Farming for soil health: assessing the impact of agricultural management systems of soil biodiversity and functioning

Farming for soil health: assessing the impact of agricultural management systems on soil biodiversity and functioning

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Declaration

I, Sean Conway, declare that this work is all of my own doing and that the results presented here are, to the best of my knowledge, correct. I have not obtained a degree from any other University or elsewhere on the basis of this work.

Signed: Seán Conway

Dated: 29/09/25

“In nature everything is connected, everything is interwoven, everything changes with everything, everything merges from one into another.” - Gotthold Ephraim Lessing

Abstract

This thesis investigated the effects of agricultural management systems on soil health in temperate grasslands. Three major gaps in knowledge were identified: (1) What impacts, if any, do different agricultural management systems (conventional intensive, organic, and extensive) have on physical and chemical health in grassland soils? (2) Does biological soil health differ between management systems? (3) Will the intensity of management, as measured by stocking rate, within these systems create a gradient of soil health in which functional trade-offs will occur?

Physical health was not affected by managements system as all sites were well drained and of similar soil types meaning they had similar physical characteristics. Chemical health was influenced by managements systems. Conventional (CON) systems had excesses of nutrients like P and K according to the Teagasc soil indexes, thus requiring other nutrient inputs (e.g. Cu, Mg), to balance chemical health. Extensive (EXT) and organic (ORG) systems had significantly lower pH, P and K than conventional systems but ORG systems were within the optimum soil index ranges while EXT systems were not. Biological health was affected by management systems with increased nitrification gene abundance in CON systems compared to ORG and EXT systems. Fungal gene abundance was increased in EXT systems at depth (15-30 cm) compared to CON systems. Alpha diversity was reduced in EXT systems for both prokaryotes (compared to CON) and fungi (compared to ORG). Beta diversity showed significant differences between systems and this was driven mainly by soil fertility (pH, P and K levels), indicating that nutrient deficiencies as well as excesses affected microbial communities. Functional trade-offs occurred across the systems, however, contrary to the hypothesis it was not a gradient of management intensity, as ORG systems had the best balance of functionality, however they did have slightly reduced soil carbon stocks compared to CON systems.

Chapter 1 General Introduction

1.1. Literature Review

1.1.1. Introduction

Soil health has increasingly become a major topic of discussion with increased international recognition that soil health is central to tackling the global challenges of food security, sustainability, climate change and environmental degradation. Most notably the United Nations Food and Agriculture Organisation (FAO) recently declared many agricultural systems as being at *'breaking point'* and there is a need to focus on long term soil health to ensure food supply (FAO, 2022b). The European Union (EU) has also recognised soil health as vital to its new agricultural policy within the Common Agricultural Policy (CAP) and The Green Deal. As part of this it has created a Soil Mission outlining its objectives for soil health (European Commission, 2020a, 2021a) as one of the five EU Missions selected for each framework period, to tackle major challenges. This has led to the development of a Soil Strategy and the drafting of a Soil Monitoring Law (European Commission, 2021b). The EU has identified in the Soil Strategy that up to 60-70% of its soils are degraded and the focus of the soil health law and soil mission is to reverse that decline. It also aims to lower the EU's contribution to climate change, as soil can be a contributor to GHG emissions, by seeking ways to lower emissions from agriculture while increasing or maintaining production levels. Ireland is unique in Europe in that agriculture is responsible for 34.3% of its GHG emissions (Duffy *et al.*, 2021). Grasslands are close to neutral or net emitters of GHGs in Ireland while most grasslands worldwide actually sequester and store carbon (Leahy, 2004). Nutrient losses to water in the form of leachate and overland flow are also partly responsible for declines in water quality in Ireland's waterways, with up to 85% of nitrates in waterways in certain catchments being attributed to agriculture (EPA Catchments Unit, 2021; EPA, 2022a). This chapter aims to examine the research that has investigated how agricultural practices affect soil health, the biodiversity within soils and the specific functions of soils in temperate grasslands. The focus was on the comparisons of different management systems, such as intensive, organic and extensive systems as there are perceptions that extensive and organic systems are better for soil health than intensive systems. Organic and extensive systems are also being promoted by the EU in the Green Deal aiming to increase land under organic agriculture to 25% of its land area by 2030 and by CAP Eco-Schemes rewarding extensive livestock production.

1.1.2. Soil health

Soil health has been defined as *"the physical, chemical and biological condition of the soil determining its capacity to function as a vital living system and to provide ecosystem services (European Commission, 2023a)*. No definitive list of soil functions exist but the most common ones discussed in literature are biomass production, water cycling, carbon cycling, nutrient cycling, and soil as a habitat (Blum, 2005; Schröder *et al.*, 2016; Wall, Bondi and O'Sullivan, 2018). These functions are often classified under Ecosystem Services, as shown in Figure 1-1, as provisioning services (food, fuel, fibre, water), regulatory services (water cycling, nutrient cycling, carbon cycling), supporting services (oxygen production, habitats) and cultural services (heritage, recreation) (Jónsson and Davíðsdóttir, 2016). Many soil functions are found under more than one Service. Water Cycling is a good example of a highly interconnected function of soil to ecosystem services. A properly functioning water cycle in soils provides good biomass production and habitats, is a source of natural resources, allows areas to remain populated preserving heritage and is of significant importance to many spiritual beliefs. Therefore healthy soils must be able to perform all these functions mentioned and contribute towards the provision of ecosystem services within the environment (Pereira *et al.*, 2018).

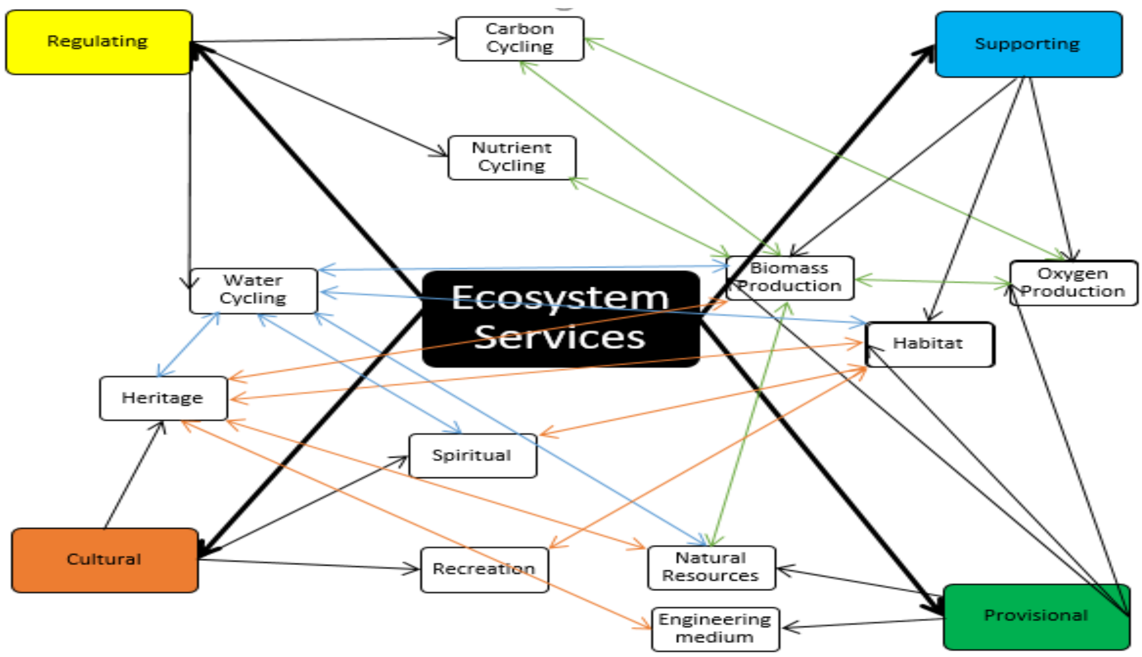

Figure 1-1 Diagram showing how soil functions and Ecosystem Services are interconnected.

Quantifying soil health has proven challenging, as many studies have recognised (Fierer, Wood and Bueno de Mesquita, 2021; Coyne *et al.*, 2022; James A. Harris, Evans and

Mooney, 2022). Soil is a complex system influenced by multiple biotic and abiotic factors. Historically, the terms soil fertility and soil quality were used to describe the chemical and physical condition of soils (Bloem et al., 2005; Schjoerring, Cakmak and White, 2019). Health, however, is a term that incorporates living organisms into the evaluation of soil condition, and the EU stated "*living system*" in its definition of soil health. For these reasons, the focus of quantifying soil health has been to include biological health alongside physical and chemical properties in soils. Harris, Evans and Mooney (2022) argue that the true assessment of a healthy soil is to take a whole systems approach, assessing the life in the soil, physical and chemical properties, the functions, and the complexity of the system. This is the approach that this chapter will focus on, looking at the biology, the physical and chemical properties, the functions of soils, and the interconnected complexity between the two.

1.1.2.1. Functions of Soils

Biomass production is the main goal of agriculture. The main ecosystem provisions from this function are food for humans, feed for animals, fibre as a raw material, and fuel. Yield is often the most common indicator used to describe the health of a soil (Romig *et al.*, 1995). However, chemical inputs can heavily skew the results of yield while reducing other soil functions (Gerzabek, 2014; Balmford *et al.*, 2018). This push for higher yields has also resulted in the decline in nutrient levels in foods mainly due to trade-offs between yield and nutrient content when breeding higher yielding varieties (Davis, Epp and Riordan, 2004; Fan *et al.*, 2008).

Nutrient cycling is a key function of soils for agricultural production. Nutrient cycling is the ability of a soil to receive nutrients, to transform them into bio-available forms, to store them within biological cells, and remove them from the system (Schröder *et al.*, 2016). Soil organisms have a major role in cycling nutrients and agricultural practices can impact these organisms either negatively or positively (Bardgett *et al.*, 1999; Kennedy *et al.*, 2004; Liliensiek, Thakuria and Clipson, 2012; Chandran, Meena and Swapnil, 2021).

Carbon cycling is one of the most important and relevant functions of soils in mitigating human GHG emissions. Plants sequester CO_2 from the atmosphere, use it for biomass growth and transfer it to the soil in the form of complex carbon molecules as exudates (Panchal *et al.*, 2022). These carbon molecules are then digested by soil microorganisms and respired as CO_2 and other carbon molecules such as CH_4 (Xu and Shang, 2016). The storing of carbon in soils as organic matter (OM) has numerous benefits to the overall functioning of soils. It provides structure by decreasing bulk density, improving infiltration and water holding capacity, retains nutrients as OM has a high cation and anion exchange capacity, helps to buffer pH, and is a source of food for soil organisms (Reeve *et al.*, 2016). Soil carbon is also the largest store of terrestrial carbon (Lal, 2004). Soil carbon levels therefore have huge impacts on atmospheric carbon dioxide levels and climate change. Estimates are that a worldwide increase of 0.01% a year in sequestration of carbon, from the atmosphere to the soil, could easily offset annual rises in CO_2 levels from human activities (Ramesh *et al.*, 2019). Thus the change in management practices of soils to store carbon instead of releasing it has the potential to reduce the effects of anthropomorphic climate change.

Water cycling is a key function of soils for sustaining human development. Soils regulate water quality, water movement through the environment, and plant and microbial growth (Garcia, Hernandez and Costa, 1994; Cheng *et al.*, 2021; Vereecken *et al.*, 2022). Agricultural practices affect how soils infiltrate, store and release water back to the environment. Soil organic matter is key to this, providing structure to soils and reducing the bulk density allowing better infiltration and storage (Lankford and Orr, 2022).

As a habitat, soils function as a home for a large diversity of terrestrial life. Soil biodiversity has been defined in an EU report as "*the variation in soil life, from genes to communities, and the variation in soil habitats, from micro-aggregates to entire landscapes"* (Turbé *et al.*, 2010). Soils as an ecosystem are one of the main sources of biological diversity worldwide as they are reservoirs of microbes, microfauna, mesofauna, macrofauna, megafauna, and plants (De Deyn and Kooistra, 2021; Thiele-Bruhn, 2021). It is estimated that 59% of all living organisms depend on soil during their life cycle (Anthony, Bender and van der Heijden, 2023). Agriculture is the largest driver of biodiversity loss worldwide (Dudley and Alexander, 2017). The change of land use and the intensification of agricultural practices has led to destruction of native vegetation and habitats worldwide (Potapov *et al.*, 2022).

The Global Soil Biodiversity Atlas identified 10 human threats to biodiversity: overgrazing, pollution, climate change, acid rain and nutrient overloading, agricultural practices, loss of aboveground biodiversity, introduction of alien species, fire, soil erosion and land degradation, and desertification (European Commission and Joint Research Centre, 2016). They have recognised that changing agricultural practices is vital in the prevention of biodiversity loss.

1.1.2.2. Life in the soil

Soil health and soil biological communities are intrinsically linked (Trivedi *et al.*, 2016; Hermans *et al.*, 2020) and one of soil's major functions is to provide habitats. Soils are estimated to support 59% of all life on earth (Anthony, Bender and van der Heijden, 2023). Soil life can be classified into 5 major groups; microflora, microfauna, mesofauna, macrofauna and megafauna (European Commission Joint Research Centre, 2016). The order Coleoptera and the phylum Annelida are the most studied macrofauna and are particularly important in the context of soils and have been described as ecosystem engineers, and have been studied extensively for their roles in soil health (Kooch *et al.*, 2025; Niero *et al.*, 2025). Nematodes are the most studied group of soil microfauna and are estimated to be the most abundant group of animals in soils and aquatic systems possibly representing 80% of all animal individuals on earth (Bongers and Bongers, 1998). There are 5 main types of nematodes; bacterivore or fungivore grazers, predators of nematodes and smaller animals, plant feeders, omnivores and animal parasites. Extensive research on nematodes has been carried out and soil health indices exist for community compositions (Sharma and Chaubey, 2024). Microflora include the groups bacteria, archaea, fungi and protists. These organisms combined are estimated to make up the bulk of all life on earth and are mostly undescribed (European Commission Joint Research Centre, 2016). They represent the greatest diversity of organisms in soils (Dervash *et al.*, 2024).

Biological communities in soils provide many functions such as carbon cycling, nutrient recycling, toxin remediation, water cycling, pest/disease control and providing beneficial compounds for plant, animal and human health (Turbé *et al.*, 2010; Harman *et al.*, 2021; Suman *et al.*, 2022). Soil communities can therefore give potential information about specific soil functions such as nitrifying and denitrifying bacteria and archaea, (Fierer, Wood and Bueno de Mesquita, 2021) giving a good indication of the functioning of the nitrogen cycle in the soil (Deveautour *et al.*, 2022). Similar approaches can be used for the other various communities to assess functionality, such as carbon cycling (Barnett *et al.*, 2021). These communities are intrinsic to the healthy functioning of soils, soil ecosystems, and can be used as a method to assess functionality.

1.1.2.3. Irish context

In Ireland agriculture is mainly grass-based livestock systems, and grassland occupied 61% of the total land use in Ireland in 2016 (Haughey, 2021). This means that grasslands are a major habitat in Ireland and management of those grasslands can affect the biodiversity within them. 33% of Irish grassland is referred to as High Nature Value (HNV) farmland, as they sustain a high level of above ground biodiversity (Moran *et al.*, 2021). These farms are mainly extensive grazing operations on semi-natural grasslands. However, in general, even intensive Irish grasslands are more diverse and contain more habitats than other European countries mainly due to the use of hedgerows and smaller field sizes (Larkin *et al.*, 2019). Soil biodiversity in Ireland is not very well understood and further research is required. The Environmental Protection Agency (EPA) attempted to assess the biodiversity in Irish soils in 2011, but only sampled 61 sites around the country (Schmidt *et al.*, 2011). This report also highlighted how little is known about the biodiversity in the soil in Ireland and the need to expand soil monitoring to include measuring of soil processes to better understand the link between biodiversity and functioning. A baseline study on biodiversity in soils under different land use types is still needed to provide an understanding on the soil functions and ecosystem services provided (Schmidt *et al.*, 2018).

1.1.3. Issues in Agriculture

1.1.3.1. Food security

Food security has become a hugely important issue as governments seek to secure their growing population's food supply by improving soil health. The main aim of food security today is to mitigate the shocks to the current food production system caused by climate extremes, conflict, economic instability, and the growing inequality in society (FAO, 2022a). Resilience to these shocks is of vital importance as the decline in several ancient civilizations can be attributed in some part to the poor agricultural management and a subsequent decline in their soil health (Montgomery, 2012; Brevik, Homburg and Sandor, 2018). As a result, their agricultural systems could not tolerate these shocks, leading to population decline and the eventual collapse of their societies.

As of 2022 Ireland is ranked 2nd in terms of food security according to a report by the Economist Group (Economist Impact, 2022). However, Ireland scored well below average in several areas of focus in the report as Ireland is actually a net importer of cereals and vegetables (CSO Ireland, 2018). This is a big factor for livestock production as many supplementary meal products for cattle, sheep and other grazing animals are made from cereal or vegetable products. A 2018 report by Teagasc showed that that year, Ireland was only 21% self-sufficient in animal concentrate feeds (Teagasc, 2020). The importing of these products is particularly vulnerable to disruption as seen in early 2022 with the invasion of Ukraine and the disruption to global cereal importation to Ireland (Raleigh, 2022).

Overall though, Ireland is a net food exporter producing high quality meat and dairy products, that are primarily sold to foreign markets (CSO Ireland, 2020), which is possible because the climate provides plentiful grass for animal grazing meaning a low cost, high quality production system which is recognised by European competitors (Bouttes, Darnhofer and Martin, 2019). However because Ireland is so dependent on the climate for its cheap production system, climate change has the potential to cause huge disruptions. Droughts, periods of intense rainfall, long periods of colder weather, and more powerful storms all have the potential to adversely affect the grass based production system, significantly increasing production costs and reducing income for farmers. Improving soil health may help to buffer some of the effects of climate change (Lal, 2011), thus limiting the impact on agricultural production.

1.1.3.2. Anthropomorphic Climate Change

Agriculture plays a big role in anthropomorphic climate change accounting for 18.4% of all greenhouse gas (GHG) emissions worldwide in 2016 (Ritchie, Roser and Rosado, 2020). 9.9% of these emissions came from livestock, manure, and soils combined, and only 0.1% came from grasslands as even when managed intensely they have low net GHG emissions (Leahy, 2004; Dangal et al., 2020). However, unlike most grasslands worldwide, Irish grasslands can be net emitters of GHGs, particularly N_2O from fertiliser use, and methane from ruminant animals, which can cancel out any sequestration of carbon dioxide (Leahy, 2004). This, however, can differ with management practices as intensive grazing systems lead to higher emissions from soils, and extensive, medium intensity grazing and Adaptive Multi-Paddock (AMP) grazing can lead to higher sequestration rates (Emmet-Booth, Dekker and O'Brien, 2019).

Methane is the main emission source, as seen in Figure 1-2, comes mainly from ruminants that graze the grassland and their manure. Agricultural soils are usually net sinks of methane except where the water table is high (Thompson and Rowntree, 2020). Work is currently being done to reduce methane produced by ruminants in their digestive tract through feed additives (Roque *et al.*, 2019; Ku-Vera *et al.*, 2020).

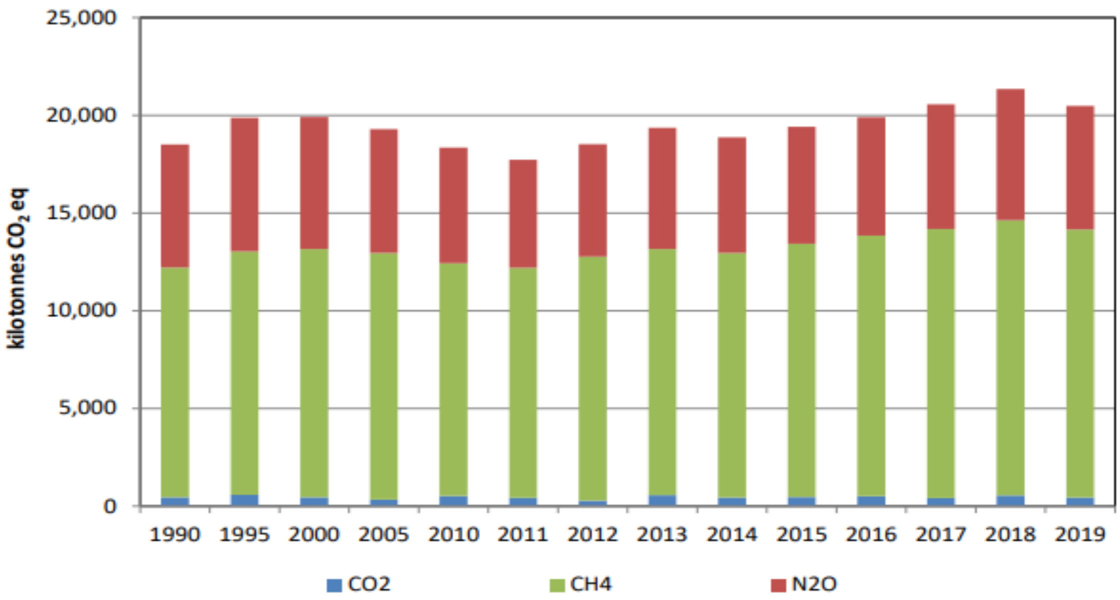

Figure 1-2.Total emissions from Agriculture in Ireland by gas from 1990-2019 (Duffy *et al.*, 2021).

Studies have also shown that higher rates of nitrogen fertilisers leads to higher N_2O emissions from soils (Rafique, Hennessy and Kiely, 2011). Several other factors impact the production of N_2O emissions from agricultural soils such as pH, temperature, moisture levels, and management practices (Wang *et al.*, 2021). Ammonia (NH_3) is also another significant emission from agriculture in Ireland with 98% of NH_3 emissions coming from agriculture. While NH_3 is not directly a GHG it can contribute indirectly as it can be converted to NOx gases in the environment. These emissions mainly come from animal manures and nitrogen fertilisers as a percentage of the nitrogen applied is lost to volatilization. Ireland has failed to meet its emission goals for NH_3 emissions for 7 of the last 9 years and will need full implementation of the AgClimatise Strategy to reduce emissions to meet the 2030 targets (EPA, 2025). This includes the widespread use of protected urea and low emissions slurry spreading, both of which significantly reduce both N_2O and NH_3 emissions. Therefore finding ways to minimise GHG emissions from soils and maximise grass growth without contributing to climate change are key challenges for Irish Agriculture.

1.1.3.3. Water security

Pollution from agriculture is a major concern for water quality as the risk of contamination of water by agricultural chemicals is of high concern in most of the world (FAO, 2022b). Major threats to water quality from agriculture are nutrients, pesticides, salts, sediments, organic carbon, pathogens, metals, and drug residues (Mateo-Sagasta, Zadeh and Turral, 2017). Under the section sustainability and adaptation in the Global Food Security Index, Ireland's oceans, rivers and lakes got the lowest possible score due to the high risk of coastal eutrophication (Economist Impact, 2022). 45% of Irish river water bodies are in moderate, poor or bad ecological quality (EPA, 2024). 42% had high nitrate concentrations (>8 mg/l NO_3) and 27% had high phosphate concentrations (>0.035 mg/l P). Other chemical pollutants related to agriculture are pesticides and herbicides with 10.3% of water quality failures related to these chemicals from 2016-2021 (EPA, 2022b).

Drought and intense rainfall events also have the capacity to disrupt agriculture in Ireland. The Economist report on food security recognised that Ireland has a lack of irrigation infrastructure (Economist Impact, 2022). In 2018, a drought affected the entire country and caused a decrease in biomass production in the most intensively farmed areas and boosted biomass production in the areas that are extensively farmed in the West and North West of the country (Falzoi *et al.*, 2019). The results of Falzoi *et al.,* (2019) seems to indicate that intensive farming negatively affects the soil's capacity to infiltrate and hold water resulting in loss of yields to drought stress, while the increased temperatures and solar irradiance during this drought period may have led to the above normal biomass production in more extensive areas where water limitations were not an issue.

1.1.4. Policies in Agriculture

1.1.4.1. EU soil policies

The EU's Green Deal aims to have no net emissions by 2050 (European Union, 2019). As part of that goal, GHG emissions from agriculture need to be reduced so that it becomes a net sink of GHGs rather than a net emitter. The EU Farm to Fork strategy, which falls under the Green Deal, lays out a pathway for agriculture to become more sustainable by aiming to reduce pesticide use by 50%, reduce fertiliser use by at least 20%, reduce antimicrobials by 50% in terms of sales, achieve 25% of EU land under Organic farming, and bring back 10% of agricultural land under high diversity landscape features by 2030 (European Commission, 2020b). These ambitious targets put pressure on farmers to change their current practices and encourages them to use alternative practices which the strategy has labelled 'eco-schemes'. These were identified under the CAP 2023-2027 to financially reward farmers for continuing to use or implementing sustainable practices (Directorate-General for Agriculture and Rural Development, 2021; EU, 2022c). The EU Soil Mission further builds on the CAP and Green Deal policies by outlining 8 objectives for soil health (European Commission: Directorate-General for Research and Innovation, 2023). These are: reduce desertification, conserve soil organic carbon stocks, stop soil sealing and increase re-use of urban soils, reduce soil pollution and enhance restoration, prevent erosion, improve soil structure to enhance soil biodiversity, reduce the EU global footprint on soils, and improve soil literacy in society. These 8 objectives will be used to guide policy and practices to improve soil health and help meet other targets such as Climate Action, Zero Pollution Action Plan, and the UN Sustainable Development Goals. Living labs and lighthouses will also be established under the proposal to research and showcase healthy soils and good practices, and communicate the ideas and practices that improve soil health to farmers and the public (Commission, 2021). The EU soil strategy aims to make sure all EU soil ecosystems are healthy and resilient so soils can continue to provide their services (European Commission, 2021b). Within the strategy they aim to do this by creating a soil monitoring law, making sustainable practices the norm by providing free soil testing, promoting sustainable practices through the CAP, preventing the draining of wetlands and peatlands and restoring degraded ones, reusing excavated soils, restoring degraded soils, remediating contaminated soils, preventing desertification, increasing research, data and monitoring of soils, encouraging societal engagement, and securing financial support. The culmination of all these policies led to the proposed Soil Monitoring Law in 2023 by the European Commission (European

Commission, 2023b) as there is currently no legal protections for soil like there is for air and water. This aimed to create a framework to protect soils and ensure they are used sustainably after the EU identified about 60-70% of its soils are degraded. As of the time of publishing the Soil Monitoring Law is still moving through the European Council and Parliament.

