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Greenhouse gas emissions when freezing and thawing sewage sludge and compost

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Preface

This report is the final result of a 4 month internship, for which I did a research project at the department of Energy and Technology of the ‘Sveriges Lantbruksuniversitat’ (Swedish University of Agricultural Sciences, SLU) in Uppsala, Sweden. This internship was the last compulsory part in order to get my Master degree in Environmental Sciences at Wageningen University, the Netherlands.

I have enjoyed both the project and my stay in Sweden. Therefore I would like to thank the SLU for giving me the opportunity to stay in Sweden and to do research here for a few months.

I would like to thank my supervisors, Evgheni Ermolaev, Agnes Willén and Håkan Jönsson for their help and advice during this project. I would also like to thank Sven Smårs, who have done a lot of practical work and helped with the set-up of the experiments.

I think this research project has given us interesting new insights, but also, the same as many other research results, quite a few new questions. I hope you will find it interesting to read.

Abstract (English)

Freezing/thawing cycles can significantly increase greenhouse gas emissions due to physical and biological mechanisms. Therefore this research was done to see if this is also the case for compost and sewage sludge. The main focus was on nitrous oxide. The set-up of the experiments was based on the outcomes of several trial experiments. 4 substrates were tested: non-nitrified mesophilic sewage sludge, nitrified mesophilic sewage sludge, 2 weeks old compost and 1 month old compost. The two composts had a clear difference in ammonium content. The substrates were first frozen at a temperature of -27 °C, and then thawed at a temperature of 5 °C. Controls were kept at a temperature of 5 °C during the whole experiment. All substrates were tested in triplicates. Emissions of carbon dioxide, methane and nitrous oxide were measured. No clear effect of freezing/thawing was observed on the greenhouse gas emissions from non-nitrified sewage sludge. From the nitrified sewage sludge the emissions of both carbon dioxide and nitrous oxide increased during thawing. The cumulative emission of carbon dioxide was 17500 µg g^{-1} initial C from the frozen and thawed sewage sludge, and 11170 µg g^{-1} initial C from the controls. The cumulative emission of nitrous oxide in the sewage sludge that had been frozen and thawed was about 3500 µg g^{-1} initial N, for the controls this was 3000 µg g^{-1} initial N. Both the increase carbon dioxide and nitrous oxide emissions were probably due to increased substrate availability, caused by the die-off of micro-organisms during freezing. The nitrous oxide probably came from denitrification. In both of the composts no effect of the freezing and thawing was observed on the emissions of carbon dioxide and methane, but it had a clear effect on the emission of nitrous oxide: in the 1 month old compost a clear emission peak was observed during the first days of thawing. For the one month compost this was probably due to a physical mechanism of trapped nitrous oxide inside the compost which could escape during the thawing period. An unexpected result was the large difference between the two composts considering the amounts of nitrous oxide emissions, which were expected to be approximately the same due to equal nitrate amounts. However, the cumulative nitrous oxide emission from the frozen and thawed 2 weeks compost was 3,00 µg g^{-1} initial N at the end, while from the 1 month compost this was 1220 µg g^{-1} initial N. Since the ammonium in the 1 month compost had decreased with 32,5%, and only 17,5% for 2 weeks compost, the large amount of nitrous oxide probably came from the conversion of ammonium. However, it is still not clear whether this comes from nitrification of the ammonium, or from denitrification of the nitrate formed during the experiment. The conclusion was that freezing/thawing increases emissions of carbon dioxide and nitrous oxide from nitrified sewage sludge during thawing, and the emissions of nitrous oxide from compost during thawing. Recommendations for further research are to find out which processes, nitrification or denitrification, are responsible for the nitrous oxide production. Also research to compost with different nitrate amounts is recommended.

Abstract (Nederlands)

Het bevriezen en daarna ontdooien van grond kan de emissie van broeikasgassen aanzienlijk verhogen door zowel fysische als biologische mechanismen. Dit onderzoek is gedaan om te onderzoeken of dit ook het geval is voor compost en zuiveringsslib. De belangrijkste focus hierbij was lachgas. De opstelling van de experimenten was gebaseerd op de resultaten van enkele proefexperimenten. 4 substraten werden uitgekozen: ongenitrificeerd mesofiel zuiveringsslib, genitrificeerd mesofiel zuiveringsslib, 2 weken oude compost, 1 maand oude compost. De twee composten verschilden aanzienlijk in ammoniumgehalte. De substraten werden eerst ingevroren bij een temperatuur van -27 °C en vervolgens ontdooid bij een temperatuur van 5 °C. De controlegroep werd gedurende het gehele experiment bij een temperatuur van 5 °C gehouden. Alle substraten werden in drievoud getest. Emissies van koolstofdioxide, methaan en lachgas werden gemeten. Geen duidelijk effect van het bevriezen en ontdooien werd waargenomen op de broeikasgasemissies uit het ongenitrificeerde zuiveringsslib. Uit het bevroren en ontdooide genitrificeerde zuiveringsslib nam de uitstoot van koolstofdioxide en lachgas duidelijk toe tijdens het ontdooien. De cumulatieve emissies aan het einde van de experimenten waren voor deze gassen hoger dan in de controlegroep. De cumulatieve emissie van koolstofdioxide was 17.500 µg g^{-1} C uit de ingevroren en ontdooide zuiveringsslib en 11.170 µg g^{-1} C van de controles. De cumulatieve emissie van lachgas uit het bevroren en ontdooide genitrificeerde zuiveringsslib was ongeveer 3500 µg g^{-1} N, voor de controles was dit 3000 µg g^{-1} N. Zowel de toename van koolstofdioxide als van stikstofoxide emissies werden waarschijnlijk veroorzaakt door een toename van beschikbaar substraat, veroorzaakt door het afsterven van micro-organismen tijdens het vriezen. De uitstoot van lachgas werd waarschijnlijk veroorzaakt door denitrificatie. In beide composten werd geen effect geobserveerd van het bevriezen en ontdooien op de uitstoot van koolstofdioxide en methaan, maar er was een duidelijk effect op de uitstoot van lachgas. Bij de 1 maand oude compost werden duidelijke emissiepieken waargenomen tijdens de eerste dagen van het ontdooien. Waarschijnlijk was dit te wijten aan een fysiek mechanisme van verdwijnende barrières tijdens het ontdooien, waardoor het lachgas kon ontsnappen. Een onverwacht resultaat was het grote verschil tussen de twee composten wat betreft de hoeveelheden lachgasemissie. Er was verwacht dat deze ongeveer gelijk zouden zijn, omdat de nitraathoeveelheden dat ook waren. Echter, de totale lachgasemissie uit de bevroren en ontdooide compost van 2 weken was 3,00 µg g^{-1} N aan het einde, voor de compost van 1 maand was dit 1220 µg g^{-1} N. Aangezien het ammoniumgehalte in de compost van 1 maand gedaald was met 32,5%, en slechts met 17,5% voor de compost van 2 weken, kan de grote hoeveelheid lachgas waarschijnlijk worden verklaard uit de omzetting van ammonium. Het is echter niet duidelijk of dit werd gevormd tijdens nitrificatie van ammonium, of dat het werd uitgestoten tijdens denitrificatie van nitraat gevormd tijdens het experiment. De conclusie was dat het bevriezen en ontdooien de uitstoot van koolstofdioxide en lachgas uit genitrificeerd zuiveringsslib, en de emissie van lachgas uit compost, verhoogt tijdens het ontdooien. Aanbevelingen voor verder onderzoek zijn om te achterhalen welke processen, nitrificatie of denitrificatie, verantwoordelijk zijn voor de productie van lachgas. Ook onderzoek naar compost met verschillende hoeveelheden nitraat wordt aanbevolen.

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Nomenclature

µg	Microgram(s)
C	Carbon
cm	Centimeter(s)
CH_4	Methane
CO_2	Carbon Dioxide
ECD	Electrical Conductivity Detector
FID	Flame Ionization Detector
g	Gram(s)
GC	Gas Chromatograph
GHG	Greenhouse gas
K	Kelvin
N	Nitrogen
N_2	Nitrogen gas
NaOH	Sodium Hydroxide
NH_3	Ammonia
NH_4^+	Ammonium
NO	Nitric oxide
NO_2^-	Nitrite

NO_3^-	Nitrate
N_2O	Nitrous oxide
O_2	Oxygen
ppm	Parts per Million
TCD	Thermal Conductivity Detector
TC	Total Carbon
TN	Total Nitrogen
TS	Total Solids
VS	Volatile Solids

1. Introduction

Nitrous oxide (N_2O) is known as a strong greenhouse gas (GHG) with nearly 300 times the global warming potential of carbon dioxide (CO_2). Therefore large emissions of this gas are undesirable. A phenomenon that may have significant influence on the emission of N_2O, and on other GHG emissions, is freezing and thawing cycles. From different studies on different soil types it becomes clear that the emissions may increase significantly during these cycles (Teepe *et al.* 2001, Goldberg *et al.* 2010, Yao *et al.* 2010). Increases of N_2O emission can occur both during freezing (Kaiser and Heinemeyer 1996, Papen and Butterbach-Bahl 1999, Teepe *et al.* 2001, Goldberg *et al.* 2010) and during a thawing period after the freezing (Kaiser and Heinemeyer 1996, Teepe *et al.* 2001, Goldberg *et al.* 2010, Yao *et al.* 2010). Freezing and thawing may also influence emissions of other greenhouse gasses. For example, methanogenesis in which methane (CH_4) is formed could increase during freezing because of more anaerobic conditions and increased substrate availability or it might decrease due to the methanogens being very temperature dependent. Also emission of CO_2 may increase due to increased substrate availability (Kim *et al.* 2012).

Both compost and sewage sludge represent substrates similar to soil and are subjected to freezing and thawing during the winter in Swedish conditions and as they are rich in nitrogen, N_2O emissions may be large when conditions that enhance N_2O emissions are present. However there were no studies found on freezing and thawing for either compost or sewage sludge. The influence of freezing and thawing cycles could be relevant for countries with cold climate, where freezing happens every winter. For smaller systems like home composting there is a high probability for the whole compost to be frozen during the winter (Ermolaev *et al.* 2011).

The objective of this study was to find how freezing and thawing cycles affect the N_2O and CH_4 emissions in relation to CO_2 from compost and sewage sludge and to investigate possible underlying mechanisms. Most attention was paid to N_2O, since this is the strongest GHG and also the one on which most literature was studied. The emissions of CO_2 were used an indicator of the amount of (heterotrophic) microbial activity.

The hypothesis was that freezing and thawing significantly influences and increases the GHG emissions from compost and sewage sludge since they are rich in carbon (C) and nitrogen (N) substrates.

1.1 N pathway in soil and formation of N_2O

During nitrification nitrogen is first hydrolyzed to ammonium (NH_4^+) and then nitrified to nitrite (NO_2^-) and nitrate (NO_3^-) in which the NO_2^- forming is the rate limiting step.
Nitrification is mostly done by autotrophic bacteria, although a small part of the NO_2^- may be produced by heterotrophic bacteria (Wallace and Nicholas 1969). Also autotrophic Achaea can play a role in nitrification (Venter *et al.* 2004). For the nitrification process enough

dissolved oxygen (O_2) is required and the optimal pH range is 7,5-8,0 (Metcalf & Eddy. *et al.* 2003). Temperature is an important factor for nitrification and at temperatures around freezing, nitrification is possible but other conditions need to be favorable (Frederick 1956, Seifert 1961). In the temperature range 8-28 °C nitrification rates can be described by the Arrhenius equation; the rate doubles with 10 °C increase in temperature (Wild *et al.* 1971, Seifert 1980) and the optimum temperature is at 30 °C (Wild *et al.* 1971).

After nitrification, denitrification can take place: NO_3^- is reduced to NO_2^-, nitric oxide (NO), N_2O and finally nitrogen gas (N_2). This happens in an anoxic environment. Denitrifying bacteria are usually heterotrophic (Knowles 1982). Denitrification has its optimum temperature around 25 °C (Saad and Conrad 1993), but can take place at various temperatures, even below 0 °C (Dorland and Beauchamp 1991). Most N_2O emitted from soils is assumed to be produced during the denitrification. In this process, N_2O as an intermediate may escape when the reduction is not complete (Poth and Focht 1985, Bateman and Baggs 2005), but also during nitrification N_2O emission can occur.

The amount of N_2O emission depends on many factors and these are different for N_2O emission during nitrification and N_2O emission during denitrification. An important factor for N_2O emission during denitrification is the amount of NO_3^- present in the soil. High NO_3^- concentrations will decrease the reduction of N_2O to N_2, because NO_3^- is preferred as electron acceptor over N_2O. Therefore large NO_3^- concentrations cause higher N_2O emissions compared to a situation in which all N_2O would be reduced (Blackmer and Bremner 1978, Weier *et al.* 1993). During denitrification a high water content and lots of easily available C can increase the N_2/N_2O ratio and therefore lower the emission of N_2O (Weier *et al.* 1993, Hwang and Hanaki 2000, Tiquia 2002). The presence of oxygen during denitrification will decrease the reduction of N_2O and therefore enhance the release of N_2O (Knowles 1982, Hwang and Hanaki 2000, Jarvis *et al.* 2009).

However the effects of water content and O_2 are different for N_2O emission during nitrification. In studies of Blackmer *et al.* (1980) and Freney *et al.* (1979) the N_2O emission increased with increasing water content when the N_2O was formed during nitrification. N_2O can also be emitted during nitrification when O_2 is limited due to a process called nitrifier denitrification. In this process NO_2^- is reduced to N_2O or N_2 by nitrifiers (Poth and Focht 1985, Bateman and Baggs 2005). This process is favored by low O_2 concentrations and low C contents (Wang *et al.* 2009). Furthermore small amounts of N_2O can be produced as byproduct during the oxidation from NH_3 to NO_2^- (Blackmer *et al.* 1980, Arp and Stein 2003).

1.2 Sewage sludge

Sewage sludge is produced during wastewater treatment. During this process liquids and solids are separated and the solids form the sewage sludge (Metcalf & Eddy. *et al.* 2003). There are different ways of sludge treatment; in this study anaerobically digested sludge at mesophilic temperatures was used. The anaerobic degradation of the sludge occurs via hydrolysis, acidogenesis and methanogenesis (Metcalf & Eddy. *et al.* 2003). After processing sewage sludge may be used on (agricultural) land, because it has fertilizer value that mainly depends on the contents of N, phosphorus, potassium, and organic matter (Metcalf & Eddy. *et al.* 2003).

The N content of sewage sludge can differ and the NO_3^--N content is usually very low in sewage sludge that has been treated anaerobically. The NH_4^+-N content is high, for example the sewage sludge used by Smith *et al.* (1998) contained TN in the range of ~40-60 g kg^{-1} total solids (TS), of which ~20-60 g kg^{-1} TS was NH_4^+-N and <0,03 g kg^{-1} TS was NO_3^--N.

No literature was found on the storage of anaerobically digested sludge, only studies on the application of sewage sludge in e.g. agricultural land. When adding the sewage sludge to the soil, the largest part of NH_4^+-N will normally be nitrified to NO_3^--N in several weeks (Terry *et al.* 1981, Smith *et al.* 1998). The organic N can be mineralized and the rate of mineralization strongly depends on the soil type to which the sewage sludge is added.

Addition of sewage sludge on agricultural land can lead to significant amounts of CO_2 and N_2O emissions compared to agricultural land with normal fertilizer use (Scott *et al.* 2000). Literature on the emissions of CH_4 from sewage sludge added to agricultural land was not found.

1.3 Compost

Composting is a natural process driven by microbial activity. It starts with the degradation of easily degradable organic compounds causing a strong increase in temperature and increased oxygen demand. Later during the process cellulose and long chain polymers are decomposed by other microorganisms at lower temperatures (de Bertoldi *et al.* 1983). Composting is an aerobic process, but anaerobic sites can develop during composting (Jarvis *et al.* 2009).

Autotrophic nitrification is usually inhibited in the early, very active stage of composting, because of the high temperature and relatively high ammonia (NH_3) production rates. Heterotrophic nitrification on the other hand is possible (de Bertoldi *et al.* 1983), but only small amounts of NO_2^- are formed during this stage of composting.

A large part of the N in compost is organic N. Initial values are in a range of 20-40 g kg^{-1} TS (Eklind and Kirchmann 2000, Tiquia 2002, Jarvis *et al.* 2009, Yamamoto *et al.* 2012) of which only a fraction is converted to NH_3-N and NH_4^+-N.

