruments, Salzburg, Austria).

Gaseous emissions of CO_2, N_2O and CH_4 were measured by a gas chromatograph (Shimadzu GC-14, Shimadzu Scientific Instruments, Columbia, USA). Nitrate and NH_4^+ concentrations in the leachates were determined on a continuous flow analyzer. Total dissolved N and TOC in the leachates were analyzed using a DIMATOC® 2000 (Dimatec Analysentechnik, Essen, Germany). Several times during the incubation, subsamples of the leachate were filtered through a 0.45 µm polyamide filter before analysis to determine the dissolved organic C content.

5.3.4 Statistical analysis

Statistical analyses were performed using SPSS 14.0.1 (SPSS 2005). The data were analyzed as a three-way ANOVA with temperature, fertilizer treatment and irrigation pattern as factors. Mean comparisons were performed using the Tukey test. Effects were considered significant for $P<0.05$. In order to avoid confounding effects due to differences in irrigation water application rates, comparisons of gaseous emissions from the different treatments were made for the six-week period following the application of BS, as the amount of irrigation water applied was the same for all soil cores during any six-week period. Effects of BS application on leaching losses became first evident after four weeks. Leaching losses from the different treatment were therefore compared for the six week-period between week four and ten after BS applications.

5.4 Results

5.4.1 Soil moisture

The gravimetric moisture content in the upper 6 cm of the cores with continuous irrigation averaged 27.5%, which corresponds to an average WFPS of 84% in the soil core (data not shown). In the treatments with cycles of strong irrigation and partial drying, the gravimetric moisture content in the upper 6 cm reached 29.5% during periods of strong irrigation and dropped to 26.5% during the five weeks without irrigation. These gravimetric moisture contents correspond to 90% and 81% WFPS in the soil core during periods of strong irrigation and partial drying, respectively. As the topsoil was loosened to incorporate the BS, the WFPS may have been lower in the topsoil after the BS application than in the rest of the core. Neither the increase in temperature after 16 weeks, nor the application of BS had a significant effect on the gravimetric moisture content (data not shown).

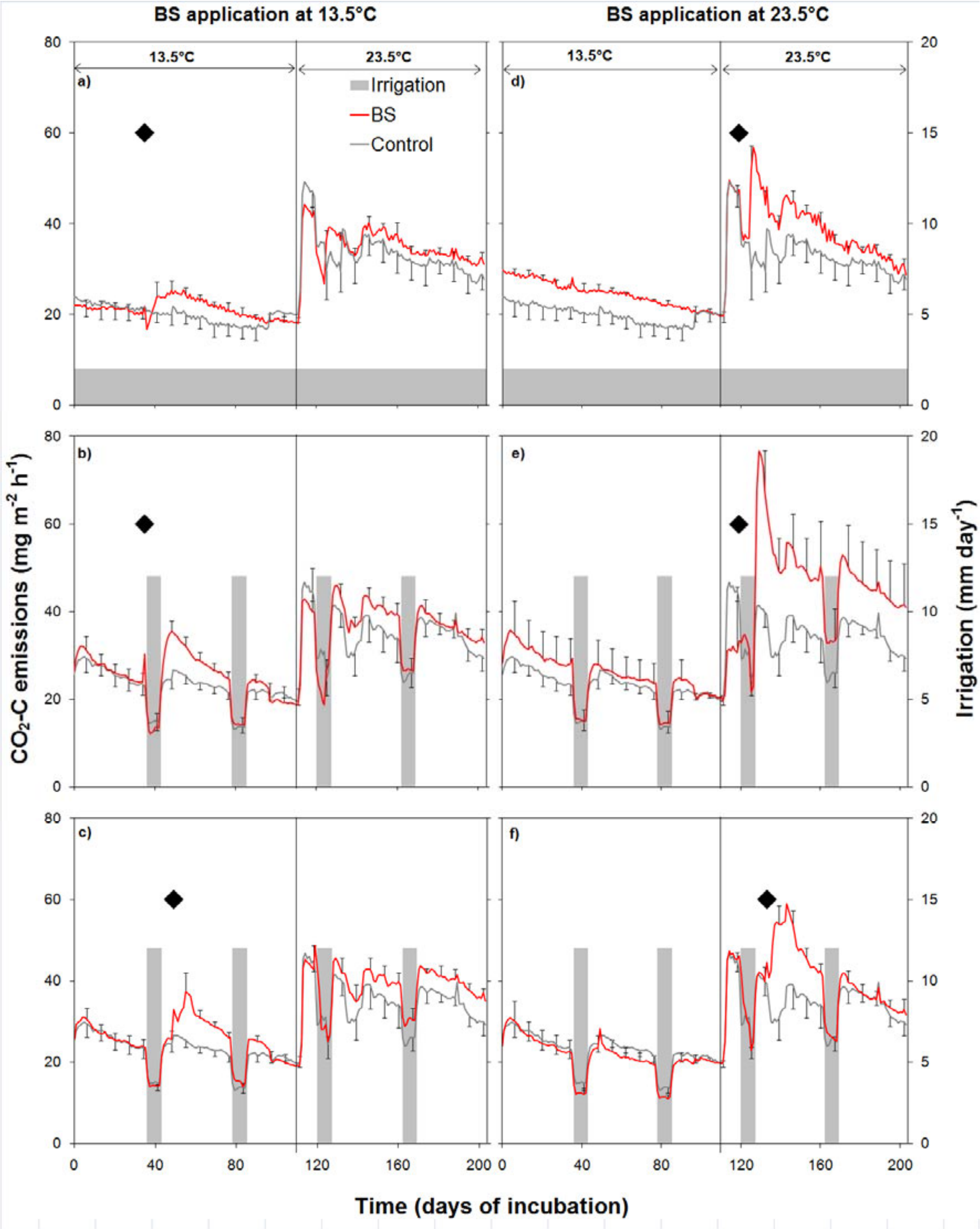

Figure 3: Average CO_2 emissions from soil cores amended with biogas slurry (BS) and unfertilized cores (control). The treatments included continuous irrigation (a, d), cycles of strong irrigation and partial drying with BS application before a wet period (b, e), and cycles of strong irrigation and partial drying with BS application during a dry period (c, f). The undisturbed soil cores were incubated at 13.5 °C and 23.5 °C. Diamonds indicate the time of BS application. Each line represents the average of four columns. The standard error of the mean (n=4) is shown for the first measurement each week.

5.4.2 Carbon dioxide production

At 13.5 °C, CO_2 emission rates from the continuously irrigated control soils averaged 20 mg CO_2-C m^{-2} h^{-1} and decreased slightly over time (Figure 3a). The temperature rise resulted in a steep and immediate increase in CO_2-C production rates up to 49 mg m^{-2} h^{-1} for one week and average emission rates at 23.5 °C were increased to 33 mg CO_2-C m^{-2} h^{-1} (Figure 3d). Therefore, the Q_{10} factor for CO_2 emissions was 1.7. The application of BS increased CO_2 emission rates at both temperatures for a few weeks. However, after 3 (when BS was applied at 13.5 °C) to 7 weeks (at 23.5°C), the CO_2 emissions had dropped to the level of the control. The amount of applied C, which evolved as CO_2 during the 6 weeks following BS application, was 13% and 34% at 13.5 °C and 23.5 °C, respectively (Figure 4).

At 13.5 °C, the average CO_2 production of control soils in the treatments with cycles of strong irrigation and partial drying was 23 mg CO_2-C m^{-2} h^{-1}, with emissions averaging 25 mg CO_2-C m^{-2} h^{-1} during partial drying periods and 14 mg CO_2-C m^{-2} h^{-1} during wetter periods (Figures 3b-c). Similar to the soils under continuous irrigation, the temperature rise led to an increase in average CO_2 production rates to 34 mg m^{-2} h^{-1} (Figures 3e-f), corresponding to a Q_{10} factor of 1.5.

When BS was applied directly before a week of stronger irrigation, the CO_2-C emissions slightly decreased during the period of stronger irrigation, but increased considerably afterwards compared to the control (Figures 3b, e). During subsequent periods of stronger irrigation, fertilization had a positive effect on CO_2 emissions only at the higher temperature (Figure 3e). The amount of applied C which evolved as CO_2 within 6 weeks after BS application was 17% and 58% at 13.5 °C and 23.5 °C, respectively, when BS was applied directly before a week of stronger irrigation. Biogas slurry applications one week after a period of strong irrigation also increased CO_2 emission rates during periods without irrigation and had little effect during periods of stronger irrigation (Figures 3c, f). The CO_2, which evolved within 6 weeks after BS application, accounted for 21% and 38% of the C applied with the BS at the lower and higher temperature, respectively (Figure 4).

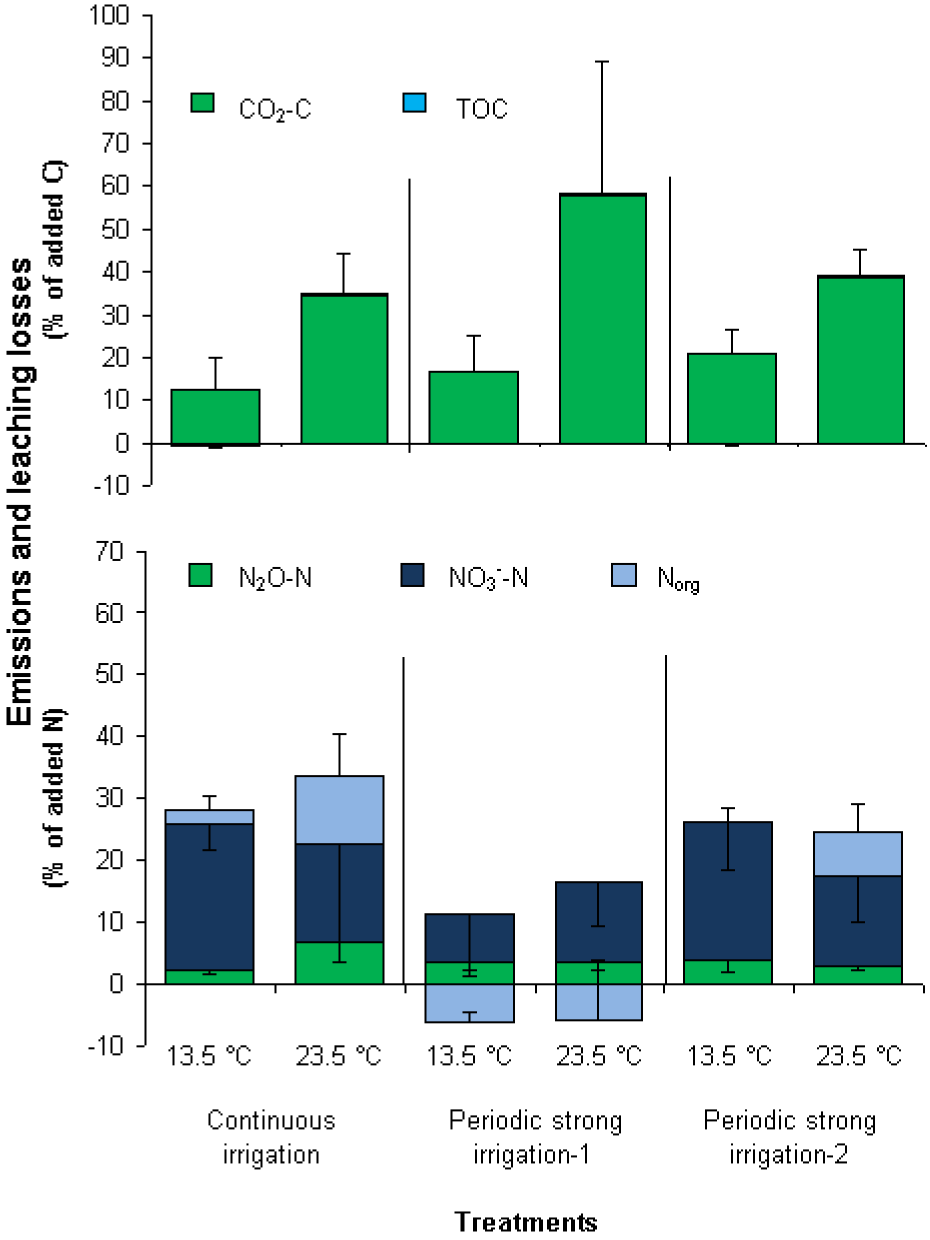

Figure 4: Proportion of biogas slurry C and N lost from soil cores incubated under continuous irrigation, and cycles of strong irrigation and partial drying with slurry applied before (periodic strong irrigation-1) and after a period of strong irrigation (periodic strong irrigation-2). The undisturbed soil cores were incubated at 13.5°C and 23.5°C. Gaseous losses include CO_2 and N_2O, and losses in leachate include TOC, NO_3^--N, and N_{org}. The difference between TDN and NO_3^--N corresponded to N_{org}. Negative values for N_{org} indicate that the losses in the biogas slurry amended cores were lower than in the control.