1.1.5. Agricultural systems and soil health

1.1.5.1. Conventional agriculture

Intensively managed agricultural grasslands rely on the use of chemical inorganic fertilisers and external inputs to maintain high levels of production. Due to the increased livestock numbers large amounts of organic manure is produced and also utilised as fertiliser. In order to boost production, feed is also imported into the system usually consisting of grains and by-products of other food industries (O'Donovan, Lewis and O'Kiely, 2011). These systems use high stocking densities and rotational grazing to maximise utilisation of grass. These systems also rely on the use of high yielding grass monocultures maintained by selective herbicides, although grass-clover swards are becoming more common due to increased nitrogen fertiliser prices and new selective herbicides that have no effect on clovers or grasses (Hennessy et al., 2022). Ireland is unique in that it is currently the only European country in receipt of a nitrates derogation allowing much higher stocking rates (250 kg organic N ha^{-1} yr^{-1}) than the EU set under the Nitrates directive (170 kg organic N ha^{-1} yr^{-1}) (European commission, 2022b). Conditions for this derogation require frequent soil testing and creation of a liming programme for the farm. This makes conventional grasslands under derogation some of the most intensively managed grasslands in Europe.

Nitrogen cycling in the soil has been shown to be affected by the application of nitrogen fertilisers (Leahy, 2004; Ouyang et al., 2018). Plants require a large amount of energy for nitrate reduction and nitrate causes changes in root growth (Marschner, 2012a). Ammonium fertilisers are more efficient than nitrate fertilisers and have been shown to reduce N_2O emissions without significant loss in yields but do produce NH_3, which is an air pollutant and can be a source of N_2O (Gebremichael *et al.*, 2021). Protected urea is now being recommended instead as it has a higher use efficiency and produces less N_2O emissions. Urea treated with nitrification inhibitors and applied to grassland soils was shown to reduced N_2O emission, had no impact on microbial composition or abundance, had no effect on nitrogen cycling, but did alter fungal community structure (Duff et al., 2022). Micronutrient applications also have significant potential to increase the efficiency of nitrogen applications.

Applying micronutrients such as sulphur can increase nitrogen use efficiency, making it more available for plants to uptake (Brown *et al.*, 2000). Long term nitrogen application in soils have been shown to cause a shift in microbial communities possibly leading to greater NO_3^- levels in soils and thus greater potential for leaching or N_2O emissions (Beat *et al.*, 2022). Applications of organic nitrogen fertilisers such as manure or slurry also affect soil microbial communities and increase microbial biomass (van der Bom *et al.*, 2018; Ma *et al.*, 2020). They can also lead to problems with N_2O and NH_3 emissions and leaching of nitrates (De Rosa *et al.*, 2018; Maris *et al.*, 2021). Slurry pits and the application of slurry are also another major source of emissions and possible contaminants such as antibiotics (Nolan *et al.*, 2020). Treatment of slurries, such as acidification, and composting of manures can reduce emissions, reduce contaminants, and increase nutrient cycling (Fangueiro *et al.*, 2015; Liu *et al.*, 2020).

Application of phosphate fertilisers modifies the soil microbial community and reduces arbuscular mycorrhizal colonization (Ikoyi, Fowler and Schmalenberger, 2018). Different P fertilisers have different effects on soil microbial communities and can be linked to the variability in bacterial and fungal communities, as P levels have significant influence on the microbial community (Ducousso-Détrez *et al.*, 2022).

Potassium fertilisers are usually applied as potassium salts such as Muriate of Potash (KCl) or Sulphate of Potash (KSO_4) and to a lesser extent Potassium nitrate (KNO_3). Applying potassium as a chloride salt can have negative effects on the soil, the biology, the surface, and groundwater especially in arid conditions and is ineffective on acid soils (Pereira *et al.*, 2019; Buvaneshwari *et al.*, 2020). Sulphate of Potash is more expensive than KCl and increases soil acidity. Potassium nitrate is rarely used as it is an expensive form of potassium fertiliser but is effective at increasing pH so can be used on acid soils (Soumare, Sarr and Diédhiou, 2022). However, potassium fertilisers have an antagonistic relationship in soils with calcium and magnesium and too much potassium causes deficiencies in these nutrients (Jakobsen, 1993).

Lime is used as a pH buffer on acidic soils to increase pH and provide calcium for microbial and plant growth. Application of nitrogen fertilisers causes acidification in soils and liming is used to counteract this pH effect. Liming, however, does not counteract the effects on the soil bacterial community caused by nitrogen fertilisation (Ma *et al.*, 2018). Liming can influence the denitrification process in the soil and reduce N_2O emissions and influences plant growth by increasing pH making certain nutrients available (Higgins, Morrison and Watson, 2012;

Žurovec, David P. Wall, *et al.*, 2021). Calcium in lime is also a very important mineral for plant growth as it is used in the membranes of cells, for osmoregulation and as a secondary messenger to initiate membrane repair, and in adaptive responses to freezing and anaerobiosis (Marschner, 2012b).

Intensive grazing systems have been shown to be net emitters of GHGs and to decrease soil functions mainly due to the use of inorganic fertilisers to drive crop growth and continuous mowing or grazing (Byrnes R.C. et al., 2018).

Application of selective herbicides or pesticides to grassland can also impact soil diversity and microbial communities as they affect the plant communities and thus the entire grassland ecosystem (E F Power, Kelly and Stout, 2013).

1.1.5.2. Introduction to sustainable systems

Sustainable agriculture is an agricultural management system that can sustain itself over a long period of time because it is economically viable, environmentally sound, and socially fair (Lichtfouse *et al.*, 2009). Sustainable agricultural practices have existed for as long as agriculture has been around, as in order to maintain a stable civilization people relied on sustaining crop production in a given area (Montgomery, 2012). Several ancient Roman texts exist on agriculture with most of them relating to how to improve agricultural production so that the soil maintained its fertility and how they sought to sustain their own agricultural production in the face of challenges they faced such as conflict, climate change, and population growth (Columella, 1941; Cato, 2014). The founding fathers in America were also interested in how to sustain agricultural production as they recognised its importance in maintaining a strong and independent country (Washington, 1847; Miller, 1942). Modern sustainable agricultural systems began in the later part of the 19th and early part of the 20th century mostly in opposition to the idea that plants could be sustained purely by inorganic chemical inputs and the development of the Bosch-Haber process (Johnson, 2022). In the 1920s after World War 1 and the widespread use of inorganic nitrogen fertiliser had begun, the first conference on what would later be called organic farming took place in Poland. This conference, run by Rudolf Steiner, was the foundation of organic and biodynamic farming systems (Paull, 2011). The term ‘organic’ was later coined by one of Steiner’s contemporaries, Sir Albert Howard (Scofield, 1986). From these systems as well as various indigenous and native practices from around the world many other agricultural systems were created such as Permaculture, Agroecology, Regenerative agriculture, and Conservation agriculture, to name a few.

1.1.5.3. Organic agriculture

Organic Agriculture was developed in the early part of the 20th century as an opposing system to the new intensive agricultural system that was focused on chemical solutions to problems. Instead it focused on returning organic matter to soil to ensure its fertility, as was done for many centuries in Asian countries which had highly productive agricultural systems (Heckman, 2006). Organic production is protected and legislated for by the EU (EU, 2022a). Certain standards have to be met and certification is required before products can be labelled as organic. The main difference with organic systems is they cannot use chemical fertilisers, pesticides, or herbicides. They are also limited by the EU nitrates directive on stocking rate and cannot exceed 170 kgs organic N ha^{-1} yr^{-1}. This generally means that organic farms are more biodiverse but this is not always the case (Stein-Bachinger *et al.*, 2021). Currently there is much debate about whether organic agriculture can provide enough food for a growing population as there is currently a production difference in yield of anywhere from 5 to 34% less compared to conventional systems (Seufert, Ramankutty and Foley, 2012). However, organic produce tends to be higher in vitamins, minerals, and secondary metabolites such as antioxidants (Chausali and Saxena, 2021). Under the EU Organic Action Plan, the EU aims to increase the area under organic production to 25% of its agricultural land by stimulating demand and consumer trust, stimulating conversion and reinforcing the value chain, and by improving the contribution of organic agriculture to sustainability (European Union, 2021). Currently, as shown in Figure 1-3, Austria is the only EU country that is meeting this target and Ireland ranks among the lowest (European Commission, 2022a).

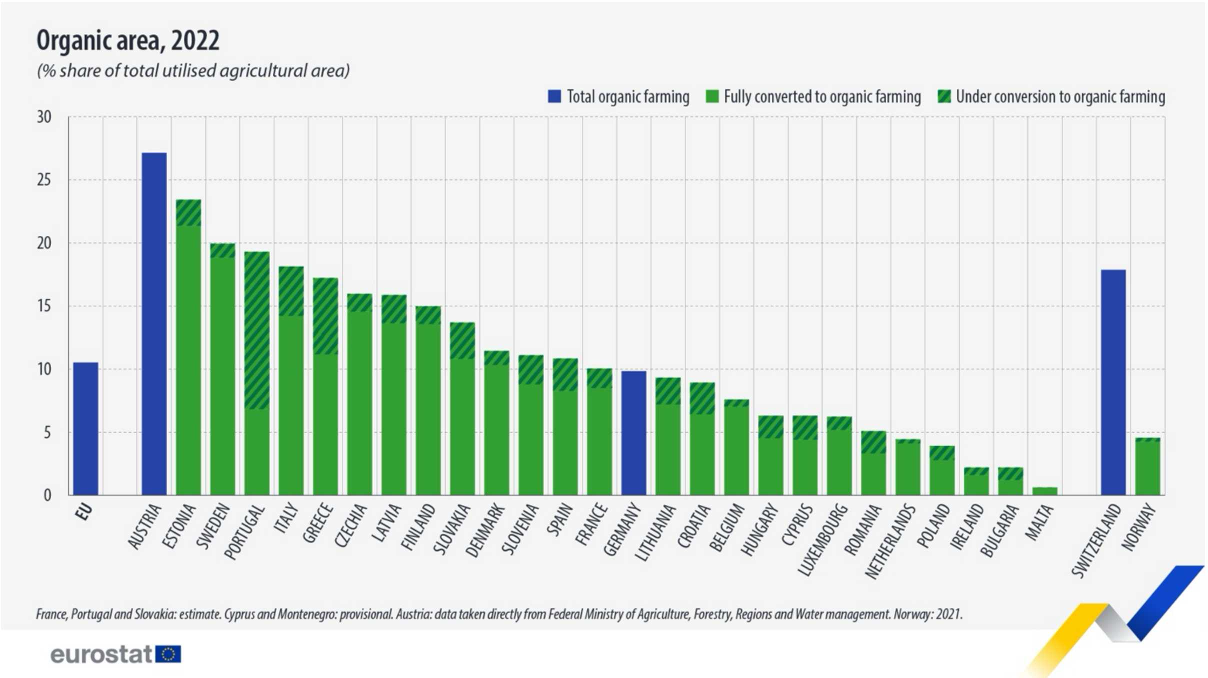

Figure 1-3. Graph showing land area practicing Organic agriculture as a percentage of total utilised agricultural area, by country, in the EU (European Commission, 2022a)**.**

Although organic systems are less productive than their conventional counterparts, they tend to be more efficient in terms of nutrient use and therefore produce more goods using less resources, however this is not the case for all systems (Smith, Williams and Pearce, 2015). This means they can be more sustainable than their conventional counterparts in terms of resource use, nutrient content and biodiversity but are still lower in production yields.

The use of clovers and other legumes in the swards fixes nitrogen from the atmosphere by the use of rhizobacteria in their root nodules, which then make it available to the plants. This is a common strategy used by organic farmers to help increase sward growth (Oberson *et al.*, 2013). Clovers also provide high protein forage for grazing animals and increases the quality of grass forage (Wilkins *et al.*, 1994). Clover is also now used by intensive farmers because of these benefits (Hennessy et al., 2022).

Composted manures showed no increase in N_2O emissions and reduced the risk of run off, leaching, and erosion as compared to fresh manure (Tejada and Gonzalez, 2008; Anwar *et al.*, 2018). Composted manure and organic wastes combined with rock phosphate has been a successful method for application for organic systems as the composting process releases phosphorus from the rock phosphate, possibly by acid production or bacteria solubilisation (Qureshi *et al.*, 2014). A common problem with rock phosphate is the high levels of cadmium and other heavy metals present as contaminants. However the composting process has proven effective at tying up these contaminants by reducing the concentrations of heavy metals in their mobile, bioavailable form (El-Ghany *et al.*, 2018; Zheng *et al.*, 2020).

Other nutrient applications are often used in organic systems such as slag, liquid or solid algae products, dairy processing sludge, animal by-products such as blood meal, fish meal, bone meal etc., compost including composted green waste, animal waste, mushroom substrate and vermicompost, several wood products, gypsum, selected bio-fertilisers and bio-char, to name a few (Irish Organic Association, 2022). The application of all these has been shown to affect growth and health in various crops including grasses, improve soil functions and improve yields (Luo *et al.*, 2018; Zarei, Jahandideh Mahjen Abadi and Moridi, 2018; Stanojković-Sebić *et al.*, 2019; Ahuja and Løes, 2020; Hassan *et al.*, 2021; Shahane and Shivay, 2021; Velusami *et al.*, 2021; Farrar *et al.*, 2022; Tawiah Croffie *et al.*, 2022).

Low intensity grazing, extensive grazing, medium intensity grazing and adaptive multi paddock grazing all have been shown to increase carbon sequestration in soils making them net GHG sinks, increase soil functions and regenerate soil food webs (Stanley *et al.*, 2018; Johnson *et al.*, 2022; Khatri-Chhetri *et al.*, 2022; Mosier *et al.*, 2022).

Since plants are the primary energy producer in the soil ecosystem they can be used as an indicator of soil health, functionality, and microbial communities. Also, plants may not only be selective in recruiting microorganisms to their rhizosphere, but are discriminatory meaning they promote species they want and suppress those they don't (Kavamura *et al.*, 2020). Plant species diversity in grasslands has been shown to increase microbial activity, increase soil carbon storage, decrease emissions, increase drought resistance, produce comparable or increased yields to monocultures, produce similar or better animal performance and increase microbial diversity and functioning (Lange, et al., 2015; Grace et al., 2019; Schaub et al., 2020; Cummins et al., 2022; Jordon et al., 2022; Lüscher et al., 2022; Ryan et al., 2023).

1.1.5.4. Extensive agriculture

Extensive agriculture has been practiced for thousands of years and involves altering the natural environment to harness local natural resources to suit low intensity agricultural production often referred to as pastoralism (Roche Ramo, 2021). This system is based on low stocking rates on permanent meadows and pastures with low external inputs which provide habitats, sequester carbon, prevent forest fires, limit the impact of floods, and prevent soil erosion. This type of agriculture is able to produce food from natural resources that may otherwise be underutilised such as mountainous areas, marginal land, and areas that are not suited to more intensive production. The European Union aims to promote the preservation of these systems as part of the CAP and Green Deal as they recognise that it helps shape European identity by preserving age-old land management practices and promotes culture and rural tourism. The use of organic fertilisers is a key part of maintaining soil fertility in these systems by improving soil fertility, structure, and water infiltration (Roche Ramo, 2021). In Ireland there has been a decline in plant species in grasslands since the 1950s when agriculture started to intensify, leading to a loss of these species rich grasslands in favour of higher yielding swards dominated by *Lolium perenne* (perennial ryegrass) and *Trifolium repens* (white clover) (Stroh et al., 2023) (Faulkner, 2023).

1.1.6. Knowledge gap

While biodiversity, farmer attitudes, economics, emissions and problems with adaption to organic systems have been assessed (Morison, Hine and Pretty, 2005; J. W. Casey and Holden, 2006; Läpple, 2010, 2013; Läpple and Cullinan, 2012; Power, Kelly and Stout, 2012; Eileen F. Power, Kelly and Stout, 2013; Läpple and Kelley, 2013; Power, Jackson and Stout, 2016), studies comparing soil health in differing grassland systems are lacking. Even across the world, studies of this kind on grasslands are rare with only a few studies in similar climates identified comparing grassland management systems and soil health (G. W. Yeates et al., 1997; L. J. Hathaway-Jenkins et al., 2011; Klaus V.H. et al., 2013a; Klaus V.H. et al., 2013b; Lori et al., 2017; Jackson, Isidore and Cates, 2019; Klaus et al., 2020; Zegler et al., 2020; Christel, Maron and Ranjard, 2021; Richter, et al., 2024a). Most studies on organic systems and soil health focus on arable crop land, arboriculture, viticulture or horticulture (Christel, Maron and Ranjard, 2021). However, management intensity is a key factor affecting soil biology in agricultural grasslands (Fox et al., 2021; Barreiro et al., 2022; Fox, Widmer and Lüscher, 2022; Richter, et al., 2024b). Yield reductions in organic systems are also expected and range from 0-30% less in grasslands compared to conventional ones (Klaus V.H. et al., 2013a; Knapp et al., 2023). Management may affect some soil functions, such as water cycling, which may be reduced in conventional grasslands compared to organic ones (L J Hathaway-Jenkins *et al.*, 2011), possibly leading to trade-offs in functionality where one function increases i.e. biomass production, while another decreases. Specifically, information on soil microbial communities in these different grassland management systems is lacking with only Richter et al., (2024b) looking at the diversity of these communities in organic and conventional grasslands. While they found only small differences in their study it is worth noting that both these systems were managed similarly, receiving similar amounts of organic manures with the conventional only receiving a small amount of inorganic fertiliser on top of this. In short, these studies looked at various aspects of soil health in these systems such as biodiversity, water cycling, and yield but none took an overall approach to soil health by looking at physical, chemical, and biological health as well as functionality. This chapter shows that very few studies have been carried out comparing the effects of conventional intensive, organic, and extensive farming systems on soil health in agricultural grassland soils.

1.1.6.1. Thesis outline

This chapter shows there is a large research gap which this thesis will aim to answer:

1. What impacts, if any, do different agricultural management systems (conventional intensive, organic, and extensive) have on physical and chemical health in grassland soils?
2. Does biological soil health differ between management systems?
3. Will the intensity of management, as measured by stocking rate, within these systems create a gradient of soil health in which functional trade-offs will occur?

These questions address the research questions identified in this literature review, healthy soils should be more biodiverse than unhealthy soils and should have increased functionality. The hypotheses are as follows:

1. Extensive systems will have better physical health than conventional and organic systems due to reduced trafficking of soils in these systems. Chemical health will be reduced in conventional systems compared to organic and extensive systems due to increased inputs.
2. Extensive systems will be more biologically diverse than organic and conventional systems due to increased plant diversity and lower inputs.
3. A gradient of management intensity is expected to be created in which it is expected that extensive systems will be more diverse and have more functionality, followed by organic systems and then the intensive systems.

This thesis focused on the sampling and analysis of soils from farms across Ireland, to establish whether a gradient of soil health exists. A range of physiochemical analysis were carried out and combined with environmental and management data to generate metadata. Soil functions were assessed such as dry matter yield, carbon stocks and fractions, nutrient levels and pH, water holding capacity, and bulk density. Microbial community abundance and structure was determined using quantitative PCR, and phylogenetic sequencing approaches (*16S rRNA*/*ITS*) respectively.

This work will contribute to improving knowledge on sustainable agricultural grassland systems and practices. The aim is to provide evidence-based advice to maintain and/or increase grassland agricultural production in a sustainable and resilient manner in the face of multiple challenges such as food security, water security and climate change.

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Chapter 2 Effect of farming systems on soil health in agriculturally managed grasslands

2.1. Introduction

In recent years soil health has become increasingly relevant as healthy soils have been recognised as the basis of agricultural production and degradation of productive agricultural soils is one of humanity's most pressing ecological issues (Bindraban *et al.*, 2012). The European Union (EU) has recognised soil health as vital to agricultural policy in the Common Agricultural Policy (CAP), The Green Deal and has created a Soil Mission outlining its objectives for soil health. Soil health has been defined as "the physical, chemical and biological condition of the soil determining its capacity to function as a vital living system and to provide ecosystem services" within the proposed Soil Monitoring Law (European Commission, 2023a). However, evaluating soil health has proven challenging (Harris, Evans and Mooney, 2022). Soil is a complex system with multiple biotic and abiotic factors that influence it. Historically soil fertility and soil quality were used to define the chemical and physical condition of soil (Bloem *et al.*, 2005; Schjoerring, Cakmak and White, 2019). However, biology plays an important role in soil, as the term 'health' can only be applied to living systems so soil biology must also be assessed to determine soil health. Soil functionality has also been identified as an indicator of soil health and it is expected that trade-offs between functions will occur, as one function increases, such as biomass production, another function like water cycling/purification will decrease.

Grasslands represent approximately 40% of the world's terrestrial area (Petermann and Buzhdygan, 2021). These ecosystems provide many services to humans such as feed, fodder, fuel and fibre, water purification, nutrient cycling, carbon cycling and are also habitats for a large range of diverse organisms. Grasslands are also one of the most threatened habitats as they are increasingly converted into cropland or abandoned, threatened by climate change, supplied with more nutrient inputs and overgrazed. Five main soil functions have been identified for grasslands 1) biomass production, 2) carbon cycling/sequestration, 3) water cycling/purification, 4) nutrient cycling and 5) a habitat for biodiversity (Schulte *et al.*, 2014). Soil health is therefore dependent on the combination of physical, chemical and biological properties of the soil as well as its ability to carry out the aforementioned functions.

Intensively managed agricultural grasslands rely on the use of chemical inorganic fertiliser to maintain high levels of production. This high level of production comes with large amounts of organic manure that is also utilised as fertiliser. These systems also use other inputs such as concentrate feeds consisting of grains combined with other feedstuffs to boost production (O'Donovan, Hennessy and Creighton, 2021). High stocking density and rotational grazing are also key parts of intensive systems allowing them to maximise utilisation of grass and reduce feeding costs. Ireland is currently the only European country that is in receipt of a nitrates derogation which allows farmers to operate at a higher stocking rate of livestock (250 kgs organic N ha^{-1} yr^{-1}) than the EU nitrates directive allows (170 kgs organic N ha^{-1} yr^{-1}) and in 2022 there were 6812 farms with a derogation, occupying ~11% of Ireland's agricultural land area making it a significant part of Irish agricultural production (Department of Agriculture Food and the Marine, 2023). These farms represent some of the most intensive grassland management systems globally.

Organic production is defined by EU legislation and prohibits the use of chemical fertilisers and pesticides. Instead, it focuses on reducing inputs, closing nutrient cycles and increasing animal welfare. Organic systems also aim to increase soil fertility using legumes, livestock manure and where this cannot be achieved, through the use of certified organic fertilisers and soil conditioners. Even within the legislation it says, "the fertility and biological activity of the soil shall be maintained and increased" (EU, 2022b). Organic systems represent a small percentage of farms worldwide at 2.1% of agricultural land with grassland representing more than two thirds of this area (Willer, Trávníček and Schlatter, 2025). In the EU that figure is 10.9% of total agricultural area and grassland represents about ~40% of that. However, the EU aims to increase the share of land under organic production to 25% of its agricultural land area (European Commission, 2021c). In Ireland only 3.6% of all farm holdings in 2023 were organic but ~93% of those were grasslands (Willer, Trávníček and Schlatter, 2025). The Irish government's goal is to increase this to 10% by 2030 (Government of Ireland, 2023). Organic production is also limited by the EU nitrates directive on stocking rates (170 kgs organic N ha^{-1} yr^{-1}).

Extensively managed temperate grasslands represent some of the most diverse habitats on earth (Wilson *et al.*, 2012). However, these grasslands are decreasing as land use intensification replaces them with arable crops or low diversity sown grasslands. Additionally increases in fertilization and overgrazing can also lead to species decline within these ecosystems (Gossner *et al.*, 2016). The National Farm Survey in Ireland shows that the average stocking rate is 102 kgs organic N ha^{-1} yr^{-1} (Dillon *et al.*, 2024). This means a large percentage of Irish farms are classified as extensive (EXT) (<125 kgs organic N ha^{-1} yr^{-1}) by the Department of Food, Agriculture and the Marine (DAFM) under the CAP Eco-Schemes (Department of Food Agriculture and Marine, 2024). These eco-schemes were set up to help farmers adopt practices that minimise the impact of agriculture on the environment and climate, while helping them move towards more sustainable systems. This system involves low levels of fertilisation, low intensity grazing and environmentally friendly management of grassland (Roche Ramo, 2021).

While there is some indication of the effects that various practices have on soil health in grasslands, the effect of different management systems has not been as clearly identified. Soil functions may vary between managements and trade-offs in functions may occur. Management intensity in grasslands is a key factor contributing to greater microbial diversity in ecosystems, when comparing extensive and intensive grasslands across Europe (Fox *et al.*, 2021; Fox, Widmer and Lüscher, 2022). Richter et al., (2024b) found no difference in microbial diversity between organic and conventional grasslands in Switzerland but did see that intensity was a significant factor affecting beta diversity in soil microbial communities. However within this study both organic and intensive grassland were managed very similarly and received similar amounts of fertilizer as organic manures with the intensive one receiving only small additions of chemical fertilizer on top of this. Another Swiss study found that organic and conventional grasslands in Switzerland were not different in terms of ecosystem services, but did find that management intensity was significant. In particular between extensive systems and non-extensive systems where ecosystem-service multifunctionality was enhanced in extensive systems (Richter, et al., 2024a). Organic grassland systems have also shown increases in soil fauna and microbial biomass compared to conventionally managed systems but this only looked at 3 sites in Wales (G W Yeates *et al.*, 1997). In New Zealand organic management of grasslands was not different to conventional management in terms of N mineralisation, pasture growth or soil micro, meso, and macrofaunal diversity across 9 sites (Parfitt *et al.*, 2005). In Germany there was no difference in biomass yields between organic and conventional systems, yet another study in Switzerland saw a 10-15% reduction in yield organic systems (Klaus et al., 2013) and in the DOK (biodynamic (D), bioorganic (O) and conventional (K; German: konventionell)) agriculture trial in Switzerland they saw a 0-30% reduction although this was in grass-clover leys (Knapp et al., 2023). Sown grass leys and grass-clover leys are often incorporated into many tillage rotation systems as they improve soil structure, conserve organic matter, enhance nutrient efficiency (Berdeni *et al.*, 2021). Thus permanent grasslands are perceived to be 'healthy' soils when compared to tillage systems and many studies, including long term ones, focus on examining soil health in different tillage systems (Krause *et al.*, 2022; Angon *et al.*, 2023). However the literature is lacking on how different management systems affect soil health in grasslands. A meta-analysis from 2021 comparing organic and conventional agriculture on soil quality showed that there were not enough studies to draw any meaningful conclusions in grasslands except that there was a trend towards higher soil biological quality in organic systems (Christel, Maron and Ranjard, 2021).

The aim of this thesis was to assess how three contrasting management systems; conventionally managed grasslands (CON), organically managed grasslands (ORG) and extensively managed grasslands (EXT) of varying intensity, as measured by stocking rate on grassland, affect soil health and its related functions in temperate agricultural grasslands. Measuring indicators of these functions as well as the physical, chemical and biological properties of the soil can provide insight into how these systems affect overall soil health within these managed grassland ecosystems.

The following hypothesis were tested; 1) physical and chemical health will be different between the management systems with CON systems having decreased physical health due to increased machinery traffic and increased stocking density and decreased chemical health due to having unbalanced nutrient and chemical inputs 2) the diversity and structure of *16S rRNA* prokaryotic, *ITS* fungal and 18S nematode communities differs between management systems, with CON systems being less diverse and having a different community structure to EXT systems, and ORG systems being somewhere in between 3) trade-offs in functionality will occur creating a gradient of intensity across the management systems with conventional farms having the highest biomass yields but with reduced functionality in carbon cycling, water cycling, nutrient cycling and diversity compared to extensive systems. Organic systems will be somewhere between the conventional and extensive systems thus creating the gradient of intensity.

