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EFFECTS OF NITROGEN AVAILABILITY ON GREENHOUSEGAS, HONO AND NO EMISSIONS FROM A HORSE PADDOCK, HAY FIELD AND GRASSLAND

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ABSTRACT

Greenhouse gas emissions remain the key driver of global climate change. The fluctuations of gaseous emissions rise through human activities such as energy production, land management and agriculture. Currently, the global health tussles with the potency of the changing climate which accommodates heat-catching emissions that continue to choke and pollute our environment, thereby driving it above a warming temperature of $3^{\circ}C$. Thus, the need to understand the biogeochemical effects of GHGs emissions from land management remains essential. Soils are important part of nature and traceable sources of CO_2, N_2O, and CH_4 – active greenhouse gases. The global economic drive to improve agricultural products through the application of N-fertilizers in soils has equipped different agricultural soils to release more atmospheric reactive nitrogen pollutant gases such as HONO and NO. This research expands the effects of nitrogen availability on economical nitrogen gases from three agricultural soil types (hay field, horse paddock and grassland), by measuring the GHGs fluxes and determining the concentration of the soil's chemical properties through clear-cut techniques. The findings show increase in emission rates of N_2O, HONO and NO gases from the soils, with effect of nitrogen availability from fertilizer application. Also, it extends the potency of horse paddocks as traceable sources of higher nitrogen gaseous emissions, with respect to soil acidity and other physiochemical properties. We conclude that GHGs emissions (especially N_2O), HONO and NO emissions increase with increasing available nitrogen. The emission rates correlate with soil NO_3^- concentration and low soil pH. NO and HONO gases behaved similarly in the soil types while N_2O emission followed a different emission pathway. HONO emissions increased with seedling length, and we found out that there were more nitrogen nutrients available for plant assimilation in the horse paddock soil than in the hay field soil.

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ABBREVIATIONS

AOB	Ammonia-oxidizing bacteria
NOB	Nitrite-oxidizing bacteria
WFPS	Water-filed pore space
GC	Gas Chromatograph
HONO	Nitrous acid
IC	Ion Chromatograph
LOPAP	Long Path Absorption Photometer
NASA	National Aeronautics & Space Administration
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
SOM	Soil Organic Matter
UV	Ultra-violet
GHG	Greenhouse gas
$H_2O_{(g)}$	Water vapor
CFCs	Chlorofluorocarbons
VOCs	Volatile organic compounds
CO_2	Carbon dioxide
N	Nitrogen
C	Carbon
O_2	Oxygen
N_2	Nitrogen gas

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1.0 INTRODUCTION

Nitrogen remains the most abundant gas in the atmosphere with a concentration of 78%, while oxygen maintains a 21% percentage by dry-air volume (IPCC, 2013). These two gases are very important to the survival of humans, animals and plants on earth. Nitrogen is a unit of every proteins found in all living organisms and it plays a key role in the nitrogen cycle where atmospheric nitrogen is transformed into different nitrogen compounds. Humans and animals inhale oxygen for survival and release carbon dioxide (CO_2), a vital gas for the plants' survival. When plants use up the essential carbon dioxide, they release oxygen which is used in nutrient formation from sunlight through a process called photosynthesis. The atmosphere also contains trace gases such as argon, hydrogen, neon, krypton, xenon, helium and neon with total concentrations of less than 2% of the whole atmospheric gases. The most essential trace gases present in the atmosphere are carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O). These trace gases are naturally essential in the sustainability of the global greenhouse effect and make the earth a conducive planet for human existence. Soil itself is important in nature, microorganism's diversity and as a source and sink of these important trace gases formation (Conrad, 1995). In the bid to increase global agricultural production, the anthropogenic impact on greenhouse gas emissions (especially N_2O) has shown strong correlation with increasing application of high amount of N-fertilizers (synthetic and natural) to crop soils over the years. This impact has led to increasing in global temperature and the destruction of the ozone layer through nitrogen compounds emissions. There are different applications of N-input in the soil such as synthetic fertilizers, animal manure, fixation of molecular nitrogen (N_2) and nitrogen deposition (Vendramini et al., 2007). The N-input in the soil affects the soil mineral nutrients which are present in form of nitrite (NO_2^-), nitrate (NO_3) and ammonium (NH_4^+) for plant assimilation. There are other factors such as soil moisture, organic matter content, soil structure and metabolites which affects the distribution of soil nutrients in various agricultural soils. Also, CO_2 and CH_4 emissions have widely been sources of agricultural activities and can mostly be traced to land use and enteric fermentation respectively. As the application of nitrogen fertilizer is expected to rise until 2050, it could contribute also to higher emissions of nitrogen gases such as nitrous oxide (N_2O), nitrous acid (HONO), and nitric oxide (NO) soils (Bhattarai et al., 2018). The direct pathways of nitrogen emission can be traced to the microbial nitrification and

denitrification of fertilizer and animal manure in some agricultural soils, while the indirect pathways could involve nitrogen emission through runoff, leaching and biomass harvest. Since soil has been sourced as a good actor in greenhouse gases (N_2O, CO_2, and CH_4) production and storage, then it poses a deeper global concern, especially in areas of land use management and general agricultural practices (Oertel et al., 2016).

Furthermore, several papers have discussed agricultural soils, especially with increasing N-input, as vital sources of N_2O, HONO and NO gaseous emissions. Emissions of N_2O and NO follow similar pattern in the soils through major microbial activities in nitrification and denitrification (Boumnan, 1990), and the amount of soil N-input is one of the crucial environmental actions promoting the emission of N_2O and NO gases (Boumnan et al., 2002). Currently, N_2O is responsible for almost 6.5% of the global increase in radiative forces, making it the third largest contributor (IPCC, 2013) as well as the key depleting matter of the stratospheric ozone layer (Zhu et al., 2013). Agricultural soils have been a major contributor of atmospheric N_2O. On the other hand, emission of HONO from agricultural soils is getting more attention in atmospheric chemistry. (Bhattarai, 2017) found that available nitrogen nutrients through application of nitrogen fertilizers to soils correlates with HONO emissions just as in N_2O and NO emissions. HONO and NO show higher increasing emission potential from boreal agricultural soils with about low 2.69% organic matter and C: N ratio of less than 10.9 (Bhattarai et al.,2017). The concentration of HONO in the atmosphere varies because its influence can mainly be ascertained in environmentally polluted air and contribution to the acid rain. Although, HONO is not a greenhouse gas but a key initiator of hydroxyl radicals (OH^-) and its emission pathway in the soil varies with contrasting reactions, but there has been a nexus between soil high NO_2^- concentration and decreasing acidity (pH) in HONO emissions (Su et al., 2011).

We carried out this study at the Biogeochemistry Research Group in Kuopio, to measure and compare how much of N_2O, HONO and NO emissions are being released from three closely-related agricultural soils (cultivated hay filed, horse paddock and grassland without significant management or horse manure input) with 10 years of steady treatment. Also, the N-gaseous emissions after the grass seeding treatments on selected soils were studied, to find the correlations of the emissions with seedling length and the effect of released nitrogen on the environment.

2.0 LITERATURE REVIEW

2.1 GREENHOUSE GASES EMISSIONS FROM AGRICULTURAL SOILS AND N-INPUT EFFECT

2.1.1 Agricultural soils and fertilization

Agriculture remains a vital part of the global economy sectors and the main source of food production for human consumption. It involves the activities of crop cultivation, animal breeding, dairy production, farm land-use management and soil cultivation. In fact, agriculture is crucial to the survival of all humans and livestock animals on the planet. Although, the depth of agricultural practices in the rural environments and developing countries such as Liberia, Bangladesh and Nigeria seem higher by about 70% to the urban areas and developed countries such as United states, Finland and other European countries. One crucial component to successful agricultural practices is the soil. The importance of agricultural soils in healthy farm produce is linked to soil features such as, nutrients availability, soil structure, moisture capacity, organic matter content and metabolites. As the effort to improve agricultural yields increases, the application of nitrogen fertilizers from organic and inorganic sources increases the processes of soil nitrification and denitrification (Macdonald et al., 2018). Nitrogen can be found in the soil as minerals in varying forms such as NO_2^-, NO_3^- and NH_4^+ for plant uptake. However, of all these nutrients, NO_3^- is the most active in soils with good aeration, while in acidic soil, NH_4^+ may be most prevalent (Miller and Cramer, 2005).

Pastures are naturally a highly nutrient rich soil which are source of food for grazing livestock such as cattle, horses, sheep, goat and pigs. It is covered with grasses and other plants species needed for energy by free-grazing animals. The soil is enriched by metabolic activities from the animal-grazing such as droplets of fecal and the urine surface wash-off. Although, over-grazing is discouraging because of its impact on the loss of biodiversity in plant species and overall effects on the ecosystem. However, controlled-grazing can remarkably play an action role in increasing

soil chemical properties (N, C, and P) and the moisture content (Wei. et al., 2011). Horse manure has been noted as a good source of important agricultural nutrients with about 0.7% and 0.8% of nitrogen and phosphate concentrations respectively, and high decomposition rate due to lower C: N ratio when compared to the cattle dungs. Overall, the pasture areas seem to be increasing because of the increasing number of animals. However, global awareness is increasing for pasture management and controlled grazing methods. Increasing fertilizer application elevates the concentration of NO and N_2O emissions significantly, but the elevation depends on the microbial activities of the available nutrients (Harrison et al., 1995). The types of fertilizers applied to agricultural soils also influence the GHGs emissions rates. For instance, reports stated that when chemical nitrogen fertilizers are applied, the emission rate of soil N_2O almost doubles compared to when animal manures are used (Bouwman et al., 2002; Stanlenga and Kawalec, 2008). Harrison et al. (1995) research implies that even as fertilizer application increases the emissions of N_2O and NO concomitantly, the emission ratio of N_2O: NO slows down.

Although, the way of nitrogen input in the soil and its effect on the environment have been studied intensively by many articles and journals, yet it is important to improve the knowledge of mineral nitrogen uptake, transport pathway, leaching, gains, nitrogen distribution and the emission of important greenhouse gases from agricultural soils.

2.1.2   Climate change and the role of soils

The global climate change is not new, yet remains a global concern. The contribution of human activities to the greenhouse effect (warming effect from trapped atmospheric heat from the sun) since the 20th century has been the driving force behind the years of elevating global warming. There are some gases in the atmosphere which are capable of trapping the heat from the sun. These gases '($H_2O_{(g)}$, CH_4, N_2O, CO_2 and CFCs)' have been identified as greenhouse gases and are responsible for 'driving' the climate change. In all the GHGs in the atmosphere, $H_2O_{(g)}$ retains the largest percentage concentration and the most responsive to the climate change. As the earth becomes warmer, the $H_2O_{(g)}$ rises in response to the atmospheric changes (NASA, 2020). This reaction is vital in the water vapor effect on the greenhouse. Greenhouse gases are responsible for

catching the atmospheric heat from the sunlight, retaining it and thereby increasing the rate of global warming. Nitrous oxide, methane (only from wet soils e.g. rice paddies and wetlands) and carbon dioxide are the major GHGs affecting the global atmosphere and are also originating at increasing rates from agricultural soils.

2.1.3 Nitrous Oxide (N2O)

N_2O is a vital GHG affecting the atmosphere in a global emission share with just about 6%, but for over two decades it exceeds to be around 290 times stronger than CO_2, with over 100 years of lifespan in the atmosphere (IPCC, 2013) The major sources of N_2O has been traced to agricultural activities such as burning fossil fuel, biomass, fertilizer use, animal husbandry and manure. Animal manure production is a utmost source of anthropogenic N_2O emissions and it accounts for more than 40% of human contribution in the depletion of the ozone stratosphere and one of the most significant human GHGs activities (Snakin et al., 2001). In the year 2000s, N_2O reportedly attained the most significant concentration from animal manure (Zhang et al., 2017).

2.1.4 Carbon dioxide (CO2)

The most important greenhouse gas is CO_2, with global emission share of over ¾th of GHGs, and a lifespan of several thousand years in the atmosphere. It recently (in 2018) attained an emission concentration of about 411ppm, which is the highest level of emission monthly recorded, according to Hawaii's Mauna LABO. Soil respiration is the major process by which CO_2 is lost from the soils into the atmosphere, contributing over 20% of the global atmospheric CO_2 emission in the air (Rastogi et al., 2002). The activity of soil respiration is found in three key biological processes such as faunal, root and microbial respiration, where they all contribute altogether to atmospheric CO_2 emission (Rastogi et al., 2002). Burning of fossil fuel contributes about 70% of the total atmospheric CO_2, while soil organic carbon loss contributes to the rest through deforestation and land cultivation for food (Ontl and Schulte, 2012). In fact, deforestation has been traced as the basic source of atmospheric CO_2 in agriculture.

