Swedish University of Agricultural Sciences
The Faculty of Natural Resources and Agricultural Sciences
Uppsala BioCenter, Department of Microbiology
Soil, Water and Environment, Department of Soil and Environment

Effects of biogas residues on respiration and denitrification in arable soil

– Evaluation of methods, microbial activity and agronomic implications

Erik Jönsson

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Sammanfattning

Jordbruksmark utgör basen för mänsklig livsmedelsförsörjning. Jordens förråd av organiskt material och växtnäring samt dess mikrobiella aktivitet är viktiga parametrar för dess förmåga att ge höga skördar av god kvalitet. Rätt gödsling är avgörande för att underhålla dessa parametrar. Framställning av biogas genom rötning av organiska substrat generar en flytande restprodukt, rötrest (RR) innehållande organiskt material samt växtnäring. Det ger den potential att användas som gödselmedel på åkermark. RR kan innehålla tungmetaller och toxiska organiska föreningar, varför den bör utvärderas innan användning.

En serie mikrobiella experiment utfördes i syfte att utvärdera fyra RR med avseende på deras gödselvärde och inverkan på jordkvalitet. Ett mer specifikt syfte var att finna en markmikrobiologisk procedur för utvärdering av RR från biogasprocessen. Jordens respiration och potentiella denitrifikationsaktivitet (PDA) vid tillsats av RR testades i två experiment. Som kontroller användes svinflytgödsel (SF) i båda experimenten samt i respirationsexperimentet en blandning av ammonium och glukos. PDA i jord efter tillsats av spårelement, extra glukos samt värmebehandlad SF testades i två uppföljande experiment. Alla tillsatser baserades på halten mineralkväve i gödseln och motsvarade 0-140 samt 0-1120 kg NH_4-N ha^{-1} i respirationsexperimentet respektive PDA-experimentet.

En respirationstopp återfanns för alla RR omedelbart eller inom tre dagar efter tillsats. Efter tio dagar hade alla RR respiration i stort sett avstannat. Skillnader fanns mellan RR men skillnaderna var större gentemot kontrollerna, vilka respirerade mer och under längre tid. Karakteristiska parametrar, användbara för att särskilja gödselmedlens kvaliteter, kunde identifieras i de uppkomna respirationskurvorna. Parametrarna inkluderade (1) utnyttjandegraden av tillsatt kol, (2) tid och (3) höjd för respirationstoppen, (4) lutningen på stigningen innan toppen samt (5) initial respiration. Praktiska problem med respirationstestet diskuterades, vilka till stor del berodde på skillnader i mängd tillsatt kol.

Alla RR stimulerade jordens PDA vid låga doser men inverkade negativt på densamma vid höga doser. SF skilde sig markant från rötresterna och stimulerade jordens PDA vid höga doser. De uppföljande experimenten indikerade att detta berodde på att SF i sig innehöll mikroorganismer och enzymer med denitrifierande förmåga, något som ej tidigare har visats men kan ge en förklaring till den i andra studier ofta uppmätta förhöjda avgång av lustgas vid spridning av SF. Innehållet av tungmetaller föreslogs som en anledning till den minskade denitrifikationen för RR.

Generellt sett stimulerade RR mikrobiell aktivitet i mindre omfattning än kontrollerna. Eftersom mikrobiell aktivitet ofta immobiliserar växtnäring kan det betyda att det blir lättare att förutsäga RR växtnäringsvärde vid spridning i fält, i jämförelse med SF. Olika RR skilde sig åt, troligen beroende på substrat och rötningsprocess. Tillsammans med ett urval av andra mikrobiologiska testmetoder, samt inkubationsexperiment och analys av kolföreningar, kan markrespiration och PDA användas för utvärdering av flytande gödselmedel.

Nyckelord: Biogas, Rötrest, Flytgödsel, Jordrespiration, Denitrifikation, PDA, Bördighet

Abstract

Agricultural soils constitute the base in human food production and soil content of organic matter and plant nutrients together with soil microbial activity are all important parameters for high crop yield of good quality. These parameters are dependant on proper fertilization. Anaerobic digestion of organic wastes for biogas production generates a liquid residue called biogas residue (BR). It contains organic material and plant nutrients which makes it a potential fertilizer for arable cropping. However, it also contains heavy metals and toxic organic compounds and it is therefore in need of evaluation before usage.

Microbial tests were performed aiming to evaluate the agronomic traits of four different BR and to find a viable procedure for evaluating slurry fertilizers. Two experiments where soil respiration and soil potential denitrification activity (PDA) was measured at fertilizer addition were performed. As controls pig slurry (PS) was used in both experiments along with a mixture of ammonium and glucose in the respiration experiment. In two follow up experiments soil PDA at addition of trace elements, extra glucose and heat treated PS were tested. All additions were based on fertilizer mineral nitrogen content and corresponded to 0-140 and 0-1120 kg NH_4-N ha^{-1} in the respiration and PDA experiment, respectively.

A respiration peak was observed for the BRs immediately or within three days after fertilizer addition. After ten days the respiration of the BR had almost stopped. There were differences between the BR but larger differences were observed when compared with the controls, which respired more and for a longer time. Characteristic data useful for determining the qualities of the fertilizers, were extracted from the respiration curves including (1) the utilization rate of carbon, (2) the time of respiration peak, (3) the height and (4) the slope of that peak and finally (5) the initial respiration rate. Practical difficulties with the experiment, mostly due to different additions of carbon with the fertilizers, were discussed.

All four BR stimulated soil PDA at low doses but inhibited soil PDA at high rates of addition. Differences between PS and BR were distinct as PS stimulated soil PDA at high rates of addition. The follow up experiment indicated that this was due to denitrifying bacteria and enzymes present in the PS itself. This has not previously been shown but offers a potential explanation to the often observed evolution of N_2O gas at PS fertilization. The heavy metal contents of the BR were proposed as the reason for their inhibition of PDA.

Overall the BR stimulated microbial processes in the soil to a lesser extent than the controls. Since microbial activity often results in immobilization of plant nutrients, it might be easier to predict nutrient dynamics for BR compared to PS when added to soil. Furthermore, the BR behaved differently probably depending on their substrates and the anaerobic digestion process. Together with a number of other microbial tests, incubation experiments and C compound analyzes, soil respiration and soil PDA can be used for evaluating slurry fertilizers.

Key Words: Biogas residue, Anaerobic digestion, Pig slurry, PDA, Respiration, Organic fertilizers

Foreword

I am studying my fifth and last year on the Master of Agriculture program with focus on Crop and Soil Science. This thesis is the result of an independent Master degree project in Soil Science, which will also count as the degree project for my Master of Agriculture. The work was performed as part of the thematic research program MicroDrivE at the Swedish University of Agricultural Sciences. The work also aimed to initialize a closer cooperation between the Department of Microbiology and the Department of Soil and Environment at SLU in Uppsala. Experiments and supervision have been performed at both of the departments.

I would like to thank my supervisors Mikael Pell (Department of Microbiology) and Sigrun Dahlin (Department of Soil and Environment). Our cooperation has worked exceptionally well and they have complemented each other in expertise and time allocation. I would also like to thank Post Doctor Harald Cederlund for help with evaluating the results of the respiration experiment. Finally, I would like to thank PhD students Jamal Abubaker and Kajsa Risberg, who dedicated time to help me interpret results and gave me immediate feed-back on my work. Jamal Abubaker deserve special gratitude for providing invaluable assistance with the statistical analysis of my results.

Thank you!

Abbreviations

AG – Ammonium Glucose
BR – Biogas Residue
C – Carbon
DM – Dry Matter
dw – Dry Weight
EC_{50} – Concentration of fertilizer lowering the microbial activity by 50%
EC_{90} – Concentration of fertilizer lowering the microbial activity by 90%
GC – Gas Chromatograph
KOH – Potassium Hydroxide
MO – Microorganism
N – Nitrogen
NMC – Nitrogen Mineralization Capacity
NOEC – No Effect Concentration
NPK – Nitrogen Phosphorus Potassium
PAO – Potential Ammonia Oxidation
PDA – Potential Denitrification Activity
PS – Pig Slurry
RoA – Rate of addition
SOM – Soil Organic Matter
UtC – Utilization rate of carbon (%)
VFA – Volatile Fatty Acid
WHC – Water Holding Capacity

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1 Introduction

Agricultural soils constitute the base for human food production and should be managed so that their quality increase or at least is maintained. The quality and the ability of soil to produce crops are dependent on its physical, biological and chemical properties. These properties determine the ability of the soil to supply the plant with water, nutrients, air and physical support. Soil content of organic matter containing carbon (C), nitrogen (N) and a wide spectrum of other elements, has often been proposed as very important for all the factors mentioned above (Eriksson et al., 2011). Thus, maintaining or increasing soil organic matter (SOM) is important for keeping the agricultural soils fertile.

Long term field trials have shown that a high crop yield along with the return of crop residues is important for maintaining SOM (Kirchmann et al., 1994). The use of mineral fertilizers can produce high crop yields, thus contributing to increase SOM. However, since the crop yield, containing many valuable plant nutrients, is removed at harvest the use of mineral fertilizer containing only a few nutrients risks to slowly deplete the soil of important micronutrients. Organic fertilizers usually contain the macronutrients as well as the micronutrients needed for crop production, and the use of such fertilizers assist in maintaining the micronutrient status of the soils. Hence, the fertility of the agricultural soils relies on both high productivity of the crops and the use of organic fertilizers and residues.

Farmyard manure has been shown to increase SOM and soil physical fertility (Blair et al., 2006). Pig slurry (PS) is a well-known and by farmers trusted organic fertilizer commonly used in Swedish crop production. However, the high content of easily available C in the fertilizer may make the crop nutrients become unavailable to plants, at least in the short term, due to immobilization by microorganisms growing on the C. This effect is most prominent with N (Delin et al., 2010; Kirchmann and Lundvall, 1992), and seems to be ruled to a large extent by the C/N ratio of the fertilizer (Chadwick et al., 2000; Morvan et al., 2006). Low C/N ratio

will result in higher N availability for the crops and thus higher N fertilizer value (Delin et al., 2010). Svensson et al. (2004) concluded that organic fertilizers with low content of mineral N and a high C/N ratio cannot produce crop yields competitive with those produced using pure mineral fertilizers. Hence, to combine the benefits of C and micronutrients added to soil with high crop yield, it is desirable to make the organic fertilizers behave more like mineral fertilizers, i.e. lowering the C/N ratio and removing the easily available C.

In recent years the production of biogas from different organic substrates has been growing (Börjesson and Mattiasson, 2007). Anaerobic digestion is a way to treat organic wastes and fertilizers in purpose of minimizing the quantities of the waste and extracting biogas for energy. The treatment removes C and thus lowers the C/N ratio and also increases the mineral N content of the product (Loria and Sawyer, 2005; Morgan and Paine, 2007). The resulting product has properties more similar to a mineral fertilizer. Both macro- and micronutrients are preserved in the process yielding the biogas residue (BR) with the potential of being used as an efficient fertilizer in crop production (Arthursson, 2009). Yu et al. (2010) showed that both BR and concentrated BR can effectively be used for fertilization of tomatoes, and Wenke et al. (2009) observed decreased nitrate levels in lettuce when fertilized with BR as compared to when fertilized with pure mineral fertilizer. Thus, both staple crops (Svensson et al., 2004) and vegetables can be suitable targets for BR application considering both quality and quantity aspects.

Soil microorganisms (MOs) are involved in many processes affecting the soil fertility such as N (Rosswall, 1982) and C (Eriksson et al., 2011) dynamics. Impact of any soil amendment on the MOs will also affect the fertility of the soil and its short term and long term potential to produce crops. The MOs of the soil need C and nutrients for maintenance and growth and since BR contain these, the BR can favor microbial growth and abundance. However, in many cases the BR also contain heavy metals, volatile fatty acids and other toxic organic compounds (Grossi et al., 1998; Angelidaki et al., 2000; Engwall and Schnürer, 2002; Leven and Schnürer, 2005), potentially counteracting the positive effects of the BR on soil MOs. Hence, there is a need for evaluation of the impact of BR on the microbial flora.

