



OLLSCOIL NA GAILLIMHÉ

UNIVERSITY OF GALWAY

Investigating changes in plant diversity
and species composition in a selection
of Irish semi-natural grasslands over the
past decade

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Declaration: I certify that all of the work in this thesis is my own, and has not been used to obtain a degree anywhere other than University of Galway.

Abstract

Semi-natural grasslands are managed through low-intensity management practices, such as extensive grazing and/or annual mowing. As a result, they are species-rich ecosystems. Europe has lost between 50–90% of the area of semi-natural grasslands, accompanied by losses in diversity and changed species composition in the extant semi-natural grasslands. This is largely due to agricultural intensification and abandonment. In Ireland, 1,192 semi-natural grassland sites were surveyed through the 2007–2012 Irish Semi-natural Grassland Survey (ISGS). Using the ISGS as a baseline, the current study aimed to determine if the species diversity and composition has changed since the ISGS. This study is part of the larger Department of Agriculture, Fisheries and the Marine funded project called ‘StableGrass’. The overall aim of this large project is to assess the impact of plant diversity on carbon storage and yield stability in semi-natural grasslands. This study forms one component of this StableGrass project. This study re-surveyed 12 sites from three habitats (4 sites each); GS1 (Dry calcareous and neutral grassland), GS3 (Dry-humid acid grassland) and GS4 (Wet grassland). Fifty-four relevés were collected that included data on the species composition and cover. Vegetation diversity indices (Species Richness, Simpson’s Diversity, and Simpson’s Evenness) were calculated for these relevés.

GS1 had the highest Species Richness and Simpson’s Diversity followed by the GS4 habitat, while GS3 had the lowest values. There was no significant difference in the diversity measures between the ISGS and StableGrass surveys. Ordination analysis suggested evidence for homogenisation in the StableGrass relevés in comparison to the ISGS relevés. This was also observed at the species level with the loss of specialist and rare species and decreased frequencies of herbaceous species across the grasslands. Subtle changes in management, namely an increase in intensity since the ISGS survey was the likely cause for the observed homogenisation. However, management may also be maintaining the diversity at these sites considering the lack of a significant difference since the ISGS. This suggests that these trends could be reversed, provided improved management practices and future monitoring are in place to ensure this.

Chapter 1: Introduction

1.1 Grasslands

Grasslands cover approximately 20–40% of the terrestrial surface of the Earth (White et al., 2000; Ramankutty et al., 2008). A wide range of definitions exist for grasslands (Gibson, 2009) but what these definitions all have in common is an emphasis on the dominance of grasses and lack of woody vegetation (Janišová et al., 2011; Dengler et al., 2014; Bardgett et al., 2021). However, many definitions differ on the percentage of woody cover that can occur while still classifying an ecosystem as a grassland. This results in often poorly delineated boundaries between grasslands and other ecosystems (Bardgett et al., 2021). Grasslands can be differentiated based on the amount of management or anthropogenic influence they receive. When the grassland vegetation exists with no requirement of anthropogenic management to suppress succession, they may be referred to as natural grasslands and are typically maintained by other factors, such as climate or other environmental conditions (Bardgett et al., 2021). Contrastingly, intensively managed grasslands that have undergone substantial changes for agricultural production are managed by intensive grazing with high stocking rates and high chemical inputs, such as fertilisers and pesticides (Bilotta et al., 2007; Grange et al., 2021). Semi-natural grasslands are in the middle of this management gradient and are extensively managed with little to no chemical inputs. This results in these grasslands being species-rich ecosystems (Bullock et al., 2011; Janišová et al., 2014).

Grasslands are typically a mosaic of graminoid (grasses, sedges and rushes) and herbaceous (forb) species (Stace, 2019), with grasses as the dominant group. Grasses belong to a large family called the Poaceae. Globally, it is estimated that there are between 771–898 accepted genera with 11,500–12,000 different species of grasses (Tzvelev, 1989; Soreng et al., 2015; 2022; Hodkinson, 2018). Most grasses, except for bamboos are herbaceous plants that can be annual or perennial. They vary greatly in their structure and growth habit, with some being very short species that rarely exceed 25 cm, while others may grow to well over a metre (Moore and Nelson, 2017). Despite this variation, grasses share a similar structure that consists of a sheath, blade, ligule, and sometimes auricles (Figure 1.1). They have leaves with parallel

venation and flowers that are considerably reduced and typically grouped into structures called spikelets (Stace, 2019). Grass species, due to their simple flower structure, are wind pollinated.

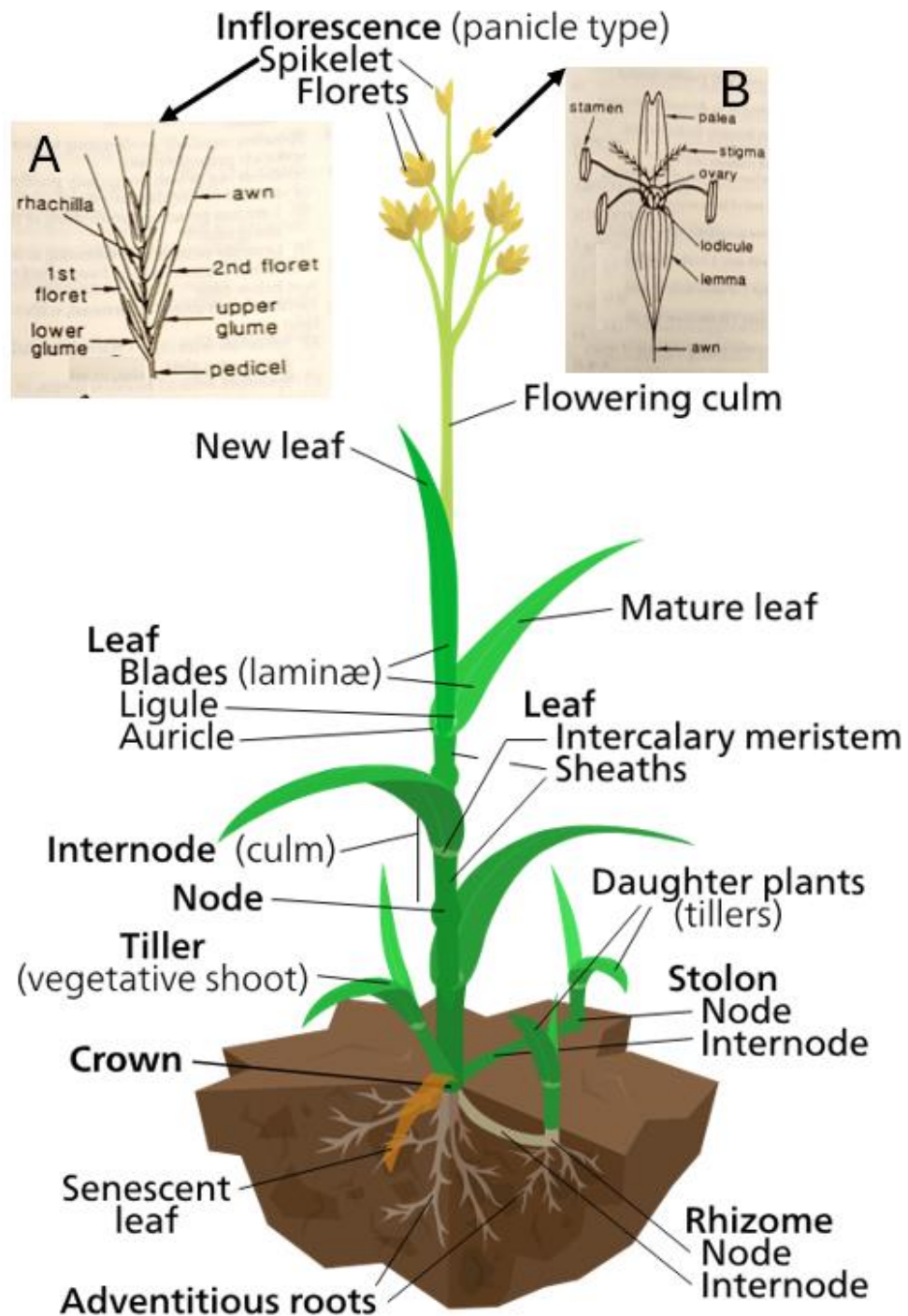


Figure 1.1: Structure of a grass (Poaceae). Flower Structures (Stace, 2019): (A) Spikelet and (B) Floret. Diagram obtained from (Kelvinsong, 2013) under licence from (Creative Commons, n.d.)

1.2 Evolutionary history of grasslands

Today's distribution of grasslands reflects the evolutionary history of grasses, environmental and climate conditions and anthropogenic activities. The earliest fossils associated with Poaceae are dated to the Early Cretaceous (112–101 Ma) (Wu et al., 2018), with other studies dating fossils of the Poaceae to between the Paleocene (~66 Ma) and the Eocene (~56 Ma) (Prasad et al., 2011; Strömberg, 2011). Along with these fossils, phylogenetic models suggest that the family evolved during the Cretaceous (~100 – 130 million year ago) (Gallaher et al., 2019; Schubert et al., 2019), with grasslands becoming more important components of some ecosystems throughout the Cenozoic Era (Jacobs et al., 1999; Strömberg, 2011; Palazzesi et al., 2022). The evolution of grasses is relatively late in comparison to other major modern plant groups. This is thought to be due to changing environmental conditions, especially increasing aridity and decreasing temperatures and CO₂ (Strömberg, 2011; Palazzesi et al., 2022). Paleocene-Eocene grasses showed adaptations to arid conditions, including increased root growth, decreased shoot growth, sunken stomata, and dense cuticles (Archibold, 1995; Christin et al., 2013; Rangan et al., 2022). These traits may have given grasses a competitive advantage compared to other plants in an increasingly arid environment (Willis and McElwain, 2014). Grass co-evolution with grazers was likely another factor in the diversification of the Poaceae (Gibson, 2009). The Cenozoic diversification of mammal groups, such as ungulates coincides with the emergence of savannahs and other grasslands that arose in response to climatically driven factors from the mid Miocene (Gibson, 2009). Currently, it is thought that very few of these natural grasslands remain outside of specific geographic locations e.g. Central European steppes and African savannahs (Hejzman et al., 2013).

Many modern-day grasslands are primarily derived from anthropogenic activities. For example, in Europe it is thought that the majority of grasslands have arisen due to early farming and pastoral systems, which can be traced back to the Neolithic period (Bredenkamp et al., 2002). There is evidence to suggest that mass woodland clearance occurred during the Neolithic with cattle-grazed pastures developing after this clearance (Sheail et al., 1974). This shift to pastoralism can be detected in the

pollen record through a change in dominant plant groups from woody species to non-woody species with crops such as *Secale cereale* and associated arable weeds being identified in sediments dating back to 5,740–5,330 BP (Kreuz, 2008; Margielewski et al., 2010; Gałka et al., 2014) in Europe. Meadows emerged with the development of iron scythes in the 7th to 6th centuries BC (Eriksson, 2020). Hay meadow enlargement is believed to have occurred during the Middle Ages (1300–1500 AD) with the development of longer scythes, like those of more modern design (Segerström and Emanuelsson, 2002). Intensification of agriculture began several hundred years later. This gradually increased from the 1700s and then accelerated greatly from the mid-1900s (Steffen et al., 2015) and again in the mid to late 1900s (Ratcliffe, 1984). Now much of European's grasslands are heavily managed grasslands for food and livestock production.