2.1.5   Methane (CH4)

Methane is another active GHG and mainly an element of industrial activities in energy production such as fossil fuel, cooking gas and land management. However, it is also produced from farm land animals (digestion of grass by livestock) and crop cultivation on wet soils (e.g. rice paddies). Its lifespan in the atmosphere is about 12 years (lesser than CO_2), but it can be effective up to 34 times than CO_2, over a period of 100 years (IPCC, 2014). It's global emission share in the GHGs is about 16%. On a global scale, up to 80% of methane is produced from biogenic sources such as wetlands cultivation, decomposing animal wastes and enteric fermentation, while human activities from production of fossil gas, landfill leakages and coal extraction contribute about 20%. (Houweling et al., 1999). Wetlands are notably good source of methane emission and some of the factors regulating methane emissions could be temperature, soil organic carbon and water gradient. Also, forest soils are potential sources of methane because the trees are capable of regulating the surface water gradient for the growth of methanotrophs. In case of a waterlogged soil condition (e.g. in boreal conditions where low temperature is limiting methane production in winter), methanogens dominate the growth of methanotrophs bacteria to produce anaerobic methane, thereby making the soil a source of methane.

The diagram below (Fig. 1) shows the sources of the crucial GHGs (CO_2, CH_4 and N_2O) emissions from the forestland, grassland, barren land, cropland and wetland. It shows the results of soil degassing following the activities of land use, fertilizer application, tillage and growing plants for food production which have contributed to over 20% of global anthropogenic GHG emissions. Wetlands, with restricted land cover area of 2.7% produced the highest total emission rate (in CO_2^{-eq}), followed by forestlands, grasslands, croplands and barren lands in a decreasing order. The figure even shows the variations in each land cover emission results. The result is also a factor of differences in land use management and climatic conditions from the five land cover types, and these differences notably are crucial to the GHGs emission from the soils. Therefore, an annual estimate of >350 Pg CO_2-eq global GHGs emission from all combined land cover areas correlates with the approximately 21% of the global amount of nitrogen and carbon in the soils (Oertel et al., 2016).
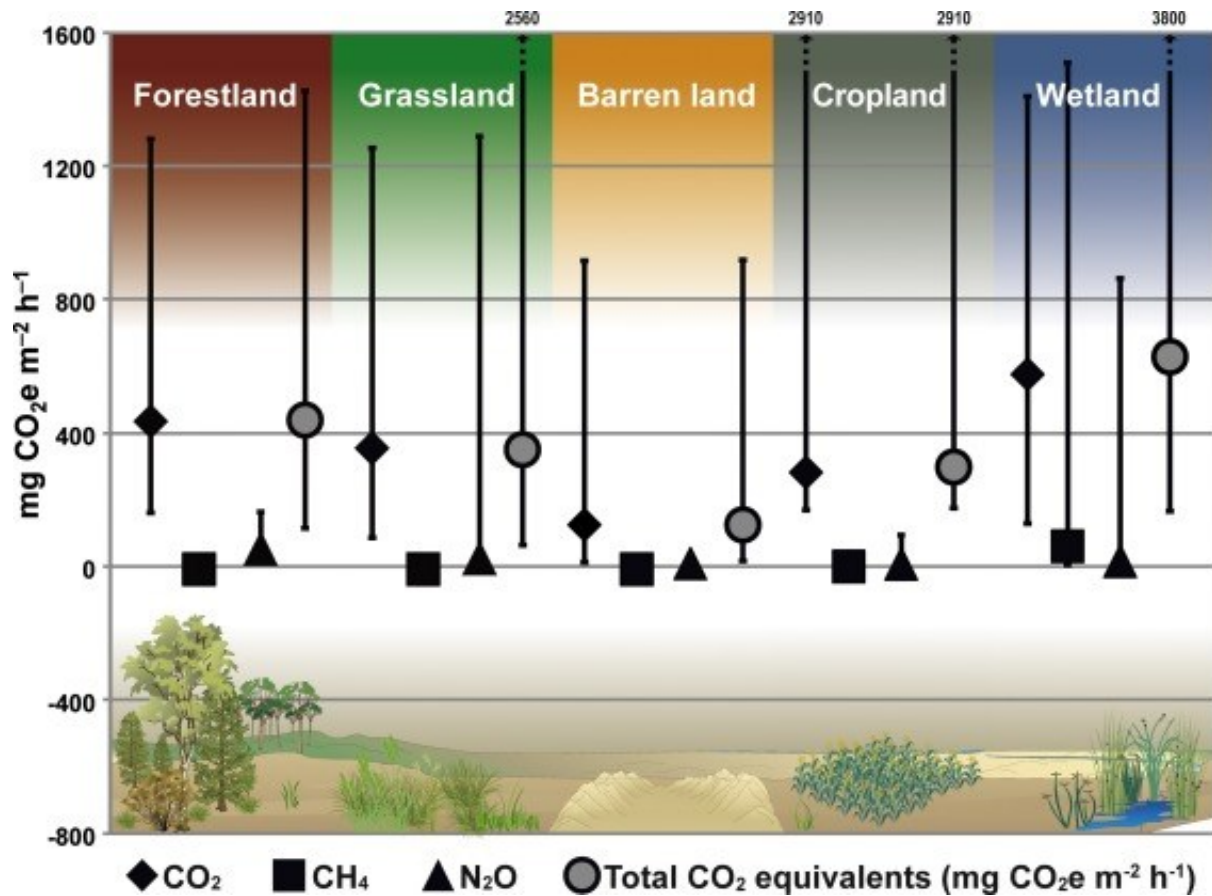
Fig. 1: The emission of greenhouse gases (CO_2, CH_4 and N_2O) from five land areas (forestland, grassland, barren land, cropland and wetland). The symbols show mean values and solid lines indicate the emission range (Source: Oertel et al., 2016)

The emission of greenhouse gases from agricultural soils remains a global concern, because an annual concentration of about 21% NO, 35% CO_2, 47% CH_4 and 53% N_2O gaseous emissions were released from the soil activities (IPCC, 2007). The major source of GHGs from soils is linked with CH_4 (methanogenesis) and N_2O (nitrification and denitrification) since post-industrialization, and agriculture has been the main source of these emissions (Forster et al., 2007). An estimation of over 13% of human contribution to GHGs, involving more than 60% of N_2O and CH_4 has been linked directly to agricultural activities and soils (Ren et al., 2017).

The key actions involved in the emissions of GHGs from agricultural soils are shown and shortly described in the diagram below (Figure 2). Soil humidity plays a vital role in the emission of gases from the soil because it is the parameter for controlling microbial activities and similar

steps (Oertel et al., 2016). The oxygen located in the soil pores are essential for nitrifying bacteria such as nitrite-oxidizing bacteria (NOB) and ammonia-oxidizing bacteria (AOB). Soils that has little water-filled pore space (WFPS) would allow more emission through nitrification, and at maximum when the WFPS is 20% (Ludwig et al., 2001). Soil temperature is another key driver because any increase in soil temperature results in increasing emission rate due to faster rate of soil respiration and positive microbial activities. A spontaneous rate of soil respiration and high soil temperature can force the emission of CH_4 and N_2O gases, with lower concentration of soil oxygen (Schindlbacher et al., 2004). The emission rate of CO_2 and NO have been reported to sporadically increase with high temperature (Tang et al., 2003). Land use management also affects the rate of GHGs emissions form the soil, especially in the cultivation of grassland, forestland and peatlands for agricultural purposes.

It's been reported that within three decades of cultivating forestland for agricultural purpose, the soil loses more than 33% of soil carbon found in about 7cm soil top layer, and no further significant changes after deep ploughing (Degryze et al., 2004). Vegetation period and varieties influences the emission rates of GHGs by altering the soil respiration rate. Vegetation impacts the emission of CH_4 and relates positively with the overall community of living organisms (Dalal and Allen, 2008). Higher concentrations of CO_2 in the soil has been linked with massive root size because of increasing CO_2 concentration in the atmosphere (Dorodnikov et al., 2009). Soil nutrients availability has valuable roles to play in plant respiration and microbial processes. Therefore, application of fertilizer or manure, amount of soil nitrogen and carbon content, and acid rain deposition are key actions involved in the soil GHGs emission. Also, soil respiration activities, soil moisture and soil temperature respond varyingly to nitrogen application (Peng et al., 2011). Overall, improving soil nitrogen content positively impacts soil respiration.
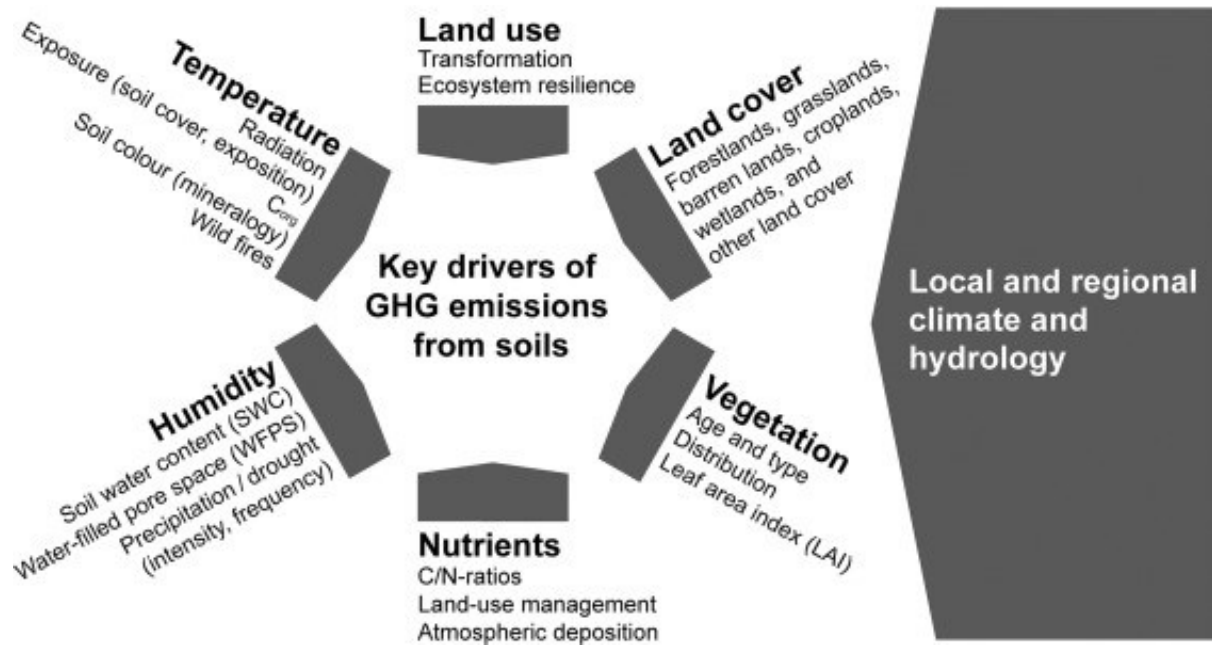
Fig. 2: Active forces of greenhouse gases emissions from soils (Source: Oertel et al., 2016)

2.2   NITROGEN CYCLE

Nitrogen remains asserted as the most abundant element found on the planet by existing in the atmosphere with a concentration of 78.09%, as the yearly global application of fertilizers rises by 15Tg (Fields, 2004). It plays a vital role as a crucial element of most biological life, especially in protein build-up and deoxyribonucleic acid (DNA). The biogeochemistry study of the complex nitrogen cycle explains how nitrogen, from its natural state (N_2) transforms into needful components required for biological use. The atmospheric state of natural nitrogen(N_2) is not easily absorbed by many organisms, therefore it requires conversion into a stable or organic form for easy assimilation. This process of conversion is known as "nitrogen fixation" and it can effectively be achieved via some biological steps. The initial step starts with the deposition of atmospheric nitrogen on the waters and soils surfaces, solely by precipitation. Then, the precipitated nitrogen undertakes some processes which breaks the nitrogen atomic bond via heat to combine with hydrogen, which ultimately forms NH_4^+. As a biological process, there are three types of microorganisms involved in the breakdown, such as the algae, symbiotic bacteria surviving on

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plants species and free active bacteria. Nitrogenase is a type of enzyme used in the N-fixation to break the molecules of inert nitrogen into separate two atoms for further reactions. Apart from the lightening spark that breaks atmospheric nitrogen into HN_4^+/NO_3^-, nitrogen fixation can also be achieved through man-made effort, which is the industrial production of N-rich fertilizers and NH_3.

