



# Mineralisation of distinct biogas digestate qualities directly after application to soil

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## Abstract

Biogas is an important energy source produced by the anaerobic fermentation of raw faecal slurries and plant residues. Separation of the total digestate increases the fertilizer quality of the liquid fraction and the carbon sequestration potential of the solid fraction. A 12-day incubation study was carried out to investigate the relationships between the chemical composition of different digestate qualities and the immediate response of soil microbial activity and biomass indices. The highest cumulative ( $\Sigma$ )  $\text{CO}_2\text{-C}$  efflux was observed after adding the solid fraction and lowest after adding the liquid fraction to soil, which was even lower than that of the control. The  $\Sigma\text{CO}_2\text{-C}$  efflux showed the strongest negative correlation with the raw ash and strong positive correlations with the raw fibre concentration and the C/N ratio of the different digestate qualities. The highest and similar  $\Sigma\text{N}_2\text{O-N}$  efflux was observed after adding the total digestate or the liquid fraction, which were equivalent to approximately 1% of added N. This relatively low percentage indicates a possible origin from nitrifier denitrification. Total digestate and its liquid fraction exhibited considerable net-N mineralisation rates, which could mainly be predicted by the C/N ratios of the different digestate qualities. Microbial biomass C did not respond to the application of any digestate quality, whereas the fungal ergosterol content increased after applying the solid and the composted solid fractions. This raw fibre-induced fungal growth led to strong net-N immobilisation in soil after applying these two digestate qualities.

**Keywords** Raw slurry · Fermented slurry · Fertilizer · Compost · C sequestration · Soil microorganisms

## Introduction

Biogas is an important energy source produced by the anaerobic fermentation of raw faecal slurries and plant residues (Blumenstein et al. 2015; Möller 2015). In the early eighties of the last century, biogas plants were installed by several

biodynamic farmers in north-east Baden-Württemberg, Germany, to gain independence from nuclear power electricity, which was strongly expanding during that time (Wentzel et al. 2015).

Biogas production is accompanied by the formation of digestates, which are usually applied to crops as fertilizer. However, odour and pathogen reduction as well as higher fertilizer quality in comparison with raw slurries were further reasons for using biogas digestates as fertilizers (Goberna et al. 2011; Möller and Müller 2012). Anaerobic digestion reduces dry matter (DM) concentrations and, thus, digestate C/N ratios, combined with increased  $\text{NH}_4^+$  concentrations and an increase in pH (Bauer et al. 2009; Möller and Müller 2012). This led to positive biogas digestate effects on plant yield in comparison with other organic fertilizers, e.g. farmyard manure (Odlare et al. 2008; Bauer et al. 2009; Sängler et al. 2010), raw slurries (Bachmann et al. 2011; Wentzel and Joergensen, 2016a) or compost (Tambone et al. 2010). In addition, biogas digestates are applied as fertilizer close nutrient cycles in stockless organic farming systems (Stinner et al. 2008).

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Separation of total biogas digestates into a liquid and a solid fraction has gained increasing importance, as the liquid phase has a higher fertilizer quality than the total digestate, due to the increased  $\text{NH}_4^+$  concentration (Bauer et al. 2009; Tambone et al. 2017). In addition, a large part of the phosphorus load is transferred to the solid fraction (Bauer et al. 2009; Insam et al. 2015; Hupfauf et al. 2016), which might enable cost-efficient transportation to areas with deficiency (Møller et al. 2000; Bauer et al. 2009; Thomas et al. 2017). The solid fraction is usually composted (Bustamante et al. 2012; de la Fuente et al. 2013) and must be applied to crops with a lower N demand, due to its N immobilising properties (Chiyoka et al. 2014).

The current study investigated two biogas plants, which were fed with mixtures of faecal manures and plant residues at similar ratios, typical for biodynamic farms (Wentzel et al. 2015). However, the size of the biogas plants and their feedstock composition differed considerably. The total digestate, the liquid and solid fraction directly after separation as well as the composted solid fraction were examined. The objective of the study was to investigate the relationships between the chemical composition of different digestate qualities and the immediate response of microbial activity and biomass. Important chemical composition indices are, for example, pH, C/N ratio, raw ash and raw fibre (Jørgensen and Jensen 2009; Wentzel and Joergensen 2016a). Important microbial activity indices are  $\text{CO}_2$  and  $\text{N}_2\text{O}$  efflux for assessing air pollution (Sänger et al. 2011) and net-N mineralisation for assessing fertilizer quality (de la Fuente et al. 2013). In particular, the threat of high  $\text{N}_2\text{O}$  effluxes after application of biogas digestates to soil has attracted considerable experimental attention (Möller 2015; Verdi et al. 2019).

