Fachgebiet Bodenbiologie und Pflanzenernährung
Fachbereich Ökologische Agrarwissenschaften

Universität Kassel

Der Einfluss langjähriger Applikation von Biogasgüllen auf die Bodenfruchtbarkeit

Dissertation

zur Erlangung des akademischen Grades eines Doktors der Agrarwissenschaften
(Dr. agr.)

im Fachbereich Ökologischen Agrarwissenschaften der Universität Kassel

vorgelegt von
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Witzenhausen, Oktober 2014

Die vorliegende Arbeit wurde vom Fachbereich Ökologische Agrarwissenschaften der Universität Kassel als Dissertation zur Erlangung des akademischen Grades eines Doktors der Naturwissenschaften (Dr. agr.) angenommen.

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Tag der mündlichen Prüfung: 12.12.2014

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(Stefanie Wentzel)

Vorwort

Die vorliegende Dissertation wurde an der Universität Kassel im Fachbereich Ökologische Agrarwissenschaften im Fachgebiet Bodenbiologie und Pflanzenernährung angefertigt, um die Anforderungen des akademischen Grades des Doktors der Agrarwissenschaften (Dr. agr.) zu erfüllen. Die Arbeit wurde von der Universität Kassel gefördert, ist mit dem DFG-Graduiertenkolleg 1397 assoziiert und beinhaltet drei wissenschaftlichen Publikationen wobei die erste schon eingereicht wurde und zwei weitere in Kürze folgen. Die Artikel sind in die Kapitel 3, 4 und 5 eingearbeitet. Kapitel 1 liefert eine generelle Einleitung zum Thema, während in Kapitel 2 die Ziele dieser Arbeit herausgestellt werden. In den Kapiteln 6 und 7 sind die Ergebnisse der Kapitel 3, 4 und 5 auf deutsch und englisch zusammengefasst, während Kapitel 8 einen Ausblick auf weitere Untersuchungen gibt.

Folgende Publikationen sind Bestandteil der vorliegenden Arbeit:

Kapitel 3

Wentzel, S., Schmidt, R., Piepho, H.P., Semmler-Busch, U., Joergensen, R.G., Response of soil fertility indices to long-term application of biogas and raw slurry under organic farming. (to be submitted to Agriculture, Ecosystems and Environment).

Kapitel 4

Wentzel, S., Joergensen, R.G., Effects of biogas and raw slurries on grass growth and soil microbial indices. (to be submitted to Journal of Plant Nutrition and Soil Science).

Kapitel 5

Wentzel, S., Joergensen, R.G., Quantitative microbial indices in biogas and raw slurries. (to be submitted to Biorecource Technology).

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AEC	Adenylate Energy Charge
ANOVA	Varianzanalyse
$BaCl_2$	Bariumdichlorid
C	Kohlenstoff
Ca	Kalzium
$CaCl_2$	Kalziumchlorid
CH_4	Methan
$CHCl_3$	Chloroform
cm	Zentimeter
C_{mik}	Mikrobiell gebundener Kohlenstoff
CO_2	Kohlenstoffdioxid
CV	Variationskoeffizient (engl. coefficient of variance)
d	Tag (engl. day)
DFG	Deutsche Forschungsgemeinschaft
DNA	Desoxyribonukleinsäure (engl. deoxyribonucleic acid**)**
DW	Trockengewicht (engl. dry weight)
E	Ost (engl. east)
e.g.	Zum Beispiel (lat. exempli gratia)
FAO-WRB	Food and Agriculture Organisation of the United Nations – World Reference Base for Soil Resources
g	Gramm
g	Beschleunigung
GalN	Galaktosamin
Glc	Glucosamin
h	Stunde (engl. hour)
H_2O	Wasser
HCl	Salzsäure
HNO_3	Salpetersäure
HPLC	Hochleistungsflüssigkeitschromatographie (engl. high performance liquid chromatography)
ICP-AES	Induktiv gekoppeltes Hochfrequenzplasma-Atomemissionspektrometer
i.e.	das heißt (lat. id est)
K	Kalium

K_2SO_4	Kaliumsulfat
k_{EC}, k_{EN}	Extrahierbarer Teil des Gesamtkohlenstoffes und -stickstoffes gebunden in der mikrobiellen Biomasse
KOH	Kaliumhydroxid
Km	Kilometer
l	Liter
m	Meter
M	Molar (mol/L)
Mg	Magnesium
Mn	Mangan
m^2	Quadratmeter
mg	Milligramm
min	Minute
ml	Milliliter
mm	Millimeter
mM	Millimolar
mmol	Millimol
MurN	Muraminsäure
N	Nord
N_2	Stickstoff
Na	Natrium
NaCl	Natriumchlorid
NaOH	Natriumhydroxid (Natronlauge)
n.d.	nicht nachweisbar (engl. not detectable)
nm	Nanometer
N_{mic}	Mikrobiell gebundener Stickstoff
O_2	Sauerstoff
OPA	ortho-Phthaldialdehyd
P	Wahrscheinlichkeit
pH	negativer dekadischer Logarithmus der Wasserstoffionenaktivität (lat. potentia Hydrogenii)
r	Korrelationskoeffizient
rev	Umdrehungen
SOC	Organischer Kohlenstoff des Bodens (engl. soil organic carbon)
SOM	Organisches Material des Bodens (engl. soil organic matter)

z. B.	Zum Beispiel
%	Prozent
µm	Mikrometer

1. Einleitung

Biogas ist eine der bedeutendsten Komponenten der heutigen Energieproduktion, die aus erneuerbaren Rohstoffen gewonnen werden kann (Møller, 2009). Biogasgülle, das zweite Produkt der anaeroben Vergärung, wird daher immer häufiger auch in der ökologischen Landwirtschaft als organischer Dünger eingesetzt (Möller, 2009; Terhoeven-Urselmans et al., 2009). Im Vergleich zu Rohgüllen und Festmist, welche hauptsächlich aus Kot, Urin, Stroh und Wasser bestehen, können in Biogasanlagen unterschiedlichste Substrate zur Produktion von Biogasgüllen verwendet werden. In den 1980ern wurde die Biogasentwicklung in Deutschland maßgeblich durch biologisch-dynamische Landwirte aus dem Nord-Osten Baden-Württembergs vorangetrieben. Deren Motivation war es hauptsächlich, Unabhängigkeit von der sich etablierenden Atomkraft zu erlangen. Weitere Gründe für den Bau von Biogasanlagen auf ökologisch bewirtschafteten Höfen waren Wärmeproduktion, verminderte Geruchsbelästigung und eine hohe Düngerqualität der Biogasgüllen (Friedel et al., 1996; Bachmann et al., 2011; Möller und Müller, 2012). Mit der Einführung des EEG (Erneuerbare Energien Gesetz) im Jahre 2000 stieg die Nachfrage von Landwirten und Beratern des ökologischen Landbaus zu den Auswirkungen der Biogasgüllen auf die Bodenfruchtbarkeit. Im Bereich der biologisch-dynamischen Landwirtschaft gab zudem Bedenken bezüglich des Lebensäthers, welcher im Wesentlichen durch die Rindermistdüngung gefördert wird (Scheller, 2006). Biogasgüllen haben bezüglich ihrer Wirkung auf Boden und Pflanze, Vor- und Nachteile (Arthurson, 2009; Möller, 2009). Die positiven Effekte auf den Pflanzenertrag und auf die chemischen, physikalischen und biologischen Eigenschaften von Acker- und Grünlandböden wurden im Rahmen von Inkubationsexperimenten (Friedel et al., 1996; Odlare et al., 2008; Sänger et al., 2011), Gefäßversuchen (Andruschkewitsch et al., 2013) und Feldexperimenten (Terhoeven-Urselmans et al., 2009; Bachmann et al., 2011; Johansen et al., 2013) wiederholt untersucht. Die anaerobe Fermentation von organischem Material führt zu einer Erhöhung der NH_4-N-Konzentration und zur Reduzierung der Trockenmasse, was wiederum zu niedrigeren C-Konzentrationen, einem engeren C/N-Verhältnis und einem höheren pH-Wert führt (Asmus et al., 1988; El-Shinnawi et al., 1989; Kirchmann and Witter, 1992; Möller and Müller, 2012). Aufgrund der erhöhten anorganischen N-Konzentration liefern Biogasgüllen mehr pflanzenverfügbaren Stickstoff als andere organische Düngemittel wie Klärschlamm, Mist (Odlare et al., 2008) oder unfermentierte Gülle (Bachmann et al., 2011). Zusätzlich enthalten Biogasgüllen höhere Konzentrationen an löslichem P und stellen somit einen guten

Phosphordünger dar (Bachmann et al., 2011). Auch in viehlosen Betrieben bieten Biogasgüllen eine Möglichkeit, um z.B. aus Gras/Kleegras organische Dünger zu produzieren (Stinner et al., 2008). Dennoch nahmen die Bedenken über möglichen Nachteile der Biogasgüllen in den letzten Jahren zu (Scheller, 2006; Möller, 2009; Terhoeven-Urselmans et al., 2009). Der niedrigere C-Input und die hohe Rekalzitranz des organischen Materials im Vergleich zu Rohgülle könnte neben dem Einfluss auf die mikrobiellen Eigenschaften des Bodens auch Einflüsse auf die Regenwurmbiomasse und langfristig auch auf den organischen C-Speicher im Boden haben (Ernst et al., 2007; Friedel et al., 1998). Auf der anderen Seite wird die höhere Rekalzitranz der organischen Substanz in Biogasgüllen als eher förderlich hinsichtlich der C-Speicherung im Boden gesehen (Asmus et al., 1988; Gutser et al., 2005).

Grundsätzlich ist die Ausbringung organischer Dünger und deren Auswirkung auf die Bodenfruchtbarkeit und das Pflanzenwachstum als positiv zu bewerten (Odlare et al., 2008; Simek et al., 1999). Hinsichtlich der Auswirkung auf die Pflanzenproduktion gibt es jedoch unterschiedliche Aussagen über die Wirkung von Biogasgüllen im Vergleich zu anderen organischen Düngemitteln oder mineralischer Dünger. Über die positiven Zusammenhänge zwischen der Düngung mit Biogasgülle und dem Pflanzenwachstum, dem daraus resultierenden höheren C-Input durch Wurzelexsudate und Rhizodeposition und die darauf folgende Stimulation von Pilzen und Bakterien in der Rhizosphäre (Knorr et al. 2005; Liu and Greaver 2010; Walsh et al., 2012) wurde schon geforscht. Es wird generell angenommen, dass die Fraktionen des pflanzenverfügbaren N in einem engen Zusammenhang mit den NH_4-N-Gehalten aus den organischen Düngern steht (Fouda, 2013; van Kessel et al., 2000; Gutser et al., 2005). Grundsätzlich ist die Art der Applikation (oberirdisch oder einarbeitend) und eine auf Gesamt-N oder Ammonium-N basierende Düngung mit organischen Substraten ausschlaggebend für den Pflanzenertrag und die Stickstoffaufnahme der Pflanze (Möller et al., 2009; Loria et al., 2007; de Boer, 2008; Möller et al., 2009; Loria et al., 2007; Möller und Müller, 2012). Hinsichtlich der Stickstoffaufnahme aus Biogasgüllen gibt es demnach unterschiedliche Aussagen. Möller et al. (2009) fanden diesbezüglich keine Unterschiede zu unfermentierten Güllen, wohingegen andere Experimente zeigten, dass die Biogasgülleapplikation zu einer höheren Stickstoffaufnahme durch die Pflanzen führten (Gutser et al., 1987; Asmus et al., 1988). In Versuchen mit Amaranth und Mais zeigte sich, dass Biogasgüllen einen positiven Effekt auf den Pflanzenertrag im Vergleich zu unfermentierten Güllen hatten, jedoch der mineralischen Düngung unterlegen waren (Bachmann et al., 2011). Andruschkewitsch et al. (2013) kamen ebenfalls zu dem Ergebnis, dass Gärreste den Pflanzenertrag positiv beeinflussen, wobei in diesem Fall die N-Wirkung

der mineralischen N-Düngung gleichzusetzen war. Des Weiteren fand man heraus, dass neben positiven Effekten auf die oberirdische Biomasse im Grünland keine Erhöhung der Wurzelbiomasse zu finden war (Andruschkewitsch et al., 2013; Kandeler et al., 1994; Salminen et al., 2001). Begründet wird dies durch die negativen Effekte der in Güllen enthaltenen organischer Säuren, die einen negativen Wirkung auf das Wurzelwachstum haben können (Salminen et al., 2001). Gegensätzliches wurde in anderer Experimenten gefunden, wo es zu einer positiven Beeinflussung der Biogasgüllen auf das Wurzelwachstum im Vergleich zu mineralischen und ungedüngten Varianten kam (Garg et al., 2005; Gunnarsson et al., 2010).

Die anaerobe Fermentation führt zu geringeren, für Mikroorganismen verfügbaren C-Mengen, was hauptsächlich durch die Umwandlung in Methan und Kohlendioxid während des Abbauprozesses zu begründen ist. Zusätzlich ist in Biogasgüllen der Anteil an Lignin im Vergleich zu Rohgüllen höher (El-Shinnawi et al., 1989). Das resultierend engere C/N-Verhältnis in Biogasgüllen führt im Boden zu einer geringeren N-Immobilisierung durch Mikroorganismen zur Zeit der Ausbringung (Messner and Amberger 1987). Dennoch ist der positive Effekt von Biogasgüllen auf Mikroorganismen im Vergleich zu ungedüngten Böden mehrfach aufgezeigt worden (Odlare et al., 2008). Grundsätzlich sind jedoch die langfristigen Auswirkungen von Biogasgüllen auf die mikrobiellen Eigenschaften des Bodens nicht ausreichend erforscht und generelle Schlussfolgerung über die Effekte auf die Bodenfruchtbarkeit nicht eindeutig, da die Bewertung von Faktoren wie der C-Menge und der C-Qualität abhängt (Ernst et al., 2012).

Organische Substrate wie z.B. Biogasgüllen enthalten neben Nährstoffen auch mikrobielle Biomasse, welche in den Boden eingetragen wird und die chemischen und biologischen Eigenschaften des Bodens ebenfalls beeinflussen kann. Derzeit gibt es jedoch nur wenige Forschungsansätze, die sich mit diesen aus Biogas- und Rohgüllen kommenden Organismen und deren Wirkung auf den Boden beschäftigen (Walsh et al., 2012). Quantitative Aussagen über die Biomasse der in Güllen vorkommenden Mikroorganismengemeinschaften sind bis heute nicht vorhanden. Zum Thema Mikroorganismen in organischen Düngern haben sich vor allem die Veterinärmedizin und Forschungsbereiche zur Risikoabschätzung auf die Umwelt hinreichend beschäftigt. In der Veterinärmedizin geht es dabei hauptsächlich um Pathogene aus tierischen Exkrementen und anderen organischen Substanzen, die als Dünger verwendet werden können (Govasmark et al., 2011; Kress and Gifford, 1984; Kudva et al., 1998; Kearney et al., 1993; Munch et al., 1987). Für den Nährstoffumsatz und die Düngerqualität sind Pathogene jedoch verglichen zur

Gesamtbiomasse der Mikroorganismen, welche die Mineralisationsprozesse während der Güllelagerung und der Anwendung im Boden beeinflussen, weniger von Interesse. Zählverfahren für Bakterienkonzentrationen und Keimzahlansätze vernachlässigen nicht kultivierbare Mikroorganismen, welche mehr als 80% an der Gesamtzahl der Arten ausmachen (Ouwerkerk and Klieve, 2001). Direkte Mikroskopieansätze unterschätzen Pilze (Joergensen and Wichern, 2008). Die DNA-Extraktion und die darauf folgende Analyse der Zusammensetzung liefert wertvolle Informationen über die Zusammensetzung der mikrobielle Gemeinschaft des Kots (van Vliet et al., 2007; Sekhavati et al., 2009). Dennoch liefert die DNA-Datenanalyse aufgrund von Verlusten während der Extraktion und unbekannter oder stark variierender Konzentrationen innerhalb der mikrobiellen Arten (Leckie et al., 2004; Joergensen and Emmerling, 2006) oder aufgrund des Vorkommens in toten Mikroorganismen (Pisz et al., 2007; Bae and Wuertz, 2009) keine Informationen über die Biomasse. ATP wurde schon in Kotproben bestimmt (Wolstrup and Jensen, 2008), jedoch ist AEC und somit die ATP Konzentration innerhalb der mikrobiellen Biomasse in der anaeroben oder mikroaeroben Umwelt von Güllen im Vergleich zum Boden unbeständiger (Jenkinson, 1988; Dyckmans et al., 2006). Im Boden sind Pilze die wichtigsten Zersetzer von komplexem organischem Material (Joergensen and Wichern, 2008). In Güllen werden Pilze jedoch aufgrund ihrer Sensibilität gegenüber einer anaerober Umwelt eher vernachlässigt (Amon, et al., 2006; Procházka et al., 2012), obwohl zum Beispiel in Rinderkot signifikante Mengen nachgewiesen werden konnten (Jost et al., 2011, 2013 a, b). In diesen Experimenten wurde die pilzliche Biomasse anhand der Ergosterol- und Glukosaminanalyse bestimmt. Ergosterol ist ein wichtiger Bestandteil der pilzlichen Zellmembran, welcher in Basidomyzeten, Askomyzeten und in der Mehrheit der Zygomyzeten vorkommt (Weete and Weber, 1980). Ergosterol konnte wiederholt in verschiedensten festen Substraten wie Böden (Joergensen and Wichern, 2008; Strickland and Rousk, 2010) und Rinderkot (Jost et al., 2011, 2013 a, b), jedoch noch nicht in Biogas- und Rohgüllen bestimmt werden. Ähnliches gilt auch für Aminozucker, obwohl Glukosamin (GlcN) früher in der Rumenflüssigkeit von Kühen zur Bestimmung von Pilzen genutzt wurde (Sekhavati et al., 2009). GlcN ist ein Bestandteil der pilzlichen, bakteriellen und archaealen Zellwand, Muramin (MurN) hingegen kommt nur in bakteriellen Zellwänden vor (Appuhn and Joergensen, 2006). Die meisten Aminozucker im Boden sind an die organische Masse als mikrobielle Residuen gebunden (Amelung, 2001; Amelung et al., 2008), wohingegen der größte Teil der Aminozucker in frischem Kot in der pilzlichen und bakteriellen Biomasse zu finden ist (Jost et al., 2011, 2013 a, b).

