Short-term effects of biogas residue addition on CO_2 and N_2O production and denitrification potentials in two contrasting soils

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Abstract

Biogas residues (digestates) are liquid slurries typically rich in ammonium (NH_4^+) and phosphorous (P) which can be used as fertilisers, thus increasing the overall sustainability of biogas production. However, the addition of mineral N and P together with easily degradable organic carbon to soils may increase carbon dioxide (CO_2) and nitrous oxide (N_2O) emissions, which could compromise the overall goal of bioenergy production (CO_2 saving). Whereas a number of studies have investigated longer term effects of residue application on plant growth, N- and P-status and greenhouse gas (GHG) emissions in cultivated soils, little is known about the immediate effects on C- and N-transformations when applying nutrient-rich slurries to bare soils. Laboratory incubation experiments was conducted with three Norwegian soils (sand, loam, silt) amended with biogas digestates from various mixtures of feedstocks (manures, wood, fish wastes, bagasse) to assess CO_2 and N_2O production potentials as affected by soil types and digestate quality. Soil type was found to strongly interact with digestate quality, resulting in soil-specific patterns of stimulation and repression in CO_2 and N_2O production across the different digestate qualities tested. This could be attributed to fundamentally different C- and N-turnover processes in the soils. The loam strongly suppressed indigenous respiration activity in the slurries and immobilised added NH_4^+ rapidly (presumably by fixation to clay minerals), resulting in little or no stimulation of CO_2 production and an overall repression of N_2O production as compared with a control only receiving water. In contrast, the silt responded with increased respiration activity, less NH_4^+ immobilisation (presumably dominated by microbial immobilisation), resulting in a clear stimulation of CO_2 production and, in some cases, also N_2O production. In a second experiment, the effect of biogas slurries on denitrification potentials and relative N_2O production was tested. Residues stimulated denitrification in both soils but strongly decreased $N_2O/(N_2O+N_2)$ ratios in the silt. Together, our results suggest that soil-specific immobilisation and stabilisation processes have to be taken into account when extrapolating environmental effects of biogas residue application to soils.

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CH_4	Methane
CO_2	Carbon dioxide
COD	Chemical oxygen demand
CSTRs	Continuous stirred-tank reactors
DI	Deionised
ECD	Electron capture detector
FIA	Flow injection analyser
FID	Flame ionization detector
GC	Gas chromatograph
GHG	Greenhouse gas
N	Nitrogen
NH_4^+	Ammonium
N_2O	Nitrous oxide
NO_3^-	Nitrate
OC_5	Oxygen consumption over 5 days
P	Phosphorous
TCD	Thermal conductivity detector
TIN	Total inorganic nitrogen
WHC	Water holding capacity

1. Introduction

Fossil energy sources are limited and offset the radiative balance of the Earth through increased emission of carbon dioxide (CO_2) and other greenhouse gasses (GHG) to the atmosphere, ultimately leading to global warming (IPCC, 2007). Gradual replacement of fossil fuels by biofuels is a promising option to mitigate CO_2 emissions, since biofuels contain carbon recently fixed from the atmosphere which is considered to be climate-neutral. Currently, biofuels are mainly produced from biomass derived from cereals, sugarcane or maize grown on fertile agricultural land (Butterbach-Bahl and Kiese, 2013). This makes biofuels a controversial issue, since the production of the source material (feed stock) competes with food production for land. Moreover, arable production of bioenergy crops requires nitrogen (N) fertilisation to replenish the N removed with the crop, which induces emissions of nitrous oxide (N_2O) through enhanced nitrification and denitrification (IPCC, 2007). N_2O is a potent GHG which, on a 100-year time horizon, is 300 times stronger than CO_2 (Ehhalt *et al.*, 2001). Crutzen *et al.* (2008) showed that N_2O emitted during the production of bioenergy crops can severely offset the GHG balance of bioethanol, or even turn it into a net GHG source. An obvious way to avoid this would be to produce "second generation biofuels" from non-edible biomass and/or organic wastes, and return the N- and P-rich residues back to soils, thus combating climate change and maintaining food production at the same time. This requires advanced process understanding of the entire production chain ranging from feedstock selection, pretreatment, fermentation technology, post-treatment to fertiliser value and GHG emission potential of biorests when applied to soil.

The most promising and versatile process involving non-edible feedstocks so far, is anaerobic fermentation of biomass to biogas (CH_4 and CO_2). Common feedstocks are silage, straw, corn stover, bagasse and animal manure (Holm-Nielsen *et al.*, 2009; Alburquerque *et al.*, 2012). In Norway, marine production is an important industry yielding significant amounts of energy-rich by-products such as fishbone meal and fish oils. Finally, woody materials such as birch and also Salix are widespread in Fennoscandia and are expected to increase in abundance as encroachment proceeds and tree lines climb due to climate and land use change (Tømmervik *et al.*, 2009). Therefore, methods have been sought to include woody materials and marine by-products in feedstocks from organic wastes, while maintaining reasonable CH_4 yields (Estevez, 2013). Recently, steam explosion has been shown to increase the digestability of highly lignocellulosic biomass by anaerobic fermentation (Horn *et al.*, 2011; Vivekanand *et al.*, 2013), and making mixtures of lignocellulosic and organic wastes is a promising feedstock for biogas production in Fennoscandia.

To ensure sustainability of the entire production chain, fate and environmental impact of biogas residue (digestates) have to be considered. Up to 80% of the organic matter in the feedstock is converted to biogas during anaerobic digestion, leaving behind a digestate high in N and phosphorous (P) with low C/N ratio (Tambone *et al.*, 2010). Apart from its fertilisation value for crop production, digestates may also serve for ameliorating soils poor in structure, and might thus have an overall positive effect on soil fertility and crop yield (Odlare *et al.*, 2011). Digestates from biogas production are typically rich in ammonium (NH_4^+), which can result in short-term N immobilisation right after application to soil, because of microbial immobilisation (Alburquerque *et al.*, 2012; Fuente *et al.*, 2013). However, the large content of NH_4^+ in combination with a high amount of liquid makes biogas digestates a potential source of N_2O due to oxygen limited nitrification of NH_4^+ and subsequent denitrification of nitrate (NO_3^-) (Odlare *et al.*, 2012; Alotaibi and Schoenau, 2013). This could counteract the idea of sustainable nutrient use and compromise the positive effect on carbon saving as a whole (Crutzen *et al.*, 2008). In general, soil C and N dynamics can be expected to be affected by digestate addition on different temporal scales through high ammonium content, change in pH, input of readily decomposable carbon leading to high biological oxygen demand and possible contamination with pollutants (e.g. heavy metals), all of which may ultimately affect CO_2 and N_2O emission from soil (Holtan-Hartwig *et al.*, 2002; Alburquerque *et al.*, 2012; Thomsen *et al.*, 2012). Digestates contain a large amount of liquid which can saturate the upper soil layer directly after application, possibly leading to transiently reductive conditions which favour N_2O formation by nitrification or denitrification. Recently conducted pot experiments with ryegrass receiving biogas digestates from various feedstock mixtures equivalent to 180 kg N ha^{-1} showed an immediate response in CO_2 and N_2O emissions for up to 10 days and a second peak in N_2O emission 20-30 days after application (Eich-Greatorex *pers.comm.*). Whereas the latter peak can be attributed to mineralisation of the digestates' solid phase, little is known about the mechanisms and controlling factors of the immediate CO_2 and N_2O emission response observed upon slurry addition.

The aim of the present study was to provide a laboratory-based assessment of instantaneous N_2O and CO_2 emissions triggered by digestates derived from various mixtures of organic wastes and lignocellulosic biomass when applied to soil. Three different soil types were used, with focus on a loam and silt soil which represent common soil types in Norwegian agriculture. For this, digestates were added to sieved soil and incubated aerobically while monitoring gas exchange. To study denitrification rate and $N_2O/(N_2O+N_2)$ product ratio as affected by digestate quality,

soil slurries amended with digestates were incubated under anoxic condition. Relationships between digestate quality and N_2O and CO_2 emission potentials are discussed.

