Nitrogen source, transformation and fate within intensive dairy systems to inform sustainable intensification

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Abstract

Contamination and deterioration of natural water quality by nitrogen (N) from agricultural sources is a major threat to the environment. Globally, there is a societal expectation that sustainable food production should be achievable. The concept of sustainable intensification is based on to the equality between production and environmental targets. For this to become a reality, increased productivity must be accompanied by provision of clean water, air, habitats for biodiversity, recycling of nutrients and mitigation against climate change.

Agriculture and food production rely heavily on external N inputs (e.g. fertilisers) and as agronomic systems generally have low use efficiency there is the risk of high N losses i.e. the leak of N excess to the environment. Agricultural landscapes contain many different soil/subsoil/bedrock typologies having heterogeneous N water attenuation capacities (intrinsic ability of soils to reduce contamination). Dairy farms represent complex environments, necessitating many techniques (isotopes, biogeochemical parameters, dissolved gases, bacterial gene abundances) used in combination, to provide a thorough characterisation of, examination of N source, transformation and fate along different subsurface pathways. These multiple techniques are currently seldom used in combination.

In Ireland, 30% of milk production occurs in high rainfall conditions and heavy textured soil areas. For better grass growth, artificial drainage systems (shallow and groundwater systems) are installed. The role of land drainage in N transfer, transformation and fate is however relatively unexplored. These systems may reduce N transformation potential by, for example, creating unsuitable conditions for denitrification leading to greater nitrate (NO_3^--N) losses or by-passing zones of high soil N attenuation capacity further compromising sustainability targets. Indeed, the potential to use drainage systems as a monitoring tool, which covers large areas of contribution, has been neglected in terms of multiple techniques that could explore N transfer, transformation and fate.

The concept of "sustainable intensification" includes all the aspects of agricultural productivity and environmental protection. The primary aim of this thesis was to examine this concept in terms of impacts and relationships of drainage systems installed at intensive sites on and with soil drainage classes, N transfer, transformation and fate and water quality to develop advises and a range of multiple techniques to improve and guide future management.

Herein, this concept has been tested within a range of different contexts in terms of scale (farm, plot and laboratory), soil characteristics (from heterogeneous soils to heavy homogeneous types), drainage designs (from random to parallel and from single to multiple, from moles to piped systems) and techniques (gaseous emissions, biogeochemical

parameters, isotopic signatures, gene abundances) in order to produce a more refined interpretation of artificial drainage systems and the role they play within the sustainable intensification framework.

As agricultural landscapes contain many different soil types with heterogeneous nitrogen (N) attenuation capacity, a zone of contribution (ZOC) surrounding a borehole and an installed drainage system was used to interpret subsurface hydro-biogeochemical functional capacity within four hydrologically isolated plots. By using the drainage system as a monitoring tool in combination with multiple techniques, a disconnectivity and complexity of the system was highlighted in terms of contamination sources uncovered and separate water attenuation functionalities. This study showed that collating isotopic, dissolved gas and biophysical data from the drainage system and groundwater locations creates a clearer conceptual model of a site showing an interpretation of source and attenuation within these areas.

Next, the study moved to five commercial farms where surface or groundwater gley soils were artificially drained (site specific designs) and monitored. This study aimed to investigate how drainage system design (e.g. shallow and groundwater) affected N transformation and how the multi-technique method could be broadened to rank commercial dairy farms in terms of their N attenuation capacity. These techniques showed the ability to divide sites into three distinct groups according to their respective water attenuation potential highlighting different sustainability for different drainage designs. A tool to compare or rank sites in terms of their N sustainability was created.

From micro-plot and field this tool was then moved to farm scale on a heterogeneous soil landscape to infer further knowledge on attenuation within drained versus un-drained areas and future management to decrease N losses. The tool was able to divide the farm into several groups with different attenuation ability which was not disrupted by the imposed artificial drainage system. The identified groups and areas could be subjected to differential management to further move towards sustainability.

The use of bacterial gene abundance was further tested as a tool to improve pour characterisation tool and lastly, an incubation experiment was conducted to examine more closely the effect of land drainage and saturation on an N problematic site and its gaseous phase component.

Major findings of the present study include:

- Techniques such as natural isotopic abundances, biogeochemical parameters, isotopomers, gaseous emissions, dissolved gasses, can be combined to elucidate sustainability of intensive dairy systems.
- Drainage systems can be used, when analysed with the above techniques, to elucidate water quality but more interestingly can be used as a monitoring tool, in combination with groundwater monitoring networks, to interpret net N source, transformation and fate, over large areas, on agricultural landscapes.
- Although surpluses of N were found to be uniform across intensive dairy sites on the present study, the soil water attenuation function and "net denitrification" varied considerably across sites. This means that there was considerable variation within dairy farms in terms of N sustainability, which will have consequences for sustainable intensification.
- Drainage systems affect this water attenuation function differently depending on their design. This means that the presence of a drainage system on agricultural landscapes does not infer poor water quality, more importantly than absence/presence is the depth and type of drainage system present.
- During this assessment the techniques used in combination with the present study worked well to characterise and rank sustainability.

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Chapter 1 - Introduction

1.1 Background

Globally, contamination of natural waters by nitrogen (N) and its reactive species from agricultural sources is a major threat to the environment (Sutton et al., 2011a). The European Union Water Framework Directive (OJEC, 2000) sets out a clear target for all water bodies, i.e. good chemical and ecological status, by certain reporting periods. No member state achieved such a target by the last reporting period in 2015. In Ireland, Food Wise 2025 (DAFM, 2015) as established by the Irish Department of Agriculture has set ambitious and equal targets for both production and achievement of sustainability for the dairy sector with milk outputs (for export) to rise in the next few years. To achieve these goals the agri-food sector is undergoing huge expansion. The dairy quota system, which placed a cap on milk production, has been abolished, and this has allowed the dairy industry to expand. A proportion of dairy farmers in Ireland are farming at intensive rates and such farmers may apply for a "derogation", which if successful, allows them to carry a higher stocking intensity than that laid out in the current Nitrates Directive (ND) (a directive put in place to reduce water contamination by nitrate). Dairy systems generally operate as an N surplus (excess of N that is either accumulated or lost) in Ireland were they tend to be relatively intensively managed (Mihailescu et al., 2014). As the efficiency of such systems are low and as intensification is occurring in different geographical areas with varied soil and climatic typologies, the inevitable fate of this surplus N is unknown. Indeed little research has been carried out, on intensive dairy farms, to characterise the N balance and then examines the N source and transformation within these systems along surface and subsurface pathway.

Presently, there is a pressure on dairy farmers to expand and take advantage of the current milk quota abolition. In some cases, farmers are buying and/or leasing land or else they are improving existing land to grow more grass. Often such land is heavy textured (high content of clay and poor permeability) and therefore in need of improvement to become profitable. Land drainage is seen as a vital component, which could maximise the potential of such land. In Ireland, several programmes are available within Teagasc (Irish Agriculture and Food Development Authority) to investigate this sub-section of farms. Within the research farm at Teagasc Johnstown Castle, a hydrologically isolated paddock facility, representing moderate to poorly drained soils, has been under-utilised in terms of N source, transformation and fate experiments. This offered a test bed in which conditions were controlled. In addition, Teagasc

has a Heavy Soils Programme and part of this programme on commercial dairy farms focused on land drainage design, installation, and knowledge transfer. A section of these farms fits the criterion just mentioned and enables a closer examination of N source, transformation and fate for the first time. To date, within Irish soils, no study has investigated the role land drainage plays in the transformation of N or indeed how it affects the fate of N, as it is an integral part of the transfer continuum.

There are now early signs of a dairy industry led examination of dairy farm sustainability. Further research is essential (and therefore this body of work) to gain a deeper understanding of N source, transformation and fate on such farms to inform future national sustainability schemes. It is therefore obvious from the outset of this body of work that the "baseline" on any intensive dairy farm for farmers and regulators and indeed the dairy industry will be to follow the ND and the Nitrates Action Plan following best management guidelines. The results of the present work aims to inform "above baseline" needs and identify where more intervention may be needed to achieve sustainability (more intervention e.g. smarter monitoring of water quality on farms with the right set of techniques to inform and guide management and where necessary implement protection measures). From the literature, it is obvious that leaks occur from agricultural systems but such losses are not equal and the role of N transformation is also important and often neglected. It should also be pointed out that this study only focuses on N whereas various contaminants (e.g. phosphorus) migrate through farming systems. The remit of the present study is therefore N migrating through surface and subsurface pathways.

In reality, this will inevitably involve a whole sector approach for now and research must provide tools that can aid in a fast determination of this sustainability within farm and catchment boundaries. The simple fact for the Irish Dairy Industry is that if dairy expansion is correlated with a loss of water quality under the EU WFD, the present derogation would come under pressure and therefore the targets pertaining to production and indeed the supply to lucrative export markets would come under pressure. On the other hand, these very lucrative export markets are asking the dairy industry to prove its sustainable credentials. Therefore, the impetus of both the dairy industry and research is to actively achieve the targets of the EU WFD. There is a need to intensify farming in a sustainable manner i.e. sustainable intensification. There is also a need to inform the dairy sector and regulators, on the complexities of the soil-subsoil-bedrock continuum and its variability to attenuate high N surpluses. To characterise such systems, a combination of techniques currently present in literature together with some new concepts must be provided. While proving water quality

sustainability through the use of decision support system tools, the industry can hope to infer positive water quality trends (e.g. nutrient management plans). However, inference versus reality will not be so straightforward due to the complexities previously mentioned.

It is interesting to note that at a national scale water status versus intensity will be monitored especially in areas that are perceived as high risk. However, at a farm scale it is not simply enough to examine N balances and concentrations at the outlet point. This simple approach does not acknowledge that soil-subsoil-bedrock has an attenuation potential, which may mitigate water quality issues even where high N surpluses exist. Therefore, sustainability at farm-scale must account for the connection between N balances, source of N, transformation of migrating N. Only then can the fate of N be determined and its likely effect on the greater environment be examined.

To combine agricultural needs and environmental requirements towards sustainable agriculture, there is a need for "Climate Smart Drainage". Through a better and smarter characterisation of soil, groundwater and drainage pathways, Climate Smart Drainage will improve our understanding of the impacts and interaction within these systems, avoiding incorrect conclusions to be made and setting the basis for a clearer intersection of both surface and subsurface pathways and design of remediation technologies. The benefits of land drainage from a grass utilisation perspective are clear, e.g. greater trafficability and extension of the grazing season (Armstrong and Garwood, 1991; Skaggs et al., 1994; Zucker and Brown, 1998; Tuohy et al., 2015). However, transfer of N along the transfer continuum occurs through distinct pathways (Mellander et al., 2014) and drainage systems have been identified as one of the main loss pathways. Drainage systems intercept infiltrating water in the soil profile and transport it quickly along with dissolved nutrients and sediment to open drains or ditches altering the chemical and ecological status of this water (Ibrahim et al., 2013). However, only specific parts of the subsurface transport nutrients in concentrations above quality thresholds (Jahangir et al., 2013a, Fenton et al., 2009) as the landscape is a mixture of high and low attenuation hotspots. Therefore, it is too simplistic to assume that all in field drainage pipes are connected to low attenuation hotspots, which in turn are transported in open drains, which lack any attenuation capacity. Difference in N-sources (e.g. organic vs. inorganic fertilizer), parallel transformational processes (e.g. nitrification, dissimilatory nitrate reduction to ammonium (DNRA), ammonification) and different N-speciation are most likely to concur in shaping these hotspots. It is essential to consider the whole system, management, water pathways and multiple N-transformational processes to comprehend hotspot distribution. Denitrification is considered a microbial process of extreme

importance for the attenuation of nitrate (NO_3^-) and for reducing NO_3^- losses before this leaches to groundwater or drainage systems (Fenton et al., 2009). During denitrification, NO_3^- is reduced to the final product di-nitrogen (N_2), via a chain of reactions (Zumft, 1997). Ecological drawbacks of denitrification arise by its multiple step nature as imperfect conditions can stop this reaction at intermediate levels causing the release of incomplete reduction products, such as the greenhouse gases nitrous oxide (N_2O) and nitric oxide (NO) (Knowles, 1982), transforming denitrification from a sink of NO_3^- to a source of air contaminants. Despite the ubiquity of denitrifiers in both soil and fresh water, denitrification requires specific environmental conditions that are primarily regulated by edaphic factors (relating to structure and composition of soil) (Hallin et al., 2009). These can be drastically affected by drainage systems, with reduction of water excess and soil moisture and increase of oxygen (O_2) concentrations at higher depth, diminishing positive outcomes of denitrification. The relationship between microbial community activity and structure, and factor affecting denitrification according to local biogeochemical conditions is still unclear (Müller and Clough, 2013) and internationally, microbial communities are still unexplored in the context of drainage characterisation. The investigation of this relationship and its bioremediation potential could lead to deeper understanding and possibly the maximisation of bioremediation capability by pushing denitrification to completeness and avoiding detrimental N leaching or emissions.

Without a better characterisation of the surrounding landscape (e.g. soil and groundwater) and the drains themselves (end-of-pipes (access point to the water within an infield drain pipe at its outlet) and open ditches) incorrect conclusions may be made about N attenuation. Agricultural systems may be leaching nutrients in excess but the landscape attenuation capacity and artificial drainage network capacity may combine to protect surface water bodies. However, where leaks do occur, these locations need to be identified and further mitigation options imposed.

A multidisciplinary characterisation, in terms of hydraulic connectivity, hydrochemistry, and isotopic analyses, is needed to understand which factors control the spatial distribution of N across agricultural landscapes (Baggs and Philippot, 2010; Bednorz et al., 2016). Gaseous emissions and physiochemical parameters give context, highlighting the presence of specific environmental factors and predicting areas of complete vs. incomplete attenuation. Isotope analyses will provide N-source identification and specific signatures of N-transformational processes and degrees of attenuation on site, from which N losses and consumption rate can be inferred. Furthermore, isotopic analyses can give information on water provenance.

Molecular techniques will improve interpretations producing a picture of the present community structure in term of common patterns, identification of microorganisms, most abundant species and activity potential. The use of this set of analyses in the context of drainage systems can lead to the creation of the concept of "Net" provenance, N source, transformation and fate where single end of pipe sample could be use to assess the origin of the water, the origin of the N, the transformation processes that have occurred from deposition to sampling point and its final outcome over the large drained area. This could eventually help us to rank farms based on sustainability and inform management.

1.2 Project information

This research was funded by the Teagasc Walsh Fellow Scheme and the Groundwater Protection and Restoration Group, Kroto Research Institute, University of Sheffield. PhD supervision at Teagasc was provided by Prof Owen Fenton and by Prof Steven F. Thornton and Dr Stephen A. Rolfe at the University of Sheffield. Advice and assistance with the isotope studies was provided by Dr Kay Knöller and Dr Naomi S. Wells at UFZ - Helmholz Centre for Environmental Research (Halle-Salle). Additional help was gained from Dr Patrick Tuohy from the Heavy Soil Program at the Moorepark - Animal and Grassland Research and Innovation Centre, Teagasc.

The PhD components were completed in seven locations:

1. Teagasc, Environmental Research Centre, Johnstown Castle, Wexford at the Beef Research Farm, specifically on the hydrologically isolated plot facility called Foals House (Chapter 3).
2. Teagasc, Environmental Research Centre, Johnstown Castle, Wexford at the Beef and Dairy Research Farm (Chapter 5 and 6)
3. Five commercial farms selected within the Heavy Soils Programme (HSP, a collaborative project between Moorepark and Johnstown Castle, Teagasc). The farms selected represent intensive dairy farms on heavy textured soils farms, which require artificial drainage to remain profitable. These are located in the south west of Ireland at the following locations:
    - Athea (Co. Limerick, Chapter 4, 6 and 7)
    - Castleisland (Co. Kerry, Chapter 4)
    - Doonbeg (Co. Clare, Chapter 4 and 6)
    - Kishkeam (Co. Cork, Chapter 4 and 6)

- Rossmore (Co. Tipperary, Chapter 4)

1.3 Dissemination of the outputs

Results from this project were presented in the following conference outputs. Underlined author was the presenting author:

1. **Oral presentation** - Beyond nitrate: developing multi-isotopic approaches to quantify the fate and transport of nitrogen within catchments. N.S. Wells, K. Knöller, E. Clagnan, O. Fenton, S.F. Thornton, S.A. Rolfe, M. Brauns. International Symposium on Isotope Hydrology: Revisiting Foundations and Exploring Frontiers – IAEA (International Atomic Energy Agency).Vienna, Austria, 11 – 15 May 2015.
2. **Oral presentation** - Nitrogen loss, source, transformation and attenuation within an intensive dairy farm in SE Ireland. O. Fenton, E. Clagnan, S.F. Thornton, S.A. Rolfe, P. Tuohy, J. Murphy, N.S. Wells, K. Knoeller. 19th Nitrogen Workshop - Sveriges Lantbruks Universitet, Skara, Sweden, 27-29 June 2016
3. **Oral presentation** - Nitrogen loss, source, transformation and attenuation on dairy farms in Ireland. O. Fenton, E. Clagnan, S.F. Thornton, S.A. Rolfe, P. Tuohy, J. Murphy, N. Wells, K. Knöller. International Drainage Symposium, University of Minnesota, Minneapolis, Minnesota, 6-9 September 2016
4. **Oral presentation** - Nitrogen loss, source, transformation and attenuation within an intensive dairy farm in SE Ireland. E. Clagnan, S.F. Thornton, S.A. Rolfe, P. Tuohy, J. Murphy, N.S. Wells, K. Knöller, O. Fenton. Groundwater Managing our Hidden Asset - Birmingham University, Birmingham, United Kingdom, 13-14 September 2016.
5. **Oral presentation** - Does drainage of poorly drained soils affect their nitrogen attenuation capacity? Evidence from six dairy farms in south Ireland. E. Clagnan, S.F. Thornton, S.A. Rolfe, P. Tuohy, J. Murphy, N.S. Wells, K. Knöller, O. Fenton. Resilience Emerging from Scarcity and Abundance - International Annual Meeting of the American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America. Phoenix, Arizona, 6-9 November 2016.

1.4 Publications

Chapter 3, 4 and 5 have been accepted/submitted as the following manuscripts for publication in peer-reviewed journals:

1. Investigating "Net" provenance, nitrogen source, transformation and fate within hydrologically isolated grassland plots. Clagnan, E., Thornton, S.F., Rolfe, S.A., Wells, N.S., Knoeller, K., Fenton, O., 2018. Agricultural Water Management, 203, 1-8.
2. Influence of artificial drainage system design on the nitrogen attenuation potential of gley soils: Evidence from hydrochemical and isotope studies under field-scale conditions. Clagnan, E., Thornton, S.F., Rolfe, S.A., Tuohy, P., Peyton, D., Wells, N.S., Fenton, O., 2018. Environmental Management, 206, 1028-1038.
3. An assessment of nitrogen source, transformation and fate within an intensive dairy system. Clagnan, E., Thornton, S.F., Rolfe, S.A., Wells, N.S., Knöller, K., Murphy, J., Tuohy, P., Fenton, O. Submitted for publication to the Journal of Agriculture, Ecosystems and Environment, August 2017. Reviewed and must be resubmitted.

1.5 Thesis aim and objectives

The aim of this thesis was to examine the "sustainable intensification" concept in terms of N loss to water on dairy farms that needed land drainage to achieve profitability targets. To achieve this aim many different water sampling locations (end-of-pipe, open ditch and groundwater) were investigated along surface and subsurface pathways. Within this thesis, multiple techniques (physiochemical parameters, stable isotope, gas, and molecular analyses) together with N balance, N surplus and N release data, were used to investigate N source, transformation and fate across multiple soil and drainage scenarios. The specific objectives and rationale of the research are presented in relation to different studies undertaken, described below.

Study 1 - Investigating "Net" provenance, N source, transformation and fate within hydrologically isolated grassland plots

In this study, water samples were taken from end-of-pipe and shallow piezometer locations across four hydrologically isolated grassland plots in the South East of Ireland. The selection of these isolated plots was selected as first study as it enables us to have a "controlled" environment in the sense that all the N inputs and transformational processes occurring in the top layers were necessarily influenced by only what is occurring on the top of these plots and not from upstream conditions. The four plots were further characterised by the same drainage design (piped and herringbone). The isolation and the similarity created the perfect condition to test on a small scale the efficiency of the use the multi techniques approach. The specific objectives of this study were to:

- Characterise N balance and surpluses within a closed (hydrologically isolated) system.
- Characterise N loss to water within the closed system using nutrient, biogeochemical and dissolved gas data.
- Characterise isotopic signatures of H_2O and NO_3^--N to elucidate provenance of water, source of N, the transformational processes and the fate of N on this multi-layered site.
- Examine the use of end-of-pipe samples as "net" provenance, source and transformation indicators for an estimated zone of contribution.
- Develop a conceptual diagram of the system to inform sustainability

Study 2- Influence of artificial drainage system design on the nitrogen attenuation potential of gley soils: Evidence from hydrochemical and isotope studies under field-scale conditions

In this study, we aimed to use the concept of study 1 (drainage system used as monitoring tool with a multi technique approach) to understand the relationship between artificial land drainage of surface and groundwater gley soils, net denitrification and water quality. From a hydrologically isolated environment we moved to a real environment. Five heavy soils farms, often neglected in Irish studies, were selected for their essential need for drainage systems for production purposes. From these farms, five plots, further characterised by soil homogeneity within each plot and five different drainage designs, were selected. This allowed further development of the concept of "net" provenance of water, source of N, transformational processes and fate of N. Specifically this allowed us to examine shallow and groundwater drainage designs and to group five commercial farms in terms of N surplus and release and "net denitrification" and comment on their respective sustainability. The objectives of the present study utilising end-of-pipe, open ditch and shallow groundwater sampling points across five sites in the southwest of Ireland were to:

- Examine N balance including surplus and release from top and subsoil, N source-transformation and fate.
- Develop a conceptual diagram of these sites and others from the literature in the context of drainage design and "net denitrification" capacity.

Study 3 - Sustainable drainage systems: gaseous and isotopic insights into the spatial and temporal variation of N on an intensive heterogeneous farm

The concept of net denitrification as a tool to "rank" sustainability and functionality of study 2 was expanded to multiple drainage classes and to farm scale: an intensive dairy farm in SW

Ireland characterised by a mosaic of soil type and an artificial drainage system designed to drain specific problematic areas (imperfectly to poorly drained soil types). The extensive drainage system on this farm was composed of an interlinked open ditch and an in-field (plastic and concrete) groundwater drainage system was mapped and sampling points were allocated. These sampling points were complemented with a vast number (38) of existing surface water and multi-level groundwater points. The sustainability of the farm was examined using the tools developed in previous chapters. The knowledge on open ditches was further improved, and a wider set of isotopic analyses was carried out. Specifically, the objectives were to:

- Deduce the farm N balance including N surplus.
- Determine the spatial and temporal variation in aqueous N-species
- Identify the provenance of water samples across the surface and subsurface monitoring network
- Determine the spatial distribution of N source and transformation
- Develop a conceptual diagram of the site to inform N fate, sustainability and future management of the system.

<u>Study 4- Further insights into N transformation processes within intensive dairy farms using bacterial gene assessment</u>

In this study an additional tool was investigated to inform sustainability and was intended to test the ability of gene abundance to improve the depth of the results for study 2 and 3 along the groundwater, end-of-pipe and open ditches continuum Here bacterial N-cycle genes in water samples from open ditch, end-of-pipe and groundwater locations were investigated to further interpret N transformation processes across locations at four sites (three heavy soil programme farms and the dairy research farm at Teagasc Johnstown Castle, Wexford).

The objectives of the present study were to:

- Examine bacterial genes involved in the N cycle using water samples taken from open ditch, end-of-pipe and groundwater locations across three HSP farms (Study 2) and the Johnstown Castle Dairy farm (Study 3). The following genes were examined: i.e. *16S rRNA* for total quantification, four bacterial denitrification genes (*nirS, nirK, nosZ1* and n*osZ2*), one for nitrification (*amoA*), one for anammox (*hzo* cluster 1) and one for DNRA (*nrfA*).

- Assess if bacterial gene abundance across these locations adds to an overall interpretation of sustainability when combined with isotope natural abundances, dissolved gases and biogeochemical parameters.

Study 5- Investigation of drained and undrained intact soil cores to examine the fate of N

Study 5 further develops from study 2, here a farm was selected in order to explore more in depth the relation between nitrification and denitrification and their influence on the gaseous phase. Here, intact soil cores to 0.5 m from an individual farm were extracted. This farm was chosen as it exhibited elevated NH_4^+ concentrations in open ditch and shallow groundwater locations. This farm therefore was interesting from an N transformation perspective and presented an opportunity to examine surface emissions as NO_3^- was being converted to NH_4^+. Cores were subjected to different saturation treatments (i.e. high (80%) and low (55%)) representing undrained and artificially drained conditions. Two fertilisers consisted of differently labelled ammonium nitrate ($^{15}NH_4{}^{15}NO_3$ and $^{14}NH_4{}^{15}NO_3$) (50% atom enrichment) and a third control consisted of non-labelled ammonium nitrate ($^{14}NH_4{}^{14}NO_3$) were then used and gaseous N losses were examined before and after application.

Specific objectives were to:

- Assess differences in N_2 and N_2O surface emissions across imposed treatments. It was hypothesised that undrained conditions (i.e. saturated conditions with high water filled pore space (WFPS)) mitigated N_2O fluxes from soil in favour of N_2.
- Examine N labelling and N_2O isotopomers to trace the fate of N and differences in transformational processes
- Investigate the microbial community and the impact of the two saturation contents on bacterial community by the analyses of *16S RNA*, *nirS*, *nirK*, *nosZ1*, *nosZ2*, *amoA*, *hzo1* and *nrfA* gene abundances.

1.6 Thesis structure

The thesis is composed of eight chapters (Fig. 1.1). This introduction is followed by Chapter 2, a literature review of global and Irish agriculture and dairy systems, which introduces the concepts of sustainability and soil functional management. This is followed by a description of soil type, soil drainage classes, drainage system design and investigation in terms of N source, transformation and fate. Chapters 3-7 contain the five experimental chapters based around the objectives of this research. Specifically, Chapter 3 examines N sustainability

within a closed system (paddock ~ 0.2 ha) and introduces the concept of "net" provenance-source-transformation using end-of-pipe monitoring locations. Chapter 4 builds on such concepts further across five HSP commercial dairy farm sites (~2 ha) that have drainage sites on groundwater and surface water gley soils. Chapter 5 assesses N sustainability on the Johnstown Castle dairy research farm (~130 ha), characterised by variable drainage class with only deep groundwater drainage system designs installed. Chapter 6 takes another look at four of the HSP farms, examining bacterial genes in water samples and their role in the conceptual model of sustainability for these farms. Finally, Chapter 7 examines a single HSP farm in greater detail, through an intact core experiment with labelled fertiliser, in which two WFPS targets are imposed representing undrained and drained conditions. The fate of N and bacterial genes in soil are examined. Chapter 8 provides a synthesis of the research findings with conclusions and suggestions for future work.

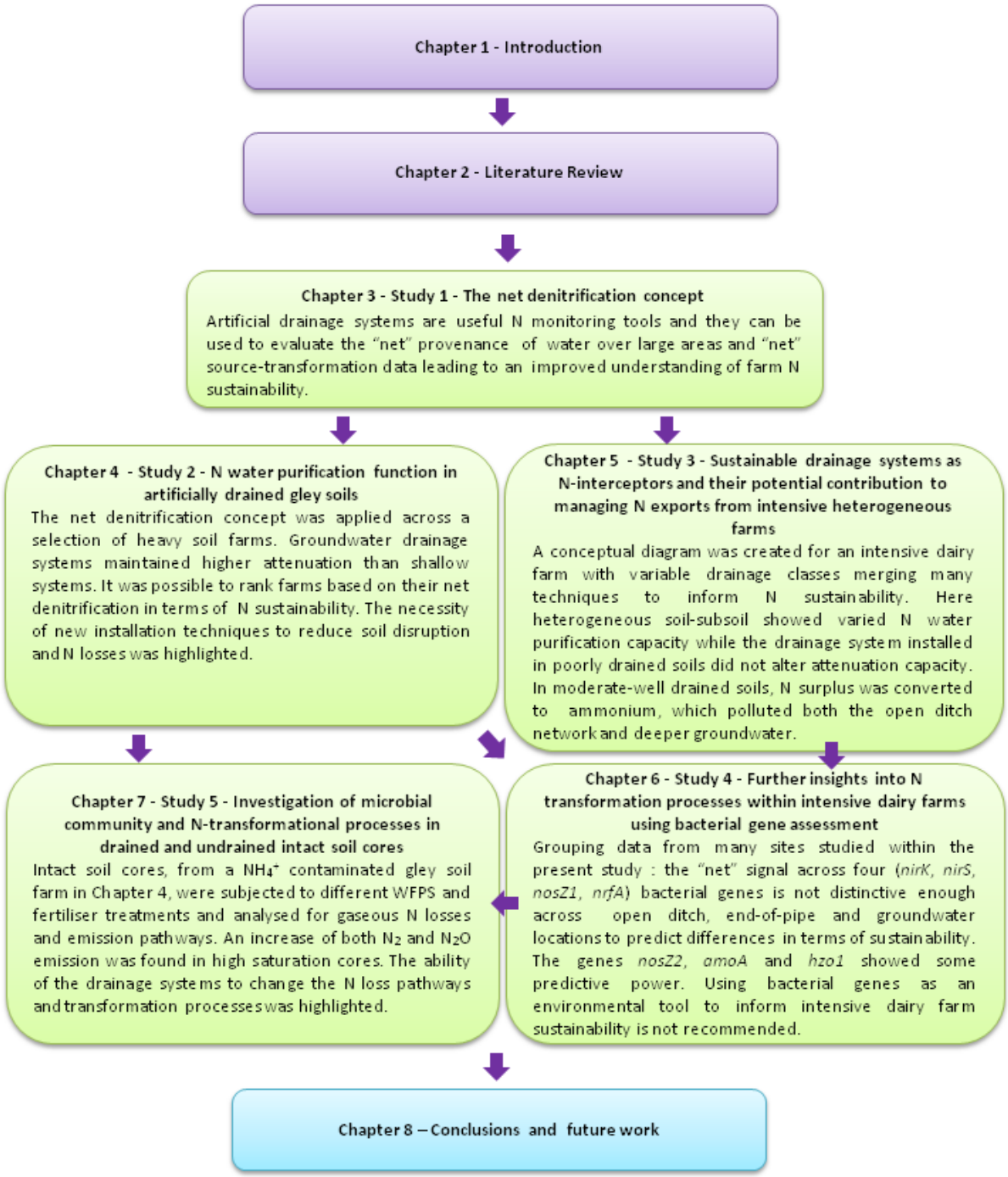


Fig. 1.1. Schematic representation of this thesis structure with highlights for each experimental chapter (WFPS, water filled pore space).

Chapter 2 – Literature Review

2.1 Overview

This chapter begins with an overview of global agriculture and introduces the concepts of sustainability and soil functional management. It refers specifically to sustainable intensification, soil type, water attenuation capacity of such soils and surface versus subsurface pathways of nitrogen (N) loss within dairying systems. As all fieldwork within the current study was conducted on intensive dairy farms (split amongst Teagasc research and commercial farms), a section relating to Irish agriculture (past, present and future) and Irish water quality is presented. This sub-section identifies a conflict between national production targets and European Union (EU) Directive environmental goals. Next, soil type and drainage classes are presented in terms of the expanding dairy sector. Considering the land that will be subjected to intensification, at one end of the scale, it will occur on high permeability free draining soils in need of no further intervention in terms of drainage installation. At the other end of the scale, intensification will occur on high-clay low-permeability heavy textured soils (i.e. 33% of all Irish dairy farms). These soils will be artificially drained to some degree to allow for better utilisation and growth of grass. In the middle, intensification will occur on highly complex soil-scapes with all drainage classes present and artificial *ad hoc* drainage systems installed (typically with no maps available to indicate their location or installation depths).

Both positive and negative aspects of drainage systems are then explored and an examination of drainage system design in Ireland is conducted. Next the N cycle is examined and especially techniques to elucidate N source, transformation and fate. Furthermore, the role of land drainage and its ability to alter the transformational processes within the soil profile is examined.

2.2 Global megatrends and drivers of change within agriculture

Global food demand is expected to increase by 100% by 2050 (Tilman et al., 2002; Godfray et al., 2010). The need for higher yield through an increased efficiency (better agricultural management and fertiliser use) to sustain a growing population and the change of dietary preferences and to face the increased incidence of extreme water events has fuelled concerns with respect to the protection of global ecosystems. For example, there is a fear that

achievement of worldwide production targets will be at the expense of water and air quality targets (Mosier et al., 1998; Foster, 2000; Lesschen et al., 2011).

Soil health and quality are important for any agricultural system, and any particular soil has an inherent ability to support certain functions. The concept of functional land management (Schulte et al. 2014) identifies that there is "a societal expectation that the agricultural sector increase productivity, and at the same time provide environmental 'ecosystem services' such as clean water, air, habitats for biodiversity, recycling of nutrients and mitigation against climate change". Different soils have different levels of efficiency in carrying out different soil functions, and certain farming practices should be targeted where more soil functions can thrive. Therefore, on agricultural landscapes, anything that alters soil function must be characterised thoroughly and a balance found across soil functions. For example, artificial land drainage increases the production function of some poorly drained soils but may be detrimental to the water attenuation function and carbon sequestration of that soil (O'Sullivan et al., 2015).

The concept of sustainability seeks to achieve increased production and meet environmental targets. This concept has two main pathways: 1) sustainable extensification and 2) sustainable intensification (Tilman et al., 2011). The problem with extensification is that it requires the exploitation of new land (expansion) for agricultural purposes. This would involve bringing land into production that could be of poor quality in terms of some soil functions e.g. production. This conversion would also reduce this soils capacity to conduct other soil functions e.g. carbon sequestration (Schulte et al., 2014). Conversely, intensification utilises the existing land bank already in production and aims to enhance production by improving management practices and functional land management, such as enhanced fertiliser spreading according to field requirements and optimised water use. Intensification has been therefore often considered the best pathway to achieve higher agricultural production (Tilman et al., 2002; Schulte et al., 2014). However, it is essential to couple intensification with sustainability to improve or maintain water quality and reduce greenhouse gas emissions, moving toward land use that optimises soil functions (Schulte et al., 2014). The application of intensification, when compared to a non intensive agriculture, will lead to an increase in economic sustainability, a deteriorating of water quality and possibly of biodiversity while maintaining the same impact on greenhouse gas emission intensity and nutrient recycling. On the other hand, extension is expected to lead to an improvement in economic sustainability and nutrient recycling while producing a negative effect on greenhouse gas emission intensity and biodiversity (neutral for water quality) (Schulte et al., 2014). Therefore, there is a case for

intensification but as documented by Schulte et al. (2014), when not sustainable, water quality will be at risk. This means that the sustainable intensification concept must be explored across different soil types with varied water attenuation potentials. The role of artificial drainage and how it affects this water attenuation function (and indeed, how to measure this potential) is important and a current knowledge gap.

2.3 Ireland as a case of study

A case in point to investigate the concept of sustainable intensification where land drainage is a vital component to increase productivity is the Ireland scenario. Irish agriculture has an expanding dairy sector with ambitious national targets for milk outputs. The agri-food sector is calculated to have 140,000 enterprises on the national territory, from farmhouse producers to large multinationals, and with 5.4% of companies accounting for 41% of employment. These enterprises export their products to more than 160 countries (10% of total Irish merchandise exports) accounting for 5.7% of the gross domestic product and with an export growth of 2.2% only in 2016 (Teagasc, 2016). In this context, 0.6% of the turnover is reused for research and development (EPA, 2016).

2.3.1 The Irish dairy sector

Approximately 81% (3.69 million ha) of Irish agricultural land is devoted to grass (silage, hay and pasture), 9% (0.45 million ha) to grazing, and the circa 9% (0.39 million ha) given to crop production (Teagasc, 2016). Nationally, approximately 18,000 (11.2%) farms are dairy farms, which produce 5.4 billion litres annually (IFA, 2017). In 2016, total milk output was estimated at 7.5 billion litres, with a decline of 1.6% on the previous year (Teagasc, 2016).

The dairy farming sector is based on the increasing the conversion of grass into animal milk and milk based products. To increase this conversion, animals are genetically selected to provide higher milk production with fewer grass inputs. This genetic selection is coupled with constant research towards more efficient management of farming practices (EPA, 2016).

On average, Irish dairy farms utilise 7.1 t of grass (dry matter) per hectare (Creighton et al., 2011), where cows graze for an average of 280 days a year, with stocking rates of over 3 cows per hectare (Shalloo et al., 2011). In other European, and world, countries emissions are expected to increase for intensive farms. European areas of high N input have been identified within the north-western countries, but also Denmark, Belgium, the Netherlands, UK, Germany and France, with highest inputs and consequently losses in correspondence of highest livestock density (e.g. areas of France, Italy, Denmark, Belgium and the Netherlands)

(Bos et al., 2013). Grazing is following a decreasing trend both in (e.g. Ireland: 2010 – 99%, 2014 – 98%, Denmark: 2001 – 84%, 2014 – 30%; France: 2011 – 95%, 2014 – 90%) and outside (e.g. New Zealand) Europe (van den Pol-van Dasselaar et al., 2015). Farm variation will depend on numerous factors (e.g. soil type and fertility, geographical position, hydrogeology, management and grass type) (Brereton, 1995, Shalloo et al., 2011). Where soil drainage class is dominated by imperfectly or poorly drained soils, artificial land drainage is often an expensive, but necessary, solution to increase production.

A national study of Irish dairy farms from 2008 to 2015 showed that efficiency in the rate of exchange between grass and milk has been constantly improving (productivity up of 29%), due to a higher number of cows and increased farm sizes (Hanrahan et al., 2017). However, fertiliser and feed usage have remained constant. With the abolition of EU milk quotas in 2015, this improvement in fertiliser usage (higher efficiency) is essential in order to sustain growth without producing a negative environmental impact, in line with EU legislation. Following this, from 2006 to 2012 Irish farms showed a decrease in P and N losses to soil and water, due to better fertiliser use inputs and higher content of milk proteins (higher N and P use efficiency) (Buckley et al., 2016).

2.3.2 Future prospects of the dairy sector

The Irish dairy sector is expected to improve further. Dairy farms are anticipated to increase. Dairy cow numbers are estimated to increase at over 100 cows per farm (current herd average size: 93 (Treacy et al., 2008)). This will produce a significant growth in the production of milk with an improved formula (expected: 3.56% protein and 4.25% butterfat), through an improved genetic pool of the herd and comparative advantage of having production based on grass and direct grazing. Future targets are shown in Table 2.1 (Teagasc, 2016). However, it is expected that sustaining this growth will require more land, which will necessitate land drainage of existing land area on those soil drainage classes that impede water infiltration e.g. surface and groundwater gleys. Therefore, less nutrient losses and reduced greenhouse gas emissions must be ensured and ecosystem biodiversity must be preserved. In this context, a list of required action points has been created. These include improvement in the efficiency of fertilizer use coupled with management optimisation, ameliorated soil fertility, increase in the use of low emissions technologies, implementation of ecological measures to improve/protect biodiversity, and reduction in point source losses in terms of nutrient release to water bodies (Teagasc, 2016).

Table 2.1. Technical performance for Irish manufacturing milk production herds (Teagasc, 2016)

	Current (av. 2013-2015)	2025	Research target
Milk Delivered (kg/cow)	5,036	5,739	5,800
Milk Solids (kg fat plus protein/cow)	372	448	475
Calving Interval (days)	394	385	365
Herd Economic Breeding Index (€)	55	180	230
Labour Input (hours/cow/year)	30	22	<16
Stocking Rate (LU/ha)	1.96	2.15	2.94
Herbage Utilised (t DM/ha)	7.36	10.0	12.7
Concentrate per Cow (kg)	1,008	750	400
GHG (kg CO_{2e}/kg m^2)	1.10	0.97	0.83
N Efficiency (%)	25.2	26.4	33.2
N Fertiliser Applied (kg/ha)	176	230	250

2.3.3 Irish agriculture and water quality: challenges

Although Irish water quality status is among the best in Europe, further measures must be taken to improve quality to a satisfactory level and protect it (EPA, 2016). There has been no improvement in water quality, with a gradual decline of high quality water bodies over the past six years (EPA, 2016). Irish rivers have shown a high loss in quality; only 21 (0.7%) sites were classified as high quality between 2013 and 2015 compared to 82 sites between 2001 and 2003. Lakes showed an increase of 3% within the moderate to worse quality category which already comprehend 54% of the monitored sites between 2007 and 2009. Only 1% of groundwater bodies showed poor status (EPA, 2016). Most contaminated areas were found in south and south-east Ireland with sources of this contamination identified within agriculture. Here, free draining soils dominate, showing concerning levels of nitrate (NO_3^-) in all types of water bodies but especially groundwater (above 10 mg N/l). A correct N use and the protection of water quality from its contamination represent one of the main challenges towards sustainability. Sources of N in agricultural systems are generally anthropogenic and heavily dependent on external addition of fertiliser (Van Grinsven et al., 2012). On heavier soils in the south-west or mixed drainage landscapes in the south and south-east, loss pathways tend to be surface driven unless such soils are artificially drained, thereby introducing infiltration capacity into these soils and preferential flow pathways for nutrient loss.

Cows are responsible for 62% and 64% of emissions of nitrous oxide (N_2O) and ammonia (NH_3) respectively (Galloway et al., 2003; Steinfeld, 2006), from the intensification perspective, the increase in stocking rates will lead to both an increase in GHG emissions and

higher N losses in water pathways due to the greater production of urine, slurry and dairy-soiled water (Selbie et al., 2015, Necpalova et al., 2012). Inefficient use of N will most likely compromise the natural N balance, leading to contamination wherever soil attenuation capacity (or water attenuation function) does not support complete bioremediation. Soil attenuation capacity is the natural ability that soils have to bioremediate contaminants and to reduce contamination. Numerous negative consequences arise from the permanently high N surpluses, such as health (e.g. methemoglobinemia and nitrosamines cancer) and environmental issues (especially eutrophication) (Smil, 1999). A widespread inefficient N use raises worries concerning the achievement of sustainable intensification for Ireland.

In order to push production the dairy sector has been expanded due to the abolition of EU milk quotas in 2015. This has enabled farmers to farm at higher stocking rates and to apply manure above 170 kg/ha, up to 250 kg/ha per year, but subject to certain conditions which comply with the Good Agricultural Practice under the application of Nitrates Derogation (DAFM, 2017). It is important to look at the exact figures and place them in context of the type of soil and drainage classes (see Section 2.4) and therefore the need for land drainage to utilise the extra grass required to carry extra cattle - 7,000 farmers availed in 2016 for derogation (exemption/relaxation from agricultural restrictions e.g. milk quotas). These farmers are predominantly located in the south-west and south-east of Ireland (the current study areas of this project), which is the same geographical area where soil has variable soil drainage classes dominated by imperfectly or poorly drained soils. This means that there is widespread installation of artificial drainage systems in such areas, but no study has characterised their effect on the achievement of sustainable intensification through achievement of good water quality. The water attenuation potential of these soils must be established, as well as the effect of different land drainage designs on this water attenuation function. Currently studies that utilise novel techniques to assess water origin within agricultural (e.g. Xue et al., 2009; Xue et al., 2013; Yan et al., 2014; Yue et al., 2014) or urban landscapes, which N species migrate through these systems, and the transformation and fate of these N species once lost to the open ditch network, are understudied in land drainage networks and the surrounding connected soil/subsoil and groundwater. Indeed some aspects are considered in the literature but results are site specific (Ibrahim et al., 2013).

The consequences of failing to meet the objectives of the EU WFD (2000/60/EC), while producing more milk, could be the loss of the present derogation. This has been illustrated by recent EU decisions. In Denmark, derogations have already been revoked (2016/2017) for not

complying with water quality standards and cuts to cow numbers (~200,000) have been applied by the Netherlands to avoid loss of a derogation.

The achievement of good status for all water bodies, as required by the EU WFD (2000/60/EC), and applied in Ireland through the third Nitrates Action Program (NAP3) of 2014, aims to protect and improve the quality of surface and groundwater, and takes precedence over national production strategies. In Ireland, the first nitrate directive national action programme came into operation in 2006, with further updates in 2009, 2010 and 2014. The NAP3 became effective with the application of a set of Nitrates Regulations, e.g. the European Communities (Good Agricultural Practice for Protection of Waters) Regulations 2009 (S.I. 101/2009). These regulations could put strict limits on production targets if positive water quality trends are not found. Additionally, in 2006 with the creation of the Groundwater Regulations, a further step was made with the inclusion of groundwater bodies within the protection and monitoring scheme (2006/118/EC). Surface water and groundwater drinking water targets for good water quality are set at 11.3 mg N/l, 0.15 mg N/l and 0.23 mg N/l for nitrate (NO_3^--N), nitrite (NO_2^--N) and ammonium (NH_4^+-N), respectively (WHO, 2008; EU, 2014a). To restrict N losses and keep their concentrations in waters below targets, a further set of Good Agricultural Practices regulations was created, with the aim of limiting contamination derived from agriculture by control of fertiliser inputs (i.e. load, timings storage and use efficiency) (Schulte et al., 2014; EU, 2014b).

2.4 Soil Drainage and artificial drainage systems employed in Ireland

Ireland is subjected to high rainfall due to its location in North Atlantic Europe (Met Eireann 2012; Fig 2.1). With managed grassland accounting for 3,178,046 ha of national land, dairy farms are spread across Ireland and on differently drained types of soils (Table 2.2; Fig 2.1). Most dairy farms are characterised by suboptimal condition for grass growth due to the high rainfall and poorly drained soil type.

It is estimated that over 33% of milk production in Ireland originates on heavy soils (Humphreys et al., 2011). To sustain population growth and higher production, intensification and the installation of artificial drainage systems on surface and groundwater gleys is vital (Fig 2.2, Fig 2.3). Gley soils are poorly drained soils that, unless drained, are saturated and waterlogged for long periods. Gleys soils are divided into two main groups: surface and groundwater. Surface water gleys are characterised by an impermeable layer (high clay content) more than 40 cm below the mineral layer that does not allow vertical water permeation (from top to underneath layers) causing stagnation of rain water and

waterlogging. On the other hand, groundwater gleys are characterised by an impermeable layer located above lower permeable layers that enable the rise (from bottom to top layer) of groundwater causing constant high watertable (Fig. 2.2). Artificial drainage systems on these gley soils are essential to avoid waterlogging, caused by a shallow watertable, due to low soil permeability and fine texture, which, combined with high rainfall events and low evapotranspiration rate (Armstrong and Garwood, 1991), leads to in management difficulties and low yields (Galvin, 1983).

Table 2.2. Drainage category across pasture farming in Ireland. Values are in ha (O'Sullivan et al., 2015).

Drainage category	Drainage characteristics	Managed grass	Other grass
Peat	- Organic layer higher than 40 cm	236,938	456,646
Poor	- Mottling present throughout the profile - Stagnation due to: a) Argic horizon: very high clay content in a layer compared with the one of an overlying layer b) Spodic horizon: high clay content layer moved by rainwater to deeper layers	797,567	87,663
Imperfectly	- Mottling 40-80 cm depth - Presence of some organic matter accumulation - Argic or spodic horizon present	157,985	44,611
Moderately	- Mottling at 40-80 cm depth - Lack of any organic matter accumulation - An argic or spodic horizon may be present.	661,375	42,447
Well	- No evidence of water-logging - No argic or spodic horizon present.	1,287,372	93,010
Excessively	- Sandy loam or sandy textural classes is dominant	10,499	803
Other		26,310	60,086
Grand total		3,178,046	785,265

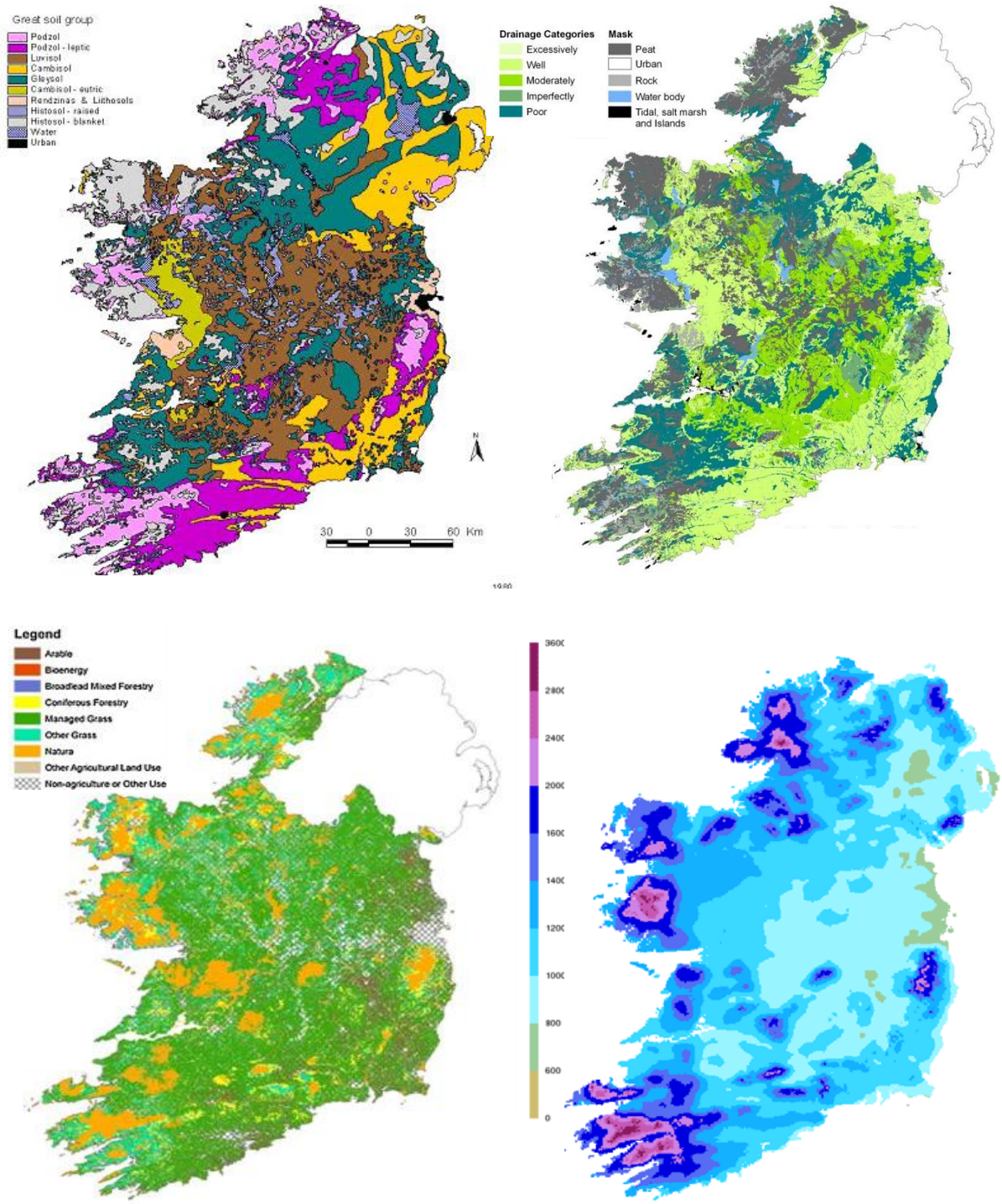


Fig. 2.1. Great Irish soils group map (top left) (Gardiner and Radford, 1980), drainage map (top right) (Schulte et al., 2014), indicative land use map of Ireland (bottom left) (O'Sullivan et al., 2015), mean annual rainfall 1981-2010 (bottom right) (Met Eireann, 2012).

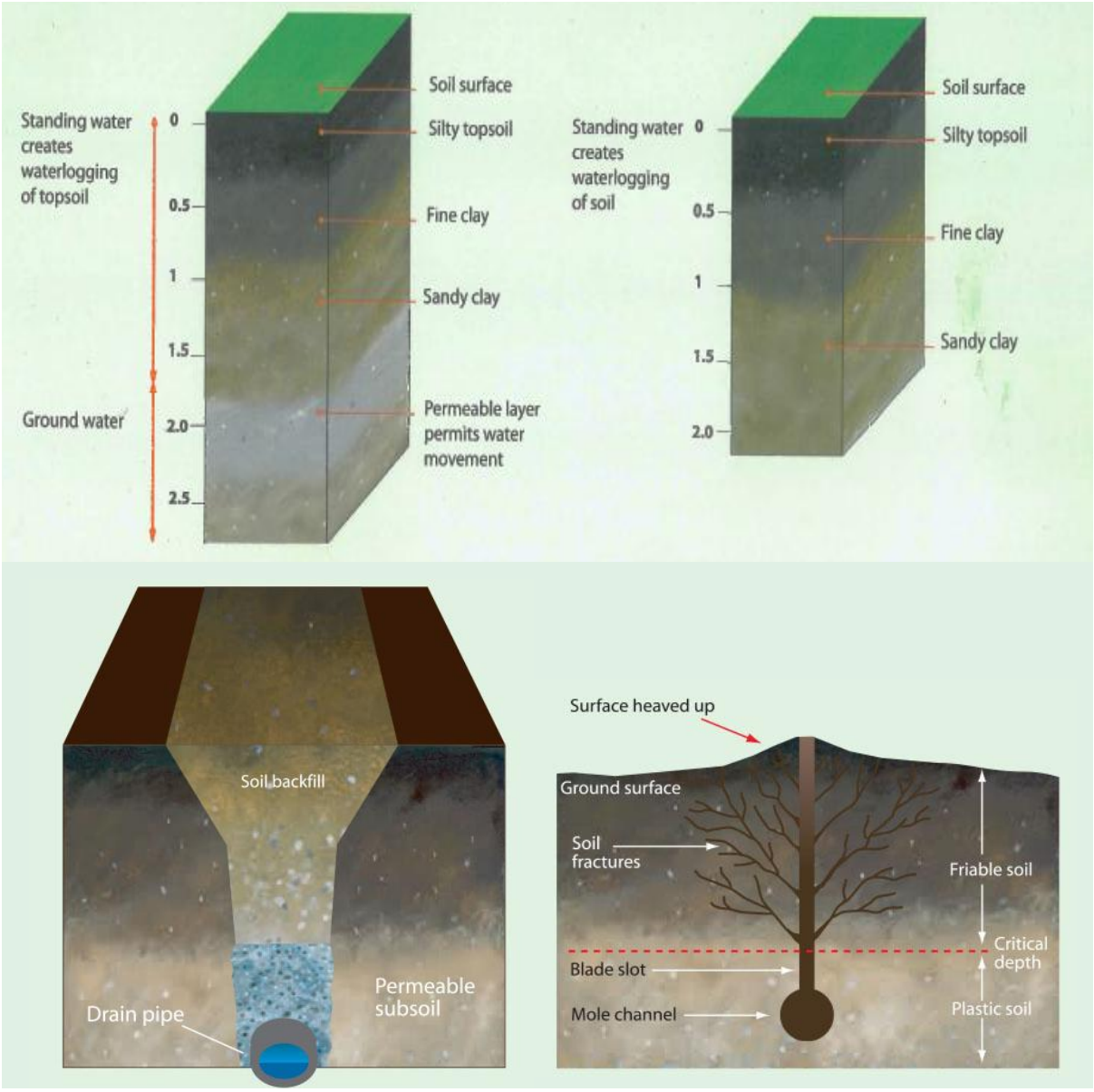


Fig. 2.2. A groundwater gley (permeable layer) (top left) and a surface water gley (no permeable layer) (top right) with relative example of artificial drainage for water removal of groundwater (bottom left) and shallow water (mole drainage) (bottom right). Fine clay and sandy clay are impermeable layers. Units are in meters) (Teagasc, 2013).

Fig. 2.3. Typical surface water gley landscape – Kishkeam Co. Cork, 52°20´, 09°13´ (top left), and cross section with impermeable layer below 40 cm (top right).Groundwater water gley landscape – Doonbeg Co. Clare, 52°43´, 09°29´ (bottom left), and cross section with impermeable layer between 30-60 cm (bottom right).

Drainage systems are generally installed to accommodate local features, as each soil type and field are characterised by specific groundwater drainage requirement, presenting therefore different designs (e.g. random (ad hoc for localised problems and heterogenic soils), herringbone or parallel) (Ritzema et al., 1996; Teagasc et al., 2013). Drainage system are generally composed of three elements: an in-field drainage system collecting excess water and regulating the water table, a secondary system of collector drains and canals conveying and transferring excess water from the field to the outside of the farm, and the outlet where the water is released into a river, lake or sea.

An in-field drainage system (with an end-of-pipe water sampling location) is divided into two main types: groundwater or shallow drainage systems (Ritzema et al., 1996) (Fig. 2.2). Groundwater systems are installed where soil texture is not impeding water vertical flow. In this drainage type drains need to be installed within or close to a permeable layer which will

determine depth and spacing of the drains (Mulqueen and Gleeson, 1982; Galvin, 1978). The shallow systems differ from the groundwater systems in that they remove water from the upper part of an impermeable soil by rupturing the soil and creating preferential flow pathways for the water. Due to their structure, surface systems respond quickly to rainfall events, removing very rapidly and efficiently the water from the surface (Leeds-Harrison et al., 1982). Shallow water systems are of three main types: pipes (same for groundwater but at lower depth, closer to the surface), moles and gravel moles. Shallow water systems can be obtained with three main designs/techniques: moles, gravel moles and sub-soiling. Mole typologies have been designed for the drainage of heavy soils with saturated hydraulic conductivity less than 0.01 m/day (high clay content, >40%). Mole drains are ploughed circular channels, very economic but with a very short life. Flow occurs through leg slots and cracks of the soil or through the soil between moles (Cavelaars et al, 1994). Due to their structure, mole drains respond quickly to rainfall events, removing water very rapidly and efficiently from the surface. If soils are not suitable for installation of a mole they can be filled with gravel or stone (gravel mole) as support to avoid collapse (Mulqueen, 1985). Sub-soiling is a technique aiming at loosening and fissuring heavy soils to improve soil aeration and water flow (Galvin, 1983).

2.4.1 Artificial drainage: advantages and disadvantages

In suboptimal drainage conditions, the installation of an adequate drainage system is an efficient instrument to control water in agricultural lands, permitting the removal of both excess surface and subsurface water. This prevents waterlogging of fields and resulting problems concerning the presence of excess water (e.g. reduced crop growth, reduced grazing periods, poor trafficability). Lowering the water table through a drainage system and the creation of a well-drained soil helps improve or maintain soil fertility, reduce compaction, thereby improving the micro-fauna and increasing the rate of crop production (Ritzema et al., 1996). Additionally, the installation of an artificial drainage system will reduce run off by providing higher water storage and by conveying excess water outside the farm, this will have beneficial effect on water quality due to reduced sediment and nutrient losses (Skaggs et al., 1994). As a consequence, fertiliser can be used more efficiently, avoiding overloading (Ritzema et al., 1996; Zucker and Brown, 1998). Production on these types of soil, poorly suitable for agriculture, is in fact often improved by the installation of artificial drainage systems, even though multiple negative effects of drain installation have been found in the literature.

