

## Effect of anaerobic digestion of manure before application to soil – benefits for nitrogen utilisation?

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### Abstract

**Purpose** Anaerobic digestion produces renewable energy, biogas, from organic residues, but also digestate, a valuable organic fertiliser. Previous studies have indicated that digestate contains ample plant available nitrogen (N), but there are also concerns about greenhouse gas (GHG) emissions after application of digestates to soil. The aim of this study was to compare digestate and undigested feedstock for fertiliser effect as well as greenhouse gas emissions during the next season.

**Method** Digestate and its feedstock, manure, were compared as N fertilisers for wheat. Mixing digestate with biochar before application was also tested. After harvest, soil samples were frozen and dried. Then GHG emissions immediately after a re-wetting of dry soil and after thawing of frozen soil were measured to determine emissions after a non-growing season (dry or cold).

**Results** All N in digestate was plant available, while there was no significant N fertiliser effect of the undigested manure. N<sub>2</sub>O emissions were higher after a dry season than after freezing, but the undigested manure showed higher emissions during thawing than those detected during thawing of soils from any of the other treatments.

**Conclusion** Anaerobic digestion makes N available to plants, and when residues with much N that is not plant available the first season are used, the risk of N<sub>2</sub>O emission next spring is high.

**Keywords** Digestate, Nitrogen Fertiliser value, Biochar, Nitrous oxide, Thawing, Re-wetting

### Introduction

Anaerobic digestion produces biogas that can replace fossil fuels. The organic residue from biogas production is called digestate. To understand the full effect of anaerobic digestion on global warming potential, also the effect on the value of the residues as fertilisers afterwards must be taken into account (Lyng et al. 2015). Digestates contain partly decomposed organic material and relatively large amounts of mineral nitrogen (N) almost exclusively on ammonium form. Digestates may

therefore be good fertilizers (Abubaker et al. 2012; Albuquerque et al. 2012a,b; Baral et al. 2017; Möller and Müller 2012; Odlare et al. 2014; Nkoa 2014; Sogn et al. 2018). There are few studies that directly compare digestates to the undigested feedstock, so the exact effect of anaerobic digestion cannot easily be established (Insam et al. 2015) and it depends on the crop grown.

Whilst all nutrients in mineral fertilizers are immediately available to plants, this is usually not the case for nutrients in organic residues, although the availability of nutrients found in digestates usually are higher than those found in undigested feedstock. The fraction of applied N used by the crop is expressed as N Use Efficiency (NUE) (Jeng et al. 2004). To get NUE as high as possible, the available N should be taken up by the crop quickly, *i.e.* before it gets lost by leaching or other loss processes. Some of the N in organic residues is usually mineralized during the growing season. This means that they can act as slow release fertilizers, but the release rate is usually not so easy to predict. If the N is released outside the growing season, it can lead to N

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losses to the environment. Digestates have more of the N immediately available than undigested feedstock and may therefore be a good fertilizer for crops that need to take up nutrients early in the season, such as grains (Abubakker et al. 2012; Kristoffersen et al. 2013; Brod et al. 2014).

Greenhouse gases (GHG's) are emitted from agricultural soil. The most important in non-flooded systems is  $N_2O$ .  $N_2O$  emission increases after fertilizer addition, but how organic residues compare to mineral fertilizers in terms of GHG emission after application to soil is not clear (Bouwman et al. 2002; Charles et al. 2017). There are a number of mechanisms  $N_2O$  can be produced by (Butterbach-Bahl et al. 2013), and it is therefore difficult to predict emissions. Particularly, emissions from digestate or combinations of residues and/or mineral fertilizers may be high and unpredictable (Baral et al. 2017; Charles et al. 2017; Hansen et al. 2019; Dietrich et al. 2020). Emissions during non-growing seasons can also be high. Especially, pulses during freezing thawing cycles are known to be important in annual budgets of  $N_2O$  emissions (Christensen and Tiedje 1990; Christensen and Christensen 1991; Stehfest and Bouwman 2006; Teepe et al. 2001; Congreves et al. 2018; Adair et al. 2019), because the microbes that produce  $N_2O$  can be active at low temperatures when there are few other processes utilizing N. Dry non-growing seasons have been less studied, but there is some evidence that re-wetting dry soil can induce a flush of microbial activity and respiration, called the Birch effect (Rey et al. 2005). However, its effects on the emissions of  $N_2O$  are less known (Congreves et al. 2018).