The amounts of different N forms depend on different properties of the compost. The amount of NH_4^+-N produced shows positive correlations with temperature, water content, pH and carbon content. The amounts of NO_2^--N and NO_3^--N show negative correlations with the same properties (Tiquia 2002). Significant parts of NH_3-N can volatilize during the process. This will reduce the rates of nitrification and thus keep the NO_3^--N concentrations low (Tiquia

2002, Jarvis *et al.* 2009). The pH and temperatures usually increase significantly during composting causing the NH_3 volatilization to increase (Emerson *et al.* 1975).

NH_4^+-N and NO_3^--N concentrations can differ a lot between different compost substrates, for example Tiquia (2002) found 4-5 g kg^{-1} TS for NH_4^+-N and 1-3 g kg^{-1} TS for NO_x^--N, and Jarvis *et al.* (2009) found ~10 g kg^{-1} TS for NH_4^+-N and ~40 mg kg^{-1} TS for NO_3^--N as highest values during the composting process.

Substantial CO_2 emissions occur during the composting process. Composting is an anaerobic process, which for a well-managed compost makes the CH_4 emission very low compared to CO_2, but CH_4 can still be produced in anaerobic sites inside the compost (Szanto *et al.* 2007, Jarvis *et al.* 2009). Anaerobic sites can also stimulate denitrification and therefore N_2O emissions (Hellmann *et al.* 1997, Jarvis *et al.* 2009)

1.4 Mechanisms influencing greenhouse gas emissions during freezing and thawing

According to a literature review of Kim *et al.* (2012) many studies on different soil types have shown increased GHG fluxes following thawing which significantly contributed to annual GHG fluxes, although there are also studies in which thawing did not show clear effects. Increases in N_2O emission in the field were in the range of ~600-4000% compared to the fluxes just before thawing.

Mechanisms behind this phenomenon are partly the same as mechanisms of increased emissions after rewetting, since both increase the availability of soil water, rehydrate cells, increase microbial metabolism, and mobilize nutrients (Kim *et al.* 2012).

There are two types of mechanisms after thawing: mechanisms related to enhanced microbial metabolism and physical mechanisms. Microbial metabolism may be enhanced because of higher nutrient availability from organisms which have died during soil freezing. These can be decomposed during thawing as the substrates become available for surviving microorganisms (de Bruijn *et al.* 2009, Kim *et al.* 2012). It is also possible that the substrate becomes available already during the time the soil is still frozen leading to significant increase in emissions during this period (Papen and Butterbach-Bahl 1999). An additional explanation for higher nutrient concentrations in the soil proposed by Papen and Butterbach-Bahl (1999) is the lack of nutrient uptake by plant roots under cold conditions.

A physical mechanism of N_2O emission increase during thawing is the prevention of N_2O release during the freezing period due to obstruction by ice layers. During thawing these ice barriers disappear and a lot of already produced N_2O can escape at once (Goldberg *et al.* 2010). According to several studies, high N_2O emissions during thawing of soil are the result of denitrification (Morkved *et al.* 2006, Sharma *et al.* 2006). For high N_2O-emissions during freezing one explanation, according to Kaiser *et al.* (1998), is that the N_2O is produced in deeper soil layers and escapes through frost-induced cracks in the soil.

The mechanisms for increased CO_2 and CH_4 emissions due to freezing and thawing cycles are partly the same as for N_2O. CH_4 emissions increase with higher temperatures, but can still occur at low temperatures, even when the soil is frozen (Friborg *et al.* 1997). Because of the availability of more substrate during soil freezing the CH_4 emission could increase during soil freezing, depending on the microbial activity under these low temperatures (Kim *et al.* 2012). The CO_2 emission depends on the amount of microbial heterotrophic activity taking place (Kim *et al.* 2012). CO_2 emissions can increase during thawing, due to decomposition of microorganisms that died during freezing. These dead cells can be decomposed during thawing, thereby causing CO_2 emissions (Kim *et al.* 2012). CO_2 emission during freezing can be caused by dissolved CO_2 that is forced out of the water when it freezes (Teepe *et al.* 2001). The same can happen to CH_4 as well, as described by Mastepanov *et al.* (2008). Another possibility, when CO_2 or CH_4 does not have the chance to emit, is that it is accumulated inside the soil and that an emission peak occurs during the first days of thawing (Friborg *et al.* 1997).

2. Material and methods

2.1 Method development

Before the main experiments, trial experiments were done in order to test the materials and methods for the main experiments and to get a rough indication of the magnitude of the gas emissions. Furthermore some analytical methods were tested for the substrates used in this study.

For the experiments with sewage sludge, fresh mesophilic anaerobically digested sewage sludge was collected at ’Kungsängsverket’, the water treatment plant at Uppsala, Sweden.
Compost was collected at the facility ‘Hovgården’, a waste treatment plant near Uppsala, Sweden. The compost was a mixture of household compostable waste and garden waste.

Both sewage sludge and compost used during the main experiments were stored at 2 °C during the time between fetching and starting of the experiments. For the composts, the boxes were left slightly opened so that it was kept aerobic.

2.1.1 First trial experiment: top-bottom temperature profile

The objective for the first trial experiment was to ensure that it was freezing and thawing from top to bottom and thus it was necessary to get a top to bottom temperature profile. A top-bottom temperature profile would simulate natural conditions.

For the first trial experiment sewage sludge was placed in a plastic container with a volume of 3770 ml as a test if the insulation worked properly. The container was approximately half-filled. The exact weight of the sludge was not measured, since the objective was only to test the insulation. The container was placed in a styrofoam box filled with wood shavings and styrofoam pieces and placed in a freezer of -27 °C. 6 temperature sensors were placed inside the material: two on top, two in the middle and two on the bottom. The temperature was measured every five minutes.

The whole set-up was kept in the freezer for three days. In Figure 1 the temperature results of three of the sensors are shown.

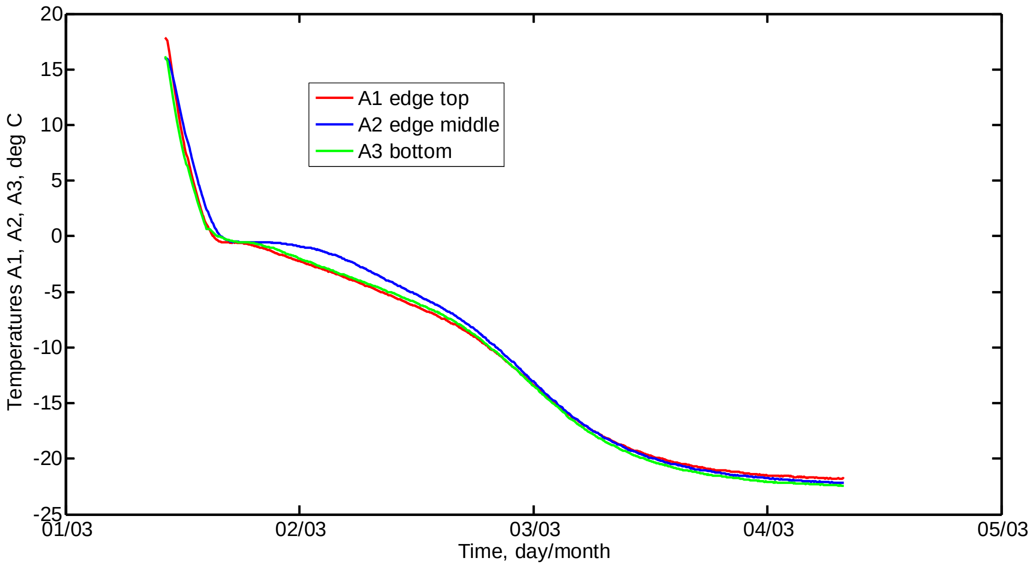

Figure 1. Temperature profile A during first trial experiment

It can be seen that there was no clear temperature profile in the sludge and it was thus not freezing from top to bottom. Based on these outcomes it was decided that better insulation was necessary to achieve the desired effect.

2.1.2 Second trial experiment: temperature profile and gas emissions from sewage sludge

The objectives of the second trial experiment were to make the insulation better and get a clear temperature profile with freezing from top to bottom and to get an indication about the gas emissions from sewage sludge during freezing and thawing.

For the second trial experiment the same sewage sludge and container were used as in the first trial experiment. 1426 g of sludge was used. The styrofoam box was made to exactly fit around the container, and a thin flexible foam layer was placed between the container walls and the styrofoam. Two insulation water bags with a weight of 1368 grams together were placed between the bottom of the container and the insulation to increase the thermal capacity below the container. 6 temperature sensors were placed in the same positions as in the first trial experiment and one sensor was placed outside the container in the freezer. Again the temperature was measured every five minutes. The sludge had been kept in the fridge before this experiment started. At the start of the experiment a gas sample was taken from the opened container to measure the background concentrations. During the experiment gas samples were taken after taking the container with the frozen sludge out of the freezer and at the end, when all the sewage sludge had thawed.

All gas samples were taken and analyzed in duplicate for CO_2, CH_4 and N_2O with a gas chromatograph (GC) as is described in chapter 2.4.2.

In Figure 2 and Figure 3 the temperature results are shown.

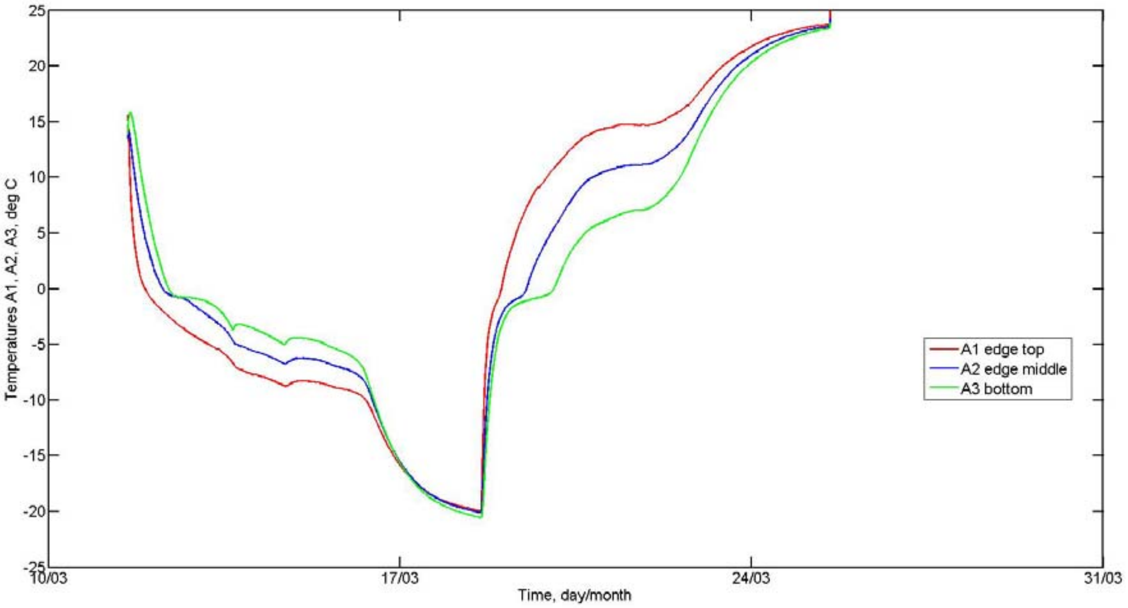

Figure 2. Temperature profile A during second trial experiment

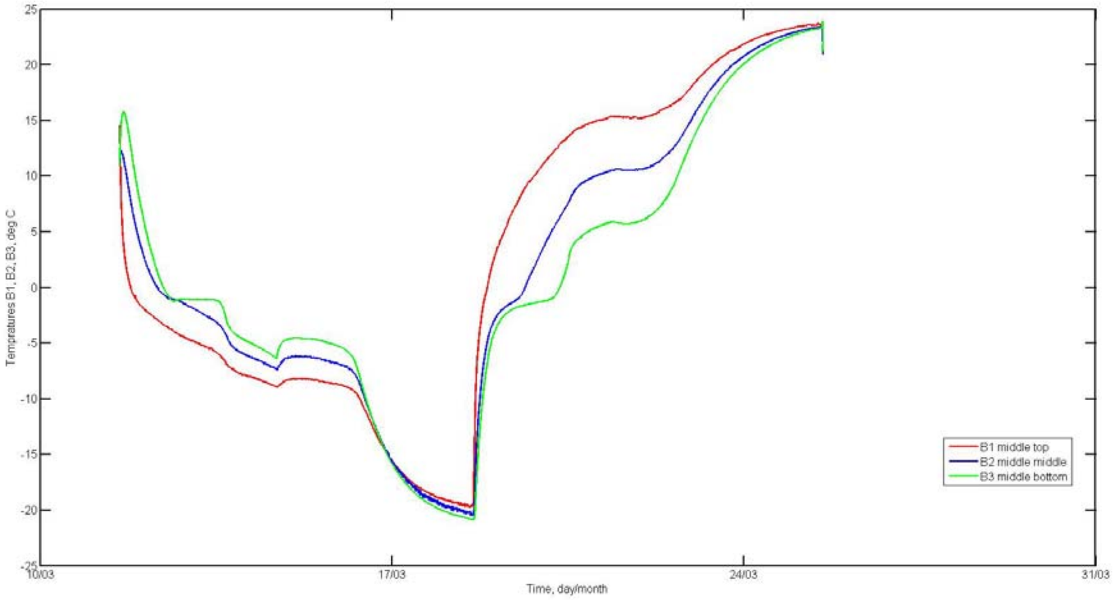

Figure 3. Temperature profile B during second trial experiment

As can be seen in both graphs that during this experiment the clear temperature profiles strived for were achieved: the top froze and thawed first, the bottom froze and thawed last. Therefore, the same insulation and procedure was applied in the main experiment.

In Figure 4, Figure 5 andFigure 6, the cumulative emissions of CO_2, CH_4 and N_2O during the second trial experiment are shown in micrograms (μg) per gram (g) of wet weight of the sewage sludge.