The application of total biogas digestates to soil is accompanied by the addition of fermenter-derived microorganisms (Abubaker et al. 2013), which might be reflected by an increased microbial biomass (Chen et al. 2012; Möller, 2015; Wentzel and Joergensen 2016b). This might be different for the liquid fraction, due to the lower organic matter and higher  $\text{NH}_4^+$  concentrations. Autochthonous saprotrophic soil fungi often respond with rapid increases to the application of organic matter that contains high concentrations of recalcitrant raw fibre (Chander et al. 2002; Joergensen and Wichern 2008). For these reasons, an incubation study was designed to investigate the following hypotheses: (1) The  $\text{N}_2\text{O}$  efflux is generally small after applying the distinct digestate qualities. (2) Soil microorganisms do not respond to the application of the liquid fraction. (3) Soil fungi increase after applying the solid and the composted solid fractions. The incubation period was restricted to 12 days, as usually most of the organic fertilizer-derived  $\text{N}_2\text{O}$  is produced directly after application to soil (Sänger et al. 2011; Jost et al. 2013).

## Materials and methods

### On-farm biogas production

Farm K is located in Künzelsau-Garnberg (49° 17' 15.22" N, 9° 42' 34.69"), Baden-Württemberg, south-central Germany, at 407 m asl. The farm has been managed according to biodynamic principles (Koeppel et al. 1990) of the Demeter organization since 1980. During sampling, the biogas plant consisted of one fermenter (300 m<sup>3</sup>) and one post-fermenter tank (300 m<sup>3</sup>), producing 30 kW h<sup>-1</sup>. The feedstock for the biogas plant was a mixture of cattle farmyard manure and clover/grass at similar proportions. The farmyard manure was loaded into the plant without pre-treatment, whereas the clover/grass was ensiled prior to loading. The raw materials were loaded with a slurry pump and a screw feeder for the solid inputs. The process temperature was maintained at mesophilic 38–40 °C without microbial inocula or further process monitoring. The liquid fraction was separated from the digestate with a screw, sieve and press. The solid fraction was composted as a pile, which was turned once every 3–4 weeks.

Farm M is located in Mühlhausen (51° 13' 24.14" N, 10° 25' 12.67" E), Thuringia, east-central Germany, at 254 m asl. The farm has been managed according to biodynamic principles of the Demeter organization since 1991. During sampling, the biogas plant consisted of one fermenter tank (1600 m<sup>3</sup>) and three post-fermenter tanks (2300 m<sup>3</sup>, 2 × 1100 m<sup>3</sup>), producing on average 220 kW h<sup>-1</sup>. The feedstock for the biogas plant was composed of chicken manure from another farm, cow and pig farmyard manure as well as ensiled energy crops, such as maize, rye and vetch, all approximately at the same percentage of volumes. The feedstock materials are loaded by a scraper and further mixed inside the tank by a blade system with slow movements. The process temperature was maintained at 30–40 °C without microbial inocula or further process monitoring. The liquid fraction was separated from the digestate with a screw press. The solid fraction was composted in open piles without further turning.

### Digestate and soil sampling

Total digestate, liquid fraction, solid fraction and composted solid fraction were sampled from each farm. Total digestate was taken from the first tank at farm K and from the second tank at farm M. The liquid and solid fractions were taken immediately after separation. The composted solid fraction samples were taken from the central part of the piles. The compost was 3–4 months old at farm K and approximately 6 months old at farm M. The four digestate qualities (total digestate, liquid fraction, solid fraction, and composted solid fraction) were each taken in quadruplicate, placed in polyethylene bags, and shock frozen with liquid  $\text{N}_2$  at -196 °C. Then,

the frozen samples were transported to the laboratory and stored at  $-18\text{ }^{\circ}\text{C}$ .