Some previous studies have reported that some sorbents (e.g., bentonite and vermiculite) were mixed with animal slurry and other wastes before application in order to reduce N losses (Redding et al. 2016; Guaya et al. 2018) and gaseous losses of ammonia and nitrous oxide (Redding 2013; Hill et al. 2016). Charred organic materials "biochar" are good sorbents and can improve soil nutrient retention (Clough et al. 2013; Lehmann and Joseph 2009; Spokas et al. 2012). It was found that mixing biochar with wet-organic residues prior to application to soil can reduce leaching losses of N (Lehmann et al. 2003; Knowles et al. 2011).

Although there have been a number of studies on organic residues as fertilizers and they have been investigated as sources of GHG emissions in recent years, most studies have focused on just comparing the different residues available, so that there is still little

understanding on how waste treatment options affect fertilizer value and GHG emissions in soil. Moreover, considerable research has been performed focusing exclusively on the first growing season, whereas determining what happens during non-growing seasons and the residual effects that occur in subsequent years are equally important.

This study therefore focused on comparing N uptake from undigested vs. digested manure and biochar addition. The effects of a simulated non-growing season (dry or cold season) were assessed by drying and freezing the soil, respectively, and then assessing nitrous oxide emissions after re-wetting and thawing.

## Materials and methods

### Residues and soil used

The cattle manure used in this work was obtained from a farm, Rådde Gård, Sweden. The manure was shredded manually to reduce the particle size of straw in it. The digestate used was a residue after biogas production from this cattle manure with straw in a textile-based bioreactor (Patinvoh et al. 2017). 86 g of straw per 5000 g of manure was added before digestion. The characteristics of the manure and the digestate are shown in Table 1. The analyses were carried out by Eurofins using their standard methods.

Biochar was from PYREG GmbH, Germany, obtained by pyrolyzing *Miscanthus giganteus* at 600 °C (C: 79.6 %, H: 8.0 %, O: 0.47 %, N: 0.31 %, pH 7.86). It has previously been found that this biochar has no N fertilizer effect (Foereid, unpublished). When biochar was mixed with the digestate, the mixture was shaken in a shaker for 2 days before it was mixed into the soil. The soil was an agricultural soil from Øsager experimental field, in Østfold in eastern Norway. The soil was collected in November 2017 and stored in an unheated cellar until the start of the experiment in February 2018. Some properties of the soil are presented in supplementary information (S1), the analyses were performed also by Eurofins, according to their standard methods.

### Growth experiment

The treatments were: no N fertilization (0N), full fertilization with N as ammonium sulphate and phosphorus and potassium (0.26 g  $KH_2PO_4$  and 0.43 g  $K_2SO_4$ ) (1N), manure fertilization (M), digested manure fertilization

**Table 1** Characteristics of the used manure and digestate

Parameters	Manure bedded with straw (M)	Digestate (DM)
Total solids	25.84 ± 0.92 %	9.60 ± 0.12 %
Volatile solids	77.21 ± 2.74 %	64.6 ± 0.06 %
Moisture	74.16 ± 0.92 %	90.40 ± 0.12 %
Total Carbon	42.89 ± 1.52 %	36.23 ± 0.73 %
Total Nitrogen	2.26 ± 0.04 %	2660 mg/L
Ammonium N	ND	1250 mg/L
Protein content	14.13 ± 0.04 %	ND
Phosphorus	ND	2300 mg/kg
Potassium	ND	1576.5 mg/L
Bulk density (kg/m <sup>3</sup> )	542 ± 26.87	ND
pH	8.72 ± 0.83	8.06 ± 0.04

\*Dry basis; ND – not determined

(DM), and digested manure fertilization with the addition of biochar (21.7 g biochar per pot) (DMB). Each treatment was carried out in three replicates. The same amount of total N, *i.e.* 0.2 g per pot, was given to all treatments receiving N. Soil and additions were mixed thoroughly in each pot, and water content adjusted to half field capacity. Wheat (variety “Bjarne”) was used as a test plant. The pot size was 2 L and 15 seeds were sown in each pot, thinned to 10 shortly after germination. The temperature in the greenhouse was kept at or above 20/12 °C day/night and 16-hour day. Plants were watered to keep water content between half and full field capacity, and pots were moved around in a random manner each time they were watered. Plants were harvested just after ear emergence, after 50 days of growth. Aboveground plant material was cut just above the soil with scissors. Plant samples were dried (70 °C) and grinded into a fine powder and weighed in for analyses of total N and C (Ogner et al. 2000). Total N and C were measured on CHN analyzer (Elementar Vario EL with TCD detector). Statistical analysis was performed with Minitab v18. ANOVA with all comparisons was used for biomass and N uptake. 5 % was used as significance level.