2. Materials and methods

2.1 Digestates

The digestates originated from anaerobic fermentation of various feedstock mixtures including woody materials, dairy co-product or fish by-product (Tab. 1). Three of the feedstocks (A, B and D) contained woody material from willow (*Salix viminali)* or birch (*Betula pubescens*) and digestate C contained seaweed (*Saccharina latissima*) and bagasse (from sugarcane, *Saccharum officinarum*). These digestates were high in lignin; in percentage of dry weight: 35.1% for digestates A and B, 46% for digestate C and 35.9% for digestate D (Tab. 1). Willow and especially birch are widespread and readily available biomass throughout Nordic Countries (Vivekanand *et al.*, 2013), which makes them interesting for biogas production, provided that the cellulose is partly hydrolyzed prior to incubation. Seaweed is another naturally occurring feedstock in Northern Europe with potential for biogas production. Much research on pre-treatment of ligneous feedstocks has been done to facilitate its fermentation, such as biological delignification (by lignin-degrading microorganisms), chemical hydrolysis (e.g. by NaOH, H_2SO_4), milling, microwave irradiation and steam pre-treatment (Gould, 1984; Singh *et al.*, 1995; Pereira, 2001). In the present study, the woody materials were pretreated by steam explosion and then co-digested with other, less recalcitrant feedstocks (Estevez *et al.*, 2012; Vivekanand *et al.*, 2013). Fish by-products are abundant in fish producing economies like Norway. Norwegian fisheries produce more than 180,000 tons of by-products annually (2011), equivalent to 30% of the fish caught and farmed in Norway (Estevez, 2013). Most of the fish by-products are used as raw materials for animal feed, such as silage. However, if contaminated by infected fish, the by-products cannot be used for feed, but may still be valuable feedstocks for biogas production. Fish by-products contain a large amount of proteins and lipids which are easily degradable, and reportedly increase methane yield (Estevez, 2013). The mixtures were prepared according to the C: N ratio. The optimal C: N ratio for biogas production is around 30 (Vivekanand *et al.*, 2013).

Digestates were obtained from continuous stirred-tank reactors (CSTRs) (Estevez, 2013; Vivekanand *pers.comm.*), except for F which was untreated manure. Digestates A and B differed from the others by having been recirculated during the fermentation process. This resulted in higher ammonium concentrations (Estevez, 2013) and a higher chemical oxygen demand (COD) (Tab. 1). All digestates showed alkaline pH with low concentration of dry matter. The lowest loss of ignition was in digestate C (66.81%), and the highest in digestate A (80.57%). Oxygen consumption over 5 days (OC_5) was determined as cumulative O_2 uptake at 15 ^oC when added to

inert quartz sand. COD was measured by chemical digestion (chapter 2.3). As a proxy for the relative biological carbon availability in the digestates, OC_5 over COD ratios were calculated. Since the amount of dry matter was small in the digestates, C/N ratios were calculated at the ratio between COD and total inorganic nitrogen (TIN) in the liquid phase (Tab. 1). All digestates were stored in 5L PVC cans at 4 ^{o}C in the dark for approximately 4 months before use.

Table 1. Description of digestates used in the study.

Digestates	Feedstock	pH	Loss of ignition	NO_3-N	NH_4-N	COD	OC_5	OC_5/COD	COD/TIN	Lignin content[a,b]	Methane yield [a,b]	Days of fermentation [a,b]
			%	mg/kg digestate	mg/kg digestate	mg O_2/L	mg O_2/L			% DW[c]	mL CH_4/ g VS[d]	
A	Manure+salix+fish	7.9	80.6	2.9	1285	8703	1246	0.1	6.8	35.1	159	132
B	Manure+salix	7.9	79.3	1.4	1400	8493	2804	0.3	6.1	35.1	141	132
C	Seaweed+bagasse	7.4	66.8	1.3	41	3601	1583	0.4	83.3	46	155	180
D	Birch+manure	7.3	79.9	0.6	625	3368	2093	0.6	5.4	35.9	127	90
E	Whey+manure	7.3	75.1	0.5	575	2548	920	0.4	4.4	—	147	90
F	Manure	7.6	71.3	2.4	615	3515	663	0.2	5.7	—	—	0

[a] data from (Estevez, 2013)
[b] data from (Vivekanand *pers.comm.*)
[c] dry weight
[d] volatile solids: substances that volatilized at 550 ^oC, indicating the organic content in materials
Note: digestate F was not anaerobically digested

2.2 Soils

Three types of soil were collected from different places in South-eastern Norway. The three soils were: Loam, from Ås (59°39′57″N 10°45′58″E); Silt, from Solør (60°23′31″N 11°54′01″E); Sand, from Elverum (60°52′54″N 11°33′44″E). These soil types cover a wide range of soil properties (Tab. 2). The silt had a somewhat higher organic matter than the loam, and the highest water holding capacity (WHC). The sand was very poor in organic matter and was biologically inert, i.e. no CO_2 production was measured. The sand soil was therefore only used to determine the OC_5 of the digestates. The silt had higher phosphorous and potassium content, and the sand had the lowest nutrient content in general. Soils were sieved through a 5 mm sieve before using them for incubation experiments.

Table 2. Description of the major physiochemical characteristics of the soils used for incubation experiments.

	pH	Organic matter %	Total N g/kg	P-Al mg/kg	K-Al mg/kg	Sand %	Silt %	Clay %	WHC % weight
Loam	5.2	3.8	2.0	28	120	45.0	34	21	48
Silt	6.5	4.0	1.1	65	145	18.5	76	5.5	56
Sand	6.0	0.3	nd	23	<10	>90	bd	bd	23

Note: bd= below detection limit.

2.3 Chemical oxygen demand

The total amount of oxidisable carbon in the digestates was determined by digestion with potassium dichromate in sulphuric acid-at 148 ^{o}C for 2 hours and measured spectrophotometrically (HACH, LANGE).

2.4 Nitrate and ammonium contents in soil after amendment with digestates

Ten grammes of air-dried soil was extracted with 25 ml 2M KCl and filtered after 1 hour of horizontal shaking through 125 mm filters (Schleicher& Schuell, Germany). The extracts were frozen prior to analysis of NO_3^- and NH_4^+ analysis by a flow injection analyser (FIA) (Tecator FIAStar 5010 Analyser).

2.5 Soil pH measurement

Soil pH was measured following a protocol for dried soil in H_2O (Ogner *et al.*, 1999) with modifications. Approximate 10 ml soil was added to screw-top conical vials, and 25 ml deionised (DI) water was added. Soil samples were shaken, and left overnight. Samples were shaken by hand once again one hour before measuring pH in the over-standing water by a glass electrode (Orion 8175BNWP, Thermo Electron Co.).

2.6 Soil water holding capacity

To determine the WHC of the three soils, air-dried soil was added into funnels equipped with paper filters so as to achieve the same height as in the experimental flasks. Soil was carefully saturated with water for > 3 hours (avoiding the inclusion of air bubbles) and then let to drain freely. The weight was recorded (W_{wet}) denoting 100% WHC. After this, the soil samples were removed from the funnels and set for drying at 50 ℃ for 72 hours (W_{dry}) to obtain the amount of water at 100% WHC. Calculation of WHC based on weight is given in equation 1:

$$\text{WHC (weight \%)} = \frac{\text{Wwet} - \text{Wdry}}{\text{Wdry}} * 100 \qquad (1)$$

2.7 Soil pre-treatment

Since the soils were air-dried, a preincubation was necessary to avoid the flush of microbial activity commonly observed upon rewetting of dry soil (Birch, 1964) which would have confound the respiration response to digestate addition. Sixty percent of WHC was chosen as moisture content for preincubation and experimentation to allow the intermediate aeration conditions supporting both nitrification and denitrification during the preincubation. Ten grammes of soil was transferred to 120 ml serum flasks and adjusted to 60% WHC by adding DI water. The bottles were covered with perforated foil (Parafilm) to avoid water loss while maintaining gas exchange. The flasks were set for preincubation at 4 ^{o}C in the dark for > 2 weeks prior to digestate addition.

2.8 Digestate addition

After equilibrating the incubation bottles at 15^{o}C, digestates in amounts equivalent to 20 - 200 kg N ha^{-1} were added by a pipette directly onto the soil surface (supplementary Tab. 1). Prior to this, the digestates were mixed thoroughly to ensure that a sample with representative dry matter content was applied. The different amounts of N added were due to the markedly different concentration of N in the digestates (Tab. 1), which prohibited addition of digestate at equal N-rate. This would have resulted in major differences in the amount of liquid added. In order to obtain the same soil moisture, water was added up to the largest amount of digestate added (3.4 ml in the loam and 3.9 ml in the silt; supplementary Tab. 1). The addition resulted in saturation of all soils, thus mimicking a condition which may be expected to occur right after application of digestates in the field. Soils without digestates (but an equivalent amount of water) were used as control. Triplicate samples were prepared for each combination of soil and digestate.

For anoxic incubation (see chapter 2.9), soil slurries were prepared from preincubated soil by adding 10 ml DI water prior to adding digestates at the same amounts as described above.

2.9 Gas kinetics under oxic and anoxic headspace conditions

Immediately after adding the digestates, the incubation bottles were crimp-sealed with butyl septa and set into a water bath holding 15 ^{o}C by means of a cryostat. The water bath is part of an automated incubation system, similar to that described by Molstad *et al.* (2007), which semi-continuously monitors headspace concentrations of CO_2, CH_4, O_2, N_2, N_2O and NO.