2.2. Materials and methods

2.2.1. Site selection

Fifty grassland sites across the southern half of Ireland (Fig. 1) were sampled between May and June 2023 (rainfall 885-1507mm per annum; temperature 9.6-11.1°C (30 year average) (Coonan, Curley and Ryan, 2024b, 2024a). Site selection focused on the inclusion of soils from the Luvisol, Brown Earth (Cambisols, WRB) and Brown Podzolic (Umbrisols, WRB) Great Soil Groups, which are the most productive agricultural soils in Ireland. Sites were selected from the well-moderately drained drainage class and were initially classified by using the Teagasc Soil Information System maps (Creamer and O'Sullivan, 2018). Site selection was restricted to pastures grazed by bovine (*Bos taurus*) animals with some being also mowed for hay or silage.

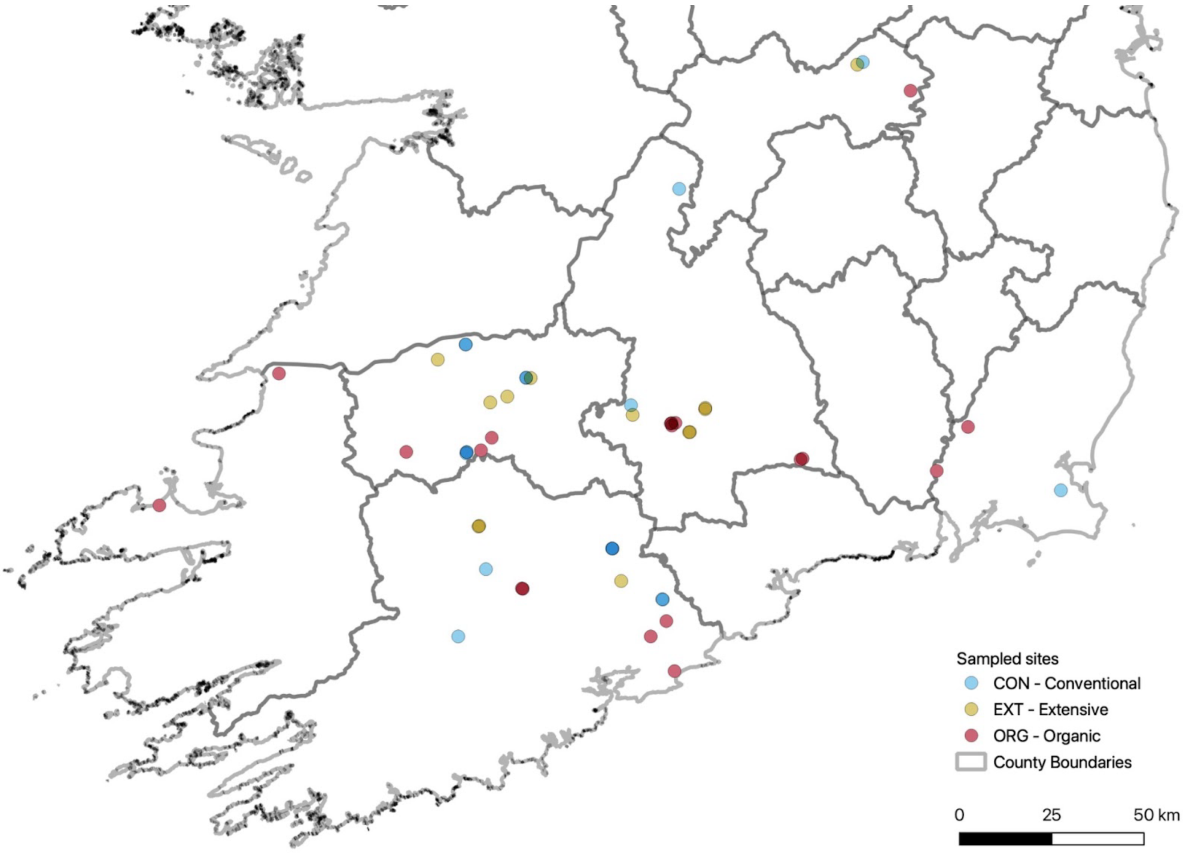

Figure 2-1. Map of the southern half of Ireland showing the location of the farms sampled in this study. Darker colour points represent multiple sites located close to each other (QGIS Development Team, 2024).

Three varying management systems were selected based on management intensity (stocking rate on grassland) and fertilisation practices. Intensive conventionally managed grasslands (CON, *n=18*) received >170 kgs organic N ha^{-1} yr^{-1} (mean=220 kgs organic N ha^{-1} yr^{-1}, range

176-250 kgs organic N $ha^{-1}yr^{-1}$) under a nitrate's directive derogation, were typically grazed at higher densities and received inorganic fertilisers alongside organic manures. Organically managed grasslands (ORG, n=19) received between 125-170 kgs organic N ha^{-1} yr^{-1} (mean=144 kgs organic N ha^{-1} yr^{-1}, range 125-162), were grazed at medium to high densities and were certified organic by either the Irish Organic Association or the Irish Organic Trust. Extensive grasslands (EXT, *n=13*) received <125 kgs organic N ha^{-1} yr^{-1} (mean=69 kgs organic N ha^{-1} yr-1, range 38-106) and were grazed at lower densities than the other two systems. Most of these EXT farms were also certified organic but some were not and did use very small quantities of inorganic fertilisers. Plant communities varied from *Lolium Perenne* (Perennial ryegrass) dominant grasslands in CON systems to *Lolium Perenne-Trifolium repens* (perennial ryegrass-white clover) dominant grasslands in ORG and EXT systems (GA1) according to the Irish National habitat classification system (Fossitt, 2000). Stocking rates, dry matter (DM) yield, lime and fertiliser application rates, grazing density and grass to concentrate ratios were determined from a detailed farmer management survey (Supplementary materials).

2.2.2. Soil Sampling

Soil samples were taken using a modified method of Fox et al., (2021). Briefly, a central point was chosen in the field, and a 20m transect was laid with two 2x2m subplots at either end (Figure 2-2). A perpendicular 10m transect, adjusted for field shape, was then laid out with subplots at both ends. Cores were taken from the four corners of each subplot, totalling sixteen samples. Each core was divided into two depths: 0–15 cm and 15–30 cm. All samples from the 0–15 cm depth were combined into a composite sample and homogenised, as were those from the 15–30 cm depth. A subsample of each composite was flash frozen in liquid nitrogen in the field for DNA analysis. Subplots were also used to do a botanical relevé of plant functional groups using a modified transformed DOMIN scale (Currall, 1987). Bulk Density cores were taken by digging a 30cmx30cm pit within the larger rectangle created by the two transects, where the top 7.5cm of soil were removed and three cores were taken. Three more cores were taken at 22.5cm depth to give three cores at 0-15cm and three at 15-30cm.

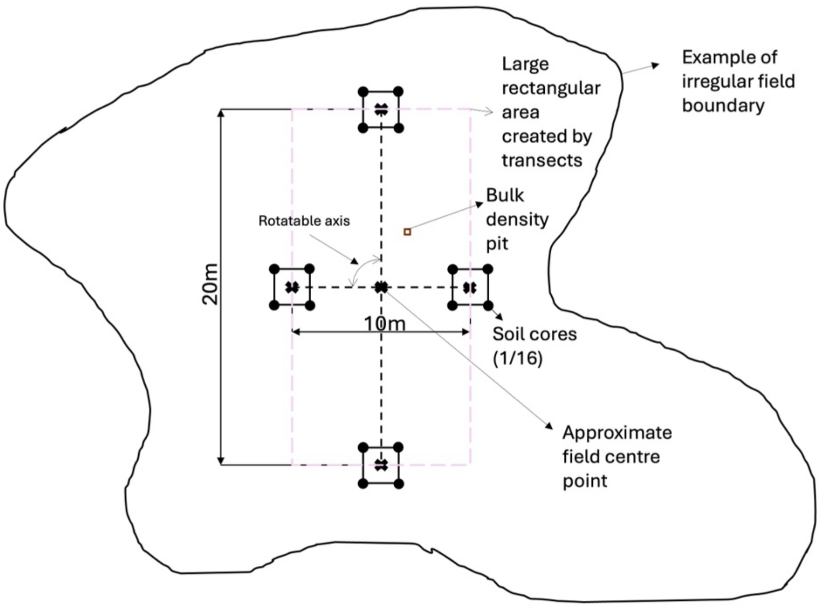

Figure 2-2. Schematic of soil sampling method based on Fox et al., (2021).

2.2.3. Soil physical and chemical properties

2.2.3.1. Soil physical properties

Bulk density and soil gravimetric water content were determined from bulk density rings (diameter 80mm x height 50mm) using the method M4 described in Fenton et al., (2024) Soil water holding capacity was determined by randomly selecting one of the 3 replicates from each depth, and soaking it in water for 2 hours with a fine mesh at the bottom to prevent loss of material. The samples were then removed from the water, allowed to drain for 2 hours and then weighed. All cores were then dried at 105ºC for 48hrs, after which they were weighed, ground up and sieved to 2mm to remove stones, which were then weighed separately. For the other physico-chemical analysis the composite bulk soils were air dried at 40°C then sieved to 2mm. Soil texture was predicted using Mid-Infrared (MIR) spectroscopy using the method and model described in de Santana et al., (2023). Soils that fell outside the range of the MIR spectroscopy library (and for which the texture could not be predicted by spectroscopy) were analysed using the Particle Size Distribution (PSD) method (Day, 1965). Aggregate stability was determined with the wet sieving apparatus model 08.13 by Eijkelkamp Inc and 0.25mm sieves using the method described in Kemper and Rosenau (1986).

2.2.3.2. Soil chemical properties

Soil $pH_{(H2O)}$ was measured using a Mettler Toledo model pH electrode and a Gibson 215 Liquid Handler auto sampler. Soil % organic matter (SOM) was determined by Loss-On-Ignition method. Morgan's P and K were analysed as described in Morgan (1941). Mehlich-3 extractions were performed according to a modified version of Wolf and Beegle (1995). Briefly, 2g of dried sieved soil were mixed with 20ml of reagent (0.2*M* CH_3COOH + 0.25*M* NH_4NO_3 + 0.015*M* NH_4F + 0.013*M* HNO_3 + 0.001*M* EDTA) and shaken at 180rpm on a gyroscopic shaker (New Brunswick Scientific G10 Gyrotory Shaker) for 5 minutes. Samples were filtered through a Whatman No. 2 filter paper and analysed using inductively coupled plasma – optical emission spectroscopy (ICP-OES). For total carbon, total nitrogen and organic carbon analysis the soil was also ball milled. Total carbon and nitrogen were assessed using the high temperature combustion method by LECO TruSpec CN analyser (Elementec, Ireland). Total organic carbon was assessed using the acidification in-situ method as described in Nieuwenhuize, Maas and Middelburg (1994) before using the high temperature combustion method by LECO TruSpec CN analyser (Elementec, Ireland).

2.2.3.3. Soil carbon fractionation

Both physical and chemical fractionation of soils were carried out to determine the stability of soil carbon fractions. Physical fractionation separates soils into different sizes, coarse (2000-250µm), fine (250µm-53µm) and mineral associated organic matter (MAOM) (>53µm) . These fractions have varying stability. The MAOM fraction which has already been decomposed and is now bound to the soil mineral matrix is a long term store of carbon. The fine fraction is a very short term store as it decomposes rapidly due to its small surface area making it essential for nutrient cycling. The coarse fraction is made up of undecomposed plant and animal residue making it a medium term store of carbon as this material usually breaks down slowly over time. Using a modified method of Lopez-Sangil and Rovira (2013) 12g of air dried 2mm-sieved soil was mixed with 24ml of deionised water and a large glass bead, then vertically rotated at 24rpm (Fisher multi-purpose tube rotator) for 1 hour. The resulting slurry was washed through a 250µm sieve with deionised (DI) water and the retentate (2000-250µm) was collected in a pre-weighed beaker and dried at 60°C. The filtrate was sonicated using a Branson SFX250 with a 19mm probe in continuous mode at 440J cm^{-3} per $200cm^3$ of soil slurry at 35% amplitude. It was then passed through a 53µm sieve, with the retentate collected in a pre-weighed beaker and the filtrate in another. A 10ml aliquot from the filtrate was taken for dissolved organic carbon (DOC) and nitrogen (DN) analysis.

This aliquot was passed through 2 separate 0.45µm SFCA 28mm filters and was analysed along with water blanks. The fractions were then dried at 60ºC. Once dried the samples were weighed, ball milled (Retsch MM200 for 90 sec at 23Hz) and analysed for total organic carbon (TOC), total carbon (TC) and total nitrogen (TN) as described in section 2.3.2. Chemical fractionation was then carried out on the resulting MAOM fraction. This results in 4 labile carbon pools of increasing stability. Tetraborate ($Na_2B_4O_7$) extracts carbon weakly bound by electrostatic forces. Pyrophosphate ($Na_4P_2O_7 \cdot 10H_2O$) extracts carbon bound by polyvalent cations. Sodium hydroxide (NaOH) is an even stronger extractant and has an unspecific extraction. The remaining insoluble carbon is the recalcitrant carbon that is highly resistant to decomposition. Briefly the 1.1g subsample was first mixed with 30ml of 0.1M Tetraborate ($Na_2B_4O_7$) adjusted to pH 9.7 by adding sodium hydroxide (NaOH) and then agitated on an orbital shaker (25mm orbital diameter) at 160rpm for 16hrs. This was then centrifuged at 5000RCF for 10mins and the supernatant was decanted. This was then repeated 2 more times but with only 1hr of agitation. The same procedure was carried out on the resulting pellet using 0.1M tetrasodium pyrophosphate decahydrate ($Na_4P_2O_7 \cdot 10H_2O$) adjusted to pH 10.2 with a few drops of 10% HCl. The final extraction was then carried out using 0.1M NaOH and a longer centrifuge time of 30mins. The resulting pellet of recalcitrant MAOM carbon was analysed for TOC, TC and TN as described in section 2.3.2. The three supernatant extractants (90ml total) were refrigerated at 4°C until analysed by taking a 5ml aliquot (1:8 dilution) for TC/Total Inorganic Carbon (TIC) and TN (Analytik Jena multi N/C 3100).

2.2.4. Soil biological properties

2.2.4.1. DNA Extraction and gene quantification

DNA extractions were performed using Qiagen DNeasy PowerSoil Kit (Qiagen, Ireland) from 0.25g of soil following the manufacturer's instructions. All extracted DNA was stored at -80°C prior to downstream analysis. DNA quality was assessed by gel electrophoresis and quantified using a Qubit 4 fluorometer with the dsDNA BR Assay Kit (Thermofisher, Ireland). The abundance of bacterial and archaeal ribosomal ribonucleic acid (*16S rRNA* rRNA) and fungal internal transcribed spacer (*ITS*) taxonomic genes were determined using quantitative polymerase chain reaction (qPCR) assays using SYBR green chemistry. The abundance of denitrification (*nirS* and *nirK*) and nitrification ammonia oxidizing archaea (AOA), ammonia oxidizing bacteria (AOB) and complete ammonia oxidation (commamox) genes were also measured. Inhibition testing was carried out on all samples as described in Duff et al., (2022) and 0.1% bovine serum albumin (BSA) was added to the reaction mixture to allow for uninhibited amplification. Each well on the 384 well plate consisted of a 10µl solution containing 1µM Takyon™ Low ROX SYBR 2X MasterMix (Eurogentec), 1 µM BSA and 1 µM of either template DNA (conc. 5ng/µl)(0.2mM or 1ng/µl conc. in the case of *16S rRNA* rRNA), negative control (DEPC and TE buffer) or positive control (1ng/µl conc.) and the concentration of forward and reverse primers are provided in supplementary materials Table S1, with the remainder was made up of diethyl pyrocarbonate (DEPC) water. Samples were run in duplicate on a CFX Connect Real-Time System (Bio-Rad) with thermal cycling conditions for each gene provided in supplementary material Table S1. Concentrations of the gene targets were determined from serial dilutions (10^7 to 10^2) of standardised DNA from target genes using CFX Maestro Software (Bio-Rad). Slopes, y-intercepts and r^2 values of standard curves are presented in supplementary materials Table S2.

2.2.4.2. Sequencing

The prokaryotes *16S rRNA* (prokaryotes) and *ITS* (fungal) communities were sequenced on Illumina NextSeq platform. Library preparation was done using the Nextera® XT DNA Library Preparation Kit following the manufacturer's instructions. A ZymoBIOMICS™ microbial Community DNA Standard was used as a control. The library preparation has 4 main steps: (1) PCR Amplification using specific primers containing overhang adaptors. For *16S rRNA* 515F (Forward overhang: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGA/GACAG [GT GYCAGCMGCCGCGGTAA]-3′) and 926R (Reverse overhang: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG [CCGYCAATTYMTTTRAGTTT]-3′) were used. For *ITS* the primers used were 86F (Forward overhang: 5′-TCGTCGGCAGCGTCAGATGTGTATAA GAGACAG [GTGAATCATCGAATCTTTGAA]-3′) and 4R (Reverse overhang: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG [TC CTCCGCTTATTGATATGC]-3′). (2) The amplified DNA was then cleaned using AMPure XP beads by combining the amplified DNA with the beads, then eluting the purified DNA from the beads. Cleaned products were checked on 1% agarose gel and visualised under UV to ensure no primer dimer was detected and amplicons were of the expected size. (3) A Nextera XT Index kit was used to attach dual indices and Illumina sequencing adaptors prior to running an Index PCR according to the manufacturer’s instructions. (4) The libraries were pooled in equal proportions and checked to verify molecular size using a Bioanalyzer DNA 100 chip.

Amplicon sequence variant (ASV) tables were created from the analysis of paired end fastq files for *16S rRNA* (prokaryotes) and *ITS* (fungi) gene libraries using the DADA2 pipeline 1.16 (Callahan *et al.*, 2016). Taxonomy was assigned using silva_ssu_r138_2019 for prokaryote sequences and UNITE_v2023_July2023 was used to assign taxonomy for fungal sequences.

2.2.5. Statistical Analysis

Physical data, chemical data, qPCR abundance and alpha diversity were analysed using an analysis of variance (ANOVA) model with a Tukey post hoc test. The assumptions of the model were checked by assessing residual normality through QQ plotting, histogram plotting, variance checks and cook's distance test. If normality assumptions were not met a Kruskal-Wallis test with pairwise Wilcox post hoc test was used. If the assumption of equal variance, assessed via Levene's test, was not met Welsh's ANOVA with Games-Howell post hoc test was used. All statistical analyses were performed using R software (v. 4.3.2) (R Core Team, 2013).

The R package phyloseq (version 1.46.0) and Vegan (2.6.8) were used to perform statistical analysis. The cleaned and filtered sequencing reads were used to conduct alpha diversity analysis for Chao and Shannon Indexes. A centre log ratio (clr) was used to transform sequencing data applying a compositional approach[75]. Aitchinson distance, which is Euclidean distance between clr transformed compositions, was used to create a distance matrix from these data. Principal component analysis (PCA) was used to perform ordination analysis. Using Vegan, multivariate comparison permutational multivariate ANOVA (perMANOVA) was performed using ADONIS (999 permutations) to investigate the effect of management on the microbial community composition. The assumptions of the perMANOVA were assessed using dispersion testing. If dispersion was significant ($p<0.05$), analysis of similarities (ANOSIM) was used instead. Posthoc testing was carried out using Pairwise Adonis with 9999 permutations and Bonferroni adjusted p values. The Vegan function env_fit was used to identify environmental variables that were correlated with microbial community composition. Non-significant variables ($p=>0.05$) were first removed and then those with moderate to weak effects ($r^2<0.3$), followed by a Pearson correlation (packages ggplot, reshape2 and stats) analysis to remove any highly correlated variables (>0.7). An ANOVA-like differential abundance analysis (ALDEx) using ALDEx2 (version 1.34.0) identified differentially abundant ASVs between management systems. A Kruskal-Wallis test with Bonferroni adjusted p-values was carried out to determine significant ($p<0.05$ or effect size >1 or <-1) differentially abundant ASVs between managements.

2.3. Results

2.3.1. Soil geo-physical parameters

First soil physical parameters were assessed, as well as some climate and geological data as these can have significant effects on soil chemical and biological properties. Weather data and elevation was taken from Met Eireann (Coonan, Curley and Ryan, 2024b, 2024a) and Copernicus DEM (Copernicus DEM, 2022) using QGIS (3.16 Hannover) (QGIS Development Team, 2024) and combined with location data from the 50 sites across the southern half of Ireland. There were no significant differences in the 30-year average rainfall or temperature as well as elevation between the systems. Across the systems there were no significant effects of management on soil physical properties at both depths (0-15cm and 15-30cm). Bulk density, porosity, water holding capacity, percentage of stones and aggregate stability were similar across the systems. There were also no significant differences in the percentages of sand, silt or clay between the systems. This result was to be expected given that the study was limited to 3 soil great groups whose major defining characteristics are associated with deeper layers (>40cm) of soil. Details can be found in the Appendix Table A-6.

2.3.2. Soil Chemical parameters

2.3.2.1. Soil pH and Nutrient levels

There was a significant difference ($p<0.05$ and $p\leq0.001$) in pH levels between the 3 systems (Table 2-1). CON systems had significantly higher pH levels (mean=6.43, range 5.94-6.83) than both ORG (mean=6.07, range 5.62-6.62) and EXT (mean=5.79, range 5.03-6.61). ORG and EXT systems were not statistically different, however, the general trend was that as farms got less intensive pH tended to decrease. PH also tended to increase with depth and there were still significant differences ($p<0.05$) between CON and EXT systems in the 15-30cm layer.

Soil nutrient analysis as shown in Table 2-1 shows that CON systems have significantly higher levels of various nutrients compared to both ORG and EXT systems. ORG and EXT systems were similar in almost all nutrients with Morgans P and M3-P being the exceptions which were higher in ORG systems at depth (15-30cm).

Table 2-1 The mean pH and nutrient values for each system (±SE): conventional intensively managed grassland (CON), organically managed grassland (ORG) and extensively managed grassland (EXT). Shown is pH_{H2O}, Morgans Phosphorus (Morgans P) and Potassium (Morgans K) and Mehlich 3 extractions for all other nutrients (M3-Aluminium, Calcium, Cobalt, Copper, Iron, Potassium, Magnesium, Manganese, Sodium, Phosphorus, Sulphur and Zinc). Significance code:'*' p≤0.001, '**' p≤0.01, '*' p≤0.05, 'ns' p>0.05. Multiple comparison of means significant differences are shown through different letters.**

Variable	Depth	F value	CON	ORG	EXT
pH	0-15cm	10.28^{***}	6.43 (±0.067)^{a}	6.07 (±0.081)^{b}	5.79 (±0.157)^{b}
	15-30cm	3.456^{*}	6.56 (±0.14)^{a}	6.21 (±0.11)^{ab}	6.03 (±0.21)^{b}
Morgans P	0-15cm	18.91^{***}	13.67 (±1.678)^{a}	5.25 (±0.817)^{b}	4.01 (±0.651)^{b}
	15-30cm	15.264^{***}	4.93 (±0.82)^{a}	2.00 (±0.36)^{b}	0.87 (±0.12)^{c}
Morgans K	0-15cm	8.471^{***}	187.87 (±18.511)^{a}	120.08 (±17.331)^{b}	84.85 (±14.855)^{b}
	15-30cm	6.038^{**}	96.3 (±12)^{a}	73.1 (±11.5)^{ab}	38.1 (±8.02)^{b}
M3-Al	0-15cm	1.511^{ns}	600.81 (±44.761)	676.75 (±47.790)	723.78 (±56.074)
	15-30cm	0.407^{ns}	669.69 (±45.040)	716.29 (±49.943)	736.79 (±67.876)
M3-Ca	0-15cm	2.203^{ns}	2552.14 (±190.626)	1942.65 (±136.997)	2265.69 (±350.494)
	15-30cm	1.341^{ns}	2283.16 (±313.146)	1649.15 (±162.258)	2176.21 (±467.875)
M3-Co	0-15cm	0.03^{ns}	0.43 (±0.052)	0.45 (±0.082)	0.43 (±0.038)
	15-30cm	0.306^{ns}	0.40 (±0.057)	0.45 (±0.087)	0.48 (±0.054)
M3-Cu	0-15cm	5.879^{**}	5.94 (±0.667)^{a}	3.8 (±0.690)^{b}	2.86 (±0.373)^{b}
	15-30cm	1.24^{ns}	3.99 (±0.516)	3.15 (±0.690)	2.61 (±0.537)
M3-Fe	0-15cm	1.733^{ns}	388.66 (±20.234)	342.86 (±20.367)	346.47 (±16.268)
	15-30cm	3.587^{*}	307.67 (±21.972)^{a}	260.23 (±15.347)^{ab}	233.82 (±20.004)^{b}
M3-K	0-15cm	6.42^{**}	260.48 (±24.982)^{a}	168.10 (±21.634)^{b}	144.43 (±25.761)^{b}
	15-30cm	4.559^{*}	139.66 (±16.562)^{a}	102.93 (±14.395)^{ab}	71.65 (±13.706)^{b}
M3-Mg	0-15cm	8.952^{***}	208.46 (±18.975)^{a}	126.14 (±12.602)^{b}	125.3 (±16.435)^{b}
	15-30cm	7.315^{**}	143.30 (±12.924)^{a}	98.32 (±11.483)^{b}	77.89 (±11.097)^{b}
M3-Mn	0-15cm	0.714^{ns}	72.46 (±10.015)	84.61 (±14.215)	64.75 (±6.545)

	15-30cm	1.19^{ns}	60.30 (±9.105)	80.89 (±13.140)	62.26 (±6.759)
M3-Na	0-15cm	1.49^{ns}	33.7 (±3.668)	26.62 (±2.027)	29.61 (±3.399)
	15-30cm	0.572^{ns}	19.88 (±2.084)	16.79 (±1.715)	18.31 (±2.870)
M3-P	0-15cm	9.171^{***}	95.55 (±10.383)^{a}	51.31 (±7.627)^{b}	40.41 (±11.144)^{b}
	15-30cm	14.92^{***}	50.60 (±8.072)^{a}	25.14 (±4.300)^{b}	10.56 (±1.871)^{c}
M3-S	0-15cm	9.081^{***}	20.99 (±1.529)^{a}	14.49 (±0.805)^{b}	15.23 (±1.184)^{b}
	15-30cm	6.082^{**}	12.59 (±1.019)^{a}	9.63 (±0.567)^{ab}	8.80 (±0.694)^{b}
M3-Zn	0-15cm	3.693^{*}	5.18 (±0.614)	3.3 (±0.443)	2.94 (±0.900)
	15-30cm	2.998^{ns}	1.88 (±0.249)	1.15 (±0.274)	0.79 (±0.463)

2.3.2.2. Carbon levels

Soil Organic Matter (SOM) levels were not different across the systems and neither were total carbon, total nitrogen, dissolved organic carbon, dissolved organic nitrogen or total organic carbon (Table 2-2). There was a significant difference ($p<0.05$) in the ratio of dissolved organic carbon to dissolved nitrogen (DOC:DN) between managements with lower values in the CON systems compared to the other two systems. Soil carbon stocks were significant ($p<0.05$) at 0-15cm between CON and ORG systems but significance was not seen at 15-30cm or when looking at the entire profile 0-30cm (Table 2-2).