2. Ziele der Arbeit

Die langjährige Applikation von Biogasgüllen und deren Wirkung auf Parameter der Bodenfruchtbarkeit kann derzeit nur durch Feld-, Gefäß-, und Inkubationsexperimente abgeschätzt werden. Vor allem in der Praxis besteht jedoch ein enormer Bedarf an der Aufklärung über die Wirkung des langjährigen Einsatzes von Biogasgüllen auf den Boden und den Ertrag. Im ersten Versuch, welcher in Kapitel 3 beschrieben ist, wurden von Flächen des biologisch-dynamischen Landbaus, die Hälfte davon wird seit über 20 Jahren mit Biogasgülle gedüngt, Bodenproben entnommen, um folgende Forschungsfragen zu klären: 1) Hat der langjährige Einsatz von Biogasgüllen Effekte auf die organische Substanz und mikrobielle Parameter im Boden welche z.B. durch die Eigenschaften der Biogasgüllen (reduzierte C-Gehalte) hervorgerufen werden? 2) Werden mögliche negative Effekte auf die Bodenfruchtbarkeit durch die positiven Effekte, wie zum Beispiel die erhöhte Nährstoffverfügbarkeit für Pflanzen kompensiert?

Um genauer auf die Wirkung von Biogasgüllen eingehen zu können, wurde nach dem On-Farm-Projekt ein Gefäßversuch mit Biogas- und Rohgüllen der Praxisbetriebe aus dem ersten Projekt durchgeführt. Zurzeit sind kaum Ergebnisse zu Untersuchungen von Biogas- und Rohgüllen unterschiedlichster Zusammensetzung und deren Wirkungen auf die Pflanze, den Boden, die Mikroorganismen und den Wechselwirkungen zwischen diesen Systemen bekannt. In diesem zweiten Versuch, welcher in Kapitel 4 beschrieben ist, wurde daher untersucht, wie sich unterschiedliche Biogas- und Rohgüllen auf die pflanzliche Biomasse und auf die Mikroorganismen im Boden und an den Wurzeln auswirken.

Da es in früheren Forschungsansätzen hauptsächlich um die chemischen Eigenschaften in organischen Düngern ging, wurde in einem dritten Projekt auf die mikrobiellen Eigenschaften der Güllen, welche ebenfalls von den Praxisbetrieben kamen, eingegangen. Bis heute gibt es keine quantitativen Ansätze zur Bestimmung der Mikroorganismen in Biogas- und Rohgüllen womit in diesem dritten Versuch, welcher in Kapitel 5 beschrieben ist, die unterschiedlichen Güllen dahingehend untersucht wurden. Ziel war es hierbei, die mikrobielle Gemeinschaft zu bewerten und einen Eindruck über die mikrobiellen Frachten, die durch die Güllen in den Boden gelangen, zu bekommen.

3. Response of soil fertility indices to long-term application of biogas and raw slurry under organic farming

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Abstract

The long-term effects of biogas slurry application on soil fertility indices were compared with raw slurry in biodynamic organic farming systems. An on-farm soil and slurry sampling was carried out to quantify the effects on stocks of soil organic matter, microbial biomass and microbial residues. Five fields with biogas slurry and five neighbouring fields with raw slurry amendments were selected at 5 different sites in the north-east of Baden-Württemberg. The long-term application of biogas slurry did not affect SOC, total N stocks or the soil C/N ratio. Biogas slurry application decreased the soil microbial biomass to SOC ratio, which indicates a reduced availability of the biogas slurry C input to soil microorganisms compared with raw slurry. At some sites, differences in clay content masked any slurry effects on the microbial activity, biomass, and residue indices. There were no general effects of biogas slurry on the ratios of ergosterol to microbial biomass C or amino sugar-based fungal C to bacterial C, whereas an increasing clay content caused a significant shift towards bacteria according to the latter ratio. The consistency in the data of all approaches strongly indicates the validity of the current on-farm study by comparing neighbouring fields.

Keywords: On-farm research; Biogas slurry; Raw slurry; Microbial biomass; Ergosterol; Amino sugars; Clay.

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3.1. Introduction

Biogas is an important component for energy production from renewable resources (Møller, 2009) and for this reason biogas slurry, the secondary product of the anaerobic digestion process, is increasingly used as fertilizer also in organic farming systems (Möller, 2009; Terhoeven-Urselmans et al., 2009). Biogas plants use a wide variety of substrates, whereas raw farmyard slurries are derived from faeces and urine, some water and rarely straw. In the early 1980s, biogas production was introduced to Germany by biodynamic organic farmers in north-east Baden-Württemberg. The basic motivation was to gain independence from nuclear power electricity, which had been strongly expanded in that period. However, heat production, odour reduction, and a higher fertilizer quality were further reasons for using biogas slurry (Friedel et al., 1996; Bachmann et al., 2011; Möller and Müller, 2012).

Biogas slurries have advantages and disadvantages in respect to their effects on soil (Arthurson, 2009; Möller, 2009). The positive effects of slurries on plant yield, soil chemical, physical and particularly soil microbial biomass characteristics have been repeatedly evaluated in highly artificial incubation (Friedel et al., 1996; Odlare et al., 2008; Sänger et al., 2011), greenhouse pot (Andruschkewitsch et al., 2013), and short-term field experiments (Terhoeven-Urselmans et al., 2009; Bachmann et al., 2011; Johansen et al., 2013). Anaerobic digestion increases the concentration of NH_4-N and reduces dry matter, leading to lower C concentrations and C/N ratio as well as to increased slurry pH values (Asmus et al., 1988; El-Shinnawi et al., 1989; Kirchmann and Witter, 1992; Möller and Müller, 2012). Due to the larger inorganic N concentrations, biogas slurries supply more plant-available N than other organic fertilizers, e.g. sewage sludge or farmyard manure (Odlare et al., 2008) or undigested slurry (Bachmann et al., 2011). Biogas slurry also contains large concentrations of soluble inorganic P and thus may represent a valuable P fertilizer (Bachmann et al., 2011).

Furthermore, in stockless farming systems, biogas slurry provides a good option for producing an organic fertilizer from grass / clover sites, which can be widely used as fertilizer on other parts of the farm (Stinner et al., 2008). However, concerns have also been raised about the use of biogas slurry (Scheller, 2006; Möller, 2009; Terhoeven-Urselmans et al., 2009). The lower C input by the biogas slurries and the higher recalcitrance of their organic matter in comparison with raw slurries may not only reduce microbial activity and biomass, but also earthworm biomass (Ernst et al., 2007) and in the long-term also soil organic C (SOC) sequestration (Friedel et al., 1998). At the same time, the higher recalcitrance of the

organic matter remaining in biogas slurries has also been considered as beneficial for SOC sequestration (Asmus et al., 1988; Gutser et al., 2005).

These conflicting results point to the need for long-term field experiments to evaluate the effects of biogas slurry application (Möller and Müller, 2012). In north-east Baden-Württemberg, there is a unique opportunity to compare biodynamic organic farmers who have been applying biogas slurry for up to 25 years with their biodynamic neighbours who have been applying raw slurry. All farms have been under organic farming management for at least 40 years and use similar crop rotations according to best organic management practice. This on-farm approach by comparing neighbouring fields with similar soil texture and soil pH has been successfully used in several investigations on the long-term effects of different land-use systems (Ahl et al., 1998; Probst et al., 2008; Bowles et al., 2014).

Microbial biomass and the fungal cell-membrane component ergosterol are sensitive indicators for the effects of organic fertilizer application to soil and thus for soil fertility (Heinze et al. 2010). As highly specific microbial cell-wall components, amino sugars are recalcitrant and, consequently, serve as slow responding indices for the contribution of microbial residues to the sequestration of SOC (Amelung 2001; Liang et al., 2011). Fungi are the main source of glucosamine (Joergensen and Wichern 2008), whereas bacteria are the exclusive source of muramic acid (Millar and Casida, 1970; Appuhn and Joergensen, 2006), making it possible to assess the specific contribution of these two main microbial groups to SOC (Joergensen et al., 2010). The specific objectives of the current study were to measure the soil fertility indices microbial activity (basal respiration), microbial biomass C and N, fungal ergosterol, microbial residues (amino sugars), and soil organic C, total N, soil pH and clay content at five neighbouring sites under biodynamic farming management using either biogas or raw slurry. The underlying hypothesis was that the long-term application of biogas slurries has no effects on soil organic matter, microbial residues or biomass indices, because their negative effects, i.e. reduced C input, are compensated by their positive effects, i.e. increased nutrient availability to plants.

3.2. Material and Methods

3.2.1. Experimental sites and soil sampling

The study area is located in the north-east of Baden-Württemberg (Germany). Here, six biodynamic farms were chosen, three with biogas plants and three using raw cattle slurry.

The first criterion for choosing a site was that the farms had the same agricultural land-use management system. The second criterion was that the climatic and geological conditions are nearly the same due to the vicinity of the farms within each site. The third and main criterion was that one farm had a biogas plant and had been using biogas slurry as fertilizer for a prolonged period of up to 25 years. Two experimental sites are located in Kirchberg (KB-I and KB-II), two in Aspach (AS-I and AS-II), and one in Künzelsau (KA) within a radius of about 86 km. Each site consisted of one field treated with biogas slurry and one field treated with raw slurry application. KB I and II as well as AS I and II had 2 fields per farm and KA only one. The mainly clayey loam soils at these sites were classified as Haplic Cambisols (36% of the study area), Stagnic Cambisols (27%), Argic Cambisols (9%), Stagnic Luvisols (18%), and Haplic Luvisols (9%) according to the FAO-WRB (2006) classification system, forming a complex mosaic in some fields. Soil samples were taken about 4 weeks after fertilization and ploughing in November 2010 (KB and KA) and April 2011 (AS) at 0-5, 5-10, 10-20 and 20-30 cm depth from 9 sampling points, resulting in 36 samples per field and 360 samples in total. The field-moist soils were sieved (< 2 mm) and stored in polyethylene bags at 4 °C. A sub-sample of each soil sample was dried and finely ground for chemical analyses. Another sub-sample was frozen at -18 °C for further analysis.

At all sites, organic farming has been practised for at least 40 years. On the farms with biogas plants, the application of biogas slurry as fertilizer has been performed for about 25 years (Table 1). Depending on crop rotation and availability, the farmers added some farmyard manure to each site, despite slurry fertilization. In each field, ploughing was carried out to a maximum of 20 cm depth, except at site KA, where reduced tillage was performed to a maximum of 15 cm depth. In general, the crop rotations on all farms consisted of legumes, grain, and root crops (Table 2).

Table 1

Fertilizer, crop rotation and amounts of fertilization at the experimental sites.

Site	Slurry (year of start)	Crop rotation	Slurry application (m^3 ha^{-1} a^{-1})	Additional Fertilization (t ha^{-1} a^{-1})
KB-I	Biogas (1996)	RC, PO, WW*, WB, AL, WW*, C, SP, WR, RC	30	Manure (~ 6)
	Raw	PO, SP*, OA, WB + AL, AL, WW, SP + RC, WB, WR	30	Manure (~13)
KB-II	Biogas (1996)	PO, RC, WW*(-stubble)	30	Manure (~ 6)
	Raw	OA, WB, GC*	30	Manure (~13)
AS-I	Biogas (1985)	SP, WW, OA*, RC	20	Manure (~ 1)
	Raw	GC, WW*	20	Manure (~ 1)
AS-II	Biogas (1985)	WT, SP, GC*	20	Manure (~ 1)
	Raw	WB*, GC	20	Manure (~ 1)
KA	Biogas (1987)	AL, WW*, SP, RC, WW, SP, SB,	25	Compost (~5)
	Raw	WW, SP*, WT, GC	30	Manure (~ 0.5)

AL = alfalfa, WW = winter wheat, SB = summer barley, WB = winter barley, RC = red clover, PO = potato, C = carrots, GC = grass / clover, WT = winter triticale, SP = spelt, OA = oats, WR = winter rye; * crop at sampling date.

Table 2

Climatic and soil characteristics (0-30 cm) of the investigated experimental sites.

Site	Slurry	Coordinates		MAT	MAP	Altitude	Clay	Silt	Sand
		North	East	(°C)	(mm)	(m ASL)		(%)	
KB-I	Biogas	49°13'0.62"	9°57'31.83"	8.7	772	388	34	47	19
	Raw	49°12'53.36"	9°57'36.15"	8.7	772	380	38	46	16
KB-II	Biogas	49°13'3.25"	9°58'35.33"	8.7	772	409	24	71	5
	Raw	49°13'44.32"	9°59'16.89"	8.7	772	427	20	75	5
AS-I	Biogas	48°58'21.30"	9°22'59.44"	8.2	1132	289	29	63	9
	Raw	48°57'57.8"	9°23'16.7"	8.2	1132	298	22	75	3
AS-II	Biogas	48°58`34.21"	9°22`25.26"	8.2	1132	284	32	63	5
	Raw	48°57'57.24"	9°23'17.22"	8.2	1132	302	22	76	4
KA	Biogas	49°17'16.03"	9°42'22.51"	8.8	937	403	22	72	8
	Raw	49°18'19.52"	9°42'5.56"	8.8	937	407	26	61	13

MAT = mean annual temperature, MAP = mean annual precipitation, ASL = above sea level.

3.2.2. Slurry sampling and analysis

All raw slurries consisted of cattle faeces, cattle urine and straw. Biogas slurry KB, added to the respective sites, was produced from 95% cattle slurry and 5% whole crop silage, a mixture of rye, wheat, and rape seed. Biogas slurry AS was produced from 70% cattle slurry and 30% grassland silage or whole crop silage, a mixture of oats, barley, beans, and clover / grass. Biogas slurry KA was composed of 60% cattle slurry and 40% clover / grass silage, followed by mechanical separation into a liquid and a dry fraction after digestion. Only the liquid fraction was collected in this study.

Raw slurries (3 farms) and biogas slurries (3 farms) were directly taken from the storage tanks with 4 replicates each. After homogenization by stirring, all samples were frozen in liquid N_2 directly after removal, kept cool during transport and stored at -18 °C until analysed. A sub-sample was dried at 60 °C and finely ground for chemical analyses using a ball-mill. In the dried slurries, total C was determined using a Vario MAX (Elementar, Hanau, Germany) elemental analyser and total P, S, Na, K, Mg, Ca, Mn, Fe, and Al were analysed after HNO_3-pressure digestion as described by Chander et al. (2008) using ICP-AES (Spectro

Analytic Instruments, Kleve, Germany). Total N was analysed in the non-dried slurries, using Kjeldahl digestion (Blume et al., 2011). Ammonium and nitrate were extracted from the slurries using 0.5 M K_2SO_4 (20 ml per gram fresh slurry) and measured on a continuous flow analyser (Evolution II, Alliance Instruments, Salzburg, Austria). The slurry pH was measured in a 0.01 M $CaCl_2$ solution (2.5 ml per gram of fresh slurry).

3.2.3 Soil chemical analyses

The soils pH was determined using a soil to water ratio of 1 to 2.5. Soil textural analysis was carried out after pre-treatment with H_2O_2, HCl and suspension in sodium polyphosphate, using a combined sieving and pipette method (Blume et al., 2011). Total C and N were determined using a Vario MAX (Elementar, Hanau, Germany) elemental analyser. Carbonate was gas-volumetrically analysed using a Scheibler apparatus (Blume et al., 2011). Then, SOC was calculated as total C minus carbonate C.

3.2.4. Microbial activity and biomass indices

For measuring basal respiration, 50 g moist soil adjusted to 40% water holding capacity were weighed into 500 ml glass jars (Schott) and pre-incubated at 25 °C for 24 h in the dark. Then, a glass beaker containing 5 ml 0.5 M NaOH was added to the glass jars, before the incubation was continued for a further 7 days. The CO_2 evolved was determined by back-titration to pH 8.3 of the excess NaOH with 0.5 M HCl after addition of saturated $BaCl_2$ solution.

Microbial biomass C and biomass N were estimated by fumigation extraction (Brookes et al., 1985; Vance et al., 1987) in the soil samples used for measuring basal respiration. A sub-sample of 20 g moist soil was separated into two portions of 10 g. One portion was fumigated at 25 °C with ethanol-free $CHCl_3$, which was removed after 24 h. Fumigated and non-fumigated samples were extracted for 30 min with 40 ml of 0.5 M K_2SO_4 by horizontal shaking at 200 rev min^{-1} and filtered (hw3, Sartorius Stedim Biotech, Göttingen, Germany). Organic C and total N in the extracts were measured via infrared and electrochemical detection, respectively, after combustion at 800 °C using a multi N/C® 2100S automatic analyser (Analytik Jena, Jena, Germany). Microbial biomass C was calculated as E_C/k_{EC}, where E_C = (organic C extracted from fumigated soil) – (organic C extracted from non-fumigated soil) and k_{EC} = 0.45 (Wu et al., 1990). Microbial biomass N was calculated as E_N/k_{EN}, where E_N = (total N extracted from fumigated soil) – (total N extracted from non-

fumigated soil) and k_{EN} = 0.54 (Brookes et al., 1985). The metabolic quotient (qCO_2 was calculated as mg CO_2-C d^{-1} g^{-1} microbial biomass C.

The fungal cell-membrane component ergosterol was extracted with 100 ml ethanol from 2 g moist soil by 30 min oscillating shaking at 250 rev min^{-1} (Djajakirana et al., 1996). Then, ergosterol was determined by reversed-phase HPLC with 100% methanol as the mobile phase and detection at 282 nm.

3.2.5. Microbial residues

Microbial residues were determined by analysing the amino sugars muramic acid (MurN), glucosamine (GlcN) and galactosamine (GalN) according to Appuhn et al. (2004) as described by Indorf et al. (2011), using OPA (o-phthalaldehyd) derivatisation. Dried soil (500 mg) was hydrolysed with 10 ml of 6 M HCl for 6 h at 105 °C. After HCl removal, chromatographic separations were performed on a Hyperclone C_{18} column (125 mm length □ 4 mm diameter) at 35 °C, using a Dionex (Germering, Germany) P 580 gradient pump, a Dionex Ultimate WPS – 3000TSL analytical autosampler with in-line split-loop injection and thermostat and a Dionex RF 2000 fluorescence detector set at 445 nm emission and 330 nm excitation wavelengths. Fungal C was calculated by subtracting bacterial GlcN from total GlcN as an index for fungal residues, assuming that MurN acid and GlcN occur at a 1 to 2 molar ratio in bacterial cells (Engelking et al., 2007): mmol fungal C g^{-1} dry weight = (mmol GlcN − 2 × mmol MurN) × 9. Bacterial C was calculated as an index for bacterial residues by multiplying the concentration of MurN by 45 (Appuhn and Joergensen, 2006). Microbial residue C was estimated as the sum of fungal C and bacterial C.