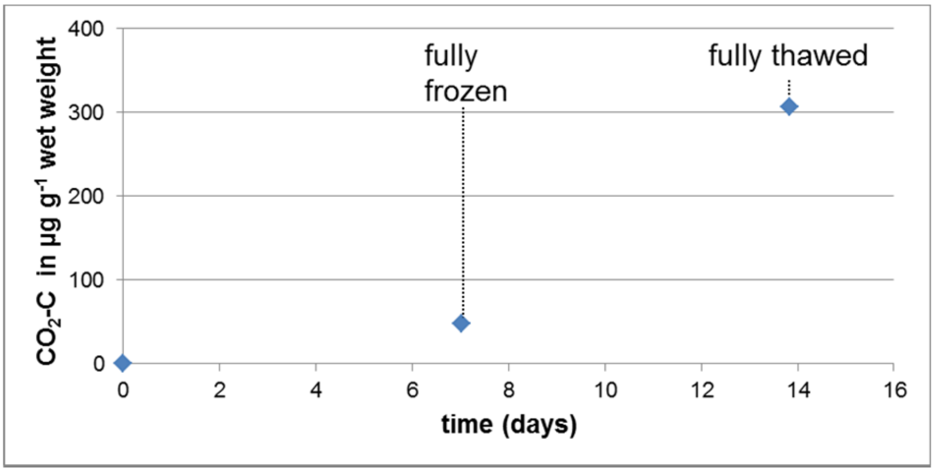

Figure 4. Cumulative CO_2 emission during second trial experiment

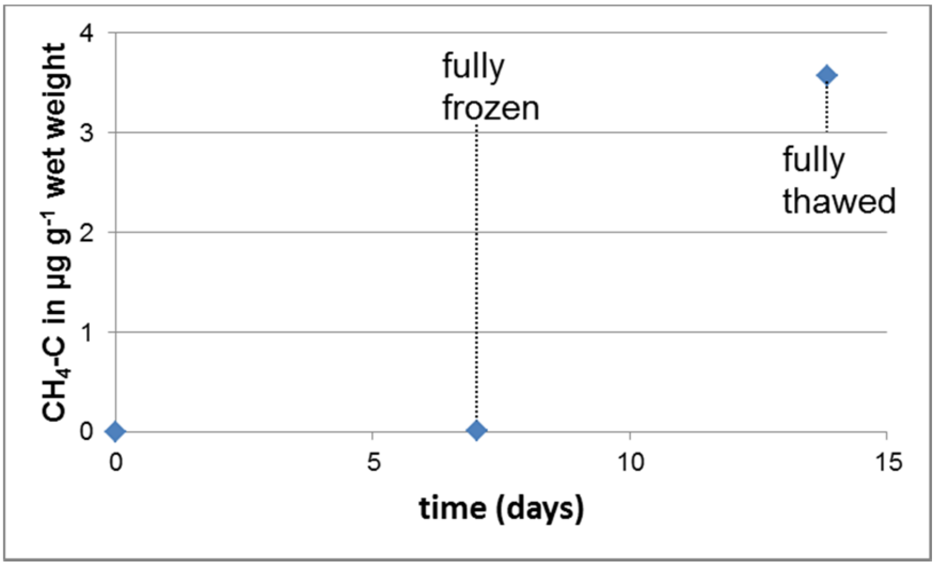

Figure 5. Cumulative CH_4 emission during second trial experiment

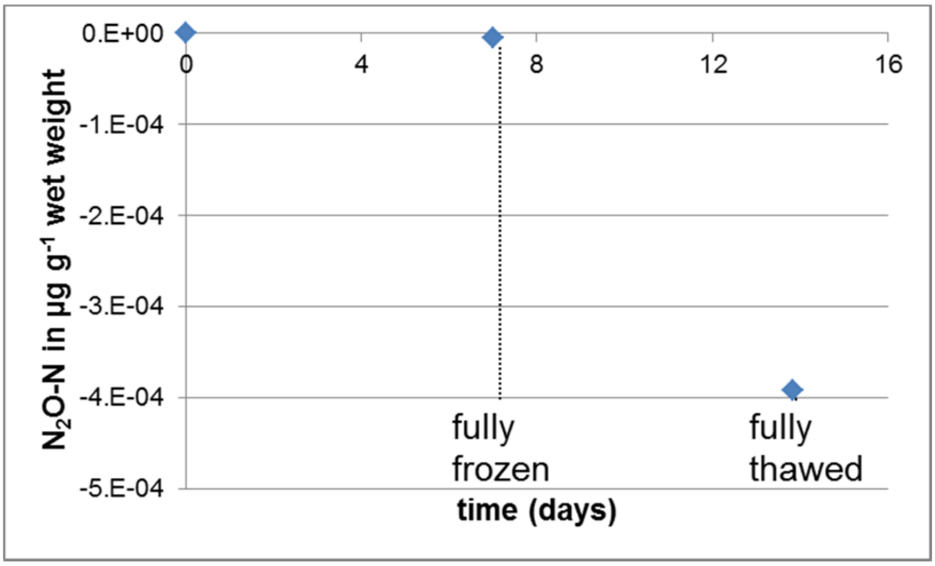

Figure 6. Cumulative N_2O emission during second trial experiment

As can be seen in the graphs the N_2O emissions were around zero, which was different from the expectations. However, the CO_2 and CH_4 emissions were high, especially the CO_2 concentration. It is likely that the high CH_4 concentration was due to the high CO_2 concentration which led to anaerobic conditions. The CO_2 concentration was about 9% at the end of the freezing period and 38% at the end of the thawing period, which means that oxygen probably was fully depleted during the thawing period. The NO_3^--N concentration of this sewage sludge was not measured but anaerobically treated sewage sludge usually contains only low concentrations of NO_3^--N, as explained in chapter 1.2. Therefore the N_2O was expected to mainly come from nitrification. However in such limited O_2 conditions, nitrification is strongly inhibited and thereby N_2O emissions due to nitrification cannot occur either. A similar pattern was observed by Smith and Patrick Jr (1983).

2.1.3 Third trial experiment: gas emissions from compost

The objectives of the third trial experiment was to get an indication of the gas emissions from compost during freezing and thawing and to test a set-up with four containers in one insulation box.

For the third trial experiment, smaller containers were used, with a volume of 1,1 liter, an inner diameter of 8,5 cm and a height of 20,5 cm.

For the containers used during the third trial experiment a leakage test was performed on four containers to test if the containers were air-tight. To these containers 200 ml of air was added. Containers 1 and 2 were incubated in the freezer of -27 °C for three days and containers 3 and 4 were left at room temperature for three days. After three days all containers were weighed again and containers 1 and 2 were left at room temperature for three more days to test the leakage during heating up again as well.

The results of the leakage test are given in Table 1.

Table 1. Weights of containers during leakage test

container	weight container without extra air	start	air weight	weight after 3 days	difference from start weight	weight after 6 days	difference from start weight
1	130,523	130,743	0,22	130,757	0,014	130,75	0,007
2	128,317	128,54	0,223	128,547	0,007	128,539	-0,001
3	127,739	127,953	0,214	127,969	0,016		
4	127,897	128,125	0,228	128,124	-0,001		

As can be seen in the table, the differences are very small every time. Since the differences are small, but above zero, the differences are probably due to measurement errors as it should not be possible to increase in weight. By putting a needle inside the container it became clear that there was still overpressure in each container, because air was coming out. Therefore the containers were considered to be air-tight and suitable for the experiments.

For the third trial experiment the containers were filled up to a height of approximately 7-8 centimeters (cm). Four containers were placed in a styrofoam box, with a thin flexible foam layer between the containers walls and the insulationFigure 1. Water bags with a weight of 120 grams were placed under each container to increase the heat capacity below the container and thus achieve the top-to-bottom freezing. Gas samples were taken from containers 1-3. At the start of the experiment one air sample was taken from all opened container as background concentrations. One gas sample was taken when the containers were taken out of the freezer, and two gas samples were taken during the thawing period. After each sampling event, the containers were opened to ventilate. New air samples from the opened containers were taken to serve as new background concentrations. Container 4 was used for temperature measurements and the assumption was that the temperatures would be the same in all containers. Three temperature sensors were used: two in the compost and one in the head space the container.

In Figure 7, Figure 8 and Figure 9, the cumulative emissions of CO_2, CH_4 and N_2O are shown in µg g^{-1} of wet weight of the compost. The standard deviations of the mean (explained in chapter 2.5) are given with black bar. The temperature graphs for this trial experiment can be found in Appendix II.

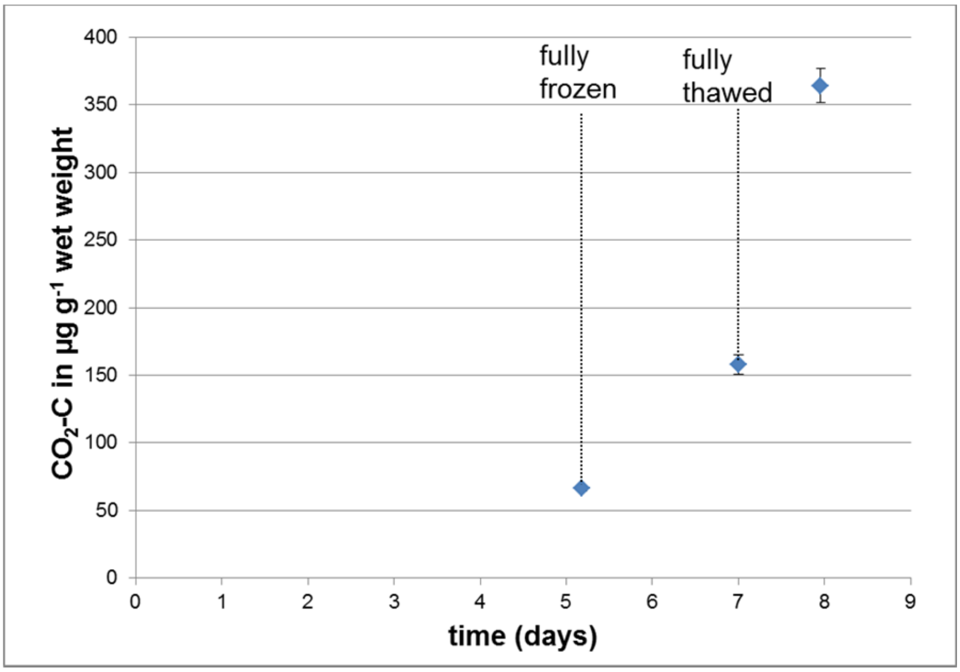

Figure 7. Cumulative CO_2 emission during third trial experiment

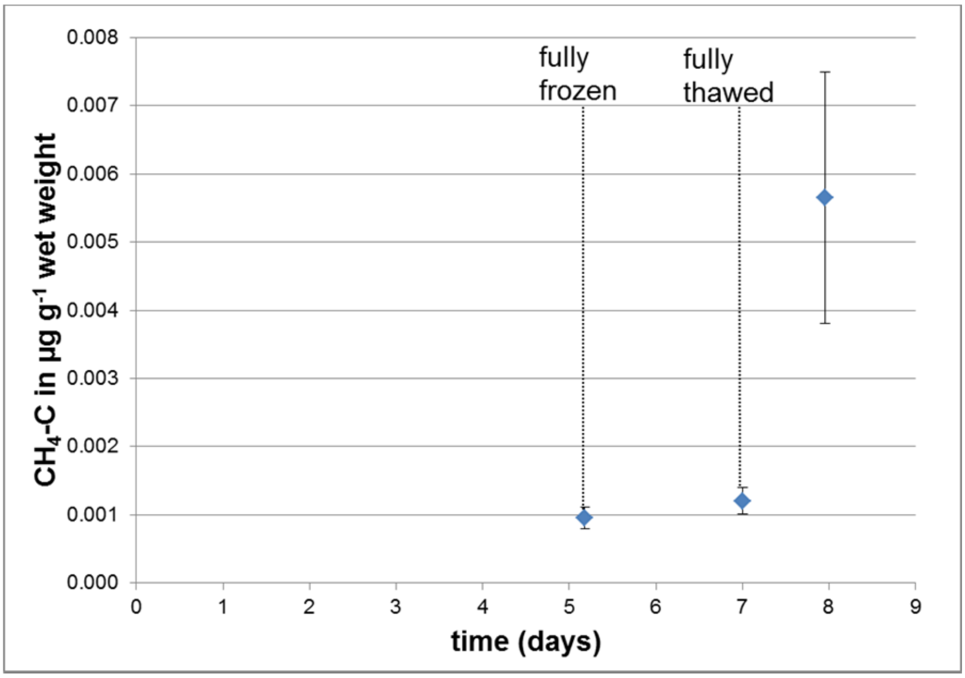

Figure 8. Cumulative CH_4 emission during third trial experiment

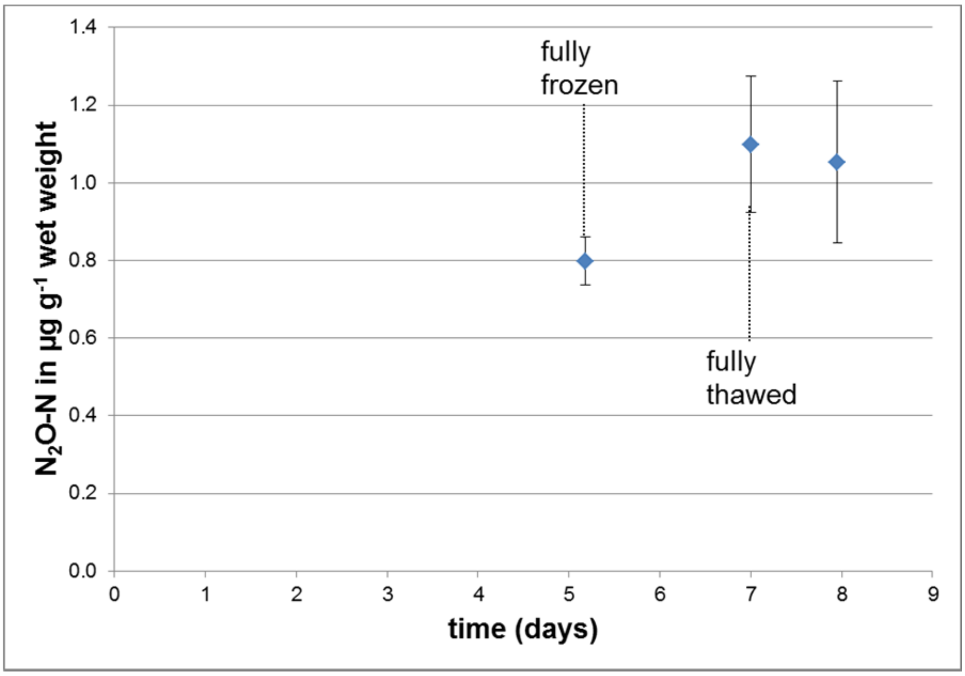

Figure 9. Cumulative N_2O emission during third trial experiment

The CO_2 emissions were again high, even higher than those from sludge during the second trial experiment. Because of ventilation every time that gas samples were taken the CO_2 concentrations were kept lower than during the second trial with the sewage sludge, but they were still very high, from about 3,5% at the end of the freezing period to about 12,5% at the end of the thawing period.

The CH_4 emissions were low compared to the second trial experiment with sewage sludge, probably due to higher oxygen concentrations and because composting is an aerobic process, so the initial concentration of anaerobic organisms was probably low. N_2O emissions were significant this time and more than 100 times higher than CH_4 emissions. For both CH_4 and N_2O the standard deviations of the mean were high.

2.2 Objective and experimental plan for the main experiment

The experimental plan for the main experiment was based on the outcomes of the trial experiments. The objectives were:

- Finding out the amount of GHG emissions under the influence of freezing and thawing from compost and sewage sludge when thawed under low temperature
- Finding out the influence of NO_3^- on the N_2O emissions
- Finding out the responsible processes (nitrification or denitrification) for the N_2O emissions

The different substrates chosen for the experiments were:

1. Non-nitrified mesophilically digested sewage sludge
2. Nitrified mesophilically digested sewage sludge
3. 2 weeks old compost
4. 1 month old compost

The 2 weeks old compost and 1 month old compost were expected to have significantly different NO_3^--N values. The two composts came from different compost piles, but from similar substrates and similar proportion according to facility standards as described by Ermolaev *et al.* (2012).

For the experiment with well-nitrified mesophilically digested sewage sludge, about 6 kg of sewage sludge was nitrified by placing the sludge in plastic boxes for 3 ½ weeks. These boxes were covered with a moist towel to prevent moisture and NH_3 loss as much as possible. The box was placed in a climate room that was kept at a constant temperature of 15 °C. Samples were taken weekly and analyzed for NO_3^--N concentration. The boxes were aerated every weekday two times for a few minutes. Towels were rewetted when they had dried out.

Before the main experiments started, samples were taken for analyzing TS, volatile solids (VS), pH, NO_3^--N and NH_4^+-N. The methods for these analyses are described in chapter 0. For the analysis of pH, NO_3^--N and NH_4^+-N on compost, only particles of smaller than ~2 cm were sampled, because it was not practical to dilute and filtrate bigger particles. Total carbon (TC), Total Nitrogen (TN) and C/N ratio were analyzed by Agrilab AB.

From the results of all the trial experiments, conclusions were drawn about what should be done different during the main experiments regarding the set-up:

- Based on the results of the first trial experiment it was decided that better insulation was necessary to make the substrates freezing from top to bottom.

- Based on the results of the second trial experiment it was decided that during the main experiment ventilation would regularly be necessary when the CO_2 concentration would become too high. Therefore it was decided to ventilate the containers every time after taking samples.
- During the third trial experiment, the CO_2 concentrations were still too high during the thawing period. It was decided to thaw at low temperature instead of room temperature. Furthermore it was decided to take samples at least once in two days during thawing and to do a quick CO_2 test after sampling. If the CO_2 would be too high then, this would be changed to sampling every day. This CO_2 test is explained in chapter 2.4.3.
- Based on the results of the second and third trial experiment, it was concluded that emissions were mainly during the thawing period, so that only 1 sample at the end of the freezing period should be enough. During the thawing period, more frequently sampling would be required.

For each substrate that was tested, four containers were placed in styrofoam boxes, in the same way as described in chapter 2.1.3. See also Figure 10Figure 1. Underneath each container, one water bag of 120 grams was placed. A foam layer of 0,5 cm thick was placed between the container wall and the styrofoam. The containers were 20,5 cm high and were filled up to 7-8 centimeters with sewage sludge or compost. In the middle of each lid, a hole with a diameter of 0,9 centimeters was made. A rubber stop was put in this hole for gas extraction. One container per box was used for temperature measurements. This time, 3 temperature sensors were placed inside the substrates, at heights of approximately 1, 4 and 7 cm from the bottom of the container. One temperature sensor was placed in the head space. In-house temperature sensors were used and measured with embedded data systems OW. For every two styrofoam boxes there was one sensor that measured the surrounding temperature. During freezing the containers were kept in a freezer at a temperature of -27 °C. During thawing they were kept in a refrigerator room with a temperature of 5 ±2 °C.