To briefly explain the crucial stages of nitrogen cycle from nitrification - assimilation – ammonification – denitrification; when N-fertilizers are applied to soils, nitrifying bacteria (e.g. nitrosomonas) helps to convert ammonium (an absorbable but toxic nutrient for many plants) into NO_3^- (which is easily absorbed by plants) through nitrification process. Then, plants assimilate the NO_3^- from the soil for protein formation. The by-products of this nitrification process are N_2O and NO gases which are released into the atmosphere. Denitrification is also a process that favors the loss of gaseous N_2O, NO and N_2 into the atmosphere. It is promoted by insufficient oxygen in the soil (e.g. water logging) and denitrifying bacteria (e.g. pseudomonas) which converts soil NO_3^- and NO_2^- into N_2O, NO and N_2 (Fig. 3). When soil organic matter is mineralized into soil nutrients, it forces the release of NH_4^+ into the soil. Then, soil microbes activities become highly activated in the presence of NH_4^+. Furthermore, NH_4^+ can easily be converted to NH_3 in the presence of high temperature and soil acidity, and released into the atmosphere through a process known as 'ammonia volatilization'. Surface conversion promotes higher loss of NH_3 into the atmosphere.

When nitrogen fertilizers are applied to soil uncontrollably, it risks washing away of the soil mineral NO_3^- below the root depth (especially during winter rainfall) through a process known as 'leaching'. The death of plants and animals releases some wastes which are decomposed by microorganisms such as decomposers, thereby recycling the nitrogen in the organic matters back into the soil for further biological transformations through a process known as 'ammonification'. The by-products of this decay process are either NH_3 or NH_4^+.

2.3 ATMOSPHERICALLY IMPORTANT N-GASES

2.3.1 Nitrous Oxide (N2O)

As mentioned earlier, N_2O is a potent GHG gas in the atmosphere with most emissions from key agricultural activities such as application of manures and fertilizers to agricultural soils. The biotic processes by which N_2O is mainly produced from soils are anaerobic denitrification and aerobic nitrification. Mostly, important factors that promote the emission of N_2O from soils are agricultural practices such as the amount of fertilizer use, types of fertilizer applied, crop varieties and soil conditions such as soil texture, acidity, soil organic matter and moisture (Hénault et al., 2012). N-fertilizers applied to agricultural soils in order to improved crop yields contain mineral nitrogen in form of HN_4^+ and NO_3^-. Plants can easily assimilate NO_3^- for growth, but they require conversion of NH_4^+ with the help of soil microbes through a nitrification process into easily absorbable NO_3^-. The nitrifying microbes take in the NH_4^+/NH_3 and exhale oxygen in the process, but they are unable to convert the total NH_4^+/NH_3 available into NO_3^-. In the process of this incomplete conversion, some amount of N_2 is loss into the atmosphere in form of N_2O gas. Denitrification on the other hand complements nitrification by converting NO_3^- into harmless N_2. However, it takes an opposite direction whereby the microbes consume carbon compounds and respire NO_3^- rather than oxygen. Just like in nitrification, denitrifiers can't completely convert all the soil NO_3^- into N_2, thereby releasing N_2O in the anaerobic process. Nitrous oxide is easily released, with higher concentration when the conversion takes place on the soil surface. Recent studies showed that in annual crops, the results of maximum N_2O produced when applied fertilizers are regulated is about 50% of total N_2O emission (Shcherbak and Robertson, 2019). High soil temperature also favors a complete production of N_2O.

In perennial crops, it was found that the total surface emission of N_2O contributes about 20% more than in the subsurface production (Shcherbak and Robertson, 2019). Grazing pastures increase the contribution of total N_2O emission because of readily available animal waste such as urine and excreta, when compared to ungrazed fields. Studies have shown that key factors involved in N_2O loss from grazed fields are animal wastes, WFPS and N-input (Saggar et al., 2004). Animal

waste such as urine increases the soil pH through urea hydrolysis, and depending on the temperature, converts urea into ammonium within few days of enzymatic activities. Then, the NH_4^+ undergoes nitrification process which results in the loss of N_2O into the atmosphere. In soil with 80% WFPS, there are usually less reductive conditions affecting the complete conversion of NO_3^- into N_2, thereby leading to significant N_2O emission (Ciarlo et al., 2007).

2.3.2   Nitrous acid (HONO)

HONO is one of the essential N-gases which power of reactivity in the atmosphere rises through the formation of OH^- in photolytic reaction and oxidizing capacity pollutants such as CH_4. Although, HONO is not a greenhouse gas but rather a potent producer of OH^- in the air with a donation of more than 53% of OH^-, especially in daylight (Elshorbany et al., 2009). It is a component source of acidic rain from the chemical reaction of highly soluble NO_2 with $H_2O_{(g)}$ and photolytic reaction of nitric acid. The importance of HONO is found in the atmospheric chemistry where it plays a critical role in light absorption and quick generation of OH^- through photolysis. Alicke et al., (2002) and Acker et al., (2006) posited that concentrations of HONO varies from 5 part per billion in urban regions to around 0.1 parts per billion in rural settlements. There are notably other pathways to HONO emissions, such as traffic exhausts, humid heterogenous surface reaction and other biochemical sources. Recent studies have identified agricultural soils as sources of HONO emission, and this emission rate has strong correlation with available NO_2^- concentration and microbial activities in soil (Maljanen et al., 2013). Although, there have been many suggestions about the relationships between N_2O, NO and HONO emissions from agricultural soils and the effects on global warming, with response to N-input (Bhattarai et al., 2018). Yet, most previous studies have not been able to pinpoint the precise pathway of HONO emissions through the application of N-fertilizers to agricultural soils. Notably, previous studies have already explained that observations in the pathway of HONO emission is a factor of relationship between the soil acidity (pH) and ammonium oxidizers. Nitrous acid and nitric oxide are products of biological actions known as nitrification and denitrification (Pilegaard, 2013). Soil nitrite has the ability to be converted into gaseous HONO, with respect to soil acidity (Fig. 3), and HONO and NO are capable of being emitted from natural soils and biocrusts (Hannah et al., 2018).

Agricultural soils with high pH are expected to have more available NH_3 for increasing nitrification rates, thereby contributing to HONO emission. Interestingly, Su et al., (2011) suggested that even at low pH, a small concentration of NO_2^- may result in high HONO emissions. Animal manure have been good sources of nitrogen nutrients for agricultural purposes (Jiang et al., 2005).

2.3.3   Nitric Oxide (NO)

Nitric oxide is a highly responsive gas that plays a crucial role in the atmosphere by contributing to the forming and depletion of the ozone stratosphere, which leads to increasing oxidizing hydroxyl radicals of the atmosphere (Pilegaard, 2013). It is a noxious colorless gas that is formed from a heated chemical reaction between nitrogen and oxygen, as a result of burning fossil fuels at high temperatures. NO is a strong air pollutant also known as nitrogen monoxide. The concentrations of NO in the atmosphere varies in the environmental air, but more background studies revealed that it is around 0.01ppm in natural air and increases by 20 times – 0.2ppm in highly polluted air. Agriculture has been identified as a crucial source of NO emissions but there are still no clear significant differences report in NO emissions between the agricultural practices in the tropical and temperate regions, however its most recent value from the universal soil source is about 21 Tg Nyr^{-1} (Davidson and Kingerlee, 1997). Globally, the anthropogenic effect on NO emission from agricultural soils is about 10%, and it is crucial in the wearing off of ozone's troposphere (Laville et al., 2009). Research has shown that the major sources of NO in the soil are nitrification, denitrification and nitrate ammonification (Baggs, 2011), but majority of soil studies suggested that the utmost source of NO emissions is through nitrification (Skiba et al., 1997). Soil microbial activities have been traced as the source of biogenic emissions of 20% global NO from nitrification.

Further experiments found a pattern where the initial concentration of NO_3^- in dry soils increases with NO emission rate, but subsequent increase in concentration of NO_3^- does not affect the emission of NO (Wang et al., 2013). Thus, it implies that NO_3^- concentration is not the sole determinant of NO emission but other soil properties such as soil acidity (pH), moisture content,

temperature and atmospheric concentration of NO are key contributors (Ludwig et al., 2001, Obia et al., 2015).
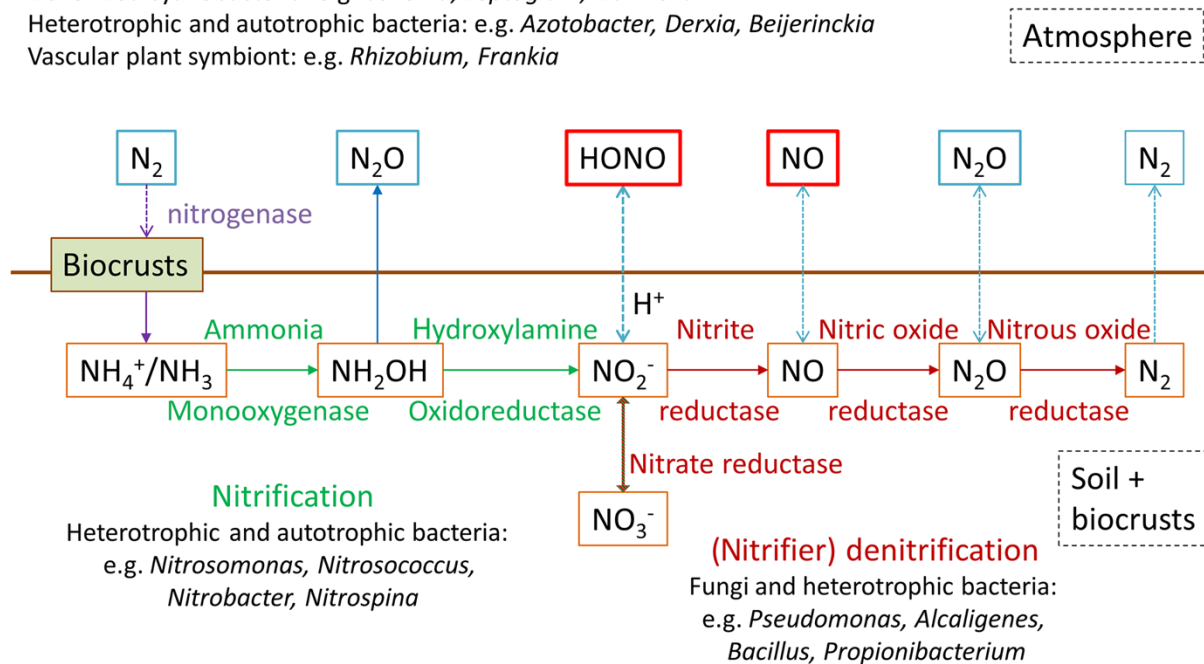
Fig. 3: The pathway of important nitrogen gases from the interphase of the atmosphere and the soil layers. Diagram reveals specific enzymes involved in the nitrification, denitrification, N-fixation and gaseous emissions. (Source: Hannah M et al., 2018)

