UNIVERSITY OF
EASTERN FINLAND

NITROGEN GAS (N_2O, NO, HONO) EMISSIONS FROM HORSE DUNG

Zafar Sajjad Gondal

MSc Thesis

University of Eastern Finland

Faculty of Science and Forestry

Department of Environmental and Biological Sciences

November 2018

UNIVERSITY OF EASTERN FINLAND, Faculty of Science and Forestry, Department of Environmental and Biological Sciences
ZAFAR SAJJAD GONDAL: Nitrogen gas (N_2O, NO, HONO) emissions from Horse dung.
Master thesis: 40 pages
Supervisors: Assoc. Prof. Marja Maljanen, MSc Hemraj Bhattarai
November 2018

Key words: nitrogen, carbon, nitrification, greenhouse gas, animal excreta

ABSTRACT

The substantial amount of nitrogen is found in horse dung which is partly lost during the storage period. The horse dung is stored in the form of stockpiles. The concrete construction is done around the stockpiles to prevent leaching of nitrogen, but still gaseous N-emissions can occur. However, there is no information about the emission rates in the literature. The aim of this thesis was to study the gaseous nitrogen emissions from horse dung and the effect of storage on emission of nitrogen gas emissions. The horse dung was selected for this study based on the age. The age of horse dung was fresh (F), one month old (M) and one year old (Y). We measured in laboratory conditions nitrous oxide (N_2O), nitric oxide (NO) and nitrous acid (HONO) emissions from all of three types of horse dung samples. The emission of NO and HONO was higher in one-year old horse dung samples demonstrated that the emission increased with the storage time of the dung. The mean emission rates of HONO and NO were from 36 to 280 ng N kg $dw^{-1}h^{-1}$ and from 15 to 3500 ng N kg $dw^{-1}h^{-1}$, respectively. N_2O emissions showed variability and (20.3 µg N kg dw^{-1} h^{-1}) was measured from the oldest samples. In conclusion, the gaseous N losses increases with longer storage of horse dung. There is a need to take measures to handle the horse dung in an environmentally friendly way.

ACKNOWLEDGMENTS

First and foremost, I would like to thank my supervisor, Assoc. Professor Marja Maljanen, for allowing me to work on this novel project. She consistently supported and guided me during this whole project. I would also like to recognize the support of my second supervisor MSc Hemraj Bhattarai who consistently guided me during lab work for this project. The Biogeochemistry Research Group in general is a team of great people, I would like to thank all of you. Finally, I would like to express my deepest gratitude to my family for their support which helped me to complete this project.

ABBREVIATIONS

AMO	Ammonia monooxygenase
AOA	Ammonia Oxidizing Achaea
AOB	Ammonia Oxidizing Bacteria
GC	Gas Chromatograph
HONO	Nitrous acid
IC	Ion Chromatograph
LOPAP	Long Path Absorption Photometer
NH_4^+/NH_3	Ammonium/ammonia
NO_2^-	Nitrite
NO_3^-	Nitrate
NO	Nitric oxide
N_2O	Nitrous oxide
NOB	Nitrite Oxidizing Bacteria
OH	Hydroxyl radicals
UV	Ultra-violet
GHG	Greenhouse gas
VOC's	Volatile organic compounds
N	Nitrogen
C	Carbon

Table of Contents

ABSTRACT ii
ACKNOWLEDGMENTS iii
ABBREVIATIONS iv
1 Introduction 1
2 Literature Review 4
2.1 Nitrogen cycle 4
2.2 Climatically important Nitrogen gases 5
2.2.1 Nitrous Oxide (N_2O) 5
2.2.2 N_2O emissions from animal manure 7
2.2.3 Nitric Oxide (NO) 8
2.2.4 Nitrous acid (HONO) 9
2.3 Uses of Horse manure and challenges 16
3 Material and Methods 20
4 Results 21
5 Discussion 25
6 Conclusion 26
7 References 27

1 Introduction

The number of horses is increasing in the EU countries as well as in Finland. There are currently 75 000 horses in Finland and the number is expected to increase (Luke, 2016). The annual amount of manure produced by these horses is more than 10^6 $m^{3,}$ out of which a small portion is left on the pastures during the summer. The main use of horse manure is to spread on the agricultural field as a fertilizer. Every year about 770 000 m^3 of manure is collected from the stables. However, the main problem is in the urban areas especially southern part of Finland. Most of these stables do not have their own agricultural field to use horse manure as a fertilizer therefore it is very expensive for stables owners to dispose of the manure. Apart from use as fertilizer the second potential option could be burning of manure at farm level and use as an energy source. The horse manure has a very high moisture contents and it should be dried before burning, this process costs a lot of money to farm owners therefore it is not always possible at farm level (Edström et al., 2011; Lundgren & Pettersson, 2009). However, in optimal conditions emission from burning horse manure for energy purposes are low (Lundgren & Pettersson, 2009) and also the ash could be used as a fertilizer. Manure could also be used in bioreactors to produce energy Böske et al. (2014) but it also requires a huge investment and as there is no standard bedding material used by every stables therefor efficiency depends on the type of bedding material used (Böske et al., 2014).

The fresh horse dung is collected from stables and stored in the form of stockpiles before any further action, for example transportation or processing. The concrete construction is done to store the horse dung to prevent leaching of nutrients (e.g. nitrate) in the soil. However, during this storage period also the gaseous N-losses e.g. as ammonia (NH_3) or nitrous oxide (N_2O) from the manure are taking place (Garlipp et al., 2011; Thomsen, 2000). The storage time and moisture content of the manure

can affect the N-gas emissions (Pratt et al., 2015) and also the type of the bedding material used (e.g. straw, wood chips, peat) used (Garlipp et al., 2011).

Nitrous oxide (N_2O) is a powerful greenhouse gas about 300 times the radiative forcing of CO2 per molecule. Nitrous oxide is predominantly emitted from agricultural soils. The other sources of greenhouse gases are livestock manure management which are linked to about 10% of greenhouse gas emissions (Owen & Silver, 2015). Apart from N_2O, there is another nitrogen gas known as nitric oxide (NO) which is not a direct greenhouse gas, but it indirectly contributes to climate change because it causes the formation of tropospheric ozone (O_3). NO is a reactive gas which cause to form smog and acid rain (Heil et al., 2016).

By nature, greenhouse gases are relatively nonreactive. Apart from NO, another atmospheric gas which is major precursor to atmospheric hydroxyl radicals known as HONO. This gas is linked to production of NO and N_2O in the atmosphere. The hydroxyl radicals are strong oxidizing molecules which can for example, oxidize atmospheric methane (CH_4) (Maljanen et al., 2013; Riedel & Lassey, 2008; Su et al., 2011). The aerosols (e.g. sulfuric acid and VOC's) formed can accelerate the HONO emissions, these are linked to air pollution and climate change (Kulmala & Petäjä, 2011). The mechanisms of HONO emissions in the soil are not well known yet. The formation of HONO in the atmosphere occur by photolytic reaction of nitrite (NO_2^-) or humic acid with nitrogen dioxide Stemmler. (2006) but some other studies showed that N-rich soils are also responsible (Maljanen et al., 2013; Oswald et al., 2013; Scharko et al., 2015; Su et al., 2011). The study by Maljanen et al. (2013) showed that large HONO emissions are linked to N-rich organic soils with low C/N ratio. The hypothesis is that animal manure with high organic matter and N-content can also be a major source of HONO as well as the other N-gases; NO and N_2O. To our knowledge there are no published data available on the gaseous N emissions (e.g. N_2O, NO or HONO) from horse manure. There are

many studies available on N-gas emissions from cattle, sheep or poultry manure e.g.(Larios et al., 2016). As the horse manure differ in terms of composition and structure than e.g. dairy cow manure, the gaseous emissions may also differ significantly. These emissions are very important to consider for environmental friendly way to handle horse manure. To study the effect of manure storage on emission of N-gas emissions we compared fresh, short term stored and long term stored horse manure.

2 Literature Review

2.1 Nitrogen cycle

Nitrogen is the most abundant pure element, found on earth, making up around 78% of our atmosphere (Fields, 2004). It is key component of many life processes, being a major constituent of DNA and proteins, however as the atmospheric nitrogen is relatively inert therefore biologically useful nitrogen is rarely available. To be of use biologically, in nature the triple covalent bond holding nitrogen atoms must be broken, which require a massive amount of energy. This triple bond is broken in nature through lightning strikes or specialized nitrogen fixing microbes a conversion called nitrogen fixation, and the product is ammonia (NH_3). Nitrogen fixation is the initial step of nitrogen transformation and whole bunch of all transformations; nitrification, denitrification, anammox, and ammonification *via* biologically facilitated and chemical processes, which may eventually lead back to N_2 in the atmosphere, are collectively known as the nitrogen cycle.

The other sources of ammonia apart from nitrogen fixation are: wet nitrogen deposition *via* dissolved in rain water and dry nitrogen deposition as a gas or particles or spreading as a synthetic fertilizer/ natural livestock manure on agricultural fields and mineralization (Fields, 2004; Pilegaard, 2013; Xiao et al., 2013).

Mineralization is a microbially facilitated process of breakdown of organic nitrogenous products into inorganic ammonia, which is the most dominant source of inorganic ammonia input in the soil (Fields, 2004; Xiao et al., 2013). Mineralization rates depend upon many factors, for example the every 10˚C increase in temperature double the mineralization rate in many recorded soils and similarly increase of moisture content of soil increase the mineralization with optimum rate around 80% WHC (water holding capacity) of soil (Guntiñas et al., 2012). Another, perhaps the most dominant factor contributing to mineralization rate in the soil is C:N ratio. The C:N ratio below 25:1 tend to favour mineralization, while soils with a C:N ratio above 25:1 tend to favour immobilization, which is the microbial assimilation of inorganic nitrogen into organic compounds (Xiao et al., 2013).