The soil used for the incubation experiment was taken (0–20 cm) with a spade from the arable site Saurasen ( $51^{\circ} 22' 35.47'' \text{N}$ ,  $9^{\circ} 53' 54.59'' \text{E}$ ) near Witzenhausen, Hesse, central Germany (Jost et al. 2013) at 280 m asl. The soil is derived from eroded loess overlying clayey sandstone and has been classified as Stagnic Luvisol (IUSS Working Group WRB 2015). The texture was 6% sand, 72% silt and 22% clay. Soil organic C and total N contents were 8.2 and 0.89 mg  $\text{g}^{-1}$  soil, respectively, with a soil pH-CaCl<sub>2</sub> of 6.4. The soil was sieved (< 2 mm) to remove stones and plant residues. Then, the soil was stored at  $4\text{ }^{\circ}\text{C}$  until the experiment started.

### Chemical digestate analysis

The pH of the four digestate qualities was measured by mixing 10-g fresh sample with 25 ml 0.01 M CaCl<sub>2</sub> solution. In 5 g of four fresh digestate qualities, total Kjeldahl N was determined by H<sub>2</sub>SO<sub>4</sub> digestion and titration as described by Althaus et al. (2013). In 25-g fresh digestate samples, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> were extracted with 70 ml 0.05 M K<sub>2</sub>SO<sub>4</sub> by 30-min horizontal shaking at 200 rev  $\text{min}^{-1}$ , followed by filtration and continuous flow analysis (Evolution 2, Alliance Instruments, Friedrichsdorf). In oven-dried digestate samples ( $60\text{ }^{\circ}\text{C}$  for 48–72 h), XF (raw fibre) and XA (raw ash) were determined by near-infrared spectroscopy (FOSS 6500, Rellingen, Germany) according to Althaus et al. (2013). Also, in oven-dried digestate samples, total C was measured in a Vario Max CHN elemental analyser (Elementar, Hanau, Germany).

### Microbial biomass and activity indices

Moist soil (300 g on an oven-dry basis) was fertilised with 10 mg digestate N, approximately equivalent to 75 kg N  $\text{ha}^{-1}$ . For this reason, the amount of added organic C varied. In farm K treatments, 80 (total digestate), 40 (liquid fraction), 210 (solid fraction) and 220 (composted solid fraction) mg C were added to 300-g soil. In farm M treatments, 50 (total digestate), 50 (liquid fraction), 200 (solid fraction) and 240 (composted solid fraction) mg C were added to 300 g soil. After fertilization, each sample was transferred into a 1500-ml hermetic glass jar. Unfertilized control soil and fertilized soil treatments were replicated four times. All samples were incubated in the dark at  $22\text{ }^{\circ}\text{C}$ .

Ergosterol was extracted at the start (day 0, directly after application of the four digestate qualities) and end (day 12) of the incubation experiment from 2-g moist soil with 100-ml ethanol for 30 min by oscillating shaking at 250 rev  $\text{min}^{-1}$  (Djajakirana et al. 1996). Then, ergosterol was measured by reversed-phase HPLC, using 100% methanol as the mobile phase and detected at a wavelength of 282 nm. Microbial biomass C (MBC) was determined at start and end of the

incubation experiment by fumigation extraction (Vance et al. 1987). Chloroform fumigated and non-fumigated portions of 10-g moist soil were extracted with 40 ml 0.5 M K<sub>2</sub>SO<sub>4</sub>. Organic C in the extracts was determined with a Multi N/C 2100S analyser (Analytik Jena, Germany). MBC was  $E_C/k_{EC}$ , where  $E_C$  = (organic C extracted from fumigated soils) - (organic C extracted from non-fumigated soils) and  $k_{EC} = 0.45$  (Wu et al. 1990).

In the extracts of the non-fumigated samples, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> were determined by continuous flow analysis to estimate net-N mineralisation. Net-N mineralised from each treatment was calculated as the sum of NH<sub>4</sub><sup>+</sup>-N + NO<sub>3</sub><sup>-</sup>-N at day 12 minus the initial sum of inorganic N.

At days 1, 2, 3, 8 and 12, two 10-ml gas samples were taken with an air tight syringe from the headspace of the incubation jars through a three-layer silicone septum (Hamilton, Reno, USA). The gas samples were immediately analysed for CO<sub>2</sub> and N<sub>2</sub>O using a GC-14B (Shimadzu, Kyoto, Japan) gas chromatograph with an electron-capture detector. For calculating cumulative ( $\Sigma$ ) fluxes, i.e.  $\Sigma\text{CO}_2\text{-C}$  efflux and  $\Sigma\text{N}_2\text{O-N}$  efflux, hourly CO<sub>2</sub>-C and N<sub>2</sub>O-N evolution rates were extrapolated to a daily rate; then, the average between two neighbouring sampling days was calculated and multiplied by the incubation period represented by these two days.