Briefly, the water bath holding up to forty-four 120 ml bottles is placed under the robotic arm of an autosampler (GC-Pal. CTC, Switzerland), which repeatedly pierces the bottles to sample headspace gas (ca. 1 ml) by a hypodermic needle and transports the gas by means of a peristaltic pump to a gas chromatograph (GC) and a chemoluminescence NOx analyser coupled in series. To avoid underpressure, an equal amount of helium (He) is returned to the bottles after each sampling, which is drawn from a He-purge line placed at the vent of the GC. The resulting dilution and leakage of O_2 and N_2 into the bottles is taken into account when calculating rates of production/consumption for each time increment (for details see Molstad *et al*. 2007). The GC (Model 7890A, Agilent, Santa Clara, CA, USA) is equipped with three sampling loops automatically injecting the same headspace sample onto a Poraplot Q capillary column (for separation of CO_2, N_2O and CH_4 from bulk gases), a 5Å capillary Molsieve column (for separation of O_2 and N_2) and a NO analyser (Model 200A; Advanced Pollution Instrumentation, San Diego, USA). The GC has three detectors: a flame ionisation detector (FID) for CH_4, a thermal conductivity detector (TCD) for CO_2, O_2, N_2 and high concentrations of N_2O (> 5 μL L^{-1}) and an electron capture detector (ECD) for low concentrations of N_2O (linear range 0-4μL L^{-1}). Bottles filled with standard gases (known concentration) were included in the measurement sequence for calibration and for evaluating dilution by sampling loss (i.e. replacement by helium) and leakage of O_2 and N_2. Assuming equal dilution and leakage for each flask, the production and consumption rates for the various gases were corrected when calculating production/consumption rates from concentration change over time. Dissolution of gases in the soil water was taken account for by applying Henry's law constants (for details see Molstad *et al.*, 2007).

Two types of incubation experiments were conducted. In the first experiment, soils were incubated with ambient air without stirring, thus mimicking field conditions to some extent. The

headspace gases in the flasks were monitored every 5th hour. Soils were incubated for 200 hours, except for sand which was only incubated for 66 hours, since the control (no digestate added) showed no measurable activity. A second experiment was conducted to measure denitrification potentials and product stoichiometries (molar ratios of denitrification gases). In this experiment, the soils were incubated under anoxic condition and constant stirring by placing the bottles with a magnetic stirring bar on a submersible stirrer in the water bath. The bottles were made anoxic by washing with He (6 cycles of evacuation and He-filling). After temperature equilibration (15 ℃), the overpressure resulting from He flushing was released by piercing the bottles with a needle attached to a 5 ml syringe without plunger but filled with 1 ml water (to avoid O_2 contamination). The headspace was monitored every 5th hour, for a period of 60-100 hours, depending of the accumulation of denitrification products.

2.10 Calculations and statistical analysis

CO_2 production was calculated from the change in the headspace gas concentrations corrected for dilution and leakage and expressed as μg C g^{-1} soil h^{-1} (Fig. 1A). The respiratory quotient (Rq) was calculated as the molar ratio of CO_2 production and O_2 uptake (not shown). Initial N_2O production was calculated from the change in headspace gas concentration within the first 50 hours and expressed as μg N g^{-1} soil h^{-1} (Fig. 1 B). After 50 hours, N_2O accumulation levelled off in some treatments, presumably because of carbon limitation, like shown in the example below (Fig. 1B)

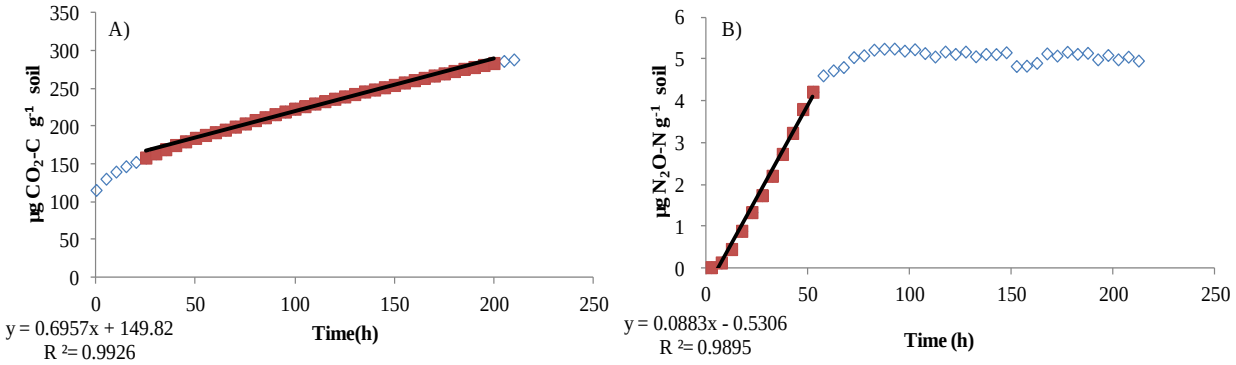

Figure 1. Accumulation of A) CO_2 and B) N_2O during "oxic" incubation (Experiment 1). The CO_2 production was calculated from concentration change over 200 hours, whereas the N_2O production rate was calculated from the first 50 hours of incubation (red symbols). The example is from the loam soil amended with digestate E.

Denitrification rate (Experiment 2) was calculated from the change of total N gasses (N_2, NO and N_2O) in the bottle before production levelled off due to exhaustion of nitrogenous electron

acceptors. The denitrification product ratio ($N_2O/ (N_2O+N_2)$) was calculated as the ratio of integrals over time (Liu *et al.*, 2010) by applying equation 2:

$$N_2O/ (N_2O+N_2) = \int_0^T N_2O/ (\int_0^T N_2O+\int_0^T N_2)*100\% \quad (2)$$

where the integrals were calculated from the area under the curve (Fig. 2). The cut off for the integrals was chosen from the accumulation curve of N-gases, which reached a plateau when electron acceptors (NO_3^-, NO_2^-) were depleted.

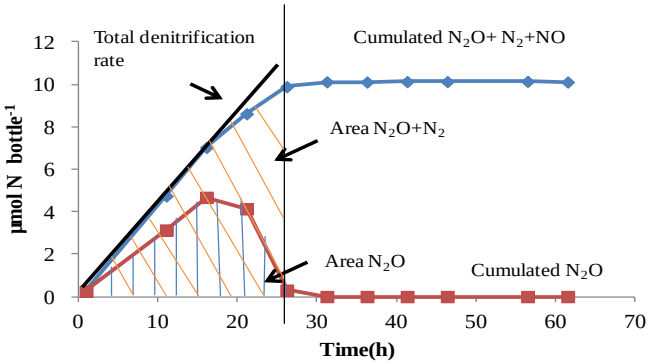

Figure 2. Net-N_2O and total N gas production and integrals used for calculating the $N_2O/(N_2O+N_2)$ ratio.

Statistical analysis was done to determine significant difference between digestates A-F in the soil. Data was tested by one-way ANOVA procedure using Minitab 16 for windows. The confidence level was $p \leq 0.05$.

3. Results

3.1 Kinetics of gas production under oxic conditions

3.1.1 CO_2 production and N_2O production in sand

The sand had negligible microbial activity (0.007 µg CO_2–C g^{-1} soil h^{-1}; Tab.3) and the addition of digestates resulted in CO_2 emission three orders of magnitude higher than in untreated sand. There was no increase in N_2O production (except for digestate D). Given the low activity of the sand, CO_2 production essentially reflected the native respiratory activity of the digestates, undisturbed by substrate-adsorption and other C-stabilising effects. The O_2 uptake data (not shown) were therefore extrapolated to 5 days and used to calculate OC_5 (Tab.1)

Table 3. Mean CO_2 production rates (n=3, SD in parentheses) and net N_2O production rates in sand soil amended with digestates A – F. Different letters indicate significance of differences ($p<0.05$) within each soil.

Treatments	CO_2 production rate (µg CO_2-C g^{-1}soil h^{-1})	Initial N_2O production rate (ng N_2O-N g^{-1}soil h^{-1})
A	0.25 (0.01)^{d}	0.015 (0.001)^{cd}
B	0.52 (0.11)^{b}	0.030 (0.011)^{b}
C	1.33 (0.09)^{a}	0.024 (0.007)^{bc}
D	0.55 (0.02)^{b}	0.163 (0.013)^{a}
E	0.41 (0.04)^{c}	0.010 (0.006)^{d}
F	0.24 (0.01)^{d}	0.013 (0.005)^{cd}
Control	0.007 (0.003)^{e}	0.007 (0.006)^{d}

3.1.2 CO_2 production and respiratory quotient in loam and silt

Table 4 shows pH-corrected CO_2 production rates and respiratory quotients in the loam and silt soil with and without digestate addition when incubated in ambient air. The CO_2 production rate of the loam (0.74 µg CO_2-C g^{-1}soil h^{-1}) without amendment was higher than that of the silt (0.5 µg CO_2-C g^{-1}soil h^{-1}). The loam also had a higher respiratory quotient than the silt (Rq=1 versus Rq=0.7; Tab. 4). Digestates significantly stimulated CO_2 production in both soils, but the pattern of stimulation differed (Fig. 3). Whereas all digestates stimulated CO_2 production between 20 and 170% in the silt soil, the stimulation was much weaker in the loam (0-40%) and only occurred with digestates B, C and D. The strongest stimulation was seen with digestate C (seaweed and bagasse) in both soils. Digestate C contained very low concentration of NH_4^+-N, but the COD value was almost the same as in digestates E and F. Thus digestate C had the highest ratio of COD/TIN in the liquid phase among the tested digestates.