The chemical and physical properties of the BR depend on the substrate used in the biogas process. A variety of substrates are currently used for biogas production in Sweden (Nordberg, 2006) yielding BR of varying quality.

Abubaker et al. (2011) performed two microbial tests on the same BR as those used in the present study and compared them with PS and mineral fertilizer. The

first test was N mineralization capacity (NMC), a tool for monitoring general impact on the microbial flora since this process is performed by a large number of species (van Beelen and Doelman, 1997). The second test was potential ammonia oxidation (PAO), which is performed by a much smaller number of microbial species, making it a more sensitive tool for toxicity evaluation (Voytek and Ward, 1995). In the NMC test it was found that most BR caused net N immobilization at low rates of addition (Abubaker et al., 2011). Despite higher C/N ratio, the PS resulted in net N mineralization at all rates of addition, which is in contradiction to the results of C/N ratio and N availability as discussed above (Delin et al., 2010; Morvan et al., 2006; Chadwick et al., 2000). Explanations offered by Abubaker et al. (2011) were that the microbial activity, e.g. immobilization of N, was restricted in the PS due to the low availability of its C and its content of toxic heavy metals. In the PAO test all the BR and especially the PS displayed inhibitory effects on the MOs. Abubaker et al. (2011) suggested further investigation to establish the impacts of the BR on the soil MOs.

Two other commonly used methods for assessing microbial response and microbial toxicity of a substrate are aerobic respiration and potential denitrification activity (PDA). During aerobic respiration, organic C compounds are metabolized to yield energy while being oxidized to CO_2. During denitrification, respiration takes place in an anaerobic environment where NO_3^- is used as terminal electron acceptor instead of oxygen in several steps (Robertson and Groffman, 2007):

$$NO_3^- \rightarrow NO_2^- \rightarrow NO_{(g)} \rightarrow N_2O_{(g)} \rightarrow N_{2(g)} \quad (1)$$

The vast majority of the soil MOs can aerobically respire easily available C. Denitrification is also performed by a large number of species (Robertson and Groffman, 2007) and the two methods are thus general measurements of the fertilizer impact on MOs. Hence, soil aerobic respiration (from here on referred to as soil respiration) and PDA can be used to complement the investigation of Abubaker et al. (2011) to further explain and understand the impact of BR on the same microbial ecosystem.

Both respiration and PDA have previously been used for evaluating acute or long-term microbial toxicity in soils contaminated with for instance pesticides or heavy metals (Johansson et al., 1998; Noredal-Throbäck et al., 2007; Holtan-Hartwig et al., 2002; Pell et al., 1998; Dahlin and Witter., 1998; ISO/DIS 17155, 2001). It may also be possible to use soil respiration and PDA for evaluation of

slurry fertilizers. To the knowledge of the author, such a study has not yet been performed.

1.1 Purpose

The overall aims of this work were to evaluate the agronomic traits of the use of biogas residues (BR) in crop production with respect to its agronomic value and impacts on soil health. The more specific aim was to evaluate the experimental methods of assaying soil respiration and potential denitrification in order to find a reliable procedure for evaluating slurry fertilizers. To do this a series of laboratory experiments were set up to study the effects of adding varying doses of four different BR and pig slurry to soil.

2 Materials and methods

2.1 Soil and fertilizers

A clay soil from an experimental field at Brunnby outside Västerås in central Sweden (59° 37' N, 16° 33' E) was used in the experiments. The 0-20 cm top soil was sampled and sieved (5 mm mesh width) and stored at -20°C until used, a procedure previously used for microbial assays (Stenberg et al., 1998). Water holding

Table 1. Chemical, physical and microbial characteristics of the soil used for testing the effects on respiration and potential denitrification (PDA) at addition of organic fertilizers (partly from Abubaker et al., 2011)

Characteristics	
Chemical	
pH	5.6
Tot C (%)	1.3
Tot N (%)	0.1
Tot P (g kg^{-1} dw)	0.7
Tot S (g kg^{-1} dw)	0.2
P AL (mg 100 g^{-1} dw)	4.8
K AL (mg 100 g^{-1} dw)	18.5
Physical	
Sand (%)	14-20
Silt (%)	36-44
Clay (%)	37-49
Water holding capacity (g water g^{-1} soil dw)	0.511
Moisture content (% of WHC)	34
Microbial	
NMC (µg N g^{-1} dw $10d^{-1}$)	21.2
PAO (ng NO_2-N g^{-1} dw min^{-1})	4.8
PDA (ng N_2O-N g^{-1} dw min^{-1})	7.8
Basal respiration (µg CO_2 g^{-1} dw h^{-1})	0.782

capacity (WHC) and initial moisture of the soil was determined through weighing soil samples after saturation with water and then drained for 24 h and finally after drying at 105°C for 12 hours. Soil characteristics are described in Table 1.

BR from four large-scale Swedish biogas plants (A, B, C and D) were used in the experiment. The biogas processes generating the different BR were operated on the following substrates: slaughterhouse waste and source sorted organic household waste (A and C); distiller waste from production of ethanol from cereals (B); silage and source sorted organic household waste (D). Pig slurry (PS) from a farm with fatteners was used as a reference. PS is a slurry fertilizer with well-known characteristics and it is trusted by farmers for use in Swedish crop production. A mixture of ammonium sulfate and glucose (AG) was used as positive control. AG was added at a C/N ratio of 20, which is favorable for microbial growth. The composition of the BR and the PS are described in Table 2.

Since all of the amendments are potential fertilizers the additions were done at rates corresponding to a certain dose per ha of mineral nitrogen. In both experiments, the rates of addition (RoA) corresponded to those made by Abubaker et al. (2011) 17.5, 35, 70, 140, 280, 560 and 1120 kg NH_4^+-N ha^{-1}. Due to practical limitations only the four lowest RoA were used in the respiration experiment. In calculation of the rates to be added, the amount of soil into which the fertilizer theoretically would be mixed when applied in the field was assumed to be the top 0.05 m.

Table 2. Chemical characteristics of the biogas residues (BR A- D) and pig slurry (PS) used in the respiration and PDA experiment. From Abubaker et al. (2011)

	BR A	**BR B**	**BR C**	**BR D**	**PS**
DM (%)	6.1	3.7	1.7	5.9	9.1
Tot C (kg ton^{-1})	19	9	7	24	40
Org N (kg ton^{-1})	2.6	2.2	0.6	2	2.9
NH_4^+-N (kg ton^{-1})	5.3	3.7	2.03	3.3	2.4
C/N	7.1	4.2	11	12.1	13.6
pH	7.9	7.9	8.0	8.7	6.6
P (kg ton^{-1})	0.9	0.7	0.2	0.4	1.4
K (kg ton^{-1})	1.6	2.8	1.1	3.7	2.5
S (kg ton^{-1})	0.6	1.22	0.12	0.26	0.47
Mg (kg ton^{-1})	0.2	0.1	0.1	0.3	0.6
Ca (kg ton^{-1})	0.6	0.1	0.3	1.3	1.7
Cr (mg kg^{-1})	13	14.9	11.1	19.5	10.7
Mn (mg kg^{-1})	200.6	266	91.8	286.5	426
Ni (mg kg^{-1})	33.8	35.5	1.7	1.7	39.6
Cu (mg kg^{-1})	69.7	69.4	39.8	97.4	217.9
Zn (mg kg^{-1})	474	465	299	395.5	801
Cd (mg kg^{-1})	0.3	0.3	1.2	0.3	0.3
Pd (mg kg^{-1})	0.1	0.7	4.6	3.4	0.1
Hg (mg kg^{-1})	0.1	0.1	0.1	0.1	0.1

2.2 Experimental setup

2.2.1 Soil respiration

Soil respiration was measured using a Respicond respirometer (Nordgren, 1988) which semi-continuously measured the evolution of carbon dioxide from the soil.

Initially, 20 g dw of thawed soil was weighed into each of the 250 ml plastic jars standardized for the Respicond and incubated at 20°C. After this initial disturbance, the soil was allowed to return to basal respiration for two weeks in the closed jars. The temperature was kept at 20°C throughout the experiment and the target moisture content of the soil after additions was 65% of WHC.

The four lowest RoA from Abubaker et al. (pers. com) were used for each of the BR and the PS, which corresponded to 17.5, 35, 70 and 140 kg NH_4^+-N ha^{-1}. No higher rate was possible since that would have brought the soil moisture above 65% of WHC for BR C which had the lowest content of mineral nitrogen. AG was added at one single RoA corresponding to 70 kg NH_4^+-N ha^{-1}.

All fertilizers were added by the use of a 10 ml automated pipette. The plastic tip of the pipette was cut off in order to allow for the heterogeneous fertilizers to

Table 3. Overview of the experimental setup and treatments used in the soil respiration experiment

Treatment	Rate of addition (kg NH_4^+-N ha^{-1})	Replicates	Name
Biogas residue A	17.5	3	BR A 17.5 RoA
	35	3	BR A 35 RoA
	70	4	BR A 70 RoA
	140	4	BR A 140 RoA
Biogas residue B	17.5	3	BR B 17.5 RoA
	35	3	BR B 35 RoA
	70	4	BR B 70 RoA
	140	4	BR B 140 RoA
Biogas residue C	17.5	3	BR C 17.5 RoA
	35	3	BR C 35 RoA
	70	4	BR C 70 RoA
	140	4	BR C 140 RoA
Biogas residue D	17.5	3	BR D 17.5 RoA
	35	3	BR D 35 RoA
	70	4	BR D 70 RoA
	140	4	BR D 140 RoA
Pig slurry	17.5	3	PS 17.5 RoA
	35	4	PS 35 RoA
	70	4	PS 70 RoA
	140	4	PS 140 RoA
Ammonium Sulfate/Glucose	70	3	AG 70 RoA
Control	0	6	Control
Empty	0	6	Empty

enter the pipette. The additions were done on a weight basis and a small Pasteur pipette was used to make small corrections. After the additions the jars were slightly tapped against the table at an angle to improve gas exchange and fertilizer distribution, while minimizing disturbance to the soil aggregates and organisms.

Due to their different initial NH_4^+-concentrations, all the fertilizers had to be diluted with different amounts of deionized water before addition to the soil. This allowed the additions to the jars to be done in a similar way, i.e. to reach both the target soil moisture of 65% WHC and desired NH_4^+-rate. This also helped spreading fertilizers homogenously onto the soil.

In practice, the additions were performed in the following way: The solution which was made for adding the highest RoA (140 kg NH_4^+-N ha^{-1}) were after addition diluted to double weight with deionized water in order to get the solution for adding the second RoA (70 kg NH_4^+-N ha^{-1}). This procedure was repeated for the third and fourth RoA. During dilution and addition the solution was stirred with a magnetic stirrer to keep it homogenous.

The 140 and 70 RoA treatments were replicated four times and the 35 and 17.5 RoA were replicated three times. However, PS at 35 RoA was also replicated four times due to the practical difficulties of adding this heterogeneous fertilizer. The single RoA (70 kg NH_4^+-N ha^{-1}) of AG was replicated three times. Six jars with soil amended with deionized water were used as negative control. Six empty jars were included in the experiment to provide a reference point when determining which values to discard after disturbance at start of the experiment and at Potassium Hydroxide (KOH) replacement (see below). This yielded a total of 86 jars, which were placed in the Respicond in a completely randomized design.

The experimental setup is described in Table 3. All the additions were done before placing the jars in the Respicond, a procedure which took approximately four hours. Thus, the time from addition to the first measurement differed for each treatment.

After the additions, the respiration was monitored every 30 minutes for twelve days (300 hours). At reading, the electric conductivity in solutions of 0.3 M KOH present in measuring cells inside the experimental jars, were determined. The KOH was replaced in all the jars when the accumulated respired CO_2 reached 50 mg in one jar, which occurred twice during the experiment.