Within catchments and agricultural lands, mixed contaminants along the transfer continuum (from source to delivery end (i.e. receptor)) migrate along distinct pathways (overland flow, interflow, artificial drainage systems, shallow and deep groundwater). Drainage systems have been identified as one of the main nutrient loss pathways, as they provide connectivity between many of the above pathways and discharge directly into surface water bodies (Mellander et al., 2014). They are responsible for the alteration of overland flow/infiltration dynamics (Ibrahim et al., 2013), soil hydrology and physicochemical properties, and can decrease soil bioremediation (e.g. saturated conductivity has been correlated with denitrification) (Skaggs et al., 1994; Blann et al., 2009; Fenton et al., 2009; Jahangir et al., 2013a). As a consequence, water quality (i.e. biological, chemical and physical feature) is the main concern and could be used as an indicator of the sustainability of a drainage system.

2.4.2 Drainage systems and nitrogen

Mobilised N is transported from agricultural fields to receiving water bodies through several pathways. These pathways can occur on the surface, an overland flow with transfer at delivery points through runoff) or on the subsurface, 1) lateral flow to the open ditch network through artificial drainage networks or highly permeable layers, 2) recharge to shallow or deeper groundwater with or without interaction after a time lag period with surface water. Depending on the quality and volume of this water, the receiving water body may be affected in terms of achievement of maximum admissible concentrations, as set out in EU legislation (Turunen et al., 2013). Tedd et al. (2014) suggested that water pathways and the governing parameters of the contribution zone are key elements when trying to understand N losses from drainage systems, their transformation and spatial distribution. Drainage systems are commonly known to cause higher N-losses than non-drained conditions (Skaggs et al. 1994). There needs to be research on grass-based farmland where soil drainage classes can be highly variable and drainage system layout and design can be quite varied. Indeed no such study has been attempted in Ireland, which covers all drainage installation types and includes both in-field drains, open ditch networks and a system of multilevel monitoring wells.

Facilitating water removal at a higher rate, drainage systems increase the connectivity from shallow groundwater to nearby receiving waterbodies (Doppler et al., 2012). This increases the amount of nutrients that may bypass the soil-subsoil water attenuation function or attenuation capacity, thereby leading to deterioration in water status. Kladivko et al. (2004) identified drainage systems as an efficient tool to evaluate N losses from an agricultural system and their efficiency in N use. That study and others highlighted appropriate drain

spacing to achieve production targets whilst still achieving the retention time needed to promote the water attenuation function of the soil being drained. However, on landscapes with varied soil types (and hence varied soil drainage classes often within the same paddock) drain spacing cannot be uniform and drainage design often shifts from optimal. Results highlighted the necessity of research on different soil types and climatic areas, to develop appropriate management strategies for sustainable intensification in the context of drainage systems.

2.5 The N cycle

In order to understand the migration of reactive nitrogen within intensive dairy systems, the N cycle is presented along with factors that affect different stages of the cycle. In addition, the techniques utilised within literature to study N cycle processes are examined. These techniques could be used in combination to inform the concept of sustainable intensification at a dairy farm site.

The N cycle is the essential group of reductive and oxidative transformational processes that controls the distribution of N compounds in the global ecosystem and is highly influenced by the water continuum (Cabello et al., 2004). Nitrogen is an essential element supporting life. However, despite its abundance, it is often found as di-nitrogen (N_2), which is not available to living organisms as an inert gas. Nitrogen forms, which are generally usable by microorganisms as substrate for a set of physical and biological processes within the N-cycle, are generally referred as reactive nitrogen (N_r). This comprises ammonia (NH_3), ammonium (NH_4^+), nitric oxide (NO), nitric acid (HNO_3), nitrous oxide (N_2O), nitrate (NO_3^-), nitrite (NO_2^-) and organic N forms (Galloway et al., 2003; Erisman et al., 2011) (Fig. 2.4). Among these, NO_3^- is the most common N contaminant in soil and groundwater. Other N species which are considered pollutants derived from agricultural sources are NH_4^+, NO_2^- and N_2O. These species are common endpoint or intermediate compounds of multiple pathways in the N-cycle (Fig. 2.4). To avoid the release of these species, two solutions have been identified: 1) decreasing N_r production or 2) increasing its depletion by pushing the N-cycle towards a complete N_2 conversion (Galloway et al., 2003). Predominant pathways of bioremediation are nitrification, followed by denitrification. Through the former, NH_4^+ is oxidised to NO_3^-, while the latter reduces NO_3^- to N_2 (Rivett et al., 2008). These two processes are carried out through a set of sequential reactions and may produce NO_2^-, nitric oxide (NO) and N_2O as undesirable intermediate compounds, which could be released in the environment. The outcome of these processes and attenuation of N-species is regulated by many environmental factors (e.g. soil

characteristics, substrate concentrations, plant coverage, management, weather) (Saggar et al. 2013) and by a large set of minor alternative pathways (i.e. dissimilatory nitrate reduction to ammonium (DNRA), anaerobic ammonium oxidation (Anammox) and co-denitrification).

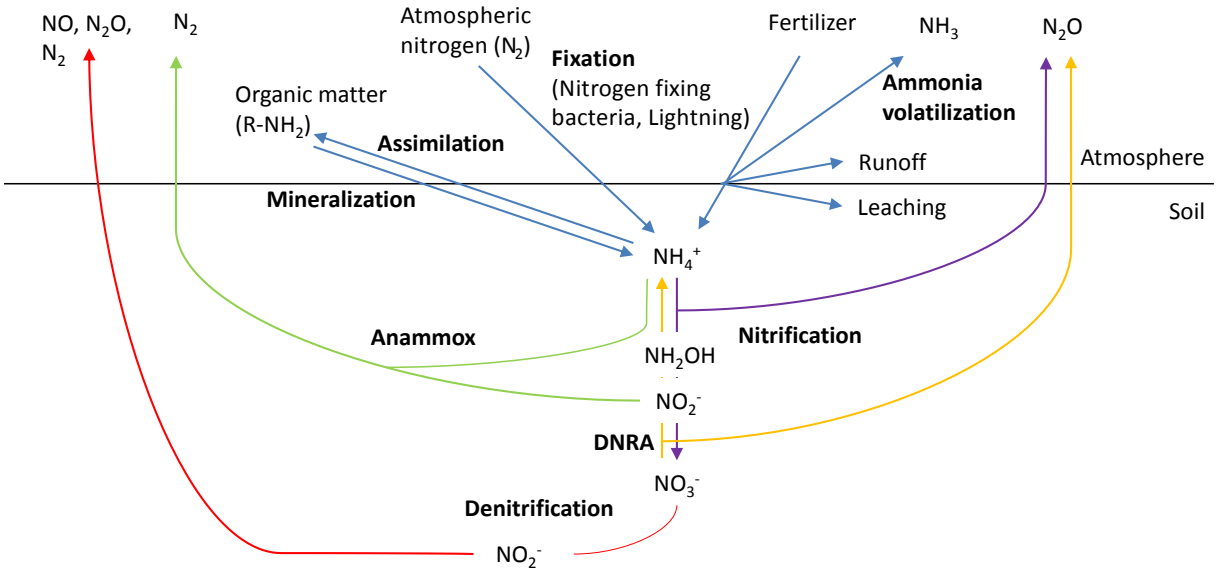


Fig. 2.4. Simplified presentation of the nitrogen cycle (DNRA, dissimilatory nitrate reduction to ammonium).

2.5.1 Nitrogen transformational processes

2.5.1.1 Nitrification

Excluding direct fertilization, N enters the soil environment via two main microorganisms mediated processes: fixation and mineralisation (Fig. 2.4). Fixation converts N_2 to NH_3 which is then immediately available for plant assimilation. On the other hand, mineralisation, or ammonification, is the decompositions of nitrogen present in organic compounds unavailable for plants such ($R\text{-}NH_2$) and releases NH_4^+ as by product (Geisseler et al., 2010). Once in the soil, NH_4^+ can be subjected to three main transformation pathways: Assimilation, the direct transformation of NH_4^+ (and NO_3^-) into organic compounds by incorporation into microorganism and plants; ammonia volatilization, an alternative process that results in the loss of NH_4^+ from the soil to the air as NH_3, and nitrification (Fig. 2.4).

Nitrification is the process through which NH_4^+ is oxidised to NO_2^- and consequently to NO_3^-. The oxidation to nitrite is an aerobic two-step reaction carried out by NH_3-oxidizers (Wood, 1986; Hollocher et al., 1981) (Eqn. 2.1). The most common group known to carry out this process is *Nitrosomonas*. However, other genera (e.g. *Nitrosospira* and *Nitrosococcus*) or subgenera (e.g. *Nitrosovibrio*) are also known to perform this activity (Hayatsu et al., 2008).

These groups can be found in both soil and water environments (Watson et al. 1981; Kim and Gad, 2008).

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + H_2O + 4H^+ \quad \text{(Eqn. 2.1)}$$

The second step, the oxidation of NO_2^- to NO_3^- (Eqn. 2.2) is carried out by NO_2^--oxidizer bacteria such as *Nitrobacter* (Watson et al., 1981), but can be performed also by fungi (Verstraete and Alexander, 1973; Watson et al., 1981).

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \quad \text{(Eqn. 2.2)}$$

An additional process combining nitrification and denitrification is nitrifier-denitrification. Nitrifying organisms are known to produce N_2O and N_2 during nitrifier-denitrification, while true nitrification only results in N_2O emissions. Nitrifier-denitrification is carried out by autotrophic NH_3^--oxidizers. After the first step of nitrification as described above, NO_2^- is reduced to N_2O and N_2 via NO. The enzyme that catalyses this reaction is a nitrite reductase (Hooper, 1968). A similar process is known within NO_2^--oxidizers, where NO_2^- can be anaerobically reduced to N_2O with pyruvate as reducing agent (Freitag et al., 1987).

2.5.1.2 Denitrification

Denitrification is a multistep process for the conversion of NO3- to N2. This process occurs in a wide range of bacteria, usually heterotrophic and facultative anaerobic. However, it can be also carried out by fungi, archea and some aerobic bacteria (Hayatsu et al, 2008). They use NO_3^- as an electron acceptor in the absence of O_2. Denitrification starts with the reduction of NO_3^- to NO_2^- which is then reduced to NO, N_2O and N_2, respectively, by the enzymes nitrate reductase (Nar), nitrite reductase (Nir), nitric oxide reductase (Nor) and nitrous oxide reductase (Nos) (Eqn. 2.3) (Hochstein and Tomlinson, 1988; Zannoni, 2004). N_2O, an important greenhouse gas, is an obligate intermediate product.

$$NO_3^- + 1.25\ (HCHO) \rightarrow 0.5\ N_2 + 0.75\ H_2O + 1.25\ CO_2 + OH \quad \text{(Eqn. 2.3)}$$

In addition to bacteria, denitrification can also be accomplished by microorganisms belonging to archea or eukarya. More than 60 genera of archea and bacteria and some species of saprophytic fungi are known denitrifiers. Denitrifiers are ubiquitous in soil but usually not

numerous, in fact they represent only 0.5 – 5% of the bacterial population (Tiedje, 1988). The majority of denitrifiers do not possess one or more enzymes required for the complete set of these four reactions and therefore microbial interactions are required to complete the process (Zumft, 1997; Wallenstein et al., 2006). Almost 33% of sequenced denitrifiers lack nitrous oxide reductase, thus they release N_2O as a final product (Philippot et al., 2011). The structure of the microbial community seems therefore to be an ultimately important factor for the release of N_2O.

The expression of the denitrificational set of enzymes is controlled both transcriptionally and post-transcriptionally. They are synthesised in response to a shortage of oxygen and presence of nitrate, while they are blocked when oxygen concentration rises (Van Spanning et al., 2007). Abiotic denitrification can occur at low pH (< 5.2), but these reactions are minor compared with biological denitrification (Rivett et al., 2008).

Typical to fungi is the process of co-denitrification, where NO_3^- and nitrogen compounds (azides, amine and NH_4^+) are combined to form hybrid N_2 and N_2O (Tanimoto et al., 1992). This process occurs under denitrifying conditions.

2.5.1.3 DNRA

DNRA (dissimilatory nitrate reduction to ammonium) is a process where NO_3^- is reduced to NO_2^- and then reduced to NH_4^+ (Eqn. 2.4) (Cruz-Garcia et al., 2007; Vermeiren et al., 2009). Environmental conditions for the occurrence of DNRA and denitrification often coincide, however it is believed that DNRA is favoured when nitrate is the limiting factor and not carbon (Cole and Brown, 1980). Additionally, bacteria able to carry out DNRA are also obligate anaerobes and therefore occupy a more limited range of environments compared with denitrification.

$$2H^+ + NO_3^- + 2CH_2O \rightarrow NH_4^+ + 2CO_2 + H_2O \qquad \text{(Eqn. 2.4)}$$

2.5.1.4 Anammox

Anammox is a three step process that is undertaken in the absence of oxygen with a limited supply of organic matter. The anammox process starts with the reduction of NO_2^- to NO, followed by the formation of hydrazine (N_2H_4) from NO and NH_4^+, which in turn, is oxidized to N_2 (Eqn. 2.5). Bacteria that carry out this process appear to exist in every system containing anoxic zones (Strous et al., 2006). These bacteria are chemolithoautotrophic and use CO_2 as a C-source.

$NO_2^- + NH_4^+ \rightarrow N_2 + 2H_2O$ (Eqn. 2.5)

2.5.2 Factors controlling the main microbial processes of the N cycle

2.5.2.1 Denitrification

Numerous factors influence and control denitrification and the N-cycle. These variables have been classified in two groups, according to their proximal or distal influence (Groffman and Tiedje, 1989; Wallenstein et al., 2006). The first group include all factors affecting denitrifying processes directly and immediately, while distal factors contribute to changes the microbial community over a larger time and spatial scale (Saggar et al., 2013).

The main factors affecting denitrification in soil and groundwater are redox potential, oxygen availability and therefore water filled pore space (WFPS), temperature, pH, soil organic matter (SOM) and management (Coyne, 2008). Denitrification can occur at temperatures above 2˚C and a pH between 3.5 and 11 (optimal pH 6-8) (Rust et al., 2000), within a typical redox potential (Eh) of +200 to +400 mV (Bailey and Beauchamp, 1973). Nitrate is the main substrate for denitrification and its presence and the rate of reduction are dependent on its accessibility and quantity. The balance between inputs and outputs regulates the NO_3^- concentration, and different rates of processes associated with transformation of nitrogen compounds play an important role in the total amount of available NO_3^- and N_r (Tiedje, 1988). Nitrate accessibility and diffusion into soil microsites, where bacteria are mainly located, is controlled by soil water content as a medium for diffusion. High NO_3^- concentrations lead to incomplete denitrification, increasing the release of N_2O, as NO_3^- reduction is more favourable than N_2O and because N_2O has an inhibiting effect on the nitrous oxide reductase (Stevens and Laughlin, 1998).

Carbon, as an electron donor, is the second required factor for denitrification to occur. Dissolved organic carbon (DOC) availability is characterised by a wide range of variability, being affected in both levels and bioavailability by factors such as temperature, pH, and oxidant concentration (Hartog et al., 2004), by processes such as mineralization, attenuation or sorption (Jacinthe et al., 2003) and by agricultural practices. Heterotrophic denitrifiers are mainly facultative anaerobes and therefore the presence of dissolved oxygen is a key limiting factor. Denitrification is known to occur at oxygen values below 1-2 mg O_2/l, as high oxygen content represses denitrifying enzymes. Under aerobic conditions rates are reduced to less than 3% of denitrification under anaerobic conditions (Parkin and Tiedje, 1984). However, the average O_2 content of a field may not reflect the environment in the microsites, where

bacteria are located (Rivett et al., 2008). Microorganism respiration, absorption, soil management and soil water content can modify oxygen values (Tiedje, 1988). Rainfall events are normally associated with high rates of denitrification as they generally reduce the amount of dissolved oxygen within soil and act as a medium for substrate transport to microbial communities. At WFPS below 20%, substrates have limited movement thereby limiting bacterial processes and N_2O emissions to anaerobic microsites. Between a WFPS of 20-35%, N_2O production increases significantly with nitrification becoming the dominant process at 35%. Nitrous oxide production peaks between 60-80%, gradually switching to higher rates of denitrification but with nitrification as the dominant process. Above a WFPS of 70%, production of N_2O is again minimal due to high rates of complete denitrification and therefore N_2 emissions (Stevens et al., 1997; Bateman and Baggs, 2005).

Differences in soil compaction can lead to variation in denitrification rates according to different soil moisture content (Luo et al., 2000). Denitrification and drainage systems are tightly linked (Deutsch et al., 2005)). Installation of drainage systems can modify the soil environment by reducing excess water under wet conditions. Increased soil temperatures, higher amounts of oxygen and reduced soil moisture, drivers of denitrification, have been described as results from the installation of drainage systems. Drainage systems, contributing to a higher level of dissolved nutrient transport and loss, change the residence time of water in the unsaturated zone and micropores thereby allowing less time for attenuation processes to occur (Ritzema et al., 1996; Zucker and Brown, 1998).

2.5.2.2 Nitrification

Nitrification, similarly to denitrification, is influenced by many factors (i.e. pH, temperature, dissolved oxygen, WFPS, and substrate availability). Nitrification is an aerobic process that occurs at an optimum pH of 7-9. However, it has been shown to occur also at low pH 3-5 (Hayatsu et al., 2008). Optimal temperature has been recorded from 25 to 35˚C (Focht and Verstraete, 1977). Nitrification is negatively affected by high clay content soils as the smaller pore space (especially when constantly waterlogged) makes N unavailable for microbial consumption. However, this can be reversed by drying-wetting cycles that cause bursts of microbial activity (Sahrawat, 2008). Most nitrifiers are obligate autotrophs and soil aeration is essential for the occurrence of nitrification, with maximum activity taking place at a soil oxygen concentration similar to the atmosphere (Tisdale and Nelson 1970).

2.5.2.3 DNRA

DNRA is an anaerobic process that can occur under similar conditions as denitrification (Kelso et al., 1997). Soil properties such as permeability, play an essential role in the control of this process (Dzakpasu et al., 2014). Reducing conditions and high DOC concentrations favour the occurrence of DNRA. The main factor controlling the occurrence of DNRA vs. denitrification is the ratio between C and NO_3^-. High C:NO_3^- (above 12, combined with a low oxidation reduction potential) favours DNRA, while low C:NO_3^- (favours denitrification (Yin et al., 1998; Rütting et al., 2011). Additionally, DNRA bacteria are obligate anaerobes and are present in a limited range of environments compared with denitrifiers (Buss et al., 2005).

2.6 Techniques to elucidate N source, transformation and fate

2.6.1 Dissolved gases

The N-cycle is known to produce N_2O and/or N_2. Both soil and water are sources of N_2O and N_2 as a result of attached and planktonic microbial community activity, with significant gas production in the saturated zone resulting in dissolved gas accumulation (Well et al., 2001).

Dissolved gasses are easily released in the atmosphere and therefore are an essential piece of information for the quantification of N-losses and evaluation of the N-amount conveyed to the receptor water bodies (Table 2.3). Agricultural dissolved N_2O losses can account for 50-67% of surface losses (Minamikawa et al., 2010). However, dissolved gasses are often overlooked due to the complexity of sampling (Harris et al., 1984; Roper et al., 2013). Dissolved N_2O is a valuable measurement to understand transformation processes and their rate (together with CO_2 and CH_4). Dissolved N_2/Ar ratios have also been used to quantify denitrification and its completion in groundwater thanks to its high throughput characteristics (Groffman et al., 2006). The assessment of N_2/Ar ratio enables to identify the N_2 produced by denitrification (excess-N_2) as the excess air trapped in solution that exceeds the air ratio (Vogel et al., 1981; Wilson et al., 1990; Wilson et al., 1994).With this measurement, values of the ratio above a certain threshold (83.5 – air) were found to be indicators of denitrification in groundwater (Weymann et al., 2008). The combination of excess-N_2 and dissolved N_2O in terms of total emissions (N_2+N_2O) and their ratio (N_2O/(N_2+N_2O)) have been used as a measure of complete vs. incomplete denitrification. For example, high spatial variability of dissolved N_2O and excess-N_2 in groundwater has been encountered by Jahangir et al. (2013a) and attributed to different levels of complete vs. incomplete denitrification. Ratios were lower on low permeable soils due to complete denitrification (Jahangir et al., 2012a). However, due to the many controls and parameters acting on the components of the N-cycle, it is often

difficult to discern the output of denitrification from the reactions which contribute to N_2O and N_2 emissions, especially when accounting for spatial and temporal variability (Butterbach-Bahl et al., 2013) and different land use (Jahangir et al., 2012a). Dissolved gasses techniques can be however biased due the evaporation or the degassing of the sample that affect gas concentration dissolved in the water. In the context of drainage systems, locations closer to in-field drains had values for dissolved N_2O that were higher, indicating a higher influence of physical rather than biological parameters on the control of N_2O (Reay et al., 2003). Therefore, when sampling drainage networks it could be recommended that sample collection from in-field (samples collected from apposite openings along the pipe) and end-of-pipe (samples collected from openings at the end of the pipe when reaching the open ditch) locations is advisable to minimise turbulent flow and degassing.

Table 2.3. Sample of the most commonly used techniques for studying the N-cycle with possible outcomes. * highlights techniques or methods that have been used within this thesis.

	Technique	Method	Data provided	Contribution to the characterization	Limitation
1	Meteorological data*	Met station	- Daily Tmax, Tmin, total rainfall, main wind speed, solar radiation (measurement used for SMD method)	- Seasonal, yearly patterns - Data for the calculation of further parameters (ED, SMD, ER, PET, AET) - Periods of the year more suitable for denitrification in terms of recharge and infiltration	- Only information related to the general environment and soil moisture deficit - Not direct measurements of transformational processes or water/soil air quality
2	Soil characteristics*	Pit excavation, Cores, Visual assessment	- Soil type - Soil drainage class - Depth to bedrock - Soil analyses (e.g. texture, SOM content, nutrient content)	- Soil type with associated drainage class - Indicative permeability - Most probable soils to have higher/lower denitrification in relation to infiltration speed, micropore dimension, preferential infiltration routes - Status of the soil	- Information related to soil qualities - Not direct measurements of transformational processes but only potential of it occurring
3	Drainage system characteristics*	Records, flux measurement	- Drainage type (i.e. surface (mole, gravel mole, piped), subsurface (piped), open ditches), spacing, length, design.	- Soil drainage with associated soil type and drainage class	- Only information related to the general environment - Not direct measurements of transformational processes or water/soil air quality
4	Agricultural management inputs*	Manual record	- Fertiliser inputs and types - Locations of yards and storage facilities - Management (i.e. ploughing, grazing, crop)	- Dictates the type of system (e.g. intensive) - Selection of possible causes of contamination and community modification - -N balances	- Only information related to the general environment and farm inputs outputs - Not direct measurements of transformational processes
5	Biogeochemical parameters*	In situ probes	- DO, Eh, EC, pH, Temperature, turb., watertable depth	- Suitable condition for the processes (e.g. anaerobicity of the system, suitable pH, temperatures) - Saturation state - Time and space related data	- No identification or quantification of transformational processes
6	Chemical species concentrations*	Quantification (e.g. photometric analyses, spectrometry)	- Nutrients (NO_3^-, NO_2^-, NH_4^+, TN, DON, P, TP) - Metals (Cu^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Mg^{2+}, K^+, S^{2-}) - Other species (TC, DOC) - Specific contaminants	- Spatial and temporal distribution of water quality parameter - Spots of contamination, pollution and dilution - Nutrient and micronutrient presence in water - Alternative electron acceptors and donors - Presence of inhibitory substances	- No identification or quantification of transformational processes

				- Speciation and possible occurring processes - Other sources of contamination - Time and space related data	
7	Dissolved gases*	Quantification (e.g. mass spectrometry, gas chromatography)	- N_2O, CH_4, CO_2 - Excess N_2, Ar	- Quantification of N and GHGs leaving (more precise farm balances) - Time and space related data - Possible completeness of denitrification processes (i.e. $N_2O/(N_2O+N_2)$) - Possible denitrification hotspots (N_2/Ar)	- Complexity of sampling - Difficult to discern the output of denitrification from other transformational processes - Biased from evaporation or degassing
8	Isotopic data*	Isotopic natural abundances, Isotopomers	- $\delta^{15}N$- NO_3^-, $\delta^{18}O$- NO_3^-, $\delta^{15}N$- NH_4^+ - $\delta^{15}N$-N_2O, $\delta^{18}O$-N_2O, $\delta^{15}N$-N_2 - $\delta^{18}O$-H_2O, δD-H_2O - $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$	- Source (organic, inorganic) and processes (denitrification, nitrification, ammonia volatilisation) controlling and affecting NO_3^- spatial distribution and attenuation - Possible rate of the processes - Provenance of H_2O and environmental connection between part of the system connection - Processes occurring over a period of time along the continuum	- Complexity of oxygen signature - Unknown fractionation factors for some transformational processes - Complex data interpretation - Semi-quantitative - No point samples but "from deposition to collection" samples
9	Molecular analyses	qPCR (for 16S and specific)*	- Quantification of bacterial/specific functional genes abundance	- More concrete distinction between nitrate transformational processes, at sampling well level, based on gene abundance (i.e. denitrification vs. DNRA)	- Based on known sequences - Semi-quantitative - No distinction between alive/active and dead/not active
		T-RFLP	- Fingerprinting and quantification of most abundant operational taxonomic unit (OTU)	- Variation in the community structure within comparative studies	- Based on known sequences - Possible limited reproducibility - Semi-quantitative - No distinction between alive/active and dead/not active
		Sequencing	- Identification of the main OTU	- ID and retrieval of OUT/species information from informatics databases	- Based on known sequences - Semi-quantitative - No distinction between alive/active and dead/not active
		Microarrays (e.g. Geochip, Philochip)	- Functional gene array - Identification of known sequences	- ID in terms of microbial taxa and gene families	- Based on known sequences - Semi-quantitative - No distinction between alive/active and dead/not active

2.6.2 Isotopic techniques

2.6.2.1 N stable isotopes - Natural abundances

Isotopic compositions are an important tool for direct source and process identification and rate semi-quantification (approximate indication of the rate given from the comparison of different samples from the same background) (Baggs, 2008) (Table 2.3). Different NO_3^--N sources and processes have characteristic isotopic signatures ($\delta^{15}N\text{-}NO_3^-$, $\delta^{18}O\text{-}NO_3^-$) that are used to track nitrogen sources, fate and transformational processes.

Natural ^{15}N-abundance has gained an essential role in the study of N transformational processes as it enables large scale field analysis and overcomes problems of other isotopic techniques concerning external addition of isotopically enriched (labelled) fertiliser to the original N-pool. Utilising one single signature (e.g. only $\delta^{15}N$ for NO_3^-) is often not sufficient to reach conclusive result (Kellan and Hillaire-Marcel, 1998; Kendall et al., 2007; Xue et al 2009). Dual approaches including both ^{15}N and ^{18}O isotopes of NO_3^- are more likely to provide additional insights and numerous techniques are available for the evaluation of $^{14/15}N$ and $^{16/18}O$ content in NO_3^- (Silva et al., 2000; Sigman et al, 2001; Casciotti et al., 2007) (Fig. 2.5). The occurring of specific processes is characterised by specific fractionation factors (Fig. 2.6). These factors leads to the creation of specific signature within the residual pools (the remaining substrate after a microbial processes occurred) that characterise specific processes. Since organisms preferentially use lighter isotopes (i.e. ^{14}N and ^{16}O, rather than ^{15}N and ^{18}O), the microbiological process cause an enrichment of heavy isotopes in the remaining source pool, with a depletion in the product signature. These signatures for denitrification and nitrification have been measured within several studies which lead to the creation of specific ranges (boxes in Fig. 2.5). Ranges are also known for several N sources (Fig. 2.5). Specifically, it was highlighted that when denitrification occur, possibly starting from the NO_3^- created by nitrification, this processes follows a linear trend generally a (1:1 - 1:2 trend). Point that fell outside these boxes can be interpreted as pools on which multiple, or of unknown signatures, sources or processes are occurring However, a dual approach is not free from bias, as *in situ* oxygen exchange between intermediate products and water, and variation of the soil $\delta^{18}O$ by respiration complicates the use of the oxygen signature (Lohse et al., 2013).

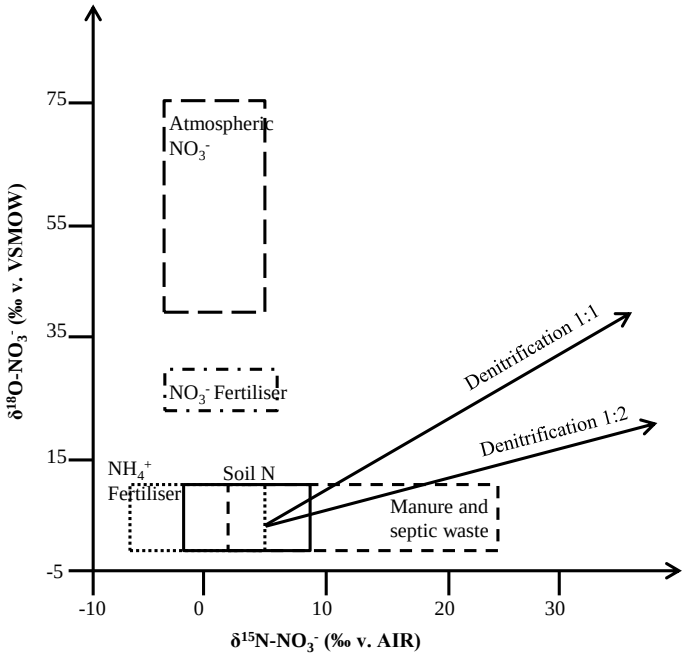
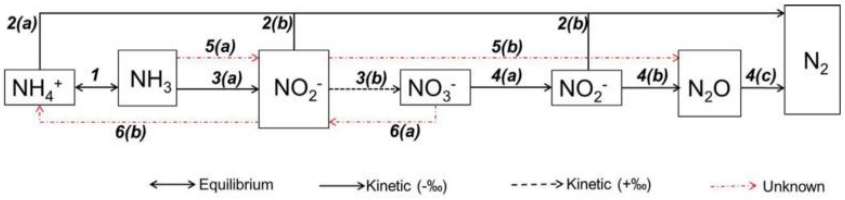


Fig. 2.5. Global NO_3^- isotopic signatures (from Kendall 1998; Sigman et al., 2001; Granger et al., 2008; Xue et al., 2009; Nestler et al., 2011).



ID	Process	Fractionation factor(s)	References
1	Chemical equilibrium	$^{15}\varepsilon_{eq} = 20‰$	Casciotti et al., 2003
2	Anammox	(a) $^{15}\varepsilon_{amx,NH4} = -27 \pm 3‰$ (b) $^{15}\varepsilon_{amx,NO2} = -16 \pm 5‰$	(a,b) Brunner et al., 2013
3	Ammonia oxidation	(a) $^{15}\varepsilon_{amo,NH3} = -14 \rightarrow -38‰$ (b) $^{15}\varepsilon_{amo,NO2} = +12.8‰$	(a) Casciotti et al., 2003 (b) Casciotti 2009
4	Denitrification/chemodenitrification[a]	(a) $^{15}\varepsilon_{denit,NO3} = -3 \rightarrow -30‰$ (b) $^{15}\varepsilon_{denit,NO2} = -5 \rightarrow -25‰$ (c) $^{15}\varepsilon_{denit,N2O} = -31 \rightarrow -25‰$	(a) Granger et al., 2008, Kritee et al., 2012, Sebilo et al., 2003, Jones et al., 2015 [a] (b) Bryan et al., 1983, Casciotti et al., 2002 (c) Sutka et al., 2003, 2004
5	Nitrifier-denitrification[b]	(a) $^{15}\varepsilon_{n\text{-}d,NH3} = ?$ (b) $^{15}\varepsilon_{n\text{-}d,NO2} = ?$	
6	DRNA[c]	(a) $^{15}\varepsilon_{DRNA,NO3} = ?$ (b) $^{15}\varepsilon_{DRNA,NO2} = ?$	

[a] Chemodenitrification causes comparable N isotope fractionation (Jones et al., 2015).
[b] Fractionation factors for nitrifier-denitrification have not been directly measured, but may reasonable be expected to be comparable to those for the NH_3 oxidation for step (a) as the same enzymes and microbial populations are involved (Kool et al., 2010; Colliver and Stephenson, 2000).
[c] There are no direct measurements of fractionation factors for DNRA, but anomalous relationships between $\delta^{15}N{-}NO_3^-$ and $\delta^{18}O{-}NO_3^-$ have been reported in regions where DNRA is known to occur (Dhondt et al., 2003).

Fig. 2.6. Overview of the microbial processes affecting N signature with relative known and unknown fractionation factors (Wells et al., 2016)

During denitrification, the enrichment of both O and N of the residual NO_3^- pool can described by the Rayleigh equation ($R/R_0 = (C/C_0)^{1/(\alpha denitr-1)}$, where R is the residual pool, R_0 is the original pool, C/C_0 is the change in concentration in NO_3^- at a constant degree of isotopic discrimination (α_{denitr}) and with continuous removal of the produced NO_3^- (Kendall and Caldwell, 1998). This NO_3^- enrichment was explained by Mariotti et al. (1981) for both $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ (2.6 and 2.7):

$$\delta^{15}N_x = \delta^{15}N_0 + \varepsilon_{denit} \times \ln(f/(1- f)) \quad \text{(Eqn. 2.6)}$$

$$\delta^{18}O_x = \delta^{15}O_0 + \varepsilon_{denit} \times \ln(f/(1- f)) \quad \text{(Eqn. 2.7)}$$

These formulas have been used to distinguish biological versus dilution processes or plant uptake (Wankel et al., 2006). However, isotopes techniques can be misleading, for example, the contribution of multiple sources and overlapping different processes complicates data interpretation (Kendall and McDonnell, 1998). Also, the isotopic signature range is still unknown for some processes (e.g. distinction between denitrification and DNRA process is still unclear as they share same NO_3^- signature) (Butterbach-Bahl et al., 2013; Wells et al., 2016) (Fig 2.8). Therefore the combination of the analyses of isotopic composition of water (δD-H_2O, δ^{18}O-H_2O) (as a source of O) and of multiple species e.g. NH_4^+ (δ^{15}N-NH_4^+) and N_2O (δ^{15}N-N_2O, δ^{18}O-N_2O) could help unravel such complexities.

The use of NO_3^- isotopic signature led Deutsch et al. (2005) to the identification of drainage systems (tile drains) as powerful tool to identify transformational processes or fertilizer effects that influence the NO_3^- concentration in drainage water. As drainage systems modify the quantity (rate) of the NO_3^- brought to the receiving waterbody and the isotopic signature of this NO_3^- is related to N-inputs or transformation processes from the deposition point to the outlet, this relation between flow rate and transformational processes can help to identify contribution areas and their attenuation capacity (Kellman and Hillaire-Marcel, 2003; Smith and Kellman, 2011). For example, in the study of Buzek et al. (2009), a double NO_3^- signature (δ^{18}O and δ^{15}N) enabled the difference in discharge source and rate (slow vs. fast response drainage) to be identified while at the same time tracking N-sources (i.e. fertiliser inputs). However, past studies mainly focused on δ^{15}N-NO_3^- (Kellman and Hillaire-Marcel, 2003; Kellman 2004; Kellman 2005), and only recently dual isotope techniques have been used for a more complete picture of source characterisation and attenuation within drainage networks installed in agricultural areas (Deutsch et al., 2005; Granger et al., 2008; Smith and Kellman 2011; Kelley et al 2013). When accompanied by appropriate chemical parameters and knowledge of the dominant flow paths, natural abundances allow for the localisation and tracking of sources and/or sinks of NO_3^- in groundwater (Pastén-Zapata et al., 2014) and are a powerful tool in mixed agricultural landscapes (Baily et al., 2011; Smith and Kellman, 2011; Minet et al., 2017). While comparing isotopic signatures in shallow groundwater and a piped in-field drainage monitoring network, Mehnert et al. (2007) showed that drainage management affected denitrification. The nitrate concentrations in groundwater showed

similar enrichment to in-field drains or a depletion, therefore indicating a combination of different recharging and denitrification degrees between the two. Data were then used to create a model to estimate denitrification within the watershed (mass of N denitrified = ΔN × groundwater recharge × watershed area; where ΔN is the difference in N concentration between start and end of the flow path).

Often natural abundances of additional N-species (i.e. $\delta^{15}N\text{-}NH_4^+$, $\delta^{15}N\text{-}N_2$, $\delta^{15}N\text{-}N_2O$ and $\delta^{18}O\text{-}N_2O$) are used together with NO_3^- to better characterise the system and to deduce different or concomitant N-cycle processes and their specific signatures and fractionation factors (Snider et al., 2012). Analysis of the isotopic composition of NH_4^+, dissolved N_2O and dissolved N_2 can lead to better identification of N processes and/or sources e.g. distinction between NH_4^+ and NO_3^- derived N_2O (Snider et al., 2012; Hood et al., 2014).

The study of $\delta^{15}N\text{-}NH_4^+$ can highlight the presence of additional processes if combined with the analyses of NO_3^- signatures (Wells et al., 2016) and further study on $\delta^{15}N\text{-}NH_4^+$ can deduce DNRA and nitrifier-denitrification, for which fractionation factors are still to be identified. As N_2O is an intermediate product of a multitude of processes, it has always been a source of interest and numerous studies have tried to unveil its apportionment. However, this is complicated due to numerous factors: 1) controls are not completely known, 2) multiple formation/consuming processes, 3) different extent of abiotic oxygen exchange, 4) unquantified fractionation factors. Currently N_2O signatures (especially $\delta^{18}O\text{-}N_2O$) are a valuable method to distinguish N_2O produced by nitrification vs. denitrification based on existing measured ranges (Snider et al., 2010; Li et al., 2014; Snider et al., 2015).

2.6.2.2 H_2O stable isotopes

The further analysis of H_2O natural isotopic abundances enables the identification of its provenance and the presence of external or anomalous sources/areas of contribution (Table 2.3). The use of both isotopes (δ^2H or $\delta^{18}O$) is recommended. However, generally only one of the two isotopes is used as these are (mistakenly) believed to carry the same information (Lyon et al., 2009; Klaus and McDonnel, 2013). In the context of surface water and groundwater, the main sources of water recharge are rainfall events. Less frequent events will affect water isotopic signatures leading to higher depletion with smaller variation associated with altitude and temperature (Darling et al., 2003). Conversely, evaporation creates enrichment in $\delta^{18}O\text{-}H_2O$ when compared to rainfall values (Klaus et al., 2015). Generally, a drainage system will transport connected groundwater from the surrounding land and therefore presents similar signatures to that of the contribution area and that of the global

meteoric water line (average relationship between δ^2H and $\delta^{18}O$ in natural terrestrial waters) (Klaus et al., 2015). The only difference is that sometimes it may possibly have a lower slope due to evaporation (especially within open drainage). However, an explorative analysis of $\delta D\text{-}H_2O$ and $\delta^{18}O\text{-}H_2O$ when the system is vast can be beneficial as it can highlight sections with a different signature of the drainage system that could reflect areas affected by lakes or rivers, generally presenting a depleted value due to evaporation.

2.6.3 Molecular methods: quantification, structure and function of microbial communities

Between 6% and 40% of prokaryotes are estimated to be found within the terrestrial subsurface (Whitman et al., 1998). Groundwater is also considered to have high microbial diversity (Gibert et al., 1990). As the N cycle mainly includes biotic processes; microbial analyses are essential to uncover the molecular basis of these pathways and the role of bacteria, archaea and fungi and to predict the attenuation capacity and resilience. Starting from the first publication on DNA soil extraction back in 1980 (Torsvik, 1980) numerous novel techniques have been developed to analyse the complex microbial environment of both the unsaturated soil zone and the saturated zone.

Culture-dependent analyses are a valid method to describe in detail bacterial function and metabolic pathways. As discussed in Pham and Kim (2012), important progress has been made in the cultivation of bacteria (i.e. transwell plates, high-throughput micro-bioreactors, diffusion chambers, culture chips, spheres with gelating agent and single cell encapsulation coupled with flow cytometry). Despite these advances, culture-dependent analyses still have a limited application and direct analysis of microbial communities using molecular approaches is still preferable, as only 1-10% of the soil community can be cultured due to lack of knowledge and difficulty to recreate the complexity of natural conditions of environmental factors and bacterial diversity (Hugenholtz, 2002; Schloss and Handelsman, 2004; Alain and Querellou, 2009).

To date most research has analysed the DNA component of soils and groundwater although there are known limitations. High rates of bacterial mutation and exchange of DNA (horizontal gene transfer) have created problems when trying to define bacterial "species". This lead to the creation of too widely comprehensive operational taxonomic units (97-99% of 16S rRNA gene similarity) and revealing different patterns at analysis with different species units highlighting the need for deep sequencing (Cohan, 2002; Koeppel and Wu, 2014). On a more methodological side, collection of samples can be a source of bias as

incorrect planning can lead to unrepresentative samples (i.e. long time lapse between collection and storage, anaerobic and aerobic conditions, etc.) and a non-significant set of replicates (CL:AIRE, 2008). Additionally, microbial (DNA or RNA) sampling must be representative of the spatial variation in the processes studied.

DNA extraction techniques vary widely with potential bias from poor extraction procedures. There have been attempts to formulate standard methodologies (e.g. ISO standard (Petric et al., 2011)) but these have their own limitations and have not been widely adopted. Most of the molecular methodologies currently used are based on PCR approaches which cannot discriminate the active section of a community by the total DNA (active cells, dormant cells, dead cells and extracellular DNA) (Josephson et al., 1993). Furthermore, PCR-based methods are inherently biased by the researchers prior knowledge and subjected to differential amplification, artefacts, contamination and partial community overlook (v. Wintzingerode et al., 1997). It is not possible to design primers that will amplify all known target genes. These PCR base analyses (e.g. qPCR) are commonly used and therefore more affordable for a preliminary or explorative study (Table 2.3). These can then be used to select more interesting samples for detailed analyses, such as whole genome or fingerprinting analyses.

Whole genome analysis of soil microbiota can be performed as this provides a direct link between microbial identity (as determined by analysis of 16S rRNA gene sequences) with potential function (as determined from the presence of genes encoding specific enzymes), but this approach is generally impractical for soils given their great microbial diversity (10^6 different 16S rRNA for soil gram) (Gans et al., 2005; Quince et al., 2008). Analysis of 16S rRNA gene diversity provides an efficient way to analyse microbial community structure (Kolbert and Persing, 1999). The 16S rRNA genes can be amplified using PCR and primers complementary to the conserved regions of the gene. The amplicons can then be either directly sequenced or subject to methods such as DGGE, TGGE or TRFLP that provide a 'fingerprint' of sequence diversity. These fingerprinting techniques allow many samples to be analysed quickly and cheaply and are often used to guide sample selection for detailed direct sequence analysis (Table 2.3). However, it is difficult to identify specific organisms using these approaches and reproducibility can be a problem (especially with gradient techniques such as DGGE or TGGE) (Janda and Abbott, 2007). Direct sequencing methods have become more popular as sequencing technologies have advanced. Whilst clone libraries typically examine 100s of sequences, pyrosequencing (454) generates tens of thousands of sequences in a single run, while Illumina sequencing generates millions. This increased sequence depth and relative affordability allows less abundant community members to be examined.

Improved primers are constantly being introduced but no primer combination can amplify all known sequences while maintaining an acceptable level of specificity.

These same approaches can also be applied to functional genes. Quantification of these genes is typically performed using q-RT-PCR techniques using 16S rRNA gene abundance as measure of total microbial abundance. However, the problem of primer bias is exacerbated with functional genes where sequence conservation is generally considerably worse (Osborn and Smith, 2005).

Focusing on denitrification, various genes coding for the enzymes of denitrification are often used since denitrifiers are phylogenetically very different. The first enzyme to be synthesised and activated under anaerobic conditions is Nar. The nitrate reductase Nar is a membrane-bound enzyme composed by three subunits while Nap is a periplasmic variant (Richardson et al., 2001). The key gene for the enzyme Nar is narG, coding for the α-subunit of the membrane-bound enzyme, and napA coding for the periplasmic enzyme (Bru et al., 2007). These two enzymes can be found also in bacteria reducing NO_3^- to NH_3 and are therefore not ideal for characterization denitrifying communities. Nevertheless they have been used for soil quantification (Philippot, 2002; Bru et al. 2007). The enzyme Nir, nitrite reductase, is a periplasmic enzyme present in two variants, NirK and NirS. NirS is found only in Gram-negative bacteria while the other form, NirK, can be found in some genera of Gram-positive denitrifiers, Gram-negative bacteria and in Archaea (Kim and Gadd, 2008). These two genes have been widely used for the identification of denitrifiers (Liu et al., 1997). The enzyme Nor, nitric oxide reductase, is composed of two subunits, NorB, the larger subunit, and NorC, the smaller subunit (Zumft, 1997). The gene for NorB is not a good target for characterization as a wide number of non-denitrifying organisms contain this gene (Richardson, 2000). The enzyme Nos, nitric oxide synthetase, is not present in all denitrifiers and some bacteria can use only N_2O as an electron acceptor. Nos is a dimeric enzyme situated in the periplasm (Zumft, 1997). The gene *nosZ* is the only gene that is known to encode for Nos (Burger and Matiasek, 2009), recently found in two clades (Jones et al., 2013). This gene is a widely used tool to characterize denitrifying populations (Philippot et al., 2009; Philippot et al., 2011). Furthermore, genes can be identified and are widely used for the measurement of other processes within the N cycle, e.g. the gene *amoA* coding for the ammonia monooxygenase of the nitrification process, hzo coding for the hydrazine oxidoreductase (bacterial annamox) and *nrfA* coding for the nitrite reductase within the DNRA process.

Considering spatial effects across soil horizons it has been found that the first 20 cm of soil seems to be the most important (and most investigated) for bacterial structure and N

attenuation compared with other soil depths (Qin et al., 2014). Denitrifiers are common in the environment, as NO_3^- is a competitive terminal electron acceptor to O_2. Across time denitrifiers communities can show different (pulsing) activity patterns. The acknowledgment of these patterns of higher and lower activity has led to the hot spot concept (areas of high attenuation) with the concept of hot moments (Groffman et al., 2009). This highlighted the need to include the analysis of variation of N attenuation processes across hours and seasons (Regan et al., 2017). Analysing denitrification patterns, Philippot et al. (2009) found that the distribution of *16S rRNA* matched that of most genes involved in denitrification (*nirK*, *nosZ* and *napA*). Interestingly, *nirS* did not follow this relationship and has been hypothesized to be the result of a reduced diversity in *nirS*, making it more responsive. Hallin et al. (2009) found a similar result, where *nirS* correlated well with edaphic factors (i.e. NO_3^-, NH_3, pH, moisture and cattle presence). Therefore, *nirS* has been considered a useful indicator and target for denitrification. The ratio between genes can also further inform the potential denitrification activity (e.g. *nosZ*/*narG*) and process completeness (e.g. *nosZ*/*16S*) (Philippot et al., 2009).

Within an agricultural context, several studies have examined the impact of land use on denitrification activity, focusing on spatial and temporal variations of denitrification genes. However, few studies have looked at microbial biodiversity especially using modern sequencing techniques. Ramirez et al. (2010) found that microbial population between grassland and crop fields differed markedly, therefore highlighting the importance of land use on microbial populations. Whereas at small scales (0.03 – 6 m) autocorrelation of microbial structure seems to occur (Franklin and Mills 2003), at larger scales Fierer and Jackson (2006) found typical populations related to edaphic factors. Common features such as N inputs and land use cause a shift in multiple cross-correlating environmental parameters e.g. NO_3 concentration, C availability and concentration, soil moisture, O_2 and pH (Philippot et al., 2009). Therefore, it is difficult to separate how single factors affect denitrification. In an agricultural context, although artificial drainage systems are a major loss pathway for surplus N, they have been neglected in terms of microbiological studies and can be considered a relatively unexplored environment.

2.7 Knowledge gaps in the research

Any assessment of intensive dairy farm sustainability in the literature typically uses N balances and its components (measure of N input and output from an agricultural system) to ascertain if a site is sustainable or not and to identify possible N losses. This is a rather crude methodology and does not acknowledge that the soil/subsoil and bedrock continuum

underneath a farm can offer varied levels of attenuation capacity. This may lead to varied water quality issues. Such sites may also be artificially drained, which once again complicates this inherent natural attenuation capacity. Indeed land drainage design may also differ greatly and therefore such differences may alter the transformation and fate of N.

In addition to N balances, surpluses and release data, an artificial drainage system if characterised correctly(e.g. position, depth of installation, lateral extent and connectivity) and studied with additional techniques from the commonly used ones (physicochemical parameters, stable isotopes, dissolved gasses, molecular techniques) could give information about a large contribution area. A single end-of-pipe sample could provide "net" information pertaining to provenance, source-transformation and fate of N. A much clearer conceptual diagram of a dairy farm could be gathered by combining "net" information from end-of-pipe samples with groundwater data (from piezometer or multilevel borehole) and surface water/open ditch data. In addition to N balance data, multiple techniques (see Table 2.3) could be applied spatially and temporally to water samples for the assessment of provenance, source, transformation and fate of N. These data could then be used to rank dairy farms in terms of sustainability and give new insights into their future management and indeed comment on whether land drainage on such sites has an effect on this ranking.

Herein, Chapters 3 to 7 tested the following hypotheses:

Hypothesis Chapter 3 (Study 1):

- End of pipe water samples can be used to examine "net" provenance, source, transformation and fate of N of a large zone of contribution (ZOC) in a similar way to that of a water sample from a screened interval of a borehole or piezometer.
- Nitrogen species monitored at different depths of the soil/subsoil continuum may be disconnected and have varied N source, transformational processes and fate.

Hypotheses Chapter 4 (Study 2):

- Heavy textured sites differ in terms of their net denitrification capacity and this can be used to rank dairy farms in terms of sustainability.
- Shallow drainage designs affect net denitrification capacity to a greater extent than groundwater designs and this affects ranking of dairy farms in terms of sustainability.

Hypotheses Chapter 5 (Study 3):

- On farms with heterogeneous drainage classes the net denitrification capacity of many different soil/subsoil profiles can be used to rank a dairy farm in terms of sustainability, as this capacity changes both spatially and is also affected by land drainage.
- Different drainage classes of a single farm vary in terms of their net denitrification capacity and therefore can be ranked in terms of sustainability.

Hypotheses Chapter 6 (Study 4):

- The analyses of bacterial gene abundance for the N-cycle in water can improve our interpretation of sustainability over and above that given by isotope natural abundances, dissolved gases and biogeochemical parameters alone.
- The bacterial genes signal is distinctive for the mobile water phase across open ditch, end-of-pipe and groundwater (to 9 m depth) locations and it can further predict differences across sites in terms of sustainability and highlight most important pathways for attenuation
- The study of bacterial genes can be an environmental tool to inform intensive dairy farm sustainability.

Hypotheses Chapter 7 (Study 5):

- Intact core analysis using labelled fertiliser of N gaseous emissions, isotopic abundances and isotopomers can give further insights to and validate the influence of each process on N_2O production/consumption.
- On gley soils, different patterns of N_2O and N_2 emissions and transformation processes are created by different water contents simulated by the installation of a drainage system with pulses of N_2O and N_2 depending on different degrees of both nitrification and denitrification.
- Undrained or saturated conditions can mitigate N_2O fluxes and instead create ideal full denitrification conditions for N_2 fluxes.

A combination of techniques, itemised previously (physiochemical, gaseous, isotopic and microbiological analyses) was deployed across surface and subsurface sampling sites to explore these hypotheses with reference to the stated objectives (Section 1.5). Drainage systems were treated as a monitoring tool and used to deduce "net water attenuation" across soil drainage classes within their respective zone of contribution. These larger systems have a

single end of pipe sampling location but the pipe that feeds this sample may be several hundreds of meter long. Such a concept has never been examined in the literature in term of a multi-technique approach and it should be investigated further across different scales (i.e. controlled to real life scenarios) and across different soil drainage classes. Such information could examine the water attenuation function in terms of N fate across vast areas of the landscape that are drained and non-drained. Non-drained sections could be investigated through the use of multi-level piezometers or boreholes. Such information would be valuable before installation at the drainage design phase and for policy makers thinking to the future where certain soil functions would need to be prioritised on a national scale e.g. preference of soil sequestration function of soils in certain areas over production could instigate rewetting of such soils. These soils could also have a higher water attenuation function if left un-drained.

Chapter 3 - Investigating "Net" provenance, N source, transformation and fate within hydrologically isolated grassland plots

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Highlights:

- End-of-pipe and groundwater N concentration and flow give "net" flux data
- Isotopic signatures within these samples give "net" provenance/origin of the water
- Isotopic signatures also give "net" source-transformation for these samples
- Interpretation improves with dissolved gases and physiochemical parameters

3.1 Abstract

Agricultural landscapes contain many different soil types with heterogeneous nitrogen (N) attenuation capacity. Typically, a zone of contribution (ZOC) surrounding a borehole is used to interpret subsurface hydro-biogeochemical functional capacity. This presents a "net" interpretation of source and attenuation within these calculated areas. Herein, we use the concept of ZOC commonly used for borehole screen intervals but for an end-of-pipe location within four hydrologically isolated plots. Water samples from end-of-pipe and piezometer locations are examined for nitrogen (N), biogeochemical, dissolved gas and isotopic viewpoints to elucidate multi-layered "net" water provenance, N source, transformations and fate. Results showed a nitrate (NO_3^--N) plume migrating in shallow groundwater (between 0.39 and 8.07 mg N/L), with low concentrations in the shallow artificial drainage system (below 3.22 mg N/L). Water provenance data showed distinct signatures of: precipitation and deep groundwater at 3-4 m below ground level (bgl) and water entering, migrating and discharging at the end of pipe location. The latter signature was caused by enrichment of $\delta^{18}O$-H_2O during migration. This means there was disconnectivity on site with no interaction between water migrating through the drainage pipe at 1 m and deeper groundwater migrating at 3-4 m depth. The analysis of NO_3^--N concentration and its isotopic signature ($\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3) identified further connections between screen interval depths and an up-gradient organic point source with elevated NO_3^--N migrating at this depth and different transformation processes occurring at different depths. Temporally NO_3^--N concentrations at this depth have decreased over time. Fenton et al. (2009) documented an average of 7.5 (±4.5) mg N/L whereas Ibrahim et al. (2013) documented an average of 6.8 (±3.7) mg N/L at this depth. The point source was removed in 2006 and NO_3^--N concentration in the present study have further reduced to an average of 3.9 (±2.8) mg N/L. End-of-pipe data at 1 m bgl highlighted connectivity with the overlying plot and showed different water attenuation functionality than the deeper system. End-of-pipe locations clustered together along the denitrification line. This highlighted a consistency of signals across the four plots in terms of what occurs in the soil profile above the drain installation depth of 1 m. At 3-4 m bgl however, samples varied spatially showing inconsistency between the end-of-pipe locations and plots indicating the occurrence of different processes.

A fuller characterisation of dairy farm N sustainability can be deemed using the "net" provenance, N source, N transformation and fate methodology presented. Future work should investigate how drainage design (shallow and groundwater) affects N transformation and the

"net" concept developed herein should be rolled out to rank dairy farms in terms of their N attenuation capacity.

3.2 Introduction

Agricultural landscapes contain many different soil types with varied drainage classes. This spatial arrangement of soils presents a soil-scape of varying soil functional capacity. All soils perform a set of functions (i.e. water purification, carbon sequestration, nutrient cycling, production of food, fibre and fuel, habitat for biodiversity). However, they differ in terms of rate (Schulte et al., 2014). One such function is water attenuation, the ability to naturally reduce water contamination, which in terms of nitrogen (N) changes across drainage classes (Coyle et al., 2016). Poorly drained soils present highest capacity while free draining soils present the lowest capacity. Nitrate (NO_3^--N) lost from agricultural systems migrates and transforms along many different subsurface pathways. Once NO_3^--N leaches to a drainage system it migrates laterally and the transformation potential becomes reduced. This pathway has been well characterised in terms of NO_3^--N concentration and flow, which of course enables flux calculations (Skaggs et al., 1994; Kladivko et al., 2004; Tiemeyer et al., 2008, Zhao et al., 2016). Attenuation over time using flux is a tool used to infer natural attenuation (Fenton et al., 2009) but gives no insights into water origin, source of N or indeed what transformation processes are involved. Others have investigated different aspects such as spatial and temporal changes in N speciation (Ibrahim et al., 2013) and indirect emissions (Weymann et al., 2008).

On pasture, artificial drainage systems are installed in imperfect or poorly drained plots, potentially altering the inherent natural attenuation or water purification function in the immediate area of the drain installation (around the pipe, mole, gravel mole, gravel pack) thereby altering transformations within the zone of contribution (ZOC) drained by this system. As used within flux calculations a single sampling point, i.e. an end-of-pipe (EOP) location, can be therefore used to examine the functional capacity of this larger area. This concept has been already explored in groundwater systems using boreholes and associated ZOCs. Typically, subsurface hydro-biogeochemical functional areas (Gonzales-Inca et al., 2015) are difficult to delineate, with studies such as Jahangir et al. (2012a, b) or Rivas et al. (2017) relying on borehole networks to identify various factors that can be used to characterise the transformational potential of these subsurface environments. For example, Fenton et al. (2009) used similar techniques (boreholes and associated ZOCs) and identified a strong correlation between NO_3^--N concentration in shallow groundwater, distance from a

known point source and subsoil saturated hydraulic conductivity (k_s). This allows prediction of natural attenuation areas but gives no further insights into origin, N source or attenuation.

An artificial drainage system, if characterised correctly in terms of position, depth of installation and lateral extent and connectivity with a set of multi-techniques (physiochemical, dissolved gasses and isotopic analyses), could provide such insights and give more characterisation power above that of flux alone. Kellman (2005) investigated EOP water samples under controlled conditions to investigate $\delta^{15}N\text{-}NO_3^-$ signature differences between inorganic and organic inputs. Whereas the signature data was distinct to a particular known source it was demonstrated that isotopic fractionation did not alter source signatures. On another isolated site (the same as the present study), Ibrahim et al. (2013) investigated N speciation in runoff, artificial land drainage installed at 1 m depth and shallow groundwater at 3-4 m depth. Tracing N losses across rainfall events that study showed that N losses were higher in runoff and groundwater with lowest losses discharging from the subsurface drains. This pointed to a multi-layered system in terms of source connectivity and the present study investigates this further. Dissolved organic nitrogen (DON) dominated losses but dissolved inorganic N was more abundant in subsurface drains. Both studies did not characterise the provenance/origin of the water within the system or the transformational processes that resulted in particularly high or low N concentrations.