2.4 EFFECTS OF INCREASING N-INPUT ON N2O, HONO AND NO EMISSIONS

The effects of increasing N-fertilizer application and production on nitrogen losses through N_2O, HONO and NO emissions and leaching continue to increase with adverse impact on the environment (Zhao et al., 2019). The extreme use of nitrogen fertilizer on crop fields such as vegetable has revealed the rate of nitrogen loss over the amount of production gained in the figure below, and this remains a global concern (Zhao et al., 2019). The figure below (Fig. 4) shows N-input effects on nitrogen losses in various control groups against the effect size (in percentage). The result revealed how increasing N-input impacts the environment with about 2.85 times of NO_3^-

leaching and 3.17 times of total N-leaching. The effect of nitrogen fertilizer can be seen in NH_3, N_2O and NO emissions with increasing percentage of about 174.95%, 200.78% and 540.50% respectively. When comparing the nitrogen application to N- losses, there seems to be a notable increase of about 23.95% nitrogen uptake and 34.95% vegetable yield. The numbers in each parenthesis were recorded from control grouped observations.
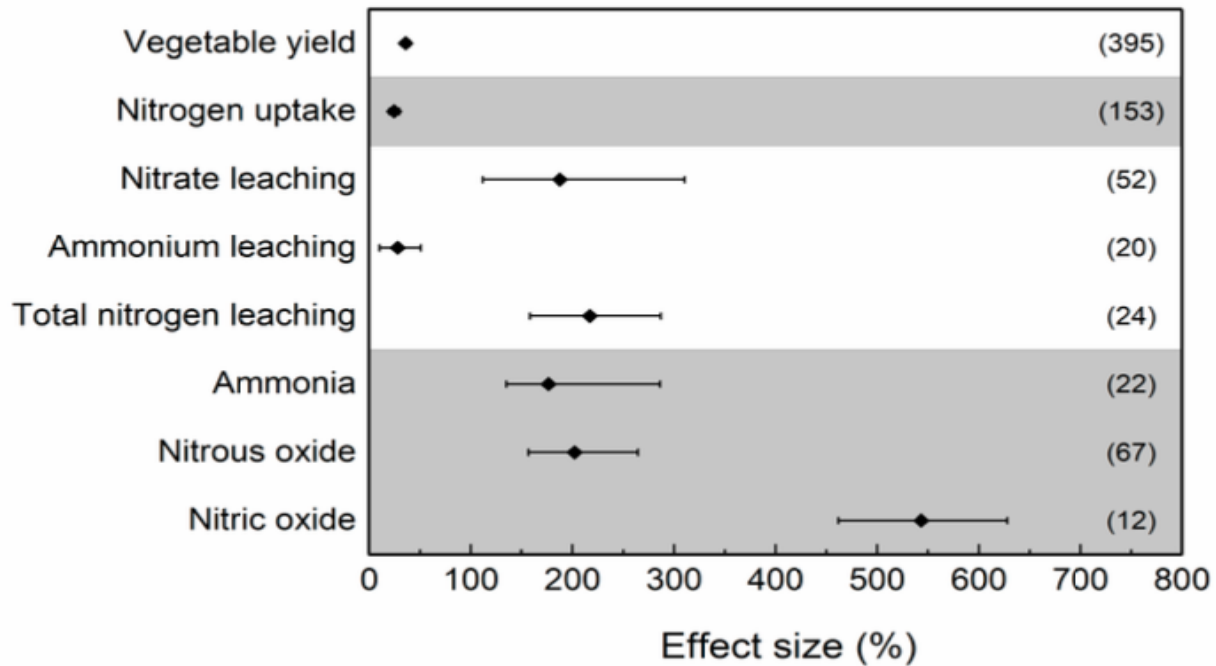

Figure 4: N-input effects on nitrogen losses and production. The percentage form in (R-1) x 100% shows the magnitude of the fertilizer effects. Letter R denotes the correlation between the results of the treatment and control groups. The bars denote 95% confidence level. The numbers in each parenthesis are specified from the grouped observations (Source: Zhao et al., 2019).

Combating the amount of nitrous oxide emission from fertilizer application would remain a global challenge because of the economic importance of N-rich fertilizers in agricultural food production and profitable revenues for farmers. However, the direct effect of higher N_2O emission from soils can be traced to increasing global-warming hazards. Soil nitrite is a crucial source of HONO, whereas nitrite is the product of nitrogen fertilizer application. The oxidizing nature of the atmosphere has intensely been influenced by agricultural practices such as fertilizer application and land management. While previous studies have reported the effects of N-input on N_2O and NO emissions from agricultural soils (Maljanen et al., 2007; Syväsalo et al., 2004; Bhattarai et al.,

2018) and recent research has shown that HONO emission can be released from germinating seeds in soils (Bhattarai et al., 2019). Thus, HONO emission becomes a vital subject within different environmental regions. Nitric oxide and nitrous acid emissions show strong correlation with soil nitrite, and their emission pathway share basic similarities (Bhattarai et al., 2018).

Bhattarai et al. (2018) stated that the tendency of boreal soils with reduced organic matter (majorly through tillage) of about 2.7% and C: N of around 10.6, to release increasing HONO and NO emissions could be linked to the mechanics of soil nitrite and acidity. Since fertilizer application increases the concentration of NO_2^- through nitrification and denitrification, thereby increasing the pool of ammonia and hydrogen ions and releasing HONO and NO in effect, hence increasing N-input is expected to correlate positively with higher HONO and NO emissions.

3.0   OBJECTIVES AND HYPOTHESIS

The main aims of this study;

- To understand the effect of nitrogen input on greenhouse gases (especially N_2O) emissions, nitrous acid (HONO) and nitric oxide (NO) emissions in three soils with different N-input (horse paddock, cultivated and fertilized hay field and grassland with minor management)
- To study the emissions of N_2O, HONO and NO during germination of hay seeds from horse paddock and hay field soils.

Research hypothesis;
- Null hypothesis (Ho): Varying nitrogen input does not affect N_2O, HONO and NO emissions in three closely related soil treatments.
- Alternative hypothesis (H_1): Varying nitrogen input affects the emission of N_2O, HONO and NO in three closely-related soil treatments

Research hypothesis (seeding experiment);
- Null hypothesis (Ho): N_2O, HONO and NO emission do not correlate with seedling length between two close-related soil treatments
- Alternative hypothesis (H_1): N_2O, HONO and NO emission correlates with seedling length between two closely-related soil treatments.

4.0   MATERIALS AND METHODS

4.1   Soil study and sampling sites

All experiments were performed at the University of Eastern Finland, Kuopio Campus. Soil samples were collected at a horse farm at Ranta-Toivala which is about 25 km from the Campus. All samples and gases were treated, analyzed and measured in the Biogeochemistry Laboratories, Kuopio.

Figure 5: Soil sampling sites from L-R (hay field in May, hay field in July, horse paddock in May, horse paddock in July and grassland in July, 2019).

The above pictures show the differences in soil conditions during spring and summer time sample collection. Temperature range was about +10ºC - 29ºC.

The soil samples were collected into sizeable well-labelled containers of about 15kg each, from three different sampling sites (Hay field, Horse Paddock and Grassland), at five different times (May 15, 2019, May 27, 2019, June 11, 2019, July 9, 2019 and July 31, 2019). Then, the samples were moved into the laboratory for different experiments.

Hay field is essentially known for growing grass for animal feeds. It is regularly ploughed and fertilized with mineral fertilizer. The sampling hay field was fertilized on May 15, 2019 with 315 kg/ha of Yara Mila (N 24.6%, P 3% and K 5.6%). Grassland with natural grassland (several

species), minor practices, only mowing couple of times during summer. Horse paddock with 4 horses daily (7-16) throughout the year. Almost all vegetation was eaten by horses. It also has heavy manure input.

4.2   Experimental Design

A 3 x 5 factorial experiment to determine the emissions of N_2O, HONO and NO from different soils, with 3 replications was used for the experiment.
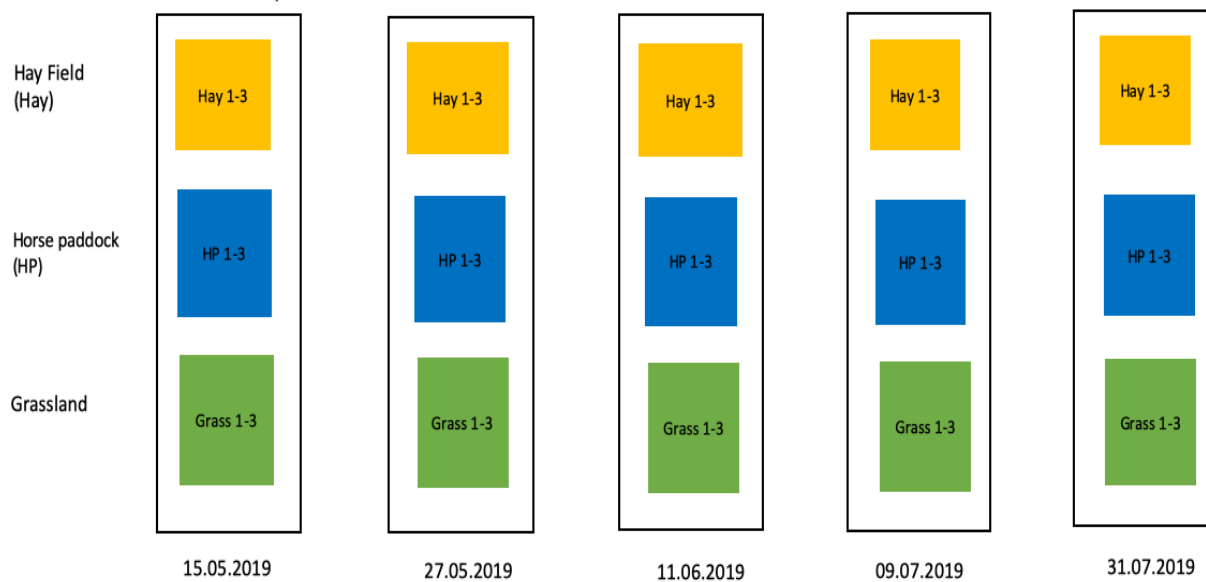
Figure 6: Experimental design showing the five sampling points with the treatments.
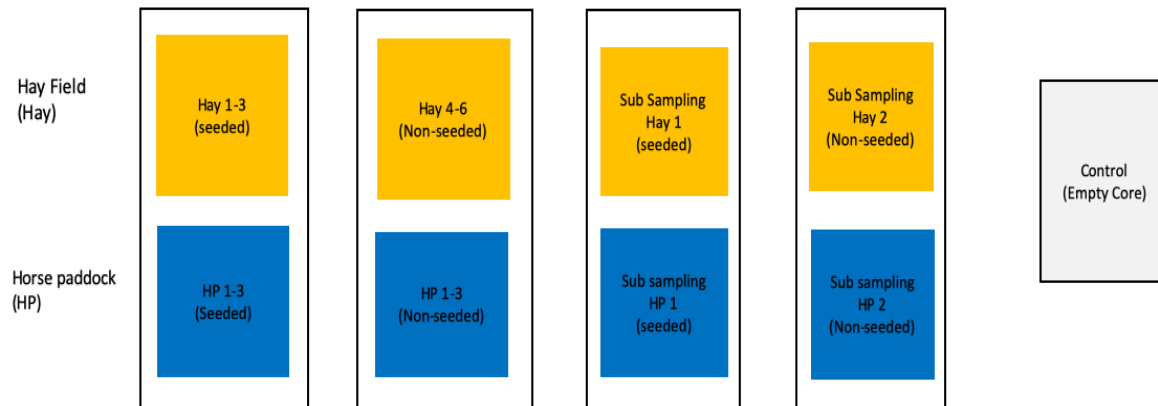
Figure 7: Seeding experimental design showing two soil types; hay field and horse paddock for July 31, 2019 sampling time.

4.3 SOIL ANALYSIS

4.3.1 Sample Preparation

The collected soil samples were carefully sieved with a manual metal sieve (12" diameter and 0.6mm mesh size).

4.3.2 Gravimetric moisture content

For the determination of gravimetric moisture content (GMC), each soil sample was weighed into well-labelled empty petri dishes of diameter 90mm/9cm. The total weight of fresh soil in dishes were recorded and gently placed an oven of temperature 105^0C (mineral soil, OMC < 20%) for 24 hours. The results were calculated using the following equations;

Calculate the percentage (%) of water and dry matter content was calculated using this equation:

Water content [%H2O] = ((fresh weight(g) – dry weight(g)) ÷ fresh weight(g)) x 100%

Dry matter content [%DW] = 100% - water content [% H_2O] = (dry weight(g) ÷ fresh weight(g)) x 100%

Gravimetric water content was calculated as water mass per mass of dry soil. Thus, the result can exceed 100% for wet soils, like peat soils.

Gravimetric water content = ((fresh weight(g) – dry weight(g)) ÷ dry weight(g)) x 100% = gH_2O/ dDW.