2.2.2 PDA

Potential denitrification activity (PDA) assay was performed according to Pell et al. (1996). In the experiment each BR and PS was assayed individually in sessions

where the RoA corresponded to 17.5, 35, 70, 140, 280, 560 and 1120 kg of NH_4^+-N ha^{-1} without replicates. The RoA used were the same as used by Abubaker et al. (2011). In addition, a control treatment, where only autoclaved water was added, was included and replicated three times in each session. This resulted in a total of ten flasks in each experimental session.

Initially, 25 g of frozen soil were weighed into ten 250 ml Duran flasks the day before the experiment and placed in a room with constant temperature of 25ºC where the soil was allowed to equilibrate overnight. Each flask was then supplied with fertilizer, water and a substrate solution to achieve a total addition of 25 ml of water. First, the fertilizer was added on a weight basis corresponding to the seven RoA. For the three lowest RoA, the fertilizers had to be diluted ten times (BR C and PS) or twenty times (BR A, BR B and BR D). Then, autoclaved water was added to the three control flasks and to the seven flasks with fertilizer. The volume of water added was different for each individual bottle as it depended on the dry matter content of the fertilizer and the amount of fertilizer added. Finally, four milliliters of an assay substrate solution (6.25 mM of KNO_3^- and 6.25 mM of $C_6H_{12}O_6$ (glucose)) was supplied to each of the ten bottles, resulting in final concentrations of 1 mM of these species (Table 4).

After substrate additions the flasks were closed airtight with lids with rubber gaskets. The lids were also provided with rubber septa for gas sampling. To make the head space anaerobic the flasks were evacuated and then flushed with N_2 five times. After this, 25 ml of acetylene per flask were injected with a syringe. The acetylene inhibited the last step in the denitrification pathway (eq. 1) making N_2O the end product of the denitrification process.

The flasks were then placed on a shake table at 175 rpm in a room with a constant temperature of 25°C. The first head space gas samples were withdrawn 15 minutes after the acetylene additions. Another six samples per flask were withdrawn at intervals of 30 minutes. The gas samples were collected with 0.5 ml syringes and injected into 20 ml gas tight vials standardized for gas chromatographs. The gas samples were analyzed using a gas chromatograph (GC) (Perkin Elmer Clarus 500, Waltham, MA, USA) provided with a head space sampler (TurboMatrix 110, Perkin Elmer). At analysis the sample vial was pressurized and the gas sample transferred to a capillary split/splitless injector (CAP) and then further into an Elite Plot Q column (length 30 m; inner diameter 0.53 mm; temperature 35°C; carrier N_2 flow 1 ml min^{-1}) for separation of the gases and N_2O detected with an electrone capture detector (ECD).

For the analyses of N_2O in each test session, two sets of vials with eleven know concentrations of N_2O were prepared. These were made by diluting pure N_2O gas to the desired concentrations in 118 ml bottles containing pure N_2. From these bottles, duplicate samples of 0.5 ml were transferred 20 ml vials. These two sets of eleven samples were analyzed along with the gas samples from the PDA to generate a standard curve.

The concentration of N_2O in the PDA assay flasks at each sampling time was then calculated by fitting the values from the GC to the standard curves.

2.2.3 PDA follow up

Two PDA follow up experiments, consisting of three and two treatments, respectively, were performed in order to provide further explanations of the results of the PDA fertilizer experiment (Table 4). The experiments were performed as described above.

The first experiment included a control treatment, a treatment with trace elements and a treatment with double concentration of glucose, all replicated three times. The control treatment was identical to the control treatment in the original PDA experiment, as described above, where only glucose and nitrate were added to the soil. The trace element treatment was supplied the same amount of nitrate and glucose as the control, but also received a modified Cohen-Bazire et al. (1957) trace element solution. Since the addition of extra nitrogen to the samples would have compromised the experimental design, the $(NH_4)_6Mo_7O_{24}$ x $4H_2O$ and $Co(NO_3)_2$ x $6H_2O$ in the Cohen-Bazire trace element solution were replaced with Na_2MoO_4 x $2H_2O$ and $CoCl_2$ x $6H_2O$, respectively. The molar concentrations of the Mo and Co ions were, however, kept the same. In the last treatment of the first experiment, the original amount of nitrate was once again supplied, but with double amount of glucose as compared to the control.

Table 4. Final concentrations of KNO_3 and glucose used in the potential denitrification activity assay (PDA) and final concentrations in modified Cohen- Bazire et al. (1957) trace elements solution in the follow up experiment

		Trace elements (mg/l)							**PDA substrate (mM)**	
Treatment		Na_2MoO_4 x$2H_2O$	$ZnSO_4$x $7H_2O$	$FeSO_4$x7 H_2O	$MnSO_4$ xH_2O	$CuSO_4$x5 H_2O	$CoCl_2$x $6H_2O$	$Na_2B_4O_7$ x$10H_2O$	KNO_3	Glucose
Original PDA	Five fertilizers	0	0	0	0	0	0	0	1	1
	Control	0	0	0	0	0	0	0	1	1
PDA follow up, experiment 1	Control	0	0	0	0	0	0	0	1	1
	Trace elements	0.254	10.95	5.00	1.54	0.392	0.203	0.177	1	1
	Double glucose	0	0	0	0	0	0	0	1	2
PDA follow up, experiment 2	PS	0	0	0	0	0	0	0	1	1
	PS heat	0	0	0	0	0	0	0	1	1

The second experiment included two treatments with PS, both without replications. The first was identical to the treatment with the highest RoA in the original PDA for PS (1120 kg NH_4^+-N ha^{-1}). The second treatment included the same RoA of PS, but the PS had been heat treated (80ºC for 15 minutes) before addition to the soil. The heat treatment aimed to denature enzymes and kill bacteria present in the PS.

2.3 Data treatment

2.3.1 Soil respiration

The primary output data attained from the respirometer were in mg CO_2 h^{-1} respired at the time of measurement. In the respiration curves obtained, the time for substrate addition was set to zero, which were between one and four hours before the first measurement. The primary respiration curves were treated in several steps in order to obtain the characteristic data, as described below. The steps were performed in the following order: (1) subtraction of basal respiration rate, (2) subtraction of respiration rate of the negative control, (3) replacement of missing values due to disturbances and finally (4) extraction of characteristic data.

In step (1) the basal respiration rate was calculated as the average respiration rate of all the 86 jars during the immediate five days prior to the fertilizer additions. This value (0.782 µg CO_2 g^{-1} dw h^{-1}), was then subtracted from each data point in each treatment, including the negative control treatment.

After subtraction of the basal respiration rate the respiration of the negative control treatment was in step (2) subtracted from each data point of the fertilizer treatments. This was done by fitting a logarithmic trend line to the average of the primary data values of the six replicates constituting the negative control treatment. This trend line provided a smoothly declining series of values to be used as a corrected negative control. Values for each sampling time were then calculated using the equation of this trend line. These, i.e. the values of the negative control, were then withdrawn from each data point of each fertilizer treatment at the corresponding time. This procedure allowed calculation of the respiration caused by the added substrates, i.e. the fertilizers.

The KOH, present in the measuring cells inside the respiration jars, had to be replaced twice during the experiment. This procedure caused disturbance in the samples for some hours afterwards leading to missing values in every treatment. Determinations of which values that were influenced by the disturbance were done

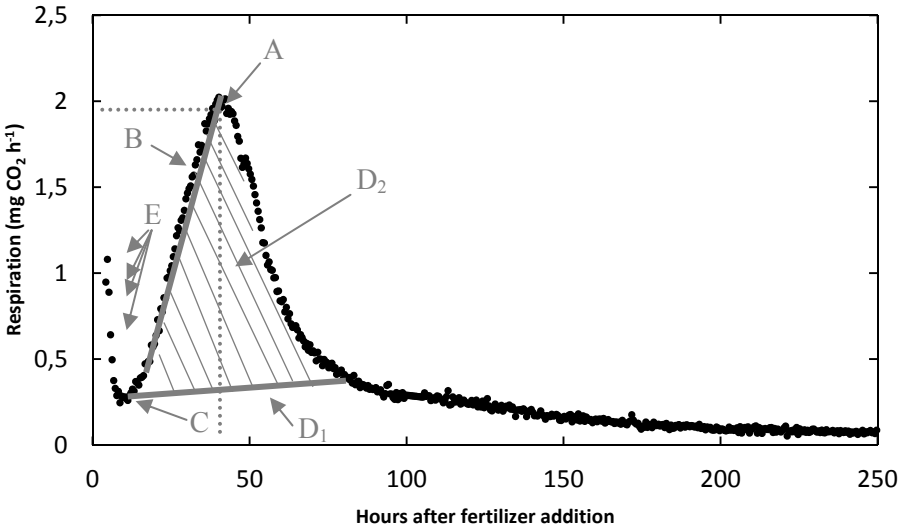

Figure 1. Schematic presentation of data extraction from a respiration curve. (A) point from which main peak time and peak height were read, (B) slope which always included peak value, (C) respiration rate at 9 hours, (D_1) the bottom line of the peak, determined through visual examination, (D_2) peak content of carbon and (E) values discarded as affected by disturbance after visual examination of empty jars (c.f. Table 5).

in step (3) by visual examination of the curves of the six empty jars. Data points in the curves from the empty jars diverging from their normal pattern were discarded as missing values. These missing values were then replaced by interpolation, i.e. by fitting a curve to the values before and after the missing values. The curve model providing the best fit in each treatment was used (linear, second order polynomial or third order polynomial). When none of these were applicable, the average values of 20 data points before and after the missing values were used.

In step (4) extraction of characteristic data from the respiration curves was done with consideration of the ISO standard for respiration curves (ISO/DIS 17155, 2001). In the ISO standard, glucose is used as the sole substrate which provides a

Table 5. Data parameters extracted from the respiration curves of the different treatments (c.f. Fig. 1)

Data	**Unit**
Utilization rate of C	%
Main peak time	h
Peak height	mg CO_2 h^{-1}
Slope	mg CO_2 h^{-2}
Respiration at 9 h	mg CO_2 h^{-1}
Peak C content	mg
C in peak	% of C added
C in peak	% of C respired

respiration curve with an appearance similar for most soils. Due to the differences between the ISO standard and the present experiment, all the characteristic values given in the ISO standard could not be attained here, but were restricted to the time for the maximum respiration (main peak time). In the ISO standard the slope of the peak is calculated from a logarithmic scale. However, due to the appearance of the respiration curves in the present experiment only the average incremental slope of the respiration peak on a regular scale was calculated. In addition, a number of other characteristic data which were considered important were extracted (Table 5 and Fig. 1).

Some of the characteristic data extracted were to a certain extent subjectively selected. The slopes of the peaks were calculated using linear trend lines based on the best fit (highest R^2). However, the single peak values of the curves were always included in these trend lines. The peak area calculations were performed through viewing the peak as a two right angled triangles. These triangles were based on the peak value and the approximated base line of the peak (Fig. 1), the latter subjectively chosen through visual examination of the respiration curve.

2.3.2 PDA

From the data on the N_2O concentration units received from the gas chromatograph, the amount of N_2O per gram of dry soil at each sampling time was calculated. This product was calculated according to Pell (1993). This provided an exponential graph, with time on the x-axis and the product on the y-axis, to which a formula (eq. 2) was fitted (Stenström et al., 1991).

$$p = p_0 + \frac{qN_0}{\mu}\left(e^{\mu t} - 1\right) \quad (2)$$

In the formula, p is the amount of N_2O at the time t, p_0 is the initial amount of N_2O, μ is the specific growth rate, q is the specific activity of the denitrifying enzymes and N_0 is the initial abundance of denitrifying bacteria. The PDA is the product of q and N_0. The obtained PDA values were then plotted against the logarithm of the RoA for each fertilizer and a linear trend line was fit to those values (subjectively) considered to be part of a regression line. From the equation of the trend line the hypothetical fertilizer rate which had no effect on the denitrifying enzymes (NOEC), lowered the activity of the enzymes by 50% (EC_{50}) and lowered the activity of the enzymes by 90% (EC_{90}) were calculated. All calculations were performed using the computer software MS Excel (ver. 2007, Microsoft, Red-

mond, WA, USA) and SigmaPlot (ver 9, Systat Software Inc., San José, CA, USA).