Across all treatments, differences due to BS application and temperature were highly significant ($p<0.01$ for both factors), while no significant differences were induced by the irrigation treatments ($p=0.34$; Table 4).

5.4.3 Nitrous oxide emissions

On average, control soils under continuous irrigation emitted 22 µg N_2O-N m^{-2} h^{-1} at 13.5 °C (Figure 5a). With the rise in temperature, N_2O production reached a peak of 408 µg m^{-2} h^{-1} within a day. The average emission rate at the higher temperature was 194 µg N_2O-N m^{-2} h^{-1} (Figure 5d), which resulted in a Q_{10} factor of 8.8. Biogas slurry application at the lower temperature in the treatment with continuous irrigation resulted in an instant increase in N_2O emissions, followed by a decrease over the next 3 weeks to the level of the control (Figure 5a). When soils were fertilized at the higher temperature, maximum N_2O emissions of 1250 µg N_2O-N m^{-2} h^{-1} were measured and the emission rates remained above the level of the control for about 6 weeks (Figure 5d). During the 6 weeks following the BS application, 2.2% and 6.8% of the N applied were lost in the form of N_2O at 13.5 °C and 23.5 °C, respectively (Figure 4).

In the treatments with cycles of strong irrigation and partial drying, N_2O emissions were highest one or two days after the beginning of the period of stronger irrigation. A second smaller peak was observed one day after the end of the period of stronger irrigation. This trend was independent of temperature and BS application time. During drier periods N_2O production was generally low, except directly after the application of BS (Figures 5b-c, e-f). Most of the BS-related N_2O emissions took place within a few weeks after the BS application. However, during periods of stronger irrigation, increased N_2O emissions could still be observed after several irrigation cycles. The increase in temperature had little effect on the proportion of the N applied lost as N_2O because N_2O emissions increased in the BS-amended and in the control soil. When BS was applied before a period of stronger irrigation, 3.5% and 3.7% of the applied N was lost as N_2O at 13.5 °C and 23.5 °C, respectively. When the application took place during the drier period, 3.9% and 3.0% of the N applied were lost at 13.5 °C and 23.5 °C, respectively (Figure 4).

As with CO_2, no significant differences were induced by irrigation pattern ($p=0.71$), as higher N_2O emissions during periods of stronger irrigation were compensated for by lower emissions during drier periods in treatments with cycles of strong irrigation and partial drying (Table 4). In contrast, the increase in temperature resulted in significantly higher N_2O emissions ($p<0.01$), with the Q_{10} factor across all treatments reaching 3.7. Slurry application also significantly increased N_2O emissions compared to the unamended control ($p<0.01$).

5.4.4 Methane consumption

Methane emission rates were constantly negative in all treatments, indicating that net CH_4 consumption took place (data not shown). The average CH_4 consumption in the treatments with continuous irrigation was 4 µg CH_4-C m^{-2} h^{-1}, which corresponds to 0.13 mg CH_4 m^{-2} day^{-1}. The same rate was measured during periods of stronger irrigation in the treatments with cycles of strong irrigation and partial drying, while CH_4 consumption reached 8 µg CH_4-C m^{-2} h^{-1} during drier periods (Table 3). No significant differences in cumulative CH_4 consumption were caused by BS application ($p=0.96$) or temperature ($p=0.33$; Table 4). In contrast, CH_4 consumption was significantly increased in the treatments with cycles of strong irrigation and partial drying compared to the continuously irrigated cores ($p<0.01$).

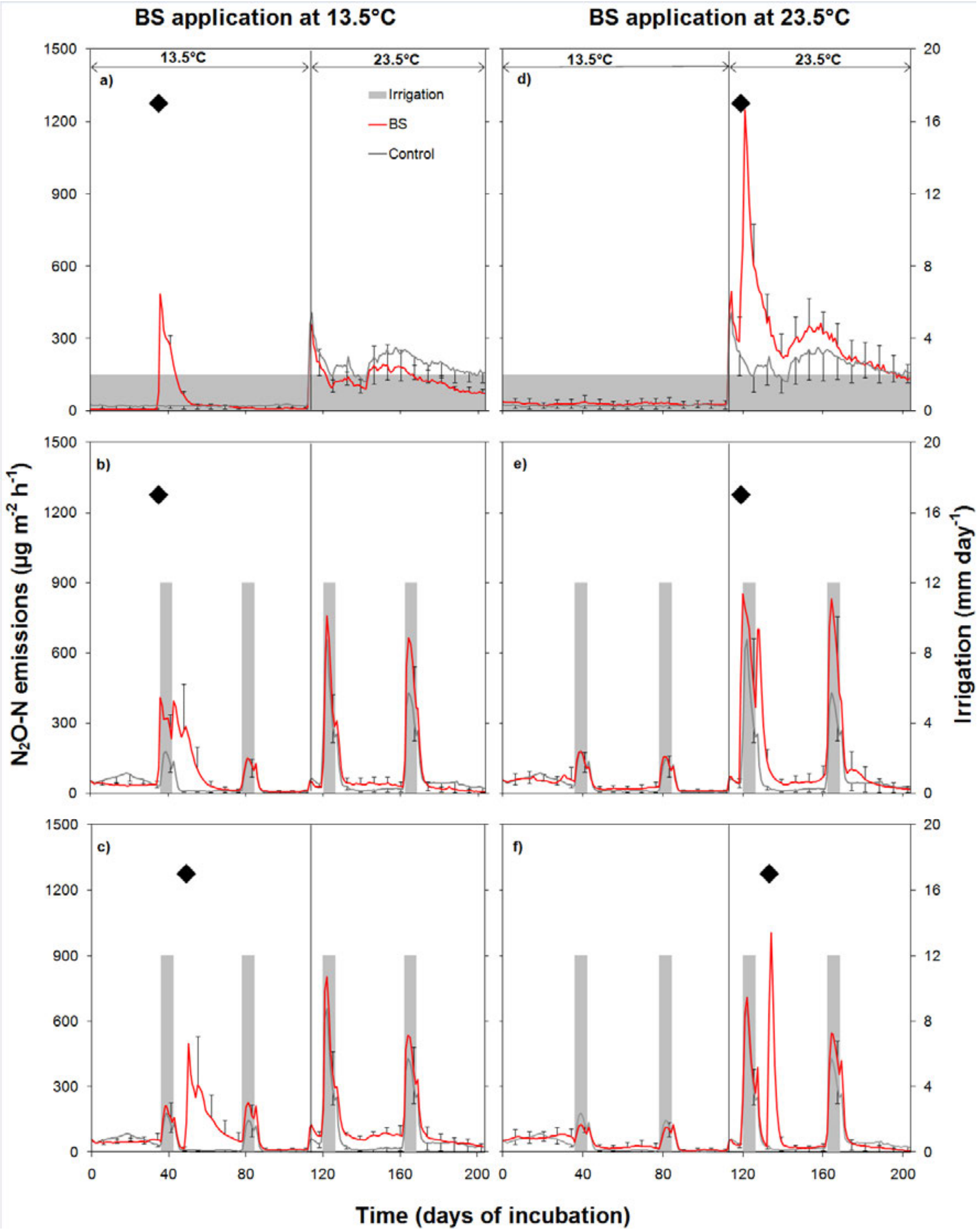

Figure 5: Average N_2O emissions from soil cores amended with biogas slurry (BS) and unfertilized cores (control). The treatments included continuous irrigation (a, d), cycles of strong irrigation and partial drying with BS application before a wet period (b, e), and cycles of strong irrigation and partial drying with BS application during a drier period (c, f). The undisturbed soil cores were incubated at 13.5 °C and 23.5 °C. Diamonds refer to time of BS application. Each line represents the average of four columns. The standard error of the mean (n=4) is shown for the first measurement each week.

5.4.5 Leaching losses

Nitrate leaching from the cores under continuous irrigation without BS application was relatively constant averaging 37 mg NO_3^--N m^{-2} day^{-1} at 13.5 °C and of 51 mg NO_3^--N m^{-2} day^{-1} at 23.5 °C, which corresponds to a Q_{10} factor of 1.38 (Figure 6). Between 60% and 70% of the NO_3^--N leached from the control soils of the treatments with cycles of strong irrigation and partial drying was lost during periods of stronger irrigation. While only 22 to 28 mg NO_3^--N m^{-2} day^{-1} were lost during drier periods, the average losses reached 257 to 368 mg NO_3^--N m^{-2} day^{-1} during periods of stronger irrigation, with the higher rates occurring at the higher temperature. Across all treatments, BS application and temperature both had highly significant effects on NO_3^- leaching ($p<0.01$), while the irrigation treatments had no significant effect ($p=0.63$). Effects of BS application on NO_3^- leaching were most pronounced between weeks 4 and 10 after the application date. During this 6-week period, cumulative NO_3^--N losses across all irrigation treatments corresponded to 18% and 14% of the N applied in the BS at 13.5 °C and 23.5 °C, respectively.

Losses of TDN closely followed NO_3^--N losses. In fact, about 80% of the N in TDN was generally in the form of NO_3^-, while the concentration of NH_4^+ in the leachate was always below the detection limit (data not shown). Thus, the difference between TDN and NO_3^--N corresponded to organic N (N_{org}). In contrast to NO_3^-, the application of BS before a period of stronger irrigation decreased leaching of organic N compared to the unamended control (Figure 4).

Leaching losses of TOC were significantly increased at the higher temperature ($p<0.01$; Table 4), with losses averaging 24 and 36 mg C m^{-2} day^{-1} at the lower and higher temperature, respectively. In contrast, the application of BS had no significant effect on TOC leaching ($p=0.73$). However, TOC losses were significantly higher under continuous irrigation than under cycles of strong irrigation and partial drying. Dissolved organic C (<0.45 µm) accounted for about 94% of the TOC leached.

