The impact of grassland sward composition on N_2O emissions and emissions intensity.

Doctor of Philosophy

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DECLARATION OF ORIGINAL AUTHORSHIP

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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ABSTRACT

The majority of intensive grassland production systems consist of perennial ryegrass (*Lolium perenne*) monocultures which are heavily reliant on inorganic nitrogen (N) fertiliser inputs. Inorganic N fertiliser drives up the carbon footprint of such systems as it is a direct source of nitrous oxide (N_2O), a potent greenhouse gas with 265 times the global warming potential of carbon dioxide. In recent years, there has been increased use of binary grass-legume and multi-species swards for livestock production. This trend is owing to the associated benefits of more diverse swards (increased yield production, drought resistance and animal performance) and the need to diverge from conventional systems in order to reduce artificial N inputs. This PhD thesis addresses the gaps in knowledge regarding the N_2O emissions and emissions intensity of multi-species swards. Annual N_2O emissions and emissions intensity of systematically varied monocultures and mixtures were assessed using a simplex experimental design and static gas chamber methodology. Furthermore, given that drought events are predicted to increase due to climate change, the impact of drought and re-wetting on N_2O emissions were also assessed according to species identity and diversity. Moving from plot to systems scale, a comparison of two production systems was also made between a) a monoculture receiving moderate N inputs and b) a grass-legume mixture receiving low N inputs and their subsequent impacts on N_2O emissions, livestock production and emissions intensity. Key findings of this thesis include highly significant reductions in N_2O emissions intensity from a 6-species mixture compared to *L. perenne* monocultures at both high and moderate N applications; no long lasting legacy effects of drought on N_2O emissions following N fertiliser application post-rewetting; and increased liveweight gain (2kg) in lambs reared on the low N grass-legume system compared with a standard production system. Overall, this thesis solidifies the role of multi-species swards in climate-smart livestock production systems.

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CHAPTER 1

GENERAL INTRODUCTION

Limiting global warming to 1.5 °C will require major cuts in greenhouse gas (GHG) emissions. Developed countries such as Ireland and the UK are legally bound to meet GHG reduction targets put in place by the Paris Agreement (2015). The primary GHGs include carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) with the agricultural, industrial and transport sectors acting as major global contributors. Regarding agriculture, the total global GHG emissions from livestock supply chains are estimated at around 7.1 Gt CO_2eq year^{-1} (Gerber et al., 2013). Of the three primary GHGs, N_2O is the most potent with 265 times the global warming potential (GWP) of CO_2 and a long atmospheric residence (120 years) (IPCC, 2014). Nitrous oxide is released to the atmosphere from various processes that constitute the terrestrial nitrogen (N) cycle, including nitrification and denitrification (Butterbach-Bahl et al., 2013). In agriculture, direct and indirect N_2O emissions are a by-product of inorganic and organic N fertiliser applications to land, crop residues, cultivation of organic soils, deposition of livestock excreta and manure management. All of these accelerate the natural N cycle and thus, increase N_2O emissions. The only sink of N_2O in the lower atmosphere is the complete reduction to N_2 in soils, otherwise N_2O is emitted to the atmosphere.

Decoupling N_2O emissions from agricultural production systems is crucial to meeting GHG reduction targets, particularly in Ireland and the UK where due to the favourable climate a large proportion of agriculture consists of intensive grass-based livestock production systems. This is reflected in the UK and Ireland's national inventory reports with agriculture being the source of 10% and 35% of GHGs, respectively (Gov.UK, 2021; EPA, 2021). Indeed, limiting global warming to 1.5 °C will limit rather than prevent climate related challenges such as drought, flooding, and sea level rise. Drought and re-wetting events further accelerate N_2O losses due to soil aggregate break down and priming of the soil system with carbon and nitrogen. Thus, future agricultural practice will need to be dual-functional providing both mitigation and adaptation to climate change.

Grass-based production systems in Ireland and the UK have typically been based on monocultures of perennial ryegrass (*Lolium perenne*) that require high levels of inorganic N fertiliser to achieve the high yields of quality forage required for optimum livestock production. In the last decade there has been increased uptake of binary grass-legume mixtures in an effort to displace artificial mineral N fertiliser with naturally available N i.e. N from legume facilitated by biological N fixation (BNF). This shift has been strategic towards improving the Nitrogen Use Efficiency (NUE) (N input/N output) of agricultural production systems as outlined by the EU Nitrogen Expert Panel Report (2015). More recently, interest in the use of multi-species sward (MSS) mixtures containing grasses, legumes and herbs has increased with numerous studies reporting increased dry matter yields, improved animal performance, drought resilience and various other agronomic and environmental benefits. There is growing evidence to suggest that diverse grassland swards containing variety of functional groups have significant positive effects on the soil N cycle and reduce N_2O emissions compared to grass monocultures. These differences could be attributed to increased N use efficiency, differential niche occupation of the rhizosphere, legume facilitated BNF, differences in plant nutrient acquisition strategies and the presence of biological nitrification inhibition compounds in certain species such as ribwort plantain (*Plantago lanceolata*). However, there are knowledge gaps surrounding the N dynamics of intensively managed binary and multi-species swards.

The aim of this PhD thesis is to provide greater insight into how grass-legume mixtures and multi-species swards affect the N cycle and reduce N_2O emissions. A key objective was to weigh N_2O losses against agronomic productivity in order to determine practical solutions for more environmentally friendly and efficient livestock production systems. Additionally, adverse climatic events are predicted to become more frequent with climate change, therefore N_2O losses from multi-species swards under environmental stressors of drought and re-wetting and compared with monocultures and binary mixtures. These objectives were achieved through two field experiments carried out at Teagasc, Johnstown Castle, Co. Wexford, Ireland and a desk study based on data collected from the North Wyke Farm Platform (NWFP), Devon, UK. The NWFP is a UK National Capability where pasture management has been tested for productivity and environmental losses. The three experimental chapters are 1) Mitigation: an annual assessment of the N_2O emissions and emissions intensity of MSS where the following hypothesis was tested: H_0: More diverse grassland swards will have lower N_2O emissions and emissions intensity than conventional monocultures. 2) Adaptation: to understand the N dynamics of MSS when subject to the environmental stressors of drought and re-wetting where the following hypothesis was tested: H_0: more diverse swards will emit lower N_2O emissions when subject to drought and re-wetting. 3) Climate-smart livestock production: a system-scale comparison of N_2O emissions, sheep production and emissions intensity of a low N input binary grass-legume based system compared with grass monoculture system receiving moderate N inputs, where the following hypothesis was tested: H_0: the low input grass-legume based system will have lower N_2O emissions, increased livestock production and lower emissions intensity than a conventional, moderate N input system.

1.1 STATEMENT OF FUNDING AND RESEARCH LOCATION

The research and experimental work carried out as part of this PhD project consists of three experiments funded by the Irish Department of Agriculture Food and the Marine (DAFM) through the Walsh Scholarships Programme. The two plot-trials were carried out on the research farm at Teagasc, Johnstown Castle, Crops, Environment and Landuse Research Centre, Co. Wexford, Ireland. All gas analyses was conducted at the Johnstown Castle GHG laboratory. The desk based study consists of data collected at the North Wyke Farm Platform (NWFP), Devon, UK, most of which is available through the NWFP online data portal.

CHAPTER 2

The role of nitrogen and sward type in intensive grassland production systems: a review

1. *General overview*

‘Climate’ refers to the average weather over a certain period of time and the term ‘climate change’ is given to lasting, extended periods of significant change in the climatic measurements such as precipitation or temperature. Climate change is considered the greatest modern day threat to humanity. Throughout our natural history, the Earth’s climate has experienced many changes, from ice ages through to extreme warming events (MET Eireann, 2020). The evidence of past climatic patterns is still evident in today’s landscape in geological features such as glacial lakes and limestone bedrock. However, the shift in climatic patterns we are observing in modern times is primarily caused by anthropogenic activities and is occurring at a much faster rate than previous, natural shifts in climate. The industrial revolution of the 18th century marked the start of mass fossil fuel combustion and intensive agriculture. These activities cause significant releases of greenhouse gases (GHGs) to the atmosphere. As the name suggests, GHGs trap heat and water vapour within the Earth’s atmosphere. The main GHGs are carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and hydrofluorocarbons, the latter of which depletes the ozone layer. Examples of human activities which act as sources of GHGs include CO_2 emissions from the burning of fossil fuels such as coal, oil and gas and peat, N_2O and CH_4 emissions from agriculture and further emissions through land-use changes i.e. deforestation and desertification. Each of the last four decades has been warmer than its preceding decade and any other decade since 1850 (IPCC, 2021) and there have been major environmental costs associated with this increase in GHG concentrations and subsequent warming. All anthropogenic activities including industry, transport and agriculture are inextricably linked and all have a major part to play in mitigating climate change. This review aims to document the role of agriculture, specifically in terms of the nitrogen (N) cycle and N_2O emissions associated with intensive farming practices.

Nitrous oxide is a highly potent GHG with 265 times the global warming potential of CO_2. It is also a relatively long-lived GHG, persisting in the atmosphere for ~120 years (IPCC, 2014). Agriculture is one of the sources of N_2O (Soussanna, 2004); in 2015, Europe produced 184,790 kilotonnes N_2O (CO_2 equivalent) from agriculture (soils and manure management) with Ireland and the UK accounting for 6,585 and 15,835 kilotons respectively (European Commission, 2020).

2. Literature review introduction

In order to understand the key sources of N_2O, N as a fundamental element and the N cycle including the many transformations N undergoes are explained in the following literature review, as well as the rules and regulations governing emissions and N usage from national to international scale are documented. The complex relationship between the N cycle, modern agricultural production and its reliance on N input, and the consequential effects this has on the environment are also covered. How plants influence the N cycle is explored along with a rundown of the current state of knowledge surrounding N_2O abatement strategies. Given the need to dramatically reduce N inputs into agricultural ecosystems, more sustainable options will need to be put into place such as N incorporation by legumes, and multi-species swards. This literature review also aims to report on multi-species swards research to date as regards yield production, animal performance, drought resilience and how more diverse swards could potentially affect the N cycle and thus, N_2O emissions. This literature review will give a thorough background to the research carried out as part of this thesis. Thus, this review explores ways of reducing N_2O emissions, while improving sustainability through yield stability, resilience to climate change and biodiversity effects.

3. Legislation surrounding GHG emissions and nitrogen use.

Efforts to combat climate change and account for and reduce anthropogenic GHG emissions are enforced by a number of legislations. The Kyoto Protocol (1997) places legal obligation upon industrial nations to provide accurate GHG inventories (including a net balance of emissions and removals). From 2021-2030, the European Commission will place greater emphasis on the compensation of GHG emissions by carbon (C) sequestration and incorporating all land-use and forestry into national inventories. Up until 2020, this was not the case as only additional C sequestration efforts from within the emitting sector were accounted for. The commitment of EU member states to combat climate change by reducing GHG emissions is bound to the Paris Agreement (2015). This agreement was put in place following the recommendations of the Intergovernmental Panel on Climate Change (IPCC) 5th assessment report (AR5) (IPCC, 2014). The Paris agreement specifies that all member states are to reduce GHG emissions by at least 40% by 2030 compared to 1990 levels (Climate Action – EC, 2020). There are also national targets in place that differ between countries that put pressure on achieving reductions in emissions.

Regarding nitrogenous based gaseous emissions specifically, the EU National Emissions Ceilings Directive (2016), places reduction targets for EU Member States as regards air pollutants such as nitrogen oxides (NO_x) and ammonia (NH_3) (European Environment Agency, 2020). There are also a number of policies at European level specifically surrounding the use of N within agricultural systems; The Nitrates Directive (1991), the Water Framework Directive (2000) and the Groundwater Directive (2006) work in conjunction to protect European water quality. The Nitrates Directive aims to reduce nitrate levels from agricultural sources from entering ground and surface water, which causes pollution and eutrophication. This is done through promoting the use of good farming practices such as constraining N application to a certain amount and to within a designated time period (where crops have a higher N requirement); enforcing the use of precision application techniques; limiting the area and environmental circumstances in which N is applied to land i.e. near watercourses, during wet periods; placing requirements for the safe storage of manures and farm yard waste. The EU Water Framework directive aims to retain the 'high' and 'good' ecological status of such surface and ground waterbodies by preventing any further deterioration while also restoring any ecologically 'poor' waters to a higher status (OECD, 2017).

4. The nitrogen cycle

Nitrogen is a primary element, critical for the survival of living organisms, and central to processes such as photosynthesis in plants and the formation of many biomolecules, including proteins and DNA (Brown, 2012). Nitrogen is a limiting resource, meaning that insufficient N availability can result in constraints to ecosystem productivity (Reich et al., 2006; Elser et al., 2007). Although N is highly abundant in the atmosphere (~80%) as nitrogen gas (N_2), it is inaccessible to primary producers in this form as the triple bond between the N molecules in N_2 is hard to break. Only when the bond between N atoms is broken, can the individual N atoms then react with other elements to form various compounds utilisable by living organisms (Galloway and Cowling, 2002). Therefore, N needs to be converted from atmospheric N_2 to terrestrial, plant available forms through N fixation in order for growth and development to take place (Crews et al. 1999). There are various forms of N, gaseous (N_2, nitrous oxide (N_2O), nitric oxide (NO), nitrogen dioxide (NO_2) and ammonia (NH_3)), organic (amino acids, nucleic acids, C/N chains) and inorganic or mineral (ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-) (Figure 1) many of which are constantly undergoing processes of transformation (Cameron, 1992). The main processes involved with the terrestrial N cycle are fixation, immobilisation, mineralisation, nitrification, complete ammonia oxidation (commamox), anaerobic ammonium oxidation (anammox) denitrification, co-denitrification, and ammonia volatilisation (Figure 2).

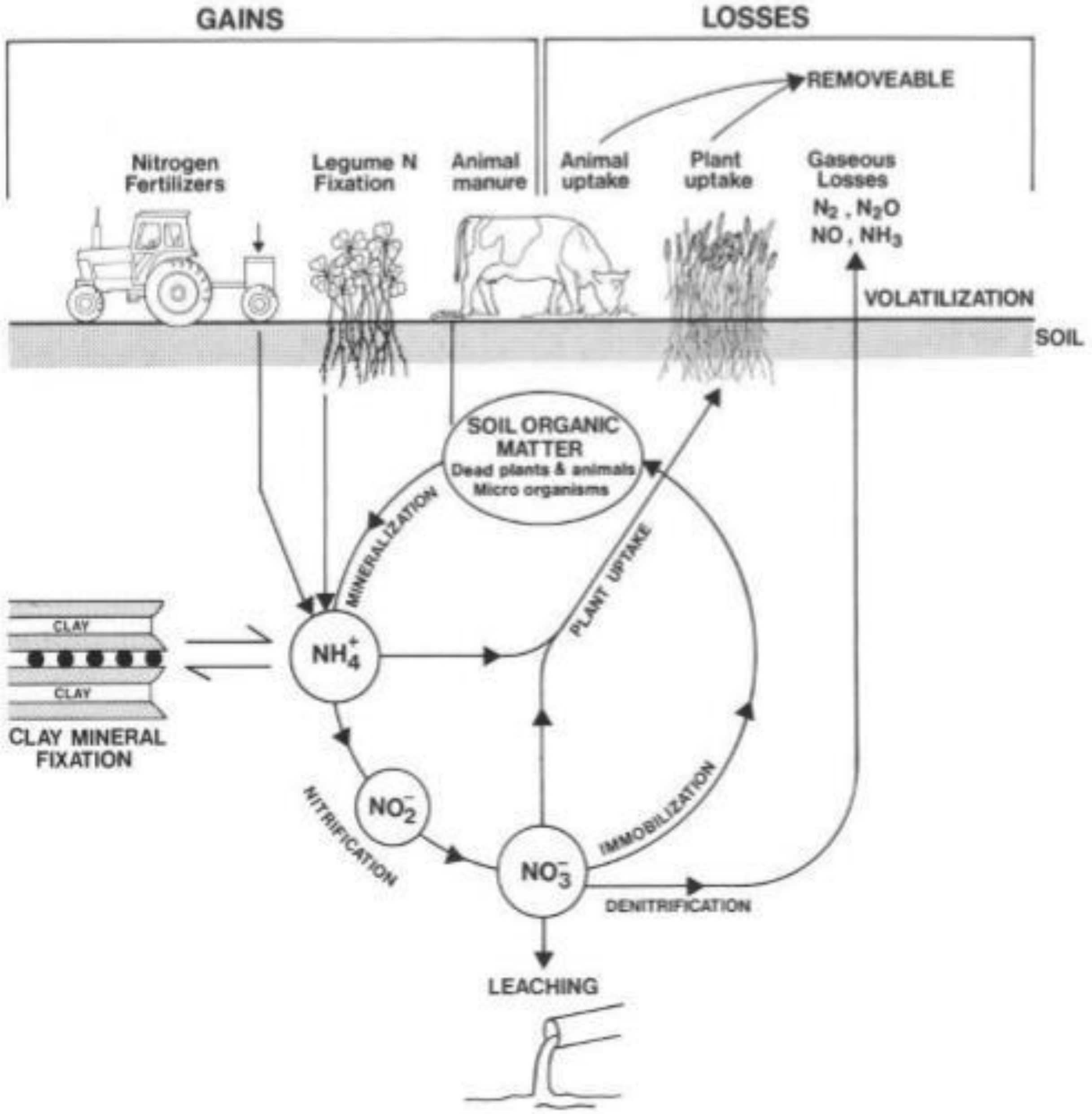


Figure 1. The agricultural N cycle (Cameron, 1992)

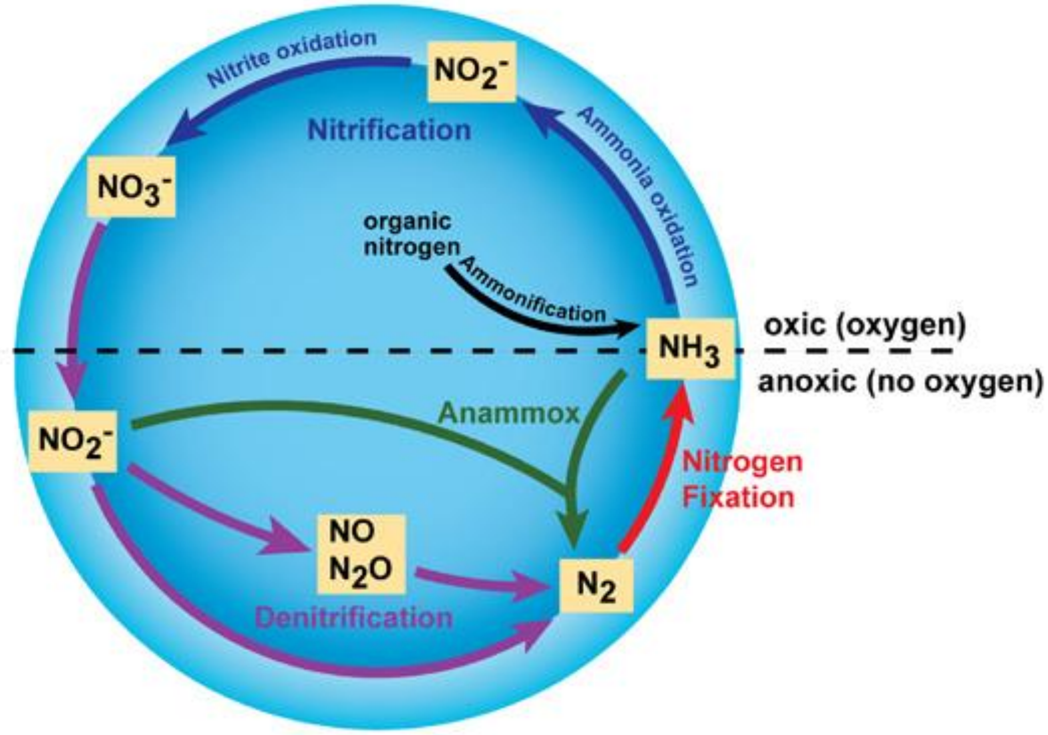


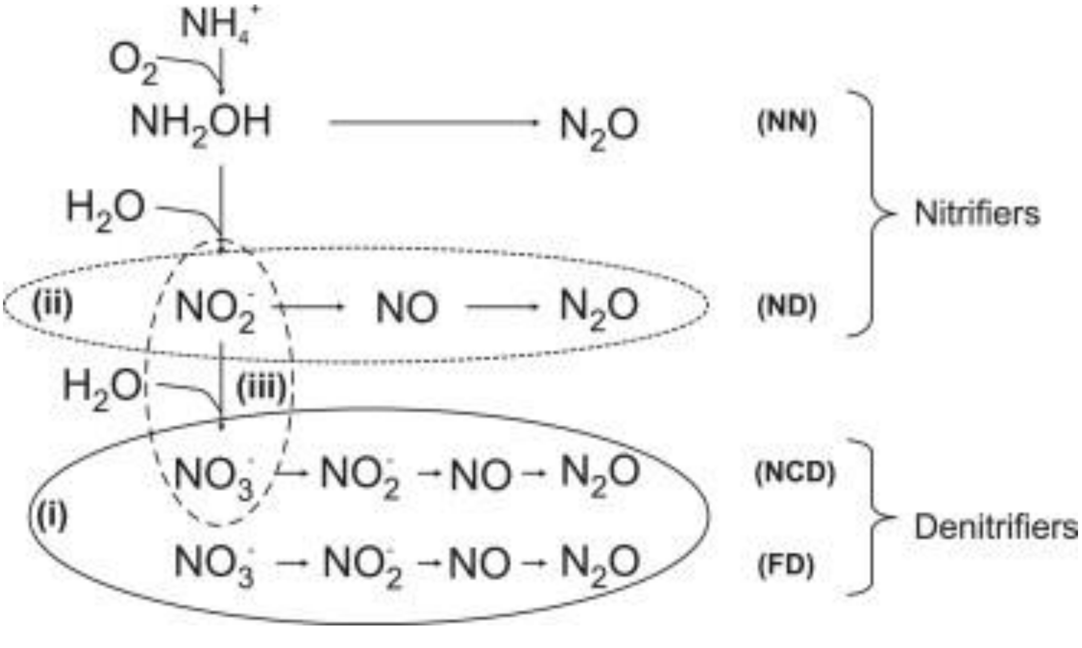
Figure 2. The processes and transformations involved in the terrestrial nitrogen cycle (Bernhard, 2010).

4.1 Nitrogen transformations

Before N can be utilised by living organisms, N-fixation and N-assimilation have to take place. These processes are carried out by a number of specialised microorganisms (both free living and symbiotic soil fungi and bacteria). Nitrogen fixation can also occur naturally during lightning and fire events (Galloway and Cowling, 2002). Biological nitrogen fixation (BNF) is primarily carried out in terrestrial/agricultural systems by symbiotic bacteria, such as *Rhizobium* and *Bradyrhizobium*, which form symbiotic relationships with legume species (Wagner, 2011). The bacteria colonize the root system of the host plant, which results in nodule formation of the root system. The host plant provides carbohydrates (by-products of photosynthesis) to the bacteria and in exchange, the bacteria fix N_2 from the atmosphere to plant available forms (Wagner, 2011). Biological nitrogen fixation is of significant importance to agricultural systems worldwide as it naturally replenishes soil N levels, promoting productivity as N is often the limiting factor in agricultural production. Yu and Zhuang (2020) found N addition to soils in temperate areas through BNF to range from 1.9 to 19.14 Tg N yr^{-1} (depending on legume coverage which ranges from 1 % to 10 % temperate land area) which accounts for 12.5 % of total BNF worldwide. Over the course of ten years from 1990-2000, the rate of anthropogenic N fixation (Haber-Bosch process) was over double that of BNF rates in natural terrestrial ecosystems (140 Tg N $year^{-1}$ vs 61.5 Tg N $year^{-1}$) (Galloway et al., 2002; Yu and Zhuang, 2020). There is also a high carbon footprint associated with the Haber-Bosch artificial N fixation process itself (Wood and Cowie, 2004). Thus, there is significant potential for BNF through legume inclusion to reduce reliance on inorganic N-fertilizer application to conventional, modern day agricultural systems (Peoples et al., 1995). This is why of recent years, white clover (*Trifolium repens)* and perennal ryegrass (*Lolium perenne)* planted in combination has become more common in temperate agricultural systems, with aim to reduce dependendy on artificial N inputs**.**

Once N enters the soil system, it goes through the processes of mineralisation and its reverse process, immobilisation. These processes can occur both alternatively and simultaneously to each other within the soil system. Both processes are highly correlated with soil carbon levels and the ratio of carbon to N within the soil system. Immobilisation is the dominant process when soil organic matter has a higher carbon/nitrogen ratio while mineralisation levels are higher when soil organic matter has higher N than carbon levels. Mineralisation involves the breakdown of organic biomolecules containing N such as amino acids and DNA until N is transformed into its ammonified, mineral form. Mineralisation predominantly determines the amount of mineral N that is available for plant uptake or accumulation in soils (Wang et al., 2001). Immobilisation is the term given to N which is taken from the soil organic matter pool for use by microorganisms where it is then incorporated into microbial biomass. The term immobilisation refers to that N is no longer available in the soil system while incorporated into microbial biomass. Once the microorganisms die, N undergoes mineralisation once again and is reincorporated into the soil system (Brady and Weil, 2013).

Once N reaches its mineral, ammonia (NH_4^+) form, it may undergo nitrification. This process is carried out by nitrifying bacteria in the soil and refers to the oxidation of NH_4^+ to produce NO_3^- via NO_2^- (Equation ii) (Kool et al., 2009). Little energy is gained by microorganisms from the breakdown of NO_2^-, therefore it is quickly transformed to NO_3^-. Nitrite is toxic to most soil organisms and is not taken up by plants in this form. Nitrous oxide is a by-product of the nitrification process, with N_2O being lost in the transformation of NH_4^+ to NO_2^- and from NO_2^- to NO_3^-. There is also a direct pathway from NH_4^+ to NO_3^- (compared to the two-step process via NO_2^-) termed complete nitrification or ‘comammox’ which is carried out by ammonia oxidizers such as *Nitrospira* bacteria (Hu and He, 2017). Nitrogen may also undergo further transformations through denitrification. The relative contribution of both nitrification and denitrification to N_2O losses is strongly related to soil moisture conditions (Morse et al., 2013). When soils have a high soil water filled pore space % (WFPS) (>70%) or are under waterlogged conditions, denitrification may occur. Denitrifying microorganisms (bacteria, fungi and yeasts) work under anaerobic conditions (i.e. waterlogged soils with low oxygen availability) (Bateman and Baggs, 2005) to utilise the oxygen molecule in NO_3^- or NO_2^- in place of O_2, as the terminal electron acceptor for the respiration process. This causes NO_3^- to be reduced to NO_2^-, nitric oxide (NO), N_2O and finally N_2 (Equation i). Incomplete denitrification results in N being lost to the atmosphere in N_2O rather than N_2 form. Codenitrification can also be carried out by certain fungi species whereby a N atom from NO_2^- is combined with a N atom from an alternate source, ultimately producing N_2 (Tanimoto et al., 1992; Laughlin and Stevens, 2002). There is also an additional pathway for N to be lost to the atmosphere as N_2 through the anaerobic oxidation of NH_4^+ simultaneously with the reduction of NO_2^- under anoxic conditions. This process is referred to as 'anammox' and is carried out by anammox bacteria of the *Planctomycetes* phylum (Wang et al., 2019). Although N_2O emissions are mostly a result of the denitrification process, N_2O production from nitrification can increase under some soil conditions e.g. low soil pH (Robinson et al., 2014). As aforementioned, N_2O losses from the soil system are of major concern due to its high global warming capacity and long-term persistence in the atmosphere. A review on the knowledge capacity surrounding N_2O emissions by Butterbach-Bahl et al. (2013) recommended building on the current understanding of the N cycle and processes involved by increasing research on N_2O fluxes at a field and landscape scale. Therefore it is vital that sustainable agronomic systems are developed along with a thorough understanding of the subsequent N_2O emissions.



Equation (i) the denitrification process carried out by denitrifying microorganisms and (ii) the nitrification process which is carried out by nitrifying microorganisms (Kool et al., 2009)

Aside from N_2O losses, ammonia (the result of ammonia volatilisation) is also of major environmental concern. Ammonia volatilisation describes the reduction of NH_4^+ in the soil (mineral) to NH_3 (gas) which in turn is lost to the atmosphere (Freney et al., 1983; Fenn et al., 1985). Although NH_3 is not a GHG, there are a number of issues with NH_3 including reduced ecosystem quality, eutrophication and issues surrounding air quality and human health (Bicer et al., 2017). Ammonia is a source of indirect GHG through N redeposition and subsequent N_2O losses (Fischer et al., 2016). Weather conditions such as temperature, solar radiation, wind and rainfall are strong drivers of volatilisation. Regarding soil specifically, Sunderlage et al (2018) found the cation exchange capacity and pH of the soil to be the main drivers behind NH_3 volatilization, with a lower exchange capacity causing an increase in NH_3 losses. Ammonia volatilisation can be prevented through proper timing of slurry application and the use of protected urea in agricultural systems (Lanigan et al., 2018).

5. *The role of nitrogen in agriculture.*

Nitrogen is a limiting factor in agricultural production. However, this limitation was overcome in the mid-20th century when the Haber-Bosch process (the industrial fixation of N from the atmosphere) was developed. This resulted in an agricultural revolution and marked the start of what we recognise as modern day agriculture. Since 1970, there has been a 120% increase in fertiliser N production and in turn a surge in food availability, along with a 78% increase in the human population (Galloway et al., 2008).

When considering conventional grassland systems for the production of beef, dairy and sheep in Northern European countries; the pasture usually consists of a grass monoculture (often perennial ryegrass *L. perenne*) receiving regular and high N applications. Many studies use an application rate of >200 kg N ha year^{-1} (Beecher et al., 2015; Crush et al., 2018; Byrne et al., 2018). Although such systems are highly productive, there is a lack of sustainability surrounding this management strategy. For example, at European scale, Quemada et al (2020) found dairy farms had a NUE of 30% and a N surplus of 155 kg N ha^{-1}. This surplus is caused by excess N application and a lack of plant uptake (Mosier et al., 1998; Bouwman et al., 2002; Hyde et al., 2006). Surplus N within the soil system creates spatial ‘hotspots’ and temporal ‘hot moments’ of environmentally reactive N (Barton et al., 2015; Wagner-Riddle et al., 2020). This enhances the aforementioned processes of nitrification, denitrification (where N_2O emissions occur), ammonia volatilisation and nitrate leaching (Galloway et al., 2002). Recognizing the need for N as a key human resource while considering alternate strategies to excessive N application to grasslands is crucial to the future of sustainable food production (Galloway et al., 2008).