2.3.3 PDA follow up

The PDA of the treatments in the two follow up experiments were calculated using the same procedure as used for the main PDA experiment.

2.4 Statistical analysis

2.4.1 Soil respiration

Data were analyzed using SAS 9.2 (SAS Institute Inc, Cary, NC, USA). To analyze differences, PROC GLM was used with mean comparisons according to Tukey's method. An ANOVA model for repeated testing of paired differences was used to analyze utilization rate of C, main peak time, peak height, slope, respiration at 9 h, peak content of C, C in peak (% of C added) and C in peak (% of C respired), where fertilizer type, fertilization rates and fertilizer type *fertilization rate interaction were considered as random effects. Comparisons were deemed statistically significant when $p \leq 0.05$.

2.4.2 PDA

For the control treatments in all experiments, mean value and standard deviation were calculated. In the first PDA follow up experiment, students t-test were performed to reveal any significant differences (MS Excel ver. 2007, Microsoft, Redmond, WA, USA). The significance level of $p \leq 0.05$ was used. No statistics were applied to the second PDA follow up experiment.

3 Results

3.1 Soil respiration

The strategy of the experiment to add the same amounts of NH_4^+-N with each of the fertilizers resulted in the addition of varying amounts of C. The total additions of C for all the fertilizers at the addition rate corresponding to 70 kg NH_4^+-N ha^{-1} ranged between 6.10 and 50.16 mg C jar^{-1} (Fig. 2). The C/N ratio of the AG was 20, which was higher than the ratios of the other fertilizers, meaning that the highest addition of C was made in the AG treatment. The PS fertilizer resulted in the second largest C addition, more than double the C addition of BR D, which was the BR with highest C/N ratio. The C addition of BR B, which had the lowest C/N ratio, was almost an order of magnitude lower than the corresponding value for AG.

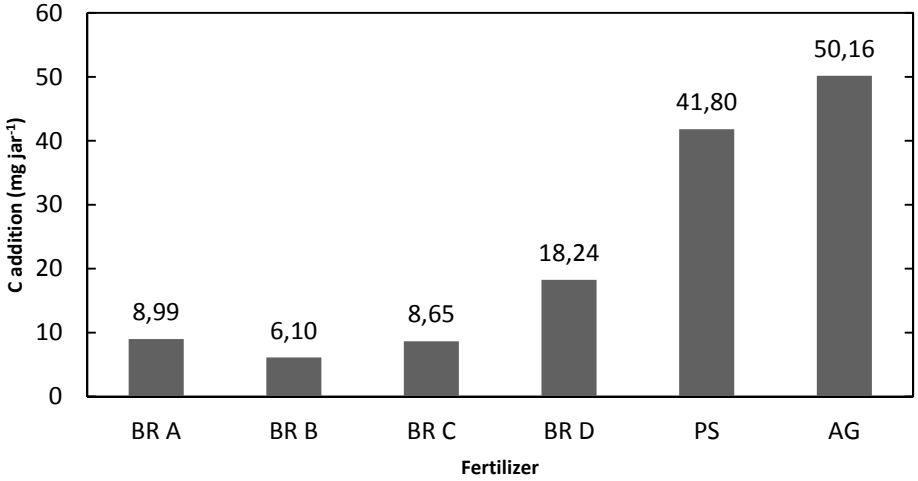

Figure 2. Comparison of C added to each jar in the respiration experiment, at the addition corresponding to 70 kg NH_4^+-N ha^{-1}.

All the data extracted from the respiration curves are presented in Table 6. Missing values indicate that the data were not attainable. For instance, there were no visible peaks in the respiration patterns for BR B at 17.5 and 35 RoA. Hence, no peak time or height could be determined for those treatments. At respiration rates below 0.1 mg CO_2 h^{-1}, the values showed large variations indicating a noise effect (Fig. 3). Due to this, unless otherwise stated, only the results of the two highest RoA are presented and discussed.

Utilization rate of C (UtC). The respiration of the C added to the soil showed different patterns for all the BR and the differences were most pronounced at the highest RoA (Fig. 3). However, the total utilization rate of C (UtC) over the twelve days was not significantly different for the BR at this RoA (Table 6). The only treatments that stood out with respect to UtC were BR D 17.5 and 35 together with BR C 17.5 and BR B 17.5, the latter two having negative values, which all were significantly lower than all or a few of the treatments (Table 6). There was a tendency for lower UtC of BR B and BR D also at higher RoA.

Peak height. When comparing the BR at 140 RoA, BR C resulted in a significantly higher respiration peak (Fig. 3 and Table 6). However, the respiration rate

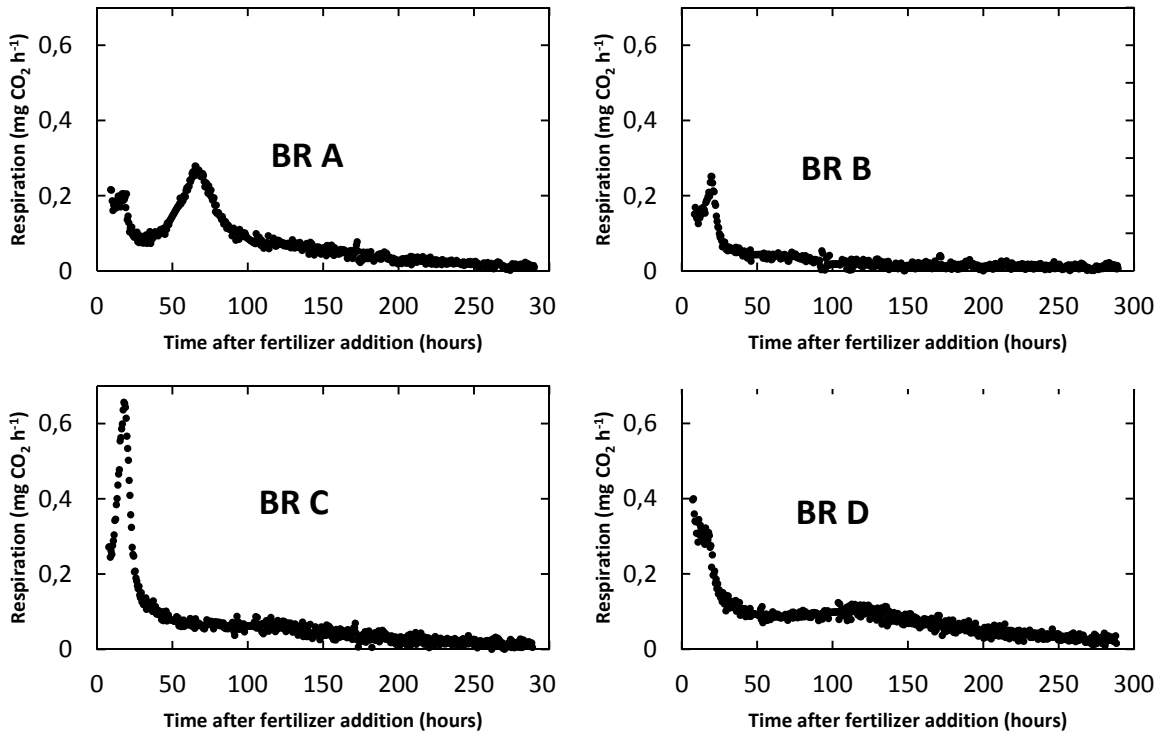

Figure 3. Respiration of four types of biogas residues (BR A-D) at the rate of addition corresponding to 140 kg NH_4^+-N ha^{-1}. (Mean, n=4).

Table 6. Extracted data from the soil respiration curves originating from additions of four types of biogas residues (BR A-D), pig slurry (PS) and ammonium-glucose mixture (AG). Values are means from three or four replications (explanation in Table 3). Values sharing the same letter are not statistically different (P > 0.05)

	Rate	Utilization rate of C (%)	Peak height (mg CO_2 h^{-1})	Main peak time (h)	Slope (mg CO_2 h^{-2})	Respiration at 9 h (mg CO_2 h^{-1})	Peak content of C (mg)	C in peak (% of C added)	C in peak (% of C respired)
BR A	17.5	28.3^{ac}	0.059^{a}	54.17^{b}	0.0008^{ac}	0.027^{cf}	0.179^{bcf}	8.0^{a}	41.1^{df}
BR A	35	28.6^{ac}	0.087^{a}	56.25^{b}	0.0018^{ac}	0.077^{cd}	0.336^{bcdf}	7.5^{ab}	27.9^{abdef}
BR A	70	30.9^{a}	0.158^{a}	61.96^{a}	0.0032^{a}	0.128^{bd}	0.635^{bd}	7.1^{ab}	22.9^{acde}
BR A	140	32.5^{a}	0.294^{abd}	66.63^{a}	0.0076^{ac}	0.217^{ah}	1.312^{a}	7.3^{ab}	22.5^{acde}
BR B	17.5	-13.3^{bc}				0.034^{cf}			
BR B	35	14.1^{ac}				0.053^{cfi}			
BR B	70	17.0^{ac}	0.123^{a}	18.67^{cdi}	0.0060^{ac}	0.105^{bdf}	0.103^{ef}	1.7^{c}	11.5^{ae}
BR B	140	19.4^{ac}	0.276^{abd}	19.46^{ci}	0.0117^{ac}	0.169^{ab}	0.289^{be}	2.4^{cd}	12.2^{ae}
BR C	17.5	-24.3^{b}				0.007^{c}			
BR C	35	15.0^{ac}	0.175^{abd}	8.33^{ef}		0.126^{bdi}	0.188^{ceik}	4.3^{def}	29.6^{adf}
BR C	70	20.2^{ac}	0.266^{abd}	14.58^{cd}	0.0209^{abc}	0.154^{abj}	0.379^{bfij}	4.4^{eg}	22.0^{adg}
BR C	140	31.4^{a}	0.668^{c}	18.13^{cdi}	0.0516^{be}	0.262^{h}	1.047^{ah}	6.1^{ae}	19.4^{ae}
BR D	17.5	8.6^{c}	0.078^{ab}	6.83^{ef}		0.033^{ck}	0.102^{cegi}	2.2^{cfg}	18.8^{adg}
BR D	35	8.7^{c}	0.110^{ab}	7.42^{ef}		0.052^{cfi}	0.209^{bfi}	1.2^{c}	7.4^{eg}
BR D	70	15.6^{ac}	0.202^{abd}	7.33^{e}		0.136^{bd}	0.542^{bil}	3.7^{dgh}	24.1^{adg}
BR D	140	17.4^{ac}	0.405^{df}	7.42^{e}		0.339^{g}	1.376^{a}	3.8^{dgh}	21.7^{adg}
PS	17.5	33.3^{a}	0.231^{abd}	13.25^{df}	0.0238^{ae}	0.153^{abd}	0.248^{bfgk}	2.4^{cfg}	7.2^{ceg}
PS	35	28.3^{ac}	0.519^{cf}	20.38^{i}	0.0432^{bce}	0.131^{bd}	0.760^{dhjl}	3.6^{dg}	12.9^{ag}
PS	70	31.2^{a}	1.108^{g}	28.92^{h}	0.0924^{f}	0.093^{dfjk}	2.340^{n}	5.6^{behi}	18.0^{ag}
PS	140	31.5^{a}	2.233^{e}	36.46^{g}	0.1931^{d}	0.133^{bd}	5.547^{m}	6.6^{ai}	21.1^{adg}
AG	70	57.1^{d}	2.198^{e}	44.16^{j}	0.0689^{bf}	0.285^{gh}	13.472^{o}	26.9^{j}	47.0^{bf}

of BR D was at start of the measurement already decreasing (Fig. 3). Thus, the real height of the BR D peak was not caught due to its early occurrence. For BR D, the first measured value was instead used as the peak. BR A, BR B and BR D had similar peak heights, but the times of occurrence for the peaks were significantly different (Table 6). A small peak between ten and twenty hours followed by a decline in respiration before the main peak could be observed for BR A (Fig. 3).