The fate of soil ammonia is carried out further by two processes nitrification and denitrification. During the process of nitrification, the biological oxidation of ammonia (NH_3) to nitrite (NO_2^-), is conducted by ammonia oxidizing microbes, and the further oxidation of NO_2^- to NO_3^- is carried out by nitrite-oxidizing microbes. Mainly during nitrification process autotrophic microorganisms, are

involved, however some heterotrophic microbes, such as *Alcaligenes faecalis,* also contribute to the nitrification process (Bateman & Baggs, 2005). The other biogeochemical mechanism of nitrification is oxidation of ammonium without oxygen, a process known as anammox (anaerobic ammonium oxidation) which has nitrite as electron acceptor. Nitrification is followed by a reduction of nitrite or nitrate, a process called denitrification. Denitrification is carried by both biological and chemical processes. The one biological process where a step-wise reduction of ($NO_3^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$) by heterotrophic microbes and fungi occur is known as heterotrophic denitrification. The other biological process in O_2 limiting conditions where nitrifying microbes use alternate substrates as electron acceptors is known as nitrifier denitrification. In addition to biological process a chemical process known as chemodenitrification is also involved in denitrification which causes chemical reduction of nitrite or hydroxylamine in acidic conditions on contact with Fe_2^+ Mg_2^+ or Cu_2^+)(Bateman & Baggs, 2005; Jones et al., 2015; Kool et al., 2011; Zhu et al., 2013).

2.2 Climatically important Nitrogen gases

2.2.1 Nitrous Oxide (N_2O)

Nitrous oxide (N_2O) is a powerful long-lived greenhouse (GHG) in the atmosphere, with an average mixing ratio of 322.5 parts per billion by volume (ppbv) in the year 2009 (IPCC, 2014). It has global warming potential of almost 300 times that of carbon dioxide (CO_2) and 12 times more potent than methane (CH_4) over a hundred year lifespan (Thomson et al., 2012). At present, nitrous oxide (N_2O) is responsible for almost 6.5% of the global increase in radiative forcing making it the third largest contributor (IPCC, 2013) as well as the key depleting substance of the stratospheric ozone layer (Zhu et al. 2013). The concentrations of N_2O in the atmosphere have increased by 19 % since pre-industrial times, by an average increase of 0.77 ppbv yr^{-1} with in the period of 2000-2009 (IPCC, 2014).

For a better understanding of N_2O soil emissions it is necessary to understand the major processes involved in N_2O production, consumption and emissions. The key multiple pathways of N_2O formation and consumption include direct release during the first step of ammonia oxidation (hydroxylamine oxidation), nitrifier denitrification (reduction of NO_2^- to N_2O, NO),nitrite oxidation, heterotrophic denitrification, anaerobic ammonium oxidation (anammox), dissimilatory nitrate reduction to ammonium (DNRA) or nitrate ammonification (Hu et al. 2015) and abiotic chemo-

denitrification (on contact with Fe, Mn or Cu ions or at low pH <5.47). There is often uncertainty about the process involved in N_2O emissions with in each soil because of difficulties to distinguish between them (Zhu et al. 2013).

Nitrification and denitrification are recognized as the principal N_2O production pathways in soils and they can occur simultaneously. However, denitrification process is generally attributed as a dominant source of N_2O emissions. The conditions favorable to denitrification often raised the magnitude of N_2O emissions (Qu et al. 2014). The major factors affecting denitrification process have been long recognized e.g. low soil oxygen level, high water contents (70% WFPS levels), pH (5-8), temperature (25 to 30°C), availability of nitrogen and carbon. In peat soils a low C/N ratio from 15 to 25 are optimum for high N_2O emissions. Freeze- thaw events as can also trigger the nitrous oxide emissions. Nitrous oxide can be accumulated in soil during winter and then rapidly released during thawing. Hydrogen sulphide (H_2S) concentrations which can alter the activity of heterotrophic denitrification and lead to N_2O emission (Harter et al. 2014; Hu et al. 2015). The other process that interact with N_2O emissions have been reported in the literature include: nitrate availability in soil, ammonification, ammonia volatilization, plant nitrogen uptake and oxidation of volatilized ammonia (NH_3) to produce N_2O on plant leaves (Hu et al. 2015).

The soil moisture is an important factor of N_2O emissions because it can affect the oxygen availability to soil microbes. At high moisture contents such as the soil water content measured by water filled pore space (WFPS) which is around 70% was often reported to link with high soil N_2O emissions (Kool et al. 2011). WFPS along with soil physical properties are linked to determine soil porosity and pore size distribution that affect the diffusion of oxygen (O_2) in the soil (Butterbach-Bahl et al. 2013). In nitrogen-fertilized soils, total cumulative N_2O emissions were significantly correlated to soil water filled pore space (WFPS) with maximum emissions at around 70% WFPS. The authors speculated that the high emissions at 70% WFPS were related to the nitrification process. As the ammonium (NH_4^+) contents were significantly higher at moisture levels (90 and 110% WFPS) than at lower moisture levels (30, 50 and 70% WFPS), whereas their NO_3^- contents were significantly lower. They concluded that nitrification process might dominate N-transformation processes at or below 70% WFPS but denitrification process might dominate N-transformation when the soil moisture level exceeds 90% WFPS. Further the application of nitrification inhibitors dicyandiamide (DCD) or 3, 4-dimethylpyrazol phosphate (DMPP) reduced the cumulative N_2O emissions by 99.2% and 97.1% respectively (Huang et al. 2014). The effect of WFPS on the role of nitrification and denitrification to N_2O emissions is very complex because of the heterogeneity of the soil environment where both

aerobic and anaerobic conditions might exist with in the soil at the same time and thus cannot be clearly correlated to oxygen (Hu et al. 2015). Soil moisture could become the crucial regulator for N_2O emission during freeze/thaw periods. As many soil microbes are active around 0 °C temperatures and freeze/thaw processes lead to pulses of N_2O emissions with significant contributions to the annual N_2O budget. This may be attributed to the release of stored carbon during the thaw (Butterbach-Bahl et al. 2013).

The global meta-analysis of field experiments showed that the amounts of N_2O increase substantially in soils with lower pH values (Shcherbak et al. 2014). The smaller emissions of N_2O were observed at alkaline conditions as the N_2 was the end product of denitrification while at acidic conditions more N_2O production was observed. This variation in emission rates was attributed to the enzymes reductases for nitrate, nitrite and nitric oxide. These enzymes are more active at pH < 7 and their efficacy is reduced at low pH. The maximum nitrification occur between pH 7 and 8. Further, N_2O production increased with decreasing pH (pH < 7) (Richardson et al. 2009). Consequently, N_2O produced in nitrification and denitrification processes is released and not reduced to N_2 (Qu et al. 2014). Acidic conditions also increase the output of N_2O *via* chemo-denitrification due to the instability of nitrite (Zhu et al. 2013).

Apart from the above-mentioned factors affecting the N_2O emissions, other factors such as soil temperature, organic matter contents greatly affect the N_2O emissions. In peat soils the organic C availability and NO_3^- concentration under anaerobic conditions can affect denitrification activity (Maljanen et al., 2007).

2.2.2 N_2O emissions from animal manure

Livestock manures are major sources of nutrients, to be used for the fertilization of cropland and grassland. The N in manure is vulnerable to losses *via* nitrification and denitrification of N from manure in the form of nitrous oxide (N_2O), nitrogen oxide (NOx) and dinitrogen (N_2) into the atmosphere (IPCC, 2014). The potential sources of nitrogen gas emissions could be housing, storage and application of animal manure on agricultural fields as well as droppings of grazing animals on soil (Camargo & Alonso, 2006). The one possible source of nitrogen loss in the form of N_2O is through storage of animal manure in the form of piles as this provide aerobic and anaerobic conditions within close proximity (Hansen et al., 2006). Emissions of total N lost in the form of N_2O in stored

cattle and pig farmyard manure (FYM) heaps, was observed about 9.8% by Webb et al. (2004) and from stored poultry manure heaps between 0.2 to 0.8% (Thorman et al., 2006).

As stated previously that animal manure application on agricultural fields could be a potential source of nitrogen gas emissions. Chadwick et al. (2011) compared N_2O emissions following application of pig slurry and dairy cow slurry at the soil with the same NH_4^+-N application rate. Higher emissions of N_2O were observed from the dairy cow slurry (2.42% of applied NH_4^+ N, 0.97% of total N applied) as compared to the pig slurry (0.94% of NH_4^+-N applied, 0.44% of total N applied). According to the author the higher N_2O emissions from the dairy cow slurry might be due to two reasons, first differences in the carbon content of the slurries as the carbon availability stimulate the denitrification activity and oxygen consumption in the soil and second the presence of fine solids in the dairy cow slurry that potentially block soil pores which favours the anaerobic soil conditions.

2.2.3 Nitric Oxide (NO)

Nitric oxide (NO) is a reactive gas, and it plays a crucial role in atmospheric chemistry by regulating oxidizing agents e.g., hydroxyl radical (OH^-) and controlling the photochemical formation of tropospheric ozone (O_3), the third largest contributor to positive radiative forcing (Zhu et al. 2013). Atmospheric NO quickly reaches photo equilibrium with nitrogen dioxide (NO_2), and the two chemicals are known collectively as NO_x (Pilegaard, 2013). NO_x can undergo a series of reaction in the presence of sunlight to form O_3, a precursor of urban photochemical smog (Zhu et al, 2013). The end photochemical products of NO: nitric acid (HNO_3) and NO_3^- cause the formation of secondary aerosols, acid rain, and nitrogen deposition. The hydroxyl radicals (OH^-), formed by NO_x often react with a great deal of chemicals in the atmosphere to control their atmospheric lifespan (Huang & Li, 2014).