4.3.3   Organic matter content

To determine the organic matter content (OMC), the collected dried soil samples from GWC experiment were grinded into finer granules and weighed (less than one-third of the crucible size) into coded crucibles. Total weight was taken with a sensitive scale of about (accuracy 0.0001mg) and gently placed (with the aid of gloves and forceps) in an oven of temperature 550^{0}C for 2 hours. After sampling collection and cooling by 0^{0}C in a desiccator, the residue of ignition was calculated using the following equation;
The residue of Ignition was calculated using the formula:

Residue of ignition = (weight of crucible + residue) – (weight of empty crucible)

The part of the soil that is left in the crucible = residue of ignition (equivalen to the ash content)

The part of the soil that has been burned (loss of ignition) = organic matter (OM).

Organic matter (g) = weight of dry soil (g) - residue on ignition (g).

The proportion (%) of organic matter was calculated using the formula:

%OM = (Loss of ignition (g)/ Weight of dry soil in crucible (g)) x 100%
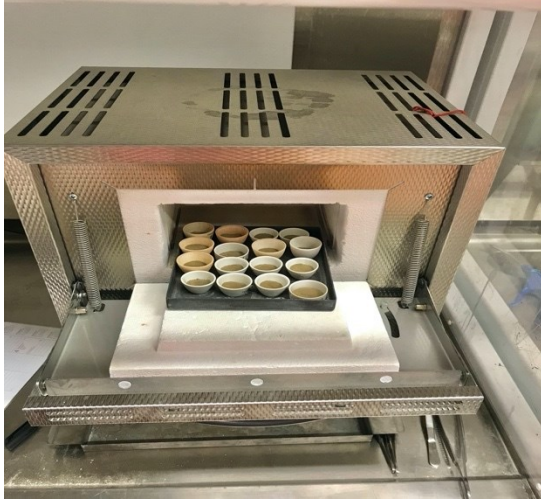
21

Figure 8: Crucible + fresh soil in the Oven

4.3.4 Analysis of pH and EC

For analysis of soil physiochemical properties, soil pH and EC (electrical conductivity) readings were taken from soil: milliQ-H_2O slurry (30:50 v/v) with a pH meter (WTW, pH340) and an EC meter (WTW pH/cond 340i), respectively.

Figure 17: Soil-liquid slurries for H_2O and KCL extractions

4.3.5   Analysis of nitrite, nitrate and ammonium

For analysis of nitrate (NO_3^-) and nitrite (NO_2^-) analysis, 30 ml of soil and 100 ml milliQ-H_2O were shaken in 250ml plastics bottles for 2hr, filtered and analyzed with an ion chromatograph (DX 120, Dionex Corporation, USA) (Maljanen et al., 2013). In the soil extraction with 1ML KCL, 15g of each fresh soil sample was weighed in 250ml plastic bottles and was shaken similarly as H2O extracts. Paper tapes were used to mark the codes on each bottle with blanks included. The solution is then filtered and analyzed. The analysis of ammonium is based on color reaction and after adding the reagents. The samples are analyzed with a spectrophotometer at wavelength of 550nm. To prepare the color reaction, 50µL of each sample and standard was pipetted into a well of microtiter plate. Two standard series line were pipetted on the 96 plates (using multi pipette). Fresh MilliQ water was added as blanks on both series line. Also, 50µL Sodium-phenate, 75µL Sodium-nitroprusside (0.01%) and 75µL Sodium-hypochlorite (0.02M)) were pipetted into the 96-plate solution.

Figure 18: Analysis of ammonium from H_2O and KCL extractions

4.3.6 Seeding experiment

Soil sampled from hay field and horse paddock samples were placed into well-labeled empty chambers treated with Teflon sheets and replicated into six (3 seeding and 3 non-seeding) treatments each. A total of 17 cores (12 sample cores, 4 sub-sampling and 1 empty core as background) were covered and kept under the light in the Biogeochemistry laboratory. In the seed experiment, each seeded core (19$\varnothing$) was treated with *Festuca perennis* (Italian raiheinä) grass seeds. It is an annual and continuing herbal grass grown for fodder and as a cover crop. It can be cultivated as ornamental plant.

Figure 9: Seeding treatment cores at the initial stage

The seeding experiment lasted a total of 10 days with daily monitoring of the seedling length and recording of the new core weight after moisture adjustment. In the moisture adjustment, the total weight of the cores before and after initial addition of water and seeds were taken. Then, every morning and evening, each core was placed on a sensitive scale and the moisture was adjusted with the aid of a water bottle containing milliQ-H_2O (see Fig. 10), until the cores return to the

initial weight (total weight of core after adding water and seeds). This process was repeated until the end of the experiment.

Figure 10: Moisture adjustment
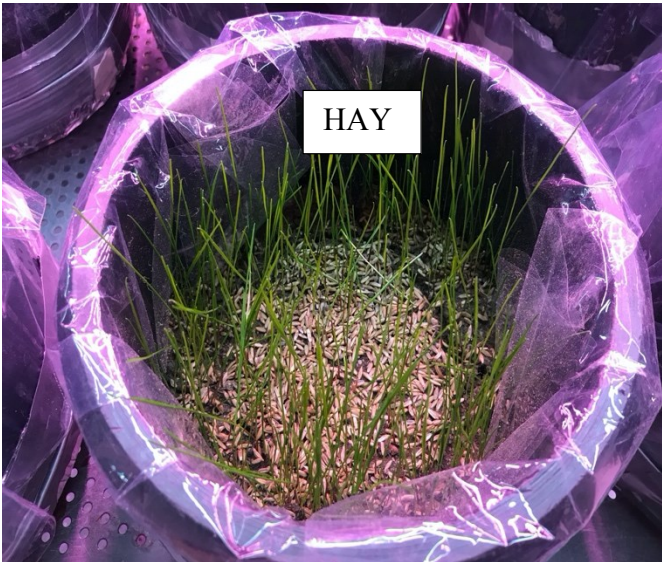
Figure 11: Germinating hay field seeded core
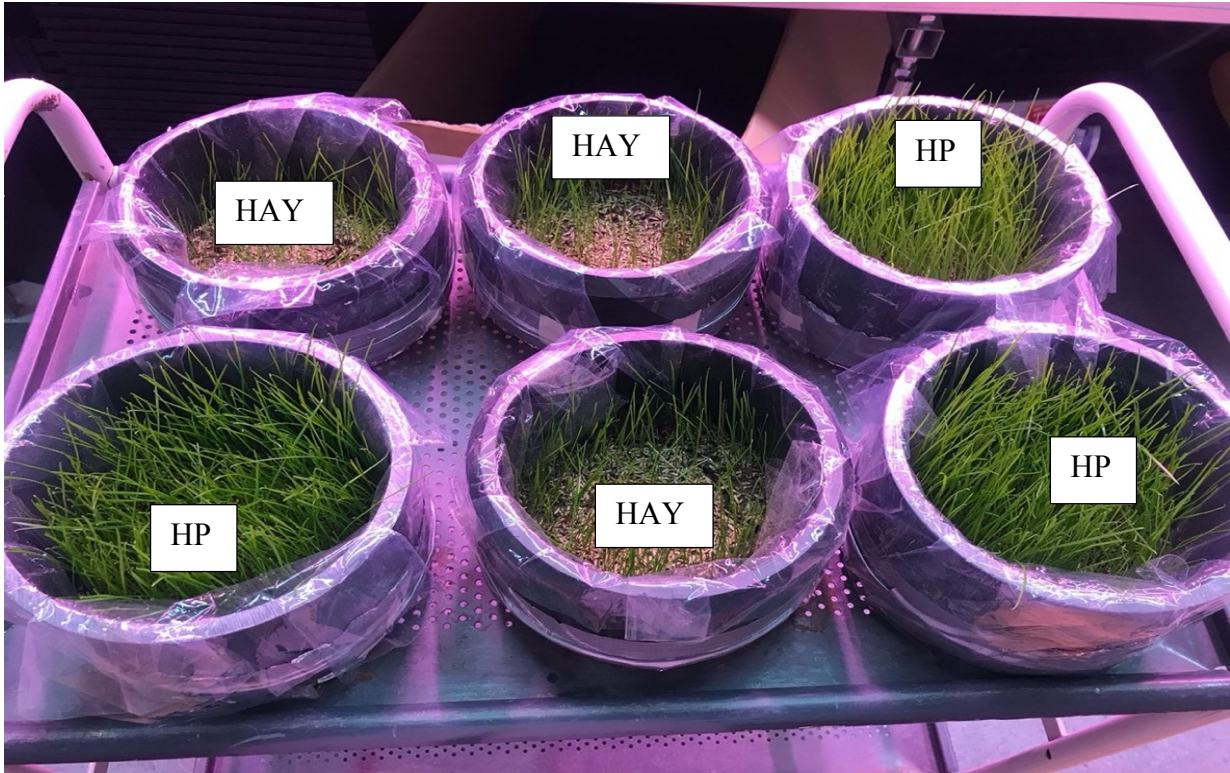
Figure 12: A germinating hay field and horse paddock seeded cores

4.3.7　Gas sampling from soil cores

The flux measurement and analysis of the greenhouse gases (CH_4, N_2O and CO_2) were carried out with a static chamber system and gas chromatography. PVC chambers ($V=6.11m^3$) was inserted over the coded soil cores and 25 ml gas samples was extracted from the chamber headspace with a 60ml terumo syringe at intervals of 5, 10, 25, 35 and 60 minutes after enclosure. Gas samples of 25 ml were injected (immediately) into evacuated 12 ml vials (Labco Exetainers, UK) and the concentration was analyzed using a GC (Agilent 7890B, Agilent Technologies, USA) provided with an auto sampler.

Figure 13: Gas flux measurement
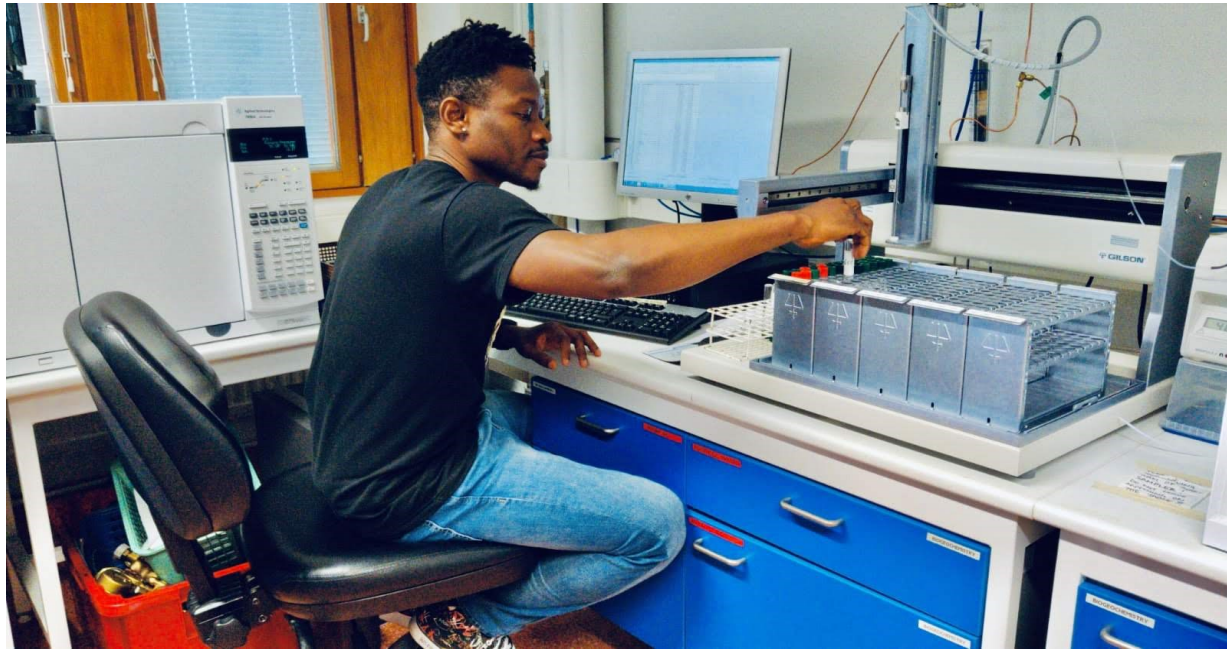
Figure 14: Gas flux analysis with gas chromatography

HONO and NO emissions were measured immediately from the collected soil sample cores from the greenhouse gas flux. HONO was measured by setting up a dynamic chamber system in a very low-light room temperature at $+21^0C$. The dynamic chamber system consisted of a 3.2 L Teflon chamber, which was connected with a LOPAP (Long Path Adsorption Photometer). At the top of the chamber, three Teflon tubes (ID: 4mm); inlet, outlet 1 and outlet 2 were used to flush the headspace purified air at a rate of 4 Lmin^{-1}, connect the external sampling unit of LOPAP, and to flush excess air from each system respectively. NO emissions were measured parallel to HONO from the same samples using the dynamic chamber system connected to a NO$_x$ analyzer (Thermo, MODEL 42i NO-NO$_2$-NO$_x$ chemiluminescent) with a detection limit of 0.4 ppb for NO. The air flow rate from the sample into the NO$_x$ analyser is about 550 ml min^{-1}. Time taken to measure each core was 50 minutes after 15 minutes flush with zero-air (synthetic air 79% N$_2$ and 21% O$_2$).