1.3 Types and distribution of grasslands across the globe

Grasslands range from natural to semi-natural and intensive agricultural grasslands. Global grasslands include tropical savannas, boreal grasslands, temperate grasslands, prairies and steppe grasslands (Buisson et al., 2022a; Strömberg and Staver, 2022; Figure 1.2). Tropical grasslands or savannas are those that are characterised by a dry winter season with regular wildfires, followed by the summer months where they get most of their annual rainfall. Savannas are characterised by vegetation of tall and tussocky grasses, with some shrubs and low trees, often occurring in clumps, but not dominating. Savannas occupy large areas of the Southern continents, for example, Africa, Australia, and South America (Gibson, 2009). The boreal grasslands occur at high latitudes and often at high altitudes and are more strongly influenced by climatic conditions than anthropogenic management. They are dominated by short grass species, e.g. *Festuca*, *Poa*, and *Agrostis* and often occur above the maximum elevation that can support woodland. In the early 1900s natural grasslands in Britain were restricted to areas of elevated topography with base-rich substrates and suffered from leaching due to their slope; and periodically flooded areas such as floodplains (Smith and Crampton, 1914). Steppe grassland are dry grasslands in temperate climates and are dominated by bunchgrasses, with small thorny trees

scattered throughout. Plants that survive in these grasslands display xerophytic traits. As a result, these grasslands do not have high productivity and would not be able to support an intensive agricultural system. Instead, these grasslands are managed through extensive grazing achieved through low grazer numbers. Temperate grasslands occur in the mid-latitudes, with an average temperature range of -3 °C and 18 °C, with 25-75 cm of rainfall a year (Gibson, 2009). Many Irish and Western and Central European grasslands fall into this category.

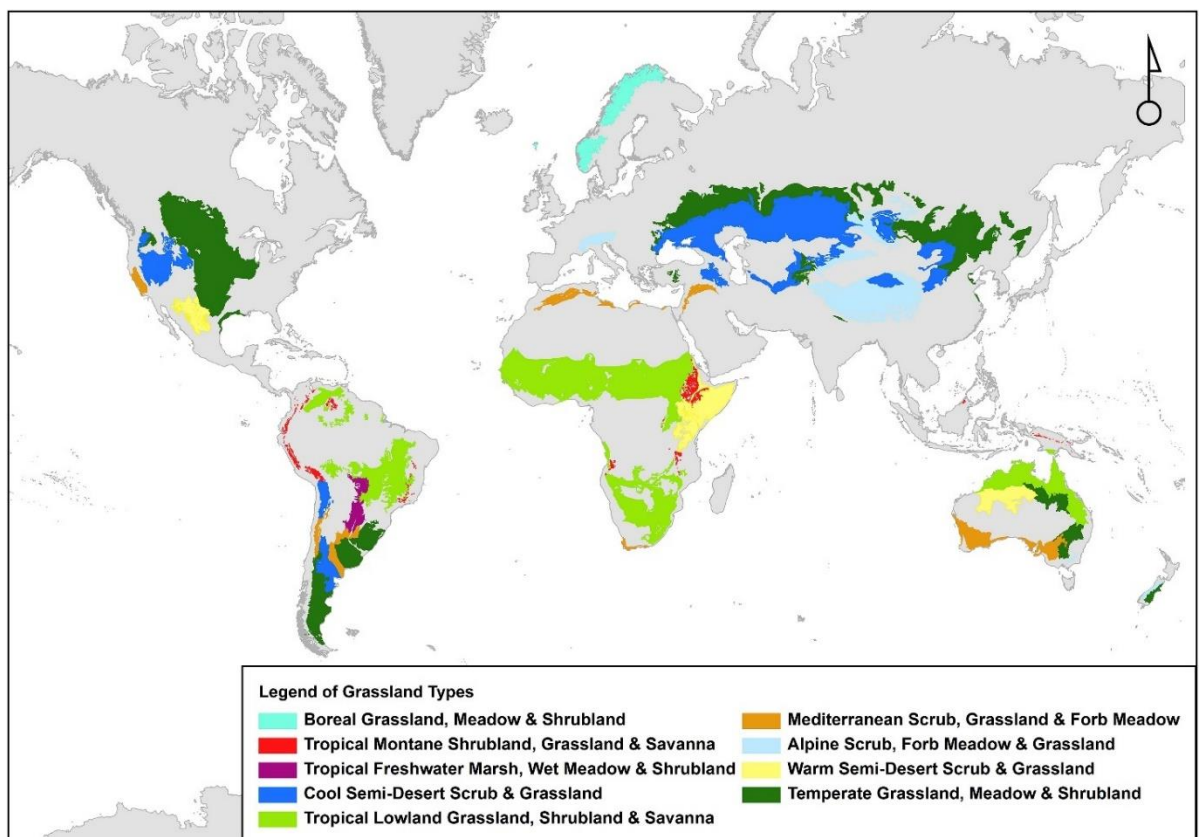


Figure 1.2: Global distribution of different grassland types (After Dixon et al., 2014); modified by (Zhou et al., 2017b)

Semi-natural grasslands are a subset of the global grassland cover and are the focus of this study. These grasslands are typically considered to be secondary grasslands, often developed after previous land clearance that may have occurred thousands of years ago (Gibson, 2009). These grasslands have not been fertilised or reseeded and are extensively managed, typically by grazing with low numbers of grazers or annual mowing (When et al., 2018; Johansen et al., 2019). As a result, they are extremely species rich and often have a high diversity of specialist grassland species that are

reliant on this long history of extensive management (Bonanomi et al., 2013; When et al., 2018). Extensive management is needed to prevent these secondary grasslands from reverting back to scrub and/or forest vegetation (Gibson, 2009). This management is what makes these grasslands “semi-natural” because they hold all the usual species of the area but are reliant on anthropogenic influences to maintain ecosystem stability.

1.4 Semi-natural grasslands

Semi-natural grasslands support a high diversity of plant species, with species richness ranging up to 81 species per 4m² plot to 89 species per m² (Bastow Wilson et al., 2012; Chytrý et al., 2015) reported globally and in Europe. European semi-natural grasslands hold 18% of Europe’s endemic species, which is twice the number of endemic species tied to woodland habitats (Hobohm and Bruchmann, 2009). This species diversity highlights the important role that semi-natural grasslands have in supporting biodiversity and occurs because of human influence, which may be unexpected considering the impact anthropogenic influences are having on global plant diversity. Anthropogenic management practices reduce the amount of aboveground biomass, but also, over time, the amount of nutrients in the soil (Al-Mufti et al., 1977; Tälle et al., 2016). As a result of the biomass and nutrient reduction, the cover of dominant grass species is suppressed resulting in reduced competition for both light and niche space (Borer et al., 2014; Pulungan et al., 2019). This allows a greater range of species, particularly forbs, to thrive in these environments. The positive effect of management on the species diversity is strongest when the management is neither too intensive nor lacking. This is consistent with the intermediate disturbance hypothesis (Grime, 1973; Horn, 1975; Moi et al., 2020). This theory states that at both low and high levels of ecological disturbance, species richness is decreased, with maximum diversity of species occurring at intermediate disturbance levels (Figure 1.3; Grime, 1973).

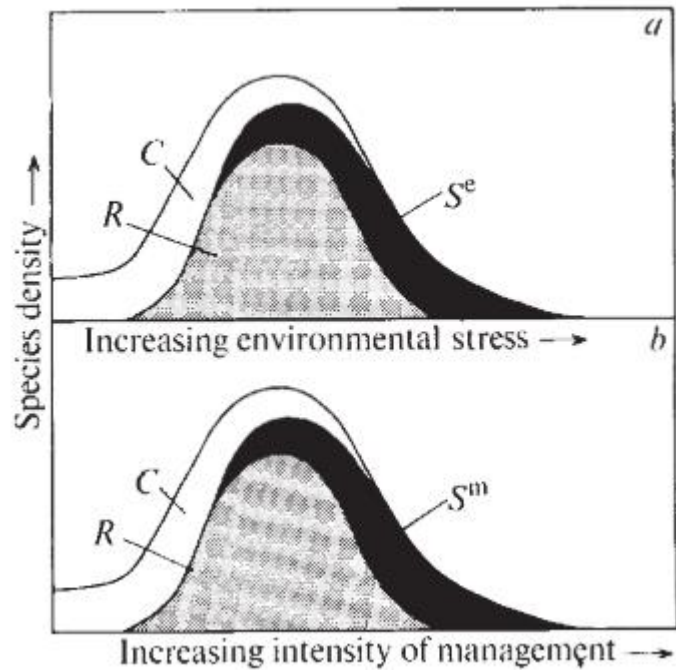


Figure 1.3: Diagram representing the Intermediate Disturbance Hypothesis (Grime, 1973). (a) intensity of environmental stress and (b) intensity of management (grazing, mowing etc.). C = Competitive species. S = Species of high resistance to stresses. R = Remaining species.

The extensive management of semi-natural grasslands benefits the diversity of plant species and species of other taxonomic groups (WallisDeVries et al., 2002; Carboni et al., 2015; Bonari et al., 2017; Fontana et al., 2020). For example, Fontana et al., (2020) found that semi-natural grasslands in the European Alps support a high biological diversity with 166 vascular plant species and 176 species of invertebrate taxa, including butterflies, grasshoppers, ants, beetles, and spiders. Semi-natural grassland species also support a large diversity of pollinator species (Bonari et al., 2017; Larkin and Stanley, 2021) and various bird species (Söderström et al., 2001). This means that these ecosystems have a high value for both conservation, for example by providing breeding sites for species of conservation concern (Vanhinsbergh et al., 2002; Tichit et al., 2005; Brüggeshemke et al., 2022) and providing a range of ecosystem services that increases the grasslands' conservation value (Zavaleta et al., 2010; Vos et al., 2014).

1.5 Ecosystem Services

Ecosystems provide a series of services for human well-being and the conservation of biodiversity, which may be direct or indirect (Parker et al., 2016). These are known as ecosystem services and can include provisioning, regulating, and cultural services (Millennium Ecosystem Assessment, 2005). Ecosystem services can have great benefits for increasing the resilience and sustainability of agricultural systems. The provision of ecosystem services is greater in biodiverse ecosystems, largely due to the greater species richness and ecological stability of these ecosystems (Naeem et al., 1994; Pywell et al., 2015; Orford et al., 2016; Dainese et al., 2019). Semi-natural grasslands promote a range of ecosystem services, namely carbon storage, yield stability, provision of habitats, flood regulation, and recreation among others (Bullock et al., 2011; Wehn et al., 2018; Peciña et al., 2019). In particular, grasslands have important roles in carbon storage and yield stability. Despite this, there are often conflicts and trade-offs that occur between the need for agricultural productivity and the conservation of grassland biodiversity. Some land managers have expressed concern that species-rich grasslands are unable to offer the same forage quality as intensively managed grasslands (Isselstein et al., 2005; Schaub et al., 2020; Lindborg et al., 2023). There are some instances where the non-intensive management was not able to support livestock production in the same way to an intensively managed grassland (Isselstein et al., 2005). For example, a review by Tallowin and Jefferson (1999) found that the production of plant biomass (in the context of a food source for grazing animals) in semi-natural grasslands was 50% lower than that of intensively managed grasslands. As a result, landowners may have concerns about integrating extensive management practices to promote biodiversity if the losses in productivity are not compensated through financial means, e.g. through agri-environmental schemes.

Semi-natural grasslands are thought to store 10–34% of the terrestrial carbon store (Zhou et al., 2017a), which is largely achieved through belowground storage in the soil (Jobbágy and Jackson, 2000; White et al., 2000a; Veldman et al., 2015b). By area, semi-natural grasslands have greater belowground carbon storage than intensively managed grasslands (Ostle et al., 2009). This has a considerable importance to

agriculture as this sector accounts for 17% of the global CO₂ emissions (FAO, 2020), and 29–37% of Ireland’s CO₂ emissions (Donnellan and Hanrahan, 2011; EPA, 2023). This increased carbon storage and allocation has been found to increase under increased species richness (Weisser et al., 2017; Chen et al., 2018; Gao et al., 2020; Wang et al., 2020; Bai and Cotrufo, 2022). As well as increased carbon storage, yield stability is another ecosystem service that is enhanced by increased plant diversity. This service can ensure resilience in the agricultural sector through the maintenance of the consistency of production even in times of future climate stress (Tilman and Downing, 1994; Olesen et al., 2011; Urruty et al., 2016; Reckling et al., 2021), and may even increase the productivity and yield of semi-natural grasslands (Schaub et al., 2020). There are two mechanisms which increased diversity of species are thought to promote this service. These include niche complementarity and the increased likelihood of the occurrence of species which have traits that greater promote this yield stability (Tilman et al., 1997; Loreau, 1998; Kahmen et al., 2006).