The main processes involved in the emission of NO are microbial nitrification, denitrification and chemo denitrification. There are several factors involved upon which the net emission of NO depends such as nitrogen availability, organic matter content, oxygen status, soil moisture, pH and temperature.

Aerobic nitrification is generally the major pathways of NO production. The soil water content play an important role in the emission of NO as the soil water controls oxygen transport in the soil and also transport of gaseous N out of the soil. Nitrification is the dominant process of NO emissions from dry well-aerated soils, because NO can diffuse out of the soil before being consumed. On the other hand, in wet soils, denitrification dominates, and much of the NO is consumed by denitrifiers before it can leave the soil; thus, N_2O is the dominating gas emitted from the soil. Generally, the process of nitrification is dominant at values of water-filled pore space (WFPS) below 60 per cent and denitrification dominates at values above 60 per cent. The ratio of NO: N_2O is often close to 1, at a WFPS of 60 per cent. At high soil moisture contents, NO is more readily consumed where the residence time is longer.

Soil pH play an important role in the emission of NO as in neutral to alkaline soils the main source of NO emissions is generally nitrification, while in acidic soils it's often denitrification (Pilegaard, 2013; Simek & Cooper, 2002). Secondly in general, NO emissions from acidic soils are lower than their alkaline/neutral counterparts (Simek & Cooper, 2002). NO undergoes chemodenitrification at low pH (especially below pH 4). Availability of N in the form of NO^-_3 and NH^+_4 in the soil, are another most important factor for NO emission in the soil. The availability of NO^-_3 and NH^+_4 depends on N input to the ecosystem and N output. The factors involved in the nitrogen input are for example: nitrogen fixation, use of fertilizer/ animal manure. The nitrogen output includes: gaseous emission and leaching. Leaching is mostly in the form of NO^-_3 and to a lesser extent dissolved organic nitrogen (Pilegaard, 2013).

2.2.4 Nitrous acid (HONO)

Nitrous acid (HONO) is the source of OH^- radicals and NO at the UV spectrum range (320 nm-400 nm) as shown in the Fig 1. HONO contribute to about 30% of OH radicals in the troposphere (Su, Chang, et al., 2011). The OH radicals are highly reactive and short lived, however due to their oxidizing properties, they act as an atmospheric detergent by reducing the level of methane Jardine et al. (2004) and are linked to ozone formation (Lelieveld et al., 2004). On the hand the increased

concentration of OH^- radicals in the environment favors formation of aerosol particles which can cause air pollution and climates change (Kulmala & Petäjä, 2011) as shown in Fig 1.

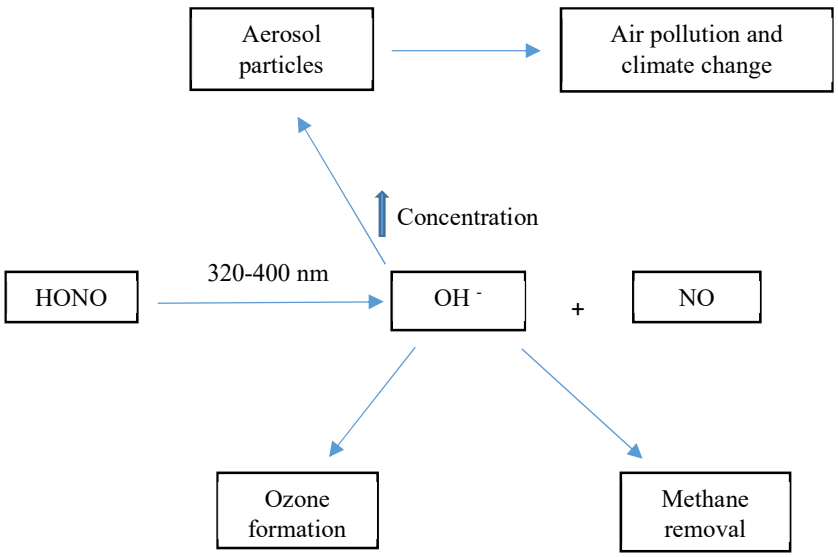

Figure. 1 Decomposition of HONO and its effects

The mechanisms of HONO formation in the atmosphere and soil are not fully understood yet. The soil NO_2^- in acidic soil, could be a potential source of elevated abiotic HONO emissions and also a source of large abiotic NO emissions (Maljanen et al., 2013; Su et al., 2011). Maljanen et al. (2013) showed the intrinsic HONO emissions from acidic soil collected from drained peatlands were higher as compared to pristine peatlands with high water table. The pH range of those drained boreal peat soil were between 3.65 to 5.05 and further C: N ratio organic matter was relatively lower than other sites suggesting that acidic soil with lower C: N ratio allow higher HONO emissions. Similarly Donaldson et al. (2014) showed high emissions of HONO from soils with surface acidity of soil minerals implying that up to 70% of global soils are capable of emitting HONO due to their acidic or close to neutral pH; suggest soil nitrite, whether microbial derived or deposited on soil might be the source of HONO emissions. The acidic snow surfaces could also be the source of HONO

emissions (Beine et al., 2008). The day time HONO sources are still under debate as photolytic lifetime of HONO is only ~10 min (Young et al., 2012). Photolysis of adsorbed nitric acid Li et al. (2012) or reduction of NO_2 on the surface of humic acid could be the source of HONO emissions (Stemmler et al., 2006).

The biogenic sources of HONO emissions might be from nitrite producing bacteria in the biological soil crust Weber et al. (2015), alkaline soil Oswald et al. (2013) as well as agricultural and urban soil Scharko et al. (2015) as shown in the table 1.

Table 1: Source of HONO emissions

Sr #	Sources	Reference
1	Surface acidity of soil minerals	(Donaldson et al., 2014)
2	Nitrite: source of HONO emissions in acidic soils	(Su, Chang, et al., 2011)
3	Drained peatland with low C:N ratio and acidic pH emits HONO in significant amount than pristine peatland and upland forest	(Maljanen et al., 2013)
4	Biological soil crusts	(Weber et al., 2015)
5	Snow packs: Upon acidification	(Beine et al., 2008)
6	Burning of biomass	(Roberts et al., 2010)
7	Ammonia oxidizing bacteria in alkaline soil.	(Oswald et al., 2013)
8	Agricultural and urban soil: Ammonia oxidizing bacteria	(Scharko et al., 2015)
9	Heterogeneous reactions: photolysis of HNO_3 (day) The reaction of OH+NO (night)	(Li et al., 2012)
10	Reduction of NO_2^- on surface of humic acid	(Stemmler et al., 2006)
11	Emissions of atmospherically important nitrous acid (HONO) gas from northern grassland soil increases in the presence of nitrite (NO_2^-)	(Bhattari et al., 2018)

Twigg et al. (2011) investigated the nitrogen containing trace gases (NH_3, HNO_3, HONO) and aerosol (NO_3^-) simultaneously after fertilizing the grassland with 164 Kg N ha^{-1} with cattle slurry in South-East Scotland by using state-of-the-art chemical analyzer's (GRAEGOR, QCLAS, PTRMS). The total ammonia loss (TAN) was estimated 33.5 % over a 5- day period after fertilization. A N budget over the 5-day period after the fertilization was estimated 17.2 kg N ha^{-1} from the fertilized field. The average trimethylamine flux in the first 31 h following the first slurry application amounted to 0.38% of the NH3-N emissions. The small HONO emission was also observed following fertilization (up to 1 ng m^{-2} s^{-1}), however the mechanism is still unclear. Further, the deposition of all compounds was observed to the adjacent unfertilized grassland. The overall summary of N budget over a 5- day period of cattle slurry application as described by Twigg et al. (2011) is shown in table 2.

Table 2. N budget over a 5-day period of cattle slurry applied above grassland (Twig et al., 2011).

Compound	Emission (g N ha^{-1})	Deposition (g N ha^{-1})
Ammonia	17.248	1.72
Nitric Acid	0.01	1.57
Nitrous Acid	0.18	0.08
Nitrate	1.57	0.68
Trimethylamine	16.38	---
Total	17.266	4.04

In the latest study by Bhattarai et al. (2018) in experiments with grassland (Phleum pratense L.), showed that HONO emissions increased up to 14 μgNm^{-2} h^{-1} in the plot receiving annually 450 kg N ha^{-1} as mineral nitrogen fertilizer. These findings were strongly linked with soil nitrite (NO_2^-) concentration and pH. The author suggested that agricultural soils after N-fertilization could be an important source of HONO and its emission. The HONO emissions in these agricultural soils are primarily dependent soil NO_2^- concentration. These findings are similar to previous studies by Maljanen et al. (2013) and Donaldson et al. (2014) where they showed that acidic soils could be potential source of HONO emissions in conjunction with higher soil nitrite concentration.

2.3. Nitrogen gas emissions from agriculture

The main source of nitrogen includes: agricultural land, livestock and poultry operations atmospheric precipitation, geological sources, and urban waste. The strong increase in agricultural emissions show due to the application of N-fertilizer to agricultural soils, spreading of animal manure and grazing of animals (Ghaly & Ramakrishnan, 2015).

In the EU states agricultural activities generated 470.6 million tons of CO_2 equivalent in 2012, about 10 % of total greenhouse gas. The agricultural EU member states tend to account the highest greenhouse gas emissions. The France and Germany together contributed just over one third (33.7 %) of the EU-28's greenhouse gas emissions from agriculture in 2012. The combined emissions of the United Kingdom (11.0 %), Italy (7.5 %), Spain (8.0 %) and Poland (7.8 %) accounted for more than one third (34.3 %) of the total. The agriculture emissions were about 30.7 % of total greenhouse gas emissions in Ireland in 2012 showed highest contribution among any of the EU Member States (Eurostat, 2016).