#### Incubation and greenhouse gas measurements after a non-growing season

To simulate a non-growing season (winter or dry season), one sample of 20 mL of soil from each pot was

taken out and air-dried and another similar sample was frozen (-20 °C). After approximately three months the frozen samples were taken out of the freezer and thawed, and the dried samples were re-wetted to close to field capacity. The 0N samples were excluded from this incubation. Samples were then kept in 100 mL bottles that were put in an incubator at 20 °C and 90 % relative humidity. Gas samples were collected twice during the first day, then once each day. Before gas sampling, the bottles were closed for +/- 1 hour. 12 mL of gas was extracted through a septum with a syringe and injected into an evacuated vial. A zero sample was taken at the opening of the bottles before closing. Bottles were then opened and kept open during the experiment. The experiment ran for 4 days, and after that the soil was air-dried and pH was measured.

The gas samples were analysed by gas chromatography mass spectrometry (GC-MS) to determine concentrations of N<sub>2</sub>O, CH<sub>4</sub> and CO<sub>2</sub>. The analysis was performed using an Agilent Technologies 7820A GC System gas chromatograph, coupled to a mass detector Agilent Technologies 5875 Series MSD and a Gilson 222 XL auto sampler. The sample was injected by a 5 ml sample loop, through a 0.5 m x 0.32 mm deactivated precolumn, into a 25 m x 0.32 mm CP-PoraPLOT Q-HT column (Chrompack), kept at 40 °C. Helium was used as carrier gas at 1.0 ml min<sup>-1</sup>.

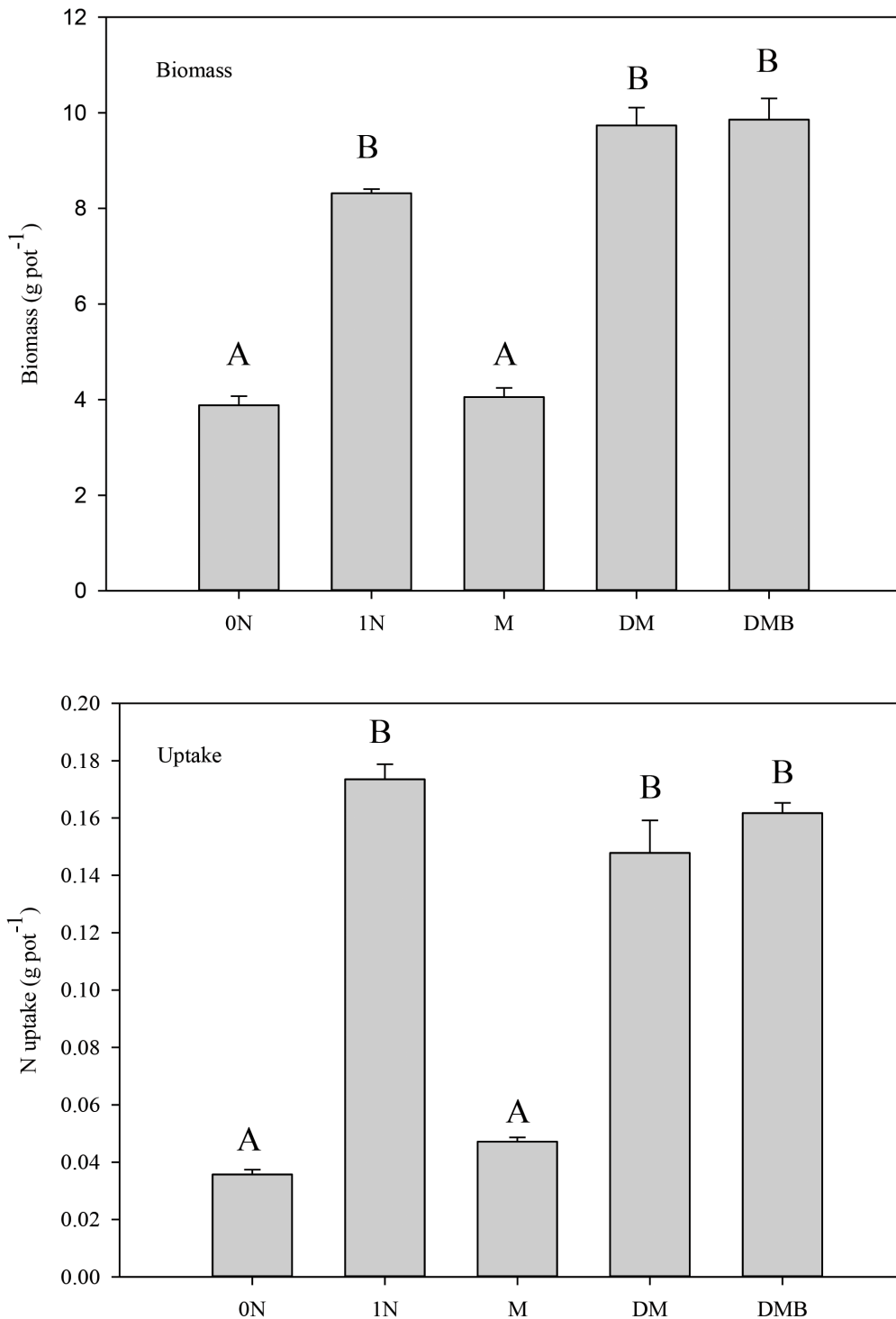
Statistical analysis of the results on gas emissions was performed using SAS<sup>®</sup> v9.4 software. A linear model with time and fertilizer treatment as factors tak-