Figure 15: HONO measurement set up with the LOPAP device

Figure 16: The NOx analyzer in action

The NO_2, HONO and NO flux rates from soil were measured from the difference in concentration, between equilibrium and ambient concentration from the chamber with the following equations;

The HONO and NO fluxes were calculated using the formula:

$$F= (fv/1000) * (1/V_m) * (T_0/ (T_0 + T) K) * (p/p_0) * \Delta c / (10^9 * 60 * h * M)/A * 10^6$$

Where;

F is the flux rate (μg N m^{-2} h^{-1})

fv is the flow rate (cm^3 min^{-1})

V_m is 22.4136 l mol^{-1}

T_0 is 273.15K

T is temperature in the chamber (K)

p is air pressure (kPa)

p_0 is 101.3 kPa

ΔC is the concentration difference of NO or HONO (ppb)

K is slope of N_2O (ppm h^{-1})

V is volume of the chamber head space (m^3)

R is the ideal gas constant (8.314 J k^{-1})

M is the molar mass (g mol^{-1})

A is the sample area (m^2)

N_2O flux rate was calculated suing the formula:

$$F = (p_0 * k * V * M)/ (R * T * A) * 60$$

5.0   RESULTS

5.1   Statistics

Statistical analysis was carried out with aid of analytical software package, IBM SPSS statistics 25. The table below shows the statistical differences of all the three soil treatments, across the sampling times and their physiochemical properties. It explains the effect of N-input, temperature change, pH (early summer and mid-summer) and organic matter content on the major soil nutrients (NH_4^+, NO_3^-, and NO_2^-). The mean values of the treatments were used to analyze the statistical differences ($P < 0.05$) between the treatments, using One-Way ANOVA.

Table S1: Physiochemical properties of soil treatments (average ± S.D) for different sampling points. EC = Soil electrical conductivity, OMC = Organic matter content (µg of H_2O in 1g of dried soil). The different superscript letters reveal the statistical difference ($P < 0.05$) within the three treatments using One-Way ANOVA.

Sampling Dates	Treatments	pH (H_2O)	EC (µS cm⁻1)	OMC (µgH_2O gdw⁻1)	NH_4^+ (µg N gw⁻1)	NO_3^- (µg N gw⁻1)	NO_2^- (µg N gw⁻1)
15.05. 2019	Grassland	5.80 ± 0.90[a]	15.67 ± 4.72 [a]	4.91 ± 0.52[a]	2.17 ± 0.39[a]	0.58 ± 0.05[a]	0.38 ± 0.15[a]
	Hay Field	5.75 ± 0.20[a]	72.00 ± 1.00[ab]	7.79 ± 0.20[ab]	13.23 ± 1.07[ab]	21.55 ± 0.73[ab]	0.19 ± 0.17[a]
	Horse Paddock	6.99 ± 0.07[b]	124.67 ± 2.08[c]	6.65 ± 1.33[c]	35.46 ± 1.21[c]	0.87 ± 0.15[a]	3.97 ± 0.62[ab]
27.05. 2019	Grassland	5.96 ± 0.08[a]	14.00 ± 2.00[a]	4.63 ± 0.02[a]	0.70 ± 0.72[a]	0.57 ± 0.07[a]	0.09 ± 0.02[a]
	Hay Field	5.54 ± 0.08[b]	65.67 ± 1.52[b]	10.31 ± 0.05[b]	4.95 ± 0.12[b]	9.02 ± 0.60[ab]	0.09 ± 0.03[a]
	Horse Paddock	7.36 ± 0.12[c]	129.67 ± 15.82[b]	5.24 ± 0.21[c]	9.39 ± 0.95[c]	0.80 ± 0.04[a]	2.82 ± 0.22[ab]
11.06. 2019	Grassland	6.11 ± 0.11[a]	19.00 ± 3.00[a]	4.04 ± 0.02[a]	0.01 ± 0.01[a]	0.32 ± 0.56[a]	0.78 ± 0.04[a]
	Hay Field	6.13 ± 0.11[a]	35.33 ± 0.57[b]	11.58 ± 0.33[b]	0.02 ± 0.03[a]	2.06 ± 0.13[b]	0.19 ± 0.26[b]
	Horse Paddock	6.49 ± 0.24[ab]	160.33 ± 6.11[c]	6.43 ± 0.07c	16.11 ± 0.92[ab]	12.37 ± 1.36[c]	2.32 ± 0.34[c]
09.07. 2019	Grassland	5.98 ± 0.11[a]	22.67 ± 9.29[a]	5.70 ± 0.09[a]	0.00 ± 0.00	0.02 ± 0.01	0.00 ± 0.00[a]
	Hay Field	5.97 ± 0.05[a]	54.00 ± 2.64[a]	9.03 ± 0.11[b]	1.21 ± 0.61	3.40 ± 0.09	0.00 ± 0.00[a]
	Horse Paddock	5.83 ± 0.55ab	494.67 ± 63.25[ab]	6.85 ± 0.22[c]	3.65 ± 1.33	55.83 ± 4.31	1.20 ± 0.12[ab]
31.07. 2019	Grassland	5.59 ± 0.06[a]	31.67 ± 1.15[a]	*	0.00 ± 0.00[a]	0.08 ± 0.07[a]	0.00 ± 0.00
	Hay Field	5.81 ± 0.01[b]	64.00 ± 1.73[b]	*	2.88 ± 0.81[ab]	2.94 ± 0.08[b]	0.00 ± 0.00
	Horse Paddock	6.05 ± 0.00[c]	298.67 ± 8.08[c]	*	0.00 ± 0.00[a]	33.61 ± 1.82[c]	0.01 ± 0.01

*OMC values were not determined.

5.2 Nitrogen gas emissions in samples taken at different timepoints during the summer

5.2.1 N$_2$O emissions

The first sampling (May, 15) of hayfield showed the highest N$_2$O emissions, with high significant difference ($P < 0.05$) within the three soil types (Fig. 19, C). The highest emission rate clearly correlates with the highest NO$_3^-$ concentration within the soil types (see Table S1). A similar pattern can be seen in the horse paddock summer sampling, June 11, where the highest N$_2$O emissions (Fig. 19 C) correlates with high NO$_3^-$ concentration, until the end of all the sampling time, and with clear significant difference ($P < 0.05$) (see Table S1). The grassland showed the least N-gaseous emission rates within the soil types and across the sampling time, with decreasing significant difference ($P < 0.05$). It also had the least (in decreasing rate) concentration of NO$_3^-$ availability across the sampling time. Another observation was that, a decreasing pH (< 7.0) concentration in each soil sampling time, correlates with increasing NO$_3^-$ concentration. There was also significant ($P < 0.05$) decrease in the soil pH (especially in the last three sampling) across all the soil samples with increasing EC and NO$_3^-$ concentration. There were not much significant differences found in the soil NO$_2^-$ concentration, except in the horse paddock sampling where most traces of soil NO$_2^-$ concentration significantly ($P < 0.001$) decreased across the all sampling times (Table S1). We observed that where NH$_4^+$ had been used up, there was increasing concentration of NO$_3^-$. In the statistical analysis, using One-way ANOVA to determine the impact of N-input on N$_2$O emissions, the results showed a significant ($P < 0.05$) test where N$_2$O emissions were not normally distributed.

5.2.2 HONO emissions

HONO emission rates steadily increased across all the horse paddock sampling, with a significant increase in the summer sampling (Fig. 19, A). We also found a significant ($P < 0.05$) increasing concentration of NO$_2^-$ in the horse paddock late sampling, whereas the NO$_2^-$ concentration in hay field and grassland was below detection limit ($< 0.01\,\mu g$ N gw$^-$1) (table S1). Although, a significant HONO emission was detected in early hay field sampling (May, 15), it however declined

progressively as the soil NO_2^- and NO_3^- concentration decrease and pH increases. A correlation between the soil pH and NO_2^- concentration was observed, especially in the horse paddock where reduced soil pH (< 7.0) favors high EC (up to 494.67 ± 63.25) and NO_2^- concentration (table S1). In the early summer grassland sampling, HONO emissions rates were significant ($P < 0.05$) and even higher than the hay field sampling (only in May 27 and June 11), before it nose-dived due to undetectable NO_2^- concentration (Fig. 19A, table S1). In the HONO emission statistical analysis, the One-way ANOVA test showed a significant ($P < 0.001$) result that HONO emission was not normally distributed.

5.2.3 NO emissions

It can be seen clearly that NO and HONO emissions followed a similar pattern throughout the sampling time (Fig 19, B), where they both significantly correlate with low pH and available NO_2^- concentration. The slight difference was in hay field (June, 11) sampling, where the NO emission increased slightly above grassland sampling, with no significant difference ($P > 0.05$). Generally, there were no significant differences in NO emission rates between the early soil sampling treatments (Fig. 19, B). In the NO emission statistical analysis, the One-way ANOVA test showed a significant ($P < 0.001$) result that NO emission was not normally distributed.
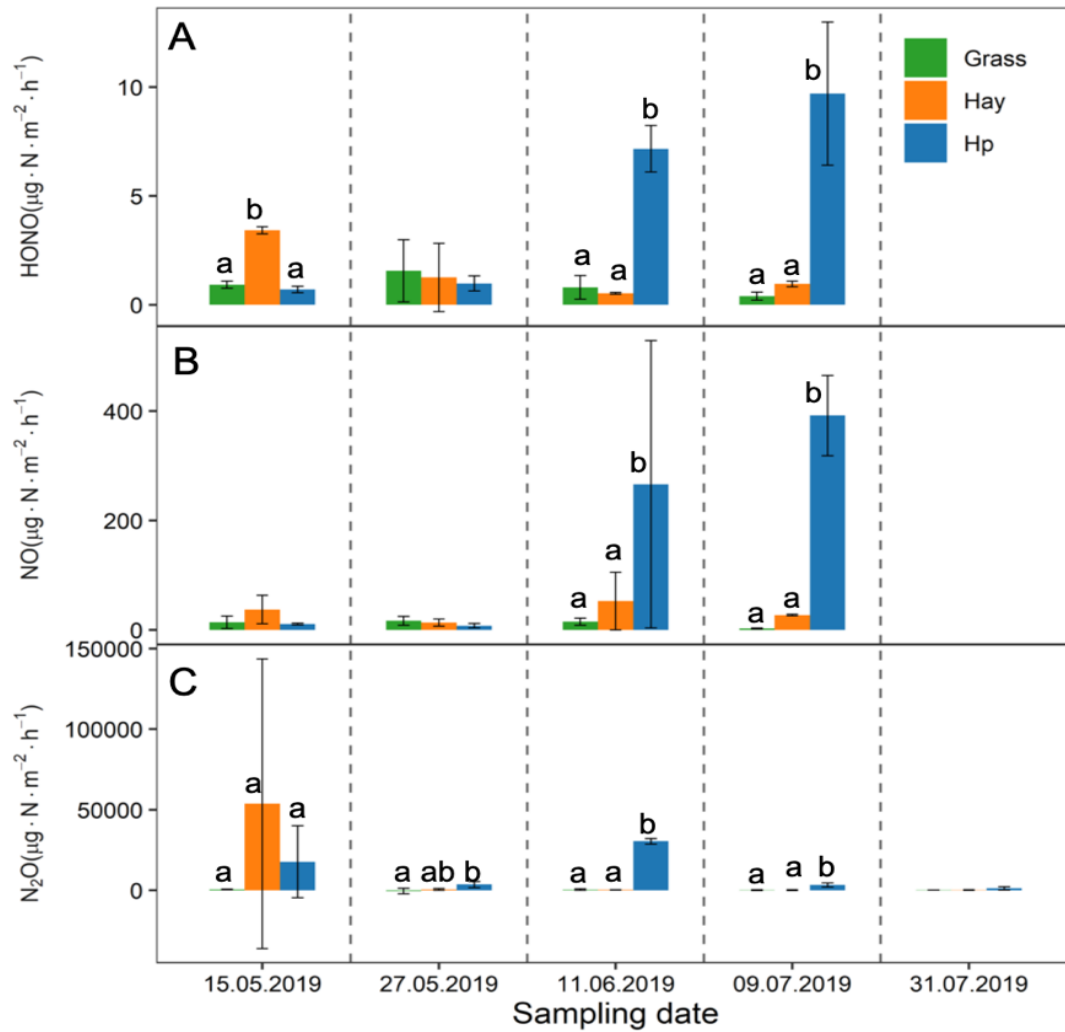
Figure 19: Gas fluxes and emission rates of HONO (A), NO (B) and N_2O (C), from the three soil treatments; Grassland (green), Hay Field (orange) and Horse Paddock (blue) during five sampling points. Error bars (S.D, n = 5) denotes the standard deviation of three replicates with different letters showing the statistical significance within the treatments and sampling dates (One-Way ANOVA, P< 0.05). The bars without letters lacks significant difference within the treatments.