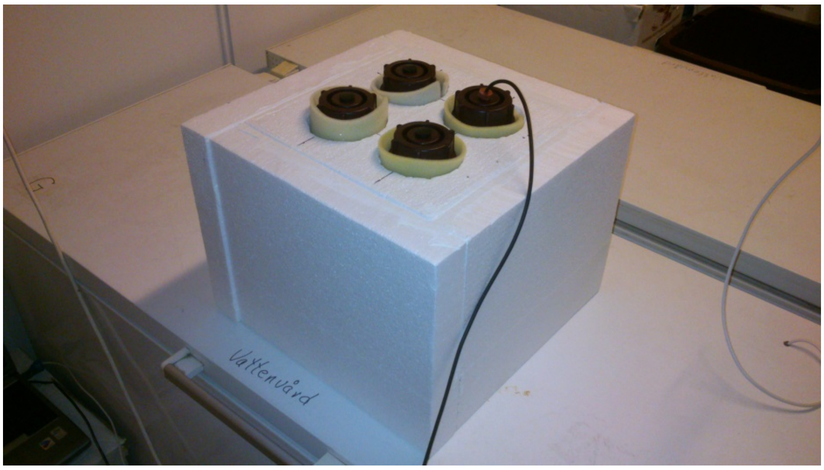

Figure 10. Set-up of the main experiments

For each substrate a control experiment was done as well. The containers with control treatments were kept in this refrigerator room from the time that the freezing started until the time that the experiments were finished. They were done in triplicate for each substrate as well. The temperatures in the containers of the controls were not measured, but assumed to be the same temperature as the temperature in the refrigerator room.

The sampling events for the frozen and thawed substrates were on the following moments:

- An air sample from the opened container at the start of the experiment.
- First gas sample at the end of the freezing period.
- Second gas samples after two days of thawing.
- After the second gas sample, every one or two days depending on the amount of CO_2.
- Last gas sample at the end of the experiment.

The sampling events for the controls were on the following moments:

- An air sample from the opened container at the start of the experiment.
- First gas sample after one day.
- After the first gas sample, every one or two days depending on the amount of CO_2.
- Last gas sample at the end of the experiment.

The procedure of the gas sampling is explained in chapter 2.4.1.

2.3 Physical and chemical analyses

2.3.1 Head space and air space measuring

The head space in the container and the air space inside the substrates were measured to calculate the total gas volume. For the second and third trial experiment the head space was simply calculated by measuring the diameter of the containers and the height of the substrates inside the container.

For the main experiments, the head space was measured at the end of the experiment: when the substrate was taken out, the height of the substrate was marked on the container. Then the container was filled up with water to this height, weighed and then filled up with water to the top of the container. Then it was weighed again. With the difference in weight the difference in water volume was calculated and this was assumed to be equal to the head space volume.

The air filled pore space in the substrate was measured by putting a weighed amount of substrate in a container and filling up the container with water until the water had reached until the top of the substrate. Then the container was weighed again; in this way the water volume per gram of substrate could be calculated. This was considered equal to the air space inside the substrate during the experiments.

The head space inside the container and air space inside the substrate together were the total air volume inside the container.

2.3.2 pH

The pH was measured by using a standard pH meter from the type PHM210, MeterLab, Radiometer, Copenhagen. At the start of the main experiments, the substrates were approximately 5 times diluted on weight base with deionized water, then left for 1 hour, and then measured. A second measurement was done 10 minutes later. After the experiments, the pH measurements were done in the same way for each container, but this time the pH was measured only once per container.

2.3.3 Nitrate and ammonium analyses

For the measuring of NO_3^--N and NH_4^+-N photometric chemical test kits of Spectroquant NOVA 60 were used, methods number 059 (NO_3^--N) and 053 (NH_4^+-N). For NO_3^--N, method number 059, which had a range of 0,5-18 mg l^{-1} NO_3^--N was used. For NH_4^+-N, methods number 053, which had a range of 5-80 mg l^{-1} NH_4^+-N, was used. The test for NO_3^--N was first tested as described in chapter 2.3.4.

The substrates were first diluted with deionized water. The dilution was chosen so that the concentration of NO_3^--N/NH_4^+-N would be in the appropriate range of the test kits. For each separate analysis a new solution from a new sample was made. The substrates were not minced when making the solution, since it was expected that mincing could influence the measured values. After dilution, the solutions were mixed for ~1 min and then filtrated. The filtrate was then analyzed in the spectrophotometer according to chosen methods.

2.3.4 Test of nitrate analysis method

The suitability of the NO_3^--N measuring method, described in chapter 2.3.3 was tested. First some sludge was nitrified for a few days and analyzed in triplicate on NO_3^--N. After these analyses two different solutions were made, consisting of 3 milliliters of diluted sludge, a known amount of NO_3^--N solution with a known concentration and some deionized water. The expected concentration in this solution was calculated and these solutions were analyzed in triplicate as well. The results were compared to the expected values. In this way it could be estimated how well the method works with sludge. If any compound in the sludge would be interfering with the measurement, the measured values in the solutions with both sludge and known amounts of NO_3^--N solution should differ significantly from the calculated values.

In Table 2 the measured values in the sludge and the standard deviation of the mean are given.

Table 2. Measured values in sewage sludge

	Measured concentration
Test 1	71,5
Test 2	69,0
Test 3	73,7
Average	71,4 ± 1,35

In Table 3 the compositions of the two solutions with added NO_3^--N are given.

Table 3. Solutions with added nitrate

	Solution 1	Solution 2
10 times diluted sludge amount (ml)	3	3
NO_3^- solution added (ml)	0,3 ml	0,4
Deionized water added	5	4
Final calculated NO_3^--N concentration (mg l^{-1})	10,7	15,1

In Table 4 the results of the test for the NO_3^--N analysis method and the standard deviations of the mean are shown.

Table 4. Results of test for nitrate analysis method

	Solution 1	Solution 2
Calculated concentration (mg l-1)	10,7	15,1
Measured concentration (mg l-1) (n=3)	10,2 ± 0,0057	14,9 ± 0
Difference (%)	-4,67	-1,32

In both solutions the standard deviations of the mean were small which shows a high accuracy of the measurement. Furthermore the differences between the calculated and actually measured concentrations were small enough to be considered as measurement errors so the NO_3^--N test was assumed to work properly for sewage sludge.

2.3.5 Total solids/volatile solids analyses

The procedure for the TS/VS analyses was as described in Eklind *et al.* (2007). For all TS analyses, about15-20 grams of material were taken and put in small aluminum foil cups. The empty cups were weighed with an analytical scale. After filling up each cup with the substrate it was weighed again. Then the cups were placed in the oven at 105 °C for 18 hours for the sewage sludge and 14 hours for the compost. For sewage sludge 18 hours period was required to reach a stable dried weight. After this the samples were placed in a desiccator to cool down to room temperature, and then weighed.

For the VS analyses, the same dry samples were placed in the oven at 550 °C for 4 hours. Then again they were taken out, placed in a desiccator to cool down to room temperature, and weighed. Before the experiments started the TS/VS analyses were done in quadruplicate for each treatment. After the experiments one TS/VS analysis was done for each container, except the containers used for temperature measurements.

2.4 Gas sampling and analyses

2.4.1 Gas sampling

At the start of the experiments gas samples were taken from inside the opened containers as background concentrations.

When a gas sample was taken, usually 50 ml of N_2 was first flushed into the container to prevent under pressure. Only for the samples taken at the end of the freezing period, 60 ml of N_2 was flushed in as more under pressure was expected due to low temperatures. The N_2 bottles were kept at 5 ± 2 °C to prevent temperature changes during the sampling procedure. After adding the N_2 50 ml of sample was extracted. A 60 ml plastic syringe was used to flush in N_2 and to extract the gas sample.

The gas samples were transferred to 22 ml glass vials initially filled with N_2 and covered with a rubber septum. When transferring the sample into the vial, another needle was put through the rubber septum so that the N_2 in the vial was flushed out by the gas sample. 2 ml overpressure of gas the gas sample was put into the vial in order to prevent surrounding air leaking into the vial. If the vials were stored longer than 2 weeks before analyzing them, they were stored in water to prevent leaking.

The containers were ventilated after sampling, and air samples from the open containers were taken as background concentrations for the next sampling event.

After each sampling event during the thawing period and after each sampling event for the controls, a quick CO_2 test was done, described in chapter 2.4.3. The goal of this test was to decide what frequency of gas sampling would be necessary: the aim was to keep the CO_2 concentration below 6%. However, as an extra safety margin it was decided that there should be sampled every day if the CO_2 concentration was above 4%.

After each sampling event, the containers were opened to ventilate. After ventilation, a new gas sample from the opened container was taken to analyze the background concentrations. In the beginning a separate sample was taken from each opened container, but after 6 days, it was decided that 1 sample per substrate per treatment was enough to save time.

2.4.2 Gas Chromatography

The GC used for analyses on CO_2 and CH_4 was a Clarus 500 system provided with a flame ionization detector (FID) and a thermal conductivity detector (TCD). The GC used for analyses on N_2O and CH_4 was a Clarus 500 Perkin Elmer gas chromatograph system provided with a FID and electrical conductivity detector (ECD). The gas concentrations of CO_2, CH_4 and N_2O in the standards that were used when running the GC's were based on the expected concentrations in the samples.

2.4.3 Quick CO_2 test

The quick CO_2 test was performed by adding 5 ml of headspace gas in a special tube filled with 7M sodium hydroxide (NaOH). No dilution with N_2 was applied when extracting this sample. A 5 ml plastic syringe was used, and directly after extracting the gas, the needle was changed by a bent needle which was more suitable for flushing the gas inside the tube. As the gas bubbled through the solution all CO_2 was dissolved, so by measuring the volume of the gas phase inside the tube the amount of CO_2 was easily determined (Schnürer and Jarvis 2010).

2.4.4 Gas calculations

The gas emissions were for CO_2 and CH_4 recalculated to the amount of µg C per g of initial organic C in the substrate and for N_2O to µg N per g of initial N in the substrate. When the measured concentrations in the gas were below zero, they were replaced by zero, as a gas concentration cannot be negative. CH_4 was analyzed in both GC systems and the average of the outcome of the two GC's was used.

For the calculation from ppm in the total air volume to grams, equations 1-6 were used:

$$P * V = n * R * T \qquad (1)$$

where:
P=pressure (bar)
V=volume (liter)
n=moles of gas (moles)
R=gas constant; 0,0821 (liter*bar mol^{-1} $Kelvin^{-1}$)
T=temperature (Kelvin)
The gas density is given by:

$$d = \frac{m}{V} \qquad (2)$$

where:
d=density (grams/liter)
m=mass (grams)

and n is given by:

$$n = \frac{m}{MW} \qquad (3)$$

where:
MW=molecular weight (grams/mole)

This gives as a final formula for the gas density:

$$d = \frac{P * MW}{R * T} \qquad (4)$$

For the pressure the normal atmospheric pressure of 1,013 bars was assumed. Due to the low temperature and the gas emissions inside the containers, this was probably not the real pressure inside, but the pressure was not measured inside the containers. For the temperature of the substrates that were frozen and thawed, the measured temperature at the time that a gas sample was taken was used in the formula. For the controls, a temperature of 278 Kelvin (K), corresponding to 5 °C, which was the average temperature of the refrigerator room, was used.

For the calculation from the concentration in ppm in the container to the emission in µg C or µg N per g of initial C or N, respectively, first the background concentration of the last air sample was subtracted.

Then the following equations were used:

$$CO_2 - C\ in\ \mu g\ g^{-1}\ C = \frac{MW_C}{MW_{CO_2}} * \frac{p{-}ppm * d * V_{air}}{g{-}TC} \qquad (5)$$

Or:

$$N_2O - N\ in\ \mu g\ g^{-1}\ N = \frac{MW_N}{MW_{N_2O}} * \frac{p{-}ppm * d * V_{air}}{g{-}TN} \qquad (6)$$

where:
p-ppm= production in ppm in container between the previous and current sampling event
d=density
V_{air}=total air volume inside the container (see chapter 2.3.1 for the calculation of this air volume
MW_C=molecular weight of C
MW_{CO2}=molecular weight of CO_2
MW_N=molecular weight of N
MW_{N2O}=molecular weight of N_2O
g-TC=total carbon present in the container (grams)
g-TN=total nitrogen present in the container (grams)

The air volume inside the container included both the head space and the air space inside the substrate. So the concentration was assumed to be the same in the air pores inside the substrates as in the head space. In reality, there is probably a difference. However, for practical reasons this possible difference was neglected.

2.5 Calculation standard deviation of the mean

For all experiments and measurements which were repeated the standard deviation of the mean was calculated to know the variation within the repetitions. The following equations were used for calculating this value:

$$\sigma_{mean} = \frac{\sigma}{\sqrt{N}} \qquad (7)$$

where:
σ_{mean}=standard deviation of the mean
σ=standard deviation
N=number of repetitions

The standard deviation was calculated by:

$$\sigma = \sqrt{\frac{\sum(x-\bar{x})^2}{(N-1)}} \qquad (8)$$

where:
x=value
$\bar{x}$=average value

3. Results and discussion

In this chapter the material properties and the results of the main experiments are given and discussed. For all analyzed gasses graphs are shown. In the graph it is shown at which point the substrate was fully frozen, and from which point it had fully thawed, which means, temperature above 0 °C and increasing. Also the increases/decreases in NO_3^--N and NH_4^+-N are shown. Standard deviations of the mean are shown in all graphs with black error bars. In most of the results the standard deviations were relatively high when the emissions were close to zero. This is because very low concentrations were lower than the lowest standards used in the GC, and thus outside the used range for the standards. This makes the results less accurate. Standard deviations were also most of the times higher in the controls than in the frozen and thawed substrates. This can be explained by the lack of activity during the frozen period. The longer time of activity in the control containers will make the variation between the triplicate containers bigger.

Although the quick CO_2 test described in chapter 2.4.2 was used in every sampling even for the controls and during the thawing period, the CO_2 concentration was often higher than 6%. However, no effects of high CO_2 concentrations on the CH_4 concentrations or on other results were observed, so it is assumed that the CO_2 concentrations did not lead to anaerobic conditions.

In all experiments the emissions of CH_4 were very low. This can be explained by the aerobic conditions and the low temperatures, as methanogens usually grow at higher temperatures.

3.1 Material properties

The properties of the substrates are described in Table 5. The numbers of measurements for TS, VS, pH, NO_3^--N and NH_4^+-N are given, as well as the standard deviations of the mean if applicable.

Table 5. Parameters of used substrates

	Non-nitrified mesophilically digested sewage sludge	Nitrified mesophilically digested sewage sludge	2 weeks compost	1 month compost
TS (%) (n=4)	0,202 ± 0,000639	0,201 ± 0.00115	0,434 ± 0.00299	0,425 ± 0,0149
VS (g g^{-1} TS) (n=4)	0,668 ± 0,000478	0,656 ± 0,000855*	0,480 ± 0,0247	0,425 ± 0.0315
pH (n=2)	7,82 ± 0	5,91 ± 0,090	8,36 ± 0,010	8,69 ± 0050
TC (g g^{-1} TS)	0,352	0,336	0,330	0,272
TN (mg g^{-1} TS)	46,5	52,5	7,98	9,32
C/N ratio	6,71	7,22	41,3	29,2
NO_3^--N (mg kg^{-1} TS) (n=3)	42,6 ± 5,33	182 ± 3,31	41,1 ± 1,39	45,7 ± 3,43
NH_4^+-N (mg kg^{-1} TS) (n=3)	7196 ± 227	2265 ± 8,74	517 ± 20,1	1160 ± 16,8

*only in triplicate, because some material was accidentally spilled just before weighing

3.2 Non-nitrified mesophilically digested sewage sludge

The carbon dioxide emission was very low during the freezing period, but increased during the thawing period, see Figure 11.