1.6 Threats to semi-natural grasslands

Globally, grasslands are threatened by a variety of challenges including land-use changes towards intensive agriculture, afforestation, and abandonment leading to the encroachment of woody species (White et al., 2000; Ramankutty et al., 2008; Świerszcz et al., 2024). The increase of nutrients in nutrient-poor systems, such as semi-natural grasslands can reduce plant species diversity through increasing the competitive advantage of grasses (Cousins and Eriksson, 2008; Bonanomi et al., 2013; Ceulemans et al., 2013). These increases in nutrients are largely due to management intensification. This increase in competitive grasses result in increased grass biomass, often by over 50% in comparison to a nutrient-poor environment (Borer et al., 2014; 2020). This occurs at the expense of less competitive species that are typical of semi-natural grasslands. The opposite of grassland intensification would be abandonment, which shares some of the negative impacts of intensification.

Grassland abandonment occurs when the extensive management that once promoted the species diversity of a semi-natural grassland has ceased or the management would be no longer economical or accessible (O’Neill et al., 2013; Valkó

et al., 2018). The effects are similar to nutrient addition where the dominance of competitive grass species is increased, resulting in litter accumulation and the subsequent competitive exclusion of forb species due to reduced light availability (Borer et al., 2014; Valkó et al., 2018; Eskelinen et al., 2022; McKeon et al., 2022). The increase of plant litter in abandoned grasslands compared to managed grasslands ranges from 45% to 106% (Kelemen et al., 2014; McKeon et al., 2022). As abandoned grasslands may be uneconomical for intensive agriculture, or inaccessible for most machinery, they are often converted to other land-uses, for example forestry (Buscardo et al., 2008; Veen et al., 2009; Mellor et al., 2021).

Globally afforestation is increasingly promoted for climate mitigation (Bárcena et al., 2014; Apellaniz et al., 2022; Tölgyesi et al., 2022). However, afforestation can occur at the expense of grasslands: with 560,000 ha of grassland in New Zealand (Huang et al., 2011) and 22% of the area of diverse grasslands in Argentina (Aguapey region) (Apellaniz et al., 2022) being lost to forestry. In Europe, a similar trend is occurring, with some examples of losses due to afforestation including a 36% loss in grassland species richness in Estonian grasslands (Prangel et al., 2023); a 96% loss of the area of semi-natural grasslands in Sweden (Cousins et al., 2015); and a 25% loss of cover in Dorset, UK (Hooftman and Bullock, 2012). In Ireland, commercial forestry cover has increased by 25% since 1950 (Buscardo et al., 2008). With afforestation, the grassland habitats can become fragmented, which has associated negative effects on the species diversity (Fischer and Stöcklin, 1997; Haddad et al., 2015; Yan et al., 2022). While much of this early afforestation has been to produce timber products, there is now a focus on afforestation as a climate mitigation measure (Doelman et al., 2020). However, this view can be controversial. For example Bastin et al., (2019) estimated that to store a significant proportion of the world's carbon, 0.9 billion hectares of open land would have to be afforested by 2050 but others (Hall et al., 2012; Temperton et al., 2019; Doelman et al., 2020; Taylor and Marconi, 2020) have criticised this view. They claim that this is an overstatement of the carbon storage potential and would be at the expense of other important habitats including grasslands.

1.7 Changes in semi-natural grasslands

Semi-natural grasslands in Europe have experienced significant declines in their area over the last 50 years. For example, in the boreal regions of Norway, Aune et al., (2018) identified a 49.1% loss in semi-natural grassland area between 1960 and 2015. Similar rates of semi-natural grassland losses of 47% over periods ranging from approximately 30–55 years have been experienced in the United Kingdom (Ridding et al., 2015). When earlier years are used as a baseline, for example 1932, it is estimated that the UK lost over 97% of unimproved grasslands (Fuller, 1987).

As well as area, the species diversity and composition of the remaining semi-natural grasslands have changed in recent decades (e.g. Duprè et al., 2010; Newton et al., 2012; Pipenbahr et al., 2013; Diekmann et al., 2014; 2019). These studies had mixed findings with regards to species diversity, with some reporting decreases in diversity (e.g. Ridding et al., 2021); no overall change in diversity measures (e.g. Diekmann et al., 2014); or even increases in species diversity, for example species richness (e.g. Mitchell et al., 2017). Changes in species composition across the literature has been more consistent than findings of changed diversity (e.g. Pykala, 2003; Duprè et al., 2010; Diekmann et al., 2014; 2019; Giarrizzo et al., 2017; Watts et al., 2019; Buzhdygan et al., 2020).

Changes in species composition can indicate that degradation of semi-natural grasslands is occurring. This degradation may be noted by a loss of specialist grassland species, increases in taller more competitive species, and increases in graminoid species that is at the expense of forb species (Berlin et al., 2000; Diekmann et al., 2014; Bauer and Albrecht, 2020). These changes are not unique to semi-natural grasslands located in mainland Europe. In a selection of UK grasslands, Ridding et al. (2020) reported a clear shift in the species composition between 1970 and 2016. These changes, given the identified degradation may suggest homogenisation is occurring. Different types of semi-natural grassland habitats may be undergoing changes at differing rates and degrees. For example, a meta-analysis by Diekmann et al. (2019) identified a decline in species richness in most wet grasslands while the other grassland types showed no consistent trend in richness despite a decline in the number of habitat specialists. Many of these changes may be attributed to the threats

described above, with agricultural intensification and land-use changes being significant drivers of change (Cousins and Eriksson, 2008; Johansson et al., 2008; Ridding et al., 2015; Aune et al., 2018; Prangel et al., 2023).

1.8 Semi-natural grasslands in Ireland

1.8.1 History of semi-natural grasslands in Ireland

Ireland's grasslands have fluctuated in area over the past 12,500 years, shaped by climate and human activity (Hall and Pilcher, 1995). There is evidence in the pollen record for the presence of grasslands after the Late-Glacial period but woodland spread rapidly and dominated for the next 5,000 years (Mitchell, 1987; Hall and Pilcher, 1995). Woodland was cleared by early humans for agriculture, creating a landscape of grassland, woodland and scrub, with a brief recovery of woodland around 4,000 years ago during the Bronze Age which lasted for about 500 years (Lucas, 1989; Molloy and O'Connell, 2016; Woodman, 2016). The end of this brief expansion of woodland cover was evidenced by a 68% decline in tree pollen in the pollen record (Hall and Pilcher, 1995). Agricultural expansion, particularly during monastic times (Hall, 1990) and later under Queen Elizabeth I, led to further woodland loss, leaving only 2% of Ireland's land area covered by forests by the end of her rule (Rackham, 2001). The area of native woodland cover in Ireland has not fully recovered to the former extent and now accounts for approximately 1% of land area (Higgins et al., 2004).

1.8.2 Current semi-natural grassland cover in Ireland

In Ireland, grasslands account for approximately 60–70% of land cover with semi-natural grasslands comprising a small component of this total grassland cover (Byrne and Jones, 2002; CSO, 2023). Many of the extant semi-natural grasslands in Ireland exist because they are either in inaccessible areas for improvement, or extensive management practices have been maintained (O' Neill et al., 2013). Based on land cover assessments, for example CORINE land cover, Ireland is reported to have approximately 48,200 hectares of semi-natural grassland, which is only

approximately 0.7% of the total land cover of Ireland, or 1% of the total area of agricultural grassland (CSO, 2023). This contrasts to the large area of the national land cover that is under intensive agricultural management. Irish semi-natural grasslands may also occur as mosaics of less-managed grasslands within intensive farm systems (Sullivan et al., 2011). These semi-natural grassland habitats in farmland often occur as High Nature Value grassland (Sullivan et al., 2010). High Nature Value farmland refers to farmland with high biodiversity value due to extensive management and includes farmlands that have a high proportion of semi-natural habitat (Paracchini et al., 2008). Prior to 2013, little was known about the extent of semi-natural grasslands in Ireland, as the majority of semi-natural grassland research before this was focused on particular areas or specific grassland sites (e.g. Ivimey-Cook and Proctor, 1966; O’Sullivan, 1968; 1982; Keane and Sheehy Skeffington, 1995; Parr et al., 2009).

1.8.3 Semi-natural grassland classification

In Ireland, prior to 2000, there were only a few limited studies into the grassland classification. Braun-Blanquet and Tüxen (1952) conducted what was probably the first phytosociological study into Irish grasslands. After this, a number of comprehensive grassland classification studies into Irish semi-natural grasslands took place and were published by O’Sullivan (e.g. 1965; 1968; 1976; 1982). These studies described three classes of grassland: Molinio-Arrhenatheretea; Calluno-Ulicetea (Nardeartea); and Festuco-Brometea. These represented improved to wet grasslands on clay or gley soils; acid grassland communities; and the base rich dry grasslands on limestone, respectively (O’Neill et al., 2013). Since these early phytosociological surveys, a nationwide habitat classification system known as ‘*A Guide to Habitats in Ireland*’ by Fossitt (2000) has been published and is colloquially referred to by many ecologists as ‘Fossitt’.

Rather than being solely a vegetation classification system, “Fossitt” is a habitat classification system that is based on characteristic plants, soils and geology, and in some cases, the management of the site (Fossitt, 2000; O’Neill et al., 2013). The system is hierarchical moving from broad groups to more specific habitats. For example, grassland habitats in this system are denoted by G, with three further

subgroups (GA – improved grassland; GS – semi-natural grassland; GM – Marsh). Under these three subgroups, there are seven grassland habitats, with five being semi-natural grassland. Overall, Fossitt (2000) describes 117 individual habitats.

Fossitt Grassland Habitats:

- GA1: Improved agricultural grassland
- GA2: Amenity grassland (improved)
- GS1: Dry calcareous and neutral grassland
- GS2: Dry meadows and grassy verges
- GS3: Dry-humid acid grassland
- GS4: Wet grassland
- GM1: Marsh

A subset of grassland habitats are of conservation importance and are defined by the EU Habitats Directive (EU Habitats Directive, 1992). These habitats are listed under Annex I of the Directive and will be referred to as ‘Annex I Habitats’ hereafter. This directive contains a set of distinct habitats with a high conservation importance and at risk of disappearing in their native range (Wilkins et al., 2016). Ireland and other EU member states are obligated to protect these habitats by identifying and including them in Special Areas of Conservation (SAC) (O’ Neill et al., 2013). Ireland is also expected to achieve a favourable conservation status of these habitats and under Article 17 of the Directive, report on the conservation status of these habitats every six years (NPWS, 2013a; Martin et al., 2018; NPWS, 2019). There are six Annex I habitats associated with semi-natural grasslands (O’ Neill et al., 2013) in Ireland and although they do not map directly onto Fossitt habitats, possible associations between the Annex I and Fossitt grasslands are given in Table 1.1.