### Statistical analysis

The results presented in the tables are arithmetic means and expressed on an oven-dry basis (about 48 h at  $60\text{ }^{\circ}\text{C}$  for the four digestate qualities and  $105\text{ }^{\circ}\text{C}$  for soil). Data were tested for normality of distribution using Shapiro-Wilks and for equal variance using the Brown-Forsyth test. Ergosterol data were ln-transformed to meet these requirements. The significance of treatment effects was analysed by a two-way ANOVA, using digestate quality and farm as independent factors and sampling date as repeated measures for ergosterol, MBC and K<sub>2</sub>SO<sub>4</sub> extractable C as well as the ergosterol/MBC ratio. ANOVA analysis was carried out with SigmaPlot 13.0 (Systat Inc., San José, USA).

### Results

The liquid fraction exhibited increased concentrations of NH<sub>4</sub><sup>+</sup>, total N and raw ash, but decreased DM and total C concentrations in comparison with the total digestate (Table 1). In the solid fraction, NH<sub>4</sub><sup>+</sup>, total N and XA concentrations were decreased, whereas DM, XF and total C concentrations were increased in comparison with the total digestate. With a mean pH of 9.3, the solid fraction exceeded the mean of all other digestate qualities by approximately a 1.0 pH step. The solid and composted solid fractions exhibited only small differences in chemical composition. DM, NH<sub>4</sub><sup>+</sup> and XF

**Table 1** Farm-specific chemical composition of different biogas digestate fractions before application to soil

	Farm	pH	DM (%)	C (mg g <sup>-1</sup> DM)	N	C/N	NO <sub>3</sub> <sup>-</sup> -N (mg g <sup>-1</sup> DM)	NH <sub>4</sub> <sup>+</sup> -N	XF (% DM)	XA
Total digestate	K	8.21	10.7	395	47	8.5	0.01	15.5	29.9	25.3
	M	8.40	9.6	351	71	5.0	0.00	49.2	21.0	31.7
Liquid fraction	K	8.25	5.0	355	101	3.5	0.08	59.7	6.9	36.7
	M	8.20	7.1	321	95	3.4	0.01	65.2	22.6	39.1
Solid fraction	K	9.33	26.7	436	20	21.8	0.00	5.7	47.4	14.0
	M	9.22	29.3	443	22	19.9	0.00	9.4	47.6	13.2
Composted solid fraction	K	8.27	13.3	416	20	21.4	0.51	0.6	40.4	19.6
	M	8.84	19.8	441	19	23.8	0.08	6.0	44.7	12.8
CV (± %)		1.0	4.5	1.0	5.9	6.1	28	15	4.1	3.3

CV, mean coefficient of variation (standard deviation/ $n \times 100$ ) between replicate samples ( $n = 4$ ), averaged over the four digestate qualities; DM, dry matter; XF, raw fibre; XA, raw ash

concentrations as well as the pH were lower in the composted than in the solid fraction, whereas the NO<sub>3</sub><sup>-</sup> and XA concentrations were higher.

The highest soil  $\Sigma$ CO<sub>2</sub>-C efflux was observed after adding the solid fraction and lowest after adding the liquid fraction (Table 2), which was even lower than that of the control. Control CO<sub>2</sub>-C efflux rates were roughly constant throughout the incubation, whereas those from the total digestate and the liquid fraction moderately declined from day 1 (Fig. 1a). In contrast, the CO<sub>2</sub>-C efflux rates from the solid and composted solid fractions continuously increased from day 2 or day 3 until the end of the experiment. The slower increase after adding the composted solid fraction from farm K led to a

$\Sigma$ CO<sub>2</sub>-C efflux was positively correlated with the XF fraction and the C/N ratio (both  $r = 0.66$ ,  $P < 0.001$ ,  $n = 32$ ) of the four digestate qualities.