5.1 Nitrous oxide emissions from agricultural ecosystems

Climatic (temperature, rainfall), landscape (slope, gradient, drainage) and soil characteristics (C/N ratio, compaction, water filled pore space and pH) are major drivers of N_2O emissions from agricultural ecosystems. Regarding the former, N_2O emissions are likely to be controlled by soil moisture and soil temperature and their concurrent effects on microbial activity and substrate availability (Luo et al., 2013). Additionally, agricultural management practices such as N fertiliser formulation and application rate, crop and sward type also play a part (Dencső et al., 2020). Urine and dung deposition from grazing livestock (both bovine and ovine) act as ‘hotspots’ of N_2O emissions in agricultural ecosystems (López-Aizpún et al., 2020). Given the potency of N_2O as a GHG, avoiding losses is critical for climate change mitigation and meeting legally bound GHG targets. There are a number of guidelines for reducing N_2O losses to the atmosphere at farm level. Overall, measures that limit N_2O emissions can potentially improve agricultural productivity or reduce N fertiliser costs.

Nitrogen fertiliser application is a major driver of N_2O emissions with global N fertiliser induced losses previously calculated at 2.8 Mtonne year^{-1} (Bouwman et al., 2002). Losses can be limited through management strategies i.e. splitting N fertiliser applications over the year and decreasing the overall N load (Burton et al., 2008; Kim et al., 2014) and only applying only when required i.e. at the start of the growing season or after silage cuts. It is recommended that fertiliser is not applied during or after drought conditions (i.e. at the re-wetting stage) as it cannot be utilised by plants and will add to increased N_2O emissions at re-wetting (Liu et al., 2014). Fertiliser application should also be avoided during very wet or waterlogged conditions as this will result in N_2O losses through denitrification (Bateman and Baggs, 2005). Fertiliser type and formulation have a major role to play in the extent to which N_2O is lost from the soil into the atmosphere. Urea based formulations are favourable over NH_4^+ and NO_3^- formulations such as calcium ammonium nitrate (CAN) as urea is more stable in the soil system. In effect, there are more pathways for urea to be emitted to the atmosphere as N_2O/N_2 than ammonia and nitrate forms of N (Harty et al., 2016). Certain N fertiliser formulations such as protected urea (urea treated with a urease inhibitor such as N-(n-butyl) thiophosphoric triamide (NBPT)) are highly effective at reducing N_2O emissions (Cantarella et al., 2018). NBPT reduces the rate of ammonification, thus stabilising N in the soil system which allows more time for plant uptake. Rahman et al (2020) found a significant decrease in the direct N_2O emission factor of protected urea (urea + NBPT) compared to both calcium ammonium nitrate (CAN) and conventional urea.

6. Grassland production systems.

Before the industrial and agricultural revolutions of the mid-20th century, in Europe traditional grassland pastures or hay meadows were used to farm livestock. Traditional systems kept less animals than modern day enterprises and consisted of permanent, mixed pasture with little to no inputs or requirements. These permanent pastures acted as fully functioning ecosystems and supported high levels of biodiversity. Today, there are strong links between high nature value farming systems and traditional agricultural approaches (O'Rourke et al., 2016). While a small amount of semi-natural grasslands still remain, the vast changes in agricultural practices throughout Europe, with greater emphasis on intensification, has resulted in a scarcity of such habitats (Garcia, 1992).

Bouwman et al (2013) record that the global nutrient surpluses increased to 36 trillion grams N year^{-1} and two trillion grams P year^{-1} between 1900 and 1950. This trend continued from 1950 to 2000 as the global surpluses increased to 138 trillion grams year^{-1} and 11 trillion grams year^{-1} of N and P, respectively. Considering Irish livestock data, from the mid-20th Century there was a major increase in livestock numbers and fertiliser use. There was also a shift from sheep to cattle based livestock production and a major increase in fertiliser land application (Teagasc National Farm Survey, 2020). These trends in agricultural production also mark a shift away from meadows to intensive grassland monocultures, highly dependent on nitrogen fertiliser input. Many species native to Ireland and the UK such as the corncrake and the curlew, are now on the brink of extinction due to this change in agricultural practice (Stowe et al., 1993; Brown et al., 2015). Thus, in recent years, the effects of human activity, agricultural intensification and climate change have led to an additional biodiversity crisis (Myers, 1996). The increased incidence of summer droughts, flooding and rising global temperatures causing major ecosystem interference and habitat loss. This combined with the rising price and environmental cost associated with inorganic fertiliser has resulted in a need for alternative, sustainable, biodiversity friendly options for livestock production.

Hopkins et al (1990) undertook a study on the productivity (yield quantity and quality) of permanent and reseeded pasture in response to N application. It was found that the maximum benefits of a reseeding a *L. perenne* monoculture were achieved with N application of 450 kg N ha^{-1} year^{-1}. However, it is worth mentioning that this study also found the *L. perenne/T.repens* reseed to be the most productive sward in the absence of fertiliser N application. More recently, there has been extensive research on perennial ryegrass (*L. perenne*) monoculture swards, as regards livestock grazing strategies and general sward management (McCarthy et al., 2013; Byrne et al., 2018). However, these studies typically consider high N application levels above the upper limitation of 170kg N ha^{-1} $year^{-1}$ and towards the upper limits of the nitrate derogation limits of 250kg N ha^{-1} $year^{-1}$ (Gov.ie, 2021). There is a lack of sustainability surrounding *L. perenne* monocultures and high levels of N use. It is also worth mentioning that as of 2021, all new reseeded pastures in Ireland will have to contain clover, in order to maintain eligibility for nitrate derogation (allowance for higher stocking rates and therefore N load <250 kg N ha^{-1} $year^{-1}$) (Gov.ie, 2021).

6.1 Legume inclusion in grasslands

The environmental issues and economic costs associated with agricultural intensification has led to an increased interest in grass-legume mixtures to improve yields and reduce N fertiliser requirements. As outlined in section 3.1 legumes such as white clover (*T. repens)* and red clover (*T. pratense*) promote N fixation, which subsequently increases soil N mineralization rates and inorganic N availability. Thus, other (non-leguminous) species can benefit from increased N availability and N sparing by legumes for transfer to neighbouring plants (Pirhofer-Walzl et al., 2012). The benefits of legume inclusion within a sward may be further promoted by the positive effect of legumes on soil microbial community structure. Harrison and Bardgett (2010) found that legume inclusion can lead to a significant shift towards a bacteria based energy channel, which is associated with higher nutrient cycling rates. It is therefore understandable why Schmeer et al. (2014) recommended legume based forage systems or grass-legume mixtures to reduce fertiliser input requirements for grass based systems, without compensating on productivity. However, it is important to highlight that higher N application rates to grass-legume swards will reduce the rate of BNF. Additionally, high N applications to mixed swards reduce the actual legume content of the sward over time as established by Nyfeler et al (2009). This can be avoided through reducing high /maintaining moderate N inputs to legume based swards. Burchill et al (2014a) found a 19, 17 and 41 % decrease in BNF over the course of two years from a *L. perenne/T.repens* sward receiving 86, 140 and 280 kg N ha^{-1} year^{-1}, respectively.

Productivity is not only uncompromised, but rather improved by legume inclusion within swards as legume plants themselves produce high yields of high protein content (Broderick et al., 1995). Guy et al (2018) compared the productivity of *L. perenne* monocultures (both diploid and tetraploid) with *L. perenne/T. repens* mixtures. All treatments were intensively grazed and received 250kg N ha^{-1} year^{-1}. Over the course of the three experimental years, it was found that white clover inclusion improved grassland productivity by means of a 1,468 kg ha^{-1} increase in DM yield production alongside increased utilization and nutritive value. The benefits of clover for productivity are further reiterated by Egan et al. (2018) who found increased grassland and animal productivity can be gained at a reduced N application rate. In this study, a *L. perenne/ T. repens* mixture receiving 150 kg N ha^{-1} year^{-1} had the same level of herbage production levels as a *L. perenne* monoculture receiving 250kg N ha^{-1} year^{-1}. There were also additional benefits associated with clover inclusion such as increased milk solids yield of dairy cows, particularly in the second half of lactation.

The proportion of legumes in a sward required to achieve beneficial results has been debated and examined across many gradients. Suter et al. (2015) found that increasing legume proportion above 30% does not increase productivity. Alternatively, in an agro-diversity experiment which measures grassland productivity across 30 sites (Brophy et al., 2017), swards saw beneficial effects of legume inclusion at a 20-50% proportion. This study also observed greater abundance and persistence of legumes grown in milder climates i.e. a higher annual minimum temperature. However, in contrast to the findings of (Brophy et al., 2017) which ascertain a positive relation between legume abundance and diversity effects, the benefits of multi-species swards are not entirely dependent on legume inclusion. Similar results were found by Nyfeler et al (2009) who examined the yield of forage monocultures & mixtures across 3 levels of N application at 50, 150 and 450 kg N ha^{-1}. Mixtures containing 40-60% clover and receiving 50-150 kg N ha^{-1} year^{-1} achieved the same yield as constituent monocultures receiving 450 kg N ha^{-1} year^{-1}.

The reduction of legume proportion in a sward over time is considered a management issue and a challenge for the real life application of clovers as a N source (Luscher et al., 2014). This issue arose in the study of Nyfeler et al. (2009) where legume proportion (both *T. repens* and *T. pratense*) within swards decreased over time and as N application was increased. This decrease was not expected from *T. repens*, and was attributed to its stoloniferous growth strategy. This may have reduced its ability to reach the upper canopy layers and therefore to compete against species with more prostrate growth habits (e.g. *L. perenne*) for light resources, especially when under high N application. The decrease in T. pratense was not surprising as persistency has been a long standing issue (Iepema et al., 2006), with many studies looking into cultivar development and genetic trait improvements, including persistence (De Vega et al., 2015; Řepková and Nedělník, 2014). Dealing with a lack of sward persistence is in need of further research, perhaps into cultivar development and management practices such as sowing proportion rates and over-sowing into established swards facing decline in legume proportion.

6.2 Multi-species swards

The severity and increased occurrence of major climatic events due to climate change will greatly impact agricultural production systems as they face increased environmental stressors. Frequent and recurrent periods of drought, flooding and waterlogging have resulted in reduced yields in grassland systems (Nelson et al., 2014). Therefore, in recent years there has been increased interest in carefully selecting forage species better able to withstand environmental stressors and climatic perturbation. Multi-species swards (sometimes called herbal leys) have been identified as a more sustainable option for grassland production systems than monocultures. A multi-species swards can be defined as a forage mixture composed of two or more forage species from different functional groups. However, it is widely accepted that the binary combination of perennial ryegrass and white clover is not considered a multi-species swards. A functional group is a non-phylogenetic grouping of species that show close similarities in their response to environmental and biotic conditions. Functional groups are derived from traits based on a species' morphology, physiology and life history (Duckworth et al., 2000). Multi-species swards usually consist of species from the grass, herb and legume functional groups in combination. Grasses are considered exploitative species, which acquire and utilise resources quickly and efficiently and in turn produce high yields of very palatable and nutritious biomass (Hubbard, 1968; Grassein et al., 2015). Plants within the herb functional group generally have high mineral content, are highly palatable and some have deep rooting system which aids in drought resistance (Germinal, 2020). Some of the most common species used within a multi-species swards include grasses such as perennial ryegrass (Lolium perenne), Timothy (Phleum pratense) and Italian ryegrass (Festuca perennis), as well as drought tolerant grass cultivars developed to be suited to sandy soils such as Festulolium (Festuca x Lolium hybrids). Legume species used include; Red clover (Trifolium pratense), white clover (Trifolium repens), crimson clover (Trifolium incarnatum) and birdsfoot trefoil (Lotus corniculatus). Two of the most common herb species used in multi-species swards include chicory (Cichorium intybus) and plantain (Plantago lanceolata). More diverse plant communities have been shown to have higher NUE, drought resilience, promote transgressive overyielding (species in mixture outperforming the highest performing monoculture) and to be less dependent on N fertiliser application (Marquard et ., 2009; Finn et al., 2017; Finn et al., 2018). This is due to niche complementarity (Mommer et al., 2010) and N fixation by legumes (Ianetta et al., 2016). However, Van Ruijven and Berendse (2003) found positive effects of plant species diversity on productivity levels in the absence of legumes. These higher levels of productivity were observed by means of increased productivity of low-productive species. This shows that the diversity effect goes beyond nutrient addition by N fixation and has more relation to exploitation of available resources by plant-plant interactions such as niche complementarity and facilitation.

6.3 Multi-species swards and productivity

Higher yield production from multi-species swards compared to monocultures has been well documented and is mainly attributed to temporal complementarity between species and greater ability to acquire and convert nutrients to above ground biomass (Husse et al., 2014). Yield of biomass has also been shown to be stable over time and across a range of spatial and geographical ranges and at different levels of N application (Connolly et al., 2009). The studies of Finn et al. (2018) and Haughey et al. (2018) display the productivity of multi-species swards to be robust and resilient to environmental stressors, particularly drought. Moloney et al. (2021) also found that multi-species swards are as ensilable as conventional grasslands, which is an important consideration for temperate climates that often rely on conserving forage as silage for winter livestock feed.

Multi-species swards outperforming the highest performing constituent species in monoculture has been observed in numerous experiments and research trials (Finn et al., 2013; Moloney et al., 2020). Transgressive-overyielding is usually observed when a combination of biological interactions occur between species in mixtures that might not be typical of the plants grown in monoculture. (Kirwan et al., 2009) presented an ecological modelling approach for such applications to diverse grassland mixtures. This study describes the net effect of these biological interactions as the total cumulative effects of both positive (synergistic) and negative (antagonistic) interactions that are occurring simultaneously between plants in a mixed sward. For example, if the net effect evens out and results in no overall effect (0) then there will be no observable diversity effect, if the net effect results in +1 there will be a synergistic response and -1 will give an antagonistic response. The effects of species interactions may be the cumulative result of a range of biological processes; therefore it is difficult to attribute diversity effects to a single biological mechanism. Community scale analysis cannot determine single biological mechanisms or processes as the driver of diversity effects. Pinpointing which interactions and mechanisms specifically drive productivity would allow for a more accurate yield prediction from multi-species swards. This would also aid in specifically designing mixtures to include plants with traits that maximise complementarity and improve the utilisation of resources and thus yields.

The amount of time for the productivity benefits of multi-species swards to become apparent has been debated in the past; Nyfeler et al. (2009) reported high levels of transgressive-overyielding from the first experimental year onwards, with mixtures displaying a strong increase in resource efficiency due to diversity effects. This is in contrast with the findings of Cardinale et al. (2007) who reported that five years were needed to build up sufficient resources in multi-species swards in order to achieve transgressive-overyielding. The positive attributes of diversity on productivity were apparent and robust in multiple years, in a number of production systems and across 33 sites in Europe and Canada in a three year multi-scale agrodiversity experiment (Finn et al., 2013). This study considered four species with each covering a separate functional group with a fast establishing grass, fast establishing legume, temporally persistent grass, and a temporally persistent legume. Transgressive-overyielding was apparent in 60% of sites and it was also found that a plant community dominated by one species (>70%) achieved the same diversity-induced benefits as an even community (equal proportions of constituent species) which is highly practical from a management point of view. This provides reassurance regarding the issues surrounding persistence and legume species as previously highlighted. The benefits seen in this experiment were not site specific. This provides further evidence (as already seen in the work of Connolly et al. (2009)) that mixtures can benefit from increases in N yield in comparison with monocultures across largely differing site productivity levels.

It is reasonable to assume that transgressive-overyielding due to increased nutrient utilisation would have a considerable effect on the geo-chemical and soil environment planted with mixtures. To date, there is a lack of research on the effects of multi-species swards on soil physical and chemical processes, which would undoubtedly influence the C and N cycles and processes of environmental concern such as N leaching (and subsequent eutrophication) and NH_3 and N_2O emissions. In order to truly understand the functions at play in multi-species swards and how they can be maximised to achieve more sustainable agricultural systems, this research area requires a multi-disciplinary approach, taking into account plant physiology, ecology, agronomy and chemistry.

Aside from quantity and quality of forage yield, the benefits of multi-species swards also extend to animal production. Livestock including both cattle and sheep, have been shown to benefit from increased performance when consuming a multi-species sward based diet. A meta-analysis by McCarthy et al. (2020) found that overall, dairy cow productivity i.e. milk production, is increased when cows graze on multi-species swards as opposed to grass monocultures or grass/legume mixtures. Grace et al. (2019) found that lambs grazing on a *L. perenne* monoculture sward required more days to reach slaughter weight than lambs grazing on more diverse swards. A two-year grazing study by Roca-Fernández et al. (2016) found higher milk production benefits (benefits included milk solids and yields) associated with a diverse grassland sward containing grasses, legumes and herbs when compared to a binary mixture of grasses and legumes only. This was attributed to the cumulative effect of improved pasture nutritive value and increased dry matter intake at pasture. Chicory inclusion also promoted increased forage intake.

Increased animal productivity is partially linked to greater overall health, which could potentially be due to increased mineral availability in multi-species swards. Hopkins (2004) found significantly higher concentrations of sodium (Na), potassium (K) and magnesium (Mg) in the herbage of multi-species swards than *L. perenne/T. repens* mixtures. Concentrations of N, calcium (Ca), Mg and Na were lower in grasses than other species. Whereas N concentrations were highest in T. repens, Ca was highest in *C. intybus* and *P. lanceolata*, Na was highest in *Achillea millefolium* (yarrow) and *P. lanceolata*, K was highest in *A. millefolium* but was low in *P. lanceolata.*

Herbs have also been shown to have anti-helminthic properties which reduce parasite burden on livestock and thus reduce veterinary costs. Grace et al. (2019) reported that lambs grazing on multi-species swards (with between six and nine species) required fewer anthelmintic treatments than lambs grazing *L. perenne* monocultures or *L. perenne/T. repens* mixtures. Such anti-helminthic benefits have been attributed to high levels of secondary metabolites i.e. tannins in forage species such as *C. intybus* (Waller et al., 2001; Foster et al., 2011). Multi-species swards also provide an opportunity to decrease N losses through animal urine deposition. Bryant et al. (2017) compared the milk yield and N excretion of dairy cattle fed on a complex multi-species sward, binary mixtures and a ryegrass monocultures. It was found that cattle grazing on multi-species swards resulted in a lower urine N concentration than those grazing binary mixtures or monocultures. This may be driven by the reduced crude protein intake associated with a multi-species swards diet (Hammond et al., 2014). The same study also found 10% reduced methane emissions from the breath of cattle fed on a diverse sward. This has great implications for reducing methane emissions and N losses through N_2O emissions and nitrate leaching.

7. *How grasslands can influence soil nutrient cycles*

Grasslands also play an important role in regulating soil nutrient cycles as they extract and store N and C as biomass or aid in locking nutrients into the soil system. Grasslands can act as both a sink and a source of GHG emissions and the balance between both functions is highly complex. Carbon sequestration is the precise term given to the extraction and storage of C from the atmosphere. This process is more prominent in productive grassland systems with high levels of soil organic matter and can be further encouraged through careful management practices (Conant, 2010). However, grasslands are also a major source of N_2O emissions and on average emit 2.0 kg N_2O-N ha^{-1} year^{-1} which is equivalent to 250 kg CO_2-C equivalent ha^{-1} year^{-1} (Soussana et al., 2004). Although, this estimate is an average across all grassland systems (extensive and intensive) and N_2O emissions are by nature, highly variable. For example, Burchill et al (2014b) found high levels of inter-annual variation in N_2O emissions from grazed a *L. perenne* and *T. repens* sward, although annual N input and weather conditions varied greatly over this three year study. Bell et al (2016) found N_2O emissions from intensive grassland production systems receiving 0-400 kg N ha^{-1} year^{-1} to range from 2.14–8.17 kg N_2O-N ha^{-1} year^{-1}. At a lower N application rate of 100 kg N ha^{-1} year^{-1}, Cardenas et al (2010) found N_2O emissions to range from 0.5 to 3.9 kg N_2O–N ha^{-1} year^{-1} across three different UK sites. Thus, the net ecosystem exchange of N_2O has the potential to offset the benefits of sequestration by atmospheric CO_2 sink activity which takes place in soils (Soussana et al., 2007). The nutrient concentration available in soil is what determines N_2O fluxes, with emissions generally reflecting high levels of available N within the soil system and a lack of plant uptake (Loick et al., 2017). Ultimately, reducing N_2O emissions from grasslands is vital to reducing absolute emissions, improving the efficacy of grasslands as a C sink and to aid with meeting GHG mitigation targets.

Many studies have found a positive correlation between plant species diversity and ecosystem N retention (in the absence and presence of legumes) (van Ruijven and Berendse, 2005; Nyfler et al., 2009) This has mainly been denoted to greater root uptake of available N, through niche complementarity and overyielding (Hooper and Vitousek, 1998; De Deyn et al., 2009; Cong et al., 2014). These attributes are the main drivers behind the aforementioned agricultural productivity associated with diverse grasslands. Albeit, when it comes to promoting N retention, plant functionality is of greater importance than the number of species present within a multi-species sward. In a mesocosm experiment, Abalos et al. (2014) measured N_2O emissions from twelve variations of four grass species (*Lolium perenne, Festuca arundinacea*, *Phleum pratense* and *Poa trivialis*) with species richness levels from 1-4. This study found no relationship between plant species richness and N_2O emissions. However, N_2O emissions were significantly reduced from specific plant species combinations, particularly the *Festuca arundinacea* and *Phleum pratense* mixture which acted as an N_2O sink. Although, it is worth noting that this experiment did not consider more than one functional group. An extensive review on N utilization in grassland swards by Vibart et al. (2016) highlights the importance of the herb functional group within diverse swards, where particularly those swards containing herbs saw increased N uptake. This study also found that herb species *P. lanceolata* can reduce the N load of urine patches and overall N losses from grazed pastures. This has also been found in numerous other studies (Box et al., 2017; Judson et al., 2018; Minee et al., 2020) and attributed to the diuretic potency and nitrification inhibition potential of *P. lanceolata*.

Certain plant functional traits within multi-species grassland mixtures can affect N utilization and microbial-mediated processes of N cycling. Ecological-economics has been used to describe and predict the behavior of plant species within mixtures (Lavorel et al., 2103; Pietsch et al., 2014; Faucon et al., 2017). These studies create a plant economics spectrum and define plant functional traits as fast (exploitative) or slow (conservative). Both exploitative and conservative plants differ in their effects on soil and ecosystem services. This is an important consideration as it allows for the performance of agricultural systems to be pre-determined. Functional group traits are the drivers of plant performance and the assembly of an ecological community. The root characteristics and nutrient foraging strategies of plants is key to predicting which species and mixtures of species that may be beneficial to the mitigation of N_2O emissions and C sequestration. Plants with exploitative traits generally have a high specific leaf area (SLA) and leaf N content and thrive in soils with high N availability (de Vries and Bardgett, 2016). Generally less N is lost from communities dominated by plants with exploitative traits due to high levels of plant uptake. Conservative species have slower growth rates and a low SLA and at lower soil N availability plant species with a conservative strategy are expected to lead to lower N_2O emissions due to their lesser impact on N mineralisation rates. Ultimately, functional diversity results from a harmonized co-existence between exploitative and conservative species as different resources can be used at different times and different rates by different plants (Gross et al., 2007). This avoids fierce competition between species of similar functionality for the same resource followed by greater microbial immobilization of N and N retention. Different functional traits may express themselves differently depending on environmental conditions and plant-soil feedback mechanisms are ultimately what shapes the rhizosphere and drives plant community dynamics. This becomes especially apparent in mixed-species pasture (Harrison and Bardgett, 2010). Plants can also behave differently depending on the soil status and nutrient availability. Klumpp and Soussanna (2009) attributed root and rhizome traits to be the most important determining factor affecting C sequestration upon disturbances in soil conditions. This is an important consideration in the N dynamics of re-seeded grasslands.

In conclusion, there are major environmental concerns surrounding intensive monoculture grassland systems and alternate strategies are required for food production to increase sustainably. Given the myriad of benefits associated with multi-species swards (yield production, lower N requirements, animal performance etc.), they will undoubtedly become more commonplace and play an important role in the future livestock farming. There has been a considerable amount of research carried out on multi-species swards regarding a number of attributes (e.g. biomass production). However, the impacts of multi-species swards on the N cycle are lesser known. Given the potency of N_2O as a GHG, it is of paramount importance that the emissions and emissions intensity associated with diverse swards are more clearly understood.

8. PROJECT AIMS AND OBJECTIVES.

As described above, it is of fundamental importance that N_2O emissions are decoupled from agricultural production systems. Thus, this project aims to develop a deeper understanding of the N dynamics and associated N_2O emissions of multi-species swards. High level hypotheses were developed based on existing knowledge and research to date on multi-species swards and the N cycle. This project sought to determine the annual N_2O losses of multi-species swards and the extent to which they are determined by the plants/functional groups within the mixtures. Given the increased likelihood of drought and flooding events, this study also sought to determine the soil mineral N dynamics and N_2O emissions from diverse grasslands when subject to environmental stressors. Additionally, this study aims to assess the N_2O losses and livestock production capacity of grass-legume mixtures at farm level. Thus, the main aims are presented through three experimental chapters 1) mitigation, 2) adaptation 3) climate smart agriculture:

Aim 1: To determine the annual N_2O emissions and emissions intensity from multi-species swards and component monocultures through:

I. Monitoring N_2O emissions for 12 months using static gas chamber methodology under an intensive management regime (5 yield cuts and 5 fertilizer applications).
II. Combining N_2O data with DM yield and N yield data to determine the emissions intensity of all communities (N_2O losses per unit output)
III. Using a simplex experimental design to determine potential species or functional group interactions affecting N_2O emissions or emissions intensity
IV. Comparing all communities receiving 150 kg N ha^{-1} $year^{-1}$ with a conventional *L. perenne* community receiving 300 kg N ha^{-1} $year^{-1}$

Hypotheses

I. N_2O emissions and emissions intensity will decrease as species and functional group numbers increase due to greater N uptake and use efficiency of plants in mixture than monoculture i.e. transgressive-overyielding.

II. There will be increased N_2O emissions with increased fertiliser levels – 150 kg N ha^{-1} $year^{-1}$ vs 300 kg N ha^{-1} $year^{-1}$

III. There will be a strong legume effect on N_2O emissions: legumes *Trifolium repens* and *Trifolium pratense* will have higher soil available N due to N fixation (highest in *Trifolium pratense* due to greater N fixation rates (~ 200 kg N ha^{-1} $year^{-1}$)

Aim 2: To assess N_2O emissions and soil mineral N dynamics of multi-species swards when subject to the environmental stressors of drought and re-wetting and to determine potential legacy effects of drought on responses from plant communities through:

I. Using rain out shelters to create an artificial 9 week drought and use the split plot experimental design to provide an ambient, rain-fed comparison.

II. Monitoring N_2O emissions using static gas chamber methodology during drought and re-wetting periods and at fertilizer application two weeks post re-wetting.

III. Taking soil samples during drought, re-wetting events and at fertilizer application two weeks post re-wetting to determine the soil mineral N (NH_4^+ and NO_3^-)

IV. Using a simplex experimental design to determine potential species or functional group interactions affecting N_2O emissions or emissions intensity

V. Comparing all communities receiving 150 kg N ha^{-1} $year^{-1}$ with a conventional *L. perenne* community receiving 300 kg N ha^{-1} $year^{-1}$

Hypotheses

I. There will be higher N_2O fluxes from the plots subject to experimental drought followed by rewetting in comparison to the normal rain-fed conditions due to increased immobilisation of soil carbon during drought and the slaking of aggregates releasing carbon and nitrogen during rewetting

II. There will be reduced N_2O emissions from more diverse swards subject to drought due to less available soil N and better ability to cope with drought conditions through differing and greater rooting depths and a greater ground cover of soil reducing water loss.

Aim 3: To determine the effects of grass-legume mixtures on N2O emissions, emissions intensity and livestock production at paddock scale.

I. Using a UK National Capability, the North Wyke Farm Platform to monitor N_2O emissions from paddock type (a) a grassland monoculture receiving 120 kg N ha^{-1} year^{-1} and (b) a grass legume mixture receiving 40 kg N ha^{-1} year^{-1} throughout an agronomic season (Jan-Sep 2018) using automated chambers.
II. Compare and contrast the lamb live weight gain and emissions intensity (N_2O losses per kg lamb LWG) of both paddocks.
III. Use UNFCCC national inventory reports to calculate N excretion rates
IV. Calculate and compare IPCC default and UK specific emissions factors for both paddocks.

Hypotheses

I. There will be greater N_2O emissions from paddock of (a) a grassland monoculture receiving 120 kg N ha^{-1} year^{-1} than paddock (b) a grass legume mixture receiving 40 kg N ha^{-1} year^{-1}
II. There will be greater lamb live weight gain and subsequently lower emissions intensity (N_2O losses per kg lamb LWG) from paddock (b) compared to paddock (a)

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CHAPTER 3

Beneficial effects of multi-species mixtures on N_2O emissions from intensively managed grassland swards.

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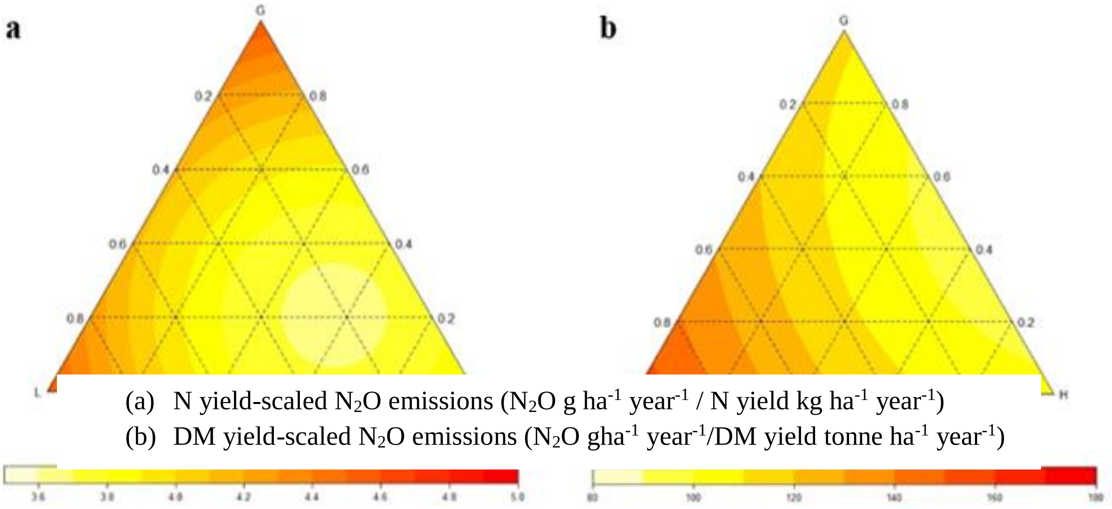
KEY WORDS

Grasslands, GHG emissions, nitrous oxide, yield-scaled N_2O emissions, multi-species mixtures, grassland production.