ing into account correlations between time-points was used. To satisfy the assumption of normal distribution and homogeneous variance Box-Cox transformations were used in all cases except for CO<sub>2</sub> from the dried soil, where no transformation was needed.

## Results and discussion

### Biomass and N uptake

Both biomass and N uptake showed that the feedstock,



**Fig. 1** Biomass and N uptake in plant growth experiment

Error bars are standard error (n=3). Different letters indicate significant differences. 0N – no nitrogen fertilization, 1N – full fertilization, M – manure fertilization, DM – digested manure fertilization, DMB – digested manure with biochar.

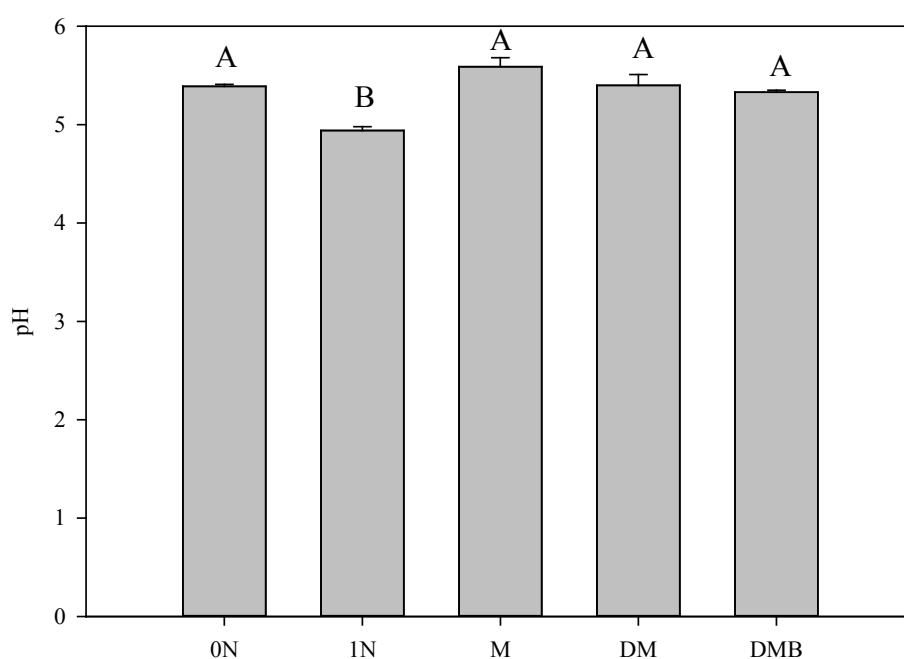
M, did not have more fertiliser effect than no fertilizer 0N, while the digestate treatments, DM and DMB, were comparable to or better than those obtained with mineral fertilizer, 1N (Fig. 1). There was a significant effect of treatment both on biomass and N uptake. Multiple comparisons showed that 0N was not significantly different from M; furthermore, treatments of 1N, DM and DMB were not significantly different, but there was a significant difference between these two groups.