The highest and similar  $\Sigma$ N<sub>2</sub>O-N efflux was observed after adding the total digestate or the liquid fraction (Table 2). The  $\Sigma$ N<sub>2</sub>O-N efflux of the solid and composted solid fractions exceeded the very low value of the control in each case. The N<sub>2</sub>O efflux rates declined from maximum values at day 1 to roughly constant values from day 8 on (Fig. 1b). An exception was the composted solid fraction from farm K, where the N<sub>2</sub>O efflux rates remained constant throughout the incubation. The  $\Sigma$ N<sub>2</sub>O-N efflux could be predicted by a linear combination of the digestate C/N ratios and the  $\Sigma$ CO<sub>2</sub>-C efflux, with an ad-

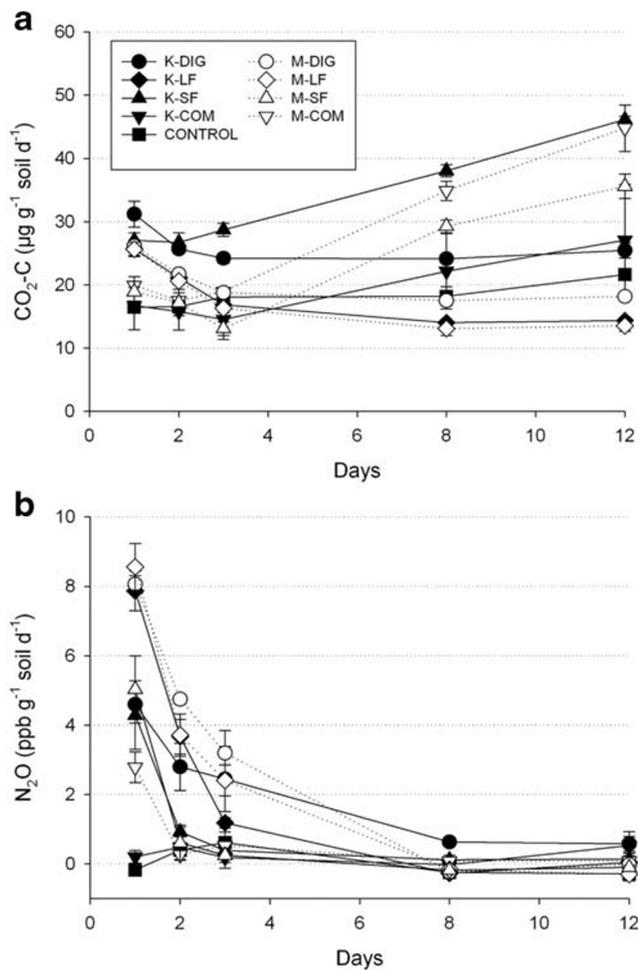
**Table 2** Mean biogas digestate quality  $\times$  sampling day interactions of the CO<sub>2</sub>-C and N<sub>2</sub>O-N sums evolved as well as of the net-N mineralised in soil amended with different biogas digestate fractions over a 12-day incubation period at 22 °C; probability levels of a two-way ANOVA,

using digestate quality and farm origin as factors and sampling day as repeated measures and excluding the control soil from statistical evaluation

	$\Sigma$ CO <sub>2</sub> (μg C g <sup>-1</sup> soil)	$\Sigma$ N <sub>2</sub> O (ng N g <sup>-1</sup> soil)	Net-N mineralisation (μg N g <sup>-1</sup> soil d <sup>-1</sup> )
Total digestate	268	31.8	28
Liquid fraction	197	31.7	59
Solid fraction	359	14.6	- 33
Composted solid fraction	303	6.5	- 44
Control soil	221	0.4	- 16
Probability levels			
Digestate quality	< 0.01	< 0.01	< 0.01
Farm	NS	0.02	NS
Digestate $\times$ farm	< 0.01	NS	NS
CV (± %)	11	28	110

CV, mean coefficient of variation (standard deviation/ $n \times 100$ ) between replicate samples ( $n = 4$ ), averaged over the five treatments; NS, not significant

significant digestate  $\times$  farm interaction (Table 3). The justed  $r^2$  of 0.80 (Fig. 2). Net-N mineralisation was higher



**Fig. 1** a CO<sub>2</sub>-C efflux and b N<sub>2</sub>O efflux from soil amended with biogas digestate fractions over a 12-day incubation at 22 °C; error bars indicate one standard error of mean; DIG, total digestate; LF, liquid fraction; SF, separated fraction; COM, composted solid fraction

after liquid fraction than after total digestate application, whereas net-N immobilisation was higher after composted solid than after fresh solid fraction application (Table 2). Net-N mineralisation was positively correlated with the XA fraction ( $r = 0.80, P < 0.0001, n = 32$ ) and negatively with the C/N ratio ( $r = - 0.74, P < 0.0001$ ) of the four digestate qualities.