GRAPHICAL ABSTRACT



(a) N yield-scaled N_2O emissions (N_2O g ha^{-1} $year^{-1}$ / N yield kg ha^{-1} $year^{-1}$)
(b) DM yield-scaled N_2O emissions (N_2O gha^{-1} $year^{-1}$/DM yield tonne ha^{-1} $year^{-1}$)

ABSTRACT

In a field experiment, annual N_2O emissions and grassland yield were measured across different plant communities, comprising systematically varying combinations of monocultures and mixtures of three functional groups (FG): grasses (*Lolium perenne, Phleum pratense),* legumes *(Trifolium pratense, Trifolium repens)* and herbs *(Cichorium intybus*, *Plantago lanceolata*). Plots received 150 kg ha^{-1} $year^{-1}$ N (150N), except *L. perenne* monocultures which received two N levels: 150N and 300N. The effect of plant diversity on N_2O emissions was derived from linear combinations of species performances' in monoculture (species identity) and not from strong interactions between species in mixtures. Increasing from 150N to 300N in *L. perenne* resulted in a highly significant increase in cumulative N_2O emissions from 1.39

to 3.18 kg N_2O-N ha^{-1} $year^{-1}$. Higher N_2O emissions were also associated with the legume FG. Emissions intensities (yield-scaled N_2O emissions) from multi-species mixture communities around the equi-proportional mixture were lowered due to interactions among species. For N_2O emissions scaled by nitrogen yield in forage, the 6-species mixture was significantly lower than *L. perenne* at both 300N and 150N. In comparison to 300N *L. perenne*, the same N yield or DM yield could have been produced with the equi-proportional 6-species mixture (150N) while reducing N_2O losses by 63% and 58% respectively. Compared to 150N *L. perenne*, the same N yield or DM yield could have been produced with the 6-species mixture while reducing N_2O losses by 41% and 24% respectively. Overall, this study found that multi-species grasslands can potentially reduce both N_2O emissions and emissions intensities, contributing to the sustainability of grassland production.

1. INTRODUCTION

Nitrous oxide (N_2O) is a potent greenhouse gas (GHG) (Ravishankara et al., 2009) with 265 times the global warming potential of carbon dioxide (CO_2) (IPCC, 2014). Large N_2O losses result from (N) fertiliser application to grasslands (Harty et al., 2016; Krol et al., 2020) and the N fertiliser production process itself (Wood and Cowie, 2004). Although conventional grassland systems for livestock production tend to use high levels of N fertiliser, they are not heavily reliant on imported concentrate feeds which have a high carbon footprint (O'Brien et al., 2011). Therefore, temperate grassland production systems have the potential to curtail N_2O losses through the displacement of N fertiliser for symbiotically produced plant-available N.

Multi-species mixtures composed of grasses, legumes and herbs provide a range of agronomic and environmental benefits in grass-based production systems. These include: increased dry matter (DM) yield production (greater biomass production from species in mixtures relative to the best performing monoculture – transgressive overyielding) (Nyfeler et al., 2009; Finn et al., 2013; Moloney et al., 2020), improved animal performance (for both cattle and sheep) (Cranston et al., 2015; Roca-Fernández et al., 2016; Bryant et al., 2017; Grace et al., 2019; Jerrentrup et al., 2020), increased N use efficiency (NUE) (Hooper et al., 2005, Suter et al., 2015), weed suppression (Suter et al., 2017; Connolly., 2018), and greater yield stability during drought events (Hofer et al., 2016; Haughey et al., 2019). Although these benefits are well established, less is known about how multi-species grasslands influence the soil N cycle and therefore N_2O fluxes.

Multi-species grasslands may affect N_2O fluxes in several ways (Gardiner et al., 2016; De Klein et al., 2019). Nitrous oxide is mainly lost during the soil-based processes of nitrification and denitrification (Bremner et al., 1997). Different plant species can influence these processes through differential niche occupation of the rhizosphere which can affect plant water uptake (Holtham et al., 2007) and soil gas diffusivity. These processes determine the nitrification and denitrification pathways and the final $N_2O:N_2$ ratio of the denitrification process (Balaine et al., 2016). Biochemical reactions may also affect N_2O production. *Plantago* species contain biological nitrification inhibition (BNI) compounds within the plant that prevent ammonium (NH_4^+) transformation to nitrate (NO_3^-); this results in stability of the soil mineral N pool and increased plant N uptake (Chapman et al., 2006; Cantarel et al., 2015). Legume inclusion within grassland swards and multi-species mixtures allows for reduced fertiliser application without adversely affecting yields (Egan et al., 2018). This is due to biological nitrogen fixation (BNF) and the transfer of N from legumes to non-legumes within a multi-species sward (Nyfeler et al., 2011; Pirhofer-Walzl et al., 2012). Legume inclusion in grasslands can increase N_2O emissions when N fertiliser is not reduced to account for symbiotically fixed N (Hakala et al., 2012; Burchill et al., 2016; Luo et al, 2018).

There can be species-specific effects of plants on N_2O emissions. A laboratory incubation by Abalos et al. (2014) quantified the N_2O fluxes from mixtures with up to four grass species including *L. perenne, Festuca arundinacea*, *P. pratense* and *Poa trivialis.* No relationship was found between plant species richness and N_2O emissions; however, there was a significant reduction in N_2O emissions when certain plant species were combined. In a field study, Luo et al. (2018) applied cattle urine and compared the N_2O fluxes from monocultures of *P. lanceolata* and *Medicago sativa* with a *T. repens* and *L. perenne* mixture. Despite seasonal variation, *P. lanceolata* had lower N_2O emissions than *L. perenne* throughout the year. Although these studies showed potential for multi-species swards to reduce N_2O emissions, the various experiments were either not monitored on an annual basis, across a range of plant communities or did not apply different N fertiliser levels. These three considerations are each important to properly understand the application of multi-species swards in livestock production systems.

Fuchs et al. (2020) modelled the N_2O mitigation potential and productivity of various combinations of legume proportions and fertilizer rates for five temperate grassland sites using two different biogeochemical models. They recommended further study of the effect of clover proportions ranging from 30–50% receiving ≤ 150 kg N ha^{-1} $year^{-1}$ input, as these were identified as best-bet climate smart agricultural practices. Our study directly responds to those research recommendations. In a year-long experiment, we investigated N_2O fluxes from six different forage types including two species from each of three FGs: grasses (*L. perenne, P. pratense),* legumes (*T. pratense*, *T. repens)* and herbs (*C. intybus* and *P. lanceolata*) in monocultures and mixtures of systematically varying proportions (Image 1). In addition, emission intensity was calculated for all treatments (van Groenigen et al., 2010) as N yield-scaled N_2O emissions (N_2O-N g ha^{-1} $year^{-1}$/ kg N yield ha^{-1} $year^{-1}$) and DM yield-scaled N_2O emissions (N_2O-N g ha^{-1} $year^{-1}$/ tonne DM yield ha^{-1} $year^{-1}$). The experimental design and analyses allowed quantification of species identity effects and species interaction effects on each of the three responses (N_2O emissions, N yield-scaled emissions and DM yield-scaled emissions). There was also a 300 kg N ha^{-1} $year^{-1}$ *L. perenne* treatment (300N *L. perenne*) to allow for a comparison between all treatments and conventional agricultural practice. The specific aims of this study were to:

1) Investigate the effect of systematically varying species and FG proportions within grassland communities on annual N_2O emissions, N yield-scaled N_2O emissions and DM yield-scaled N_2O emissions.

2) Compare annual N_2O emissions and yield-scaled N_2O emissions from 150N forage communities with the 300N *L. perenne* community.

Image 1. An experimental plot containing two herb (*C. intybus* and *P. lanceolata*) and two legume species (*T. repens* and *T. pretense*) at the JC field trial, 2018.

2. MATERIALS & METHODS

2.1 Experimental site

The year-long field experiment took place at Teagasc, Johnstown Castle, Co. Wexford, Ireland 52°18′27 N between March 2018 – March 2019. The climate is temperate maritime and meteorological data (precipitation, air and soil temperature) was recorded at the Johnstown Castle weather station. The soil type at the field site was a stagnic brown podzolic. Soil texture was sandy loam, pH was 5.7 and the average bulk density of the plots on the trial site at 5-10 cm depth was 1.35 g cm^{-3}.

2.2 Experimental design

The experimental site was treated with herbicide, ploughed, and reseeded in spring 2017. The experiment followed a simplex design (Scheffe, 1963) for use in conjunction with the statistical modelling described in Section 2.5. Experimental plots, each measuring 5 m x 7 m were sown with grassland communities (*Appendix A and Image 1*) comprising one to six species that systematically varied FG composition and relative abundance. The six species comprised two species from each of three FGs: two grasses (*L. perenne* and *P. pratense),* two clovers (*T. repens* and *T. pratense)* and two deep-rooting herbs (*C. intybus* and *P. lanceolata*). There were 20 different communities with between one to four replicates per treatment (*Appendix B)* resulting in 43 experimental plots in total. Each main plot was divided into two 5 m x 3.5 m sub plots, and two water supply treatments were applied at random to the two halves. One split plot (randomly chosen) received natural water supply over the year ('rain fed'), while a two-month summer drought was simulated on the other half, using rainout shelters ('drought'). Here, we only report the measured N_2O emissions from the rain fed sub plots. Due to the natural drought conditions during the summer of 2018, the rain fed sub plots were irrigated on three occasions with 30mm of water, to match historical rainfall records (Met Éireann, 2020).

Fertiliser N application was divided into five applications of varying rate from March-September 2018 *(Appendix C)*. Maintenance levels of P and K fertilisers were applied in line with soil test recommendations at the beginning of the growing season. Calcium ammonium nitrate (CAN) fertiliser was applied at rates of 150 kg ha^{-1} year^{-1} (150N; communities 1-19, *Appendix B)* and 300 kg ha^{-1} year^{-1} (300N *L. perenne;* community 20, *Appendix B*).

2.3 Nitrous oxide measurements

Nitrous oxide emissions were monitored from 13th March 2018 – 21st March 2019. To capture fertiliser-induced effects on N_2O fluxes, a high resolution N_2O sampling strategy was put in place for six months (March to September 2018) in order to coincide with fertiliser application - the time that emissions were expected to be highest. Sampling took place four days a week for two weeks immediately following each fertiliser application, two days a week in the next two weeks (weeks three and four) and once per week up until the next fertiliser application date. High-resolution N_2O sampling was followed by six months of low-resolution sampling at a frequency of once a month (October 2018 to March 2019). The less intensive sampling approach is reflective of the low N_2O fluxes expected during this period due to a combination of no N fertiliser application and low soil temperature (Maire et al., 2020).

Nitrous oxide was measured using static chamber methodology (De Klein and Harvey, 2012) (Image 2), with a single chamber placed in each plot giving a total of 43 chambers. Chambers consisted of square, stainless steel collars 40 cm (length) × 40 cm (breadth) x 10cm (height) lined with a neoprene strip and inserted to 5 cm soil depth with matching steel covers creating an approximately 16 litre headspace. A 10 kg weight was placed on top of the covers at sampling times to ensure an airtight headspace for an enclosure time of 40 minutes. A 10 ml air sample was removed through a 16 mm rubber septum using a 10 ml polypropylene syringe and hypodermic needle. The syringe was filled and emptied twice within the chamber to mix the headspace air prior to sampling. The gas samples were injected into pre-evacuated (–1,000 mbar) 7 ml screw-cap septum glass vials. Gas samples were taken from each chamber at 0, 20 and 40 minutes to measure N_2O concentration over time. Sampling events took place between the hours of 10:00 and 13:00 to obtain measurements representative of the average hourly flux of the day (De Klein & Harvey, 2012). Nitrous oxide concentrations were analysed using a gas chromatograph (GC, Varian CP 3800 GC, Varian, USA) fitted with an electron capture detector using high-purity helium as the carrier gas. Quality control N_2O standards, which were representative of the upper N_2O concentration limit expected, were analysed alongside N_2O field samples. Linear regression of the increase in N_2O gas concentrations over time (0, 20 and 40 minutes) was used to calculate daily fluxes (g N ha^{-1} day^{-1}). A single annual cumulative N_2O value was calculated per plot by integration of daily fluxes and linear interpolation between measurements (Burchill et al., 2014; De Klein and Harvey, 2012). Yield scaled-N_2O emissions (van Groenigen et al., 2010; Sanz-Cobena et al., 2014) were calculated by dividing annual cumulative g N_2O-N (g ha^{-1} $year^{-1}$) by 1) aboveground N yield (kg ha^{-1} $year^{-1}$) and 2) DM yield (tonnes ha^{-1} $year^{-1}$).

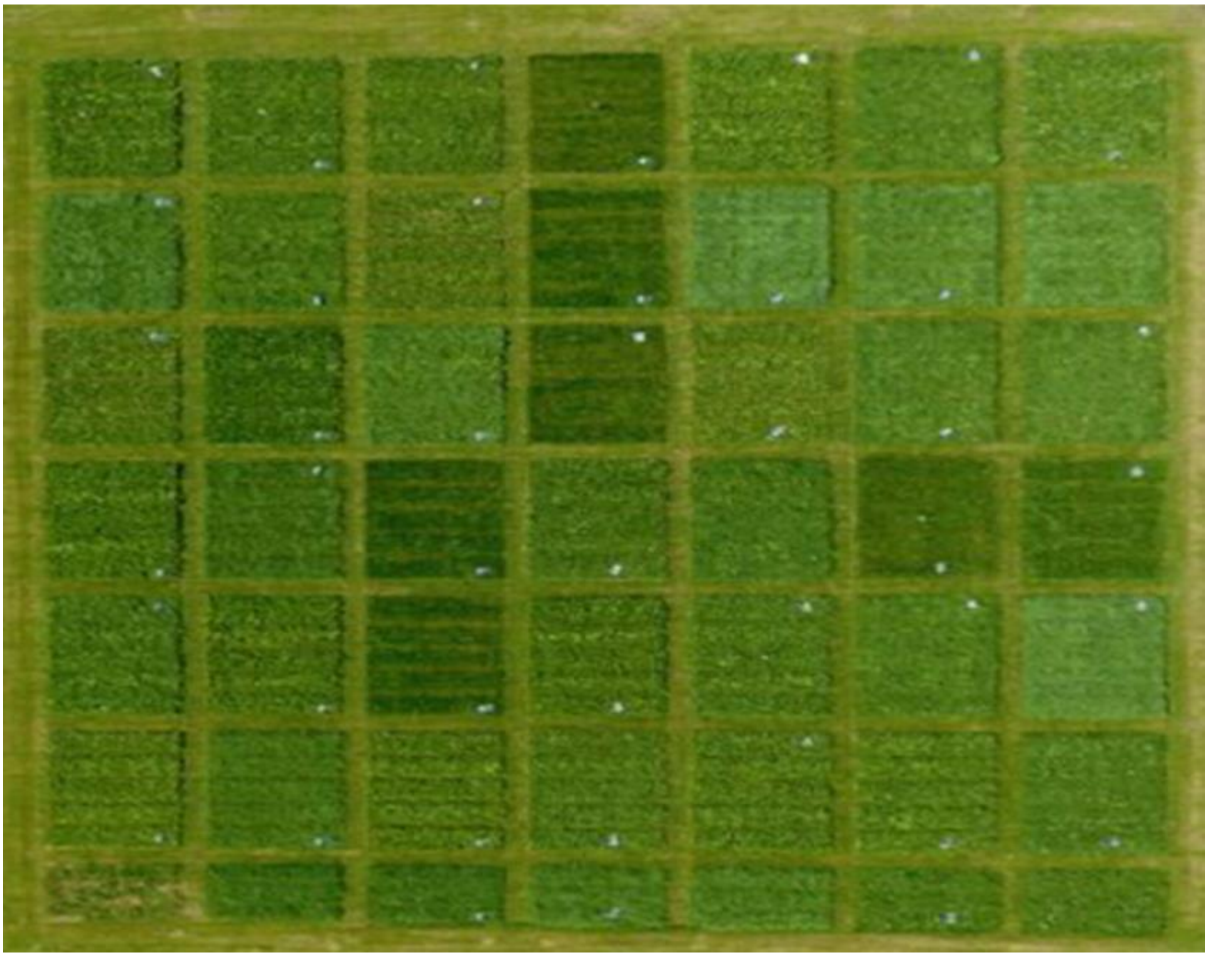


Image 1. Aerial photograph of the experimental plot layout.

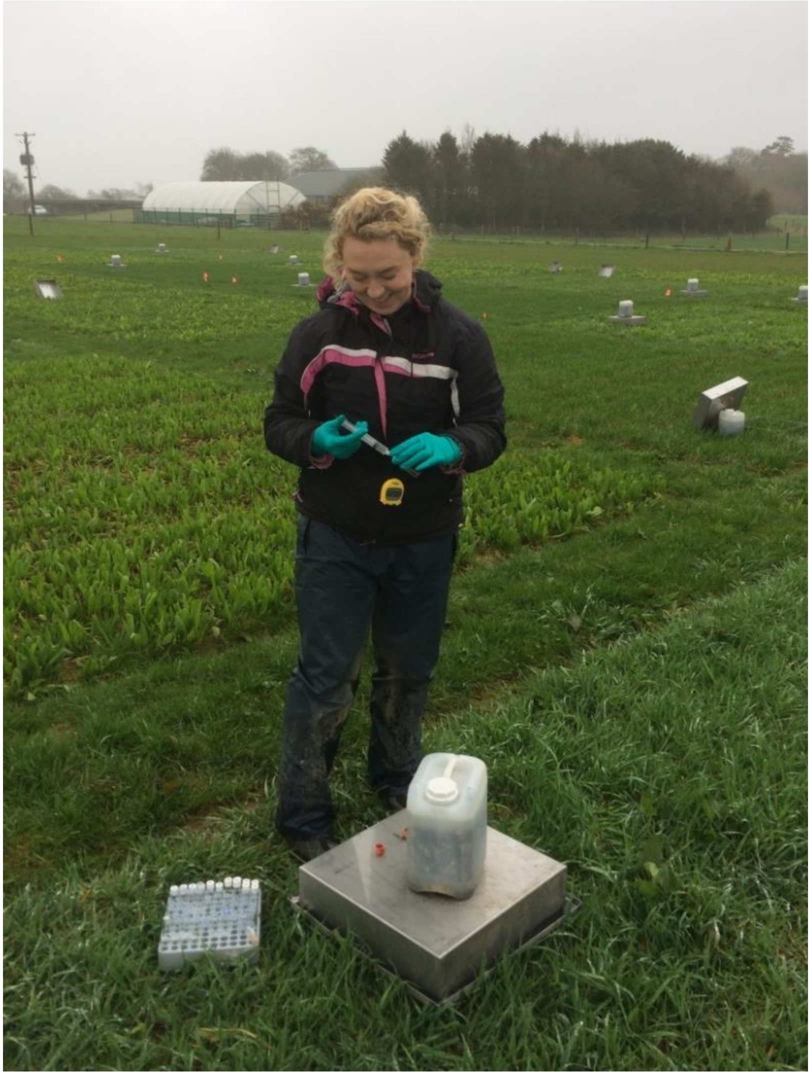


Image 2. Nitrous oxide sampling using static gas chamber methodology

2.4 Ancillary measurements

An area of the experimental plots was designated for ancillary measurements. A meteorological station was located approximately 500 m from the experimental site. Air temperature and atmospheric pressure were noted at each N_2O sampling occasion along with volumetric soil water content using a Theta probe (type ML2; Delta-T Devices, Cambridge, UK). Soil moisture measurements were used to calculate the water filled pore space % (WFPS) (Equation 1):

Equation 1. Where SWC = volumetric soil water content, BD = bulk density and PD = particle density (Fichtner et al., 2019).

$$WFPS\,\% = \frac{SWC}{1 - \frac{BD}{PD}} \times 100$$

2.5 Data analyses

The three response variables (y) were: N_2O-N emissions (kg ha^{-1} $year^{-1}$), N yield-scaled N_2O emissions (N_2O-N g ha^{-1} $year^{-1}$/ kg N yield ha^{-1} $year^{-1}$) and DM yield-scaled N_2O emissions (N_2O-N g ha^{-1} $year^{-1}$/ tonne DM yield ha^{-1} $year^{-1}$). Using the regression-based Diversity-Interactions modelling approach (Kirwan et al., 2009), we regressed responses on the sown proportional contributions of the six species as follows:

Equation 2. Regression model equation

$$y = \sum_{i=1}^{6} \beta_i P_i + \beta_7 P_{Lp300N} + \delta \sum_{\substack{i,j=1 \\ i<j}}^{6} P_i P_j + \varepsilon$$

Where y is the response variable (model fitted separately to each of our three responses), P_i represents the sown proportion of a species in a community (for i: 1 = *L. perenne*, 2 = *P. pratense*, 3 = *T. pratense*, 4 = *T. repens*, 5 = *C. intybus* and 6 = *P. lanceolata*). The β_1 to β_6 coefficient are the identity effects of each species (under 150N fertiliser); if $P_i = 1$, the β_i coefficient is the expected monoculture response of species i, while if $P_i < 1$, the expected contribution of that species to the mixture is $\beta_i P_i$. An extra term (β_7) was included for the 300N *L. perenne* monoculture plots ($P_{Lp300N} = 1$ for these plots and 0 otherwise). Equation 2 assumes that all pairs of species interact in the same way (captured by the coefficient δ). We tested various forms of the interactions, including no interaction effects and whether pairwise interactions were determined by FG membership (Kirwan et al., 2009). The error term ε was initially assumed to be normally distributed with zero mean and constant variance σ^2. However, exploratory analysis indicated that responses from plots with 100% legume were considerably more variable than all other plots, therefore we assumed that the error was normally distributed with zero mean and with two variance terms depending on the sown proportion of legume (100% or <100%). The Diversity-Interactions modelling approach allows prediction of the response for a wide range of communities from this six-species pool, based on the relative proportions of the component species. The overall response is based on the linear combinations of the identity effects, plus the sum of the interaction effects as required. Thus, for example, for a 50:50 grass-legume mixture of *L. perenne* and *T. pratense*, the expected response is $(\beta_1)0.5 + (\beta_3)0.5 + (\delta)(0.5*0.5)$. We predicted from the final fitted model to assess the effects on our three response variables across the monocultures and selected communities, which included the 6-species mixture and 300N *L. perenne* monoculture. The analysis was performed using SAS software version the software package SAS version 9.4 (SAS Institute, Cary, North Carolina, USA).

3. RESULTS

3.1 Climatic conditions

The highest average daily temperature (Fig. 1) recorded at the field site was in July at 20.4°C. The lowest daily average temperature was 18th March 2018 at 0.1°C. These are in contrast to long-term climatic averages recorded at the Rosslare Co. Wexford station (10 km away). Between 1978 and 2007, on average the highest daily temperature was 13.1°C and the lowest was 8.1°C. The long term mean annual rainfall for Johnstown Castle was 905.5 mm, with 49.9 mm for July, whereas the total annual rainfall for 2018-2019 (Fig. 1) was 1089.4 mm, with the average monthly rainfall for July 2018 being 1.7 mm (not including irrigation). The WFPS of soil at the experimental site averaged 48% over the experimental year (Fig. 2). Following high levels of precipitation in early 2018 (Fig. 1), the WFPS stayed at ~70% until early May, whereas during June, July and August the WFPS declined to 20-30%.

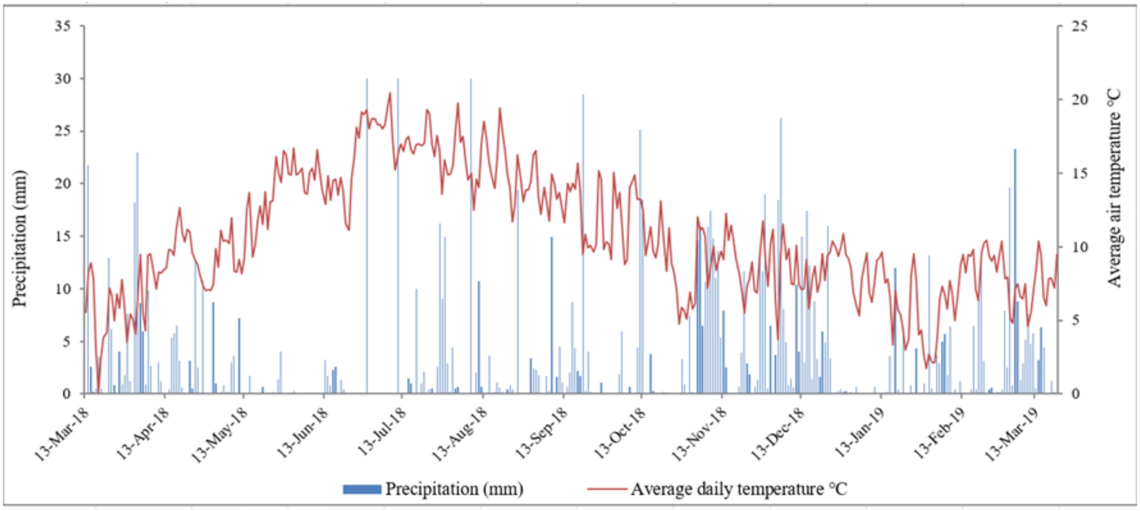


Fig. 1. Precipitation (mm) and temperature (°C) meteorological data for the experimental site collected from the JC meteorological station. Graph includes 3 x 30mm irrigation events which took place during summer 2018 of (due to drought).

3.2 Nitrous oxide emissions

3.2.1 Seasonal patterns in N_2O emissions

High N_2O fluxes were measured in April 2018, coinciding with high soil WFPS during March 2018 (Fig. 2). Conversely, low daily average N_2O fluxes were recorded during the drought period from June-August 2018 (Fig. 2). The highest daily average N_2O flux recorded (cumulative flux/number of days within the period) during the experimental year was from 300N *L. perenne* at 17.85 g ha^{-1} day^{-1} and the lowest was from *P. lanceolata* at 4.07 g N_2O ha^{-1} day^{-1}. The 150N *L. perenne* had a daily average N_2O flux of 5.40 g ha^{-1} day^{-1}. The highest individual N_2O measurement was from 300N *L. perenne* at 112 g ha^{-1} day^{-1} (Fig. 2) on 18th April. From April to May, (Fig. 2) the daily average N_2O flux of 300N *L. perenne* was nine times higher than the 6-species mixture and five times greater than 150N *L. perenne*. No N fertiliser was applied after September 2018 (in line with the Nitrates Directive), resulting in little to no N_2O fluxes during the autumn and winter period, except for the legume monocultures. Both clover species (Image 4) continued to produce N_2O emissions in the autumn/winter period (Fig. 2) with 36% of legume N_2O emissions occurring from August 2018 to January 2019, a time usually associated with low emissions.

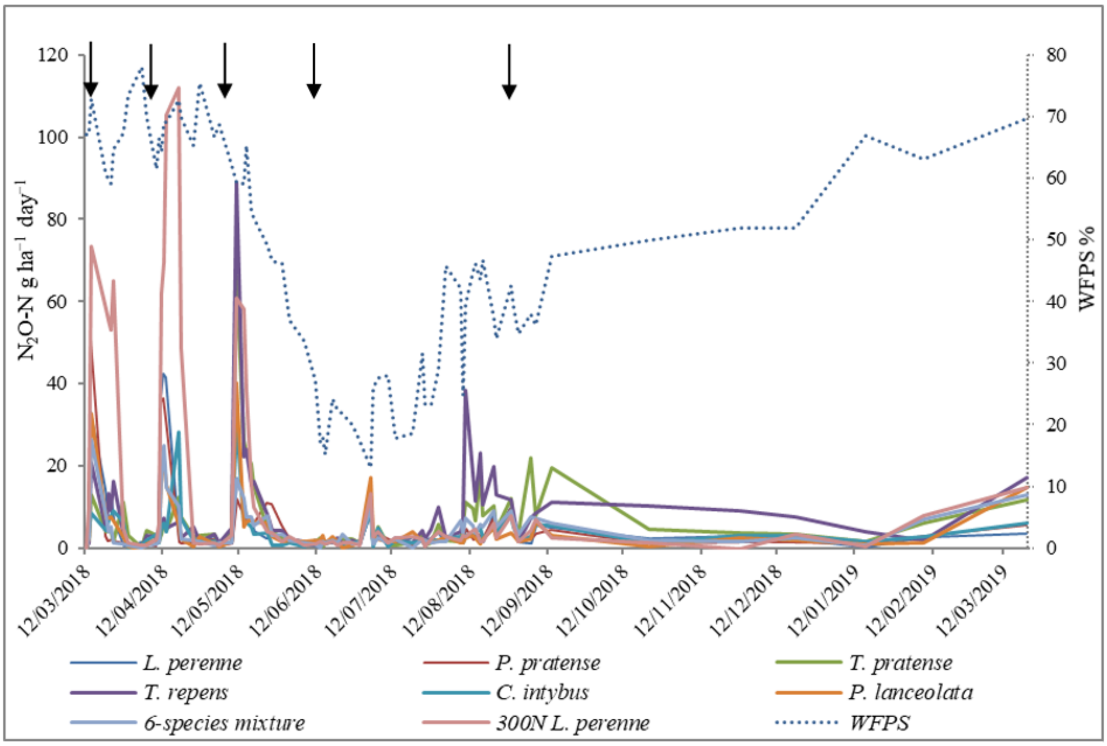


Fig. 2. Nitrous oxide emissions and corresponding water filled pore space (WFPS %) of the experimental site for each sampling occasion. Emissions are displayed for the 6-species mixture, monocultures of the individual species (*L. perenne, P. pratense, T. pratense, T. repens, C. intybus* and *P. lanceolata*) and the 300N *L. perenne* monoculture. All communities received 150 kg ha^{-1} year^{-1} nitrogen fertiliser, except for the 300N *L. perenne* community that received 300 kg ha^{-1} year^{-1} of inorganic nitrogen fertiliser. Arrows indicate fertiliser application dates (*Appendix C)*.

3.2.2 Effects of plant diversity on cumulative N_2O emissions

According to model comparisons, the best model for the N_2O emissions included species identity effects for each species and a term for 300N *L. perenne*, but no effects of species interactions were detected (model coefficient estimates shown in Table 1, first column). Thus, the effects of plant diversity on N_2O emissions were derived from species identity effects and their linear combination in mixtures, rather than from synergistic or antagonistic species interaction effects in mixtures (as shown in equation 2, but with the last term involving δ omitted). There was no significant difference between the 6-species mixture and any of the 150N monocultures, with the exception of the *C. intybus* monoculture (Fig. 3). The N_2O emissions from 300N *L. perenne* (3.18 kg ha^{-1} $year^{-1}$) were over twice that of the 6-species mixture (1.52 kg ha^{-1} $year^{-1}$) (Table 1, Fig. 3).

Table 1. (a) Coefficient estimates ± standard errors for the identity effects (β) and interaction estimates (δ) from equation 2, and (b) predictions for the average monoculture and the equi-proportional 6-species mixtures. These are presented for the models fitted to each of the three responses: N_2O-N emissions, N yield-scaled N_2O emissions and DM yield-scaled N_2O emissions.