Table 4. Mean CO_2 production rates (n=3, SD in parentheses), respiratory quotient and initial N_2O production rates in the loam and silt soil amended with digestates A – F. Different letters indicate significance of differences ($p<0.05$) within each soil.

Treatment	Oxic incubation					
	CO_2 production rate ($\mu g\ CO_2$-C g^{-1}soil h^{-1})		Rq (nmol CO_2/nmol O_2)		Initial N_2O production rate (ngN_2O-N g^{-1}soil h^{-1})	
	Loam	Silt	Loam	Silt	Loam	Silt
Digestate A	0.68^{c} (0.05)	0.85^{b} (0.04)	0.90^{c} (0.02)	1.28^{b} (0.08)	17.8^{cd} (3.1)	11.4^{c} (0.8)
Digestate B	0.96^{b} (0.10)	0.89^{b} (0.02)	0.99^{abc} (0.13)	1.14^{c} (0.03)	38.0^{cd} (8.6)	2.2^{d} (0.6)
Digestate C	1.05^{a} (0.01)	1.33^{a} (0.35)	1.09^{a} (0.07)	1.28^{b} (0.02)	10.8^{d} (6.8)	22.8^{b} (0.9)
Digestate D	1.01^{ab} (0.01)	0.74^{c} (0.04)	0.92^{bc} (0.04)	1.11^{c} (0.05)	35.5^{c} (3.4)	66.0^{a} (7.1)
Digestate E	0.73^{c} (0.02)	0.65^{d} (0.04)	1.04^{a} (0.02)	1.30^{b} (0.04)	105.1^{a} (10.2)	12.7^{c} (0.7)
Digestate F	0.69^{c} (0.01)	0.59^{d} (0.02)	0.98^{abc} (0.06)	1.60^{a} (0.05)	19.3^{cd} (7.5)	2.5^{d} (0.2)
Control	0.74^{c} (0.02)	0.50^{e} (0.01)	1.03^{ab} (0.07)	0.71^{d} (0.05)	76.9^{b} (19.6)	13.5^{c} (0.6)

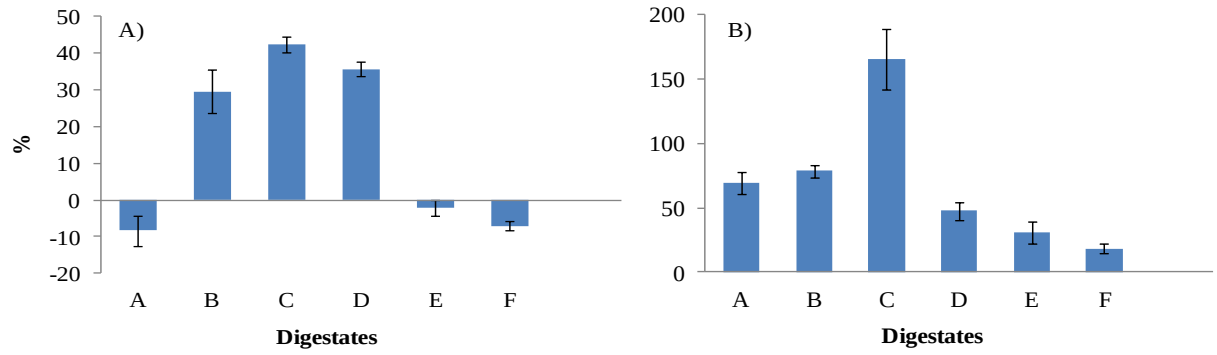

Figure 3. Stimulation of CO_2 production by digestates in A) loam and B) silt.
Note the difference in scale

Digestates affected Rq differently in the two soil types (Tab.4). No significant change relative to the control was observed in the loam. In contrast, digestates significantly increased Rq in the silt which was less active than the loam in terms of respiration (Tab. 4). Digestate F amended to silt resulted in the highest Rq (1.60). Figure 4 shows the relative changes of Rq in the loam and silt after amendment with digestates.

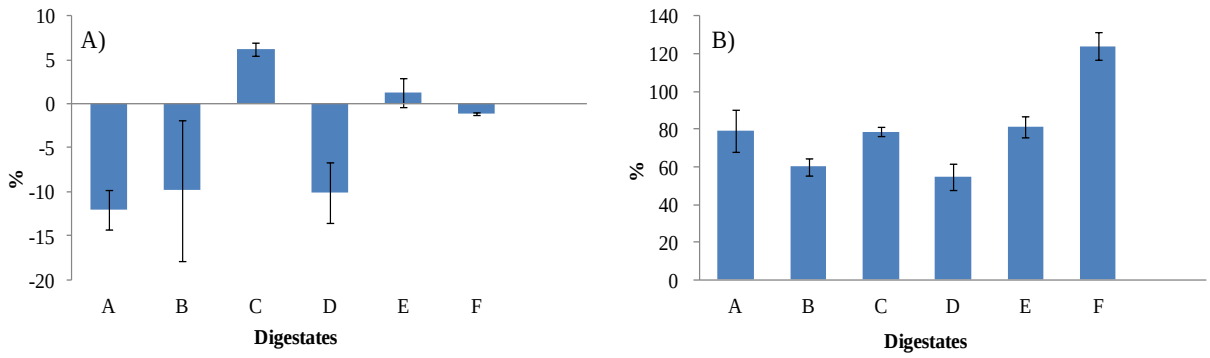

Figure 4. Change of respiratory quotient relative to the control by digestate addition in A) loam and B) silt.

3.1.3 N_2O production in loam and silt

Even though ample amounts of O_2 were present in the headspace of the incubation bottles, significant rates of N_2O production were measured (Tab. 4). This was most likely due to partially anoxic conditions in the water saturated soil after adding the digestate (or water for the controls), resulting in a water content equivalent to 130% of soil water holding capacity. The N_2O production in the non-treated loam was 6 times higher than that in the non-treated silt.

Figure 5 shows the kinetics of the net N_2O accumulation for both loam and silt soil. Distinct kinetic patterns were observed: the loam without amendment and with digestates D and E showed exponential N_2O accumulation until a plateau was reached which remained more or less stable. Other digestates triggered a brief period of enhanced N_2O production (at around 25 hours into the incubation, in both soils), after which N_2O production stabilised at a slower pace, probably because of increasing N_2O reductase activity. A biphasic pattern was seen with digestate C in silt; N_2O production rates rose initially, slowed down at around 25 hours and increased again at 50 hours. Maximum N_2O accumulation was generally smaller in silt except for digestate C, which showed a strong concurrent stimulation of CO_2 production (Fig. 3). N_2O emission rates were calculated for the initial part of constant N_2O accumulation (0-50 hrs). Except for digestate E, this initial N_2O production rate was lower with all digestates than that of the control in the loam, resulting in a marked repression of initial N_2O production after digestate addition. However, digestate D increased N_2O production later during the incubation (> 50 hrs), resulting in a similar net accumulation as in the control (Fig. 5A). In the silt, initial N_2O production rates were generally lower than that in the loam, except for digestate C and D which strongly stimulated initial N_2O production by 80 and 400%, respectively (Fig. 6B). In the loam and silt, digestates A, B and F resulted in an inhibition of initial N_2O accumulation.