The mean K<sub>2</sub>SO<sub>4</sub> extractable C content was generally higher after adding the four digestate qualities from farm K than from farm M and significantly declined during the incubation (Table 3). This was true for all digestate qualities, but not for the control soil (Fig. 3a). Mean MBC generally increased during the incubation but did not specifically respond to the addition of any digestate quality (Table 3). Like MBC, the soil ergosterol content increased during the incubation, mainly after adding the solid and composted solid fractions, but not after adding the total digestate or the liquid fraction (Fig. 3b). This led to a significant digestate quality × day

**Table 3** Main effects of sampling day ( $n = 2$ ), farm ( $n = 2$ ), and digestate quality ( $n = 4 +$  control) on the contents of K<sub>2</sub>SO<sub>4</sub> extractable C, MBC and ergosterol amended with four biogas digestate qualities at the start (day 0) and end (day 12) of an incubation at 22 °C; probability levels of a two-way ANOVA, using digestate quality and farm origin as factors and sampling day as repeated measures and excluding the control soil from statistical evaluation

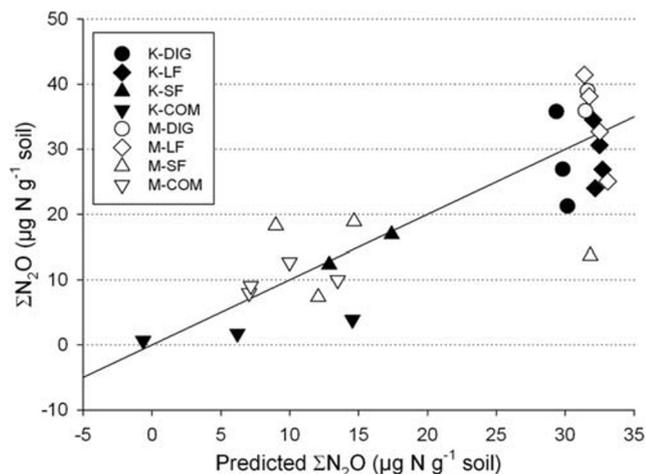
	K <sub>2</sub> SO <sub>4</sub> extractable C (µg g <sup>-1</sup> soil)	MBC	Ergosterol
Day 0	48.8	218	0.42
Day 12	42.1	264	0.51
Farm K	46.7	240	0.48
Farm M	43.3	243	0.45
Total digestate	42.3	247	0.40
Liquid fraction	42.3	217	0.38
Solid fraction	46.8	231	0.58
Composted solid fraction	48.6	270	0.51
Control soil	42.8	244	0.54
Probability values			
Digestate quality	<0.01	NS	0.01
Farm	0.01	NS	NS
Incubation day	<0.01	0.01	0.01
Digestate × farm	NS	NS	NS
Digestate × day	NS	NS	0.02
Farm × day	NS	NS	NS
CV (± %)	11	24	20

CV, mean coefficient of variation (standard deviation/ $n \times 100$ ) between replicate samples ( $n = 4$ ), averaged over the five treatments; NS, not significant