In 2014, agricultural activities in United States were responsible for emissions 8.3 percent of total U.S. greenhouse gas emissions. The primary greenhouse gases emitted by agricultural activities were methane and nitrous oxide. The fertilizer uses and other cropping practices, were the largest source of U.S. N_2O emissions in 2014, accounting for 78.9 percent (USEPA, 2016). In Canada emissions directly related to animal and crop production are about 8.0% of total 2014 GHG emissions (Environment Canada, 2016).

Soil is a major source of nitrous oxide (N_2O), nitric oxide (NO) and molecular nitrogen (N2), and human activities lead to an increase in emissions. From natural and agricultural soil nitrogen oxide (NOx) emissions are estimated at 7.3 and 3.7 tera grams (TgN year $^{-1}$), respectively, amounting to about 23% of total global NOx emissions (Ciais et al., 2013). The agricultural soil, has been identified

as the major source of N_2O, with an approximately 50–60% of global N_2O emissions (USEPA, 2010). The increase in global demand for food and use of synthetic and organic fertilizers will further increase N_2O emissions (Wuebbles, 2009).

N-fertilizer use, animal manure applications, might be the possible source of nitrite accumulation in the soil as shown in Fig 2. N-Fertilization caused more NO_2^- accumulation in the soil compared to the natural conditions. Application of N-fertilizers such as urea might cause the large NO_2^- concentrations in the soil due to the formation of alkaline solution on hydrolysis. Alkaline conditions promote dissociation of NH_4^+ to NH_3 (pK_a = 9.3). The freer more NH_3 and high soil pH adversely affect the activity of *Nitrobacter Spp* and nitrite accumulate because of slow conversion of nitrite to nitrate (Burns et al., 1996; Heil et al., 2016).

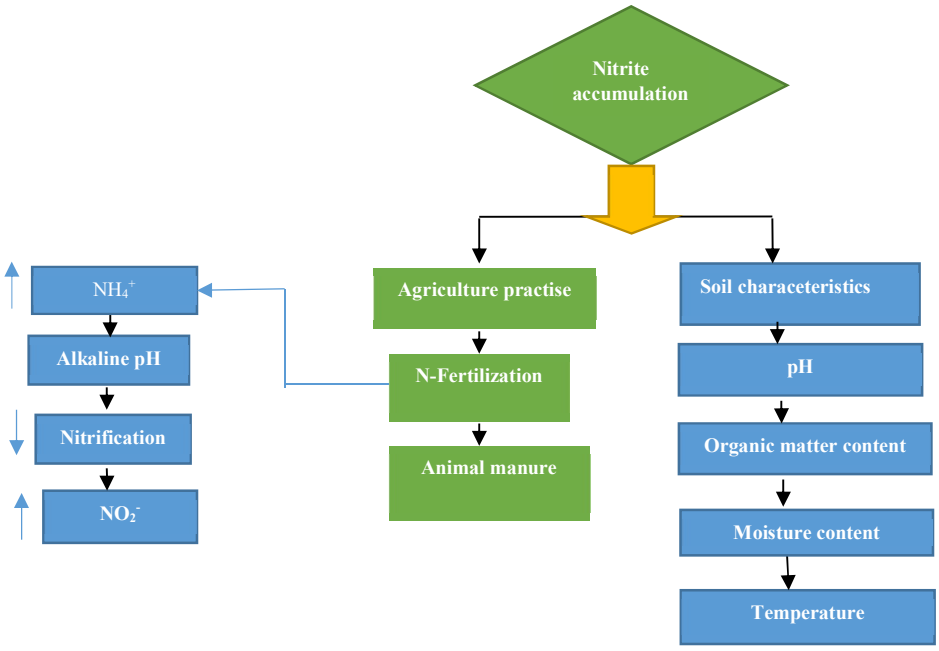

Figure. 2 Factors affecting the nitrite accumulation in the soil

Microbial nitrification and denitrification are the key processes in regulating the emissions of NO and N_2O from agricultural soils. During nitrification NH_4^+ oxidizing bacteria transform $NH4^+$ to nitrite (NO_2^-) which is further converted into NO_3^- by *Nitrobacter sp.* and *Nitrospira sp.* bacteria. Under oxygen-deficient conditions, nitrous oxide and nitric oxide (NO) are minor by-products of the transformation from nitrite (NO_2^-) when nitrifier bacteria's use NO_2^- as a terminal electron acceptor (FAO/IFA, 2001).

Nitrification is used to determine the form of nitrogen present and its mechanism of absorption, utilization, or emission into the environment. The nitrification converts immobile $NH4^+$ to the highly mobile NO_3^-. Conversion of NH_4^+ to NO_3^- strongly affects N utilization by plants, because is often the major uptake form of nitrogen (Subbarao et al., 2006). During denitrification the NO_3^- is transformed to dinitrogen (N_2) gas as shown below.

$NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2$

During the transformation of NO_3^- to N_2, a small and variable portion of the N is emitted as N_2O gas. This occur especially when the well aerated soils got moistened or saturated with water (Robertsone & Groffman, 2007). The microbial denitrification is studied well but the abiotic denitrification knowledge is still lacking. The coupled biotic-abiotic processes are not well studies yet, although they can occur over a wide range of soil properties. On the other hand, existing analytical methods are not well enough to differentiate among biotic and abiotic sources and sinks satisfactorily (Heil et al., 2016).

The abiotic denitrification pathways for NO_2^- loss from soil are known; such as nitrite self-decomposition in the acidic condition, reactions with organic matter and chemo denitrification. Self-decomposition appears to be the dominant process of NO_2^- loss at pH $\leq$ 5 as shown in the figure 3 below. At acidic conditions, NO_2^- converts to nitrous acid, which then self-decomposes mainly to NO. The NO further may be oxidized to NO_2 by atmospheric O_2 as shown in figure 3 (Mørkved et al., 2007). The other mechanism of abiotic denitrification can be direct oxidation of ammonium

hydroxide (NH_2OH) leading to nitrous oxide (N_2O) emissions as described in the figure 3 (Udert et al., 2005). The potential to oxidize NH_2OH depends mainly on soil pH, and soil organic matter (SOM) giving an evidence of coupled biotic–abiotic N_2O production (Jannis et al., 2015).

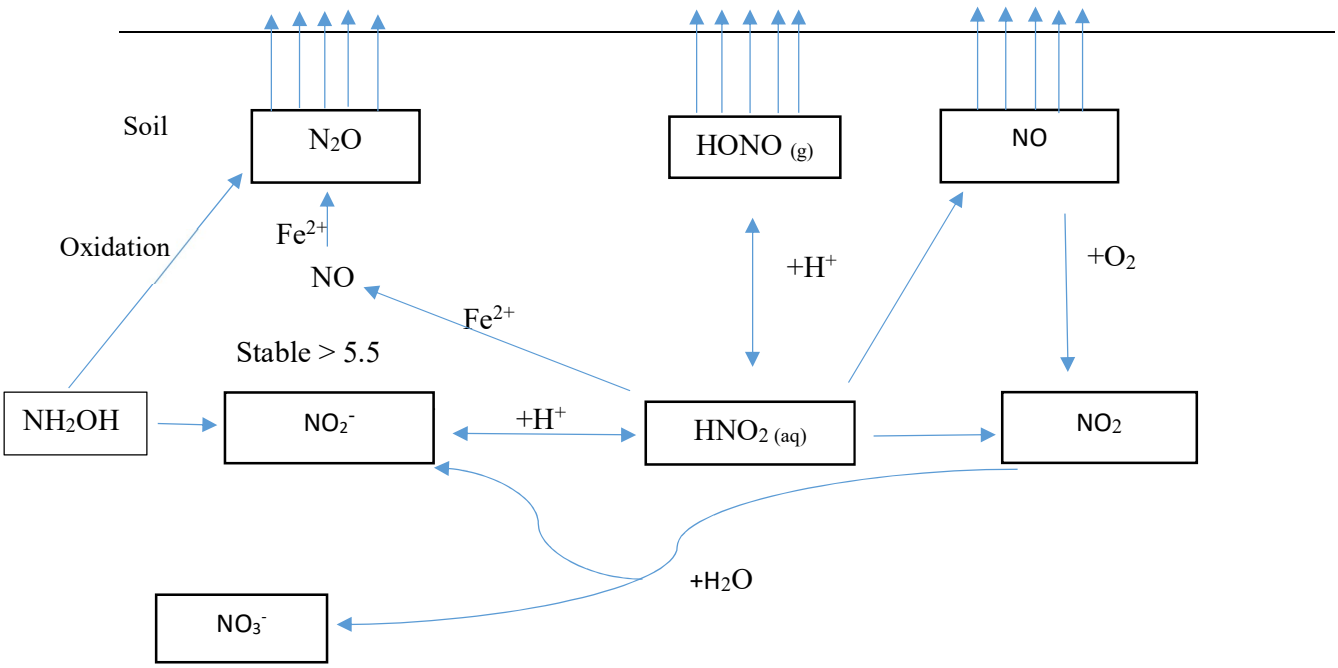

Figure.3 Schematic diagram of NO_2^- self-decomposition, chemodenitrification and NH_2OH oxidation in the soil (modified from Heil et al., 2016)

2.3 Uses of Horse manure and challenges

The horse manure consists of about 60% of solids and 40% of liquids portion and mainly consists of faeces, urine, and used bedding material. Storage of manure causes emissions to air, soil, and water Oenema et al.(2007) in the form of ammonia (NH_3), nitrous oxide (N_2O), carbon dioxide (CO_2) and

methane (CH_4) (Garlipp et al., 2011). During storage, emissions to air occur from the pile of manure. Storing manure without a proper cover for a long time permits losses of nutrients to soil and water as shown in Fig 4.The improper manure storage caused environmental impacts mainly due to nitrogen leaching to the subsoil, ground water and emissions to the air e.g. of CH_4 and N_2O (Oenema et al., 2007).