The peak heights at 70 RoA showed the same patterns as those at 140 RoA and differences between the BR and the positive controls (PS and AG) were prominent (Fig. 4 and Table 6). The addition of AG resulted in the highest peak. The PS had the second highest peak, but the respiration from this fertilizer was slower than that of the BR in the start (Fig. 4). The BR all resulted in respiration peaks lower than those of PS and AG (Table 6), but there were no significant differences between the individual BR at the 70 RoA.

Main peak time. Differences in peak time were significant between all fertilizers at 70 RoA, except between BR B and BR C. The peak time also differed among the fertilizers at different RoA. Lower RoA yielded earlier peak times, which was statistically significant for BR A, BR C and PS (Table 6).

Slope. No slope was attained for BR D, as the peak occurred before the first measured point (Fig. 4). At 140 RoA the slope was significantly steepest for PS

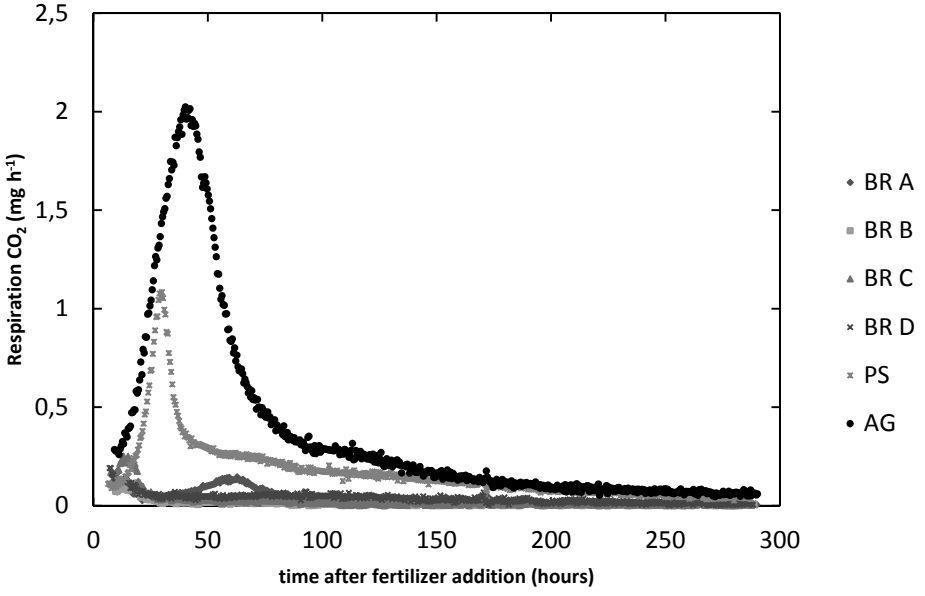

Figure 4. Soil respiration at addition of four types of biogas residues (BR A-D) and pig slurry (PS) corresponding to 70 kg NH_4^+-N ha^{-1}. Mean, n=4).

followed by BR C (Table 6). At 70 RoA, PS yielded the steepest slope together with AG. The slopes of all the three BR where a slope could be derived, were significantly flatter than the slope of PS at 70 RoA, but the BR did not differ among themselves.

Respiration at 9 h. The C in the PS tended to initially be decomposed more slowly in the start of the experiment than the C in the BR at all RoA, with significant differences at the highest RoA. BR D had the significantly highest respiration at 9h at 140 RoA and the tendency was the same at 70 RoA, although the latter relationship was not significant. At 70 RoA, the only RoA where AG were present, AG had the significantly highest respiration at 9 h. In comparing the BR, the respiration at 9 h was in most cases significantly higher at RoA 70 and 140 than at 17.5 and 35. For PS, there were no differences in respiration at 9 h between the different RoA.

C content in peak (mg C, % of C added and % of C respired). The largest content of C in peak was found in the AG treatment, followed by the PS. All the BR had lower amounts of C in their peaks. Generally, the amount of C in the peak increased with increasing C addition. The C content of the peak (in % of C added) of BR B was significantly lower compared to the corresponding values of the other BR, the PS and AG, except for BR D at 140 RoA, for which parts of the peak were missed in the measurements (Table 6). For the same parameter BR D had the second lowest values. BR A stood out by having the highest proportion of added C respired in the peak in comparison with the BR and the PS, which was significant at all RoA but 140. The C content in the peak of AG was more than 25% of the added, which was significantly more in comparison with the other fertilizers. When considering the total amount of C respired during the 12 days (% of C respired), no significant differences were observed, except for AG which had almost half of its respired C in the peak (Table 6). There were no differences between different RoA for any of the other fertilizers with respect to % of C respired in the peak.

3.2 PDA

Two examples of N_2O curves from the PDA assays are presented in Fig. 5. The calculated PDA values at the different RoA for all the fertilizers are shown in Fig. 6. Due to a mistake in the test session of PS, an eighth RoA was included in this treatment corresponding to 644 kg NH_4^+-N ha^{-1}. All the fertilizers showed a positive response to the lowest two or three doses, which means that the initial activity

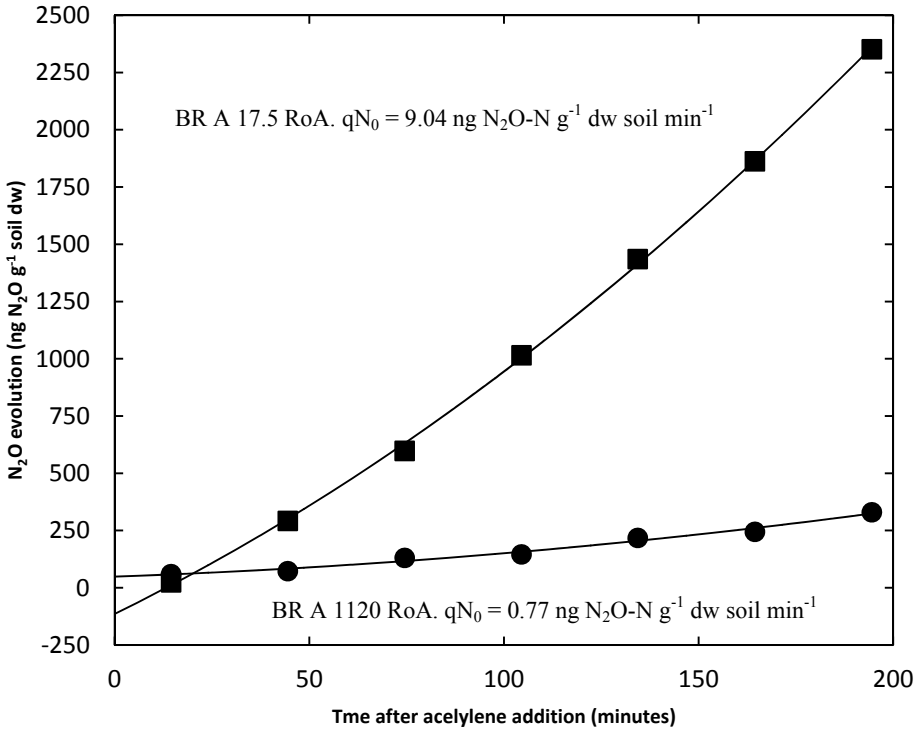

Figure 5. Examples of curves from potential denitrification assays (PDA) on a soil amended with BR A at rates of additions (RoA) corresponding to 17.5 and 1120 kg NH_4^+-N ha^{-1}. Lines are fitted values to a product equation and indicate the evolution of N_2O as a product of time in the assay flasks. From the lines PDA (qN0) was derived.

of the denitrifying enzymes was higher than in the control. Especially the addition of fertilizer corresponding to 35 kg NH_4^+-N ha^{-1} had a positive effect on the enzymes. Higher RoA than 70 for the four BR resulted in a decrease of the PDA. From the linear regression lines NOEC, EC_{50} and EC_{90} were calculated for each fertilizer (Table 7). The NOEC values of BR C and especially BR D stood out as they were lower than the corresponding values of the other two BR.

The PS showed a completely different response pattern in comparison with the BR. Instead of a decrease in PDA there was an increase of enzyme activity with

Table 7. No effect concentrations (NOEC) 50% (EC_{50}) and 90% (EC_{90}) reduction of PDA as response to seven rates of addition (RoA) of four types of biogas residues (BR A-D) and pig slurry (PS) in a dose response test (Fig. 6).

	Fertilizer				
	BR A	**BR B**	**BR C**	**BR D**	**PS**
NOEC (kg NH_4^+-N ha^{-1})	160	162	53	10	n/a
EC_{50} (kg NH_4^+-N ha^{-1})	431	487	491	429	n/a
EC_{90} (kg NH_4^+-N ha^{-1})	648	747	841	765	n/a

n/a = Values not attainable (explanation in text)

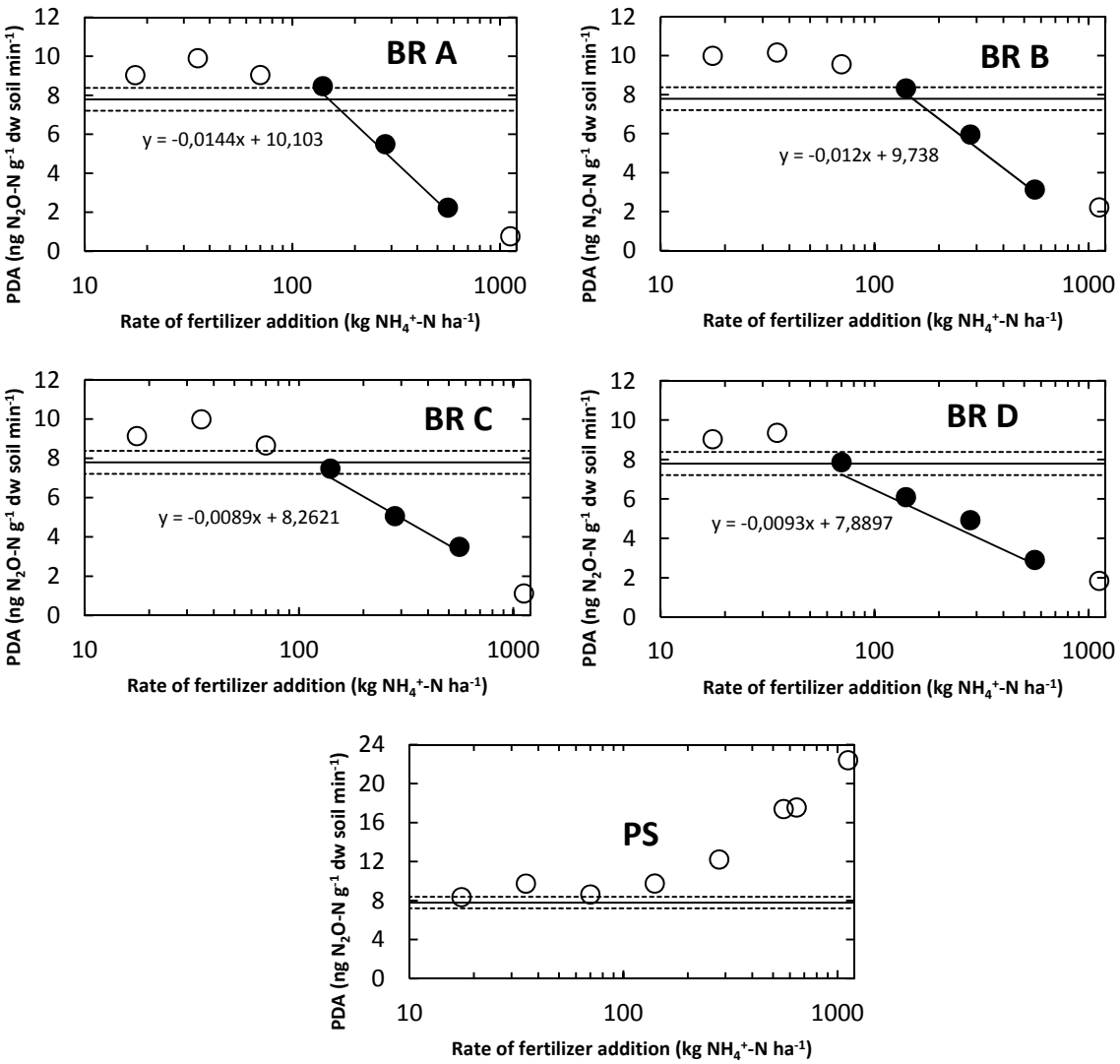

Figure 6. Potential denitrification activities (PDA) at seven rates of addition of four types of bio-gas residues (BR A-D) and pig slurry (PS). Horizontal solid line (———) indicate the mean value of control treatment (n = 14), and dashed line (---------) indicate standard deviation of control treatment. Closed (●) and open (○) circles indicate data points included and excluded in a linear regression, respectively.