5.3   Sampling time experiment for soil analysis

High soil NH_4^+ concentration was significantly (P < 0.05) detected, especially across the horse paddock sampling. The horse paddock had the highest NH_4^+ concentration, followed by the hay field and the grassland respectively. Overall, soil NH_4^+ concentration decreased significantly (P < 0.05) from the early sampling until the last sampling time across all soil types (Fig. 20A, table S1).

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Soil NO_2^- concentration clearly showed a steady reduction across the horse paddock sampling, until a non-significant (P > 0.05) concentration in the final sampling. This shows that the early horse paddock sampling had higher NO_2^- concentration and the concentration decreased as the soil temperature changes and the soil pH fluctuates significantly (P < 0.05). A sharp increase in NO_2^- concentration was noticed in the grassland summer (temperature of about 29^oC) sampling (June, 11), but it faded away in mid-summer sampling and even went under detection limit (< 0.01µg N gw-1) (Fig. 20, B). In the early grassland sampling, the NO_2^- concentration fluctuates slightly as the pH changes, until it went under detection limit of < 0.01 µg N gw-1 in the final sampling times (see table S1).

The NO_3^- concentration followed almost a reverse pattern to the NO_2^- concentration, except in the grassland sampling where the concentration was lowest with non-significant (P > 0.05) differences across the sampling time (Fig. 20, C). The soil pH in the grassland appeared to increase as the NO_3^- concentration in the grassland decreases and slightly fluctuates non-significantly (P > 0.05) as the NO_3^- concentration went below detection limit (< 0.01µg N gw-1) (see table S1). In the early summer hay field sampling (May, 15 and 27), a highly significant (P < 0.001) NO_3^- concentration was detected, when compared to the horse paddock and grassland. However, we noticed that as the soil moisture changes (due to increasing summer temperature), the soil NO_3^- concentration fluctuates slightly in hay field (Fig. 20C, table S1). The soil NO_3^- concentration clearly increased significantly (P < 0.001) in the horse paddock summer sampling until the end. The highest NO_3^- concentration (55.83 ± 4.31 µg N gw-1) was noted in the July, 9 sampling (table S1).
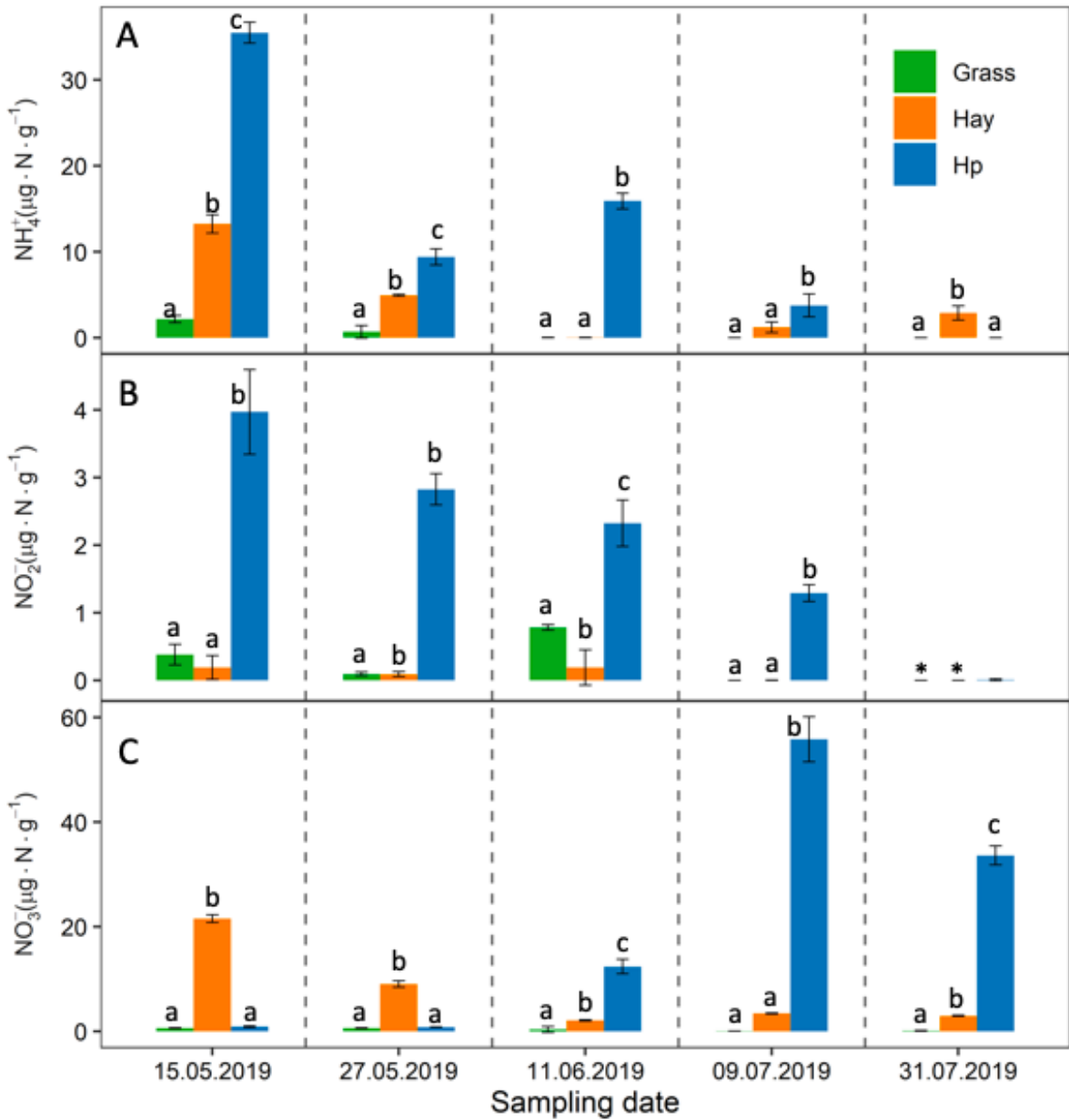
Figure 20: Concentrations of soil chemical properties NH_4^+ (A), NO_2^- (B) and NO_3^- (C) from three soil treatments; Grassland (green), Hay Field (orange) and Horse paddock (blue) during five sampling points. Error bars (S.D, n = 5) denotes the statistical difference within the three treatments (One-way ANOVA < 0.05), while lack of letters indicates no significant difference. *Shows the values under the detection limit of the device (* < DL, 0.01µg N gw-1).

5.4   Seeding experiment for gas fluxes

The seeding experiment which was completed within a total of 10 days (August, 4 -13) shows the NO and HONO emission rates with seedling length (in both the horse paddock and hay field's seeded and non-seeded treatments) from the initial measurement (August, 4) to the final measurement (August 13) (Fig. 21). The NO emission rate in the hay field seeded and non-seeded sampling remained about the same (about 12.0 µg $Nm^{-2}h^{-1}$) during the initial measurement (zero germination in the seeded hay field). However, during the final measurement, the emission rate of NO in the seeded hay field decreased to about 4.0 µg $Nm^{-2}h^{-1}$ against the increasing seedling length (of about 9cm), while in the non-seeded hay field, NO emission rate increased significantly (P < 0.05) to about 29.0 µg $Nm^{-2}h^{-1}$ from the initial emission rate (Fig. 21, A). A similar pattern was observed in the horse paddock seeded and non-seeded NO emission rates (Fig. 21, C). At the initial measurement, the NO emission rate in the horse paddock seeded treatment was around 12.4 µg $Nm^{-2}h^{-1}$ while in the non-seeded treatment, the emission rate was lower (about 7.5 µg $Nm^{-2}h^{-1}$). As the seedling length increases to about 11.8cm, the NO emission rate in the seeded treatment decreased significantly by 7.0 µg $Nm^{-2}h^{-1}$, while the non-seeded increased by about 2.9 µg $Nm^{-2}h^{-1}$ (Fig. 21, C).

In the HONO measurement, both the seeded and non-seeded hay field treatment maintained an emission rate of about 0.9 µg $Nm^{-2}h^{-1}$ at the initial stage (before seedling germination). Then, when the seedling length increased to about 9.0 cm, the final HONO emission rate in the non-seeded and seeded treatment increased to about 2.8 µg $Nm^{-2}h^{-1}$ and 0.9 µg $Nm^{-2}h^{-1}$ respectively (Fig. 21, B). The horse paddock HONO emission rate in the seeded and non-seeded treatments showed an initial emission of about 3.6 µg $Nm^{-2}h^{-1}$ and 1.5 µg $Nm^{-2}h^{-1}$ respectively. Then, we noticed an increasing emission rate of HONO as the seedling length increased to about 11.7cm. HONO emission rates in the seeded and non-seeded horse paddock treatment increased significantly (P < 0.05) to about 5.5 µg $Nm^{-2}h^{-1}$ and 4.2 µg $Nm^{-2}h^{-1}$ respectively (Fig. 21, D). HONO emission rates clearly increased as the seedling length increases in both soil treatments.
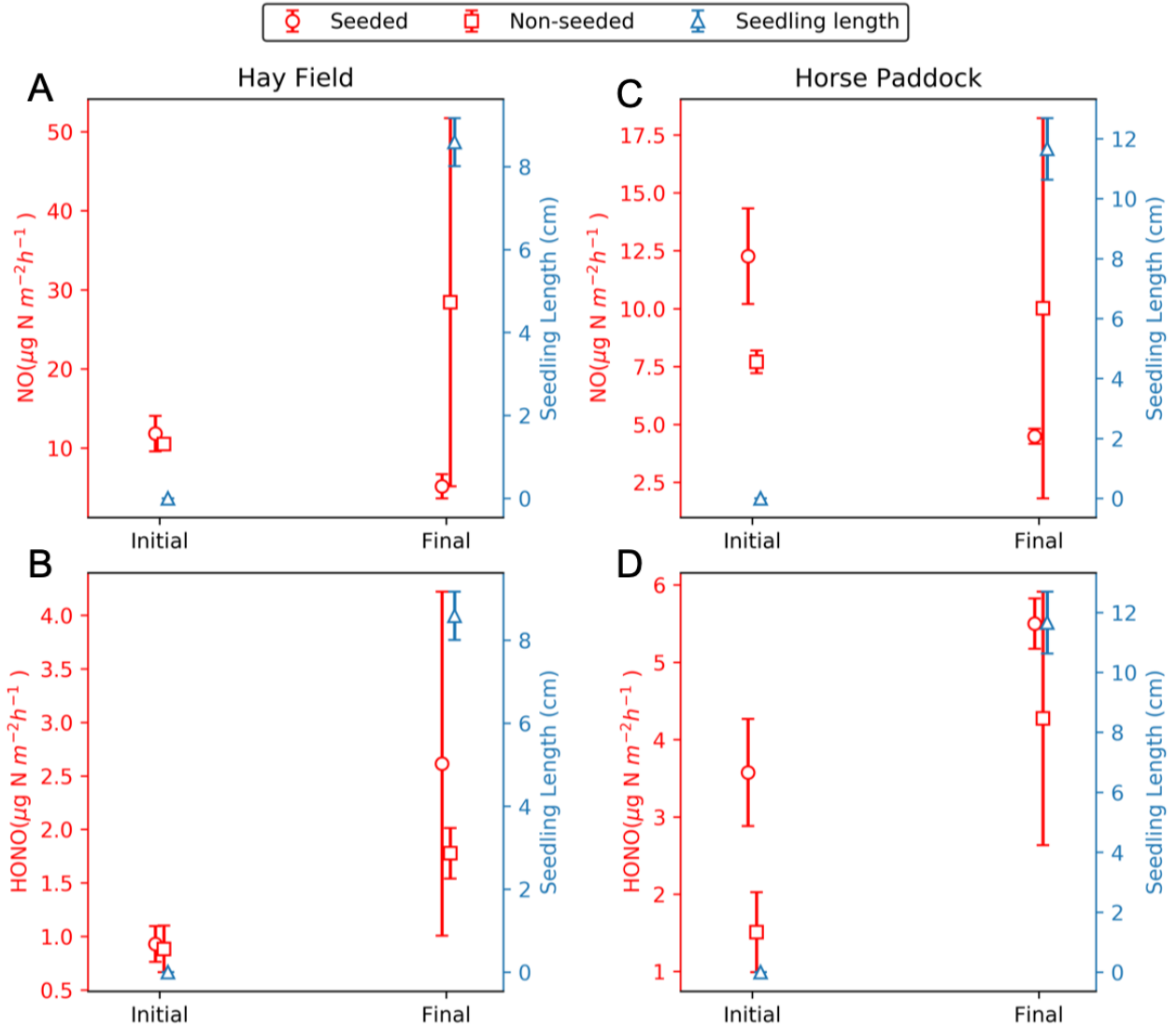
Figure 21: Emission rates of NO (A, C) and HONO (B, D) between two seeding soil treatments, Hay Field (A, B) and Horse Paddock (C, D) with seedling length, from initial gas measurement to the final stage. The plot shows the relationship between the gaseous emissions on the left x-axis (red) and the seedling length on the right x-axis (blue) against the stages of gas measurement, y-axis. Error bars shows the standard deviation of three replicates of each soil treatments and five points of the seedling height. Initial stage indicates the immediate planting of the seed, while the final stage indicates the fully germinated seedling.