The results clearly show that digestate is a good fertilizer with N fertiliser effect comparable to mineral fertiliser, this has also been found by others (Abubaker et al. 2012; Alburquerque et al. 2012a, b; Baral et al. 2017; Kristoffersen et al. 2013; Sogn et al. 2018). However, this study also shows that the digestion process improves N availability dramatically. While N uptake from the undigested manure was not significantly different from that where no fertilizer was applied, there was no significant difference between N uptake when either digestate or the mineral fertilizer was used. During the digestion process, organic N is mineralised to Ammonium-N, which can immediately be utilised by the plants. Although organic N in the manure can also be mineralised in the soil, this process will take a longer time, hence might be too late for the plants to uptake. Grain crops need to take up most of the N early in their growth cycle (Kristoffersen et al. 2013), consequently

a large fraction of the N should be available as mineral N at that time. It is not likely that such a dramatic effect can be found after digestion of all other organic feedstocks, but the results of this study clearly point out that anaerobic digestion is a suitable treatment for organic residues not just in terms of renewable energy generation, but also in terms of improving the fertilizer value of the residues. Anaerobic digestion can particularly be recommended on organic farms where they depend on high N utilisation of the residues applied (Hansen et al. 2019).

No clear effect of biochar was found. There was no leaching loss in this experiment, but previous studies have indicated that mixing manure with sorbents can also reduce ammonia volatilisation (Redding 2013) and thereby make more N available to plants. There was no sign of that in this experiment. This may indicate that biochar is a poorer sorbent than the sorbent used in the previous experiment (bentonite), or that the digestate used here had a relatively high dry matter content, meaning that there was ample sorption in the digestate itself. The high N uptake from digestate also without biochar indicates that ammonia volatilisation was unimportant as a loss of N in all treatments.

There was little difference in pH between the soils from each treatment after the growth (Fig. 2) except 1N treatment that was significantly different from the rest.