3.2.6. Statistical analysis

The results for soil chemical and biological indices are presented in tables 4, 5, 6 and expressed on an oven-dry basis (24 h at 105 °C). The stocks of the soil chemical properties and soil biological indices were calculated on a volume basis, using the mean bulk density of 0-30 cm soil depth. The values of nine sampling points per field were used to calculate mean stocks or mean concentrations (n = 9). An independent-samples t-test was used to test for differences between slurry types within each site ($P \leq 0.05$). The statistical calculations were carried out using SPSS 17.0. (SPSS Inc., Chicago, USA). To calculate and compare the overall effect of the slurry, a mixed model was fitted to account for both fixed and random

effects of treatments and the sampling design of this on-farm trial (Piepho et al., 2011), taking the nine single values per field into account. The calculation was based on the nine single values per field. Analyses were performed using the MIXED procedure of SAS (Statistical Analysis System, version 9.2; SAS Institute, Cary, NC, USA). Fixed effects were fitted for the fertilizer treatment (field (mean biogas slurry, mean raw slurry)) and clay, and the random effects were site and field within each site. The LSMEANS (Least squares means) was used to compute adjusted treatment means and the PDIFF option was used to compare treatments ($P < 0.05$). A check of studentized residuals revealed no problematic departures from normality or homogeneity of variance.

3.3. Results

3.3.1. Slurry properties

All fertilizers were alkaline, with a mean pH of 8 and a dry matter (DM) concentration ranging from 5% to 7% (Table 3). On average, the OC concentration was significantly 10% higher in the raw than in the biogas slurries. In all slurries, the total N concentration varied between 3.4 and 8.3% DM. The mean N concentration was 40% higher in the biogas than in the raw slurries, with a maximum in the separated biogas slurry KA. Ammonium accounted for 35 to 50% of total N in the raw slurries and for 44 to 62% in the biogas slurries, with the highest proportion in the separated biogas slurry KA. The C/N ratio varied between 4.4 and 13 and was significantly lower in the biogas than in the raw slurries.

Table 3

Physical and chemical characteristics of biogas and raw slurries from sites Kirchberg (KB), Aspach (AS) and Künzelsau (KA).

	Raw slurry			Biogas slurry			CV
Property	KB	AS	KA	KB	AS	KA	(± %)
pH ($CaCl_2$)	8.1	8.2	8.1	8.2	8.2	8.3	0.7
DM (% FM)	5.5	6.6	7.4	5.0	6.6	6.0	7.2
OM (% DM)	76	76	74	74	69	61	3.3
OC (% DM)	43	42	42	41	38	36	1.2
Total N (% DM)	4.1	3.9	3.4	5.3	4.5	8.3	9.9
NH_4-N (% DM)	2.0	1.7	1.2	2.3	2.2	5.1	8.2
NH_4-N (% total N)	50	43	35	44	49	62	16
C/N	10	10	13	7.8	8.4	4.4	7.0
Nutrients (mg g^{-1} DM)							
P	7.7	5.6	6.4	7.5	10.3	4.0	2.5
S	4.3	3.3	4.1	4.6	4.4	5.6	2.1
Na	2.8	1.8	3.0	1.5	3.9	2.5	1.5
K	71	41	42	77	61	107	1.0
Ca	24	24	21	33	26	22	3.2
Mg	8.4	4.8	7.1	8.8	8.6	3.9	2.1

n = 4 for dry matter (DM), organic matter (OM), pH, organic carbon (OC), Kjeldahl total N, NH_4-N, and C/N; n = 3 for all other properties.

3.3.2. Soil physical and chemical properties

Clay contents at 0-30 cm depth varied between 22% and 38% (Table 4). The mean of the biogas and the raw slurry sites did not differ significantly, although at three of the five sites significant differences occurred between the neighbouring fields (AS-I, AS-II, and KA). The soil pH ranged from 6.1 (KB-II) to 7.1 (KB-I) and showed significant differences between the neighbouring fields at sites KB-I and KB-II, with higher pH in the biogas slurry and raw slurry fields, respectively (Table 4).

Table 4

Clay content and soil pH, mean stocks of soil organic C (SOC) and total N as well as the C/N ratio in soils of ten investigated fields at 0-30 cm depth, mean values for the slurry types and probability values based on the MIXED procedure (n = 45).

Site	Field	Clay (%)	pH (H_2O)	SOC (t ha^{-1})	Total N (t ha^{-1})	C/N
KB-I	Biogas	34	7.1 a	65 b	6.2	10.9
	Raw	38	6.7 b	76 a	7.2	10.7
KB-II	Biogas	25	6.1 b	55	5.4	10.4
	Raw	22	6.7 a	47	4.4	10.5
AS-I	Biogas	30 a	7.0	54 a	5.6 a	9.6
	Raw	20 b	6.5	43 b	4.3 b	10.0
AS-II	Biogas	32 a	6.8	57 a	6.1 a	9.6 b
	Raw	22 b	6.4	46 b	4.4 b	10.3 a
KA	Biogas	22 b	6.9	48 b	4.5 b	10.6 b
	Raw	28 a	6.8	62 a	5.5 a	11.3 a
Mean	Biogas	29	6.7	55	5.6	10.2
Mean	Raw	26	6.7	56	5.2	10.5
Probability values						
Fertilizer		NS	NS	NS	NS	NS
Clay		ND	0.01	0.01	0.01	NS
CV (± %)		16	5	14	14	4

NS = not significant; ND = not determined; CV = mean coefficient of variation within one field (n = 9); different small letters within a column show significant differences within each site (t-test, $P < 0.05$, n = 9); all fertilizer × clay interaction were insignificant.

Mean SOC and total N stocks varied around 55.5 and 5.4 t ha^{-1}, respectively, and did not differ between the biogas and raw slurry fields (Table 4). At the site KA, significantly higher stocks of SOC (approx. 30%) and total N (approx. 20%) were found with raw than with biogas slurry application. In contrast, at the sites AS-I and AS-II, significantly higher stocks of SOC (approx. 20%) and total N (approx. 30%) were observed with biogas than with raw

slurry application. Consequently, significantly higher soil C/N ratios were measured with raw slurry application at the sites KA, AS-I and AS-II. Soil pH, SOC, and total N were affected by the clay content.

3.3.3. Soil microbial biomass and activity

Microbial biomass C stocks varied between 850 and 1580 kg ha^{-1} (Table 5). They were on average 16% lower with biogas than with raw slurry application. Clay significantly lowered the microbial biomass C stocks (Table 5). At the sites KA (approx. 50%) and KB-I (approx. 35%), significantly higher stocks were found with raw slurry than with biogas slurry application. In contrast, significantly 30% larger stocks were measured with biogas slurry than with raw slurry application at the site AS-I. Microbial biomass C and SOC were highly correlated, considering the data from all sites (Fig. 1). Biogas slurry application significantly lowered the microbial biomass C to SOC ratio (Table 5). Microbial biomass N stocks ranged from 130 to 300 kg ha^{-1} and did not generally differ between the biogas and raw slurry fields (Table 5). The lowest microbial biomass C/N ratios were observed at the site KA and also for the biogas slurry site KB-II. Here, the microbial biomass C/N ratio was significantly higher with raw slurry than with biogas slurry application. A higher C/N ratio was also found at the biogas slurry field at site AS-I. The mean ergosterol stock of the biogas slurry fields was 22% lower than that of the raw slurry fields, mainly due to the site KA.

Table 5

Stocks of soil microbial biomass C, N, and ergosterol, the biomass C/N ratio, the ergosterol to microbial biomass C ratio and the basal respiration in soils of ten investigated fields at 0-30 cm depth, mean values for the slurry types and probability values based on the MIXED procedure (n = 45).

Site	Field	Microbial biomass				Ergosterol	Ergosterol / microbial biomass C	CO_2-C
		C	N	C/N	C			
		(kg ha^{-1})			(% SOC)	(kg ha^{-1})	(%)	(kg d^{-1} ha^{-1})
KB-I	Biogas	1129 b	144 b	8.7	1.8	1.7	0.14	41 b
	Raw	1542 a	195 a	8.5	2.1	1.9	0.13	44 a
KB-II	Biogas	848	184 a	4.6 b	1.6 b	2.5 b	0.37	53 a
	Raw	1073	131 b	8.4 a	2.4 a	3.0 a	0.31	41 b
AS-I	Biogas	1254 a	159	8.4 a	2.3	1.9	0.17	46 a
	Raw	947 b	145	6.5 b	2.2	1.6	0.17	34 b
AS-II	Biogas	1139	169	6.7 a	2.0	1.7	0.17	42
	Raw	1064	174	6.6 b	2.3	2.0	0.20	44
KA	Biogas	947 b	208 b	5.3	2.1 b	1.1 b	0.13 b	44 b
	Raw	1403 a	296 a	5.0	2.3 a	3.0 a	0.25 a	49 a
Mean	Biogas	1039	170	6.7	1.9 B	1.8	0.20	45
	Raw	1230	191	7.0	2.3 A	2.3	0.21	43
Probability values								
Fertilizer		NS	NS	NS	0.05	NS	NS	NS
Clay		0.05	NS	NS	NS	NS	NS	NS
CV (± %)		18	20	16	13	20	23	15

NS = not significant; ND = not determined; CV = mean coefficient of variation within on field (n = 9); different small letters within a column show significant differences within each site (t-test, $P < 0.05$, n = 9); capital letters within a column show a significant difference between the slurry type-specific mean of all sites (LS-means, $P < 0.05$, n = 45); all fertilizer × clay interaction were insignificant.

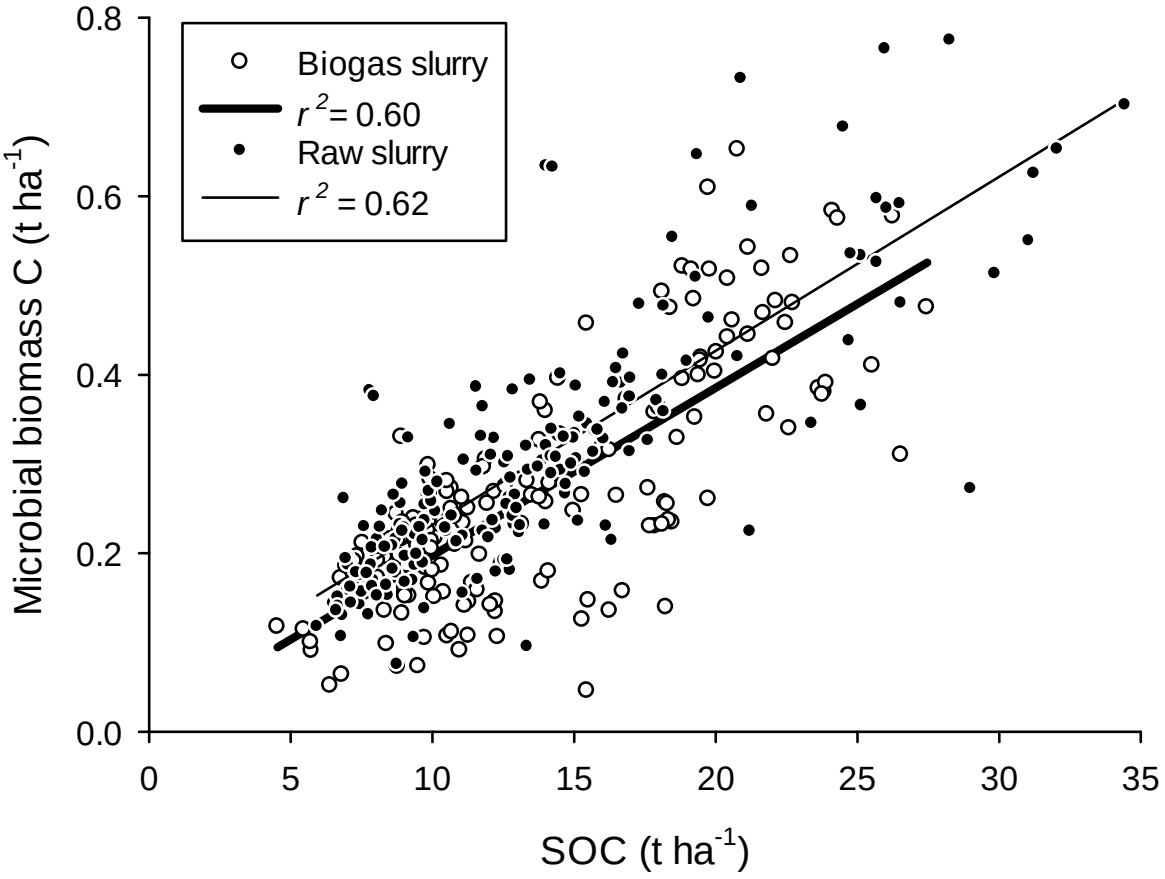

Fig. 1. The relationship between soil microbial biomass C and soil organic C, data from 10 fields, both slurry types, 4 sampling depths and field replicates are combined (n = 360).

Mean CO_2-C evolved varied around 44 kg d^{-1} ha^{-1} and did not differ between the biogas and raw slurry fields. However, significant differences were observed in the raw slurry field at site KA, with 14% more CO_2-C, and in the biogas slurry field at site KB-II with 30% more CO_2-C, compared with the respective neighbouring field. Significantly 20 and 40% higher metabolic quotients qCO_2 were found in the biogas slurry field at sites KB-I and KB-II, respectively (Fig. 2). The qCO_2 was significantly reduced by the clay content ($P < 0.05$).

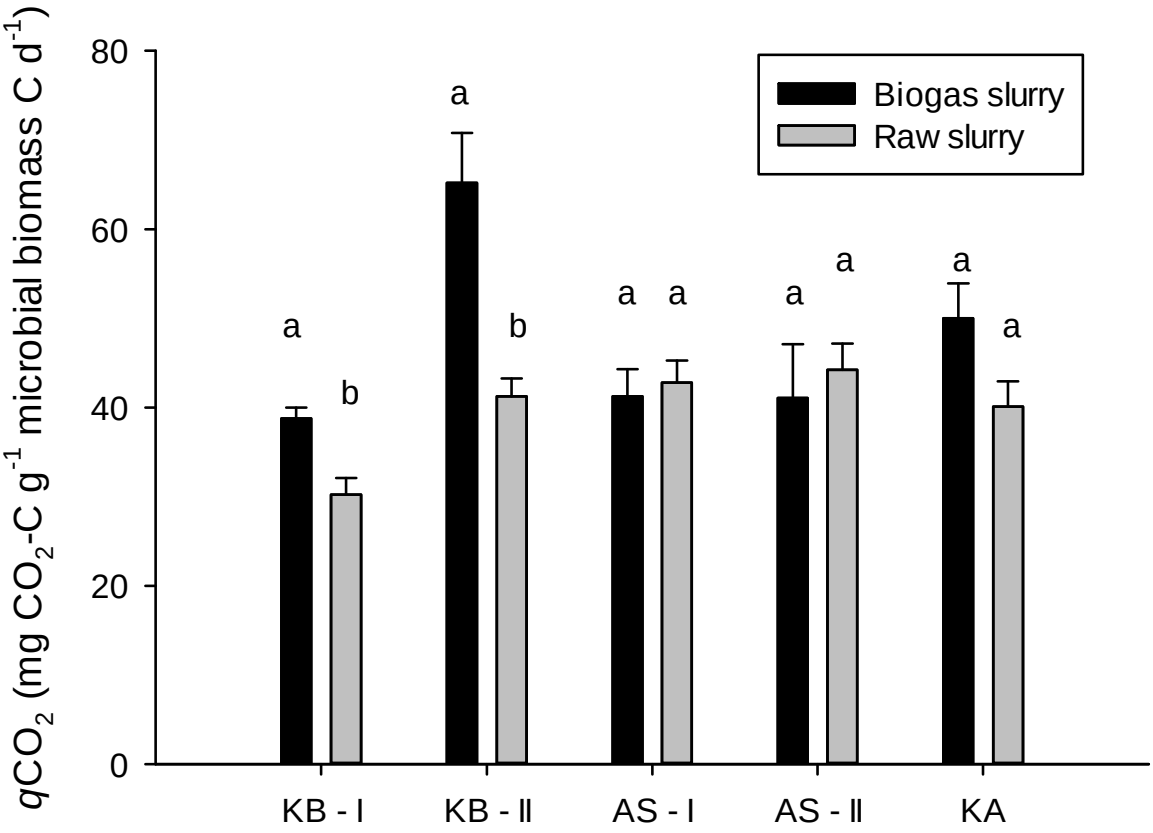

Fig. 2. Metabolic quotient at all sites for both slurry types at 0-30 cm depth; different letters show significant differences within each site (t-test, $P < 0.05$, n = 9).

3.3.4 Amino sugars

The MurN, GalN, and fungal GlcN stocks varied around 0.30, 2.15, and 2.85 t ha^{-1}, respectively (Table 6). They were all on average roughly 16% lower with biogas than with raw slurry application, due to differences at the sites KB-I, AS-II, and KA. At the site AS-I, there were no significant differences between the slurry treatments for any amino sugar. At the site KB-II, the MurN and GalN stocks with biogas slurry exceeded those with raw slurry, whereas again no differences were observed for the GlcN stocks. A significant negative relationship was found between the fungal C to bacterial C ratio and the clay content (Fig. 3). Microbial residue C stocks varied round 74% SOC and were larger with raw than with biogas slurry. This was true for most of the sites, with the exception of the site KB-II. The contribution of microbial residue C was especially large at the sites AS-I and AS-II. Clay significantly affected the fungal C to bacterial C ratio and the contribution of microbial C to soil organic matter (Table 6).