Table 2-2 The mean SOM, carbon and nitrogen values for each system (±SE): conventional intensively managed grassland (CON), organically managed grassland (ORG) and extensively managed grassland (EXT). Shown are the percentages of soil organic matter (SOM), total carbon (C_{tot}), total nitrogen (N_{tot}), organic carbon (C_{org}), dissolved organic carbon (DOC), dissolved nitrogen (DN) & the ratio of dissolved organic carbon to dissolved nitrogen (DOC:DN). Also shown are the carbon stocks for the layers measured in Megagrams per hectare. Significance code:'*' p≤0.001, '**' p≤0.01, '*' p≤0.05, 'ns' p>0.05. Multiple comparison of means significant differences are shown through different letters.**

Variable	Depth	F value	CON	ORG	EXT
SOM %	0-15cm	1.901^{ns}	9.52 (±0.568)	8.46 (±0.484)	10.33 (±1.030)
	15-30cm	0.121^{ns}	5.94 (±0.394)	5.67 (±0.313)	5.87 (0.598)
C_{tot} %	0-15cm	1.993^{ns}	4.25 (±0.257)	3.56 (±0.266)	4.36 (±0.445)
	15-30cm	0.304^{ns}	2.41 (±0.194)	5.14 (±0.148)	2.27 (±0.358)
N_{tot} %	0-15cm	1.896^{ns}	0.37 (±0.028)	0.3 (±0.025)	0.39 (±0.051)
	15-30cm	0.345^{ns}	0.19 (±0.019)	0.17 (±0.012)	0.17 (0.026)
C_{org} %	0-15cm	2.412^{ns}	3.92 (±0.268)	3.11 (±0.232)	3.82 (±0.416)
	15-30cm	0.758^{ns}	2.03 (±0.174)	1.78 (±0.131)	1.75 (±0.254)
C:N	0-15cm	0.288^{ns}	10.6 (±0.213)	10.5 (±0.323)	10.3 (±0.310)
	15-30cm	0.522^{ns}	11.29 (±0.502)	10.89 (0.672)	10.44 (0.348)
DOC %	0-15cm	0.434^{ns}	0.95 (±0.083)	0.87 (±0.087)	0.99 (±0.087)
	15-30cm	0.121^{ns}	0.64 (±0.064)	0.66 (0.064)	0.69 (0.096)
DN %	0-15cm	2.894^{ns}	0.13 (±0.010)	0.10 (±0.008)	0.11 (±0.012)
	15-30cm	0.502^{ns}	0.07 (±0.007)	0.06 (±0.005)	0.07 (0.008)
DOC:DN	0-15cm	5.394^{*}	7.33 (±0.321)^{a}	9.15 (±0.540)^{b}	9.3 (±0.868)^{ab}
	15-30cm	1.185^{ns}	9.28 (±0.672)	10.68 (±0.610)	10.71 (1.106)
Carbon stocks (Mg/ha)	0-15cm	3.450^{*}	62.13 (±4.033)^{a}	48.54 (±3.122)^{b}	58.62 (±5.129)^{ab}
	15-30cm	0.512^{ns}	34.42 (±2.765)	30.85 (±2.396)	31.03 (±3.722)
	0-30cm	2.04^{ns}	96.54 (±6.342)	79.39 (±4.765)	89.65 (±8.646)

2.3.2.3. Soil carbon fractions

Following soil carbon fractionation there were no significant differences in the carbon or nitrogen levels in the physical fractions between the systems. After chemical fractionation on the resulting MAOM physical fraction, significant differences in chemically extracted carbon fractions were seen between the systems as shown in Figure 2-3. For the Borax extracted fraction, which is the weakest bound C fraction, EXT had significantly higher levels ($p<0.05$) of extracted TOC compared to ORG but was not significantly different from CON. EXT also had significantly higher levels ($p<0.05$) of extracted TN compared to both ORG and CON in this fraction. For the pyrophosphate extracted fraction there were no significant differences (Figure 2-3). For the NaOH extracted fraction, which is the least labile carbon pool, CON had significantly higher levels ($p<0.05$) of TOC and TN compared to ORG but not EXT who were not significantly different to either (Figure 2-3). The recalcitrant insoluble carbon was significantly different between the systems with CON being significantly higher ($p<0.05$) than ORG but not EXT which was not different to either. There were no significant differences in carbon to nitrogen ratios in any of the fractions and there were no significant differences at depth but the trends could still be seen (Figure 2-3). However when viewed as percentages of the total bulk soil organic carbon pool or as percentages of the MAOM total organic carbon pool, the chemical fractions were no longer significantly different between systems as shown in Figure 2-4.

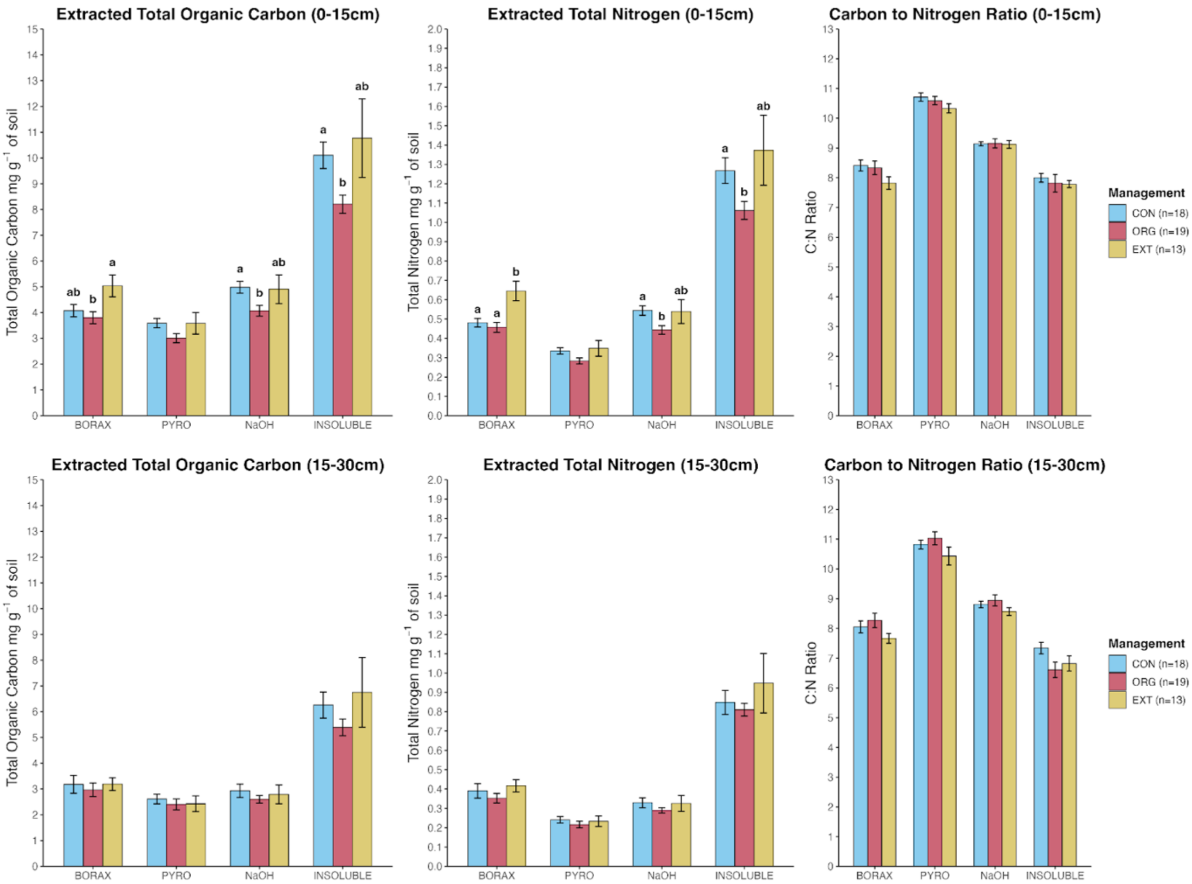

Figure 2-3 Soil chemical fractionation shown at both depths 0-15cm and 15-30cm. Multiple comparison of means significant differences are shown through different letters. Absence of letters indicates no significant differences were seen.

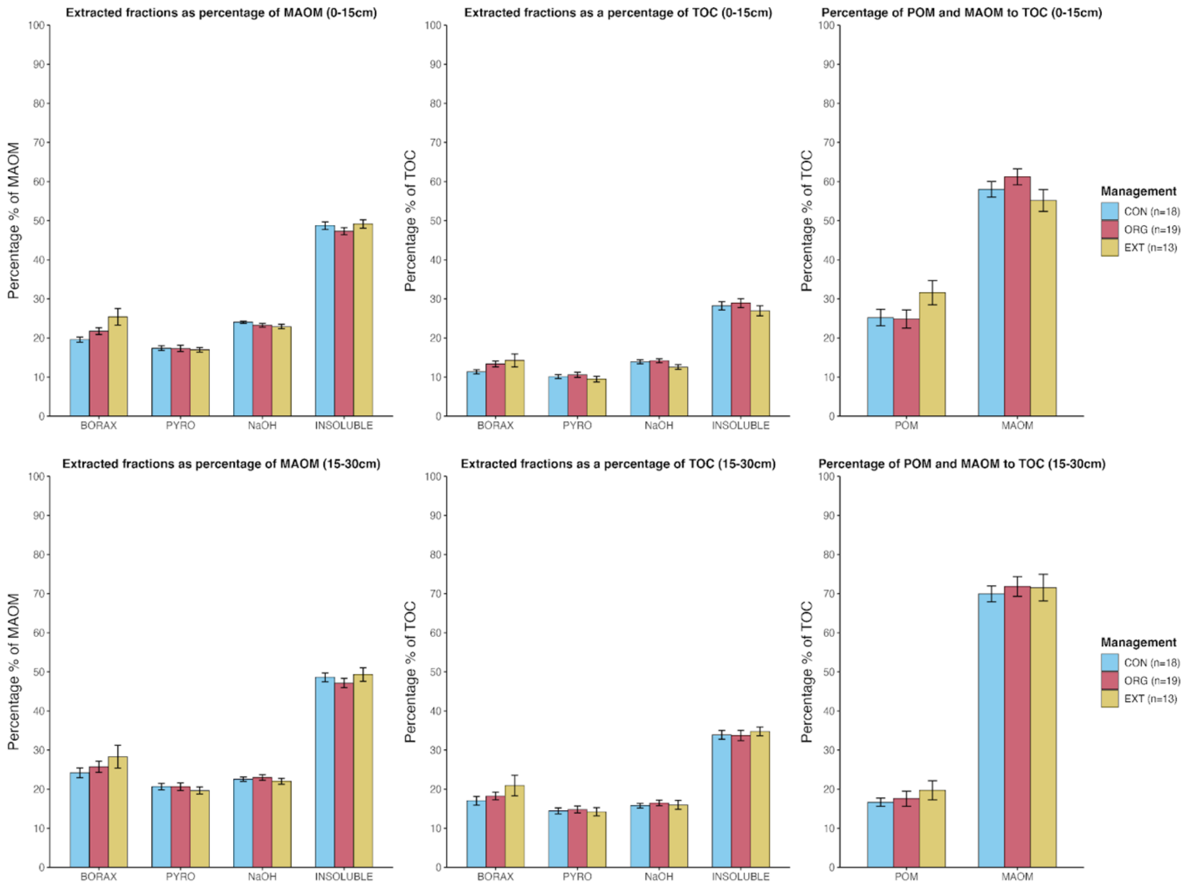

Figure 2-4 Soil chemical fractionation shown as percentages of mineral associated organic matter (MOAM) and total organic carbon (TOC) of bulk soil. Also shown are the physical fractions, particulate organic matter (POM) and MAOM, as percentages of TOC of bulk soil at both depths 0-15cm and 15-30cm. Multiple comparison of means significant differences are shown through different letters. Absence of letters indicates no significant differences were seen.

2.3.3. Soil biological parameters

2.3.3.1. qPCR results

Nitrogen cycling gene abundance across systems was evaluated with qPCR. There was significantly increased nitrifying AOA ($p<0.001$) gene copy numbers in CON systems (mean=949, range 275-2477) compared to ORG (mean=392, range 84-1260) and EXT (mean=325, range 41-1019) systems, who were not different to each other, as shown in Figure 2-5. AOB gene copy numbers were also significantly higher ($p<0.01$, $p<0.001$) in CON systems (mean=742, range 81-1780) compared to ORG systems (mean=312, range 108-825) and EXT systems (mean=162, range 69-231) with ORG and EXT being significantly different ($p<0.05$) to each other also. For COMMAMOX gene copy numbers, significant differences ($p<0.01$) were seen between CON (mean 498, range 12-1236) and EXT systems (mean=284, range 55-686) with ORG systems not being different to either (mean=379, range169-842). The same differences were also seen in the 15-30cm layer for the nitrifying genes with AOB being the only exception where ORG and EXT were not significantly different at depth. There was also a significant increase in denitrification genes *nirS* ($p<0.01$) and *nirK* ($p<0.05$) between CON and EXT systems but ORG systems were not significantly different to either. In the 0-15cm layer *nirS* was more abundant in CON systems (mean=2044, range 711-4480) than EXT systems (mean=1104, range 445-1799) but in the 15-30cm *nirK* was more abundant in EXT systems (mean=2911, range 973-5067) compared to CON systems (mean=1872, range 258-4342). Lastly there were no differences in abundance of *ITS* fungal genes in the 0-15cm layer between the systems but at 15-30cm there was a significant increase ($p<0.01$) in EXT systems (mean=12610, range 7650-20448) compared to CON systems (mean=7527, range 4070-13395), with ORG systems (mean=9740, range 4256-18671) not significantly different to either.

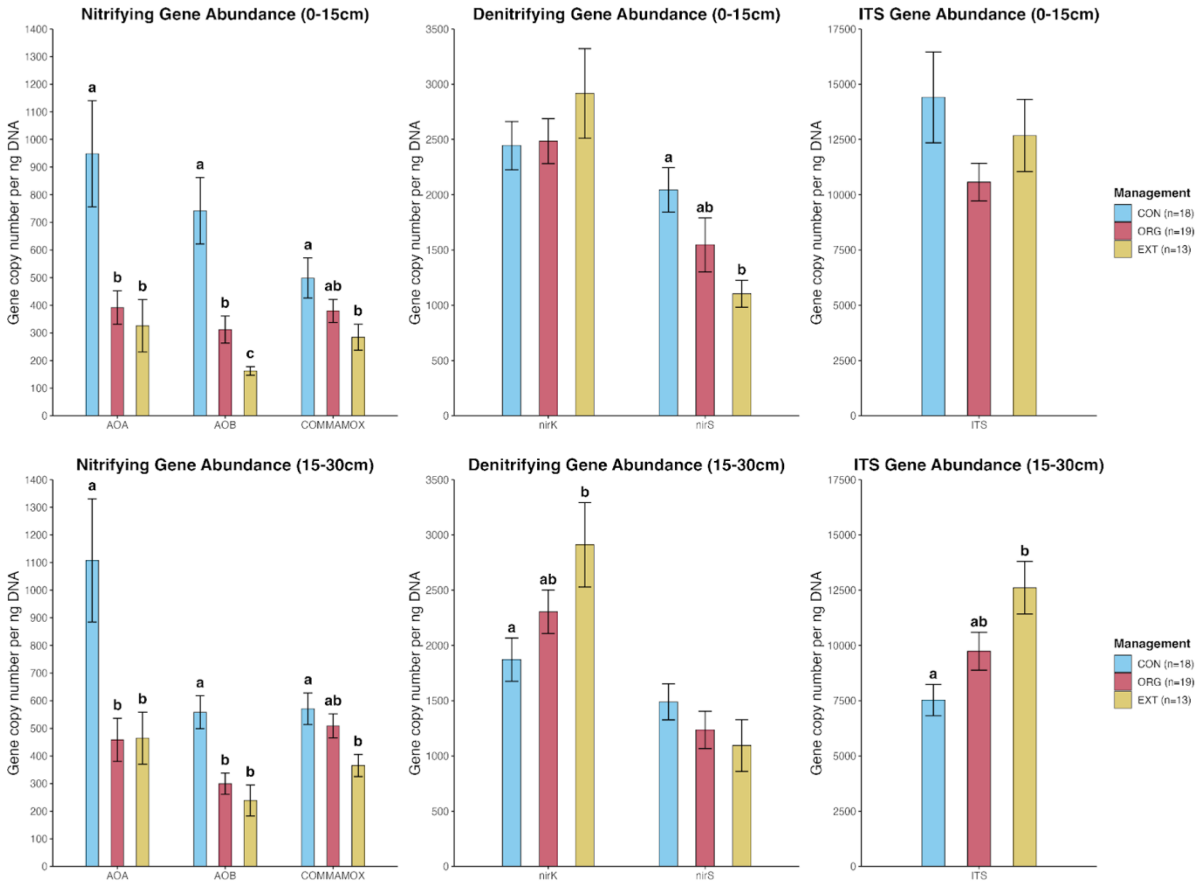

Figure 2-5 qPCR gene abundances. Multiple comparison of means significant differences are shown through different letters.

2.3.3.2. Alpha diversity

After cleaning of amplicon sequence variants (ASVs) there were a total of 22162 ASVs for *16S rRNA* and for *ITS* there were 11938 ASVs identified to domain/kingdom level. There were significant differences in alpha diversity between managements with CON having significantly higher Shannon diversity scores than EXT for *16S rRNA* ($p<0.05$) and ORG having significantly higher Shannon diversity scores than EXT for *ITS* ($p<0.01$) in the 0-15cm layer (Figure 2-6). There were no significant differences in the 15-30cm layer. There were no significant differences in Chao1 diversity between managements for *16S rRNA* or *ITS* in both the 0-15cm and 15-30cm layers (data not shown).

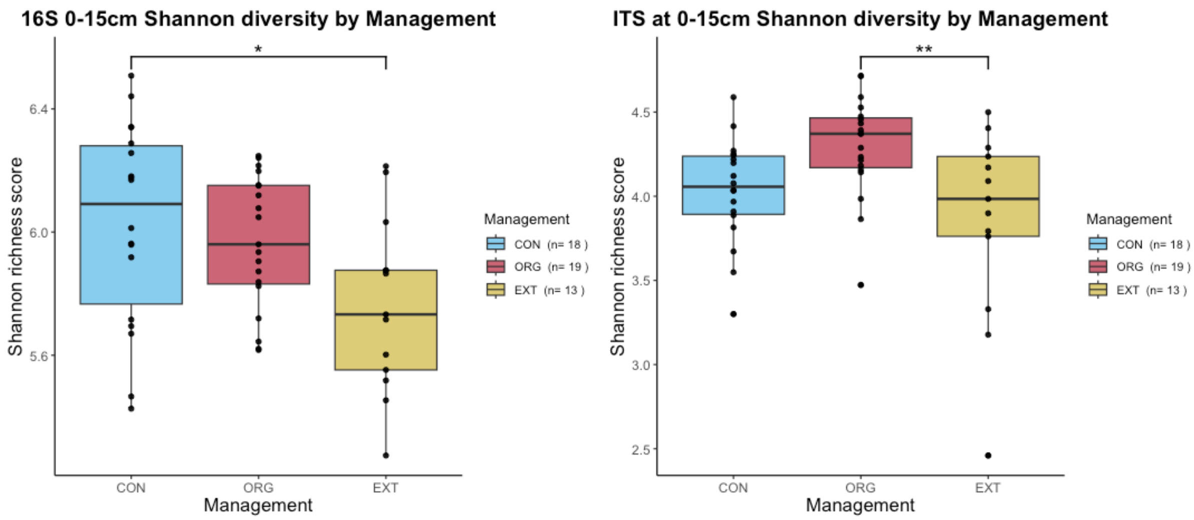

Figure 2-6 Alpha diversity between the 3 management systems for *16S rRNA* and *ITS* at 0-15cm. Significance code:'*' p≤0.001, '**' p≤0.01, '*' p≤0.05, ' ' p>0.05.**

2.3.3.3. Community structure beta diversity

Looking at the microbial community structure perMANOVA results show that the community composition was significantly different between the management systems at both 0-15cm and 15-30cm for *16S rRNA* ($p>0.001$) (Table 2-4). For *ITS,* Management was not significant due to dispersion. After plotting of the environmental variables it was seen that pH, Morgans P and Morgans K were among the main drivers for both *16S rRNA* and *ITS* at both depths, as seen in Figure 2-7 and Figure 2-8. Given that there exists agronomic Index levels for Morgans P and K as well as recommended pH values, sites were then grouped based on these under the name fertility status. Morgans P and K were categorised into 4 index levels based on the Irish Soil Index system (Plunkett, Murphy and Wall, 2021) Index 3 or higher (Morgans P >5mg L^{-1})(Morgans K >100mg L^{-1}) are considered agronomically optimal and lower than these are considered deficient. Small margins of error were factored in for Morgans values resulting in values of >4.9mg L^{-1} Morgans P and >90mg L^{-1} Morgans K being used instead as these values as there can be large variation between subsamples (Gallagher and Herlihy, 1963). A pH threshold of <5.7 was chosen as a cut off between deficient and optimal based on several factors, mainly due to its effect on microbial metabolism (Jin and Kirk, 2018) but also due to nutrient tie up, particularly phosphorus (Corbett *et al.*, 2021). Sites were grouped based on these with optimal not being deficient in any of the categories (pH, P or K), deficient was deficient in one or two but not all three categories and being deficient in all three categories simultaneously being considered severely deficient (Table 2-3). These groupings matched up with management groups having similar F values, p values, similar r^2 values and were significant for *ITS* at both depths ($p<0.001$) and *16S rRNA* in the 15-30cm layer ($p<0.001$) (Table 2-4).

Table 2-3 Fertility status groupings based on Morgans P and K values as well as pH.

Category	**pH**	**Morgans P**	**Morgans K**
Optimal	>5.7	>4.9 mg L^{-1}	>90 mg L^{-1}
Deficient	>5.7	>4.9 mg L^{-1}	<90 mg L^{-1}
Deficient	>5.7	<4.9 mg L^{-1}	>90 mg L^{-1}
Deficient	<5.7	<4.9 mg L^{-1}	>90 mg L^{-1}
Deficient	<5.7	<4.9 mg L^{-1}	<90 mg L^{-1}
Severe deficiency	<5.7	<4.9 mg L^{-1}	<90 mg L^{-1}

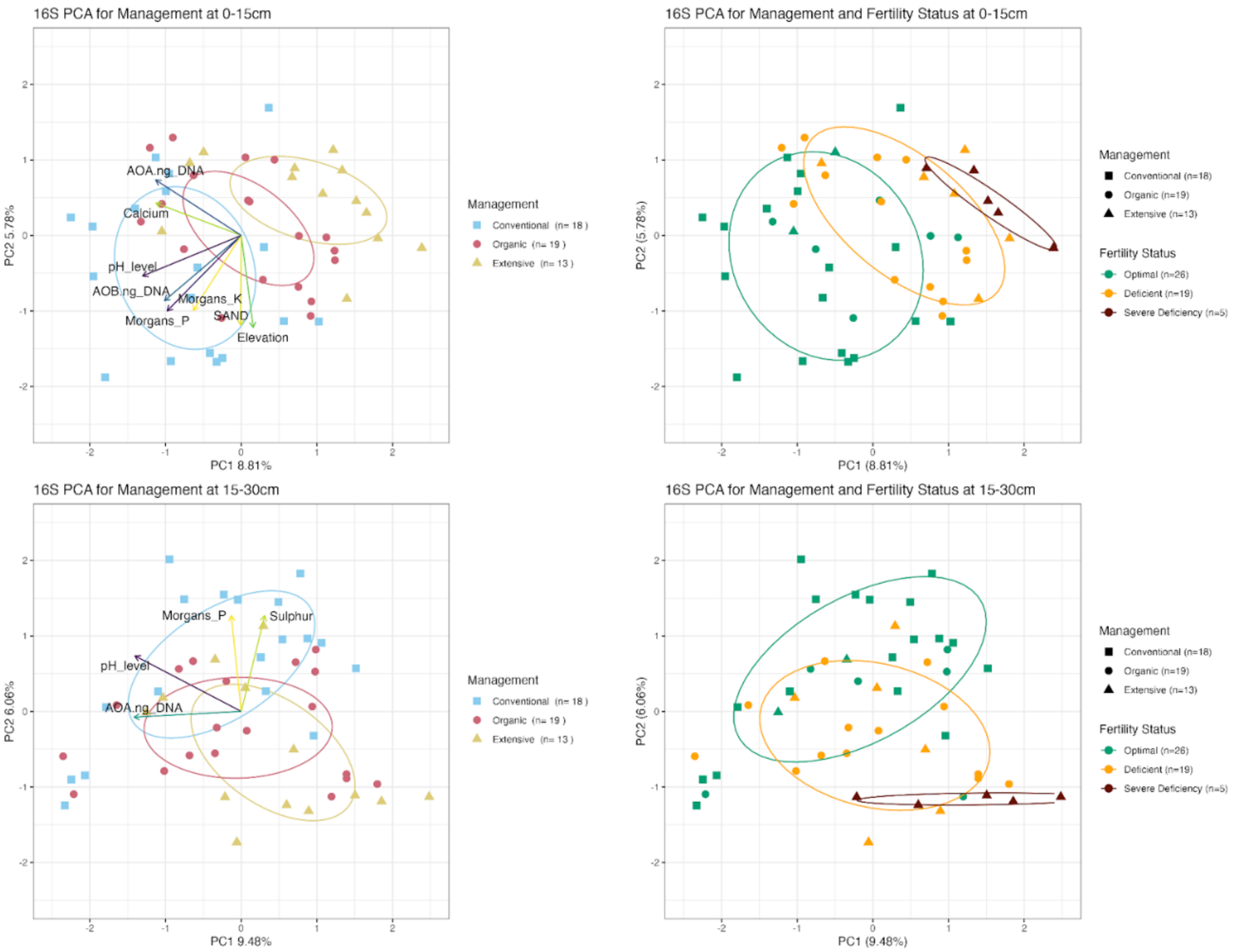

Figure 2-7 PCA plot showing clr transformed sequencing data beta diversity for *16S rRNA* at 0-15cm and 15-30cm including environmental variables plotted using scaling k=2. Also shown is the fertility status grouping.