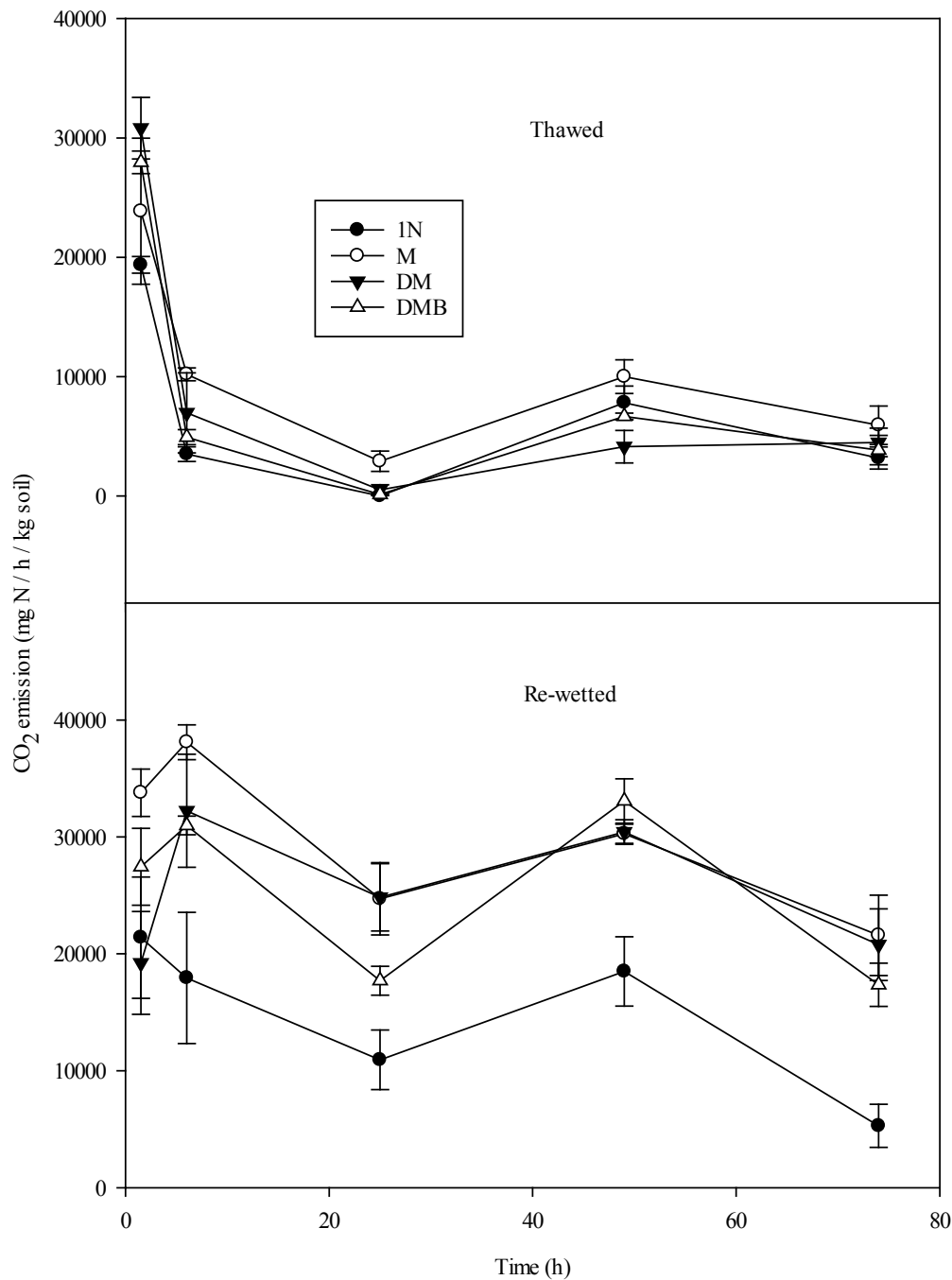
**Fig. 2** pH in soil at the end of the experiment

Error bars are standard error (n=3). Different letters indicate significant differences. 0N – no nitrogen fertilization, 1N – full fertilization, M – manure fertilization, DM – digested manure fertilization, DMB – digested manure with biochar.

This shows that the differences observed could not simply be explained as the effects of pH, since there was no significant difference found between the effects of mineral fertiliser and the two other treatments (DM and DMB) on plant growth (Fig. 1). Differences in pH in this range are also not expected to affect plant growth, although it might have some effect on nutrient availability (Oburger et al. 2011).

### Greenhouse gas emissions after a non-growing season

No methane emission was detected in any of the treatments. CO<sub>2</sub> emission (respiration) was relatively large in the first few hours during thawing, but then it went down to almost zero in all treatments (Fig. 3). In the re-wetting experiment, respiration rates started at about