Natural isotopic techniques ($\delta D\text{-}H_2O$, $\delta^{18}O\text{-}H_2O$, $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$) can be used for the identification of water provenance, processes such as nitrification and denitrification and sources. This procedure can be carried out at a single moment, whereas N concentrations need to be taken over time. When combined with other methods these natural isotopic techniques, e.g. dissolved gas in water samples and biogeochemical parameters, could give greater insight into N source and transformational processes at a given site (Baily et al., 2011; Jahangir et al., 2012a; Pasten-Zapata et al., 2014; Wells et al., 2016). To our knowledge, the use of a combination of multiple techniques, e.g. physiochemical, dissolved gasses and isotopic analyses, has never been attempted under hydrologically isolated field conditions. Such controlled conditions enable a more defined interpretation of results and therefore present an opportunity to examine a) single EOP water samples as "net" provenance, source and transformation indicators for a large ZOC and b) single shallow groundwater samples from screen intervals as "net" provenance, source and transformation indicators for an associated ZOC Knowledge pertaining to on site, EOP and shallow groundwater NO_3-N flux has already been established in various publication (Fenton et al., 2009; Ibrahim et al., 2013). Therefore, the objectives of the present study using historic and additional fieldwork were to

utilise EOP and piezometer "Net" approaches across four isolated grassland plots in the South East of Ireland to 1) characterise N migration through the multi-layered site using dissolved gases, N species and biogeochemical parameters and 2) to characterise isotopic signatures of H_2O and NO_3^--N to elucidate the "net" provenance of water, source of N and the transformational processes and the interaction of fate of N on this multi-layered site.

3.3 Materials and methods

3.3.1 Site description

The site is located on the beef farm at the Teagasc, Johnstown Castle, Environmental Research Centre, Co. Wexford, SE Ireland (52˚17′36″ N, 6˚31′6″ W) (Fig. 3.1a). It has a cool maritime climate with mean annual precipitation of 1002 mm and annual temperature 9.6˚C (Ibrahim et al., 2013). The isolated plots (~4.2 ha in total, 2% slope) were installed in 2005 and contain six plots separated by 4 m deep open ditches that intersect shallow groundwater. To understand the overburden (soil and subsoil) lateral variations and thicknesses, an electromagnetic (EM) conductivity and resistivity survey was conducted on site in September 2009 (APEX Geoservices Ltd., IE) (Fig. 3.1b). For the EM survey, values ranged from 15 to 38 mS/m and were interpreted as > 26 mainly silt clay, 20 - 26 mainly gravelly clay and < 20 represent silty-clayey gravel lenses within the gravelly clay. This means that in Fig. 3.1b plots 1-2 are dominated by >20 (green, yellow red, heavier in terms of soil texture) with some < 20 (blue, lighter in texture and better drainage) whereas plots 3-4 are dominated by <20 (blue) with some > 20 (green). From a NO_3^--N distribution perspective this interpretation matches that of Fenton et al. (2009) where higher NO_3^--N concentration migrates in blue areas of Fig. 3.1b, which have a high k_s and a lower water attenuation capacity (i.e. natural ability of the soil to bioremediate contaminants, in this case NO_3^--N, depending upon hydrologic and biological factors). Plots 1 and 2 are grouped as poorly drained whereas plots 4 and 5 are grouped as imperfectly drained.

In terms of geology, the Cullenstown Formation is present on site except in the south-west where rocks of the Shelmaliere Formation are indicated. The Cullenstown Formation is described as grey-green metagreywacke and slate and ranges from 6.5 m to 16.5 m depth. The Geological Survey of Ireland subsoils map (GSI, 2018) indicates till derived from metamorphic rocks. A narrow strip of alluvium is indicated along the western boundary of the survey area and also along the stream valley to the south. The Geological Survey of Ireland vulnerability map for the area indicates that the groundwater vulnerability rating of the site is "High". The bedrock is listed as a locally important aquifer.

All plots had three installed piezometers at top, middle and bottom locations drilled to 4 m depth with a 1 m screen section (sample depth 3-4 m) at the bottom of each installation (plot 2 only middle and bottom due to damage at the top position) (Fig. 3.1). A herring bone drainage design (primary drain with side laterals 10 m spacing, with a single end of pipe discharge point, which can be sampled) installed at 1 m depth in each plot intercepted infiltrating water but also drained shallow groundwater when the water table raised above the position of the drains. An important aspect of the present study is to use isotopes to elucidate the origin of EOP water when it is sampled and to find out if it contains a distinct rainwater signal or a groundwater signal or indeed both. Due to the slope on site, the water table position was shallow at the bottom field position and deepened towards the top position. Each plot had 4 sampling locations a) three piezometer screened sampling points and b) a single EOP location that gave a composite of the entire paddock at 1 m depth. For the purposes of the present study objectives (September 2014), four paddocks were sampled as identified in Fig. 3.1a-b. These plots were not grazed and used for silage with N inputs of 358 kg N/ha (262 kg N/ha - inorganic fertilizer, 38 kg N/ha - organic fertiliser and 58 kg N/ha - feed concentrate) and N surpluses of 219 kg N/ha, calculated as the difference between inputs and outputs (123 kg N/ha milk plus 16 kg N/ha slurry) (data from 2014 annual farm balance). Fenton et al. (2009) calculated a ZOC from piezometer screen data on the present study site, which connected these monitoring points with a point source (a soiled water irrigation system) in an up-gradient field (Sand Hill). Excessive irrigation hydraulic loads promoted leaching of N, which was subsequently mineralised and currently migrates as NO_3-N in shallow groundwater underneath the isolated plots. For the drainage system ZOC, the lateral length of the drain multiplied by the spacing involved (10 m) was used. In the current study, this equated to a ZOC larger than the plots themselves i.e. plots 1, 2, 3 and 4 have a ZOC of 1.0, 0.9, 0.4 and 0.4 ha, respectively. This indicated that all infiltrating water within each plot discharged through EOP locations and the isolated nature of the plots (open ditches were deeper than the installation depth on all sides of each plot) ensured no up-gradient or lateral run-on. Therefore, the ZOC for each of the plots was the actual surface area of each plot down to 1 m depth.

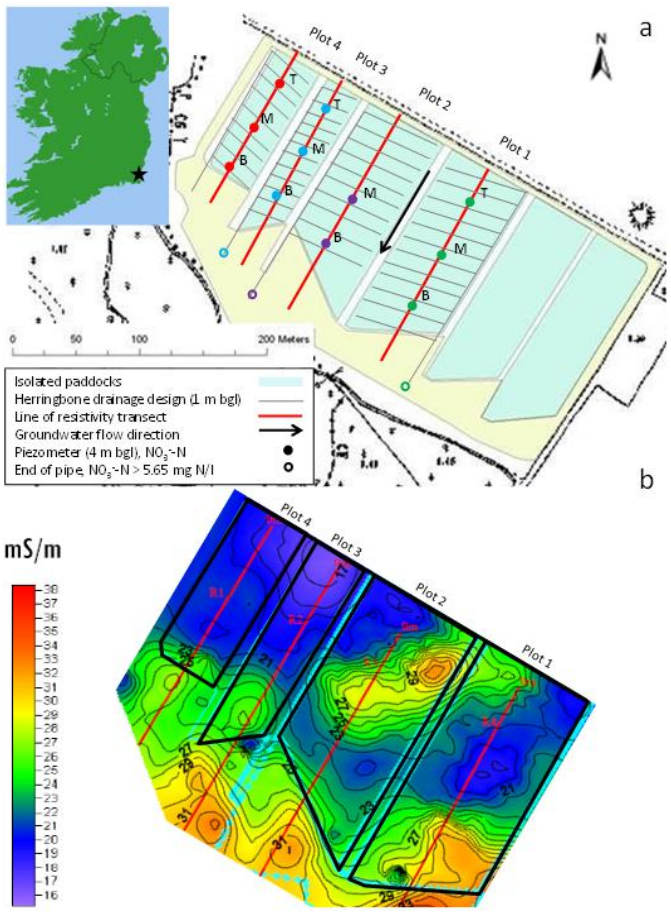

Fig. 3.1.a. Field site with sample locations (T: top, M: middle, B: bottom) (EOP: end of pipe) and b. Electromagnetic survey of the site with > 26 mS/m mainly silt clay, 20 – 26 mS/m mainly sandy gravelly clay and < 20 mS/m represent clayey sand gravel lenses within the gravelly clay. R1-4 represents resistivity lines on each of the plots and help with depth to bedrock measurement.

3.3.2 Data collection

Water samples were collected in October 2014. Samples from piezometers were collected using a bladder pump (flow rate of 100 ml/min) (Geotech Environmental Equipment, Inc., USA) as it minimised sample degassing (Jahangir et al., 2012a). A low-flow micro-purging protocol for piezometers was followed (CL:AIRE, 2008). Water samples from EOP locations were collected manually. Duplicate 50 ml water samples were collected in HDPE screw top bottles and filtered in field through 0.45 µm cellulose acetate filters (Sartorius Stedim Biotech GmbH, Germany). Samples were then stored at 4°C and analysed within 2 weeks from collection. Water table depths at top, middle and bottom locations in each plot were measured with an electronic dipper (Van Walt Ltd., Surrey, UK). An in-situ Multi-parameter Probe (In Situ Inc., USA) was used to measure pH, temperature (T), electrical conductivity (EC), turbidity (Turb.), dissolved oxygen (DO) and redox potential (Eh) in water samples.

3.3.3 Nutrient and biogeochemical parameters

Water samples were analysed at Teagasc Laboratories, Johnstown Castle for NO_2^--N, NH_4^+-N, Total Oxidised Nitrogen (TON) using an Aquakem 600 Discrete Analyser (Aquakem 600A, 01621 Vantaa, Finland). Concentrations of NO_3^--N were calculated by subtraction of NO_2^--N from TON (NO_3^--N + NO_2^--N). Total Nitrogen (TN) was determined by alkaline persulfate oxidation (Askew and Smith, 2005).

3.3.4 Dissolved gases

Dissolved gasses are an essential piece of information for the quantification of N-losses and evaluation and apportionment of the N-amount conveyed from the ZOC to the receptor water body by a drainage system. Dissolved N_2O and excess-N_2 are a valuable measurement to further understand N transformation processes (e.g. denitrification), their rate and completeness at a specific time and space point (e.g. ZOC of a piezometer) especially when in contraposition with isotopic data which measure these changes not as singular points but as the sum of what is occurring from deposition to end point.

Water samples were collected from each location for excess-N_2 quantification. The duplicate 12 ml exetainers (LabcoWycomb Ltd., UK) were sealed after overflow of 10 ml without headspace using double septum (butyl rubber and teflon) stoppers. The exetainers were transported in water-filled containers at groundwater temperature and stored at 4˚C submerged upside down in water to prevent gas diffusion across the septa. Samples were analysed within one week from collection. Excess-N_2 quantification was carried out through a high precision membrane inlet mass spectrometer (MIMS) (Pfeiffer Vacuum ^{TM}QMS 200 quadrupole mass spectrometer). The MIMS was set at the groundwater temperature of the time of sample collection (Kana et al., 1994) and was calibrated before the initial reading and after every 10 samples to correct analytical drift. Deionized water, previously equilibrated with air at constant temperature and pressure, was used as standard (Kana et al., 1994). Gaseous N_2 concentrations were calculated as per Weymann et al. (2008).

For the detection of dissolved N_2O, duplicate groundwater samples were collected in 160 ml serum bottles capped without headspace, after an overflow of 150 ml, with butyl rubber septa and aluminium crimp caps (Wheaton, USA) and stored as for N_2-excess samples. Within one week from collection, samples were degassed by simultaneous water extraction and addition of high purity helium (He:water 1:3; v/v) (BOC, Linde Group, Germany). A further 40 ml headspace was created (Lemon, 1981) and samples were agitated at 400 rpm (Gyrotory shaker G-10, New Brunswick Scientific, USA) for 5 minutes before being left to stand for 30

minutes. The gas in the headspace was then transferred into evacuated 12 ml exetainers. N_2O was quantified by auto-sampler gas chromatography (CP-3800, Varian Inc. USA) and final concentrations were calculated using Henry's Law for the ambient groundwater temperature. Indirect N_2O-N emission factor for groundwater was calculated as per Weymann et al. (2008) following the equation 3.1.

$$EF_5g(1) = (N_2O\text{-}N)/(N_2O\text{-}N + N_2\text{-}N + NH_4^+\text{-}N + NO_3^-\text{-}N + NO_2^-\text{-}N + DON) \quad \text{(Eqn. 3.1)}$$

3.3.5 Isotopes

For isotopic measurements at piezometer and EOP locations, water samples (40 ml) were collected, filtered in the field through 0.2 µm polyethersulfone filters (Sartorius Stedim Biotech GmbH, Germany), and stored at -20˚C in 50 ml polyethylene screw cap tubes. Within two months from collection, samples were analysed (Dept. of Catchment Hydrology, UFZ, Germany) for the isotopic composition of NO_3^- ($^{15/14}N$ and $^{18/16}O$) and H_2O ($^{2/1}H$ and $^{18/16}O$). On the day of EOP collection the water table in plot 4 remained under the drainage system (top: 2.93 m, middle: -1.52 m, bottom: -1.58 m) and therefore no sample was gained. Isotope values were reported in δ‰ relative to international standards (AIR for N and VSMOW (Vienna Standard Mean Ocean Water) for O and H). $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ were obtained through the bacterial denitrification method (McIlvin and Casciotti, 2011). Briefly, Pseudomonas chloraphis (ATCC #13985) was used to quantitatively produce N_2O from NO_3^-. The $\delta^{15}N$ and $\delta^{18}O$ composition of the produced N_2O was measured using mass spectrometry (DeltaPlus IR-MS) (method precision: ±0.3‰). For NO_3^-, triplicate international standards (IRMS-standard NO_3-1, IRMS-standard USGS-34, IRMS-standard USGS-35) and water blanks were used to calibrate results. Water $\delta^{18}O$ and δD (δ^2H) signatures for H_2O were analysed on a Los Gatos liquid water isotope analyser (analytical precision <0.15‰ for $\delta^{18}O$ and <0.5‰ for δD) using a 5x replicate analysis with discard of the first two samples. Normalisation to the VSMOW scale was based on replicate (20x) analysis of internal standards (MAST, PES and HAD, certified to Standard Light Antarctic Precipitation (SLAP) reference materials).

3.3.6 Statistics

Methods such as t-test, one way ANOVA and Tukey's HSD test (IBM SPSS Statistics version 24) were used to analyse differences between plots and between groundwater and EOP

samples and possible relationships between nutrient, isotopic and gaseous data and other variables.

3.4 Results and discussion

3.4.1 Nitrogen distribution, dissolved gases and water provenance

Due to the high N surpluses (219 kg N/ha) identified with the partial N balance, this site has the potential for high N leaching from fields to the drainage system and groundwater. Groundwater sampled within plot 1 showed increasing NO_3^--N values from top to bottom (T: 0.89, M: 1.90 and B: 2.55 mg N/L). Plot 2 (B: 0.39 mg N/L) contained lowest NO_3^--N concentrations. Plot 3 showed higher NO_3^--N concentrations (T: 5.34, M: 4.71 and B: 6.46 mg N/L) with one piezometer breaching the contamination threshold of 5.65 mg N/L (Daly, 2000). This threshold indicates the presence of a significant contamination but not pollution due to either inorganic fertiliser or an organic waste source. In plot 4, each piezometer showed values above the threshold (T: 6.31, M: 8.07 and B: 6.05 mg N/L). No sample was above the maximum admissible concentrations (MAC) of 11.3 mg NO_3^--N/l (EU, 2014a). From a temporal side, the average NO_3^--N concentration within groundwater has been declining over time. For example it was 7.5 (±4.5) mg N/L from 2006 to 2007 (Fenton et al., 2009), but a later study by Ibrahim et al. (2013) showed that this average concentration decreased further during the 2007-2008 sampling period to 6.8 (±3.7) mg NO_3^--N /L and continued to be low at EOP locations with an average of only 0.45 ± 0.63 mg N/L. Natural attenuation and removal of the point source in 2006 has enabled average groundwater concentrations to reach 3.9 (±2.8) mg N/L and 1.18 ± 1.78 mg N/L for EOP locations in the present study.

The water table position for top-middle-bottom locations for plots 1, 2, 3 were 2.2-1.1-2.3 m below ground level (bgl), n/a-0.45-0.85 m bgl and 2.8-1.5-0.8 respectively. This equates with the EM survey on the site where plot 2 contained heavier textured soils (with higher water attenuation function) and a corresponding shallow water table. Plot 2 showed a much greater groundwater interaction with the artificial drainage system than the other plots. End of pipe locations showed NO_3^--N value of 3.22 mg N/L - plot 1, 0.00 mg N/L - plot 2 and 0.31 mg N/L plot 3 (Table 3.1). In agreement with Ibrahim et al. (2013), the high concentrations in the groundwater did not express themselves at EOP locations which generally had lower values than the groundwater samples (i.e. from 0.02 to 1.34 mg N/L).

Table 3.1. In-situ parameters and N species concentration from samples collected in October 2014 from piezometer and end-of-pipe (EOP) locations at the site (temperature (T), electrical conductivity (EC), turbidity (Turb.), dissolved oxygen (DO) and redox potential (Eh), below ground water level (bgl)).

Sample	**Water table**	**pH**	**EC**	**Eh**	**DO**	**T**	**Dissolved N_2O**	**Excess-N_2**	**EF5g(1)**	**EF5g(2)**	**NH_4^+-N**	**NO_2^--N**	**NO_3^--N**
	(m bgl)		(mS)	(mV)	(mg/L)	(˚C)	(mg N/L)	(mg/L)			(mg N/L)	(mg N/L)	(mg N/L)
Plot 1 Top	2.24	6.3	239	1343	5.3	17.3	0.0035	0.0000	0.0020	0.0039	0.01	0.00	0.89
Plot 1 Middle	1.10	8.6	182	302	8.6	13.6	0.0405	0.2787	0.0098	0.0213	0.00	0.00	1.90
Plot 1 Bottom	2.30	6.4	178	1357	5.5	14.3	0.0365	0.9954	0.0060	0.0143	0.00	0.00	2.55
EOP 1	-	7.4	181	291	10.1	13.3	0.0049	0.0223	0.0008	0.0015	0.00	0.00	3.22
Plot 2 Middle	0.45	6.6	445	274	6.3	12.5	-	-	-	-	-	-	-
Plot 2 Bottom	0.85	7.2	279	274	5.2	13.1	0.0101	0.2872	0.0094	0.0261	0.00	0.00	0.39
EOP 2	-	7.1	511	1318	8.1	16.3	0.0013	0.1945	0.0057	0.3291	0.03	0.00	0.00
Plot 3 Top	2.83	7.3	155	313	8.2	12.7	0.0612	0.1417	0.0056	0.0115	0.00	0.00	5.34
Plot 3 Middle	1.50	6.2	161	1352	4.9	14.2	0.0648	0.4245	0.0065	0.0138	0.00	0.00	4.71
Plot 3 Bottom	0.86	6.2	192	136	5.3	14.4	0.0580	0.9836	0.0041	0.0090	0.00	0.00	6.46
EOP 3	-	7.4	172	244	10.2	13.1	0.0009	0.1067	0.0012	0.0029	0.00	0.00	0.31
Plot 4 Top	2.93	6.0	193	1340	7.4	17.9	0.0332	0.2041	0.0026	0.0053	0.00	0.00	6.31
Plot 4 Middle	1.52	7.1	310	1352	6.2	15.2	0.0739	0.0000	0.0046	0.0092	0.00	0.00	8.07
Plot 4 Bottom	1.58	6.9	216	1349	5.7	15.8	0.0449	0.3447	0.0036	0.0074	0.00	0.00	6.05
EOP 4	-	-	-	-	-	-	-	-	-	-	-	-	-

Dissolved gas data aids with "net" transformation interpretation and indeed indirect emissions relate to the fate of N. Groundwater was characterised by dissolved N_2O values in the range of 0.0035 and 0.0739 mg N/L while excess-N_2 ranged from 0.1417 to 0.9954 mg N/L. Values for $EF_5g(1)$ ranged from 0.0098 to 0.0020 ($EF_5g(2)$: 0.0261 to 0.0039) with only one shallow groundwater location showing values below 0.0025 (IPCC set default value for groundwater N_2O emission) (IPCC, 2006) (Table 3.1).

Dissolved N_2O average (0.0427 mg N/L) was higher than previous values recorded on another site within the same farm (0.024 mg N/L (Jahangir et al., 2013a) while excess-N_2 was lower (0.4575 vs. 2.28 mg N/l). Plots 1 and 2 showed lower ($p = 0.01$) dissolved N_2O (av. 0.0227 mg N/L) when compared with plots 3 and 4 (showing sign of contamination) (av. 0.0560 mg N/L). Excess-N_2 on plots 1 and 2 averaged 0.5204 mg N/L slightly higher than plots 3 and 4 (av. 0.4197 mg N/L). Plots 1-2 showed similar "total emissions" (dissolved N_2O + excess-N_2) to plots 3-4 (0.4130 and 0.4097 mg N/L, respectively). However, plots 3 and 4 showed higher values of $N_2O/(N_2O+N_2)$ which might indicate a higher component of incomplete denitrification. End-of-pipe locations showed dissolved N_2O values between 0.0009 and 0.0049 mg N/L while for excess-N_2 between 0.0223 and 0.1945 mg N/L. End-of-pipe samples in plot 3 showed $EF_5g(1)$ values above the IPCC standard while these were below the standard in plots 1 and 2.

In terms of "net" origin of water in samples, groundwater piezometer samples exhibited variability across the site with H_2O stable isotopes ranging from -3.2 and -8.5‰ for $\delta^{18}O$-H_2O and from -33.4 and -41.5‰ for δD-H_2O (Fig. 3.2). Samples within groundwater showed a signature consistent with the high-humidity climate for the British Isles ($\delta^{18}O$-H_2O from -8.5 to -5, δD-H_2O from -30 to -55; specifically for the Wexford region $\delta^{18}O$-H_2O from -6.5 to -5.5, δD-H_2O from -35 to -45) (Darling et al., 2003). This means that precipitation (Irish long-term weighted mean for precipitation: -5.5 for $\delta^{18}O$-H_2O and -36 for δD-H_2O) and groundwater at 3-4 m have a distinctive signal. Water entering the drainage pipe (a mix of shallow groundwater surrounding the pipe within the ZOC and infiltrating water from the soil profile above) has a second distinctive signal that can be seen in the depleted values for $\delta^{18}O$-H_2O within plot 2 and 3. Groundwater has been found to generally reflect a rainfall signature. However, evaporation from soil and "surface detention storage" can produce evaporative enrichment and local evaporation lines (Gibson et al., 2005; Kim and Lee, 2011; Klaus et al., 2015). In Fig. 3.2, two out of three EOP samples show a shift towards the left i.e. enrichment. This enrichment could be due to migration from deposition to the EOP location with possible evaporation from soil and within the drainage system itself. The EOP samples from plot 1

however show a common signature with groundwater samples that could be signifying a reduction of evaporation or a higher interaction with groundwater within this field and/or pipe. The drainage design was therefore insufficient to control the water table below the drainage systems for all locations in the field.

Ibrahim et al. (2013) noticed a reduction in terms of NO_3^--N concentration from groundwater to EOP samples at this same site. Here both the H_2O signature and NO_3^--N concentration data are highlighting disconnectivity with subsurface transfer pathways and dissolved reactive N (N_r) migration pathways on the site potentially are as follows: 1) infiltration of rainwater directly to the drainage system, 2) recharge of infiltrating water from the plot which does not go into the drainage system but recharges to shallow groundwater and 3) groundwater that interacts with the drainage system as the water table rises and could come from an offsite location and/or the paddock. However, shallow groundwater at 3-4 m depth (piezometer samples - 1 m screen interval) is not likely to interact with the drainage system at 1 m depth due to the heavy texture of the soil. Only groundwater around the drainage pipe ends up as a part of the end-of-pipe sample. This gives a multi-layered system that exhibits disconnectivity on site between a) a nitrate plume associated with an up-gradient source migrating at depth and b) low levels of leached N or high attenuation capacity above the drainage system within the isolated paddock thereby resulting in unpolluted water discharging from the end-of-pipe location.

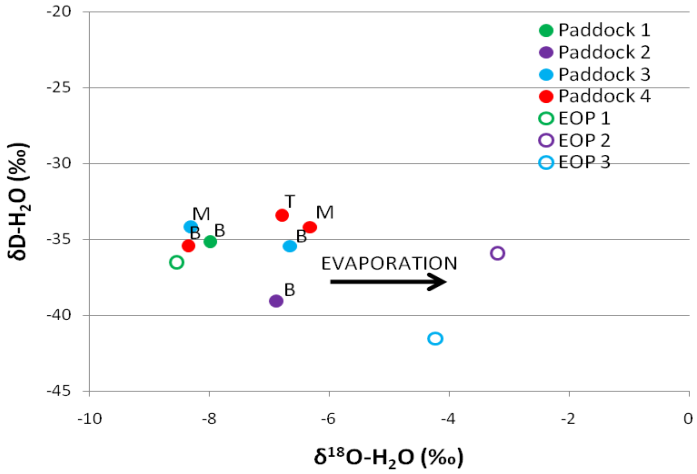

Fig. 3.2. $\delta^{18}O$ versus δD-H_2O values for samples collected within the four plots. Groundwater samples are indicated by the full circle, End-of-pipe samples (EOP) are indicated by the empty circles.

3.4.2 N source and transformation processes

NO_3^--N isotopic signatures were determined by the combination of N-sources and transformational processes affecting the original pool of NO_3^--N (Xue et al., 2009). Shallow groundwater and EOP samples from the four plots, showed values between the ranges typically associated with a manure/sewage source (Kendall, 1998). The occurrence of specific processes is characterised by specific fractionation factors which leads to the creation of specific signatures within the residual pools. Since organisms preferentially use lighter isotopes, the microbiological process cause an enrichment of heavy isotopes in the remaining source pool, with a depletion in the product signature (Kendall, 1998). Ranges are known for several N sources and processes (e.g. denitrification and nitrification) and a recent review has summarised these for agricultural areas (Nikolenko et al., 2017). Specifically, it was highlighted that when denitrification occurs, this process follows a linear trend generally a (1:1 - 1:2 trend) (Granger et al., 2008, Granger and Wankel, 2016, Hernandez-del Amo, et al. 2018). Both piezometers and EOP locations showed mainly a pattern clustering along a 1:1 - 1:2 line indicating an influence of denitrification in the transformation of NO_3^--N (Fig. 3.3). This was consistent with the study of Baily et al. (2011) on a neighbouring dairy site and was consistent across seasons and in terms of the source. The absence of NH_4^+-N contamination within the field seems to exclude dissimilatory nitrate reduction to ammonium (DNRA, process that reduces NO_3^--N to NO_2^--N and then to NH_4^+-N). However, distinguishing between denitrification and DNRA is difficult as the isotope effect of DNRA has still not been investigated (Alkhatib et al., 2012, Wells et al., 2016).

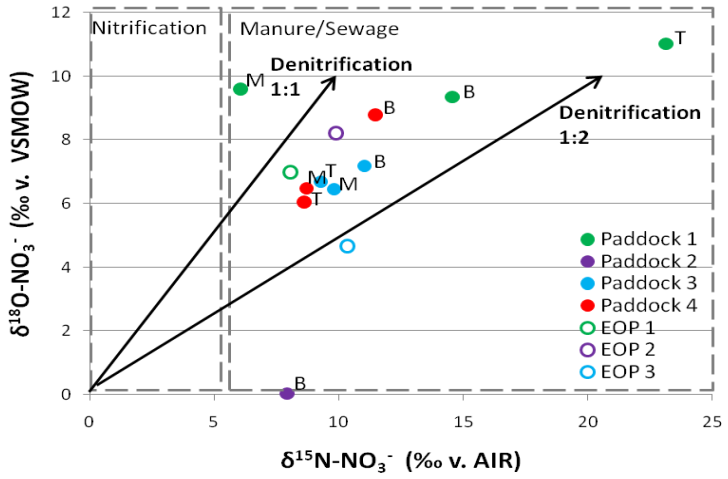

Fig. 3.3. $\delta^{18}O$ versus $\delta^{15}N$-NO_3^- values for samples collected within the four plots. Also showing 1:1 and 1:2 denitrification slope and $\delta^{18}O$ and $\delta^{15}N$ ranges for N-sources (after Kendall, 1998).

The NO_3-N isotopic composition showed low spatial variability. Shallow groundwater average δ^{15}N-NO_3^- was 11.1‰ (maximum: 23.2‰, minimum: 6.0‰) whereas average δ^{18}O-NO_3^- was 7.2‰ (maximum: 11.0‰, minimum: 0.0‰). Plots 1-2 showed larger spatial variability, whereas Plots 3-4 showed less variability with data clustering as in Fig. 3.3. End-of-pipe locations had average δ^{15}N-NO_3- of 9.4 ± 1.2 and 6.6 ± 1.8 for δ^{18}O-NO_3^- (Fig. 3.3). End-of-pipe locations shared the same transformation signature as for groundwater from plots 3 and 4 (Fig. 3.3). This indicated a consistency of signal across all four plots in terms of which microbial process occurred within the soil profile above the drain installation depth of 1 m. The inconsistency of signal within the piezometer monitoring system for plots 1-2 i.e. high spatial variation and no indication of clustering along the denitrification line; indicates the occurrence of a different or a mix of processes (e.g. nitrification, DNRA, anammox).

Fenton et al (2009) identified k_s, as one of the main explanatory parameters for NO_3^--N concentration, suggesting that lower k_s equates with higher attenuation capacity and therefore low concentrations of NO_3-N. Therefore, k_s areas present lower attenuation areas and therefore give rise to higher NO_3-N concentrations in groundwater. Herein, groundwater samples, which clustered along the denitrification line, had a higher enrichment of both δ^{15}N-NO_3^- and δ^{18}O-NO_3^-, matching lower k_s areas (Fig. 3.4). As k_s and NO_3^- enrichment correlate well, NO_3^--N fractionation shows potential to be used to identify denitrification hotspots within agricultural areas.

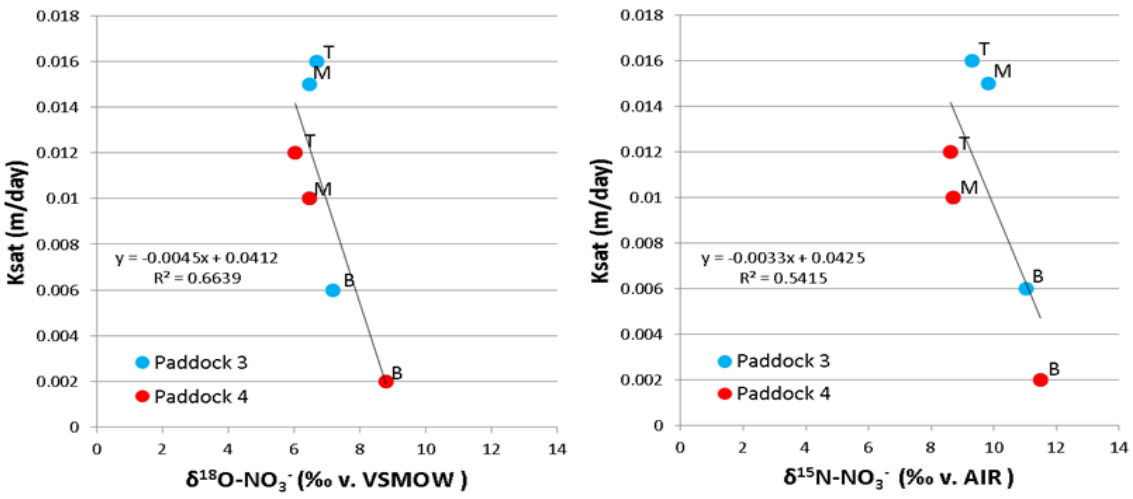

Fig. 3.4. Graphs showing the correlations of δ^{18}O and δ_{15}N-NO_3^- values vs. k_s.

As can be seen from the present study, in addition to N flux, EOP water samples can also provide information to elucidate "net" water origin, N source and give further insights into N

transformational processes. When combining data from different monitoring networks at the same site data can be used to investigate the connectivity or indeed disconnectivity as found in the present study between these two monitoring depths. At the present site dissolved gas in EOP samples allowed for greater interpretation of shallow subsurface "net denitrification" processes above and around a drainage system. The classification of an artificial drainage system, location and design (type, depth and spacing), and their study in terms of physiochemical, gaseous and isotopic parameters, are essential to understand the fate of N and guide future installation and management. Future work should consider the different types of land drainage design including shallow disruptive techniques and their role in the water attenuation function of soils on agricultural landscapes. A multi-level piezometer network could then be used to compliment such interpretations for all other areas and depths outside the influence of the drainage system. Indeed the open ditch network should be investigated further as part of this system. Broadening out this type of N characterisation across dairy farms in specific geographical locations with specific rainfall and soil conditions (e.g. poorly drained soils) could enable the ranking of farms based on their N attenuation capacity. This would aid specific components of water quality sustainability on these sites.

3.5 Conclusions

The concept of flux is well established using concentration and flow data from land drainage EOP or borehole screen intervals. However, this data tells very little about the "net" origin of a water sample, the source of N in that sample or indeed the transformational processes responsible for the N concentrations in that sample. This study showed that collating isotopic, dissolved gas and biophysical data from EOP and groundwater locations creates a clearer conceptual model of a site. Water origin results indicated disconnectivity between the two sample depths studied. Groundwater at 3-4 m depth was connected with an up-gradient dairy soiled water irrigation point source with elevated nitrate concentrations migrating at this depth. End-of-pipe water at 1 m depth had low nitrate concentrations. Multi-techniques highlighted connectivity with the overlying plot with a different water attenuation functionality than the deeper system. Denitrification was the main process of attenuation which was correlated with subsoil k_S. Land drainage systems in connection with a multi-technique analysis can be used to examine the water attenuation function of soils over larger areas. Future work should investigate how drainage system design (e.g. shallow and groundwater) affects N transformation and this method should be broadened to rank commercial dairy farms in terms of their N attenuation capacity.

Within this Chapter 3 the hypotheses created in Chapter 2 were met. End of pipe water samples were used to examine "net" provenance, source, transformation and fate of N of a large zone of contribution (ZOC) in a similar way to that of a water sample from a screened interval of a borehole or piezometer. Nitrogen species were monitored at different depths of the soil/subsoil continuum and showed: a) disconnection between drainage system (1 m bgl) and piezometers (3-4 m bgl), b) these two depth showed a varied origin of N source. Transformational processes and fate differ in process type and grate of attenuation.

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Chapter 4 - Influence of artificial drainage system design on the nitrogen attenuation potential of gley soils: Evidence from hydrochemical and isotope studies under field-scale conditions

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Highlights:

- N attenuation capacity is altered by drainage installation and design
- Groundwater drainage systems maintain higher attenuation than shallow systems
- Isotopic measurements provide quantified data for net denitrification
- Net denitrification is an efficient monitoring tool to rank farm sustainability

4.1 Abstract

In North Atlantic Europe intensive dairy farms have a low nitrogen (N) use efficiency, with high N surpluses often negatively affecting water quality. Low feed input systems on heavy textured soils often need artificial drainage to utilise low cost grassland and remain profitable. Heavy textured soils have high but variable N attenuation potential, due to soil heterogeneity. Furthermore, drainage system design can influence the potential for N attenuation and subsequent N loadings in waters receiving drainage from such soils. The present study utilises end of pipe, open ditch and shallow groundwater sampling points across five sites in SW Ireland to compare and rank sites based on N surplus, water quality and "net denitrification", and to develop a conceptual framework for the improved management of heavy textured dairy sites to inform water quality N sustainability. This includes both drainage design and "net denitrification" criterion, as developed within this study. N surplus ranged from 211 to 292 kg N/ha (mean of 252 kg N/ha) with a common source of organic N across all locations. The predicted soil organic matter (SOM) N release potential from top-subsoil layers was high, ranging from 115 to >146 kg N/ha. Stable isotopes analyses showed spatial variation in the extent of specific N-biotransformation processes, according to drainage location and design. Across all sites, nitrate (NO_3^--N) was converted to ammonium (NH_4^+-N), which migrated offsite through open ditch and shallow groundwater pathways. Using the ensemble data the potential for soil N attenuation could be discriminated by 3 distinct groups reflecting the relative dominance of in situ N-biotransformation processes deduced from water composition: Group 1 (2 farms, ranked with high sustainability, NH_4^+-N < 0.23 mg N/l, $\delta^{15}N\text{-}NO_3^-$ > 5‰ and $\delta^{18}O\text{-}NO_3^-$ > 10‰), low NH_4^+-N concentration coupled with a high denitrification potential; Group 2 (1 farm with moderate sustainability, NH_4^+-N < 0.23 mg N/l, $\delta^{15}N\text{-}NO_3$- < 8‰ and $\delta^{18}O\text{-}NO_3^-$ < 8‰), low NH_4^+-N concentration with a high nitrification potential and a small component of complete denitrification; Group 3 (2 farms, ranked with low sustainability, NH_4^+-N > 0.23 mg N/l, 14‰ > $\delta^{15}N\text{-}NO_3^-$ > 5‰ and 25‰ > $\delta^{18}O\text{-}NO_3^-$ > -2‰), high NH_4^+-N concentration due to low denitrification. The installation of a shallow drainage system (e.g. mole or gravel moles at 0.4 m depth) reduced the "net denitrification" ranking of a site, leading to water quality issues. From this detailed work an N sustainability tool for any site, which presents the relationship between drainage class, drainage design (if present), completeness of denitrification, rate of denitrification and NH_4^+-N attenuation was developed. This tool allows a comparison or ranking of sites in terms of their N sustainability. The tool can also be used pre-land drainage and presents the

consequences of future artificial land drainage on water quality and gaseous emissions at a given site.

4.2 Introduction

Global food demand is expected to increase by 100% by 2050 (Tilman et al., 2002; Godfray et al., 2010). The need for higher yields, in order to sustain a growing population, has fuelled fears that achievement of worldwide production targets will be at the expense of water and air quality targets (Mosier et al., 1998; Foster, 2000; Lesschen et al., 2011). The ambition for sustainable food production implies that increased productivity must be carefully managed to reduce negative externalities, such as impacts on soil and water quality, increased greenhouse gas emissions and reduction in habitat biodiversity (Schulte et al., 2014).

Agricultural landscapes are typically heterogeneous, in which soils have various important functions and capabilities supporting the in situ transformation of nutrients such as N. For example, soil texture can influence N attenuation and typically heavier textured gley soils have optimal conditions for N-biotransformation pro- cesses such as denitrification, which reduces NO_3^--N to N_2O and N_2 (Saggar et al., 2013). Artificial land drainage, as a tool to manage water table levels and reduce the duration of soil saturation, plays an important role in improving crop yields and maintaining on- farm profitability but drainage system design can influence the potential for N attenuation and subsequent N loadings in waters receiving drainage discharge from such soils. In an 11 year study in Denmark, Ernstsen et al. (2015) found varied N-fluxes from tile drains (depth: 1.1 m bgl, spacing: 10-20 m) installed in heavy textured clay tills, inferring natural attenuation or "net denitrification" gradient across sites due to site-specific hydrological set- tings (e.g. watertable elevation, length and intensity of the drainage) and crop cover.

Gley soils are either surface water gleys (fed by surface rainfall, where relatively impermeable horizons impede drainage causing periodic or permanent wetness), or groundwater gleys (wherein the substrata is seasonally or permanently wet and affected by free groundwater) (Thomasson, 1975). However, clay loam pseudo-gley soils are typically unprofitable due to annual grass yield deficits of 3e31% when subjected to continuous saturation (e.g. watertable of 0 m bgl) rather than at lower saturation (1.15 m bgl) (Mulqueen, 1985) and require the installation of artificial land drainage systems to increase the soil profile permeability as a management measure to improve their productivity. The fundamental aim of land drainage is to remove excess groundwater, thus lowering the water table and reducing the period of waterlogging (Armstrong and Garwood, 1991; Nijland et al.,

2005). This provides suitable conditions for the cultivation, growth and harvesting of a crop. The design of land drainage entails the specification and installation of drains in the soil at such a depth and spacing to control the water table at a predetermined depth below ground level under a particular intensity of rainfall (Mulqueen, 1998). Various techniques have been developed to suit different soil types and conditions with associated drainage characteristics, with this end in mind. The type of drainage system installed could potentially alter the natural attenuation or "net denitrification" of a soil profile by modification of the soil water saturation and drainage characteristics (e.g. rate, permanence time, by-pass of the soil layers).

On dairy farms N originates from inorganic or organic fertilizer (e.g. cattle slurry and soiled water), with potential ammonium (NH_4^+-N) and/or nitrate (NO_3^--N) losses along surface or leached pathways. These two N-species are the main substrate for N-biotransformation processes (i.e. denitrification, nitrification, anaerobic ammonium oxidation (anammox) and dissimilatory nitrate reduction to ammonium (DNRA)), which can lead to the production of nitrous oxide (N_2O), a potent greenhouse gas, and di- nitrogen gas (N_2), effectively removing reactive N from biological cycling (Rütting et al., 2011; Burgin et al., 2013) (Fig.4.1). Most studies still consider NO_3^--N the main species for N losses and focus attention only on denitrification when addressing sustainability targets and land use (e.g. Coyle et al., 2016). Soil type and physicochemical properties are generally the main factors which define the soil microbial community structure, with the first 20 cm of soil being the most important (and most investigated) in shaping the bacterial community of the underlying groundwater (Qin et al., 2014).

While it is well documented that land drainage can circumvent the N attenuation capacity of a soil, leading to nutrient losses (Skaggs et al., 1994; Billy et al., 2013), the effect of drainage system design on soil function, N-biotransformation processes and N-cycling "hotspots" is poorly understood. Poorly-drained soils amended with fertilizer can result in high N-losses, via increased N_2O emissions, due to favourable conditions for denitrification and a high NO_3^--N content (Nash et al., 2012). Periods of extended saturation support denitrification by retaining the substrate for longer, favouring complete reduction to N_2 (Bergsma et al., 2002).

Combining chemical analysis of drainage water samples with stable isotope characterisation of N-species (e.g. NO_3^--N and N_2O concentrations and isotopes and excess-N_2) provides a convenient and effective approach to understand the complex interactions within the soil N-cycle of an agricultural system and the relation- ship with the drainage system.

The analysis of these N species (concentrations) in soil pore water and gas can indicate system outputs, e.g. total biological N_2 production and dissolved N_2O, but cannot distinguish

between production processes, which could include (anammox, DNRA, nitrification and denitrification) (Jahangir et al., 2012a, 2012b and 2013). Therefore these complementary analytical techniques must be used simultaneously to gain a full understanding of N-biotransformation in soils. Stable isotope analysis (e.g. quantification of $\delta^{15}N$ and $\delta^{18}O$) has been widely used to deduce sources, biotransformation processes and rates of turnover for NO_3^--N in soil environments (Smith and Kellman, 2011; Pasten-Zapata et al., 2014; Snider et al., 2015; Wells et al., 2016). However, N-biotransformation processes which do not originate with NO_3^--N (nitrifier-denitrification and anammox) can be overlooked even though they produce N_2O and N_2.

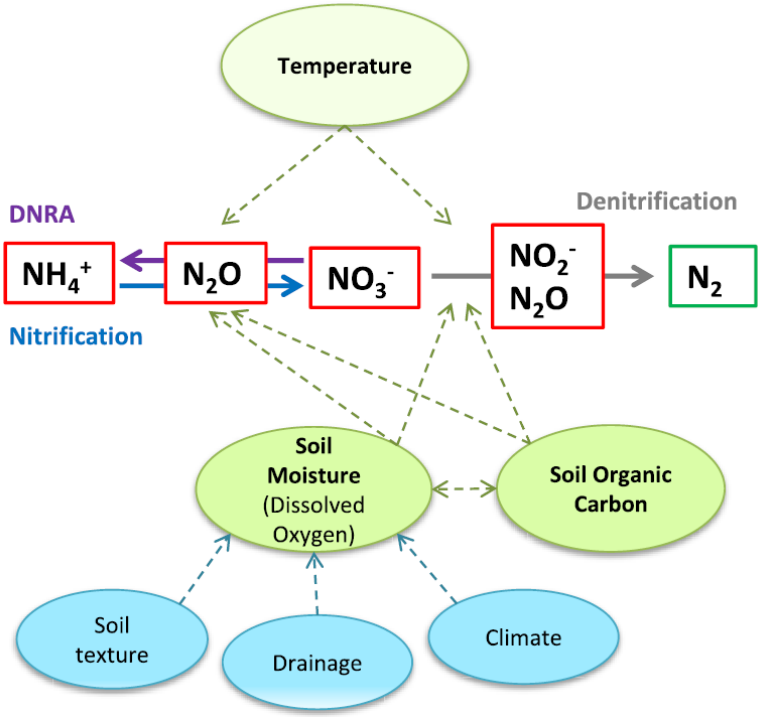

Fig. 4.1. Key parameters affecting N attenuation and speciation in soil and groundwater. Red boxes represent all species that might be lost causing the deterioration of water quality; Green box represent a favourable outcome; green circles represent proximal factors affecting these processes; Blue circles represent distal factors (from Coyle et al., 2016).

Further studies are needed to understand the relationship between the design of artificial land drainage systems and the N-attenuation potential of host gley soils. This must encompass the characterisation of the hydraulic connectivity of an agricultural system, its hydrochemistry, gas and isotopic signature in order to identify which factors control the spatial distribution of N biotransformation potential across agricultural landscapes, and the N release to the drainage waters and environment (Baggs and Philippot, 2010; Bednorz et al., 2016). Therefore, the objectives of the present study utilising end of pipe, open ditch and shallow groundwater sampling points across five sites in the southwest of Ireland were to: a) compare

and rank sites based on N surplus, water quality and "net denitrification", and b) develop a conceptual framework for the management of heavy textured dairy sites, which includes the results of the present site and the literature, to inform water quality N sustainability.

4.3 Materials and methods

4.3.1 Study sites

Five permanent grassland sites were selected in SW Ireland as part of the Teagasc Heavy Soils Programme (HSP): Kishkeam (KM), Doonbeg (DG), Castleisland (CD), Athea (AA) and Rossmore (RE). Before drainage installation, each site was soil mapped at 1:25,000 scale and divided into surface and groundwater gleys. At each site a site assessment including excavation of soil profiles and examination of the soil profile was conducted. Then various soil horizons were sampled and a drainage design was constructed, including drain spacing, depth of installations, materials to be used. A bespoke artificial drainage system was installed in a paddock at each site, comprising either a shallow drainage design or a groundwater drainage design (Tuohy et al., 2016). To compare sites in terms of soil and drainage design specification consult Table 4.1. The layout and location of the sites are presented in Fig. 4.2, replication within plots is achieved by the presence of multiple sampling points (end-of pipes).

Individual meteorological stations (Campbell Scientific Ltd., Loughborough, U.K.) were installed at all locations to estimate and compare a water balance for each site. Average daily rainfall (mm), wind speed and hours of sunshine were used in the hybrid soil moisture deficit (SMD) grassland model of Schulte et al. (2005) to estimate a daily effective drainage (ED, mm) value (Table 4.1).

Farm N balances (2015) were calculated following the methodology of Treacy et al. (2008), which utilises stocking rate, N inputs (chemical and organic fertilisers), concentrate feed (volume and composition) and milk production (volume and composition).

Milking was conducted at 07.30 h each morning and 15.30 h each evening. Milk yield per cow (kg) was recorded at each milking. Milk composition (fat, protein and lactose concentrations) for each cow was measured twice fortnightly on a successive morning and evening milking using a Milkoscan 203 (Foss Electric DK-3400, Hillerød, Denmark) following normal quality controls protocols. Solids corrected milk yield was calculated using the equation of Tyrell and Reid (1965). The N value in concentrates fed, and in milk

produced is an average across the farm while the N (fertilizer plus slurry) is in one paddock only (the drained paddock).

Estimated N release was calculated from soil organic matter (SOM) for each soil horizon of every farm (Brookside Laboratories Inc. OH, USA) (Pastor and Binkley, 1998). This is a computed estimate of the N that may be released annually through OM decomposition. The calculation is based on the loss on ignition method at 360 °C (Schulte and Hopkins, 1996).

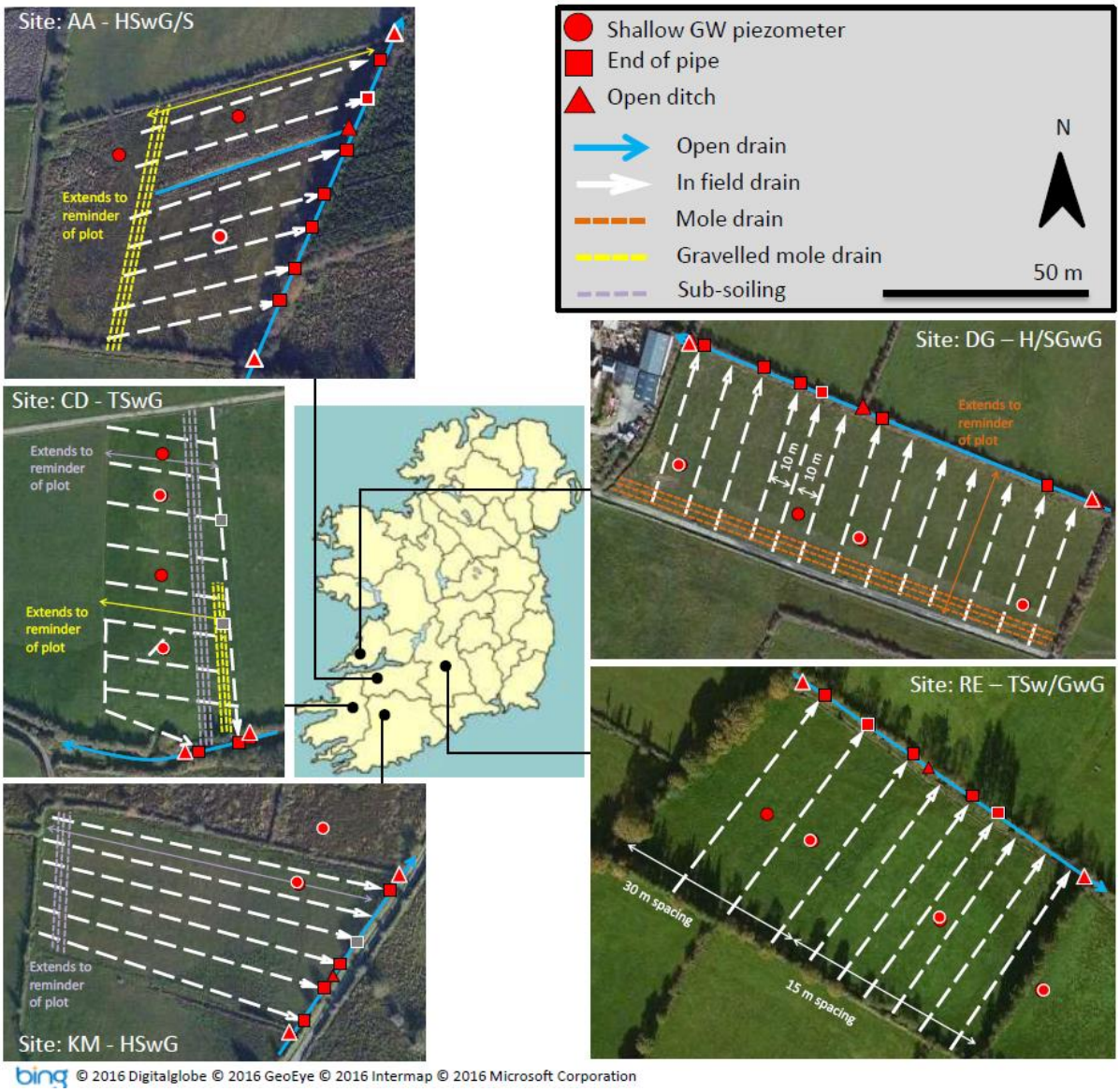

Fig. 4.2. Site locations, drainage design layouts (details are in Table 4.1) and sampling positions at end of pipe, open ditch and shallow groundwater (GW) piezometer locations. Symbols with a white outline indicate location in common between monthly sampling (Table S4.1) and Oct-Nov 2015 sampling (Table 4.3). Grey symbols with a white outline indicate location of monthly sampling but not for Oct-Nov 2015.

4.3.2 Water samples

Monthly water samples were taken from shallow groundwater piezometers, end-of-pipe, and open ditch locations (Fig. 4.2) from August 2015 to August 2016. Additional fieldwork was conducted between October and November 2015. The end-of-pipe samples give a "net" representation of water provenance, source, N-transformation processes over their entire length and zone of contribution. The zone of contribution of each paddock was calculated by multiplying the length of the piped drainage system for the spacing of the system. This equated approximately to 1.4, 1.7, 2.4, 1.7 and 1.1 ha for KM, AA, CD, RE and DG, respectively. In addition, due to the high number of drainage sections installed and presence of open ditches, these paddocks can be considered isolated from horizontal water flow from adjacent paddocks. The drainage water is therefore representative of the superficial layers only of the paddocks studied.

Open ditch water samples represent water from the drained paddock and other areas of the farm. Shallow piezometers were installed to different depths (see Table 4.1) at various locations (Fig. 4.2) to measure continuous water table depth (electronic dipper, Van Walt Ltd., Surrey, UK). Shallow groundwater samples (Fig. 4.2) were collected using low-flow micro-purging of the piezometers, following standard protocols (CL:AIRE, 2008). A peristaltic pump (Model 410, Solinst Canada Ltd.) fitted with Teflon outlet tubing (Ø 0.6 cm) was used to collect these water samples. End-of-pipe and open ditchwater samples were collected in duplicate (50 ml, HDPE screw top bottles). One replicate was filtered in the field through 0.45 mm cellulose acetate filters (total recoverable vs. dissolved analytes) (Sartorius Stedim Biotech GmbH, Germany). A Multi-parameter Probe (In Situ Inc., USA) was used to measure pH, temperature (T), electrical conductivity (EC), turbidity (Turb.), dissolved oxygen (DO) and redox potential (Eh) of each water sample.

Water quality maximum admissible concentrations (MAC) provided within the EU WFD were used as baseline threshold values to identify N impacts. It should be noted that some of these MACs are for surface water or drinking water and therefore are not necessary applicable to land drainage discharges. However, this approach provides a consistent basis to compare water quality data for the different samples, given the emphasis on deducing potential impacts to receiving waters. For N species MAC were for NO_3^--N (surface drinking water): 11.3 mg NO_3^--N/l MAC (OJEC, 2006; EU, 2014a); NO_2^--N: 0.15 mg NO_2^--N/l (EU, 2014a), NH_4^+-N: 0.23 mg NH_4^+-N/l (EU, 2014a). MAC for other chemical parameters were 12 mg/l for potassium (K^+), 2.2 mg/l for dissolved reactive phosphorus (P) and 250 mg/l for chloride (Cl^-) (all surface water standards (EC, 1998; EU, 2014a)). Additional thresholds

have been further highlighted to assess the degree of contamination of groundwater (and therefore are drinking water standards) and are indicative of early signs of contamination. These concentration limits are: 4 mg/l for K^+, 25 mg/l for Cl^-, 0.4 mg/l for potassium and sodium ratio (K/ Na), 0.1 mg/l for P and 5.65 mg NO_3^--N/l for NO_3^--N (organic contamination limit) (Daly, 2000; OECD, 2001).

All water samples were analysed for NO_2^--N, NH_4^+-N, Total Oxidised Nitrogen (TON) and Cl^- using an Aquakem 600 Discrete Analyser (Aquakem 600A, 01621 Vantaa, Finland). Method detection limits (MDL) were 0.006 mg/l, 0.05 mg/l, 0.25 mg/l and 0.8 mg/ l, respectively. Concentrations of NO_3^--N were calculated by subtraction of NO_2^--N from TON (NO_3^--N + NO_2^--N). Total Nitrogen (TN) was determined by alkaline persulfate oxidation (Askew and Smith, 2005). Dissolved Ca^{2+}, Cu^{2+}, Fe^{2+}, K^+, Mg^{2+}, Mn^{2+}, Na^+ and Zn^+ were quantified by inductively coupled plasma spectrophotometer ICP-OES (Varian, CA, USA) following manufacturer's procedures (Szikla, 2001), with (MDL: 1 mg/l, 1 mg/l, 1 mg/l, 185 mg/l, 2 mg/l, 0.4 mg/l, 17 mg/l and 1 mg/l, respectively). Dissolved SO_4^- was determined turbidimetrically using the method of Askew and Smith (2005) with an MDL of 0.25 mg/l. Dissolved organic carbon (DOC) and TOC was measured as Non-Purgeable Organic Carbon using through a Total Organic Carbon Analyser (Shimadzu Corporation, Japan) (MDL 0.06 mg/l). Quality control (QC) samples were analysed with each run in the following order; start, after every 10 samples and at the end. All QC samples are made from stock solutions certified to ISO 17025 or traceable to NIST certified reference material. Quality control values were set at approximately 30% of the calibration range for each analyte, e.g. TON, range 10 mg/l, routine QC 3 mg/l. Results were rejected if QC values were outside ±10%, and all samples, back to the previous correct QC, reanalysed. Sample results over range were diluted automatically or ran on a higher range calibration.

Duplicate water samples for dissolved gas analyses were taken at the same locations as nutrient samples. For excess-N_2 estimation samples were taken in 12 ml exetainers (LabcoWycomb Ltd., UK) after overflow of 10 ml. Exetainers were sealed without headspace using double septum (butyl rubber and teflon) stoppers. The exetainers were transported in water-filled containers at groundwater temperature (12 °C) and stored at 4 °C submerged inverted in water to prevent gas diffusion across the septa. N_2 quantification was carried out within one week using a high precision membrane inlet mass spectrometer (MIMS) (Pfeiffer Vacuum TMQMS 200 quadrupole mass spectrometer) set at the groundwater temperature of the time of sample collection (Kana et al., 1994) (MDL:< 0.03% (N_2/Ar), QCS: standard tap water was air-equilibrated at known temperature close to that of the samples). MIMS was

calibrated before the initial reading and after every 10 samples to correct analytical drift. Deionised water previously equilibrated with air in a condition of constant temperature and pressure was used as standard (Kana et al., 1994). Gaseous N_2 concentrations were calculated as per Weymann et al. (2008).