5.5   Seed experiment for soil analysis

The soil analysis was done at the end of seeding experiment and after collecting the plant biomasses from the final day of seedling germination. We noticed a low soil NO_3^- concentration of about 2.92 µg N gw^{-1} and 0.66 µg N gw^{-1} in seeded hay field (S-Hay) and seeded horse paddock (S-Hp) treatments respectively, when compared to the high NO_3^- concentration found in non-seeded hay field (N-Hay) and non-seeded horse paddock (N-Hp) (Fig. 22, A). Also, the seeded horse paddock (S-Hp) showed a significant difference ($P < 0.05$) of higher NO_3^- concentration and seedling length than in the seeded hay field (Fig. 22, A). For the soil NO_2^- concentration, we noticed a general increase in soil NO_2^- concentration in both S-Hay and S-Hp treatments. The S-hay treatment showed a significant ($P < 0.05$) higher soil NO_2^- concentration with lower seedling height (about 8.59 cm), when compared to the S-Hp with lower soil NO_2^- concentration but significantly ($P < 0.05$) higher seedling length of about 11.66 cm (Fig. 22, B). However, the soil NH_4^+ concentration was below detection limit of 0.01 µg N gw^{-1} for all the treatments, hence its absence from the results figures.
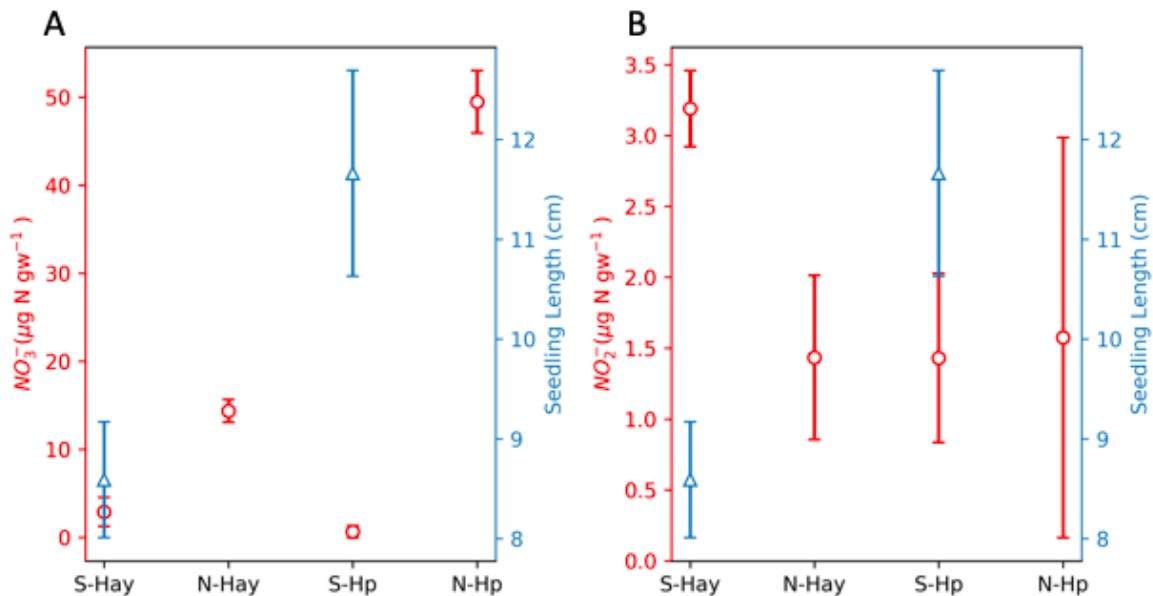

Figure 22: Concentration of NO_3^- (A) and NO_2^- (B) between in two seeded (S-hay field and S-horse paddock) and non-seeded (N-hay field and N-horse paddock) soil treatments with the seedling length. The plot shows the relationship between the soil nutrients concentrations on the left x-axis

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(red) and final seedling length on the right x-axis (blue) against seeded and non-seeded soil treatments, y-axis. Error bars denotes the standard deviation of three replicates of each soil treatments and five points measurement of the seedling height on the final day of full germination.

*NH_4^+ was below detection limit for all the treatments (< DL, 0.01µg N gw-1), reason for its absence.

6.0   DISCUSSION

The result from the gas fluxes revealed that the horse manure had the most significant HONO, NO, and N_2O emissions when compared to hay field and grassland. This can be traced to (1) ammonium oxidation, due to the notable highest concentration of NH_4^+, (2) hydrolysis of nitrite because of the traceable highest NO_2^- concentration, and (3) reduction of the highly present NO_3^- concentration. This suggests that nitrogen availability in the horse dung has positive correlation with traceable nitrogen input in the horse feeds. The gaseous emissions can also be traced to low pH and the presence of NO_3^- and NO_2^-. However, a disappearance in NO_2^- concentration as HONO, NO and N_2O increased was noticed throughout the sampling points. Previous studies have shown that NO_2^- pool has the potential to form NO and HONO gases, as most of their emissions have been linked to the presence of NO_2^- (Bhattarai et al., 2018).

Although, the hay field and grassland treatments showed very similar total nitrogen gas emission rates and nutrients concentrations between each other, yet more nitrogen appeared to be available in the hay field than in the grassland. This acknowledges the effect of higher nitrogen input in the hay field through the 24.6% N of 315 kg/ha fertilizer applied, shortly before the sampling collection. Also, the emissions of all the gases have a positive correlation with decreasing soil pH and the presence of NO_2^- and NO_3^- concentration across the sampling times. Our results show that N_2O emissions through denitrification process correlates with soil available NO_3^- concentration, and nitrification process through conversion of NH_4^+ to NO_3^- availability (Bhattarai et al., 2018). It is also logical to say that the NO and HONO gases behave similarly while N_2O emissions followed a different emission pathway within the tree soil treatments. An observation

from the last sampling point was that, as the soil moisture decreases, pH decreases and the N-gaseous emissions rates increase (Henault et al., 1998).

In the seed experiment, only two soil samples (hay field and horse paddock) were compared. In both the hay field (Fig. 21, A), and the horse paddock (Fig. 21, C) seeded treatments, it was observed that the NO emission decreases with increasing seed length. Nitric oxide has been identified as a dormancy-releasing candidate in seed germination by releasing major nitrogen nutrients such as NO_3^- and NO_2^- which are essential for seedling growth (Arc et al., 2013). We assume that majority of NO concentration has been converted into soil NO_3^- and NO_2^- for seed germination and seedling growth. However, in the HONO emissions we observed a positive correlation with the seedling length in both the hay field (Fig. 21, B) and horse paddock (Fig. 21, D) treatments. HONO emission increased totally as the seedling length increased, but the emission rates differ in the two soil treatments. The horse paddock had the highest emission rate of about 5.5 μg Nm^{-2} h^{-1} and seedling length (of about 11.7cm), while the hay field had lower emission rate of about 2.7 μg Nm^{-2} h^{-1} and seedling length (of about 8.8cm) respectively (Fig. 21 B, D). Past studies have shown that HONO emissions have been linked to soil NO_2^- and pH (Su et al., 2011). Therefore, we noticed a high presence of soil NO_2^- concentration in both the hay field and the horse paddock seeded cores (Fig. 22, B). However, the effect of N-input can still be noticed due to the higher concentration of soil NO_3^- and NO_2^- in the hay field treatment compared to the horse paddock (Fig. 22). When comparing this study with recent research, where maximum HONO emission was noticed in early stage of germinating wheat seed with decreasing emission rate as the shoot length increases (Bhattarai et. al 2019), this result showed that experimenting with a different seed and perhaps at different timing has clearly influenced the HONO emission rates. We hope that further studies would help to clarify the effect of N-input on HONO emissions in seed varieties.

The soil nutrient concentration after the collection of biomasses from the seedling growth shows the uptake of soil NO_3^- and NO_2^- for seedling growth (Fig. 22). In fact, it was clear that seedling length increases with the reduction of NO_3^- concentration when comparing the seeded and non-seeded treatments of each soil types. The disappearance of soil NO_3^- supports the literature review that most of the soil NO_3^- have been used up during the seedling growth and some were converted into NO and N_2O. However, NO_2^- showed some irregular variation with a sharp increasing concentration in S-Hay and a slight decreasing concentration in S-Hp. This may be a

good reason why the S-Hp has more NO and HONO emission rates compared to S-Hay, because previous results have shown that HONO emissions have strong correlation with soil NO_2^- availability (Su et al., 2011). Majority of the soil NO_2^- concentration in S-Hp may have been converted into increasing the concentrations of NO and HONO in the soil.

Finally, clearly well-germinated, taller and greener seedling length in the horse paddock cores can be observed when compared to the hay filed (Fig. 12) This indicates that the horse paddock has more available nitrogen for seedling assimilation, with stronger affinity to emit more greenhouse gases (especially N_2O), HONO and NO emissions when compared to the hay field. The biomass yield from the horse manure seed treatment was at least twice the size of that from hay field, and thicker. We assume that the biomass yield responded to the rate of nitrogen input in the horse paddock. The organic C and N concentration in N-fertilized soils tends to increase through high biomass yield and crop residues (Russell et al., 2005)

7.0   CONCLUSIONS

- Greenhouse gas (especially N_2O), HONO and NO emissions increased with increasing available nitrogen in a horse paddock, hay field and grassland. The emission rates correlate positively with soil NO_3^- and NO_2^- concentration and reduced soil pH.

- NO and HONO gases behaved the same way in all the soil treatments with response to soil NO_2^- across the sampling times, while N_2O emission followed a different pathway with response to soil NO_3^-.

- The horse paddock had the most N_2O, HONO and NO emission rate amongst the three closely-related agricultural soils, with respect to soil pH.

- HONO emission increased with seedling length in both hay field and horse paddock treatments, with respect to soil NO_2^-.

- More nitrogen nutrients were available for plant assimilation in the horse paddock, than in the hay field.

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