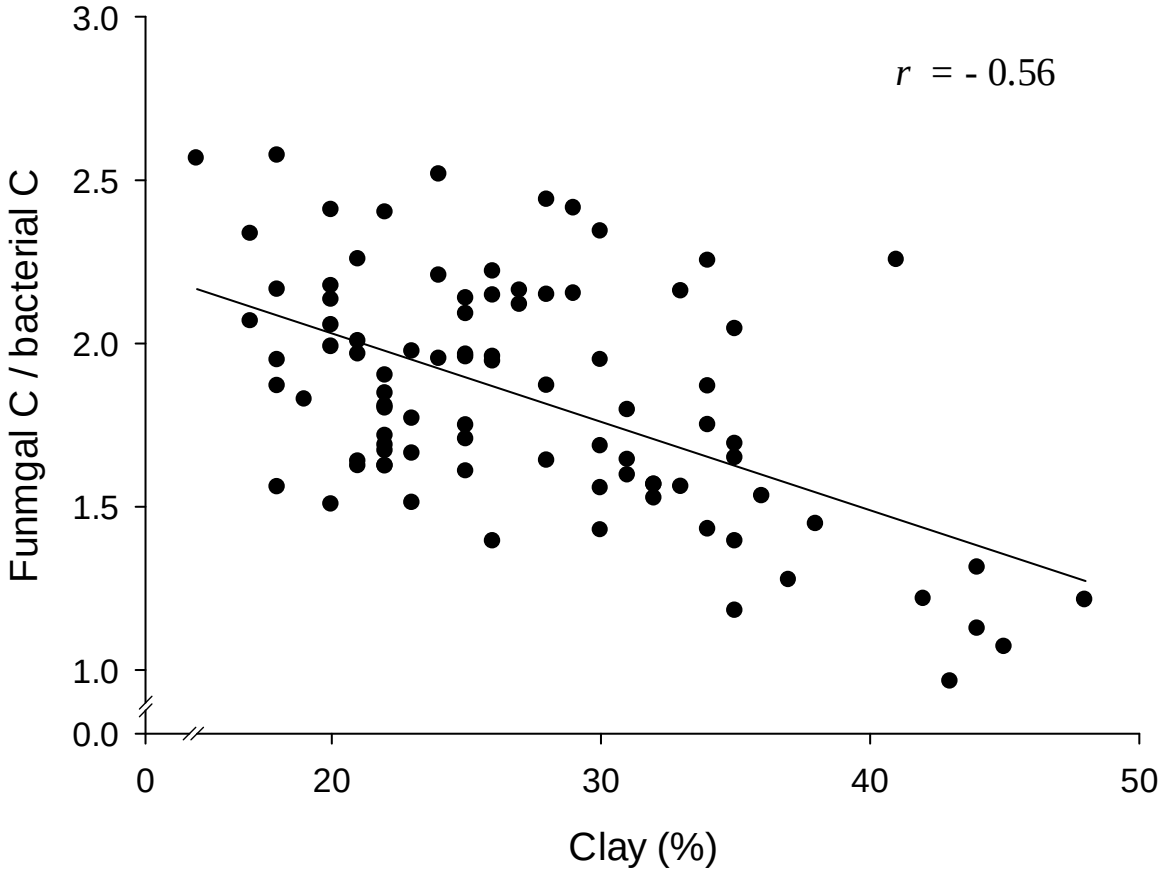

Fig. 3. The relationship between fungal C to bacterial C ratio and clay, data from 10 sites, 10 fields, both slurry types and field replicates are combined (n = 90).

Table 6

Stocks of muramic acid (MurN), galactosamine (GalN) and fungal glucosamine (GlcN), the fungal C to bacterial C ratio and the microbial residual C in soils of ten investigated fields at 0-30 cm depth, mean values for the slurry types and probability values based on the MIXED procedure (n = 45).

Site	Field	MurN	GalN	Fungal GlcN	Fungal C/	Microbial residue C
		($t\ ha^{-1}$)			Bacterial C	(% SOC)
KB-I	Biogas	0.29 b	1.8 b	2.3 b	1.7	54 b
	Raw	0.46 a	2.7 a	3.5 a	1.5	68 a
KB-II	Biogas	0.24 a	1.8 a	2.4 a	1.9	58 a
	Raw	0.18 b	1.0 b	1.6 b	1.8	50 b
AS-I	Biogas	0.36	2.2	3.1	1.7 b	85
	Raw	0.34	2.4	3.7	2.2 a	112
AS-II	Biogas	0.25 b	2.1 b	3.1	2.4	72 b
	Raw	0.33 a	2.3 a	3.5	2.2	104 a
KA	Biogas	0.19 b	1.4 b	2.1 b	2.0	56 b
	Raw	0.35 a	2.3 a	3.4 a	1.9	75 a
Mean	Biogas	0.27	1.9	2.6	2.0	66
	Raw	0.33	2.2	3.1	1.9	80
Probability values						
Fertilizer		NS	NS	NS	NS	NS
Clay		NS	NS	NS	0.01	0.05
CV (± %)		21	25	24	16	20

NS = not significant; ND = not determined; CV = mean coefficient of variation within on field (n = 9); different small letters within a column show significant differences within each site (t-test, $P < 0.05$, n = 9); all fertilizer × clay interaction were insignificant.

3.4. Discussion

3.4.1. Slurry effects on soil organic matter

The current biogas slurries applied to biodynamic fields always had lower organic matter and higher ammonium concentrations than the raw slurries applied to the neighbouring fields, despite the differences between feedstock and biogas plant process, resulting in a clear categorisation of biogas and raw slurries. Long-term application of these biogas slurries had no specific effects on SOC, total N or C to N ratio. The lower C input by the biogas slurries apparently had no effect on SOC contents. This might be due to the high clay contents counteracting small differences in C input. In addition, the high amounts of ammonium applied with biogas slurry are known to increase crop yields (Dick, 1992; Kirchmann and Witter, 1992; Svensson et al., 2004) and, thus, to increase C input by litter and root exudates (Odlare et al., 2008). Furthermore, Reinhold et al. (1991) suggested that the higher presence of recalcitrant components such as lignin in biogas slurry fully compensates for the lower C input rates in their effects on SOC sequestration in comparison with raw slurry.

In general, all investigated fields showed relatively high SOC and total N stocks in comparison with other arable fields in Germany (Anderson and Domsch, 1989; Höper and Kleefisch, 2001). This leads to the assumption that biogas slurries had no detectable negative effects on these soil fertility indices under biodynamic organic farming practice, contrasting the view stated by Scheller (2006) and allaying the concerns of the participating farmers. However, no information is available on the trajectory of SOC and total N stocks. Biodynamic farming systems are not only characterized by the use of organic fertilizers but also by a highly diverse crop rotation, including several legumes (Höper and Kleefisch, 2001; Mäder et al., 2002; Heinze et al., 2010).

In the short term, biogas slurries have negligible effects on microbial biomass in comparison with raw slurries (Andruschkewitsch et al., 2013). This is even more true for the less variable stocks of SOC and microbial residues. Also, differences in the actual crop have insignificant effects on soil microbial biomass (Haynes and Francis, 1993; Kaiser et al., 1995). Consequently, long-term observations are necessary to reveal differences resulting from organic fertilizer application (Heinze et al., 2010; 2011) or crop rotation (Dick et al., 1992). On-farm research gives immediate access to information on long-term effects of relevant and urgent questions asked by farmers, in the present case whether long-term application of biogas slurry has negative effects on soil fertility. Another advantage of our on-farm experiment is that all treatments are carried out according to best practice by the

participating farmers. Although all pairs of the current neighbouring fields were similar in climate, soil pH and land-use management, the problem remains that the pairs might already have significantly differed in the soil properties analysed when farming practices changed, as no data are available from this period. However, many long-term experiments investigating organic fertilizer effects also suffer from the problem that the baseline data were not appropriately determined (Mäder et al., 2002; Heinze et al., 2010), i.e. by measuring the soil properties from each single plot and not only from a "representative" bulk sample taken from the whole site. Other problems are the costs of running replicated field experiments with organic fertilizer application for long-periods without results and with the risk that current questions might not be of any practical relevance in 20 to 30 years' time.

3.4.2. Slurry effects on microbial biomass indices

Long-term biogas slurry application resulted in a significantly lower microbial biomass C to SOC ratio, an important indicator for C availability to soil microorganisms (Anderson and Domsch, 1989). This suggests that C availability is reduced with biogas compared with raw slurry application, which is in line with the higher presence of recalcitrant components (Reinhold et al., 1991) and the absence of effects on SOC stocks (Möller and Müller, 2012). Our results are in contrast with the short-term incubation study by Stumpe et al. (2012), who did not observe any strong impact of biogas slurry on the SOC dynamics in comparison with liquid manure and sewage sludge, despite the differences of these amendments in many properties, e.g. organic matter, NH_4-N, and pH. In some fields, a high clay content seems to have masked the effects of slurry quality, as an increasing clay content reduces the microbial turnover, leading to an increased microbial biomass (van Veen et al., 1985; Müller and Höper, 2004) and also to an increased microbial biomass C to SOC ratio (Kaiser et al., 1992). The absence of clear effects on the ergosterol to microbial biomass C ratio reveal the absence of slurry effects on the microbial community structure, i.e. in terms of a shift in the ratio of fungal to bacterial biomass. This contradicts the results of Walsh et al. (2012), who observed that biogas slurry application changes the microbial community towards bacteria in comparison with raw slurry.

The metabolic quotient qCO_2 indicates the amount of energy, i.e. the SOC respired as CO_2, necessary to maintain a certain content of microbial biomass C (Anderson and Domsch, 1990). It is thus an important indirect measure of the substrate use efficiency and is often negatively related to the microbial biomass C to SOC ratio (Anderson and Domsch, 1990;

Heinze et al., 2010; Murugan et al., 2014). Higher values, e.g. down the profile (Meyer et al., 1997; Murugan et al., 2014), indicate an increasing recalcitrance of SOC. This reduced availability to soil microorganisms must lead to lower microbial biomass contents in the long term (Anderson and Domsch, 1990). The strong clay effect on the qCO_2 values might have masked the negative, i.e. increasing effects of biogas slurry application on this important soil ecophysiological index.

3.4.3. Slurry effects on microbial residues

Long-term biogas slurry application did not affect any of the microbial residue indices. A high average contribution of microbial residue C to SOC was observed in the current study. This is similar to Murugan et al. (2014) and had already been proposed by Liang et al. (2011). They used an Absorbing Markov Chain approach, which differs completely from the current calculation procedure and is based on empirically determined conversion values (Appuhn and Joergensen, 2006; Engelking et al., 2007). However, microbial residue values exceeding 100% SOC indicate some limitation of the current calculation approach. This might be caused by higher concentrations of amino sugars within the microbial cells, especially by a higher contribution of Gram positive bacteria to the total bacterial community than the 65% proposed by Joergensen and Potthoff (2005). Another possibility might be the unknown contribution of GlcN derived from extracellular microbial polysaccharides (Zippel and Neu, 2011), containing higher GlcN concentrations than fungal biomass to the microbial residue pool.

The absence of slurry effects on the fungal C to bacterial C ratio again suggests that biogas slurry has no general strong effects on the microbial community structure, which is absolutely in line with the current results of the ergosterol to microbial biomass C ratio. This supports the view that there is a close relationship between the living biomass and dead microbial residues in long-term equilibrated agricultural ecosystems (Appuhn et al., 2006). The ratios of fungal C to bacterial C and ergosterol to microbial biomass C are very low in comparison with those from other arable soils (Djajakirana et al., 1996; Joergensen and Wichern, 2008). This indicates that N-rich raw animal and also biogas slurries promote bacteria more than saprotrophic fungi in comparison with the application of N-poor and lignin-rich straw (Scheller and Joergensen, 2008; Ding et al., 2011). Also, the view that clay promotes bacteria more than fungi is supported by negative effects on the fungal C to bacterial C ratio. Clay has been repeatedly shown to protect bacteria by adsorption (Wu et al.,

2012), by reducing the pore-size diameter, by reducing competition with fungi (Sessitsch et al., 2001), and by excluding soil animals that feed on bacteria, such as protozoa and nematodes (Rutherford and Juma, 1992; Görres et al., 1999). Consequently, higher contents of bacterial MurN were repeatedly found with increasing clay content (Zhang et al., 1998, 1999; Kandeler et al., 2000; Liang et al., 2013).

3.4.4. Conclusions

The long-term application of biogas slurry did not affect SOC or total N stocks and had no general negative effects on soil fertility. Biogas slurry application reduced the microbial biomass C to SOC ratio, indicating a reduced availability of the biogas slurry C to the soil microorganisms compared with raw slurry. Biogas slurry application tended to decrease the stocks of microbial residues, but differences in clay content masked any slurry effects on the microbial activity, biomass, and residues at some sites. This suggests that it would be necessary to increase the number of sites to obtain statistical significance. There was no general effect of biogas slurry on the microbial community structure in terms of the ratio fungi to bacteria, whereas increasing clay content caused a significant shift towards bacteria. The consistency in the data of all approaches strongly indicates the validity of the current on-farm study by comparing neighbouring fields.

Acknowledgements

We would like to thank the farmers Karl Kuch, Gerhard Kuch, Martin Frank, Ernst Bürkert, Marianne Holz, and Martin Lachenmaier for their good cooperation. We highly appreciate Gabriele Dormann for her skilled technical assistance. We dedicate this study to the late Edwin Scheller, whose pioneering ideas strongly motivated us to carry out this research.

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4. Effects of biogas and raw slurries on grass growth and soil microbial indices

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ABSTRACT

Biogas slurry, the secondary product of the anaerobic digestion process, is increasingly used as fertilizer. For this reason, biogas and raw slurries obtained from 6 biodynamic farms were added to a soil, which was then cultivated with Italian rye-grass (*Lolium multiflorum, var.* Ligrande) for 70 days to investigate the effects on plant growth, soil microbial biomass, soil fungi, and root colonizing microorganisms. Biogas slurries increased the mean total aboveground plant biomass by 69% and raw slurries by 36% in comparison with the unfertilized control. The total aboveground biomass had a strong non-linear relationship with the NH_4-N input. In contrast to biogas slurries, the raw slurries significantly increased microbial biomass C and N by roughly 25% in comparison with the unfertilized control. The application of biogas slurries significantly decreased the soil ergosterol content in comparison with raw slurry and control treatments, leading to a significantly lower ergosterol to microbial biomass C ratio. In the root DM, biogas and raw slurry application significantly decreased the concentrations of the amino sugars muramic acid, galactosamine, and glucosamine by 24, 39, and 27%, respectively, but not that of ergosterol in comparison with the control. This was most likely due to a reduced colonization with arbuscular mycorrhizal fungi in the presence of highly available plant nutrients. The differences between biogas and raw slurries but also those within the slurry types were small and non-significant in most cases.

Keywords: Biogas slurry, organic fertilizer, soil fertility, soil microorganism, plant growth, root amino sugar

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4.1. Introduction

Biogas is an important component for energy production from renewable resources (Møller, 2009) and for this reason biogas slurry, the secondary product of the anaerobic digestion process, is increasingly used as fertilizer (Möller, 2009). In biogas plants a wide variety of substrates is used, whereas raw slurries are derived from faeces and urine, some water and rarely straw. Biogas slurries provide a way of closing nutrient cycles in livestock and especially in stockless organic farming systems (Stinner et al., 2008). The positive effects of biogas slurries on plant yield and soil properties have been repeatedly evaluated (Terhoeven-Urselmans et al., 2009; Sänger et al., 2011; Andruschkewitsch et al., 2013). Anaerobic digestion increases the concentration of NH_4-N and reduces dry matter (DM), leading to lower C concentrations and C/N ratio as well as to increased slurry pH values (Gutser et al., 1987; Möller et al., 2009; Möller and Müller, 2012). Due to the larger inorganic N concentrations, biogas slurries supply more plant-available N than other organic fertilizers, e.g. farmyard manure (Odlare et al., 2008) or undigested slurry (Bachmann et al., 2011).
Some studies have reported close positive relationships between biogas slurry application and plant growth, while belowground C inputs by root exudates and root turnover are also increased, thus stimulating rhizosphere fungi and bacteria (Knorr et al. 2005; Liu and Greaver 2010; Walsh et al., 2012). On the other hand, biogas slurries contain less organic C due to the anaerobic fermentation, leading to the concern that this may have negative effects on soil organic matter contents (Scheller, 2006; Arthurson et al. 2009; Möller, 2009). However, the long-term application of biogas slurry for 15 years and more to arable soils in the north-east of Baden-Württemberg did not affect soil organic C (SOC) or total N stocks or the soil C/N ratio (Wentzel et al., 2015). Biogas slurry application also tended to reduce the stocks of microbial biomass C, fungal ergosterol, and those of microbial residues, i.e. muramic acid (MurN), galactosamine (GalN), and fungal glucosamine (GlcN). The resulting decrease in the contribution of microbial tissue to SOC indicates a reduced availability of the C input by biogas slurries to soil microorganisms compared with that of raw slurries. In this field observation, the absence of general biogas slurry effects on the ratios of ergosterol to microbial biomass C and amino sugar-based fungal C to bacterial C might be masked by differences in clay content (Wentzel et al., 2015).

For this reason, biogas and raw slurries obtained from the 6 farms, participating in the study of Wentzel et al. (2015) were added to one soil, which was then planted with Italian rye-grass (Lolium multiflorum, var. Ligrande) to invetigate the following hypotheses; (1)

Biogas slurries have stronger increasing effects on plant growth than raw slurries and (2) Biogas slurries have less positive effects on soil microbial biomass, soil fungi and root colonizing microorganisms than raw slurries. The microbial colonisation of ryegrass roots was analysed using the fungal cell-membrane component ergosterol, the fungal cell-wall component GlcN, and the bacterial cell-wall component MurN. This may provide an early indication of any difference in the future development of microbial communities fertilized with biogas and raw slurries. In freshly excised roots, amino sugars can be used as indices for fungal and bacterial biomass (Frey et al., 1994; Kortemaa et al., 1997; Appuhn and Joergensen, 2006), in contrast to soil, where GlcN and MurN are accumulated as microbial residues in SOC (Amelung, 2001; Liang and Balser, 2011).

4.2. Material and Methods

4.2.1. Soil and slurry sampling

Soil sampling was conducted at site "Saurasen" (51°22'34.5" N, 9°53'53.0" E) which is located in Neu-Eichenberg near Witzenhausen (Northern Hessia, Germany) at 0-20 cm depth. The site is located at 280 m above sea level, with an average annual precipitation of 625 mm and temperature of 6.5 °C. Developed from eroded loess overlying clayey sandstone, the soil is classified as Stagnic Luvisol (FAO-WRB, 2006; Quintern et al., 2006). Soil organic C and total N content was 9.7 mg g^{-1} and 1.9 mg g^{-1}, respectively. The soil had a water holding capacity of 57% and a pH ($CaCl_2$) of 7. After sampling, plant tissue and stones were removed by hand and the soil was sieved (< 2 mm) and stored in polyethylene bags at room temperature until the experiment started. A sub-sample of the soil was dried and finely ground for chemical analysis. Another sub-sample was frozen at -18 °C for further analysis. Slurry sampling and analyses are described in Chapter 3.2.2.