For the detection of dissolved N_2O, carbon dioxide (CO_2) and methane (CH_4) duplicate groundwater samples were collected in 160 ml serum bottles after an overflow of 150 ml. Bottles were capped without headspace with butyl rubber septa and aluminium crimp caps (Wheaton, USA) and stored as above. Samples were degassed by simultaneous water extraction and addition of high purity helium (He:water 1:3; v/v) (BOC, Linde Group, Germany), creating a 40 ml headspace (Lemon, 1981). Samples were agitated at 400 rpm (Gyrotory shaker G-10, New Brunswick Scientific, USA) for 5 min before being left to stand for 30 min. The gas in the headspace was then transferred into evacuated 12 ml exetainers. Extra 12 ml exetainers, two replicates for each sample, were conserved and used for $\delta^{15}N$ and $\delta^{18}O$ composition of dissolved N_2O. N_2O, CO_2 and CH_4 were quantified by auto-sampler gas chromatography (CP-3800, Varian Inc. USA) (MDL for N_2O, CH_4 and CO_2 was 0.02, 0.74 and 62 ppm respectively, QCS used were ARGO International standards at different and known N_2O, CH_4 and CO_2 concentrations) and final concentrations were calculated using Henry's Law for the ambient groundwater temperature.

The indirect N_2O-N emission factor for groundwater (N_2O-N EF5g) was calculated from the relationship between dissolved N_2O and N inputs, as per Weymann et al. (2008), using equation 4.1.

$$EF5g(1) = (N_2O\text{-}N)/(\text{dissolved } N_2O + \text{Excess-}N_2 + NH_4^+\text{-}N + NO_3^-\text{-}N + NO_2^-\text{-}N + DON) \quad \text{(Eqn. 4.1)}$$

The alternative equation (Eqn. 4.2) used by the intergovernmental panel on climate change (IPCC, 2006),was also used, although it assumes no processing of NO_3^--N and N_2O-N throughout the system (Weymann et al., 2008; Jahangir et al., 2013a).

$$EF5g(2) = (N_2O\text{-}N)/(NO_3^-\text{-}N)) \quad \text{(Eqn. 4.2)}$$

Table 4.1. Site parameters pertaining to drainage system and soil profile (based on data from Tuohy et al., 2016).

Site	Soil	Horizon Depth: Type (Texture)	Weather data, Water in situ parameter, Drainage design and sampling depths.
KM - 1.59 ha, Co. Cork, 52°12´,09°08´	Humic SW Gley	0-32 cm: AO (silty clay loam), 33-70 cm: Btg (silt loam), 71-97 cm: Cg (loam), 98-125 cm: Cr (loam), 126-190 cm: R (shale)	- Average annual rainfall 1629 mm, Av. AE: 0.6 mm/day, Av. ED: 3.9 mm/day, Av. SMD: -8.2 mm, T: 8.5˚C - WT: 0.6 m bgl, pH: 6.8, Eh: 726 mV, DO: 8.9 mg/l, T: 11.0˚C (Av. for site Oct-Nov 2015) - Drainage system: Subsoiling (0.6 m bgl, 1.5 m spacing), In-field pipes (1.1 m bgl, 15 m spacing) - End of pipe samples equate with 0-1.1 m bgl; Groundwater water samples equate with 1.9 m bgl depth of the soil profile; Open ditch samples equate to 1.5 m bgl
AA - 2 ha, Co. Limerick, 52°27', 09°19'	Humic SW Gley/Shale	0-40 cm: Ap/O (clay loam), 41-62 cm: Btg (silty clay), 63-140 cm: Cg1 (silty clay loam), 140-170 cm: Cg2 (silty clay loam)	- Average annual rainfall 1444 mm, Av. AE: 1.1 mm/day, Av. ED: 2.9 mm/day, Av. SMD: -7.1 mm, T: 9.1˚C - WT: 0.1 m bgl, pH: 6.7, Eh: 433 mV, DO: 9.7 mg/l, T: 11.5˚C (Av. for site Oct-Nov 2015) - Drainage system: Gravel moles (0.45 m bgl, 1.5 m spacing) ,In-field (0.9 m bgl, 20 m spacing) - End of pipe samples equate with 0-0.9 m bgl; Groundwater water samples equate with 1.8 m bgl depth of the soil profile; Open ditch samples equate to 1.5 m bgl
CD -1.31 ha, Co. Kerry, 52°13', 09°28'	Typical SW Gley	0-36 cm: Ap (silty clay loam), 37-100 cm: BCtg (silty clay loam), 101-190 cm: Cr (loam)	- Average annual rainfall 1148 mm, Av. AE: 1.1 mm/day, Av. ED: 1.8 mm/day, Av. SMD: -8.2 mm, T: 10.0˚C (missing values: 12-14/04/15, 04-07/05/15) - WT: 0.8 m bgl, pH: 7.2, Eh: 582 mV, DO: 9.4 mg/l, T: 11.6˚C (Av. for site Oct-Nov 2015) - Drainage system: subsoiling at 0.5 m bgl with 1.5 m spacing), then gravel moles at 0.45 m bgl with 1.5 m spacing), in-field pipes (0.9 m bgl, 20 m spacing). - End of pipe samples equate with 0-0.9 m bgl; Groundwater water samples equate with 1.8 m bgl depth of the soil profile; Open ditch samples equate to 1.2 m bgl
RE -2.56 ha, Co. Tipperary, 52°36', 08°01'	Paddock 1: Typical SW Gley Paddock 2 GW Gley	Paddock 1: 0-28 cm: Apg (loam), 29-50 cm: Eg (sandy loam), 51-90 cm: C (sandy clay loam), 91-140 cm: Cr (typical old red sandstone) Paddock 2: 0-30 cm: Apg (loam), 31-53 cm: Eg (sandy loam), 54-70 cm: Btg (sandy clay loam), 70- 100 cm: C1 (Sandy clay loam) 100-140 cm: C2 (Sandy loam)	- Average annual rainfall 852 mm, Av. AE: 1.1 mm/day, Av. ED: 1.7 mm/day, Av. SMD: -1.8 mm, T: 9.9˚C (missing values: 10/12/14-04/02/15, 20-21/11/15). - WT: 1.2 m bgl, pH: 7.2, Eh: 319 mV, DO: 8.7 mg/l, T: 11.3˚C - Drainage system: In-field pipes (1.6 m bgl, 15 m spacing in paddock 1, 30 m spacing in paddock 2) - End of pipe samples equate with 0-1.6 m bgl of the soil profile; Groundwater water samples equate with 2.0 m bgl depth of the soil profile; Open ditch samples equate to 0.6 m bgl
DG - 2.09 ha, Co. Clare, 52°44´, 09°30´	Humic Stagnic GW Gley	0-26 cm: Apg (silty clay loam), 27-48 cm: Btg (clay loam), 49-75 cm: Cg1 (silt loam), 76-140 cm: Cg2 (clay loam)	- Average annual rainfall 1144 mm, Av. AE: 1.2 mm/day, Av. ED: 2.0 mm/day, Av. SMD: -4.1 mm, T: 9.8˚C (Weather station not on the farm, 25 km away, similar climate). - WT: 0.2 m bgl, pH: 7.2, Eh: 308 mV, DO: 7.7 mg/l, T: 10.5˚C (Av. for site Oct-Nov 2015) - Drainage system: naked moles (0.60 m bgl, 1.5 m spacing) In-field (0.9 m bgl with 10 and 15 m spacing)

	- End of pipe samples equate with 0-0.9 m bgl; Groundwater water samples equate with 1.8 m bgl depth of the soil profile; Open ditch samples equate to 1.2 m bgl

4.3.3 Stable isotope analysis

For isotopic measurements of NO_3^-, water samples (40 ml) were collected at the same locations as other parameters, filtered in the field through 0.2 mm polyethersulfone filters (Sartorius Stedim Biotech GmbH, Germany), and stored at -20 °C in 50 ml polyethylene screw cap tubes. Gas exetainers (12 ml) from the previous section were additionally used for measurement of dissolved N_2O isotopic abundances. Isotopic compositions ($^{15/14}N$ and $^{18/16}O$) of NO_3^--N were determined using the denitrifier method at the UC Davis Stable Isotope Facility, Davis, California (McIlvin and Casciotti, 2011).

Isotope values for both NO_3^--N and dissolved N_2O were determined by using a Thermo Finnigan Gas Bench + PreCon trace gas concentration system interfaced to a Thermo Scientific Delta V Plus isotope-ratio mass spectrometer (Bremen, Germany). The calibration standards used were the nitrates USGS 32, USGS 34, and USGS 35 while additional laboratory reference materials are included in each batch to monitor and correct for instrumental drift and linearity. Limits of quantitation for ^{15}N and ^{18}O of N_2O from NO_3^- are 2-1500 µM NO_3^- in water. For ^{15}N and ^{18}O of N_2O, a calibration was carried out by thermally decomposing N_2O to convert N_2O to N_2 and O_2. The resulting N_2 was calibrated against the Oztech N_2 standard, and the O_2 was calibrated against an Oztech O_2 standard ($\delta^{18}O$ vs. VSMOW = 27.48). Limit of Quantitation for N_2O are approx. 150 pmol. Isotopes values were reported in δ‰ relative to international standards (AIR for N and VSMOW (Vienna Standard Mean OceanWater) for O).

4.3.4 Statistics

Different methods (t-test, oneway ANOVA and Tukey's HSD test (IBM SPSS Statistics version 24)) were used to determine if relationships existed between nutrient and gaseous data and other measured variables to identify significant differences amongst the main variables controlling processes and attenuation rates.

4.4 Results

4.4.1 Farm N balances

The five farms had similar stocking rates and grazing periods. The N-inputs ranged from 261 kg N/ha at AA to 341 kg N/ha at DG with an average of 307 kg N/ha (Table 4.2). Milk outputs ranged from 46 kg N/ha (CD) to 69 kg N/ha (DG). Mean excess N was 252 kg N/ ha; CD had high excess (292 kg N/ha) together with KM and DG (both 272 kg N/ha), while AA and RE had lower outputs (both 211 kg N/ha). The highest potential for N that can be

released by SOM decomposition from superficial layers was found in AA and KM, respectively an estimated N release of >146 kg N/ha and 144 kg N/ha. Lowest values were found at DG (120 kg N/ha) (Table 4.2).

4.4.2 Water quality

Longer term NO_3^--N, NO_2^--N and NH_4^+-N across sites and sampling locations is presented in Table S4.1. Both spatial and temporal NO_3^--N and NO_2^--N concentrations were all consistently below MAC (Table S4.1). Ammonium-N concentrations appear to be elevated and exceeded MAC across sites but not in all sampling locations (see Table S4.1 for number of sample events and % breaches).

Data for the more intensive sampling period in October 2015 is presented in Table 4.3 (see also Fig. S4.1). KM had NH_4^+-N concentration of 0.05 ± 0.05 mg NH_4^+-N /l, with a 0.14 ± 0.03 mg NH_4^+-N/l value in groundwater, AA had a concentrations of 0.31 ± 0.12 mg NH_4^+-N/l over the threshold in groundwater, CD had average concentrations above MAC (0.43 ± 0.46 mg NH_4^+-N), with EOP (0.86 ± 0.39 mg NH_4^+-N/l) and GW (0.28 ± 0.31 mg NH_4^+-N/l) locations exceeding MAC, RE had low average concentrations (0.09 ± 0.15 mg NH_4^+-N/l) but elevated groundwater concentrations (0.22 ± 0.21 mg NH_4^+-N/l) and DG had low average concentrations (0.07 ± 0.06 mg NH_4^+-N/l) with groundwater concentrations of 0.15 ± 0.04 mg NH_4^+-N/l.

Dissolved organic carbon showed high inter-farm variability. The highest concentration was found at DG (22.35 mg C/l), with lowest at KM and RE (5.91 and 4.73 mg C/l). AA had an intermediate average concentration, i.e. 14.22 mg C/l, similar too CD at 15.00 mg C/l (Table S4.2; Fig. S4.2).

The K^+ concentration ranged from 0.51 to 25.23 mg/l. AA had the highest K^+ concentration (14.65 mg/l), with all end-of-pipe and one piezometer locations above MAC. AA, together with one piezometer at DG (19.77 mg/l), was the only other paddocks with a K^+ concentration above MAC. DG, CD and KM showed organic contamination in most locations (farm averages for K^+ were 6.76, 6.89, 4.79 mg/l, respectively), while K^+ was only detected in two piezometers at RE (2.74 mg/l) (Fig. S4.3). Cl^- values ranged from 12.92 to 68.01 mg/l, with DG (53.10 mg/l) and AA (48.78 mg/l) having the highest farm averages. Most piezometer locations were above those concentrations, indicating some organic contamination. RE (19.18 mg/l) and KM (20.72 mg/l) had the lowest concentrations, with only a few locations indicating contamination, while CD (35.69 mg/l) had intermediate

values (Fig. S4.3). AA had the highest K/Na ratio (0.74), indicating organic waste influences. RE had a high concentration in piezometers (0.99 and 0.77), while end-of-pipe and open ditch locations remained unpolluted. CD (0.44) and KM (0.43) indicated contamination of open ditches, whereas DG (0.22) only exceeded the threshold in one piezometer and end-of-pipe sample location (Fig. S4.3).

4.4.3 Dissolved gasses

Dissolved N_2O concentrations ranged from 0.106 mg N/l to 0.001 mg N/l. The highest values were at CD (av. 0.026 mg N/l) and lowest at DG (av. 0.002 mg N/l) (Table 4.4). The N_2O concentration was generally higher in end-of-pipe locations than in groundwater or in open ditches. CD had the greatest variation in dissolved N_2O values, with highest concentrations in a piezometer location characterised by low NH_4^+-N. RE had high N_2O values within end-of-pipe locations. (Table 4.4, Fig. S4.4).

In most of the farms, excess-N_2 was below background levels. Therefore the values ranged from below background levels to 0.859 (DG) mg N/l. On sites where excess-N_2 was above background level this range was from 0.053 (RE) to 0.859 (DG) mg N/l. The highest excess-N_2 was found in DG, and lowest in RE (0.05 mg/l) (Table 4.4, Fig. S4.4). Due to the presence of excess-N_2 values below background levels limited data were available for the EF5g(1) calculation. EF5g(1) ranged between 0.0010 (AA) to 0.0288 (CD) (IPCC set default value: 0.0025). When looking at the EF5g(2) (data not shown) emission values were from 0.0008 to 0.0980, with 87% of locations above the IPCC set default value; every field site had averages above limits, with the highest concentration at RE (0.0296) and lowest at KM (0.0115) (data not shown). Dissolved CO_2 values were between 2.3 (KM) and 108.3mg C/l (RE). Higher dissolved CO_2 concentrations were found in groundwater and in-field pipes, rather than in open drains (Table S4.2, Fig. S4.5). Values for CH_4 varied between 1.45 and 38.00 mg C/l, except for two extreme values in AA groundwater (58 and 650 mg C/l) (Table S4.2, Fig. S4.5).

4.4.4 Stable isotopes

The NO_3^--N isotopic values ranged from 25.5 to -4.8‰ for $\delta^{15}N\text{-}NO_3^-$ (av. 10.1‰) and from 23.3 to -1.7‰ for $\delta^{18}O\text{-}NO_3^-$ (av. 5.7‰). Different farms showed specific and significantly different $\delta^{15}N\text{-}NO_3^-$ enrichment ($p < 0.005$), with KM (av. 20.0‰ $\delta^{15}N\text{-}NO_3^-$ and 8.1‰ $\delta^{18}O\text{-}NO_3^-$) and RE (av. 12.5‰ $\delta^{15}N\text{-}NO_3^-$ and 7.9‰ $\delta^{18}O\text{-}NO_3^-$) showing the highest enrichment, whereas DG had the least enriched values (av. 4.7‰ and 4.2‰ for $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$

respectively). CD and AA showed similar $\delta^{15}N\text{-}NO_3^-$ enrichment (av. 8.4‰ and 8.3‰ respectively) ($p > 0.05$). However CD showed lower $\delta^{18}O\text{-}NO_3^-$ values (av. 2.2‰) than AA (6.5‰) (Fig. 4.3).

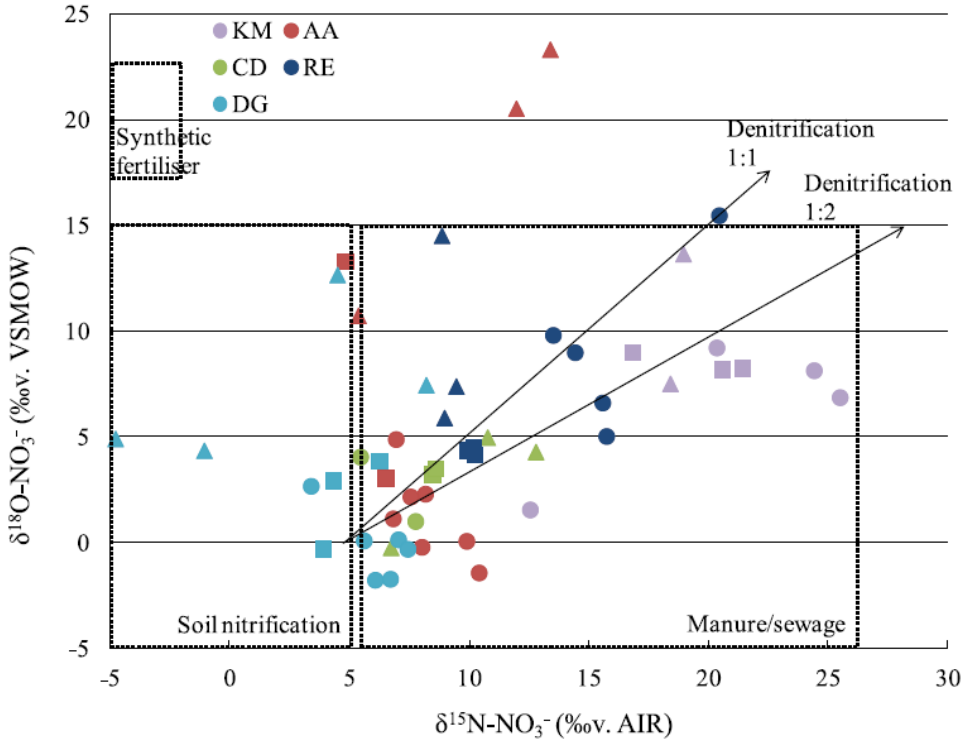

Fig. 4.3. $\delta^{18}O\text{-}NO_3^-$ versus $\delta^{15}N\text{-}NO_3^-$ values for the sites, also showing 1:1 and 1:2 denitrification slope and $\delta^{18}O$ and $\delta^{15}N$ ranges for N-sources (after Kendall,1998). Open ditch (OD): squares, end of pipe (EOP): circles and shallow groundwater piezometers (GW): triangles.

The $\delta^{18}O\text{-}NO_3^-$ was higher in piezometer (10.0‰) than end-of-pipe (3.2‰; $p < 0.005$) and open ditch (5.2‰; $p < 0.05$) locations. End-of-pipe locations at RE had a higher $\delta^{15}N\text{-}NO_3^-$ (15.9‰) than open ditches and piezometers (10.1 and 9.1‰ respectively). The highest values of $\delta^{18}O\text{-}NO_3^-$ were in a piezometer (14.5‰) and the end-of-pipe location (15.5‰). These locations also had highest the NH_4^+-N and NO_3^--N concentrations at time of sampling. Two AA piezometers had the highest $\delta^{18}O\text{-}NO_3^-$ values (23.3 and 20.5‰). At DG piezometers showed high variability, with alternatively low $\delta^{15}N\text{-}NO_3^-$ or high $\delta^{18}O\text{-}NO_3^-$ values. The two DG piezometer locations had the lowest $\delta^{15}N\text{-}NO_3^-$ values (-4.8 and -1.1‰) (Fig. 4.3).

The $\delta^{15}N\text{-}N_2O$ values ranged from 4.3 to -20.3‰ while $\delta^{18}O\text{-}N_2O$ was 68.2 to 27.2‰. No difference was evident in $\delta^{15}N\text{-}N_2O$ values between the farms (Fig. 4.4).

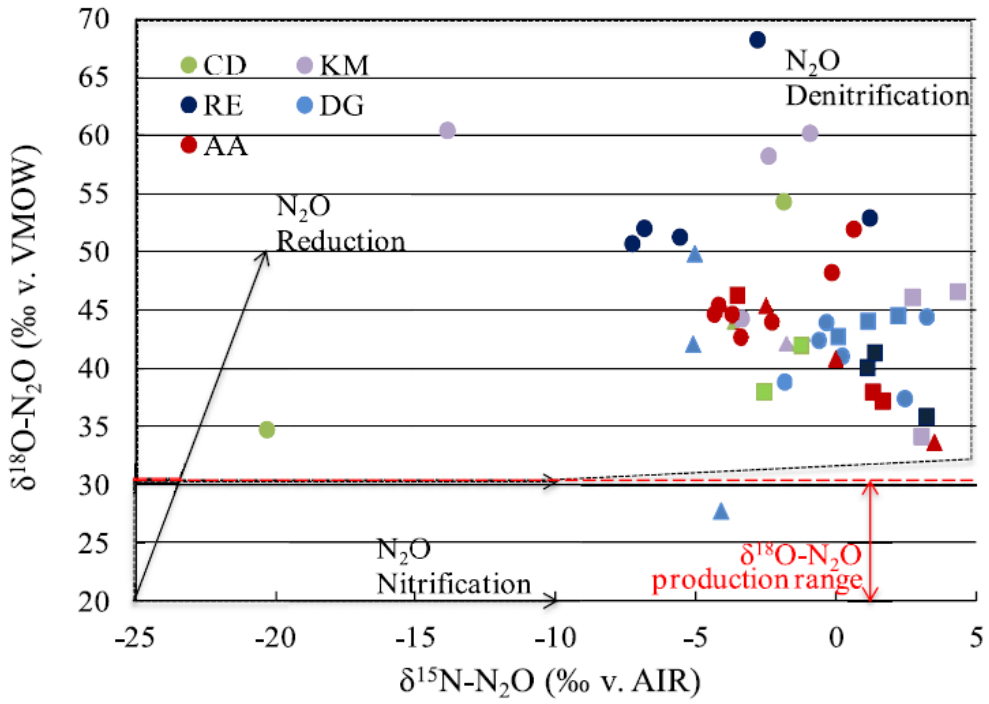

Fig. 4.4. $\delta^{18}O\text{-}N_2O$ versus $\delta^{15}N\text{-}N_2O$ values for the farms, also showing 2.5:1 N_2O reduction slope and source boxed as identified by Li et al. (2014). Red line represents the limit for N_2O production calculated for the sites. Open ditch (OD): squares, end-of-pipe (EOP): circles and shallow groundwater piezometers (GW).

Table 4.2. N annual balance and management for the five Paddocks in 2015. N input included fertilizer (chemical and organic) and concentrates; N output corresponds to milk; N surplus was calculated subtracting N outputs from N inputs. N release for other soil layers represent the average (±standard deviation) calculated for the soil layers underlying the top layer.

Site	Stocking rate	Grazing period	Management	N input	N output	N surplus	Estimated N release	
							Top soil	Other soil layers
	(LU/ha)	(days)		(kg N/ha)				
KM	2.38	251	Synthetic fertilizer (monthly)	329	57	272	144	54 (±13)
AA	2.46	254	Synthetic fertilizer (monthly), cattle slurry (Feb., Apr., Oct.)	261	50	211	>146	68 (±34)
CD	2.59	229	Synthetic fertilizer (monthly), cattle slurry (Feb., Apr., Oct.)	338	46	292	131	52 (±5)
RE	2.56	251	Synthetic fertilizer (monthly), cattle slurry (Mar., May, Sep.) and parlour washings (Oct.)	264	53	211	115	25 (±10)
DG	2.37	249	Synthetic fertilizer (monthly), urea (Jul.) and parlour washings (Sep.)	341	69	272	120	52 (±18)

Table 4.3. Average values for NO_3^--N and NH_4^+-N within the five paddocks in October 2015 (NO_2^--N was below 0.04 mg NO_2^--N/l at all locations); open ditches (OD), end-of-pipes (EOP) and shallow groundwater piezometers (GW) at the five sites.

	NO_3^--N (mg NO_3^--N/l)				NH_4^+-N (mg NH_4^+-N/l)			
Site	Site	OD	EOP	GW	Site	OD	EOP	GW
KM	0.80 ± 0.90	0.76 ± 0.43	1.19 ± 1.24	0.10 ± 0.03	0.05 ± 0.05	0.03 ± 0.01	0.01 ± 0.01	0.14 ± 0.03
AA	0.47 ± 0.37	0.42 ± 0.56	0.66 ± 0.28	0.08 ± 0.07	0.17 ± 0.18	0.08 ± 0.04	0.13 ± 0.20	0.31 ± 0.12
CD	1.78 ± 1.29	0.60 ± 0.00	2.92 ± 1.46	1.43 ± 0.36	0.43 ± 0.46	0.02 ± 0.01	0.86 ± 0.39	0.28 ± 0.31
RE	0.76 ± 0.80	1.97 ± 0.02	0.35 ± 0.40	0.38 ± 0.42	0.09 ± 0.15	0.02 ± 0.02	0.02 ± 0.02	0.22 ± 0.21
DG	0.22 ± 0.13	0.23 ± 0.14	0.30 ± 0.11	0.09 ± 0.05	0.07 ± 0.06	0.03 ± 0.02	0.04 ± 0.02	0.15 ± 0.04

Table 4.4. Mean values for excess-N_2 and dissolved N_2O for whole farm, open ditches (OD), end-of-pipes (EOP) and shallow groundwater piezometers (GW) at the five sites.

	Excess-N_2 (mg N/l)				Dissolved-N_2O (µg N/l)			
Site	Site	OD	EOP	GW	Site	EOP	FD	GW
KM	0.34 ± N/A	N/A	N/A	0.34 ± N/A	6.67 ± 6.83	1.95 ± 0.40	11.27 ± 7.24	2.48 ± N/A
AA	0.42 ± 0.25	0.13 ± N/A	N/A	0.56 ± 0.05	3.30 ± 1.50	2.01 ± 0.27	4.44 ± 0.68	1.94 ± 1.48
CD	0.17 ± N/A	N/A	N/A	0.17 ± N/A	25.95 ± 43.21	2.28 ± 0.18	9.42 ± 6.31	0.11 ± N/A
RE	0.19 ± 0.12	N/A	0.19 ± 0.12	N/A	6.31 ± 4.68	1.74± 0.18	9.06 ± 3.65	N/A
DG	0.35 ± 0.26	0.44 ± 0.24	0.13 ± 0.041	0.61 ± 0.21	1.99 ± 0.65	2.17 ± 0.84	2.17 ± 0.64	1.46 ± 0.21

4.5 Discussion

4.5.1 Farm N balances

A high input of N on these farms is necessary to sustain milk production. However, inputs on these paddocks farm are well above the average (223-228 kg N/ha) for Irish intensive farms (Treacy et al., 2008; Mihailescu et al., 2014). These paddocks have low efficiency with respect to N utilisation (between 14 and 20%) (averages for dairy farms: 20% (Treacy et al., 2008), 28% (Mihailescu et al., 2014)) and high N-surplus (between 211 and 292 kg N/ha) (average for Irish farms (227 kg N/ha (Treacy et al., 2008), 175 kg N/ ha (Mihailescu et al., 2014))). In addition, soil from these field sites has a high estimated N release potential, suggesting high N storage by SOM, with high leached losses expected as decomposition occurs (Table 4.2). Nitrogen is more likely to accumulate and be retained by SOM in soil when it is not lost through denitrification or leaching (Jarvis et al., 1996). Hence, the N balances for these farms indicate a high potential for N-losses. However, the high N inputs and low N efficiency indicates that simple improvement related to nutrient use efficiency could decrease environmental impact without significantly affecting yields (Mihailescu et al., 2014).

4.5.2 Water quality

Ammonium is the pollutant of concern across the sites. Low NO_3^--N concentrations occurred in shallow groundwater and end- of-pipe locations, indicating a high NO_3^--N attenuation potential in the upper 1 m of the soil profile, but with pollution swapping also evident (see Stevens and Quinton, 2009). The high saturation, poor aeration and low permeability of soil profiles on the farms increase the potential for denitrification (Hanson et al., 1994). In addition, weather data showed, from the biogeochemical stand- point, that the systems could promote high rates of anaerobic N reduction processes (e.g. denitrification, DNRA) (Giles et al., 2012; Cardenas et al., 2017) (Table 4.1).

Incomplete denitrification is likely due to excess fertilizer, which leads to high N_2O emissions. However, Burchill et al. (2014) studied groundwater gleys with deep groundwater drainage designs and showed that a high water-filled pore space still remained in topsoil layers, creating conditions for complete denitrification and a corresponding increased release of N_2 rather than N_2O.

The high C content of these soils also creates conditions for pollution swapping, leading to an increased amount of N being transformed back to NH_4^+-N by DNRA, as this process is thought to dominate under low O_2, high C conditions (Rütting et al., 2011). Highly anaerobic

conditions could also increase NH_4^+-N concentrations, by inhibiting nitrification (aerobic conversion of NH_4^+-N to NO_3^--N (Redding et al., 2016). However, at some sites with high saturation content, the installation of artificial drainage systems could encourage nitrification and NH_4^+-N attenuation, due to greater DO infiltration to deeper levels. This could also have caused an increase in NO_3^--N losses, with lower levels of complete denitrification.

At AA, where waterlogged areas persist, the high concentration of NH_4^+-N is attributed to the suppression of nitrification (Redding et al., 2016). CD has a general contamination problem, with NH_4^+-N values above MAC, whereas at RE only the groundwater sampling location within the wider spaced i.e. 30 m shows NH_4^+-N contamination. The elevated NH_4^+-N concentration at these locations is persistent and does not originate from farm management or application of organic or inorganic fertilizer. The 30 m treatment was installed on a groundwater gley site (some higher permeability at depth); whereas the 15 m treatment was installed on the adjoining surface-water gley (limited permeability through the profile). However, a groundwater-type drainage system was installed across the entire site with no disruption techniques deployed on the surface water gley section. This is interesting as a shallow drainage system in the surface water gley site would create conditions for increased N losses. However, the tighter spacing achieved drainage production goals by controlling the water table and preventing water quality issues.

Tighter spacing of pipes, rather than connecting an 80 mm pipe at 1 m with a disruption technique (e.g. mole or gravel moles) should be explored as a water quality sustainability measure. The purpose of shallow drainage designs is to increase infiltration in the first metre of impermeable soil profiles (Tuohy et al., 2015; Filipovic et al., 2014), but this soil disruption will decrease the N attenuation potential of this soil layer.

The dissolved gas surveys show that there is no significant difference between contaminated and uncontaminated locations at the AA and DG sites, while CD has the highest dissolved N_2O values in groundwater characterised by a low NH_4^+-N concentration. Jahangir et al. (2012a) examined GHGs emissions on farms with low and high permeability characteristics. Results from comparable sites to the present study (same soil drainage class) had mean values for groundwater dissolved N_2O of 0.024 and 0.011 mg N/l. The present study found lower averages for dissolved N_2O, from 0.002 to 0.006 mg N/l. Herein, CD had the highest average of 0.022 mg N/l. A lower N_2O value in groundwater could be caused by decreased denitrification, nitrification, and/or a higher enhanced reduction of N_2O to N_2 however this result alone is not sufficient to discriminate which process is responsible (Jurado et al., 2017)

(Table 4.4, Fig. S4.4). Reduction of N_2O to N_2 is favoured under the low NO_3^--N and high saturation conditions at the five study sites here.

Excess-N_2 is below background levels in most of the farm, possibly implying in situ degassing of water and N_2 formation below solubility (Weymann et al., 2008; Well et al., 2012). However, no indications of degassing due to sampling errors were found (decreasing Ar concentration within a group). Excess-N_2 values (farm av. between 0.171 and 0.346 mg/l) are higher than those previously reported for the low permeability farms (2.28 and 2.33 mg/l) in Jahangir et al. (2012a). With a higher number of piezometer locations having excess-N_2, DG had a higher level of N_2 production, potentially due to complete denitrification or other N_2 production process, i.e. anammox (Table 4.4, Fig. S4.4). The CO_2 in shallow groundwater ranged from 2.3 to 108.3 mg C/l, compared with 19-45 mg C/l in Jahangir et al. (2012b). The present sites have a CH_4 concentration mostly between 1.4 and 57 mg C/l, which are generally in the range of those (1.7-1001 mg C/l) found by Jahangir et al. (2012b) (Table S4.2, Fig. S4.5).

4.5.3 Isotopes

The NO_3^--N isotope values in most locations are within the range attributed to organic fertilisers (Kendall,1998; Xue et al., 2009), and more recently recognised as characteristic of a "mixed source", represented by NO_3^--N leached from pasture soils (Wells et al., 2014). Two samples had a $\delta^{18}O\text{-}NO_3^-$ signature within the range of synthetic fertilizer (Fig. 4.3). Overall, the isotope data plotted along a $\delta^{18}O\text{-}NO_3^-$:$\delta^{15}N\text{-}NO_3^-$ ratio between 1:1 and 1:2, suggesting that variable degrees of denitrification affect the NO_3^--N pool across the sampled locations (Kendall, 1998; Wells et al., 2014) (Fig. 4.3). A shift from this denitrification line can arise from a variation in the degree of nitrification relative to denitrification, which creates NO_3^--N with relatively low $\delta^{15}N$ but consistent $\delta^{18}O$ values (Granger and Wankel, 2016).

Different field sites have different isotopic signatures and dispositions along the denitrification line (Fig. 4.3). KM and RE have NO_3^--N derived from organic sources, with the highest enrichment values due to denitrification. In contrast, DG has the least isotopically enriched values, with locations mainly characterised by a nitrification signal. The higher enrichment at KM with respect to RE, may indicate a higher net denitrification at KM and therefore an enrichment in both $\delta^{18}O\text{-}NO_3^-$ and $\delta^{15}N\text{-}NO_3^-$, with a shift upwards along the denitrification line (Wells et al., 2016). However, it could also result from variability (e.g. a slightly different "starting point" of the NO_3^--N signature between the two farms) due to a different history of mixing processes which modify the isotopic composition. Most locations

in AA lie near the intercept of the denitrification line, indicating a homogenous organic source and negligible net denitrification. However, two AA piezometers have $\delta^{18}O\text{-}NO_3^-$ values high enough to be attributed to synthetic fertilizer, while a third piezometer and section of the open drainage shows a predominance of nitrification processes, with a shift towards lower $\delta^{15}N\text{-}NO_3^-$ values from the denitrification line. CD is similar to AA, with a homogeneous organic NO_3^--N and low/absent net denitrification.

The N_2O isotope data fall within the range of values for freshwaters (Snider et al., 2015) and further confirm the importance of denitrification across the farms. Farm N_2O signatures can be attributed to reduction (Li et al., 2014), indicating that denitrification occurs on every farm, but to different extents.

4.5.4 Ranking the N attenuation potential of the sites

As Fig. 4.5 includes both nitrate and ammonium attenuation it goes beyond the present conceptual diagram of Coyle et al. (2016). After collating all datasets from the present study, three groups emerge. As can be seen from Fig. 4.5 there is a spread in the location of these sites within both figures. Groups emerge as follows: 1) (KM and RE) Low NH_4^--N concentration and high denitrification potential, 2) (DG) Low NH_4^--N concentration and high nitrification potential, 3) (AA and CD) High NH_4^--N concentration and low denitrification potential. This means that the highest ranked sites in terms of N attenuation were those in Group 1 i.e. KM and RE. From Fig. 4.5 (left) it can be seen that this group has a higher complete denitrification capacity and from Fig. 4.5 (right) such sites have a higher attenuation of NH_4^--N. The lowest ranking sites in terms of N sustainability are those in Group 3. The conceptual diagram clearly shows that shallow disruption techniques (e.g. moles and gravel moles) installed within the top 1 m of the soil profile negatively affect the N attenuation potential of the soil profile. Deeper groundwater systems do not negatively affect the N attenuation potential of the soil profile.

Other studies should utilise Fig. 4.5 and include data on drainage class, drainage design (if present), completeness of denitrification, rate of denitrification and NH_4^--N attenuation. For example, Jahangir et al. (2012a, HS) results have been added to Fig. 4.5. These results were from a moderately drained site without land drainage. Plotted results from that study exhibit another type of signal with less complete denitrification and greater N_2O losses and some NH_4^--N losses. The conceptual diagrams can be used as a tool to highlight the consequences of draining the HS site (both cases can be considered i.e. GW or SW). If drainage was installed on the HS site the tool shows that the levels of N_2O are likely to increase with higher

associated NH_4^--N losses. The conceptual diagram can therefore be used to rank any site in terms of N sustainability and in addition be used as a management tool to inform likely outcomes with respect to installation of land drainage (GW versus SW) on any site.

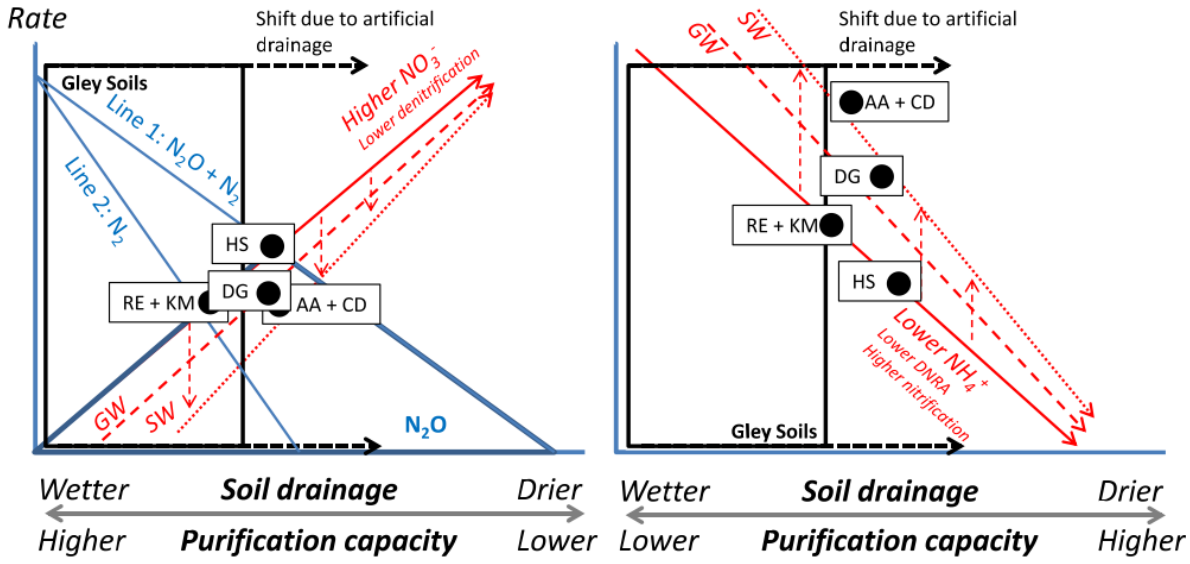

Fig. 4.5. Left. Conceptual diagram showing NO_3^--N water purification capacity represented by denitrification in relation to soil drainage. Red line shows NO_3^--N loss; dotted red line shows NO_3^--N loss in water from artificial drainage systems (GW: groundwater design; SW: surface water design) enhanced by soil bypass; line 1 indicates the first step of denitrification where NO_3^--N is converted to N_2O (incomplete denitrification); line 2 represents the second step of denitrification where N_2O is converted to N_2 (complete denitrification); HS indicates the low permeability sites from Jahangir et al. (2012a). **Right.** Conceptual diagram showing NH_4^+-N water purification capacity represented by nitrification in relation to soil drainage. Red line shows NH_4^+-N loss; dotted red line shows NH_4^+-N loss in water from artificial drainage systems (GW: groundwater design; SW: surface water design) enhanced by soil bypass (from Coyle et al., 2016).

4.6 Conclusions

Five gley soils were artificially drained and water from end-of-pipe, shallow groundwater and open ditch locations sampled for dissolved gas (N_2O), hydrochemical species and stable isotopes (NO_3^- and N_2O). Both soil N surpluses and (organic) source were consistent across the sites, but the soil N attenuation potential differed across sites. Deep groundwater drainage systems maintain their soil N attenuation potential but installation of shallow drainage systems can cause a negative shift, resulting in loss of this function, pollution swapping and increased water quality impacts from nutrient loadings in drainage. From this detailed work an N sustainability tool for any site, which presents the relationship between drainage class, drainage design (if present), completeness of denitrification, rate of denitrification and NH_4^+-N attenuation was developed. This tool allows a comparison or ranking of sites in terms of their N sustainability. The tool can also be used pre-land drainage and presents the

consequences of future artificial land drainage on water quality and gaseous emissions at a given site.

Within this Chapter 4 the hypotheses created in Chapter 2 were met. Heavy textured sites did differ in terms of their net denitrification capacity showing higher rates of denitrification. The multi-techniques approach was used to create a conceptual model and to rank dairy farms in terms of sustainability. Shallow drainage designs affected net denitrification capacity to a greater extent than groundwater designs and this affects ranking of dairy farms in terms of sustainability.

4.8 Acknowledgements

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Chapter 5 - An assessment of nitrogen source, transformation and fate within an intensive dairy system

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Highlights:

- A conceptual diagram created from many techniques informed sustainability
- Heterogeneous soil-subsoil showed varied nitrogen water purification capacity
- A drainage system in poorly drained soils did not alter attenuation capacity
- In moderate-well drained soils nitrogen surplus was converted to ammonium

5.1 Abstract

On intensive dairy farms, soil heterogeneity presents a landscape of varied water purification functionality. The overall sustainability and fate of reactive nitrogen (N_r) depends on the connectivity of various surface and subsurface pathways of loss and the "net" N source-transformation of these pathways. The present study takes place on an intensive dairy farm and collates long term management, nutrient, biogeochemical, isotopic and dissolved gas data across an extensive land drainage and multi-level groundwater monitoring network to examine: a) the farm N balance, b) the spatio-temporal distribution of N_r and dissolved gases c) the provenance of water samples within the monitoring network and d) the N source and transformation of N. Furthermore interpretation of this entire dataset was used to provide a conceptual diagram of the dairy farm to inform the sustainability of the agronomic system and the fate of N. Results showed a high N-surplus of 219 kg N ha^{-1}. Stable isotope compositions of water samples showed low spatial variability (-7.2‰<H_2O-$\delta^{18}O$<-3.4‰, -40.4<H_2O-δD<-32.4‰) with end-of-pipe and multi-level groundwater samples exhibiting the same signal. Open ditch samples presented a different signal as enrichment in $\delta^{18}O$ but not δD indicated higher accentuated evaporation. By combining datasets and maximum admissible concentration (MAC) thresholds four groups of locations emerged on-site: Group 1: had good water quality with NH_4^+-N and NO_3^--N below threshold concentrations (i.e. <0.23 mg NH_4^+-N l^{-1} and 5.65 mg NO_3^--N l^{-1}); located on imperfectly to moderately-well drained soils with high denitrification ($\delta^{15}N$-NO_3^->12‰,$\delta^{18}O$-NO_3^->10‰) and low N_2O emissions inferring completeness of the process (<0.01 mg N_2O-N l^{-1}). Group 2: showed poor water quality with NO_3^--N>5.65 mg l^{-1}; these soils were well to moderately-well drained, with low denitrification ($\delta^{15}N$-NO_3^-<12‰, $\delta^{18}O$-NO_3^-<10‰) and high N_2O emissions (>0.01 mg N_2O-N l^{-1}) indicating incompleteness of the process. Group 3: on well to moderately drained soils, showed low NO_3^--N concentration (NO_3^--N<5.65 mg l^{-1}) as in Group 2, but exhibited high N_2O production. Group 4, located on well to imperfectly drained soil, had NH_4^+-N>0.23 mg l^{-1} and high N_2O emissions and low potential for denitrification. Conceptually the dairy farm is a two tiered system: a) in artificially drained poorly drained or imperfectly drained soils the water purification functionality is high and where connected conveys clean water from large areas of the farm to the open ditch network and outlet of the farm and b) in un-drained moderately and well drained soils the water purification function is lower and leached N is converted at depth to NH_4^+-N and migrates off site along deep groundwater pathways. Such knowledge should inform future management on site thereby decreasing N_r losses.

5.2 Introduction

Agriculture and food production rely heavily on external N inputs (Van Grinsven et al., 2012) and as agronomic systems they can pass high N surpluses to the environment. The movement of N_r along surface and subsurface pathways affects water quality (Mosier et al., 1998; Foster, 2000; Lesschen et al., 2011) and contributes to greenhouse gas emissions (Sutton et al., 2011a). The distribution of soil-subsoil and bedrock at a given intensive dairy site may or may not offer natural attenuation against N surpluses. Research on soil functions has indicated that the N water purification function (identified as: "the amount of denitrification required to ensure that the N surplus leaving the rooting zone does not lead to groundwater N concentrations in excess, by Schulte et al. (2014)) of soil is higher in poorly drained or lower conductivity soils and lowest in freely drained or high conductivity soils (Fenton et al., 2009; Coyle et al., 2016). Across Ireland the N water purification function of agricultural soils has been indicated as being significant with denitrification as the main agent (Jahangir et al., 2012 a,b). The N speciation and extent of attenuation is regulated by many other environmental factors e.g. edaphic factors, substrate concentrations, plant coverage, management and weather (Saggar et al., 2013).

Nitrogen surpluses from animal excretion and fertiliser inputs (inorganic: urea, calcium ammonium nitrate (CAN), or organic: manure, slurry and urine) can migrate through heterogeneous subsurface pathways. This N_r can be transformed through mainly biological processes within the N cycle and especially nitrification and denitrification through which ammonium (NH_4^+-N) is oxidised to nitrate (NO_3^--N) and then reduced to di-nitrogen gas (N_2) (Rivett et al., 2008). This and other pathways (e.g. nitrification, DNRA (dissimilatory nitrate reduction to ammonium), anammox) are composed of sequential reactions, with the production and possible release of intermediate and undesirable compounds to the environment e.g. NO_3^--N, nitrite (NO_2^--N) and nitrous oxide (N_2O).

The role of an installed drainage system on N_r transfer, transformation and migration on such intensive dairy farms with variable soil type and drainage classes remains unclear. A drainage pipe installed within the sub-soil is likely to connect denitrification "cold" and "hot" spots, depending on soil functionality characteristics (Schulte et al., 2014), i.e. soil zones characterised by relatively low and high capacity for N transformation, respectively, according to the area drained. Indeed, it is important to note that water sampled at an end-of-pipe location reflects the composite attenuation capacity of the subsoil draining into this pipe (e.g. 100 m length). However, these systems may reduce N transformation potential by, for example, creating unsuitable conditions for denitrification (higher aerobicity, lower water

saturation and shorter residence time) leading to greater NO_3^--N losses. Equally, zones of high soil N attenuation capacity may be by-passed by the drainage system.

Utilising drainage systems to gather information on N_r source, fate and net transformation is novel on heterogeneous farms as current models of nutrient fate in drainage systems are simplified and assume soil homogeneity. Other N species such as NH_4^+-N, NO_2^--N and N_2O are seldom considered. There is a need to examine whether drainage system pathways in heterogeneous soils can utilise areas which support N attenuation capacity or bypass and nullify this capacity, with increased reactive N losses from the system. There have been limited investigations of NO_3^--N distributions in shallow groundwater systems under such intensive dairy farms (Fenton et al., 2009; Fenton et al., 2011). On the same site as the present study Baily et al. (2011) used NO_3^--N natural isotopic abundances for three sampling periods (April, August and December) to deduce the role of denitrification on soil N dynamics. Jahangir et al. (2012a; 2013a) added to such information and estimated such N-losses to be approximately 106 kg N ha^{-1}, which could be explained by hydrological and geochemical factors (e.g. availability of dissolved oxygen (DO) concentration and redox potential (Eh)). While these studies offered insight into the fate of NO_3-N in shallow groundwater and travel time to groundwater below heterogeneous soil farms, such studies gave no insight into N provenance, multi-level spatial distribution, the transformation process along shallow (including the extensive land drainage network on site and its connectivity to the surface loss pathway) and deep pathways or the ultimate fate of the lost N. Nutrient concentrations (e.g. NO_3^--N, NO_2^--N and NH_4^+-N), physiochemical parameters (e.g. pH, DO, Eh) and soil properties (e.g. saturated hydraulic conductivity, k_s) can provide qualitative evidence of spatial and temporal variation in N-transformation processes and the soil conditions supporting these.

Additional gas analyses increase the understanding of local N-transformation processes, which can be quantified (in term of sinks and sources of N_2O) by the release of their final gaseous products (i.e. N_2 and N_2O). However, since multiple N-transformation processes can contribute to N_2 and N_2O production, further analysis using isotopic abundances can help elucidate the different N sources and transformation processes (e.g. Xue et al., 2009).

The objectives of the present study on an intensive and heterogeneous dairy farm drained with a random drainage design were to use a combined nitrogen, biogeochemical, isotopic and dissolved gas dataset to examine: a) the farm N balance, b) spatial and temporal variation in aqueous N-species, c) provenance of water samples within a surface and subsurface monitoring network d) spatial distribution of N source and transformation and finally e) to

present a conceptual diagram of the site to inform the sustainability of the agronomic system and the fate of N.

5.3 Materials and Methods

5.3.1 Study site

The study site is located at the Teagasc research centre in Johnstown Castle, Co. Wexford, South-east Ireland (52˚17'30″N, 6˚29'50″W) (Fig. 5.1). The site comprises two units, one of 72.95 ha (terms down-gradient unit) and an up-gradient unit of 50.80 ha separated by a road way. The up-gradient subsurface drainage system is connected to the down-gradient system through an underground connector pipe. The farm is intensive and operates at 3.1 Livestock units (LU) per hectare (LU ha^{-1}). Nitrogen inputs arise from urea (spread February to April), calcium ammonium nitrate (CAN) (May to September) and manure (spring) for a total of 257 kg N ha^{-1} inputs of inorganic fertiliser and 103 kg N ha^{-1} of organic (5 years average). The central area of the dairy farm receives dairy soiled water (DSW consisting of rainwater, yard and milk parlour washings) from February to October through an irrigation system (Roto-Rainer, Briggs, New Zealand)) in (Fig. 5.1, former spreading area: plot with locations 11 and 14; current spreading area: between the Met station and location 19). Farm partial N balances for each year were calculated as per Treacy et al. (2008), utilising stocking rates, N inputs (inorganic and organic fertilisers and concentrate feed (volume and composition) and N exports (milk production (volume and composition) and slurry). Cows were milked twice daily (07·30 h and 15·30 h) with milk yield registered for each cow (kg) each time. The milk composition (fat, protein and lactose concentrations) for each cow was tested on a successive morning and evening every two weeks using a Milkoscan 203 (Foss Electric DK-3400, Hillerød, Denmark). Milk solids were calculated using the method of Tyrell and Reid (1965). For both milk and concentrates fed the N value is averaged across the farm. This region has a cool maritime climate with an average annual air temperature of 10˚C (1981-2011). Average annual rainfall (1981-2011) is 1037.5 mm with maximum intensity between September and November (Rosslare synoptic station, 52°15'00"N, 6°20'5"W). There is an in-situ synoptic meteorological station (Fig. 1) on the dairy farm which records daily rainfall, wind speed and hours of sunshine which can then be inputted into the grassland hybrid soil moisture deficit (HSMD) model of Schulte et al. (2005) to estimate effective drainage (combined runoff and recharge amount, mm day^{-1}). To examine the differences in recharge across drainage classes (see Fig. 1) on site the modelling was conducted for well, moderately and poorly-drained soils using input data from 2008 to 2014. Soil texture varies from fine loam to clay loam

(Brown Earth, Gleyic Cambisol with Irish Sea till origin (Gardiner and Ryan, 1964), Fig 1), with small areas of sandy textured soils. Subsoil is of moderate permeability (0.2-10 m; 5 x 10^{-8} m s^{-1} <k_s< 5 x 10^{-4} m s^{-1}), but can be interspersed with high permeability gravel and/or sand lenses (Fenton et al., 2009; Jahangir et al., 2013b). Residence time of water in the unsaturated zone is from 1 (shallowest areas) to 3 (deepest areas) years with probability analysis indicating 1.5 years 85% of the time (Baily et al., 2011). This means there is a mismatch between best management practice intervention at farm level, when nutrients are lost, when such nutrients are stored and mineralised and when leaching affects N_r concentrations along subsurface pathways. Due to this heterogeneity the average watertable on site from 2005-2014 is at 2.8 (±1.7) m below ground level (bgl) (deepest from July to September i.e. 3.0 m bgl and shallowest from December to February i.e.2.3 m bgl). The low permeability bedrock is Pre-Cambrian greywacke mixed with quartzite at a depth of 10 to 12 m (k_s, 3.6 x 10^{-6} m s^{-1}), containing a poorly productive aquifer, which is classified as receiving 1 to 50 mm yr^{-1} (Baily et al., 2011).

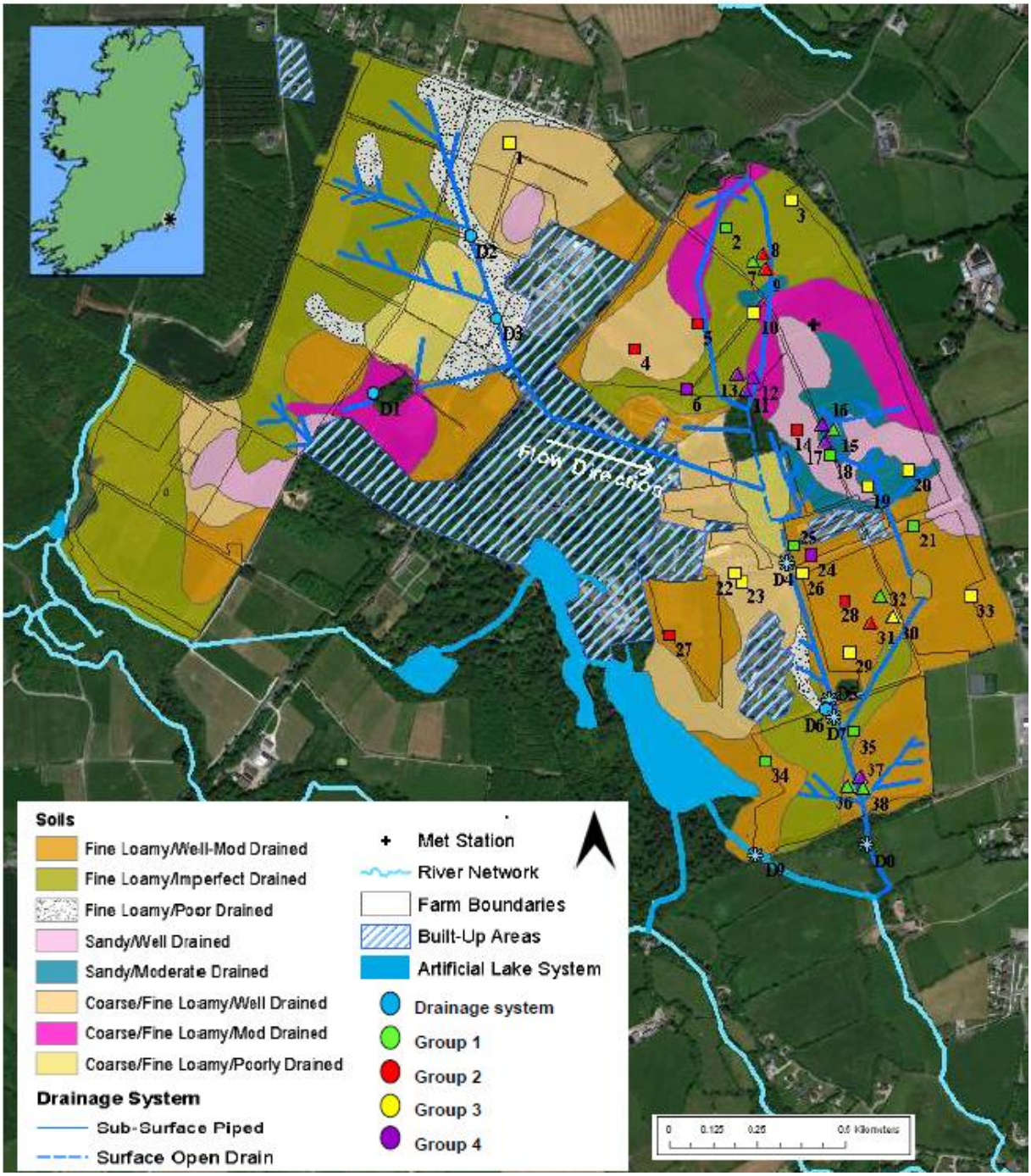

Fig. 5.1. Map of the intensive farm merging soil texture, drainage class, position of the surface and subsurface drainage networks including the lake system and outlet and groundwater monitoring locations (Squares indicate piezometers, multilevel boreholes are indicated by triangles, end-of-pipe locations by circles and open ditch locations by asterisks).

5.3.2 Surface and subsurface monitoring network

To examine the spatial and temporal variation in aqueous N-species, provenance of water and N source, transformations and fate of the intensive dairy system a large monitoring surface and subsurface network needed to be collated in GIS and a map developed. The open ditch network and groundwater monitoring components of the system were well documented but

the subsurface drainage system remained unmapped. Examination of historical maps and discussions with present and retired farm staff mapped out likely positions of in-field drains. Field work validated or refined such positions (for more information see Table S5.1, Fig 5.1). Both up and down-gradient units contain poorly-imperfectly drained soils (79% and 28%, respectively) (Fig. 1). These areas are all artificially drained composing of an in-field piped network of 10.1 km (Fig. 1). The in-field piped system is composed of either corrugated slotted plastic (predominantly 80 or 100 mm at 0.5-1 m depth) or concrete non-perforated pipes (600 mm at 1 m depth act as connectors).

The components of the surface and subsurface drainage system are as follows: 1) Up-gradient component: a herring bone drainage network (80 mm, variable depth) conveys drainage water from an area of 24 ha, to an underground outlet (D2, Fig. 5.1) and joins up with another outlet at D3, which passes to a junction also fed by a drainage system passing through D1. This composite migrates in a fully cased concrete pipe and eventually discharges into the open ditch in the down-gradient unit. 2) Down-gradient component including open ditch: the subsurface drain that transfers water from locations 2, 3, 7, 8, 9 and 10 is an ad-hoc drain developed over time with many unknown extensions and discharges to the start of the open ditch. A short herring bone system discharges water into the open ditch adjacent to location 14. The open ditch extends from 11 to D4, before being piped underground to the outlet at D8 (Fig. 5.1, Table S5.2). There is an access point in the underground section at D5-6-7. Individual drains connect to the underground part of this primary drain at location 30 (fully cased draining a marl pond) and 36. 3) Unconnected components - an in-field herring bone drain in the area of 15, 16 and 17 flows in the opposite direction to the open drain and have an offsite outlet as does the system around 19 and 20. Any discharge from the nearby artificial lake system (D9) does not affect D8. Prior to the 2014 sampling campaign, water sampling locations were added to this system i.e. three positions along the subsurface drainage system (D1, D2, D6), one position along the surface stream (D5) and three within the groundwater network (1, 22, 23) (Fig. 5.1).