The most recent vegetation classification system to be used in Ireland is the Irish Vegetation Classification System (IVC). This was introduced in 2015 and aimed to classify, describe and map the natural and semi-natural vegetation of Ireland (Perrin, 2015). This classification system is similar to the UK National Vegetation Classification System (NVC) (Rodwell et al., 1992). The system uses an online web tool known as ERICA (Engine for Relevés to Irish Communities Assignment) to apply a vegetation

community to relevé data, using the percentage cover values assigned for each species (Perrin, 2019). This differs to Fossitt as it utilises quantitative plot data to assign the plot to a vegetation community, rather than being qualitative. Multivariate analysis (e.g. fuzzy analysis) is used to assign each vegetation relevé to the closest matching IVC based on the similarity of the floristic composition and species abundances to an existing database of vegetation relevés, from which the IVC has been developed. This system captures the finer scale variation that occurs in the vegetation that is not captured by Fossitt. For example, there are a total of 19 IVCs for grasslands, compared to only six Fossitt grassland habitats. For each IVC, the Fossitt habitat that the community is most likely to represent is provided in Table 1.1. In most cases, a community is represented by one Fossitt habitat, but in other cases, the one IVC may be represented by one or more Fossitt habitats. For example, GL4C (Table 1.1) may represent vegetation that is of the habitats GS1, GS3 or GS4). In the IVC, there are four major groups that can be used to describe the GS1, GS3, and GS4 grasslands. Groups GL1 and GL2 can correspond with the GS4 grasslands, GL3 with the GS1 grasslands, and GL4 with the GS3 grasslands. The GS2 and GM1 habitats are also represented with the GL grassland division. Each group has approximately 4–6 communities, which demonstrates the greater resolution in which the IVC describes the vegetation when compared to Fossitt.

Table 1.1: Comparison of semi-natural grasslands across different classification systems. Abbreviation explanations provided in notes below table.

Habitat	Description	IVC	Annex I
GS1 – Dry calcareous and neutral grassland	Unimproved and semi-improved dry grassland, associated with well drained soils.	GL3A, GL3B, GL3C*, GL3D, GL3E*, GL3F, GL4A*, GL4C*#	6210[*], 5130, 6130
GS2 – Dry meadows and grassy verges	Found on free draining soils and is often ungrazed. Maintained by mowing. Can range	GL3C*, GL3E*, GL3G*	6510

	from hay meadows to road verges and banks.		
GS3 – Dry-humid acid grassland	Found on free-draining soils, that are acidic. Can be of upland grasslands, but also occur in lowlands where siliceous soils occur.	GL4A*, GL4B, GL4C*#, GL4D	6230, 6130
GS4 – Wet grassland	Found on poorly drained, often waterlogged soils. These grasslands may also be seasonally flooded. Can be variable in species composition from rush dominated pastures to diverse callow grasslands.	GL1A*, GL1B*, GL1C, GL1D*, GL1E, GL2A*, GL2B, GL2C*, GL2D, GL3G*, GL4A*, GL4C*#, GL4D*,	6410
GM1 – Freshwater marsh	Found where waterlogged soils occur and are near lake and river edges, where the water table remains close to the surface.	GL1A*, GL1B*, GL2A*	6430

Notes:

Annex I Habitats:

5130 = ‘*Juniperus communis* formations on heaths or calcareous grasslands’

6130 = ‘Calaminarian grasslands of the *Violetalia calaminariae*’

6210[*] = ‘Semi-natural dry grasslands and scrubland facies on calcareous substrates (*Festuco-Brometea*) (* Priority habitat)’

6230 = ‘*Species-rich *Nardus* grasslands on siliceous substrates in mountain areas’

6410 = ‘*Molinia* meadows on calcareous, peaty or clayey-silt-laden soils (*Molinion caeruleae*)’

6430 = ‘Hydrophilous tall herb fringe communities of plains and of the montane to alpine levels’

6510 = ‘Lowland hay meadows (*Alopecurus pratensis*, *Sanguisorba officinalis*)’

IVC Communities

<https://biodiversityireland.ie/ivc-classification-explorer/>

* = The IVC could be represented by two or more different Fossitt habitats (e.g. GL1A = GM1 Marsh/GS4 Wet Grassland). *# May be represented by three Fossitt habitats.

Full names for IVCs encountered in StableGrass and ISGS surveys are given in Appendix 1.

1.8.4 Threats to Irish Semi-natural grasslands

Despite few detailed studies focusing on threats such as agricultural intensification, abandonment, and afforestation to Irish semi-natural grasslands, the impacts described in Section 1.6 are anticipated to be similar. Land-use changes, for example, including agricultural intensification and afforestation are two of the greatest threats faced by Irish semi-natural grasslands. Over 70% of Ireland’s Annex I habitats are reported as being negatively impacted by agricultural activities (NPWS, 2019a). Buscardo et al., (2008) investigated the impact of afforestation on Irish wet grasslands and found that the plant diversity was greatest in grasslands prior to afforestation, with five years’ post-afforestation showing significant declines in plant richness and changes to the species composition. Abandonment, as discussed in Section 1.6.2, is also a significant threat to Irish semi-natural grasslands, especially in areas that are hard to reach with machinery. In Ireland, this can also result in increased land-use change, with abandoned grasslands having a greater likelihood of being converted to forestry (Buscardo et al., 2008). Unproductive grasslands, such as semi-natural grasslands are also more likely to be afforested (Heritage Council, 1999) and it is estimated that approximately 46,200 hectares of grassland were converted to woodland between 2000–2018 (CSO, 2023).

Agricultural intensification since the 1900s, largely driven by increase in mechanisation, creation of arterial drainage schemes, and the application of fertiliser (O’ Neill et al., 2013) and by policies such as the 1962 EU Common Agricultural Policy, led to over 92% of Ireland’s current agricultural area being devoted to grasslands and

utilised for dairy or silage production (DAFM, 2023). This land-use change had significant effects on the area, quality and diversity of semi-natural grassland habitats. McKeon et al., (2022) demonstrated that when grasslands of the Burren were abandoned and subjected to increased nutrients, the species richness and Shannon's Diversity reduced significantly. Keane and Sheehy Skeffington (1995) reported that changes to the plant species composition, as well as reduced species diversity were demonstrated in semi-natural grasslands of the Burren that were undergoing regular fertiliser addition and re-seeding. These studies corroborate the findings of many European studies on the impacts of abandonment and eutrophication on semi-natural grasslands (e.g. Bobbink, 1991; Habel et al., 2013; Harpole et al., 2016; Prangel et al., 2023). While most existing research that describes the threats faced by semi-natural grasslands being of a European or global context, these threats and their effects are occurring in similar ways in Irish semi-natural grasslands.

1.8.5 The Irish Semi-Natural Grassland Survey 2007–2012

The Irish Semi-natural Grassland Survey 2007–2012 (ISGS) surveyed 1,192 semi-natural grassland sites (Figure 1.4) throughout Ireland, and collected 4,544 grassland relevés (O' Neill et al., 2013). This was the first nationwide survey of Ireland's semi-natural grasslands. The ISGS provides an overview of the different types and extent of semi-natural grasslands in Ireland and a conservation assessment for each of the assessed grassland types. As well as providing this valuable information on Ireland's semi-natural grasslands, the survey also identified and assessed 324 Annex I grassland sites. Over the past decade, a considerable amount of baseline data on Irish semi-natural grasslands and their conservation status, in both an Irish and EU context has been collected.

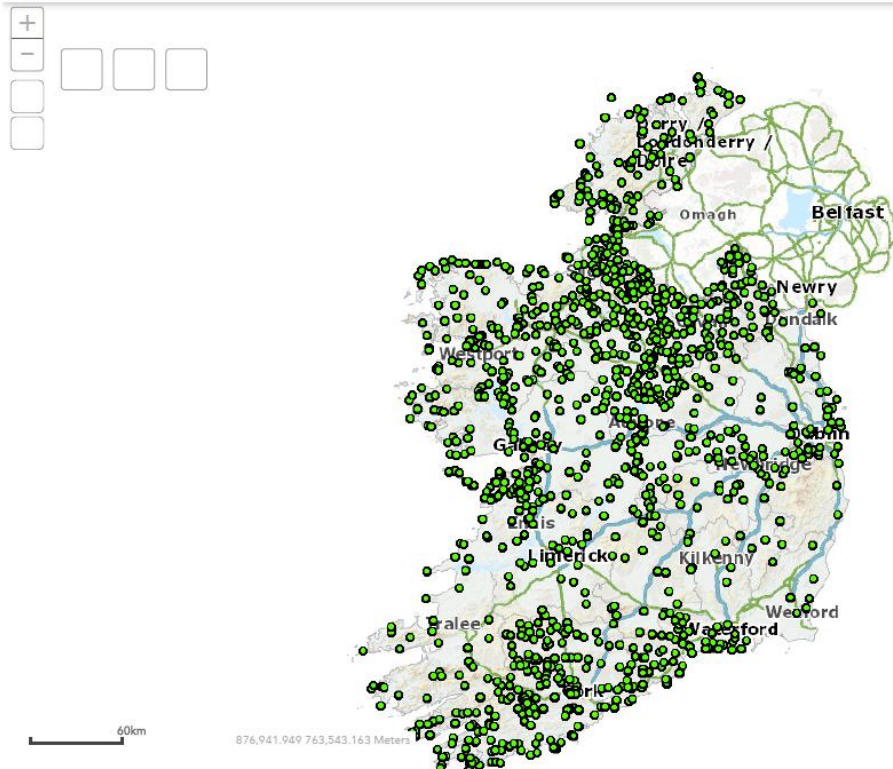


Figure 1.4: Locations of ISGS Study Sites in Ireland (O' Neill et al., 2013). Map courtesy of NPWS.

The ISGS substantially increased the knowledge of Irish semi-natural grasslands and their locations (Figure 1.4); however, this is unlikely to account for the full coverage of semi-natural grassland in Ireland. The ISGS surveyed 23,188.1 hectares of semi-natural grassland (O' Neill et al., 2013), which is approximately 48% of the total area of semi-natural grassland in Ireland according to the CORINE land cover (imagery of land-uses in Ireland and Europe through the Copernicus Land Monitoring Service) (CSO, 2023). From a conservation perspective, the area of semi-natural grassland that receives protection is small and often restricted to the Annex I protected grasslands (O' Neill et al., 2013; Lynn and O' Neill, 2019). As of the 2019 Article 17 reporting, the six Annex I grassland habitats had a total area of 2,879.6 hectares (12.4% of the area of grassland identified in ISGS) (NPWS, 2019b), compared to the 23,188.1 hectares of semi-natural grassland habitat identified in the ISGS (O' Neill et al., 2013). Also, very few of the semi-natural grassland sites identified in the ISGS have been re-visited and monitored. For example, only 110 of the 1,192 ISGS semi-natural grassland sites (Annex I sites) have been re-surveyed during the 2015–2017 Grassland Monitoring

Survey (Martin et al., 2018). As a result, a considerable area of semi-natural grasslands in Ireland is expected to be without official protection. This means there is a sizable research gap, especially for the future assessment of the condition of non-Annex I semi-natural grasslands in Ireland. Using the ISGS as a baseline study can help to determine if the grasslands that have not been re-surveyed are also facing the same threats and on similar trajectories as the Annex I grasslands that have been repeatedly monitored.

1.8.6 Status of grasslands in Ireland

The status of semi-natural grasslands in Ireland is largely assessed through the network of six Annex I grassland habitats (O' Neill et al., 2013; Table 1.1), which would encompass some of the Fossitt grassland habitats described in Table 1.1 (Fossitt, 2000). These Annex I grasslands have experienced a decline in conservation status through the various monitoring periods (e.g. 2013, 2015–2017, and 2019) (NPWS, 2013b; 2019b; Martin et al., 2018). In the 2013 Article 17 reporting by NPWS, five of the six Annex I grassland habitats were found to have a Bad overall status, with four of these having decreasing trends (NPWS, 2013b). In the most recent Article 17 reporting in 2019, all six of the Annex I grasslands had a bad overall status with trends that continued to be on the decline (NPWS, 2019a). The changes may be less defined between the two monitoring periods when each parameter is assessed. For example, the Range of '*Semi-natural dry grasslands and scrubland facies on calcareous substrates (Festuco-Brometalia)*' (6210) remained stable in both monitoring periods. The Area of this habitat went from stable in 2013 to decreasing in 2019. In the interim between these two reporting periods, an assessment and monitoring survey was conducted on the 6210, 6410 (*Molinia* meadows on calcareous, peaty or clayey-silt laden soils (*Molinion caeruleae*)), and 6510 (Lowland hay meadows (*Alopecurus pratensis*, *Sanguisorba officinalis*) Annex I grasslands, repeating approximately 110 of the ISGS sites. As with the previous 2013 reporting, and the subsequent 2019 reporting, the trends in range, area, and structure and functions were largely Unfavourable–Bad (Martin et al., 2018). This survey found that the 6210 habitat was the most threatened by habitat loss, with 31% of the area of surveyed 6210 being lost

due to agricultural intensification. In the 6410 habitat, the largest threats were habitat degradation, caused by both abandonment and intensification, as well as afforestation. The 6510 habitat were the most threatened of the three, with only three sites receiving Favourable Structures & Functions.