		Modelled estimates		
		N_2O emissions	**N yield-scaled N_2O emissions**	**Dm yield-scaled N_2O emissions**
		(N_2O-N kg ha^{-1} $year^{-1}$)	(N_2O-N g ha^{-1} $year^{-1}$/N yield kg ha^{-1} $year^{-1}$)	(N_2O-N g ha^{-1} $year^{-1}$/DM yield tonne ha^{-1} $year^{-1}$)
(a)	300N *L. perenne*	3.18 ± 0.196	10.14 ± 0.603	259.3 ± 17.46
	150N *L. perenne*	1.39 ± 0.198	6.39 ± 0.644	144.4 ± 18.68
	P. pratense	1.33 ± 0.206	4.65 ± 0.659	124.1 ± 19.11
	T. pratense	2.18 ± 0.428	5.21 ± 1.269	183.6 ± 44.95
	T. repens	1.87 ± 0.428	5.76 ± 1.269	174.6 ± 44.95
	C. intybus	1.10 ± 0.197	5.04 ± 0.640	133.2 ± 18.57
	P. lanceolata	1.23 ± 0.197	4.66 ± 0.640	115.0 ± 18.57
	Species interaction effect δ	n/a	-3.69 ± 1.394	-87.1 ± 43.98
(b)	6-species mixture	1.52 ± 0.083	3.75 ± 0.356	109.5 ± 10.33
	Mean of 6 monocultures	1.52 ± 0.083	5.29 ± 0.401	145.8 ± 13.51

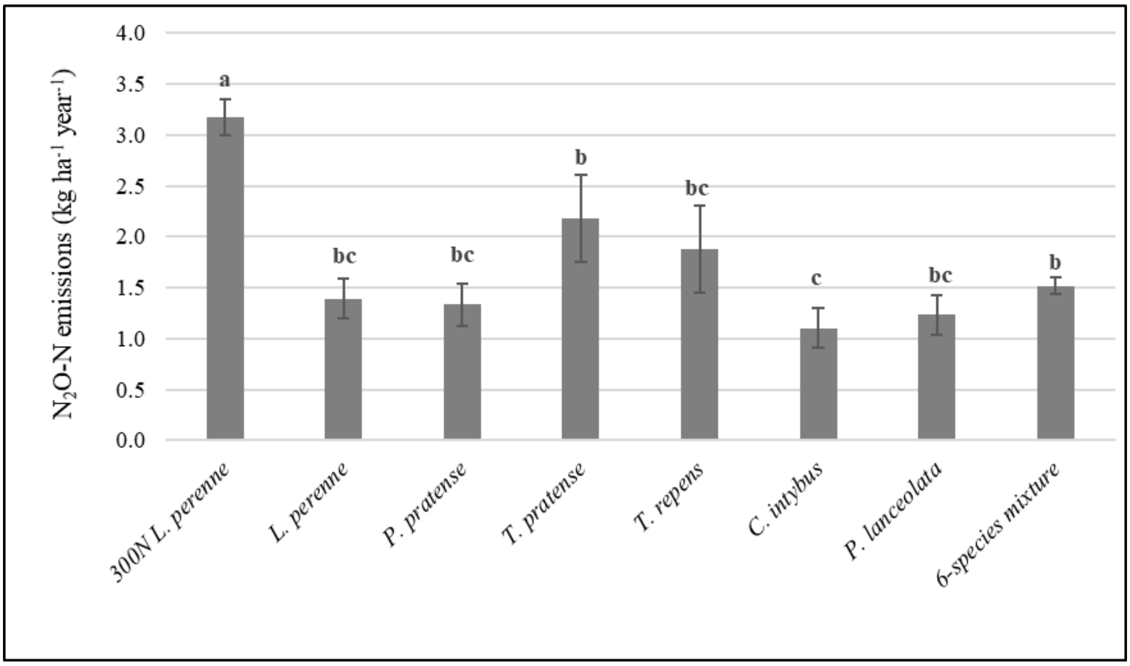


Fig. 3. Comparison of predicted N_2O-N emissions (kg ha^{-1} year^{-1}) from monocultures and the 6-species mixture. Values that share the same letter are not significantly different ($\alpha = 0.05$).

The highest N_2O emissions were from 300N *L. perenne* (significantly higher than all other treatments). Increasing fertiliser application to a *L. perenne* monoculture from 150N to 300N increased ($P < 0.001$) cumulative N_2O emissions from 1.39 to 3.18 N_2O-N kg ha^{-1} year^{-1} (Table 1, Fig. 3). N_2O emissions from 300N *L. perenne* were nearly three times higher than those from the *C. intybus* monoculture, the latter having the lowest estimated annual emissions at 1.1 kg ha^{-1} year^{-1}.

The ternary diagram (Fig. 4a) displays how variation in FG proportion (grass, herb and legume) affected the predicted annual N_2O emissions; higher N_2O emissions resulted from increased legume proportion while lower emissions were associated with communities dominated by grasses and/or herbs. The annual N_2O emissions from the community comprising 100% legume FG (50% *T. pratense* and 50% *T. repens*) were significantly higher ($P = 0.033$ and $P = 0.007$) than those from the equi-proportional community of either the grass (150N *L. perenne* and *P. pratense*) or herb (*C. intybus* and *P. lanceolata*) FGs, respectively (Fig. 4b).

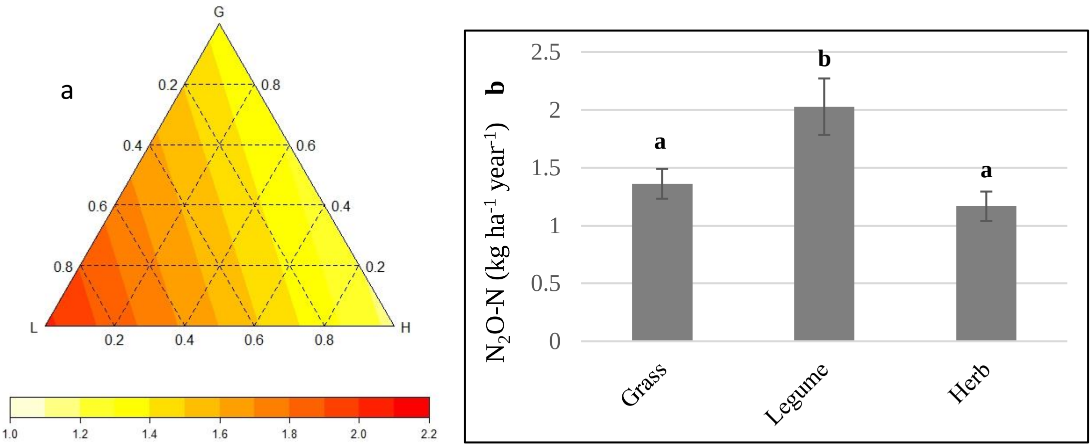


Fig. 4 a) Predicted annual N_2O emissions (N_2O-N kg ha^{-1} year^{-1}) in response to variation in the proportion of the grass (G), herb (H) and legume (L) FGs within grassland communities. The communities represented in this ternary diagram are based on the equi-proportional contribution of each of the two species within a FG. Thus, each vertex represents a 50:50 mixture of the two component species in the respective FG; the sides represent communities with varying proportions of two FGs (comprising four species), and the interior points represent varying proportions of three FGs (comprising six species). Thus, for example, the predicted N_2O emissions for the community comprising 10% grass, 40% legume and 50% herb is calculated from the species-level composition comprising 5% *L. perenne,* 5% *P. pratense,* 20% *T. pratense*, 20% *T. repens*, 25% *C. intybus* and 25% *P. lanceolata.* **b)** Predicted annual N_2O emissions (N_2O-N kg ha^{-1} year^{-1}) for a 50:50 mixture of the two species from each FG: grass, legume and herb (these predictions correspond to the vertices in the ternary diagram). For example, the legume FG contains 50% *T. pratense* and 50% *T. repens*. Bars that share a letter are not significantly different ($\alpha = 0.05$).

3.3 Yield-scaled N_2O analyses (emissions intensity)

For 2018, the average N-yield in harvested forage of the 6-species mixture, 150N *L. perenne* and 300N *L. perenne* was 40.5 kg ha^{-1} year^{-1}, 19.9 kg ha^{-1} year^{-1} and 28.5 kg ha^{-1} year^{-1} respectively. The 6-species mixture produced an average DM yield of 12.4 tonnes DM ha^{-1}

year^{-1}, and *L. perenne* at 150N and 300N produced 9.2 and 10.7 tonnes DM ha^{-1} year^{-1} respectively (Grange et al., in review). This yield data was combined with N_2O data to calculate two measures of yield-scaled N_2O emissions; as outlined in section 2, the two measures of emission intensity analysed were N yield-scaled N_2O emissions (N_2O-N g ha^{-1} year^{-1}/ kg N yield ha^{-1} year^{-1}) and DM yield-scaled N_2O emissions (N_2O-N g ha^{-1} year^{-1}/ tonne DM yield ha^{-1} year^{-1}).

For both measures of emission intensity, the best model included species identity effects for each species, and a negative average pairwise interaction effect that resulted in an additional suppressive effect on yield-scaled N_2O emissions in mixtures (as in equation 2, with model estimates in Table 1). The suppressive interaction term was only borderline significant for the DM yield-scaled emissions ($P = 0.056$), however, diagnostic analysis of the model with and without the interaction term indicated a superior fit when the interaction term was included and it was kept in the final model. Emissions from the 6-species mixture were lower than the mean of the six 150N monocultures for N yield-scaled emissions ($P = 0.012$) and there was a similar indication for DM yield-scaled emissions ($P = 0.056$), demonstrating that increasing species diversity in multi-species mixtures suppressed yield-scaled N_2O emissions (Fig. 5a and 5b). The 300N *L. perenne* treatment had higher N yield-scaled emissions (Fig. 5a) than all other 150N communities. This result was similar for DM yield-scaled emissions (Fig. 5b), with the exception of both legume monocultures.

As the proportions of grasses, legumes and herbs change, communities with high proportions of legumes and/or grasses showed an increase in N yield-scaled N_2O emissions (Fig. 6a). Communities with high legume proportion showed an increase in DM yield-scaled N_2O emissions (Fig. 6b) compared with herbs and grasses.

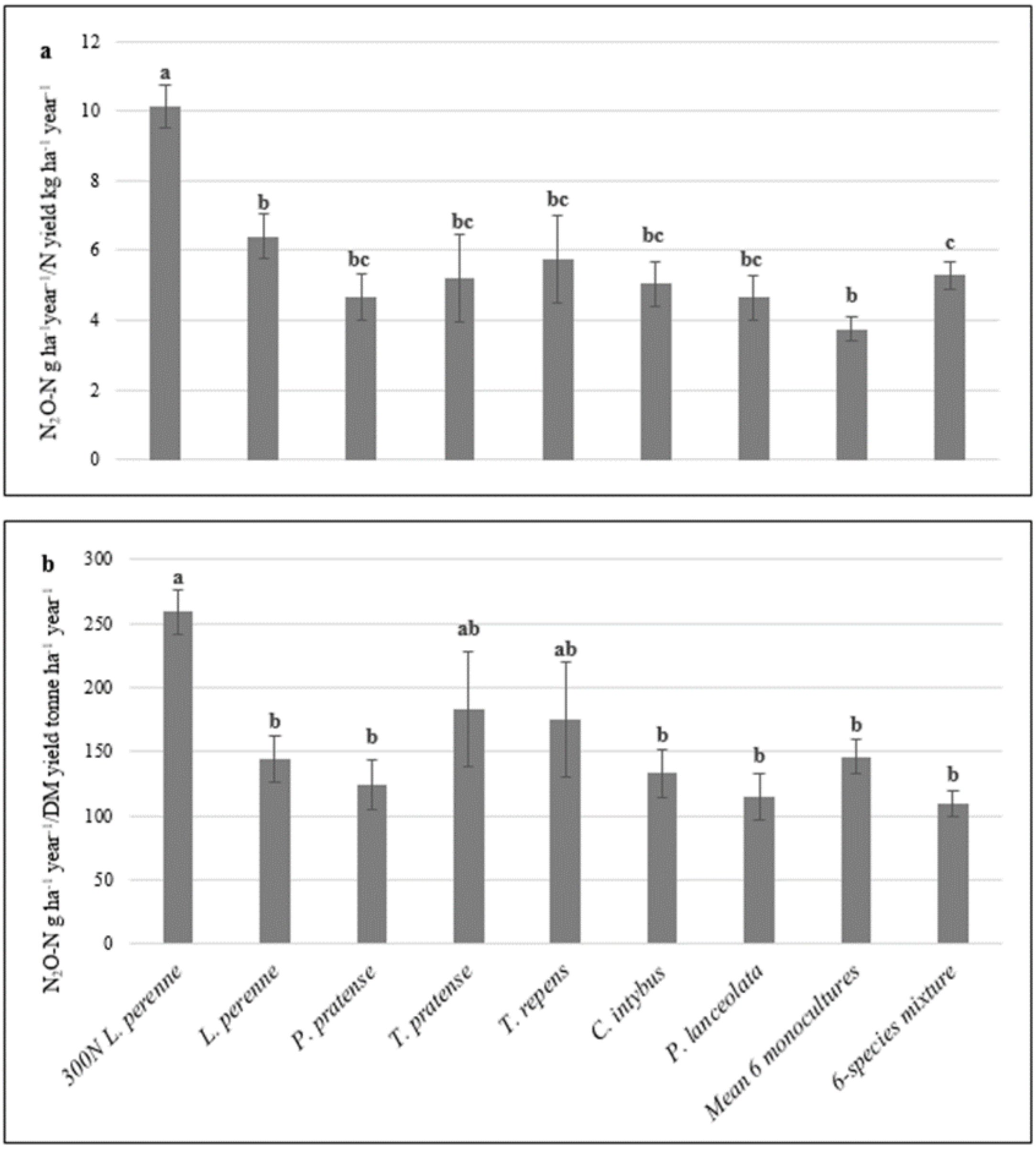


Fig. 5. a and b Comparison of yield-scaled N_2O emissions from forage monocultures, the 6-species mixture and the mean of the six 150N monocultures for **a)** N yield-scaled N_2O emissions (N_2O-N g ha^{-1}year^{-1}/N yield kg ha^{-1} year^{-1}) and **b)** DM yield-scaled N_2O emissions (N_2O-N g ha^{-1} year^{-1}/DM yield tonne ha^{-1} year^{-1}). Values that share the same letter are not significantly different ($\alpha = 0.05$).

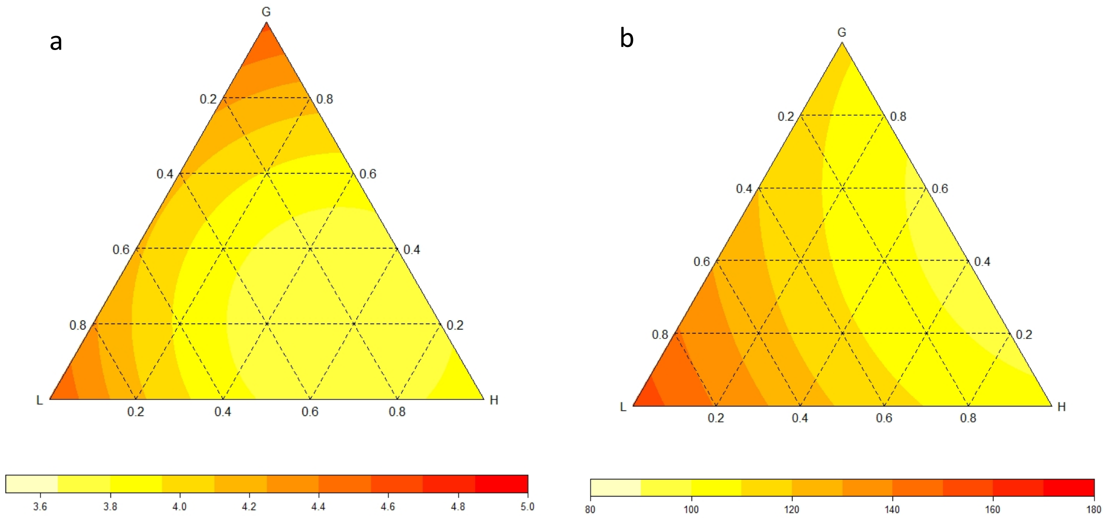


Fig. 6. Estimated emission intensity analyses for **a)** N yield-scaled N_2O emissions (N_2O g ha^{-1} year^{-1} / N yield kg ha^{-1} year^{-1}) and **b)** DM yield-scaled N_2O emissions (N_2O gha^{-1} year^{-1}/DM yield tonne ha^{-1} year^{-1}) in response to variation in the proportion of grass (G), herb (H) and legume (L) FGs within grassland communities. The communities represented in this ternary diagram are based on an equal proportional contribution of each of the two species within a FG. Thus, each vertex indicates the average of the two component species in the respective FG; the sides represent communities with varying proportions of two FGs (comprising four species), and the interior points represent varying proportions of three FGs (comprising six species).

4. DISCUSSION

4.1 N_2O emissions

There were no net synergistic or antagonistic effects due to interspecific interactions; nevertheless, there was still a benefit from multi-species mixtures through a reduction in the proportions of higher-emitting species in the mixture, i.e., legumes (Fig. 4a and b).

Our study highlights the potential for the multi-functional benefits associated with diverse grassland communities (e.g., see Introduction) to be gained without an associated increase in N_2O emissions.

Overall, increasing the proportion of both grasses and herbs within a grassland community resulted in lower N_2O emissions than in legume-dominated communities (Fig. 4a). When comparing FGs, legumes had significantly higher N_2O emissions than both herb and grass communities (Fig. 4b). When looking at selected monocultures, there was no significant difference in N_2O emissions between *P. lanceolata* and 150N *L. perenne* (Fig. 3). This is surprising given that many studies have found that swards dominated by *Plantago* species can directly reduce N_2O emissions in comparison with *L. perenne* monocultures (Gardiner et al., 2018; Luo et al., 2018). Bracken et al. (2020) found evidence that *P. lanceolata* potentially inhibits nitrification when included in a mixed sward. In contrast to aforementioned studies, the lowest N_2O emissions in our experiment were from the *C. intybus* monoculture rather than *P. lanceolata.*

When looking at annual N_2O emissions from selected individual monocultures (Fig. 3), each of *T. repens* and *T. pratense* did not have significantly higher N_2O emissions than other monocultures (with the exception of *T. pratense* being higher than *C. intybus* (Fig. 3)). This is strongly related to the much higher variability associated with legume monocultures in this experiment, compared to the other species (Table 1).

Greater variation in N_2O emissions from legumes may stem from the high levels of variation associated with BNF (and therefore N_2O emissions from legume stands) (Rochette et al., 2004; Unkovich et al., 2008; Evers., 2011; Nyfeler et al., 2011;). Across studies, the rate of BNF can range from 100 to 380 kg N ha^{-1} $year^{-1}$ in northern temperate pastures and is dependent on multiple factors including environmental conditions, grassland management practices and legume species/cultivar type and proportion (Ledgard and Steele 1992; Hansen and Vinther 2001; Fox et al., 2019). Nitrous oxide fluxes associated with legumes can be attributed to N release from root exudates and from crop residue decomposition, rather than from the BNF process itself (Rochette and Jansen., 2005). The latter results were pivotal in the removal of the BNF process from the Intergovernmental Panel on Climate Change (IPCC) N_2O inventory methodology. Legume inclusion within a grassland community allows for N addition into the soil system by BNF, lowering reliance on N fertilizer application without compromising on yield (Egan et al., 2018). Legume residues can improve the quality and quantity of soil organic matter over time, providing benefits for following crops (termed ‘legacy effect’). Fox et al. (2019) assessed legacy effects over a range of legume proportions and N application levels, and found maximum legacy benefits on a *L. multiflorum* crop from a prior grassland ley comprising 50% legume proportion and receiving 150N. Our study should be considered when using legumes as ley cover crops as environmental benefits of N addition to soil (i.e., less fertilizer N requirement) may be compromised by high N_2O losses.

4.2 Yield-scaled N_2O emissions (emissions intensity)

The effects of multi-species swards were more pronounced when considering yield-scaled N_2O emissions (expressed as either N_2O-N g ha^{-1} $year^{-1}$ / N yield kg ha^{-1} $year^{-1}$ or N_2O-N g ha^{-1} year⁻

^{1} / DM yield tonne ha^{-1} year^{-1}). Looking at both responses, the performance of mixtures was best explained as a linear combination of the identity effects, and an additional antagonistic interaction between species that acted to suppress emissions intensity. Thus, compared to the yield-scaled N_2O emissions predicted from the average of the six monocultures, the yield-scaled N_2O emissions of the 6-species mixture were 29.1 % lower ($P = 0.012$) and 24.9% lower ($P = 0.056$) for the N- and DM yield-scaled measures respectively (Table 1). Given the differences among the species, mixture compositions at and around the 6-species mixture tended to have considerably lower yield-scaled N_2O emissions (Fig. 6a and b). As both yield-scaled N_2O responses had the same numerator (N_2O emissions) the significant diversity effect must be related to a strong effect of plant diversity (interspecific interactions) on each of the denominators, total N yield and total DM yield (presented elsewhere for both the former (Grange et al., unpubl.) and latter (Grange et al., in review)). Transgressive over yielding, whereby mixtures outperform the highest performing constituent monoculture, is driven by resource use efficiency and complementarity among species in mixtures (Mason et al., 2020).

Our study confirms that reduced emissions intensity can now be considered one of the many multi-functional benefits associated with multi-species swards. The 6-species mixture was more efficient, because more yield was produced with reductions in N_2O losses to the environment. Overall, the six-species mixture significantly reduced N yield-scaled N_2O emissions compared with *L. perenne* (both 150N and 300N) and 150N legume monocultures (Fig. 5a) and lower DM yield-scaled N_2O emissions than 300N *L. perenne* (Fig. 5b). These results accord with the agronomic assessment of N_2O emissions by van Groenigen et al (2010), where yield-scaled N_2O emissions of non-leguminous crops increased rapidly at higher N application levels (>190 kg N ha^{-1} year-^{1}).

5. CONCLUSION

Overall, the effect of plant diversity on N_2O emissions was derived from linear combinations of the species' performance in monoculture (species' identity). The effects of multi-species mixtures on N_2O emissions intensity included species identity effects, and a net interspecific interaction that suppressed emissions intensity. The conventional 300N *L. perenne* community produced over double the N_2O emissions as the 150N six-species mixture (3.18 vs 1.52 kg N_2O-N ha^{-1} $year^{-1}$). Considering emissions intensity, the same N yield and DM yield of 300N *L. perenne* could have been produced with the 6-species mixture using half the fertiliser and reduced N_2O losses of 63% and 58% respectively. In comparison to 150N *L. perenne*, the same N yield and DM yield could have been produced with a 6-species mixture while producing 41% and 24% less N_2O emissions. Communities dominated by legumes significantly increased N_2O emissions, this should be considered when using legumes as cover crops. Overall, the manipulation of grassland composition is a practical, farm-scale management action that can reduce both N_2O emissions and yield-scaled N_2O emissions, and contribute to the sustainability of grassland production.

6. ACKNOWLEDGEMENTS

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7. APPENDICES

Appendix A Seeding rates of the multi-species experimental field trial. Species include *L. perenne* (Lp) *P. pratense* (Pp), *T. pratense* (Tr), *T. repens* (Tr), *P. lanceolata* (Pl), and *C. intybus* (Ci).

Species	**Lp**	**Pp**	**Tp**	**Tr**	**Pl**	**Ci**
Seed (Kg ha^{-1})	28	12	12	15	10	8

Appendix B Experimental design indicating the composition and relative abundance of the sown communities. Also indicated are functional group richness (FGs), species richness (Species) and number of replicates (Reps). Species include *L. perenne* (Lp) *P. pratense* (Pp), *T. pratense* (Tr), *T. repens* (Tr), *P. lanceolata* (Pl), and *C. intybus* (Ci).

Community	**Reps**	**FGs**	**Species**	**FG(G)**	**FG(H)**	**FG(L)**	**Lp**	**Pp**	**Tp**	**Tr**	**Pl**	**Ci**
1	3	1	1	1	0	0	1	0	0	0	0	0
2	3	1	1	1	0	0	0	1	0	0	0	0
3	3	1	1	0	1	0	0	0	1	0	0	0
4	3	1	1	0	1	0	0	0	0	1	0	0
5	3	1	1	0	0	1	0	0	0	0	1	0
6	3	1	1	0	0	1	0	0	0	0	0	1
7	2	1	2	1	0	0	0.5	0.5	0	0	0	0
8	2	1	2	0	1	0	0	0	0.5	0.5	0	0
9	2	1	2	0	0	1	0	0	0	0	0.5	0.5
10	2	2	4	0.5	0.5	0	0.25	0.25	0.25	0.25	0	0
11	2	2	4	0.5	0	0.5	0.25	0.25	0	0	0.25	0.25
12	2	2	4	0	0.5	0.5	0	0	0.25	0.25	0.25	0.25
13	1	3	5	0.6	0.2	0.2	0.6	0	0.1	0.1	0.1	0.1
14	1	3	5	0.6	0.2	0.2	0	0.6	0.1	0.1	0.1	0.1
15	1	3	5	0.2	0.6	0.2	0.1	0.1	0.6	0	0.1	0.1
16	1	3	5	0.2	0.6	0.2	0.1	0.1	0	0.6	0.1	0.1
17	1	3	5	0.2	0.2	0.6	0.1	0.1	0.1	0.1	0.6	0
18	1	3	5	0.2	0.2	0.6	0.1	0.1	0.1	0.1	0	0.6
19	3	3	6	0.33	0.33	0.33	0.17	0.17	0.17	0.17	0.17	0.17
20	4	1	1	1	0	0	1	0	0	0	0	0

Appendix C Fertiliser application rate equivalents to each plot over the agronomic year. (The community numbers are as listed in Appendix B.)

Split	Date	Fertiliser application: communities 1-19	Fertiliser application: community 20
1	12-Mar-2018	30 kg N ha^{-1}	60 kg N ha^{-1}
2	09-Apr-2018	30 kg N ha^{-1}	60 kg N ha^{-1}
3	09-May-2018	30 kg N ha^{-1}	60 kg N ha^{-1}
4	11-Jun-2018	20 kg N ha^{-1}	40 kg N ha^{-1}
5	20-Aug-2018	40 kg N ha^{-1}	80 kg N ha^{-1}

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CHAPTER 4

The effect of sward composition on N_2O emissions during drought and re-wetting events.

Intended for submission to Science of the Total Environment

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ABSTRACT

Extreme weather events such as drought and floods are predicted to occur more frequently as a consequence of ongoing climate change. The drying and re-wetting of soils causes results in slaking of soil aggregates and a priming effect due to the release of carbon and nitrogen and a flush of microbial activity, which results in elevated nitrous oxide (N_2O) emissions. Multi-species swards have shown increased nitrogen (N) use efficiency and greater drought resilience compared to conventional monocultures. However, the impacts of drought and re-wetting have not been assessed regarding the N dynamics of intensively managed multi-species swards. This experiment aimed to quantify the effects of drought and re-wetting on the N dynamics across a gradient of grassland species diversity. This was done by measuring N_2O emissions during an eight week drought simulation and after the re-wetting event. The legacy effect of drought was assessed by analysing responses post fertiliser application, two weeks after the re-wetting event. Re-wetting drought induced plots resulted in much greater N_2O losses than when fertiliser was applied two weeks after-rewetting. Drought resulted in a significant increase in soil NH_4^+ levels, no significant increase in NO_3^- and N_2O fluxes did not escalate above background levels. Following re-wetting of dry soils, NO_3^- concentrations were significantly higher than from control soils ($P > 0.05$), indicating resumption of the nitrification process. There were no lasting legacy effects of drought and re-wetting on elevated N_2O emissions. This indicates that fertilizer applied two weeks after a re-wetting event is an effective management strategy and does not further increase the elevated N_2O emissions associated with a re-wetting event.

1. INTRODUCTION

‘Continued global warming is projected to further increase... the severity of wet and dry events’ and the occurrence of every major drought once in 10 years is predicted to double with a 1.5 °C warming and increase to 2.4 times with a 2 °C warming (IPCC, 2021). Emissions of nitrous oxide (N_2O), a potent greenhouse gas (GHG) are also predicted to increase from grasslands in line with agricultural intensification and climate change (Field et al., 2012, de Klein et al., 2014). An example of a climate related effect on soil nutrient cycling the elevated pulses of N_2O from soils following drought and re-wetting events. The nitrification, denitrification and mineralisation processes slow down as soils dry out. Re-wetting dry soil results in aggregate slaking which releases previously protected organic substrates and N compounds. These newly available substrates can then be utilized by microbial communities thus increasing the rates of N transformation in the soil system. The length and extremity of the drought and rewetting phases influence the extent of total N_2O losses (Guo et al., 2014). Additionally, there is evidence to suggest that plant responses to drought can further influence the soil N dynamics and N_2O pulses upon rewetting (Liu et al., 2014). Plants directly impact N_2O production as they determine the N availability and thus nitrification and denitrification (the processes where N_2O losses primarily occur). This is due to competition for resources, oxygen consumption, root structure, root exudates, changes in pH and carbon availability (Abalos et al., 2014).

Multi-species swards are considered a drought tolerant and resilient substitute to conventional monocultures in intensive production systems (Hofer et al., 2016; Haughey et al., 2018). Intensive multi-species mixtures generally contain combinations of species within distinct functional groups (FGs) including grasses, legumes and herbs. A functional group contains species that share morphological and physiological traits. Grouping species by functional groups provides a link between plant traits and processes which occur at ecosystem level

(Pokorny et al., 2005). The rate of biomass production from multi-species swards has been shown to recover quickly after drought impairment compared to monocultures (Finn et al., 2018). Drought resilience has been attributed to diversity effects, high N use efficiency and niche complementarity, the latter being the main driver of grassland ecosystem productivity (Finn et al., 2013; Isbell et al., 2011). It has also been found that higher species diversity promotes increased stability of above ground vegetative carbon (Bloor and Bardgett, 2012). To date there are gaps in knowledge surrounding N cycling and N_2O emissions from multi-species swards, especially regarding environmental stressors such as drought and re-wetting. Sward resistance and resilience to drought is more effective when containing deep-rooting species such as chicory (*Cichorium intybus)*. Belonging to the herb FG, chicory has a deep tap root that can acquire resources from the deeper soil layers (Hoekstra et al., 2015).

However, increased N use efficiency, drought tolerance and resilience (Husse et al., 2017, Hoekstra et al., 2015) indicate that multi-species grasslands may also influence the N dynamics during a drought and re-wetting event i.e. soil mineral N (NH_4^+ and NO_3^-) and N_2O fluxes (Figure 1). This study monitored the effect of grassland species identity and diversity on N_2O emissions and soil mineral N during drought, rewetting and subsequent fertiliser application events. Thus, the objective of this experiment was to quantify, compare and determine any potential species or FG interaction on the N_2O and mineral N responses during the three experimental periods. Additionally, from a management perspective we aimed to determine an appropriate fertiliser application strategy to avoid exaggerating the increased N_2O emissions associated with re-wetting. The experimental hypotheses are summarised in Figure 1 and the specific aims of this study were to:

1) Investigate the effect of species identity and diversity on N_2O emissions and soil mineral N dynamics throughout three distinct experimental periods: Drought, Re-wetting (irrigation) and Fertiliser application two weeks post re-wetting.