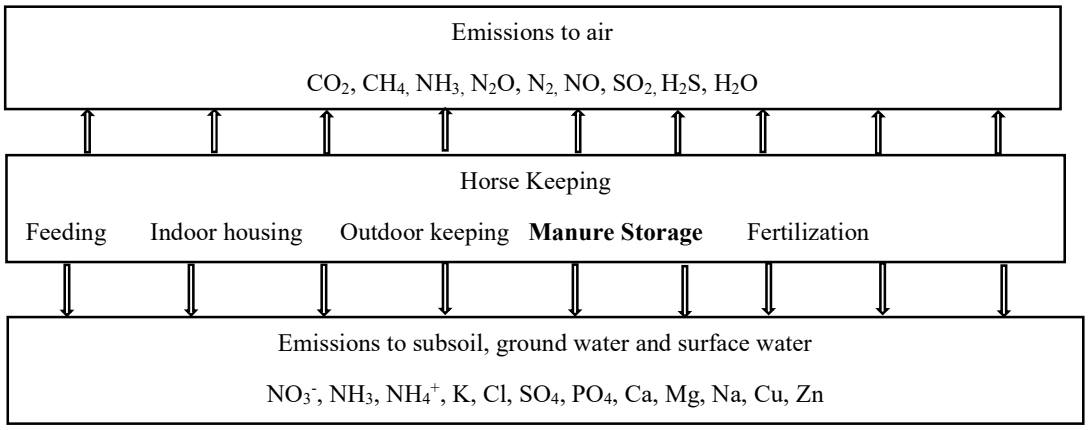

Figure 4. Environmental impacts from horse keeping with focus on horse keeping (Hadin et al., 2016)

The possible uses of horse manure are to spread on the fields as a fertilizer or as an energy production. The horse manure has a slow spontaneous decomposition as it is dry and has a low bulk density and high carbon-nitrogen ratio due to a high proportion of bedding material. It is recommended to compost the manure before to use as a fertilizer as it enhance nitrogen (N) availability to plants and reduces the amount of manure, to spread (Hadin et al., 2016). The rate of decomposition of horse manure depends on the type of bedding material used. The study by Airaksinen et al. (2001) showed, the horse manure where peat was used as a bedding material was ready to spread after the composting period. The use of composted horse manure as a fertilizer on arable land near horse stables is a viable alternative for many of the horse stable owners. However, cereal farmer often not interested to use composted horse manure as fertilizer since it may contain oat weeds that can put an extra burden on the framer to weed the field manually. On the other hand, there is a rumor, the spreading of composted

manure with wood shaving tend to restrain the plants growth because of its lignin and terpene contents (Lundgren & Pettersson, 2009). In Finland, horse manure is a growing problem for horse stables, as it is prohibited to spread or utilize on sloping fields at the beginning of 2016 (Paalanen, 2016). Because of these issues as described above this is a huge problem for stable owners to manage horse manure in Finland. However, there was news in media on behalf of Finland Maa- ja metsätalousministeriö that regulations for burning horse manure at farm level will not be so strict , but there are still some rules, e.g. the burning temperature must be over 850^{o}C for at least 2 seconds. This is a good new for the horse farm owners in southern Finland where the number of horses is high. However still, the major part of horse manure will be used as a fertilizer, if it is possible ("Hevosenlannan poltto helpottuu – Suomesta löytyy jo monta sille soveltuvaa kaukolämpökattilaa," 2017 accessed date 21.11.2018).

The global need of alternative energy sources is getting momentum because of increasing energy demand, rising fuel prices, decline in energy reservoirs, greenhouse gas emissions and global warming (Nanda et al., 2015).The high ash fusibility (melting /sintering ash at lower temperature) is the most common problem of non-wood biomass. To overcome this behavior, the temperature inside the combustion bed should be limited. The incomplete combustion at low temperature leads to the carbon monoxide (CO) emissions. On the other hand, the combustion of fuels with high ash contents also shows high particle contents in the flue gas. Finally, the high particle level increases the fouling on the heat exchanger surfaces. Further additional measures such as filter are required to fulfill the emission limits (Edström et al., 2011).

Aims

The aims of this study were

1. To study the nitrogen gas (N_2O, NO, HONO) emissions from horse manure
2. To study the effect of storage on the emission of nitrogen gases

3 Material and Methods

The horse manure was sampled from a horse stable (Tapsan talli, Ranta-Toivala) which is located in Eastern Finland (mean annual T 3.2. °C, annual precipitation 630 mm (Pirinen et al., 2012). Fresh manure (F) samples (< 12 h) were collected in baskets directly from inside the stable. The short term stored (about 1 month, M) and older samples (stored at least one year, Y) were collected from outdoor stockpiles. The stockpiles have concrete floor and were covered with a roof. Wood chip pellets were used as a bedding material. During sampling it was tried to remove bedding material as possible. The samples were mixed manually. Four replicates of each sample were prepared as shown in Fig. 5 below. Four replicates of each type of manure (150 g dw) were placed in PVC rings (diameter 18 cm). The rings were sealed from the bottom with an aluminum foil as shown in Fig. 5 below. Cores were incubated at room temperature (20 °C) before first flux measurements. Gas fluxes (N_2O, NO and HONO) were measured first at original moisture content (Table 2) and then after adjusting all samples to same moisture level (2 g H_2O g dw^{-1}). After moisture adjustment the fluxes were measured after incubation of 13 days.

Figure. 5 Horse dung replicates

pH and electric conductivity (EC) were measured from sample:water slurry (30: 50 v/v). Total C and N were analysed with vario MAX elementar analyser (Elementar Analysensysteme GmbH, Germany). For analysis of nitrate (NO_3^-), nitrite (NO_2^-) and organic carbon (TOC) 30 ml sample and 100 ml milliQ-H_2O were shaken at 175 rpm for one hour and then filtered and analyzed with an ion chromatograph (DX 120, Dionex Corporation, USA) for NO_3^- and NO_2^- and with Shimadzu TOC-VCPH/CPN analyzer (Shimadzu, Japan) for DOC.

A sample of 30 ml manure and 100 ml 1M KCl was used to extract ammonium (NH_4^+). The ammonium (NH_4^+) was analyzed with spectrophotometer. The samples were dried for 24 h at 65 °C to measure gravimetric moisture content. Loss on ignition was used to determine Organic matter (OM) concentration at 550 °C. HONO fluxes were measured by dynamic flow chamber method which was connected with a commercial HONO analyzer (LOPAP, QUMA Elektronik & Analytik GmbH, Germany). NO emissions were also measured by dynamic chamber which was connected with Thermo 42i NOx analyzer (Thermo Fisher Scientific). N_2O fluxes were measured with a static chamber method. The samples were taken with syringes from the headspace of the chamber at intervals of 5, 10, 15 and 20 min after closing the chamber. In addition to N_2O also CH_4 and CO_2 fluxes at initial moisture content were measured with the same method. Gas samples were injected into 12 ml Exetainers (Labco, UK) and were analyzed with a gas chromatograph (Agilent 7890B, Agilent Technologies, USA).

4 Results

Samples properties were measured from each type of four replicates. The pH in all samples was above 7 and EC above 800 µS cm^{-1} as shown in (Table 2). Organic matter (OM) content, carbon concentration, and C/N ratio were significantly lower in older one-year (Y) samples as compared to fresh (F) and one month (M) old samples. Dissolved organic carbon (DOC) concentration decreased

with increasing age of manure as shown in table 2. The initial moisture content was higher in fresh (F) and one month old (M) samples than in one year old (Y) sample, as expected.

Table 2. Sample properties (average ± standard deviation) measured from 4 replicate samples. EC = electrical conductivity (µS cm^{-1}), OM = organic matter concentration (%), C = total carbon concentration (%), N = total nitrogen concentration (%), C/N = carbon and nitrogen ratio, DOC = water extractable dissolved organic carbon (mg g dw^{-1}), GM = initial and adjusted gravimetric moisture (g H_2O g dw^{-1}), CH_4 = methane production rate (µg g dw^{-1} h^{-1}) and CO_2 = carbon dioxide production rate (mg g dw^{-1} h^{-1}) measured at initial moisture.

	Fresh (F)	One month old (M)	One year old (Y)	F-value
pH	7.5	7.9	7.7	1.64
EC	810 ± 180[a]	1670 ± 330[b]	1630 ± 350[b]	10.43**
OM	90.1 ± 0.4[a]	90.6 ± 1.0[a]	65.7 ± 8.1[b]	36.42**
C	44 ± 0.1[a]	43 ± 0.4[a]	31.8 ± 4.6[b]	27.31**
N	1.8 ± 0.1	1.6 ± 0.1	1.8 ± 0.3	1.07
C/N	25.0 ± 0.7[a]	27.6 ± 0.8[b]	18.1 ± 1.5[c]	83.61**
DOC	18.8 ± 0.6[a]	13.5 ± 0.9[b]	11.3 ± 1.8[b]	97.43**
$GM_{initial}$	3.63 ± 0.12[a]	3.05 ± 0.15[a]	0.56 ± 0.13[b]	12.29**
$GM_{adjusted}$	1.98 ± 0.10	1.94 ± 0.05	1.58 ± 0.40	1.85
CH_4	20.8 ± 4.1[a]	10.0 ± 3.3[b]	0.61 ± 2.4[c]	36.76**
CO_2	1140 ± 280[a]	1940 ± 400[a]	120 ± 54[b]	41.61**

The statistical difference between sample types were analyzed by One-Way ANOVA. Values with a common letter as superscript do not differ as a statistical significance of $p < 0.05$ in Tukey's test.