The conservation status of some semi-natural grasslands in Ireland, in particular the Annex I grasslands are well known, through the series of monitoring and reporting in the past decade. However, as this has been very focused on Annex I areas, little is known about the status or composition of the large area of semi-natural grasslands that occur outside the conservation areas or are not of Annex I habitat quality (approximately 95% of the ISGS grasslands). For example, in the ISGS, only 324 sites were mapped as Annex I grassland, with the remaining 868 sites having no Annex I grassland habitat (O' Neill et al., 2013). To the best of the author's knowledge, the majority, if not all the non-Annex I grassland sites have not been re-surveyed or monitored since the last ISGS (Pers., Comm., Long, 2024). This means that potential changes in species diversity, and plant species composition may be going unchecked since the last ISGS, be it from agricultural intensification, abandonment, or other unseen drivers of change.

1.9 Conclusion

While Ireland has a large coverage of grasslands, only a small percentage of these are semi-natural grasslands. These grasslands are of importance globally and nationally for biodiversity and provide a range of ecosystem services. Elevated plant species diversity is a key component of these grasslands, and higher diversity is associated with improved ecosystem functioning and resilience. In Ireland, it is likely that most semi-natural grasslands are experiencing declines in species diversity, or will in the future, but the research into this has been limited to grasslands of conservation importance.

In recent years, Irish semi-natural grasslands have received some attention in terms of the conservation value and the threats that they face. This was highlighted by the 2007–2012 Irish Semi-natural Grassland Survey. This survey provided baseline

information on the extent of semi-natural grasslands in Ireland, their conservation status, and the Annex I grassland habitats present. As described above, recent monitoring has been focused on protected grassland habitats. These protected habitats, while often the best examples of good quality grassland, may not adequately cover the national range of Ireland's semi-natural grasslands. Hence, additional work is needed to assess the current diversity and species diversity of these unvisited grasslands.

1.10 Aims & Objectives

This project forms a component of the larger Department of Agriculture, Food and the Marine-funded project StableGrass (Impact of plant diversity on carbon storage and yield stability in semi-natural grasslands). StableGrass aims to address the question of how plant diversity affects the below-ground carbon storage and yield stability of semi-natural grasslands in Ireland. This masters project focuses on the plant diversity aspect of StableGrass. The findings of this study regarding grassland diversity will be related to the functional traits of the grasslands and yield and productivity data collected by remote sensing technology by other team members.

The overall aim of this thesis specifically is to assess whether changes in plant species diversity and species composition have occurred in selected Irish semi-natural grasslands since the previous 2007–2012 Irish Semi-natural Grassland Survey (ISGS) and to begin to fill the gap in knowledge of how plant species diversity non-Annex I grasslands may have changed since the initial survey period. This will be achieved through the following:

1. Twelve sites representing three habitat types (GS1, GS3, GS4) will be selected from the ISGS and resurveyed to determine their current diversity and composition.
2. Detailed vegetation classification will be conducted to describe the vegetation types observed in the sites.
3. Changes in diversity and species composition since the ISGS will be assessed.

4. The implications of these findings on the conservation and structure of the grassland will be discussed.

Chapter 2 - Methodology

2.1 Overview

This study uses the 2007–2012 Irish Semi-natural Grassland Survey (ISGS) (O’ Neill et al., 2013) as a diversity baseline and involves the selection of a subset of the ISGS sites for repeat surveying of the relevés collected in the ISGS survey. These repeat field surveys largely followed the field methodology used in the ISGS. This chapter presents the site selection process, field survey methodology, soil analysis, and subsequent data analysis for the project. From here-on-in, the 2007–2012 ISGS survey will be referred to as the ‘ISGS’ survey and the 2023 StableGrass survey will be referred to as the ‘StableGrass’ survey.

2.1.1 Study Area

The selected study area for this project encompasses Leitrim, Mayo, Roscommon, Galway, Clare, Limerick, Cork, and Kerry. Ireland has a mild temperate and oceanic climate but there is localised variation, especially when comparing the two extremes of the county range in the study (Leitrim – Kerry). The average 1991–2020 July temperature for Leitrim was 14.9 °C (Belmullet weather station) and 15.3 °C for Co. Kerry (Valentia weather station). The average 1991–2020 July rainfall was 85.9 mm, and 117.2 mm respectively for these two counties (Met Éireann, 2023). While variations in weather and microclimate cannot be discounted, they are not anticipated to have a major impact on vegetation across the sites in the study. The sites also have variations in geography, e.g. elevation and topology (lowland vs upland sites), geology and other environmental factors, namely soil type, and hydrological conditions. Twelve sites were selected across these counties.

2.1.2 Site Selection

Twelve sites were selected from the ISGS (O'Neill et al., 2013), which is available as a GIS resource and as an Access database¹. Three habitat types: GS1 – Dry calcareous & neutral grassland, GS3 – Dry-humid acid grassland, and GS4 – Wet grassland (Fossitt, 2000) were selected (Figure 2.1). These were the three most frequently recorded habitats in the ISGS and were also expected to be more representative of the semi-natural grassland cover in Ireland. The remaining two grassland habitats in the ISGS (GS2 and GM1) have relatively low cover in Ireland and were not included in this study. For each of the three selected habitats, four sites were chosen. A subjective approach was taken to the site selection process due to practical constraints on the project.

¹ <https://data.gov.ie/dataset/irish-semi-natural-grassland-survey-2007-2012>



Figure 2.1: The three Fossitt habitat types selected for this project. A) GS1: Dry calcareous and neutral grassland (Site 3 (ISGS ID 1624), Burren, Co. Clare). B) GS3: Dry-humid acid grassland (upland) (Site 6 (ISGS ID 2429), Lauragh, Co. Kerry). C) GS4: Wet grassland (Site 10 (ISGS ID 2719), Lough Gur, Co. Limerick)

The criteria for site selection were: 1) Located in the 12 counties listed above; 2) The sites had an area greater than 9 hectares to ensure that they were suitable for the remote sensing and aerial imagery analysis for StableGrass WP4; 3) Had a semi-natural grassland habitat cover of >55% of the target habitat type; and 4) Consisted of a large continuous area of habitat as opposed to some sites containing numerous, separated grassland parcels. Neither existing diversity nor Annex I habitat status were considered during the site selection process to ensure bias was minimised and that a range of diversities were encountered. After these criteria were applied to the site list from the ISGS, the site list was grouped by habitat (GS1, GS3, GS4). From each habitat, 12 sites were randomly selected, giving a total of 36 provisional sites. A total of eight sites for each habitat were selected from this list based on its suitability for remote sensing (as determined by Dr. Sam Hayes, Post-doctoral Researcher in UCC). Four of these sites were the intended study sites, with the remaining four sites acting as back-up sites in the event of refusal of landowner permission, or the site was agriculturally improved since the ISGS.

Prior to commencement of field surveys, the Land Direct (www.landdirect.ie) public land registry was consulted to establish land ownership details to obtain permission to access the sites. Initial site visits were also made to locate landowners. For 10 sites, permission to survey the site was received on the first instance. To fill the remaining two sites, three back-up sites were required. However, these were also deemed unsuitable, so the site selection process was repeated to include counties Leitrim and Mayo and resulted in the selection of the final two sites (Sites 11 & 12, respectively).

Other permissions also had to be considered. For example, some sites had national monuments on site, and/or were in Special Areas of Conservation. This means that the survey work became an Activity Requiring Consent (ARC) (European Communities (Birds and Natural Habitats) Regulations 2011). To comply with Section 5 (8) of the 1987 Act (Register of Historic Monuments) and the National Monuments (Amendment) Act 1994, a Section 12 (3) 'Notification to the Minister for Housing, Local Government and Heritage' form had to be completed. This specifically concerned the collection of a soil sample from relevés. Permission to conduct soil analysis was received for most relevés, with some conditions. For sites that were in

an SAC (e.g. Sites 4 & 12), a form to request permission to perform the survey work had been completed to comply with Regulation 30 of the European Communities (Birds and Natural Habitats) Regulations 2011 (No. 477 of 2011). Permission documentation is listed in Appendix 2. A final site list with their locations can be found in Table 2.1, and Figure 2.2. The 12 sites ranged in area from 20.112 – 178.821 hectares, and in elevation, on average 10 – 820 metres.

Table 2.1: Location of selected sites used in the study including date surveyed in the ISGS, county, location, the primary (dominant) ISGS habitat and the area and elevation

Site	ISGS ID	ISGS Date	County	Location	Primary Habitat	Area (Ha)	Elevation (Max, m)
1	215	18/07/2007	Roscommon	G 82500 01400	GS1	29.5	110
2	227	31/07/2007	Roscommon	M 87700 74900	GS1	21.63	60
3	1624	09/05/2011	Clare	M 25254 00909	GS1	27.35	210
4	2702	21/05/2012	Limerick	R 84850 24237	GS3	103.02	820
5	642	21/07/2008	Cork	W 21319 80511	GS3	28.01	470
6	2429	13/08/2012	Kerry	V 76302 54261	GS3	22.63	380
7	2344	10/09/2012	Galway	M 86313 04265	GS4	49.77	40
8	1604	05/09/2011	Clare	R 53123 87045	GS4	64.26	200
9	2722	09/05/2012	Limerick	R 20388 44482	GS4	36.06	100
10	2719	12/06/2012	Limerick	R 60614 40960	GS4	35.10	80
11	825	31/08/2009	Leitrim	G 82159 38391	GS1	178.82	380
12	1854	17/05/2011	Mayo	M 13204 60423	GS3	26.11	20