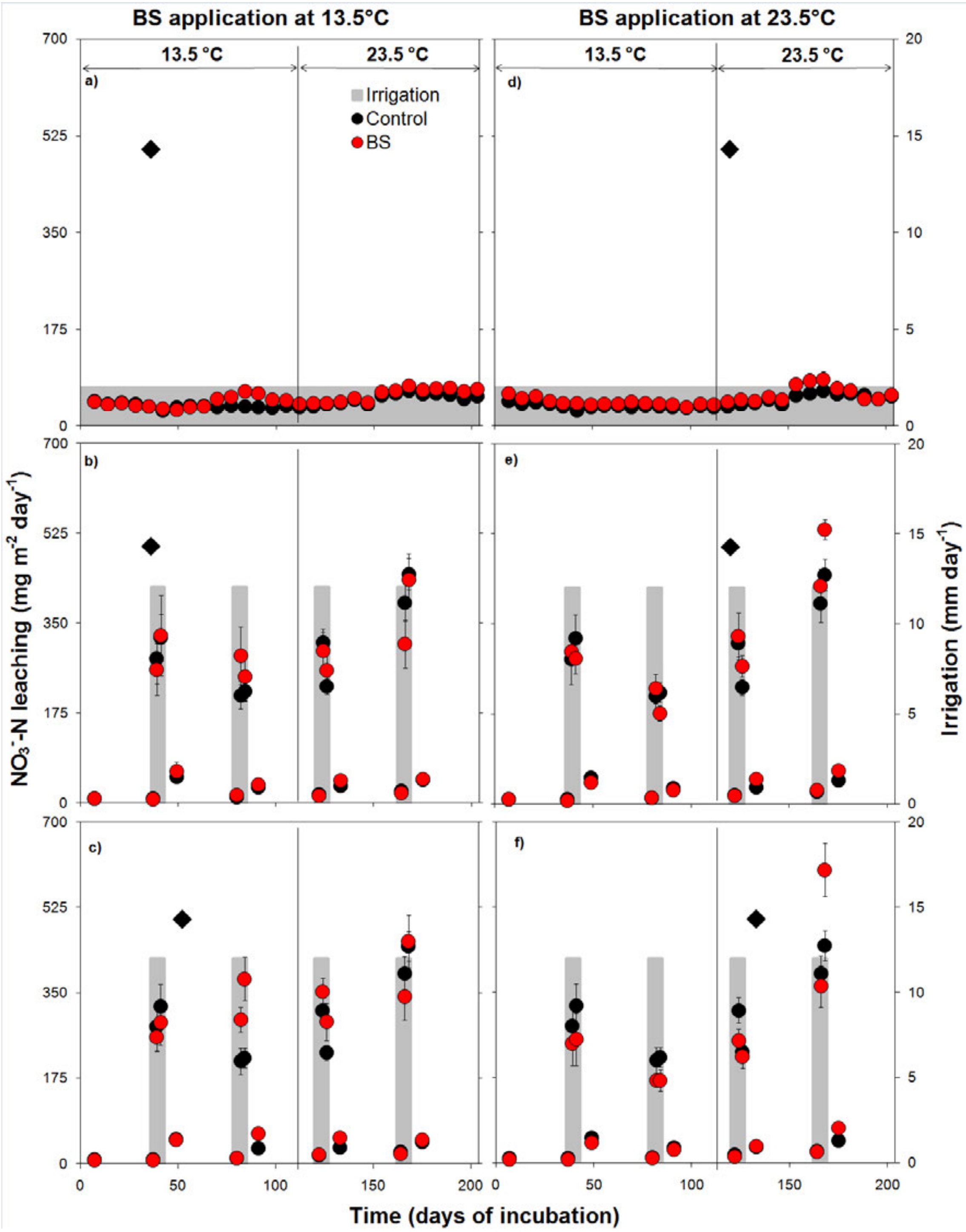

Figure 6: Average leaching losses of NO_3^--N from soil cores amended with biogas slurry (BS) and unfertilized cores (Control). The treatments included continuous irrigation (a, d), cycles of strong irrigation and partial drying with BS application before a wet period (b, e), and cycles of strong irrigation and partial drying with BS application during a dry period (c, f). The undisturbed soil cores were incubated at 13.5 °C and 23.5 °C. Diamonds refer to time of BS application. Symbols represent means (n=4) with standard error of the mean.

Table 3: Cumulative gas emissions and leaching losses from soils amended with biogas slurry (BS) or without BS application (Control).

Emissions and leaching losses ($g\ m^{-2}$)		Continuous irrigation		Periodic strong irrigation - 1		Periodic strong irrigation - 2	
		Control	BS	Control	BS	Control	BS
N_2O-N	13.5°C	0.021 (0.010)	0.091 (0.015)	0.034 (0.009)	0.147 (0.036)	0.028 (0.009)	0.155 (0.063)
	23.5°C	0.197 (0.089)	0.418 (0.107)	0.094 (0.021)	0.214 (0.048)	0.078 (0.021)	0.177 (0.028)
CO_2-C	13.5°C	19.62 (2.37)	22.95 (1.91)	22.53 (1.61)	26.97 (2.13)	22.01 (1.51)	27.49 (1.46)
	23.5°C	34.99 (4.58)	44.06 (2.63)	35.09 (3.74)	50.30 (8.29)	33.78 (4.76)	43.89 (1.81)
CH_4-C	13.5°C	-3.25 (1.01)	-3.47 (0.60)	-6.00 (0.67)	-5.97 (0.46)	-6.29 (0.66)	-6.08 (0.34)
	23.5°C	-4.34 (1.27)	-3.27 (0.72)	-7.24 (1.40)	-6.93 (0.43)	-7.05 (1.34)	-7.31 (1.19)
NO_3-N	13.5°C	1.47 (0.17)	2.24 (0.13)	1.51 (0.13)	1.77 (0.33)	1.59 (0.13)	2.31 (0.25)
	23.5°C	2.47 (0.45)	2.98 (0.52)	2.72 (0.17)	3.14 (0.24)	2.70 (0.15)	3.18 (0.25)
N_{org}	13.5°C	0.29 (0.06)	0.36 (0.08)	0.57 (0.06)	0.38 (0.05)	0.62 (0.06)	0.62 (0.08)
	23.5°C	0.79 (0.11)	1.16 (0.22)	0.91 (0.05)	0.72 (0.32)	0.98 (0.08)	1.20 (0.15)
TOC	13.5°C	1.11 (0.21)	0.95 (0.11)	0.86 (0.03)	0.81 (0.05)	0.88 (0.04)	0.81 (0.05)
	23.5°C	1.54 (0.31)	1.69 (0.19)	0.97 (0.02)	1.14 (0.09)	0.99 (0.02)	1.11 (0.09)

Undisturbed soil cores were incubated at two different temperatures and subjected to continuous or periodic irrigation patterns (Periodic strong irrigation-1: BS applied immediately before one week of strong irrigation; Periodic strong irrigation-2: BS applied one week after period of strong irrigation). Cumulative gas emissions were calculated for 6 weeks following BS application. Cumulative leaching losses were calculated between week 5 and 10 after applications. Values shown are means (n=4) and standard errors.

Table 4: Statistical analysis of the data presented in Table 1.

	IRR	BS	T	IRR x BS	IRR x T	BS x T
	p-values					
N_2O emissions	0.71	<0.01	<0.01	0.85	0.02	0.12
CO_2 emissions	0.34	<0.01	<0.01	0.9	0.49	0.27
CH_4 emissions	<0.01	0.96	0.33	0.93	0.97	0.75
NO_3^- leaching	0.63	<0.01	<0.01	0.68	0.56	0.7
N_{org} leaching	0.03	0.47	<0.01	0.06	0.22	0.21
TOC leaching	<0.01	0.73	<0.01	0.94	0.08	0.12

The datasets were analyzed as a three-factor analysis of variance. The factor irrigation (IRR) includes the levels continuous irrigation, cycles of strong irrigation and partial drying with biogas slurry application before a period of strong irrigation, and cycles of strong irrigation and partial drying with biogas slurry application after a period of strong irrigation. The factor biogas slurry (BS) includes with and without BS application, while the factor temperature (T) includes 13.5 °C and 23.5 °C.

5.5 Discussion

Under the conditions of our experiment, C and N turnover in undisturbed soil cores were strongly affected by BS application and soil temperature. In contrast, the irrigation pattern and time of BS application had minor effects. In addition, only a few weak interactions were observed between these factors (Table 4).

5.5.1 Biogas slurry application

With the application of BS, CO_2 production increased immediately. Within 6 weeks, on average 30% of the C applied with BS was respired as CO_2. These results indicate that a considerable proportion of the BS was readily available. In some microsites, O_2 demand likely exceeded supply by diffusion, resulting in anaerobic conditions favourable for denitrification to occur.

Across all treatments, 3.8% of the N applied with the BS was lost as N_2O during the 6 weeks following its application. In a study carried out at 13.5 °C with a similar soil type and BS from the same fermenter, Sänger et al. (2010) found that about 1.2% of the added N was lost as N_2O. Surprisingly, this proportion is lower than the average of 3.2% found in the present study at the lower temperature even though the application rate in the present study was three times lower. This suggests that the application rate had only a minor effect on the proportion of N lost as N_2O. In fact, in a pot experiment over 35 days with soil held at 85% WFPS, Senbayram et al. (2009) found that about 1.7% and 1.5% of the applied BS were lost as N_2O with application rates of 90 and 360 kg N ha^{-1}, respectively. When applied to grassland at a rate of 480 kg N ha^{-1}, less than 0.5% of the BS-N was lost in the form of N_2O (Senbayram et al. 2009).

In a review of the literature, de Klein et al. (2001) found that the N_2O emissions reported for mineral fertilizers generally ranged from 0.1% to about 2% of the N applied; however, a few short-term studies using nitrate-based fertilizers reported emissions of up to 12%. For a wide variety of animal manures, emissions ranged from 0% to about 5% of the manure N applied (de Klein et al. 2001). This comparison suggests that the N_2O emissions from BS in our study are similar to those reported for animal waste and slightly higher than emissions from mineral fertilizers. However, in the studies reviewed by de Klein et al. (2001), N fertilizer was applied to crops in the field. The fact that no N uptake by plants took place in our study most likely resulted in higher N_2O emissions compared to field studies.

The application of BS increased leaching losses of NO_3^- but not of TOC, indicating that the microorganisms were limited by the availability of C while N availability exceeded demand. This result is not surprising given the narrow C to N ratio of BS, which was 8, and the high content of NH_4^+. On average, about 16% of the N applied with the BS was leached from the cores between week 5 and 10 after application.

In summary, a large proportion of the C and N were readily available to soil microorganisms. The excess N, which leached as NO_3^- in our study, could have been taken up by crops in a planted field. In contrast, as with slurry from animal husbandry, the application of BS to fallow fields may result in high NO_3^--leaching losses.

5.5.2 Soil moisture effects

In general, soil moisture has a strong effect on the decomposition of organic material. While at low moisture water is the limiting factor, excess moisture inhibits aerobic metabolism because of O_2 diffusion limitations. In an incubation study with different soils, Franzluebbers (1999) found that C mineralization was highest when the WFPS was between 53% and 66%. In the present study, WFPS ranged between 80% and 90%, which was likely at the upper limit for optimal aerobic respiration. This is evident from the fact that CO_2 evolution strongly decreased during rewetting periods. However, while the irrigation treatment affected the temporal pattern of CO_2 evolution, it had no significant effect on the cumulative CO_2 production. This is likely due to the fact that the variation in soil moisture observed in our study was relatively small.