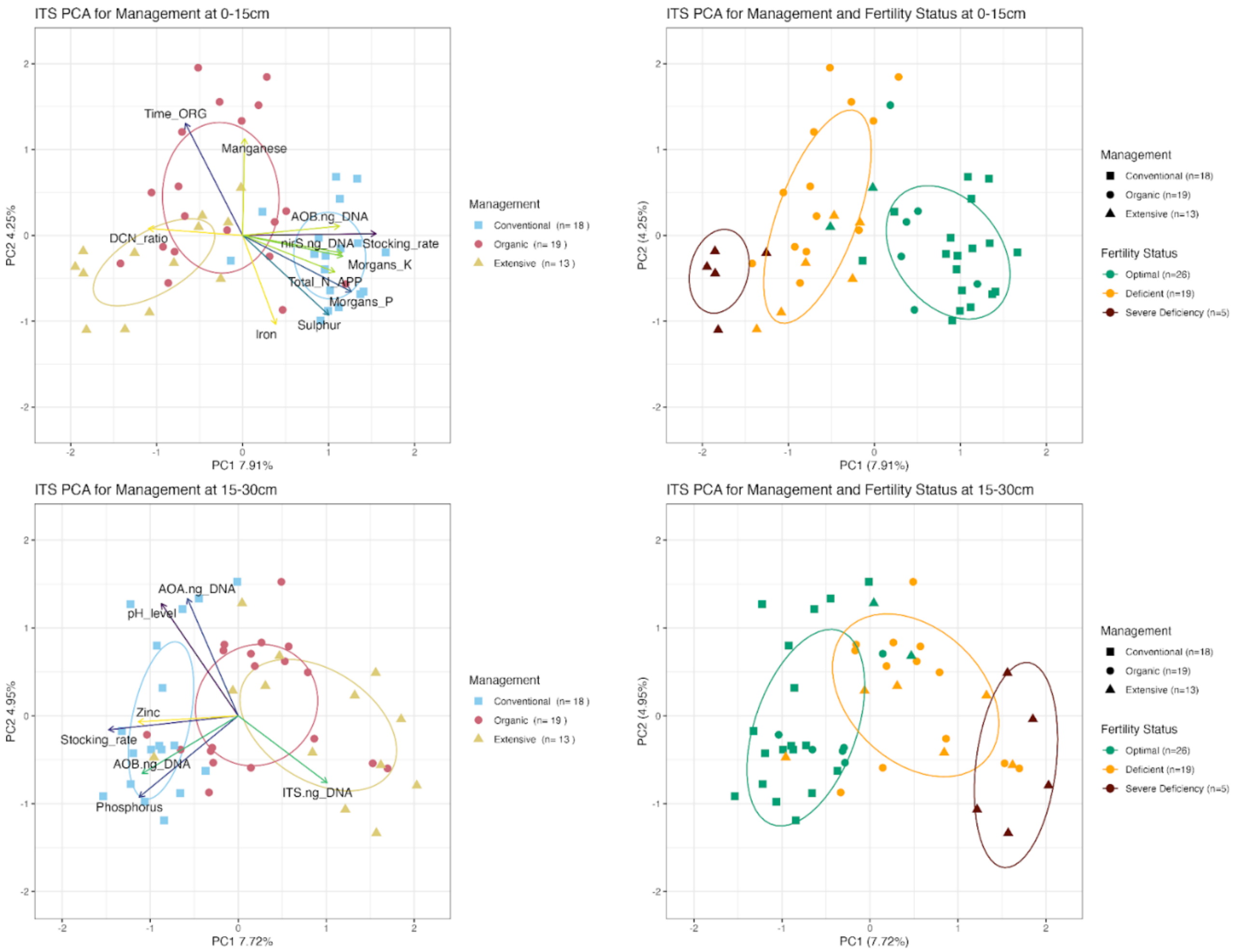

Figure 2-8 PCA plot showing clr transformed sequencing data beta diversity for *ITS* at 0-15cm and 15-30cm including environmental variables plotted using scaling k=2. Also shown is the fertility status grouping.

Table 2-4 PerMANOVA results for *16S rRNA* and *ITS* communities at 0-15cm and 15-30cm for management and fertility status groupings. Significance code:'*' p≤0.001, '**' p≤0.01, '*' p≤0.05, 'ns' p>0.05. Multiple comparison of means significant differences are shown through different letters. Underlined values failed the dispersion test (p<0.05) and thus aren't reliable p values.**

	Depth	*Group*	*R2*	*F value*	*Pr (>F)*
16S rRNA	*0-15cm*	*Management*	*0.07106*	*1.7976*	*0.001****
		Fertility status	*0.07217*	*1.8279*	*0.001****
	15-30cm	*Management*	*0.05667*	*1.4117*	*0.003***
		Fertility status	*0.06301*	*1.5803*	*0.001****
ITS	*0-15cm*	*Management*	*0.08941*	*2.3074*	*0.001****
		Fertility status	*0.08945*	*2.3086*	*0.001****
	15-30cm	*Management*	*0.07826*	*1.9953*	*0.001****
		Fertility status	*0.08197*	*2.0984*	*0.001****

2.3.3.4. Differential abundance analysis

A differential abundance analysis was carried out to determine which ASVs were significant ($p<0.05$ or effect size >1 or <-1) between management groups. For *16S rRNA* only 24 ASVs were significant in the 0-15cm layer (Figure 2-9) and only 2 ASVs were significant in the 15-30cm layer (Figure 2-10). For the 0-15cm layer there was no difference between EXT and ORG for any ASV. The main differences were between the EXT and CON sites, however there were 5 ASVs that were different between CON and ORG and these ASVs were also significantly different between CON and EXT (Figure 2-9). *Xanthobactereaceae,* a family of nitrogen fixing bacteria associated with legumes with some species being able to degrade toxic compounds (Oren, 2014), had 3 ASVs that were different between managements. Two of these ASVs increased in relative abundance in CON systems relative to EXT systems, and one increased in EXT and ORG systems relative to CON systems (Figure 2-9). *Gaiellaceae*, a family of bacteria that can reduce nitrate (Albuquerque and da Costa, 2014) were found to be more abundant in CON systems for 2 ASV and less abundant than both ORG and EXT for 1 ASV (Figure 2-9). *Nitrospiraceae,* a family of nitrite oxidizing bacteria, were significantly less abundant in CON systems compared to both ORG and EXT systems (Figure 2-9). *Hyphomicrobiaceae,* is a family of aerobic chemoheterotrophic bacteria that can reduce nitrate, with some species that can even photosynthesise. The genus *Pedomicrobium,* in this family, accumulates oxidized iron and manganese and these were more abundant in CON systems than EXT systems with ORG being not different to either (Figure 2-9). *Nitrososphaeraceae,* a family of ammonia oxidizing archaea which can also fix atmospheric CO_2, were more abundant in EXT and ORG systems than CON systems (Figure 2-9). This family also can use nitrifier denitrification pathways producing nitrous oxide using *nirK* genes (Stieglmeier, et al., 2014a). Most other ASVs that were significant were either novel with no known function, were generalist organic matter degraders or were not classified to family level. At 15-30cm the only significant ASVs were of the family *Nocardiaceae* which is a generalist involved in organic matter turnover and the family *Nitrosomondaceae* which are an ammonia oxidizing bacteria that also use *nirK* genes (Prosser, Head and Stein, 2014) Both of these genes were higher in the EXT systems than CON systems and *Nocardiaceae* was also more abundant in ORG systems than CON systems (Figure 2-10).

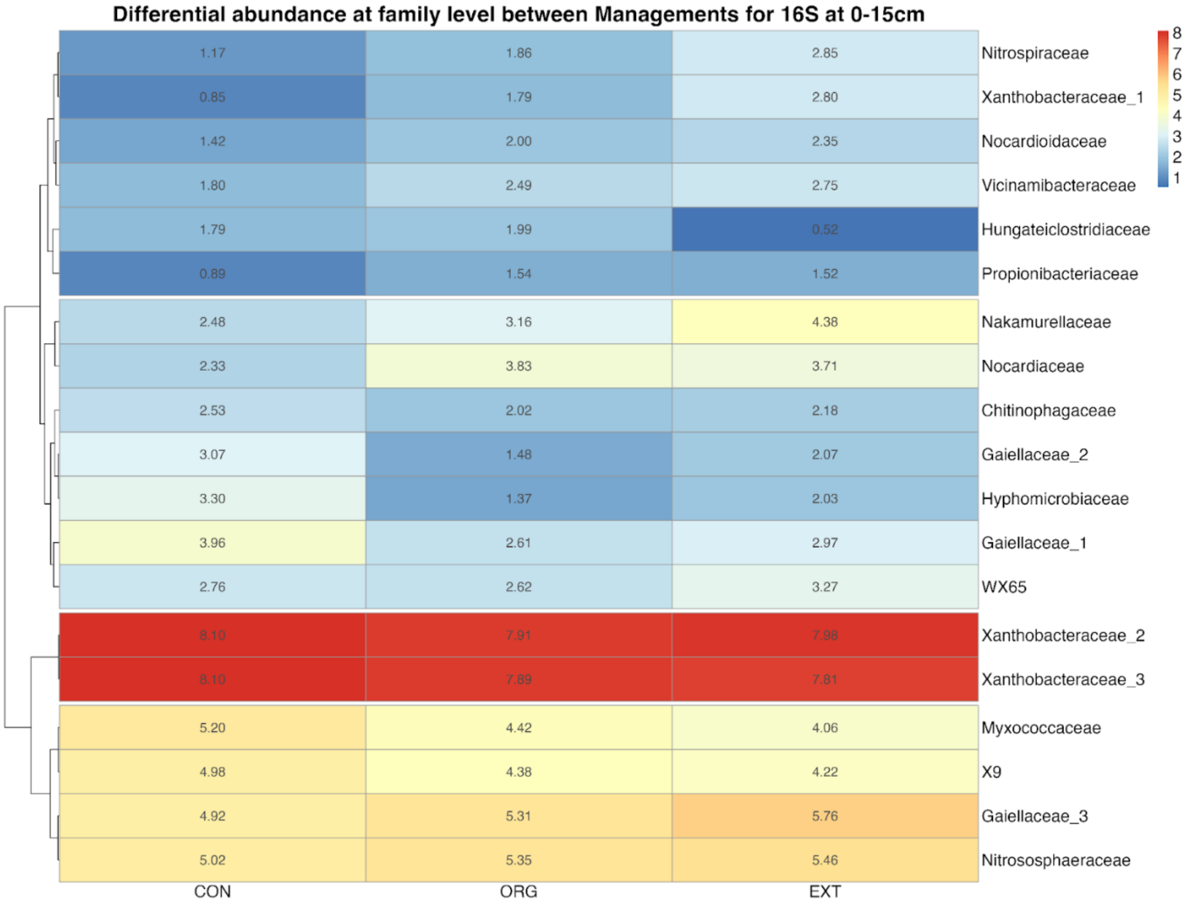

Figure 2-9 Differential abundance (ALDEx2) results showing the significant ASVs at family level (p<0.05 and effect size >1 or <-1) for *16S rRNA* at 0-15cm.

Figure 2-10 Differential abundance (ALDEx2) results showing the significant ASVs at family level (p<0.05 and effect size >1 or <-1) for *16S rRNA* at 15-30cm.

For *ITS* there were 40 significant ASVs in the 0-15cm layer with 13 ASVs that were unclassified at family level, 10 of these were unclassified at order level and 4 were unclassified all the way to phylum level (Figure 2-11). Most ASVs that were differentially abundant were saprophytes. The majority of ASVs were different between CON and EXT systems but ORG and CON also had significantly different ASVs. There was a single ASV that was different between ORG and EXT, *Tylosporaceae*, which are a family of ectomycorrhizal fungi (Figure 2-11). Another ASV of the same family was also identified as being differentially abundant between CON and both ORG and EXT systems. Four ASVs of the order *Hypocreales* were identified, two were unidentified at family level, one was identified to family level and one was identified to genus level (Figure 2-11). The family *Hypocreaceae* can include plant pathogens as well as mutualistic endophytes. The ASV identified to genus level was *Fusarium,* a well-known pathogen of plants but depending on species can also be a harmless saprobe in soils. The family *Pleosporales* was differentially abundant between both EXT and CON, and ORG and CON (Figure 2-11). This fungal family contains necrotrophic pathogens of grasses as well as human pathogens and pathogens of many other agricultural crops. Only 4 ASVs were increased in abundance in CON systems, one in the family *Clavariaceae,* and one in the family *Chytridiaceae* with two other unidentified ASVs. The 2 identified ASVs were saprobes of organic matter (Figure 2-11). At the 15-30 cm layer similar trends were seen where CON only had 4 ASVs that were more abundant than EXT or ORG, while ORG and EXT were more different at this level than at 0-15cm (Figure 2-12).

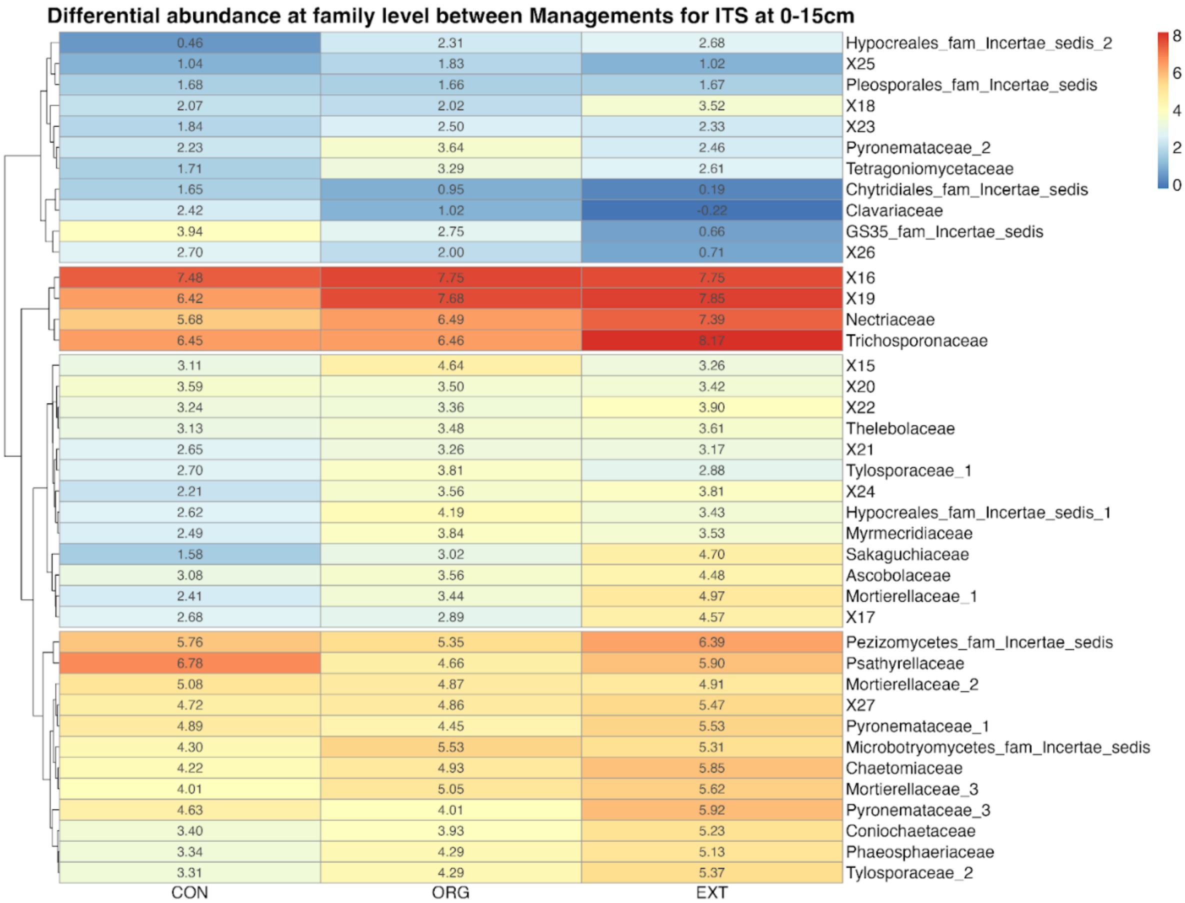

Figure 2-11 Differential abundance (ALDEx2) results showing the significant ASVs at family level (p<0.05 and effect size >1 or <-1) for *ITS* at 0-15cm.

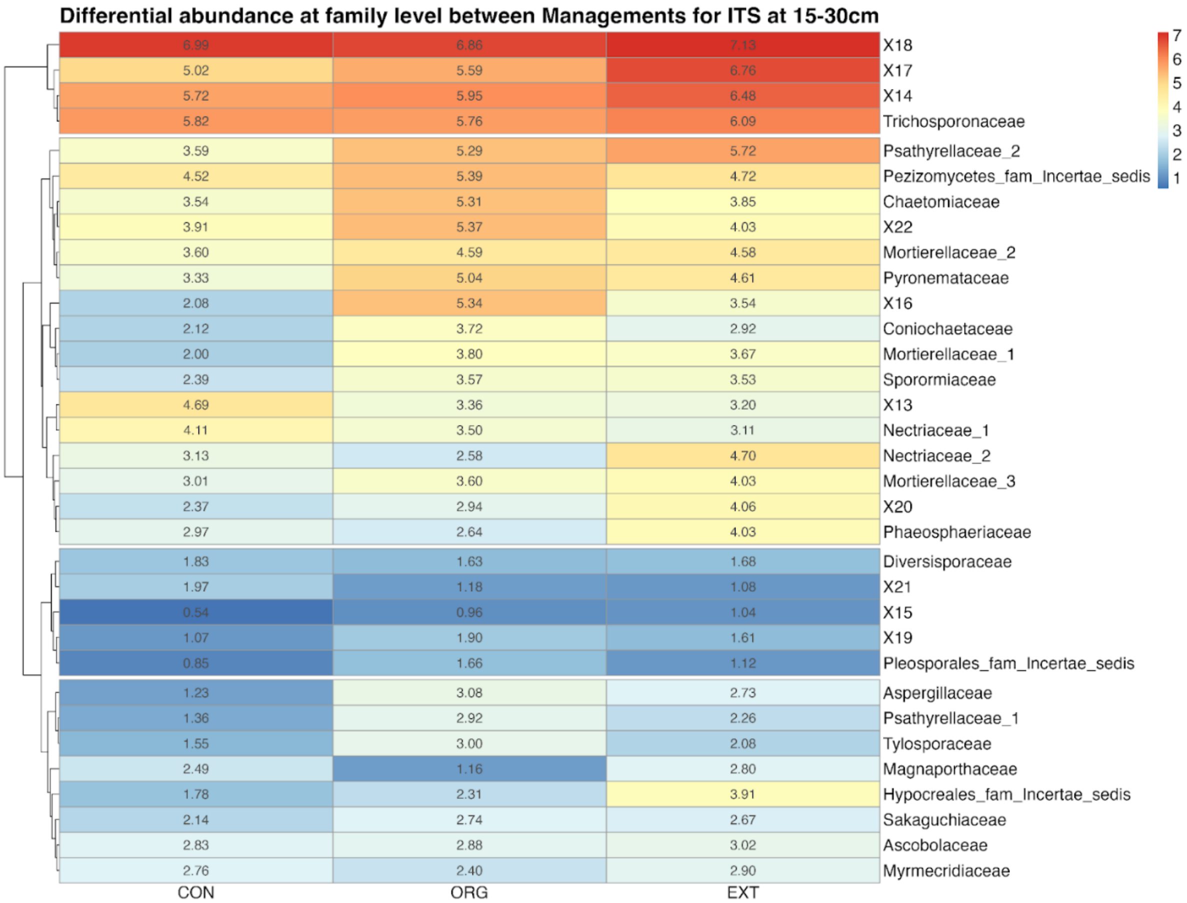

Figure 2-12 Differential abundance (ALDEx2) results showing the significant ASVs at family level (p<0.05 and effect size >1 or <-1) for *16S rRNA* at 15-30cm.

2.3.3.5. Sward diversity

Plant functional communities differed significantly between management systems with ORG and EXT being significantly ($p<0.001$) higher in Forbs than CON sites as shown in Table 2-5. CON sites also had significantly ($p<0.05$) more bare soil exposed than both ORG and EXT sites and although not significant there was a trend towards higher legume cover in ORG systems.

Table 2-5 The mean percentage cover of sward functional groups for each system (mean ±SE), conventional intensively managed grassland (CON), organically managed grassland (ORG) and extensively managed grassland (EXT). Shown are the transformed percentage cover of grass, legumes, forbs, bare soil, litter and dung pats. Significance code:'*' p≤0.001, '**' p≤0.01, '*' p≤0.05, 'ns' p>0.05. Multiple comparison of means significant differences are shown through different letters. Underlined values are chi-squared values from a non-parametric (Kruskal-Wallis) test.**

Variable	F value	CON	ORG	EXT
Grass %	0.669^{ns}	75.9 (±4.36)	70.5 (±3.43)	70.6 (±3.38)
Legume %	2.633^{ns}	7.9 (±2.56)	13.4 (±1.92)	6.9 (±1.76)
Forb %	10.056^{***}	3.3 (±1.16)^{a}	12.7 (±2.76)^{b}	18.7 (±4.12)^{b}
Bare Soil %	4.659^{*}	11.1 (±2.88)^{a}	2.0 (±1.07)^{b}	1.9 (±1.11)^{b}
Litter %	<u>0.3124</u>^{ns}	0.39 (±0.164)	1.00 (±0.653)	1.77 (±1.160)
Dung Pats %	2.952^{ns}	1.17 (±0.487)	0.16 (±0.086)	0.31 (±0.237)

2.3.3.6. Sward yield

Approximate Dry Matter (DM) yield was determined from a detailed farmer management survey. DM yield was calculated based on animal DM requirements over the year using previously calculated figures for Irish farms (O'Mara, 1996; O'Brien, Moran and Shalloo, 2018). Values for dairy cows were then adjusted based on new dairy banding rates based on milk yields (European Union, 2022). Values for different animals are given in supplementary materials Table A-3. The resulting difference was then divided by the hectares farmed to give average DM yield per hectare over the whole farm. An average standard DM equivalent in grass was assumed for all purchased concentrates where 1kg of concentrate feed equals 1.04kgs DM grass. These results, as shown in Figure 2-13, show that between CON and ORG systems the yield difference is 29%, which is similar to what was seen in other studies (Klaus V.H. et al., 2013; Knapp et al., 2023). Furthermore, EXT systems had much lower yields than both CON and ORG systems.

$$\Bigg(\left(Average\ feeding\ value\ for\ animals\ \left(kgs\frac{DM\ grass}{year}\right)\right.$$
$$\left.\times\left(\frac{Banding\ rate\ \left(kgs\ Org\,\frac{N}{ha}\right)^{*}}{Previous\ dairy\ cow\ stocking\ rate\ \left(kgs\ Org\,\frac{N}{ha}\right)^{**}}\right)^{***}\right)\times(Livestock\ numbers)$$
$$-\Bigg(Amount\ of\ purchased\ feed\ (kgs)$$
$$\times\ average\ equivilant\ DM\ grass\ for\ purchased\ feed\ type\ \left(kgs\frac{DM}{kg}\right)\Bigg)\Bigg)$$

Equation 2-1 showing how DM yield was calculated from information gathered in the survey. * Dairy Banding Rates: Band 1 <4500kg Milk Yield = 80kg N/cow/year, Band 2 4501-6500kg Milk Yield = 92kg N/cow/year, Band 3 >6500kg Milk yield = 106kg N /cow/year. ** Previous value for all dairy cows = 89kgs N/cow/year. * Where applicable, only applies to dairy cows.**

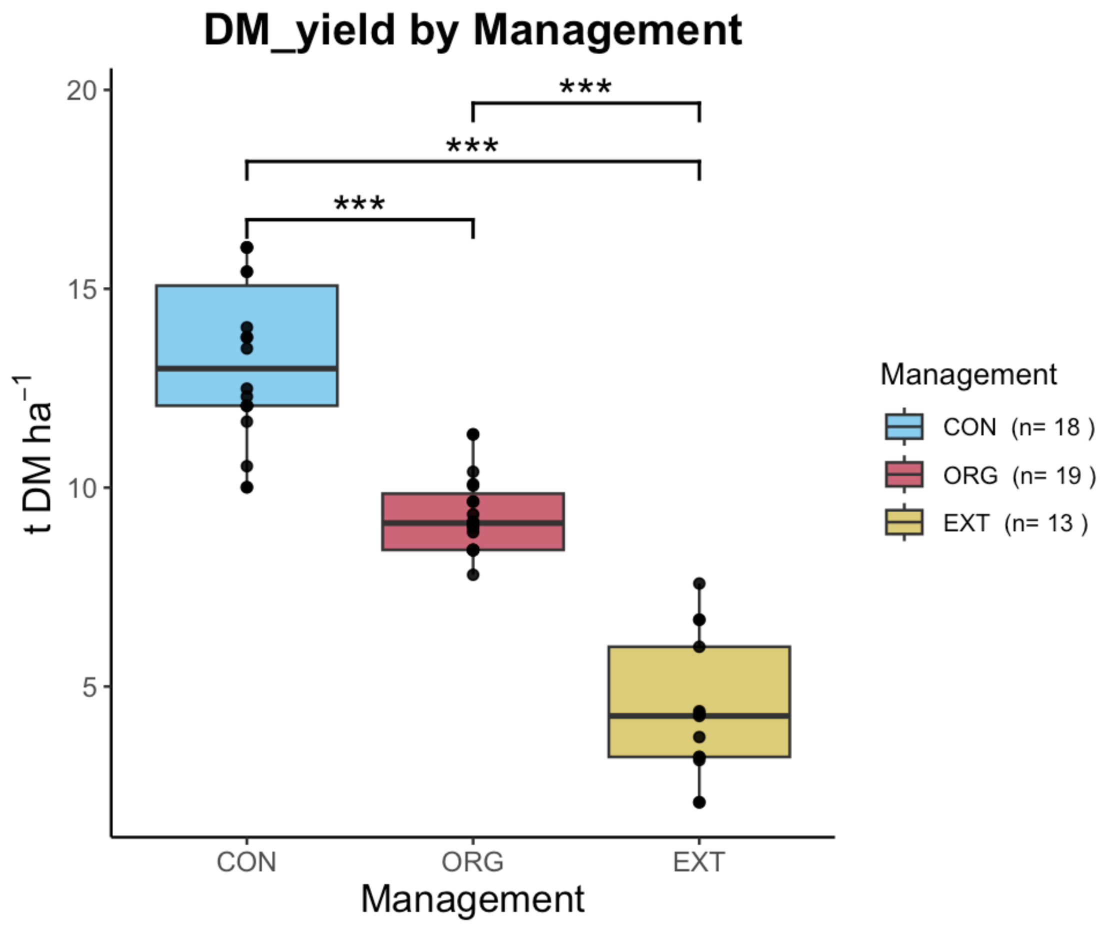

Figure 2-13 Mean biomass yields in t/ha of DM for each system. Significance code:'*' p≤0.001, '**' p≤0.01, '*' p≤0.05, ' ' p>0.05.**

2.4. Discussion

2.4.1. Physical indicators of soil health

The lack of differences in physical health between farming systems leads us to reject the first part of our first hypothesis that the increased machinery traffic and grazing density of CON systems would have some detrimental effect on bulk density and as a result water holding capacity and soil porosity. However Bondi et al., (2021) showed increased trafficking intensity in grasslands was less likely to cause compaction and structural issues on well drained soils, which this study was limited to. Using only 3 great soil groups (Luvisol, Cambisols and Umbrisols) whose defining characteristics are associated with deeper soil layers (>40cm) meant that physical properties in the topsoil (0-30cm) were similar.