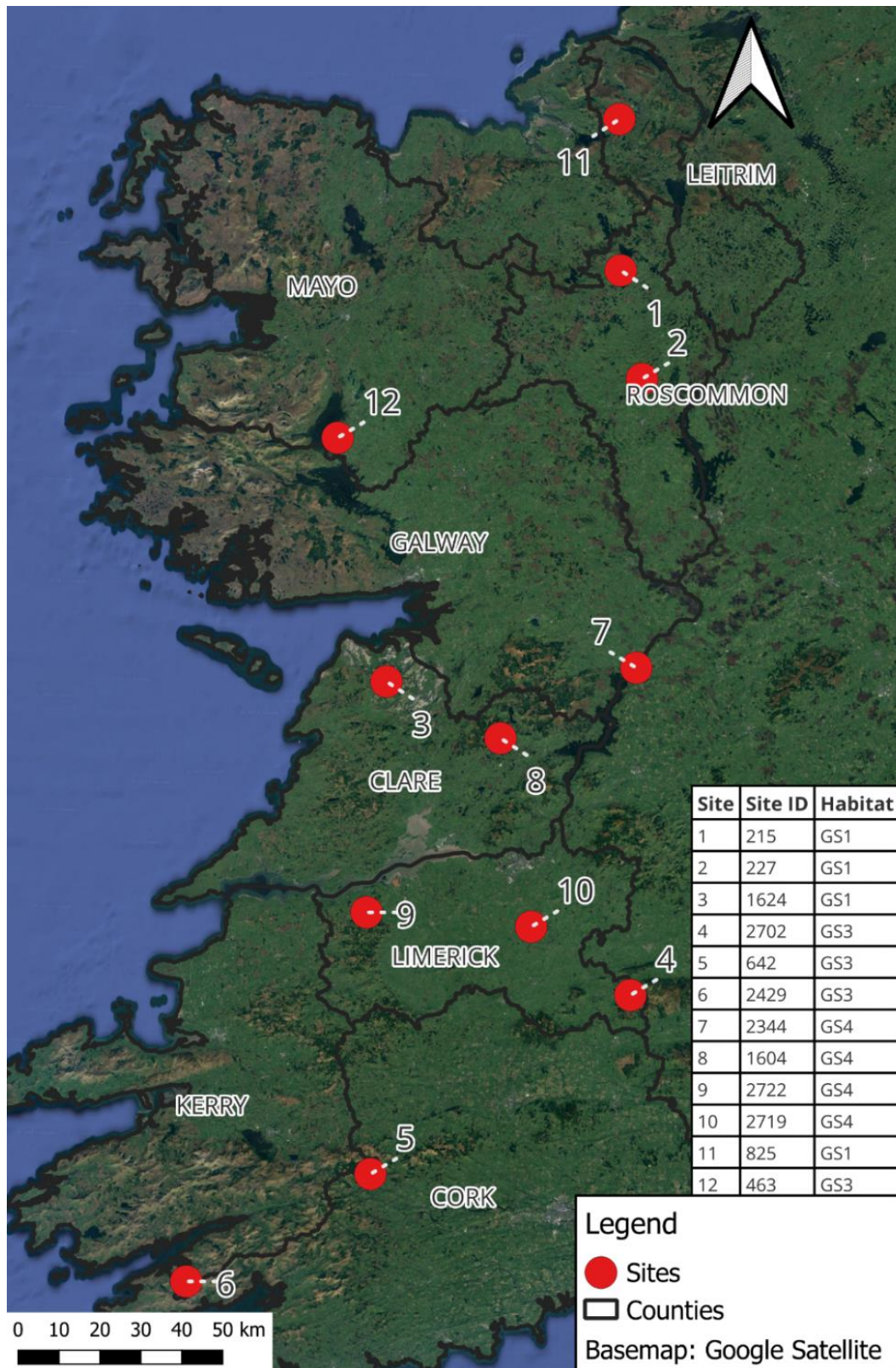


Figure 2.2: Map showing the distribution and approximate location of the 12 study sites is (GS1 = 4 sites, GS3 = 4 sites, GS4 = 4 sites). ISGS and habitat for each site is included on the figure. Individual site maps are presented in Appendix 3

2.2 Field Surveys

2.2.1 General Site Survey

Brief, but more detailed site descriptions for each individual grassland study site can be found in Appendix 4. These notes detail the site location, dominant habitat types, total number of relevés recorded, and other notes of ecological interest. Notes are also supplemented from information gathered from the ISGS survey (O' Neill et al., 2013), such as variables such as Soil Types, Geology, elevation etc, were already established from desk studies during the ISGS and are not expected to have changed between the two survey periods. Detailed location information, such as grid references can be found in Table 2.1.

Fieldwork was conducted between the 12th of June 2023 and the 08th of August 2023, with a day given for each site survey, resulting in a total of twelve field days in this period. The field surveys were assisted by Phoebe O'Brien (Independent botanical consultant, with a particular expertise in grasslands) to ensure that plant identifications were accurate and that the methodology from the ISGS was followed consistently. A decision was made on the day whether the site was suitable to survey as a 'semi-natural grassland' habitat and had not been improved/afforested/completely abandoned. Each site was deemed suitable to survey. A weather resistant Android tablet was used for data collection. Microsoft Excel was loaded on the tablet for data entry.

During the site survey some general information was collected to describe the site. This information included other internal and adjacent habitats (according to (Fossitt, 2000)), site management (from visual inferences), damaging operations, and general site notes (Appendix 5). Unlike the ISGS survey, habitat mapping was not conducted in the StableGrass survey, as this was not the objective of this study. It was not expected that the Fossitt habitat types would have changed considerably between the surveys. However, instances such as scrub encroachment, where scrub becomes the dominant habitat (WS1) or conversion to intensive agriculture were noted.

2.2.2 Relevé Survey

Relevés (2 m x 2 m quadrats) were collected to describe the current vegetation composition and diversity at the selected StableGrass sites. This is a common method used for both phytosociological investigations and repeat monitoring surveys (Weekes and FitzPatrick, 2010). A four m² relevé is the standard unit for grassland vegetation studies (Weekes and FitzPatrick, 2010) and was the relevé size used in the ISGS (O' Neill et al., 2013). As the StableGrass study is a repeat survey of the ISGS sites, the relevés from the ISGS were selected and repeated, where possible. Additional relevés were conducted on some sites, for one of the following reasons: a relevé no longer being accessible; a relevé being improved; or because an extra relevé(s) was deemed beneficial to further describe the vegetation at the site. This has implications for data analysis, which are described below in Section 2.5. The relevés were relocated using a GPS unit (Garmin GPSmap 62st). These GPS units are reported to be accurate to be within 15 meters (95% of the time), with an accuracy of 5-10 meters to be more typical. This may have implications for the relocation of the ISGS relevés, as described in this section, but also in Section 4.6.1. An updated 10-figure grid reference was recorded for each relevé collected during the StableGrass survey (Appendix 6, Sheet 3). This grid reference was taken from the South-west corner of the relevé. A total of 54 relevés were collected across the 12 sites, with 45 of these being replications of those recorded in the ISGS.

There may have been a degree of relocation error when using the GPS unit to relocate these non-permanent relevés (Kapfer et al., 2017). The distance between the original ISGS relevé locations and the StableGrass relevé locations was assessed using QGIS. The distances between the two locations was approximately 1-9 metres, which is considerably lower than in other studies that focused on relocation error (e.g. Verheyen et al., 2018). Statistical analysis (Wilcoxon-signed Rank Test) was conducted to determine if the X and Y coordinates differed significantly between the two surveys to determine if relocation error may have occurred. There was no significant difference found in the position of the X coordinates in comparison to the ISGS ($p = 0.718$). However, the position of the Y coordinates did differ significantly ($p = 0.005$). This suggests on the East–West axis there was no change in the position of the

relocated plots, but they did change position on the North–South axis. However, as discussed above this was not expected to impact the results too greatly because other measures, such as using the ISGS species lists, and site notes will have helped to relocate the relevé in an area of vegetation that was representative of the initial survey.

For each relevé, all vascular plants and bryophyte species were identified, following the nomenclatures of Stace (2019) and Atherton (2010), respectively. All species were recorded to species level, except for particularly difficult species, for example, *Hieracium spp*, non-flowering *Viola riviniana/reichenbachiana*, non-flowering *Carex spp* (where vegetative ID would have been doubtful). Bryophytes were recorded in the field, when possible, but for difficult taxa, specimens were vouchered and identified in the lab. For each species, a percentage cover scale was applied. This scale was as follows: Covers below 10%: 0.1%, 0.3%, 0.5%, 0.7%, 1%, 3%, 5%, and 7%. Covers above 10% were applied in 5% intervals. This scale was recommended by O’Neill et al., (2013). The DOMIN cover was also applied.

Environmental variables were collected within each relevé to describe the sward structure (Table 2.2). These variables were used to indicate the level of management at the sites, with sward height being an indicator for grazing intensity for example (Parr et al., 2009). A single soil sample of approximately 300–400 grams per relevé was taken from the centre to a maximum depth of 15 cm. The soil sample was collected from below the vegetation surface. In the case of very shallow soils, a couple of smaller sample points within the relevé would be taken to get an adequate quantity of soil. Soil samples were refrigerated on the day of collection until they were analysed.

Table 2.2: List of environmental variables collected for each relevé. Units are given in brackets. If calculations are required, formulas are also included.

Forb Cover (%)	Bryophyte Cover (%)
Graminoid Cover (%)	Litter (%)
Forb:Graminoid Ratio (forb cover/(forb cover + graminoid cover) x 100)	Bracken (%)
Median sward height (cm) (of 4 random locations in the relevé)	Scrub (%)
Bare rock (%)	

2.3 Vegetation classification and assignment of Ellenberg values

Two vegetation classification systems were used during this study: 1) The original Fossitt habitat applied to the relevés during the ISGS and 2) The Irish Vegetation Classification (IVC) system. These have been described in Section 1.8.3. The Irish Vegetation Classification (IVC) system was used to classify each relevé as it provides a more comprehensive classification system than Fossitt. It is also more suitable for vegetation monitoring because it is more sensitive to changes due to a greater range of communities (Perrin, 2015). ERICA (Engine for Relevés to Irish Communities Assignment: <https://biodiversityireland.shinyapps.io/vegetation-classification/>) is an online webapp provided by the IVC. This system utilises fuzzy clustering to assign the relevé to the best match out of an existing database of grassland relevés (Perrin, 2019). This clustering system assigned each relevé to a community for which the vegetation in the relevé has the closest membership to, in comparison to the existing database of relevés and vegetation communities. A degree of membership is used to determine the goodness of the fit of the community to the relevé. For example, a plot membership of ≥ 0.5 for a vegetation community, and refers to the core definition of the vegetation community is an 'Assigned' plot; plot membership of ≥ 0.5 , but is poorly represented by the classification scheme is 'Unassigned'; and a plot membership of < 0.5 , but the community falls within the scope of the current classification system is 'Transitional' (Perrin, 2019). A full description of the analysis

conducted for ERICA is provided in Perrin (2019). IVCs were applied to both the StableGrass relevés, and the ISGS relevés. Prior to using ERICA, all species nomenclature was adjusted to follow the 2008 Irish Plant Checklist (Ireland2008) (Weekes and FitzPatrick, 2010). Species that were recorded to genus level (i.e. *Carex spp*) were removed from the dataset prior to analysis in ERICA.

As well as assigning an IVC to each relevé, the ERICA app was used to assign each relevé with an Ellenberg Indicator Value (EIVs). These included values for Wetness, Light, Reaction (pH), Nitrogen and Salinity. These values attempt to make inferences on the environmental conditions present in the plot, based on a set of indicator values assigned to each species. These values are available for most plant species in Britain & Ireland and when applied to each species, reflects the realised niche of that species, in particular for the typical light, soil pH, moisture and nutrient conditions that the species grows under (Hill et al., 1999). The Ellenberg Indicator Values (EIVs) are scale values, with a scale of increasing availability of the indicator (e.g. Moisture: 1: Indicator of extreme dryness → 12: Submerged plant, permanently or almost constantly under water). See (Hill et al., 1999, pp.5–7)² for the scale used for each of the EIVs. They can be used as proxies for the environmental conditions based on the flora present (Diekmann, 2003). For each relevé, ERICA presented the mean EIV for the four indicators listed above. To account for the influence of species abundances, the abundance-weighted mean was reported for each EIV for each relevé.

2.4 Soil analysis

Soil samples were collected for all relevés in seven sites (Sites 2, 4, 6, 8, 9, 10 and 12). The remaining five sites were not sampled for soil in 2023 due to delays with gaining permission from the National Monuments Service and NPWS (see Section 2.1.2). A complete soil sampling programme was conducted in 2024 by Eoin Halpin (StableGrass, UCC), with one subset of these samples being sent to an external laboratory (IAS Labs Ltd.) and the other set analysed internally in UCC. The results

² <https://nora.nerc.ac.uk/id/eprint/6411/1/ECOFAC2a.pdf>

from the 2024 soil sampling protocol, and subsequent analysis do not form part of this thesis.

The soil samples collected from the seven sites were analysed for the soil pH, % Soil Organic Matter (SOM), and nutrient status estimates internally at the University of Galway. To measure the soil pH, a 1:2.5 soil to deionised water solution was created, stirred and left to settle overnight, as recommended by various methodologies (e.g. van Reeuwijk, 2002). Once the solution settled, the pH was measured by placing a calibrated pH probe into the first 2 cm of the solution, above the settled solids.