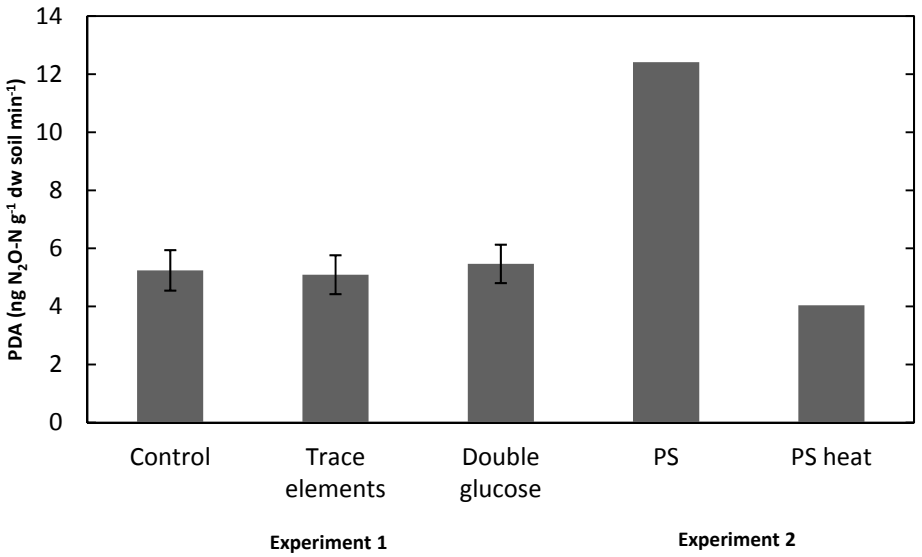

Figure 7. Potential denitrification acitivities (PDA) of the follow up experiments. For experiment 1, where no slurry was added, mean values (n=3) for control, trace elements and double glucose are presented. Error bars indicate standard deviation. For experiment 2 single values for pig slurry (PS) and heat treated PS are presented.

increasing RoA. The increase in PDA appeared to be proportional to the increase in RoA. Since no inhibitory effect on the potential denitrification was observed for PS, no values on NOEC, EC_{50} and EC_{90} were calculated for this fertilizer.

3.3 PDA follow up

Mean values and standard deviation for the first follow-up experiment (control, trace elements and glucose) are presented in Fig. 7 together with the two additions of PS (not heat treated and heat treated, respectively) from the second follow up experiment. No significant differences between the treatments in the first experiment were revealed when compared (Students t-test, $p \leq 0.05$, Table 8).

Table 8. P-values from the statistical analysis (students t-test) of the PDA follow up experiment (n=3)

	Control (1)	**Trace elements (2)**	**Double glucose (3)**
Control (1)		0.49	0.64
Trace elements (2)	0.49		0.56
Double glucose (3)	0.64	0.56	

4 Discussion

4.1 Soil respiration

In arable cropping, fertilizers such as those tested in the present experiments would be spread according to their content of easily available N. Monitoring the C and N dynamics based on the application rate of mineral N therefore should provide a relevant evaluation of the agronomic traits these fertilizers will have when used for crop production. Also, since the same fertilizers have been tested for effects on NMC and PAO (Abubaker et al., 2011) based on their content of mineral N, the strategy to use the same RoA for PDA and respiration makes the present experiment a good complement to those experiments.

However, the fact that the fertilizers were added in amounts based on their content of NH_4^+-N, resulting in large differences in C additions, made the respiration results partly difficult to interpret. It also caused practical problems with the experiment itself, which is further discussed in the section 4.4.1 below. The results from the two highest RoA (70 and 140) gave the most reliable results and were also closer to application rates likely to be used in the field, and the following discussion is therefore based on these two RoA.

Looking at 70 and 140 RoA with respect to UtC, AG was the only treatment significantly higher than the other fertilizers, which means that the C in the BR and the PS was present in compounds less accessible to the microbes within the time frame of the experiment. This is also logical since anaerobic digestion for biogas production (El-Shinnawi et al., 1989) and digestion in the pig gastrointestinal system followed by anaerobic storage (Kirchmann and Witter, 1992) should have consumed the most easily available carbohydrates of the biogas substrate and pig feed, leaving the more recalcitrant compounds in the residues.

Angers et al. (2007) observed, in a field study of PS decomposition, a UtC of 22% in 22 days, which should be compared with the 31% in 12 days found in the present study. This difference could probably be attributed to differences in soil characteristics, temperature regimes and moisture content of the soils. Also, the PS was mixed into a larger soil volume in the field experiment (Angers et al., 2007). The UtC of the PS at all RoA after twelve days were in the range of that reported by Kirchmann and Lundvall (1992) for anaerobically stored PS.

De Neve et al. (2003) found the mineralization of C from a BR to be rapid during the first week after application and then almost stopped completely after 240 h during similar temperature regime but with higher soil moisture (80% WHC). At the end about 15% of the C of the BR had been mineralized, i.e. considerably less than what was found for all the four BR tested in the present experiment.

After 70 days of incubation in the study by Kirchmann and Lundvall (1992), the PS had lost 45% of its C. The PS in the present study was still showing higher respiration at the end of the experiment when compared to the BR (Fig. 4). In the case of BR B for instance, the respiration was retarded and had almost returned to zero already after 100 hours (Fig. 3). This indicated that differences in UtC between the PS and the BR might occur in time.

The time of the respiration peak was the parameter which separated the fertilizers most clearly. However, since the peak time differed also within the fertilizers with respect to RoA, the time of peak was clearly also related to the amount of C added. This implies that the significant differences between the fertilizers might, to some degree, have been an artifact of the varying C additions. The high addition of C was probably the reason for the significantly later peak of AG (except when compared to BR A). However, the different additions of C cannot solely explain the differences in peak time observed between the fertilizers.

Since the main peak of BR A appeared late, it was probably derived from compounds not very rapidly metabolized. Another explanation to the lag could be that the MOs needed time to adapt to the new environment before being able to mineralize a new type of easily available C. However, the initial small respiration peak and decline at 10 to 20 h for BR A, showed that early respiration took place. The time variation of the respiration could be due to the fact that some microbial enzymes, degrading frequently occurring substrates, are constitutive (i.e. always produced) and some microbial enzymes, degrading less frequently occurring substrates, have to be induced by the presence of these substrates. Furthermore, some compounds are readily taken up by the cell(s) of the MOs and some compounds need to be degraded by exoenzymes outside the cell before it can be taken up and

metabolized inside the cell. Both these mechanisms affect the time needed to degrade different organic C compounds.

The flat slope and the late time of occurrence of the main peak of BR A indicate that a C compound which was not immediately accessible for the microbes was being degraded and generated this peak. Proteins have been shown to be decomposed slower than free sugars and amino acids (Gunnarsson et al., 2008). A high protein content of BR A was indicated by the high content of organic N (Table 2). In a work by Gunnarsson (pers. comm.), where decomposition of a range of pure compounds were monitored, the protein albumin was found to have a peak of similar appearance and timing as the main peak of BR A, supporting the theory that this peak was derived from the decomposition of proteins.

The substrates used for the biogas process of BR B were distiller waste from production of ethanol from grains. BR B showed a rapid peak and decline, which suggests that the C degraded was derived from rapidly metabolized compounds. The timing and shape of the respiration peak are similar to peaks derived from decomposition of amino acids under similar conditions (Gunnarsson, pers. comm.). Distiller waste has been shown to contain amino acids for which the deamination is oxidative (Dahiya and Prabhu, 1984). These amino acids would thus be preserved during anaerobic treatment and it is therefore probable that amino acids were present in BR B.

Although derived from slaughterhouse waste, BR C had by far the lowest content of organic N (Table 2) and the initial rapid respiration must have been derived from N poor, but easily available C compounds. This was in contrast to BR A, where the substrate also partly constituted of slaughterhouse waste and yielded more N in the residue. Due to the differences in C quality, the peak was significantly later and also lower for BR A than for BR C. This showed how two BR, although derived from similar substrates, can be altered in quite different ways depending on the biogas process or by using differing amounts of the substrates.

BR D showed an early peak (probably within hours, although not detected) and a second, later and broader peak at 100 to 150 hours (Fig. 3). The slope of the early (main) peak of BR D must have been very steep in order to achieve the high respiration rate measured at 9 h (Table 6, Fig. 3). The substrate for BR D constituted partly of silage, i.e. grass. However, the early peak of BR D was not matched by the pure grass decomposition as reported by Gunnarsson et al. (2008), suggesting that the anaerobic digestion had altered some of the C into more labile forms, since decomposition in the BR occurred earlier, i.e. immediately at the start of the experiment. Concerning the later peak in BR D, Gunnarsson et al. (2008) found

similar respiration patterns when looking at decomposition of six grass species. In that study, the respiration during day 1-9 was closely related to the high content of xylose and the low content of arabinose in the grasses. However, the respiration of xylose and arabinose in the study by Gunnarsson (pers. comm.) did not result in the same pattern as the one observed in the present study. Instead, the compounds pectin and to some extent cellulose seem to better correspond to the late respiration by BR D. In any case, it is obvious that some of the decomposition characteristics of the grass were still prevailing in BR D, even though the substrate had gone through anaerobic digestion.

The fact that higher RoA of PS did not increase respiration at 9 h (as it did for the BR) and that PS showed (along with BR B) the lowest respiration at 9 h, despite a higher C content, suggest that the microbial activity in the soil of the PS treatment was somewhat restricted during the early stages of the experiment. The slow start of the respiration at PS addition, followed by a rapid development with a steep slope (Table 6, Fig. 3), could be due to a need for adaption for the MOs when exposed to high rates of PS before the quite easily available substrate could be decomposed. The slope of the respiration peak of PS was not different from that of AG (Table 6) indicating that the respiration lag of PS was due to a need for adaptation rather than inaccessible C compounds. Anaerobically stored manures, such as the PS used in the present experiment, most likely contain volatile fatty acids (VFAs) which can affect the microbes (Sørensen, 1998). VFAs have been shown to both be utilized as energy source by some MOs, contributing to soil respiration, (Kirchmann and Lundvall, 1993) and kill others (Conn et al., 2005).

Since the additions of C were different between the treatments, it becomes difficult to draw any conclusions on the content of C in the peaks on a weight basis. The fraction of the C respired (C as % of respired) found in the peaks seemed to be similar for all the fertilizers and all application rates. Thus the differences in C content of the peaks appeared to be more related to the total C addition rates rather than the different concentrations of individual C compounds. The uncertainty of these results due to subjectively chosen data for the calculations of the peak (based on visual examination of the curves), which also yielded few statistically significant differences, make this parameter hard to interpret further. However, it is clear that the BR and the PS contained a ray of different C compounds, which decomposed heterogeneously in comparison with AG where most C was respired together.

4.2 PDA

All the BR inhibited denitrification at high RoA. This means that there was something in the fertilizers which was toxic to the MOs. The opposite was observed for the PS, which resulted in increased PDA with increasing RoA. Hence, in this aspect there were large differences between the BR and the PS.