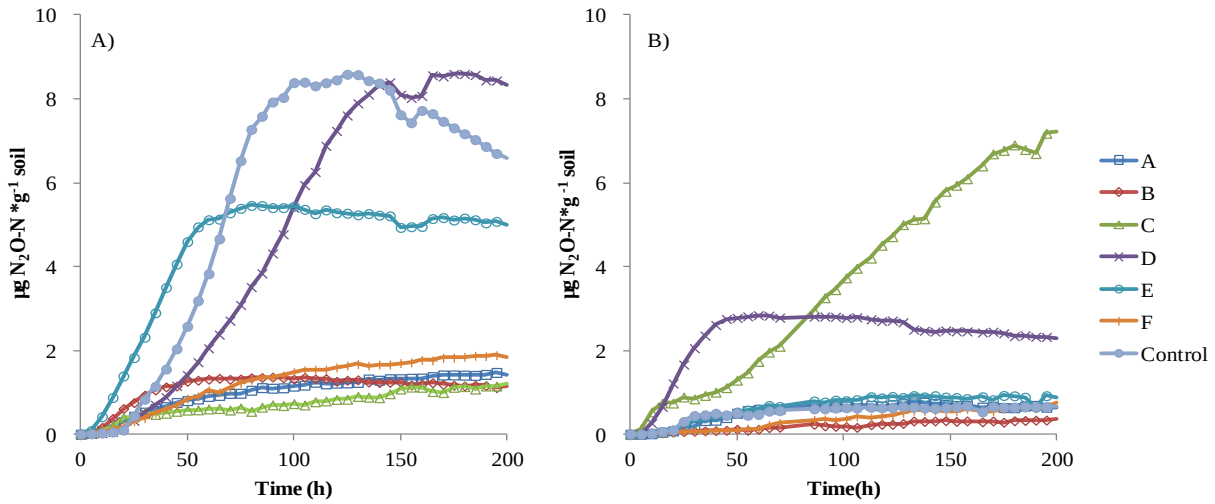

Figure 5. Kinetics of average N_2O net accumulation (n=3) in A) loam and B) silt in Experiment 1 ("oxic incubation").

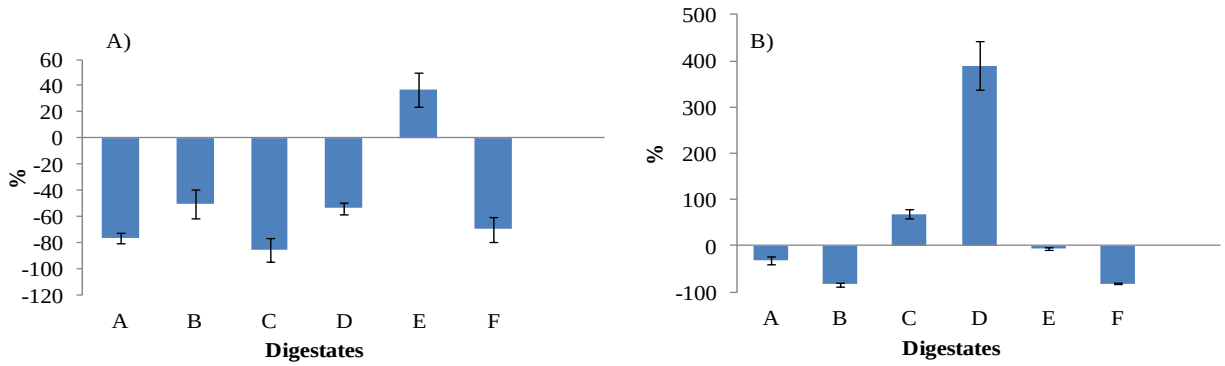

Figure 6. Effect of digestates on initial N_2O production rate relative to the control in A) loam and B) silt.

Figure 7 shows the cumulative N_2O production for the treatments. The total N_2O accumulation ranged from 1.1 to 8.3 µg N g^{-1} soil. Surplus N_2O accumulated in the loam with digestate D accounted for 4.68 % of the added N (Tab. 5). In the silt, the accumulation of N_2O ranged from 0.4 to 8.9 µg N g^{-1}. The N_2O-N which could be related to the added N accounted for 0.03 to 53.91% N. Digestate D induced higher N_2O-N accumulation in the loam and digestate C showed high N_2O-N accumulation in the silt.

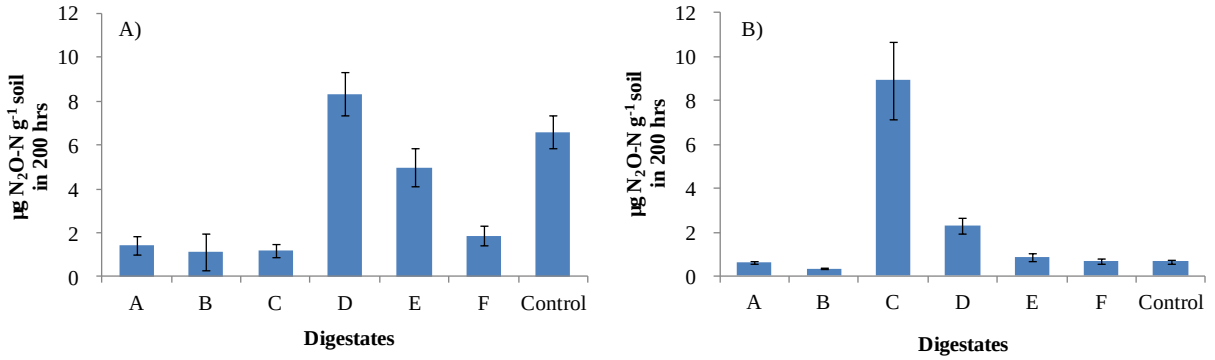

Figure 7. Effect of digestates on cumulative net N_2O production throughout 200 hours in A) loam and B) silt.

Table 5. Cumulative N_2O production (n=3, SD in parentheses) and amount of N accumulated as N_2O relative to N added with digestates in loam and silt. Different letters indicate significant differences ($p<0.05$) within each soil

Treatments	Cumulative N_2O production ($\mu g\ N_2O\text{-}N\ g^{-1}$ soil)		Equivalent to N from digestates (%)	
	Loam	Silt	Loam	Silt
A	1.43 (0.4)^{d}	0.65(0.06)^{b}	—	—
B	1.11 (0.4)^{b}	0.36(0.02)^{b}	—	—
C	1.20 (0.3)^{d}	8.92(1.7)^{a}	—	53.91
D	8.33 (0.09)^{a}	2.29(0.4)^{b}	4.68	3.78
E	4.99 (0.8)^{c}	0.88(0.2)^{b}	—	0.48
F	1.85 (0.5)^{d}	0.68(0.1)^{b}	—	0.03
Control	6.59 (0.7)^{b}	0.66(0.09)^{b}	—	—

The effects of digestates on initial N_2O emission were not consistent for the two soils. The same digestate (for example C, D and E) could have opposite effects in the loam and silt (Fig.6). In the loam, digestate E had a strong increasing effect on initial N_2O production, whereas in silt it was digestate C and D which stimulated N_2O production. Conversely, these two digestates inhibited initial N_2O production in the loam. However, looking at the net N_2O production over the entire incubation period, digestate D would result in overall high N_2O accumulation in the loam despite its initially low production rate. In summary, the effect of digestate C, D and E on N_2O production rate was soil dependent. The effect of the other three digestates on N_2O production was inhibiting initial N_2O production in both soils with clear (loam) or no effect on overall N_2O accumulation.

The initial N_2O production rates were positively related to available C (here expressed as OC_5/COD) in the digestates (Fig. 8), more strongly so in the silt than in the loam. This finding corresponds to the overall greater response in CO_2 production to digestate additions(Fig. 3, 4) Likewise, the cumulative N_2O production throughout 200 hours was positively related to available C in the digestates, and this positive relation was observed in both soils, more strongly in the loam (Fig.9).

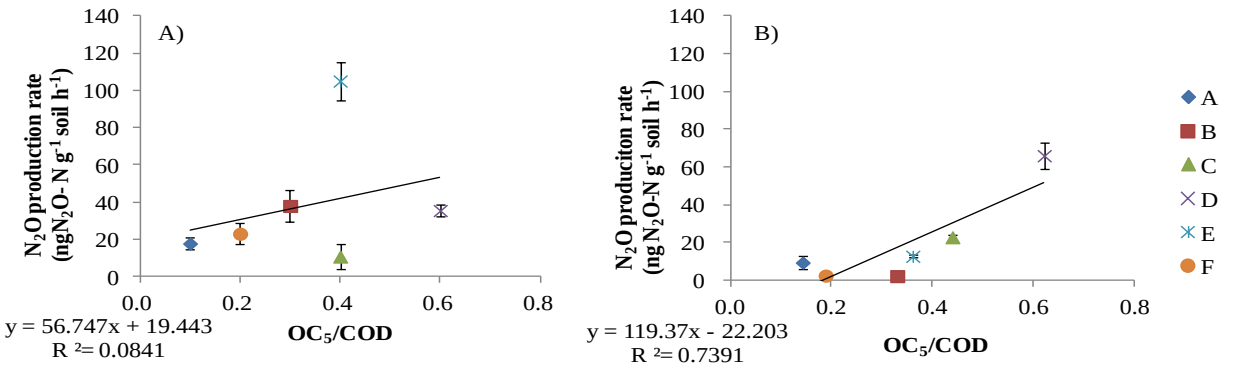

Figure 8. Initial N_2O productions as a function of OC_5/COD. Shown are average N_2O production rates for the first 50 hours of incubation as a function of the relative amount of biologically available C in the digestates in A) loam and B) silt.