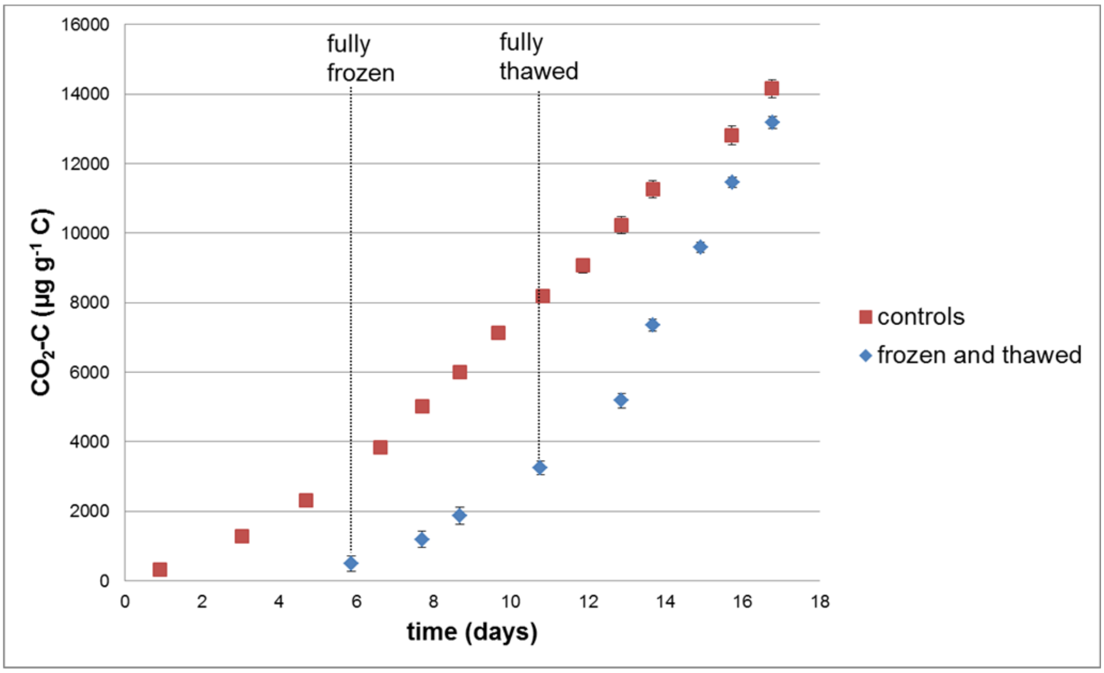

Figure 11. Cumulative CO_2-C emission in µg g^{-1} of initial C from the non-nitrified mesophilically digested sewage sludge

From the moment that the sewage sludge had fully thawed the emissions increased faster. However at the end of the experiment the accumulative emissions were almost the same for the frozen and thawed sewage sludge and the controls. During the last days of the thawing period the emissions increased fast, but the experiment should have continued longer to see if the cumulative emission of the frozen and thawed sludge would exceed the cumulative emission of the controls.

Compared to the CO_2 emissions, the CH_4 emissions are low; at the end of the experiment, the CO_2-C/CH_4-C ratio was 956 for the frozen and thawed sewage sludge, and 329 for the controls, which shows that only a small fraction of the degradation was anaerobic, see Figure 12.

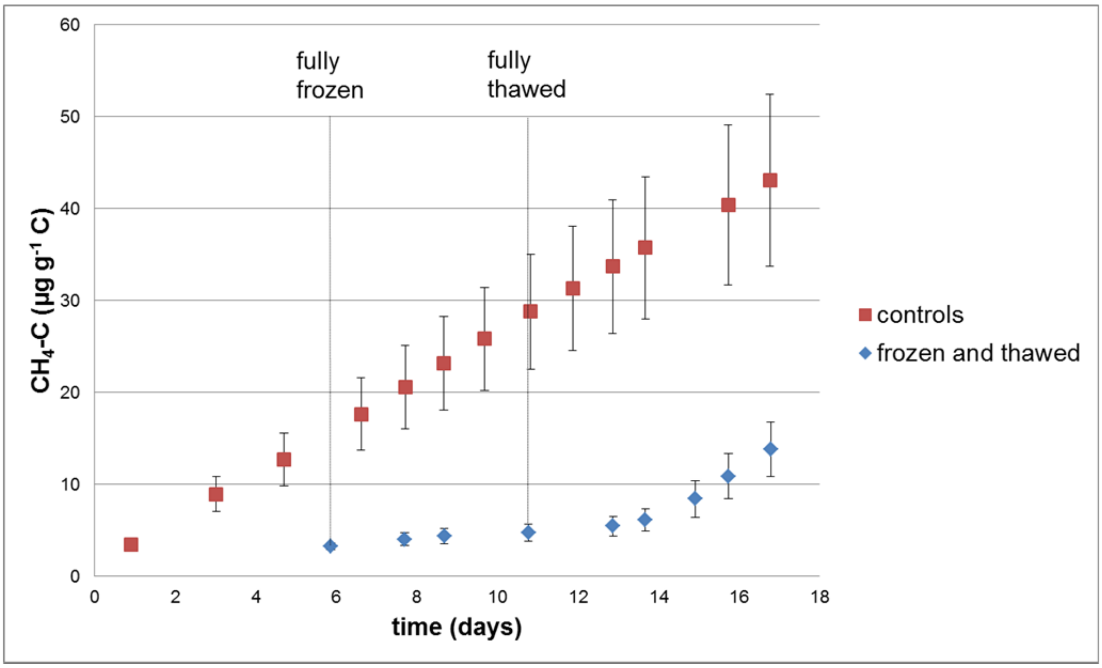

Figure 12. Cumulative CH_4-C emission in µg g^{-1} of initial C from the non-nitrified mesophilically digested sewage sludge

The CH_4 emission was low during freezing, but still higher than during the first few days of the thawing period; during the first days of the thawing period it was almost zero. The emission during the freezing period can be explained by dissolved gas being forced out of the water during freezing as described in chapter 1.4. During the last days of thawing the cumulative emission in the sludge that had been frozen and thawed increased at approximately the same rate as the controls, which indicates that on a long term the freezing and thawing does not have any clear effect. However in the controls, the standard deviations are very high which makes the evaluation of the effect of freezing less reliable.

The N_2O emissions were very low. In the controls, N_2O consumption was measured most of the time. In the sewage sludge that had frozen and thawed some N_2O was emitted during freezing, see Figure 13.

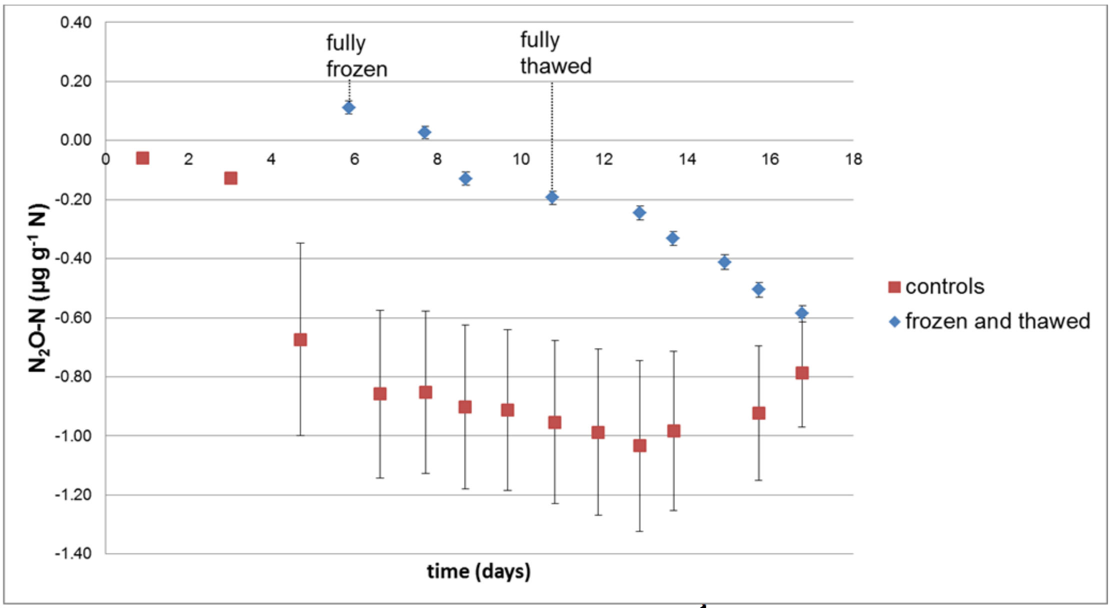

Figure 13. Cumulative N_2O-N emission in µg g^{-1} of initial N from the non-nitrified mesophilically digested sewage sludge

The emission of N_2O during freezing could be explained by better conditions for denitrification, as explained in chapter 1.4, and by the N_2O being forced out of the liquid during freezing. During thawing, some N_2O consumption took place all of the time.

The NO_3^--N and NH_4^+-N values, shown in Figure 14 and Figure 15, were not very different at the start and end of the experiment.

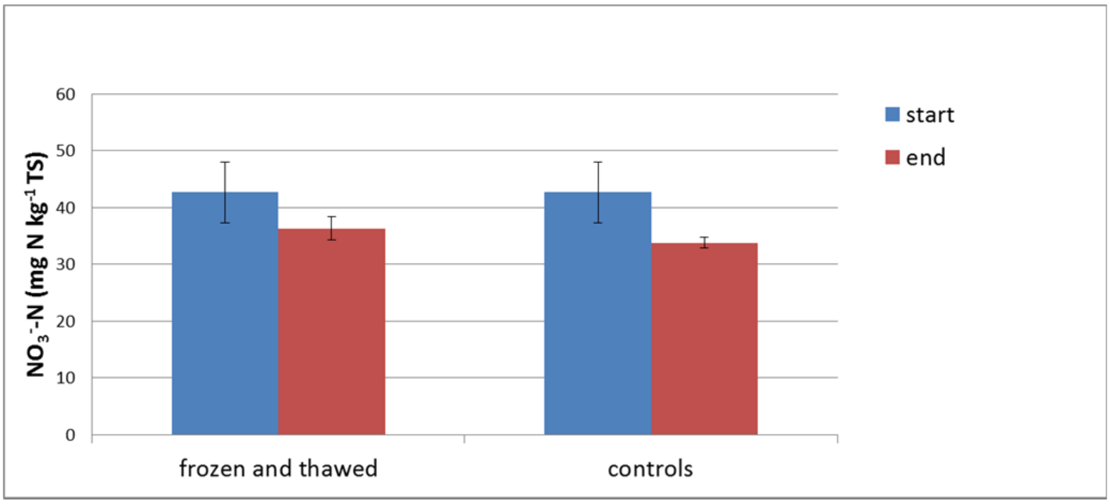

Figure 14. NO_3^--N in non-nitrified mesophilically digested sewage sludge at the start and end in mg kg^{-1} of initial TS

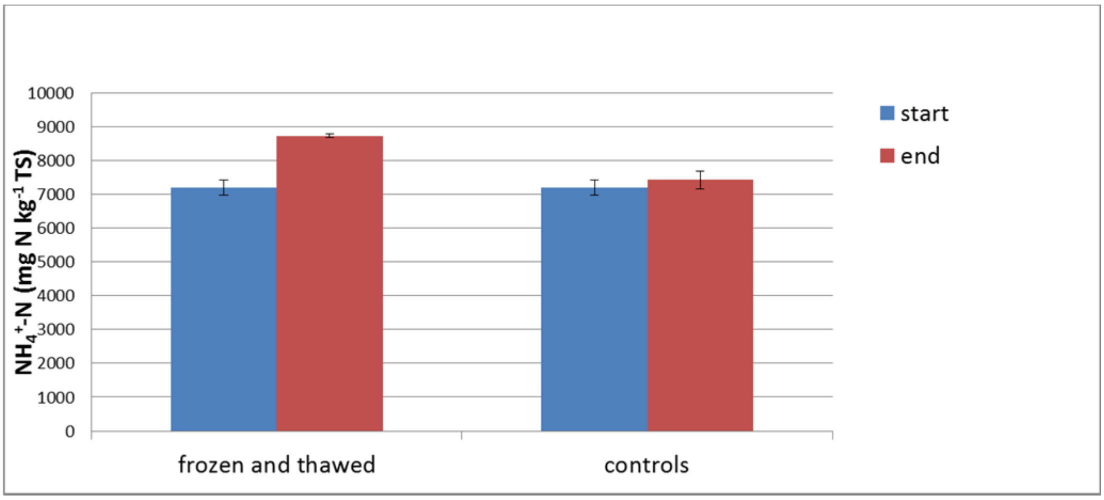

Figure 15. NH_4^+-N in non-nitrified mesophilically digested sewage sludge at the start and end in mg kg^{-1} of initial TS

The NO_3^--N amount decreased during the experiment according to the average values, but taken into account the standard deviations of the mean it cannot be said for sure that there was a difference. This is the same as for the NH_4^+-N in the controls. In the frozen and thawed sewage sludge, the NH_4^+-N concentration increased a bit. This can be explained by mineralization of substrate coming from die-off of bacteria.

3.3 Nitrified mesophilically digested sewage sludge

The cumulative CO_2 emissions from nitrified mesophilically digested sewage sludge increased sharply from the moment that the sewage sludge had fully thawed and continued increasing even above the emission observed in the controls, see Figure 16.

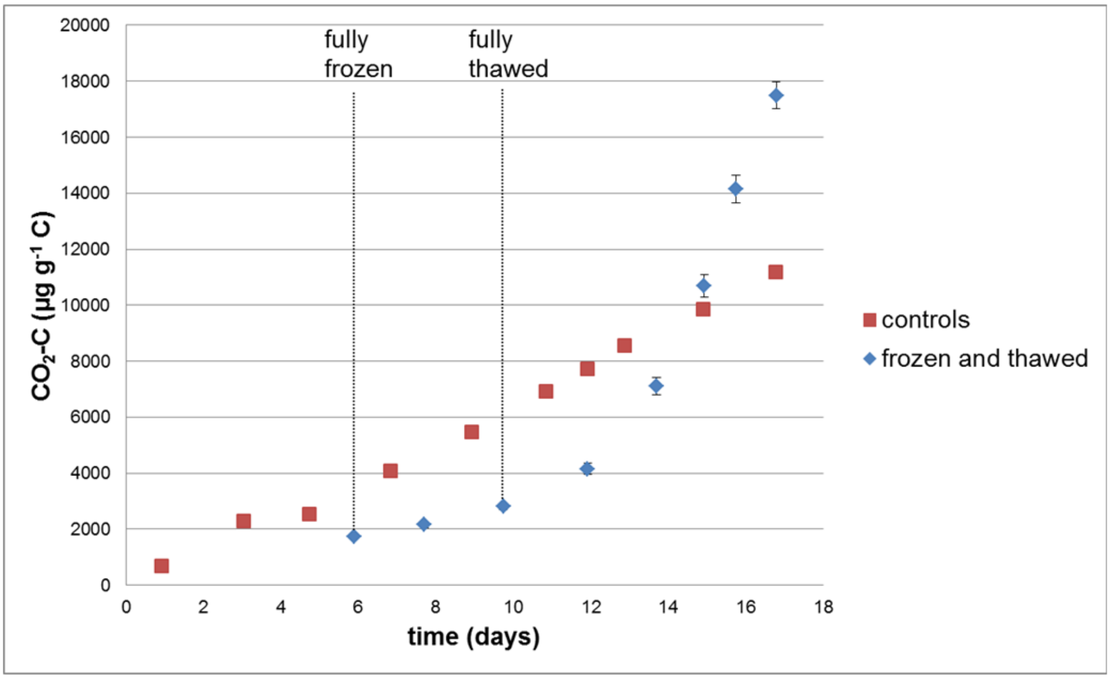

Figure 16. Cumulative CO_2-C emission in µg g^{-1} of initial C from the nitrified mesophilically digested sewage sludge

Such a sharp increase in CO_2 production is most likely related to the die-off of micro-organisms and increased substrate availability as described in chapter 1.4. A similar effect was also observed for the non-nitrified sewage sludge, but it was more prominent for nitrified sewage sludge. This might be explained by the biomass accumulation during nitrification incubation and then rapid die-off during freezing.

The methane emissions, shown in Figure 17, were almost zero in both the frozen and thawed sewage sludge and the controls. This can be explained by the low temperatures and aerobic conditions during the experiment. Furthermore, since the sewage sludge had also been under aerobic conditions during the nitrification process before the experiment started there were probably hardly any methanogens present.