4.2.2. Greenhouse pot experiment

A 70-day completely randomized greenhouse experiment was carried out in plastic pots (2.7 l, 13 ×13 cm) placed in a greenhouse chamber with a peak temperatures of 23 °C (day) and 15 °C (night). Biogas and raw slurries were defrosted and thoroughly mixed with soil before filling into the pots. After mixing, each pot was filled with moist soil at 50% of the soil water holding capacity equivalent to 2.6 kg dry soil with a bulk density of 1.1 g m^{-3}. In 4 replicates, the soil-filled pots were either treated with one of three different biogas slurries,

one of three different raw slurries or served as untreated controls. The positions of the pots in the greenhouse chamber were changed weekly. The calculation for the application rate was based on Total Kjeldahl Nitrogen (TKN) and equivalent to 120 kg N ha^{-1}. The application rate ranged from 13 to 27 g freshly thawed slurries kg^{-1} soil. This amount was equivalent to 0.8% of the soil dry weight and comprised an addition 0.9 to 2.5 g C per pot (0.3-1.0 g C kg^{-1} soil) (Table 7) and 200 mg N per pot (76.9 mg N kg^{-1} soil).

Seeding took place 5 days after fertilization to avoid a possible seed acid burn. During these five days, the pots were covered with perforated film to prevent desiccation. Italian ryegrass (*Lolium multiflorum*, var. Ligrande) was sown at a density of 200 seeds per pot, with 1.2 seeds cm^{-2}. The first cut of the aboveground biomass was performed 38 days after germination, with a mean plant height of 25 cm, and the second cut after 70 days, with a mean plant height of 23 cm. Also, after 70 days, the root biomass was separated by hand, removed from soil and afterwards washed over sieves (1 mm). Plants and a subsample of the roots were dried at 40 °C for determination of dry matter and finely ground for total C and N analysis by combustion in a CNS Analyzer (Elementar Vario EL, Elementar, Hanau, Germany). Another subsample of the root biomass was stored at -18 °C for ergosterol and root amino sugar analysis.

Table 7

C and N input by slurry amendment per pot.

	Biogas slurry			Raw slurry			Mean		CV
	1	2	3	1	2	3	Biogas-SL	Raw-SL	(±%)
Organic C (g pot^{-1})	1.6 a	1.6 a	0.9 b	2.0 b	2.1 b	2.5 a	1.4 B	2.2 A	7.2
NH_4-N (mg pot^{-1})	84 b	90 b	117 a	87 a	79 b	67 c	97 A	87 B	3.9

CV = pooled coefficient of variation between treatments (n = 4); small letters indicate differences within slurry treatments ($P < 0.05$, Scheffé-test, n = 4); capitals indicate differences between means of biogas and raw slurries (Mann-Whitney-U Test, $P < 0.05$, n = 12).

4.2.3. Analytical procedures

Soil textural analysis was carried out after pre-treatment with H2O2, HCl and suspension in sodium polyphosphate, using a combined sieving and pipette method (Blume et al., 2011). Soil total C (and slurry total C) as well as soil total N was determined using a Vario MAX (Elementar, Hanau, Germany) elemental analyzer. For soil mineral N analysis, 30 g of fresh soil were extracted with 70 ml 0.05 M K_2SO_4 within 2 days after harvest, shaken for 30 min (200 rev min-1) and centrifuged at 4000 g. The supernatant was filtered and analyzed for inorganic N (NH_4^+ and NO_3^-) using a continuous flow analyser (CFA).

Slurry total N was analysed by H_2SO_4 digestion (Kjeldahl, 1983), using a 323 digester (Büchi, Essen, Germany). Ammonium and nitrate were extracted from the slurries using 0.5 M K_2SO_4 (20 ml g^{-1} fresh slurry) and measured on a continuous flow analyser (Evolution II auto-analyzer, Alliance Instruments, Salzburg, Austria). Total P, S, Na, K, Mg, and Ca were analysed after HNO_3-pressure digestion as described by Chander et al. (2008) using ICP-AES (Spectro Analytic Instruments, Kleve, Germany). The slurry pH was measured in a 0.01 M $CaCl_2$ solution (2.5 ml g^{-1} fresh slurry).

For the microbial biomass C and N estimation, first a pre-extraction of the soil was made to minimize the soil inorganic N content (Widmer et al., 1989). Briefly, 30 g fresh soil was pre-extracted with 70 ml 0.05 M K_2SO_4 by 30 min horizontal shaking at 200 rev min-1 and centrifuging at 2000 *g*. Microbial biomass C and biomass N were estimated by fumigation extraction (Brookes et al., 1985; Vance et al., 1987). Then, microbial biomass C and N was estimated by fumigation extraction from two portions equivalent to 10 g of the pre-extracted soil. One portion was fumigated at 25 °C with ethanol-free $CHCl_3$, which was removed after 24 h. Fumigated and non-fumigated samples were extracted for 30 min with 40 ml of 0.5 M K2SO4 by horizontal shaking at 200 rev min^{-1} and filtered (hw3, Sartorius Stedim Biotech, Göttingen, Germany). Organic C and total N in the extracts were measured using a multi N/C® 2100S automatic analyser (Analytik Jena, Germany). Microbial biomass C was calculated as *EC/kEC*, where *EC* = (organic C extracted from fumigated soil) – (organic C extracted from non-fumigated soil) and *kEC* = 0.45 (Wu et al., 1990). Microbial biomass N was calculated as EN/kEN, where *EN* = (total N extracted from fumigated soil) – (total N extracted from non-fumigated soil) and *kEN* = 0.54 (Brookes et al., 1985).

The fungal cell-membrane component ergosterol was extracted from 2 g moist soil with 100 ml ethanol (96%) by 30 min oscillating shaking at 250 rev min^{-1} (Djajakirana et al., 1996). Quantitative determination of ergosterol was performed by reversed-phase HPLC

analysis with 100% methanol as the mobile phase and detected at a wavelength of 282 nm (Dionex UVD 170 L, Germering, Germany).

The amino sugars muramic acid (MurN), glucosamine (GlcN) and galactosamine (GalN) were determined according to Appuhn et al. (2004) using OPA (o-phthalaldehyd) derivatisation. 500 mg of oven-dried (60 °C) soil and 500 mg of fresh roots were hydrolysed with 10 ml of 6 M HCl for 6 h and 3 h, respectively, at 105 °C. Chromatographic separations were performed on a Hyperclone C18 column (125 mm length × 4 mm diameter) at 35 °C, using a Dionex P 580 gradient pump, a Dionex Ultimate WPS – 3000TSL analytical auto-sampler with in-line split-loop injection and thermostat and a Dionex RF 2000 fluorescence detector set at 445 nm emission and 330 nm excitation wavelengths. Fungal C was calculated by subtracting bacterial GlcN from total GlcN as an index for fungal residues, assuming that MurN and GlcN occur at a 1/2 molar ratio in bacterial cells (Engelking et al., 2007): mmol fungal C g-1 dry weight = (mmol GlcN − 2 × mmol MurN) × 9. Bacterial C was calculated as an index for bacterial residues by multiplying the concentration of MurN by 45 (Appuhn and Joergensen, 2006). Microbial residue C was the sum of fungal C and bacterial C.

4.2.4. Statistical analysis

The results presented in the tables are arithmetic means and expressed on an oven-dry basis (24 h at 105 °C). Normality of data distribution was tested using the Kolmogorov-Smirnoff and Shapiro-Wilk test and data were log transformed when appropriate. Mann-Whitney U test was used for analyzing the significance of differences between means of the 3 biogas and the means of the 3 raw slurries (n = 12, $P < 0.05$). The significance of differences within the two slurry types was tested by one way analyses of variance using post hoc Scheffé test (n = 4, $P < 0.05$). The significance of differences between the fertilization treatments was tested by one-way analysis of variance (ANOVA) using the Scheffé test ($P < 0.05$). Additionally, Pearson product moment correlation analyses were performed to determine relationships between selected indices. All statistical calculations were performed by SPSS Statistics 20.0 (SPSS Inc., Chicago, USA).

4.3. Results

On average, the biogas slurries had significantly higher pH values, higher concentrations of total N and NH_4-N, but lower concentrations of organic C than the raw slurries. This led to

a significantly lower C/N ratio of the biogas slurries in comparison with the raw slurries. The N related slurry properties showed a strong variability, leading to several significant differences within a slurry type (Table 3).

Biogas slurries increased the mean total aboveground plant biomass by 66% and raw slurries by 35% in comparison with the unfertilized control (Fig. 3a). In the biogas slurry treatments, the positive effects on plant growth were stronger after the first and also after the second cut. The plant biomass of the second cut contributed on average 48% to the total biomass of both cuts. The highest plant biomass was found after the application of biogas slurry 3. The biogas slurries increased the mean total aboveground N uptake by 166% and the raw slurries by 65% in comparison with the unfertilized control (Fig. 3b). The N uptake in the plant biomass of the second cut contributed on average only 29% to the total N uptake of both cuts. The total aboveground biomass had a strong non-linear relationship with the NH_4-N input (r^2 = 0.77) (Fig. 4).

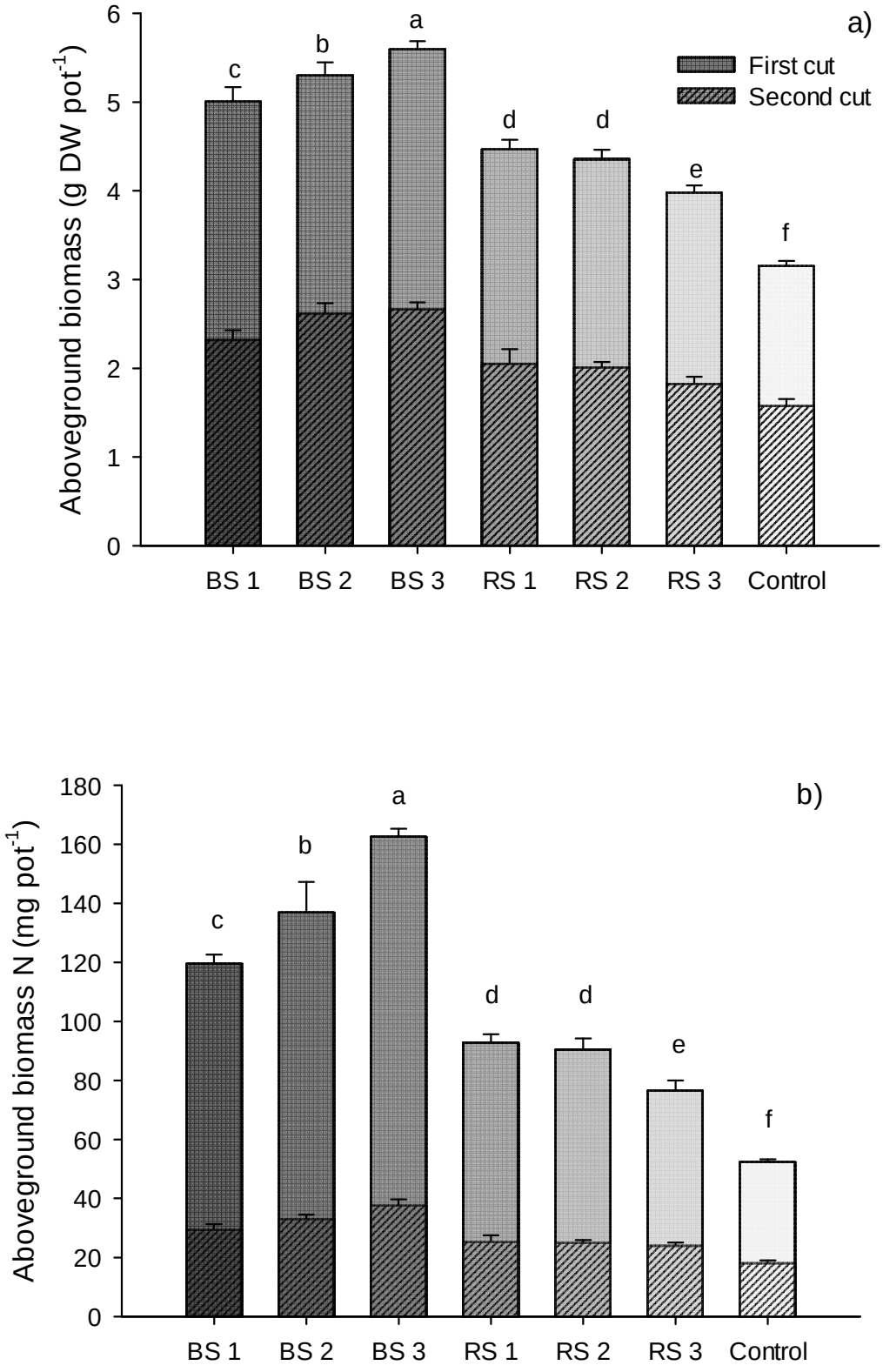

Fig. 4. (a) Aboveground biomass and (b) aboveground biomass N after first and second cut, different small letter indicates significant differences between treatments (Tukey HSD-test, $P < 0.05$, n = 4), BS = biogas slurry, RS = raw slurry.

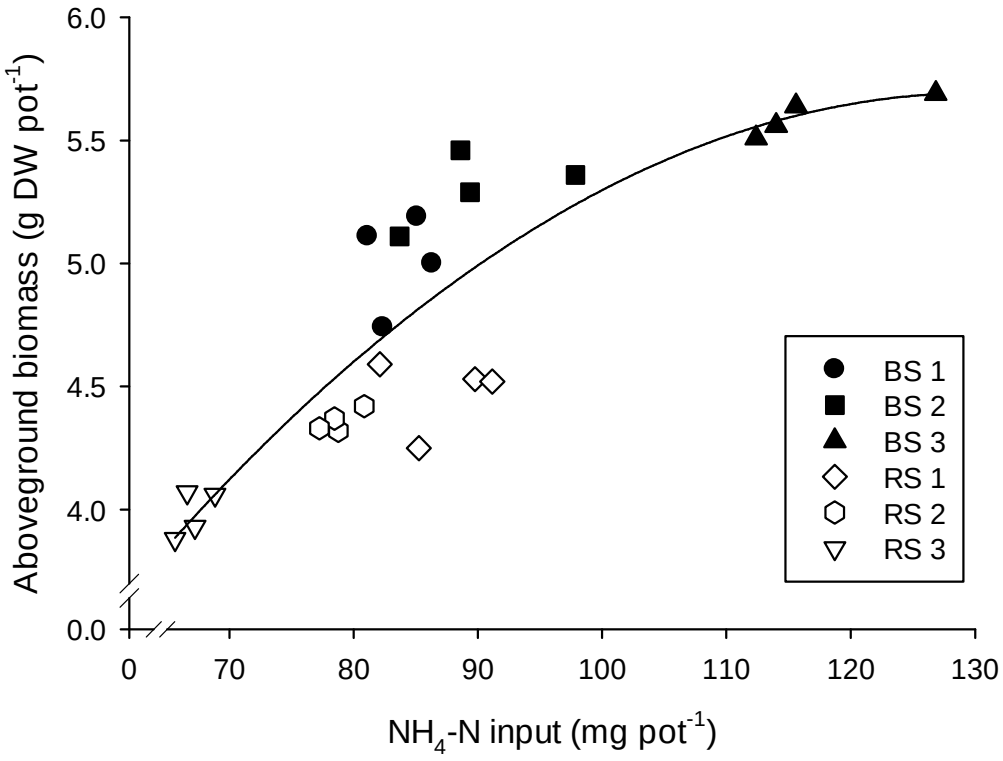

Fig. 5. Relationship between NH_4-N supply through slurries and plant yield ($y = -0.0004x^2 + 0.113x - 1.6611$, $r = 0.77$, n = 24, $P < 0.05$).

The mean root DM did not significantly differ between the slurries and the control pots and varied around 344 mg pot^{-1}. The biogas slurry treatments led to the significantly highest root N concentration (8.8 mg N g^{-1} root DM) in comparison with the raw slurry (8.3 mg N g^{-1} root DM) and the control treatments (7.7 mg N g^{-1} root DM) ($P < 0.05$, n = 28). In the root DM, slurry application significantly decreased the concentrations of MurN, GalN, and GlcN by 27, 39, and 27%, respectively, but not that of ergosterol compared to the control (Table 8). The differences between biogas and raw slurries but also those with the slurry types were small and non-significant in most cases. The ratio of fungal C to bacterial C ranged from 3.2 to 4.3 and was not affected by any treatment. However, the fungal C to bacterial C ratio was significantly correlated with the slurry NH_4-N concentration ($r = 0.42$, $P < 0.05$, n = 24). The application of biogas slurries resulted in small but nom-significant increases in soil microbial biomass C and N (Table 9). The application of raw slurries significantly increased microbial biomass C and N by roughly 27% in comparison with the unfertilized control.

Table 8

Contents of amino sugars and ergosterol as well as the ratio fungal C to bacterial C in rye grass roots after the second harvest in a slurry application experiment.

Slurry	MurN	GalN	GlcN	Fungal C/	Ergosterol
	($\mu g\ g^{-1}$ DW)		($mg\ g^{-1}$ DW)	bacterial C	($\mu g\ g^{-1}$ DW)
Biogas-SL1	55	135	1.1	3.7	18 a
Biogas-SL2	64	160	1.3	3.8	17 a
Biogas-SL3	49	184	1.1	4.3	11 b
Raw-SL1	99	181	1.7	3.0 b	15
Raw-SL2	69	170	1.5	4.1 a	16
Raw-SL3	64	169	1.2	3.6 ab	17
Mean biogas-SL	56 B	160 B	1.2 B	4.0	15
Mean raw-SL	78 AB	174 B	1.5 B	3.6	16
Control	124 A	396 A	2.4 A	3.5	17
CV (± %)	19	23	16	20	16

SL = slurry; CV = pooled coefficient of variation between the replicates (n = 4); different capitals indicate significant differences between the mean of the treatments, biogas slurry and raw slurry (n = 12), control (n = 4), different small letters indicate a significance within the slurries (n = 4, Scheffé, $P < 0.05$).

The application of biogas slurries significantly decreased the fungal ergosterol content in comparison with raw slurry and control treatments, leading to a significantly lower ergosterol to microbial biomass C ratio. The application of raw slurry had no effects on ergosterol content or the ergosterol to microbial biomass C ratio. In the slurry treatments, the soil ergosterol content was positively correlated with the C/N ratio of the slurries ($r = 0.60$, $P < 0.01$, n=24) and the DM content of the slurries ($r = 0.58$, $P < 0.01$, n=24). The amino sugar-based ratio of fungal C to bacterial C varied around 1.7 and was not affected by any treatment.