5.3.3 Historic data

The groundwater monitoring network on site is extensive and for the purposes of the present study 10 years of data for the dairy farm were collated (Table S5.1, No 5). No such data was available before the current study for the up-gradient farm. The groundwater monitoring system consists of three components: 1) five sets of multilevel boreholes (Fig. 5.1) representing three depths: subsoil (4.0 - 7.5 m bgl), bedrock (16.8 - 23.0 m bgl) and the

subsoil-bedrock interface (11.0 - 13.0 m bgl); 2) a network of piezometers and boreholes (Fig. 5.1), with screen depths from 1.95 to 8.95 m bgl installed to sample shallow groundwater; 3) a single borehole drilled to 37 m bgl was installed to sample deep groundwater (18, Fig. 1).

A fieldwork campaign was conducted from December 2005 to June 2014 (Table S5.1, No.5). During this period, grab samples were taken from open ditch and end-of-pipe locations at locations indicated in Fig 1. Groundwater samples were collected after purging three well volumes (CL:AIRE, 2008) with a peristaltic pump (Model 410, Solinst Canada Ltd.) and teflon outlet. Duplicate 50 ml water samples were collected in HDPE screw top bottles and filtered through 0.45 µm cellulose acetate filters (Sartorius Stedim Biotech GmbH, Germany) To elucidate groundwater flow direction the watertable depth was measured with an electronic dipper (Van Walt Ltd., Surrey, UK). An In-Situ Multi-parameter Probe (In Situ Inc., USA) with flow through cell was used to measure pH, temperature (T), electrical conductivity (EC), dissolved oxygen (DO) and redox potential (Eh) in water samples.

5.3.4 Current data

After the ten year water dataset, additional fieldwork was undertaken in September 2014 to collect water samples for all locations in Fig. 5.1.This sampling campaign was carried out to merge different techniques (i.e. stable isotopes isotope and dissolved gaseous analyses) and all the elements of the water continuum (i.e. shallow and groundwater, in field drainage and open ditches). Only one single sampling campaign was carried out as Bailey et al. (2011) highlighted the high stability of the signatures on this farm (within shallow water), while the pattern of dissolved gas was examined by Jahangir et al. (2012a,b) across the multilevel wells (Table S5.1). During this fieldwork, water was sampled using a bladder pump (flow rate of 100 ml min^{-1}) with a Teflon outlet tube (diameter: 0.6 cm) (Geotech Environmental Equipment, Inc., USA) following a low-flow micro-purging protocol for piezometers (CL:AIRE, 2008). The bladder pump minimises sample mixing and degassing (Jahangir et al., 2012a). Where a bladder pump could not be used, due to spatial constraint, a peristaltic pump and 20 ml syringe connected to a Teflon tube (diameter: 0.5 cm) with 3-way stopcock was used. Dissolved gases analysis showed that there was no significant difference between the two methods (data not shown). Triplicate 50 ml surface water samples from pipes or open drains were collected manually in HDPE screw top bottles and stored at 4°C until analysed. One replicate was filtered in the field (0.45µm cellulose acetate filter). As per historic data an electronic dipper (Van Walt Ltd., Surrey, UK) was used to elucidate watertable depth and an

In-Situ Multi-parameter Probe (In Situ Inc., USA) to measure pH, temperature (T), electrical conductivity (EC), DO and Eh in water samples.

5.3.5 Water analyses

Water samples (both current and historical) were analysed within two weeks from collection at Teagasc Johnstown Castle for the following: NO_2^--N, NH_4^+-N, Total Oxidised Nitrogen (TON) and dissolved organic carbon (DOC) were quantified using an Aquakem 600 Discrete Analyser (Aquakem 600A, 01621 Vantaa, Finland). Concentrations of NO_3^--N were calculated by subtraction of NO_2^--N from TON (NO_3^--N + NO_2^--N). Total Nitrogen (TN) was determined by alkaline persulfate oxidation (Askew and Smith, 2005).

Only for the current fieldwork, duplicate water samples were collected from each location for excess-N_2 estimation in 12 ml exetainers (LabcoWycomb Ltd., UK) after overflow of 10 ml. Double septum stoppers made of butyl rubber and teflon were used to seal the exetainers without headspace. Samples were submerged and inverted within groundwater-filled containers to prevent gas diffusion and stored at 4°C. A high precision membrane inlet mass spectrometer (MIMS) (Pfeiffer Vacuum ^{TM}QMS 200 quadrupole mass spectrometer) was used for excess-N_2 quantification. Analyses were carried out within one week from collection. The MIMS was set at the groundwater temperature of the time of sample collection (Kana et al., 1994) and calibrated before the initial reading and after every 10 samples to correct analytical drift. Standard used was deionized water equilibrated with air at constant temperature and pressure (Kana et al., 1994). Gaseous N_2 concentrations were calculated following the protocol of Weymann et al. (2008).

Duplicate groundwater samples were collected for the quantification of dissolved N_2O, carbon dioxide (CO_2) and methane (CH_4). Samples were collected in 160 ml serum bottles after an overflow of 150 ml and capped without headspace with butyl rubber septa and aluminium crimp caps (Wheaton, USA) and stored as above. Within one week from collection samples were degassed by simultaneous water extraction and addition of high purity helium (He:water 1:3; v/v) (BOC, Linde Group, Germany (Lemon, 1981). An additional 40 ml headspace was created and bottles were agitated at 400 rpm (Gyrotory shaker G-10, New Brunswick Scientific, USA) for 5 minutes and then left to stand for 30 minutes. The gas in the headspace was then collected with gas tight syringes and injected into evacuated 12 ml exetainers (one 12 exetainer was conserved and used for $\delta^{15}N$ and $\delta^{18}O$ composition of dissolved-N_2O). An auto-sampler gas chromatography (CP-3800, Varian Inc.

USA) was used to analyse N_2O and final concentrations were calculated following Henry's Law at groundwater temperature for the moment of collection.

The indirect N_2O-N emission factor (N_2O-N EF_5g) for groundwater was calculated from the relationship between dissolved-N_2O and N-inputs, as per Weymann et al. (2008) following equation 5.1.

$$EF_5g(1) = (N_2O\text{-}N)/(N_2O\text{-}N + N_2\text{-}N + NH_4^+\text{-}N + NO_3^-\text{-}N + NO_2^-\text{-}N + DON). \quad \text{(Eqn. 5.1)}$$

The alternative equation (5.2) used by the intergovernmental panel on climate change (IPCC, 2006), was not used as it assumes no processing of NO_3^- and N_2O throughout the system (Weymann et al., 2008; Jahangir et al., 2013a).

$$EF_5g(2)\ (EF_5g(2) = (N_2O\text{-}N)/(NO_3^-\text{-}N)) \quad \text{(Eqn. 5.2)}$$

5.3.6 Stable isotope analysis

Groundwater samples (40 ml) were collected and filtered in the field through 0.2 µm polyethersulfone filters (Sartorius Stedim Biotech GmbH, Germany), and stored at -20˚C in 50 ml polyethylene screw cap tubes. Samples were analysed (Dept. of Catchment Hydrology, UFZ, Germany) for the isotopic composition of NO_3^- ($^{15/14}N$ and $^{18/16}O$), NH_4^+ ($^{15/14}N$), and H_2O ($^{2/1}H$ and $^{18/16}O$). Gas exetainers of 12 ml were additionally used for dissolved-N_2O ($^{15/14}N$ and $^{18/16}O$).Isotope values were reported in δ‰ relative to international standards (AIR for N and VSMOW (Vienna Standard Mean Ocean Water) for O and H). Water $\delta^{18}O$ and δD (δ^2H) signatures for H_2O were analysed on a Los Gatos liquid water isotope analyser (analytical precision <0.15‰ for $\delta^{18}O$ and <0.5‰ for δD) using a 5x replicate analysis after discarding the first two samples. Normalisation to the VSMOW scale was based on replicate (20x) analysis of internal standards (MAST, PES, and HAD, certified to SLAP reference materials). The results are interpreted according to a modified Rayleigh equation 5.3, where *f* is the fraction of substrate remaining, ε the isotope enrichment factor, δs the isotopic composition of the residual substrate and δs0 of the initial substrate.

$$f = 1 - e^{(\delta s - \delta s0)/\varepsilon} \quad \text{(Eqn. 5.3)}$$

The $\delta^{15}N\text{-}NH_4^+$ was measured in subsurface locations with detectable NH_4^+-N concentration. The $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ were obtained through the bacterial denitrification method

(McIlvin and Casciotti, 2011). Briefly, *Pseudomonas chloraphis* (ATCC #13985) was used to quantitatively produce N_2O from NO_3^-. The $\delta^{15}N$ signature for NH_4^+ was obtained through the method described by Zhang et al. (2007). N_2O derived by a BrO^- oxidation of NH_4^+ to NO_2^- followed by reaction with sodium azide to create N_2O. The $\delta^{15}N$ and $\delta^{18}O$ composition of the produced N_2O (plus that of the dissolved N_2O samples) was measured using mass spectrometry (DeltaPlus IR-MS) (method precision: ±0.3‰). For NO_3^-, triplicate international standards (IRMS-standard NO3-1, IRMS-standard USGS-34, IRMS-standard USGS-35,) and water blanks were used to calibrate results. For NH_4^+, triplicate international standards (USGS25, USGS26), an internal $(NH_4)_2SO_4$ standard and water blanks were used for calibration.

5.4 Results and discussion

5.4.1 Partial nitrogen balance and surplus N

The annual partial N balance for total N-inputs was estimated to be 297 kg N ha^{-1} from fertilizers and 63 kg N ha^{-1} from feed concentrates, giving a total of 360 kg N ha^{-1}, compared with an average of 223 kg N ha^{-1} for Irish intensive farms (see Tracey et al. 2008) (Table 5.1). With an N-output of 141 kg N ha^{-1} exported in milk and slurry, the estimated N-surplus is 219 kg N ha^{-1}, which may potentially be stored in soil or leach from the system in the absence of loss by ammonia volatilisation (urine N volatilized as gaseous ammonia was calculated, as an average of three studies, at 15% for grassland by Scholefield et al. (1991). NH_4^+-N and/or NO_3^--N are the main N-species which are lost along surface or leaching pathways. These two N-species are the main substrate for N-biotransformation processes (i.e. denitrification, nitrification, anaerobic ammonium oxidation (anammox) and dissimilatory nitrate reduction to ammonium (DNRA)), and can further produce greenhouse gases such as N_2O when not efficiently removing reactive N from biological cycling (Rütting et al., 2011; Burgin et al., 2013).

Table 5.1. Five years annual N balance for the farm.

Year	**2011**	**2012**	**2013**	**2014**	**2015**	**Average**
Stocking Rate (per ha)	3.4	3.0	3.1	3.0	3.1	3.1
Grazing season (days)	238	215	244	256	263	243
N Inputs (kg N ha^{-1})						
Inorganic Fertilizer	232	270	266	262	256	257
Organic Fertilizer	39	44	36	38	41	40
Feed Concentrates	80	58	60	58	61	63
Total	351	372	362	358	358	360

N Exported from the system (kg N ha^{-1})						
Milk	132	124	122	123	123	125
Slurry	19	17	15	16	15	16
Total	151	141	137	139	138	141
N Balance (kg N ha^{-1})						
Surplus	200	231	225	219	220	219
N efficiency %	23	26	26	26	26	26
N Efficiency/t Fat and protein	184	224	196	175	172	190
Milk production						
Volume	23.3	22	21.5	21.7	21.9	22.1
Milk solids	1.78	1.63	1.61	1.65	1.67	2.00
Total denitrification (N_2O-N + excess N_2-N (mg N l^{-1})) (Jahangir et al., 2013a)						
Subsoil	1.76 ± 0.04					
Bedrock-interface	2.64 ± 0.43					
Bedrock	2.50 ± 0.33					
Site mean	2.30 ± 0.27					

5.4.2 Spatial and temporal variation in aqueous N-species

Considering all locations from 2005 to 2014, NO_3^--N concentrations varied from a maximum of 25.31 mg NO_3^--N l^{-1} to below the detection limit. Over time the farm average decreased from 4.56 to 3.00 mg NO_3^--N l^{-1} from 2005 to 2010, respectively. In 2014 the average was 3.66 mg NO_3^--N l^{-1} (Fig. S1).

In September 2014, NO_3^--N concentrations in the drainage system were below the significant contamination threshold of 5.65 mg NO_3^--N l^{-1} as set out by Daly (2000) and OECD (2001). Sample locations in the up-gradient unit were below 2.9 mg NO_3^--N l^{-1} at D3 and close to the contamination threshold for D1 at 5.2 mg NO_3^--N l^{-1}. In the down-gradient unit, the average NO_3^--N drainage concentration leaving the farm and being discharged to the receiving water body was 3.4 mg NO_3^--N l^{-1} (Fig. 5.2). In the up-gradient unit (dominated by low permeability), shallow groundwater had a NO_3^--N concentration of 3.4 mg NO_3^--N l^{-1}, whereas in the down-gradient unit (variable permeability) three distinct shallow groundwater signatures emerge: a) in the north, shallow samples ranged from 6.2 to 8.3 mg NO_3^--N l^{-1}, and deeper layers ranged from 5.7 to 7.0 mg NO_3^--N l^{-1}; b) well drained soils close to sampling location 14 (see Fig 5.1) where concentrations ranged from 4.03 to 7.2 mg NO_3^--N l^{-1}; c) central part of the farm on a well-moderately drained soil exhibited concentrations from 6.3 mg NO_3^--N l^{-1} (5.47 m bgl) to 7.6 mg NO_3^--N l^{-1} (Fig. 5.2). The deepest well on the farm (location 18) had a concentration below NO_3^--N MAC, indicating the vertical extent of the NO_3- plume to be around 16 m. There were no elevated concentrations in wells at the south

border of the farm on imperfectly drained soil (Fig. 5.1 and 5.2). The NO_2^--N concentration was on average 0.008 mg NO_2^--N l^{-1}, with a maximum of 1.56 mg NO_2^--N l^{-1} in July 2007. Average values for both the farm and the drainage system only exceeded the NO_2^--N MAC (0.15 mg NO_2^--N l^{-1}; EU, 2014a) between July and August 2007.In September 2014, the NO_2^--N concentration averaged 0.018 mg NO_2^--N l^{-1}, with only one well (6) having a value above NO_2^--N MAC (Fig. S5.1).

The average farm NH_4^+-N concentration was 0.10 mg NH_4^+-N l^{-1}, with a maximum of 14.54 mg NH_4^+-N l^{-1} in October 2013. The drainage system of both units had an average value above NH_4^+-N MAC (0.23 mg NH_4^+-N l^{-1}) (EU, 2014a), with 90% of locations showing at least one sporadic concentration above the NH_4^+-N MAC over the years. In September 2014, the farm average was 0.98 mg NH_4^+-N l^{-1}, while the drainage system average was 0.010 mg NH_4^+-N l^{-1}. The highest NH_4^+-N concentration, i.e. 22.74 mg l^{-1}, occurred at location 37 in the southern reaches of the farm (Fig. S5.1). Across time this location showed the most persistent NH_4^+-N contamination. At this sampling time the drainage system, with a concentration of 0.00 mg NH_4^+-N/l, showed no sign of impact on the receiving surface waterbody (0.01 mg NH_4^+-N l^{-1}). There was no elevated NH_4^+-N concentrations at shallow depths (0 - 4.5 m bgl), but at deeper sampling depths higher concentrations occurred in the central area of the dairy where groundwater concentrations ranged from 0.16 to 8.98 mg NH_4^+-N l^{-1} (Fig. 5.3). These differences between shallow and deeper screen intervals and their consistently elevated NH_4^+-N concentration at deeper screen intervals infer the presence of different transformational processes along the vertical path of N from deposition to collection as no changes of management occurred.

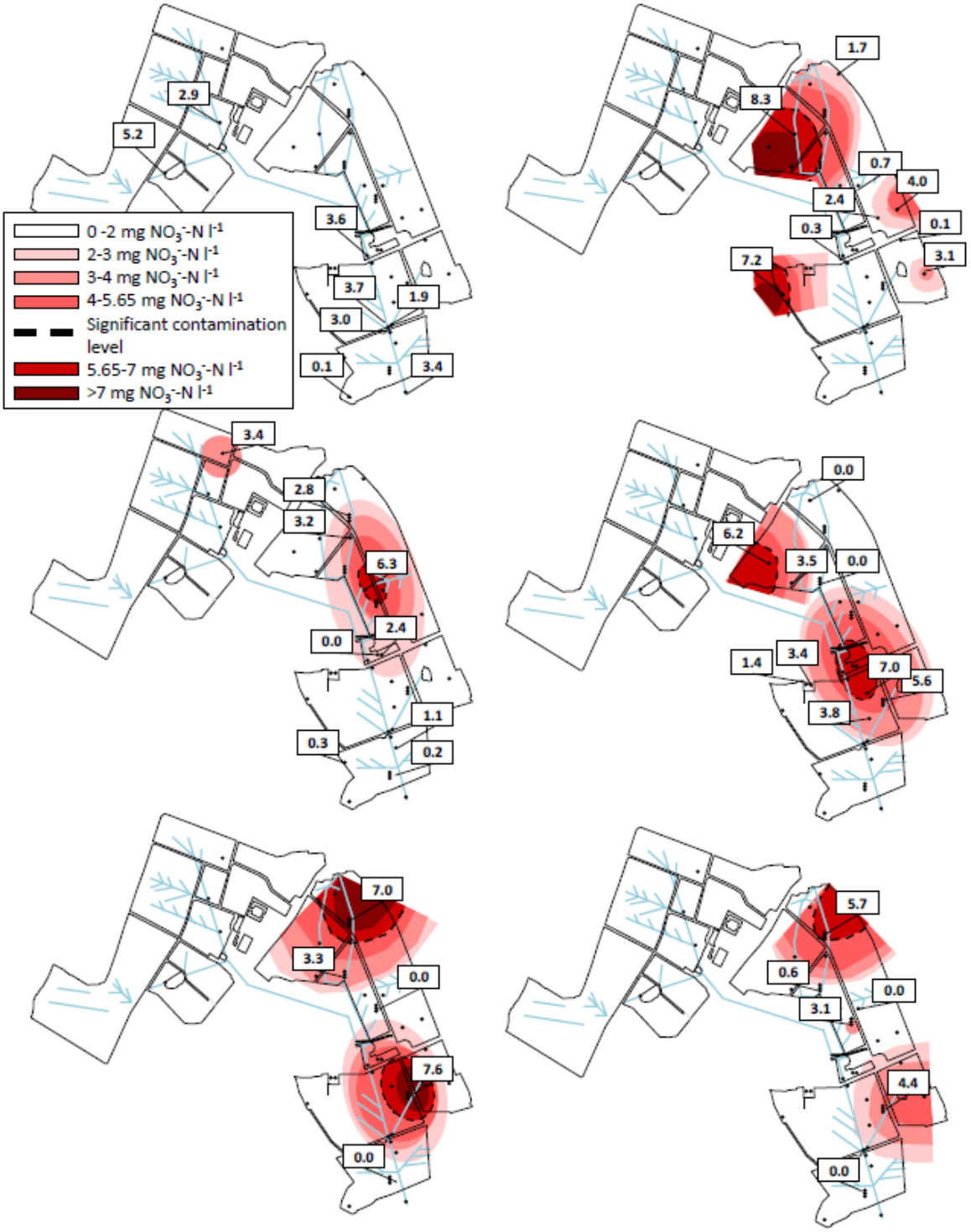

Fig. 5.2. Depth specific NO_3^--N concentration on the farm collected on September 2014. Top left: drainage system, top right: 2.95-4.5 m bgl, middle left 4.5-6 m bgl, middle right 6-9 m bgl, bottom left 11-13 m bgl, bottom right: below 16 m bgl.

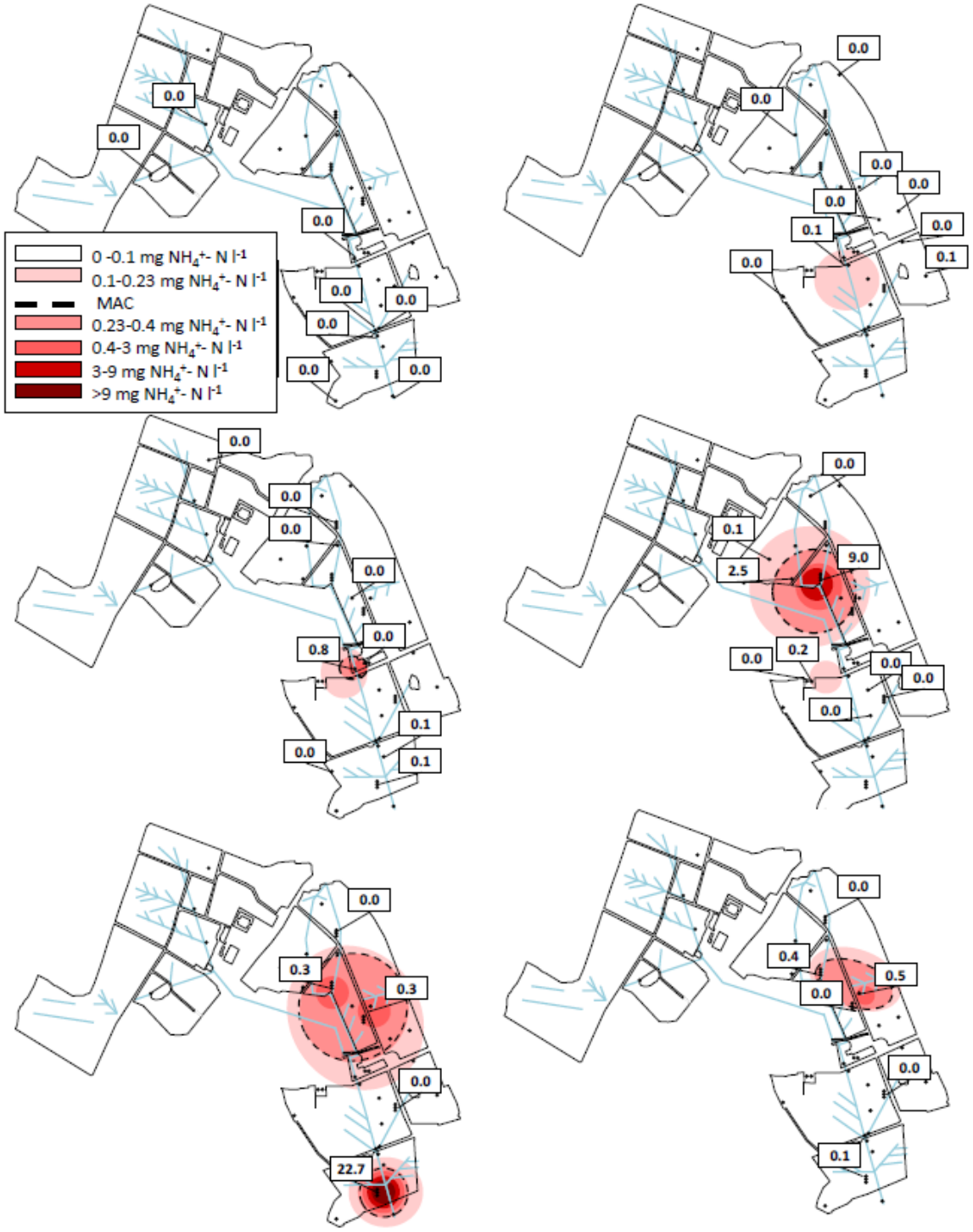

Fig. 5.3. Depth specific NH_4^+-N concentration on the farm collected on September 2014. Top left: drainage system, top right: 2.95-4.5 m bgl, middle left 4.5-6 m bgl, middle right 6-9 m bgl, bottom left 11-13 m bgl, bottom right: below 16 m bgl.

In terms of dissolved gases (dissolved-N_2O and excess-N_2) in water samples, the excess-N_2 concentration averaged 2.28 mg N_2-N l^{-1}, with higher values at the interface and bedrock layer, while dissolved N_2O was 0.024 mg N_2O-N l^{-1}, with higher values in the subsoil layer (Jahangir et al., 2013a). In September 2014, the farm excess-N_2 average was 1.90 mg N_2-N l^{-}

^{1}, with a maximum of 6.82 mg N_2-N l^{-1} and a minimum of 0.0004 mg N_2-N l^{-1}, while average for the drainage system was 0.076 mg N_2-N l^{-1} (max, 0.18 mg N_2-N l^{-1}; min, 0.006 mg N_2-N l^{-1}). The drainage system did not generally show excess-N_2 emissions, with only the last section showing excesses between 0.006 and 0.18 mg N_2-N l^{-1} (Fig. S5.2). The up-gradient unit was characterised by emissions of 0.54 mg N_2-N l^{-1}. . At shallow depth in the down-gradient unit excess-N_2 was found at higher rates in the south eastern area on well to moderately drained soils, with peak concentrations of 3.36 mg N_2-N l^{-1} at 2.95 - 4.5 m bgl and 4.32 mg N_2-N l-1 at 4.5 - 6 m bgl. Excess-N_2 occurred below these depths on imperfectly drained soils in the northern area, with a peak excess of 4.12 mg N_2-N l^{-1} at 6 - 9 m bgl, and on moderately drained soils in more central areas with maximum excess values of 6.52 mg N_2-N l^{-1} and 6.82 mg N_2-N l^{-1} at 11-13 and 16 m bgl, respectively (Fig. S5.2).Dissolved-N_2O values averaged 0.03 mg N_2O-N l^{-1}, ranging from a maximum of 0.036 to a minimum of 0.0002 mg N_2O-N l^{-1}. The farm value for $EF_5g(1)$ was 0.0039 mg N_2O-N/mg N input, compared with the IPCC set default value of 0.0025 mg N_2O-N/mg N input for groundwater N_2O emissions. Both the drainage and surface water emission were below this default value. Only one infield drain (D1, 0.0057 mg N_2O-N/mg N input) had a higher value than the default (Fig. S5.3). The up-gradient unit was characterised by a high emission factor (0.0243 mg N_2O-N/mg N input), while the down-gradient unit (0 – 4.5 m bgl) had a high number of wells exceeding default values in the central area, reaching a maximum of 0.0081 mg N_2O-N/mg N on well to moderately drained soil. At intermediate depths (4.5 – 9 m bgl) values were above the default values towards the north (6, 0.0325 mg N_2O-N/mg N input) and south (23, 0.0114 mg N_2O-N/mg N input; 37, 0.0097 mg N_2O-N/mg N input). In contrast to excess-N_2, N_2O decreased with increasing depth, with almost no monitoring well above the default values below 11 m bgl (Fig. S5.3).

Dissolved-N_2O and excess-N_2 distribution data can help with the identification of denitrification hot-spots. On the present site a wide range of dissolved N_2O vs. total emissions (N_2O + N_2) were found, which indicates variable total gas emissions and rate of denitrification, thus highlighting different degrees of denitrification. When the denitrification rate is high enough to keep the NO_3^--N concentration below the contamination threshold, excess-N_2 is released due to completion of the process. Conversely, where denitrification is limited, high dissolved-N_2O occurs, probably due to incompleteness (Rivett et al., 2008).

Nitrification is an important process which can contribute to N_2O production, stable isotopes ($\delta^{18}O$ and $\delta^{15}N$) of N_2O can elucidate discrepancies in gas production and identify N_2O sources (Kool et al., 2007; Snider et al., 2012; Snider et al., 2013; Lewicka-Szczebak et al.,

2016). N_2O-N was found to be produced *in situ* and correlated with the watertable depth and k_s (Fenton, et al., 2011; Jahangir et al., 2013a; Jahangir et al., 2013b). Based on Snider et al. (2012) the expected range of $\delta^{18}O$-N_2O produced by nitrification-denitrification on this farm was calculated between 0 and 20‰ (Fig. S5.4). This range included 75% of the wells. Wells with a relative enrichment of $\delta^{18}O$-N_2O above these values are presumably only influenced by N_2O reduction via denitrification. In addition, using the $\delta^{18}O$-N_2O and $\delta^{15}N$-N_2O ranges reported by Li et al. (2014), in particular $\delta^{15}N$-N_2O enrichment values, almost all locations have a N_2O signature characterised by relative enrichment in $\delta^{15}N$-N_2O. This probably reflects enrichment in the NH_4^+ source due to its consumption by microbial processes.

5.4.3 Provenance of water

Stable isotope compositions for all water samples showed low spatial variability, with values between -7.2 and -3.4‰ for H_2O-$\delta^{18}O$ and between -40.4 and -32.4‰ for H_2O-δD (Fig. 5.4). To examine the groundwater interaction with the drainage system, water stable isotope values were compared with the Global Meteoric Water Line (GMWL, $\delta D = 8*\delta^{18}O+10$) (Craig, 1961) to infer their composition and provenance (Fig. 4). Farm values had a lower slope than the GMWL. Water samples (from screened intervals of piezometers and boreholes) had stable isotopic values close to the GMWL, between -6.4 and -5.0‰ for $\delta^{18}O$ and between -40.3 and -32.4‰ for δD. Some sub-surface locations had the same water isotope values as many groundwater boreholes and piezometers. However, for other locations e.g. along the surface open system, there was an enrichment in $\delta^{18}O$ but not δD relative GMWL (Fig. 5.4) therefore showing a second separated signature. A third isotope signature representing water from the lake system exhibited enrichment in both $\delta^{18}O$ and δD-H_2O (Fig. 5.4).

The groundwater flow direction on site is from north to south mirroring topography (Baily et al., 2011). The isotopic composition of the groundwater samples collected in September 2014 plots on a lower slope of the GMWL identified by Craig. (1961) (Fig. 5.4). This indicated a relative enrichment consistent with the high-humidity climate of the British Isles (i.e. higher enrichment with higher rainfall) and findings of Darling et al. (2003). Samples from the end-of-pipe locations had the same water isotopic signature as for most groundwater samples, suggesting a common origin and interaction between groundwater and the drainage system. However, other locations belonging to the drainage system located along the open ditch were relatively enriched in $\delta^{18}O$ but not δD, resulting in shifted values from the main group. This most likely reflects migration and accentuated evaporation (enrichment of $\delta^{18}O$-H_2O) of the first signal within the drainage pipe in these surface waters with the creation of a second

signal (Gibson et al., 2005). Additionally, the further absence of data showing mixed values between the two separated signatures of 1) non-evaporated and 2) the evaporated points suggests that evaporation is only significant along the old surface open drains and that there is no mixing. Water from the outlet of the lake systems had a different signature, suggesting a different origin (Fig. 5.4). However, this water with a third signal is isolated from the field systems. It is possible that this sample from the lake system will however show an alteration of its signature temporally in relation to upstream events.

Overall, the assessment of continuity from groundwater to surface open drain water passing though the in-field drainage system highlighted a good hydraulic connection between surface and deeper soil horizons, with an established drainage through the soil to groundwater and in-field drainage system. It also indicated the absence of multiple sources of water, with common recharge for the entire area, enabling the system to be treated as a single influence area. This reduces sourced of variability for the isotopic signatures.

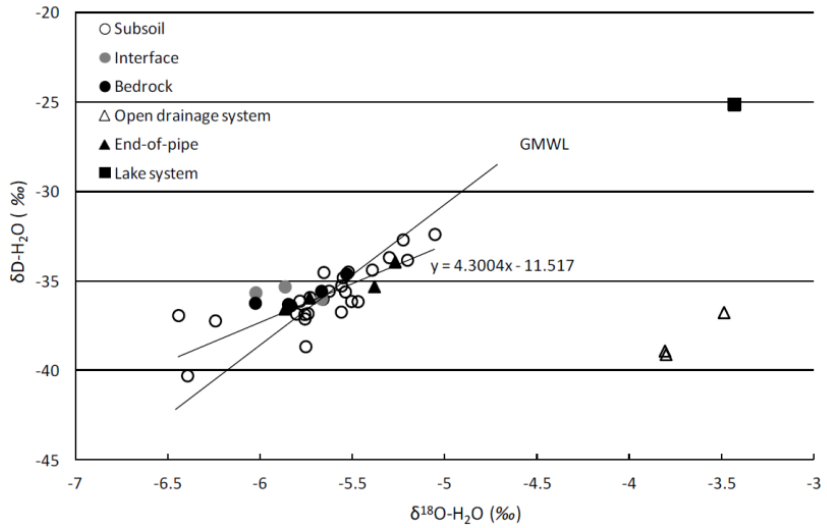

Fig. 5.4. $\delta^{18}O$ versus $\delta D\text{-}H_2O$ values for samples collected in September 2014 at the sampling locations.

5.4.4 Spatial variation in nitrate $\delta^{18}O$ and $\delta^{15}N$ in water samples

The average $\delta^{15}N$ composition of NO_3^- in water samples was 14.2‰, with a range from 6.2 to 54.9‰ (Fig. 5.5). The average $\delta^{18}O$ composition of NO_3^- in water samples was 9.7‰, with a range of 2.3‰ to 28.2‰. Isotopic values within the drainage system varied between 5.86 and 7.86‰ for $\delta^{18}O\text{-}NO_3^-$ and between 9.72and 12.89 ‰ for $\delta^{15}N\text{-}NO_3^-$. The up-gradient unit was characterised by isotopic values of 3.2 and 13.2‰ for $\delta^{18}O$ and $\delta^{15}N\text{-}NO_3^-$ respectively. From 0 - 4.5 m bgl the down-gradient unit showed higher enrichment in the central and south area close to location 20 (17.1 and 25.6‰ for $\delta^{18}O$ and $\delta^{15}N\text{-}NO_3^-$) moderately drained soil and

location 26 (17.6 and 52.7‰ for $\delta^{18}O$ and $\delta^{15}N\text{-}NO_3^-$) poorly drained soil, while location 21, well moderately drained soil, showed an enrichment only for $\delta^{15}N\text{-}NO_3^-$ (54.9‰). Relatively enriched values occur between 4.5-6 m bgl in the south area (locations 24 and 25 poorly drained soils) and one near locations 35 and 36 imperfectly drained soils. Between 6 - 9 m bgl there is also a relative enrichment for $\delta^{15}N\text{-}NO_3^-$ in the north area (11, 28.2 and 13.0‰ for $\delta^{18}O$ and $\delta^{15}N\text{-}NO_3^-$) and in the south area. Similar enrichments occur between 11-13 and 13-16 m bgl for both $\delta^{18}O$ and $\delta^{15}N\text{-}NO_3^-$ at location 13 (19.5 and 21.9‰ for $\delta^{18}O$and $\delta^{15}N\text{-}NO_3^-$) and for $\delta^{15}N\text{-}NO_3^-$ at location 38 (20.9‰).

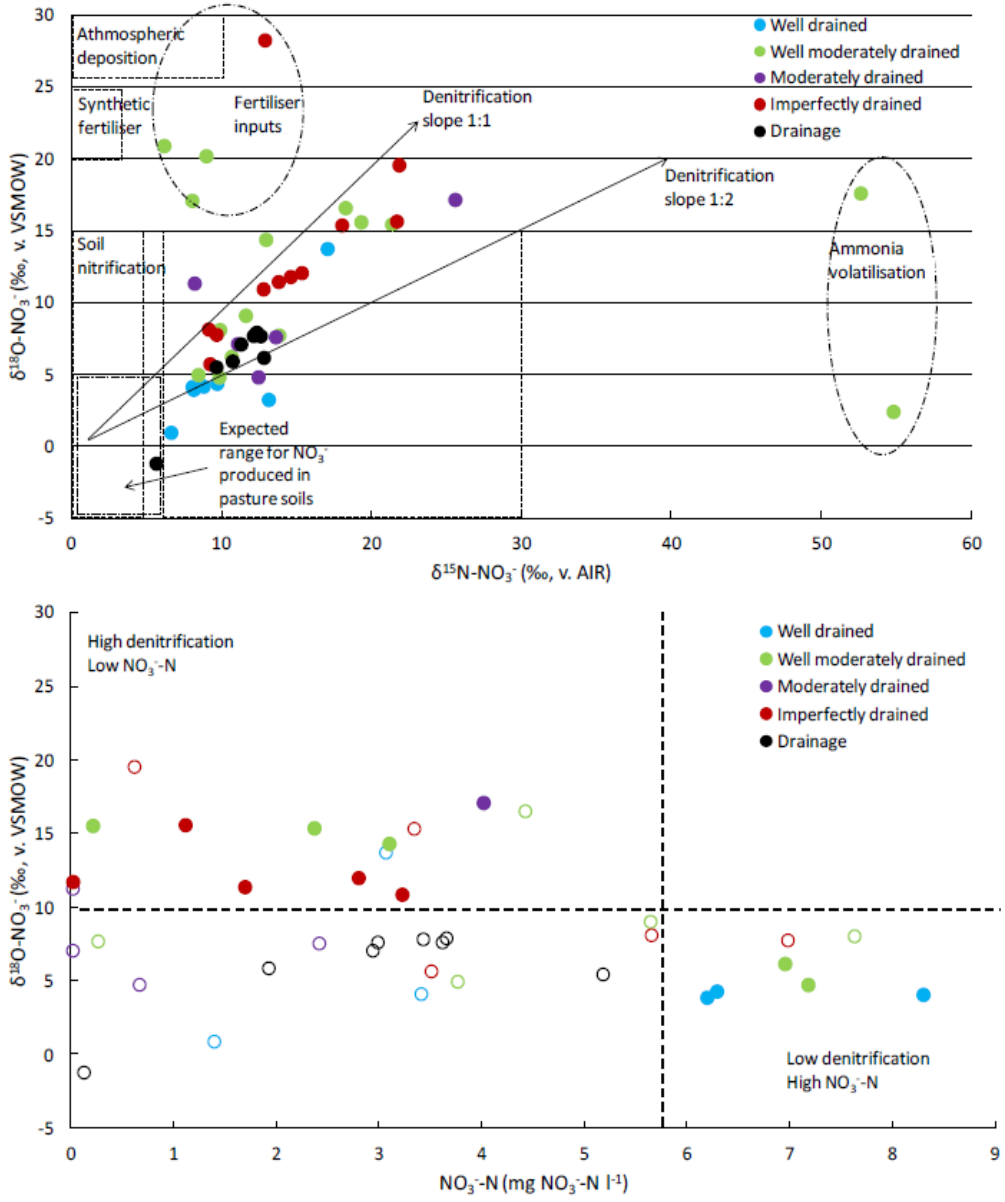

Fig. 5.5. Top: scatterplot showing $\delta^{18}O\text{-}NO_3^-$ vs. $\delta^{15}N\text{-}NO_3^-$ for water samples collected in September 2014 superimposed onto $\delta^{18}O$ and $\delta^{15}N$ ranges for N-sources and processes by Kendall (1998) and Baily et al (2011). Bottom: scatterplot showing $\delta^{18}O\text{-}NO_3^-$ vs. NO_3^-N values, identifying condition of inputs and denitrification rate. Whole circles identify wells with alternatively 1) NO_3^-N concentration <5.65 mg NO_3^-N l-1 and high denitrification

isotope signature (>10‰) i.e. exhibiting excess inputs of NO_3^--N that has been denitrified or 2) NO_3^--N >5.65 mg NO_3^--N l-1 and low denitrification isotopic signature (<10‰) i.e. exhibiting contamination due to an insufficient rate of denitrification. Open circles identify wells that were discarded due to depth or where the NO_3^--N concentration was <5.65 mg NO_3^--N l-1 in combination with a low denitrification isotopic signature (<10‰) i.e. exhibiting a situation of limited denitrification and low inputs.

The spatial distribution of NO_3^--N was highly variable with some locations exceeding 5.65 mg l^{-1} (Fig. 5.2). Comparing these values with those reported for 2008 (Baily et al., 2011) for the same wells, most of the NO_3^- isotopic signatures were consistent over time, with 75% having $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values within 3‰ of the 2008 values. In 2008, the NO_3^--N concentration above the NO_3^--N MAC or significant contamination level (5.65 mg NO_3^--N l^{-1}) occurred on the down-gradient unit in three main areas: the north end (surrounding 5, well drained soil), with a value of 9.5 mg NO_3^--N l^{-1} (±2.9); the central area (surrounding 14, well drained soil), with a value of 10.3 mg NO_3^--N l^{-1} (±4.5); and the south end (surrounding 28 well moderately drained soil), with a value of 7.3 mg NO_3^--N l^{-1} (±2.4) (Baily et al., 2011). An elevated NO_3^--N concentration was attributed to old DSW irrigation areas and farmyard leachate (i.e. milking parlour, winter-housing and out-wintering pads) (Baily et al., 2011). In September 2014, areas characterised by NO_3^--N below the contamination threshold (5.65 mg NO_3^--N l^{-1}) were present on all types of soil (Fig. 5.5), and the drainage system generally had low levels of NO_3^--N. An explanation of the low NO_3^--N concentration within the drainage system requires an understanding of the potential origins and processes affecting this N species. NO_3^--N isotopic values are determined by N-sources (i.e. organic and inorganic fertilizer) and biotransformation processes (i.e. denitrification, nitrification and DNRA) affecting the NO_3^--N pool (Xue et al., 2009). The groundwater NO_3^--N isotopic composition clustered within the manure/sewage value range and along a 1:1 – 1:2 slope, suggesting a common organic source for these, and denitrification as the main biotransformation process (Fig. 5.5) (Granger et al., 2008, Granger and Wankel, 2016, Hernandez-del Amo, et al. 2018). However, distinguishing between denitrification and DNRA is difficult as the isotope effect of DNRA has still not been investigated (Alkhatib et al., 2012, Wells et al., 2016). Locations falling along the isotopic denitrification line are inferred to have a generally consistent NO_3^- source, given the similar fertiliser management across the farm and shared drainage system. The absence of mixing of water and sources therefore does not invalidate the use of a modified Rayleigh equation to estimate attenuation of NO_3^--N (Ostrom et al., 2002). Values between -3 to -30‰ have been reported for ε_{denit} (Sebilo et al., 2003; Granger et al., 2008), but on this site the ratio of $\delta^{18}O{:}\delta^{15}N$ enrichment during denitrification remained constant.

Under this condition, assuming a single source and value of ε, movement up the 1:1 denitrification slope (enrichment in both $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$) can be assumed to reflect biotransformation of NO_3^--N and is directly proportional to the degree of denitrification (*f*) (Ostrom et al. 2002, Wells et al., 2016). Fractionation was therefore used to classify the magnitude of biotransformation (i.e. higher attenuation producing greater enrichment) and assess the NO_3^--N that was denitrified at different locations. NO_3^--N fractionation therefore represented a useful tool to identify and locate hot spots of denitrification across the farms. However, it is important to note that a 'high attenuation' value may not imply exactly where the denitrification occurred, as NO_3^- isotopes data reflect cumulative processes during transport (between deposition and measurement point, therefore from surface to well and mixing of 'upstream' water).

Nonetheless, the consistency of isotopic signatures over time (six years from 2008 to 2014) may imply that these values reflect location and soil intrinsic denitrification ability rather than the immediate product of a 'hot spot' or 'hot moment' activity (for more detail see Baily et al., 2011). This consistency supports the use of the single sampling event in the present study. Only a few wells showed higher temporal variability compared with values collected in 2008 (Baily et al., 2011). Distinct signatures characterised these small groups of wells, which showed alternatively high values in $\delta^{18}O\text{-}NO_3^-$ or $\delta^{15}N\text{-}NO_3^-$, with a shift from the 1:1-1:2 slope (Fig. 5.5) (Kendall, 1998). Two main alternative signatures were identified: a high enrichment of $\delta^{15}N\text{-}NO_3^-$ possibly from surface NH_3 volatilization, and high $\delta^{18}O\text{-}NO_3^-$ values, possibly due to an atmospheric source or synthetic fertilizer as nitrate source (Fig. 5.5).

The subset of the data points close to the 1:1 isotope ratio line, where denitrification was the dominant process, was further examined by eliminating points with a fertiliser and ammonia volatilisation signature (Fig. 5.5). As subsoil extended from 0.2 to 10 m bgl, therefore having higher similarity with the soil map, wells below 10 m bgl were further eliminated. A plot of $\delta^{18}O\text{-}NO_3^-$ (as measure of denitrification) versus NO_3^--N, revealed three main groups, each identifying a specific condition of inputs and denitrification rate (Fig. 5.5). The first group had a low NO_3^--N concentration (below 5.65 mg NO_3^--N l^{-1}) associated with low denitrification isotopic signature (<10‰); locations in this group were therefore characterised by limited denitrification and low inputs. The second group had a low NO_3^--N concentration but high denitrification isotope signature (>10‰), indicating that excess inputs of NO_3^--N have been denitrified. The final group had high NO_3^--N (>5.65 mg NO_3^--N l^{-1}) and low denitrification isotopic signature (<10‰), highlighting contamination derived by a rate of

denitrification which was not sufficient to ensure concentration below the contamination threshold. Group one was discarded from analysis as not influential in terms of denitrification and contamination, while groups with NO_3^--N above 5.65 mg NO_3^--N l^{-1} or $\delta^{18}O$-NO_3^- above 10‰ were selected. The extremes of soil drainage categories (e.g. well-drained vs. poorly drained) showed a relationship with poorly drained soils characterised by high denitrification potential and low NO_3^--N, while well drained soils had low denitrification potential and a higher NO_3^--N concentration. An isotopic enrichment pattern with higher values on lower permeability soils, but less enrichment on highly permeable soils was consistent with the findings of Fenton et al. (2011) of higher denitrification rates at lower values of soil k_S (Fig. 5.5).

Natural isotopic abundance for NH_4^+-N was measured in piezometer and borehole water samples with detectable NH_4^+-N concentration. The average $\delta^{15}N$-NH_4^+farm concentration was 18.5‰ with a maximum value of 34.3‰ and a minimum of 3.3‰. The most enriched $\delta^{15}N$-NH_4^+ values were observed in piezometers and boreholes with NH_4^+-N concentrations above NH_4^+-N MAC (e.g. 24, 34.3‰, imperfectly drained soil). The site average N_2O stable isotope composition was 14.2‰ for $\delta^{18}O$-N_2O and -17.1‰ for $\delta^{15}N$-N_2O respectively. Maximum values were 46.7 and 21.4‰ for $\delta^{18}O$-N_2O and $\delta^{15}N$-N_2O respectively, while minimum values were -14.5 and -32.5‰. NH_4^+-N contaminated wells were found mainly in the central area of the farm (Fig. 5.3) with moderate to imperfectly drained soil. A high NH_4^+-N concentration was evident in wells showing relative enrichment in $\delta^{18}O$-NO_3^- and with a synthetic fertilizer source signature (Fig. 5.5). However, a high NH_4^+-N concentration could occur without this signature, due to *in-situ* production of NO_3^- to NH_4^+ by DNRA (Jahangir et al., 2012b; Jahangir et al., 2013a). Even though denitrification and DNRA occur under similar environmental conditions, DNRA has rarely been observed with respect to denitrification but is an important process under anaerobic conditions with high (>12) C/NO_3^- ratio (Yin et al., 1998, Rütting et al., 2011). Since no direct fractionation factors exist for DNRA (Dhondt et al., 2003), $\delta^{15}N$-NH_4^+ values were measured to assess a possible role of DNRA. As per Rayleigh fractionation, we hypothesised that DNRA would lead to a $\delta^{15}N$-NH_4^+ value significantly less enriched than at a location where denitrification was the dominant process. With only four locations having a C/NO_3^- ratio >12, $\delta^{15}N$-NH_4^+ signatures did not show any distinct patterns. This indicated that DNRA was of secondary importance and restricted to a few locations at high depth or micropores within the soil profile (Fig. S5.5).

5.4.5 Conceptual diagram of the site to inform the sustainability of the agronomic system and the fate of N

Bringing together all of the data (Fig. 5.6) from the present study four main groups of locations emerged which were (Fig. 5.1., Table S5.2):

1: locations with good water quality, based on NH_4^+-N and NO_3^--N concentrations which were below both NH_4^+-N and NO_3^--N MAC and significant contamination respectively. These soils are imperfectly to moderately-well drained and drainage from these does not represent a threat to water contamination. This was most likely the result of relatively high NO_3^--N attenuation rates, via denitrification. Generally low N_2O emissions inferred completeness of denitrification, although some locally elevated N_2O production suggested concurrent secondary processes (e.g. nitrification), with a possible threat to air quality.(Av. from 2009 to 2014, WT: 3.5 m bgl; pH: 7.2; EC: 518 µS; Eh 428 mV; DO: 2.2 mg l^{-1}).

2: locations with poor water quality, based on a NO_3^--N concentration above significant contamination. These soils were mainly well to moderately-well drained, with a higher permeability than those in Group 1. Drainage from these locations represented a threat to groundwater quality and air quality (due to high N_2O emissions, which indicated incomplete denitrification). This most likely resulted from a medium-low potential for NO_3^--N attenuation by denitrification and if drained will present a threat to groundwater and GHG (greenhouse gases) emissions (Av. from 2009 to 2014, WT: 4.2 m bgl; pH: 7.2; EC: 335 µS; Eh 126 mV; DO: 5.4 mg l^{-1}).

3: locations with intermediate water quality, based on a NO_3^--N concentration which was below the contamination threshold, but with high N_2O production. These soils were classified as having well to moderately drained classes. Drainage from these soils did not represent a threat to groundwater, but instead was a problem in terms of GHG emissions. This occurred due to a low capacity for denitrification, resulting in low NO_3^--N, possibly coupled with additional N_2O emissions from nitrification. (av. from 2009 to 2014, WT: 3.1 m bgl; pH: 7.0; EC: 497 µS; Eh 90 mV; DO: 3.2 mg l^{-1}).

4: locations on well to imperfectly drained soil, based on low NO_3^--N but with NH_4^+-N above NH_4^+-N MAC and high N_2O emissions. Soils under this condition had generally low potential for denitrification and drainage presented a threat to groundwater and GHG emissions. (Av. from 2009 to 2014, WT: 3.9 m bgl; pH: 6.9; EC: 441 µS; Eh 81 mV; DO: 2.6 mg l^{-1}).

De Klein et al. (2017) observed that more than 50% of EU dairy farms had high N surpluses (>200 kg N ha^{-1}) with only 7% showing values below 100 kg N ha^{-1}. The N surplus on our Irish farm was high and was above national averages (i.e. 175 kg N ha^{-1} (Mihailescu et al.,

2014)). This surplus could be lost across the site along two distinct pathways (Fig. 5.6). The consequences in terms of water quality sustainability and the fate of N are different for these two pathways: a) a shallow migration pathway in poorly drained or imperfectly drained soils with high water purification functionality. The artificial drainage system does not disrupt this capacity but instead conveys clean water through the drainage system to the exit point of the farm and b) a deep migration pathway in moderately and well drained soils where the water purification function is lower. This facilitates leaching of N, which is then converted at depth to NH_4^+-N and migrates off site along deep groundwater pathways creating a two layered system. To prevent future N_r losses in groundwater, management should be cognisant of this two tiered system. The farm could be considered sustainable in relation to N losses from the drainage system as the high attenuation within the first 5 m of poorly/moderately drained soils protects water quality. Furthermore, the installation of this drainage system did not interrupt the normal process of soil attenuation within the farm. The management however cannot be considered as sustainable in the area where NO_3^--N was converted to NH_4^+-N and groundwater contamination by NH_4^+-N represents a concern. Further studies must be carried out in order to understand the fate of this NH_4^+-N in the deeper layer and verify its attenuation or its loss during the migration to the river.

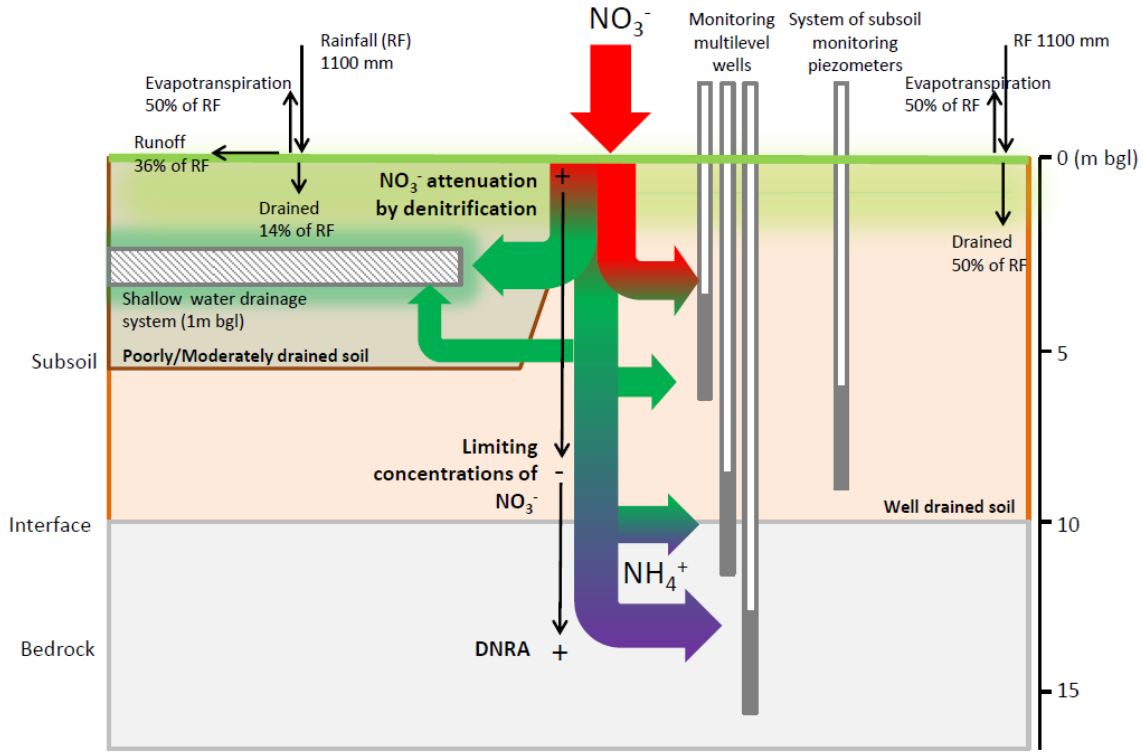

Fig 5.6. Conceptual diagram of the two tiered system beneath the site: 1) a shallow migration pathway in poorly-imperfectly drained soils with high NO_3^--N attenuation which is not disrupted by the artificial drainage system to the outlet; 2) a deep migration pathway under

moderately-well drained conditions where NO_3^--N attenuation is lower therefore leading to its transformation in NH_4^+-N.

5.5 Conclusions

Coupling multi-level data on nutrition, biogeochemistry, isotopes and dissolved gases enabled a conceptual diagram of an intensive dairy system to be created. Four groups of locations emerged based on these factors, which could be further distilled into a two tiered system: a shallow system with a high water purification function that is not disrupted by the extensive artificial drainage system and which allows attenuated water to leave the farm, and a deep groundwater system where nitrate was converted to ammonium which subsequently could migrate off site. Future management on the present or other sites, which aims to attain sustainable intensification credentials, must consider such complexity.

Within this Chapter 5 the hypotheses created in Chapter 2 were met. The concepts of Chapter 3 and 4 were expanded to a wider area, a more varied range of drainage classes, the totality of pathways along the water transfer continuum and additional isotopical analyses. On this farm with heterogeneous drainage classes the net denitrification capacity of different soil/subsoil profiles was used to rank areas of the dairy farm in terms of sustainability (N attenuation) as the different drainage classes of this farm varied in terms of their net denitrification capacity. However, the random artificial land drainage created in order to remediate specific problems in the targeted areas did not affect water attenuation while they seemed to act as a conduit to transport attenuated water.

5.6 Acknowledgements

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Chapter 6 - Further insights into N transformation processes within intensive dairy farms using bacterial gene assessment

6.1 Introduction

For nearly a century, mankind caused unprecedented changes to the nitrogen cycle by more than doubling the transformation of non-reactive atmospheric di-nitrogen (N_2) into reactive nitrogen (N_r) forms, which cascade through the environment (Galloway et al., 2003). On the one hand, the production of N_r for crops through fertilisation has been essential, enabling population growth, but on the other hand, this growth has come with a very high environmental and societal cost. This has highlighted N_r as one of the three "planetary boundaries" that have been exceeded as a result of human activities (Sutton et al., 2011a, b). These boundaries included threshold breaches for drinking water quality and air quality, with consequences such as the eutrophication of fresh waters, the depletion of coastal waters ecosystems, climate change and ozone layer depletion, biodiversity loss, soil acidification and loss (Ayres et al., 1994; Sutton et al., 2011b). As nutrient use efficiencies on intensive dairy systems continue to be low (Mihailescu et al., 2014), N surpluses continue to be lost and migrate along varied pathways (surface (Ibrahim et al., 2013) and subsurface (Selbie et al., 2015)) thereby negatively affecting water quality and sustainable intensification credentials. Leached losses are converted through mineralisation in the unsaturated zone to nitrate (NO_3^-), which depending on the water attenuation capacity (Schulte et al., 2014) of the soil-subsoil-bedrock continuum (Jahangir et al., 2013a), can either have a positive or negative effect on the water quality of connected water bodies. An assessment of "sustainable intensification" must elucidate which N transformational processes occur at different points along this transfer continuum. In previous chapter water samples were used to elucidate N source, transformation and fate, here we examined other constituents in these sample e.g. bacterial genes. Bacterial genes could shed further light on sustainability as they could highlight difference in terms of most probable N transformational processes to occur and the differences of occurring processes within water continuum, soil drainage classes and attenuation groups (e.g. sustainability groups identified in Chapter 4 and 5). Spatial distributions of ammonium (NH_4^+) and NO_3^- concentrations along surface and subsurface pathways vary according to the prevailing conditions at that point or within the zone of contribution to that point. As can be seen from previous chapters, several methods, which when combined, can be used to investigate the N source, transformation and fate and thereby

inform a conceptual model of a dairy system and therefore comment on the sustainability of that system (see Chapters 3, 4 and 5). Further insights pertaining to a conceptual diagram of any intensive dairy field site may or may not be achieved by adding further layers of complexity. However, the microbial analysis of water samples at distinct breakthrough points (hydrologically sensitive areas as highlighted by Thomas et al. (2016)) e.g. end-of-pipe (EOP) locations of an artificial drainage system, may improve the overall interpretation of isotopic transformational data. Also, such information may support conclusions based on biogeochemical data for various layers of the conceptual diagram formed.

Nitrification and denitrification are the main processes that attenuate NH_4^+ and NO_3^- contamination. Denitrification is a multiple step process, therefore it can release various intermediate products depending on environmental condition e.g. too high NO_3^- concentration or dissolved oxygen might lead to the release of greenhouse gases (GHG) such as nitrous oxide (N_2O) and nitric oxide (NO) (Knowles, 1982). Even though denitrifiers are ubiquitous in both soil and fresh water, denitrification requires specific environmental conditions controlled by edaphic factors to occur (Hallin et al., 2009). Other processes of the N cycle occur under similar conditions as denitrification (DNRA) while others affect denitrification in terms of availability of substrates (e.g. nitrification, anammox).