While soil moisture had a strong effect on the temporal pattern of N_2O emissions, there was no significant effect of the different irrigation treatments on cumulative N_2O emissions. These results were unexpected and differ from Clayton et al. (1997) and Smith et al. (1998) who found in a field study that N_2O emissions depend particularly on WFPS and rainfall at the time of fertilization. However, the WFPS in these two studies ranged from 40% to 90%, while the fluctuations in WFPS in our study were much smaller. This is most likely the reason for the small effects the irrigation treatments had in our study.

Uptake of atmospheric CH_4 through biological oxidation has been reported in a variety of upland soil ecosystems. Methane oxidation generally occurs in well aerated soils, although an anaerobic pathway for CH_4 oxidation has also been described (Segers 1998). Boeckx and van Cleemput (2001) summarized experimentally determined CH_4 uptake rates from different ecosystems and from different parts of the world. For arable soils, the calculated average of the minimum and maximum values from these data was 0.25–0.83 mg CH_4 m^{-2} day^{-1}. With 0.13 to 0.26 mg CH_4 m^{-2} day^{-1}, the CH_4 consumption measured in our study was therefore at the lower end of this range. In contrast to N_2O, soil moisture significantly affected CH_4 consumption. Methane consumption rates were increased during drier periods which may be due to the fact that CH_4 consumption in aerated soils is mainly controlled by soil moisture, as CH_4 diffusion is much slower in water than in air (Dörr et al. 1993). However, as soils contain both methanotrophic and methanogenic soil microorganisms, it is not possible to determine with the data available whether this difference was due to increased CH_4 consumption or decreased CH_4 production.

5.5.3 Temperature

While the irrigation treatment and BS application time had only minor effects on the proportion of BS-N lost in the form of N_2O, the effect of temperature was much stronger. Across all treatments, the ten-degree increase in temperature increased N_2O losses by a factor of 3.7, which is more than twice as high as the factor for CO_2 (1.7). The Q_{10} factor for CO_2 found in our study is within the range of values reported in a number of laboratory studies summarized by von Lützow and Kögel-Knabner (2009). High Q_{10} factors for N_2O have been reported in several studies. Abdalla et al. (2009) found Q_{10} factors ranging from 4.4 to 6.2 for a temperature range of 10–25 °C in an anaerobic laboratory incubation with pasture soil amended with mineral fertilizer. In an incubation with soil cores of an imperfectly drained Gleysol fertilized with NH_4NO_3, Dobbie and Smith (2001) reported Q_{10} factors for an arable soil of 8.9 for the 12-18 °C interval. These results indicate that N_2O emissions are very sensitive to increasing temperature.

5.5.4 Aerobic and anaerobic processes

The high N_2O emission rates indicate that anaerobic conditions were dominant in the soil cores, especially after the addition of BS and at the higher temperature. However, the high CO_2 production rate, CH_4 consumption and nitrification suggest aerobic conditions.

The greatly increased N_2O emissions observed after the application of BS suggest that microorganisms used NO_3^- as an alternative electron acceptor when O_2 became limiting. As the BS contained almost no NO_3^-, denitrifying bacteria must have either used NO_3^- from the soil solution or NH_4^+ from the BS must have been nitrified in aerobic microsites and diffused to anaerobic sites where denitrification took place. However, nitrification may also result in the production of N_2O. In a summary of published data, Mathieu et al. (2006) found that the proportion of nitrified N lost as N_2O generally ranged between 0.05% and 0.5% for well-aerated soils while it may increase to values higher than 1% under O_2 limiting conditions. In the present study, about 37 and 51 mg NO_3^--N m^{-2} day^{-1} were leached from the unamended cores under continuous irrigation at 13.5 °C and 23.5 °C, respectively. Under the assumption that most of the NO_3^- produced was leached during the course of the experiment and that between 0.05% and 1% of the N was lost as N_2O during nitrification, the production of the NO_3^- leached would have caused N_2O-N emissions of 19 to 371 µg m^{-2} day^{-1} at 13.5 °C, and of 26 to 510 µg m^{-2} day^{-1} at 23.5 °C. Therefore, at the lower temperature, between 4% and 76% of the N_2O emissions may have been produced through nitrification, while the contribution of nitrification was below 11% at the higher temperature. Even though these calculations are rough estimates, they indicate that nitrification may have considerably contributed to the N_2O emissions even though the soils were relatively moist. However, denitrification was most likely the predominant source of N_2O in our study, especially at the higher temperature. Applying the same calculations for the other treatments, some general patterns emerge. The contribution of nitrification was likely higher at the lower temperature than at the higher temperature. In addition, nitrification likely contributed less to N_2O emissions in the BS amended soils compared to the control. In both cases, these findings can be explained by the increased O_2 demand at the higher temperature and in BS amended soil, which resulted in more anaerobic microsites.

Our finding that net CH_4 consumption took place throughout the experiment in all treatments also indicates that aerobic microsites existed, as aerobic CH_4 oxidation, requires both oxygen and CH_4. Anaerobic methane oxidation is also possible, however, little is known about this process in the soil environment. Its contribution to CH_4 consumption seems low (Segers 1998). These results highlight the complexity of biochemical reactions in soil, which is a heterogeneous environment. During the incubation aerobic and anaerobic microsites likely existed on a temporal and on a spatial scale. The application of an organic C and N source, such as BS, may strongly affect the O_2 balance in the soil and with it the rates of the biochemical processes taking place.

5.6 Conclusion

The use of maize and other crops for CH_4 production is likely to increase in the future and increasing quantities of maize-derived BS will become available as fertilizer. So far, only a few studies have dealt with the effects of BS applications on C and N turnover in soil.

Our results indicate that N_2O emissions may be relatively large when BS is applied to moist soil. However, the proportion of N lost as N_2O is within the range reported for slurries from animal husbandry. The small effect of soil moisture at the time of application was at least partly due to the high initial moisture contents and subsequent small fluctuations in soil moisture content observed in our study and cannot be generalized.

Our results also indicate that a relatively large proportion of the N is mineralized and nitrified during the first few weeks after BS application and could be susceptible to N leaching in fallow fields. However, our study was carried out under controlled conditions and there is still little known about the proportion of N which becomes available during the cropping season. In addition, we used BS from only one fermenter in our study, and the properties of BS from other fermenters and production processes may be quite variable.

5.7 Acknowledgement

We would like to thank Anja Sawallisch and Margit Rode for technical assistance, two anonymous reviewers and the editor-in-chief for their valuable comments on the manuscript as well as the Institute for Anaerobic Technology from the University of Applied Sciences of Giessen for the BS. This project is financed by the Deutsche Forschungsgemeinschaft (DFG-Research Training Group 1397 ‘‘Regulation of soil organic matter and nutrient turnover in organic agriculture’’).

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6 C and N dynamics of a range of biogas slurries as a function of application rate and soil texture: a laboratory experiment

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6.1 Abstract

Biogas slurries (BS's) vary widely in raw material and composition and studies regarding their impacts on carbon (C) and nitrogen (N) dynamics after application to soil are scarce. The objective of this study was to evaluate the effect of different BS's and their application rates on the C and N dynamics after incorporation into a silty Anthrosol and a sandy Cambisol adjusted to different water contents. Additionally, those slurry parameters related to the amounts of nitrate (NO_3^-), ammonia (NH_3) and nitrous oxide (N_2O) produced were determined. Ten BS's from different biogas plants using various substrates were characterized and mixed with the two soils at a rate of 0.5 g N (kg soil)$^{-1}$, respectively, and incubated for 6 weeks at 25°C and 60% of the soils' water holding capacity (WHC). Three BS's were additionally incubated for 4 weeks at 25°C (60% of WHC) with varying fertilizer levels of 0.1, 0.3 and 0.5 g N (kg soil)$^{-1}$. Six weeks after application the N_2O emission factors ranged between 0.1 and 0.8%. On average 21% of the C supplied was emitted as carbon dioxide (CO_2) and potential NH_3 emissions during the first 10 days of incubation ranged between 6% and 12% of ammonium (NH_4^+) present in the slurries. The emission factors in the sandy Cambisol were on average 1.7, 1.2 and 2 times higher for N_2O, CO_2 and potential NH_3, respectively, compared to the silty Anthrosol. Losses of NH_3 were significantly positively correlated (Pearson) with NH_4^+ supplied by BS's ($r = 0.4$, $p < 0.01$) and negatively with the total C (C_t) content of BS's ($r = -0.3$, $p < 0.01$). Highest nitrification

rates in both soils developed during the first 7 days of incubation as well to a greater extent in the sandy Cambisol. Increasing biogas slurry (BS) N rates increased the N_2O emissions in the sandy Cambisol from 0.04 to 1.5% of added N. Our results indicated that the type of substrate input to biogas plants influences the properties of the by-product BS and therefore the mineralization dynamics of C and N. However, differences in the C and N dynamics induced by soil texture exceeded differences induced by varying properties of BS.

6.2 Introduction

There were 5905 biogas plants used in Germany in 2010 and this number is increasing, with expected 7000 biogas plants in use in 2011 (*German Biogas Association 2011*). Biogas may be produced from manure, bio waste, energy crops and residual materials from industry and agriculture. Animal excrements and energy crops represent the main inputs for biogas production in Germany, being 45% and 46%, respectively. The main energy crop used, amounting to 76% is maize silage, since it offers the highest biogas yield (202 m^3 t^{-1} fresh matter) with a methane content of 52%. In comparison, cow slurry, pig slurry and grass silage offer methane yields of 25, 28 and 172 m^3 t^{-1} fresh matter with methane contents of 60%, 65% and 60%, respectively (*Agency for Renewable Resources 2011*). During the anaerobic fermentation process the content of dry matter (DM), C_t, the biological oxygen demand and C to N ratio decrease whereas the content of NH_4^+ and the pH increase (*Asmus* et al. 1988; *Gutser* et al. 2005; *Möller* et al. 2008).

Biogas slurries may serve as a valuable fertilizer because of the increased availability of N and the better short-term fertilization effect compared to undigested slurries (*Gutser* et al. 2005; *Weiland* 2010). However, BS's show a high variability in their nutrient composition because of different feed substrates and conditions of digestion used (*Möller* et al. 2010; *Sensel* and *Wragge* 2008; *Wendland* et al. 2011).

Thus, the term “biogas slurry” refers to a wide range of by-products produced by anaerobic fermentation of biodegradable materials.

Biogas slurries may have varying C and N mineralization dynamics when applied to soils. For example, mineralization of N is influenced by the amount and quality of organic matter (*Heumann* et al. 2002). Large amounts of readily decomposable organic matter enhance immobilization of N (*Griffin* et al. 2005; *Sörensen* 1998) and losses due to denitrification (*Flowers* and *Arnold* 1983). Due to the high content of NH_4^+ in BS’s the risk of NH_3 emissions after application to soil is high. In addition to the loss of plant available N, NH_3 is an indirect greenhouse gas which deposits in a dry or wet form and may induce eutrophication of other ecosystems. Studies dealing with NH_3 emissions after BS application to soil are rare.

Few studies have reported the effects of biogas wastes and BS’s on C and N dynamics in soils. For instance, field and laboratory studies (*Dittert* et al. 2009; *Senbayram* et al. 2009) indicated similar N_2O emissions after the application of ammonium nitrate, cattle/pig slurry and biogas waste at 65% of water WHC. However, with increasing water contents up to 85% of WHC N_2O emissions of soils amended with biogas waste exceeded emissions of mineral fertilized soils (*Senbayram* et al. 2009). Comparing BS’s exclusively made from maize silage with composted cattle manure, *Sänger* et al. (2010) found that the application of BS to bare soil at a rate of 100 kg N ha^{-1} resulted in four times higher cumulative N_2O emissions (162 mg N_2O-N m^{-2} corresponding to 1.6% of applied N) than composted cattle manure during 140 days of incubation. This difference was explained by the higher amount of readily available N in the BS.