The denitrifying bacteria use easily available C as energy source when reducing the NO_3^-, and high content of C in the PS was a parameter which separated it from the BR. This C was easily available for respiration, which was shown in the respiration experiment. Thus, the higher supply of C with the PS was a potential explanation for the higher PDA. However, the treatment with double amount of glucose did not show increased PDA in comparison with the control which means that the substrate, containing glucose and nitrate, which was supplied to all treatments saturated the MOs. Hence, C was not the limiting factor or the reason for the positive and different response of PS.

Another possible explanation for the positive response of PDA to PS could have been the micronutrients supplied with the PS. However, the nutrient profile in the PS was not different in comparison with the BR and the supplementary treatment with a micronutrient solution showed no increase in PDA. Thus, the soil was able to supply all nutrients by itself and the macro- and micronutrient profiles of the fertilizers could not explain the differences in the PDA response to these substrates.

Heat treated PS resulted in lower PDA than the treatment with original PS. This result could not be statistically confirmed since only one replicate of each was tested. However, the result indicated that the PS itself contained MOs or enzymes with denitrifying abilities. If this was in fact the case it offers a plausible explanation to the PDA response of the PS, since larger addition of PS would result in larger addition of denitrifiers, thus raising the N_0 and therefore also the qN_0 (eq. 2), which is the PDA. To the knowledge of the author, this is the first time such a stimulatory affect on PDA by PS has been reported. From these results it could be discussed whether a large share of the generally observed N_2O release at PS fertilization (Jongebreur and Monteny, 2001; Petersen, 1999) could origin from denitrifying activity in the fertilizer itself. This is a topic in need of further investigation.

The increased supply of C and micronutrients with BR could explain that all the BR resulted in positive PDA response for the two or three lowest RoA, but, as discussed above, the experiments with double glucose and trace elements contradict

that conclusion. Instead, the increased PDA at low doses could either be the effect of adding MOs and enzymes with the BR, as discussed for the PS, or possibly the effect of increased microbial activity due to stress.

It has been shown that stress caused by heavy metal toxicity can increase the maintenance energy required by the MOs (Dahlin and Witter, 1998; Bardgett and Saggar, 1994), i.e. lead to increased activity by a set number of MOs. The basic energy requirement of MOs also increased when subjected to stress (Fernandes et al., 2005). All of these studies measured aerobic respiration during a longer time than what was used in the PDA experiment. The MOs of the PDA was respiring anaerobically, but can be expected to respond in the same way since the denitrifying bacteria are facultative anaerobes meaning that they respire with oxygen as final electron acceptor if oxygen is available. However, it remains uncertain whether this stress would occur to such an extent that it would show after the limited amount of time used in the present PDA experiment.

The PDA increase caused by the BR at low RoA can also originate from the mechanisms discussed above for PS. Also, since denitrifying MOs and enzymes are adapted to working in an anaerobic environment, it is likely that they survived the anaerobic digestion process and were present in the BR after that treatment.

The fertilizers contained heavy metals, predominantly Mn, Cu and Zn. Ag was not believed to be a component of importance in the fertilizers of the present experiment and was therefore not analyzed for. However, Ag within the range of the concentrations of other heavy metals in the present experiment (~100 ppm) have been shown to decrease PDA (Johansson et al., 1998; Noredal-Throbäck et al., 2007) causing similar response patterns as those observed for PDA at additions of the BR in the present study.

Holtan-Hartwig et al. (2002) observed inhibitory effects of Cu, Cd and Zn on denitrification. In an experimental setup similar to that of the present experiment, a severe decrease of the PDA was observed when the heavy metals were present. The highest Cu addition in the present experiment occurred with the PS 1120 where 171 mg Cu were added kg^{-1} dry soil (171 ppm), an addition exceeding the highest dose of Holtan-Hartwig et al. (2002).

In most experiments where severe decreases of PDA have been observed the metals have been added as chloride or sulfate salts (Johansson et al., 1998; Noredal-Throbäck et al., 2007; Holtan-Hartwig et al., 2002), which, in the short term, have made them highly bioavailable as compared to adding heavy metals with organic slurries.

Despite the high levels of Cu and Zn added to the soil with PS, no inhibitory effect was found on denitrification in the present experiment. A plausible explanation for this is the high organic C content of the PS, which may have contributed to decreasing the bioavailability of the heavy metals through complex binding. The BR inhibited denitrification at high RoA, despite the lower concentrations of heavy metals in the BR compared to PS. However, the BR contained comparatively less organic C which may have resulted in higher bioavailability of the present Cu and Zn, explaining the inhibition of denitrification by the BR.

Holtan-Hartwig et al. (2002) also found that the last step of denitrification (N_2O reduction to N_2) was more severely affected than the NO_3^- reduction to N_2O. In the present experiment, only the reduction of NO_3^- to N_2O was measured as the last step of the denitrification was inhibited by acetylene (eq. 1). Hence, there are reasons to believe that a stronger inhibition of denitrification by the BR would have been found in the present experiment if the full reaction, from NO_3^- to N_2, would have been taken into account. Since no inhibition on denitrification was caused by PS, this phenomenon might be less significant for this fertilizer.

It is more than likely that the NOEC of BR C and BR D will be exceeded at most RoA used in practice, especially for BR D. BR A and BR B have similar NOEC values and when used as the sole fertilizer in crops with a high demand for N, the NOEC can be exceeded. Since the positive response of PDA by PS addition were most prominent at the highest RoA, only a moderate increase of denitrification can be expected at RoA of PS which can be expected to be used in practice (up to 150 kg NH_4^+-N ha^{-1}).

4.3 Implication of N fertilization to the arable soil ecosystem

Generally, when the soil MOs run out of easily decomposable C, they decompose and mineralize other C compounds, also containing N which subsequently become mineralized. According to the present respiration experiment, the MOs became limited by easily decomposable C already after a few days when BR were added to the soil. Thus, the mineral N content of the soil amended with BR should have increased already after a few days. Even though the C/N ratios of the BR were lower than that of PS, the NMC of the soil was lower when amended with most BR in comparison with PS ten days after amendment (Abubaker et al., 2011). This showed that the N dynamics of an organic fertilizer cannot be predicted solely by its C/N ratio. Instead it is the nature of the compounds being decomposed which regulates the N mineralization/immobilization.

Looking at the data from this perspective the results by Abubaker et al. (2011) can be explained. In their study, BR C showed the clearest pattern of N immobilization. As discussed above, the C compounds respired in this fertilizer were probably poor in N, and thus the MOs had to use mineral N for growth. The respiration of BR A was largely attributed to decomposition of proteins and the NMC attained by Abubaker et al. (2011) for BR A mostly displayed positive values. The corresponding discussions are also valid for BR B and BR D.

The PS had the highest content of organic N, which probably led to decomposition of N rich compounds. The rapid growth of the MOs during the first days after C addition yielded a microbial flora too big to be sustained by the soil C after consumption of the easily decomposable C compounds of the PS. Thus, a large amount of C starved MOs was, after the respiration peak, mineralizing N as they tried to get C (C which was affiliated with N) from less available C compounds. Furthermore, since the MOs probably grew out of physical hiding places in the soil during the respiration peak, they became more accessible for predation. Altogether this should have yielded N mineralization, potentially explaining the high NMC observed for PS by Abubaker et al. (2011).

Incubation experiments where N immobilization and mineralization were monitored on a daily to weekly basis in soil when amended with PS were performed by Kirchmann and Lundvall (1992) and Delin et al. (2010) and showed no net mineralization of N after 10 days at PS RoA corresponding to approximately 450 (adapted and calculated following the methods used for the fertilizers in the present experiment) and 70 kg NH_4^+-N ha^{-1}, respectively. Delin et al. (2010) compared a variety of organic fertilizers, including both PS and a BR, with respect to N dynamics and found that the BR did show net N mineralization after 10 days. However, the BR of Delin et al. (2010) had a considerably lower C/N ratio (4) compared to the BR used in the present study (4.2 - 12.1). Such incubation experiments would be useful to further investigate the N transformations of the BR used in the present study.

In an arable field the small content of easily available C present in the BR will in most cases be consumed by the MOs and mineralized to CO_2 quite rapidly, while the mineralization in soil amended with PS will be intensive during the first week and probably go on for another week (Fig. 3; Table 6; Kirchmann and Lundvall, 1992). This means that the initial immobilization of the N in the fertilizers would be much lower for the BR in comparison with the PS, but that the following remineralization would be more intensive for the PS. Altogether this means that the N value of the fertilizers would be similar when applied to an agricultural field

but that the N from a BR would be available for the plants earlier than the N from PS.

From an agronomical point of view, the decrease in PDA in response to the additions of BR is not necessarily undesirable since it would prevent losses of valuable N to the atmosphere. The reduced PDA reported in the present study (Fig. 6) was a measure of the acute toxicity in the short term. It is not impossible that onset of anaerobiosis can be rapid (within hours) when wet fertilizer is spread to an agricultural field resulting in similar environment as in the laboratory. If this is true, the PS will more likely result in losses of N as N_2O or N_2. Although the N of the fertilizer itself will be in a reduced form (NH_4^+) the NO_3^- present in the soil at the time of spreading will still be available for the denitrifiers.

There is, however, another aspect to consider if the heavy metals are responsible for the PDA response caused by the BR. In the present experiment, inhibition of denitrification was observed when adding BR. In context of the results of Holtan-Hartwig et al. (2002) where the reduction of N_2O to N_2 was even more sensitive to heavy metals than the reduction of NO_3^- to N_2O, the overall effect of using BR as fertilizers, besides less total denitrification, might be that N_2O to a larger extent become the end product of the reaction.

Holtan-Hartwig et al. (2002) found that soil denitrifying MOs were able to acclimatize to the exposure of heavy metals in time of months, and reach almost the same PDA as the control, although severely affected at short time exposure. This would mean that only small long term effects on PDA can be expected from the addition of BR to soil.

The PS stimulation of the MOs, by the additions of both C and MOs, seems to be the factor separating it from the BR, explaining most of the differences in N dynamics observed. In a waterlogged and anaerobic soil, the C added with PS can be used as energy source for denitrifying and anaerobically respiring MOs, resulting in N losses to the air. In an aerobic soil, the C can be used for aerobic respiration, making the N unavailable both for plants and denitrifying organisms through incorporation in MO biomass (immobilization). However, abiotic factors, such as temperature and moisture regimes in an arable field, will have large impacts on these microbial processes, which can alter the conclusions of a laboratory study. When applied in spring time, low soil temperature would make both denitrification and respiration a lot slower than found in the present laboratory study.

PS seems to stimulate microbial processes to a larger extent compared to BR. Therefore, the fate of the nutrients in the BR will be less dependent on MOs. As a result of that, abiotic factors (such as weather) which are hard to predict but never-

theless drive the microbial processes of the soil ecosystem, will have less influence of the nutrient dynamics of BR when compared to the nutrient dynamics of PS. This relationship between organic fertilizers, MOs and abiotic factors might make the nutrient dynamics easier to predict for BR as compared to PS in an agricultural field. This would in turn make it easier to match the nutrient supply by the fertilizer by the nutrient demand of a crop. In a field study, Möller and Stinner (2009) evaluated the use of slurry and crop residues as fertilizers versus performing anaerobic digestion on the same materials before using the resulting BR as fertilizers. They found that anaerobic digestion of the organic fertilizers reduced the N_2O emissions by a third and increased the overall N use efficiency.

4.4 Some considerations of the experimental methods

4.4.1 Respiration

After performing a pre-test with PS and one of the BR, it was decided that the concentration of KOH in the cells inside the jars in the Respicond (Nordgren, 1998) had to be set to the relatively high value of 0.3 M in order to handle the large CO_2 production from mineralization of the added PS and AG. Using this concentration unfortunately also resulted in lower accuracy of the instrument, especially at low rates of C mineralization. Due to this, the data from the respiration experiment contained considerable amount of noise. This became particularly evident for the treatments where low rates of BR were added, and the data from these treatments therefore had to be interpreted with caution.