2) To identify potential legacy effects of drought on elevated N_2O emissions following fertiliser application two weeks after re-wetting across a range of sward compositions.

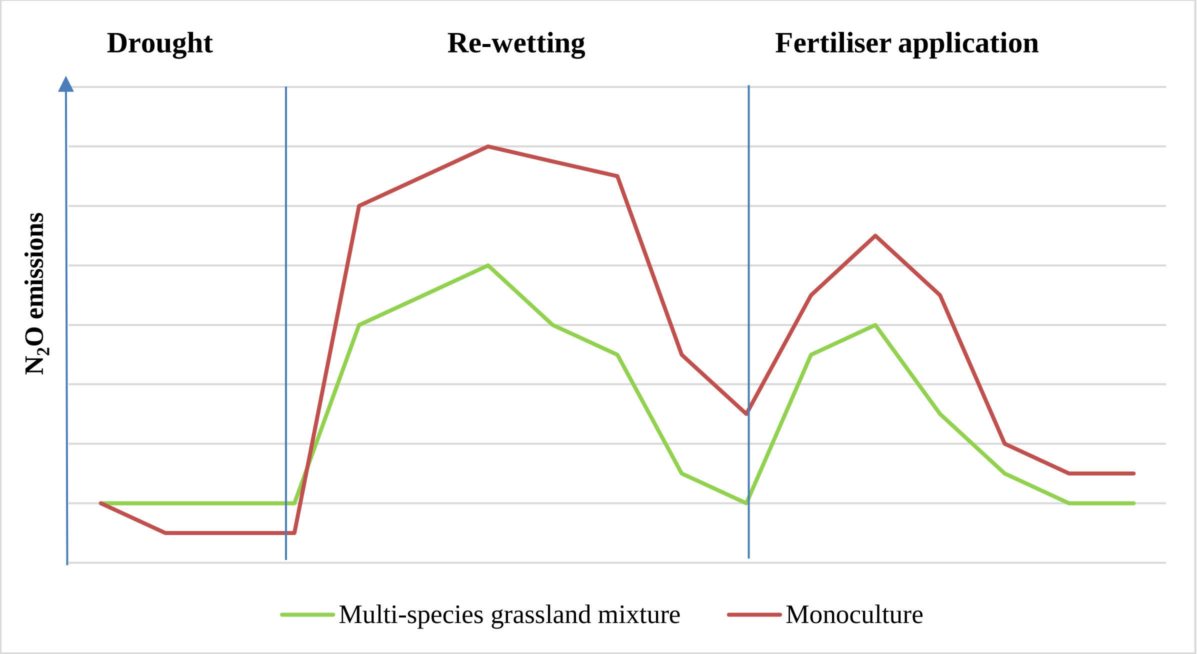

Figure 1: Graphical representation of hypotheses: More diverse swards will not be as adversely affected by drought and re-wetting events as the monoculture. Higher N_2O fluxes during the re-wetting and fertiliser application period are indicative of reduced N use efficiency and drought resilience. Therefore, this experiment aims to quantify this expected difference.

2. MATERIALS AND METHODS

The experimental site was located at Teagasc, Johnstown Castle, Co. Wexford, Ireland (JC) 52°18′27N. Ireland has a temperate, maritime climate, with an average annual air temperature of 10.4°C and average cumulative rainfall of 1,037 mm (1981–2010, 30 year average). Rainfall, air and soil temperature were recorded at the JC meteorological station located within one kilometre of the experimental site. The experimental field is a stagnic brown podzolic soil which corresponds to a stagnic podzol within the WRB classification (IUSS Working Group WRB, 2006). Soil pH is 5.7 and average bulk density of the plots at 5-10 cm depth is 1.35 g cm^3. The experiment followed a simplex design (Scheffe, 1963) including 43 main plots measuring five x ten metres. The 'split-plot' divided the main plot into two treatments: a drought and control treatment. Plots were sown with one to six species of varying proportions including two productive grasses (*Lolium perenne* and *Phleum pratense*), two clovers (*Trifolium repens* and *Trifolium pratense*) and two deep rooting herbs (*Cichorium intybus* and *Plantago lanceolata*) with between one and four replicates per treatment appendix 1. A *L. perenne* (HN Lp) monoculture community receiving double N fertiliser levels was included (n = 4) as a high N reference. Calcium ammonium nitrate (CAN) fertiliser was applied in June (20 kg N ha^{-1} for all mixtures and 40 kg N ha^{-1} for HN Lp) and in August, two weeks after re-wetting (40 kg N ha^{-1} and 80 kg N ha^{-1} for HN Lp) Nitrous oxide emissions were monitored between June-September 2018, both during an eight week experimental drought and a six week re-wetting period.

2.1 Drought treatment

An experimental drought was applied to one half of each plot of the multi-species field trial for eight weeks (57 days) between 11/06/2018 and 07/08/2018. This timeframe was in line with seasonal rainfall and soil moisture deficit predictions. Artificial drought conditions were created through the use of rainout shelters which consisted of metal frames and polypropylene plastic covers. These were placed on subplots of the main field trial as per the methodology described in Finn et al. (2018) (Image 1). Fertilisation occurred two weeks after the re-wetting event (08/08/2018) on the 20/08/2018.

Due to a naturally occurring drought in summer 2018, the control plots were irrigated to maintain a reasonable rain-fed comparison. The control plots were irrigated on three occasions (28/06/2018, 04/07/2018 and 18/07/2018) with 30 mm rainfall equivalent (a total of 90 mm rainfall equivalent). At the re-wetting event on 08/08/2018, drought shelters were removed and all plots (drought impaired and control) were irrigated with 30 mm water. Natural rainfall occurred on 10/08//2018 and continued intermittently for the rest of the experiment (Figure 2).

2.2 Nitrous oxide sampling

Nitrous oxide was monitored using the static chamber methodology (De Klein and Harvey, 2012). An individual stainless steel gas chamber was placed in each of the 86 experimental plots. The static gas chamber design and protocol followed that of Harty et al (2016) and Krol et al (2016). Chambers consisted of 40 cm × 40 cm collars lined with neoprene and inserted to a 5 cm depth in the soil. Matching chamber lids (10 cm high) and a 10 kg weight was placed on top of the collars at sampling to create an airtight headspace. A 10 ml air sample was taken through a 16 mm suba-seal rubber septum using a 10 ml polypropylene syringe fitted with a hypodermic needle. The syringe was plunged twice to mix the gas inside the chamber before the final sample was taken. The gas samples were injected into individual pre-evacuated (to – 1,000 mbar) 7 ml double-wadded screw-cap septum glass vials. Gas sampling took place between 10:00 and 13:00 for an enclosure time of 40 min to obtain measurements representative of the average hourly flux of the day. Daily N_2O flux calculations (g N $ha^{-1}day^{-1}$) were calculated using the linear regression determining change in N_2O concentrations in the chamber between sampling times at 0, 20 and 40 minutes (Chadwick et al., 2014). Cumulative N_2O emissions were obtained by integration of daily fluxes and linear interpolation between measurement points (De Klein and Harvey, 2012; Burchill et al., 2014).

Nitrous oxide samples were taken once a week during the drought phase from the *L. perenne* monoculture and the six-species mixture from both drought and rain-fed plots. This was to monitor the low N_2O fluxes that previous research indicated were to be expected from soils under drought conditions (Hartmann and Niklaus, 2012; Davidson et al., 2008; Van Haren et al., 2005). High resolution N_2O sampling took place immediately following re-wetting on 08/08/2018 on all 86 plots and continued for two weeks. High resolution sampling continued again after fertiliser application on the 20/08/2018 for four weeks until the 13/09/2018. Sampling took place three days a week for both two week intensive sampling periods (i.e. post re-wetting or two weeks after fertiliser application) and two days a week for weeks three and four following fertiliser application until background level emissions were reached.

Nitrous oxide gas was analysed in the Johnstown Castle Gas laboratory by gas chromatography (GC) (Varian CP 3800 GC; Varian, Walnut Creek, CA, USA) fitted with a 63Ni electron capture detector (ECD) with high-purity helium as a carrier gas. Areas under N_2O peaks were integrated using Star Chromatography Workstation (Varian). A 0.5 ppm N_2O standard (within the range of expected results) was placed with field gas samples as an extra quality assurance method.

Image 1. Drought shelters in place at the experimental site to create drought conditions on one half of each split plot.

2.3 Ancillary soil measurements.

An area of experimental plots was designated for soil sampling and ancillary measurements. Air temperature and atmospheric pressure were monitored at each N_2O sampling occasion at a nearby meteorological station approximately 600m away. Soil cores were taken on 25/06/2019 to establish the bulk density of the experimental site. Soil moisture at 10cm depth was monitored using soil water potential probes on both drought and control sides of five randomly chosen plots of the *L. perenne* and six-species communities. Water potential was converted to volumetric soil moisture content, using HYPROP equipment (METER group (UMS 2015)), fitted with Fredlund-Xing model. Soil moisture was used to calculate soil water-filled pore space % (WFPS) (Equation 1). Soil samples were taken on 02/08/2108 at the end of the drought period and on 09/08/2018 following re-wetting and analysed for mineral N by KCl extraction. On both soil sampling occasions, five soil cores were taken from across each plot using a 10 cm depth soil corer and stored in a cool box. Samples were sieved within 24 hours using a 4 mm sieve and extracted in 2 M KCl (5:1 ratio and shaken for 1 h). The extract was analysed for ammonium (NH_4^+) and total oxidised N (nitrite NO_2^- and nitrate NO_3^-) by colorimetric analysis using an Aquakem 600 discrete analyser (Thermo Electron OY, Vantaa, Finland). The gravimetric moisture content was determined by drying 20g subsamples 24 h for at 105°C and weighing before and after (Krol et al., 2017).

$$WFPS\,\% = \frac{SWC}{1 - \frac{BD}{PD}} \times 100$$

Equation 1. Where SWC = volumetric soil water content, BD = bulk density and PD = particle density (Fichtner et al., 2019).

2.4 Statistical analyses

The response variables were N_2O-N emissions (cumulative over the rewetting and fertiliser periods) and mineral N responses (NH_4^+ and NO_3^-) during the drought and re-wetting events. Some responses were log-transformed to correct for non-normal distribution of data (Moulin et al., 2014). The effect of treatment on the N_2O and soil mineral N responses were analysed using regression-based modelling techniques for the simplex design of the experiment under control and drought conditions using SAS 9.4 (Kirwan et al., 2009). This approach regressed responses on the sown proportions of the six species, HN *L. perenne* and the drought treatment, as follows:

$$Drt\ {*}[\beta_1 P_{Lp} + \beta_2 P_{Pp} + \beta_3 P_{Tp} + \beta_4 P_{Tr} + \beta_5 P_{Ci} + \beta_6 P_{Pl} + \beta_7 P_{HNLp} \qquad \text{(identity effects)}$$

$$+ \delta_1 P_{Lp}{*}P_{Pp} + \delta_2 P_{Ci}{*}_{Pl} + \delta_3 P_{Tp}{*}_{Tr} \qquad \text{(within-FG interaction effects)}$$

$$+ \delta_4 P_G{*}P_L + \delta_5 P_G{*}_H + \delta_6 P_H{*}_L] + \varepsilon \qquad \text{(across-FG interaction effects)}$$

Equation 1.

Where P represents the sown species proportions (Lp = *L. perenne*, Pp = *P. prate*nse, Tp = *T. pratense*, Tr = *T. repens* Ci = *C. intybus*, Pl = *P. lanceolata* and HNLp = high nitrogen *L. perenne*) of the sward. The identity effect of each species is thus estimated by β_1 to β_6, and, if P = 1, β coefficients estimate responses of a species grown in monoculture. Pairwise interactions between species were grouped to quantify diversity effects (δ) for within functional group interactions ($\delta_1 - \delta_3$) and between functional group interactions ($\delta_4 - \delta_6$). The between functional group interactions were among the three FGs (G = Grass, H = Herb and L = Legume, where $P_G = P_{Lp}+P_{Pp}$, $P_H = P_{Ci}+P_{Pl}$ and $P_L = P_{Tp}+P_{Tr}$). The effect of drought (*Drt*) includes two levels: 0 for control, 1 for drought) and was crossed with each term. Equation 1 was extended to a linear mixed model by specifying each pair of control and drought plots as a random unit (modelled as random intercept). There was also an extra term (β_7) for the high nitrogen *L. perenne* comparison (HNLp). There was an additional variance term for 100% legume monocultures as exploratory analysis indicated that responses from plots with 100% legume proportion were considerably more variable than all other treatments. The starting model (Equation 1) included all interactions and subsequent model selection identified the model best suited to each response.

3. RESULTS

3.1 Environmental and soil conditions

The mean daily temperature throughout the experimental period (11/06/2018 -13/09/2018) was 15.7°C which is in line with the long-term average (Met Eireann, 2018) (Figure 2). The highest and lowest daily temperatures were recorded at 24.9°C on 08/07/2018 and 7.2°C on the 22/06/2018. The experiment coincided with natural drought conditions; total rainfall over the experimental period was 105 mm with a daily average rainfall of 1.4 mm (excludes irrigation to control plots). Soil moisture deficits for moderately drained soils in Johnstown Castle reached the lowest point of 47 mm on 01/08/2018. The average bulk density of experimental plots at 5-10 cm depth was 1.35 g cm^{-3} (n = 9). At the end of the drought period, the WFPS% was three times higher in the control compared to drought plots (26% vs 82% on 07/08/2018). The WFPS% of the drought/re-wet plots rose to 81% by 16/08/2018 one week post re-wetting. The difference in WFPS% evened out by 09/09/2018 when both drought/re-wet and control plots had an average WFPS of 88% (Figure 2).

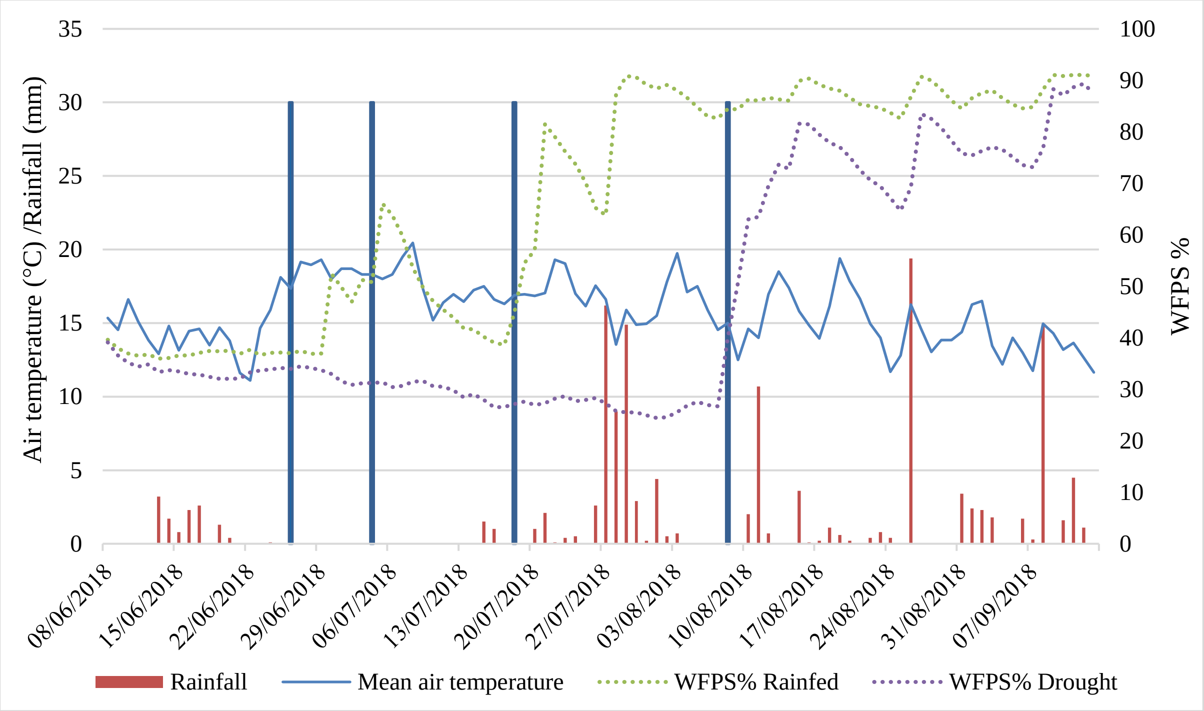

Figure 2. Water filled pore space (WFPS) % of plots under drought/re-wet and control conditions displayed alongside rainfall (mm) and mean air temperature (°C) during the course of the drought, irrigation and fertiliser application periods. The dark blue bar represents irrigation events of 30mm to control plots on 28/06/2018, 04/07/2018 and 18/07/2018 and to all plots on 08/08/2018.

3.2 Nitrous oxide emissions

Daily average N_2O emissions were calculated for a subset of grassland communities (*L. perenne* and the 6-species mixture) under drought/re-wet and control conditions across all three experimental periods (drought, re-wetting and fertiliser application). Regarding the control grassland communities, N_2O emissions were higher during the fertiliser application period than post-irrigation. The control 6-species mixture had higher daily average N_2O emissions during the irrigation and fertiliser application periods compared to control *L. perenne* (Figure 3). The highest daily average N_2O emissions from the drought/re-wet communities occurred post-irrigation. There was a 200% and 150% increase in daily average N_2O emissions produced post irrigation from the drought/re-wet vs control *L. perenne* and 6-species mixture communities, respectively (Figure 3)

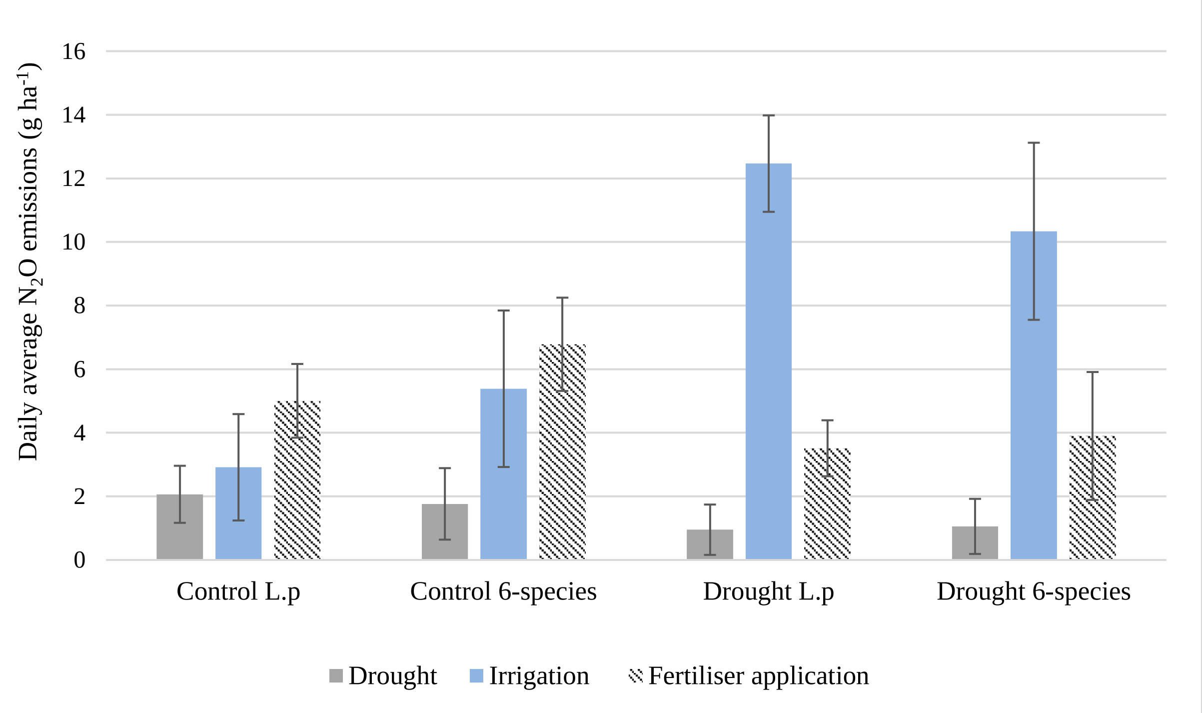

Figure 3. Daily average N_2O emissions (raw data - cumulative N_2O-N g ha^{-1}/ days within the experimental period) for the *L. perenne* (L.p) and 6-species communities under drought/re-wet and control conditions during the drought, irrigation and fertiliser application experimental periods. Error bars indicate the standard error (n=3).

3.2.1 Drought (resilience of treatments to drought).

During the eight week drought period (11/06/2018 to 07/08/2018) (Figure 4) daily N_2O emissions remained low for all four grassland communities not rising above 2 g N_2O-N ha^{-1} day^{-1} from the drought *L. perenne* community and 3.7 g N_2O-N ha^{-1} day^{-1} from the control *L. perenne* community.

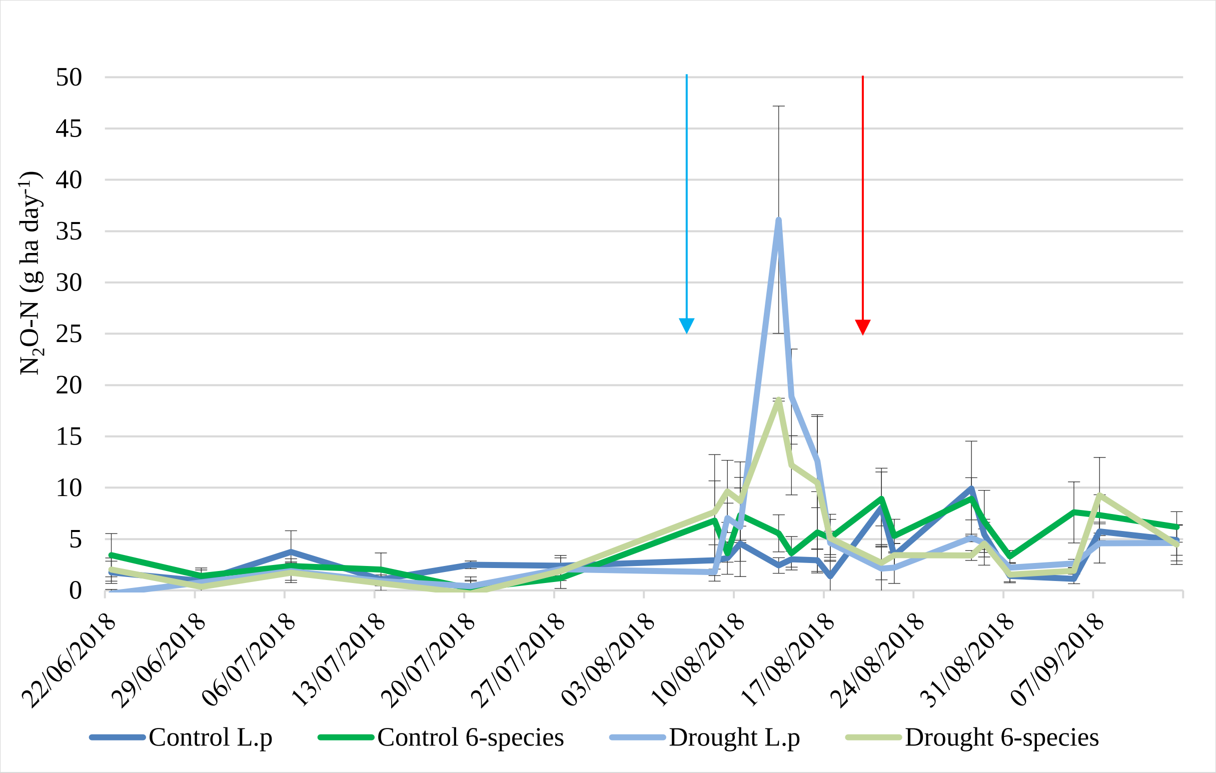

Figure 4. Nitrous oxide emissions (g N_2O-N ha^{-1} day^{-1}) from the *L. perenne* (L.p) and the 6-species mixture under control and drought/re-wet conditions. The blue arrow indicates the irrigation event post-drought with 30mm water to all grassland communities. The red arrow indicates a fertiliser application of 40 kg N ha^{-1} to all grassland communities. Error bars represent the standard error (n = 3).

3.2.2 Re-wetting (Recovery from drought)

Nitrous oxide emissions were monitored during the recovery period following drought for nine days post irrigation (08/08/2018-17/08/2018) from all grassland communities, which were previously either subject to drought or kept under ambient, control conditions (Figure 5). Figure 5b displays the elevated pulses of N_2O across all grassland communities once re-wetted after drought. The first grassland community to react to irrigation (i.e. emit pulses of N_2O) was *C. intybus* on 09/08/2018. The highest response from drought/re-wet communities post irrigation was from HN L.p five days after irrigation (13/08/2018) at 43.8 g N_2O-N ha^{-1} day^{-1} and followed by *L. perenne* the same day at 36.1 g N_2O-N ha^{-1} day^{-1}. The drought/re-wet *P.*

lanceolata community produced considerably lower N_2O emissions than other communities. This community produced a delayed response to irrigation with a peak of 12.4 g N_2O-N ha^{-1} day^{-1} six days after irrigation (14/08/2018) (Figure 5B).The highest response in daily N_2O emissions post irrigation from the control grassland communities was with *T. repens* at 17 g N_2O-N ha^{-1} day^{-1}. Both control legume communities (*T. repens* and *T. pratense)* produced higher daily N_2O emissions than all other grassland communities following the irrigation event. Conversely, there was no obvious response in elevated N_2O fluxes from the control HN *L. perenne* community (Figure 5A).

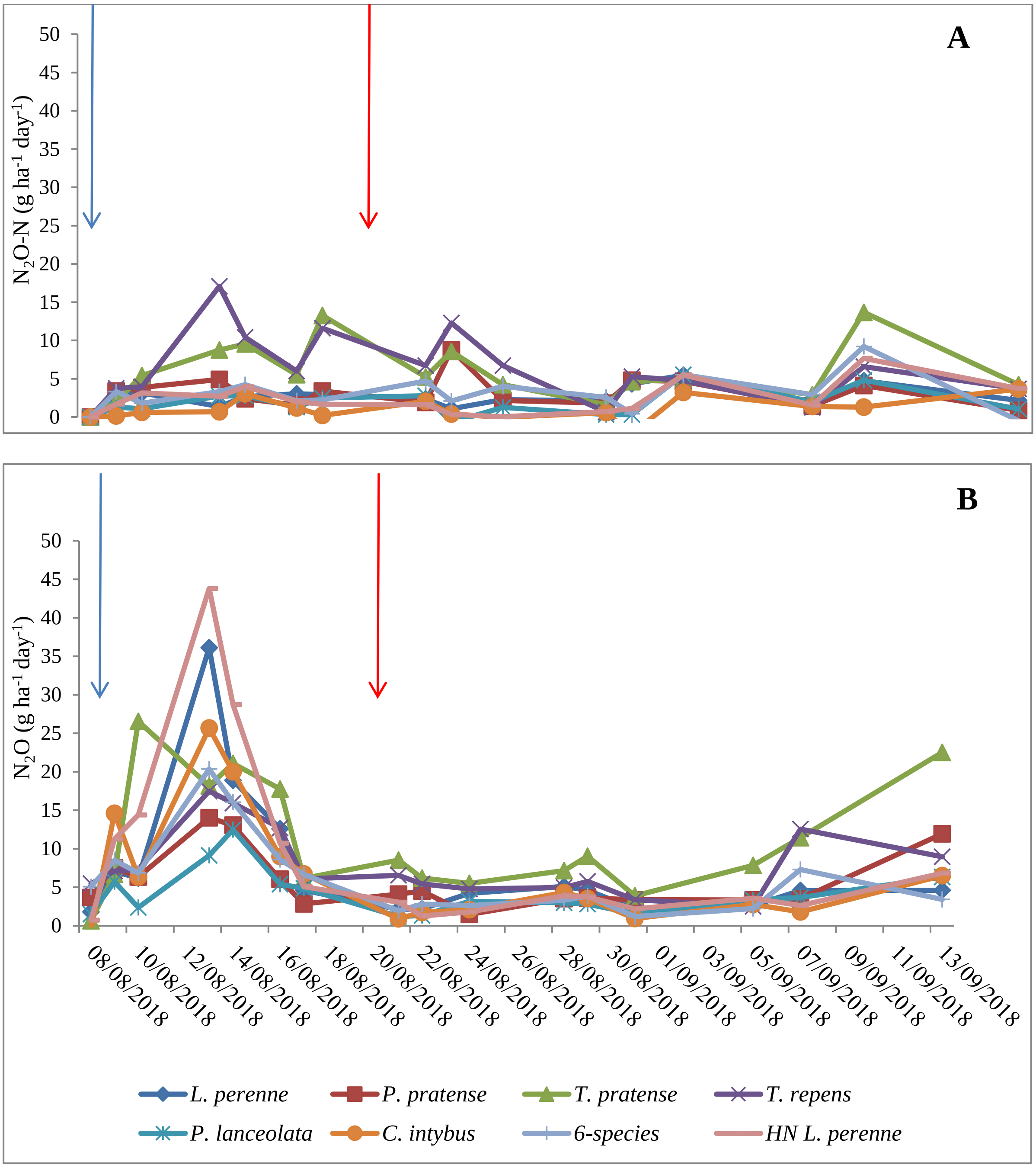

Figure 5. (A) Temporal trace of N_2O fluxes (g N_2O-N ha^{-1} day^{-1}) from control conditions. (B) Temporal trace of N_2O fluxes (g N_2O-N ha^{-1} day^{-1}) after drought and rewetting. The blue arrow indicates 30mm irrigation after an eight week drought to both drought/re-wet and control treatments. Red arrow indicates a fertiliser application of 40 kg N ha^{-1} (80 kg N ha^{-1} for HN Lp).

Cumulative N_2O emissions for the period post irrigation (08/08/2018-17/08/2018) were assessed across all grassland communities (drought/re-wet and control) (Figure 6). Data was log transformed and the final model included species identity effects only as there were no additional interactions between species or functional groups. All drought/re-wet grassland communities had significantly higher cumulative N_2O emissions (N_2O-N g ha^{-1}) compared to the controls ($P<0.05$) (Figure 5A and Table 1), bar the two legume monocultures *T. repens* and *T. pratense*. The drought/re-wet HN L.p, *T. repens* and *T. pratense* communities had significantly higher ($P <0.05$) N_2O fluxes at the irrigation event than all other drought/re-wet communities (Figure 5A).