Table 9

Mean contents of microbial biomass C, N, ergosterol and the ergosterol to microbial biomass C ratio of the soils with different fertilizer treatments after harvest.

Slurry	Microbial biomass C ($\mu g\ g^{-1}$ soil)	Microbial biomass N ($\mu g\ g^{-1}$ soil)	Ergosterol ($\mu g\ g^{-1}$ soil)	Ergosterol/ Microbial biomass C (%)	Fungal C/ bacterial C
Mean biogas-SL	305 ab	65 ab	0.34 b	0.13 b	1.8
Mean raw-SL	330 a	70 a	0.50 a	0.16 ab	1.7
Control	263 b	55 b	0.45 a	0.20 a	1.6
CV (± %)	15	14	11	26	10

CV = pooled coefficient of variation between the replicates (n = 4); different letters indicate significant differences between the treatments, biogas slurry and raw slurry (n = 12), control (n = 4), (Scheffé-test, $P < 0.05$).

4.4. Discussion

The application of slurries to the soil generally increased plant yield, but the application of the biogas slurries revealed much stronger positive effects than that of the raw slurries. Fermentation of plant residues and co-substrates such as animal manures increases the availability of nutrients to plants, thus increasing plant yield (Khalil et al., 2000; Svensson et al., 2004). The difference between biogas and raw slurry was much stronger than the differences within the slurry types, although the slurries markedly differed in feedstock and co-substrate input. The increase in plant yield was mainly related to the amount of NH_4-N applied, which is in accordance with Fouda et al. (2013), studying the effects of different biogas slurries on ryegrass. However, the relationship between NH_4-N applied and aboveground plant yield was not linear, suggesting that other slurry components, e.g. the contents of plant-available P (Bachmann et al., 2011) or the absence of toxic organic acids (Lee et al., 1977; Salminen et al., 2001), may have additional positive or negative effects on plant growth. This might be the reason for the observation that the positive slurry effects on plant biomass observed after the first cut were maintained after second cut, although virtually all slurry-derived NH_4-N was apparently taken up by the rye-grass. This was indicated by the 6 times higher N concentration of the plant biomass after the first cut in comparison with the second cut, whereas negligible differences were observed for the plant biomass.

In contrast to the aboveground biomass, the root biomass did not differ between the treatments. This indicates that the high availability of plant nutrients after slurry application did not force the plants to form an extended root system. Microbial root colonization was significantly reduced by slurry application as shown by reduced concentrations of GalN and GlcN, but also MurN. This suggests a lower root colonization arbuscular mycorrhizal fungi (AMF) and AMF helper bacteria after slurry application. This assumption is in line with the observation that ergosterol, which does not occur in AMF (Olsson et al., 2003), remained unaffected by slurry application. High contents of plant available nutrients, especially P have been repeatedly observed to suppress AMF colonization (Nagahashi et al., 1996; Liu et al., 2000; Kahiluoto et al., 2000). The low P contents in the experimental soil were increased by slurry application, containing on average 7 mg P g^{-1} DW (Wentzel et al., 2015).

An average fungal C to bacterial C ratio of 3.7 in freshly colonized roots after slurry application means that the microbial tissue consisted of 79% fungal and 21% bacterial material. The fungal dominance within the root colonizing microbial community was even stronger than that within the organic matter of the experimental soil with 63% fungal and 37% bacterial contribution to microbial residues. In the root material, a large percentage of GlcN was located within the fungal biomass as indicated by a low GlcN to ergosterol ratio, varying between 80 and 140. This is in the range observed by Appuhn and Joergensen (2006) and Appuhn et al. (2006). In the experimental soil, the GlcN to ergosterol ratio exceeded 1000, suggesting strong accumulation of fungal residues in soil organic matter.

The application of biogas slurry did not affect the soil microbial biomass in comparison to raw slurry, supporting the results of Wentzel et al. (2015), obtained from arable soils after long-term application of biogas and raw slurries for 15 and more years. However, the significant decrease in soil ergosterol in the current biogas slurry treatments in comparison with the raw slurry application could not be statistically confirmed by the study of Wentzel et al. (2015), presumably masked by the clay effects and the high spatial variability of their field study. Higher ergosterol contents in the raw slurry than in the biogas slurry treatments were also found by Walsh et al. (2012) and explained by fungi promoting substances. Differences in the C availability to the soil microbial community could be another reason (Ernst et al., 2008; Clemens et al., 2006).

4.4.1. Conclusions

Biogas slurries have generally stronger plant growth promoting properties than raw slurries, mainly due to higher concentrations of NH_4-N. Biogas slurries did not affect microbial biomass C and N, but have negative effects on saprotrophic fungi which might be explained by the poorer C quality. Biogas and raw slurries both have similar negative effects on the microbial colonization of roots, most likely due to a reduced colonization with arbuscular mycorrhizal fungi in the presence of highly available plant nutrients.

Acknowledgements

We would like to thank the farmers Karl Kuch, Gerhard Kuch, Martin Frank, Ernst Bürkert, Marianne Holz, and Martin Lachenmaier for their good cooperation. Furthermore, we are very thankful for the help and great support of Reiner Schmidt and highly appreciate Gabriele Dormann for her technical assistance.

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5. Quantitative microbial indices in biogas and raw cattle slurries

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Abstract

Biogas slurry, the secondary product of the anaerobic digestion process, is increasingly used as fertilizer. For this reason, biogas and raw slurries obtained from 6 farms were analyzed for their ergosterol and amino sugar concentrations as indices for microbial biomass. A reliable, precise method for determining ergosterol in biogas and raw slurries is presented. Biogas slurries contained significantly less ergosterol (-34%), MurN (-42%), GalN (-32%), and fungal GlcN (-40%) than raw slurries. The mean fungal GlcN to ergosterol ratio (50) and also the mean fungal C to bacterial C ratio (0.29) did not significantly differ between the slurry types. The mean microbial C concentration in the biogas slurries was significantly lower than in the raw slurries. Consequently, the contribution of microbial C to slurry organic C was 3.6% in the biogas slurries and 5.7% in the raw slurries. Microbial C revealed significant non-linear relationships with the fiber and ash concentration, pH as well as the C/N ratio of the slurries.

Keywords: Organic fertilizer, Microorganisms; Biogas slurry; Raw cattle slurry; Ergosterol; Amino sugars

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5.1. Introduction

Biogas and raw cattle slurries are common organic fertilizers and an important source of nutrients in conventional as well as in organic farming systems (Odlare et al., 2011; Svenson et al., 2004; Möller et al., 2009). Chemical and physical characteristics of these slurries have been repeatedly investigated (Petz, 2000; Stinner et al., 2008). Anaerobic digestion in the biogas fermenter increases the concentration of NH_4-N and reduces dry matter (DM), leading to lower C concentrations and C/N ratio as well as to increased slurry pH values (Gutser et al., 1987; Möller et al., 2008; Möller and Müller, 2012). Information on microbial properties in biogas and raw slurries is mostly restricted to questions concerning pathogens in slurries (Munch et al., 1987; Kearney et al., 1993; Kudva et al., 1998; Govasmark et al., 2011) and not regarding the biomass of the total slurry inhabiting community.

For questions related to nutrient turnover and fertilizer value, the presence of pathogens is less important than the total biomass of the microorganisms that control further mineralization processes during storage and after application to soil (Jost et al., 2011). Feces contain a highly dynamic community of bacteria, archaea and fungi that has yet to be sufficiently quantified by the methods currently available. Most probable number and plate count approaches discriminate non-cultivable microorganisms, which contribute more than 80% to the total number of species (Ouwerkerk and Klieve, 2001). Direct microscopic approaches often severely underestimate fungi (Joergensen and Wichern, 2008). The extraction of DNA followed by analysis of its composition provides important information on the microbial community composition of feces (van Vliet et al., 2007; Sekhavati et al., 2009). However, DNA data usually provides no information on the biomass, due to losses during extraction, due to unknown or highly variable concentrations within different microbial species (Leckie et al., 2004; Joergensen and Emmerling, 2006) or due to occurrence in dead microorganisms (Pisz et al., 2007; Bae and Wuertz, 2009). ATP has been estimated in fecal samples (Wolstrup and Jensen, 2008), but the AEC and thus the ATP concentration within the microbial biomass is more variable in the anaerobic or micro-aerobic environment of slurries than in soil (Jenkinson, 1988; Dyckmans et al., 2006).

In soil, fungi are the principal decomposer of complex organic matter (Joergensen and Wichern, 2008). In slurries, fungi are neglected due to their sensitivity to anaerobic environments (Among, et al., 2006; Procházka et al., 2012), although e.g. cattle feces contain significant amounts (Jost et al., 2011, 2013). In their experiments, fungal biomass was estimated by ergosterol and glucosamine (GlcN) analysis. Ergosterol is an important

component of fungal cell membranes, occurring in Basidiomycota, Ascomycota, and the majority of Zygomycota, and is responsible for their stability (Weete and Weber, 1980). Ergosterol has been successfully determined in a variety of solid substrates such as soils (Joergensen and Wichern, 2008; Strickland and Rousk, 2010), but not in biogas and raw cattle slurries.

The same is almost true for amino sugars, although GlcN has previously been used in rumen fluid of cattle for detecting fungi (Sekhavati et al., 2009). GlcN occurs in the cell walls of fungi, bacteria and archaea, muramic acid (MurN) exclusively in the cell walls of all bacteria, especially those of Gram positive bacteria (Appuhn and Joergensen, 2006). In soil, most amino sugars are bound to soil organic matter as microbial residues (Amelung, 2001; Amelung et al., 2008). However, this was not the case in freshly and dynamically decaying feces, where the largest percentage of GlcN and MurN was still in the fungal and bacterial biomass (Jost et al., 2011, 2013). For this reason, ergosterol and amino sugars were analyzed in biogas and raw slurries obtained from the 6 farms participating in the study of Wentzel et al. (2015), to investigate the following hypotheses: (1) Biogas slurries contain less ergosterol and amino sugars than raw slurries. (2) Fungi contribute less biomass to the total microbial tissue in biogas slurries than in raw slurries, because they respond sensitively to the strongly anaerobic environment of the biogas fermenter. A special focus of the present investigation was to modify the ergosterol extraction procedure to obtain reliable results in slurries containing large amounts of water.

5.2. Material and Methods

5.2.1. Slurry sampling and chemical analysis

The slurry sampling and analyses are described in chapter 3.2.2. In addition, crude ash and crude fiber was analyzed according to the conventional Weende procedure (Naumann and Bassler, 1997).

5.2.2. Ergosterol analysis

To determine ergosterol in the slurries, 0.5 g of freeze-dried sample material (equivalent to 6-12 g slurry fresh matter) was weighed into a 30 ml test tube. Then, 1 g KOH, 10 ml of methanol and 2.5 ml ethanol were added to each sample, thoroughly vortexed, and refluxed for 90 min at 70 °C (Zelles et al. 1987; Jost et al., 2011). After cooling, the non-polar fraction

was separated from the alkaline extractant by adding 10 ml n-hexane and swinging 10 times, before the top 5 ml of the supernatant were removed and transferred to a round brown glass flask. Then, 10 ml n-hexane was added again to the alkaline extractant and the separation procedure was repeated. The combined supernatants were evaporated to dryness in a rotary evaporator at 40 °C. Afterwards, the glass flask was rinsed 3 times with 3 ml of methanol, this being transferred to a volumetric flask after each step and then topped up to 10 ml with methanol. The extract was filtered through a syringe filter into 10-ml plastic tubes and stored for a maximum of 7 days at 4 °C until measurement with the Gynkotek 480 HPLC (Dionex, Germering, Germany). Ergosterol was separated with 100% methanol as the mobile phase at a flow rate of 0.5 ml h^{-1}, and detected with a diode-array detector (Dionex 170 S) at 282 nm, with a 30-min run and 50 µl sample injected. The column was conditioned with the mobile phase for 0.5 h at a flow rate of 1.0 ml min^{-1} before each measurement. The runtime extension, the higher flow rate and the change of injection quantity led to a better integration of ergosterol peaks during HPLC measurement (Fig. 7).

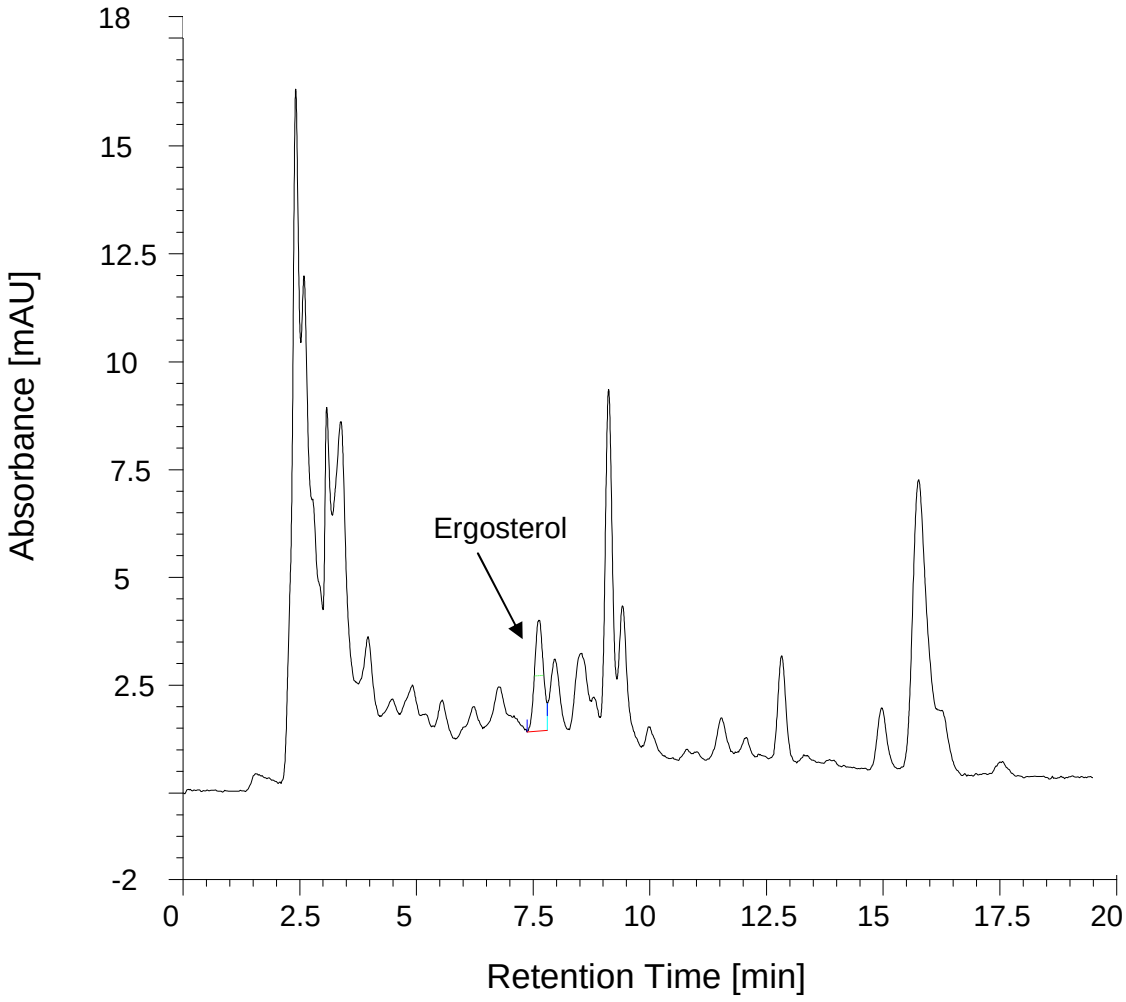

Fig. 6. Chromatogram of an ergosterol peak from HPLC measurement and the appearance of ghost-peaks while testing different runtimes.

5.2.3. Amino sugar analysis

The amino sugars muramic acid (MurN), glucosamine (GlcN) and galactosamine (GalN) were determined according to Appuhn et al. (2004) using OPA (o-phthalaldehyd) derivatisation. 500 mg of freeze-dried slurries were hydrolyzed with 10 ml of 6 M HCl for 3 h at 105 °C and filtered. Chromatographic separations were performed on a Hyperclone C_{18} column (125 mm length $\times$ 4 mm diameter) at 35 °C, using a Dionex P 580 gradient pump, a Dionex Ultimate WPS – 3000TSL analytical autosampler with in-line split-loop injection and thermostat and a Dionex RF 2000 fluorescence detector set at 445 nm emission and 330 nm excitation wavelengths. Fungal C was calculated by subtracting bacterial GlcN from total GlcN as an index for fungal residues, assuming that MurN and GlcN occur at a 1–2 molar ratio in bacterial cells (Engelking et al., 2007): mmol fungal C g^{-1} dry weight = (mmol GlcN −

2 × mmol MurN) × 9. Bacterial C was calculated as an index for bacterial residues by multiplying the concentration of MurN by 45 (Appuhn and Joergensen, 2006). Microbial residue C was estimated as the sum of fungal C and bacterial C.

5.2.4. Statistical analysis

The results presented in the tables are arithmetic means and expressed on an oven-dry basis (24 h at 105 °C). The Mann-Whitney U-test was used for analyzing the significance of differences between the two slurry types. The significance of differences within the slurry types was tested by one way analyses of variance, using the Scheffé post hoc test. The relationships between microbial C and fiber, ash, pH and C to N ratio were tested by regression analysis. All statistical calculations were carried out using SPSS 17.0 (SPSS Inc., Chicago, USA).

5.3. Results

On average, the biogas slurries had a significantly higher pH as well as higher concentrations of total N, NH_4-N and crude ash, but a lower C/N ratio and a lower concentration of crude fiber than the raw slurries. In the separated biogas slurry 3, C/N ratio and crude fiber concentration were lowest and crude ash concentration highest (Table 3, 10).
The ergosterol concentration in the slurries ranged from 6.9 to 16 µg g^{-1} DM (Table 11). In the biogas slurries, the mean ergosterol concentration was 34% lower than in the raw slurries. Significant differences were also found within the slurry types. Ergosterol and slurry pH were negatively correlated (r = 0.68, P < 0.01). In the biogas slurries, the mean concentrations of MurN, GalN and fungal GlcN were 0.24, 0.62, and 0.35 mg g^{-1} DM, respectively (Table 11). These values were roughly 40% lower for MurN and GlcN as well as 30% lower for GalN than the respective mean values in the raw slurry. The mean fungal GlcN to ergosterol ratio (50) and also the mean fungal C to bacterial C ratio (0.29) did not differ significantly between the slurry types. The mean microbial C concentration in the biogas slurries (14 mg g^{-1} DM) was significantly lower than in the raw slurries (24 mg g^{-1} DM). Consequently, the contribution of microbial C to slurry organic C was 3.6% in the biogas slurries and 5.7% in the raw slurries. Microbial C showed significant non-linear relationships with the fiber concentration (r^2 = 0.59, Fig. 7a), the ash concentration (r^2 = -0.61, Fig. 7b), the pH (r^2 = 0.58, Fig. 7c) and the C/N ratio (r^2 = 0.71, Fig. 7d) of the slurries.