2.4.2. Chemical indicators of soil health

The differences in soil chemistry between the management systems may be partially an effect of pH with the use of lime to increase pH. This practice has become more common in recent years due to its beneficial effect in reducing N_2O emissions from fertilisers and the push to lower agricultural greenhouse gas emissions (Žurovec, et al., 2021). Lime also has numerous other benefits such as releasing tied up nutrients and providing calcium (Corbett *et al.*, 2021). While ORG farms can and do also use lime they tended to apply it less often. EXT farms were a mix of farms who applied it regularly, those that only applied it when yield started to drop off and those that never applied lime meaning there was much more variability within this system. The main reason for this is that ORG and EXT farms tend to only get soils tested periodically whereas CON farms must follow a liming programme as part of new derogation requirements (European Union, 2022). Soil pH increased with depth for all systems due to a combination of leaching of cations and soil parent material.

All CON farms were in the Index 3 or 4 range for soil available phosphorus (>5mg L-1 Morgans) according to the Irish Soil Index system and were therefore restricted from applying P fertiliser to only maintenance applications. Most of this P therefore is coming from manure applications and is cycled through the system (Figure A-4). ORG farms can apply certain P containing fertilisers and the most commonly used was dairy sludge. Dairy sludge is a high P organic fertiliser that is approved by certifying bodies as long as it comes with a nutrient content report (Irish Organic Association, 2022). Research has shown that this is a suitable alternative to chemical P fertilisers for increasing soil Morgans P levels in grasslands (Tawiah Croffie *et al.*, 2022) and this can be seen in the results where ORG farms had a mean value in the Index 3 range for Morgans P. EXT farms also used dairy sludge but in general tended to limit external inputs to lower costs. Increased P at depth may be indicating that available P is leaching downward in CON systems into lower soil layers due to historical over application and for ORG this increased level compared to EXT systems at depth may be due to frequent inversion ploughing, required in these systems to maintain production, mixing P into the lower layers.

Some inorganic K fertilisers were used in CON and EXT systems to increase soil available potassium. However organic certifying bodies also allow the use of some inorganic K fertilisers (sulphate of potash, K_2SO_4) if soil tests show a deficiency. These fertilisers however tend to be more expensive and can only be applied to reach optimal levels (Index 3 in this case) preventing over application. Therefore most of the K in ORG systems is coming from organic manures (Figure A-5). As livestock numbers increase, more manure is cycled through the systems leading to higher soil K levels and in EXT systems the decreased animal numbers leads to decreased available K levels.

Magnesium is often applied in CON systems as dolomitic lime to pastures to combat grass tetany which is an overdose of potassium in fresh spring grass(Martens, 2016). This tends to be less of a problem in ORG and EXT systems who are applying much less potassium fertilisers. There are also some fertiliser blends that include it as it is an important nutrient for photosynthesis and helps combat acidity if in the oxide form. Leaching of cations such as magnesium tends to occur causing them to accumulate at depth. Sulphur is becoming increasingly common as an additive in inorganic nitrogen fertilisers used in CON systems as it increases nitrogen use efficiency (Brown *et al.*, 2000). Sulphur is also prone to leaching, especially on sandier soils, leading to increasing levels at depth (Table 2-1). Copper and zinc are important micronutrients for ruminant health (Hill and Shannon, 2019) and are often added to concentrate blends and used in mineral licks which are frequently used in CON systems to maintain productivity and animal health (O'Donovan *et al.*, 2022). These micronutrients end up being cycled through manure into the soil. They are also increasingly added to some fertiliser blends as copper is involved in photosynthesis and zinc is primarily involved in enzymatic activity and protein synthesis in plants (Marschner, 2012c). The increase in iron levels in CON systems at depth may be a result of increasing pH at depth which may trigger plants to exudate compounds that extract iron from the soil into the rhizosphere (Colombo *et al.*, 2014). This increase in available iron is possibly related to nitrate and nitrite reductase as both require iron in enzymes and cofactors (Marschner, 2012c) Another possible cause of this is leaching of iron from the upper layers into the subsoil known as podzolization. The use of chemical fertilisers in CON systems are known to be acidifying and can be a contributing factor to leaching of nutrients such as iron and aluminium (Haynes and Swift, 1986).

Decreased soil carbon stocks at 0-15cm in ORG systems may be due to the use of the plough in these systems to maintain production as they often are mixed enterprises where they produce tillage crops as well as dairy or beef cattle. Therefore a majority of grasslands in ORG systems are part of a tillage rotation. However this rotation is usually 5-6 years grass with 1-2 years crops, depending on the farm, meaning that these grasslands are not leys as the main system is still grassland production. This system is used because organically certified concentrate feeds are expensive to purchase and on farm production is economically viable. This periodic use of the plough may be partly behind the lower levels of carbon in the soil (Linsler *et al.*, 2013). Another factor contributing to declining carbon stocks may be increased levels of legumes in swards in ORG systems as once legume concentration reaches above a certain level in the sward it can actually have a detrimental effect on soil organic carbon (SOC) levels (Rodríguez, Canals and Sebastià, 2022). This difference however was not seen when looking at the entire soil profile (0-30cm) meaning that this decrease may not be a long term effect and may be related to the shorter length of time in pasture (Lin *et al.*, 2020). CON systems showed possible signs of increased mineralisation of organic nitrogen within the soil, as seen by the reduced dissolved carbon to nitrogen ratio (Table 2-2). This could indicate potential increase in nitrate leaching (Yang *et al.*, 2023) and potential increases in denitrifying activity as microbes consume labile DOC to reduce nitrate and nitrite (Saggar *et al.*, 2013).

Borax extracts relatively labile carbon and nitrogen from the mineral associated organic matter (MAOM) fraction. The increase in plant species richness in EXT grasslands may contribute to this increased borax fraction (Lange et al., 2015). While in CON systems this increase in the borax fraction may be from exudation due to increased biomass levels and therefore increased photosynthesis (Eze, Palmer and Chapman, 2018). These differences are related to the differences in the TOC levels between the systems. In EXT systems the higher SOC levels, although not significant, in the particulate organic matter (POM) fraction shows that the overall higher SOC levels in EXT systems are possibly related to the slower decay of plant litter and detritus due to the lower levels of grazing and nitrogen applications. In CON and ORG systems the higher intensity of grazing and fertilisation with organic manures means this carbon is cycled through animals digestive systems leading to quicker assimilation into the more stable MAOM fraction.

These results show that the first hypothesis can be rejected, that the physical health would be reduced and chemical health of soils would be decreased in CON systems. Soil physical properties were not different between the systems and that there were increased available nutrient levels in CON systems compared to both ORG and EXT systems. While there may have been imbalances in soil chemistry in CON systems overall they had higher amounts of most nutrients, more optimum pH levels, increased soil carbon stocks and had similar proportions of stable long term carbon to ORG and EXT systems. However there is evidence of increased susceptibility to leaching of nutrients with excessively high levels of available P in both layers, decreased C:N ratios in the dissolved fraction meaning mineralisation of soil N was occurring and the need to supplement nutrients to prevent deficiencies due to over supplying other nutrients.

2.4.3. Biological indicators of soil health

The increased nitrification potential within the soil is linked to higher fertiliser inputs from both organic manures and chemical nitrogen (Raglin *et al.*, 2022) and AOA and AOB abundance has been linked to increased N_2O emissions and NO_3^- leaching in soils (You *et al.*, 2022; Liu *et al.*, 2025). Denitrification was also affected by management, however these results were unusual in that increased *nirS* levels are usually seen in wet, low oxygen environments. The increase in *nirS* is most likely related to application of higher amounts of organic manures in CON and ORG systems (Ju *et al.*, 2025). The increase in *nirK* may be related to the significantly higher abundance of *ITS* fungal genes, as shown in Figure 2-5, in the lower layer (15-30cm) of EXT systems as certain species of fungi have been shown to use the *nirK* copper nitrite reductase gene in soils (Maeda *et al.*, 2015). The higher *ITS* abundance in EXT systems is most likely related to pasture age and low N fertilisation, as most sites in this category were long term permanent pastures with low N inputs (de Vries *et al.*, 2007).

Soil α-diversity may be related to pH where more neutral pH as found in CON systems favours more diverse bacterial communities compared to more acidic conditions like those found in EXT systems (Kaiser *et al.*, 2016). For *ITS* diversity similar results were seen in Europe where the diversity of arbuscular mycorrhizal fungi (AMF) of organic tillage fields was similar or higher than those found in natural grassland sites (Ryan and Graham, 2018).

This is most likely related to the increased abundance of clover in the swards of ORG grasslands as it has a higher colonisation rate of fungi than grasses alone (Ryan, Small and Ash, 2000).

Soil β-diversity showed environmental variables like percentage sand and elevation were significantly affecting *16S rRNA* community structure meaning that bacteria are more affected by climate and environment as seen by Barreiro et al., (2022) than fungi, who were only significantly affected by variables related to management such as stocking rate, available P and the length of time that a site was organically certified. However almost all of the strongest drivers of community structure in *16S rRNA* and *ITS* communities were related to management such as pH, available P and K, abundance of nitrification genes, which are related to nitrogen inputs, and levels of other nutrients. This result shows that long term management and most significantly the input of nutrients has a direct effect on community structure in grassland soils.

Differential abundance analysis showed that most 16S rRNA prokaryote ASVs that were significantly different between managements were related to the nitrogen cycle in soils. The increase in Nitrosomondaceae abundance is most likely related to the increased nirK gene abundance, as shown in Figure 2-5, in the lower layer (15-30cm) of EXT systems (Prosser, Head and Stein, 2014) and may indicate that the nitrogen cycle is more interconnected in EXT systems preventing leaching of a nutrient that is limiting within the system. Increased abundance of Nitrospiraceae in EXT systems, who are a family of nitrite oxidizing bacteria and thought to be K-strategists, may be a sign of a stable and more connected nitrogen cycle since nitrite is only an intermediary molecule of the nitrogen cycle and hardly ever accumulates in natural ecosystems (Daims, 2014). An ASV in the family Nitrososphaeraceae was also increased in abundance in EXT and ORG systems compared to CON systems. Archaea in this family are highly efficient at fixing atmospheric CO2 as bicarbonate and some are more suited to low nutrient environments than other AOA (Stieglmeier, et al., 2014b). While this result appears contradictory to the qPCR results which showed more AOA genes in CON systems, further analysis of the abundance of archaeal ASVs in the sequencing data shows that archaea make up a much more significant percentage of the total ASVs in CON systems than ORG or EXT systems, which were not significantly different to each other (supplementary materials Figure A-6). So while this ASV was increased in EXT and ORG systems, archaeal ASVs were more abundant in CON systems overall but individual ASVs may not have been significant enough to be detected by the differential abundance analysis. Interestingly as seen with most other results ORG and EXT management showed no significant difference in abundance to each other indicating that intensity of management, (as most EXT farms were also organic) may not be causing the differences and it may in fact be related to inputs rather than the direct result of intensifying production i.e. producing more organic manure. The increased abundance of the genus Pedomicrobium, which accumulates oxidized iron and manganese, may also be a sign that CON soils are more oxidized than ORG or EXT soils possibly resulting in leaching of nutrients down through the soil profile (Oren and Xu, 2014). *ITS* differential abundance showed that the majority of ASVs were related to organic matter turnover. EXT and CON systems were the most different with ORG and CON having a large amount of differentially abundant ASVs also.

These results indicate that our second hypothesis can be accepted that the *16S rRNA* and *ITS* community diversity and structure were different between the systems, however the α-diversity of the systems was not as originally hypothesised, where EXT systems were actually the least diverse of the 3 systems.

2.4.4. Functions and trade-offs

2.4.4.1. Nutrient cycling

High soil organic matter levels within grassland ecosystems (n=50, mean=9.33±0.39) mean that most nutrients that are applied to the soil will stay within the soil matrix as they will be bound to the organic matter (Lal, 2020). This makes grassland soils' nutrient storage capacity relatively high. Although this may be the case, many soils were still deficient in various nutrients according to the Soil Index system used by Teagasc in Ireland as shown in Table A-7. This is in line with previous work done on Soil Fertility in Ireland which found only 17% of soils with optimum fertility (Nyhan *et al.*, 2025). Of the 50 sites sampled in this study 52% had optimum pH (6.2 to 7.0), 64% had optimum P levels (>5 mg L-1 Morgans P) and 62% had optimum K levels (>100 mg L-1 Morgans K). Overall only 32% of sites sampled had optimal levels of all three. CON sites were selected for their optimum fertility as this represents the most intensively managed fields and is the benchmark for agricultural productivity aka biomass production, although some of the CON sites were just marginally outside the optimum range for pH and K, the majority (67%) were optimum for all three.

The use of P fertilisers used in CON systems to achieve high P Index levels has many issues, such as water quality degradation, contamination of soils with heavy metals, as well as the issues around the production of phosphorus fertilisers (Ahmad *et al.*, 2023). CON farmers are also limited in the amount of fertiliser P they can apply, meaning that the majority of P in these systems is cycled through animal manures. However the use of organic manures to supply these nutrients also has its own issues such as losses to water and the atmosphere, accumulation of heavy metals, antibiotic resistance and declines in soil fauna due to antiparasitic compounds present in manure of treated animals (Köninger *et al.*, 2021). All systems used some form of these manures such as cattle slurry, farm yard manure or dairy processing sludge. However there are stricter regulations around the use of these in organic systems and issues such as antibiotic use, anti-parasitic drugs and metal content is strictly controlled by certifying bodies meaning there should be less of these in manures used on ORG and EXT farms (EU, 2022b). P fertilisers are also tightly controlled in organic production meaning they can only be applied if a deficiency (<5mg L-1 Morgans P) is shown on a soil test (Irish Organic Association, 2022). The amount applied is also limited meaning over application is also prevented. P levels are naturally low in Irish soils making fertilisation or cycling through animal manures required (Whitehead, 2000) and the decreased P levels in EXT systems is most likely related to P leaving the farm in animal produce, that is not being replaced sufficiently. Cycling of P in these systems is therefore reduced which is likely one cause of the decreased biomass production in these systems.

Heavy use of K fertilisers, in particular potassium chloride (KCl) which is the preferred source of K fertiliser in Ireland (Alexander *et al.*, 2020) means that soil K levels in CON systems are kept high as there is no limit on application rates of K. KCl is prohibited under organic production standards and potassium sulphate (K_2SO_4) is often used instead. However a recent soil test must show a deficiency (<100mg L-1 Morgans K) in order to justify applying it. This explains why ORG systems had sufficient K levels as they can purchase and apply it should they show deficiency and are prevented from over applying it. However it would seem that K levels are generally maintained by the application of organic manures as K_2SO_4 is expensive to apply. Thus K cycling in ORG systems is a result of nutrient cycling through animal manures and to some extent this is also true in CON systems as they also apply a lot of organic manure (Figure A-5). K cycling was also reduced in EXT systems with only 38% of sites being in Index 3 or higher. This again is related to nutrients leaving the farm in animal produce not being sufficiently replaced in these systems (McCarthy *et al.*, 2024).

CON systems imported a significant percentage of their animals feed requirements, as shown in Figure A-2, (14.94%) as concentrate feeds compared to ORG (3.57%) and EXT (1.54%), and this also imports a substantial amount of nutrients into the system (Gulati *et al.*, 2018; Magan *et al.*, 2021). A study of milk from conventional and organic dairy farms in north west Spain found higher levels of trace minerals in conventional milk as they were fed concentrate mixes with added minerals like zinc, copper, selenium and iodine (Rey-Crespo, Miranda and López-Alonso, 2013). The excess of these minerals then end up in animal manures which are then spread onto the fields. In EXT and ORG systems concentrates tend to be basic mixes of grains and legumes and do not usually contain added micronutrients. This means that micronutrient concentrations in ORG and EXT farms tend to be the result of natural nutrient cycling within the farm which is one of the core principles of organic production. However as (Rey-Crespo, Miranda and López-Alonso, 2013) have noted this could lead to hidden deficiencies in these systems if soils are naturally low in certain micronutrients.

The inclusion of these micronutrients may be related to the increased available P levels in CON soils which can cause copper, zinc, selenium and iron deficiencies. This excessive P level may be the reason that CON farms need to add some of these micronutrients to their concentrate feeds as their uptake is suppressed in soils with excessive P. Excessive K levels also suppress the uptake of other cations and some micronutrients such as zinc and iron. To offset these imbalances calcium and magnesium are also added to some of these concentrate blends, as well as to soils through liming. This supports the hypothesis that CON systems would have decreased chemical health and would be imbalanced due to excess application of nutrients.

2.4.4.2. Carbon cycling

Grasslands tend to have high soil carbon levels in the surface soil (0-30cm) and in this study similar results were seen (n=50, carbon stocks mean=88.24±3.752). However this varied widely (range = 43.86-154.84) depending on soil type, texture and management practices. While all sites were free draining, some sites were slightly heavier in texture than others leading to overall higher carbon stocks on these sites. Soils with high clay content can accumulate more organic matter and there is a direct relationship between clay content and soil carbon (Churchman *et al.*, 2020). However since there was no statistical difference in soil texture between the managements this should not be a factor.

Increased use of organic manures has been shown to increase soil carbon in Irish grasslands (Fornara *et al.*, 2016). CON systems due to their increased livestock numbers have increased organic manure to use in their systems. While EXT farms have less organic manure, the longer recovery time and decreased grazing can lead to more plant litter being available for decomposition. Evidence of this can be seen in the soil carbon fractions where EXT systems have a higher, although not significant, percentage of POM than the other two systems. This increased plant material is then decomposed slowly by soil microorganisms into the MAOM fraction. Evidence of increased exudation in EXT systems is possibly also seen in the borax fraction of the MAOM. The increased exudation is likely related to nutrient deficiencies, as plants increase their exudation in nutrient deficient soils (Parasar, Sharma and Agarwala, 2024). This then stimulate microorganisms such as fungi, who can acquire P from the recalcitrant P fraction, and this may also explain the increased *ITS* gene abundance at depth in this system.

Sites on CON farms consisted of paddocks on the grazing platform and were rotationally grazed at high densities for short periods, a practice that can increase SOC levels (R.C Byrnes et al., 2018). On ORG farms this rotational system was also practiced but with slightly lower stocking densities. While on EXT farms some form of rotation was still practiced just with lower grazing densities, longer grazing times and longer recovery times which also can increase SOC levels.

Many ORG farms were diversified operations often incorporating tillage into the system. Usually this was either for grain crops to sell or to produce concentrates for the farm. The length of time from being in arable influences carbon stocks and it can take about 5 years in grassland to recover to permanent pasture levels (Lin *et al.*, 2020). This means that while ORG systems had lower carbon stocks in the 0-15cm layer as a result of tillage, sequestration of carbon should be occurring once the field was put back into pasture again. The frequency of reseeding in grasslands has also been shown to not have any impact on carbon stocks in Ireland (Carolan and Fornara, 2016) so while CON farms did reseed regularly the resowing back into grassland immediately means the loss of soil carbon was minimal. Another study however showed that when reseeding frequency increases from every 10 or so years to 8, 7 or 5 years, reduced soil carbon levels are seen (Fornara and Higgins, 2022) and this may explain the reduced carbon in the 0-15cm layer of the ORG systems as they tend to use 5-7 year reseeding schedules to control weeds.

While all CON sites were grazing fields, some of the ORG and EXT farms had fields that were used regularly for silage with only minimal grazing usually immediately after cutting or in the autumn or spring when grass supply was short. This frequent mowing has been linked to SOC degradation in grassland (Gilmullina *et al.*, 2020). Grass growth sometimes exceeds grazing capacity in ORG and EXT systems, prompting farmers to close off fields for silage. All systems typically used fields located further from the farmyard for silage for logistical reasons. ORG and EXT farms often opt for arable or red clover silage, as these crops provide high yields with minimal inputs. However this means that regular ploughing and reseeding is required. The combined frequent cutting, ploughing and limited organic manure return, meant some of these ORG and EXT sites had the lowest soil carbon stocks. This is in line with Fornara and Higgins (2022) who reported that increasing reseeding frequency leads to decreased carbon levels particularly if those fields are mowed for silage and not grazed rotationally.

Fertiliser application increases biomass production in grasslands which also increases photosynthesis and thus exudation (Eze, Palmer and Chapman, 2018) which in turn can lead to higher soil carbon stocks. However higher chemical fertiliser use increases N_2O and NH_3 emissions in Irish grasslands (Krol *et al.*, 2020). Cashman, Casey and Humphreys, (2024) showed that by using zero chemical nitrogen fertiliser and instead using biologically fixed nitrogen from clover, overall emissions can be reduced from grassland soils while still maintaining productivity. So while CON farms had higher carbon stocks they also had increased emissions from those soils compared to ORG and EXT farms due to the use of chemical nitrogen fertilizer.

2.4.4.3. Water cycling and purification

While no physical differences were seen, CON systems had significantly more bare soil exposed and had fewer plant species present in swards as seen in Table 2-5. Increased vegetation cover and plant species have been linked to increased infiltration and hydraulic conductivity in soil (Fullen, 1991; Leimer *et al.*, 2021). A study looking at infiltration rates in organic and conventionally managed grasslands in the UK reported that infiltration was higher in the organic grasslands even though the soils had similar physical properties (Hathaway-Jenkins et al., 2011). This may be related to the use of KCl fertilisers as they can cause changes in soil structure reducing water holding capacity and cation exchange capacity (CEC) in the surface of soil (Khan, Mulvaney and Ellsworth, 2014) as KCl is a cementing agent to improve mechanical properties of clays (Helle, Aagaard and Nordal, 2017). The use of urea could also be responsible for this cementing effect, as it causes precipitation of calcium carbonate once it is hydrolysed which can make soil pores close up (Krajewska, 2018). This could potentially lead to increased surface runoff from CON farms. While infiltration rate was not measured in this study, this warrants further investigation as derogation from the nitrates directive depends on surface water quality being maintained or improved.

Nitrogen when supplied to plants in nitrate form may have a negative effect on plant resistance to drought. This may be related to the higher energy requirement for nitrate reduction and assimilation which is 20 moles of ATP versus only 5 for ammonium (Marschner, 2012a). Nitrate is also xylem dependent meaning as respiration increases so too does nitrate uptake, so when respiration is increased in drought conditions, more and more nitrate is taken up by plants and sent to the leaves to be reduced and assimilated. Urea, the main fertiliser used by CON systems, when applied to soil is eventually converted to nitrate and leaches through the soil profile (Forrestal *et al.*, 2025). Matimati, Verboom and Cramer, (2014) showed that gas exchange and stomatal conductance was increased and water use efficiency was decreased when plants were grown without direct access to nitrogen fertiliser and thus had to acquire them through mass flow indicating that plants increased their respiration to pull more nitrogen in from the soil. Grasses, in particular *Lolium perenne* which is the dominant species in most sown grasslands in Ireland, have a high affinity for uptake of nitrate (Weigelt, Bol and Bardgett, 2005) and in grasslands drought has also been shown to increase soil nitrate levels (Oram *et al.*, 2023). This may lead to grasses in fertilised swards increasing respiration in order to acquire more nitrate as that nitrate moves further down the soil profile away from the root zone. This may also explain why plants grown in organic systems have reduced water use than those in conventional systems (Schärer *et al.*, 2022) potentially leading to decreased soil water during drought conditions.

Most CON farmers used calcium ammonium nitrate (CAN) or protected urea as chemical nitrogen and both of these were shown to result in similar leaching of nitrates from well drained soils (Forrestal *et al.*, 2025). Given that ORG and most EXT farmers used none (or very little in the case of some EXT sites, see Figure A-3) of these fertilisers and used only organic manures, mainly in the form of slurry or farmyard manure (FYM), these applications should have very little effect on nitrate leaching (Gibbons, Rodgers and Mulqueen, 2007). CON farmers however also used slurry as a fertiliser and applied heavier rates than both ORG and EXT systems as shown in Figure A-3 and Figure A-4. The increased AOA and

AOB abundance in CON systems has also been linked to increased nitrate leaching (Liu *et al.*, 2025) and lower dissolved C:N ratios in CON soils may indicate that mineralization of SOM is occurring, increasing soil inorganic N. These all indicate that CON systems are much more susceptible to nitrate leaching.

Increased phosphorus in CON systems in both soil layers gives increased risk of loss to waterways. In 0-15cm the average Morgans P levels as shown in Table 2-1 in CON systems were more than twice the agronomic optimum levels (Index 3, >5mg L-1) and at 15-30cm they were only just below this. This is most likely a result of historic over application as CON farmers are now limited in how much P fertilizer they can apply. However they can still apply slurry to Index 4 (>8mg L-1) fields if they have already applied slurry to all other paddocks and still have some left over. This potentially could lead to over application of P on some fields as this P is continuously cycled through the system and more is imported through concentrate feeds.

Overall CON farms may be much more susceptible to nutrient loss to water than ORG or EXT systems. These results suggest that decreased infiltration rates, increased nitrate levels, and increased phosphorus levels may all contribute to this. With decreased infiltration, loss overland to water is increased, possibly bringing nutrients to drains or waterways. Application of chemical fertilizers such as urea or CAN increases the risk of potential losses to groundwater and surface water and potentially increases water use of plants leading to decreased soil water levels in drought.

2.4.4.4. Biomass Production

Biomass production increased with increasing intensity as expected as shown in Figure 2-13. This difference would indicate a 29% reduction in biomass production between the systems. However ORG systems produced biomass levels similar to the national average (9.1t DM ha^{-1}) for Ireland as most farms operate below the nitrate limit (M. O'Donovan, Hennessy and Creighton, 2021). The difference between systems is mostly related to fertility management and chemical fertilizer use however as some recent studies have shown increased biomass production is possible with very little to no added chemical N on Irish farms through the use of biological nitrogen fixation from legumes (Cashman, Casey and Humphreys, 2024). This means the yield potential for ORG systems could be much higher if they were not restricted by organic production standards and could also receive a derogation from the nitrates limit like CON farms can. However increased production in ORG systems comes with reduced carbon stocks in the top 15cm of soil due to the increased use of the plough and legumes, although this may actually be a result of not having enough livestock and thus manure to cycle the carbon back to the soil.

EXT systems produced only enough biomass to support the animals they carried and this is mostly related to the reduced nutrient cycling occurring within these systems. Many of these systems were classified as severely deficient in terms of fertility. Increasing soil fertility in these systems could potentially increase biomass production leading to overall better soil health in these systems. The increased Borax extracted carbon in EXT systems as shown in Figure 2-3 may also show increased exudation in these systems of more complex carbon molecules that are only recently bound to the soil matrix by weak forces (Lopez-Sangil and Rovira, 2013). Nutrient deficient plants produce more exudates to recruit microbes that can extract the required nutrients from the soil matrix (Lekberg *et al.*, 2021; Parasar, Sharma and Agarwala, 2024). However, this may favour certain species over others in these systems, leading to reduced diversity as plants will select species to address the deficiency. This also contributes to reduced biomass as more energy is sent out as exudates to acquire nutrients rather than put into new growth.