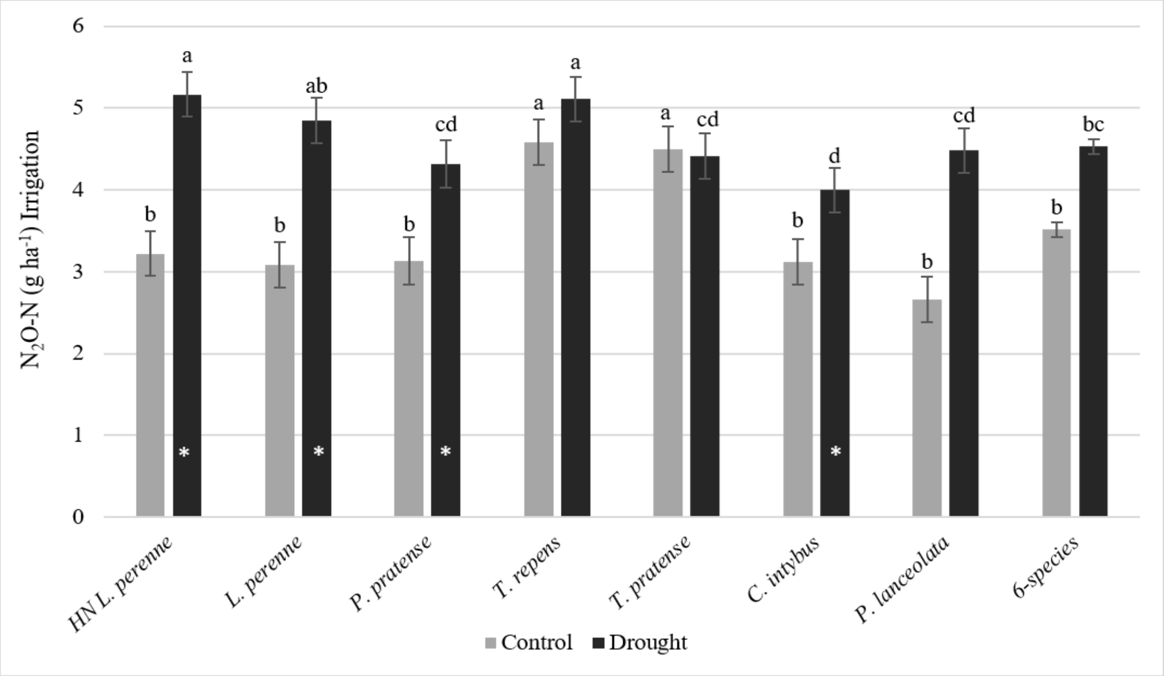

Figure 6. Cumulative N_2O-N g ha^{-1} for the irrigation period (08/08/2018-17/08/2018). The stars indicate that the N_2O emissions from drought/re-wet grassland community is significantly different to its control ($P < 0.05$). Error bars represent the standard error and communities with the same letter are not significantly different from one another (comparisons are only between communities of the same moisture level)

3.2.3 Fertiliser application post re-wetting (legacy effects of drought).

Daily N_2O emissions following fertiliser application were highest from the drought/re-wet legume monocultures (Figure 5B and Table 1). Both *T. repens* and *T. pratense* emitted 12 g N_2O-N ha day^{-1} on 07/09/2018 which coincides with WFPS levelling out (Figures 2 and 5B). Cumulative N_2O emissions were also calculated for the fertiliser application period (21/08/2018-13/09/2018) for all grassland communities (drought/re-wet and control) (Figure 7). According to model comparisons, the best model fit for N_2O emissions post fertiliser application included species identity effects only as no interactions between species were detected. Overall, there was no significant legacy effect of drought on elevated N_2O fluxes from any grassland community post fertiliser application. However, the drought/re-wet *L. perenne*, *T. pratense* and 6-species community had significantly lower N_2O emissions than the controls. Both drought/re-wet legume monocultures (*T. repens* and *T. pratense*) had significantly higher N_2O emissions than the controls during the fertiliser application period (Figure 7).

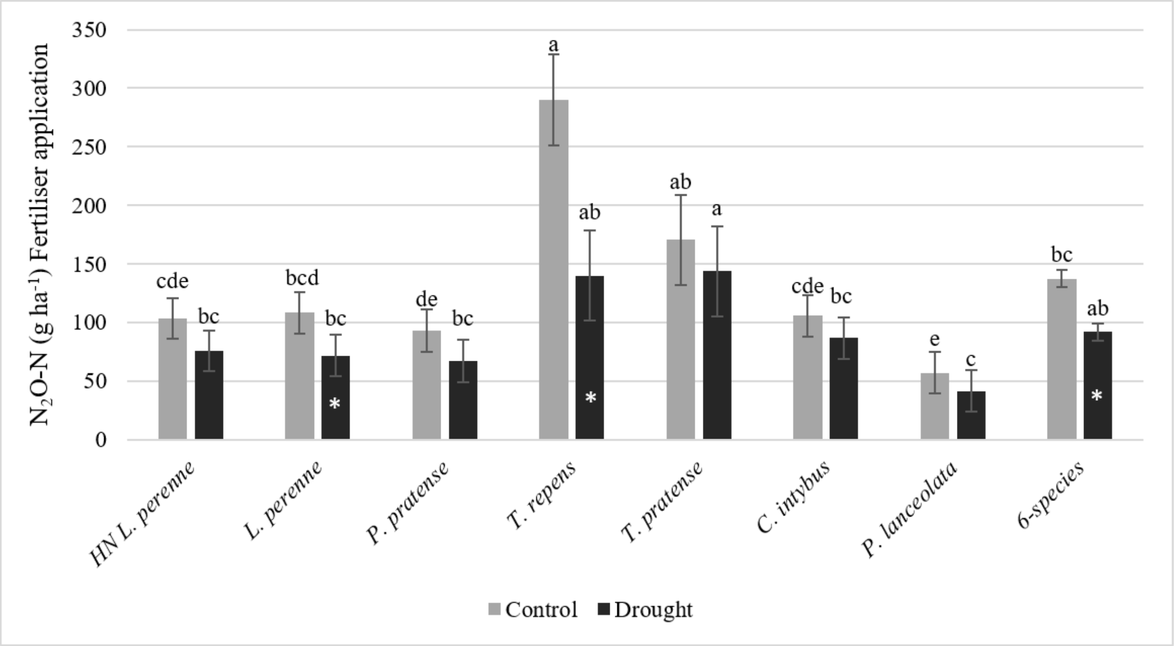

Figure 7. Cumulative N_2O-N g ha^{-1} for the fertiliser application post irrigation (21/08/2018-13/09/2018). The stars indicate that the drought/re-wet grassland community is significantly different to the control ($P > 0.05$). Error bars represent the standard error and communities with the same letter are not significantly different from one another (comparisons are only between communities of the same moisture level).

Table 1. Coefficient estimates ± standard errors for the identity effects for $\beta_{1\text{-}7}$ (equation 2) and predictions for the 6-species mixture. These are presented for the models fitted to each of the control and drought nitrous oxide responses during the irrigation (log transformed) and fertiliser application periods.

	N_2O emissions (N_2O-N g ha^{-1})			
	Irrigation		**Fertiliser application**	
	Control	*Drought*	*Control*	*Drought*
HN *L. perenne*	3.2 ± 0.27	5.2 ± 0.27	103.5 ± 17.51	75.7 ± 17.51
L. perenne	3.1 ± 0.28	4.8 ± 0.28	108.6 ± 17.51	71.6 ± 17.51
P. pratense	3.1 ± 0.29	4.3 ± 0.29	93 ± 18.41	67.1 ± 18.41
T. repens	4.6 ± 0.27	5.1 ± 0.27	290 ± 38.54	139.9 ± 38.54
T. pratense	4.5 ± 0.27	4.4 ± 0.27	170.5 ± 38.54	143.8 ± 38.54
C. intybus	3.1 ± 0.27	4 ± 0.27	105.9 ± 17.66	86.7 ± 17.66
P. lanceolata	2.7 ± 0.27	4.5 ± 0.27	56.9 ± 17.66	41.6 ± 17.66
6-species	3.5± 0.09	4.5 ± 0.09	137.5 ± 7.46	91.8 ± 7.46

3.3 Soil mineral nitrogen

All soil mineral N responses: NH_4^+ during drought, NO_3^- during drought, NH_4^+ at rewetting bar NO_3^- at rewetting were log transformed for statistical analyses purposes. The best model fit included identity effects only with an additional within FG interaction for both NH_4^+ responses. Regarding the drought period, there were significantly ($P < 0.05$) higher levels of ammonium (NH_4^+) present in soil from all grassland communities under drought compared to control bar *L. perenne* and *T. repens* (Figure 8A). There was no significant difference in soil nitrate (NO_3^-) from grassland treatments under drought compared with control communities other than at high N application rates (HN Lp), *P. lanceolata* and the 6-species mixture (Figure 8B).

Trends in soil mineral N levels were reversed at the irrigation event; there was no significant difference in NH_4^+ levels between control and re-wet bar HN Lp, *P. pratense* and the 6-species mixture (Figure 8C) Conversely, there was significantly higher ($P < 0.05$) levels of NO_3^- in re-wet soils compared to control regarding all grassland communities bar *P. lanceolata* (Figure 8D).

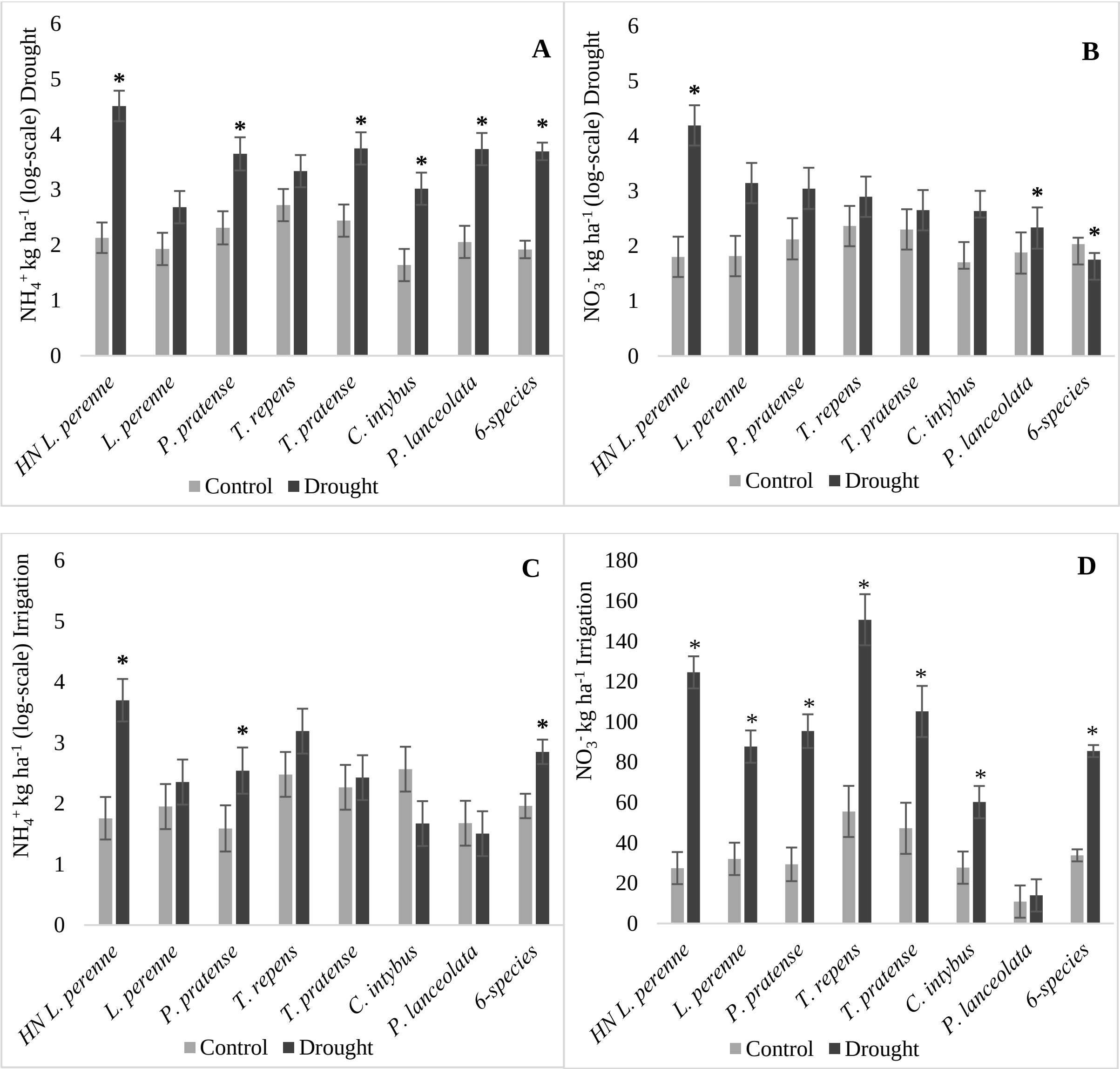

Figure 8. Soil mineral nitrogen levels for **A)** soil ammonium (NH_4^+) kg ha^{-1} (log-scale) during drought conditions **B)** soil nitrate (NO_3^-) kg ha^{-1} (log-scale) during drought conditions **C)** soil ammonium (NH_4^+) kg ha^{-1} (log-scale) after the irrigation event **D)** soil nitrate (NO_3^-) kg ha^{-1} after the irrigation event. Control treatments did not undergo drought stress and received 30mm water at the irrigation event. The star indicates a significant ($P<0.05$) difference between drought and control treatments and error bars represent the standard error.

4. DISCUSSION

The low N_2O fluxes observed during the eight week drought are correlated with the rapid decline in soil WFPS % (Figure 2). Soil moisture levels were below the optimum WFPS % threshold for nitrification and denitrification (the main pathways through which N_2O emissions are lost) to occur. This ranges from 30 to 60% for nitrification after which denitrification dominates (>70%) (Bateman and Baggs, 2005; Cardenas et al., 2007). The studies of Hartmann and Niklaus (2012) and Goldberg and Gebauer (2009) also found N_2O production to be significantly impaired by drought conditions. The latter study found that N_2O produced in the subsoil was consumed during upward diffusion through the soil profile resulting in sub ambient N_2O levels in upper soil horizons which indicates that soils can serve as a sink of N_2O during drought impairment. An absence of the nitrification process during drought is evident from the significantly higher amounts of NH_4^+ across treatments (compared to control). Overall, high soil NH_4^+ levels are likely the result of reductions in the net nitrification rate during drought rather than an increase in mineralisation as mineralisation rates slow down as soils become drier (Larsen et al., 2011; Morillas et al., 2015; Borken and Matzner, 2009). Additionally, drought reduces plant N uptake and biomass N production, which further increases soil mineral N availability (He & Dijkstra, 2014). The lack of elevated NH_4^+ levels in the control compared to drought *Lolium perenne* (150N) community is likely due to high levels of plant N uptake from control treatments. Grass FG traits are considered ‘fast’ on the plant economics spectrum (Reich and Cornelissen, 2014) which link to high rates of nutrient acquisition and processing. Additionally, grass root systems have small diameters, high specific root length and density and occupy the surface horizon which allows vigorous capacity for water uptake and nutrient acquisition (Fort et al., 2016).

A highly significant effect of irrigation on N_2O fluxes was observed from all species (bar legumes) due to re-wetting. These high N_2O pulses are reflective of the severity and length of the drought (eight weeks). Severe drought impairment results in a burst of decomposition, mineralization and release of inorganic N following rewetting and thus accelerated microbial responses (Jarvis et al., 2007; Unger et al., 2010). Overall, there was variation in the amount of N_2O emitted between species during the recovery period i.e. following irrigation after drought. The first species to react to re-wetting was *C. intybus* the day following irrigation. This quick response may be indicative of the species resilience to drought. Hoekstra et al (2014) using $\delta^{18}O$ natural abundance, found that *C. intybus* can switch from water uptake at 0– 10 cm soil depth to deeper and less dry soil layers during drought. Similarly, *C. intybus* was the only species that displayed drought tolerance in a previous experiment on the same experimental site (Haughey et al., 2018). The lack of diversity interaction effects on N_2O during the irrigation phase is most likely due to the irrigation reaction being soil chemical, microbial and mechanical rather than plant influenced

There was rapid change in the soil mineral N dynamics of drought plots following irrigation with significant increases in soil NO_3^- found across drought treatments. This is indicative of an increase in the rate of nitrification during the recovery period after drought. This increase in NO_3^- availability upon the re-wetting of dry soils was also observed by Schaeffer et al. (2017). In our study, all species had a significantly higher level of NO_3^- upon soil re-wetting after drought compared to controls bar *P. lanceolata*. This could be due to the presence of biological nitrification inhibition (BNI) compounds such as aucubin and catalpol which are present in plantago and slow down the nitrification process and stabilise soil mineral N in its NH_4^+ form (Gardiner et al., 2018; de Klein et al., 2020; Gardiner et al., 2020). This is reflected in the N_2O emissions from *P. lanceolata* which are significantly lower than other forage treatments (control and re-wet) post fertiliser application (Figures 4 and 5). The differences in N_2O

emissions across species identity similar to the results of Luo et al. (2018), who found significantly higher N_2O fluxes from *T. repens* and lower N_2O emissions from *P. lanceolata* in comparison with other forage species.

Nitrous oxide emissions of legume monocultures were significantly higher than emissions from other treatments both at irrigation (recovery from drought)and at fertiliser application (legacy following drought) (Figures 5 A and B). This is owing to excess N availability in the soil system due to biological nitrogen fixation (BNF). Thus, the non-significant difference between control and re-wet legume monocultures is likely due to the increased emissions from the control treatment as the BNF process would not have been impaired by drought. Therefore, N_2O emissions resulting from N incorporation through BNF and fertiliser application are similar to heightened emissions associated with re-wetting dry soils. High soil N availability (be it from BNF or fertiliser application) are further reflected in the higher soil mineral N levels from HN Lp and both legume species at the irrigation event (Figure 6 C and D).

An important finding from a grassland management perspective was that there were no lasting legacy effect of drought/re-wetting on elevated N_2O emissions across a variety of grassland monocultures and mixtures. Therefore, it is acceptable to apply fertiliser two weeks post irrigation without further exaggerating the heightened emissions associated with re-wetting. Limiting fertiliser application directly after re-wetting also reduces the risk of NO_3^- leaching which is more likely to occur once dry soils are re-wet due to excess mineral N availability (Shepherd et al., 2018). This is an important from a grassland management perspective as it considers diverse grasslands which are likely to be used more regularly as a climate adaptation measure in for drought prone production systems due to yield stability benefits (Grange et al., 2021).

5. CONCLUSION

As climate change will bring more frequent and extreme drought events, it is vital that suitable grass management strategies are put in place to maintain production levels. Thus, this study provides insight regarding suitable fertiliser application strategies to minimise N_2O losses 1) fertiliser should not be applied during drought periods as it cannot be utilised and may lead to excessive soil mineral N pools 2) Fertiliser application should be delayed at least two weeks post re-wetting to avoid adding to the already high emissions associated with this period. This study also highlights the suitability of Plantain species to drought prone climates as it had more stable N dynamics (less fluctuations in N_2O emissions and soil mineral N levels) throughout the drought and re-wetting events. Conversely, legume monocultures will further increase N_2O emissions during extreme climatic events such as drought and re-wetting. Long term monitoring of N dynamics and N_2O fluxes are needed to further understand the N dynamics of multi-species swards as this experiment may not have discerned the potential interactions at play between species and functional groups. Isotope tracing methods could potentially be used to further understand the N dynamics from multi-species swards during drought and re-wetting periods.

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7. APPENDICES

Community	FG	Species	Grasses	Legumes	Herbs	L. p	P. p	T. p	T.r	P. l	C. i	Reps
1	1	1	1	0	0	1	0	0	0	0	0	3
2	1	1	1	0	0	0	1	0	0	0	0	3
3	1	1	0	1	0	0	0	1	0	0	0	3
4	1	1	0	1	0	0	0	0	1	0	0	3
5	1	1	0	0	1	0	0	0	0	1	0	3
6	1	1	0	0	1	0	0	0	0	0	1	3
7	1	2	1	0	0	0.5	0.5	0	0	0	0	2
8	1	2	0	1	0	0	0	0.5	0.5	0	0	2
9	1	2	0	0	1	0	0	0	0	0.5	0.5	2
10	2	4	0.5	0.5	0	0.25	0.25	0.25	0.25	0	0	2
11	2	4	0.5	0	0.5	0.25	0.25	0	0	0.25	0.25	2
12	2	4	0	0.5	0.5	0	0	0.25	0.25	0.25	0.25	2
13	3	5	0.6	0.2	0.2	0.6	0	0.1	0.1	0.1	0.1	1
14	3	5	0.6	0.2	0.2	0	0.6	0.1	0.1	0.1	0.1	1
15	3	5	0.2	0.6	0.2	0.1	0.1	0.6	0	0.1	0.1	1
16	3	5	0.2	0.6	0.2	0.1	0.1	0	0.6	0.1	0.1	1
17	3	5	0.2	0.2	0.6	0.1	0.1	0.1	0.1	0.6	0	1
18	3	5	0.2	0.2	0.6	0.1	0.1	0.1	0.1	0	0.6	1
19	3	6	0.33	0.33	0.33	0.167	0.167	0.167	0.167	0.167	0.167	3
20	1	1	1	0	0	1	0	0	0	0	0	4

Appendix 1: Community composition: number of functional groups (FG), species and replicates. Species include *L. perenne* (L. p) *P. pratense* (P. p), *T. pratense* (T. r), *T. repens* (T. r), *P. Lanceo*lata (P. l), and *C. intybus* (C. i).

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CHAPTER 5

A comparison of the N_2O emissions and production efficiency of contrasting livestock systems on the North Wyke Farm Platform.

Intended for submission to Agriculture, Ecosystems and Environment.

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ABSTRACT

Nitrous oxide (N_2O) is a powerful greenhouse gas with 265 times the global warming potential of carbon dioxide. Intensive grassland livestock systems are a key source of N_2O emissions, owing to high nitrogen inputs through fertiliser application and livestock excreta. There are a number of mitigation strategies to reduce absolute emissions and emissions intensity (emissions per unit product) from intensive grassland systems such as improving animal performance and substituting synthetic fertiliser N for N originating from grass-legume (e.g. clover) based swards. While many studies have previously assessed the impacts of legume inclusion, fertiliser application and livestock N excretion on N_2O emissions at plot scale, there is a notable lack of system-scale assessments. This is important for determining farm level N balances and refining GHG inventories. To date, there has been no N_2O emissions intensity assessment of livestock (bovine or ovine) carried out. This study considered two paddocks supporting two different sheep production systems on the North Wyke Farm Platform (a *Festulolium* [FL] monoculture receiving 120 kg N ha^{-1} and a *Festulolium/T. repens* [FL + WC] mixture receiving 40 kg N ha^{-1}) whereby we monitored lamb live weight gain and N_2O emissions using automated chambers. Nitrogen excretion was estimated according to UNFCCC UK national inventory reports and subsequent N_2O losses were calculated using IPCC and UK-derived emission factors (EF). This study highlights the requirement to sufficiently capture spatial and temporal variation in N_2O emissions in order to make comparisons at the farm system level. There was a decrease in absolute N_2O emissions and N_2O emissions intensity from the FL+WC paddock compared to the FL paddock. Additionally, lambs gained an average of 2 kg extra over the season on the FL+WC system. There was a stark contrast between N_2O inventory accounting when using the default IPCC N_2O EF for livestock excreta and fertiliser application compared with the UK country-specific EF.

1. INTRODUCTION

Agriculture, is a key source of greenhouse gas (GHG) emissions and major contributor to climate change, as are other anthropogenic activities such as industrial processing and fossil fuel combustion. In effort to curb GHG emissions and to limit global warming to below 2°C, the Paris agreement (2015) places requirements on industrial countries to lower GHG emissions by 10-20% by 2030. Further efforts will be required to maintain a downward trajectory to reach carbon neutrality by 2050 (UNFCCC, 2021). Livestock, albeit a major source of GHG emissions, are directly responsible for the sustenance and livelihoods of 1 billion people worldwide (McLeod, 2011). Agriculture in Ireland and the UK are important sources of total GHG emissions, accounting for 35 and 10%, respectively, (EPA, 2021; Gov.UK, 2021). Of these emissions, nitrous oxide (N_2O) is a particularly potent GHG, having 265 times the global warming capacity of carbon dioxide and an atmospheric persistence of 120 years (IPCC, 2014). Nitrous oxide emissions are soil derived, mainly produced during the nitrification (NH_4^+ to NO_3^-) and denitrification (NO_3^- to N_2O/N_2) processes (Thomson et al., 2012). Intensive livestock production systems are responsible for high N_2O emissions as high concentrations of N within soil systems result from livestock urine and dung deposition, and applications of manure and inorganic N fertiliser to grassland swards in order to maintain yields (López-Aizpún et al., 2020; Maire et al., 2020).

There are a number of measures which can be incorporated into agricultural production systems to mitigate and reduce GHGs. Regarding N_2O, efforts such as improved manure management, low emission N fertilisers, limiting and correctly timing N fertiliser application and clover incorporation into grassland swards can reduce emissions (Lanigan et al., 2018). Incorporation of clover has the potential to reduce N_2O emissions by reducing dependency on inorganic N fertiliser application through symbiotic N fixation by rhizobia bacteria, facilitated by legumes.

White clover (*T. repens*) inclusion in a grassland sward can supply between 50-200 kg N ha^{-1} $year^{-1}$ for plant uptake (Teagasc, 2021). However, if N fixation is not accounted for during fertiliser application, annual N fixation rates may decrease (Ledgard et al., 1996) and N_2O emissions may increase due to excess N availability within the soil system (Reinsch et al., 2020a). Plot scale studies (e.g. Burchill et al., 2014; Reinsch et al., 2020b) have been useful for understanding how legumes affect the N cycle, however, they do not take into account real life grazing events or agronomic output as can only be fully assessed at a field and/or system scale. The benefits of clover for increasing grassland productivity and forage quality and quantity are well established (Andrews et al., 2007; Ledgard et al., 2009; Egan et al 2018). Clover inclusion within grassland swards has also been shown to improve livestock productivity, especially sheep (Somasiri et al., 2014; Cranston et al., 2015; Hurley et al., 2021). Animal performance is often factored into assessments of enteric methane (CH_4) emissions by means of an emission intensity analyses, i.e. g CH_4/ kg output (milk or meat) (Gerber et al., 2011; Hayes et al., 2013). However, to date, a similar assessment of the N_2O losses per unit livestock production at the field or system scale has not been reported.

Static gas chamber methodology has been extensively used in quantifying N_2O fluxes in response to various treatments. However, this method is highly labour intensive, which places limitations on the scale and amount of measurements that can be taken. Alternatively, automated N_2O sampling provides high frequency temporal measurements. This allows for higher resolution temporal sampling than possible with manually sampled chambers. Automated chambers also provide an in-depth understanding of real time emissions and the development of accurate N_2O inventories and effective mitigation strategies (Grace et al., 2020).

The North Wyke Farm Platform (NWFP) is a UK National Capability, initially designed to assess the productivity and environmental sustainability of finishing beef and sheep production on grassland at the systems scale (Orr et al., 2016; Takahashi et al., 2018). In this study, data from the NWFP were used to compare N_2O emissions and emission intensity (i.e. emission per unit of lamb live-weight gain) from two contrasting management systems: 1) a grass-clover mixture receiving 40 kg N ha^{-1} year and, 2) a grass monoculture receiving 120 kg N ha^{-1} year^{-1}. We hypothesised that at paddock scale, substituting a moderate inorganic N fertiliser input (120 kg N ha^{-1} year^{-1}) monoculture sward for a low (40 kg N ha^{-1} year^{-1}) inorganic N fertiliser input grass-clover sward would result in lower N_2O emissions and emissions intensity.

2. MATERIALS AND METHODS

2.1. Research location

The NWFP, established in 2010, is a farm-scale experimental system located at Rothamsted Research, Devon, SW England (50°45′N, 3°50′W). The soil type is a slightly stony clay loam topsoil overlying a mottled stony clay (Orr, 2016). The research platform consists of three ~21 ha farmlets, with each farmlet consisting of seven paddocks of consistent grassland compositions within five hydrologically isolated catchments. The present study focussed on a single paddock of each of the two grassland treatments reseeded in 2017 (paddocks 14 and 15, Fig. 1) on which the N_2O emission measurements were made. These reseeded treatments were a monoculture grass and a monoculture grass combined with white clover. Specifically, for paddock 15 (treatment FL) this was a deep rooting grass (*Festulolium*) and for paddock 14, *Festulolium* and white clover (FL+WC). Each farmlet maintains and grazes a flock of ~75 ewes and their lambs, 30 finishing beef cattle and produces silage. The paddocks used in this study were only grazed by sheep, with the following pasture management:

I. FL Farmlet: (Paddock 15- Longlands East) previously under permanent pasture (PP) it was reseeded in 2017 with a deep rooting *Festulolium* (cv. Prior) monoculture (FL). Ammonium nitrate fertiliser has been applied at 120 kg N ha^{-1} $year^{-1}$ (3 x 40 kg N splits), following recommendations for the system and region. Animals (ewes and lambs) were grazed for 104 days of the year. *Festulolium* (cv. Prior) is a hybrid cross between *Festuca* and *Lolium grasses*. This cultivar was developed in order to improve drought resistance and prevent waterlogging as it has deeper rooting traits.

II. FL+WC farmlet: the sward (Paddock 14 – Longlands North) that was previously PP was reseeded in 2017 with deep rooting *Festulolium* (cv. Prior) and a white clover *(Trifolium repens)* mixture (FL+WC) (30% clover). A reduced fertiliser level of 40kg ha^{-1} $year^{-1}$ has been applied at the start of each growing season to account for N fixation.

Botanical composition assessments measured the legume proportion of the sward on an annual basis to ensure clover proportion remained ~30%. Animals (ewes and lambs) were grazed for 77 days of the year.