Table 10

Crude fiber and crude ash of different biogas- and raw slurries.

	Biogas slurry			Raw slurry			Mean		CV
	1	2	3	1	2	3	Biogas-SL	Raw-SL	(±%)
Crude fibre (% DM)	23 a	24 a	8.1 b	24 b	26 ab	27 a	18 B	26 A	10
Crude ash (% DM)	26 c	31 b	39 a	26	24	22	32 A	24 B	5

CV = pooled coefficient of variation between treatments (n = 4); small letters indicate differences within slurry treatments ($P < 0.05$, Scheffé-test, n = 4); capitals indicate differences between means of biogas and raw slurries (Mann-Whitney-U Test, $P < 0.05$, n = 12).

Table 11

Contents of muramic acid (MurN), galactosamine (GalN), fungal glucosamine (fungal GlcN) and ergosterol in biogas and raw slurries.

	MurN	GalN	Fungal GlcN	Ergosterol
	(mg g^{-1} DM)			(µg g^{-1} DM)
Biogas slurry 1	0.26 a	0.63 ab	0.45 a	6.9 b
Biogas slurry 2	0.27 a	0.68 a	0.40 a	10 a
Biogas slurry 3	0.20 b	0.54 b	0.25 b	7.2 b
Raw slurry 1	0.46	0.91	0.55	16 a
Raw slurry 2	0.40	0.91	0.63	7.3 c
Raw slurry 3	0.39	0.89	0.60	14 b
Mean biogas slurry	0.24 B	0.62 B	0.37 B	8.0 B
Mean raw slurry	0.42 A	0.90 A	0.59 A	12 A
CV (± %)	10	6	14	7

DM = dry matter; CV = pooled coefficient of variation between the replicates (n = 3); small letters indicate differences within slurries treatments ($P < 0.05$, Scheffé-test, n = 4); capitals indicate differences between means of biogas and raw slurries (Mann-Whitney-U Test $P < 0.05$, n =12).

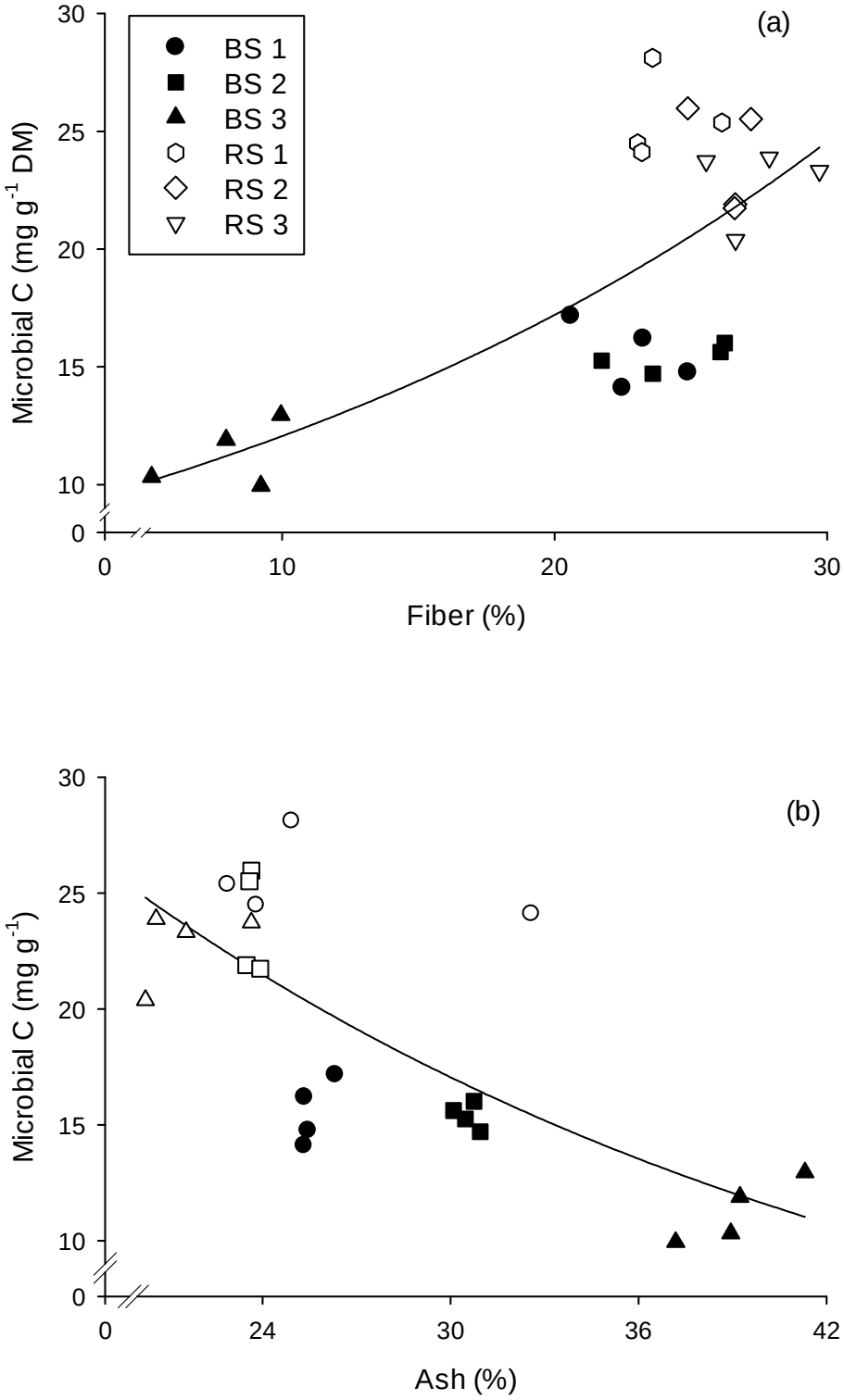

Fig. 7a+b. Regression analysis of microbial C against (a) slurry fiber, $y = 8.4013e^{0.0349x}$, r^2 = 0.59 and (b) slurry ash, $y = 53.922e^{-0.039x}$, r^2 = 0.61 for all slurries investigated; data points represent values of each replicate.

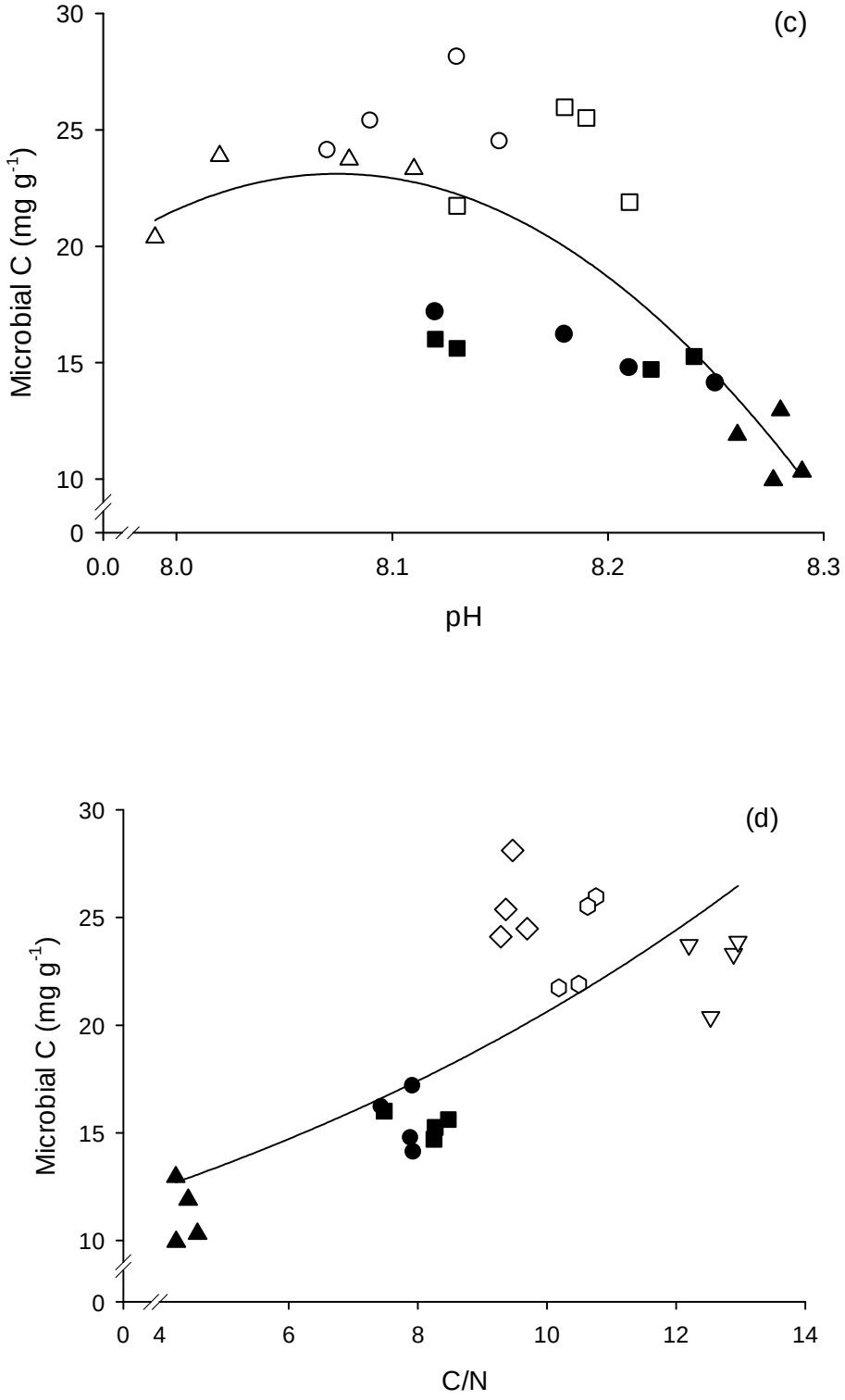

Fig. 7c+d. Regression analysis of microbial C against (c) slurry pH, $y = -279.88x^2 + 4519.6x - 18223$, $r^2 = 0.58$ and (d) the slurry C to N ratio, $y = 7.5461^{e0.1001x}$, $r^2 = 0.71$ for all slurries investigated; data points represent values of each replicate.

5.4. Discussion

5.4.1. Modifications of the ergosterol method

The method of Djajakirana et al. (1996) for determining ergosterol in soil by using ethanol as extractant is not suitable for organic materials like feces (Jost et al., 2011). They used the saponification method of Zelles et al. (1987), where petroleum ether was used for separating the non-polar fraction from the alkaline methanol / ethanol extractant. However, this method was also unreliable for determining ergosterol in biogas and raw cattle slurries, due to the strong variability between replicates and difficulties in integrating the ergosterol peak. Zill et al. (1988) tested n-hexane as a substitute for petroleum ether, resulting in fewer impurities during the HPLC measurements. Extension of the HPLC runtime from originally 13 min for soil and cattle feces to 40 min for slurries, and an increase in the flow rate from 0.5 to 0.8 ml min^{-1} was necessary to remove impurities from the HPLC column. Finally, the injection volume was reduced from 100 to 50 µl to reduce peak width and peak tailing. Biogas slurries and raw slurries have higher water contents in comparison with soil, increasing the variability between replicates. Consequently, samples were freeze-dried for ergosterol analysis. As a consequence, the coefficient of variation was markedly reduced compared with the data presented by Jost et al. (2011, 2013).

5.4.2. Ergosterol and amino sugar concentrations in slurries

Biogas slurries contain markedly lower ergosterol and amino sugar concentrations than raw slurries, leading also to a significantly smaller contribution of amino sugar-based microbial C to slurry organic C. The relative decrease in the microbial indices analyzed strongly suggest a preferential turnover of microbial tissue in comparison with the fermenter feedstock, i.e. feces, manure, plant residues, etc. The contribution of microbial C to slurry organic C did not exceed 15% in any case, contrasting the situation of aerobic soil environments, where microbial C reaches maximum contributions of 80% and more to soil organic C (Murugan et al., 2014; Wentzel et al., 2015). The absence of microbial residue formation during storage seems to be a striking feature of raw slurries, but especially of biogas slurries. The positive relationships between the fiber content and the C/N ratio of the slurries indicates that the quality of the feedstock in combination with differences in the fermentation process, e.g. temperature, water content, and residence time, affect slurry microorganisms (Weiland, 2010; Sahlström, 2003). The stronger the processing and decomposition of the fermenter feedstock, the lower the C/N ratio, the higher the NH_4^+

concentration and pH, and consequently the lower the concentration of microbial indices in the remaining slurries.

Concentrations of the fungal cell-membrane component ergosterol in the current biogas and raw slurries are similar to those in the feces of heifers and dairy cows reported by Jost et al. (2011, 2013 a, b), who measured a range from 2.2 to 13.2 µg ergosterol g^{-1} DM. The concentrations of cell wall-derived microbial C was also similar, but at the lower end of the concentrations obtained by Jost et al. (2011, 2013 a, b), who measured a range from 21 to 50 mg microbial C g^{-1} DM. Consequently, the ratio of fungal GlcN to ergosterol is smaller in the current slurries than in cattle feces and only slightly above the chitin to ergosterol ratio of 40 obtained by Matcham et al. (1985) in liquid cultures of *Agaricus bisporus*. This suggests that not only ergosterol but also fungal GlcN occurs predominantly in fungal biomass in biogas and raw slurries. The relatively low fungal GlcN to ergosterol ratio is remarkable, considering the fact that ergosterol does not occur in all fungal species of anaerobic environments. Of anaerobic fungi, yeasts such as *Candida* sp. (Ahmad et al., 2010) or *Saccharomyces cerevisia* (Aguilera et al., 2006) contain high concentrations of ergosterol, also food spoiling *Mucor plumbeus* (Taniwaki et al., 2009). In contrast, no ergosterol was measured in chytridiomycetes (Weete et al., 1989; Kagami et al., 2007). The same might be true for anaerobic fungal species found in cattle rumen such as Anaeromyces, Orpinomyces, Caecomyces, or Piromyces (Griffith et al., 2009).

The similarity of the ratios fungal glucosamine to ergosterol and fungal C to bacterial C is another striking feature of the current slurries. The differences in the chemical composition of the feedstock and the differences in the processing of the feedstock in biogas fermenters did not lead to strong differences in the basic composition of the two main decomposing microbial groups, neither in comparison to the current raw slurries nor to cattle feces (Jost et al., 2011, 2013 a, b). The average fungal C to bacterial C ratio for biogas slurries and raw slurries was 0.29, which means that microbial C consists of 23% fungal C and 77% bacterial C, neglecting the possible presence of archaea. The slurry microbial community is clearly dominated by bacteria, but contains a significant fungal minority. This is in line with results reported for cattle feces by Jost et al. (2013 a, b), who measured a range in fungal C to bacterial C ratios from 0.34 to 1.1. The current results are consistent with to the view of McGranaghan et al. (2006) that many fungi remain viable in the anaerobic environment of cattle feces, slurries and manures for long periods. In contrast, Procházka et al. (2012) observed that rumen cultivable fungi do not exhibit long-term survival in biogas fermenters.

5.4.3. Conclusions

A reliable, precise method for determining ergosterol in biogas and raw slurries has been presented. Biogas slurries contain markedly lower ergosterol, MurN, GalN, and fungal GlcN concentrations than raw slurries, which results in a significantly smaller contribution of amino sugar-based microbial C to slurry organic C. However, the ratios fungal glucosamine to ergosterol and fungal C to bacterial C were almost identical in biogas and raw cattle slurries, despite the strong anaerobic organic matter turnover in the biogas fermenters.

Acknowledgements

We would like to thank the farmers Karl and Gerhard Kuch, Martin Frank, Ernst Bürkert, Marianne Holz, and Martin Lachenmaier for their good cooperation. We highly appreciate Gabriele Dormann and Nicole Gaus for their skilled technical assistance.

5.5. References

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6. Zusammenfassung

Biogasgüllen haben bezüglich ihrer Wirkung auf die Bodenfruchtbarkeit, das Pflanzenwachstum und die Umwelt, Vor- und Nachteile. Als Folge der intensiven Nutzung von Biogas zur Energieerzeugung wurde die Biogasgülle eine der wichtigsten organischen Dünger, mit zunehmender Relevanz für den konventionellen aber auch den ökologischen Landbau. Aufgrund der frühen Entwicklung von Biogas und dem Einsatz von Biogasgüllen in der ökologischen Landwirtschaft in den achtziger Jahren im Nord-Osten Baden-Württembergs sind heute Ackerflächen vorhanden, die seit mehr als 25 Jahren mit Biogasgülle gedüngt werden. Somit bestand hier eine Möglichkeit, Informationen über die Langzeiteffekte von Biogasgüllen auf Parameter der Bodenfruchtbarkeit in der ökologischen, landwirtschaftlichen Praxis zu erlangen.

Im ersten Projekt wurde daher eine On-Farm Boden- und Gülleprobenahme durchgeführt. Ziel war es, Parameter der Bodenfruchtbarkeit wie die mikrobielle Aktivität (Basalatmung), den mikrobiellen Biomasse C und N, pilzliches Ergosterol, mikrobielle Residuen (Aminozucker), organischen C, gesamt N und den pH-Wert im Boden auf fünf Flächenpaaren des biologisch-dynamischen Landbaus zu messen. Die sich daraus ergebende Hypothese war hierbei, dass der langjährige Einsatz von Biogasgülle im Vergleich zu Rohgüllen keinen Effekt auf die organische Bodensubstanz und die mikrobiellen Eigenschaften des Bodens hat, da die negativen Effekte wie zum Beispiel der reduzierte C-Input durch positive Effekte wie z.B. die erhöhte Nährstoffverfügbarkeit für Pflanzen kompensiert wird. Die Ergebnisse zeigten, dass die Langzeitanwendung von Biogasgülle keinen negativen Einfluss auf die C- und N-Vorräte im Boden hatte. Der Einsatz von Biogasgülle führte jedoch zu einem engeren Verhältnis von mikrobiellem C zu organischem C im Boden, was auf eine reduzierte C-Verfügbarkeit für Mikroorganismen im Vergleich zu Rohgüllen schließen lässt. Die Biogasgülleanwendung führte zu einer tendenziellen Abnahme der mikrobiellen Residuen, wobei die unterschiedlichen Tongehalte der untersuchten Flächen mögliche signifikante Gülleeffekte auf die mikrobiellen Eigenschaften verdeckt haben. Es gab keine generellen Effekte der Biogasgülle auf das Verhältnis von Pilzen zu Bakterien, wohingegen ein zunehmender Tongehalt eine signifikante Verlagerung in Richtung der Bakterien verursachte. Die Übereinstimmung der erhobenen Daten aller angewendeten Methoden weist auf die starke Aussagekraft dieser On-Farm-Studie zum Vergleich benachbarter Flächen hin.