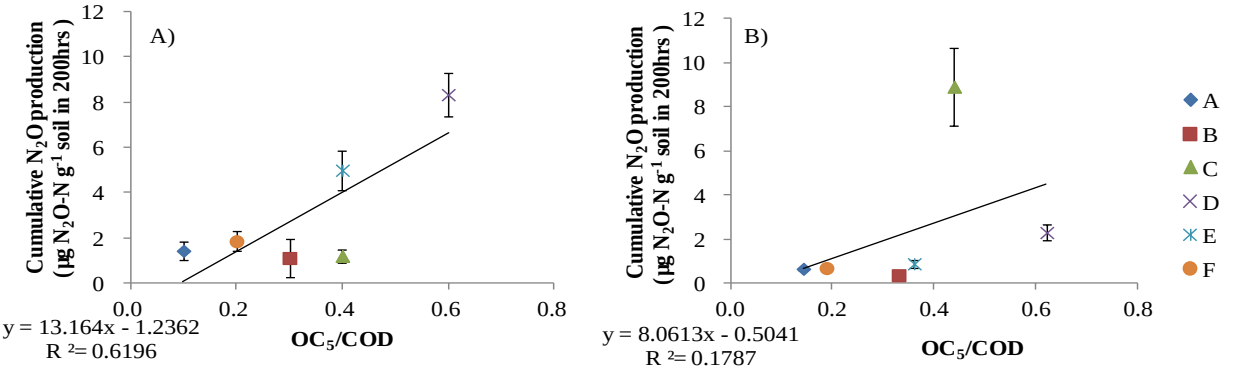

Figure 9. Cumulative N_2O productions as a function of OC_5/COD. Shown are average cumulative N_2O production rates for 200 hours of incubation as a function of the relative amount of biologically available C in the digestates in A) loam and B) silt.

3.2 Denitrification and kinetics of N_2O production under anoxic condition

The effect of digestates on denitrification rate and product ratio ($N_2O/(N_2O+N_2)$) was studied in constantly stirred anoxic soil slurries. The amounts of soil incubated and of digestates added were the same as in the Experiment 1 with sieved soil under ambient atmosphere. Like with CO_2 and N_2O production in Experiment 1, the denitrification rate without addition of digestates was higher in the loam (0.60 µg N g^{-1} soil h^{-1}) than in silt (0.44 µg N g^{-1} soil h^{-1}) (Tab. 6). In contrast to the first experiment, all digestates stimulated denitrification. Denitrification was increased by 10% to 84% in the loam and by 28% to 213% in the silt (Fig.10). The increasing effect was stronger in the silt than that in the loam. Digestate C showed the greatest increase in denitrification in both soils.

Table 6. Mean denitrification (n=3, SD in parentheses) and denitrification product ratio ($N_2O/(N_2O+N_2)$) in loam and silt soil amended with digestates A – F. Different letters indicate significance of differences ($p<0.05$) within each soil.

Treatment	Anoxic incubation			
	Denitrification ($\mu g\ N\ g^{-1}\ soil\ h^{-1}$)		$N_2O/(N_2O+N_2)$ (%)	
	Loam	Silt	Loam	Silt
Digestate A	0.77^{b} (0.07)	0.69^{bc} (0.10)	4.24^{c} (0.54)	0.21^{b} (0.09)
Digestate B	0.79^{b} (0.04)	0.80^{b} (0.15)	4.16^{c} (0.33)	0.09^{b} (0.02)
Digestate C	1.11^{a} (0.09)	1.37^{a} (0.16)	39.2^{a} (1.14)	0.11^{b} (0.01)
Digestate D	0.66^{cd} (0.04)	0.77^{b} (0.04)	2.99^{c} (0.27)	0.21^{b} (0.13)
Digestate E	1.07^{a} (0.04)	0.85^{b} (0.03)	12.3^{b} (1.84)	0.07^{b} (0.002)
Digestate F	0.71^{bc} (0.02)	0.56^{cd} (0.04)	3.37^{c} (0.09)	0.34^{b} (0.03)
Control	0.60^{d} (0.04)	0.44^{d} (0.05)	3.86^{c} (0.27)	1.40^{a} (0.69)

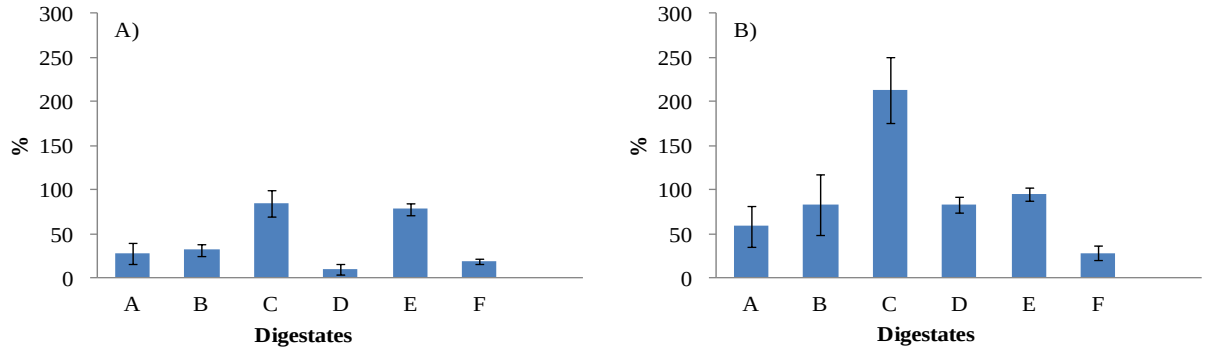

Figure 10. Change in denitrification relative to control in A) loam and B) silt amended with digestates A – F.

A positive correlation between denitrification rate and anoxic respiration (CO_2 production) was found for the silt soil but not for the loam (Fig.11).

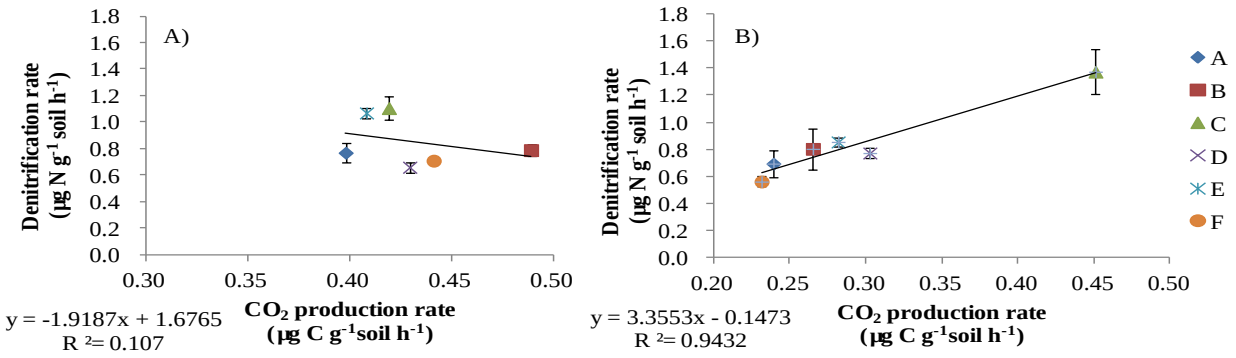

Figure 11. Correlation between denitrification rate and anoxic respiration in A) loam and B) silt. Dots represent single bottle values of denitrification and CO_2 production in experiment (anoxic)

Denitrification was only weakly negatively related to pH when measured after anoxic incubation in the loam (Fig.12). No such effect was found in the silt soil, which had a higher native pH.

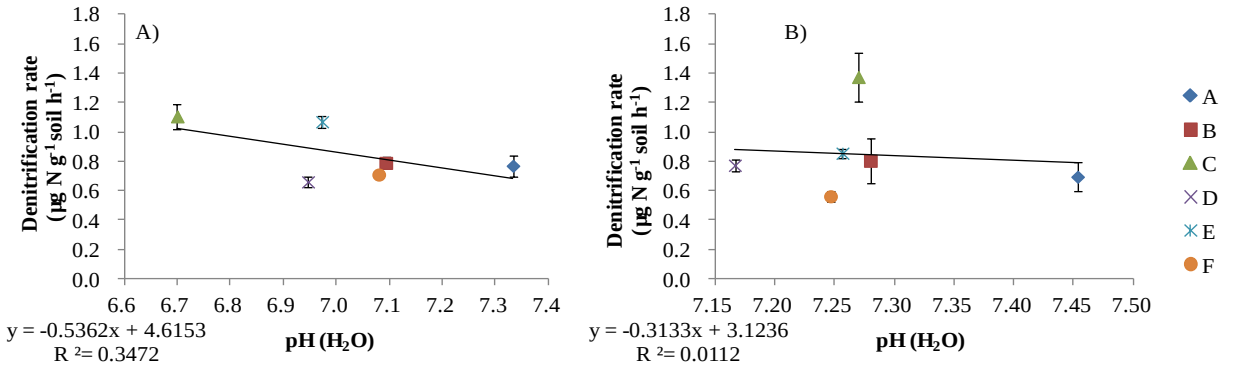

Figure 12. Correlation between pH and denitrification rate. Note: the pH was measured in the soil slurries after 60-100 hours of anoxic incubations and is to some extend affected by alkalinisation through denitrification.