HONO and NO were emitted from fresh (F), one-month (M) and one-year old (Y) samples. The nitrous oxide was emitted only from fresh (F) and one-year old (Y) samples. The emission rates of HONO and NO were statistically different among fresh (F), one-month old (M) and one-year old (Y) samples as shown in (Fig. 6). The emissions of HONO and NO were dependent on the age of manure as shown in (Fig. 6). HONO emissions were five times and NO emissions 60 times higher in one-year old sample in comparison to fresh manure (F) at original moisture level. After adjusting moisture 30 and 500 times, respectively. Fresh (F) and one-year old (Y) samples also emitted N_2O. The one-month (M) samples had negative mean N_2O emission (uptake). The mean N_2O uptake rate in one-month (M) samples was 0.80 ± 5.2 and 0.59 ± 0.39 µg N kg dw $^{-1}$ at original and adjusted moisture levels, respectively (not shown in Fig. 6 with log-scale in y-axis). The mean N_2O emission from one-year old (Y) samples were from 40 to 50 times as compared to fresh (F) samples.

NO_3^- concentration was observed to be dependent on the age of the manure at initial moisture content as shown in (Fig. 6). At the adjusted moisture content and after incubation of 13 days concentrations of NO_3^- were lower than the detection limit (0.01 µg g^{-1}) in fresh (F) and one-month (M) samples. NO_2^- was detectable (> 0.01 µg g^{-1}) only from one-year old (Y) samples as shown in (Fig. 6). Ammonium (NH_4^+) concentration was lower in fresh (F) samples in comparison to one month (M) or one year old (Y) at original moisture content but also there was not significant difference after adjusting the moisture.

Methane emissions were observed to be decreased with the age of manure as shown in (Table 2). Some of the one-year old (Y) samples even consumed some methane (CH_4). CO_2 emissions were significantly higher from fresh (F) and one month (M) samples as compared to one year old (Y = samples as shown in (Table 2).

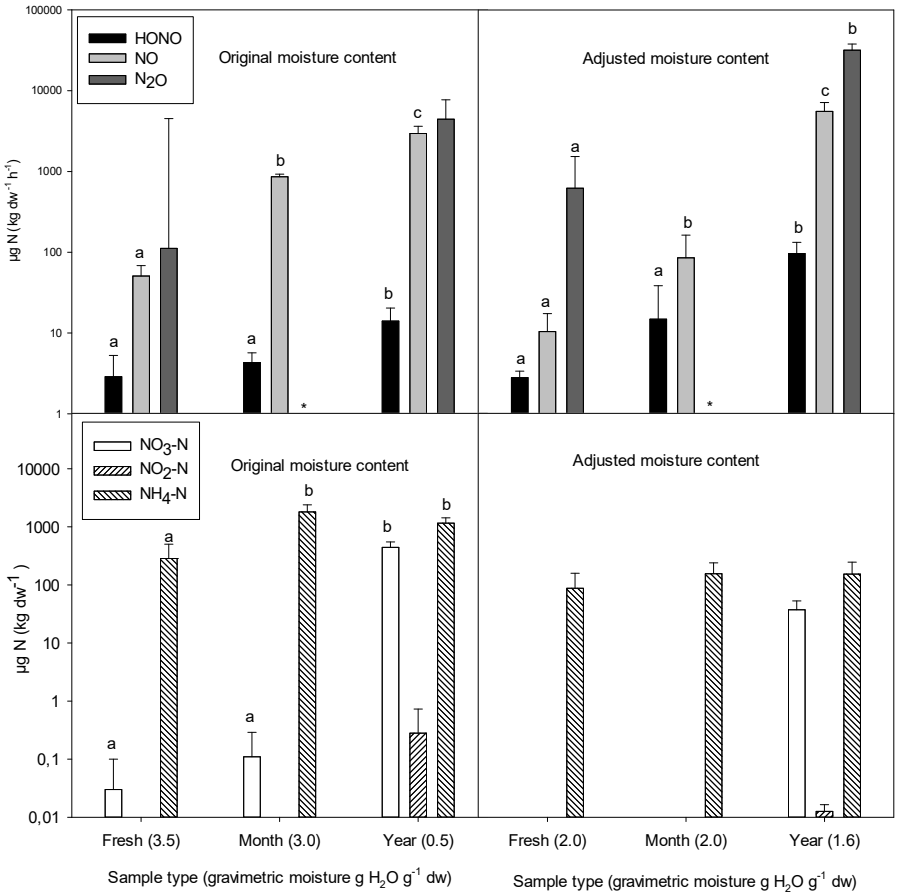

Figure 6. HONO, NO and N_2O emissions and mineral nitrogen concentrations from samples in a) original moisture content and b) adjusted moisture content. *) The average N_2O emission from M sample was negative (mean N_2O consumption rate 0.80 ± 5.2 and 0.59 ± 0.39 ng N kg dw $^{-1}$ at original and adjusted moisture) and therefore it is not shown in the figure with a log-scale. Different letters indicate statistical difference between sample types (Mann Whitney U-test).

5 Discussion

NO and HONO were emitted from all samples types (fresh, one month old, one year old). The emissions were observed to be dependent on the age of manure. Fresh (F) and one-year old (Y) samples emitted N_2O. From one-month old (M) net N_2O uptake was measured despite emissions. This was surprising for us. The reason behind net uptake measured in one-month old (M) samples was not clear. As the samples were collected in the winter with temperature range (2^{o}C-15 ^{o}C), the storage of dung at low temperature might had decreased nitrogen mineralization. We assumed that higher emissions from one-year old (Y) samples is a result of increased N mineralization/ nitrification rate during storage in stockpiles. When manure is stored in stockpile, a portion of the organic N is converted by soil microbes to ammonium (NH_4^+). NH_4^+ is then nitrified by different microbes to nitrate which is then converted to gaseous N (including N_2, N_2O, NO and possibly HONO) in denitrification. It was observed that ammonium (NH_4^+) was not accumulating in the one-year old manure samples. This observation indicated that it was being used rapidly in nitrification. Detectable amounts on NO_2-was found only from one-year old (Y) manure. The higher HONO emission can be linked to nitrite concentration as previous study by Su et al. (2011) showed that NO_2^- can be a potential source of HONO emission chemically.

There is no published data available about direct greenhouse gas (GHG) emissions from horse manure. However, there are some studies Borhan et al. (2014); Garlipp et al. (2011) showing that horse manure emitted N_2O but the emission fluxes were not reported. If we compare the one year (Y) horse manure emissions calculated per area (300 µg N_2O-N m^{-2} h^{-1} and 52 µg NO-N m^{-2} h^{-1}) with those from dairy cow manure patches measured with similar chamber and analyzer systems in the field in Eastern Finland (110 ± 20 µg N_2O–N m^{-2} h^{-1} and 15 ± 2 µg NO–N m^{-2} h^{-1}) by Maljanen et al. (2007)) it can be seen that NO and N_2O emissions from horse manure are almost three and ten times

higher as compared to the mean emissions from dairy cow manure. However, our study was conducted in laboratory conditions with controlled temperature and humidity. The realistic result of N-gas emission from horse dung, potentially can be get by conducting experiments in the agricultural fields where the manure is spread as a fertilizer. It seems that horse manure is very potential source of the N-gases, including still rather unknown HONO gas. The mean HONO-N emissions were only 0.3% of the mean N_2O-N emissions and therefore there the positive climate effect of HONO cannot compensate the warming effect of N_2O from horse manure. However, the HONO emissions measured here from the one-year-old manure were among the highest "soil" related HONO sources published so far.

6 Conclusion

- Horse manure is a source of nitrous acid (HONO) as well as nitric oxide (NO) and nitrous oxide (N_2O)
- Storage in an outdoor pile increases nitrification, nitrate accumulation and emissions of HONO, NO and N_2O
- Accumulation of nitrite was found after one-year storage of manure could be associated to enhanced emissions of HONO

7 References

Airaksinen, S., Heinonen-Tanski, H., & Heiskanen, M. L. (2001). Quality of different bedding materials and their influence on the compostability of horse manure. *Journal of Equine Veterinary Science*, *21*(3), 125–130.

Bateman, E. J., & Baggs, E. M. (2005). Contributions of nitrification and denitrification to N2O emissions from soils at different water-filled pore space. *Biology and Fertility of Soils*, *41*(6), 379–388. https://doi.org/10.1007/s00374-005-0858-3

Beine, H., Colussi, A. J., Amoroso, A., Esposito, G., Montagnoli, M., & Hoffmann, M. R. (2008). HONO emissions from snow surfaces. *Environmental Research Letters*, *3*(4), 045005. https://doi.org/10.1088/1748-9326/3/4/045005

Bhattarai, H. R., Virkajärvi, P., Yli-Pirilä, P., & Maljanen, M. (2018). Emissions of atmospherically important nitrous acid (HONO) gas from northern grassland soil increases in the presence of nitrite (NO2−). *Agriculture, Ecosystems & Environment*, *256*, 194–199. https://doi.org/10.1016/j.agee.2018.01.017

Borhan, S., Rahman, S., & Hammer, C. (2014). Water Absorption Capacity of Flax and Pine Horse Beddings and Gaseous Concentrations in Bedded Stalls. *Journal of Equine Veterinary Science*, *34*, 611–618.