Overall, investigations of BS’s obtained by maize silage as co-substrate (which is common due to its highest biogas yields) with a view to its varying chemical properties and use as fertilizer are still scarce and only little quantification of C and N mineralization dynamics after application to soils exists. Therefore, the research questions of the present microcosm study were:

(1) To what extent do the C and N dynamics of different BS's differ after application to sandy or silty soils and which slurry parameters are related to the amounts of NO_3^-, NH_3 and N_2O produced?
(2) What quantitative effect do different N rates and water contents in soils have on the C and N dynamics when BS is applied to bare soils of different texture?

6.3 Material and methods

6.3.1 Biogas slurries

Ten BS's were collected from biogas plants in Brandenburg ($P_{57.1}$), Thuringia (E_{90}, E_{88}), Lower Saxony (P_{66}, P_{58}) and Hesse (E_{83}, P_{62}, P_{63}, $P_{57.2}$, P_{100}). These BS's represented a wide range of different substrate inputs. The acronyms refer to the main type of the feeding material (P - plant based and E - animal based) and the percentage of the main feeding material. The amount of feeding material added daily to the biogas plant varied between 7 and 238 t, the rate of maize silage between 10 – 100%. Samples were taken directly from the digester shortly before storage (BS's $P_{57.1}$, E_{90}, E_{88} and $P_{57.2}$) or from the storage (BS's P_{66}, P_{58}, E_{83}, P_{62}, P_{63} and P_{100}).
Chemical properties of the ten BS's are shown in Table 5. The highest variations were found in the contents of NH_4^+-N and DM. A product-moment correlation analysis (Pearson) showed a relationship between increasing amounts of maize silage and higher C_t contents ($r = 0.8$, $p < 0.01$). This was possibly due to higher concentrations of lignin, cellulose and hemi-cellulose than in BS's mainly based on animal excrements. Overall, the chemical characteristics of the BS's were in accordance with those of fermented slurries studied by *Ernst* (2008), *Möller* et al. (2010), *Senbayram* et al. (2009) and *Sensel* and *Wragge* (2008).

6.3.2 Soils

Two soils differing in texture and type were used for incubation. A silty Anthrosol (5% sand, 75% silt, 20% clay) was sampled in June 2010 from the upper 25 cm of a field margin at the organic Hessian State Domain Frankenhausen near Kassel. The contents of C_t and total N (N_t) were 1.3 and 0.1%, respectively, pH ($CaCl_2$) was 6.5, the content of NO_3^- was 133 mg kg^{-1} and the content of NH_4^+ was below detection limit. The WHC was 50%. A sandy Cambisol (46% sand, 39% silt, 15% clay) derived from loess was collected from the same soil depth in a field of winter wheat in Reinhausen near Göttingen in May 2010. The contents of C_t and N_t were 0.8 and 0.1%, respectively, pH ($CaCl_2$) was 7.5, the content of NO_3^- was 44 mg kg^{-1} and the content of NH_4^+ was below detection limit. The WHC was 47%. Before incubation both soils were dried at room temperature and sieved to 5 mm.

Table 5: Substrate inputs (in % mass) of sampled biogas plants and characterization of biogas slurries used.

		Biogas slurries									
		Mainly excrement based			Mainly plant based						
		E_{90}	E_{88}	E_{83}	$P_{57.1}$	$P_{57.2}$	P_{58}	P_{62}	P_{63}	P_{66}	P_{100}
Substrate input											
Maize silage	(%)	10	11	17	35	50	33	52	24	61	100
Grass silage	(%)	-		-	11	-	-	8	31	-	-
Rye silage (whole plant)	(%)	-	-	-	-	-	25	-	8	-	-
Sorghum	(%)	-	-	-	8	-	-	-	-	-	-
Grain	(%)	-	2	-	3	7[b]	-	2[a]	-	5[a]	-
Pig slurry	(%)	-	87	19	43	-	20	35	-	34	-
Cattle slurry	(%)	90	-	64	-	43	-	-	37	-	-
Farmyard manure[c]	(%)	-	-	-	-	-	22	3	-	-	-
Properties of biogas slurries											
DM	(%)	5.7	4.7	5.5	5.4	8.1	9.6	7.2	9.2	5.8	6.8
pH ($CaCl_2$)		8.1	7.9	8.0	7.8	7.6	8.0	7.7	7.8	7.9	7.7
C_t	(% DM)	38.8	39.8	38.4	42.1	42.0	41.3	40.7	39.2	42.0	43.2
Total Kjeldahl-N	(% DM)	8.3	6.7	6.3	6.2	7.2	5.0	7.7	6.5	8.2	7.2
C/N		4.7	6.0	6.1	6.8	5.8	8.3	5.3	6.0	5.1	6.0
NH_4^+-N	(% DM)	5.5	3.6	2.9	2.6	3.4	2.0	4.3	3.5	5.5	2.9

NO_3-N in biogas slurry not detected

[a] shredded grain, [b] squeezed grain, [c] mixed farmyard manure with 80% of cattle

6.3.3 Experimental design

Trial 1: C and N dynamics of a range of biogas slurries

Each of the ten fresh BS's (1.14 – 3.45 g, depending on the N content) was mixed with the sandy Cambisol and silty Anthrosol (20 g dry soil) at a rate of 0.5 g N (kg soil)$^{-1}$ (corresponding to 250 kg N ha^{-1}) in a plastic beaker. Additionally, plastic beakers were filled solely with the silty or sandy soil (controls) and without any substrates (blanks). Thus, the total number of treatments was 23. The water contents of all samples were adjusted to 60% of WHC - corresponding to 49% and 45% water filled pore space (WFPS) in the silty and sandy soils, respectively - by adding distilled water to provide ideal moisture conditions for microbial activity. In total, 24 beakers (sampling on six dates with four replicates) were used for each of the 23 treatments resulting in 552 samples. The samples were then placed in preserving jars with a volume of 1.6 l which were hermetically closed immediately. Four jars per treatment were equipped with a valve for gas sampling. Samples were incubated for 6 weeks in a climate chamber at 25°C. During the first 2 weeks, gas samples were taken daily and every second to fourth day thereafter. One gas sample of 50 ml was taken from each valve-equipped jar with a pre-evacuated glass vial after circulating the air in the 1.5 l headspace of the jar using a 50 ml syringe. After taking one sample per preserving jar, the jars were aerated and closed again until next gas sampling time. The remaining jars were aerated regularly to prevent excessive concentrations of CO_2. On days 0, 3, 7, 14, 28 and 41 four plastic beakers of each treatment were harvested destructively to determine NH_4^+ and NO_3^-. For the determination of potential NH_3-N losses traps with 25 ml 0.05 M H_2SO_4 were placed with the samples into the preserving jars. During the first 17 days of incubation, traps were replaced at an interval of 18 h to 72 h and analyzed for NH_4^+-N (*DIN 38406/5* 1983). A preliminary trial with an alkalized NH_4^+ solution showed that after 24 h 95% of the volatized NH_3 was captured in the H_2SO_4 traps.

Trials 2 and 3: The effect of different N-rates and water contents on the C and N dynamics

The BS's P_{66}, P_{62} and E_{83} were selected for further investigations because they covered a wide range of N_t and NH_4^+-N contents. These BS's were incubated for 4 weeks at 25°C. Treatments included varying fertilizer levels of 0.1, 0.3 and 0.5 g N (kg soil)$^{-1}$ (corresponding to 50, 150 and 250 kg N ha^{-1}) and water contents of 60% of WHC in all samples (trial 2). Additional treatments included a fertilizer rate of 0.3 g N (kg soil)$^{-1}$ and varying water contents of 50%, 75% and 100% of WHC (trial 3). These water contents corresponded to 20% and 19% WFPS (50% of WHC), 61% and 56% WFPS (75% of WHC) and 81% and 75% WFPS (100% of WHC) in the silty and sandy soils, respectively. The experimental setup and gas sampling procedure corresponded to trial 1, with the exception of potential NH_3 emissions, which were not measured. However, NH_4^+ and NO_3^- in all treatments were sampled destructively at days 3, 6, 14 and 27 in 4 replicates.

6.3.4 Analyses of biogas slurries and soils

Properties of the BS's (Table 5) were determined as follows: Biogas slurries were dried at 105°C and subsequently at 550°C to determine their total DM content and organic DM, respectively. Ammonium and NO_3^- were extracted from BS's with 0.5 M K_2SO_4 (20 ml per gram of fresh BS) and measured on a continuous flow analyzer (Evolution II auto-analyzer, Alliance Instruments, Salzburg, Austria). The pH values were measured in a 0.01 M $CaCl_2$ solution (2.5 ml solution per gram of fresh BS). Total content of C was determined by dry combustion on a CN Elemental Analyzer (Elementar Vario El, Heraeus, Hanau, Germany). Total N was analyzed by the Kjeldahl method on a Büchi 323 (Büchi Labortechnik, Essen, Germany).

Soil DM contents (total and organic), pH values, and mineral N (N_{min}) (NH_4^+ and NO_3^-) were determined in the same way as for BS's, except that for N_{min} determination

5 ml 0.5 M K_2SO_4 per gram of soil was used. The content of C_t and N_t of soils was determined by dry combustion on a CN Elemental Analyzer. Particle size distribution was ascertained with the pipet method according to *DIN ISO 11277* (2002). For the determination of the WHC a funnel equipped with a folded filter was filled with fresh soil and placed in a beaker filled with distilled water reaching to the bottom of the folded filter. After 30 minutes the soil samples were weighed, dried for 24 h at 105°C and weighed again.

6.3.5 Determination of gaseous emissions and mineral N

Gas samples taken with pre-evacuated glass vials were placed on a pressure-controlled autosampler for 64 glass vials and analyzed by a gas chromatograph (Shimadzu GC-14B, Shimadzu Scientific Instruments, Columbia, USA) equipped with an electron-capture detector (ECD) and a flame ionization detector (FID) to determine CO_2 and N_2O, respectively (*Loftfield* et al. 1997). Nitrate and NH_4^+ were extracted from samples with 0.5 M K_2SO_4 (20 ml per gram of sample) and determined on a continuous flow analyzer (Evolution II auto-analyzer, Alliance Instruments, Salzburg, Austria). Cumulative net nitrification was calculated by subtracting the NO_3^- content on day 0 from the NO_3^- content at sampling time. Net N mineralization and N immobilization was calculated by subtracting the N_{min} content on day 0 from the N_{min} content at sampling time.

6.3.6 Statistical analysis

Statistical analyses were performed using SPSS 14.0.1 (*SPSS* 2005). Data sets were calculated for each treatment and dependent variable for days 3, 7, 28 and in trial 1 also for day 41. Mean values of the different treatments were analyzed as a two-way ANOVA with the factors BS and soil. Data of trial 2 and 3 were analyzed as a three-way ANOVA both with factors BS and soil plus N rate and water content, respectively.

Mean comparison was performed using the Tukey test. Effects were considered significant for $p < 0.05$. Pearson correlation coefficients were determined for relations between input material and BS's properties as well as for BS's properties (incl. C and NH_4 applied with BS's).