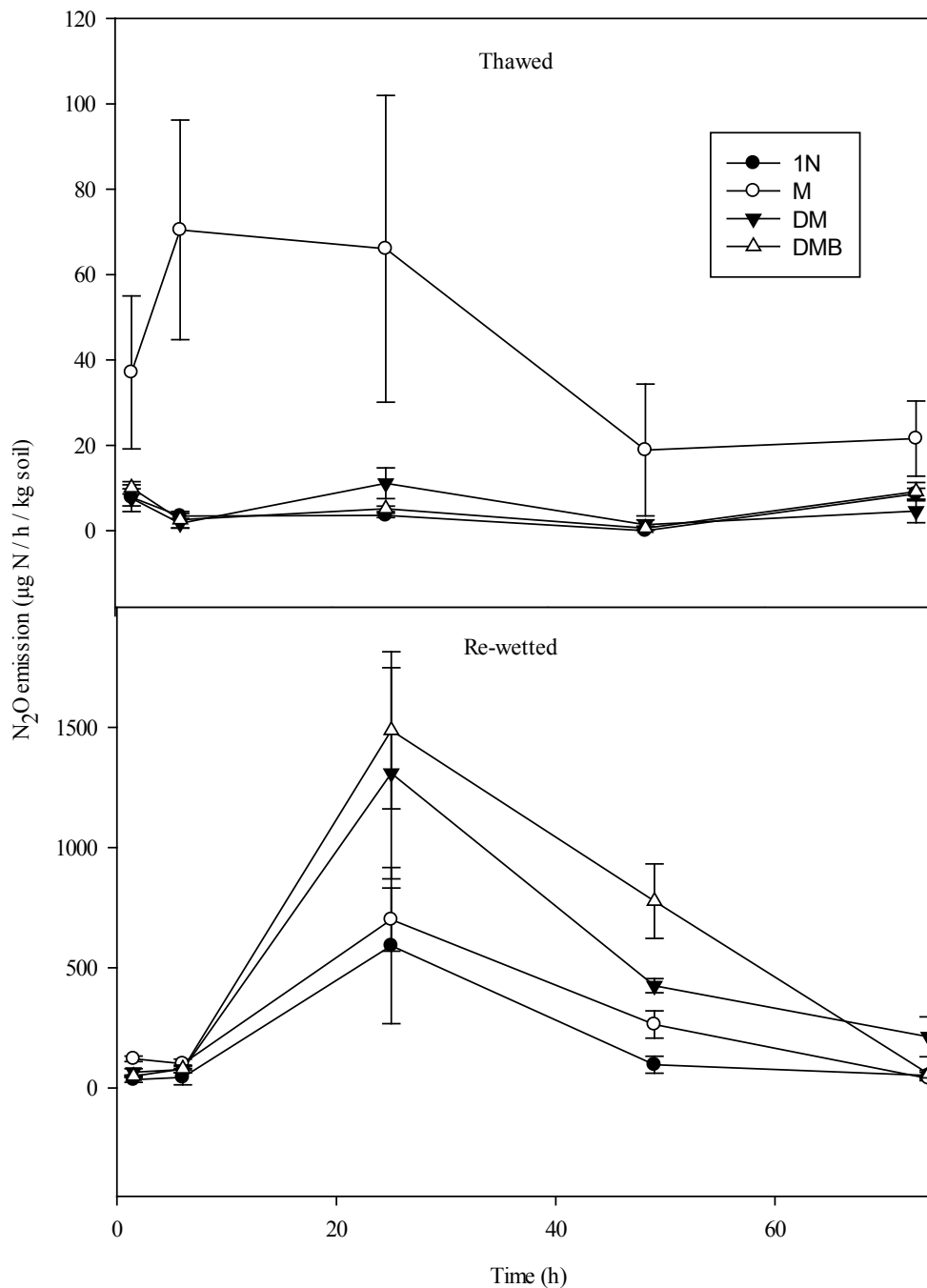


**Fig. 3** CO<sub>2</sub> emission after frozen soil was taken out of freezer and dried soil re-wetted

Error bars are standard error (n=3). The symbols represent 1N – full fertilization, manure fertilization (●), M – manure fertilization (○), DM – digested manure fertilisation (▼) and DMB – digested manure with biochar (△)

the same level as after thawing, but stayed there, with only a slow decline towards the end. The effect of treatment was significant ( $p < 0.5$ ) in the re-wetting experiment, and almost significant in the thawing experiment (Table 2). The effect of time was always highly significant. In the re-wetting experiment, 1N was significantly different from all the other treatments. In the thawing experiment, M was significantly different from DM and 1N (Table 2).

$N_2O$  emissions were very low in most treatments during thawing (Fig. 4), but  $N_2O$  emission from M was significantly higher than all the other treatments (Table 2).  $N_2O$  emissions after re-wetting were low during the first few hours, but then increased, and became much larger than those from the thawing samples (Fig. 4). The overall effect of treatment was significant ( $p < 0.05$ ) in both the thawing and re-wetting experiments (Table 2). The effect of time was always highly significant.



**Fig. 4**  $N_2O$  emission after frozen soil was taken out of freezer and dried soil re-wetted

Error bars are standard error ( $n=3$ ). The symbols represent 1N – full fertilization, manure fertilization (●), M – manure fertilization (○), DM – digested manure (▼) and DMB – digested manure with biochar (△)