Böske, J., Wirth, B., Garlipp, F., Mumme, J., & Van den Weghe, H. (2014). Anaerobic digestion of horse dung mixed with different bedding materials in an upflow solid-state (UASS) reactor at mesophilic conditions. *Bioresource Technology*, *158*, 111–118. https://doi.org/10.1016/j.biortech.2014.02.034

Burns, L. ., Stevens, R. J., & Laughlin, R. . (1996). Production of nitrite in soil by simultaneous nitrification and denitrification. *Soil Biology & Biochemistry*, *28*, 609–616.

Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R., & Zechmeister-Boltenstern, S. (2013). Nitrous oxide emissions from soils: how well do we understand the processes and their controls? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1621), 20130122–20130122. https://doi.org/10.1098/rstb.2013.0122

Camargo, J. A., & Alonso, Á. (2006). Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environment International*, *32*(6), 831–849. https://doi.org/10.1016/j.envint.2006.05.002

Chadwick, D., Sommer, S., Thorman, R., Fangueiro, D., Cardenas, L., Amon, B., & Misselbrook, T. (2011). Manure management: Implications for greenhouse gas emissions. *Animal Feed Science and Technology*, *166–167*, 514–531. https://doi.org/10.1016/j.anifeedsci.2011.04.036

Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., … others. (2013). Carbon and other biogeochemical cycles. In *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 465–570). Cambridge University Press. Retrieved from http://pubman.mpdl.mpg.de/pubman/item/escidoc:2058766/component/escidoc:2058768/WG1AR5_Chapter06_FINAL.pdf

Donaldson, M. A., Bish, D. L., & Raff, J. D. (2014). Soil surface acidity plays a determining role in the atmospheric-terrestrial exchange of nitrous acid. *Proceedings of the National Academy of Sciences*, *111*(52), 18472–18477. https://doi.org/10.1073/pnas.1418545112

Edström, M., Schü\s sler, I., Luostarinen, S., & others. (2011). Combustion of manure: manure as fuel in a heating plant. *Baltic MANURE WP6 Energy Potentials*. Retrieved from http://balticmanure.eu/download/Reports/baltic_manure_combustion_final_2_2011_total.pdf

Environmenta Canada. (2016). *National Inventory Report 1990-2014: Greenhouse Gas Sources and Sinks in Canada - Executive Summary*. Retrieved from https://www.ec.gc.ca/ges-ghg/default.asp?lang=En&n=662F9C56-1#agriculture

Eurostat. (2016). *Agriculture - greenhouse gas emission statistics* (p. 8). European commission. Retrieved from Statistics Explained (http://ec.europa.eu/eurostat/statistics-explained/)

FAO/IFA. (2001). *Global estimates gaseous emissions of NH3, NO and N2O from agricultural land* (p. 66). International Fertilizer Industry Association. Retrieved from ftp://ftp.fao.org/agl/agll/docs/globest.pdf

Fields, S. (2004). Global Nitrogen: Cycling out of Control. *Environmental Health Perspectives*, *112*(10), A556–A563.

Garlipp, F., Hessel, E. F., & van den Weghe, H. F. A. (2011). Characteristics of Gas Generation (NH3, CH4, N2O, CO2, H2O) From Horse Manure Added to Different Bedding Materials Used in Deep Litter Bedding Systems. *Journal of Equine Veterinary Science*, *31*(7), 383–395. https://doi.org/10.1016/j.jevs.2011.01.007

Ghaly, A., & Ramakrishnan, A. (2015). Nitrogen Sources and Cycling in the Ecosystem and its Role in Air, Water and Soil Pollution: A Critical Review. *Journal of Pollution Effects & Control*, *03*(02). https://doi.org/10.4172/2375-4397.1000136

Guntiñas, M. E., Leirós, M. C., Trasar-Cepeda, C., & Gil-Sotres, F. (2012). Effects of moisture and temperature on net soil nitrogen mineralization: A laboratory study. *European Journal of Soil Biology*, *48*, 73–80. https://doi.org/10.1016/j.ejsobi.2011.07.015

Hadin, Å., Eriksson, O., & Hillman, K. (2016). A review of potential critical factors in horse keeping for anaerobic digestion of horse manure. *Renewable and Sustainable Energy Reviews*, *65*, 432–442. https://doi.org/10.1016/j.rser.2016.06.058

Hansen, M. N., Henriksen, K., & Sommer, S. G. (2006). Observations of production and emission of greenhouse gases and ammonia during storage of solids separated from pig slurry: Effects of covering. *Atmospheric Environment*, *40*(22), 4172–4181. https://doi.org/10.1016/j.atmosenv.2006.02.013

Harter, J., Krause, H.-M., Schuettler, S., Ruser, R., Fromme, M., Scholten, T., … Behrens, S. (2014). Linking N2O emissions from biochar-amended soil to the structure and function of the N-cycling microbial community. *The ISME Journal*, *8*(3), 660–674.

Heil, J., Vereecken, H., & Brüggemann, N. (2016). A review of chemical reactions of nitrification intermediates and their role in nitrogen cycling and nitrogen trace gas formation in soil: Chemical reactions of nitrification intermediates in soil. *European Journal of Soil Science*, *67*(1), 23–39. https://doi.org/10.1111/ejss.12306

Heil, Jannis, Liu, S., Vereecken, H., & Brüggemann, N. (2015). Abiotic nitrous oxide production from hydroxylamine in soils and their dependence on soil properties. *Soil Biology and Biochemistry*, *84*, 107–115. https://doi.org/10.1016/j.soilbio.2015.02.022

Hevosenlannan poltto helpottuu – Suomesta löytyy jo monta sille soveltuvaa kaukolämpökattilaa. (2017). Retrieved November 21, 2018, from https://www.maaseuduntulevaisuus.fi/cm/2.176/maatalous/hevosenlannan-poltto-helpottuu-suomesta-l%C3%B6ytyy-jo-monta-sille-soveltuvaa-kaukol%C3%A4mp%C3%B6kattilaa-1.175814

Hu, H.-W., Chen, D., & He, J.-Z. (2015). Microbial regulation of terrestrial nitrous oxide formation: understanding the biological pathways for prediction of emission rates. *FEMS Microbiology Reviews*, *39*(5), 729–749. https://doi.org/10.1093/femsre/fuv021

Huang, T., Gao, B., Hu, X.-K., Lu, X., Well, R., Christie, P., … Ju, X.-T. (2014). Ammonia-oxidation as an engine to generate nitrous oxide in an intensively managed calcareous Fluvo-aquic soil. *Scientific Reports*, *4*. https://doi.org/10.1038/srep03950

Huang, Y., & Li, D. (2014). Soil nitric oxide emissions from terrestrial ecosystems in China: a synthesis of modeling and measurements. *Scientific Reports*, *4*, 7406. https://doi.org/10.1038/srep07406

Intergovernmental Panel on Climate change. (2013). *Intergovernmental Panel on Climate Change 2013* (IPCC) (p. 1552). Retrieved from http://www.climatechange2013.org/images/report/WG1AR5_ALL_FINAL.pdf

IPCC (Ed.). (2014). *Climate Change 2013 - The Physical Science Basis: Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge: Cambridge University Press. https://doi.org/10.1017/CBO9781107415324

Jardine, C. N., Boardman, B., Osman, A., Vowles, J., & Palmer, J. (2004). methane UK. *The Environmental Change Institute*. Retrieved from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.127.1871&rep=rep1&type=pdf

Jones, L. C., Peters, B., Lezama Pacheco, J. S., Casciotti, K. L., & Fendorf, S. (2015). Stable isotopes and iron oxide mineral products as markers of chemodenitrification. *Environmental Science & Technology*, *49*(6), 3444–3452. https://doi.org/10.1021/es504862x

Kool, D. M., Dolfing, J., Wrage, N., & Van Groenigen, J. W. (2011). Nitrifier denitrification as a distinct and significant source of nitrous oxide from soil. *Soil Biology and Biochemistry*, *43*(1), 174–178. https://doi.org/10.1016/j.soilbio.2010.09.030

Kulmala, M., & Petäjä, K. (2011). Soil nitrites influence atmospheiric chemistry. *Science*, *333*(6049), 1584–1586. https://doi.org/10.1126/science.1208527

Larios, A. D., Kaur Brar, S., Avalos Ramírez, A., Godbout, S., Sandoval-Salas, F., & Palacios, J. H. (2016). Challenges in the measurement of emissions of nitrous oxide and methane from livestock sector. *Reviews in Environmental Science and Bio/Technology*, *15*(2), 285–297. https://doi.org/10.1007/s11157-016-9394-x

Lelieveld, J., Dentener, F. J., Peters, W., & Krol, M. C. (2004). On the role of hydroxyl radicals in the self-cleansing capacity of the troposphere. *Atmospheric Chemistry and Physics*, *4*(9/10), 2337–2344. https://doi.org/10.5194/acp-4-2337-2004

Li, X., Brauers, T., Häseler, R., Bohn, B., Fuchs, H., Hofzumahaus, A., … Wahner, A. (2012). Exploring the atmospheric chemistry of nitrous acid (HONO) at a rural site in Southern China. *Atmospheric Chemistry and Physics*, *12*(3), 1497–1513. https://doi.org/10.5194/acp-12-1497-2012

Luke. (2016, January 14). Lannanpoltto ei ratkaise kaikkien tallien jätehuoltoa. Retrieved November 17, 2018, from https://www.luke.fi/lannanpoltto-ei-ratkaise-kaikkien-tallien-jatehuoltoa/

Lundgren, J., & Pettersson, E. (2009). Combustion of horse manure for heat production. *Bioresource Technology*, *100*(12), 3121–3126. https://doi.org/10.1016/j.biortech.2009.01.050