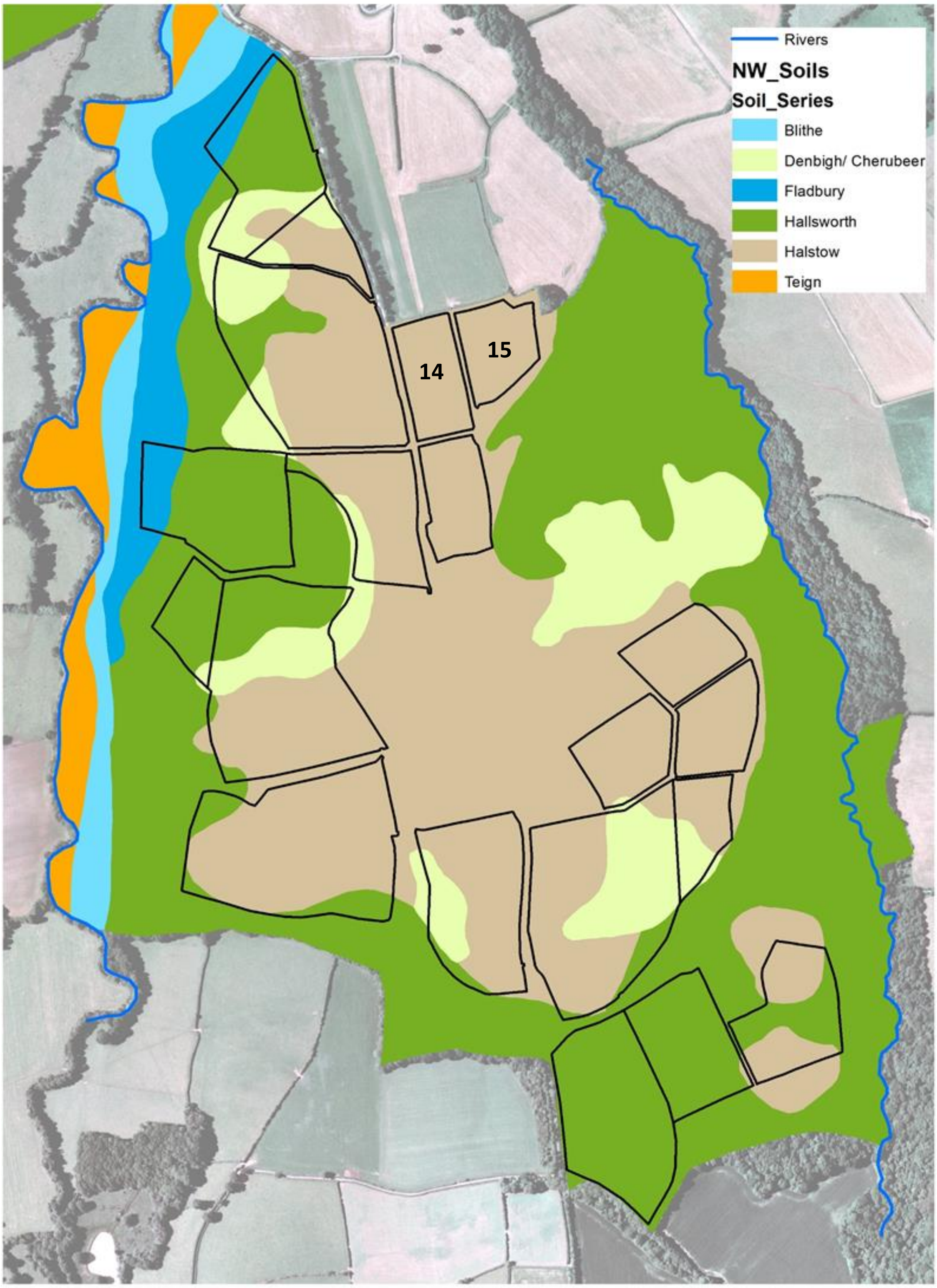

Figure 1. Map of the North Wyke Farm Platform, surrounding catchment area and associated soil types. The automated chamber laboratories were situated in paddock 14 and 15.

2.2 Nitrous oxide emissions

Nitrous oxide emissions were measured using automated soil CO_2 Licor flux system (Model LI8100-104) and an INNOVA Photoacoustic Analyser (Model 1412) field gas monitor to measure CO_2, N_2O and CH_4. Twelve automated chambers (48.3 cm L x 38.1 cm W x 33.0 cm H) were placed in each paddock (total 24) and attached to both analysers. Gas flux measurements (mol $m^{-2}s^{-1}$) were taken simultaneously at 20-minute intervals from each chamber. Four sampling areas were designated in each paddock, with collars pre-installed into the soil at the chamber sampling positions, and the system being moved around these areas in turn every two weeks. When in position at a given sampling location, the chambers and analysers were fenced off to prevent damage or interference by the grazing sheep. A full description is provided in Cardenas et al. (submitted).

In this study we assessed the N_2O data during the 2018 agronomic season from 01/01/2018 – 31/08/2018, with the exception of April when the analysers malfunctioned. A daily average flux was calculated for each of the 12 chambers, this value was then averaged across chambers and a singular daily flux was then converted from N_2O-N mol $m^{-2}s^{-1}$ to N_2O-N g ha day. A cumulative N_2O flux was calculated for each experimental month as well as a total cumulative value for the entire experimental period. This was done through integration of daily fluxes and linear interpolation between measurements (Burchill et al., 2014; De Klein and Harvey, 2012). A daily average (cumulative N_2O flux/ number of days within the cumulative period) was calculated for each experimental month in order to normalise the data and allow for a fair comparison between time points.

2.3 Emissions intensity

A flock of ewes and their lambs (Suffolk-Mule × Charollais crosses) were continuously set-stocked, based on sward surface height, from the 18th April 2018 onwards. An emissions intensity value was calculated for both paddocks to determine and compare the efficiency of both systems (i.e. N_2O emission per unit output), and was derived by dividing the total cumulative N_2O emissions from 1st May to 31st August 2018 by the total lamb LWG from birth to 31st August 2018 (g N_2O-N/Tonne total lamb LWG).

2.4 N inputs and N_2O emission factors

Nitrogen excretion levels were calculated using standard sheep N excretion values as used in the UK National Inventory Reports (UNFCCC, 2021). This data was then multiplied by the average number of animals in each paddock and the total number of days at grazing (Table 2). Nitrogen losses were partitioned according to a 60%–40% split in excretion of N to urine and dung, respectively (Webb and Misselbrook, 2004). Nitrous oxide losses were then estimated for excreta from grazing livestock according to the IPCC default EF_3 of 0.3% (IPCC, 2019) and the UK country-specific EF_3 of 0.629% (urine) and 0.193% for dung (Brown et al., 2020). Nitrous oxide emissions from applied inorganic N fertiliser were calculated according to the IPCC EF_1 (1.6% for wet soils) and the UK site specific EF_1 (Brown et al., 2020) *equation 1.*

$$\mathrm{Ln}\ (N_2O+1) = 0.1616\ (\pm 0.13526) + 0.00006093\ (\pm 0.000240365) * N + 0.0005187\ (\pm 0.00016259) * R + 0.00000354\ (\pm 0.0000002785) * N * R$$

Equation 1. Total N_2O emissions (kg N_2O-N) in the year following ammonium nitrate fertiliser application are calculated according to: N = the annual total fertiliser application (40/120 kg N ha^{-1}). R = the long-term average annual rainfall (1015 mm) (Figure 2) at the NWFP. Values in brackets are the standard errors of the model parameters and a bias corrector of 1.074 is in place for the ammonium nitrate model. Model predictions are made with and without fertiliser N and the EF is the difference.

2.5 Ancillary measurements

Rainfall, gravimetric soil moisture (%) at 10 cm depth and soil temperature at 15 cm depth were monitored using real time data via telemetry, a rain gauge and a relay station in the centre of each paddock. A botanical survey was carried out in September 2018 to determine the post-grazing percentage cover of Festulolium, *T. repens*, weeds and bare ground.

2.6 Statistical analyses

A one way ANOVA which included daily average N_2O emissions (cumulative N_2O emissions/number of days within cumulative period) as the variate, sward composition (FL and FL+WC) as the factor with experimental months as replicates was used. Analyses took place using Genstat 19th edition.

3. RESULTS

3.1 Meteorological and soil moisture data

The NWFP is situated in Devon, South West England which is considered to have a temperate, maritime climate (Avery, 1980). Daily precipitation and temperature data from the Met Office weather station situated on-site were sourced from the NWFP data portal. From 1st January 2018 - 31st August 2018 (excluding April) there was a total of 586 mm precipitation. Precipitation was highest during March with a total of 164 mm while June was the driest month with a total of <1 mm. The sampling period was both wetter and hotter than the long-term climatic conditions for the site (Figure 2). The maximum recorded air temperature for the experimental site was on the 8th July at 29.3°C while the lowest temperature was on the 28th February at -7.5°C (Figure 3). Soil gravimetric moisture content (%) was marginally, although consistently, lower in the FL than the FL+WC paddock. Soil moisture content was ~40% from January to May 2018 before dropping in the summer months to as low as 15% in July and August. Average soil temperature across both paddocks was 11°C over the experimental period. Soil temperature never fell below 0°C, the lowest measurement was on the 3rd of March at 0.8°C while the highest soil temperature was on 9th July 2018 in the FL paddock at 23.1°C (Figure 4).

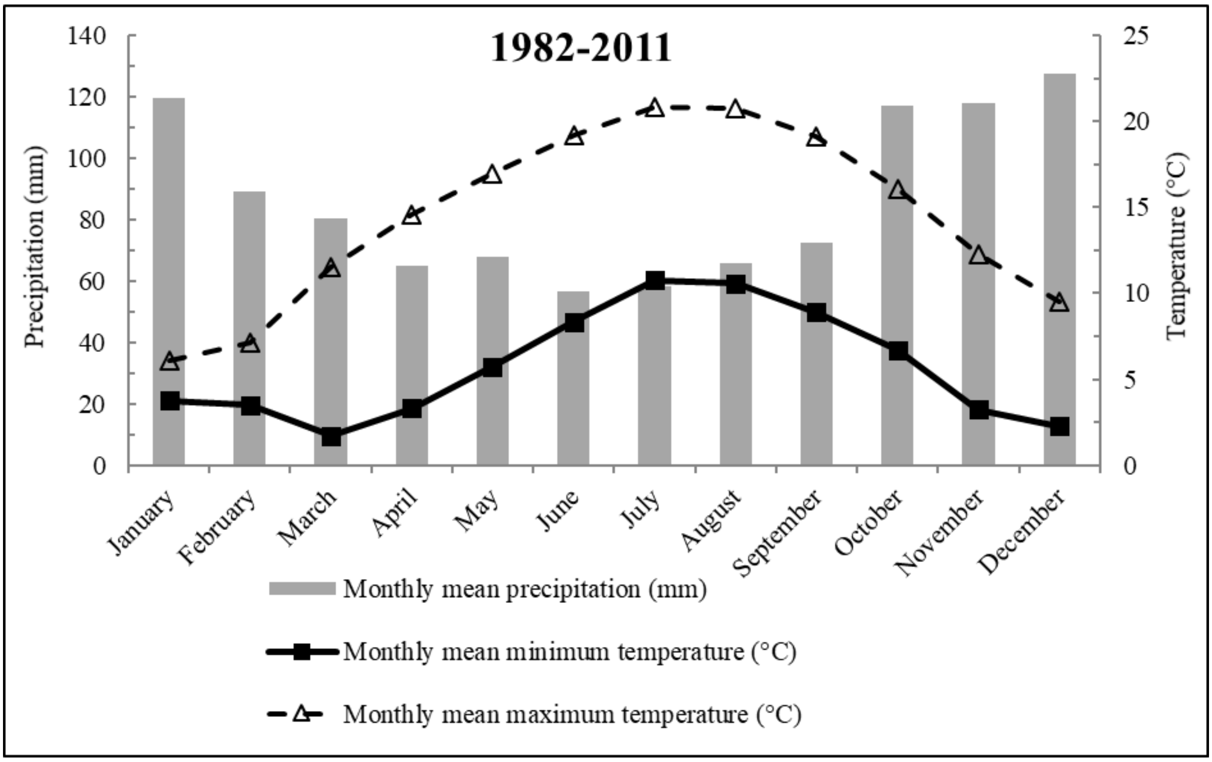

Figure 2. Long term climatic data from the NWFP site including mean monthly precipitation, minimum and maximum air temperatures.

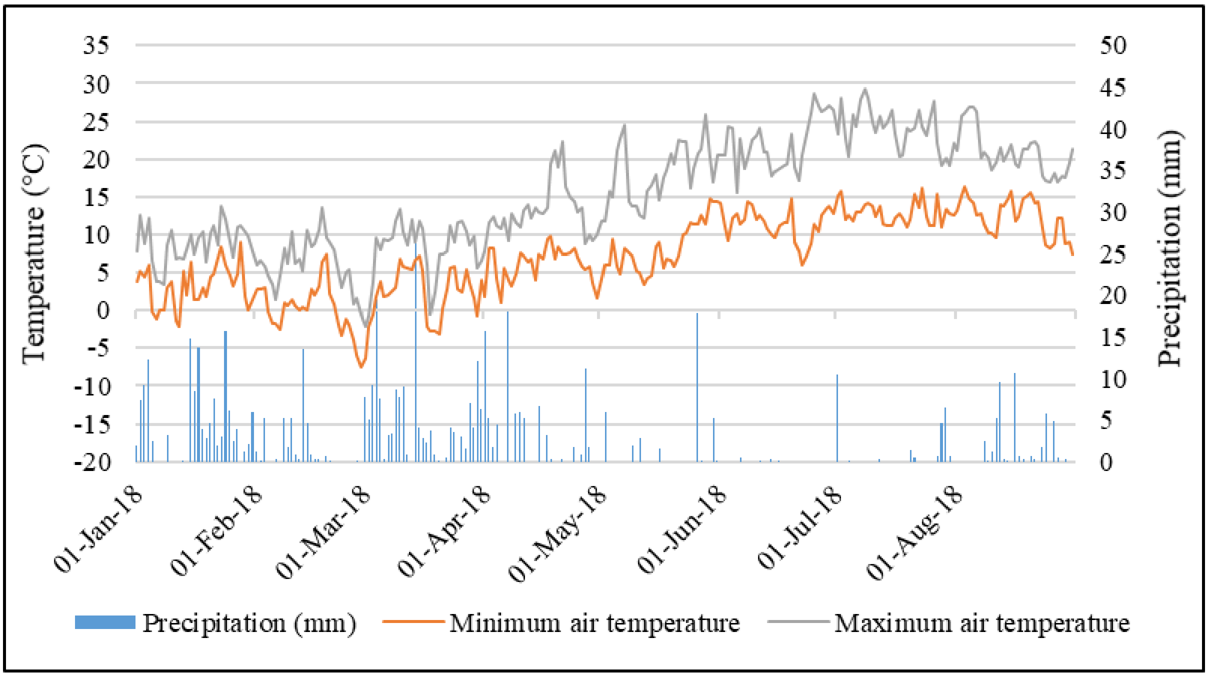

Figure 3. Meteorological data for the NWFP from January – August 2018 including precipitation (mm) and minimum and maximum air temperature (°C).

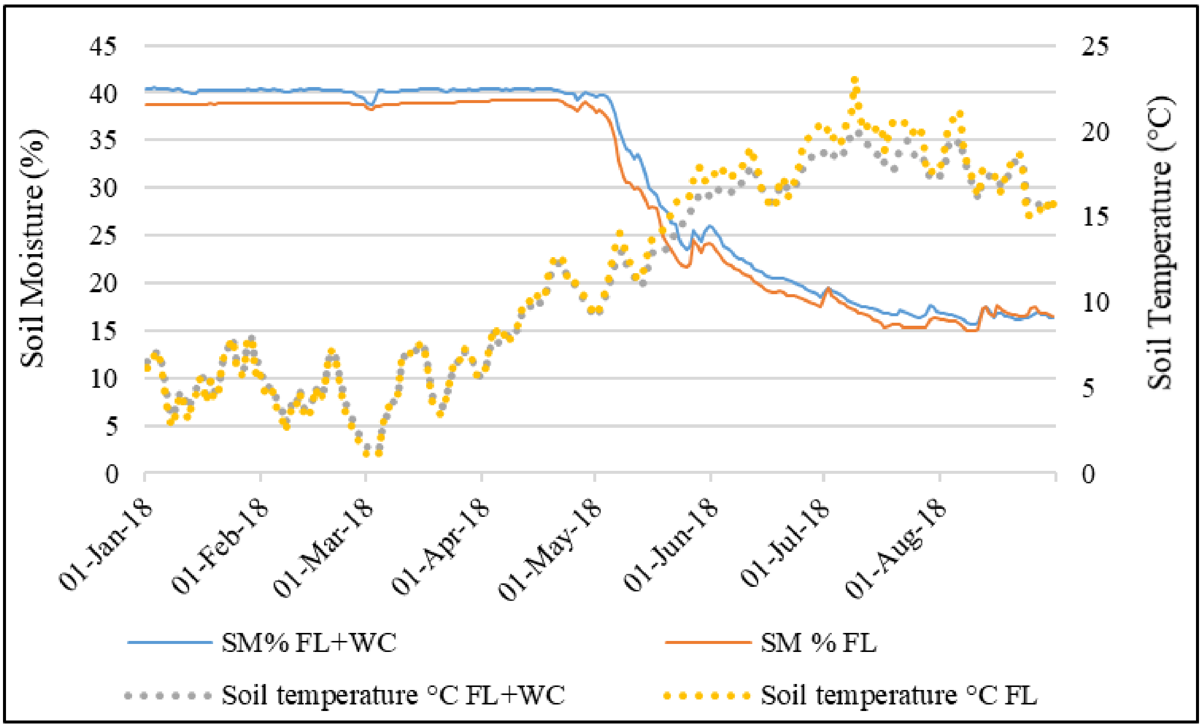

Figure 4. Soils data for the FL and FL+WC paddocks including soil moisture content (% gravimetric) at 10 cm depth and mean daily soil temperature (°C) at 15 cm depth over the experimental period of January to August 2018.

3.2 Botanical composition

Grass species other than those sown accounted for >10% of ground cover in both paddocks (Figure 5). Both paddocks also had a considerable amount of bare ground cover (~15%). The FL+WC paddock was initially sown in 2017 at 70% *Festulolium* and 30% *T. repens*; by September 2018 the proportion of *Festulolium* had dropped to 65% and *T. repens* to 11.5%. The FL paddock was initially sown as a *Festulolium* monoculture, but by September 2018 this had dropped to 70% of the ground cover.

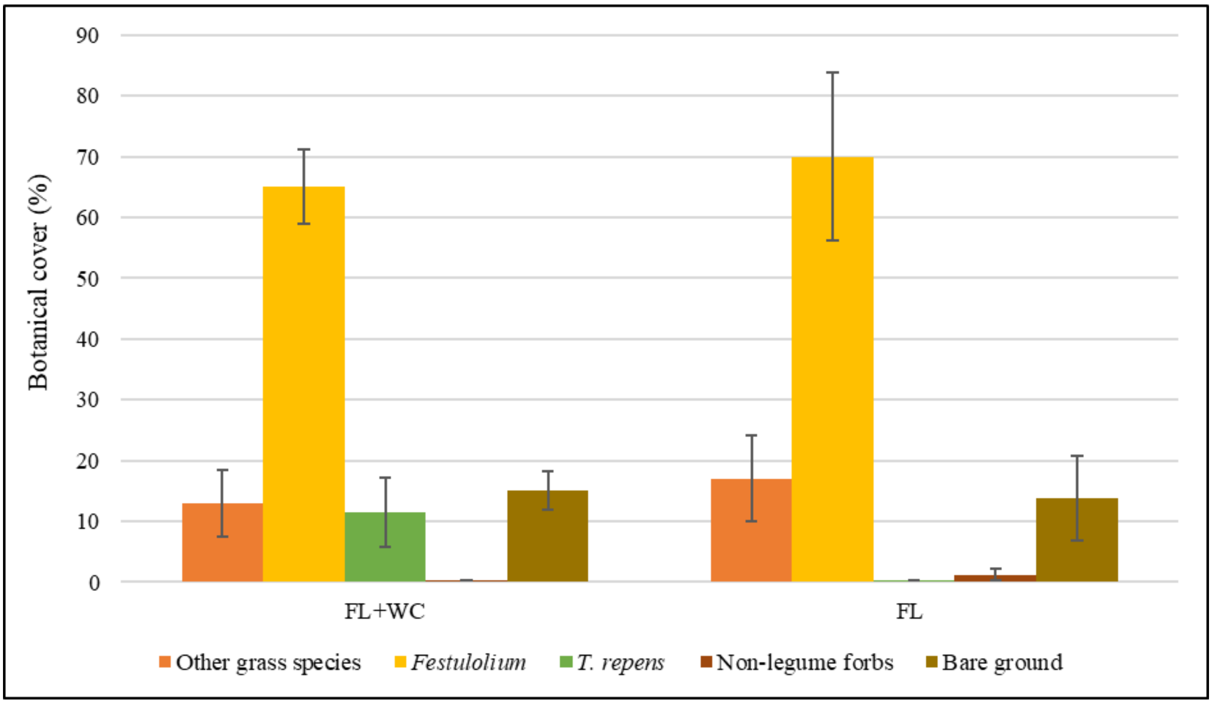

Figure 5. Botanical compositon (% ground cover) of the FL+WC and FL paddocks including *Festulolium*, *T. repens*, other grass and non-legume forb species and bare ground as of September 2018.

3.3 Nitrous oxide emissions

Over the experimental period 01/01/2018 – 31/08/2018, N_2O emissions from the FL paddock were three times greater than from the FL+WC paddock (1401 vs 462 g N_2O-N ha^{-1}) (Table 1). Generally speaking, the FL+WC paddock had low N_2O emissions, with a peak emission of 12 g N_2O-N g ha^{-1} day^{-1} on the 31st May (Figure 5). Peak emission from the FL paddock was also on this day at 68 N_2O-N g ha^{-1} day^{-1} (Figure 6). These peak events in daily emissions are following the initial (and only for FL+WC) fertiliser application of 40 kg N ha^{-1} (Figures 5 and 6). As regards daily average N_2O emissions across individual experimental months, the minimum value was 0.6 g ha^{-1} day^{-1} from FL+WC in January, the maximum daily average value was 55 g ha^{-1} day^{-1} from FL during May with a mean daily average value across all paddocks and months of 7.3 g ha^{-1} day^{-1}. Overall, there was no significant difference in N_2O emissions between treatments (P = 0.304), although emissions from the FL paddock were greater in May and June (Figures 5 and 6). It is worth noting that there are large standard errors associated with emissions from both paddocks during these periods (Figure 7).

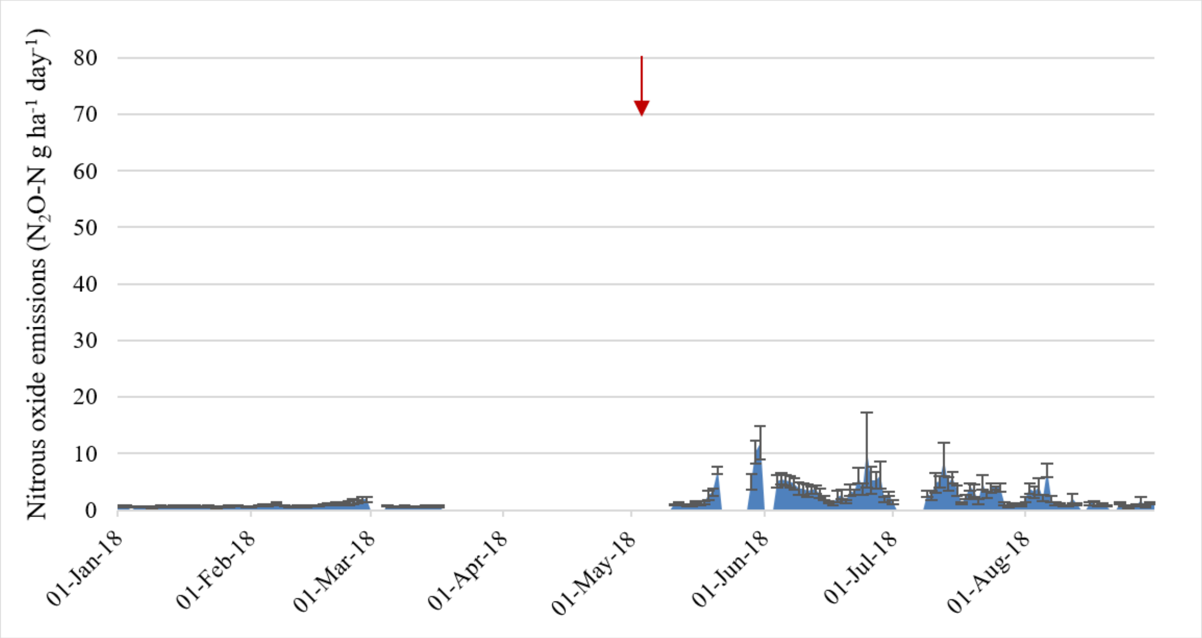

Figure 6. Temporal trace for nitrous oxide emissions (N_2O-N g ha^{-1} day^{-1}) for the FL+WC paddock. The arrow indicates a fertiliser application of 40 kg N ha^{-1} equivalent on 08/05/2018.

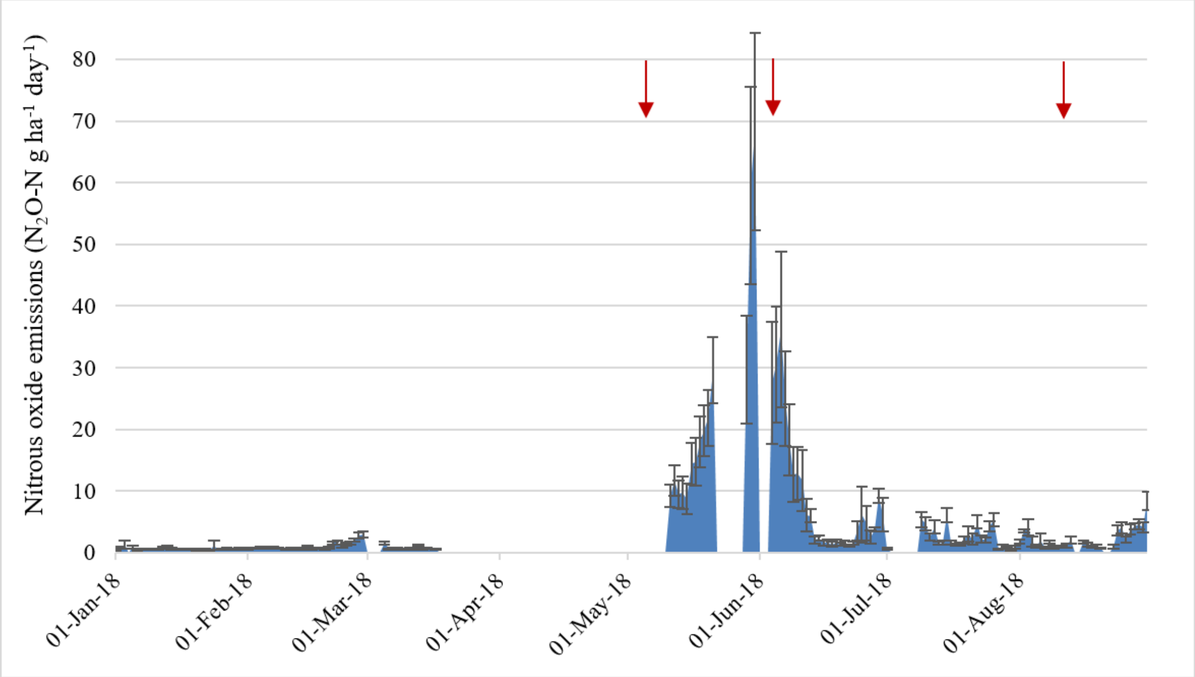

Figure 7. Temporal trace for nitrous oxide emissions (N_2O-N g ha^{-1} day^{-1}) for the FL paddock.

The arrow indicates fertiliser applications of 40 kg N ha^{-1} equivalent on each of the 08/05/2018, 05/06/2018 and 17/08/2018.

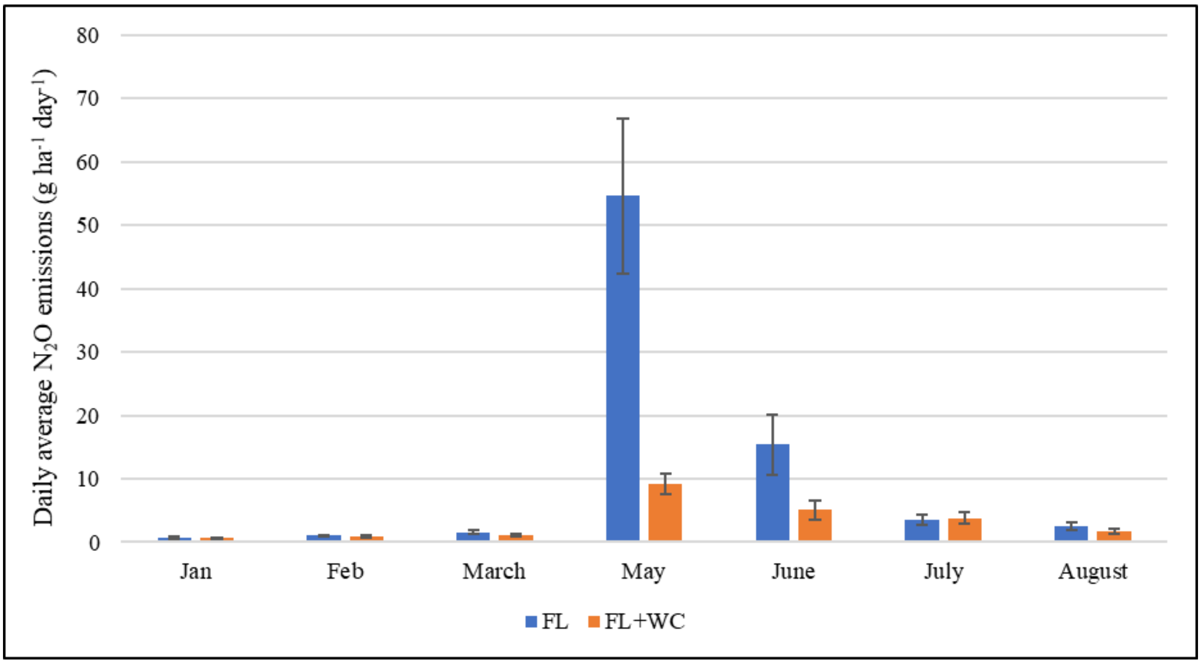

Figure 8. Daily average N_2O emissions (g ha^{-1} day^{-1}) for each month for the FL+WC and FL paddocks. Cumulative emissions and number of days of data per month listed in Table 1.

Table 1. Total cumulative N_2O emissions (g ha^{-1}) per month (± standard error) and for the total experimental period (01/01/2018 - 31/08/2018) for the FL+WC and FL paddocks**.**

Month	**FL (g N_2O-N ha^{-1})**	**FL+WC (g N_2O-N ha^{-1})**	**Number of days of data**
Jan	20 ± 4.2	17 ± 2.9	30
Feb	28 ± 4.2	25 ± 4.5	28
March	20 ± 3.3	13 ± 2.4	13
May	764 ± 171.2	129 ± 22.3	14
June	414 ± 126.1	135 ± 38.5	27
July	85 ± 20.5	91 ± 21.2	24
August	67 ± 14.5	48 ± 11.4	27
Total	1400 ± 344.1	461 ± 103.2	163

3.4 Emissions Intensity and N excretion.

The FL+WC paddock held 7% more animals which included 20% more lambs than the FL paddock (Table 2). Although, the FL paddock held animals for more days at grazing than the FL+WC paddock. This resulted in total estimated N addition from excreta 12.8% greater (10.7 kg N) for FL compared to FL+WC. Overall, the FL+WC paddock supported 27% more total LWG. Thus, on average, lambs gained 2 kg extra from birth to 31/08/2018 when grazing on the FL+WC paddock. Regarding N_2O emissions intensity, the FL paddock produced 291 g N_2O-N ha^{-1} more per tonne lamb LWG from birth to 31/08/2018 than the FL+WC paddock (Table 2). This equates to a 63% decrease in emissions intensity associated with lamb LWG from grazing sheep on the FL+WC paddock.