Im Anschluss an das On-Farm-Projekt, in dem die Langzeitwirkung von Biogasgülle auf den Boden untersucht wurde, sollten die Auswirkungen der Düngung mit unterschiedlichen Biogas-und Rohgüllen unter kontrollierten Bedingungen geprüft werden. Daher wurde ein 70-

tägiger Gewächshausversuch mit den Güllen der biologisch-dynamischen Betriebe auf einem tonigen Schluff und unter Weidelgras (*Lolium multiflorum,* var. Ligrande) durchgeführt. Ziel war es, die Effekte unterschiedlicher Güllen auf das Pflanzenwachstum und die mikrobiellen Eigenschaften im Boden und an Wurzeln zu untersuchen. Die Düngung erhöhte die durchschnittliche oberirdische Pflanzenbiomasse um 69% unter Biogasgülle und um 36% unter Rohgülle im Vergleich zur ungedüngten Kontrolle. Zwischen der oberirdischen Biomasse und dem zugeführten NH_4-N wurde ein stark linearer Zusammenhang festgestellt. Im Gegensatz zu den Biogasgüllen gab es unter den Rohgüllen einen signifikanten Anstieg des mikrobiellen Biomasse C und N um etwa 25% verglichen zur ungedüngten Kontrollvariante. Der Einsatz von Biogasgüllen führte gegenüber der Rohgülle und Kontrolle zu geringeren Ergosterolgehalten im Boden, was ein engeres Verhältnis von Ergosterol zu mikrobieller Biomasse C zur Folge hatte. Bezogen auf die Wurzeltrockenmasse verringerte die Düngung die Konzentrationen der Aminozucker Muraminsäure, Galaktosamin und Glukosamin um 24, 29 und 37%, gleichzeitig wurde jedoch kein Einfluss auf die Ergosterolgehalte festgestellt. Dies war höchstwahrscheinlich Folge der reduzierten Kolonisierung mit arbuskulärer Mykorrhiza in Gegenwart ausreichend verfügbarer Pflanzennährstoffen.

Um einen Eindruck über die mikrobielle Biomasse und die mikrobielle Gemeinschaft in Biogas- und Rohgüllen zu bekommen, wurde ein drittes Projekt durchgeführt. Die von den 6 biologisch-dynamischen Betrieben erhaltenen Güllen wurden auf ihre Ergosterol- und Aminozuckerkonzentrationen untersucht. Hierbei entstand eine zuverlässige und präzise Methode zur Bestimmung von Ergosterol in Biogas- und Roghüllen. Die Biogasgüllen enthielten signifikant geringere Konzentrationen an Ergosterol (-34%), MurN (-42%), GalN (-32%) und pilzlichem GlcN (-40%) im Vergleich zu den Rohgüllen. Die durchschnittlichen Verhältnisse von pilzlichem GlcN zu Ergosterol (50) und pilzlichem C zu bakteriellem C (0.29) zeigten keine signifikanten Unterschiede zwischen den Gülletypen. Die durchschnittliche Konzentration von mikrobiellem C war in Biogasgülle signifikant geringer als in Rohgülle. Demzufolge lag der Anteil des mikrobiellen C am organischen C bei 3.6% in den Biogasgüllen und 5.7% in den Rohgüllen. Zwischen dem mikrobiellen C der Güllen und deren Faser- und Aschegehalte, sowie den pH-Werten und den C/N-Verhältnissen konnten nicht-lineare Zusammenhänge festgestellt werden.

7. Summary

Biogas slurries are known to have advantages and disadvantages regarding soil fertility, plant growth and environment. Since the increasing use of biogas for energy production, biogas slurry has become one of the main organic fertilizers today, with growing relevance also in organic farming systems. Due to the early development of biogas in organic farming in the eighties in the north-east of Baden-Württemberg, arable fields which were fertilized since more than 25 years with biogas slurries exist. Hence, there is a possibility to get information about the long-term effects of biogas slurry application on soil fertility indices in organic farming practice.

An on-farm soil and slurry sampling was carried out in the first project. The objectives were to measure the soil fertility indices, e.g. microbial activity (basal respiration), microbial biomass C and N, fungal ergosterol, microbial residues (amino sugars), soil organic C, total N, soil pH and clay content at five neighbouring sites under biodynamic management using either biogas or raw slurries. The underlying hypothesis was that the long-term application of biogas slurries has no effects on soil organic matter, microbial residues and biomass indices, because their negative effects, i.e. reduced C input, are compensated by their positive effects, i.e. increased nutrient availability to plants. The results showed that long-term application of biogas slurry did not affect SOC and total N stocks and had no general negative effects on soil fertility. Biogas slurry application reduced the microbial biomass C to SOC ratio, indicating a reduced availability of the biogas slurry C to the soil microorganisms compared with raw slurry. Biogas slurry application tended to decrease the stocks of microbial residues, but differences in clay content masked any slurry effects on the microbial activity, biomass and residues at some sites. There was no general effect of biogas slurry on the microbial community structure in terms of the ratio of fungi to bacteria, whereas increasing clay content caused a significant shift towards bacteria. The consistency in the data of all approaches strongly indicates the validity of the current on-farm study by comparing neighbouring fields.

After the on-farm research, where we examined soil after many years of biogas slurry application, there was the need to focus even more on the slurries when conditions like soil texture, climatic parameters and plants are the same. For this reason, biogas and raw slurries obtained from 6 biodynamic farms were added to a soil, planted for 70 days with Italian rye-grass (*Lolium multiflorum*, var. Ligrande) to investigate the effects on plant growth, soil microbial biomass, soil fungi, and root colonizing microorganisms. Biogas slurries and raw slurries increased the mean total aboveground plant biomass by 69% and 36%, respectively, in comparison with the unfertilized control. The total aboveground biomass had a strong non-

linear relationship with the NH_4-N input. In contrast to biogas slurries, the raw slurries significantly increased microbial biomass C and N by roughly 25% in comparison with the unfertilized control. The application of biogas slurries significantly decreased the soil ergosterol content in comparison with raw slurry and control treatments, leading to a significant lower ergosterol to microbial biomass C ratio. In the root DM, biogas and raw slurry application significantly decreased the concentrations of the amino sugars muramic acid, galactosamine and glucosamine by 24, 39, and 27%, respectively, but not that of ergosterol in comparison with the control. This was most likely due to a reduced colonization with arbuscular mycorrhizal fungi in the presence of highly available plant nutrients. The differences between biogas and raw slurries but also those within the slurry types were small and nin-significant in most cases.

Trying to get an impression about the slurry microbial biomass and community composition a third project was conducted. Biogas and raw slurries obtained from 6 farms were analyzed for their ergosterol and amino sugar concentrations as indices for microbial biomass. A reliable, precise method for determining ergosterol in biogas and raw slurries has been presented. Biogas slurries contained significantly lower ergosterol (-34%), MurN (-42%), GalN (-32%), and fungal GlcN (-40%) concentrations than raw slurries. The mean fungal GlcN to ergosterol ratio (50) and also the mean fungal C to bacterial C ratio (0.29) did not significantly differ between the slurry types. The mean microbial C concentration in the biogas slurries was significantly lower than in the raw slurries. Consequently, the contribution of microbial C to slurry organic C was 3.6% in the biogas slurries and 5.7% in the raw slurries. Microbial C revealed significant non-linear relationships with the fiber and ash concentration, pH as well as the C/N ratio of the slurries.

8. Schlussfolgerungen und Ausblick

In der vorliegenden Arbeit konnte gezeigt werden, dass der langjährige Einsatz von Biogasgüllen auf Flächen des biologisch-dynamischen Landbaus, keinen Effekt auf die C- und N-Vorräte im Boden und somit auch keine sichtbare negative Wirkung auf die Bodenfruchtbarkeit hatte. Jedoch verringerte sich der Anteil des mikrobiellen C am organischen C im Vergleich zu den Rohgülleflächen was auf eine reduzierte Verfügbarkeit des Biogasgülle C hindeuten könnte. Nicht zu vernachlässigen war der Effekt des Tongehalts auf einigen Flächen, welcher bestimmte Gülleeffekte auf mikrobielle Parameter maskiert hat. Generell hatten die Biogasgülle keinen Einfluss auf die mikrobielle Gemeinschaft während ein Anstieg des Tongehaltes eine signifikante Verschiebung hinzu Bakterien zur Folge hatte. Die Plausibilität der erhobenen Daten weist stark auf die Eignung des On-farm-Versuches zum Vergleich benachbarter, unterschiedlich gedüngter Flächen hin. Es sollten jedoch zukünftig mehr Forschungsansätze auf Praxisflächen zur Wirkung von Biogasgüllen auf Parameter der Bodenfruchtbarkeit durchgeführt werden, um die hier erhaltenen Ergebnisse auch für andere Standorte und Landwirtschaftssysteme abzusichern. Des Weiteren würde die Analyse zur Auswirkung von Biogasgüllen auf die biotrophen, arbuskulären Mykorrhizapilze weitere Erkenntnisse liefern, da diese einen wesentlichen Beitrag zur Nährstoffversorgung in landwirtschaftlichen Systemen liefern, jedoch durch Ergosterolmessungen nicht erfasst werden können.

Der Gefäßversuch im zweiten Projekt lieferte weitere Einblicke zur Wirkung von Biogas- und Rohgüllen auf Parameter der Bodenfruchtbarkeit. Vorteil dieser Untersuchungen war die Anlehnung an das erste Projekt, da hier die Biogas- und Rohgüllen der beteiligten Betriebe und deren Auswirkungen auf Boden, Mikroorganismen und Pflanze unter gleichen Bedingungen untersucht werden konnten. Alle eingesetzten Biogasgüllen führten zu einem signifikant höheren Pflanzenertrag und höheren Nährstoffgehalten in den Pflanzen verglichen zu den Rohgüllen. Die erhöhten NH_4-N-Gehalte in den Biogasgüllen haben hierbei die Erträge stark beeinflusst. Wie schon im ersten Projekt, wurden zwischen den beiden Güllevarianten nur minimale Unterschiede in der mikrobiellen Biomasse im Boden gefunden. Der Einsatz von Biogasgülle verringerte den Anteil der Saprotrophen Pilze an der mikrobiellen Biomasse im Boden, was mit der Qualität des zugeführten C erklärt werden könnte. Man konnte jedoch beobachten, dass für die Einzelbetrachtung der Biogasgüllen die generellen Effekte unterschiedlich stark zu sehen waren. Es lässt sich deshalb annehmen, dass neben dem Fermentationsprozess auch der Substratinput und/oder nachgeschaltete Verfahren

wie die Separierung der Biogasgüllen einen starken Einfluss auf mikrobielle Parameter im Boden haben.

Um Erkenntnisse über die Mikrobiologie von Güllen zu bekommen, wurden quantitative Methoden, die ursprünglich in Böden, Wurzeln und Rinderkot angewendet werden, durchgeführt. Die Bestimmung der Ergosterolgehalte erzielte nach kleinen Veränderungen der schon etablierten Methode, wiederholbare und repräsentative Ergebnisse. Diese zeigten geringere Ergosterolgehalte in den Biogasgüllen im Vergleich zu den Rohgüllen, was in Zusammenhang mit den chemischen Eigenschaften gebracht werden konnte, jedoch auch eine Folge der Fermentation sein dürfte. Neben den geringeren Ergosterolgehalten wurden auch geringere Gehalte an Aminozuckern festgestellt. Gleichzeitig zeigten beide Gülletypen eine ähnliche Zusammensetzung der mikrobiellen Gemeinschaft welche in beiden Fällen von Bakterien dominiert wird. Aufgrund der guten Wiederholbarkeit der Ergosterol- und Aminozuckerbestimmung, bieten diese Methoden im Rahmen von Gülleuntersuchungen einen zusätzlichen Informationsinput. Hierzu wären jedoch weitere Forschungsansätze sinnvoll, die zur Aufklärung über die Ursachen reduzierter, mikrobieller Biomasse in Biogasgüllen beitragen.

Eine der wichtigsten Mikroorganismengruppen, die Archaeen, wurde in den 3 Projekten vernachlässigt und sollte zukünftig weiter in den Fokus rücken. Weitere Methoden zur Quantifizierung der mikrobiellen Biomasse in Güllen sollte ebenfalls etabliert werden, da man davon ausgehen kann, dass bestimmte Mikroorganismen aus den Güllen im Boden überleben und die Folgen der applizierten Mikroorganismenfrachten noch nicht erforscht sind. Hierzu wäre die Bestimmung der mikrobiellen Biomasse mit Hilfe die CFE-Methode (Brookes et al., 1985; Vance et al., 1987) eine Möglichkeit, da hierbei auch die Archaeenbiomasse erfasst wird. Die luminometrische Bestimmung von Adenosintriphosphat (ATP) (Lucifirin-Luciferase-Assay nach Jenkinson und Oades, 1979) bietet einen weiteren Ansatz zur Abschätzung der mikrobiellen Biomasse und sollte in Anlehnung an Untersuchungen im Boden (Jenkinson und Ladd, 1981) und ersten, schon durchgeführten Untersuchungen im Kot durch Jost et al. (2011) auch in Biogas- und Rohgüllen durchgeführt werden. Bezüglich der CFE- und ATP-Methode, sollte jedoch über mögliche Schwierigkeiten dieser Methoden in sehr flüssigen Substraten und über eine eventuell erforderliche Neuberechnung der Extraktionsverhältnisse nachgedacht werden. Durch die hohe Artspezifität von PLFA (Phospholipidfettsäuren) bietet sich eine weitere Möglichkeit zur Bestimmung spezifische Organismengruppen im Boden (Tunlid und White, 1992) und könnte, angewendet in Biogas- und Rohgüllen, zur weiteren Charakterisierung der mikrobiellen Eigenschaften beitragen. Zudem ermöglicht die Summe aller identifizierbaren PLFAs eine zusätzliche Möglichkeit zur

Bestimmung der mikrobiellen Biomasse (Zelles, 1999). Darüber hinaus ist bis heute noch nicht geklärt, welche Mikroorganismen in gelagerten Biogas- und Rohgüllen vorkommen und wie viel Masse sie an der organischen Substanz ausmachen. Aufgrund der sehr flüssigen Substrate würden sich hierbei eventuell auch Methoden aus der Gewässerforschung anbieten.

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10. Danksagung

Mein besonderer Dank gilt meinem Betreuer, Prof. Dr. Rainer Georg Jörgensen, Reiner Schmidt und den Landwirten in Baden-Württemberg. Herrn Jörgensen danke ich für das entgegengebrachte Vertrauen, die fachliche Unterstützung und die Möglichkeit, dieses Thema bearbeiten und ohne Finanzierungslücken auch über den Antragszeitraum hinaus fertig stellen zu können. Reiner Schmidt danke ich für sein ausnahmsloses, großes Engagement und das Ermöglichen der Zusammenarbeit mit den Landwirten in Baden-Württemberg. Er war menschlich wie fachlich eine Bereicherung, schuf eine optimale Verbindung zwischen Wissenschaft und Praxis, bot Unterkunft bei Probenahmen und jeder Zeit ein offenes Ohr für jegliche Befindlichkeiten. Den Landwirtinnen und Landwirten, Marianne Holz, Armin Rapps, der Familie Kuch, Familie Bürkert, Familie Frank und Martin Lachemaier danke ich vor allem für die uneingeschränkte Zusammenarbeit, ihre Gastfreundlichkeit und ihre Geduld. Ihr Interesse an der Wissenschaft und der unkomplizierte Umgang mit mir und meinen Kollegen habe sehr vieles erleichtert, eine Menge Spaß gebracht und den Horizont erweitert.

Großer Dank gebührt vor allem Gabriele Dormann die neben ihrer fachlichen Kompetenz, ihres breiten Erfahrungsschatzes aus Landwirtschaft, Wissenschaft und Laborarbeit, jedoch vor allem wegen ihrer menschlichen Größe, meine Arbeit und meine persönliche Entwicklung maßgeblich beeinflusst hat. Großen Dank aussprechen möchte ich auch Nicole Gauß und Luisa Bierwirth die nicht nur fachlich, sondern auch menschlich einen bleibenden Eindruck hinterlassen haben. Für die Unterstützung in der Laborarbeit möchte ich mich ebenfalls bei Anja Sawallisch, Margit Rode, und Sabine Ahlers bedanken. Des Weiteren danke ich meinen Kollegen des Fachbereichs Bodenbiologie und Pflanzenernährung Caroline Indorf, Cahrlotte Tönshof, Nicole Hein, Andre Sradnik, Ramia Jannoura, Juliane Strücker, Sybille Faust, Josephine Möller und Raja Murugan für viele unterhaltsame Frühstücks- und Mittagspausen. Ein großes Dankeschön geht auch an alle ehemaligen und aktuellen Auszubildenden: Sophie Trümper, Matthias Wollrath, Ann-Katrin Becker, Sabine Schröter, Sinan Önder und Jawad Sadiqi. Ein besonderer Dank geht an meine lieben Kollegen Daphne Jost und Stephanie Meyer die nicht nur Projektbegleitend eine große Hilfe waren, sondern mit ihrem großen Herz auch schwierigere Zeiten erträglich gemacht haben. Ein ganz besonderer Dank geht an meinen Kollegen und Partner Stefan Lukas. Ihm danke ich für das Aushalten auch schwieriger Zeiten, für seine Hilfe und für eine schöne Zeit. Schließlich möchte ich noch meiner geliebten Schwester und meiner geliebten Mama für die Kraft und den Zuspruch danken.

Diese Arbeit wurde durch die Universität Kassel finanziert und ist mit dem DFG-Graduiertenkolleg 1397 assoziiert.