BR B 17.5 and BR C 17.5 both showed a negative UtC. This means that the basal respiration together with the control respiration were larger than the respiration of the jar with BR addition, on average throughout the experiment. This might have been a result of the BR having inhibitory effects on the soil MOs, but the possibility that it was an effect of noise, due to high concentration of the KOH solution, and differing accuracy of the cells cannot be discarded. Also, these two treatments were not significantly different from BR D 17.5 and BR D 35, which showed positive UtC.

In order to correct for background respiration, the mean basal respiration (the average of all jars in the experiment over the five days preceding the additions) was subtracted from each measured value. When, instead, the individual basal respiration rate (the average basal respiration for a specific jar over the five days preceding the additions) was subtracted from each measured value in the correspond-

ing jar, the negative UtC of BR C 17.5 and BR B 17.5 became less negative. However, instead the UtC of BR B 35 and BR D 17.5 became negative. In the end, the mean basal respiration of all the jars was chosen to be the better representative for the background respiration of the soil since the disturbance at addition might have affected both that and the cell. This showed the vulnerability of the data at the low rates of respiration attained from these treatments.

During field conditions, a farmer would firstly consider the easily available N in a fertilizer before applying it to an agricultural field. If the purpose of a laboratory study mainly is to evaluate the agronomic traits of the fertilizer, the strategy of adding it to soil based on its content of NH_4^+-N is very relevant. This strategy makes it possible to predict short term N dynamics in the soil of the BR, which is highly relevant when the fertilizer is used to stimulate short term crop production.

However, when the C contents of the fertilizers vary to the extent as it did in the present study, there would be a need for a slightly different experimental setup to achieve better results. Since the respirometer used in the present experiment is not able to deliver accuracy at low respiration rates in some measuring cells while simultaneously measuring large C mineralization peaks in others, it seems to be necessary to divide the experiment into two parts where two suitable concentrations of KOH can be used. This is of course a matter of time and resources, but in order to attain reliable results for all RoA of BR there is a need for better accuracy. A suitable amount of glucose, which would work for different concentrations of KOH, could be added in large numbers of replicates in both experiments. These would provide references, enabling the comparison between the two parts of the experiment.

If, on the other hand, the purpose of the laboratory study is to compare and contrast the C quality of the BR, and the impact on the overall microbial community, it would be more suitable to add the fertilizers on the basis of their C content. This would enable a more practical comparison of the BR impacts on the SOM and soil health, and also allow the use of only one concentration of KOH without accuracy difficulties.

Gunnarsson et al. (2008) suggested a set of standard compounds to be analyzed which would assist in explaining the decomposition of plant materials. The same principal, but maybe with another set of compounds, could be useful as a complement to performing a respiration experiment when evaluating slurry fertilizers.

The *UtC* provided only few statistically significant differences, which could partly be attributed to low instrument accuracy. However, UtC seemed still to be a useful parameter, especially since the RoA of C were differing.

Main peak time gave differences between the fertilizers and separated them in the clearest way. Together with the *peak height* and the *slope* of the peak, the parameter seemed to be partly related to the quantity of C added, potentially compromising the validity of the differences since the C additions were different for each treatment. However, these three parameters provided profiles of the fertilizers and helped explain the C decomposition, both in time and quantity and as a result also assisted when discussing N dynamics.

The *respiration at 9 h* was chosen as a parameter as this was the first measuring point after the initial disturbance where all the fertilizers were represented by a valid value. It provided data on the initial respiration of the fertilizers and revealed whether or not a lag phase was present.

The three parameters where *C in peak* was calculated were difficult to interpret, mostly due to difficulties in objectively deciding what values to include in the peak area. The values which were to be considered as the baseline of the peak had to be chosen by visual examination. This was probably a considerable source of error. For future reference, an alternative approach could be to use the x-axis as the base of the peak. This approach would make the data also to include C which was not a part of the peak, although respired during the peak. However, it could provide data, more reliable for comparison between the fertilizers.

4.4.2 PDA

For evaluating the acute toxicity of a slurry fertilizer on the soil MOs, a PDA assay seems to be an applicable approach. Since the experimental design already is based on transforming the soil and substrate into a slurry, the low DM content of the fertilizers is not an obstacle. The method displayed sensitivity of toxic elements present in the slurry fertilizers and proved to be a valuable instrument for measuring acute toxicity.

It was, however, difficult to use PDA for evaluating agronomic characteristics since the environment created in the experimental setup with unlimited supply of glucose and nitrate along with completely anaerobic conditions would be unrealistic on a field scale.

5 Conclusions

There were differences between all the BR and the PS evaluated in the present experiments, both regarding the effects on the soil microbial community and agronomic impacts. The differences were most prominent between the BR and the PS, but smaller differences among the individual BR were also revealed. In comparison with the conventional organic fertilizer PS, the BR generally acted more inhibitory, or less stimulatory, on both respiration and PDA. These differences are likely to make it easier to predict the nutrient dynamics of a BR.

The respiration patterns of the BR in soil were to a large extent determined by both the substrate composition and the process parameters used in the biogas process. Despite the different origins and compositions of the fertilizers, only AG stood out with respect to the utilization rate by the soil MOs of the C added (UtC). However, there were large differences between the BR and PS in the overall respiration patterns.

The respiration curve parameters which provided best contrast of the fertilizers were *main peak time*, *peak height* and the *slope* of the increase in respiration before the peak. Together with the *respiration at 9h* and the *utilization rate of C* these parameters seemed to represent the most characteristic data attainable from the respiration curves.

Differences between the BR and the PS were distinct with respect to denitrification. BR inhibited denitrification at high rates of addition, most likely due to heavy metal toxicity. PS stimulated denitrification at high rates of addition, possibly due to the presence of denitrifying MOs and enzymes in the slurry itself and less availability of the heavy metals due to higher content of organic C. The presence of microorganisms in the PS was a new finding and future research on that topic can provide an explanation to the generally observed N_2O evolution at PS application to soil.

There is a need for further investigations on exactly how the nutrient dynamics of the soil are altered by different BR in comparison with the conventional organic fertilizer PS, especially on field level. Incubation studies can be proposed as a potentially useful tool to further complement microbial toxicity studies and the evaluation of nutrient dynamics. Analysis of C compounds in the fertilizers responsible for the respiration patterns could provide another useful tool for the evaluation of the fertilizers. Determining which C compounds to analyze for is an area in need of further investigation.

If the purpose of a future laboratory study is to investigate the short term agronomic traits, such as N dynamics, a respiration study based on equal additions of N, such as the one performed in the present study, should be the method of choice. However, there will be a need for better adapting the instrument of use to match the C content of the fertilizers, and maybe also a need for dividing the experiment into two parts. The experimental setup of the PDA experiment worked well with low DM of the slurry fertilizers, and the method proved to be useful in evaluating acute toxicity of the fertilizers.

Together the two experimental methods soil respiration and PDA complement each other and provide a good overview of different impacts of any liquid organic fertilizer, especially if accompanied by a complementary set of experiments such as assessment of potential ammonia oxidation (PAO), nitrogen mineralization capacity (NMC), incubation studies and C compound analysis.

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7 Restprodukter från biogasprocessen påverkar markens mikroorganismer

Det mesta av maten vi äter till vardags är producerad på åkermark. Åkermarken är en begränsad resurs som måste behandlas så att dess produktionsförmåga inte försämras. Genom att gödsla på rätt sätt så att förrådet av växtnäringsämnen och organiskt material inte minskar kan jordens kvalitet bibehållas eller förbättras. Organiskt material är dels viktigt för markens struktur men också för att underhålla markens mikroorganismer som både frigör bunden växtnäring och bidrar till uppbyggnad av markens struktur vilket stimulerar växtproduktionen.

I Sverige produceras idag biogas som kan ersätta fossil olja. Biogasen produceras ofta från olika organiska avfallsprodukter så som matavfall från hushåll, slakteriavfall eller stallgödsel från djuruppfödning. I vissa fall används även gräs eller spannmål. Produktion av biogas genererar förutom gasen också en flytande restprodukt som innehåller växtnäringsämnen och organiskt material. Denna kallas rötrest och kan användas på åkermark för att gödsla t.ex. grönsaker och spannmål. Rötrester kan dock innehålla ämnen som är potentiellt skadliga för marken och dess mikroorganismer varför de bör utvärderas innan användning.

I en studie samlades rötrester in från fyra stora biogasanläggningar i Sverige. I två olika laboratorieexperiment tillsattes rötresterna till en svensk åkerjord i stigande mängd upp till motsvarande 1120 kg ammoniumkväve per hektar. Genom att mäta mängden CO_2 som producerades bestämdes sedan i det ena experimentet hur mikroorganismerna respirerade det organiska materialet i gödseln. I det andra experimentet undersöktes påverkan på de mikroorganismer som utför denitrifikation, d.v.s. omvandlar nitrat till kvävgas när de respirerar utan syre. I båda experimenten jämfördes rötresterna med svinflytgödsel. Ett syfte var att testa metoder och utveckla en standardprocedur för utvärdering av flytande gödselmedel. Ett

annat syfte var att söka förstå hur olika rötrester och svinflytgödsel uppför sig och påverkar markens mikroorganismer när de sprids i fält.

Respirationsförsöket visade att mer organiskt material från gödseln bröts ner i jorden då svinflytgödsel tillsattes jämfört med tillsats av rötrester. Detta berodde till största delen på att svinflytgödseln innehöll mer organiskt material än rötresterna. Försöket visade också att det substrat som rötresten härstammade från hade betydelse för hur det organiska materialet i rötresten omsattes. Alla fem gödselmedlen uppvisade olika respirationsmönster och ett antal karaktäristiska mätvärden kunde bestämmas ur de uppkomna respirationskurvorna vilka kan användas för att beskriva det organiska materialets kvalitet hos olika rötrester.

I det andra försöket, där gödselmedlens påverkan på denitrifikation testades, visade det sig att stora tillsatser av alla rötrester till jorden hämmade denna process. Anledningen till denna respons kunde inte klarläggas fullt ut men en möjlig förklaring skulle kunna vara rötresternas innehåll av metaller. Svinflytgödseln skilde sig markant från rötresterna då den i stället ledde till högre denitrifikation vid stora tillsatser. Detta berodde troligen på att svinflytgödseln i sig själv innehöll mikroorganismer som kunde samverka med jordens mikroorganismer. Det här är en helt ny upptäckt som kan hjälpa till att förklara tidigare forskning runt växthusgasemissioner vid gödsling med svinflytgödsel.

Slutsatsen är att det finns skillnader mellan hur rötrester och svinflytgödsel påverkar mikrobiella processer i marken. Mindre skillnader kunde även påvisas mellan de olika rötresterna vilka till stor del kunde härledas till det substrat som använts i biogasprocessen. Aktiviteten av jordens mikroorganismer var större vid tillsats av svinflytgödsel jämfört med vid tillsats av rötrester. Mikroorganismers omsättning av organiska gödselmedel är allmänt svår att förutsäga på grund av att den påverkas av yttre faktorer såsom väder. I förlängningen gör detta rötresterna mindre känsliga än konventionella flytgödsel för dessa yttre faktorer och rötresternas växtnäringsvärde blir därmed lättare att förutsäga.

Experimentet visade också att olika gödselmedel med stora skillnader i innehåll av organiskt material kan orsaka praktiska problem med respirationstestets mätutrustning. För att i framtiden kunna kalibrera mätutrustningen för bästa känslighet vid liknande experiment rekommenderas det därför att gödselmedlen delas i två grupper efter innehåll av organiskt material. Rent praktiskt fungerade testmetoden för denitrifikation väl med de flytande gödselmedlen. Både respirationstestet och denitrifikationstestet kunde klassa de testade gödselmedlen med avseende på effekt på markens mikrobiella ekosystem och båda metoderna kan rekommenderas ingå i ett testbatteri för värdering av rötrester som gödselmedel och jordförbättrare.