Table 2. The range and average number of ewes and lambs grazed, live-weight gain (LWG) (kg) and subsequent emissions intensity (N_2O-N g ha^{-1}/ Tonne total LWG) for FL+WC and FL paddocks during the experimental period. Estimates of animal N excretion (total, urine and dung) are presented for both paddocks throughout the agronomic season.

01/05/2018 – 31/08/2018	**FL**	**FL+WC**
Number of animals/paddock	148	158
Number of ewes/paddock	71	66
Number of lambs/paddock	77	92
Total lamb LWG/paddock(Tonne)	2.4	2.9
Average lamb LWG (kg)	30 ± 0.5	32 ± 0.6
Average number ewes/paddock	37	44
Range ewes/paddock	16-70	21-64
Ewe days grazing/paddock	104	77
Average number lambs/paddock	43	61
Range lambs/paddock	3-49	3-77
Lambs days grazing/paddock	76	49
Emissions Intensity (g N_2O-N/Tonne Total LWG)	459	169
Average N excretion/ewe(kg)	65.7	57.7
Average urine N excretion/ewe (kg)	39.4	34.6
Average dung N excretion/ewe (kg)	26.3	23.1
Average N excretion/lamb (kg)	28.1	25.5
Average urine N excretion/lamb (kg)	16.9	15.3
Average dung N excretion/lamb (kg)	11.2	10.2

Total estimated N excretion/paddock	93.8	83.2

The IPCC default EF_3 (2019) for excreta from grazing livestock underestimated N_2O-N losses from excreta from both paddocks compared to the UK refined EF_3 by 0.14 and 0.12 kg N_2O-N ha^{-1} for the FL and FL+WC paddocks respectively. Conversely, the IPCC default EF_1 of 1.6% for wet soils overestimated N_2O-N losses from ammonium nitrate fertiliser compared to the UK refined EF by 1.49 and 0.51 kg N_2O-N ha^{-1} for the FL and FL+WC paddocks respectively. Overall, it was found that using the IPCC default EFs rather than the UK refined EF resulted in a cumulative overestimation of 1.35 (FL) and 0.38 (FL+WC) kg N_2O-N ha^{-1} for each paddock (Table 3).

Table 3. Estimate N_2O-N emissions calculated using the IPCC default and UK specific EFs.

1st May - 31st August 2018	**FL**	**FL+WC**	**Difference**
Excreta kg N_2O-N (IPCC 0.3%)	0.28	0.25	-0.03
EF3 sheep urine kg N_2O-N (UK 0.629%)	0.35	0.31	-0.04
EF3 sheep dung kg N_2O-N (UK 0.193%)	0.07	0.06	-0.01
Fertiliser kg N_2O-N losses (IPCC EF1 1.6%)	1.92	0.64	-1.28
Fertiliser kg N_2O-N losses (UK EF1)	0.43	0.13	-0.29
Total kg N_2O-N emissions (IPCC EF)	2.20	0.89	-1.31
Total kg N_2O-N emissions (UK EF)	0.85	0.51	-0.34
Difference between IPCC and UK inventory methods (kg N_2O)	1.35	0.38	na

4. DISCUSSION

The higher daily average N_2O emissions in the FL paddock compared to the FL+WC paddock over the experimental period (particularly in May and June) are in line with the long established trend linking heightened N_2O emissions to higher fertiliser application levels (Mosier et al., 1998; van Groenigen et al., 2010). The highest daily average N_2O flux was in May for both paddocks, most likely driven by the first fertiliser application event on the 12th May. The effect of higher fertiliser application (120 kg ha^{-1} year^{-1} for FL vs 40 kg ha^{-1} year^{-1} for FL+WC) on increased N_2O emissions from the FL paddock was potentially amplified by the management practices which have been in place since 2013. The long-term application of manures and fertilisers can result in an accumulation of N in the soil system, particularly in the top 30 cm layer (Sommerfeldt et al., 1988; Edmeades, 2003) where the nitrification process primarily takes place (Persson and Wirén, 1995).

It was surprising that although N_2O emissions were three times greater from FL compared with FL+WC (Table 1), there was no significant difference between the two treatments, or effect of sward type. This is a reflection of the high levels of variability associated with measurements for both paddocks as regards daily average emissions (Figure 7) and overall cumulative emissions (Table 1). Soil N_2O fluxes are by nature, extremely spatially and temporally variable (Charteris et al., 2020). Although automated chambers are better able to reduce temporal variability through increased measurements, the Global Research Alliance recommends supplementing automated systems with a low-temporal-frequency manual sampling network for increased robustness and model validation (Grace et al., 2020). This practice of combining both automated and manual N_2O sampling efforts has been used in previous studies such as Smith and Dobbie (2001) and Rowlings et al (2012). This may be more important at farm and paddock scale, where replication of treatments is more difficult to achieve. However, it is worth noting the logistical difficulties of conducting manual chamber measurements when livestock are present.

From an agronomic perspective, the FL+WC paddock was more productive as it held more animals, with lambs gaining an average of 2kg more than lambs grazing the FL monoculture (Table 2). This agrees with the results from a two year study by Grace et al. (2019), who found that lambs grazing a *Lolium perenne* monoculture sward took significantly longer to reach slaughter weight than lambs grazing on more diverse swards including a *L. perenne/ T. repens* mixture, a 6-species mixture and a 9-species mixture. This was attributed to improved ewe milk composition (up until weaning ~42 days old) and thereafter to improved herbage quality and increased availability. Similar results on increased lamb performance regarding legume-based pastures are also reported by Golding et al. (2011) and Somasiri (2015).

The emissions intensity analysis (Table 2) provides a more in-depth interpretation of sustainability than N_2O emissions alone (Table 1). At paddock scale, FL+WC proved a more sustainable livestock system owing to the 63% decrease in N_2O emissions intensity relative to FL. This is an important consideration for livestock production as there is increased demand from the growing population for food to be produced in a sustainable manner (Pretty and Bharucha, 2014). Although the FL+WC paddock held more animals, the longer grazing period (most likely due to forage availability) (Table 2) was the main contributor to the increased excreta N loading to the FL paddock throughout the season. Urine deposition by grazing livestock results in an immediate increase in N_2O emissions (Carter, 2007). The total N levels in sheep urine range from 3.0 to 13.7 g litre^{-1} of which an average of 83 % N is as urea and the rest consisting of organic N compounds (Bristow et al., 1992). Urea hydrolyses and enters the nitrification pathway more quickly than organic N, and Carter (2007) found nitrification as opposed to denitrification to be the most important factor explaining the high initial N_2O fluxes following urine patch deposition to well-aerated soils. However, a 20% increase in livestock numbers over the agronomic period on the FL+WC paddock did not outweigh the effects of a 66% increase in fertiliser application on the FL paddock regarding heightened N_2O emissions or emissions intensity.

The standard UK N excretion values (used to estimate the paddock excretal N loadings (10 g N lamb^{-1} day^{-1} and 20 g N ewe^{-1} day^{-1}) (Brown et al, 2020) (Table 2) are an intermediate between those reported by Marsden et al. (2018) at 26.7 ± 2.3 g N sheep^{-1} day $^{-1}$ for improved grassland and 16.7 ± 1.2 g N sheep^{-1} day^{-1} for semi-improved pasture. Sward composition, digestibility and protein content, in addition to sheep size and energy requirement, will influence N excretion in dung and urine (Lagrange and Villalba, 2019; Lagrange et al., 2020). Therefore, the actual N excreta to paddocks in this specific study may have varied compared to what was estimated using the standard values. In addition, we cannot directly link the N_2O field measurements to N excretion events as the chambers were fenced from the animals, meaning N_2O 'hotspots' from N excreta were not captured directly after urination (Marsden et al., 2017). From our estimations, it is worth noting that the IPCC default EF_3 (excreta from grazing livestock) underestimated N_2O emissions compared to the UK specific EF_3. Conversely, the IPCC default EF for ammonium nitrate fertiliser application majorly overestimated N_2O emissions compared to the UK NIR site specific EF calculation for ammonium nitrate fertiliser application. Overall, using the default EFs overestimated emissions from both paddocks. This equated to a 357 kg CO_2 eq and 100 kg CO_2 eq overestimation of N_2O emissions from the FL and FL+WC paddocks, respectively. Therefore, our study highlights a) the importance of specific estimates of important parameters in EF calculations (N excretion, fertiliser N, specific EF) and b) the importance of disaggregated or modelled (Tier 2 or Tier 3 calculations) EF for sheep N excreta, which should be developed for the management practices commonly used in specific countries or regions to provide more accurate inventory accounting.

5. CONCLUSION

In a comparison of sheep grazing a *Festulolium* monoculture and a *Festulolium* + white clover mixture, N_2O emissions (absolute and per kg LWG) were much lower for the system including white clover and livestock productivity was greater. The greater N fertiliser application to the grass monoculture was the most important factor in the observed increase in emissions (albeit non-significant due to high variability) as there were minimal differences in soil characteristics or ancillary measurements between both paddocks. This study emphasises the necessity of reducing artificial N inputs into livestock systems to reduce emissions as outlined in the EU Farm to Fork Strategy.

While automated chambers enable better representation of the temporal variability in emissions, accounting for the high spatial variability remains a challenge with limited chamber numbers. Additionally, analysing N_2O at the paddock scale gave issues as regards replication i.e. comparisons were only made between two paddocks. Increased monitoring of emissions across replicate paddocks, potentially through more limited measurement frequency using manual chambers, would provide a more robust system comparison. It The study also highlighted the importance of developing country and region specific (Tier 2 or Tier 3) EFs for accurate system-level GHG accounting.

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CHAPTER 6

GENERAL DISCUSSION

Research implications

Trends in N_2O emissions

Many previous studies have monitored N_2O emissions during short term experiments, at incubation scale or from less complex or binary swards (Abalos et al., 2014; Bracken et al., 2020; Reinsch et al., 2020). However, the work carried out as part of this PhD thesis is novel in that it takes into account a full yearlong agronomic assessment of N_2O emissions from multi-species swards using a full simplex design to assess for diversity interactions. The findings of this PhD thesis reiterate the importance of applying inorganic N fertiliser at moderate levels to limit N_2O losses. This has been proven to be of particular importance during and following summer drought periods (Chapter 4), which are predicted to become more commonplace in line with climate change (IPCC, 2021). All N_2O emissions observed in the three experimental chapters of this PhD thesis are within, although towards the lower end, of the average emissions (0.04–21.21 N_2O-N kg ha^{-1} $year^{-1}$) reported for European grasslands (Rees et al., 2013). The study of Rees et al. (2013) also found nitrogen addition to be the greatest determinant of N_2O emissions. In this PhD, the highest annual cumulative N_2O emissions observed in Chapter 3 (3.18 kg ha^{-1} $year^{-1}$ from 300N *L. perenne*) are lower than the annual emissions reported in the study of Žurovec et al. (2021), which ranged from 4 to 6.6 kg N_2O-N ha^{-1} $year^{-1}$ for *L. perenne* monocultures under different phosphorous management regimes and received the same level of N (300 kg N ha^{-1} $year^{-1}$) at the same location (the following year, 2019 at Teagasc, Johnstown Castle). The highest daily flux observed was reported in Chapter 3 at 112 g N_2O-N ha^{-1} day^{-1} from the 300N *L. perenne* treatment on the 18/04/2018 following fertiliser application of 60 kg N ha^{-1} on the 09/04/2018 (with soil WFPS at 72%). Fertiliser application was also the driver of the highest daily N_2O peak in Chapter 5 from the *Festulolium* monoculture paddock at 68g N_2O-N ha^{-1} day^{-1} on 31/05/2018 following the initial fertiliser application of 40 kg N ha^{-1} (volumetric soil moisture 24%). Fertiliser application further exaggerated the high N_2O

emissions caused by re-wetting in Chapter 4 with the highest daily N_2O flux of 43.8 g N_2O-N ha day^{-1} on 13/08/2018 from the high N *L. perenne* treatment six days post rewetting after the eight-week drought. The irrigation event on 08/08/2018 brought soil WFPS from 7% to 48% WFPS by 13/08/2018 when this pulse in N_2O emissions occurred.

Legume proportion and N_2O emissions

Legume inclusion within a sward can increase soil mineral N pools and thus N_2O emissions (Niklaus et al., 2006; Abalos et al., 2021). The proportion at which legume inclusion begins to increase N_2O emissions from an intensively managed temperate grassland sward is clarified through the experimental work carried out in Chapter 3. Nitrous oxide emissions were assessed across a gradient of sward legume proportions and it was found that N_2O emissions begin to significantly increase above $^1/_3$ sward proportion when receiving 150 kg N ha^{-1} year^{-1}. Nitrous oxide emissions most likely increased as the N requirement of the sward was exceeded and excess soil N was available to undergo nitrification and denitrification. However, this study only considered red and white clover and observations may vary according to legume species or under different climatic conditions. Nonetheless, the high N_2O emissions associated with increased legume proportion should be noted during the use of legume-based swards as ley cover crops. Fox et al. (2020) found lasting, beneficial legacy effects on crops grown following legume cover crops of 50-100% inclusion, however given the high N_2O emissions observed in both Chapters 3 and 4 with an increasing legume proportion, this should be recognised as a potential source of emissions indicating that swards would ideally have lower legume proportions. It was also evident from experimental Chapters 3 and 4 that inorganic fertiliser addition to high legume proportion swards further exaggerates the N_2O emissions associated with BNF and legume N residue incorporation into the soil (de Neergaard et al., 2002). Thus, lower N application to legume-based swards (such as cover crops) will aid in avoiding excess N_2O emissions. The analyses carried out in Chapter 5 subsequently follows on from these findings as N application was tailored to account for clover inclusion within the sward (30% *T. repens*). As a result, the grass/clover sward receiving 40 kg N ha^{-1} year^{-1} produced 33% less N_2O emissions than the grass monoculture receiving 120 kg N ha^{-1} year^{-1}. Importantly, these savings in N_2O emissions were achieved without compromising on productivity. Additionally, as reported in both Chapters 3 and 4 there is high variability in N_2O emissions from legume monocultures. This could be due to a number of factors such as differences in the rate of BNF, soil C:N ratios or the amount of N rich crop residue incorporated into the soil (Rochette and Janzen, 2005). The associated variability reported in this PhD thesis should be considered in future studies that aim to monitor N_2O emissions from legume-based swards and dealt with by increasing replication within the experimental design.

Emissions intensity as a measure of sustainability

The sustainable expansion of agriculture will require higher yields on the same land area with less impact on the environment. Thus, emissions intensity is an appropriate measurement of sustainability i.e. the cost of emissions per unit product. This is highlighted in the Irish Marginal Abatement Cost Curve (MACC) as an important metric in order to maintain future sustained demand for agricultural produce (Lanigan et al., 2019). This PhD thesis provides strong evidence at both plot and paddock scale that there does not need to be a trade-off between mitigation N_2O emissions through reducing N inputs and maintaining productivity. Regarding the latter, I carried out a novel N_2O emissions intensity analyses of yield production across systematically varying levels of grassland diversity in Chapter 3. It was found that MSS can significantly reduce N_2O emissions while maintaining/increasing yields (N biomass and DM yield). This is further explored in Chapter 5, which directly builds on the experimental work carried out in Chapter 3; the productivity of grass-legume mixtures was assessed at paddock scale and considered livestock productivity. In this experiment, I found that the N_2O emissions and emissions intensity from the binary grass-legume mixture (40 kg N ha^{-1} year^{-1})

were comparably lower than the grass monoculture (120 kg N ha^{-1} $year^{-1}$). Of notable importance is the increased productivity with lambs reared on a grass/clover sward gaining an average of 2kg more than lambs grazing a grass monoculture. This is in line with Grace et al. (2019), where lambs reared on more diverse swards (>2 species) took two weeks less to reach age at slaughter (45 kg target slaughter weight). There were also additional animal health benefits associated with MSS in this study including anti-helminthic properties i.e. lambs grazing on MSS required fewer worming doses. Regarding dairy cattle, Loza et al (2021) established the methane emissions intensity of multi-species swards across a full grazing dairy system. It was found that grazing on diverse swards resulted in less methane emissions per unit milk produced. Similar results were previously reported by Roca-Fernández et al (2016) where significant increases in milk production, milk solids production and DM intake were observed with dairy cattle grazing on diverse swards (>4 species and >3 FGs). Our findings and the aforementioned emissions intensity studies point towards the use of low N grass-legume swards as having dual GHG abatement potential: 1) Reduced inorganic N requirements will reduced N_2O emissions and emissions intensity 2) The earlier age of livestock at slaughter will reduce cumulative methane emissions over the animal's lifetime 3) improved animal performance can reduce methane emissions intensity (Cummins et al., in review). Overall, it is evident from the emissions intensity work carried out in both Chapters 3 and 5 that MSS and binary grass legume swards could greatly contribute to climate smart livestock production.

The annual assessment of N_2O emissions across a gradient of species diversity (Chapter 3) adds lower N_2O emissions intensity to the myriad of multi-functional benefits associated with MSS. Such pre-established benefits include yield production, yield stability, weed suppression and nitrogen use efficiency (NUE) as highlighted in the study of Suter et al. (2021). Suter et al. (2021) found that the multi-functional benefits of a 4-species mixture equated to that of a productive grass monoculture receiving 450 kg N ha^{-1} $year^{-1}$.That particular study did not consider N_2O emissions, but in our study we found that emissions were highest from the *L. perenne* monoculture receiving 300 kg N ha^{-1} $year^{-1}$ emitting 440 kg CO_2 equivalent more than 6-species mixture. Therefore, the multifunctional benefits of the 4-species mixture in Suter et al. (2021) are more pronounced in light of the finding of this PhD thesis i.e. decreased N_2O emissions and emissions intensity. The emissions intensity results of Chapter 3 partly mirror the work of Nyameasem et al. (2021) who found no significant effect of sward species diversity on N_2O emissions or emissions intensity. Nyameasem et al (2021) assessed an 8-species mixture containing five herb species which may not have driven productivity thus not lowered the emissions intensity. Conversely to Nyameasem et al. (2021) there were significant reductions in N_2O emissions intensity from the 6-species mixture in Chapter 3 compared to both 300N and 150N *L. perenne* monocultures. Fuchs et al. (2018) measured N_2O emissions and emissions intensities from a low-clover (14% clover in 2015 and 4% clover in 2016) receiving high N input (296 kg N ha^{-1} in 2015 and 181 kg N ha^{-1} in 2016) paddock with a high-clover paddock (21% clover in 2015 and 44% clover in 2016) receiving zero N input paddock. Both paddocks were over-sown with clover at the start of each agronomic season. It was found that the latter grassland treatment had >40% lower N_2O emissions than its low-clover, high-N counterpart. Additionally, the lower N_2O emissions of the high-clover, zero-N paddock resulted in reduced emissions intensity. The results of this study are reiterated in Chapter 3 where it was found that a multi-species sward containing 33% clover reduced N_2O emissions and emissions intensities at 150kg N ha^{-1} $year^{-1}$ input compared to a *L. perenne* monoculture receiving 300kg N ha^{-1} $year^{-1}$. Thus, an important practical recommendation in light of these experimental findings is that inorganic N fertiliser is not applied in excess of 150 kg N ha^{-1} $year^{1}$ to avoid excessive N_2O losses without compensating on N biomass or DM yield production. However, as reported in Chapter 3, N application to swards containing legumes was not reduced to adjust for N input through BNF. Thus, a recommendation for further research emerging from this PhD thesis would be to determine the minimum N requirement for diverse swards that does not compromise on yield benefits. This could be done through assessing N_2O emissions and N_2O emissions intensity from multi-species swards across a gradient of N application levels from high to moderate to reduce e.g. 0-150/300 kg N ha^{-1} $year^{-1}$.

This thesis established a number of ways through which more diverse, legume based swards can reduce certain measures of emissions intensity. However, in addition to these, there are a number of additional avenues through which the use of more diverse swards could lower the overall carbon footprint of livestock production systems: Lowering overall N requirements; providing natural N input without the use of industrially fixated N as the N fertiliser production process being a major form of off-farm GHG emissions (O'Brien et al (2010); Increasing C sequestration by including deep rooting and highly productive species; improving animal performance and thus output i.e. milk/beef.

Multi-species swards and climate adaptation

Climate projections predict more frequent and severe drought events throughout Europe especially after 2070 (Spinoni et al., 2018). It is likely that multi-species swards will be used in place of conventional monocultures as a climate adaptive measure owing to drought resilience and recovery benefits regarding yield production (Haughey et al., 2018; Grange et al., 2021). Drought resistance traits are driven by functional group diversity and the inclusion of deep rooting herb species within a grassland mixture (Komainda et al., 2020). The experimental work carried out in Chapter 4 assessed the soil mineral N dynamics of different monocultures and mixtures in face of these predictions. The results provide the required clarification surrounding fertiliser application during summer drought and re-wetting conditions i.e. to limit fertiliser application during drought periods and to delay fertiliser application at least two weeks post re-wetting. The soil mineral N dynamics were typical of what would be expected during drought (inhibition of nitrification - significantly higher NH_4^+ than ambient) and re-wetting events (nitrification resumes – leading to significantly higher NO_3^- than ambient). Legume monocultures had no significantly elevated N_2O emissions at re-wetting, this was due to the high emissions from the ambient treatment rather than low re-wet emissions. The only treatments which did not have higher N_2O emissions due to re-wetting were the 6-species mixture and the *P. lanceolata* monoculture. This reflects the drought recovery and resilience benefits of *P. lanceolata* and multi-species swards as more stable N dynamics in face of environmental stressors will see less extreme fluctuations in N_2O emissions and soil mineral N (NH_4^+ and NO_3^-). The presence of BNI compounds in plantain may have prevented the increased rates of nitrification associated with the rewetting process. Given that the 6-species mixture contained 15% plantain proportion, it is very likely that this contributed to the lower N_2O emissions at re-wetting. The results presented in Chapter 4 and the findings of Bracken et al. (2020) both highlight that *P. lanceolata* inclusion within a multi-species swards could potentially inhibit the nitrification process and thus reduce N_2O losses. The same mechanisms were shown to be responsible for reductions in nitrate leaching following irrigation (Carlton et al., 2019). Thus, the trends observed in Chapter 4 build on previous research that all point towards *P. lanceolata* inclusion within a multi-species swards contributing to a reduction in N losses to the environment. This requires further exploration perhaps through the use of ^{15}N tracing techniques and the quantification of BNI compounds (e.g. aucubin, catalpol and acteoside) (Tamura and Nishibe, 2002; Gardiner et al., 2018). Overall, it is clear from Chapter 4 that consideration should be given to sward type and grassland management strategies depending on yield requirements and environmental conditions.

Emission factor disaggregation/refinement

Achieving GHG policy objectives will require the implementation of mitigation strategies within agricultural production systems such as those highlighted in Lanigan et al. (2018). Such strategies include the use of protected fertiliser, selecting for higher genetic merit livestock and importantly, reducing fertiliser N inputs through the use of clover based swards. It is crucial that such mitigation efforts are clearly reflected within National Inventory Reports. In Chapter 5, Tier 1 and 3 calculations for N_2O emissions were compared. The comparison highlighted the inaccuracy of using default EFs. There were major overestimations of N_2O emissions when solely using IPCC default vs the refined UK EFs. This overestimation of N_2O losses equated to a 357 kg and 100 kg CO_2 equivalent from the *Festulolium* monoculture and *Festulolium*/white clover paddocks, respectively. The former UK GHG research platform (2010 – 2015) made considerable advances in refining N_2O EFs (Rees et al., 2016). Similarly in Ireland, the studies of Harty et al (2016), Roche et al (2016) and Krol et al (2016) played a pivotal role in disaggregating EF calculations for inorganic N fertilisers and livestock excreta. However, the Irish NIR reports direct N_2O emissions from managed soils using the Tier 1 approach as per the 2006 IPCC Guidelines. Thus, more detailed EFs and corresponding activity data will be required to provide a more accurate Tier 2 inventory report. Model development work would be required to formulate Tier 3 methodology such as the UKs EF_1 calculation (Chapter 5, equation 1). Such models are useful as they consider the relationship between soil characteristics, environmental variables (rainfall, temperature etc.) and N_2O fluxes. Tier 3 models can thus be utilized in estimating N_2O emissions at regional/country scale where direct experimental measurements are impracticable (IPCC, 2019). Additionally, disaggregation of EFs (i.e. IPCC default methods) is also required for a number of other agricultural GHG sources in Ireland i.e. N_2O emissions from sheep excreta and enteric methane from sheep.

Model refinement

The data generated for this thesis directly responds to gaps in knowledge identified by world renowned authors in N_2O emissions (Fuchs et al., 2020). This study compared the N_2O flux simulations from common biogeochemical models such as DayCent, PaSim and APSIM. The study identified a number of inaccuracies in simulated N_2O emissions across all models vs GHG inventory calculations. Following on from this analyses, Fuchs et al. (2020) recommends thorough model evaluations against experimental data of N_2O emissions particularly regarding legume-based swards in order to validate and improve model performance. Therefore, the data generated throughout this PhD thesis will be useful in improving such models as all three experimental Chapters provide N_2O data from legume based swards at different fertiliser levels and climatic conditions.

Nitrous oxide measurement methodologies

This PhD thesis provides insight towards the measurement of N_2O emissions from diverse swards, which have not been as widely researched as grass monocultures. Manual gas chambers were used in Chapters 3 and 4 while automated chambers were used to measure N_2O emissions in Chapter 5. It seems that there are advantages and disadvantages to both methodologies. For example, the use of automated chambers could have provided better temporal resolution of N_2O emissions at re-wetting if used in Chapter 4. Conversely, the high levels of uncertainty associated with automated measurements in Chapter 5 may have benefited from additional manual measurements to improve experimental robustness. More recently, the Global Research Alliance has added supplementing automated systems with a low-temporal-frequency manual sampling network into the automated N_2O measurement guidelines (Grace et al., 2020). However, taking manual measurements in paddocks with livestock is both difficult and impractical. Overall, Chapter 5 displays the positive effect of clover based swards on lamb LWG and shows the real-life application of GHG mitigation practices at farm scale successfully reducing emissions and maintaining productivity. Going forward, better quantification of systems scale emissions could be done through the use of decision support tools e.g. Farm Carbon Navigator, Overseer, Cool Farm Tool etc.

Recommendations for further research

- To assess N_2O emissions and emissions intensity from multi-species swards across a gradient of N application levels e.g. 0-150/300 kg N ha^{-1} $year^{-1}$.
- To establish specific emission factors for fertiliser N application to multi-species swards at various levels of species and functional group diversity.
- To assess the carbon sequestration potential of multi-species swards and the use of life cycle assessment to determine carbon balances of multi-species based production systems
- To use isotope tracing techniques to determine if nitrification or denitrification are the main pathways of N_2O losses across gradients of species diversity at re-wetting events
- To further assess the potency and effects of BNI compounds from *P. lanceolata* on N_2O emissions and at various sowing proportions
- To assess N_2O emissions from diverse swards in face of other extreme climatic events i.e. freeze-thaw and flooding
- To conduct emissions intensity analyses of drought induced swards
- To assess farm scale productivity and N inputs through life cycle assessments
- Assessing paddock scale N_2O emissions with increased paddock replication to allow for repeated measures analyses with manual chambers to increase experimental robustness.
- The use of Decision Support tools to assess the farm carbon balance of MSS and clover based production systems
- To assess the N_2O emissions intensity of other livestock systems i.e. dairy or beef when grazing binary or multi-species swards.
- To determine Tier 2/3 EFs for N application and livestock N excreta from grazing animals where required i.e. Ireland for grazing sheep excreta.

GENERAL CONCLUSIONS

There has been increased interest in the use of multi-species swards in both extensive and intensive livestock production systems in recent years. This PhD thesis builds on existing knowledge and adds novel insights into how grassland diversity affects N_2O emissions and emissions intensity. The overarching aim of this PhD thesis was to determine whether or not there was potential for increased NUE and savings in N_2O emissions by replacing high N input monocultures with moderate to low N input grass-legume mixtures/multi-species swards. Nitrous oxide emissions from MSS at moderate N applications were not higher than those from monocultures on the same N rates. Although, it was proven that MSS directly lower N_2O emissions intensity in comparison with grass monocultures receiving both high and moderate N application levels. Additional benefits to MSS were identified throughout this PhD thesis including increased Lamb LWG, increased, less emissions at re-wetting events and no lasting legacy effects of drought on increased N_2O emissions. The urgency for emission factor refinement and accurate GHG inventory accounting was also highlighted in this thesis. This is especially important to ensure mitigation efforts are reflected in National Inventory Reports. Additionally, the data generated and research findings from this PhD thesis will be useful for biogeochemical model and decision support tool validation. I conclude that N_2O emissions from intensively managed grasslands can be effectively reduced without reducing productivity (DM yield, N biomass or lamb LWG). This can be done through reducing inorganic N inputs and increasing grassland functional group diversity i.e. including legumes and herb. Thus, grass-legume mixtures and multi-species swards receiving moderate to low N fertiliser inputs are a practical option for the sustainable intensification of agriculture.

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APPENDIX

ABOUT THE AUTHOR

Saoirse began her education and was first introduced to the importance of on farm GHG mitigation at University College Dublin where she studied Agri-Environmental Science. She graduated in 2017 with an honours degree and subsequently began her PhD at Teagasc Johnstown Castle, Co Wexford. Her PhD project was carried out in collaboration with the University of Reading and Rothamsted Research, North Wyke. As part of her PhD, Saoirse carried out a novel assessment on the relationship between grassland diversity, N_2O emissions and emissions intensity. These results were presented at the 13th British Grassland Society Research Conference where Saoirse presented as a speaker. Saoirse has a keen interest in science communication, the findings of her PhD project have been disseminated and received with keen interest by various stakeholders through publication, national and international conferences, open days, farm walks and science week events. During her final year, Saoirse also worked as a Technologist on the Irish Agricultural GHG Centre of Excellence Scoping Study. During which she reviewed the GHG research infrastructure in Ireland, conducted a review on solutions to enteric-methane abatement in Ireland, ran numerous national and international stakeholder development workshops, investigated international GHG research approaches and established a roadmap for the development of a national agricultural GHG research centre. Furthermore, Saoirse has recently began a Research Officer position on the MELS (Mitigating Emissions from Livestock Systems) ERA-GAS project. As part of this project, she has begun working on the development of a prototype farm-scale GHG decision support tool and will contribute to national and international GHG inventory refinement through data collation and component modelling.

A LIST OF THE AUTHOR'S PUBLICATIONS

Journal articles

Published

Cummins, S., Finn, J.A., Richards, K.G., Lanigan, G.J., Grange, G., Brophy, C., Cardenas, L.M., Misselbrook, T.H., Reynolds, C.K. and Krol, D.J., 2021. Beneficial effects of multi-species mixtures on N_2O emissions from intensively managed grassland swards. *Science of The Total Environment*, *792*, p.148163.

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