**Table 2** Results of statistical analysis of greenhouse gas measurements

<b>Main effects</b>				
	Treatment		Time	Interaction
CO <sub>2</sub> re-wetted	<b>0.0090</b>		<b>&lt;.0001</b>	0.1944
N <sub>2</sub> O re-wetted	<b>0.0048</b>		<b>&lt;.0001</b>	0.3140
CO <sub>2</sub> thawed	0.0576		<b>&lt;.0001</b>	0.2006
N <sub>2</sub> O thawed	<b>0.0211</b>		<b>&lt;.0001</b>	0.5079
<b>Pairwise comparisons CO<sub>2</sub> re-wetted</b>				
	M	DM	DMB	
1N	<b>0.0016</b>	<b>0.0104</b>	<b>0.0114</b>	
M		0.2328	0.2143	
DM			0.9554	
<b>Pairwise comparisons N<sub>2</sub>O re-wetted</b>				
	M	DM	DMB	
1N	<b>0.0128</b>	<b>0.0015</b>	<b>0.0018</b>	
M		0.3127	0.3440	
DM			0.9469	
<b>Pairwise comparisons CO<sub>2</sub> thawed</b>				
	M	DM	DMB	
1N	<b>0.0118</b>	0.5631	0.4112	
M		<b>0.0370</b>	0.0595	
<b>Pairwise comparisons N<sub>2</sub>O thawed</b>				
	M	DM	DMB	
1N	<b>0.0128</b>	0.6777	0.9419	
M		<b>0.0057</b>	<b>0.0148</b>	
DM			0.6258	

1N = full fertilization, M = manure fertilization, DM = digested manure fertilization, DMB = digested manure with biochar. Significant results are shown in bold; p-value < 0.05.

Gas emissions after a non-growing season seems to indicate that winter (with frost) is more serious for microbial processes than a dry season. Respiration recovered much faster and emissions of both CO<sub>2</sub> and N<sub>2</sub>O were much larger after re-wetting of dry soil than after thawing of frozen soil, this has also been observed by Congreves et al. (2018). The initial high respiration rate detected right after thawing may indicate microbial utilization of bacterial cells killed by the frost (Christensen and Tiedje 1990). Denitrifying enzyme activity increases quickly after frost (Haider and Schneider 1992). Dörsch et al. (2004) also found that microbial biomass was highly variable during freezing-thawing cycles, indicating cell death and regrowth.

The results also clearly show that low NUE the year before, as when undigested manure was used as fertilizer, gave rise to higher N<sub>2</sub>O emissions during thaw-

ing than the other treatments, that all had little residual N. This effect was not seen after re-wetting dry soil, but the emissions from all treatments were higher after re-wetting than during thawing. The mechanism for the large effect of freezing and thawing in this case is not known. A possible explanation is that a lot of the N bound in microbial cells, which in turn were killed by the frost, were released after thawing, making the N available. This explanation is frequently invoked, and it was discussed above. Microbial life in manure has developed without frost, so that it may have a larger effect on manure than on e.g., soil. However, Petersen et al. (2013) did not find any indication that higher organic input gave higher emissions, probably because they also can affect soil aeration.

The low pH in the mineral N treatment could affect decomposition and carbon loss (Foerid et al. 2006;



Leifeld et al. 2013). It could also affect N<sub>2</sub>O emissions, although it is yet not clear how (Liu et al. 2010; Simek and Cooper 2002). However, the important result showing the difference between untreated and digested manure is unaffected by this.

Often farms have excess manure and spread as much as possible on the field. This study shows that only a small part of the N in this manure will be utilised, at least by grain crops. In addition, nutrient losses during winter can be expected. Therefore, it is recommended to treat the manure by anaerobic digestion in biogas plants on these farms, since it would improve the quality of the manure as fertiliser.

The simulated winter in this study was very cold (-20°C) and stable. However, the winter in most agricultural areas is more variable, often with a number of freezing and thawing cycles. This may increase the yearly total N<sub>2</sub>O emission from residues (Adair et al. 2019). Moreover, the released N may also be lost by leaching. Winter crops or cover crops grown over the winter may reduce losses, but the very fast start of N<sub>2</sub>O emission when thawing may indicate that this would not solve the problems with the N<sub>2</sub>O emissions detected in this study.

## Conclusion

The results indicate that slow release of N from organic manure can be a problem, both from an agronomic and environmental perspective. No N was available from the manure in the first growing season, but it induced GHG emissions after a winter. It appears that particularly freezing makes this N available, while a dry season will not have as large effect.

The study also points at anaerobic digestion as a solution to make the N in organic residues available the first growing season, and both increase NUE and reduce losses and pollution.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that there are no conflicts of interest associated with this study.

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