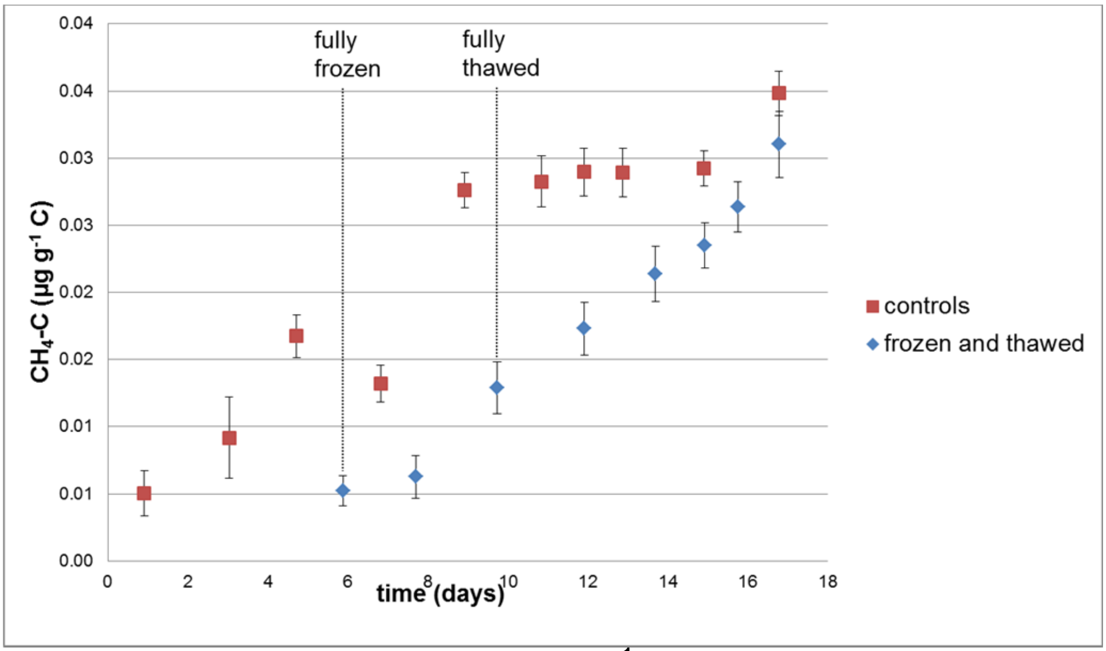

Figure 17. Cumulative CH_4-C emission in µg g^{-1} of initial C from the nitrified mesophilically digested sewage sludge

There was a clear effect of NO_3^- on the N_2O emissions. The N_2O were much higher in this nitrified sewage sludge than in the non-nitrified mesophilically digested sewage sludge for both the frozen and thawed sewage sludge and the controls, see Figure 18.

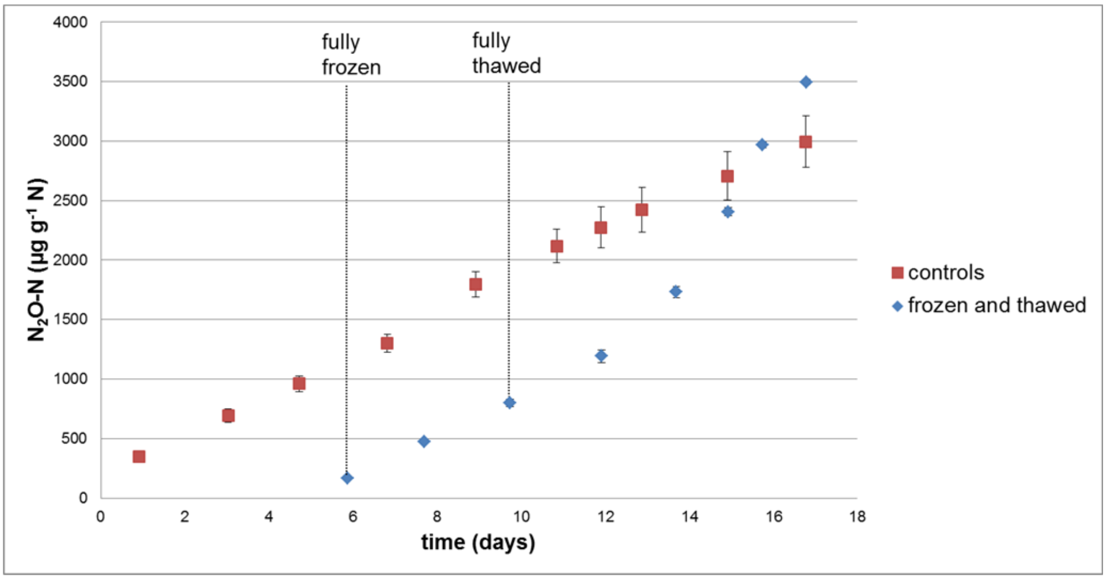

Figure 18. Cumulative N_2O-N emission in µg g^{-1} of initial N from the nitrified mesophilically digested sewage sludge

The cumulative emissions at the end were 3000-3500 µg g^{-1} N, equal to 0,3-0,35% of the initial TN. The average cumulative emission from the frozen and thawed sewage sludge was even higher than for the controls, but the difference is small, especially when taking into account the standard deviations of the mean of the controls. However, for the frozen and thawed sewage sludge it should be remarked that for the last four points in the graph the N_2O concentrations in the gas samples in all of the triplicates were so high that they were above the detection range for the GC, which means that the points shown in the graph are minimum values and they should in fact be even higher. The same was the case for the sample of one of the three triplicates on day 12, and for the sample of one of the three controls taken on day 11. In Appendix I, the raw data of the gas measurements in ppm in which N_2O was not fully detected are shown.

Both the NH_4^+-N and the NO_3^--N increased during the experiment, see Figure 19 and Figure 20.

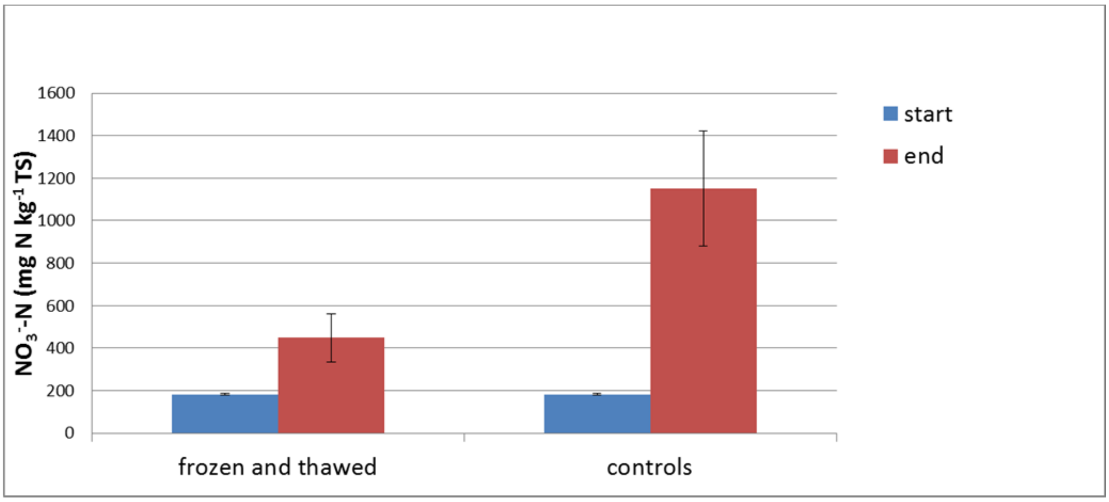

Figure 19. NO_3^--N in nitrified mesophilically digested sewage sludge at the start and end in mg kg^{-1} of initial TS

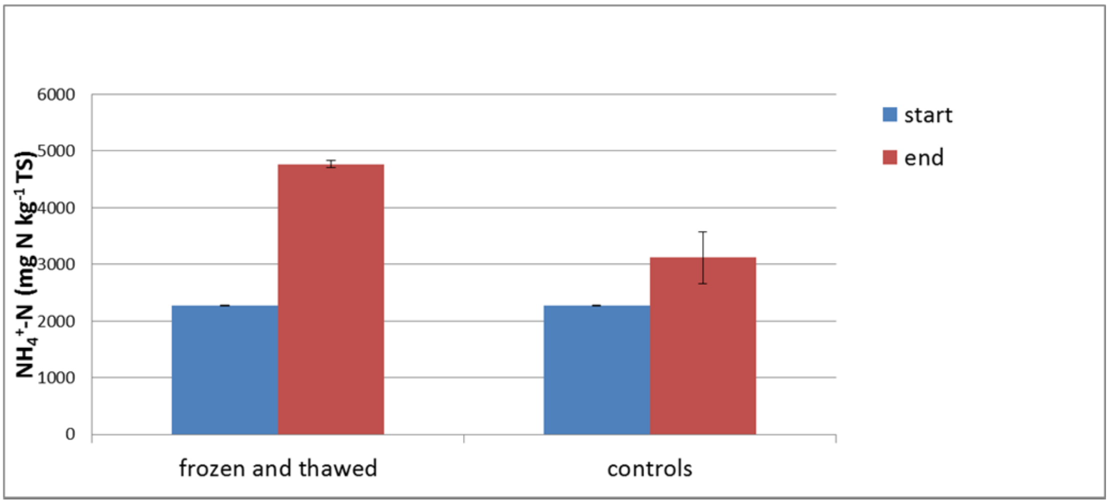

Figure 20. NH_4^+-N in nitrified mesophilically digested sewage sludge at the start and end in mg kg^{-1} of initial TS

NO_3^--N increased for both the frozen and thawed sewage sludge and the controls, although the increase was much higher in the controls. This can be explained by the high amount of nitrifying bacteria grown during the nitrification of the sewage sludge before the experiments started. The NH_4^+-N concentration clearly increased for the frozen and thawed sludge, which was not the case for the controls. This is probably due to increased mineralization, which is supported by the higher CO_2 emissions from the frozen and thawed sewage sludge.

The results for NO_3^--N and NH_4^--N make it hard to estimate whether the N_2O comes from nitrification or denitrification. However, since NO_3^- has been proven to increase N_2O emissions during denitrification, it is most likely that denitrification, or parallel nitrification and denitrification are responsible. This is also supported by the lower NO_3^--N values, and higher CO_2 emissions from the frozen and thawed sewage sludge, which indicates that more denitrification took place here, causing higher N_2O emissions.

3.4 2 weeks compost

During the first sampling event with the controls, in 1 of the containers the sampling failed due to a broken needle. For these missing values the average values of the other two triplicates were used.

The CO_2 emission was low during freezing, but quickly increased after the thawing had ended and was then about of the same rate as in the controls, see Figure 21.

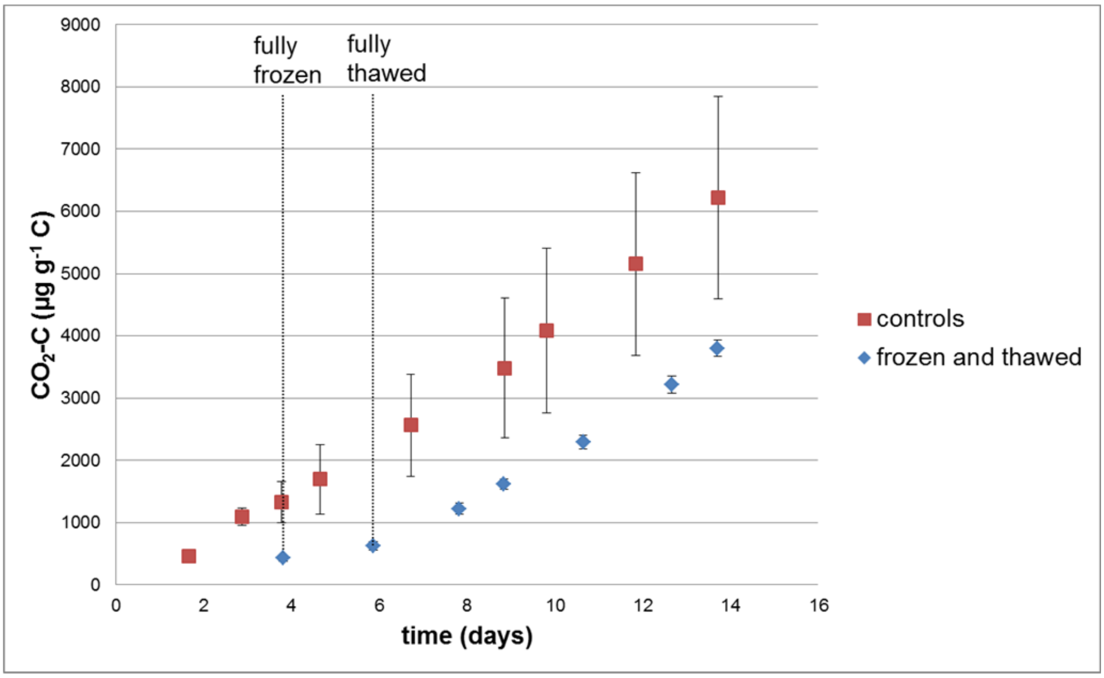

Figure 21. Cumulative CO_2-C emission in µg g^{-1} of initial C from the 2 weeks compost

The high standard deviation of the mean in the controls, which is about 26% of the average at the last point, makes it hard to draw a reliable conclusion. This was not just due to one ‘outsider’ in the triplicates; the CO_2 emissions in were all three very different from each other. This might be explained by independent development of microorganisms in different containers of controls maintaining different levels of activity between them.

The CH_4 emissions were almost zero in both the frozen and thawed compost and in the controls, which shows that there were almost no methanogens present see Figure 22.

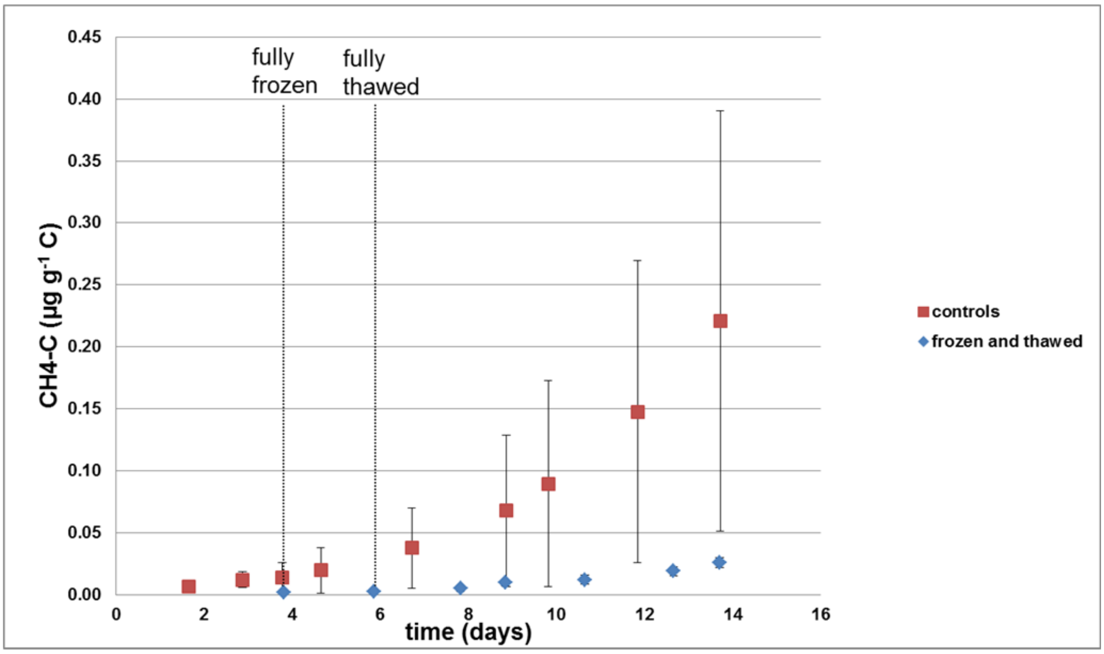

Figure 22. Cumulative CH_4-C emission in µg g^{-1} of initial C from the 2 weeks compost

The low amount of methanogens is logical, because composting is an aerobic process and the temperatures were low.

The N_2O emissions show a clear effect of the freezing and thawing: while in the controls almost no emission of N_2O and even some consumption during the last days occurred., a clear N_2O emission peak during the thawing period can be observed, see Figure 23.

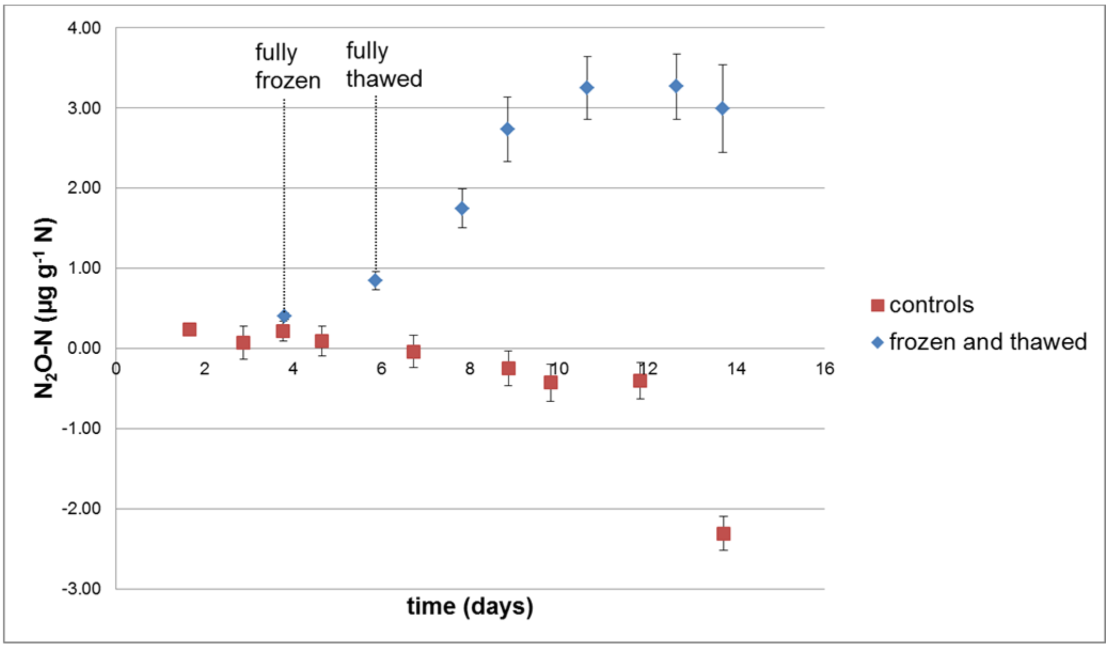

Figure 23. Cumulative N_2O-N emission in µg g^{-1} of initial N from the 2 weeks compost

The N_2O emission from the frozen and thawed compost stayed for a few days after the compost had fully thawed. From day 11, there was a small amount of N_2O consumption as in the controls.