The % Soil Organic Matter was determined through the Loss on Ignition (LOI) procedure (Howard and Howard, 1990; Heiri et al., 2001). Prior to this, the 100 grams of fresh soil were oven dried at 100 °C overnight. Once cooled, they were sieved using a 2x2 mm sieve to ensure no material such as plant fragments or roots were present. Five grams of the oven dried and sieved soil was measured into a crucible (that was previously put through a muffle furnace cycle to remove any residue) and placed into a muffle furnace for 2 hours at 500 °C. LOI is calculated using the following formula $LOI = ((W_1 - W_2)/W_1) * 100$, where W_1 is the weight of the sample before LOI, and W_2 is the weight of the sample, minus the weight of the crucible after LOI.

2.5 Data Analysis

2.5.0 Overview and data treatment

Two datasets were used for data analysis: 1) ISGS relevé data for selected sites (n = 51 relevés) and 2) StableGrass relevé data collected for the 12 sites in 2023 (n = 54 relevés). As described in Section 2.2.2, the relevés from the ISGS were repeated when possible. However, this was not always the case. A total of 44 relevés were directly comparable between the ISGS and StableGrass surveys. The additional relevés which are not paired, are not included in certain analyses (Sections 3.2.3, 3.3.1 and 3.3.2). Some data treatment was required prior to analysis, especially regarding the ISGS data. As the ISGS used the DOMIN scale to assign cover to the relevé data, conversion to percentage cover was required to allow for quantitative analysis, and for comparisons to be made to the StableGrass data. The conversion was done using mid-

range values (Table 2.3), as recommended by O’ Neill et al., (2013). All data analysis was conducted in the R Statistical Environment (R Core Team, 2024) (Appendix 7), with the exception of the assignment of IVCs, which was conducted in ERICA.

Table 2.3: Conversion of DOMIN cover values to percentage cover (O’ Neill et al., 2013).

DOMIN Scale	Range (%)	Mid-range value (%)
10	91-100	96
9	76-90	83
8	51-75	63
7	34-50	42
6	26-33	30
5	11-25	18
4	5-10	8
3	1-4	3
2	<1	0.5
1	<1	0.3
+	<1	0.1

2.5.1 Calculation of diversity indices

Diversity indices, namely Species Richness, Shannon’s Diversity, Shannon’s Evenness, Gini-Simpson’s Diversity, and Simpson’s Evenness were calculated for all relevés. From here on in, the ‘Gini-Simpson Diversity index’ will be referred to as the ‘Simpson’s Index’. These indices have been calculated using the ‘specnumber’ (Species Richness) and ‘diversity’ functions of the ‘Vegan’ package (Oksanen et al., 2024). In this case, Species Richness refers to the number of unique species present in the relevé. As the Simpson’s Diversity and Simpson’s Evenness are more robust to smaller sample sizes, and are easier to interpret (Magurran, 2004), these two indices, along with Species Richness are presented in Section 3.2. The Shannon’s Diversity and Evenness indices are presented in Appendix 6 (Sheet 2). The formulas used in these calculations are provided in Table 2.4.

Table 2.4: Formulas used to calculate the Shannon’s Diversity, Shannon’s Evenness, Simpson’s Diversity and Simpson’s Evenness diversity measures (Hill, 1973)

Diversity Measure	Formula
Shannon’s Diversity	$H = -\sum_{j=1}^S p_j \ln p_j$
Shannon’s Evenness	$E_H = H/\ln(S)$
Simpson’s Diversity (Gini-Simpson is used as ‘Simpson’s Diversity’ here-on-in)	$D = \sum_{i=1}^S p_i^2 \rightarrow \text{Gini-Simpson} = (1/D)$
Simpson’s Evenness	$E = \frac{(1/D)}{S}$
<p>Notes: p_i = the proportion of species (i) out of the total number of individuals. \ln refers to the natural log. H = Shannon’s Diversity. E_H = Shannon’s Evenness. S = number of species. D = Simpson’s Diversity. E = Simpson’s Evenness.</p>	

2.5.2 Differences in diversity across habitats, sites and surveys

To determine the differences in the three selected diversity measures (Species Richness, Simpson’s Diversity and Simpson’s Evenness), analysis using ANOVA tests (normal data) or Kruskal-Wallis test (non-normal data) was conducted across both the three habitats (in the case of the Relevé and Site habitats), and the 12 sites. A Shapiro-Wilk test was conducted on the diversity data to assess the normality and determine the appropriate test. A Kruskal-Wallis test was used to test for differences in Species Richness across the relevé habitats. An ANOVA test was used to test these differences in the Simpson’s Diversity and Simpson’s Evenness indices. As well as testing for a significant difference in the diversity indices across the three habitats, pairwise analysis was conducted. For Species Richness, a Pairwise Wilcoxon Rank Sum test (for non-normally distributed data) was used for the pairwise analysis. This test compared the Species Richness of GS1 to GS3, GS1 to GS4 and GS3 to GS4, and determined if any of these pairwise comparisons were significant. This pairwise analysis was repeated for the Simpson’s Diversity measure by using the Tukey’s HSD test (for normally distributed data).

The differences in diversity across the relevés within each habitat were also tested. The relevés of the GS1, GS3 and GS4 habitats were divided into habitat specific datasets. Within each habitat dataset a Kruskal-Wallis test was used to test for differences in the Species Richness and Simpson's Diversity across the four sites within each habitat type using 'Site' as a grouping variable, and the same procedure, using an ANOVA for the Simpson's Evenness. For the analysis of differences in the three diversity measures across sites, a Kruskal-Wallis test was used to test Species Richness and Simpson's Diversity, and an ANOVA test for the Simpson's Evenness measure.

The differences in the three diversity measures between the ISGS and StableGrass surveys was assessed using paired analysis. This analysis was conducted on the diversity indices calculated for the paired relevés of the two surveys (n = 44 relevés for both surveys). Shapiro-Wilk tests were conducted to determine the normality of the data with Paired T-tests used when the differences in the diversity measure between the two surveys were normally distributed; and a Wilcoxon signed-rank test if the differences were not normally distributed. A Paired T-test was used to test for significant differences in the Species Richness and Simpson's Diversity between the two surveys. A Wilcoxon signed-rank test was used to test the differences in the Simpson's Evenness between the two surveys. Differences in diversity of each habitat between the two survey periods were also assessed. This was conducted by analysing differences in the diversity measures by separating the dataset into three separate subsets. A subset was created for each habitat containing all the relevés of that habitat (GS1, n = 17; GS3, n = 13; GS4, n = 24). Within each habitat subset paired analysis was conducted, with the test dependent on the data normality. For the GS1 and GS3 datasets, Paired T-tests were used to test for significant differences in the Species Richness, Simpson's Diversity and Simpson's Evenness measures of these habitats between the two survey periods. For the GS4 dataset a paired t-test tested for significant differences in the Species Richness and Simpson's Diversity measures of the GS4 habitat between the two survey periods. A Wilcoxon signed-rank test was used to test for differences in the Simpson's Evenness of the GS4 habitat between surveys.

2.5.2.2 Definitions pertinent to interpretation of differences in diversity

Some definitions are provided here to describe the different scales at which the differences in diversity are assessed. These definitions are pertinent to Section 3.2.

- 1) Site:** A defined parcel of land that was surveyed as part of the ISGS study. These land parcels in the ISGS are demarcated by the polygons mapped by the ISGS to denote each area of semi-natural grassland habitat. In StableGrass there are 12 Sites each of which were previously surveyed during the ISGS. Each Site has an individual Site ID that is the same as during the ISGS and associated site boundaries that were mapped in the ISGS. The sites all also have a StableGrass number between 1 and 12. A site may have many land parcels or may be one large continuous area with no divisions in land parcels. Within each Site, relevés were collected to assess the vegetation composition and diversity.
- 2) Site Habitat:** During site selection a habitat type which aimed to encompass the Site was applied to each site. This allowed for the selection of four sites for each of the three habitats mentioned above. For each site surveyed by the ISGS, data were provided on the percentage cover of each semi-natural grassland habitat within each site (e.g. 60% GS1, 20% GS4, 20% GM1). For the purposes of site selection, these data were used to assist in selecting the four GS1, GS3 and GS4 sites. A site was selected for each habitat if it had a habitat cover of >55% of the desired habitat (predominant habitat type) (e.g. if Site 215 had a 60% cover of GS1 habitat, the Site was selected as a GS1 site). This is referred to as the Site Habitat (SiteHab) and has been applied to each site to result in four sites each of GS1, GS3 and GS4. This is irrespective of any other habitat types that may have occurred within the site but were of low coverage in comparison to the predominant habitat type. This could have resulted in some relevés being collected within the site that had a habitat type different to the habitat for which the site was selected.
- 3) Relevé:** The individual unit or 'plot' used to assess the vegetation within each Site. The size used for the relevés was 2 m x 2 m (4 m²) square. In most cases, these were the original relevés collected in the ISGS. However, extras were

collected during the StableGrass surveys if the ISGS relevé was no longer representative of semi-natural grassland habitat, if the site had only one previous relevé, or if access to a particular relevé taken during the ISGS was no longer available.

4) Relevé Habitat: This refers to the original habitat assigned to each individual relevé during the ISGS. Each ISGS relevé was assigned to a Fossitt habitat based on the grassland habitat assessed (e.g. a GS4 relevé was taken in an area of grassland that was identified and mapped as the GS4 habitat, and so on for other grassland habitats). The Relevé Habitat may differ to the Site Habitat. For example, while three relevés in a GS1 site may have been surveyed for the GS1 habitat, one relevé may have been collected in an area of GS4 habitat resulting in the presence of a GS4 habitat in a Site with a Site Habitat of GS1. This distinction can influence the analysis of differences in diversity across habitats but is explained when necessary.

2.5.3 Species composition through ordination

2.5.3.1 Species composition of the ISGS and StableGrass surveys

Ordination analysis, using Non-Metric Multidimensional Scaling (NMDS) was conducted using the 'metaMDS' function of the Vegan package (Oksanen et al., 2024). A scree plot to determine the number of dimensions (k) for the NMDS was run using the 'dimcheckMDS' function of the goeveg package (von Lampe and Schellenberg, 2024). The Bray-Curtis distance measure was used for the ordination as it is the most appropriate distance measure for community data (McCune and Grace, 2003). All the default settings in the metaMDS function were used, with the number of dimensions (k) being set as per the scree plot above. The 'autotransform' criterion was set to TRUE, which applied a Wisconsin, square-root transformation, as recommended for community data. This method was used to create separate plots containing the ISGS relevés and the StableGrass relevés with convex hulls showing habitat type fitted. Convex hulls are vectors which allow for the visualisation of the ordination space occupied by each category of a grouping variable, in this case, habitat. In R, the 'chull' function was used to compute the convex hull, based on the habitat grouping

variable. The 'polygon' function was used to draw the hulls for each habitat on the NMDS plot. The ISGS and StableGrass datasets were aggregated to site by calculating the mean abundance of all species across the relevés to give an overall data point for each site in the ISGS and StableGrass surveys. These aggregated data points were plotted on an NMDS together to show the separation in the composition of the StableGrass sites in comparison to the ISGS. The significance of the differences observed in the species composition on both the relevé and site-level NMDS plots was tested using a Multi-Response Permutation Procedure (MRPP). This analysis is suitable for vegetation data, which is typically not normally distributed (McCune and Grace, 2003). Both a p-value and A-value are produced. The p-value expresses the significance, and the A-value describes the within-group heterogeneity.

2.5.3.2 Influences of environmental factors on species composition

The 'Envfit' function of the Vegan package was used to apply both the measured soil variables and the calculated Ellenberg values (Section 2.3) to the ordination plots. This allows for correlations to be conducted between these variables and the species composition and to identify gradients that may explain the variation in the composition across the data points. Only variables that had a significant correlation with the NMDS axes ($p < 0.05$) were plotted.

2.5.4 Soil analysis

For the soil data analysis, only seven sites had associated soil variables, meaning the following analyses were conducted on a subset of relevés that had associated soil variables. These variables included the soil pH, Soil Organic Matter (% SOM), and Soil Organic Matter, weight (SOM (g)). Three different analyses were conducted to determine the soil properties. Firstly, analyses were conducted to determine whether the soil properties differed significantly across the three habitat types. As the pH and % SOM were normally distributed across the three habitats, an ANOVA was used to test for differences across the habitat types. A Kruskal-Wallis test was used to test for significant differences across habitats for SOM (g), as this variable was not normally

distributed. Secondly, analysis was conducted to determine whether the soil variables influenced the diversity indices, or vice-versa. Spearman rank correlations were used for this analysis, since none of the comparisons were normally distributed.