Numerous studies have analysed the spatial pattern of the denitrifier community. Some studies analysed water samples however the vast majority focused on soil. For example, Philippot et al. (2009) showed spatial patterns of denitrifiers abundances based upon soil properties and land management criterion, with *nosZ1* emerging as a strong predictor of denitrification i.e. $N_2O/(N_2+N_2O)$. The *nosZ* gene was found to be divided in two physiologically different clusters *nosZ1* and *nosZ2* (Jones et al., 2013). Results correlated negatively with N_2O, which indicates that the more *nosZ1* present the more full denitrification occurs i.e. di-nitrogen (N_2) production and greater sustainability. However, further studies need to be carried out to assess the impact of these two clusters on N_2O emissions. In a Swedish study (Enwall et al., 2010), which utilised soil from an organic research farm, spatial autocorrelation was found for denitrifier community structure, size and activity. Important here from a sustainability perspective was that *nirK* and *nirS* correlated with the potential rate of conversion of nitrite (NO_2^-) to N_2O and with the *nirS*/*nirK* ratio identifying a particular environmental niche.

Using wetland sediments in Ohio, USA, Song et al. (2010) showed that bacterial structure changed due to long term wet/dry cycles rather than short lived episodic periods and *nirS* abundance was affected by such wet-dry cycles. However, the structure was not found to be a

determinant of denitrification rates due to the redundancy of this process. Regan et al. (2011), utilising grassland soils in Germany, found that the ratio of *nosZ*/*nirK* could be used as an indicator of N_2O emissions and therefore could be used to interpret the level of completeness of denitrification. Utilising multi-level water samples (6, 12 and 15 m depths), Barrett et al. (2013) explored the bacterial community of groundwater in terms of *nirK*, *nirS* and *nosZ1* abundance across multiple Irish research farms (including intensive dairy farms). Results showed that groundwater abundance of *nirK*, *nirS*, and *nosZ* was variable within sites but constant across sites. Indeed no changes in abundance were found based on farm management change, even though such changes did in fact alter groundwater quality. Across sites, differences were explained by in-situ variable environmental conditions and these explained a switch mechanism in communities between active/dormant phases.

Most importantly and contrary to Enwall et al. (2010) and Regan et al. (2011), *nirK* and *nirS* was not correlated to N_2O production in the Barrett et al. (2013) study. Additionally, N_2O production and n*osZ* did not show any correlation with N_2 production. This potentially shows the mobile phase as represented by water samples from a multi-level borehole network in low to moderately permeable subsoils and bedrock as not having the same predictive potential in terms of other N cycle processes as the soil phase. Instead, groundwater migrating down hydraulic gradients interacts with soil/subsoil horizons and acts in effect, as a highway for the distribution of particulate and dissolved constituents to different soil/subsoil horizons e.g. dissolved organic carbon (DOC), nitrogen, oxygen. These soils horizons in turn also receive inputs from the agronomic system, which can leach and build up in the unsaturated zone. In addition, an area may or may not be artificially drained depending on soil type and drainage class. This additional pathway controls the height of the water table in the zone of contribution of that system and influences wet/dry cycles in the surrounding soil profile. For example, an increase of dissolved organic concentrations in water appeared to be the main driver of *nirK*, *nirS* and *nosZ* abundances with depth in the Barrett et al. (2013) study.

The *amoA* gene and the potential nitrification rates have been previously positively correlated in soil with increase in the production intensities and N-inputs (Stempfhuber et al., 2014). Anammox bacteria are common in water-saturated agricultural soils with high N availability (Humbert et al., 2010). Even though abundant they are generally present at lower GCC concentration than the *nosZ* gene and not considered to be a main N_2 production process within agricultural soils (Long et al., 2013). Limited research with respect to the DNRA process (exception here is Morrissey et al., 2013) is available within the literature and where present conclusions are inconclusive or contradict each other e.g. Welsh et al. (2014). Bu et

al. (2017), for example, analysed sediment samples from a river estuary in China. They correlated DNRA rates with the organic content of the sediment and NH_4^+ concentration but found weak correlations of DNRA with nrfA gene copies concentration (GCC). This was possibly due to the limited success of the amplification of the *nrfA* gene (Song et al., 2014).

The differences in outcomes across such studies could be due to a number of factors such as sample locations and type (surface versus subsurface environments). In the literature, there is a lack of studies which simultaneously investigate bacterial genes in open ditch, end-of-pipe and groundwater locations. As we have investigated "net" source, transformation and fate, we attempt here to elucidate whether bacterial genes can be used in the same way.

Therefore, objectives of the present study were to: 1) Examine bacterial genes involved in the N cycle using water samples taken from open ditch, end-of-pipe (EOP) and groundwater locations across three Heavy Soil Program (HSP) farms (see Chapters 4) and the Johnstown Castle Dairy farm (see Chapter 5). The following genes were examined: i.e. *16S rRNA* for total quantification, four bacterial denitrification genes (*nirS, nirK, nosZ1* and n*osZ2*), one for nitrification (*amoA*), one for anammox (*hzo* cluster 1) and one for DNRA (*nrfA*). 2) Assess if bacterial gene abundance across these locations adds to an overall interpretation of sustainability when combined with isotope natural abundances, dissolved gases and biogeochemical parameters.

6.2 Materials and methods

6.2.1 Study sites

Three of the five HSP farms ((Kishkeam (KM), Doonbeg (DG) and Athea (AA) (see Chapter 3)) were selected (Fig 6.1). Only shallow groundwater (< 2 m) and EOP samples were taken on these sites. Athea was indicative of a site with poor water quality where NH_4^+-N water contamination (>0.23 mg NH_4^+-N/l) occurred along with a low denitrification signature. Sampling locations at AA were as follows: 1) three shallow groundwater locations - one below the NH_4^+-N MAC and two above the MAC, 2) three EOP locations - two below the NH_4^+-N MAC and one above the MAC. Doonbeg was indicative of a site with good water quality and a NO_3^- signature of nitrification. Two groundwater locations were selected and four EOP locations (all of these had no NH_4^+-N contamination). Kishkeam was selected as representative of farms with no NH_4^+-N contamination and a signature of high denitrification. Here one groundwater location and three EOP locations were selected (Fig 4.2, Fig 6.1).

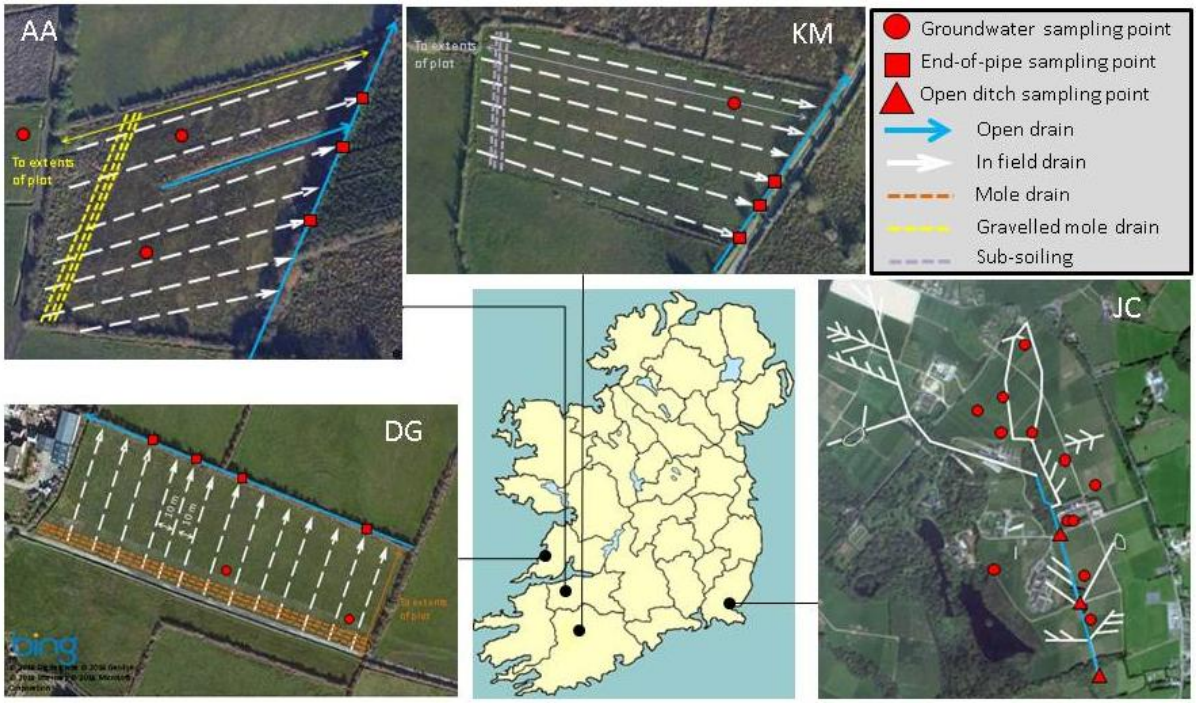

Fig 6.1. Schematic of the four study sites (AA, Athea; KM, Kishkeam; DG, Doonbeg; JC, Johnstown Castle) selected with a representation of the drainage system layout and sampling locations.

Additionally, the intensive dairy farm at Teagasc research centre in Johnstown Castle (JC, Co. Wexford, South-east Ireland, see Chapter 3 and 5)was used to take open ditch water samples at three locations (D4, D7, and D8, Fig 6.1) and groundwater samples limited to the subsoil section to a depth of 9 m (Fig 6.1). Three samples were collected for each of the four groundwater groups identified in Chapter 5:

- Group 1 (G1): piezometers 2, 25 and 35, characteristics: good water quality, NH_4^+-N and NO_3^--N below MAC and organic contamination limit, imperfectly to moderately-well drained soils, high denitrification ($\delta^{15}N\text{-}NO_3^-$>12‰, $\delta^{18}O\text{-}NO_3^-$>10‰), low N_2O emissions (<0.01 mg N_2O-N/l), completeness of denitrification;
- Group 2 (G2): piezometers: 4, 5 and 27, characteristics: poor water quality, NO_3^--N>5.65 mg/l, well to moderately-well drained soils, low denitrification ($\delta^{15}N\text{-}NO_3^-$ <12‰, $\delta^{18}O\text{-}NO_3^-$<10‰), high N_2O emissions (>0.01 mg N_2O-N/l mg/l), incompleteness of denitrification);
- Group 3 (G3): piezometers: 15, 19 and 29, characteristics: low NO_3^--N concentration, well to moderately drained soils, high N_2O production;
- Group 4 (G4): piezometers: 6, 11 and 24, characteristics: NH_4^+-N>0.23 mg NH_4^+-N/l, high N_2O emissions, low potential for denitrification).

For interpretation purposes the open ditch samples were collated into an additional group i.e. Group 5 (G5).

6.2.2 Water samples collection and DNA extraction

Water samples were collected during the same sampling campaign as in Chapters 4 (AA, KM, and DG, Oct-Nov 2015) and 5 (JC, Sep-Oct 2014). Open ditch and EOP samples were taken manually, whereas groundwater samples were collected after first well purging (CL:AIRE, 2008) with a low flow peristaltic pump (Model 410, Solinst Canada Ltd.) and Teflon outlet tube (⌀ 0.6cm). For each sampling point (open ditch, EOP and groundwater) (Fig. 6.1), one litre of water was collected in polypropylene bottles (VWR, International GmbH, Germany) and immediately stored in an ice-box. On each collection day after agitating to achieve a homogeneous sample, 200 ml of water was filtered (0.2 µm polycarbonate filter, Sartorius Stedim Biotech GmbH, Germany) using a Microsart® e.jet vacuum laboratory pump (Sartorius Stedim Biotech GmbH, Germany) (Barrett et al., 2013). Triplicate filters were produced for each location. Filters were stored at -80˚C until analysis. DNA from each filter was extracted using an UltraClean® Microbial DNA Isolation Kit (MoBio Laboratories, Inc, USA) according to manufacturer guidance in Oct 2016. Samples were visualized on 1% (w/v) 1×TAE agarose gels and DNA was stored at -80 °C until analyses (Nov 2016 - May 2017).

6.2.3 16S and functional genes quantitative real time polymerase chain reaction (q-RT-PCR)

Extracts were diluted 1:10 in Ambion® nuclease-free water (Thermo Fisher Scientific, Inc, USA) to reduce possible inhibition. Amplifications were realised using the SYBR Green PCR kit master mix (QIAGEN, Netherlands) according to manufacturer's instruction in a total volume of 15 µl. An aliquot of 3µl of the 1:10 solution of template were added per reaction to the PCR master mix. Condition of the PCR followed the protocols outlined in the references of Table 6.1. The q-RT-PCR quantification was performed in triplicate for standards and in duplicate for extracts using an AB700 real-time PCR cycler according to manufacturer's instructions. Duplicates that showed a difference between threshold cycles (ΔCt) below 1 were considered acceptable. Samples that showed low amplification were purified using Microcon® PCR grade filters (Merck Millipore, USA) No inhibition was however encountered.

Standard curves were produced for absolute quantifications of 16S rRNA, four bacterial denitrification genes (*nirS*, *nirK*, *nosZ1* and *nosZ2*), one for bacterial nitrification (*amoA*), one for bacterial anammox (*hzo cluster 1*) and one for bacterial DNRA (*nrfA*). Plasmid (pGEMt for 16S rRNA, *nirS*, *nirK*, *nosZ1*, *nosZ2* and *amoA* while PCR4-Topo for *hzo*) with an insert of the target genes, and genomic *E. Coli* MG1655 DNA for *nrfA*, were used as standards. Standard plasmid was quantified though the use of Qubit dsDNA BR Assay Kit following manufacturer's instructions. Triplicate curves were created using corresponding standards (from 10^9 to 10^1 copy numbers, 10-fold serial dilution series) and primer sets (Table 6.1). For all bacterial genes, results are presented as GCC per litre (GCC/l).

6.2.4 Statistical analyses

Significant differences between abundance of N cycling genes and the 16S RNA gene were tested within and between sites and among their water contaminations groups through the use of one way ANOVA and Tukey's HSD test (IBM SPSS Statistics version 24) ($\alpha = 0.05$). When variance was not equal, Dunnett's T3 test was used instead of Tukey's. Data was log transformed using prior to statistical testing to ensure normality.

Table 6.1. Genes and primer sets used for the qPCR of the water samples collected at the four sites in Oct-Nov 2015 (Athea, Kishkeam, and Doonbeg) and Sep-Oct 2014 (Johnstown Castle).

Gene	Primer	Sequence (5'-3')	Amplicon size (bp)	Reaction condition	Reference
16S	F: 341F R: 518R	CCTACGGGAGGCAGCAG ATTACCGCGGCTGCTGG	194	95˚C-15 min; 40 cycles of 95˚C-20 sec, 54˚C-20 sec, acquisition at 72˚C-30 sec; 95˚C-10 sec; 60˚C-15 sec; dissociation curve. (Improved from Daniell et al., 2012)	Muyzer et al. (1993)
*nir*K	F: nirK876 R: nirK1040	ATYGGCGGVCAYGGCGA GCCTCGATCAGRTTRTGGTT	164	95˚C-15 min; 6 cycles of 95˚C-15 sec, 63to58˚C-30 sec with a decrease of 1˚C every cycle, 72˚C-30 sec, 80˚C-15 sec; 40 cycles of 95˚C-15 sec, 60˚C-30 sec, 72˚C-30 sec, acquisition at 80˚C-30 sec; 95˚C-15 sec; dissociation curve. (Henry et al., 2004)	Hallin et al. (2009)
*nir*S	F: Cd3aF R: R3cd	GTSAACGTSAAGGARACSGG GASTTCGGRTGSGTCTTGA	416	95˚C-10 min; 40 cycles of 95˚C-30 sec, 57˚C-20 sec, acquisition at 72˚C-30 sec; 95˚C-15 sec; dissociation curve. (Thompson et al., 2016)	Michotey et al. (2000) Throback et al. (2004)
*nos*Z1	F: nosZ2F R: nosZ2R	CGCRACGGCAASAAGGTSMSSGT CAKRTGCAKSGCRTGGCAGAA	267	95˚C-15 min; 6 cycles of 95˚C-15 sec, 65to60˚C-30 sec with a decrease of 1˚C every cycle, 72˚C-30 sec, 80˚C-15 sec; 40 cycles of 95˚C-15 sec, 60˚C-15 sec, 72˚C-30 sec, acquisition at 80˚C-30 sec; 95˚C-15 sec; dissociation curve. (Henry et al., 2006)	Henry et al. (2006)
*nos*Z2	F: nosZ-II-F R: nosZ-II-R	CTIGGICCIYTKCAYAC GCIGARCARAAITCBGTRC	683	95˚C-15 min; 40 cycles of 95˚C-15 sec, 60˚C-30 sec, 72˚C-30 sec acquisition at 80˚C-30 sec; 95˚C-15 sec; dissociation curve. (Jones et al., 2013)	Jones et al. (2013)
amoA	F: amoA-1F R: amoA-2R	GGGGTTTCTACTGGTGGT CCCCTCKGSAAAGCCTTCTTC	491	95˚C-10 min; 45 cycles of 95˚C-1 min, 54˚C-1 min, acquisition at 72˚C-1 min; 72˚C-10 min; dissociation curve. (Segal et al., 2017)	Rotthauwe et al. (1997)
*hzo*1	F: hzoF1 R: hzoR1	TGTGCATGGTCAATTGAAAG CAACCTCTTCWGCAGGTGCATG	740	95˚C-10 min; 40 cycles of 95˚C-30 sec, 56˚C-20 sec, acquisition at 72˚C-40 sec; 95˚C-15 sec; dissociation curve. (Kong et al., 2013))	Kong et al. (2013)
nrfA	F: nrfAF2aw R: nrfAR1	CARTGYCAYGTBGARTA TWNGGCATRTGRCARTC	269	95˚C-10 min; 50 cycles of 95˚C-15 sec, 52˚C-45 sec, 72˚C-20 sec acquisition at 80˚C-35 sec; 95˚C-15 sec; dissociation curve. (Song et al., 2014)	Welsh et al. (2014)

6.3 Results

6.3.1 Inter-farm variations of GCCs of genes across all sites

Between the sites investigated, GCCs followed similar patterns and were relatively constant indicating a low influence of soil type and management on the abundance of denitrifiers (Fig. 6.2). The genes *nirS*, *nirK*, *nosZ1*, *hzo1*, *nrfA* and *16S* did not show significant differences across sites. Denitrification genes for nitrite reductase, *nirS* and *nirK*, averaged between 2.3×10^6 GCC/l (JC) and 6.9×10^6 GCC/l (DG) and 4.2×10^7 GCC/l (JC) and 1.4×10^8 GCC/l (AA) respectively; genes for the nitrous oxide reductase genes for the two clusters *nosZ1* and *nosZ2* averaged between 2.4×10^9 GCC/l (JC) and 4.2×10^9 GCC/l (DG) for *nosZ1* while *nosZ2* was significantly lower ($p<0.05$) within JC (1.4×10^6 GCC/l) than in DG (1.2×10^7 GCC/l). The gene for the ammonia monooxygenase (*amoA*) was again significantly higher at DG (6.7×10^6 GCC/l) than in KM (2.9×10^5 GCC/l). The bacterial anammox gene *hzo1* (*hzo* cluster 1) showed max 2.3×10^6 GCC/l average at JC and min 2.2×10^5 GCC/l at AA. The GCC for the gene for the DNRA process (*nrfA*) showed averages between 4.6×10^5 GCC/l at DG and 1.2×10^6 GCC/l at JC, respectively. Values for *16S* gene averaged between 1.4×10^8 GCC/l (JC) and 2.5×10^8 GCC/l (DG).

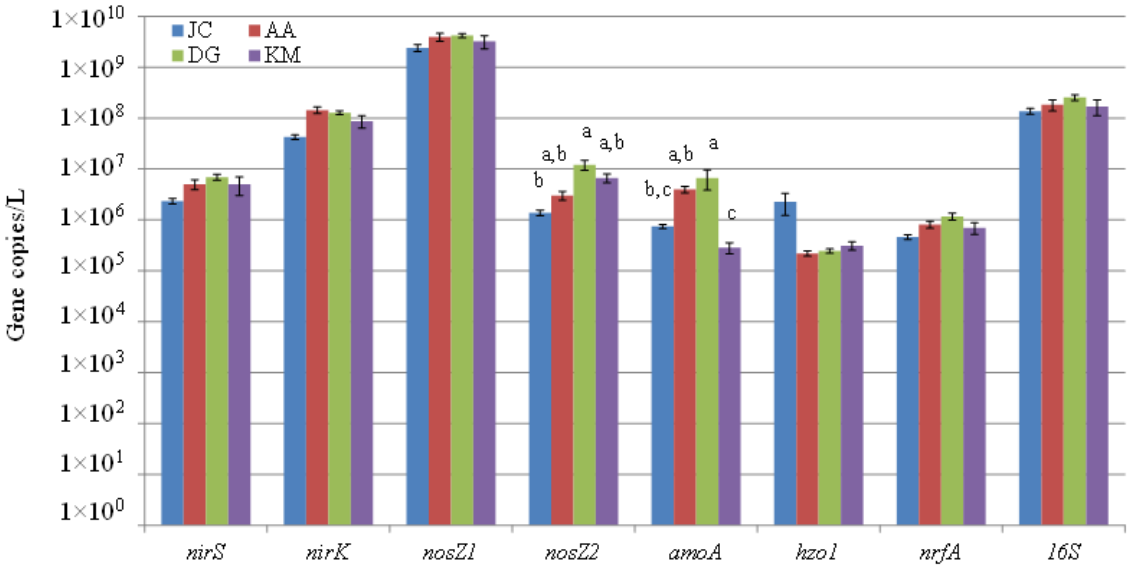

Fig. 6.2. Variation in gene copy concentrations (GCC/l) across the four selected farms for the analysed genes. Standard errors are indicated for each separate group. Statistical differences ($p<0.05$) between GCC within sites are indicated by different letter.

Gene ratio for *nosZ1/nosZ2*, *nirS/nirK*, *nosZ1/nirS*, *nosZ1/ nirK*, *nosZ2/nirS* and *nosZ2/ nirK* were further analysed. Only significant differences were found for *nosZ2/nirS* and *nosZ2/ nirK*. The ratio *nosZ2/nirS* was found significantly higher at KM (7.96) than JC, AA and DG (0.26, 0.25 and 0.32 respectively) which showed similar ratios. The ratio *nosZ2/nirK* was

again found significantly higher at KM (0.22) than JC and AA (both showing a ration of 0.01) while DG showed a ratio of 0.03.

6.3.2 Inter HSP farm variation of GCC of genes

Results showed that similar patterns of GCC for each gene were present across the three farms studied and between groundwater and EOP locations (Fig. 6.3, Table 6.2).

In general the HSP farms did not show any significant difference within groundwater versus drainage water. More into details, no significant differences were found for the GCC of the genes *16S*, *nirS*, *nirK*, *nosZ1*, *nosZ2*, *hzo1* and *nrfA*. The only gene that showed significant differences ($p<0.05$) across HSP farms was *amoA* within groundwater (Fig. 6.3). More specifically at DG, the gene for the ammonia monooxygenase showed an average of 8.1×10^6 GCC/l for EOP locations. At AA, the *amoA* gene averaged 4.7×10^6 GCC/l for EOP locations. Here, however, NH_4^+-N contaminated water did not show difference with good quality locations (Fig. 6.4). At KM, *amoA* showed an average of 1.6×10^5 GCC/l in EOP locations (Fig. 6.3).

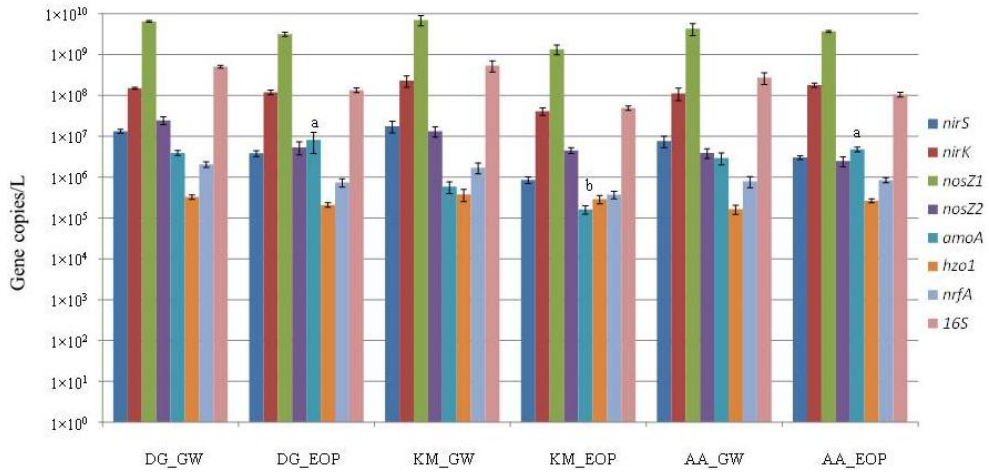

Fig. 6.3. Variation in gene copy concentration (GCC/l) between groundwater (GW) and end-of-pipes (EOP) water across the heavy soil farms for the analysed genes. Standard errors are indicated for each separated group. Statistical differences ($p<0.05$) between GCC within sites are indicated by different letter.

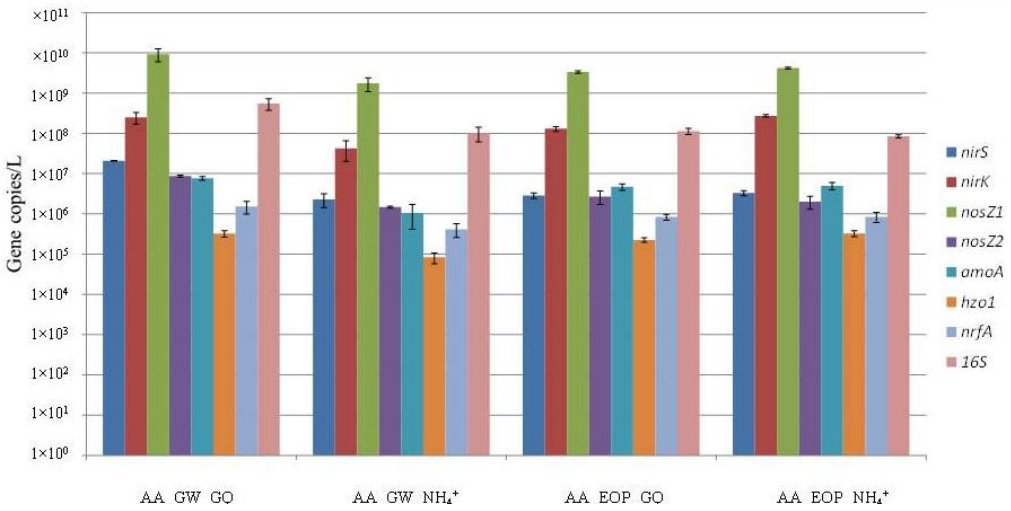

Fig. 6.4. Variation in gene copy concentration (GCC/l) between NH_4^+-N contaminated and not contaminated groundwater and drainage water across Athea (AA). GQ indicates good water quality locations while NH_4^+ indicates locations where NH_4^+-N was above MAC. Standard errors are indicated for each separated group.

6.3.3 Variation of GCC of genes on Johnstown Castle Dairy Farm

Results across the four groundwater groups showed that the GCC for the *16S*, *nirS*, *nirK*, *nosZ1*, *nosZ2*, *amoA* and *nrfA* genes were similar within all locations (groundwater (G1, G2, G3 and G4) and open ditches (G5)). The GCCs for gene hzo1 however showed significant differences between the groups. More specifically, the *hzo1* gene showed significantly higher GCC for group G1 and G4 than G2 (G1: 7.3×10^6 GCC/l; G2: 4.7×10^4 GCC/l; G3: 2.4×10^5 GCC/l; G4: 4.1×10^5 GCC/land G5: 2.4×10^5 GCC/l).

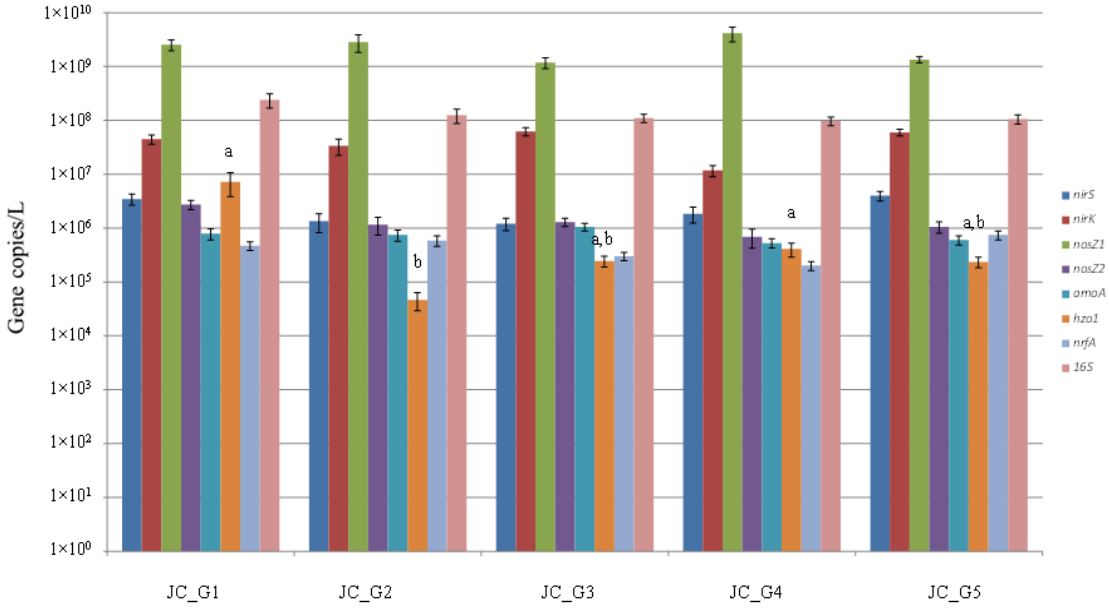

Fig. 6.5. Variation in gene copy concentration (GCC/l) between groundwater (G1, G2, G3 and G4) and open ditches (G5) across Johnstown castle (JC) farm for the analysed genes. Standard errors are indicated for each separated group. Statistical differences ($p<0.05$) between GCC within sites are indicated by different letter.

Table 6.2. Shallow groundwater (piezometer), open ditch and end-of-pipe GCCs, N-gaseous emissions*, NO_3^- isotopic compositions* and NH_4^+-N and NO_3^--N concentrations*. *values retrieved from Chapter 4 and 5.

Farm	Location	Water quality	*nirS*	*nirK*	*nosZ1*	*nosZ2*	*amoA*	*hzo1*	*nrfA*	*16S*	N_2	N_2O-N	$d^{15}N$-NO_3	$d^{18}O$-NO_3	NH_4^+-N	NO_3^--N
			(GCC/l)								(mg N/l)		(‰)		(mg N/l)	
AA	Piezometer	Good	2.1×10^{7}	2.5×10^{8}	9.2×10^{9}	8.7×10^{6}	7.6×10^{6}	3.2×10^{5}	1.5×10^{6}	5.5×10^{8}	0.60	0.00	11.97	20.53	0.17	0.04
AA	Piezometer	NH_4^+-N >MAC	2.2×10^{6}	7.1×10^{7}	2.1×10^{9}	1.6×10^{6}	1.7×10^{6}	1.1×10^{5}	3.7×10^{5}	9.2×10^{7}	0.00	0.00	5.38	10.74	0.38	0.16
AA	Piezometer	NH_4^+-N> MAC	2.4×10^{6}	1.4×10^{7}	1.4×10^{9}	1.4×10^{6}	5.1×10^{4}	5.9×10^{4}	4.6×10^{5}	1.2×10^{8}	0.52	0.00	13.38	23.34	0.39	0.04
AA	EOP	Good	1.7×10^{6}	9.7×10^{7}	2.9×10^{9}	5.1×10^{5}	2.8×10^{6}	1.4×10^{5}	5.5×10^{5}	7.1×10^{7}	0.00	0.01	7.55	2.20	0.01	0.87
AA	EOP	Good	4.0×10^{6}	1.6×10^{8}	3.8×10^{9}	4.9×10^{6}	6.6×10^{6}	2.8×10^{5}	1.1×10^{6}	1.5×10^{8}	0.00	0.00	10.41	-1.40	0.57	0.68
AA	EOP	NH_4^+-N> MAC	3.3×10^{6}	2.7×10^{8}	4.2×10^{9}	2.0×10^{6}	5.0×10^{6}	3.2×10^{5}	8.4×10^{5}	8.4×10^{7}	0.00	0.00	6.94	4.92	0.09	0.85
DG	Piezometer	Good	1.6×10^{7}	1.4×10^{8}	5.9×10^{9}	1.7×10^{7}	2.5×10^{6}	2.8×10^{5}	1.5×10^{6}	4.6×10^{8}	0.47	0.00	8.22	7.46	0.14	0.09
DG	Piezometer	Good	1.0×10^{7}	1.5×10^{8}	6.9×10^{9}	3.1×10^{7}	5.3×10^{6}	3.7×10^{5}	2.6×10^{6}	5.3×10^{8}	0.86	0.00	4.52	12.67	0.16	0.05
DG	EOP	Good	2.3×10^{6}	1.4×10^{8}	3.4×10^{9}	2.5×10^{6}	2.3×10^{6}	1.7×10^{5}	6.2×10^{5}	1.0×10^{8}	0.09	0.00	7.03	0.17	0.03	0.36
DG	EOP	Good	5.0×10^{6}	1.8×10^{8}	3.6×10^{9}	3.2×10^{6}	2.7×10^{7}	3.0×10^{5}	5.1×10^{5}	1.5×10^{8}	0.18	0.00	3.38	2.70	0.05	0.22
DG	EOP	Good	6.9×10^{6}	1.0×10^{8}	4.2×10^{9}	1.4×10^{7}	1.1×10^{6}	2.9×10^{5}	1.5×10^{6}	2.2×10^{8}	0.00	0.00	5.60	0.12	0.02	0.15
DG	EOP	Good	9.6×10^{5}	4.9×10^{7}	1.2×10^{9}	2.2×10^{5}	1.4×10^{6}	6.9×10^{4}	2.8×10^{5}	6.0×10^{7}	0.16	0.00	6.06	-1.74	0.02	0.32
KM	Piezometer	Good	1.7×10^{7}	2.3×10^{8}	7.0×10^{9}	1.3×10^{7}	5.8×10^{5}	3.7×10^{5}	1.7×10^{6}	5.3×10^{8}	0.34	0.00	18.98	13.68	0.16	0.12
KM	EOP	Good	4.2×10^{5}	1.4×10^{7}	4.9×10^{8}	3.3×10^{6}	9.2×10^{4}	2.3×10^{5}	3.2×10^{5}	3.1×10^{7}	0.00	0.01	25.50	6.90	0.01	0.32
KM	EOP	Good	5.2×10^{5}	2.0×10^{7}	5.8×10^{8}	3.1×10^{6}	2.9×10^{4}	3.1×10^{5}	6.0×10^{4}	4.0×10^{7}	0.00	0.02	20.35	9.25	0.01	3.02
KM	EOP	Good	1.6×10^{6}	8.8×10^{7}	2.9×10^{9}	7.0×10^{6}	2.7×10^{5}	3.0×10^{5}	7.2×10^{5}	7.5×10^{7}	0.00	0.00	12.55	1.58	0.01	0.81
JC	Piezometer 2	Group 1	6.1×10^{6}	5.1×10^{7}	4.4×10^{9}	4.4×10^{6}	4.6×10^{5}	2.0×10^{6}	4.9×10^{5}	3.0×10^{8}	3.29	0.00	14.67	11.74	0.00	0.02
JC	Piezometer 25	Group 1	3.6×10^{6}	6.7×10^{7}	2.8×10^{9}	2.4×10^{6}	9.2×10^{5}	2.0×10^{6}	4.5×10^{5}	3.8×10^{8}	2.99	0.00	21.42	15.38	0.00	2.37
JC	Piezometer 35	Group 1	6.4×10^{5}	1.6×10^{7}	3.9×10^{8}	1.3×10^{6}	9.8×10^{5}	1.8×10^{7}	4.7×10^{5}	3.4×10^{7}	2.40	0.00	21.75	15.60	0.08	1.11
JC	Piezometer 4	Group 2	2.2×10^{5}	1.0×10^{7}	1.2×10^{9}	1.6×10^{5}	5.6×10^{5}	6.4×10^{3}	6.4×10^{5}	7.3×10^{7}	0.00	0.02	8.20	3.88	0.11	6.20
JC	Piezometer 5	Group 2	5.8×10^{5}	1.2×10^{7}	3.1×10^{8}	5.0×10^{5}	3.9×10^{5}	4.7×10^{4}	2.2×10^{5}	1.9×10^{7}	0.00	0.03	8.12	4.08	0.00	8.31
JC	Piezometer 27	Group 2	3.2×10^{6}	7.9×10^{7}	7.0×10^{9}	2.8×10^{6}	1.3×10^{6}	8.7×10^{4}	8.9×10^{5}	2.8×10^{8}	0.00	0.05	9.92	4.75	0.00	7.19
JC	Piezometer 15	Group 3	1.3×10^{6}	1.0×10^{8}	1.4×10^{9}	1.7×10^{6}	1.2×10^{6}	3.9×10^{5}	4.8×10^{5}	1.7×10^{8}	0.14	0.01	12.52	4.75	0.00	0.66
JC	Piezometer 19	Group 3	2.3×10^{5}	2.5×10^{7}	3.8×10^{8}	9.0×10^{5}	6.0×10^{5}	1.9×10^{5}	1.5×10^{5}	3.9×10^{7}	0.43	0.02	13.68	7.54	0.00	2.42
JC	Piezometer 29	Group 3	2.1×10^{6}	5.8×10^{7}	1.8×10^{9}	1.2×10^{6}	1.4×10^{6}	1.3×10^{5}	2.7×10^{5}	1.2×10^{8}	0.25	0.01	8.52	4.91	0.00	3.76
JC	Piezometer 6	Group 4	1.8×10^{5}	4.5×10^{6}	9.1×10^{8}	8.2×10^{4}	1.7×10^{5}	7.9×10^{4}	7.2×10^{4}	7.2×10^{7}	4.12	0.36	9.30	5.67	2.55	3.51

JC	Piezometer 11	Group 4	1.6×10^{5}	2.8×10^{6}	4.4×10^{8}	1.8×10^{5}	3.1×10^{5}	8.7×10^{3}	1.9×10^{5}	3.5×10^{7}	2.47	0.00	12.96	28.21	8.98	0.00
JC	Piezometer 24	Group 4	5.2×10^{6}	2.8×10^{7}	1.1×10^{10}	1.8×10^{6}	1.1×10^{6}	6.5×10^{5}	3.4×10^{5}	1.6×10^{8}	4.32	0.00	8.10	17.04	0.77	0.04
JC	Open ditch D4	Good	4.4×10^{6}	6.2×10^{7}	1.7×10^{9}	7.7×10^{5}	1.0×10^{6}	1.7×10^{5}	6.5×10^{5}	1.3×10^{8}	0.00	0.00	12.67	7.61	0.00	3.62
JC	Open ditch D7	Good	5.5×10^{6}	7.2×10^{7}	1.7×10^{9}	2.1×10^{6}	5.5×10^{5}	4.8×10^{5}	1.4×10^{6}	1.7×10^{8}	0.18	0.00	12.20	7.63	0.00	2.98
JC	Open ditch D8	Good	1.1×10^{6}	4.3×10^{7}	5.8×10^{8}	3.1×10^{5}	6.9×10^{4}	1.8×10^{5}	2.3×10^{5}	2.2×10^{7}	0.00	0.00	12.36	7.80	0.00	3.44

6.4 Discussion

Conceptual diagram: incorporation of bacterial gene abundance

As can be seen from Fig 6.6, when considering all four sites together there was no difference in GCC of bacterial genes (*16S*, *nirK*, *nirS*, *nosZ1* and *nrfA*) across open ditch, shallow piezometer sampling to 9 m and EOP locations. Significant differences were however found for *nosZ2* and *amoA* across sites, for *amoA* among HSP farms (AA, DG and KM) and for *hzo1* between groups at JC. This means that the use of bacterial gene abundance in water samples across these locations adds little to the overall interpretation of sustainability over and above that of the interpretation gained through the use of isotope natural abundance, dissolved gases and biogeochemical parameters. Only *nosZ2* and *amoA* were significantly different for DG, indicating high nitrification levels and a higher potential to complete the process of denitrification. These results concurred with previous outcomes in Chapter 4 and therefore backed up the sustainable credentials at that site. More specifically, due to the low variability *nosZ1* did not emerge as a strong predictor of denitrification i.e. $N_2O/(N_2+N_2O)$ and did not negatively correlate with N_2O. In terms *nirK* and *nirS* there was no evident correlation with the potential rate of conversion of NO_2^- to N_2O. However, when analysing gene ratios for the *nir* and *nosZ* genes (*nosZ1/nosZ2*, *nirS/nirK*, *nosZ1/nirS*, *nosZ1/nirK*, *nosZ2/nirS* and *nosZ2/ nirK*), *nosZ2/nirK* and *nosZ2/nirS* showed some predictive power. The two ratios were found significantly higher at KM which could indicate a higher potential at KM for complete denitrification.

In terms of GCCs, when comparing the present results (Table 6.2) with that of Barrett et al. (2013) and Jahangir et al. (2013a), numbers within these studies are quite high i.e. samples taken at the JC dairy farm in May-June 2009 were as follows: *16S* (10^3 GCC/l), *nirK* (10^4 GCC/l), *nirS* (10^4 GCC/l) and *nosZ*1 (10^1 GCC/l) genes. As no other studies were carried out utilising groundwater and open ditch/EOP samples within Irish studies, it is difficult to compare the results in the present study with those from other intensive dairy systems. The differences between this study and Barrett et al., (2013) could be due to temporal differences and/or protocol differences (i.e. sampling method (pumping, sampling of boreholes vs. piezometers), storage, extraction method, set of primers, qPCR conditions). Due to the modularity of processes (e.g. denitrification), the full set of enzymes necessary for the completion of the process is not possessed by each organism, which are performing only sections of the full pathway and therefore with possible different outcomes (Zumft, 1997). In addition, the presence of a gene does not assume its expression and the production of the correlated enzyme. However, abundance has often been used as a proxy for process rate

(Zhang et al., 2013). The farms and groups of this study showed the same patterns of genes therefore it was assumed that all these groups and farms are characterised by the potential to carry out the same N-cycle related processes.

The ratio *nirS*/*nirK* has been showed to correlate with the capacity of soils and waters to act as a sink of N_2O (Jones et al., 2014). Across the analysed farms, the GCC of *nirK* was found to be higher than *nirS* and pointing towards a high production of N_2O. In Barrett et al. (2013) however, *nirS* seemed to be present at slightly higher GCC than *nirK*. The genes *nirK* and *nirS* were thought to be mutually exclusive however a limited amount of denitrifying organisms have been shown to possess both genes (Graf et al., 2014). The genes *nirK* and *nirS* have been proven to respond to different environmental conditions and having different ecological niches (Philippot et al., 2009; Jones and Hallin, 2010, Azziz et al., 2017). The gene *nirS* has generally showed high GCC within soil and extreme habitats while *nirK* occurred in a wider range of environments but often underrepresented (Graf et al., 2014). However, within fresh waters, *nirK* seemed to be predominant with higher GGC for *nirK* found in groundwater beneath grassland (Graf et al., 2014; Peter et al., 2012).

The gene *nosZ* is commonly used as a signal for the ability to reduce N_2O to N_2 and to bring the process of denitrification to completeness. The *nos*/*nir* ratio seems in fact to be a factor affecting the completeness of the denitrification process (Philippot et al., 2011). The gene *nosZ* was shown to be spread within organisms both alone or associated with *nirK* and *nirS*. However, *nirS* genes are assumed to be more capable of complete denitrification than *nirK* harbouring organisms (Graf et al., 2014). On these farms and within groups, *nosZ1* was present at significantly higher GCC than *nosZ2*. The *nosZ1* and *nosZ2* genes were found at high GCC when compared with *nirS* and *nirK*. While *nosZ1* did not show any differences in GCC across the four sites, the significantly higher GCC at DG than JC for *nosZ2* could indicate a slightly higher potential for N_2O reduction to N_2 at DG. Additionally the highest *nosZ2/ nirK* and *nosZ2/nirS* ratios found significantly higher at KM could indicate a high potential for complete denitrification also at KM.

Focusing on the nitrification process, the rate limiting reaction is catalysed by the ammonia monooxygenase enzyme. In terms of the *amoA* gene and potential nitrification rates, DG showed a higher GCC for *amoA* than the other farms and significantly higher than JC and KM, which could be a sign of a higher predisposition towards the occurrence of nitrification. This could correspond with the nitrification signature found at DG (Chapter 4).

As per Long et al., (2013) the *hzo1* was found at largely lower GCC than the *nosZ* gene, however the *hzo1* gene did not differ across farms. At JC the differences in the GCC of *hzo1*

within groups showed a higher abundance in G1 (group characterised by good water quality) and G4 (NH_4^+-N>MAC) while lower in G2 (NO_3^--N>MAC). The higher concentration of *hzo1* within G1 and G4 could be indicator of a higher attenuation potential for NH_4^+-N depending on environmental conditions (e.g. dissolved oxygen) suggesting that anammox bacteria could be an important group to attenuate groundwater clean where anoxic condition occur.

The nrfA gene again did not show any GCC differences among the locations, this gene showed lowest representation.

This qPCR analysis confirmed some of the findings from previous isotopic and gaseous data e.g. higher denitrification occurring at DG and highlighted the importance of the anammox process for higher attenuation rates. The low variability along the water continuum and within farms and contamination groups did not lead to further insides into N-cycle clarification described in Chapters 3, 4 and 5. However, the present analysis did not exclude the possibility that more in depth and specific analyses (e.g. metagenomics, T-RFLP or the use of microchips) and the further use of primers for the analyses of Archea and Eukariotes communities could produce further insights pertaining to the processes occurring under these farms. However, such analyses due to their complexity of execution, data analyses and high cost and labour are less relevant for exploratory or monitoring analyses to be carried out routinely on farms or as an early field characterisation to guide drainage installation.

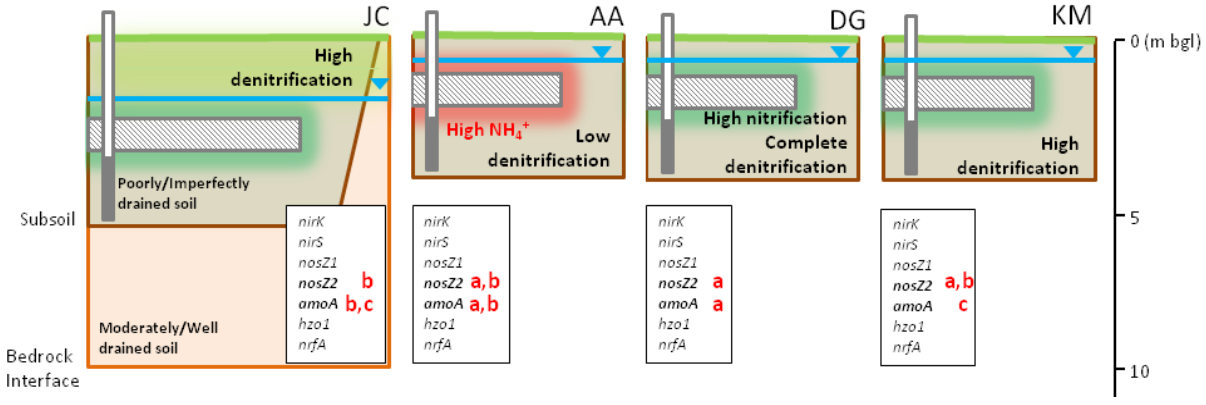

Fig. 6.6. Conceptual diagram of the four farms with highlighted the occurring processes identified within Chapter 4 and 5 and the significant differences in GCC found within this study indicated with different letters as per Fig. 6.2.

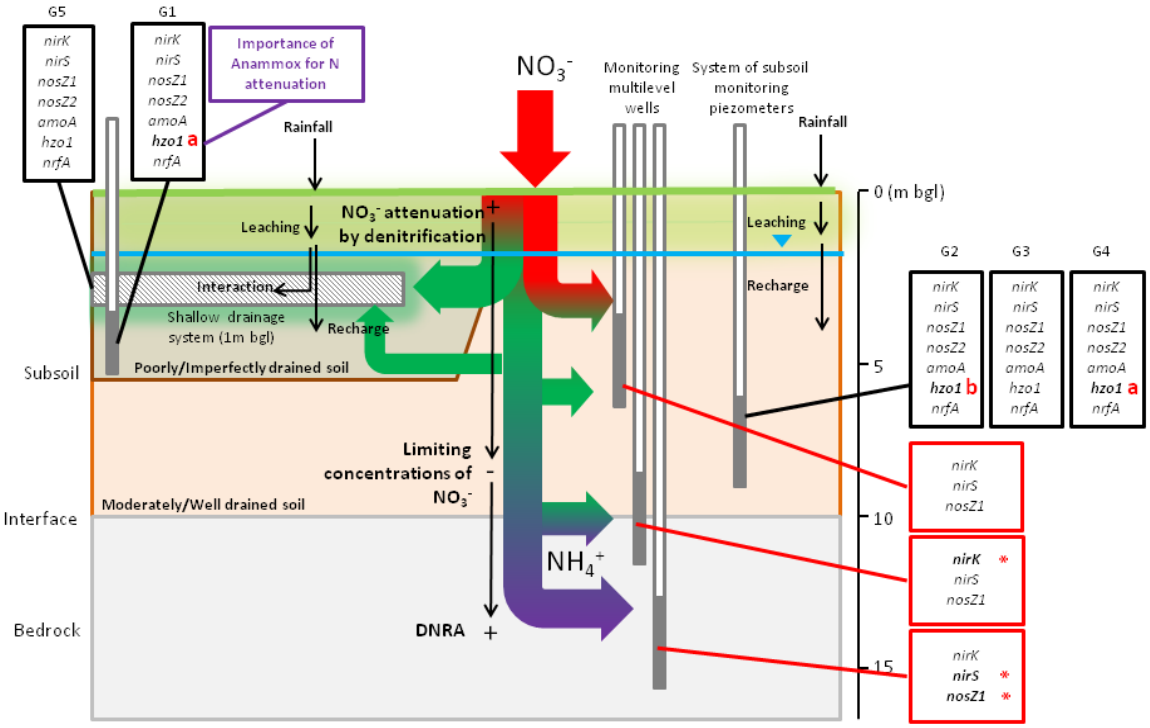

Fig. 6.7. Improved conceptual diagram (from Fig. 5.6) of JC dairy farms with the significant GCC differences found within this study indicated by different letters as per Fig. 6.5 and the significant differences in GCC found by Barrett et al. (2013) indicated with an asterisk within the red boxes. The knowledge improvement achieved with the qPCR analyses of the N-genes is highlighted within the purple box.

6.5 Conclusions

The "net" signal across four (*nirK*, *nirS*, *nosZ1* and *nrfA*) bacterial genes was not distinctive enough in the highly mobile water phase across open ditch, EOP and groundwater (to 9 m depth) locations to predict differences across sites in terms of N sustainability in the water phase from in addition to what gathered in Chapters 4 and 5. The only bacterial genes that showed some predictive power were *nosZ2*, *amoA* and *hzo1*. With few exceptions, across bacterial genes, the GCC were slightly higher within groundwater than EOP or open ditch locations. The gene *amoA*, specifically at EOP locations, showed significantly lower GCC's at KM than for example at DG and AA. Between the two variants of nitrite reductase, *nirK* was favoured over *nirS* while when considering the nitrous oxide reductase gene, the gene for cluster 1 (*nosZ1*) was clearly preferred over cluster 2 (*nosZ2*). The gene *hzo1* showed variability across JC indicating the importance of anammox for N attenuation. Using bacterial genes as an environmental tool to inform intensive dairy farm sustainability is not recommended.

Within this Chapter 6 the hypotheses created in Chapter 2 were partially met. The concepts and datasets gathered within Chapter 4 and 5 where implemented and expanded. The analyses of bacterial gene abundance for the N-cycle in water was used to try to improve our interpretation of N sustainability in the water phase over and above that given by isotope natural abundances, dissolved gases and biogeochemical parameters alone. However, the bacterial genes signal was not significantly distinctive for the mobile water phase across open ditch, end-of-pipe and groundwater (to 9 m depth) locations. No significant differences were further identified across sites in terms of sustainability and highlight of most important pathways for attenuation. Although some genes showed some predictive power, this was a limited environmental tool to inform intensive dairy farm sustainability.

Acknowledgements

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Chapter 7 - Investigation of drained and undrained intact soil cores to examine the fate of N

7.1 Introduction

Shallow fluctuation zones in soils are typically within the first few metres of the profile and are highly dynamic due to changes in water content. Superficial layers, which are the most affected by variations in water content, are zones with higher bacterial gene abundances and have highest impact on nitrogen (N) removal and bioremediation (Qin et al., 2014). In dairy systems to increase grass utilisation on heavy textured soils, drainage solutions are necessary (Fig 7.1). These include either a shallow disruptive technique (sub-soiling, mole or gravel moles (installation depth: 0.45-0.60 m) connected through a gravel pack to an underlying pipe system at ~1m depth) or a deeper piped groundwater drainage system. Drainage systems modify the water filled pore space (WFPS) and therefore the bioremediation capacity of the soil profile due to an alteration of physiochemical parameters, which in turn modify microbial activities and transformational processes (Ruehle et al., 2015). Furthermore, the modification of soil profile saturation level can alter the amount of gases emitted from the soil surface (e.g. di-nitrogen (N_2) or nitrous oxide (N_2O)). Since N_2 is not a GHG and it is difficult to measure, few studies document N_2 emissions (Bergstermann et al., 2011; Cardenas et al., 2017). However, many studies have analysed N_2O emissions across different WFPS scenarios (Bateman and Baggs, 2005; Rafique et al., 2011; Decock and Six, 2013). In an extensive study in Ireland across multiple grassland sites, high N_2O emissions were registered in concomitance with high WFPS, high soil temperature and fertiliser application (Rafique et al., 2011).

Fig 7.1. Heavy soil farm showing a drained (left) and undrained surface water gley soil (right) (October 2015).

A combination of flux analysis, isotopic labelled fertiliser, isotopomers and molecular techniques have been suggested to have the potential to improve our understanding and

validate the influence of each process involved in N_2O production/consumption (e.g. nitrification, denitrification) (Decock and Six, 2013). Studies with labelled fertiliser have been able to detect the contribution of nitrification and denitrification processes to N_2O emissions. Bateman and Baggs (2005) showed in a labelled fertiliser silt loam incubation study, that WFPS below 20% limited substrate movement thereby limiting bacterial processes and N_2O emissions to anaerobic microsites. Between a WFPS of 20-35%, N_2O production increased significantly with nitrification becoming the dominant process at 35%. N_2O production peaked between 60-80%, with gradually increasing rates of denitrification but still nitrification as the dominant process. However, Cardenas et al. (2017) showed that these thresholds vary across soil textural classes and therefore comparison of results must factor in soil type. In Ireland, Baily et al. (2011) measured N_2O and N_2 fluxes on a moderately well-drained fine loam textured soil with a gas chamber experiment using labelled fertilizer ($^{14}NH_4{}^{15}NO_3$: 100 kg N/ha). Results showed that mean values for N_2O and N_2 emissions for the first five days after fertilisation were dominated by N_2O produced through denitrification. However, outside of direct fertilization application timings, nitrification was dominant under milder and wetter conditions.

Isotopomers studies can give additional information as these methods have the advantage of being quantitative, independent from precursor isotopic signatures and non-invasive (Yoshida and Toyoda 2000; Stein and Yung, 2003; Well et al., 2006; Ostrom, 2011; Yamazaki et al., 2014). Due to the preferential location of ^{15}N within the N_2O molecule, the analyses of natural isotopomer ratios can specifically discriminate the percentage of N_2O produced by denitrification or nitrification. That is, different degrees of site preference depends on enzyme specificity and microbial groups (Decock and Six, 2013). However, to date, isotopomer studies are carried out at laboratory scale with disturbed soil and pure bacterial cultures.

The WFPS is also a key parameter driving microbial community structure (Fierer et al., 2003). Microbial communities are sensitive to environmental disturbance with changes in community structure followed by variation in process rates (Allison and Martiny, 2008). In wetland (silty clay loam) and terrestrial ecosystems (silt loam; peat), major differences have been encountered, in terms of both microbial communities and N_2O emissions, comparing saturated and unsaturated soil areas with the former enhancing denitrification (Well et al., 2001; Peralta et al., 2013). Variations in soil water content and consequently in dissolved oxygen (DO) concentration modifies nutrient and chemical species ratio, supply and distribution, resulting in a variation in the control of processes and community structure and therefore of gas emissions (Giles et al., 2012).

Additionally, in un-drained soil profiles, conditions in heavy textured soils are anaerobic resulting in the suppression of nitrification and complete attenuation of ammonium (NH_4^+) (Aulakh et al. 1991). After the installation of drainage systems, an increased drainage property of the soil to siphon off more water allows deeper infiltration of water and DO, which may induce contamination and/or pollution swapping. On a previous study on five Irish Heavy Soil Farms (see Chapter 4), it has been shown that NH_4^+ is the reactive nitrogen (N_r) species that presents water quality issues at end-of-pipe and groundwater locations. Within this study, we selected a farm from the Heavy Soil Programme farms outlined in Chapter 4. Moving closer to field conditions by utilising intact soil cores, we investigated N_2O emissions, with and without labelled fertiliser applications, to elucidate the N transformation processes. Intact cores were excavated from the AA (Co. Limerick) farm, which was chosen as it exhibited a high level of NH_4^+-N contamination (above maximum admissible concentration (MAC) > 0.23 mg NH_4^+-N/l) and a mixed $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ signature pointing towards low rates of denitrification mostly incomplete. The objectives of the present study were to: 1) assess differences in N_2 and N_2O emissions, 2) examine N labelling and N_2O isotopomers to trace the fate of nitrogen and differences in transformational processes 3) investigate the microbial community and the impact of the two saturation contents on bacterial community by the analyses of *16S RNA*, *nirS*, *nirK*, *nosZ1*, *nosZ2*, *amoA*, *hzo1* and *nrfA* gene abundances.

Note: Although samples were taken for isotopomers, the results were not received during the time of the project. These will be added later for a publication.