Figure 24 and Figure 25 suggest that the nitrous oxide emissions come from denitrification, since a large decrease in NO_3^--N and hardly any difference in NH_4^+-N can be observed.

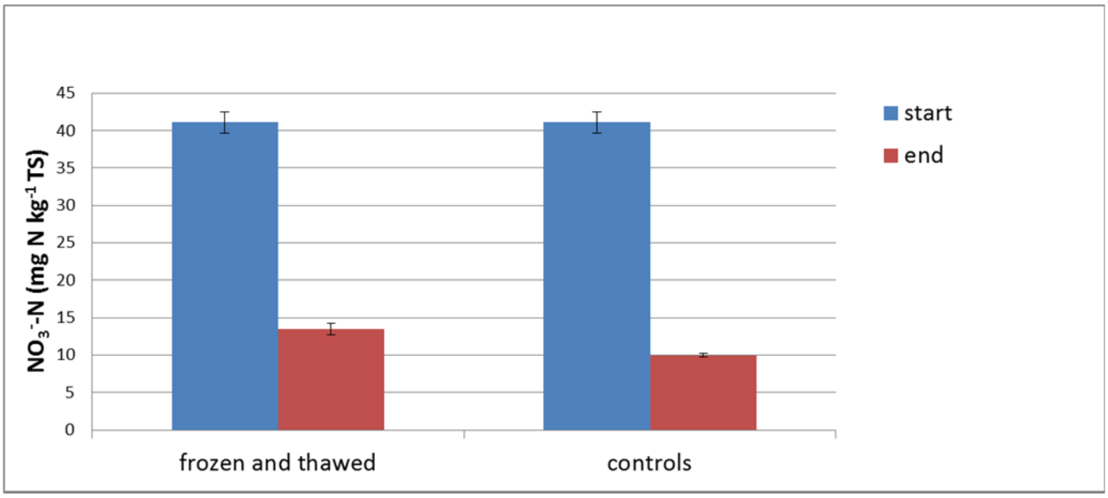

Figure 24. NO_3^--N in 2 weeks compost at the start and end in mg kg^{-1} of initial TS

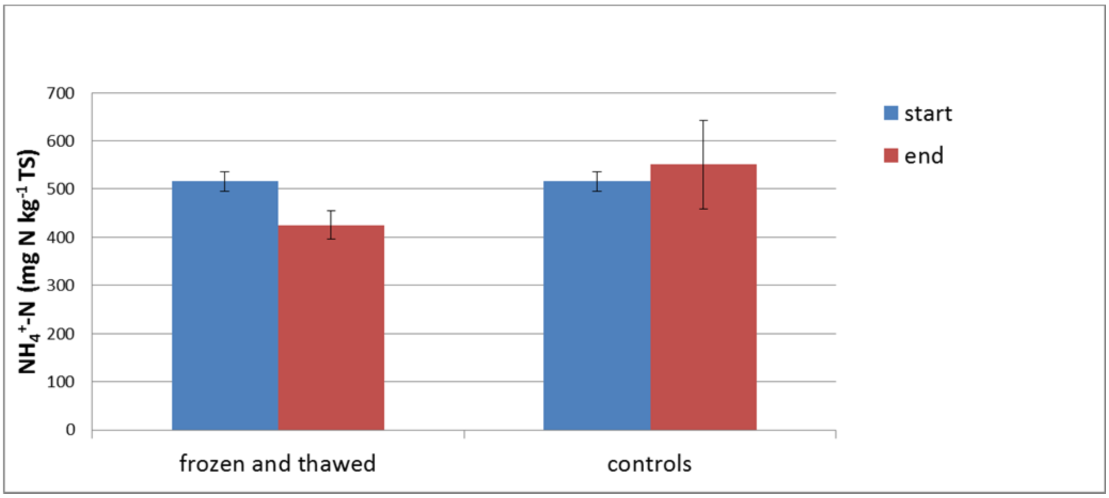

Figure 25. NH_4^+-N in 2 weeks compost at the start and end in mg kg^{-1} of initial TS

The relatively large amount of denitrification is surprising, since composting is an aerobic process and also the conditions during the experiment were aerobic. So, it would be expected that nitrification, and not a lot of denitrification would take place. This would result in higher NO_3^--N and lower NH_4^+-N at the end. However, as is stated in chapter 1.3**Error! Reference source not found.** there can be anaerobic parts in compost which could be responsible for the denitrification. The cause for the higher emissions of N_2O from the 2 weeks compost is unclear: it could be explained by higher substrate availability, but this is not supported by the CO_2 emissions. However, since the numbers were so small it is possible that this was why such an effect was not observed.

3.5 1 month compost

The CO_2 emissions from the frozen and thawed 1 month compost differed from the controls during the freezing period, but not during the thawing period, see Figure 26.

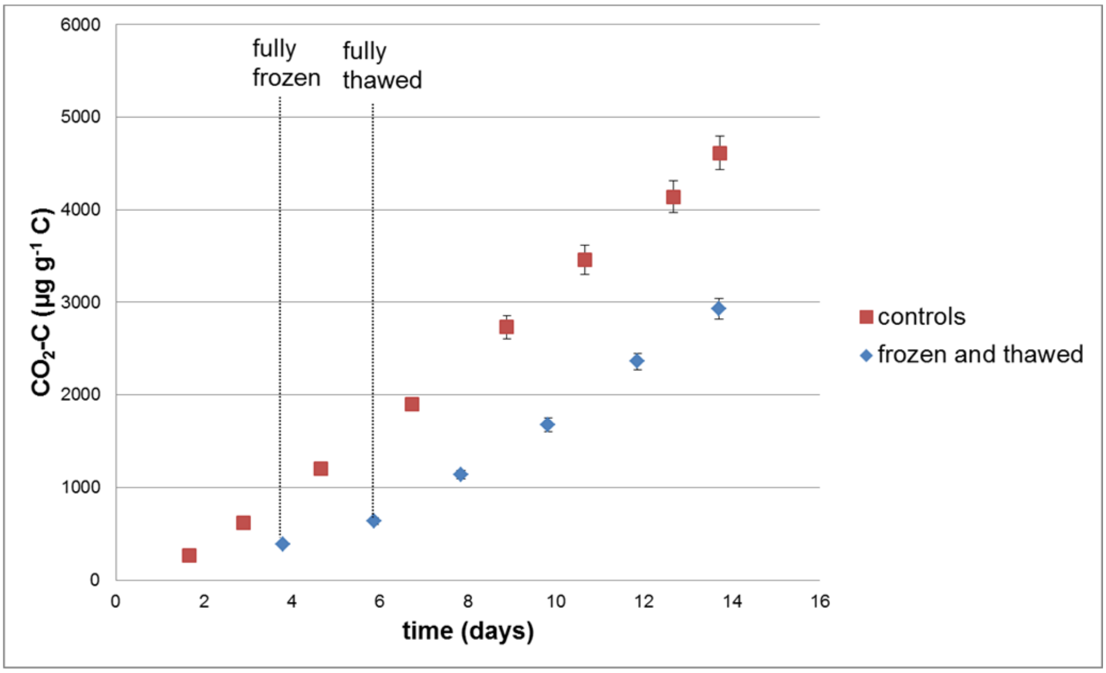

Figure 26. Cumulative CO_2-C emission in µg g^{-1} of initial C from the 1 month compost

The graph shows the same pattern as for the CO_2 emission from 2 weeks old compost, although the emissions were a bit lower. During the thawing period, the rate of CO_2 emissions from the frozen and thawed compost were similar as in the controls.

As was also the case in the 2 weeks compost, the CH_4 emissions were close to zero in both the frozen and thawed compost and in the controls, which shows that there were almost no methanogens present, see Figure 27.

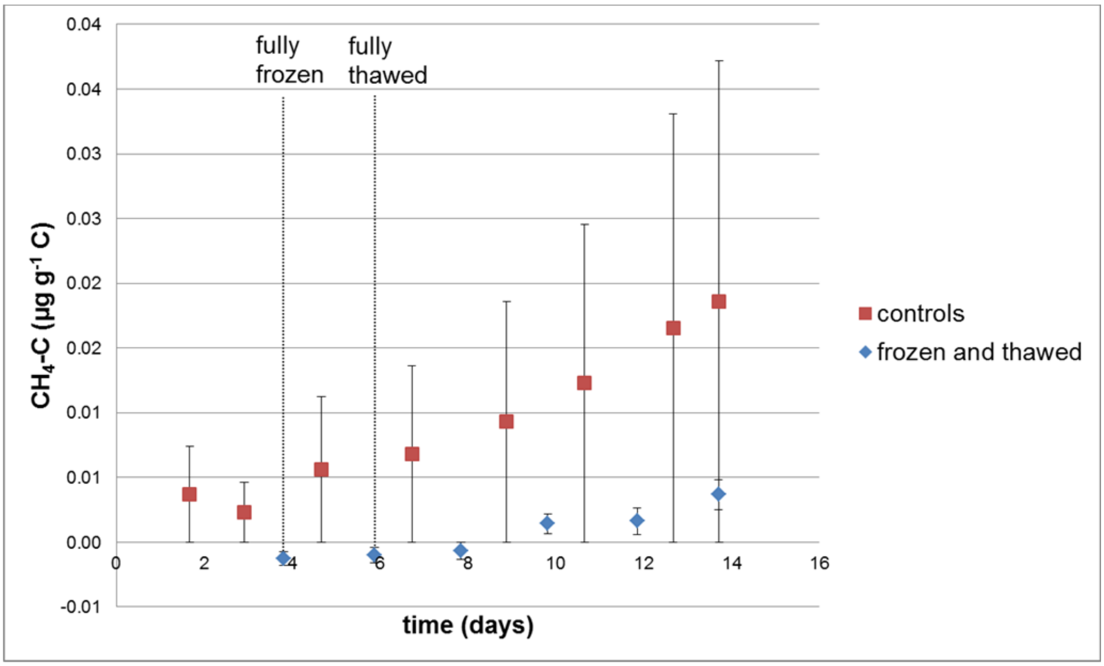

Figure 27. Cumulative CH_4-C emission in µg g^{-1} of initial C from the 1 month compost

The low amount of methanogens can be explained by aerobic conditions and low temperatures. Furthermore composting is an aerobic process.

The N_2O emissions were much higher than for the 2 weeks compost, see Figure 28. This is interesting, because the NO_3^--N amount did not differ a lot between these composts.

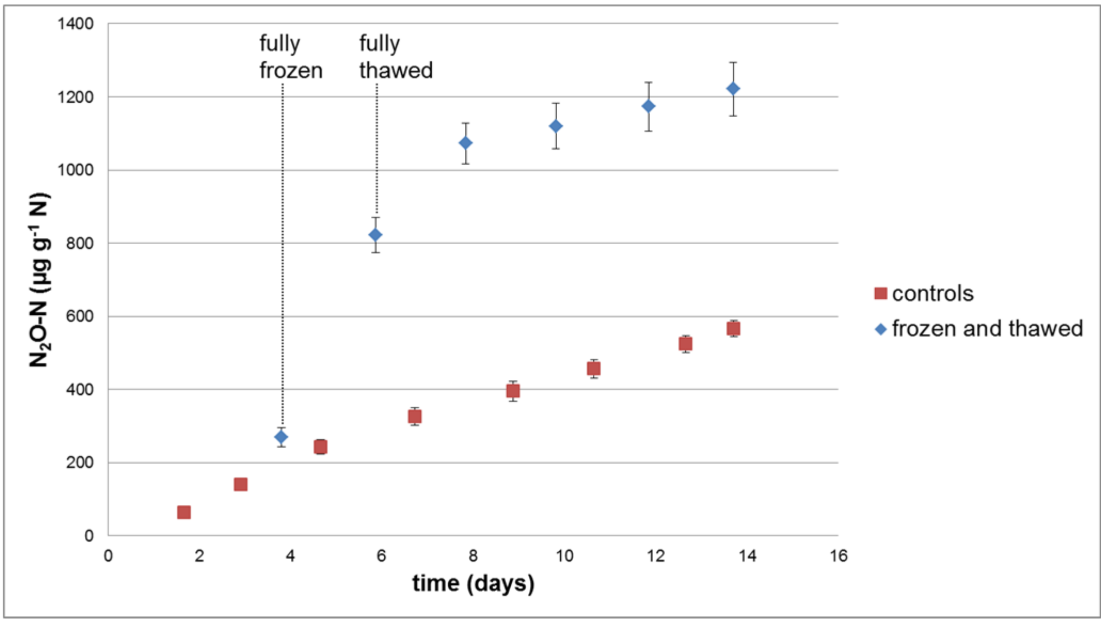

Figure 28. Cumulative N_2O-N emission in µg g^{-1} of initial N from the 1 month compost

From this compost the cumulative N_2O emission in the frozen and thawed sludge was much higher than in the controls, with a large peak during the first days of thawing. This indicates that in this compost it was probably a physical mechanism of the emission of trapped gas responsible for the N_2O emission.

The decrease in NO_3^--N was about the same as in the 2 weeks compost. As the N_2O emission was much higher than in the 2 weeks compost, it suggests that it was not only denitrification responsible for the high emissions, see Figure 29 and Figure 30.

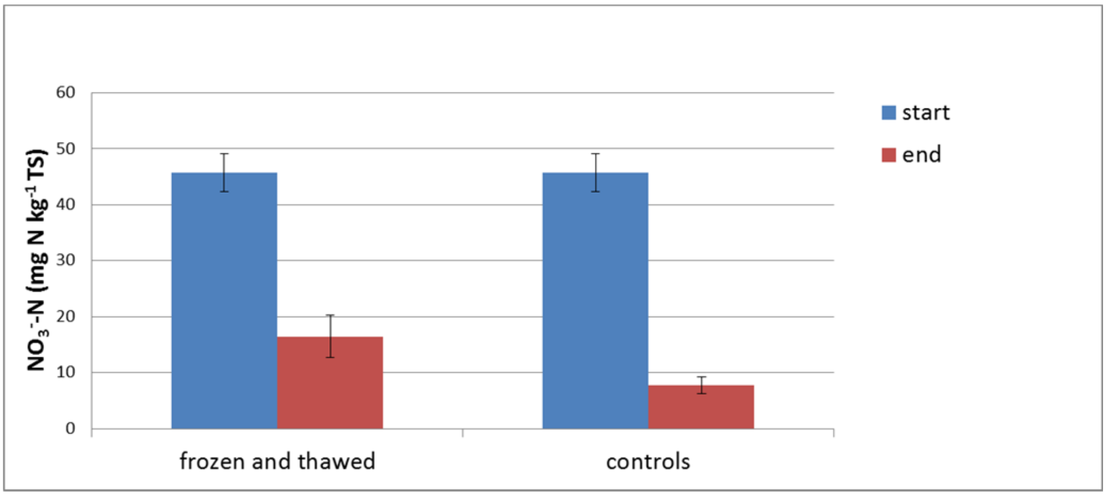

Figure 29. NO_3^--N in 1 month compost at the start and end in mg kg^{-1} of initial TS

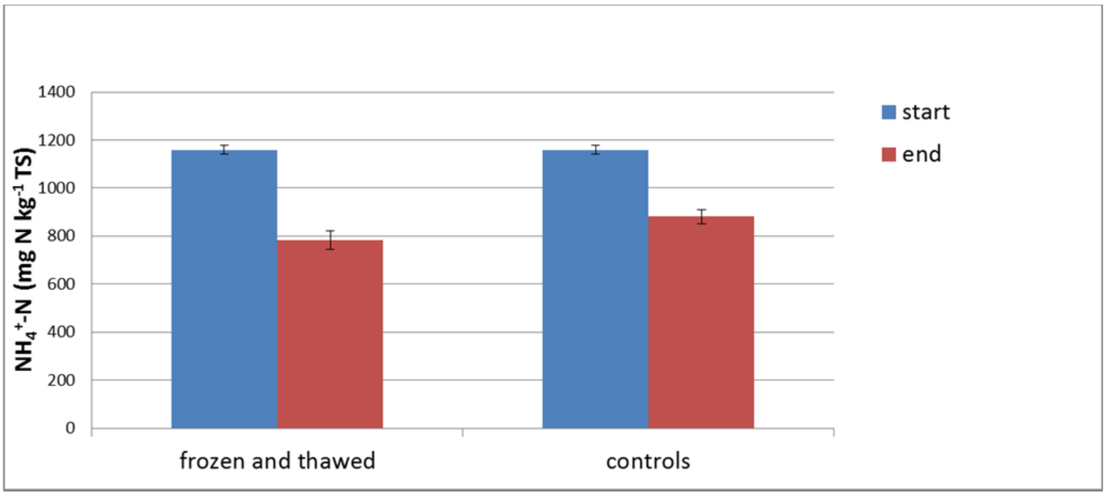

Figure 30. NH_4^+-N in 1 month compost at the start and end in mg kg^{-1} of initial TS

Also the TC did not differ a lot between the two composts. The TN was slightly higher in the 1 month old compost, but not much. See Table 5 for the values of TC and TN.