6.4 Results and Discussion

Trial 1: C and N dynamics and NH_3 volatilization of a range of biogas slurries added to soils

Cumulative CO_2 evolution after 6 weeks in unamended control soils reached 318 mg C (kg soil)$^{-1}$ in the silty Anthrosol and 516 mg C (kg soil)$^{-1}$ in the sandy Cambisol. In the treatments with BS application production of CO_2 averaged 871 mg C (kg soil)$^{-1}$ (ranging from 709 to 1114 mg C (kg soil)$^{-1}$) and 1161 mg C (kg soil)$^{-1}$ (975 to 1461 mg C (kg soil)$^{-1}$) in silty and sandy soils, respectively. On average 19% and 22% of the C supplied were emitted as CO_2 in silty and sandy soils (Table 6), respectively, which was slightly lower compared to other studies (*Jarecki* et al. 2008; *Sänger* et al. 2010; *Sänger* et al. 2011; *Terhoeven-Urselmans* et al. 2009).

Figure 7 illustrates exemplarily the development of cumulative CO_2 emissions of the silty Anthrosol and the sandy Cambisol amended with BS's E_{90} and P_{100}, respectively. Application of BS's to sandy soils resulted for each slurry in a greater C mineralization from the 10th day onwards (Figure 7) compared to silty soils, probably because of a greater organic matter stabilization of silty soils due to organo-mineral interactions (*von Lützow* et al. 2006) as well as due to the higher pH in sandy soils promoting the mineralization.

For both soils the BS treatments E_{88} and P_{66} had highest CO_2 emissions with values from 23.4% to 30.6% of added C (Table 6), whereas BS treatment P_{100} emitted lowest CO_2 (12.5 - 14.3 % of added C) compared to the other BS's. However, no correlation between CO_2 emissions and BS properties or operating parameters of biogas plants could be identified.

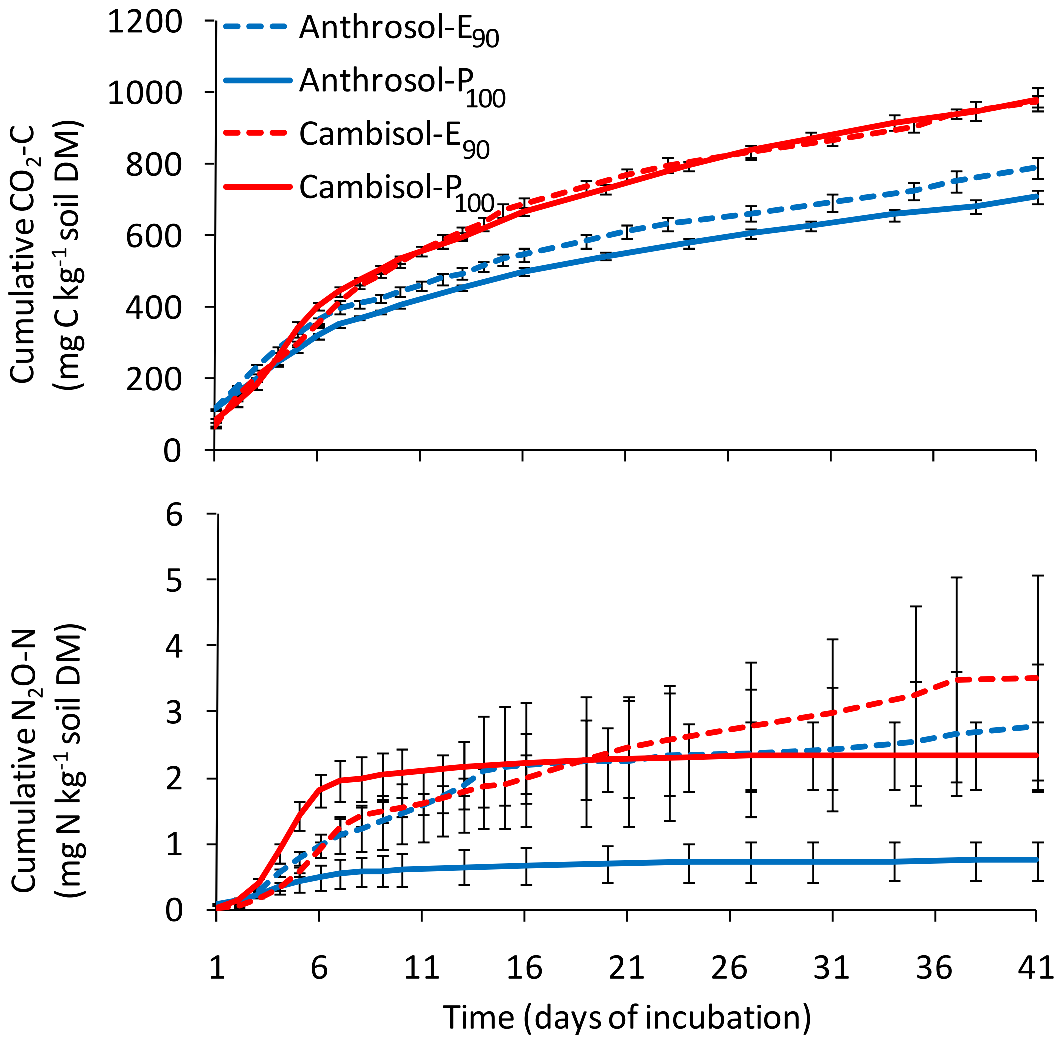

Figure 7: Average cumulative CO_2 and N_2O emissions from silty Anthrosol (solid line) and sandy Cambisol (broken line) amended with biogas slurries E_{90} (black) and P_{100} (grey). The samples were incubated for 6 weeks at 25°C. Values shown are means (n=4) and standard errors.

After application, the large amounts of NH_4^+ present in the BS's (Table 5) were nitrified quickly and the net nitrification rate was highest during the first week of incubation. The amount of NO_3^--N recovered after 6 weeks of incubation was between

51% and 65% of N supplied with BS in the silty Anthrosol and between 35% and 62% of N supplied in the sandy Cambisol (data not shown).

The addition of BS generally resulted in net N immobilization during the first half of the incubation (Figure 8). After 4 weeks, the N_{min} content in the soils with BS had been decreased by about 20 mg (kg soil)$^{-1}$ (corrected by the N mineralized in the control soils), which corresponds to 2 and 4% of N_t and NH_4^+-N added, respectively. During the second half of the incubation, net N mineralization was observed with most BS's added to the silty Anthrosol. So that by the end of the 6-week incubation, the N_{min} content in the soils with BS addition, when corrected by the N mineralized in the control soils, had reached again the initial level in both soils. Net N immobilization in the sandy soils continued during the second half of the incubation and resulted in a total decrease in N_{min} of about 40 mg (kg soil)$^{-1}$ after 6 weeks (Figure 8). The difference between soils may be due to the high mineralization rate in the control of the sandy soil between days 28 and 42. This increase has a huge standard error.

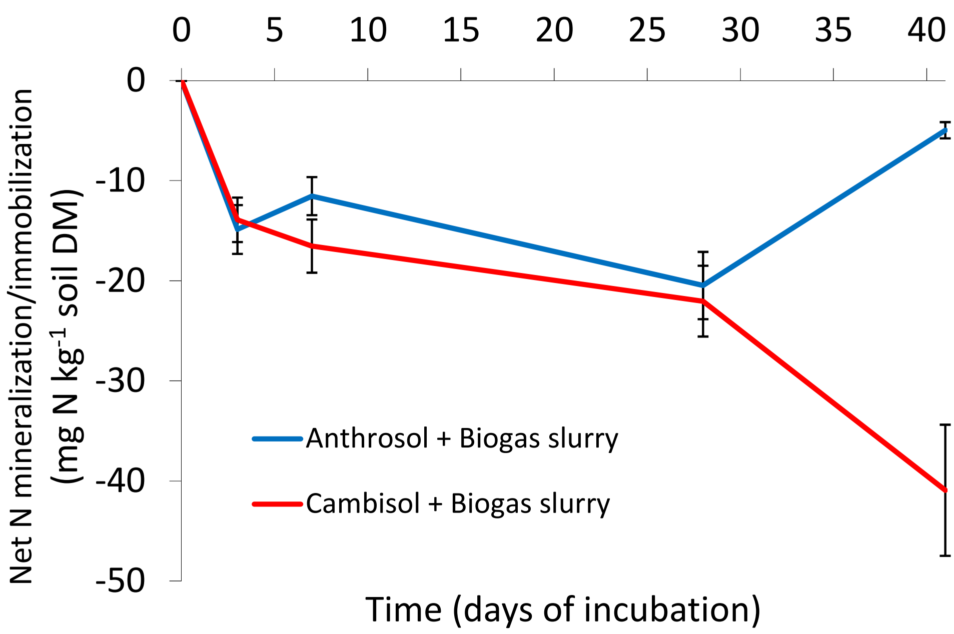

Figure 8: Net N mineralization and immobilization from samples amended with biogas slurries. The samples were incubated for 6 weeks at 25°C. Values shown are means (n=40) and standard errors.

Cumulative N_2O emissions from control soils after 6 weeks reached 0.02 and 0.01 mg N (kg soil)$^{-1}$ in the silty Anthrosol and the sandy Cambisol, respectively. Application of BS's resulted in an increased N_2O production of 0.7-2.8 mg N (kg soil)$^{-1}$ in the silty Anthrosol and 1.7-3.8 mg N (kg soil)$^{-1}$ in the sandy Cambisol. Overall, between 0.1 and 0.8% of the amount of N added with BS's was emitted as N_2O (Table 6). In comparison, Velthof et al. (2003) reported considerably higher N_2O emissions after the application of liquid pig manure and cattle slurry to soil after an incubation of 98 days. However, in their study, a large proportion of N_2O was emitted after the addition of water at day 57 to restore the initial moisture content. While the N_2O emissions ranged from 1.8 to 3.0% of the applied N with cattle slurries, they ranged from 7.3 to 13.9% when liquid pig manure was applied (*Velthof* et al., 2003). The authors attributed this difference to the availability of readily degradable C, especially of volatile fatty acids.

Cumulative N_2O emissions were on average 1.7 times higher in sandy soil compared to silty soil. This could be attributed to the higher clay content in silty soil reducing the N availability due to partial adsorption of the added NH_4^+ at the cation exchange sites (*Jarecki* et al. 2008) and the protection of organic matter against decomposition. However, cumulative N_2O emissions of the silty Anthrosol amended with BS E_{90} were in the range of amended sandy soils amended with BS P_{100} (Figure 7) due to the high amount of NH_4^+ caused by the predominant share of animal excrements in BS E_{90}.

Table 6: Average cumulative CO_2, N_2O and potential NH_3 emissions and N_{min} concentrations relative to control and applied C and N with biogas slurries, respectively. Silty and sandy soils were amended with each of the biogas slurries at a rate of 0.5 g N (kg soil)$^{-1}$, respectively. The samples were adjusted to a water content corresponding to 60% of WHC and incubated at 25°C for 6 weeks. Values shown are means (n = 4) and standard errors.