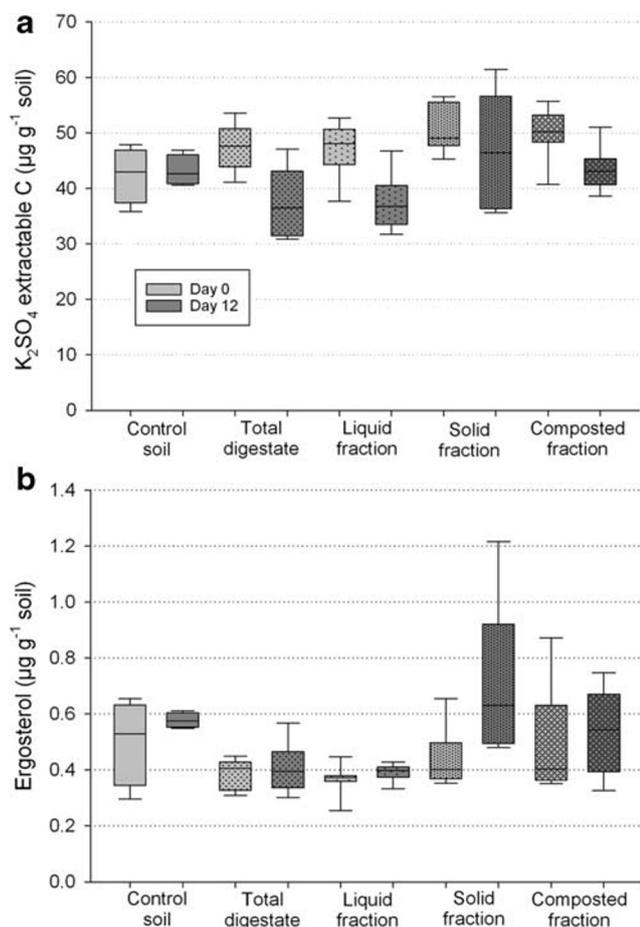
interaction (Table 3). The ergosterol content at day 12 was positively correlated with the ΣCO<sub>2</sub>-C efflux ( $r = 0.60, P < 0.05, n = 32$ ) and the XF fraction ( $r = - 0.64, P < 0.001$ ) but negatively with net-N mineralisation ( $r = - 0.65, P < 0.001$ ).

## Discussion

Each of the four digestate qualities behaved similarly after application to soil, although obtained from two very different production systems, based on animal slurries and plant residues. In the current study, the separation of the total biogas digestate resulted on average in an 81% liquid fraction with 6% DM and in a 19% solid fraction with 28% DM. These data are in line with Bauer et al. (2009), who reported that separation produced a 79.2% fluid phase with 4.5% DM and a 20.8% solid phase with 19.3% DM. Some liquid digestate properties of farm K were already analysed 3 years before (Wentzel and Joergensen 2016a). The similarity of these data with the current chemical composition gives confidence that



**Fig. 2** Measured and predicted  $\Sigma\text{N}_2\text{O}$ -N efflux from soil amended with four biogas digestate qualities over a 12-day incubation at 22 °C; linear prediction:  $\Sigma\text{N}_2\text{O}\text{-N} = 28.085^{***} + (0.0476^* \times \Sigma\text{CO}_2\text{-C}) - (1.512^{***} \times \text{digestate C/N})$ , adjusted  $r^2 = 0.80$ , \*  $P < 0.05$ , \*\*\*  $P < 0.001$ , variance inflation factor = 1.773, Shapiro-Wilk test passed, constant variance test passed; DIG, total digestate; LF, liquid fraction; SF, separated fraction; COM, composted solid fraction



**Fig. 3** Boxplots for mean contents of  $\text{K}_2\text{SO}_4$  extractable C and ergosterol in soil amended with four biogas digestate qualities at the start (day 0) and end (day 12) of an incubation at 22 °C

sampling and chemical analytical procedures lead to reliable data on the composition of digestates.

Nearly 1% of the added digestate N was lost as  $\text{N}_2\text{O}$  during the incubation. This loss is lower than the percentages obtained in previous studies. Sanger et al. (2010) reported an  $\text{N}_2\text{O}$  loss of 1.2% from a soil amended with 100 kg N  $\text{ha}^{-1}$  and Senbayram et al. (2009) observed an  $\text{N}_2\text{O}$  loss of approximately 1.7% from a soil amended with 90 kg N  $\text{ha}^{-1}$  applied as biogas digestate. Such low loss rates are in the range observed after inorganic fertilizer application (de Klein et al. 2001). This indicates that  $\text{N}_2\text{O}$  is mainly produced during aerobic ammonium oxidation (Zhou et al. 2020), i.e. nitrifier denitrification (Kool et al. 2011; Wrage-Monnig et al. 2018). Consequently, no additional threat of  $\text{N}_2\text{O}$  production by denitrifier denitrification emerges from the application of the four digestate qualities to soil (Moller and Stinner 2009; Nicholson et al. 2017).