2.4.4.5. Diversity/Soil as a Habitat

Microbial communities were mainly driven by inputs. Nitrogen and phosphorus fertilisers are known to increase fungal pathogens and suppress mutualists, while nutrient limitation (particularly P) promotes Arbuscular Mycorrhizal Fungal (AMF) abundance in grasslands (Lekberg *et al.*, 2021). The increased abundance of *ITS* genes at depth in EXT systems may be a result of the low nutrient inputs into these systems stimulating these AMF. This increased *ITS* gene abundance may also be related to the increase in plant diversity within these systems (Hoeksema *et al.*, 2010) as they have increased forb abundance. However, EXT systems also had lower Shannon alpha diversity scores and richness for *ITS* compared to ORG systems. This lower diversity may mean that EXT systems are favouring species that can acquire the nutrients the soil is deficient in, from the soil (Behie and Bidochka, 2014). While in CON systems the excess of nutrients, such as nitrogen, favours increased nitrifying bacteria and archaea gene abundance.

Plant functional groups were more diverse in ORG and EXT systems containing significantly more forbs and tending towards higher clover abundance. Plant species have significant effects on both prokaryotic and fungal communities in grassland soils as shown in Ryan et al., (2023), however, no variables associated with plant communities were observed to have a significant effect on the microbial communities in this study. This is possibly due to soil type, texture, chemistry etc differing between all sites making the overall contribution of plant functional groups to the variation very small. While CON systems did have some grass clover and multispecies swards (6 species mixes containing 2 grasses, 2 legumes and 2 forbs) the majority of these sites were grass monocultures dominated by *Lolium perenne*. The shallow rooting system of these grasses may also be why EXT systems had higher *ITS* abundance at depth as shown in Table 2-5, as they contained more forbs with deeper rooting structures.

ORG and EXT systems tended to be more diverse in terms of livestock and enterprise. CON systems were all dairy farms with only dairy cattle. While ORG and EXT systems were a mix of beef and dairy farms with some keeping sheep, some practicing tillage and some having other livestock (pigs, chickens and horses). The increased diversity of grazing animals may also influence soil diversity through diverse manure inputs (Zhelezova *et al.*, 2024) and selective grazing of plant species by these livestock (Tälle *et al.*, 2016).

2.5. Conclusions

Physical health was found to be similar in soil types of similar physical characteristics regardless of trafficking and grazing density. Chemical health was found to be imbalanced in CON systems requiring more inputs to keep the system balanced. Biological health is driven by these inputs and increased nitrogen inputs, particularly inorganic fertilisers, was shown to drive changes in nitrogen cycling gene abundance disrupting the soil nitrogen cycle and increasing the risk of nutrient losses. Management had a significant effect on bacterial communities mostly due to increased pH and inputs in CON systems. Fungal communities were affected by soil fertility, increasing soil P and K levels and by nitrogen inputs. Functional trade-offs existed between the systems as increased biomass production in CON systems came with decreased water cycling/purification functionality and diversity of plants. ORG systems had decreased carbon cycling due to frequent ploughing and biomass removal. EXT systems had decreased nutrient cycling and as a result lower biomass production. These results indicate that nutrient deficiencies as well as excesses lead to altered soil microbial communities. These results indicate that a balance between productivity and overall functionality is best achieved through ORG systems while CON systems have the potential to retain their increased productivity and provide the same level of functionality with more careful management of inputs.

2.6. References

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Chapter 3 Conclusions and future opportunities

3.1. Conclusions

3.1.1. Soil physical and chemical health

The results of this study show that in similar soil types, different management systems have similar soil physical health. This result was surprising as it was expected that increased trafficking and stocking density would have some impact on soil physical properties. However, as previously stated, Bondi et al., (2021) also saw that trafficking intensity had no significant effect on soil physical health in well-drained soils. This means that for the soil types chosen, as long as soils were relatively dry when trafficked, no physical damage is to be expected. Lepore et al., (2024) also showed that soil moisture was the key factor in moderately drained soils for compaction and showed that bulk density alone was not a sufficient predictor of physical damage and that aggregate stability was also necessary. The percentage of water stable aggregates also showed no significant differences between management systems. This means in these well-drained soils physical damage is much less likely to occur from trafficking thus the first hypothesis can be rejected, although the experimental design did not allow appropriate testing of this hypothesis.

Soil chemical health was found to be affected by the management system. pH, Morgans P, Morgans K as well as Mehlich-3 Cu, Mg and S were all significantly higher in CON systems than both ORG and EXT systems. Lime is a big factor in this pH increase as CON farms are required to have a liming plan while both ORG and EXT farms only apply lime irregularly. High soil P levels in CON systems put them in the Index 4 range meaning they have excessive soil P levels. This can cause issues with availability of other nutrients like copper and zinc which is why they need to be supplemented in these systems. In contrast, in EXT systems available P was low partly due to low pH making it unavailable as well as lack of inputs to the system. High K levels in CON systems were also the reason why higher Mg levels were seen, to prevent grass tetany. S is now applied with nitrogen fertilisers to improve use efficiency which is why CON systems had higher levels. For carbon levels ORG systems had lower carbon stocks than CON systems. This may be the result of increased legume content, the more frequent use of the plough, more frequent mowing, inclusion of tillage rotations in ORG systems or the increased organic manure use in CON systems. So while CON systems had higher nutrient and pH levels than ORG and EXT systems these required the use of inputs to maintain the balance in the systems and prevent excesses and deficiencies. However in EXT systems and ORG systems hidden deficiencies may occur if nutrients that are leaving the farm are not replaced.

3.1.2. Soil biological health

While ORG systems had increased *ITS* fungal alpha diversity than EXT systems, CON systems had increased *16S rRNA* bacterial alpha diversity than EXT systems. This decreased diversity in EXT systems is most likely related to pH and P levels in these systems as more neutral pH supports more diversity and low P levels causes plants to select for species of fungi that can extract it from the soil matrix. Lack of disturbance may also play a part as fungi are more susceptible to soil disturbance (de Vries *et al.*, 2007). Beta diversity was significantly different between systems for *16S rRNA* at both depths and for *ITS* management was not significant. However, management and fertility status showed similar results and environmental variables showed that pH, P and K were among the main drivers of the community structure for *ITS*. Nitrogen cycling genes are significantly increased in CON systems due to the use of chemical fertilisers and increased organic manure use. The increased *nirK* abundance in EXT systems may be related to the increased fungal *ITS* gene abundance at depth as fungi can use this gene to produce energy (Maeda *et al.*, 2015). This may also be a way of preventing a limiting nutrient from leaving the system through leaching. All of the *16S rRNA* ASVs identified were involved in the nitrogen cycle and were mostly different between CON and EXT systems with ORG systems being similar to EXT systems. For *ITS* the ASVs were related to organic matter turnover with the vast majority being saprophytes. Finally sward diversity showed that ORG and EXT systems had significantly higher levels of forbs than CON systems and while not significant ORG systems tended towards higher legume abundance. CON systems also had significantly more bare soil than both ORG and EXT systems.

3.1.3. Soil functions and trade-offs

3.1.3.1. Biomass production

Biomass production as expected was lower in ORG and EXT systems with the difference being 29% lower in ORG systems compared to CON systems. While this was the case, ORG systems still had yields in line with the national average for Ireland (O’Donovan, Hennessy and Creighton, 2021) as most conventional farms are not in derogation and are subject to the same restrictions on stocking rates as ORG farms (<170kg N ha^{-1} yr^{-1}). While ORG systems could produce higher yields without nitrogen fertiliser if they were not restricted by stocking rates, as Cashman et al., (2025) showed in Solohead research farm. However, ORG systems have no need to increase production so long as they can feed all their animals sufficiently.

3.1.3.2. Water cycling

While no direct measures taken in this study showed any differences in water cycling functionality, several indirect measures indicate that CON systems could have reduced water cycling functionality. Excessive P levels in soils could lead to surface water decline if run-off was to occur. Even at 15-30 cm soil P levels were high meaning that leaching through the soil profile could be occurring. While bulk density, porosity and water holding capacity were not different between the systems it is worth noting that L J Hathaway-Jenkins et al., (2011) found that infiltration was reduced in CON grasslands compared to ORG ones. The sward survey also showed that CON systems have a higher percentage bare soil exposed which has been shown to reduce infiltration rates (Fullen, 1991). Urea and KCl fertilisers have cementing effects on soil and causing macropores to close (Helle, Aagaard and Nordal, 2017; Krajewska, 2018). This may mean that this may be a surface phenomenon where soil porosity and thus infiltration at the very surface is reduced due to inorganic fertiliser use, increased bare soil levels due to increased stocking density, and increased traffic. Chemical fertiliser also increases plant water use (Schärer *et al.*, 2022) possibly leading to negative effects in drought conditions in CON farms.

3.1.3.3. Carbon cycling

Carbon cycling was impacted by management systems with ORG farms having slightly lower carbon stocks than CON systems. This decrease while small is significant and is likely a result of increased legume content, the more frequent use of the plough, more frequent mowing, inclusion of tillage rotations in ORG systems or the increased organic manure use in CON systems. However this result does not include emissions or energy use on farm and a life cycle assessment is needed to reach further conclusions on this. In Europe it was shown that ORG systems were lower than CON and EXT in terms of global warming potential and environmental impact (Haas, Wetterich and Köpke, 2001; Nemecek *et al.*, 2011).

3.1.3.4. Nutrient cycling

Nutrient cycling was reduced in EXT systems compared to both ORG and CON systems. CON systems tended to have nutrients like P and K in excess leading to the need to increase Copper and Zinc supplementation to prevent deficiency and Magnesium to prevent grass tetany. ORG systems were more balanced but still required some nutrients as well as lime to bring pH to more optimum levels. The microbial communities were also driven by these nutrient excesses and deficiencies.

3.1.3.5. Diversity/soil as a habitat

Soil alpha diversity was reduced in EXT systems compared to CON systems for *16S rRNA* bacterial communities while for *ITS* fungi EXT systems were less diverse than ORG systems. This reduced diversity is in part related to pH but may also be due nutrient deficiencies causing selective species to be favoured. Inputs seemed to be the main driving factors for *ITS* while for *16S rRNA* it was also inputs but some geophysical parameters like sand content and elevation also significantly affected communities. Results from the plant functional diversity within swards also showed that CON systems had lower plant diversity and contained fewer forbs than both ORG and EXT systems.

3.1.3.6. Trade-offs

EXT systems had reduced nutrient cycling within their systems due to biomass removal and low input levels. This reduced nutrient level may cause the observed reduced diversity in *16S rRNA* as lime applications, to maintain pH at more neutral levels, were not regular. Reduced pH also affects P availability so this may reflect the low P levels seen in these systems. *ITS* diversity was most likely related to reduced P levels favouring fungi that can acquire P from the soil matrix in these systems. K was also low in these systems and as it is also an important nutrient for plant growth low levels can also alter microbial communities (Soumare, Sarr and Diédhiou, 2023). Reduced nutrient cycling within these systems was most likely the cause of both decreased soil diversity and biomass production.

ORG systems had decreased biomass production compared to CON systems but were actually in line with conventional farms of similar stocking rates across Ireland. This decreased biomass production also came with a slight decrease in soil carbon stocks. ORG systems seemed to have the best balance of functionality, they maintained high biomass production (relatively speaking) while only having slightly reduced carbon stocks.

CON systems had increased biomass production but that came at a decrease in water cycling functionality. More bare soil, excessive nutrients, use of chemical fertilisers, and reduced plant diversity all give CON systems much more risk of both run-off and leaching of nutrients to water.

3.1.4. Overall conclusions

Physical health in soils is determined mostly by soil type. Well drained soils are very resilient to physical damage and can tolerate heavy trafficking and stocking density. However care should still be taken when soils are very wet as damage can still occur and some compaction was seen on individual farms, particularly on silage ground.

Chemical health in soils is very much determined by management. pH, P and K are all managed for in grasslands and have indexes. However more careful management of nutrients is needed in CON systems as excesses were seen for both P and K resulting in supplementation to prevent deficiencies in other nutrients. In EXT systems pH needs to be more carefully monitored and lime applied to prevent nutrients being tied up. Increased soil testing in ORG systems could also help with monitoring pH and nutrient levels, as there were still some deficiencies seen in these systems.

Biological health was driven by inputs. N cycling genes were increased where organic and chemical fertiliser was used in large amounts potentially indicating increased emissions or leaching. Nutrient excess as well as deficiencies shaped the microbial communities as well as some geophysical factors like sand content and elevation. Alpha diversity was most likely influenced by pH and P availability.

Soil functions only differed slightly between systems. CON systems had the highest biomass production, while ORG had similar levels to the national average and EXT systems were less than half of that. Carbon cycling only showed a slight difference in carbon stocks and no other carbon values measured were significantly different. Nutrient cycling was reduced in EXT systems compared to the other two systems. Diversity of plant functional groups was reduced in CON systems but microbial communities showed no difference in alpha diversity between CON and ORG systems while EXT systems had reduced *16S rRNA* diversity compared to CON systems and reduced *ITS* diversity compared to ORG systems. Beta diversity showed all systems had significantly different communities and these were related to inputs and soil fertility. Water cycling was potentially reduced in CON systems due to more bare soil in swards, reduced plant diversity, excessive nutrient levels and chemical fertiliser use.

ORG systems provided the best balance of functionality of the 3 systems. They produced relatively high biomass for their stocking rate with only a small negative impact on soil carbon stocks. EXT systems need to improve soil fertility as improving nutrient cycling would potentially increase biomass production and improve microbial diversity. CON systems need to manage nutrients more carefully to prevent excesses and to prevent loss to the environment.

3.1.5. Limitations

The main limitations in this study were related to soil physical properties. While basic measures like bulk density, aggregate stability and water holding capacity were measured, infiltration, pore structure and rooting architecture were not. These are all important factors in measuring soils capacity to infiltrate and store water. While these measurements are time consuming and can be expensive to carry out, they can provide valuable information on soil structure. It would also be interesting to look at the top 5cm of the soil surface, to see if as hypothesised, this layer is subject to more damage and thus infiltration is slowed in this layer creating potential run-off issues.

The use of only 3 soil types in this study is also a limitation as there are many different soil types across Ireland. This was done to keep the study comparable as EXT and ORG farms are much easier to find on heavier, poorly draining soils as this system suits the carrying capacity of these soils.

However having more soil types included would give a greater understanding of soil health in different soil types and how management affects them. Some soils do not suit intensive production as they cannot produce the biomass needed. However information is still needed on these soils and how management systems affect them.

While the nitrogen cycle was shown to be affected in CON soils from the use of chemical nitrogen more information is needed to confirm what is actually occurring. While gene copy numbers from qPCR assays give an indication of nitrogen cycling, functional assays could provide a better indicator of potential functionality. Time restraints when collecting samples meant that analysing fresh soil samples was very difficult and so functional assays were not carried out. Genes related to nitrogen fixation and denitrification if assessed could have given a more complete picture of the nitrogen cycle in the soil.

Sequencing results, while informative, show that the vast majority of organisms are undescribed, in which case it is difficult to discern with confidence anything other than community diversity, unless a specific function is conserved with a genus. This means that although one system has a distinct community compared to another system, we cannot say anything other than the communities are different. Tying community structure to other functions or overall soil health is therefore not possible with the current limitations of the sequencing approach used here.

3.2. Future Opportunities

3.2.1. Infiltration and water use

While there was no differences in any of the indicators measures for soil physical health in this study, results elsewhere have shown that there are differences in infiltration and water use in organic systems compared to conventional ones (L J Hathaway-Jenkins *et al.*, 2011; Schärer *et al.*, 2022). Research into this in Irish grasslands could be vital to ensure resilience to droughts and flooding as well as preventing loss to waterways from surface run off.

3.2.2. Improving nutrient management

While CON systems in this study had optimum nutrient levels, many of these were actually in excess for P and K. These nutrients are vital for producing large amounts of biomass but also have an antagonistic effect on other nutrients such as copper, zinc and magnesium requiring them to be supplemented or applied in fertilisers. Therefore more research into the effects of nutrient excesses is required to determine what the optimum level is for each nutrient before it starts to become antagonistic. This would create an upper limit for nutrients as well as a lower one and thus help farmers more accurately determine fertiliser applications and reduce input costs, while also protecting from losses to the environment.

3.2.3. Liming and traditional grasslands

Acidification of grassland soil results in plant diversity loss as plant species can only tolerate certain pH levels (Goulding, 2016). Lime has a long historical use in Ireland on traditional grassland meadows and pastures (Collins, 2008). However as the results of this study show, EXT farmers seem to not be applying lime regularly enough to combat acidification. More research needs to be carried out on traditional grasslands in Ireland examining the effects of liming vs not liming on these grasslands. While acidification is usually not a desired property in grasslands it does create unique habitats and alters soil diversity. However no evidence exists to establish which is actually better for soil health, liming or allowing soil to naturally acidify over time.

3.2.4. Closing the yield gap

ORG systems were shown to produce 29% less biomass per hectare than CON farms. While ORG farms produce enough biomass to support their stocking rate as seen by the fact that they import very little feed into their system, there is evidence to suggest that they could produce more biomass without the use of chemical fertilisers (Cashman *et al.*, 2025). While ORG farms are restricted by European legislation on their stocking rate and cannot get a derogation from the Nitrates directive, research on producing more biomass using less fertiliser could benefit CON farmers by helping them reduce their impact on the environment.

3.2.5. Life cycle assessment

While all CON systems were dairy operations, many of the ORG and EXT systems were mixed where they had more than one enterprise. Many were also tillage farmers producing crops either for human or animal consumption, often growing their own concentrate feed to reduce costs. This system of producing concentrates on farm as well as the lack of chemical fertiliser and pesticide use may lower overall emissions from the system. While other studies have shown that organic systems reduce impact on the environment (Haas, Wetterich and Köpke, 2001; J W Casey and Holden, 2006; Nemecek *et al.*, 2011), a life cycle assessment is required to determine if this mixed farming system does lower the impact in Irish systems. This may also be of benefit to CON farmers as if the nitrates derogation is not granted in the future, producing their own concentrates on tillage ground may be a way to keep their stocking rate below the nitrate limit and reduce emissions.

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Appenix A

Table A-1 qPCR primers and cycle conditions used in this study

Primer Name	**Primer Sequence (5'-3')**	**Conc.**	**Target gene**	**Thermal profile**	**Reference**
T7F	*TAATACGACTCACTATAGGG*	*0.2μM*	*Plasmid spike*	*95°C, 5 min; 39X (95°C, 30 s; 57°C 30 s, 72°C, 30 s, 85°C 1s with plate read) Melt curve 65°C to 95°C, increment 0.5°C, 0:05+ plate read*	(Duff et al., 2022)
M13R	*GGATAACAATTTCACACAGG*	*0.2μM*	*Plasmid spike*		
341F	*CCTACGGGNGGCWGCAG*	*1.5 uM*	*Bacterial 16S rRNA gene*	*95 °C, 5 min; 39 x(95°C, 40 s; 55.6°C, 1 min; 72°C, 30 s, 80°C 3s with plate read) Melt curve 65°C to 95°C, increment 0.5°C, 0:05+ plate read*	(Klindworth *et al.*, 2013)
785R	*GACTACHVGGGTATCTAATCC*	*1.5 uM*	*Bacterial 16S rRNA gene*		

ITS4R	*TCCTCCGC TTATTGATA TGC*	*0.2 uM*	*ITS*	*95 °C, 2 min; 39 x(95°C, 30 s; 54°C, 30 s; 72°C 30 s with plate read) Melt curve 65°C to 95°C, increment 0.5°C, 0:05+ plate read*	(Op De Beeck *et al.*, 2014)
ITS86F	*GTGAATCA TCGAATCT TTGAA*	*0.2 uM*	*ITS*		
Arch-amoA-23F	*ATGGTCTG GCTWAGAC G*	*1 uM*	*Archaeal amoA gene*	*95°C, 5 min; 39X (94°C, 45 s; 50°C 45 s, 72°C, 45s, 80°C 15s with plate read) Melt curve 65°C to 95°C, increment 0.5°C, 0:05+ plate read*	(Tourna *et al.*, 2008)
Arch-amoA-616R	*GCCATCCA TCTGTATG TCCA*	*1 uM*	*Archaeal amoA gene*		
BacamoA-1F	*GGGGTTTC TACTGGTG GT*	*1 uM*	*Bacterial amoA gene*	*94°C, 5 min; 39X (94°C, 30 s; 55°C 45 s, 72°C, 45 s, 82°C 2s with plate read) Melt curve 65°C to*	*(Pereira e Silva, 2012)*
BacamoA-2R	*CCCCTCKG SAAAGCCT TCTTC*	*1 uM*	*Bacterial amoA gene*		

				95°C, increment 0.5°C, 0:05+ plate read	
Ntsp_amoA_162F	*GGATTTCTGGNTSGATTGGA*	*1 uM*	*Commamox gene*	*95°C, 5 min; 39X (95°C, 30 s; 56°C 30 s, 72°C, 45 s, 80°C 2s with plate read) Melt curve 65°C to 95°C, increment 0.5°C, 0:05+ plate read*	(Fowler *et al.*, 2018)
Ntsp_amoA_359R	*WAGTTNGACCACCASTACCA*	*1 uM*	*Commamox gene*		
nirK1040	*GCCTCGATCAGRTTRTGGTT*	*0.2 uM*	*nirK*	*95°C, 10 min; 39X (95°C, 10 s; 60°C 30 s, 72°C, 10 s, 85°C 2s with plate read) Melt curve 65°C to 95°C, increment 0.5°C, 0:05+ plate read*	(Hallin *et al.*, 2009)
nirK876	*ATYGGCGGVCAYGGCGA*	*0.2 uM*	*nirK*		

cd3AF	*GTSAACGTSAAGGARACSGG*	*0.5uM*	*nirS*	*95°C, 15 min; 6X (95°C, 15 s; 66°C-1°C per cycle 30 s, 72°C 30 s, 80°C 30s with plate read); 40X (95°C, 15 s; 58°C 30 s, 72°C 30 s, 80°C 30s with plate read) Melt curve 65°C 31s, 65°C to 95°C, increment 0.5°C, 0:05+ plate read*	(Throbäck *et al.*, 2004)
R3cd	*GASTTCGGRTGSGTCTTGA*	*0.5uM*	*nirS*		

Table A-2 Slopes, y-intercepts and r^2 values of *qPCR* standard curves

Genetic Target	Slope	% Efficiency	Y intercept	R^2	NTC	PTC
Bacterial 16S rRNA	*3.282*	*101.7%*	*36.329*	*0.988*	*33.16*	*15.46*
ITS fungi	*3.528*	*92.1%*	*39.821*	*0.997*	*0*	*N/A*
Archaeal amoA	*3.587*	*90.0%*	*33.280*	*0.987*	*0*	*13.13*
Bacterial amoA	*3.532*	*91.9%*	*35.653*	*0.993*	*0*	*N/A*
Comammox	*3.504*	*92.9%*	*34.208*	*0.995*	*0*	*N/A*
nirS	*3.626*	*88.7%*	*28.400*	*0.998*	*27.84*	*12.87*
nirK	*3.520*	*92.4%*	*34.135*	*1.000*	*0*	*20.05*

Table A-3 Animal DM kgs requirements per annum

	Dairy Cows	**Suckler Cows**	**Calves <1year**	**Yearlings 1-2 years**	**2+ years**	**Sheep**	**Small Equines**	**References**
kgs DM per annum	5000	4017	1369	2515	2938	1000	2737.50	(O'Mara, 1996; O'Donovan, Lewis and O'Kiely, 2011; Teagasc Equine Specialist Unit, 2018; M O'Donovan, Hennessy and Creighton, 2021; O'Donovan *et al.*, 2022)

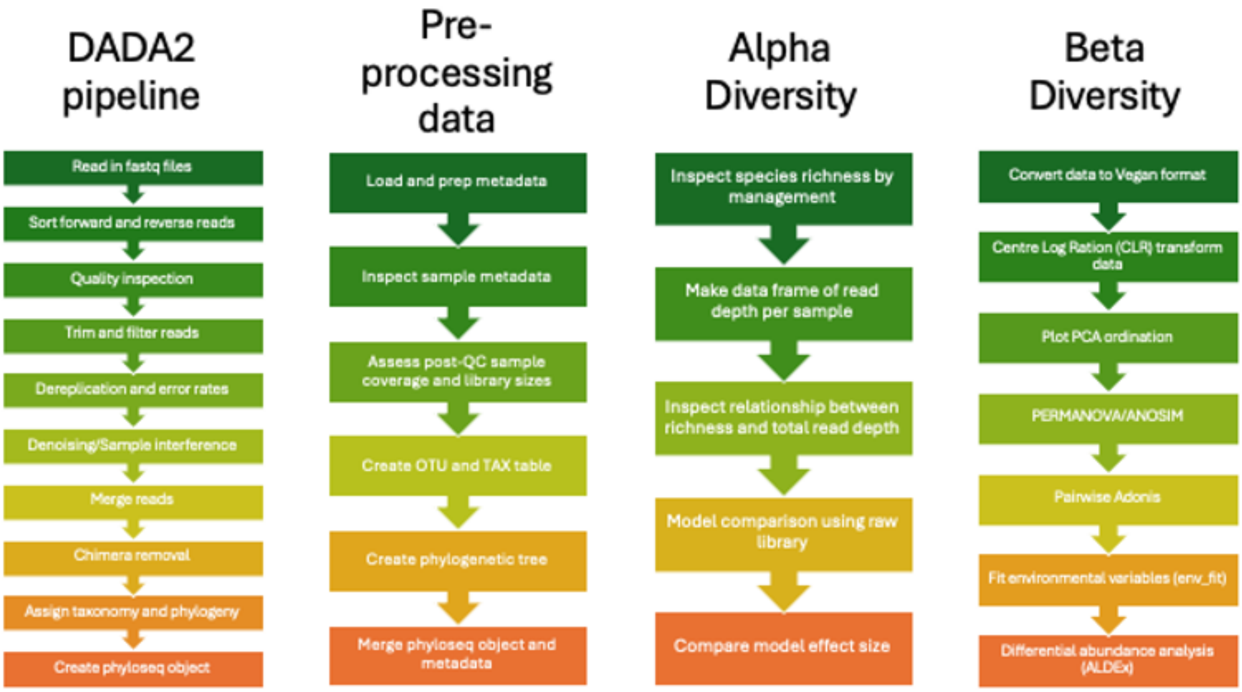

Figure A-1 DADA2 pipeline

Nitrogen, phosphorus, potassium and other fertilisers/amendment rates

Using the management survey the amounts of organic and inorganic fertiliser applied was calculated from the end of the fertiliser closing period (January 29th 2023) up until the time of sampling in 2023. Organic fertiliser nutrient values were calculated from Teagasc figures and other literature sources (Alexander *et al.*, 2020; Shi *et al.*, 2021). All lime and other soil amendments applied were also recorded.