	Biogas slurry	CO_2-C (% of added C)		N_2O-N (% of added N)		NH_3-N (% of added N)[1)]		N_{min} (% of added N)		N_{min} (% of added NH_4^+-N)	
		silty soil	sandy soil	silty soil	sandy soil	silty soil	sandy soil	silty soil	sandy soil	silty soil	sandy soil
Trial 1	E_{90}	20.8 (1.3)	21.3 (0.6)	0.6 (0.2)	0.7 (0.3)	4.6 (0.3)	7.4 (0.1)	63.9 (1.4)	52.7 (1.0)	95.9 (2.1)	79.1 (1.5)
	E_{88}	23.4 (2.1)	30.6 (2.0)	0.5 (0.1)	0.8 (0.2)	3.3 (0.5)	7.3 (0.2)	55.6 (3.6)	49.3 (1.2)	102.9 (6.7)	91.2 (2.3)
	E_{83}	17.0 (1.1)	24.6 (1.1)	0.4 (0.1)	0.6 (0.1)	3.7 (0.2)	6.4 (0.1)	55.2 (1.2)	40.6 (2.7)	119.0 (2.7)	87.5 (5.8)
	$P_{57.1}$	18.2 (0.8)	21.8 (0.3)	0.5 (0.1)	0.3 (0.0)	1.0 (0.1)	3.1 (0.3)	49.2 (0.7)	38.7 (2.4)	117.5 (1.7)	92.5 (5.8)
	$P_{57.2}$	19.0 (1.0)	22.1 (0.7)	0.2 (0.1)	0.5 (0.1)	-[2)]	-[2)]	57.2 (1.0)	51.7 (3.6)	121.1 (2.1)	109.4 (7.7)
	P_{58}	19.5 (1.1)	23.7 (0.7)	0.2 (0.0)	0.4 (0.1)	2.7 (0.2)	5.5 (0.5)	50.4 (1.2)	29.6 (2.7)	127.1 (3.0)	74.5 (6.7)
	P_{62}	17.0 (1.1)	19.3 (0.8)	0.2 (0.0)	0.4 (0.1)	3.6 (0.2)	7.0 (0.2)	62.2 (0.5)	47.8 (0.8)	110.7 (1.0)	85.0 (1.3)
	P_{63}	16.1 (0.7)	17.7 (0.9)	0.2 (0.1)	0.4 (0.1)	3.3 (0.4)	6.8 (0.0)	54.3 (1.2)	52.3 (0.7)	99.9 (2.2)	77.9 (1.2)
	P_{66}	24.4 (1.3)	26.8 (1.6)	0.3 (0.1)	0.5 (0.1)	3.7 (0.3)	7.2 (0.1)	64.7 (0.9)	54.2 (0.7)	95.4 (1.4)	80.0 (1.1)
	P_{100}	12.5 (0.6)	14.3(1.1)	0.1 (0.1)	0.5 (0.1)	-[2)]	-[2)]	52.7 (0.6)	43.5 (1.1)	132.7 (1.6)	109.4 (2.6)
	Average	18.8	22.2	0.3	0.5	3.2	6.3	56.5	46.0	112.2	88.7
	CV	0.2	0.2	0.5	0.3	0.3	0.2	0.1	0.2	0.1	0.1

[1)] amount of N applied with biogas slurry emitted as NH_3 during the first week of incubation, subsequently no NH_3 emissions were detected

[2)] NH_3 emissions were not determined in these samples

Under the conditions of the experiment, potential NH_3 emissions during the first week in the sandy Cambisol were twice the emissions in the silty Anthrosol (Figure 9). Overall, potential NH_3 emissions accounted on average for 3.2% and 6.3% of added N (Table 6) or 6% and 12% of NH_4^+ present in the slurries in silty and sandy soils, respectively, during the first week after application of BS. Thereafter, potential NH_3 volatilization was negligible. The silty Anthrosol offered a higher NH_4^+ exchange capacity due to higher clay contents whereas the pH of 7.5 in the sandy Cambisol additionally promoted NH_3 volatilization. Emissions of NH_3 in our study were in the range of NH_3 volatilization reported by other studies where slurry was incorporated (*Bussink* and *Oenema* 1998, *Thompson* and *Meisinger* 2002; *Huijsmans* et al. 2003). Losses of NH_3 were significantly positively correlated (Pearson) with NH_4^+ supplied by BS's ($r = 0.4$, $p < 0.01$) and negatively with the C_t content of BS's ($r = -0.3$, $p < 0.01$).

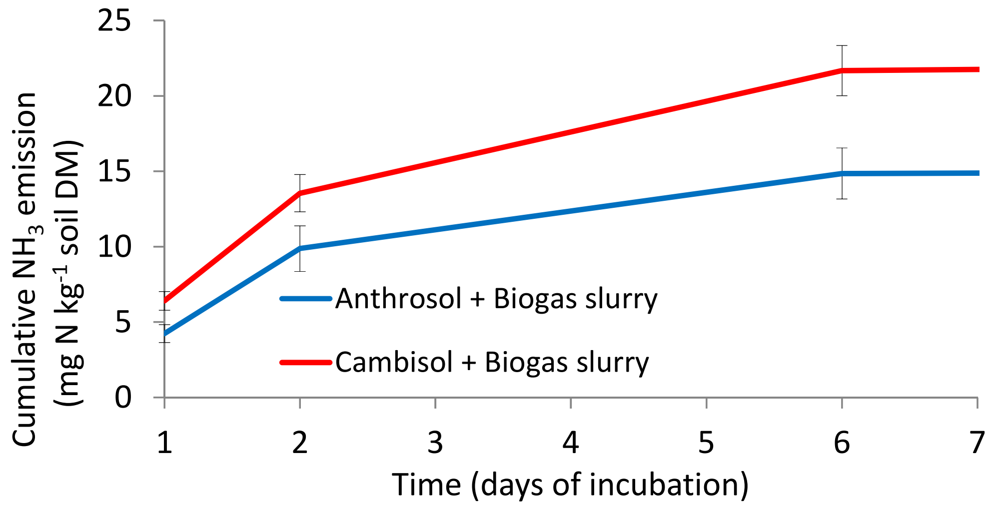

Figure 9: Average cumulative potential NH_3 emissions from samples amended with biogas slurries. Ammonia emissions are shown for the first week of incubation, since no marked changes occurred later on. Samples were incubated at 25°C. Values shown are means (n=32) and standard errors.

Trial 2: The effect of different N-rates on the C and N dynamics

Averaged over soils and BS's, the amount of emitted CO_2-C after 4 weeks of incubation was 368, 626 and 836 mg C (kg soil)$^{-1}$ for low, medium and high N rates, respectively.

The average increase by a factor of 1.3 and 1.7 between low and medium N rate and medium and high N rate applied to both soils. However, relating the CO_2 emissions to the C applied showed differences merely in the silty Anthrosol between low and medium N rate and low and high N rate (Table 7) possibly because the potential for C sequestration was not exhausted at low N rate (low amount of added C) and consequently increased until medium N rate (medium amount of added C). Another reason may be the difficulty to weigh out representative samples and mix them homogeneously with the soil at the lowest fertilizer level (0.1 g N (kg soil)$^{-1}$) due to the consistencies of the BS's, especially of the BS P_{62}.

The cumulative net nitrification in both soils at the end of incubation increased linearly with values of 57, 182 and 282 mg N (kg soil)$^{-1}$ at low, medium and high N rate, respectively (Figure 9). Increasing nitrification with increased availability of NH_4^+ is in line with existing findings (*Barnard* et al. 2005).

Likewise, the average cumulative N_2O emissions increased significantly with increasing BS N rate from 0.3 to 3.3 mg N (kg soil)$^{-1}$. However, the amount of added N with BS's emitted as N_2O stayed relatively constant in the silty Anthrosol (0.4% of added N) but showed an increase with N rates in the sandy Cambisol (0.04 – 1.5% of added N) (Table 7). The results from the sandy Cambisol are comparable with the results from *Senbayram* et al. (2009) and *Cardenas* et al. (2010), who also reported increasing N_2O emissions with increasing application rates. *Senbayram* et al. (2009) reported a linear increase in N_2O emissions from sandy soils with increasing application rates of biogas waste and mineral fertilizers, whereas *Cardenas* et al. (2010) found that the proportion of applied N being lost as N_2O from loams and clay loams increased exponentially with increasing inorganic N application rates to grazed grasslands. The proportion of N lost as N_2O increased especially when application rates exceeded 175 kg N ha^{-1}.

Table 7: Average cumulative CO_2 and N_2O emissions and N_{min} concentrations relative to control and applied C and N with biogas slurries. For trial 2 silty and sandy soils were amended with biogas slurries E_{83}, P_{62} and P_{66} at a rate of 0.1, 0.3 and 0.5 g N (kg soil)$^{-1}$, respectively, and adjusted to a water content corresponding to 60% of WHC. For trial 3 silty and sandy soils were amended with biogas slurries E_{83}, P_{62} and P_{66} at a rate of 0.3 g N (kg soil)$^{-1}$ and adjusted to a water content corresponding to 50, 75 and 100% of WHC, respectively. Values shown are means (n = 4) and standard errors.

	Biogas slurry	N Rate applied g N (kg soil)$^{-1}$	CO_2-C (% of added C)		N_2O-N (% of added N)		N_{min} (% of added N)		N_{min} (% of added NH_4^+-N)	
			silty soil	sandy soil	silty soil	sandy soil	silty soil	sandy soil	silty soil	sandy soil
Trial 2	E_{83}	0.1	12.6 (1.5)	27.1 (3.1)	0.6 (0.6)	0.1 (0.1)	35 (11)	46 (9)	76 (23)	99 (20)
	E_{83}	0.3	20.4 (0.9)	24.3 (1.9)	0.2 (0.1)	0.1 (0.0)	58 (1)	52 (4)	125 (2)	112 (9)
	E_{83}	0.5	16.2 (1.2)	19.5 (0.6)	0.6 (0.3)	0.3 (0.1)	56 (1)	54 (3)	122 (2)	116 (6)
	P_{62}	0.1	1.7 (3.4)	22.4 (2.0)	0.4 (0.3)	0.1 (0.0)	58 (2)	64 (2)	104 (3)	114 (4)
	P_{62}	0.3	10.7 (1.5)	20.7 (1.4)	0.3 (0.1)	0.8 (0.4)	63 (2)	51 (5)	113 (4)	91 (9)
	P_{62}	0.5	12.5 (1.4)	21.8 (1.2)	0.3 (0.1)	0.9 (0.4)	55 (5)	61 (1)	98 (9)	108 (1)
	P_{66}	0.1	8.4 (3.2)	26.1 (3.7)	0.4 (0.4)	0.04 (0.01)	58 (4)	73 (1)	86 (6)	108 (2)
	P_{66}	0.3	18.6 (2.3)	32.0 (3.3)	0.3 (0.1)	0.4 (0.1)	59 (5)	59 (5)	87 (8)	87 (8)
	P_{66}	0.5	21.2 (0.9)	31.6 (1.1)	0.4 (0.2)	1.5 (0.1)	61 (2)	59 (3)	90 (4)	86 (4)
	Biogas slurry	**water content (% of WHC)**								
Trial 3	E_{83}	50	15.7 (1.3)	11.7 (1.5)	0.0 (0.0)	0.4 (0.4)	51 (1)	43 (2)	110 (1)	94 (3)
	E_{83}	75	21.1 (1.0)	25.7 (1.4)	0.7 (0.2)	1.3 (0.1)	50 (2)	53 (2)	109 (5)	115 (4)
	E_{83}	100	16.1 (1.8)	12.7 (2.2)	3.0 (1.3)	1.1 (0.4)	15 (5)	2 (1)	32 (10)	5 (1)
	P_{62}	50	11.4 (1.6)	13.6 (2.1)	0.0 (0.0)	0.2 (0.2)	58 (2)	47 (1)	104 (3)	84 (1)
	P_{62}	75	17.1 (1.2)	23.7 (2.5)	0.1 (0.1)	2.6 (1.3)	60 (1)	53 (4)	107 (3)	94 (7)
	P_{62}	100	13.0 (1.5)	16.0 (1.8)	2.2 (1.1)	4.3 (1.6)	30 (1)	10 (2)	54 (3)	18 (3)
	P_{66}	50	16.4 (0.3)	16.2 (3.0)	0.1 (0.0)	0.7 (0.7)	60 (1)	55 (0)	88 (2)	81 (1)
	P_{66}	75	24.4 (1.4)	32.1 (2.5)	0.9 (0.2)	3.8 (2.5)	60 (3)	59 (5)	89 (4)	87 (7)
	P_{66}	100	20.7 (1.7)	22.7 (3.1)	1.5 (1.7)	4.4 (2.5)	24 (6)	10 (3)	36 (10)	15 (4)