An explanation might be indicated by the significant decrease in NH_4^+-N. Two explanations are possible: the emissions come from nitrifier denitrification, explained in chapter 1.1. In He *et al.* (2001) a correlation between NO_2^--N and N_2O was observed and it was suggested that the N_2O here came from nitrifier denitrification. NO_2^--N was not measured, but it is known that it is formed during the nitrification process, so it must have been present if nitrification took place. Thereby it is possible that N_2O could not escape during the freezing period, but escaped at once during thawing, which would explain the peak at the start of the thawing period.

Another possibility is that during the experiment the NH_4^+ was both nitrified and denitrified, and that the N_2O comes from denitrification. It could be that during the first part of the freezing still O_2 will be available in the lower part of the compost, so that nitrification could take place. At a certain moment, the O_2 will be depleted, while the lower part is not totally frozen yet. However, the upper part with already frozen compost forms a barrier for O_2 to be transported to the lower part. This would lead to anaerobic conditions in which denitrification might take place. During the denitrification process N_2O can have accumulated, leading to the peak emission during the first days of thawing. This last explanation seems more reliable, as most N_2O emissions peaks come from denitrification according to literature, which was mentioned in chapter 1.4. A combination of both processes, nitrifier denitrification and nitrification followed by denitrification, is also a possibility.

4. Conclusions

First it can be concluded that, although temperatures were very low, still a significant amount of GHG emission took place, which indicates that still a lot of activity was going on. In all of the different substrates the CH_4 emissions were low, probably both due to aerobic conditions and low temperatures.

For the sewage sludge it can be concluded that the NO_3^--N concentration clearly increased the N_2O emissions: 3500 µg g^{-1} initial N in the sewage sludge that had been frozen and thawed 3000 µg g^{-1} initial N in the controls at the end of the experiments, compared to about zero from the non-nitrified sewage sludge. It can also be concluded that for nitrified mesophilically digested sewage sludge, both the CO_2 and N_2O emissions increased after some days of thawing, which resulted in higher accumulated emissions at the end of the experiments. This was probably because of increased substrate availability. For non-nitrified mesophilically digested sewage sludge, no clear effect was observed of the freezing and thawing on any of the greenhouse gasses.

For the CO_2 emissions from compost, the only effect observed was a very low rate during freezing, after that, during thawing, the rates were the same as in the controls. The freezing and thawing had the strongest effect on the N_2O emissions during thawing from both composts, although the amounts of emission were in very different ranges: the N_2O emission from the 1 month compost was much higher than those from the 2 weeks compost, but the reason for this is unclear. For both composts the emissions from the frozen and thawed treatments were clearly higher than those from the controls during the first days of the thawing. For the 2 weeks compost it is not certain what kind of mechanism was responsible for the emission during thawing. For the 1 month compost, it is very probable that a physical mechanism of trapped N_2O that was emitted during thawing plays a role, as the highest peak took place around the time that the compost had been fully thawed.

5. Recommendations for further research

Although it is clear that freezing and thawing cycles can significantly influence N_2O emissions in compost and sewage sludge, it is not certain which processes are behind this. It would therefore be interesting to measure the nitrification and denitrification potentials before and after the experiment to investigate this further.

Some of the emissions were not constant at the end of the experiment, which shows that still an effect of the freezing and thawing could be observed during long periods after thawing than applied in this experiment. Because of this, it could not be said exactly how much effect the freezing and thawing had. Therefore it would be better to continue the experiment until the emissions are constant.

It is important to make sure that enough head space is present in the container. In these experiments, sometimes the CO_2 concentrations became a bit high, indicating a risk for depletion of oxygen, and furthermore some concentrations of N_2O were very high, making their analysis difficult.

A last important recommendation is to research the effect of different NO_3^--N concentrations in compost, since in sewage sludge this clearly influenced the N_2O emissions.

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Appendix I. Not fully detected gas concentrations

In Table 6 and Table 7 the ppm concentrations from the nitrified sewage sludge are given for all three triplicates. The red marked values are the concentrations that were too high to be fully detected.

Table 6. PPM values for the N_2O from the frozen and thawed mesophilically digested nitrified sewage sludge in the containers

1		2		3	
time (days)	N_2O concentration (ppm)	time (days)	N_2O concentration (ppm)	time (days)	N_2O concentration (ppm)
5,87	420	5,88	390	5,88	379
7,70	863	7,70	860	7,70	972
9,73	942	9,73	831	9,74	1087
11,92	1098	11,92	1003	11,92	1252
13,69	1576	13,69	1528	13,70	1545
14,93	1843	14,93	1884	14,93	1853
15,75	1759	15,75	1769	15,75	1741
16,79	1710	16,79	1696	16,79	1731

Table 7. PPM values for the controls of the nitrified mesophilically digested sewage sludge in the containers

1		2		3	
time (days)	N_2O concentration (ppm)	time (days)	N_2O concentration (ppm)	time (days)	N_2O concentration (ppm)
0,90	917	0,91	922	0,91	774
3,03	977	3,03	938	3,04	672
4,72	903	4,72	881	4,72	684
6,82	1077	6,82	1068	6,82	888
8,92	1340	8,92	1276	8,92	1073
10,84	1208	10,84	1141	10,85	917
11,90	790	11,90	761	11,90	539
12,88	510	12,88	461	12,88	368
14,91	742	14,91	774	14,91	666
16,77	794	16,77	816	16,78	707

Appendix II. Temperature graphs

In Figure 31 the temperature graph of the third trial experiment is shown.

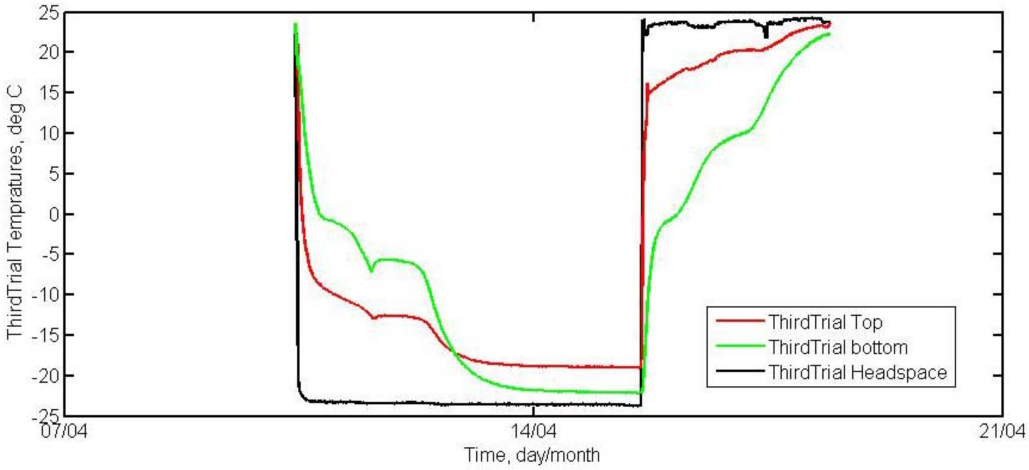

Figure 31. Temperature graph third trial experiment

The graph looks a bit strange: the temperature at the bottom first decreases the slowest as expected, but gets a lower temperature at the end of the freezing period. There is no explanation for this other than that there might be something wrong with the temperature sensors. Since other temperature sensors were used during the main experiments, no further attention was given to it.

In Figure 32, Figure 33, Figure 34 and Figure 35, respectively, the temperature graphs of the main experiments with non-nitrified mesophilically digested sewage sludge, nitrified mesophilically digested sewage sludge, 2 weeks compost and 1 month compost are shown. The temperatures in the head space are constantly going up and down, because of the variation of the temperature in the refrigerator room. The peaks in the temperature come from the moments of sampling, since the containers were then shortly taken out of the refrigerator room.

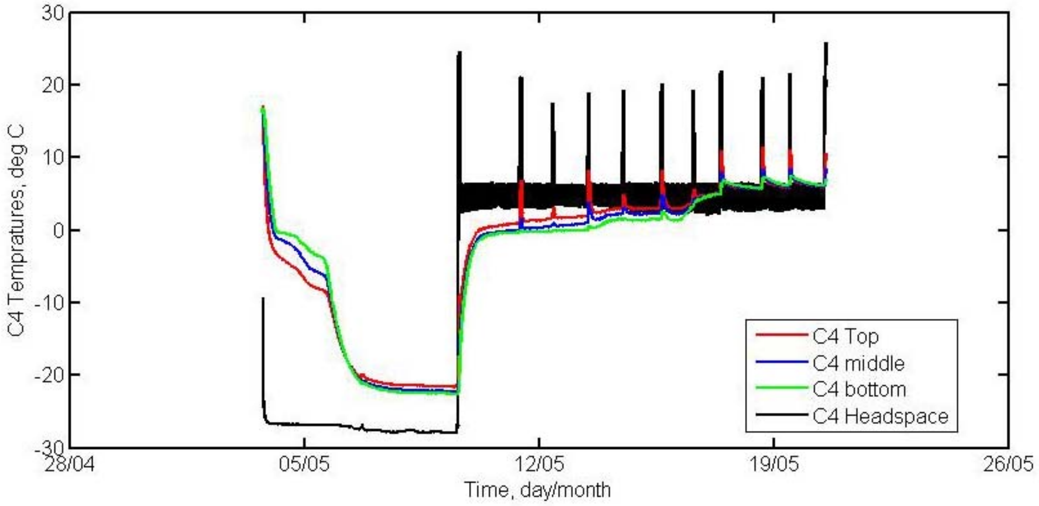

Figure 32. Temperatures in non-nitrified mesophilically digested sewage sludge

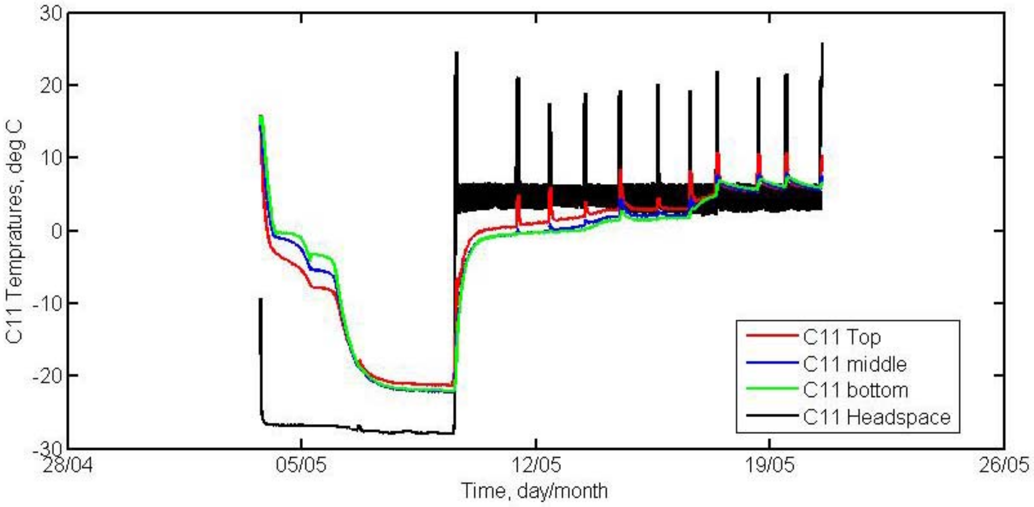

Figure 33. Temperatures in nitrified mesophilically digested sewage sludge

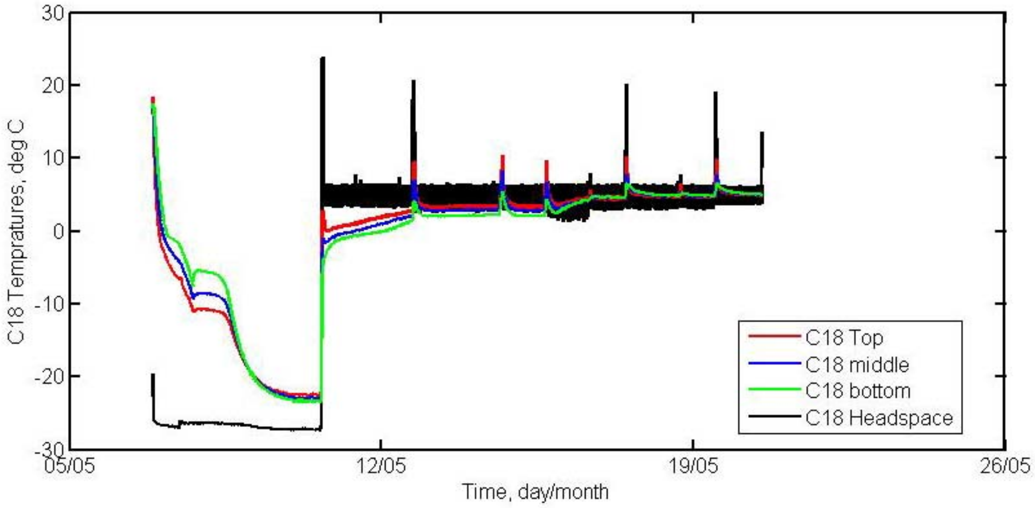

Figure 34. Temperatures in 2 weeks compost

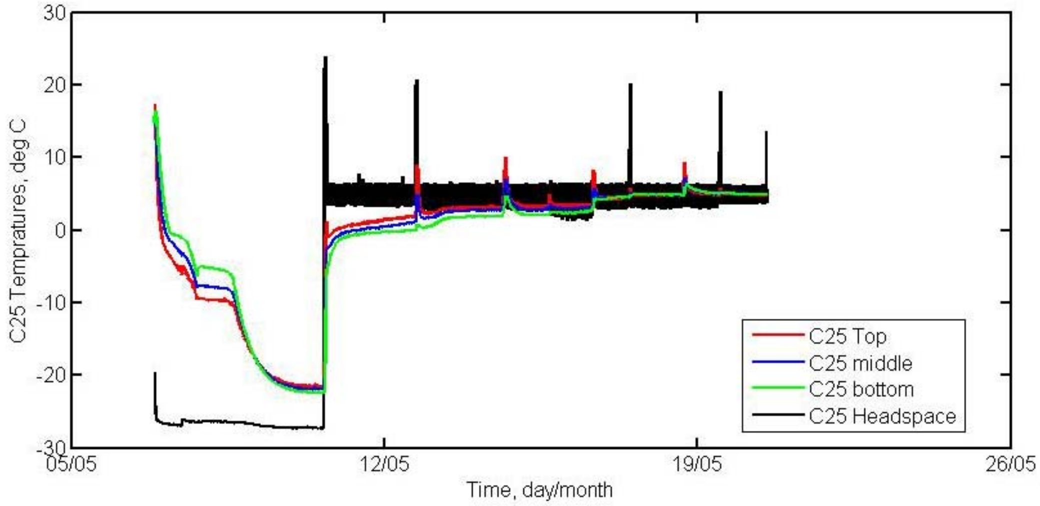

Figure 35. Temperatures in 1 month compost