Table A-4 The mean physical fraction carbon and nitrogen values for each system (mean ±SE): conventional intensively managed grassland (CON), organically managed grassland (ORG) and extensively managed grassland (EXT). Shown are the physically separated carbon fractions, percentage coarse (2mm-250mm) carbon (Coarse C_{tot}) and nitrogen (Coarse N_{tot}), coarse organic carbon (Coarse C_{org}), the carbon nitrogen ratio of the coarse fraction (Coarse C:N, percentage fine (250mm-53mm) carbon (Fine C_{tot}) and nitrogen (Fine N_{tot}), Fine organic carbon (Fine C_{org}), the carbon nitrogen ratio of the Fine fraction (Fine C:N), percentage Mineral Associated Organic Matter (MAOM) (>53mm) carbon (MAOM C_{tot}) and nitrogen (MAOM N_{tot}), MAOM organic carbon (MAOM C_{org}), the carbon nitrogen ratio of the MAOM fraction (MAOM C:N).. Significance code:'*' p≤0.001, '**' p≤0.01, '*' p≤0.05, 'ns' p>0.05. Multiple comparison of means significant differences are shown through different letters.**

Variable	Depth	F value	CON	ORG	EXT
Coarse C_{tot} %	0-15cm	2.187^{ns}	3.02 (±0.439)	2.74 (±0.385)	4.28 (±0.806)
	15-30cm	0.234^{ns}	2.77 (±0.432)	2.74 (±0.519)	3.21 (0.575)
Coarse C_{org} %	0-15cm	3.108^{ns}	2.57 (±0.359)	2.10 (±0.287)	3.75 (±0.725)
	15-30cm	1.413^{ns}	1.30 (±0.173)	1.06 (±0.138)	1.82 (0.494)
Coarse N_{tot} %	0-15cm	0.503^{ns}	0.23 (±0.031)	0.22 (±0.031)	0.30 (±0.078)
	15-30cm	1.030^{ns}	0.26 (±0.050)	0.28 (±0.045)	0.36 (±0.044)
Coarse C:N	0-15cm	0.827^{ns}	20.97 (±4.093)	18.92 (±4.295)	28.05 (±7.040)
	15-30cm	0.283^{ns}	5.57 (±0.762)	5.92 (1.293)	4.76 (0.902)
Fine C_{tot} %	0-15cm	1.885^{ns}	1.01 (±0.055)	0.9 (±0.051)	1.16 (±0.156)
	15-30cm	0.172^{ns}	0.64 (±0.048)	0.63 (±0.034)	0.98 (±0.256)
Fine C_{org} %	0-15cm	1.243^{ns}	0.43 (±0.069)	0.38 (±0.056)	0.62 (±0.135)
	15-30cm	1.595^{ns}	0.14 (±0.023)	0.14 (±0.018)	0.30 (±0.088)
Fine N_{tot} %	0-15cm	1.207^{ns}	0.08 (±0.004)	0.07 (±0.004)	0.09 (±0.010)
	15-30cm	3.090^{ns}	0.05 (±0.003)	0.06 (±0.003)	0.06 (±0.006)
Fine C:N	0-15cm	0.801^{ns}	4.96 (±0.692)	5.08 (±0.634)	6.26 (±0.981)
	15-30cm	1.543^{ns}	3.20 (±0.707)	2.38 (±0.304)	3.92 (±0.795)
MAOM C_{tot} %	0-15cm	0.563^{ns}	5.39 (±0.246)	5.01 (±0.309)	5.49 (±0.498)
	15-30cm	0.177^{ns}	3.33 (±0.187)	3.21 (±0.246)	3.47 (±0.166)
MAOM C_{org} %	0-15cm	1.161^{ns}	4.8 (±0.254)	4.17 (±0.269)	4.54 (±0.443)
	15-30cm	0.298^{ns}	2.82 (±0.209)	2.57 (±0.212)	2.63 (±0.364)
MAOM N_{tot} %	0-15cm	1.088^{ns}	0.6 (±0.031)	0.53 (±0.034)	0.53 (±0.064)
	15-30cm	0.525^{ns}	0.38 (±0.024)	0.36 (±0.019)	0.65 (±0.284)
MAOM C:N	0-15cm	4.801^{ns}	7.96 (±0.112)	8.21 (±0.678)	6.94 (±0.597)
	15-30cm	3.965^{ns}	7.34 (±0.139)	6.91 (±0.275)	6.41 (±0.181)

Table A-5 The mean chemically extracted fraction carbon and nitrogen values for each system (±SE): conventional intensively managed grassland (CON), organically managed grassland (ORG) and extensively managed grassland (EXT). Shown are the chemically extracted carbon fractions, percentage Borax ($Na_2B_4O_7$) extracted organic carbon (Borax C_{org}) and nitrogen (Borax N_{tot}), the carbon nitrogen ratio of the Borax fraction (Borax C:N), percentage tetra sodium Pyrophosphate ($Na_4P_2O_7 \cdot 10H_2O$) carbon (Pyro C_{org}) and nitrogen (Pyro N_{tot}), the carbon nitrogen ratio of the Pyrophosphate fraction (Pyro C:N), percentage insoluble carbon (Insoluble C_{org}) and nitrogen (Insoluble N_{tot}), and the carbon nitrogen ratio of the insoluble fraction (Insoluble C:N). Significance code:'*' p≤0.001, '**' p≤0.01, '*' p≤0.05, 'ns' p>0.05. Multiple comparison of means significant differences are shown through different letters.**

Variable	Depth	F value	CON (n=18)	ORG (n=19)	EXT (n=13)
Borax C_{org} mg g soil^{-1}	0-15cm	5.526^{*}	4.08 (±0.239)^{ab}	3.81 (±0.232)^{b}	5.04 (±0.428)^{a}
	15-30cm	0.260^{ns}	3.19 (±0.347)	2.97 (±0.261)	3.19 (±0.245)
Borax N_{tot} mg g soil^{-1}	0-15cm	5.348^{*}	0.48 (±0.022)^{a}	0.46 (±0.036)^{a}	0.65 (±0.051)^{b}
	15-30cm	1.296^{ns}	0.39 (±0.037)	0.35 (±0.024)	0.42 (±0.032)
Borax C:N	0-15cm	2.058^{ns}	8.41 (±0.183)	8.34 (±0.225)	7.82 (±0.210)
	15-30cm	2.511^{ns}	8.05 (0.203)	8.27 (±0.242)	7.67 (±0.165)
Pyro C_{org} mg g soil^{-1}	0-15cm	1.950^{ns}	3.59 (±0.182)	3.01 (±0.177)	3.59 (±0.417)
	15-30cm	0.399^{ns}	2.62 (±0.191)	2.41 (±0.214)	2.44 (±0.306)
Pyro N_{tot} mg g soil^{-1}	0-15cm	2.226^{ns}	0.34 (±0.016)	0.28 (±0.015)	0.35 (±0.041)
	15-30cm	0.298^{ns}	0.24 (±0.017)	0.22 (±0.018)	0.23 (±0.027)
Pyro C:N	0-15cm	1.595^{ns}	10.71 (±0.143)	10.59 (±0.138)	10.33 (±0.157)
	15-30cm	2.854^{ns}	10.82 (±0.148)	11.03 (±0.223)	10.43 (±0.129)
NaOH C_{org} mg g soil^{-1}	0-15cm	4.431^{*}	4.98 (±0.232)^{a}	4.07 (±0.210)^{b}	4.91 (±0.558)^{ab}
	15-30cm	0.447^{ns}	2.94 (±0.260)	2.61 (±0.147)	2.80 (±0.364)
NaOH N_{tot} mg g soil^{-1}	0-15cm	4.867^{*}	0.54 (±0.024)^{a}	0.44 (±0.022)^{b}	0.54 (±0.061)^{ab}
	15-30cm	0.662^{ns}	0.33 (±0.026)	0.29 (±0.014)	0.33 (±0.042)
NaOH C:N	0-15cm	0.021^{ns}	9.14 (±0.068)	9.16 (±0.153)	9.12 (±0.133)
	15-30cm	1.746^{ns}	8.81 (±0.113)	8.94 (±0.186)	8.57 (±0.134)
Insoluble C_{org} mg g soil^{-1}	0-15cm	5.242^{*}	10.10 (±0.514)^{a}	8.21 (±0.355)^{b}	10.77 (±1.527)^{ab}
	15-30cm	0.868^{ns}	6.26 (±0.508)	5.40 (±0.326)	6.75 (±1.351)
Insoluble N_{tot} mg g soil^{-1}	0-15cm	3.953^{ns}	1.27 (±0.066)^{a}	1.062 (±0.047)^{b}	1.37 (±0.181)^{ab}
	15-30cm	0.505^{ns}	0.85 (±0.062)	0.81 (±0.032)	0.95 (±0.154)
	0-15cm	0.261^{ns}	8.00 (±0.151)	7.82 (±0.296)	7.79 (±0.120)

Insoluble C:N	15-30cm	2.886^{ns}	7.34 (±0.194)	6.61 (±0.261)	6.82 (±0.253)

Table A-6 The mean geo-physical values for each system; (mean ±SE) conventional intensively managed grassland (CON), organically managed grassland (ORG) and extensively managed grassland (EXT). Shown are Elevation, the 30 year average rainfall per annum and 30 year average temperature per annum, well as the percentage of sand, silt and clay, the bulk density, soil porosity, the gravimetric water content, water holding capacity, the volume of stones as a percentage of total soil volume and the percentage of water stable aggregates. Significance code:'*' p≤0.001, '**' p≤0.01, '*' p≤0.05, 'ns' p>0.05. Multiple comparison of means significant differences are shown through different letters.**

Variable	Depth	F Value	CON	ORG	EXT
Elevation (m)	N/A	0.394^{ns}	85.90 (±11.627)	78.88 (±11.763)	70.61 (±10.376)
30 year avg rainfall (mm)	N/A	2.937^{ns}	1087.55 (±31.243)	1141.42 (±35.965)	1028.37 (±18.609)
30 year avg temp (°C)	N/A	0.807^{ns}	10.14 (±0.060)	10.28 (±0.091)	10.23 (±0.078)
Sand %	0-15cm	2.760^{ns}	37.94 (±2.431)	35.9 (±1.929)	30.19 (±2.433)
	15-30cm	1.474^{ns}	37.04 (±2.383)	36.69 (±2.302)	31.69 (±1.914)
Silt %	0-15cm	2.646^{ns}	39.96 (±2.083)	41.64 (±1.630)	46.04 (±1.443)
	15-30cm	1.455^{ns}	42.90 (±2.129)	43.19 (±1.612)	47.05 (±1.199)
Clay %	0-15cm	1.045^{ns}	22.1 (±0.717)	22.46 (±0.524)	23.76 (±1.262)
	15-30cm	0.473^{ns}	20.06 (±0.672)	20.12 (±0.872)	21.27 (±1.297)
Bulk Density (g/cm^3)	0-15cm	0.593^{ns}	1.13 (±0.024)	1.13 (±0.026)	1.1 (±0.029)
	15-30cm	1.578^{ns}	1.24 (±0.036)	1.26 (±0.033)	1.33 (±0.030)
Porosity %	0-15cm	0.669^{ns}	75.9 (±4.36)	70.5 (±3.43)	70.6 (±3.38)
	15-30cm	2.111^{ns}	51.4 (±1.36)	50.9 (±1.24)	47.8 (±1.09)
Gravimetric water content %	0-15cm	3.099^{ns}	23.2 (±1.58)	22.9 (±1.45)	28.1 (±1.55)
	15-30cm	0.200^{ns}	19.9 (±1.18)	19.2 (±1.21)	20.1 (±0.64)
Water holding capacity %	0-15cm	0.797^{ns}	43.4 (±2.55)	39.4 (±1.55)	43.2 (±3.92)
	15-30cm	0.765^{ns}	31.7 (±1.54)	31.1 (±1.73)	28.8 (±1.36)
Stoniness % volume	0-15cm	1.520^{ns}	5.83 (±0.79)	6.53 (±1.08)	4 (±1.12)
	15-30cm	0.017^{ns}	7.56 (±1.330)	7.26 (±0.878)	7.46 (±1.490)

Water Stable Aggregates %	0-15cm	1.250^{ns}	95.26 (±0.31)	95.78 (±0.25)	95.28 (±0.25)
	15-30cm	0.783^{ns}	94.12 (±0.788)	94.28 (±0.709)	92.55 (±1.673)

Table A-7 Showing the percentage of sites in each Management category that had optimum fertility according to the Teagasc Index system for agricultural production in grasslands. Also shown is the percentage of the total sites with the optimum values.

	n=	**pH (6.2-7.0)**	**P (>5mg L^{-1})**	**K (>100mg L^{-1})**	**Overall**
CON	18	83%	100%	83%	72%
ORG	19	47%	47%	58%	16%
EXT	13	38%	46%	38%	15%
Total	50	52%	64%	62%	32%

Table A-8 Showing the mean stocking rate values in livestock units per hectare, kilograms of organic nitrogen per hectare and grazing density in livestock units per hectare for each of the systems.

Management	Stocking Rate (LU ha^{-1})	kgs Org N ha^{-1}	Grazing density (kgs Org N per grazing ha^{-1})
CON (*n=18*)	2.6 (range 2.07-2.97)	220 (range 176-250)	28 (range 9-45)
ORG (*n=19)*	1.7 (range 1.4-1.91)	144 (range 125-162)	22 (range 2-83)
EXT (*n=13)*	0.8 (range 0.45-1.25)	69 (range 38-106)	17 (range 0-50)

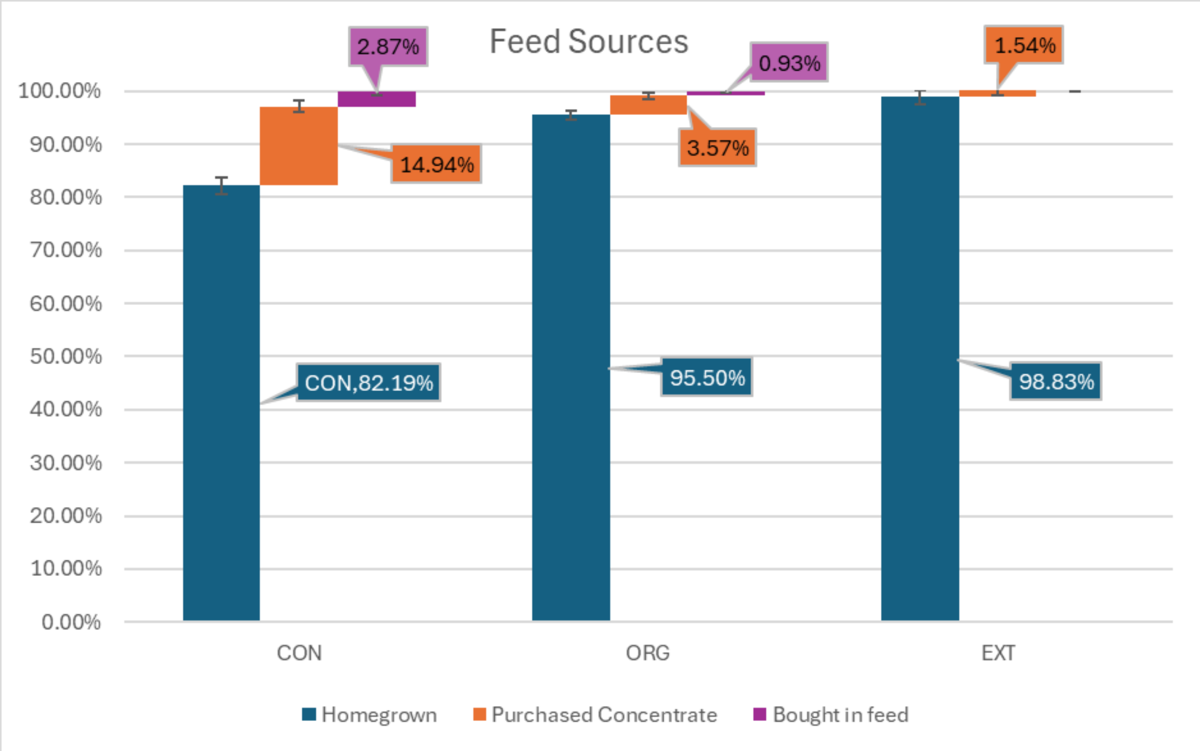

Figure A-2 Showing the percentage of energy from feed sources in each system. Homegrown represents forage grown on the farm either grazed or cut and consumed as silage. Purchased concentrates are concentrate feeds brought in from outside the farm. Bought in feed is any silage, hay etc. bought in to supplement the animals.

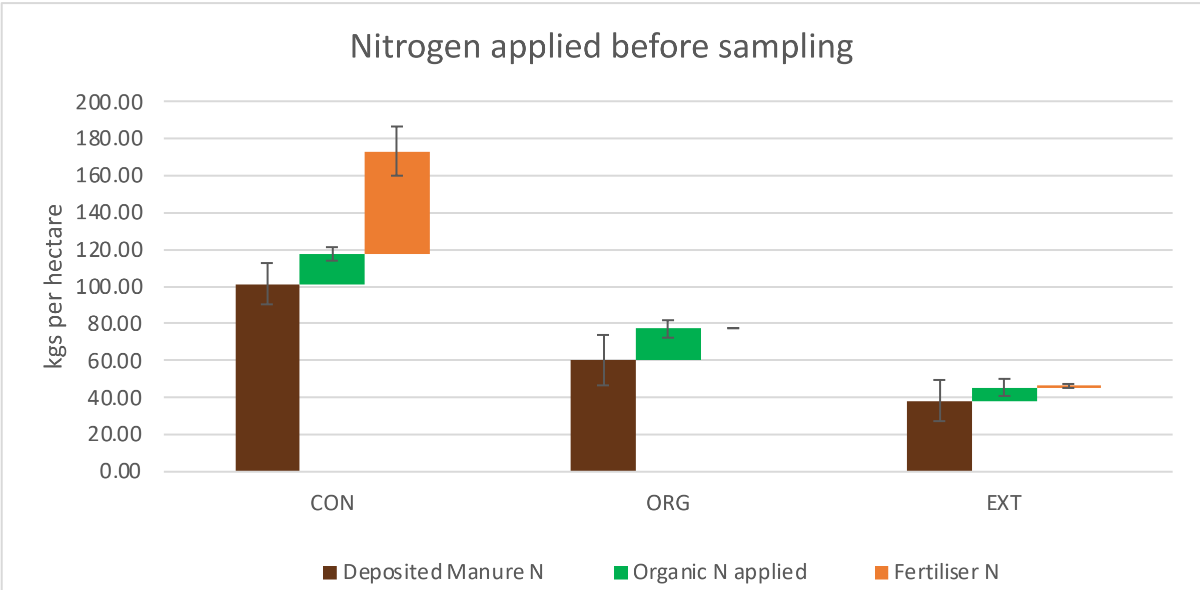

Figure A-3 Nitrogen applied to field before sampling took place from the end of the closing period.

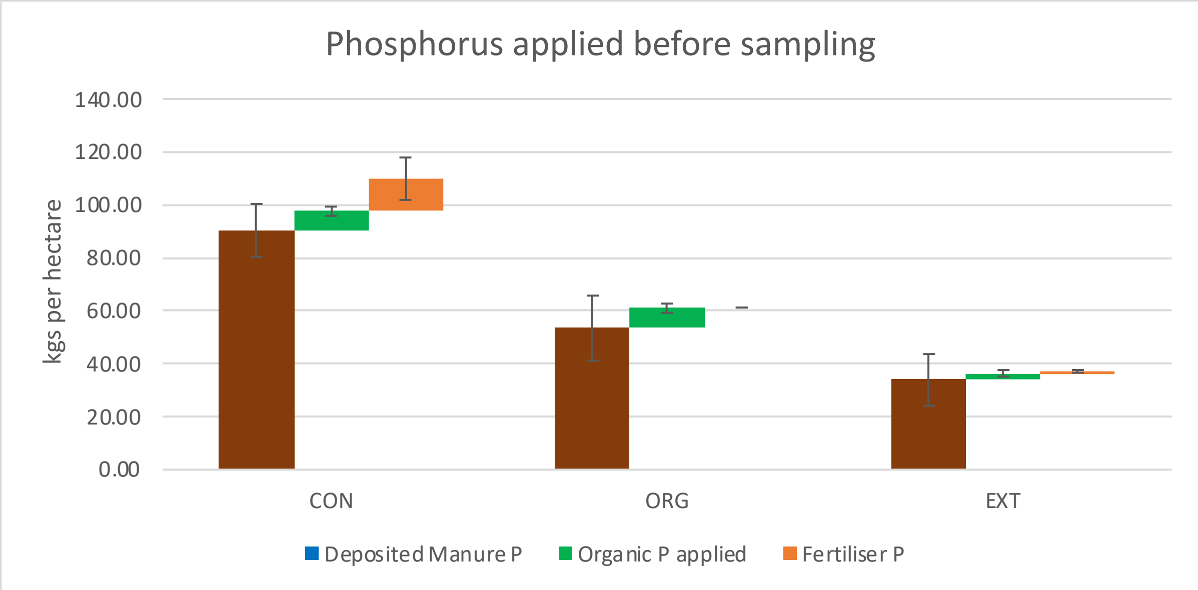

Figure A-4 Phosphorus applied to field before sampling took place from the end of the closing period.

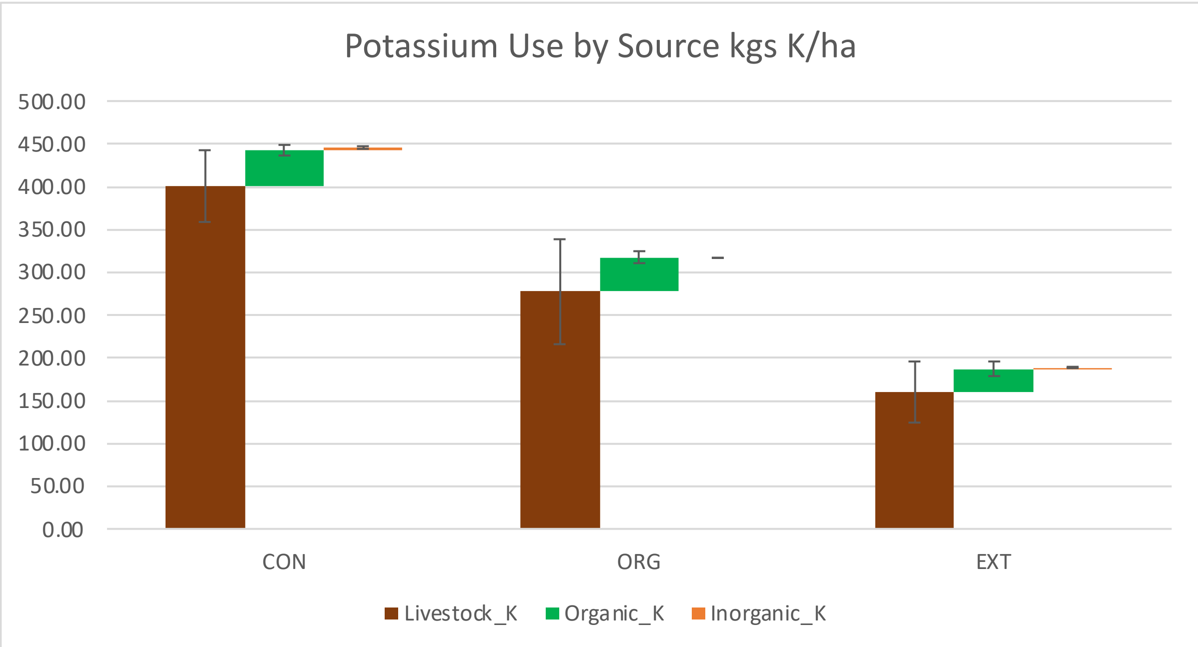

Figure A-5 Potassium applied to field before sampling took place from the end of the closing period.

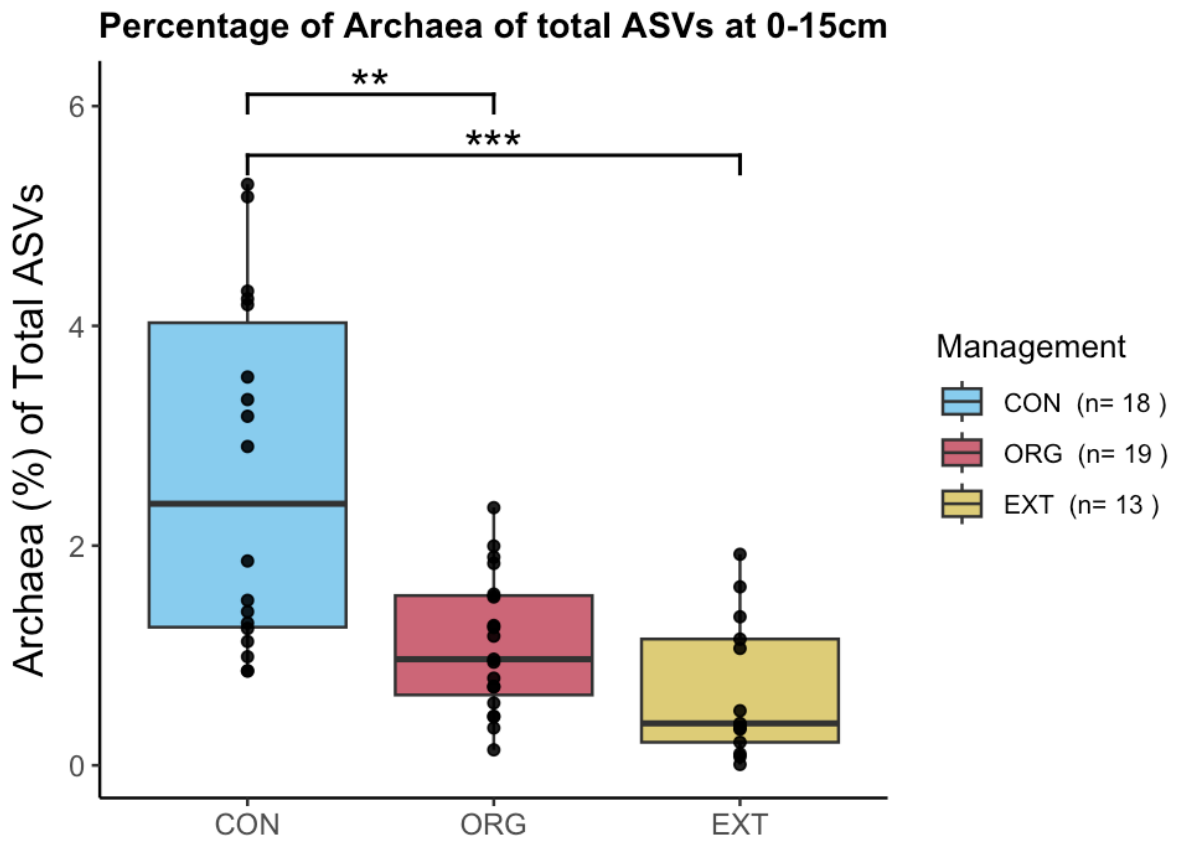

Figure A-6 Abundance of archaea as a percentage of total ASVs from sequencing results.

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