[1)] Due to the consistency of the biogas slurry P_{62} (which contained higher amounts of solids) it was particularly difficult to weigh out homogeneous samples at the lowest fertilizer level (0.1 g N (kg soil)$^{-1}$) which may have contributed to the exceptional small CO_2-C emission

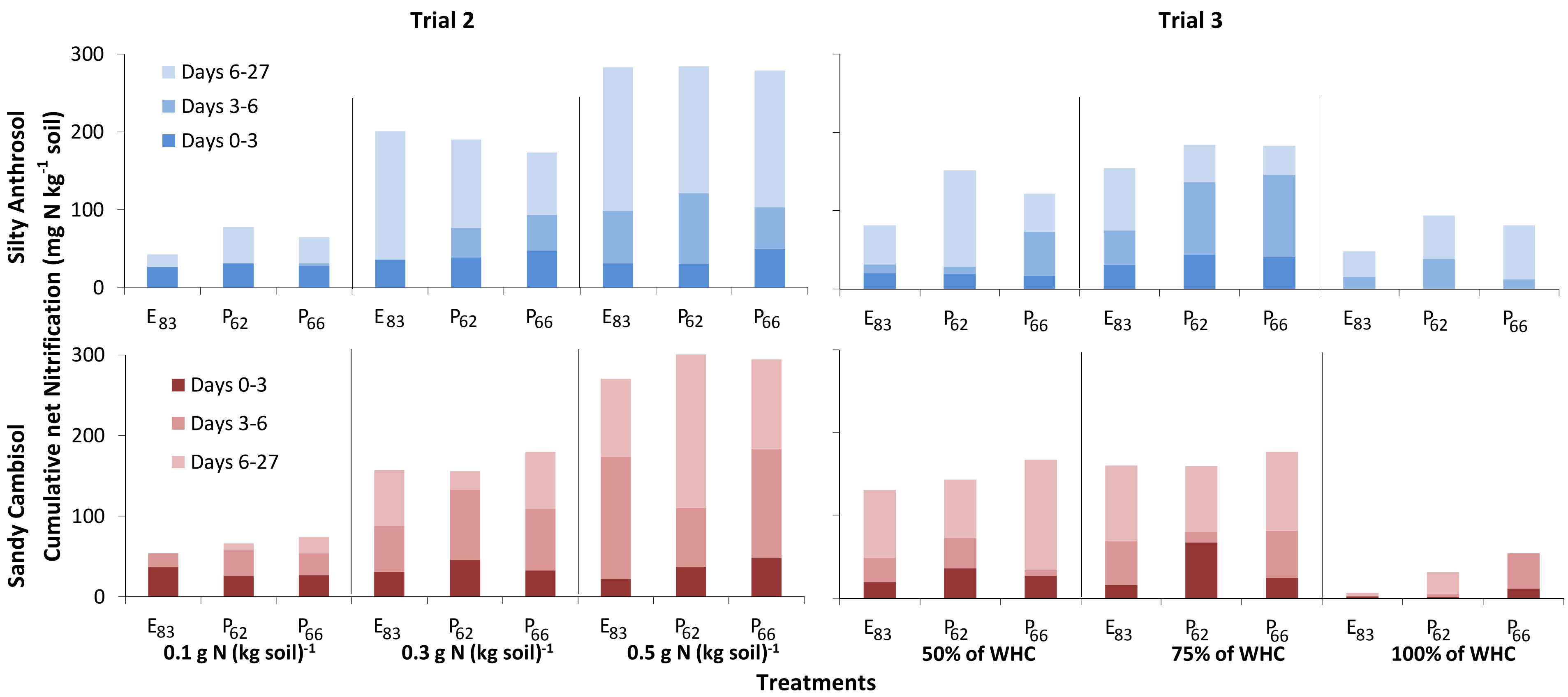

Figure 10: Cumulative net nitrification on day 3 (grey), 6 (light grey) and 27 (medium grey). Sandy soil and silty soil were amended with biogas slurries E_{83}, P_{62} and P_{66}. In trial 2 at a rate of 0.1, 0.3 and 0.5 g N (kg soil)$^{-1}$, respectively, and a water content corresponding to 60% of WHC. In trial 3 at a rate of 0.3 g N (kg soil)$^{-1}$ and water contents corresponding to 50%, 75% and 100% of WHC, respectively. Samples were incubated for 4 weeks at 25°C. Values shown are means (n = 4).

In contrast, *Velthof* et al. (2003) reported for sandy soils no differences in N_2O emission factors with increasing N rates of liquid pig manure, which is more in line with our results from the silty Anthrosol. These results indicate that the effects of N application rates on N_2O emissions depend more on other factors (water content, available C, N_{min}) than on the soil texture.

Trial 3: The effect of water contents on the C and N dynamics

Cumulative emissions of CO_2 from unamended control soils after 4 weeks of incubation decreased in the order 254 (at 75% of WHC) > 214 (100% of WHC) > 159 (50% of WHC) mg C (kg soil)$^{-1}$. The same order was observed for the cumulative emissions of fertilized soils with average levels of 650 (75% of WHC) > 490 (100% of WHC) > 393 (50% of WHC) mg C (kg soil)$^{-1}$. Overall, 24 (75% of WHC), 17 (100% WHC) and 14% (50% of WHC) of applied C was respired and the differences may be explained by an inhibition of oxygen supply at 100% of WHC and by a limited substrate supply at a water content of 50% of WHC and therefore restricted microbial activity (*Franzluebbers* 1999).

The highest cumulative net nitrification was measured at 75% and 50% of WHC, with the differences being more pronounced in the silty Anthrosol, whereas the lowest cumulative net nitrification was found at 100% of WHC especially in the sandy Cambisol (Figure 10) caused by limited oxygen supply.

Cumulative emissions of N_2O after a 4-week incubation increased with increasing water contents from 0.1 to 3 to 12 mg N (kg soil)$^{-1}$ in the slurry-amended soils. Control soils emitted 0.02, 0.7 and 2 mg N (kg soil)$^{-1}$. At 50 and 75% of WHC, the N_2O emissions from the silty Anthrosol were very small (Table 7). Averaged over both soils 0.2%, 1.6% and 2.8% of N supplied was emitted in the form of N_2O at 50%, 75% and 100% of WHC, respectively. *Senbayram* et al. (2009) reported 5-fold higher N_2O emissions at 85% than at 65% of WHC. Comparatively, in our study the factor between

75% and 100% of WHC was only 4, but it can be assumed, that an increasing proportion of N lost through denitrification was emitted as molecular nitrogen at the high water content in our study.

6.5 Conclusions

Our results indicated that the types of substrate input to biogas plants influence the properties of the by-product BS and also the C and N dynamics after application of the slurries. The losses of NH_3 were significantly positively correlated with NH_4^+ supplied by BS's (r = 0.4) and negatively with the C_t content of BS's (r = -0.3). Moreover, soil properties affected emissions markedly. Both N_2O and NH_3 emissions were increased in the sandy Cambisol, which had a lower clay content and a higher pH than the silty Anthrosol.

Increasing rates of BS N resulted in an increase of N_2O emissions from 0.04 to 1.5% of added N in the sandy Cambisol, whereas no effect was found for the silty Anthrosol. Nitrous oxide emissions were also affected by soil moisture, increasing strongly with increasing moisture content from 50 to 100% WHC. In contrast, CO_2 emissions were highest at the intermediate moisture content of 75% WHC.

6.6 Acknowledgments

We would like to thank the operators of the sampled biogas plants for providing the BS's for this study, A. Sawallisch for the technical assistance and two anonymous reviewers for their valuable comments on the manuscript. This project was financed by the Deutsche Forschungsgemeinschaft (DFG-Research Training Group 1397 "Regulation of soil organic matter and nutrient turnover in organic agriculture").

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7 General conclusion

The results of the present work highlighted that BS's exhibit a high N fertilizer value due to the high proportion of plant available N after the fermentation process, independent of the feed material even if BS is exclusively obtained from plant material. Compared to aerobically treated cattle manure the emissions of CO_2 were on the same level indicating similarly amounts of readily available C after composting and fermentation of organic material. However, the application of BS induced considerably higher N_2O emissions caused by a higher content of NH_4^+ but these emissions were in the range of unfermented animal slurries. Consequently, the legislation which is valid for the application of manure or slurry of animal excrements should also be valid for each kind of fermented substrate. Nitrogen was predominantly lost with percolating water in the form of NO_3^-, also to a greater extent in BS amended soils compared to CM.

The rainfall patterns in these studies merely affected the temporal production of CO_2 and N_2O emissions but showed no effect on the cumulative emissions after 6 and 20 weeks of incubation (chapter 4 and 5). Temporary effects within periods of stronger irrigation were reduced CO_2 and enhanced N_2O emissions due to the constantly high moisture contents in the undisturbed soil cores. A significant increase of CH_4 consumption under periodic strong irrigation events was also observed (chapter 5). The time of fertilization either directly before a strong irrigation event or one week after stopping of irrigation showed no influence on the overall C and N dynamics (chapter 5) which can also be associated with the high moisture contents during the incubation experiments.

An increase of the temperature by 10°C enhanced the CO_2 and N_2O emissions by a factor of 1.7 and 3.7, respectively. Leaching of NO_3^- was also significantly affected by the temperature rise whereas the consumption of CH_4 was not influenced. Consequently, the time and type of application should be well chosen to match plant needs and to avoid excessive N losses by leaching, gaseous emissions or volatilization.

The feeding materials of biogas plants affected the properties of the resulting BS's. In particular the contents of DM and NH_4^+ were influenced by the amount of added plant biomass and excrement based biomass, respectively. Correlations between BS properties and CO_2 or N_2O emissions were not detected. Solely the NH_3 emissions showed a positive correlation with NH_4^+ content in BS's as well as a negative correlation with the C_t content (chapter 6). The BS-N rates affected the relative CO_2 emissions (% of C supplied with BS) when applied to the silty Anthrosol as well as the relative N_2O emissions (% of N supplied with BS) when applied to the sandy Cambisol. The impacts on the C and N dynamics induced by BS application were exceeded by the differences induced by soil texture. Presumably, due to the higher clay content in silty soils, organic matter was stabilized by organo-mineral interactions and NH_4^+ was adsorbed at the cation exchange sites. Different water contents induced highest CO_2 emissions and therefore optimal conditions for microbial activity at 75% of WHC in both soils. Cumulative nitrification was also highest at 75% and 50% of WHC whereas the relative N_2O emissions increased with the water content and showed higher N_2O losses in sandy soils.

The trial for validating the extent of captured volatilized NH_3 with an alkalized NH_4^+ solution showed that after 24 hours 95% of the volatized NH_3 was captured in the H_2SO_4 traps.

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