Whereas all digestates increased denitrification rates in the soils (Tab.6), the denitrification product ratio $N_2O/(N_2O+N_2)$ was decreased by digestates in the silt and loam, except for digestates C and E which increased the product ratio in the loam (Tab.6). The N_2O emission potential arising from denitrification can be roughly calculated as the product of denitrification times the N_2O product ratio (Fig. 13). The loam showed clearly higher emission potentials with all digestates than the silt, mainly because of exceptionally low product ratios in the silt observed after the addition of digestates. The effect of digestates on emission potentials was consistent in both soils. Highest emission potentials were associated with digestate C and E (Fig. 13).

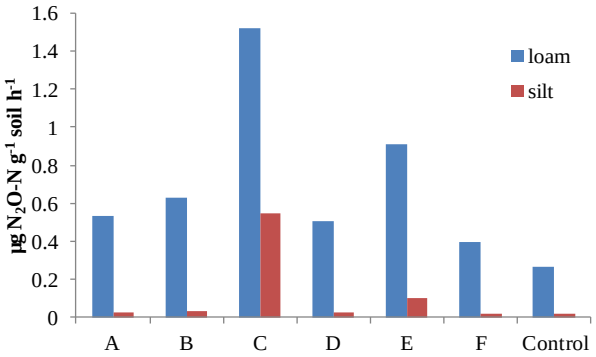

Figure 13. Effect of digestates on N_2O emission potentials from denitrification

3.3 Fate of added ammonium

The fate of NH_4^+ added with the digestates (14-140 µg N g^{-1} soil, depending on the digestate; supplementary table 1) was studied by incubating soils adjusted to 60% WHC for 8 days under ambient atmosphere. Identical amounts of digestates were added as in Experiment 1 and 2 and mineral N (NH_4^+ and NO_3^-) was measured by extraction right after addition and after 8 days of incubation. In the loam soil, the addition of digestates did not affect the KCl-extractable NH_4^+-N

at t=0, and all NH_4^+ was immobilised after 8 days (Fig. 14A). In contrast, the addition of digestate resulted in an immediate increase of NO_3^--N (t=0) relative to the control with most of the digestates (Fig. 14B). NO_3^--N content increased somewhat throughout the incubation, but this increase could not account for the disappearance of NH_4^+-N observed in the same period. In the silt, the picture was similar to the loam, with the difference that NH_4^+-N was still detected after 8 days of incubation (Fig.14C), suggesting that immobilisation was slower than in the loam. Addition of digestates to the silt resulted in a practically identically pattern of NO_3^--N concentrations at t=0 as in the loam and the concentration increase was negligible as in the loam (Fig.14D). For the silt, digestates had an effect on the amount of NH_4^+ immobilised; immobilisation was greatest with digestate C, which was the digestate with the lowest NH_4^+ content and the highest carbon availability (Fig.14 C). For both soils, the amount of N added as NH_4^+ with the digestates was in the range of (loam) or higher (silt) than the NH_4^+ recovered at T_o. This suggests that the KCl-extractable NH_4^+ was controlled by soil-dependent immobilisation processes to a large extent.

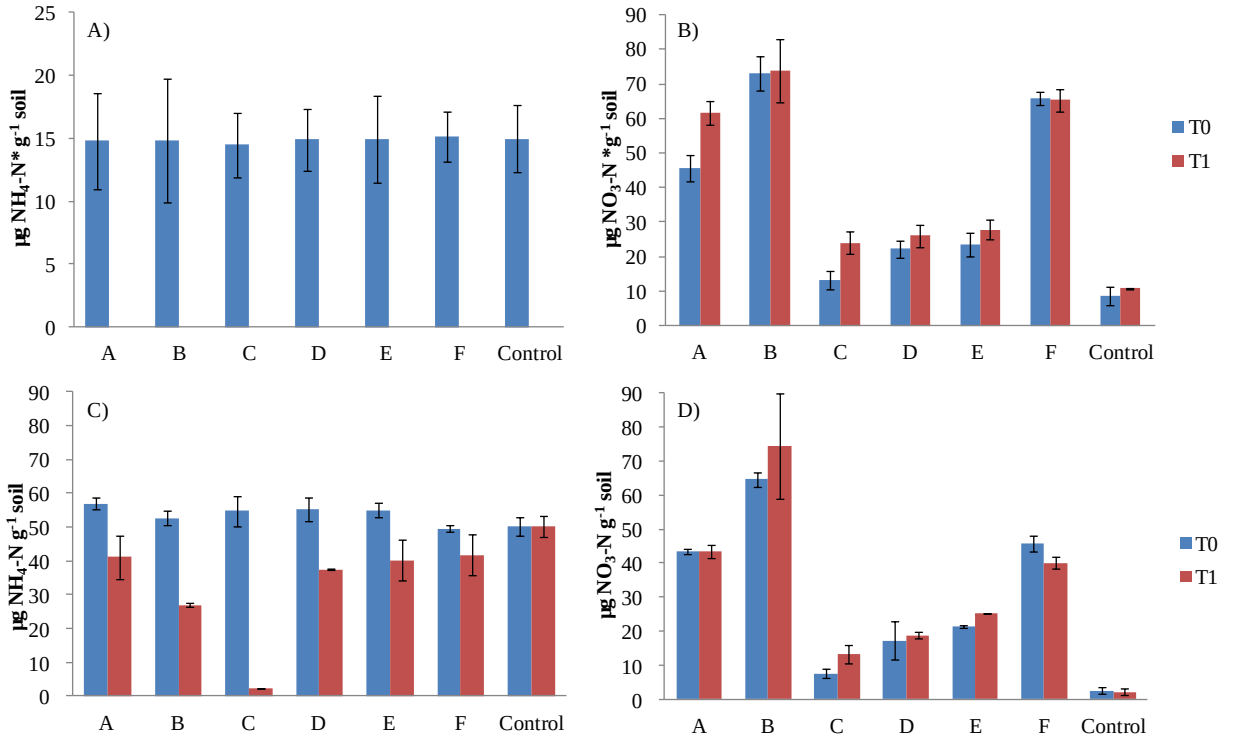

Figure 14. Recovery of NH_4^+ added with the digestates one hour after addition (T_o) and after 8 days of oxic incubation (T_1) at15°C. A) NH_4-N in loam. B) NO_3-N in loam. C) NH_4-N in silt. D) NO_3-N in silt.

4. Discussion

The objective of the present study was to provide a laboratory-based assessment of instantaneous CO_2 and N_2O production triggered by the addition of various biogas digestates to soil and to establish relationships between digestate quality and N_2O and CO_2 emission potentials. In previous pot experiments with the same soils as used in the present study (Eich-Greatorex *pers.comm.*), instant N_2O emission were observed within 5 days after setting up the experiment, raising the question which processes drive these emissions and how the quality of the digestates would affect them. Therefore, short-term incubations (ca. 200 hrs) were carried out adding similar amounts of digestates as used in the pot experiments. However, in contrast to the above-mentioned pot experiments, which were set up with air-dried soil, remoistened and preincubated soils were used in the present study. Therefore, results cannot be compared directly.

The digestates had soil-dependent effects on CO_2 and N-gas production; whereas addition of digestates resulted in persistently higher CO_2 production relative to the control (particularly in the silt), the effect on N-gas production was less clear. Addition of digestates resulted in reduction of initial N_2O production from the saturated soils used in Experiment 1. Reduction of initial N_2O production was found for four (loam) and three (silt) out of five digestates (Tab. 4). This is in contrast to findings from previous experiments which reported increased N_2O emissions after digestate application (Odlare *et al.*, 2012; Alotaibi and Schoenau, 2013). In the present study, addition of digestates resulted in saturated conditions, likely inducing hypoxic conditions which would have supported N_2O production from the added ammonium by both nitrification and subsequent denitrification (Siciliano *et al.*, 2007). The observation of reduced N_2O production upon addition of digestates can therefore not be explained by soil conditions not being favourable for N_2O production by denitrification. The amount of NH_4^+ added with the digestates was similar to that extractable by KCl from the untreated soil both for the loam and silt (compare controls and digestate treatments at t=0 in Fig.14A and C) and therefore unlikely limiting for N_2O production by nitrification. However, partially anoxic condition in the saturated soil may have inhibited nitrification and may therefore be responsible for the lower initial production rates observed directly after digestate addition. Increasing N_2O production later during incubation (after ~20 hours, Fig.5) may then be interpreted as a result of increasing denitrification, being induced after O_2 depletion in microsites within the soil (Parkin, 1987).