The fungal biomarker ergosterol was highly interrelated with  $\Sigma\text{CO}_2\text{-C}$  efflux and XF fraction. Fungi are the principal decomposers of cell wall components (Schneider et al. 2012), especially lignin and lignin-cellulose complexes (Baldrian et al. 2011). In contrast,  $\Sigma\text{N}_2\text{O}$  efflux and net-N mineralisation were closely related to the C/N ratio of the four digestate fractions. Also, the N turnover model CANDY emphasised the C/N ratio as the most important parameter for predicting net-N mineralisation of digestates (Prays et al. 2018). However, the importance of this index should not be overestimated, as low C/N ratios of digestates are caused by high concentrations of easily available  $\text{NH}_4^+$  and low concentrations of organic C components. In highly processed materials such as faeces (Jost et al. 2013) or sugar cane filter cake (Rasul et al. 2009), low C/N ratios indicate an increased complexity of organic matter (Maynaud et al. 2017), i.e. biodegradability, and, thus, net-N mineralisation is reduced.

The application of the liquid fraction reduced the  $\Sigma\text{CO}_2$  efflux, presumably due to its high  $\text{NH}_4^+$  concentration. Another reason might be negative interactions of the biogas-derived microbial community and autochthonous soil microorganisms. The liquid fraction of farm K contained 0.25 mg fungal GlcN  $\text{g}^{-1}$  DM and 0.20 mg MurN  $\text{g}^{-1}$  DM (Wentzel and Joergensen 2016a). Multiplying these two values by 9 and 45 (Joergensen 2018), respectively, results in 2.3-mg fungal biomass C and 9.0-mg bacterial biomass C  $\text{g}^{-1}$  DM, i.e. a fungal/bacterial ratio of 0.25. This bacterial dominance might be a reason for the absence of any positive effects on microbial activity and biomass indices after applying the liquid fraction. The current observations are in line with the distinct physiological profiles of microbial communities, determined by the multi-substrate-induced respiration

approach, after supplying the solid or the liquid fraction to soil (Hupfauf et al. 2016).

The  $K_2SO_4$  extractable fraction has sometimes been considered an indicator for easily available SOC (Badalucco et al. 2010). This view has been repeatedly challenged (Wolters and Joergensen 1991; Poeplau et al. 2018), because  $K_2SO_4$  extractable C contents remained constant throughout incubations, as in the current non-amended control. However, in all treatments with digestate addition, the extractable  $K_2SO_4$  contents declined in soil, indicating that this fraction initially contained some organic components that are mineralised during incubation.

In contrast to total digestate application (Wentzel et al. 2015), the composted solid fraction has the potential to increase SOC stocks (Brito et al. 2008; Möller 2015). However, composting of the solid fraction with a pH of 9.3 has to deal with the serious threat of  $NH_3$  volatilization during storage (Brito et al. 2008). This risk might be reduced by adding clay to adsorb the  $NH_3$  (Chen et al. 2018). Another possibility is to add elemental sulphur (Roig et al. 2004; Gielli et al. 2016) or sulphuric acid (Pantelopoulos et al. 2017) to decrease slurry pH.

The decreasing DM concentration at farm K indicates the accumulation of water in the absence of rain-shelter roofs, which might increase the structural problems of the solid fraction. These problems could be reduced by co-composting with fibrous organic bulking material (Bustamante et al. 2012; Zeng et al. 2016), which cannot be added to biogas fermenters. A sufficient  $O_2$  supply to the solid fraction in compost piles lowers  $N_2O$  and  $NH_3$  emissions (Nicholson et al. 2017). A compost site with a solid concrete floor under a roofed structure reduces excessive rewetting of the compost by rain as well as nitrate and potassium leaching into the environment (Larney and Hao 2007; Luck et al. 2008).

## Conclusion

Application of the total biogas digestate or the liquid fraction to soil caused only a small  $N_2O$  efflux, which was mainly derived from nitrifier denitrification. These two digestate qualities exhibited considerable net-N mineralisation rates, which could be predicted by their low C/N ratios. Saprotrophic soil fungi were promoted after applying the solid and the composted solid fractions, both containing high concentrations of raw fibre. However, this recalcitrant organic matter led to strong net-N immobilisation induced by fungal growth. Soil microbial activity ( $CO_2$  efflux) and biomass indices (MBC and fungal ergosterol) did not respond to the application of the liquid fraction, due to its low organic matter and high  $NH_4^+$  concentrations. Additional negative effects of the biogas-derived microbial community in the liquid fraction on

autochthonous soil microorganisms could not be excluded and warrant further investigations.

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