Maljanen, M., Martikkala, M., Koponen, H. T., Virkajärvi, P., & Martikainen, P. J. (2007). Fluxes of nitrous oxide and nitric oxide from experimental excreta patches in boreal agricultural soil. *Soil Biology and Biochemistry*, *39*(4), 914–920. https://doi.org/10.1016/j.soilbio.2006.11.001

Maljanen, M., Yli-Pirilä, P., Hytönen, J., Joutsensaari, J., & Martikainen, P. J. (2013). Acidic northern soils as sources of atmospheric nitrous acid (HONO). *Soil Biology and Biochemistry*, *67*, 94–97. https://doi.org/10.1016/j.soilbio.2013.08.013

Mørkved, P. T., Dörsch, P., & Bakken, L. R. (2007). The N2O product ratio of nitrification and its dependence on long-term changes in soil pH. *Soil Biology and Biochemistry*, *39*(8), 2048–2057. https://doi.org/10.1016/j.soilbio.2007.03.006

Nanda, S., Azargohar, R., Dalai, A. K., & Kozinski, J. A. (2015). An assessment on the sustainability of lignocellulosic biomass for biorefining. *Renewable and Sustainable Energy Reviews*, *50*, 925–941. https://doi.org/10.1016/j.rser.2015.05.058

Oenema, O., Oudendag, D., & Velthof, G. L. (2007). Nutrient losses from manure management in the European Union. *Livestock Science*, *112*(3), 261–272. https://doi.org/10.1016/j.livsci.2007.09.007

Oswald, R., Behrendt, T., Ermel, M., Wu, D., Su, H., Cheng, Y., … others. (2013). HONO emissions from soil bacteria as a major source of atmospheric reactive nitrogen. *Science*, *341*(6151), 1233–1235.

Owen, J. J., & Silver, W. L. (2015). Greenhouse gas emissions from dairy manure management: a review of field-based studies. *Global Change Biology*, *21*(2), 550–565. https://doi.org/10.1111/gcb.12687

Paalanen, A. (2016, February 19). Manure service for stables. Retrieved from http://www.fortum.com/en/products-and-services/horsepower/pages/default.aspx

Pachauri, R. K., Mayer, L., & Intergovernmental Panel on Climate Change (Eds.). (2014). *Climate change 2014: synthesis report*. Geneva, Switzerland: Intergovernmental Panel on Climate Change.

Pilegaard, K. (2013). Processes regulating nitric oxide emissions from soils. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1621), 20130126–20130126. https://doi.org/10.1098/rstb.2013.0126

Qu, Z., Wang, J., Almøy, T., & Bakken, L. R. (2014). Excessive use of nitrogen in Chinese agriculture results in high $N_2O/(N_2O+N_2)$ product ratio of denitrification, primarily due to acidification of the soils. *Global Change Biology*, *20*(5), 1685–1698. https://doi.org/10.1111/gcb.12461

Richardson, D., Felgate, H., Watmough, N., Thomson, A., & Baggs, E. (2009). Mitigating release of the potent greenhouse gas N2O from the nitrogen cycle – could enzymic regulation hold the key? *Trends in Biotechnology*, *27*(7), 388–397. https://doi.org/10.1016/j.tibtech.2009.03.009

Riedel, K., & Lassey, K. (2008). Detergent of the atmosphere. *Water & Atmosphere 16(1) 2008*.

Roberts, J. M., Veres, P., Warneke, C., Neuman, J. A., Washenfelder, R. A., Brown, S. S., … others. (2010). Measurement of HONO, HNCO, and other inorganic acids by negative-ion proton-transfer chemical-ionization mass spectrometry (NI-PT-CIMS): Application to biomass burning emissions. *Atmospheric Measurement Techniques*, *3*, 981.

Robertsone, & Groffman. (2007). Nitrogen Transformations (pp. 341–364). Retrieved from http://35.8.7.173/images/stories/docs/robertson/robertson__groffman_2007_soil_microbiology.pdf

Scharko, N. K., Schütte, U. M. E., Berke, A. E., Banina, L., Peel, H. R., Donaldson, M. A., … Raff, J. D. (2015). Combined Flux Chamber and Genomics Approach Links Nitrous Acid Emissions to

Ammonia Oxidizing Bacteria and Archaea in Urban and Agricultural Soil. *Environmental Science & Technology*, *49*(23), 13825–13834. https://doi.org/10.1021/acs.est.5b00838

Shcherbak, I., Millar, N., & Robertson, G. P. (2014). Global metaanalysis of the nonlinear response of soil nitrous oxide (N2O) emissions to fertilizer nitrogen. *Proceedings of the National Academy of Sciences*, *111*(25), 9199–9204. https://doi.org/10.1073/pnas.1322434111

ŠImek, M., & Cooper, J. E. (2002). The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years. *European Journal of Soil Science*, *53*(3), 345–354. https://doi.org/10.1046/j.1365-2389.2002.00461.x

Stemmler, K., Ammann, M., Donders, C., Kleffmann, J., & George, C. (2006). Photosensitized reduction of nitrogen dioxide on humic acid as a source of nitrous acid. *Nature*, *440*(7081), 195–198. https://doi.org/10.1038/nature04603

Su, H., Chang, Y., Oswal, R., Behrendt, T., Trebs, I., & Meixner, F. . (2011). Soil Nitrite as a source of atmospheric HONO and OH radicals. *Science*, *333*(5963), 1616–1618. https://doi.org/10.1126/science.1207687

Su, H., Cheng, Y., Oswald, R., Behrendt, T., Trebs, I., Meixner, F. X., … Pöschl, U. (2011). Soil nitrite as a source of atmospheric HONO and OH radicals. *Science (New York, N.Y.)*, *333*(6049), 1616–1618. https://doi.org/10.1126/science.1207687

Subbarao, G. V., Ito, O., Sahrawat, K. L., Berry, W. L., Nakahara, K., Ishikawa, T., … Rao, I. M. (2006). Scope and Strategies for Regulation of Nitrification in Agricultural Systems—Challenges and Opportunities. *Critical Reviews in Plant Sciences*, *25*(4), 303–335. https://doi.org/10.1080/07352680600794232

Thomsen, I. K. (2000). C and N transformations in 15N cross-labelled solid ruminant manure during anaerobic and aerobic storage. *Bioresource Technology*, *72*(3), 267–274. https://doi.org/10.1016/S0960-8524(99)00114-5

Thomson, A. J., Giannopoulos, G., Pretty, J., Baggs, E. M., & Richardson, D. J. (2012). Biological sources and sinks of nitrous oxide and strategies to mitigate emissions. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367*(1593), 1157–1168. https://doi.org/10.1098/rstb.2011.0415

Thorman, R. E., Chadwick, D. R., Boyles, L. O., Matthews, R., Sagoo, E., & Harrison, R. (2006). Nitrous oxide emissions during storage of broiler litter and following application to arable land. *International Congress Series*, *1293*, 355–358. https://doi.org/10.1016/j.ics.2006.04.005

Twigg, M. M., House, E., Thomas, R., Whitehead, J., Phillips, G. J., Famulari, D., … Nemitz, E. (2011). Surface/atmosphere exchange and chemical interactions of reactive nitrogen compounds above a manured grassland. *Agricultural and Forest Meteorology*, *151*(12), 1488–1503. https://doi.org/10.1016/j.agrformet.2011.06.005

Udert, K. M., Larsen, T. A., & Gujer, W. (2005). Chemical Nitrite Oxidation in Acid Solutions as a Consequence of Microbial Ammonium Oxidation. *Environmental Science & Technology*, *39*(11), 4066–4075. https://doi.org/10.1021/es048422m

USEPA. (2010). *Methane and Nitrous Oxide Emissions From Natural Sources*. Retrieved from nepis.epa.gov

USEPA. (2016). *Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990 –2014* (p. 34). Environmental protection agency USA. Retrieved from https://www3.epa.gov/climatechange/Downloads/ghgemissions/US-GHG-Inventory-2016-Chapter-2-Trends.pdf

Webb, J., Chadwick, D., & Ellis, S. (2004). Emissions of ammonia and nitrous oxide following incorporation into the soil of farmyard manures stored at different densities. *Nutrient Cycling in Agroecosystems*, *70*(1), 67–76.

Weber, B., Wu, D., Tamm, A., Ruckteschler, N., Rodríguez-Caballero, E., Steinkamp, J., … Pöschl, U. (2015). Biological soil crusts accelerate the nitrogen cycle through large NO and HONO emissions in drylands. *Proceedings of the National Academy of Sciences*, *112*(50), 15384–15389. https://doi.org/10.1073/pnas.1515818112

Wuebbles. (2009). Nitrous Oxide: No Laughing Matter. *Atmosphere*, *326*, 55–56. https://doi.org/10.1126/science.1181034

Xiao, K., Xu, J., Tang, C., Zhang, J., & Brookes, P. C. (2013). Differences in carbon and nitrogen mineralization in soils of differing initial pH induced by electrokinesis and receiving crop residue

amendments. *Soil Biology and Biochemistry*, *67*, 70–84. https://doi.org/10.1016/j.soilbio.2013.08.012

Young, C. J., Washenfelder, R. A., Roberts, J. M., Mielke, L. H., Osthoff, H. D., Tsai, C., … Brown, S. S. (2012). Vertically Resolved Measurements of Nighttime Radical Reservoirs in Los Angeles and Their Contribution to the Urban Radical Budget. *Environmental Science & Technology*, *46*(20), 10965–10973. https://doi.org/10.1021/es302206a

Zhu, X., Burger, M., Doane, T. A., & Horwath, W. R. (2013). Ammonia oxidation pathways and nitrifier denitrification are significant sources of N2O and NO under low oxygen availability. *Proceedings of the National Academy of Sciences*, *110*(16), 6328–6333. https://doi.org/10.1073/pnas.1219993110