Another process which may have affected N_2O production in Experiment 1 and denitrification in Experiment 2, is the strong observed immobilisation of NH_4^+ (Fig. 14). The loam immobilised NH_4^+ completely and the silt partially immobilised NH_4^+ within 8 days of incubation under the same conditions as in the oxic Experiment 1. In the silt, the immobilisation was clearly higher with digestates than without (Fig. 14C). This points towards microbial NH_4^+ uptake in the silt, as readily available carbon was added with the digestates, stimulating microbial respiration in the silt (Fig. 3B). In the loam, the stimulation of microbial respiration was much less (Fig, 3A), but overall greater immobilisation of NH_4^+ was observed (Fig. 14A), most likely because of NH_4^+-fixation to clay (Black and Waring, 1972). The loam had 21% clay whereas the silt only contained 5.5% clay (Tab. 2). This suggests different fates of added ammonium in the two soils: strong abiotic NH_4^+ fixation by clays in the loam and predominately biotic immobilisation by microbial growth in the silt. This is consistent with the stronger positive response to digestates of the silt than loam in oxic respiration and cumulative N_2O production in Experiment 1 as well as with the greater stimulation of denitrification observed in Experiment 2. Rapid immobilisation of NH_4^+ after digestate addition to soil has been reported previously (e.g. Alburquerque *et al.*, 2012; Fuente *et al.*, 2013).

Estevez (2013) found no NH_4^+ inhibition of fermenters in CSTRs with recirculation, indicating that the microbial communities added with the digestates are well-adapted to high NH_4^+ concentrations. In fact, the CO_2 production rates measured in the more or less inert sand with digestates suggest that much of the respiration activity observed in the loam and the silt originated from the digestates (compare Table 3 and 4). However, the surplus of respiration by adding digestates to the silt was clearly higher than in the loam, indicating that the loam inactivated the activity of added microbes more than the silt. Hence, growth of microbes added to the silt with the digestates may have contributed to the observed microbial immobilisation (Fuente *et al.*, 2013).

Cumulative N_2O production in Experiment 1 was positively related to the OC_5/COD ratio (Fig.8 and Fig.9). Assuming that the OC_5/COD is a proxy for dissolved organic carbon (DOC), this finding is in accordance with results of Huang *et al.* (2004). On the basis of their results, Huang et al (2004) suggested to use DOC content as an index of C availability in digestates. The present study shows that OC_5/COD could be used for the same purpose. A similar approach was shown to be used for assessing biodegradability of organic matter in waste water (Henze *et al*., 2008). However, the effect of carbon availability seemed to be soil-dependent. For example, a positive

correlation between denitrification and anoxic CO_2 production was found in the silt (Fig.11B) but not in the loam (Fig. 11A). This is consistent with the overall greater inactivation of microbial activity after digestate addition to the loam, confirming that C availability (and its effect on denitrification activity) is strongly soil dependent.

The digestates used here contained different amounts of lignin (Table 1) in the dry matter, which should have influenced their degradability in the incubations trails (Chen *et al.*, 2012). However, dry matter accounted for only a small fraction of the digestates, thus having little effect on the overall quality. Also in term of COD/TIN ratios for the liquid phase, the digestates showed little difference, except for digestate C which had an extremely high COD/TIN ratio (Tab.1). As seen with NH_4^+ in the loam, soil may greatly modify the biological availability of carbon in organic amendments by adsorption of organic carbon to minerals and subsequent stabilisation (Saidy *et al.*, 2012).

Denitrification rate and product ratio ($N_2O/(N_2O+N_2)$) were studied in a separated experiment by anoxic incubation of continuously stirred soil slurries. Unlike in the first experiment, total N-gas production was stimulated by all digestates. In contrast, the effects of digestates on denitrification product ratio differed for the two soils (Tab. 6). In both soils, denitrification product ratio was reduced in most cases, but digestates C and E in the loam increased the product ratio. The possible explanation for this is that addition of readily degradable C leads to more complete denitrification (i.e. reduction of NO_3^- all the way to N_2), and an increase in available C has been reported to decrease the $N_2O/(N_2O+N_2)$ ratio (Senbayram *et al.*, 2012). Next to C availability, pH plays an important role for the denitrification product ratio (ŠImek and Cooper, 2002). Addition of alkaline digestates may increase soil pH and result in lower N_2O product ratios (Liu *et al.*, 2010). When digestates were added to the loam, soil pH was increased from 5.3 to 6.5 and from 6.5 to 7.3 in the loam and silt respectively (data not shown). However, digestate specific pH measured after the anoxic incubation had no effect on the N_2O product ratio (data not shown), suggesting that pH change by adding digestates to soil play a minor role for N_2O emissions

In the present study the product of denitrification rate and product ratio was used as an indicator for "N_2O emission potential" driven by denitrification (Fig. 13). Unlike in the first experiment, in which digestate addition caused soil-dependent effects on N_2O production, digestates had a consistent effect on the N_2O emission potentials in both soils. This may have to do with the fact

that Experiment 2 (stirred, anoxic soil slurries) selected for only one N_2O producing process (denitrification), whereas in Experiment 1, nitrification and denitrification may have proceeded at the same time or subsequently. Digestates C stimulated the emission potential greatly in both soils. Digestate C was the digestate with the lowest NH_4^+ content and the highest COD/TIN ratio (Tab. 1). This shows that increased N_2O emissions from denitrification can be expected after application of digestates with low NH_4^+ ammonium contents and high COD/TIN ratios in the liquid phase.

5. Conclusion

Unlike in pot experiments with plants which aim at assessing long-term effects of digestate addition on plant growth, N-status and GHG emission, the present study focused on processes of C and N turnover occurring directly after the addition of digestate to soils. Addition of digestates representing realistic N rates (20 – 120 kg N ha^{-1}) stimulated respiration depending on the soil but inhibited initial N_2O production, probably because microorganisms coming with the digestate competed with the soil microbes for resources. However, N_2O accumulation rates increased after approximately 50 hours to those of amended soils, suggesting that soil microbial functions recovered. The added N contained in the digestates was mainly in the form of NH_4^+ that was quickly immobilised in both soils. However, the mechanism of immobilisation seemed to differ between the two soils. While NH_4^+ in the loam was most likely immobilised by fixation to clay, added NH_4^+ in the silt seemed to be immobilised predominately by microbial growth. This was consistent with a greater stimulation of respiration and denitrification by digestates in the silt than in the loam. Overall, differences in soil type seemed to have a greater influence on the GHG production potentials of the biogas residues than the chemical composition of the residues. The silt, which immobilised N and stabilised C to a lesser extent than the loam, had a CO_2 and N_2O emission response that was clearly related to C availability. Thus, the short-term emission response seems to be mainly controlled by soil-dependent immobilisation and stabilisation processes, which could also play a role for longer term effects of digestate addition on GHG emissions. Surprisingly, the digestate with the lowest N content (and highest COD/TIN ratio) induced the highest N_2O emission potential independently of soil type, showing that labile carbon in digestates may play a more important role for N_2O emission from denitrification than N amount.

Denitrification typically occurs in wet soils, and digestates from biogas production contain substantial amounts of water. This seems to be a notorious problem with respect to storage and transportation, which will prevail in future because removing water is costly and providing the energy to do so causes CO_2 emissions. Saturation of soils by biogas slurry can be avoided by ploughing the soil directly after the addition. However, this may counteract C sequestration by breaking down stored soil organic matter. On the other hand, digestates themselves might contribute to carbon sequestration in soil. The surplus in soil respiration observed after adding digestates to the loam and silt was clearly lower than the respiration of the digestate itself (as measured on inert sand), indicating that a substantial fraction of the added carbon was quickly stabilised in the soil, particularly in the loam. This could contribute positively to the overall goal

of bioenergy production which is to save CO_2, and probably balance N_2O emissions associated with returning biogas residues to soils.

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Supplementary

Table 1. Volume of digestates, volume of water and equivalent NH_4^+ added with digestates.

Soil	Treatments	Volume of digestates (ml)	Volume of water (ml)	NH_4-N added µg/g soil
Loam	Digestate A	0.7	2.7	90
	Digestate B	0.9	2.5	126
	Digestate C	0.8	2.6	14
	Digestate D	3.2	0.2	69
	Digestate E	1.4	2.0	75
	Digestate F	1	2.4	62
	Control	0	3.4	0
Silt	Digestate A	0.8	3.1	103
	Digestate B	1.0	2.9	140
	Digestate C	3.9	0	16
	Digestate D	1.3	2.6	81
	Digestate E	1.6	2.3	92
	Digestate F	1.1	2.8	68
	Control	0	3.9	0