7.2 Materials and methods

7.2.1 Study site

The AA (Co. Limerick, 52°45', 09°30') site is outlined in Fig. 7.2 and further details can be found in Chapter 4. Low permeability is derived from a Humic Surface Water Gley, underlain by a poorly productive Shale aquifer. Humic here refers to a soil which contains an A horizon with significantly more organic matter (OM) than mineral matter. The soil profile consisted of the following depth/horizon classification and textures: 0-40 cm: Ap/O (clay loam), 41-62 cm: Btg (silty clay), 63-140 cm: Cg1 (silty clay loam) and 140-170 cm: Cg2 (silty clay loam)) (Tuohy et al., 2016; full details in Chapter 4). The site was characterised by high annual rainfall (e.g. 1443.6 mm in 2015). On the 2.11 ha site, a shallow drainage system, consisting of a gravel mole was installed at 0.45 m connected to a pipe drain system at 0.9 m

bgl with 20 m spacing. Discharge is to an open ditch network (Tuohy et al., 2016) (Fig. 7.2). Athea was managed as an intensive site. Management data, N balance and annual N-inputs can be found in Chapter 4. Again in Chapter 4, this paddock was identified as having poor signs of attenuation by both nitrification and denitrification, and a low water attenuation capacity with pollution swapping occurring, where NO_3^- was converted to NH_4^+ above MAC (0.23 mg N/l (EU, 2014a)) (see Chapter 4, Table. 7.1 and Fig. 7.2).

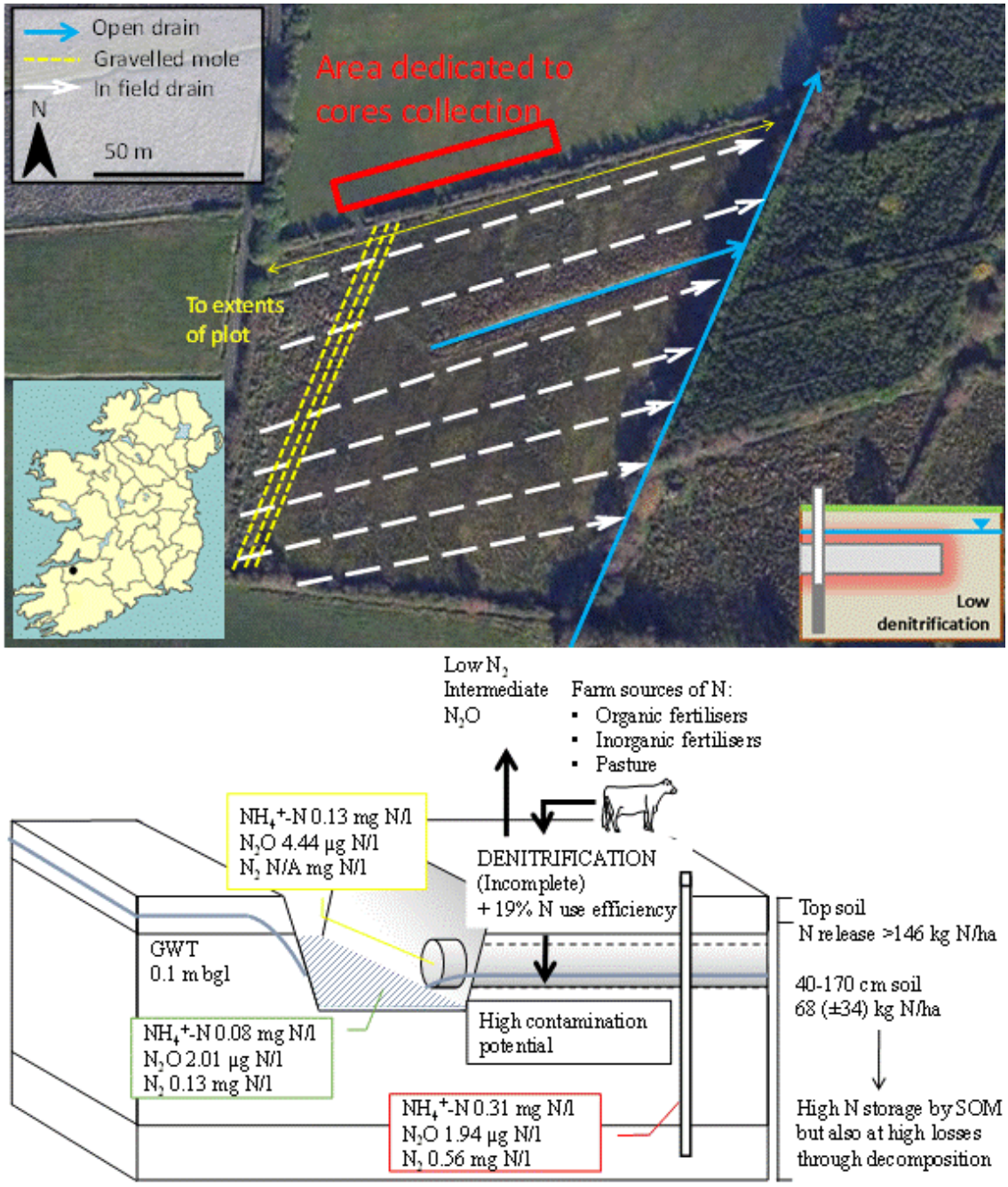


Fig. 7.2. Paddock drainage setup with indication of the soil collection area (top). Soil cores were collected in a neighbouring field from the one in Chapter 4. This field had the characteristics of the pristine drained site. Conceptual site model as developed in Chapters 4 and 6 (bottom), Estimated N is a computed estimate of the N that may be released annually through OM decomposition (more details can be found in Table 7.1 or Chapter 4).

Table 7.1. Mean values for NO_3^--N, NO_2^--N, NH_4^+-N, dissolves-N_2O and excess-N_2 at the site (plot), open ditch (OD), end-of-pipe (EOP) and shallow groundwater (GW) in October 2015 (more details on methods and results can be found in Chapter 4).

	Plot	OD	EOP	GW
NO_3^--N (mg N/l)	0.47 ± 0.37	0.42 ± 0.56	0.66 ± 0.28	0.08 ± 0.07
NO_2^--N (mg N/l)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
NH_4^+-N (mg N/l)	0.17 ± 0.18	0.08 ± 0.04	0.13 ± 0.20	0.31 ± 0.12
Dissolved-N_2O (µg N/l)	3.30 ± 1.50	2.01 ± 0.27	4.44 ± 0.68	1.94 ± 1.48
Excess-N_2 (mg N/l)	0.42 ± 0.25	0.13 ± N/A	N/A	0.56 ± 0.05

7.2.2 Intact core collection

A bespoke intact core excavation kit was designed and fashioned with the help of technical staff in University of Sheffield and transported to the field site (Fig. 7.3 and 7.4). In total 20 intact soil cores were collected in February 2017 to a depth of 0.45 m (see Fig 7.2 for locations). Cores were taken outside the drained field of Chapter 4. This field was selected as having the same pristine conditions of drained site and soil type and as excavation here would not have caused damage to the existing drainage system. Each core consisted of a PVC tube (45 cm length and 15 cm of internal diameter). In the field, the farmer operated a digger in conjunction with the apparatus. Cores were excavated with the use of an excavator. The locations for these cores collection were selected to insure homogeneity within the grass cover layer. Cores were positioned carefully on the grass sod to cause minimal disturbance of the topsoil and grass cover during excavation. Cores were then capped and transported to the Teagasc Johnstown Castle glasshouse facility. Here, grass was trimmed. The last 10 cm of soil profile was removed from the cores so that the top soil layer (Ap/O, clay loam) and a portion of the second soil layer (Btg, silty clay) were preserved. Three cm of gravel were then added to the bottom part of the cores and end caps were sealed to the bottom of each core using silicone. To monitor the water level within the core, a hole was drilled to house a detachable transparent side fitting tube, which was then sealed with silicone. On the surface, petroleum jelly was heated and then poured down the sides of the soil core to seal any possible gap between the perimeter of the core and the PVC tube for the top 5 cm, and to ensure water flow through the soil and not along the sides. This has been used in lysimeter studies at the Teagasc research centre in other studies e.g. Selbie et al. (2015). Three 2 cm diameter holes were created on the side of the intact cores and these were used to create the varied saturated conditions. Cores were then left to condition for a period of 1 month to achieve two targeted saturations (80% and 55% WFPS) through a differential watering. For the saturated treatment, the holes were sealed for the duration of the experiment; for the

unsaturated treatment, the holes were left open for the duration of the experiment. Stainless steel mesh was used to cover the open holes to ensure no soil loss (Fig. 7.4).

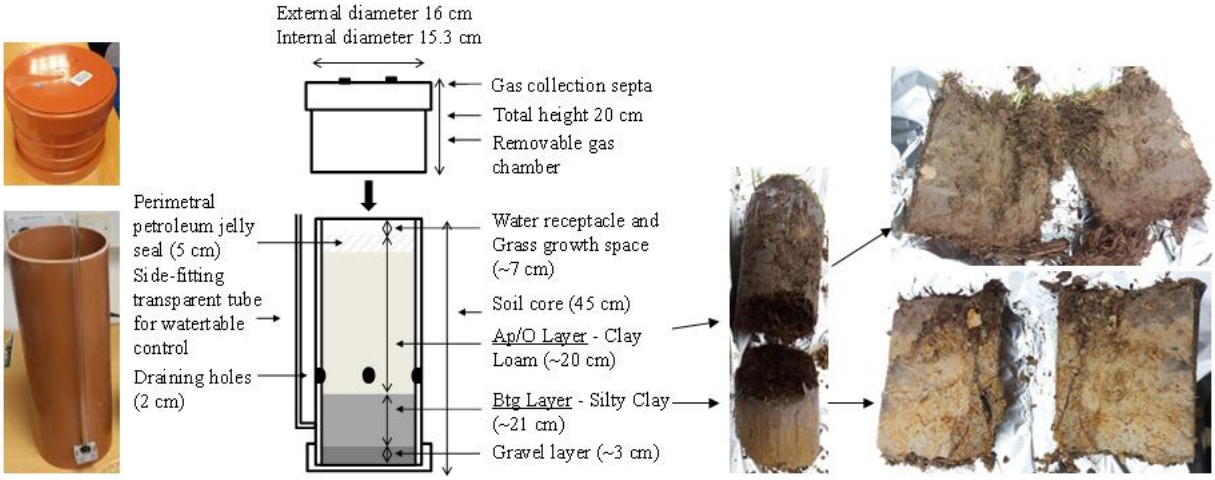
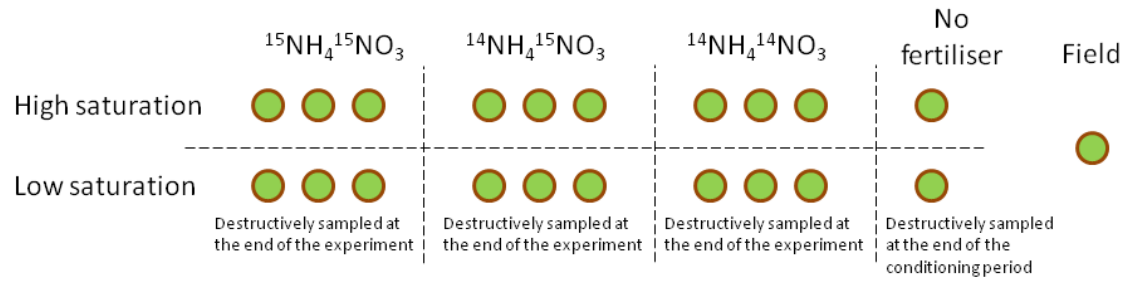


Fig. 7.3. Core design and soil cores highlighting the different texture of the two layers.

Fig. 7.4. Steps for the collection of intact soil cores in the field and laboratory setup.1) A metal sleeve was created to contain the plastic PVC pipe of Fig 7.2; 2) The plastic PVC pipe was inserted within the metal sleeve and secured with a metal cap (indicated by the red arrow); 3) The metal sleeve was carefully positioned on the location selected for coring; 4) With the use of a JCB the metal sleeve was pushed in the soil; 5) The chain connected to the metal sleeve was secured to the JCB; 6) The metal sleeve was slowly pulled out from the soil (being careful not to damage the internal soil core; 7) The metal sleeve was carefully dropped to the soil and prepared for transported to JC; 8) The cores were cleaned and assembled at the JC facility; 9) A petroleum jelly seal was created on the top of the cores; 10&11) The two water treatments were installed, low saturation cores (10) and high saturation cores (11);12) Final laboratory setup.

7.2.3 Experimental design and analyses

The experimental design is presented in Fig 7.5, showing saturation level (high, low) and when and what parameters were assessed over time.



	$^{15}NH_4{}^{15}NO_3$	$^{14}NH_4{}^{15}NO_3$	$^{14}NH_4{}^{14}NO_3$	No fertiliser	Field
Gas sampling					
N_2O, N_2 emissions	Days 0, 1, 2, 3, 5, 7, 10, 14, 24	Days 0, 1, 2, 3, 5, 7, 10, 14, 24	Days 0, 1, 2, 3, 5, 7, 10, 14, 24	Day 0	-
N_2O, N_2 isotopes	Days 0, 1, 2, 3, 5, 7, 10, 14, 24	Days 0, 1, 2, 3, 5, 7, 10, 14, 24	Days 0, 1, 2, 3, 5, 7, 10, 14, 24	-	-
N_2O isotopomers	Days 0, 1, 2, 3, 5, 7, 10, 14, 24	Days 0, 1, 2, 3, 5, 7, 10, 14, 24	Days 0, 1, 2, 3, 5, 7, 10, 14, 24	-	-
CO_2 emissions	Days 0, 1, 2, 3, 5, 7, 10, 14, 24	Days 0, 1, 2, 3, 5, 7, 10, 14, 24	Days 0, 1, 2, 3, 5, 7, 10, 14, 24	-	-
Water content					
In situ probe	Every 2 days	Every 2 days	Every 2 days	Every 2 days	-
Oven	End of the experiment	End of the experiment	End of the experiment	Day 1	-
Soil sampling					
^{15}N enrichment	End of the experiment	End of the experiment	End of the experiment	Day 1	-
DNA extraction	End of the experiment	End of the experiment	End of the experiment	Day 1	Collection day
Total N and C, pH and SOM	End of the experiment	End of the experiment	End of the experiment	Day 1	-
Grass sampling					
^{15}N enrichment	End of the experiment	End of the experiment	End of the experiment	Day 1	-
Total N and C	End of the experiment	End of the experiment	End of the experiment	Day 1	-

Fig. 7.5. Schematic documenting the treatment design, parameters tested and frequency of sampling.

During the running of the experiment, ambient temperature ranged between 9.6 and 23.0 ˚C with a similar variation encountered within the 0-5 cm of the soil cores (max: 23.9 ˚C, min: 8.1 ˚C).

At the field site, BD was calculated at 1.11 g/cm^3 in the top soil horizon. WFPS during the experiment averaged 79% for high saturation (HS) cores (max: 100%, min: 58%) while 53% for low saturation (LS) cores (max: 76%, min: 40%). The selected targeted saturations were 80% and 55% WFPS. The highest saturation was selected as in an Irish study on eight Irish farms, Rafique et al. (2011) found that the WFPS ranged from 30.4% to 85.2% over the summer months while it ranged from 49.1% to 99.5% over the winter months, with highest values recorded within heavy textured gley soils. The two saturations were calculated using the following equation:

$$WFPS = (GSMC*BD)/(1-(BD/PD))*100 \quad \text{(Eqn. 7.1)}$$

Where GMSC is the gravimetric soil moisture content (VSMC/BD), BD is bulk density (g/cm^3) and PD is particle density (2.65 g/cm^3). The depth of water inside the cores was monitored daily using the outside tubing and volumetric soil moisture content (VSMC). Surface soil temperature and electrical conductivity (EC) were measured every 2 days for a month before and after fertiliser application using a ProCheck 5TE in-situ probe.

Different core sets were subjected to three different fertiliser amendments. Two fertilisers consisted of differently labelled ammonium nitrate ($^{15}NH_4{}^{15}NO_3$ and $^{14}NH_4{}^{15}NO_3$) (50% atom enrichment) and a third control consisted of non-labelled ammonium nitrate ($^{14}NH_4{}^{14}NO_3$) fertilisation (Bateman and Baggs, 2005). The rate of fertiliser was 250 N kg/ha, dissolved in distilled water and 30 ml manually applied to each core with a 50 ml plastic syringe (different set of syringes were used for each treatment). Three cores for each treatment were used for each amendment and fertiliser was applied between day 0 and day 1 of sampling.

Gas samples were collected before (day 0) and at 1, 2, 3, 5, 7, 10, 14, 24 days after fertilisation (Fig. 7.4). Gas chambers (15 cm diameter, 20 cm height) were created for gas sampling following guidelines for N_2O chambers by Klein and Harvey (2012). Air tight gas chambers were fitted onto the top of the cores and samples were collected though rubber septa using 20 ml plastic syringes and needles. For N_2O, 20 ml gas samples were taken from the gas chambers of all the cores using gas tight syringes, at 0, 15, 30 minutes after chamber deposition. Samples were stored in 12 ml exetainers (LabcoWycomb Ltd., UK) which were previously evacuated with He. The N_2O was quantified by gas chromatography (CP-3800, Varian Inc. USA). Additional 20 ml samples were collected 2 hours after chamber deposition, with the same methodology as for previous samples. These additional samples were collected from both the labelled and non-labelled cores for the analysis of $^{15}N\text{-}N_2O$ and $^{15}N\text{-}N_2$. Samples were stored in 12 ml serum bottles previously evacuated with He. Isotopic compositions ($^{15/14}N$) for N_2O and N_2 and N_2 quantification were determined at the UC Davis Stable Isotope Facility, Davis, California. In addition, 20 ml samples were collected from each core 3 hours after chamber deposition for the analyses of N_2O isotopomers at University College Dublin (these samples were not analysed within the present work). Additional 20 ml atmospheric samples were collected at the same time as the 2 and 3 hour samples and represents background values.

N_2O fluxes were calculated following the following equation:

$$\text{Flux} = (dGas/dt)\times10^{-6}\times(V_{chamber}\times p\times100\times MW)/(R\times T)\times10^{3}\times(1/A) \quad \text{(Eqn. 7.2)}$$

Where, dGas is the gas concentration change over time (dt) (ppm/h), $V_{chamber}$ is the volume of the gas chamber used (0.003 m^3 in this study), p is the atmospheric pressure (hPa, measured with an EGM-4 Environmental Gas Monitor (PP Systems)), MW molecular weight (g/mol), R gas constant (8.314 J/mol/K), T is the temperature (K, measured in this study by the EGM-4 Environmental Gas Monitor (PP Systems)) and A is the area of the chamber. Enrichments of N_2O and N_2 were calculated following the methods illustrated by Mosier and Schimel (1993), Stevens and Laughlin (1998) and Bateman and Baggs (2005).

Soil samples were collected by destructively sampling the cores at multiple time periods (end of the conditioning for the two cores that did not received fertiliser while end of the experiment for the other cores) (Fig 7.4). Early samples were collected on site to assess site conditions at the moment of sampling and two cores were sacrificed at the end of the conditioning period with the remainder of cores destructively sampled at the end of the experiment. Two samples were collected for each core: one in the upper organic rich clay loam (Ap/O, SOM: 59.6%) horizon and one in the lower heavier silty clay (Btg, SOM: 4.54%) horizon. All samples were dried for one week at 60˚C and then manually sieved (≤2 mm) and then ball milled to produce a fine powder. Chemical analyses were conducted at the Teagasc Laboratories, Johnstown Castle (Ireland) for pH, soil organic matter (SOM) and C and N % contents. A soil to solution ratio of 1:2.5 suspension of soil in water was created by mixing deionised water (25 ml) with the milled soil samples (10 ml) in a 50 ml polyethene tube, which was then shaken for 2 hours on an orbital shaker (set to 160 rotations/min) (Reeuwijk, 2002) and then pH was measured. For SOM, ceramic crucibles were dried overnight at 105˚C, 4 g were added to the crucible, and the contents were weighted again. This process was repeated and then the samples were placed in a furnace (Nabertherm, Germany), burned at 500˚C, and weighted again. For quality control, Teagasc has a range of soil SOM standards for comparison. SOM was then calculated following the following formula:

$$SOM\ (\%\ w/w) = (((Soil_{105^{\circ}C}\ (g) + Crucible\ (g)) - (Soil_{500^{\circ}C} + Crucible\ (g))/(Soil_{105^{\circ}C}\ (g) - Crucible\ (g)))*100 \quad \text{(Eqn. 7.3)}$$

For C% and N%, samples (approximately 0.2 g) were transferred into tin foil cups and then analysed through a LECO TruSpec CN elemental analyser. Soil samples at known C% and N% were used as standards. Soil C% and N% were then used to optimise sample weight for

soil enrichment (^{15}N) analyses. Samples where then encapsulated in tin capsules and ^{15}N contents were determined at the UC Davis Stable Isotope Facility, Davis, California, through a PDZ Europa ANCA-GSL elemental analyser interfaced with a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Enrichment in soil was calculated following the method of Mosier and Shimel (1993).

Additional soil samples were collected with a sterile trowel for the two horizons from the holes left by the core extraction in the field. Three subsamples were taken randomly spaced across the exposed horizon layer and combined in a sterile sealable bag to create a composite soil sample. After homogenisation, these were immediately frozen in dry ice while in the field and stored at -80°C at the end of each sampling day. Further soil samples were collected for the treatment of $^{15}NH_4{}^{15}NO_3$. Three replicates for each soil sample were extracted using a PowerSoil® DNA Isolation Kit (MoBio Laboratories, Inc, USA) according to manufacturer guidance. Samples were visualized on 1% (w/v) 1×TAE agarose gels and DNA was stored at -80 °C until analysis within 2 months from extraction. To quantify DNA from soil (number of copies per gram of dry soil), the dry-weight of the soil and the proportion of water to soil was accounted for through soil moisture analyses. To create a multiplication factor specific for each sample to convert the absolute estimation of copies into an estimation of copies per gram dry soil, samples of soil were weighted before extraction and replicates of these samples were weighted before and after a period of 2 weeks at 80˚C. Samples were then subjected to the same analyses as per (Section 6.2.4).

Grass samples were collected at multiple time periods; 1) from the two cores that were destructed at the end of the conditioning period and 2) from all cores destructively sampled at the end of the experiment. A composite sample was created for each treatment. Grass was dried at 60˚C for 5 days within perforated plastic bags and then ground (≤0.2 mm) through a grass grinder. Samples were analysed for C% and N%. Samples (approximately 0.1 g) were transferred into tin foil cups and then analysed through a LECO TruSpec CN elemental analyser as per soil. Soil C% and N% were then used to optimise sample weight for soil enrichment (^{15}N) analyses as per soil. Enrichment in grass was calculated following the method of illustrated in Mosier and Shimel (1993).

7.2.4 Statistical analyses

Significant differences between abundance of N cycling genes and the 16S RNA gene was tested between treatments through the use of one way ANOVA and Tukey's HSD test (IBM SPSS Statistics version 24)) ($\alpha = 0.05$). When an equal variance was not assumed, Dunnett's

T3 test was used instead of Tukey's. Data was logarithmically transformed to ensure normality before analyses.

7.3 Results

7.3.1 N_2O emissions

Prior to the addition of fertiliser, background values of N_2O emission were in the range of 0.01 and 0.02 mg N_2O-N/m^2h and 0.10 and 0.00 mg N_2O-N/m^2h for the HS and LS cores, respectively. Emissions of N_2O did not significantly differ before fertiliser application. Following fertilisation (27/3/2017 between day 0 and day1), a steep increase was seen in the N_2O emission rate for the HS cores but a slower increase was observed for the low saturation treatment. HS cores had an immediate increase in N_2O emissions on day 1 (7.36 mg N_2O-/m^2h ± 0.00) with a peak after 5 days (11.97 mg N_2O-N/m^2h ± 2.16). After day 5, emissions decreased up to day 24 reaching an average of 0.04 mg N_2O-N/m^2h ± 0.04. The LS treatment showed a slower increase in the N_2O emission rate at a lower magnitude, which peaked between day 7 (1.64 mg N_2O-N/m^2h ± 0.99) and 14 (1.63 mg N_2O-N/m^2h ± 1.10) and decreased again on day 24 (0.22 mg N_2O-N/m^2h ± 0.19) (Fig. 7.6).

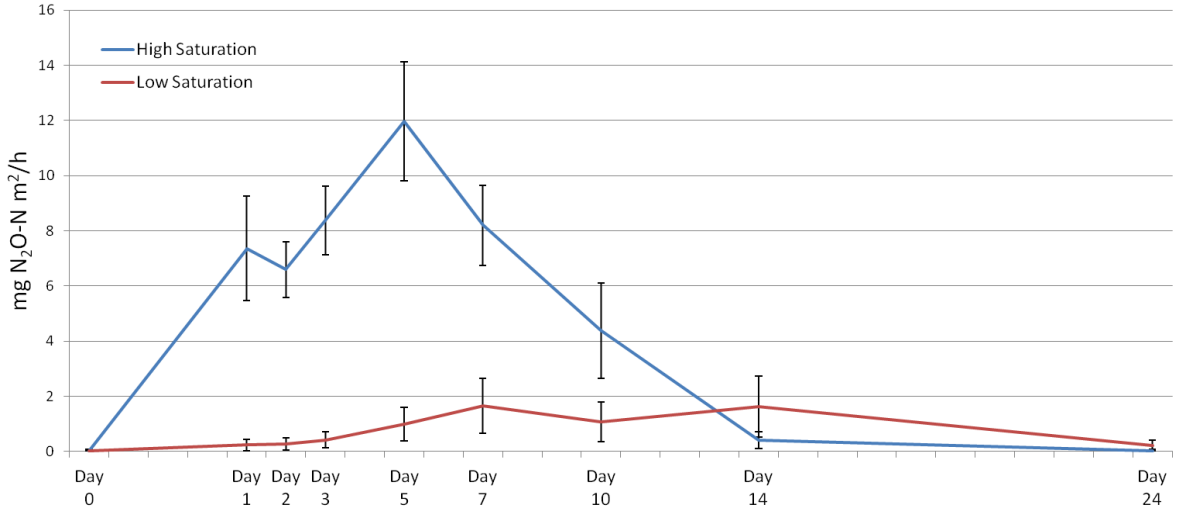


Fig. 7.6. Temporal patterns of N_2O-N emission rates from the high (80% WFPS) and low (50% WFPS) saturation treatments. After fertilisation (between day 0 and 1) with 250 N kg/ha of NH_4NO_3. Standard deviations are indicated for high and low saturation treatment (n=9).

7.3.2 N_2O and N_2 gas enrichment

The use of fertiliser with different isotopic labels was necessary in order to assess the contribution of denitrification and nitrification (Fig. 7.7). Compared with N_2O flux data, enrichment data showed a net predominance of nitrification in the LS treatment. These cores showed a production from denitrification between a minimum of 2.8% of the total N_2O

emission and a maximum of 25.2% while nitrification accounted for values between 74.8% and 97.2%. The ratio between these two processes remained almost constant across the duration of the experiment, with a slight decrease in denitrification after day 5 following fertilisation. The HS treatment showed a higher contribution of the denitrification process when compared with the LS treatment. In the day following fertilisation, denitrification accounted for 72.5-73.4% of the emission. Denitrification decreased constantly from the start of the experiment, reaching minimum values of 18.8% at day 24 after fertilisation. Denitrification was the main producer of N_2O for this treatment.

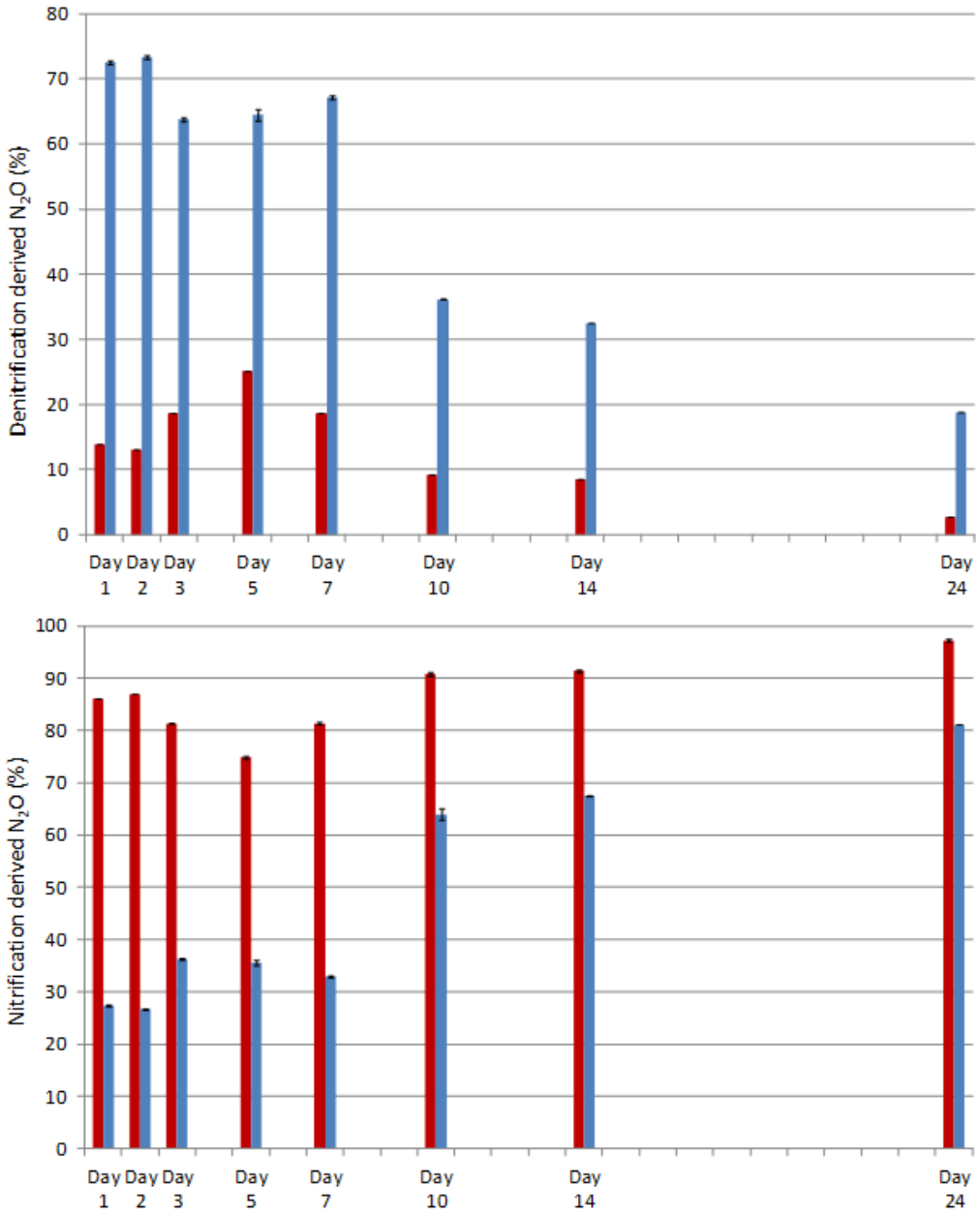


Fig. 7.7. Percentage of N_2O emissions created by denitrification (top) and nitrification (bottom) for low saturation (red) and high saturation (blue) treatments for the days following fertilisation. Standard deviations are indicated for high and low saturation treatment (n=3).

Values for N_2 emission were calculated from the enrichment factor. N_2 flux increased with the proceeding of the experiment within HS cores, Day 1 showed a N_2 flux of 6.3 mg N/m^{2}h reaching a flux of 30.3 mg N/m^{2}h on day 10 (Fig. 7.8). However, only usable data were obtained from the HS cores ($^{15}NH_4{}^{15}NO_3$). Low saturation cores did not produce detectable N_2 amounts (only one recordable data of 0.8 (±0.1) mg N/m^{2}h on Day 1). Additionally, no data were also recorded for HS after Day 10 possibly due to a reduction of the producing enriched pool.

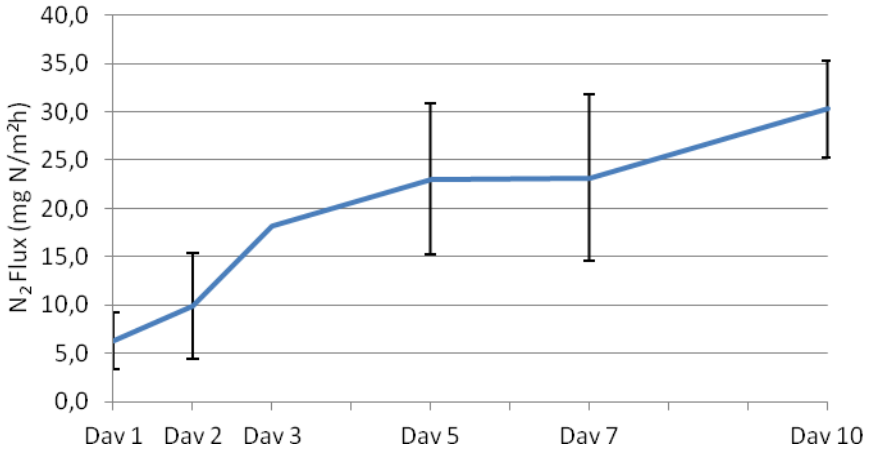


Fig. 7.8. Temporal patterns of N_2 emission rates from the high (80% WFPS) saturation treatment. After fertilisation (between day 0 and 1) with 250 N kg/ha of $^{15}NH_4{}^{15}NO_3$. Standard deviations are indicated (n=3).

7.3.3 Soil and grass enrichment and recovery rates

The amount of ^{15}N in soil derived from the fertiliser was calculated for both soil (within the top 0-5 cm) and grass for both HS and LS treatments (Fig. 7.9). Within the $^{14}NH_4{}^{15}NO_3$ treatment, HS cores averaged 0.007 g ^{15}N while LS 0.026 g ^{15}N. The same pattern evident for the $^{15}NH_4{}^{15}NO_3$ treatment, HS had values of 0.051 g ^{15}N while LS of 0.064 g ^{15}N. The same trend of higher enrichment within LS cores was exhibited for grass, with values of 0.03 g ^{15}N and 0.36 g ^{15}N for the HS and LS cores of the $^{14}NH_4{}^{15}NO_3$ treatment, respectively. Results for the HS and LS core of the $^{15}NH_4{}^{15}NO_3$ treatment were 0.16 g ^{15}N and 0.55 g ^{15}N, respectively. These data were further used to calculate the ^{15}N fertiliser recovery rates for both soil and grass for the treatment $^{14}NH_4{}^{15}NO_3$ recovery rates were of 0.7% and of 6.1% for HS and LS, respectively. Results for $^{14}NH_4{}^{15}NO_3$ they were of 1.7% and of 4.9% for HS and LS treatments, respectively.

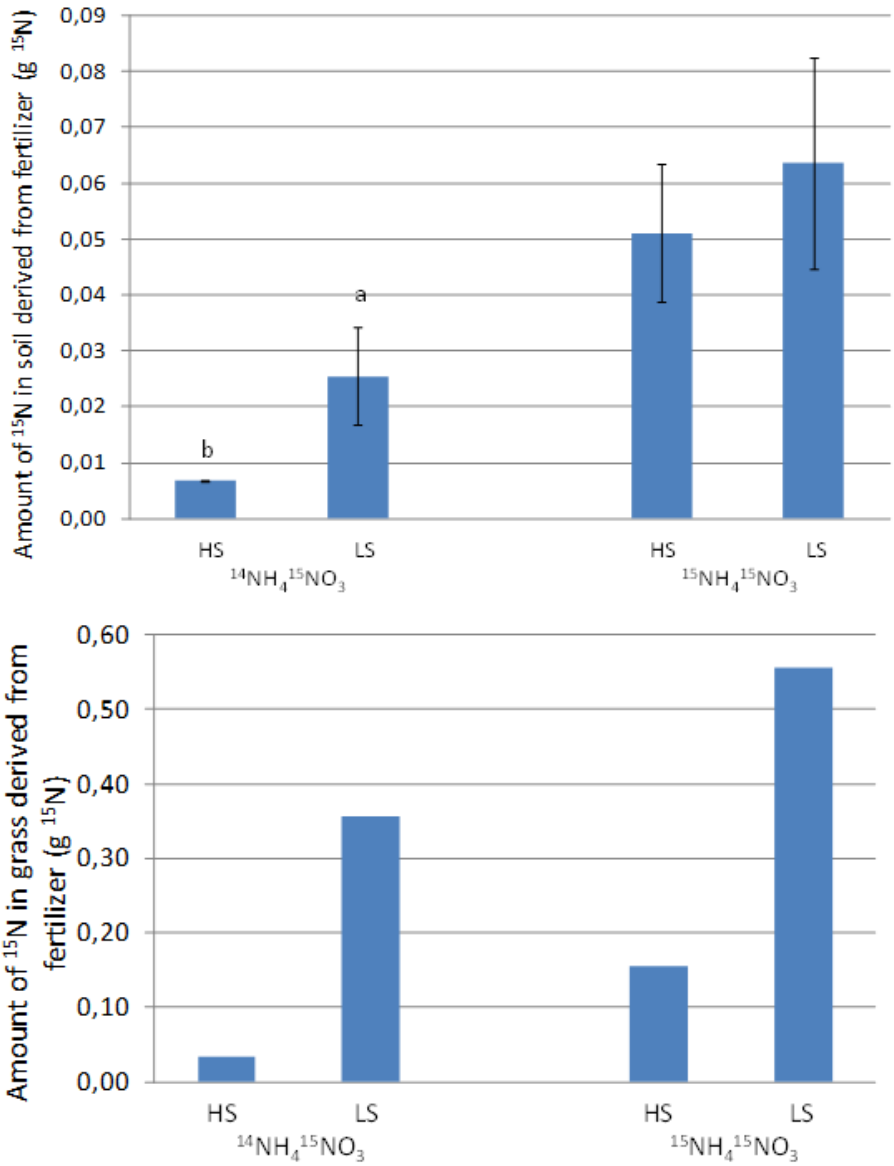


Fig. 7.9. Top: total amount of ^{15}N in soil (top 0-5 cm) derived from the fertiliser per core. Standard deviations are indicated for each group (n=3). Statistical differences ($P<0.05$) are indicated by different letters. Bottom: total amount of ^{15}N in grass derived from the fertiliser per core. Standard deviations are not indicated for as grass was analysed as a composite samples merging the three cores for each treatment together.

Considering all components, the two treatments showed a very different pattern of apportionment (Fig. 7.10). The HS cores and LS cores showed similar N_2O emissions from nitrification (4.6% and 3.5% respectively) ($p>0.05$). However, HS had a high component of N_2O derived from denitrification (6.0% HS vs. 0.4% LS)($p<0.05$). A significant amount of N_2O was transformed to N_2 within HS cores that showed 62.9% of the N lost through N_2 production compared with 3.7% within LS cores. From these balances, a portion of N resulted unaccounted. High saturation cores showed a lower proportion (23.9%) of unaccounted N compared with a high proportion for LS (84.7%). The unaccounted N could

be lost through NH_3 volatilisation or possibly leached along subsurface pathways out of the 5 cm thickness that was analysed (NH_3 volatilisation and leaching pathways were not measured within this study).

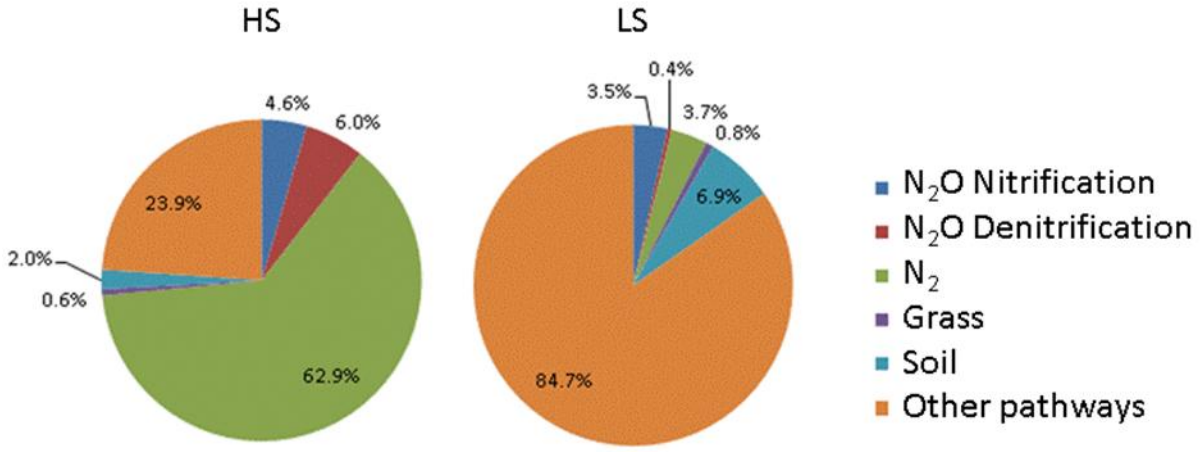


Fig. 7.10. Contribution of each N loss pathways for the high (HS) and low (LS) saturated treatments.

7.3.4 Variation of GCC of genes across treatments

Gene abundances were analysed for 1) samples collected within the field at the moment of core extraction (F), 2) samples collected before the addition of the fertiliser from high (HS-i) and low saturation cores (LS-i) and at the end of the experiment again from high (HS-f) and low saturation cores (LS-f).

Soils from the top layer showed higher GCC for the *16S* gene within LS-i treatment while significantly lower concentrations were found in HS-f and LS-f (Fig. 7.11, Table 7.2). The gene *nirS* showed lowest GCCs within F. The gene *nirK* showed higher GCCs than nirS; lowest GCC were found in F while LS-i presented highest GCC. Treatments LS-f and HS-f were significant different from LS-i. The gene for *nosZ1* was favoured over *nosZ2*. The gene *nosZ1* did not showed significantly different GCCs values across treatments except for F. The gene *nosZ2* showed significantly higher GCC in HS-i while lower in HS-f. The gene for *amoA* had higher GCC at LS-f while lowest at HS-f; other groups did not exhibit any statistical differences. The gene for *hzo1* showed no significant differences across groups. The gene for nrfA showed higher GCC in group LS-f and lower within group HS-f.

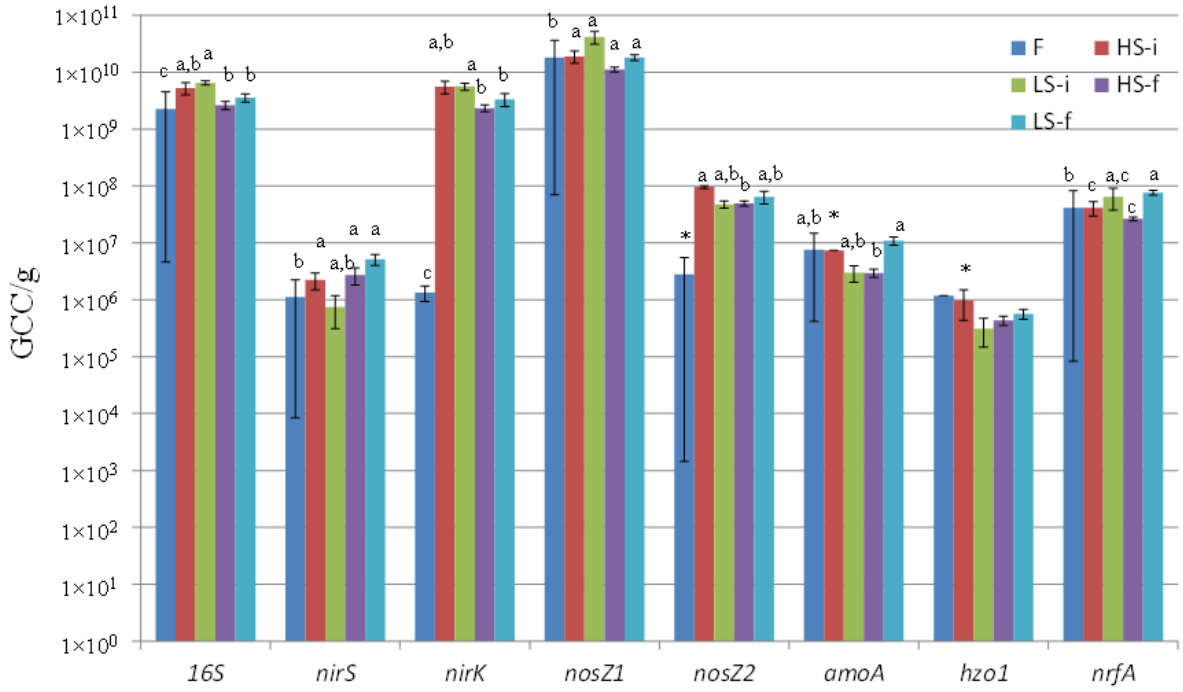


Fig. 7.11. Variation in gene copy concentration (GCC/l) in the topsoil among: samples collected within the field(F; n=3) at the moment of core extraction, samples collected before the addition of fertiliser from high saturation treatment (HS-i; n=3) and low saturation treatment (LS-i; n=3) and at the end of the experiment again from high saturation cores (HS-f; n=9) and low saturation cores (LS-f; n=9). Standard errors are indicated for each separated gene group. Statistical differences ($p<0.05$) between GCC are indicated by different letter within each gene group. Groups excluded from the analyses are indicated with *.

Table 7.2. Copy concentration (GCC/l) in the topsoil among: samples collected within the field (F) at the moment of core extraction, samples collected before the addition of fertiliser from high saturation treatment (HS-i) and low saturation treatment (LS-i) and at the end of the experiment again from high saturation cores (HS-f) and low saturation cores (LS-f).

	GCC averages (GCC/g)							
Treatments	***16S***	***nirS***	***nirK***	***nosZ1***	***nosZ2***	***amoA***	***hzo1***	***nrfA***
F	2.3×10^{9}	1.1×10^{6}	1.3×10^{6}	1.8×10^{10}	2.8×10^{6}	7.6×10^{6}	1.2×10^{6}	4.1×10^{7}
HS-i	5.3×10^{9}	2.2×10^{6}	5.6×10^{9}	1.9×10^{10}	9.6×10^{7}	7.4×10^{6}	9.6×10^{5}	4.1×10^{7}
LS-i	6.5×10^{9}	7.5×10^{6}	5.6×10^{9}	4.2×10^{10}	4.7×10^{7}	3.0×10^{6}	3.1×10^{5}	6.5×10^{7}
HS-f	2.6×10^{9}	2.7×10^{6}	2.3×10^{9}	1.1×10^{10}	4.9×10^{7}	3.0×10^{6}	4.4×10^{5}	2.7×10^{7}
LS-f	3.6×10^{9}	5.2×10^{6}	3.4×10^{9}	1.8×10^{10}	6.5×10^{7}	1.1×10^{7}	5.7×10^{5}	7.7×10^{7}

Soils from the bottom layer showed a lower GCC for the *16S* gene when compared to those from the top layer ($p<0.05$). The GCC of *16S* varied between groups, higher GCCs were found within F, whilst lowest equivalent were found in HS-f and LS-f (Fig. 7.12, Table 7.3). Due to the low abundance found for the *16S* gene, analysis of the bottom layer was restricted to the most abundant genes found within the top layer (*nirK*, *nosZ1*, *amoA* and *nrfA*). The gene *nirK* followed the same pattern as for*16S*. The gene *nosZ1* had a similar pattern to *16S* with highest GCC at F and lowest only at HS-f. For the gene *amoA*, F, HS-f and LS-f were

found to have statistically different GCC. The gene *nrfA* showed higher GCC in F, lower in HS-f.

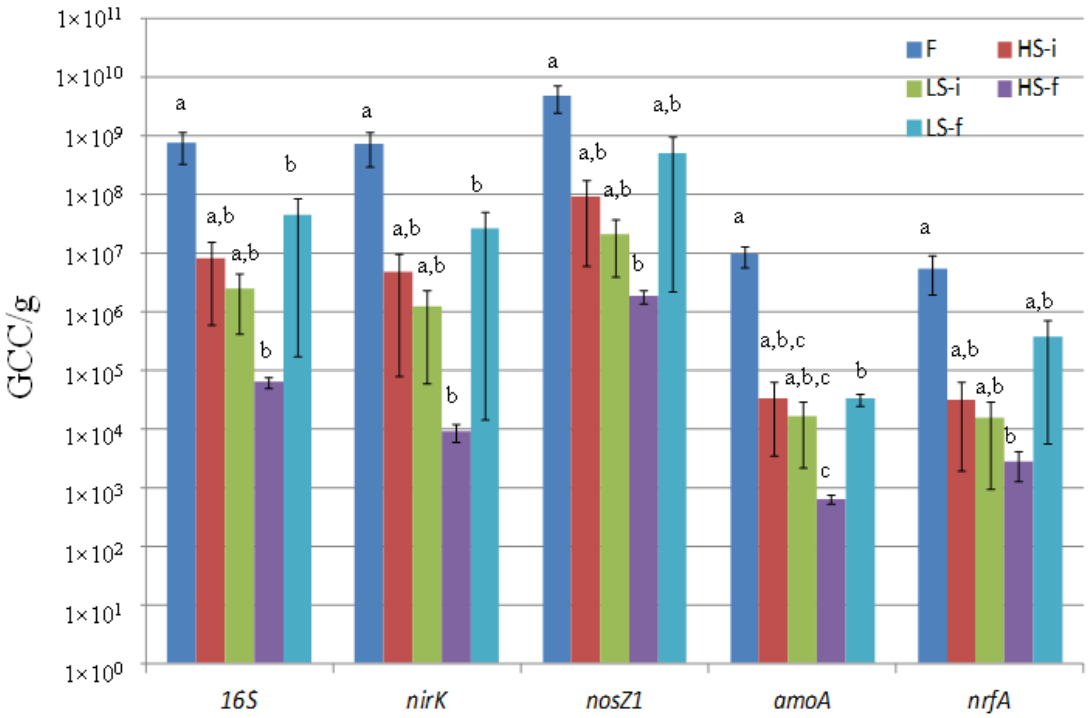


Fig. 7.12. Variation in gene copy concentration (GCC/l) at the base of the soil profile: samples collected within the field(F; n=3) at the moment of core extraction, samples collected before the addition of fertiliser from high saturation treatment (HS-i; n=3) and low saturation treatment (LS-i; n=3) and at the end of the experiment again from high saturation cores (HS-f; n=9) and low saturation cores (LS-f; n=9). Standard errors are indicated for each separated gene group. Statistical differences ($p<0.05$) between GCC are indicated by different letter within each gene group.

Table 7.3. Copy concentration (GCC/l) in at the base of the soil profile: samples collected within the field (F) at the moment of core extraction, samples collected before the addition of fertiliser from high saturation treatment (HS-i) and low saturation treatment (LS-i) and at the end of the experiment again from high saturation cores (HS-f) and low saturation cores (LS-f).

	GCC averages (GCC/g)				
Treatments	***16S***	***nirK***	***nosZ1***	***amoA***	***nrfA***
F	7.7×10^{8}	7.3×10^{8}	4.9×10^{9}	9.5×10^{6}	5.5×10^{6}
HS-i	8.0×10^{6}	4.9×10^{6}	9.1×10^{7}	3.4×10^{4}	3.2×10^{4}
LS-i	2.5×10^{6}	1.2×10^{6}	2.1×10^{7}	1.6×10^{4}	1.5×10^{4}
HS-f	6.4×10^{4}	9.1×10^{3}	1.8×10^{6}	6.3×10^{2}	$2{,}7\times10^{3}$
LS-f	4.4×10^{7}	2.6×10^{7}	5.1×10^{8}	3.3×10^{4}	3.7×10^{5}

7.4 Discussion

7.4.1 WFPS and fertilizer application versus N_2O and N_2 fluxes

In terms of N_2O emissions, Rafique et al. (2011) found high variation in N_2O emissions among eight Irish intensive grasslands as thresholds tend to vary among soil types and

structures (e.g. highest N_2O fluxes found at 70% in a silt loam soil while at 80% in a silty clay loam (Cardenas et al., 2017)). A WFPS below 20% was shown to be limiting for N_2O emissions while a WFPS between 35% and 60% (range common to the LS cores) had N_2O production constantly increasing with a peak between 60% and 80% (range common to the HS cores) (Rafique et al., 2011). In an incubation study, Bateman and Baggs (2005) further found that almost only N_2 was produced above WFPS of 90%. In this study, WFPS of 90% could not be achieved and the cores were characterised by high N_2O emission under extreme WFPS conditions (HS, non-drained cores). Background N_2O-N emission fluxes found within this study were slightly lower than the background values registered by Rafique et al. (2011) for grassland on Irish gley soils (Rafique et al. (2011) average values: min. -0.054, max. 0.668 mg N_2O-N/m^2h) and by Abdalla et al. (2009) from a sandy loam grassland (Abdalla et al. (2009) average values: min. -0.03, max 0.06 mg N_2O-N/m^2h). High N_2O fluxes were generally recorded (from clay soil cores) immediately after fertiliser application (Scholefeld et al., 1997). Within this study, background values showed a spike in N_2O emission values, especially within the HS treatment following fertiliser application. Here the spike was reported from day one to day five after fertilisation for the HS treatment while from day 3 to day 14 for the LS treatment. This coincides with what found by Hyde et al. (2006), which recorded an increase in N_2O emission within 1-2 weeks after fertilisation.

Di-nitrogen is not considered a GHG or a contamination. Its measurement is challenging due to the high atmospheric background concentration and not many studies include N_2 emissions measurements (Bergstermann et al., 2011; Cardenas et al., 2017). In a study on Irish moderately well-drained fine loam soil, Baily et al. (2011) reported N_2 fluxes (8780 mg N/m^2h (297 st.err.; Jun 2009) and 940 mg N/m^2h (330 st.err.; Mar 2010)) higher than the one of this study after the addition of 100 kg N/ha of fertilizer ($^{14}NH_4{}^{15}NO_3$).

Within the present study, the saturation contents achieved, especially for HS treatment, were conducive to N_2O dominance over N_2 and possibly incomplete denitrification.

7.4.2 N transformation apportionment

As the WFPS was kept constant over the duration of the experiment, N transformation apportionment could be assessed after fertilisation. The LS treatments showed a high prevalence of a nitrification signal throughout the experiment. Significantly higher rates of denitrification were found in HS treatments during the days (1 to 7) immediately following fertilisation. Denitrification was replaced by higher levels of nitrification on the last days (10 to 24) of the experiment coinciding with the drop in N_2O emissions of the initial spike after

fertilisation. The N transformation apportionment changed therefore due to the management of the cores. Mathieu et al. (2006) highlighted that, while during unsaturated conditions 60% of N_2O is produced by nitrification, under saturated conditions N_2O production by nitrification decreases to 10-15%. The LS treatment in the present study showed denitrification and nitrification rates values similar to the ones presented by Bateman and Baggs (2005) for 50% WFPS. As expected, the HS treatment showed high denitrification rates. However, the achieved rates were of 73% and only in the initial stage of the experiment and not of 100% for the whole duration of the experiment. Such a difference highlighted in this study could be due to the use of intact rather than disturbed cores. Intact cores have more variable texture and micropores than sieved equivalents and represent emissions from the natural environment. Most laboratory scale studies investigating the role of soil moisture and fluxes have been designed using disturbed sieved soils (Stres et al., 2008). This means that the structure of the soil column has been removed and represents non-field conditions (Banerjee et al., 2016). Furthermore, some studies utilise small cores limiting the soil profile to specific soil horizons (Stres et al., 2008), which does not reflect the multi layered heterogeneity and complexity of the unsaturated zone.

Although nitrification was higher within LS cores and during the terminal phase (possibly after the effect of fertiliser application) of the experiment within the HS cores, the pulses of denitrification that followed the fertiliser application within the HS cores was responsible for more than a double N_2O concentration within HS cores. The ^{15}N apportionment and recovery rates further highlighted the different ratios of the pathways of N transformation due to the denitrification spike within the HS vs. LS cores. The ^{15}N that did not leave the farm through gaseous emission of N_2O or N_2 was recovered within soil and grass, with a higher percentage of ^{15}N retained in soil within LS core, or possibly lost through groundwater (i.e. other unaccounted pathways).

7.4.3 Variation of GCC of genes across treatments

The influence of water content and flow velocities on microbial GCC was shown to be a driver for the definition of community structure and bacterial transport (Ruehle et al., 2015). The saturation level in natural systems varies continuously and is dependent on temporal changes (i.e. seasonal and meteorological patterns) and management, which create difficulties when demonstrating the link between communities, activity and environmental factors (Giles et al., 2012). Therefore, controlled laboratory experiments offer more stable conditions to examine processes without such variability. The constant change of water conditions and

saturation seem to select microbial populations with high resilience characteristics. These will maintain their structure over the long term but quickly respond to daily variation (i.e. respiration pulses) and seasonal dynamics (Waldrop and Firestone, 2006, Cruz-Martinez et al., 2009; Peralta et al., 2013). An increasing frequency of extreme weather events and changes in baseline conditions to levels outside the normal range can initiate longer-term changes in microbial population composition with the creation of distinct communities (Cruz-Martinez et al., 2009; Peralta et al., 2013).

Herein, differences in GCCs were highlighted across most analysed genes (*16S*, *nirS*, *nirK*, *nosZ1*, *nosZ2*, *amoA* and *nrfA*) with the exception of and *hzo1*. As in Chapter 6, *nirK* was favoured over *nirS*, with *nosZ1* preferred over *nosZ2*. Both HS and LS treatments showed similar *nirK* GCCs and therefore similar potentials for N_2O production. The similar values for *nosZ1* gene highlighted within the HS and LS cores suggested a similar ability to transform N_2O to N_2, however *nosZ2* seemed to indicate a reduction of this ability from HS-i to HS-f cores. A reduction of GCCs for the HS-f cores when compared to LS-f cores was seen for the genes *amoA* and *nrfA*. Therefore, this highlighted a reduced potential for both nitrification and DNRA within HS-f cores.

7.4.4 Implementation of AA conceptual site model

Taking all the interpretative components from the field site and the intact cores a series of conceptual diagrams were developed as in Fig 7.13.

In Chapter 4, this farm was characterised by high NH_4^+-N concentration. Isotopic analyses indicated a homogenous organic source. This contamination was possibly explained by both low denitrification and nitrification processes. Low dissolved-N_2O concentrations but high N_2-excess were found when compared with other HSP farms. Further groundwater gene abundances within Chapter 6 did not give any further insights into the present site characterisation.

Information collected from LS treatments showed that low WFPS produced low N_2O and N_2 emissions with a shift towards higher losses of N in groundwater (indicated by the large amount of non-apportioned N). HS cores showed a reduced potential for nitrification, complete denitrification and DNRA (lower GCCs than LS cores). However, the vast majority of N emissions were in the form of N_2 with a high component of N_2O due to pulses of denitrification when compared with LS cores. HS cores further showed a lower amount of unaccounted N, which highlights lower losses.

In some countries re-wetting (no farming scenario) or the installation of control structures to manage water table heights (continue farming scenario) have been shown to be effective at controlling N_2O emissions to decrease the N_2O:N_2 ratio in favour of more complete denitrification and N_2 production (Elmi et al., 2005). The drainage of heavy soils is two-fold: it can reduce complete denitrification, thereby reducing N_2 transformation. However, it can also avoid high N_2O emissions. To prevent emissions at the present site re-wetting due to low permeability soils and long recovery times (not conducive to water table control) is a probable mitigation measure. Remediation in terms of re-wetting is a possibility and would push the system towards complete denitrification with only N_2 production (WFPS ~100%) but may present risks in terms of NH_3 emissions to the atmosphere and NH_4^+ losses to water. This should be investigated further in terms of willingness for farmers to adopt such a mitigation measure and cost effectiveness. However, there is no regulatory mechanism to introduce such a measure in Irish agri-environmental programmes although such a strategy appears in National Mitigation Abatement cost curve analyses by Teagasc.

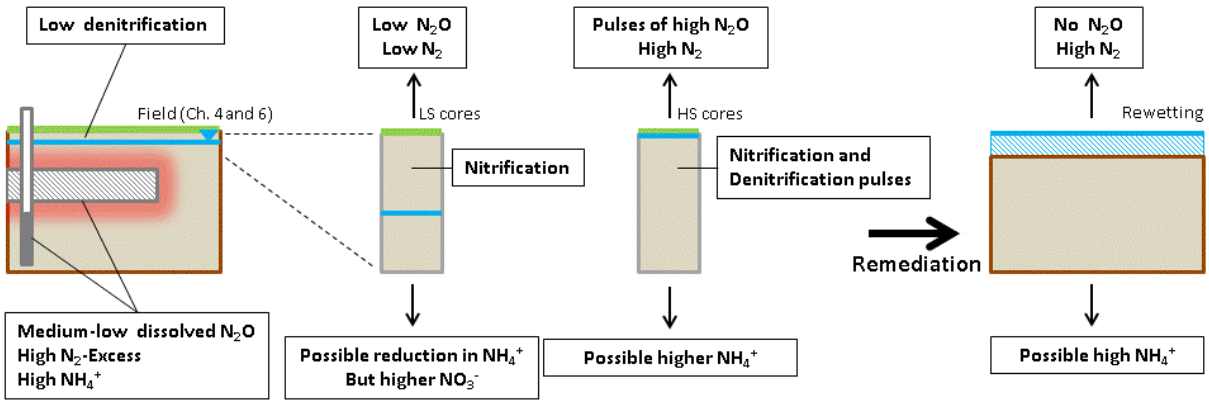


Fig. 7.13. Improvement on the conceptual model achieved within this chapter.

7.5 Conclusions

Different patterns of N_2O and N_2 emissions and transformation processes were evident in the HS cores. Pulses of N_2O and N_2 occurred and both nitrification and denitrification signals were identified. There was a definite increase in denitrification after fertilisation. This could lead to high ammonium concentration in the leached N pathway. In the LS treatment, the transformation process was dominated by nitrification with low N_2O and N_2 emissions. In the leached N pathway, there could be a reduction in ammonium but a higher concentration of nitrate.

This study shows that installation of different WFPS (due to an artificial drainage system) on the present site altered transformation processes, gaseous N emissions and leached N. If the site is left as is and farmed (undrained) the reduction in the nitrification process could enhance NH_4^+ losses with pulses of high N_2O emissions in correspondence with fertilisation. However, if the site is drained and farmed (drained) the reduction of denitrification could cause higher N losses in particular an excess of NO_3^-. The drainage of heavy soils is two-fold: it can reduce complete denitrification, thereby reducing N_2 transformation however, it can also avoid high N_2O emissions (pulses). Remediation in terms of re-wetting is a possibility and would push the system towards complete denitrification with only N_2 production (WFPS ~100%) but may present risks in terms of NH_3 emissions to the atmosphere and NH_4^+ losses to water. This should be investigated further in terms of willingness for farmers to adopt such a mitigation measure and cost effectiveness.

Within this Chapter 7 the hypotheses created in Chapter 2 were partially met. The concepts and datasets gathered within Chapter 4 and 6 where implemented and expanded. This intact core analysis using labelled fertiliser of N gaseous emissions, isotopic abundances and isotopomers gave further insights to and characterised the influence of each process on N_2O production/consumption. The different patterns of N_2O and N_2 emissions and transformation processes were created by different water contents with pulses of N_2O and N_2 depending on different degrees of both nitrification and denitrification following fertilisation. Undrained or saturated conditions however could not mitigate N_2O when fertiliser was added however high rates of N_2 were recorded.

7.6 Acknowledgements

The study was funded by the Teagasc Walsh Fellowship Scheme under the RMIS No. 6707JC in collaboration with the Department of Civil and Structural Engineering, University of Sheffield. Special thank goes to the landowner Mr. J. Leahy for facilitating access to the farm and helping with core collection. Thanks also to the Heavy Soil Programme team in Moorepark. To Mr. P. Blackbourn at the University of Sheffield for the creation of the core extraction device. Thanks to C. O'Connor for laboratory assistance and soil analyses in Johnstown Castle and to Dr K. Richards, Dr G. Lanigan, Dr W. Burchill and Dr D. Krol for advice during the experimental design phase of this experiment.

Chapter 8-Conclusions and future recommendations

8.1 General discussion

This final chapter synthesises the findings of the results chapters (from 3 and 7) and reviews how the experimental results relate to the objectives outlined with conclusive remarks.

Chapter 3 - Investigating "Net" provenance, nitrogen source, transformation and fate within hydrologically isolated grassland plots.

Here the objectives were to:

- Characterise N migration through the system using dissolved gases, N species and biogeochemical parameters using both an end-of-pipe and piezometer approach across four isolated grassland plots in the South East of Ireland.
- Characterise isotopic signatures of H_2O and NO_3^--N to elucidate the "net" provenance of water, source of N, the transformational processes and the fate of N on this multi-tiered site.

Results in brief for each of these objectives were as follows:

- A NO_3^--N plume was found migrating in shallow groundwater but low concentrations occurred in the shallow artificial drainage system at 1 m depth. Higher values of $N_2O/(N_2O+N_2)$ were detected within this shallow groundwater plume pointing to a higher component of incomplete denitrification within this plume.
- Water provenance data showed three distinct signatures indicating disconnectivity on site with no interaction between water migrating through the drainage pipe and deeper groundwater. Source tracking identified further connections between screen interval depths and an up-gradient organic source with elevated NO_3^--N migrating at this depth. End-of-pipe data highlighted connectivity with the overlying plot and showed different water attenuation functionality than the deeper system. End-of-pipe locations clustered together along the denitrification line showing a consistency of signals across the four plots in terms of what occurred in the soil profile above the drain installation depth, while groundwater samples varied spatially showing inconsistency between the end-of-pipe locations and plots indicating the occurrence of different processes.

Here the conclusions were:

- Collating isotopic, dissolved gas and biophysical data from EOP and groundwater locations creates a clearer conceptual model of a site and can be used to examine the water attenuation function of soils over larger areas "net denitrification".
- The "multi-layeredness" of agricultural and drainage systems should be considered when carrying out monitoring campaigns and it should be further studied how drainage system design (e.g. shallow and groundwater) affects N transformation. This multi-technique and multi-layered method should be broadened to rank commercial dairy farms in terms of their N attenuation capacity. Water samples should be taken by the local advisor where water quality issues at the water body or catchment scale have already been identified through the EPA catchment characterisation tool.

Chapter 4 - Influence of artificial drainage system design on assessment of the nitrogen attenuation potential water purification function of artificially drained gley soils: Evidence from hydrochemical and isotope studies under field-scale conditions

Here the objectives were to:

- Examine the N balance, source, transformation and fate of end-of-pipe, open ditch and shallow groundwater sampling points across five sites in the southwest of Ireland.
- Develop a conceptual diagram of these sites and another from the literature in the context of drainage design and water attenuation capacity.

Results in brief for each of these objectives were as follows:

- N surplus and source (organic N) were uniform across the five sites but water attenuation capacity and the fate of N differed. Across the sites NO_3^--N was converted to NH_4^+-N. Three distinct water attenuation capacity groups emerged.
- The developed conceptual diagram merged purification function and drainage design highlighting that the installation of shallow drainage systems, create conditions for transformation of NO_3^--N to NH_4^+-N, negate the soil profiles water attenuation function and create problems from a sustainability perspective. Low concentrations occurred from deep groundwater drainage designs where the water attenuation capacity remained high resulting in good water quality.

Here the conclusions were:

- Deep groundwater drainage systems were classified as more sustainable as water attenuation function was not disrupted by drainage installation

- Deep groundwater drainage systems maintain their soil N attenuation potential but installation of shallow drainage systems can cause a negative shift, resulting in loss of this function, pollution swapping and increased water quality impacts from nutrient loadings in drainage.
- The N sustainability tool based on net denitrification can be used for the comparison or ranking of sites in terms of their N sustainability and it can also be used pre-land drainage to present the consequences of future artificial land drainage on water quality and gaseous emissions at a given site.

Chapter 5 - An assessment of nitrogen source, transformation and fate within an intensive dairy system.

Here the objectives were to:

- Examine the farm N balance on an intensive dairy farm, the spatial and temporal variation in aqueous N-species and the provenance of water samples within a surface and subsurface monitoring network and the spatial distribution of N source and transformation using a combined nitrogen, biogeochemical, isotopic and dissolved gas dataset
- Present a conceptual diagram of the site to inform the sustainability of the agronomic system and the fate of N.

Results in brief for each of these objectives were as follows:

- High N-surplus of 219 kg N/ha were found from organic source. Water signature ($\delta^{18}O$ and δD) showed low spatial variability. End-of-pipe and multi-level groundwater samples exhibiting the same signal while open ditch samples presented a different signal with an enrichment in $\delta^{18}O$ indicating evaporation.
- By combining datasets, four groups of different soil functionality emerged on-site. The sustainability of the dairy farm in terms of N loss could be considered as a two tiered system, in poorly drained or imperfectly drained soils with high water attenuation functionality an artificial drainage system does not disrupt this capacity but it conveyed clean water through the drainage system to the exit point of the farm. In moderately and well drained soils the water attenuation function is lower, facilitating leaching of N, which is then converted at depth to NH_4^+-N and migrates off site along deep groundwater pathways. To prevent future N_r losses in groundwater, management should be cognisant of this two-tiered system, for example movement of dairy soil water to poor drained areas.

Here the conclusions were:

- The installation of an extensive artificial drainage system targeting poorly drained areas in a heterogeneous farm was not disrupting attenuation and it was conveying attenuated water off site while the deep groundwater system showed NO_3^- conversion to NH_4^+.

Chapter 6 - Further insights into N transformation processes within intensive dairy farms using bacterial gene assessment.

Here the objectives were to:

- Examine bacterial genes involved in the N cycle using water samples taken from open ditch, end-of-pipe and groundwater locations across three HSP farms (see Chapters 4) and the Johnstown Castle Dairy farm (see Chapter 5). The following genes were examined: i.e. *16S rRNA* for total quantification, four bacterial denitrification genes (*nirS, nirK, nosZ1* and n*osZ2*), one for nitrification (*amoA*), one for anammox (*hzo* cluster 1) and one for DNRA (*nrfA*).
- Assess if bacterial gene abundance across these locations adds to an overall interpretation of sustainability when combined with isotope natural abundances, dissolved gases and biogeochemical parameters.

Results in brief for each of these objectives were as follows:

- When considering all four sites together, no difference in GCC of bacterial genes (*16S, nirK, nirS, nosZ1* and *nrfA*) were found across open ditch, shallow piezometer sampling to 9 m and EOP locations. The exception being *nosZ2* and *amoA* showing across sites differences, *amoA* among HSP farms (AA, DG and KM) and *hzo1* between groups at JC. The gene *hzo1* indicated the possible importance of anammox for N attenuation.
- The use of bacterial gene abundance in water samples across these locations added little to the overall interpretation of sustainability above that of the interpretation gained through the use of isotope natural abundance, dissolved gases and biogeochemical parameters.

Here the conclusions were:

- Bacterial genes quantification of water samples is not an efficient environmental tool to inform intensive dairy farm sustainability.
- The gene *hzo1* showed the importance of anammox for N attenuation, which requires further investigation.

Chapter 7- Investigation of drained and undrained intact soil cores to examine the fate of N.

Here the objectives were to:

- Assess differences in N_2 and N_2O emissions and examine N labelling and N_2O isotopomers to trace the fate of nitrogen and differences in transformational processes.
- Investigate the microbial community and the impact of the two saturation contents on bacterial community by the analyses of *16S RNA*, *nirS*, *nirK*, *nosZ1*, *nosZ2*, *amoA*, *hzo1* and *nrfA* gene abundances following N fertiliser addiction.

Results in brief for each of these objectives were as follows:

- In the high saturation treatment, pulses of N_2O and N_2 were registered and both nitrification and denitrification signals were evident. A definite increase in denitrification followed fertilisation. In the low saturation treatment, the transformation process was dominated by nitrification although N_2O and N_2 emissions were relatively low.
- Differences in gene abundances were highlighted across most analysed genes (*16S*, *nirS*, *nirK*, *nosZ1*, *nosZ2*, *amoA* and *nrfA*) with the exception of *hzo1* with high saturation treatment showing a reduced potential for nitrification and DNRA.

Here the conclusions were:

- Different water filled pore space led to differences in N apportionment, which highlighted the capacity of drainage systems, as simulated with two different water saturation conditions, to change the N loss pathways.

Overall conclusions from the study were as follows:

- Not all dairy farms even within the same soil type and drainage class range can be treated the same in terms of N source, transformation and fate. This becomes more complicated as different pathways within these farms also vary.
- Farms with variable soil drainage classes present varied source transformation and fate dynamics and are highly complex
- The presence of different land drainage systems alters N apportionment. Knowing what the purification or attenuation of a soil-subsoil water continuum and measuring how this is actually affected by a drainage design is necessary to design and install drainage systems to support sustainability.
- The combination of nutrient, biogeochemical, isotopes and dissolved gases analyses can be used effectively to assess the sustainability of dairy systems. However, the quantification of N cycle genes abundance is not effective in informing intensive dairy farm sustainability.

8.2 Implications of research

From this thesis the following implications could be drawn:

- Drainage systems can be used to elucidate water quality but more interestingly can be used as a monitoring tool, in combination with groundwater monitoring networks, to interpret net N source, transformation and fate, over large areas, on agricultural landscapes. This has implications for environmental research at all scale. Basically, water quality research should incorporate the land drainage pathway to field from catchment scale. Such information is useful as it is a composite for large scale contribution and not a point location. This will be helpful in the future for catchment characterisation studies and future studies that involve sustainability that only allow for few sampling locations.
- Techniques such as natural isotopic abundances, biogeochemical parameters, isotopomers, gaseous emissions, dissolved gasses, can be combined to elucidate multi-layered sustainability of intensive dairy systems. In the present study, a tool box of techniques has been developed within this study to assess N sustainability on intensive dairy farms. Such a combination of techniques should be use at dairy farm worldwide. The tools provided could be in fact utilised on any site where it is possible to collect water samples and give insight into N source, transformation, and water origin. Although flux measurement are important but need temporal monitoring, moment in time tools, such as nitrate concentrations and isotopes, can be used to show if a site offers any protection at all and if a drainage system has interrupted this ability or not. Such tools could divide our landscape into safe and non safe areas. Areas that could or should not be drained. These tools move beyond flux or load and actually examine and characterise the system. The decision not to drain, or even farm, on specific land may become a reality in years to come and if this functional land concept is to become a reality, the types of tools developed herein will be important to develop further.
- Although surpluses of N were found to be uniform across intensive dairy sites on the present study, the soil water attenuation function and "net denitrification" varied considerably across sites. There was considerable variation within dairy farms in terms of N sustainability, which will have consequences for sustainable intensification. Such research findings will have implication for regulatory and policy development as each dairy farm needs a bespoke sustainability plan that tailors site specific soil-subsoil-bedrock conditions. Such information must be included in decision support systems that assess the efficacy of problems of measures in determining and improving water quality in the

future. The result of the present study will have implication for farm management e.g. soil specific farming where right source, right rate, right time and right place are increasingly more important.

- Transformation processes due to inherent conditions can lead to a conversion of NO_3^--N to NH_4^+-N losses in deeper horizons even when high attenuation is present in shallower layers therefore highlighting the need of multi-level monitoring of ground, drainage and surface water for water quality improvement. For example, a single open ditch discharge water sample leaving an intensive dairy farm will not give any information on the groundwater pathway, which could be polluted and linked at depth to a surface water body. To move beyond baseline assessment of sustainability, measures such as multilevel water samples should be considered. During this assessment the techniques, used in combination, worked well to characterise N sustainability.

  In the future, it is likely that the dairy sector will provide a decision supporting system to guide farmers towards sustainability. However, there needs to be another level of complexity attached to this tool to prove sustainability; this will have cost implications.
- Drainage systems affect the water attenuation function differently depending on their design. In the present study, shallow drainage systems removed the natural attenuation capacity of the soil profile, whereas deeper groundwater drainage designs enabled the soil profile to function as normal. The presence of a drainage system on agricultural landscapes does not infer poor water quality. More important than absence/presence of a drainage system is the depth and type of the drainage system present.
- This means that there is the need to rethink how to deal with soil profiles that necessitate shallow drainage systems in Ireland. This may involve: developing new installation techniques that do not negate the natural attenuation capacity of the soil profile, avoidance in terms of land drainage in these areas, consideration of rewetting of these areas through subsidising agri-environmental programs. In fact, on many of these farms correction of soil fertility may achieve production targets without the need for land drainage of new areas.

8.3 Suggestions for future work

Some of the key research questions and points arising from this project are:

- There needs to be a knowledge transfer program initiated with respect to land drainage and water quality issues in Ireland. In addition, research on land drainage designs and

installation as a tool to mitigate negative water quality consequence needs to be promoted by regulators and subsidised within agri-environment programs. This is currently not available.

- How will we change the way we design shallow drainage systems in Ireland to avoid N losses to water and the atmosphere? There needs to be a national study covering all the major soil types and drainage designs to highlight N losses from drainage systems. Such a study should utilise high resolution flow and nutrient data to across the farm calendar to pick up influences of fertilizer application. For example, could mole and gravel mole be replaced by closer spaced tile drainage?
- At present, the concept of N balances is used to infer N sustainability but this is not enough as this tells us nothing about the source, transformation and fate of N. How will we get the tools developed in the present study to be used by the dairy industry to assess N sustainability out on dairy farms where water quality is an issue? How can we refine and simplify these tools to make them cost effective and to be adopted?
- High resolution soil maps at national scale or at least on intensive dairy farm that have a derogation need to be created. All the soil maps used in this study were mapped at a scale of 1:25,000. Without these soil maps the landscape could not be divided into functional land management parcels. Dividing the landscape up in terms of hydraulic conductivity could also be an option. There is a good correlation between ks and water purification and landscape could be divided into units with the ability retain and attenuate N and areas that will always not attenuate and lose N. Where land drainage is installed this dynamic changes and in the future avoiding N losses will become more important that simply remediation of N at distinct points. Many proxies are also being developed in terms of unmanned air craft that could facilitate this production of maps.
- Further investigations need to be carried out on the unsaturation zone down to 1 m in terms of gas emissions, pore water stable isotopes across rainfall event at high resolution. The same investigation of this study needs to be conducted across season and linked to fertiliser management. The same exercise needs to be carried out for phosphorus and N and P sustainability, at the same site, needs to be linked.
- Although here the analysis of gene abundances did only gave small additional information, the present analysis does not exclude the possibility that more in depth and specific analyses (e.g. metagenomics, T-RFLP or the use of microchips) and the further use of primers for the analyses of Archea and Eukariotes communities could produce further

insights pertaining to the processes occurring under these farms to further increase sustainability. However, such analyses due to their complexity of execution, data analyses and high cost and labour are less relevant for exploratory or monitoring analyses to be carried out routinely on farms or as an early field characterisation to guide drainage installation.

Supplementary pictures

Chapter 4

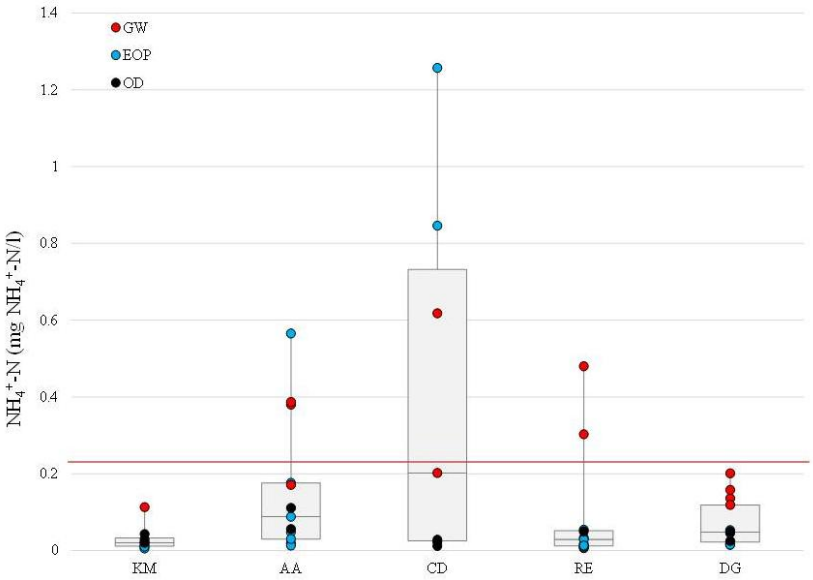


Fig. S4.1. NH_4^+-N concentrations and distribution for open ditches (OD), end of pipes (EOP) and shallow groundwater piezometers (GW) at the five sites. Red line indicates NH_4^+-N MAC.

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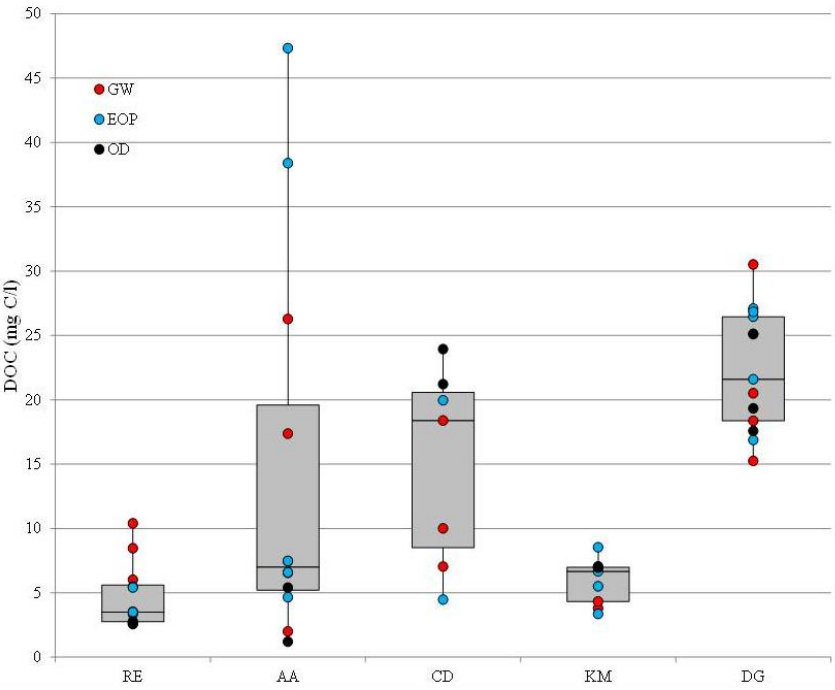

Fig. S4.2. DOC concentrations and distribution for open ditches (OD), end of pipes (EOP) and shallow groundwater piezometers (GW) at the five sites

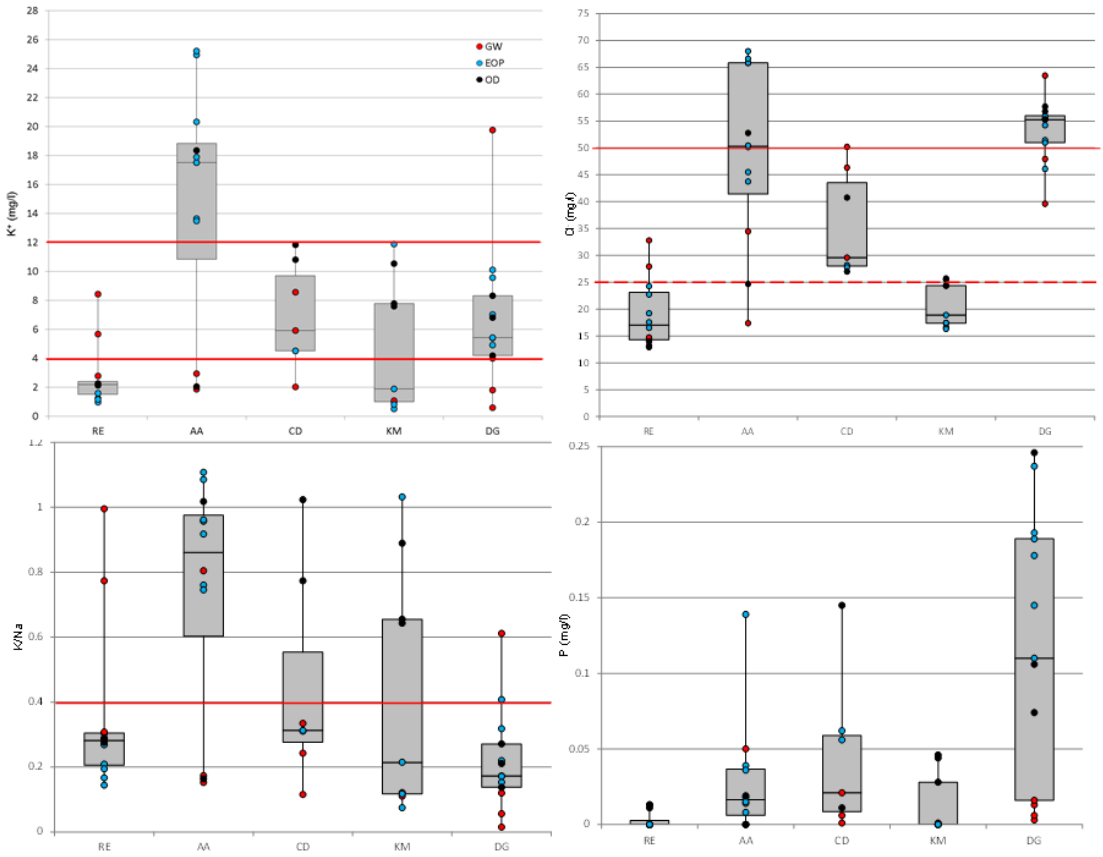

Fig. S4.3. Boxplots for K^+, Cl^-, K/Na and P contamination for open ditches (OD), end of pipes (EOP) and shallow groundwater piezometers (GW) at the five sites. Red lines show limits of contamination (Daly, 2000).

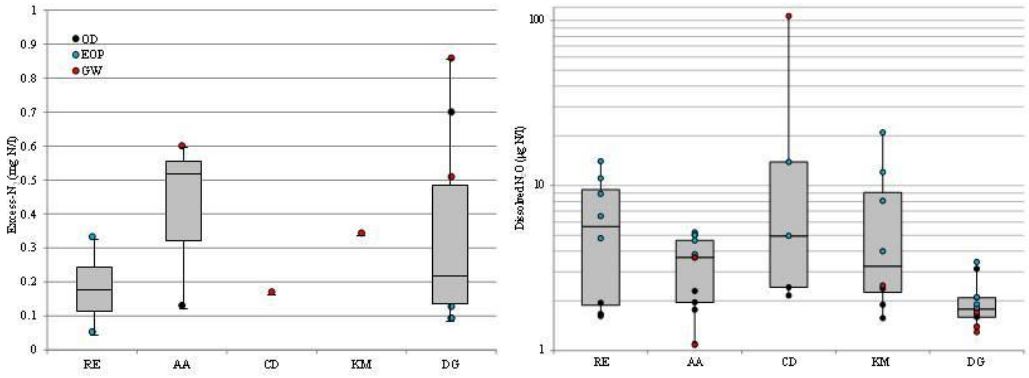

Fig. S4.4. Concentrations and distribution of dissolved-N_2O and excess-$N_2$for open ditches (OD), end of pipes (EOP) and shallow groundwater piezometers (GW) at the five sites

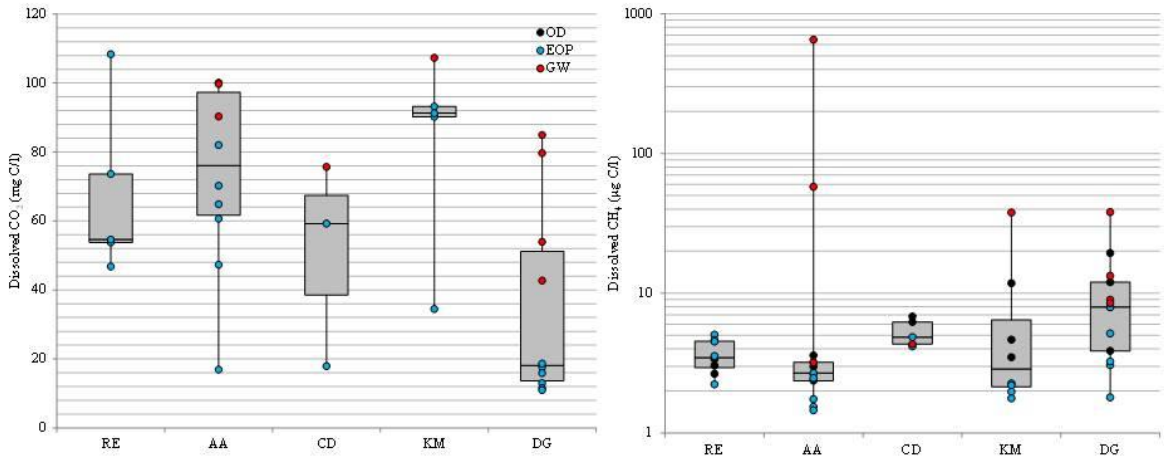

Fig. S4.5. Concentrations and distribution sitesfordissolved-CO_2 and dissolved-$CH_4$for open ditches (OD), end of pipes (EOP) and shallow groundwater piezometers (GW) at the five sites.

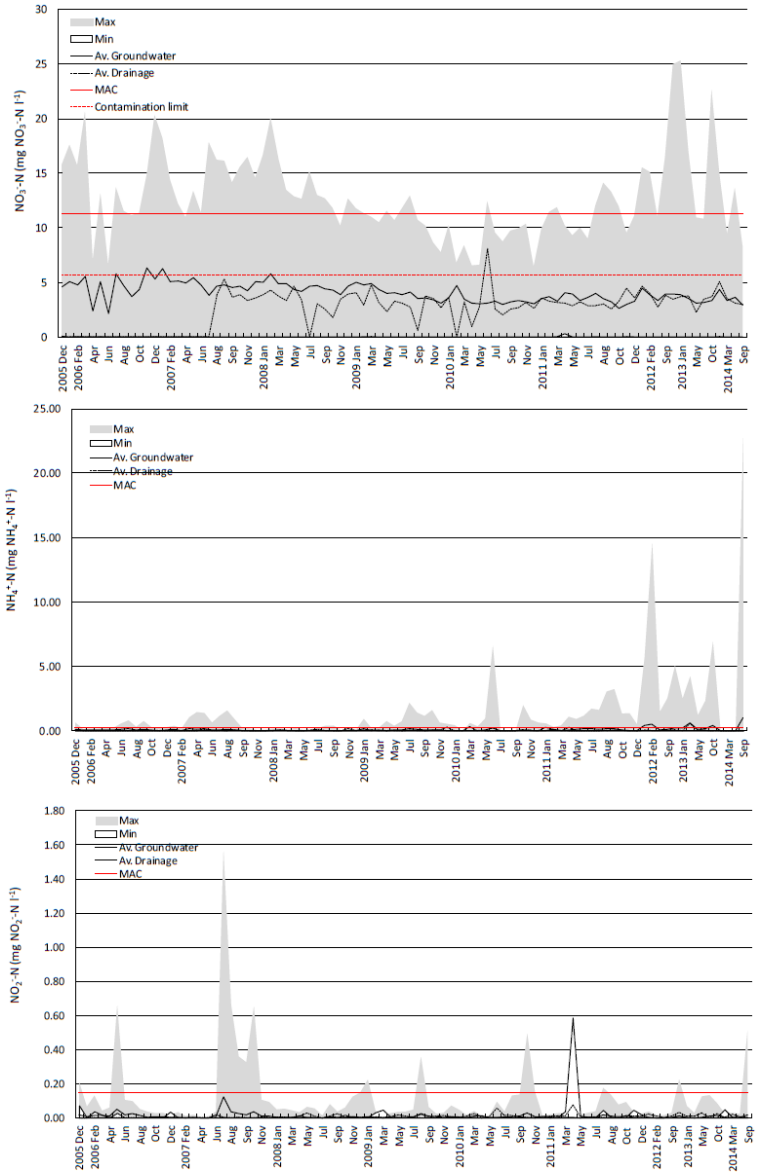

Fig. S5.1. NO_3^--N, NH_4^+-N and NO_2^--N variation across the farm from December 2005 to September 2014 (Combination of data from the September 2014 sampling campaign and the historic dataset).

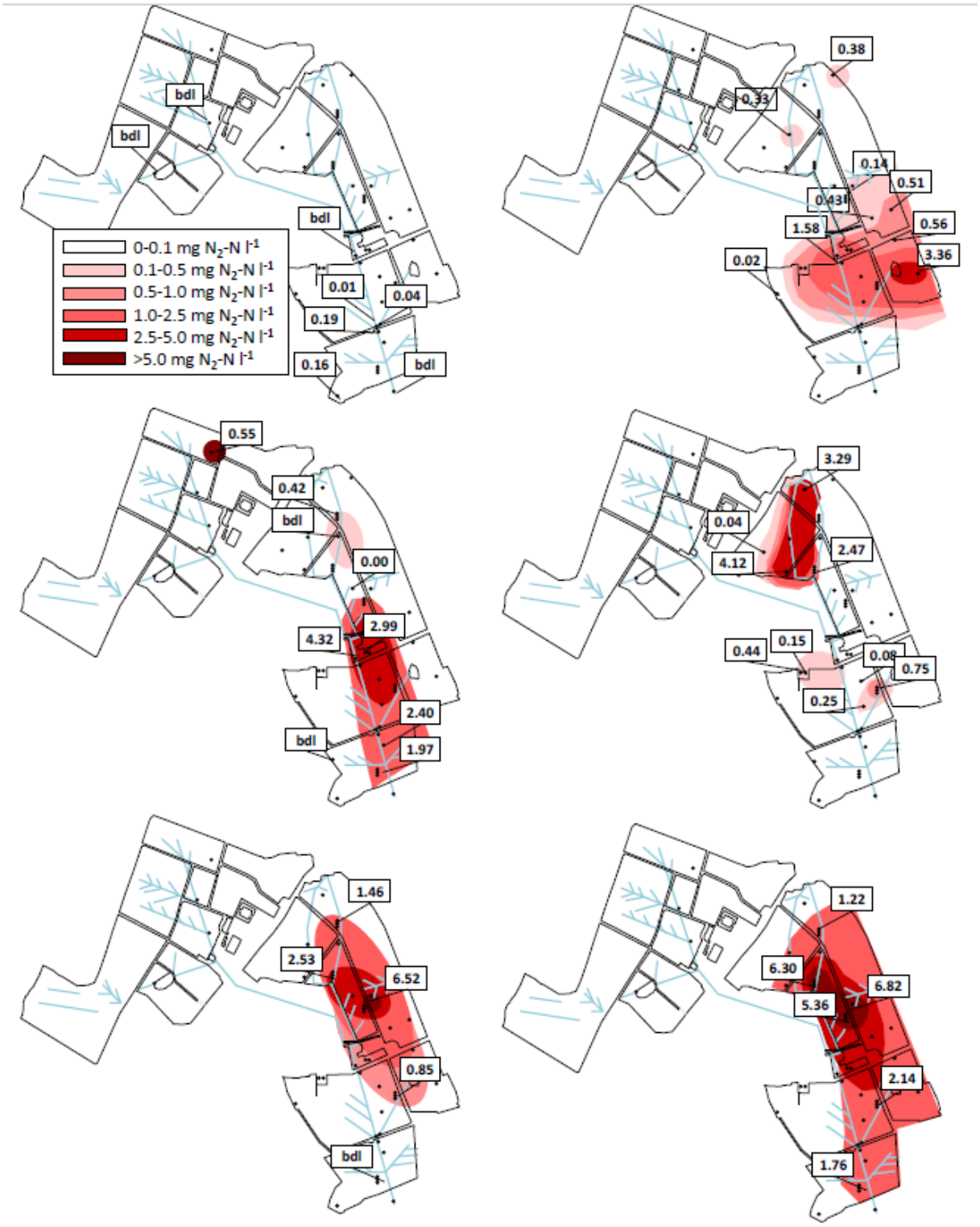

Fig. S5.2. Depth specific Excess-N_2 concentration on the farm collected on September 2014. Top left: drainage system, top right: 2.95-4.5 m bgl, middle left 4.5-6 m bgl, middle right 6-9 m bgl, bottom left 11-13 m bgl, bottom right: below 16 m bgl.

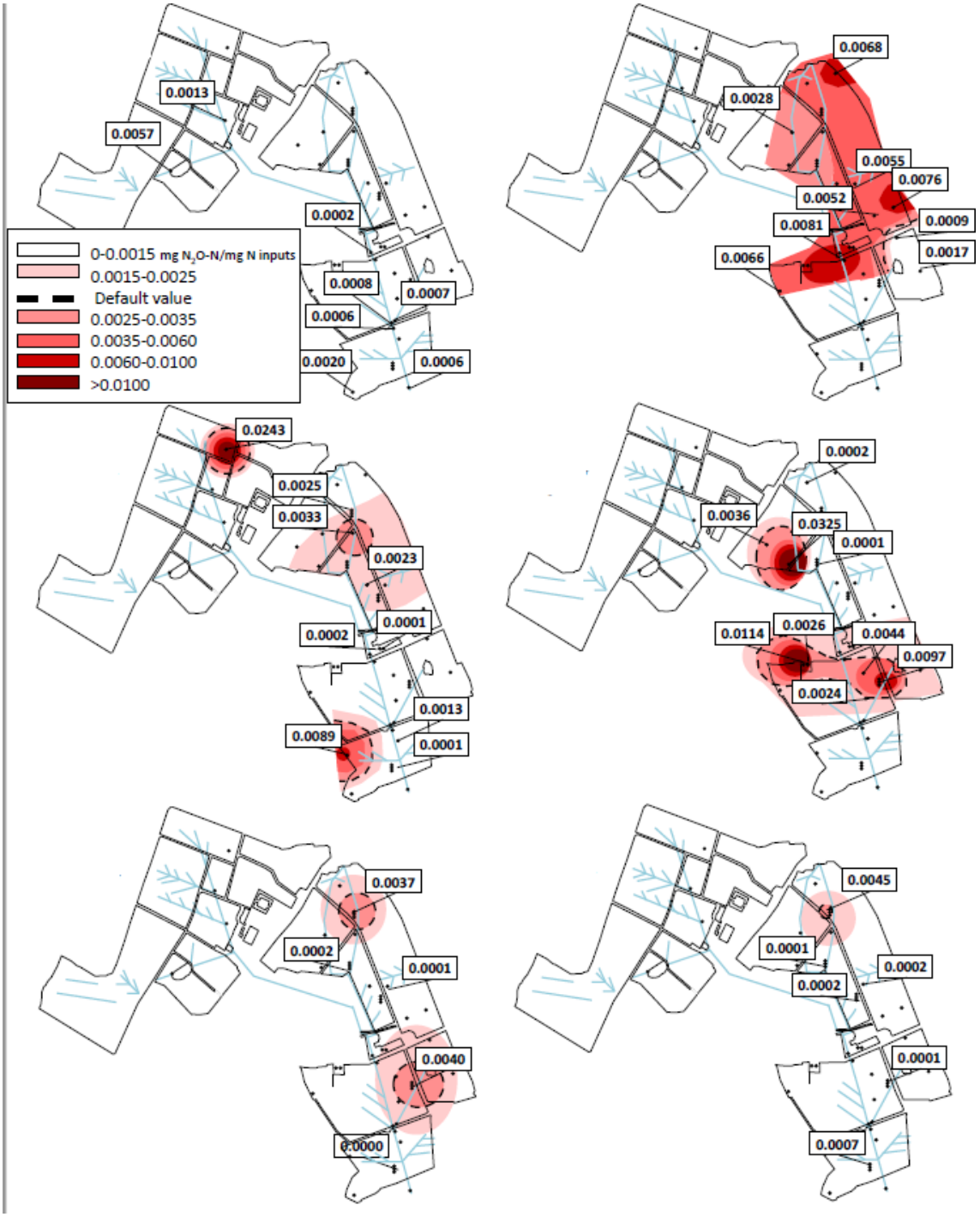

Fig. S5.3. Depth specific EF5g (1) concentration on the farm collected on September 2014. Top left: drainage system, top right: 2.95-4.5 m bgl, middle left 4.5-6 m bgl, middle right 6-9 m bgl, bottom left 11-13 m bgl, bottom right: below 16 m bgl.

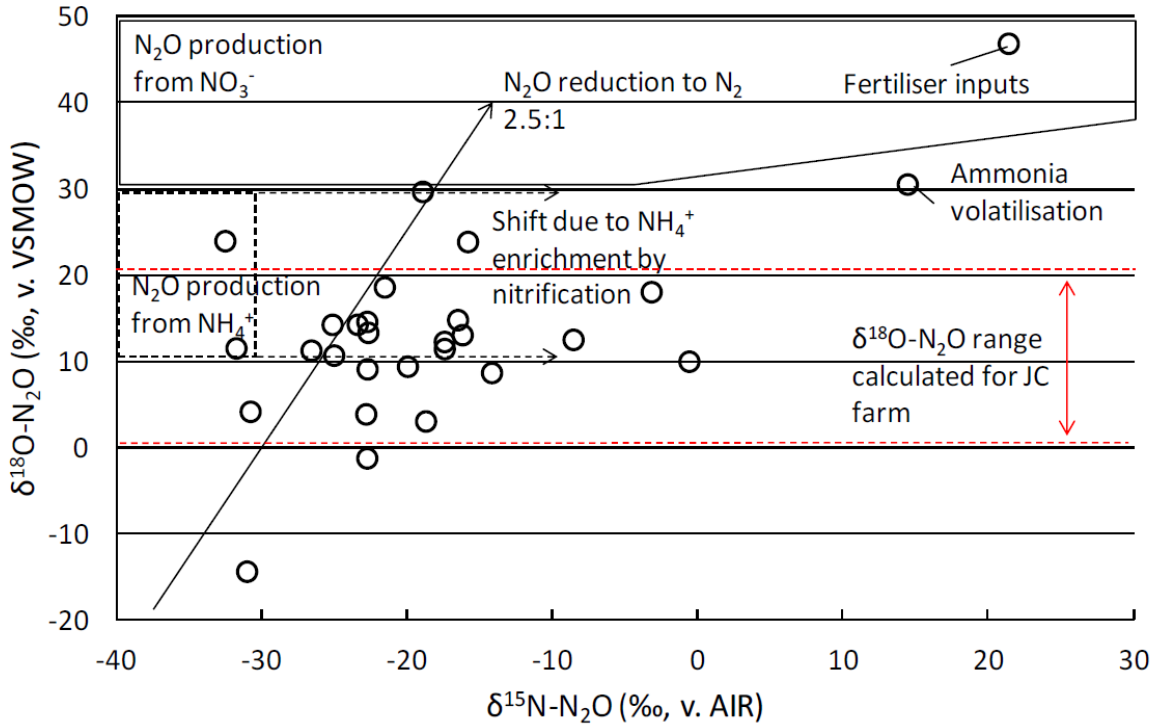

Fig. S5.4. $\delta^{18}O$ versus $\delta^{15}N\text{-}N_2O$ values for samples collected in September 2014. Red lines represent the limits for N_2O production calculated for the farm (JC site). Black squares represent source as delineated by (Li et al., 2014).

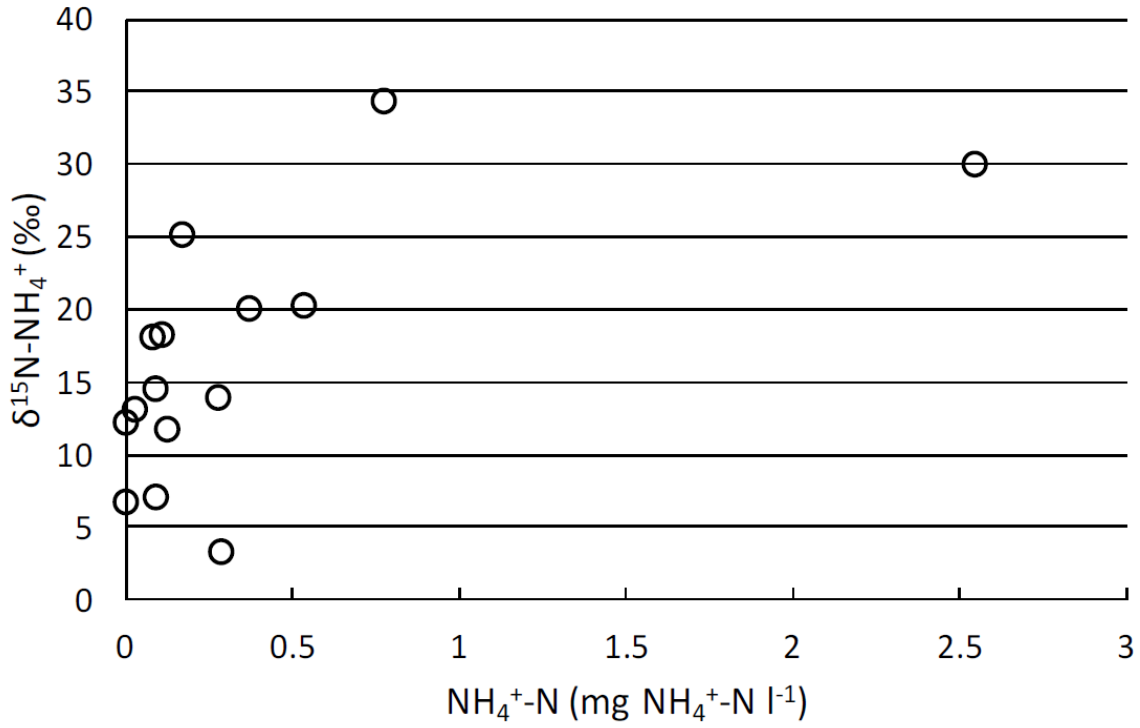

Fig. S5.5. $NH_4^+\text{-}N$ concentration vs. $\delta^{15}N\text{-}NH_4^+$ values for samples collected in September 2014.

Supplementary tables

Chapter 4

Table S4.1. Annual averages for NO_3^--N, NO_2^--N, NH_4^+-N within the five paddocks between 2015-2016

Site	NO_3^--N	NO_2^--N	NH_4^+-N		
	Mean (mg NO_3^--N/l)	Mean (mg NO_2^--N/l)	No of Samples	Breaches (%)	Mean (mg NH_4^+-N/l)
RE					
EOP, paddock 1	0.64 ± 0.57	0.00 ± 0.00	11	0	0.06 ± 0.06
EOP, paddock 2	0.40 ± 0.63	0.00 ± 0.00	12	0	0.07 ± 0.06
GW, Paddock 1	0.57 ± 0.45	0.01 ± 0.01	11	36	0.27 ± 0.40
GW, Paddock 2	0.33 ± 0.39	0.00 ± 0.01	12	58	0.92 ± 2.03
GW 3 (ctrl)	0.36 ± 0.66	0.00 ± 0.00	8	0	0.12 ± 0.06
CD					
EOP 1	1.13 ± 1.09	0.01 ± 0.02	12	8	0.16 ± 0.35
EOP 2	0.80 ± 0.22	0.00 ± 0.00	8	13	0.17 ± 0.28
GW 1	0.19 ± 0.36	0.00 ± 0.00	13	23	0.21 ± 0.17
GW 2	0.33 ± 0.36	0.00 ± 0.00	13	62	0.28 ± 0.12
KM					
EOP 1	0.89 ± 0.57	0.00 ± 0.00	8	13	0.13 ± 0.21
GW 1	0.25 ± 0.24	0.00 ± 0.00	11	18	0.21 ± 0.27
GW 2 (ctrl)	0.45 ± 0.81	0.01 ± 0.01	10	90	0.51 ± 0.29
DB					
EOP 1	0.26 ± 0.11	0.01 ± 0.01	8	25	0.39 ± 0.58
GW 1	0.06 ± 0.04	0.00 ± 0.00	9	44	0.43 ± 0.42
GW 2 (ctrl)	0.13 ± 0.16	0.00 ± 0.00	8	50	0.56 ± 0.79

AA						
EOP 1	0.47 ± 0.38	0.00 ± 0.00	11	0	0.08 ± 0.07	
GW 1	0.09 ± 0.16	0.00 ± 0.01	11	82	0.44 ± 0.25	
GW 2 (ctrl)	0.01 ± 0.00	0.00 ± 0.00	1	100	0.43 ± 0.00	

Table S4.2. Mean values for DOC, dissolved-CH_4 and dissolved-CO_2 for whole farm, open ditches (OD), end of pipes (EOP) and shallow groundwater piezometers (GW) at the five sites.

	DOC (mg C/l)				**Dissolved-CH_4** (µg C/l)				**Dissolved-CO_2** (mg C/l)			
Site	**Site**	**OD**	**EOP**	**GW**	**Site**	**OD**	**EOP**	**GW**	**Site**	**OD**	**EOP**	**GW**
KM	5.91 ± 1.76	7.00 ± 0.05	6.02 ± 2.17	4.06 ± 0.36	8.24 ± 12.38	6.64 ± 4.50	2.05 ± 0.22	37.76 ± N/A	54.44 ± 45.16	6.38 ± 3.49	77.27 ± 28.55	107.30 ± N/A
CD	15.00 ± 7.68	22.57 ± 1.92	8.14 ± 1.92	11.81 ± 5.89	52.80 ± 11.80	65.21 ± 0.42	4.51 ± 0.49	4.33 ± N/A	32.41 ± 32.95	4.64 ± 0.15	38.56 ± 29.24	75.66 ± N/A
AA	14.22 ± 15.15	3.29 ± 2.97	16.92 ± 17.93	15.21 ± 12.28	56.60 ± 179.00	2.98 ± 0.61	2.25 ± 0.67	237.05 ± 358.83	57.13 ± 38.47	3.59 ± 0.51	63.13 ± 26.34	96.66 ± 5.52
RE	4.73 ± 2.53	2.68 ± 0.10	4.06 ± 1.30	7.10 ± 2.98	3.63 ± 1.01	3.02 ± 0.37	3.99 ± 1.13	N/A	43.32 ± 38.24	3.15 ± 0.71	67.43 ± 24.94	N/A
DG	22.35 ± 4.77	20.67 ± 3.94	23.99 ± 4.03	21.16 ± 6.60	10.39 ± 10.07	11.75 ± 7.76	4.86 ± 2.63	20.10 ± 15.65	25.26 ± 21.91	13.05 ± 2.18	14.60 ± 3.24	58.78 ± 18.96

Chapter 5

Table S5.1. Data sources used in addition to the present fieldwork.
Nutrient concentrations: nitrate-N concentration (NO_3^--N), nitrite-N concentration (NO_2^--N), ammonium-N concentration (NH_4^+-N), total nitrogen (TN), total organic nitrogen (TON), phosphorus (PO_4^{3-}), total phosphorus (TP), dissolved reactive phosphorus (DRP). Physiochemistry: dissolved oxygen (DO), electrical conductivity (EC), redox potential (Eh), pH, calcium (Ca^{2+}), chloride (Cl^-), copper (Cu^{2+}), potassium (K^+), iron (Fe^{2+}), manganese (Mn^{2+}), magnesium (Mg^{2+}), sodium (Na^-), sulphide (S^{2-}), sulphate (SO_4^+), zinc (Zn^{2+}), dissolved organic carbon (DOC). Isotope: δ^{18}O-NO_3^- values,δ^{15}N-NO_3^- values. Dissolved gasses: nitrous oxide (N_2O), molecular nitrogen (N_2), carbon dioxide (CO_2), methane (CH_4). Others: Water table (WT) , vertical travel time (Tt), Effective rainfall (ER), effective drainage (ED), potential evapotranspiration (PET), actual evapotranspiration (AET), soil moisture deficit (SMD), saturated hydraulic conductivity (k_s).

Source	Data collected	Approach	Contribution to present work	Times and locations
1.Met station	- Daily Tmax, Tmin, total rainfall, main wind speed, solar radiation	Download	Annual Rainfall and national weather condition, used in conjunction with soil drainage class to elucidate ED	Jan. 2008 – Nov.2014 Dairy farm
2.Fertiliser dataset (unpublished data)	- Inputs: N, K and P (urea, CAN, farmyard manure, dirty water, slurry, woodchip, MOP, Super phosphate)	Farm records	Fertiliser inputs, types, locations of yards and storage facilities for DSW	Jan. 2007 – Oct. 2014 Beef and dairy farms
3.Johnstown Castle Soil Map	- Soil type (1-20 cm bgl) - Soil drainage class - Depth to bedrock	Map and report available	Soil type with associated drainage class; Indicative permeability	Beef and dairy farms
4.Kurz et al., 2005	- Nutrients (NH_4^+-N, TON, DRP, K^+). - Fertilizers use (N, P, K). - Runoff	Field work	Nutrient concentration at limited locations along the old sub-surface piped drainage system; Correlation between management and water chemistry in drainage sections; Proportion of overland flow vs. drainage flow	Nov. 1996 - May 1997 Some sections of Dairy and beef farm
5.Groundwater quality dataset (unpublished data)	- GWT (Dec. 2005 - Jun. 2014). - pH, T, EC, Turb., DO and Eh (Mar. 2009- Jun. 2014). - Ca^{2+}, Mg^{2+}, K^+, DRP, TP and Na^- (Dec. 2005 - Sep. 2010). - Cu^{2+}, Fe^{2+}, Mn^{2+} and Zn^{2+} (Dec. 2008 - Sep. 2010). - Cl^-, NO_3^- -N, NO_2^--N, NH_4^+-N, TN, DON and DOC (Dec. 2005-Jun. 2014).	Grab water samples collection (manually, peristaltic, bladder pump), In situ probe and Physiochemical analyses	Spatial and temporal distribution of water quality parameter	Dec. 2005 – Jun. 2014 (monthly, Dec. 2005 – Dec. 2011, bimonthly, Jan. 2012 – Jun. 2014) Dairy farm: 2 multilevel boreholes (11-13; 36, 37, 38), 17 shallow piezometer (2, 3, 4, 5, 6, 10, 19, 20, 21, 26, 27, 28, 29, 33, 34, 35) 3 boreholes (18, 24, 25) and 4 surface locations D4, D7, D8, D9) (n. 2, 11, 12, 13, 19, 20, 21, 24, 28, 36, 37, 38, Dec. 2005 – Jun. 2014; n. 3, 4, 5, 6, 10, 26, 27, 29, 33, 34, 35, Jul. 2007 – Jun. 2014, D4, D7, D8 May 2007 – Jun. 2014, D9 Jul. 2007 – Jun. 2014)
6. Baily et al., 2011	- Nutrients (NO_3^--N). - Hydrochemistry (Tt, Cl^-, DO). - Isotopes ($\delta^{18}O$-NO_3^-, $\delta^{15}N$-NO_3^-). - GWT, k_s.	Natural isotopic abundance	Correlates nitrates with a source e.g. organic fertilizer; Transformational processes; location of hotspots Vertical travel time to shallow groundwater in site varied from months to years.	Apr., Aug., Dec. 2008 Dairy farm, shallow piezometer network (L3, L5, L8, L9, L11, L13, L14, L16, L17, L18, H1, H2, H5, H6, H9, A1)
7.Jahangir et al., 2012a	- Nutrients (NO_3^--N, TN, DON). - Hydrochemistry (DOC, TC). - Dissolved gasses (N_2O, N_2, CO_2, CH_4). - GWT, ER, PET, AET, k_s.	Physiochemical and gaseous analyses	Farm N-balance with surplus; Quantification of farm scale indirect GHG emissions; Role of site characteristics in the partitioning of N losses	Feb. 2009 – Jan. 2011 (monthly) Dairy farm, multi-level boreholes (7, 8, 9, 11, 12, 13, 15, 16, 17, 22, 24, 18, 25, 30, 31, 32, 36, 37, 38)
8.Jahangir et al., 2012b	- Nutrients (NO_3^--N, NO_2^--N, NH_4^+-N, TN). - Physiochemistry (DO, ORP, pH, Cl^-, Fe^{2+}, Mn^{2+}, S^{2-}, SO_4^+, DOC). - Dissolved gasses (CO_2, CH_4). - GWT, SMD, ER, PET, AET, k_s.	Physiochemical and gaseous analyses	Soil type and bedrock geology; Physiochemical variations and correlation with denitrification rates; Localisation of hot spot due to waste water irrigation practices	Feb. 2009 – Jan. 2011 (monthly) Dairy farm, multi-level boreholes (7, 8, 9, 11, 12, 13, 15, 16, 17, 22, 24, 18, 25, 30, 31, 32, 36, 37, 38)

9.Jahangir et al., 2013a	- Nutrients (NO_3^--N, NO_2^--N, NH_4^+-N, TN, PO_4^{3-}). - Physiochemistry (DO, ORP, pH, Cl^-, Fe^{2+}, Mn^{2+}, S^{2-}, SO_4^+, DOC). - Dissolved gasses (N_2O, N_2). - GWT, SMD, ER, PET, AET, k_s.	Physiochemical and gaseous analyses	Farm scale N balance with surplus; Indirect gaseous emissions trends according to hydrology and depth	Feb. 2009 – Jan. 2011 (monthly) Dairy farm, multi-level boreholes (7, 8, 9, 11, 12, 13, 15, 16, 17, 22, 24, 18, 25, 30, 31, 32, 36, 37, 38)
10. Jahangir et al., 2013b	- NO_3^--N in groundwater - Physiochemistry (DO, ORP, pH, Fe^{2+}, S^{2-}, SO_4^+, DOC). - Dissolved gasses (N_2O, N_2). - GWT, SMD, ER, PET, AET, k_s	Push and Pull	Soil; Groundwater denitrification rates	Oct., Dec. 2010 Dairy farm, multi-level boreholes (7, 8, 9, 15, 16, 17, 30, 31, 32)

Table S5.2. Sustainability groups

Depth (m bgl)	**Samples**			
	Groundwater			
	Group 1	**Group 2**	**Group 3**	**Group 4**
2.95 - 4.5	7, 15, 21	5, 27	3, 19, 20, 26, 33	
4.5 - 6	25, 34, 35, 36	14	1, 10	24
6 - 9		4, 28	22, 23, 29, 30	6
11-13		8, 31		12, 16, 37
> 16	18, 32, 38	9		13, 17
	Drainage system			
--	D1, D2, D3, D4, D5, D6, D7, D8, D9			

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