2.5.5 Changes in plant species and frequency since the ISGS

Changes that occurred in individual plant species between the two surveys were also assessed through changes in frequency between the two survey periods. Frequency in this project was calculated at two scales: the **relevé** and the **site** level. Frequency at the relevé level refers to the number of relevés (out of the total number of relevés collected) the species has been recorded in. Frequency at the site level refers to the number of sites (out of a total of 12) the species was recorded in. The distinction of frequencies into Site and Relevé has allowed for inferences to be made on species that may have increased/decreased in frequency across sites, and those that were recorded in more/less relevés than the ISGS. The rate of change (StableGrass frequency – ISGS frequency) in the frequency of all species between the surveys was calculated for the relevé and site frequency. Paired T-tests/Wilcoxon-signed Rank Tests were used to test for significant differences between the site and relevé level species frequencies for the StableGrass and ISGS surveys. A functional group approach was also taken as well through the division of the species lists in both surveys into Grasses, Forbs, Legumes, Woody/shrub, Pteridophyte (ferns and lycophytes), and Bryophytes. This allowed for an assessment into which groups were increasing/decreasing in frequency between the two surveys.

Chapter 3: Results

3.1 Overview

This chapter consists of five subsections that present findings on: (i) the current diversity of the 12 selected Irish Semi-natural Grasslands sites and how it has changed; (ii) species compositional changes through ordination; (iii) Influences of soil variables on vegetation; (iv) changes to the species frequency since the ISGS; and (v) vegetation classification and changes since ISGS.

Between June and August 2023, 54 grassland relevés were surveyed at 12 sites across eight counties (Section 2.1.2). The number of relevés surveyed per site ranged from three to seven, with a mean of 4.5 and a median of four relevés per site. Three habitat types, each represented by four sites, were assessed: GS1: Dry calcareous & neutral grasslands; GS3: Dry-humid acid grasslands; and GS4: Wet grasslands. For the three habitats, a total of 17, 13, and 24 relevés were collected, respectively (Table 3.1). A total of 173 species were recorded in the StableGrass survey conducted for this MSc project in 2023 (Appendix 8).

As mentioned, this project is part of the larger StableGrass project. However, the results presented and discussed here on in, are those data that have been collected, analysed and presented for this specific MSc project, which fulfilled the task which aimed to described and investigate the diversity of the semi-natural grasslands, and assess how this has changed over time.

3.2 Grassland diversity

3.2.1 Diversity across the three grassland habitats

For this section, the differences in the Species Richness, Simpson's Diversity, and Simpson's Evenness measures between the three habitat types (GS1, GS3, or GS4) were assessed. A Fossitt habitat has been applied to each relevé within each site by the ISGS. It is important to note that while this relevé habitat was the same as that of the habitat for which the site was initially selected (SiteHab) (Section 2.1.2) in most cases, there were some exceptions. Some sites were a mosaic of different grassland habitats and although one type was common, smaller areas of additional habitats

made up the remainder of semi-natural grassland cover in that site. This means that the Fossitt habitat type assigned to a relevé may not always be the same as the Fossitt habitat of the overall site.

3.2.1.1 Differences in species diversity across relevés of different habitat types

Table 3.1 shows considerable variation in the diversity measures across the three different habitat types in the StableGrass surveys. The Species Richness differed significantly across the three habitats (Kruskal-Wallis, $p = 0.000$) (Figure 3.1A). The Species Richness ranged from 21 to 38 for the GS1 habitat, 12 to 35 for the GS3 habitat, and 7 to 34 for the GS4 habitat. The largest differences in Species Richness occurred in the minimum end of the range, with the three habitats having a similar maximum Species Richness. The Species Richness of the GS1 habitat differed significantly to those of the GS3 and GS4 habitats (Pairwise Wilcoxon Rank Sum Test, $p = 0.000$ in each test) (Figure 3.1A). The Species Richness of the GS3 habitat did not differ significantly to the GS4 habitat (Pairwise Wilcoxon Rank Sum Test, $p = 0.496$) (Figure 3.1A).

Table 3.1: Summary Statistics for the three diversity indices summarised across Habitat. Habitat in this case refers to the Relevé Habitat.

		GS1	GS3	GS4
No of relevés		17	13	24
Species Richness	Mean	30.647	19.077	20.875
	Std. Dev	5.314	6.898	6.327
	Min	21.000	12.000	7.000
	Max	38.000	35.000	34.000
Simpson's Diversity	Mean	11.274	7.484	7.685
	Std. Dev	4.132	4.283	3.696
	Min	4.560	2.727	2.322
	Max	22.184	14.910	16.683
Simpson's Evenness	Mean	0.369	0.380	0.367
	Std. Dev	0.128	0.136	0.122
	Min	0.175	0.173	0.175
	Max	0.716	0.548	0.610

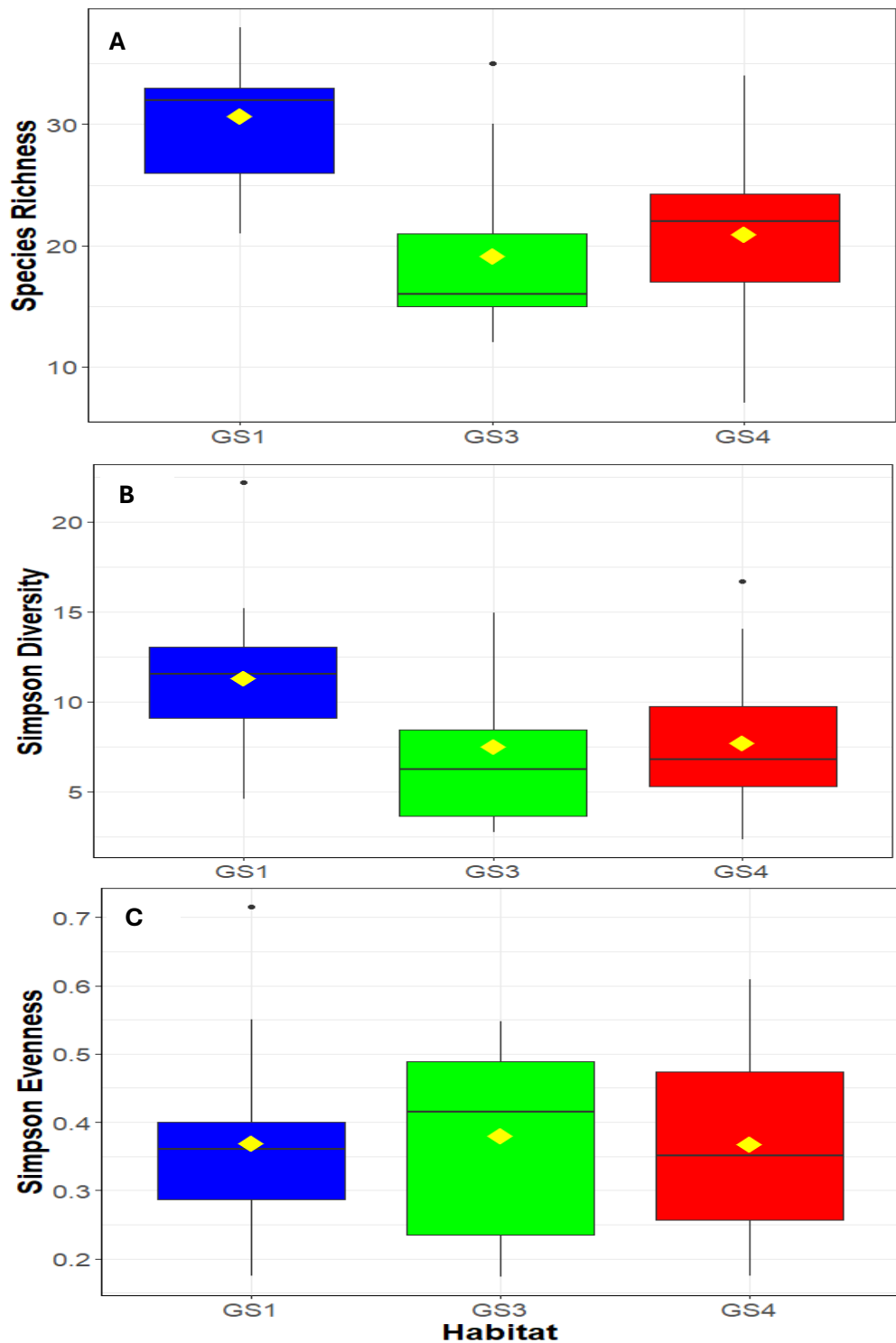


Figure 3.1: Boxplots showing the three diversity measures for relevés assigned to the three Fossitt habitats (RelHab) (GS1: n = 17; GS3: n = 13; GS4: n = 24). A) Species Richness, B) Simpson's Diversity and C) Simpson's Evenness. Mean superimposed on figure as yellow diamonds. The boxes refer to the interquartile range (Top line = Q1, Centre line = Median, Bottom line = Q3). The whiskers refer to the Minimum (bottom) and Maximum (top). Outliers are represented by black dots. Statistical Tests: A: Kruskal-Wallis, $p = 0.000$; B = ANOVA, $p = 0.011$; C = ANOVA, $p = 0.955$

As with Species Richness, the mean Simpson's diversity values were highest for the GS1 habitat, followed by GS4 and GS3 (Table 3.1, Figure 3.1B). The difference in Simpson's Diversity across the three habitats was significant (ANOVA, $p = 0.011$). The Simpson's Diversity of relevés of the GS1 habitat differed significantly from the GS3 relevés (Tukey's HSD, $p = 0.033$) and the GS4 relevés (Tukey's HSD, $p = 0.017$). This index did not differ between the GS3 and GS4 habitat (Tukey's HSD, $p = 0.9888$). In contrast to the Species Richness, and Simpson's Diversity values, the mean Simpson's Evenness was highest for the GS3 habitat, followed by GS1, and closely by GS4 (Table 3.1, Figure 3.1C). However, this difference was not significant (ANOVA, $p = 0.955$). While the mean Evenness was highest for GS3, the GS1 had both the highest range in values, but also the highest maximum Evenness. On average, relevés for the three habitats indicate the three habitat types are moderately even, though with some unevenness occurring across the habitat.

3.2.1.2 Differences in species diversity across the Site Habitat

The diversity indices were summarised across the three habitat types, as in Section 3.2.1.1. However, this section differs in that it describes the differences in the diversity measures across the Site Habitat. This analysis has been conducted irrespective of the Fossitt habitats assigned to the individual relevés across the sites, meaning that all relevés for this analysis, were assumed to be the habitat type of that the site was selected for. For each habitat, GS1, GS3, and GS4, four sites were surveyed, with 20, 15, and 19 relevés collected, respectively per habitat.

Table 3.2: Summary statistics for Species Richness, Simpson's Diversity, and Simpson's Evenness across the three Fossitt habitats (GS1, GS3, GS4), for which Sites (n = 4 sites each) were selected for. The number of relevés per habitat differs.

		GS1	GS3	GS4
	No. of Relevés	20	15	19
Species Richness	Mean	27.850	20.200	21.579
	Std. Dev	7.184	8.082	6.345
	Min	10.000	12.000	7.000
	Max	38.000	38.000	34.000
Simpson's Diversity	Mean	10.020	7.843	8.176
	Std. Dev	4.387	4.372	3.941
	Min	3.493	2.727	2.322
	Max	22.184	14.910	16.683
Simpson's Evenness	Mean	0.360	0.378	0.376
	Std. Dev	0.124	0.126	0.131
	Min	0.175	0.173	0.175
	Max	0.716	0.548	0.610

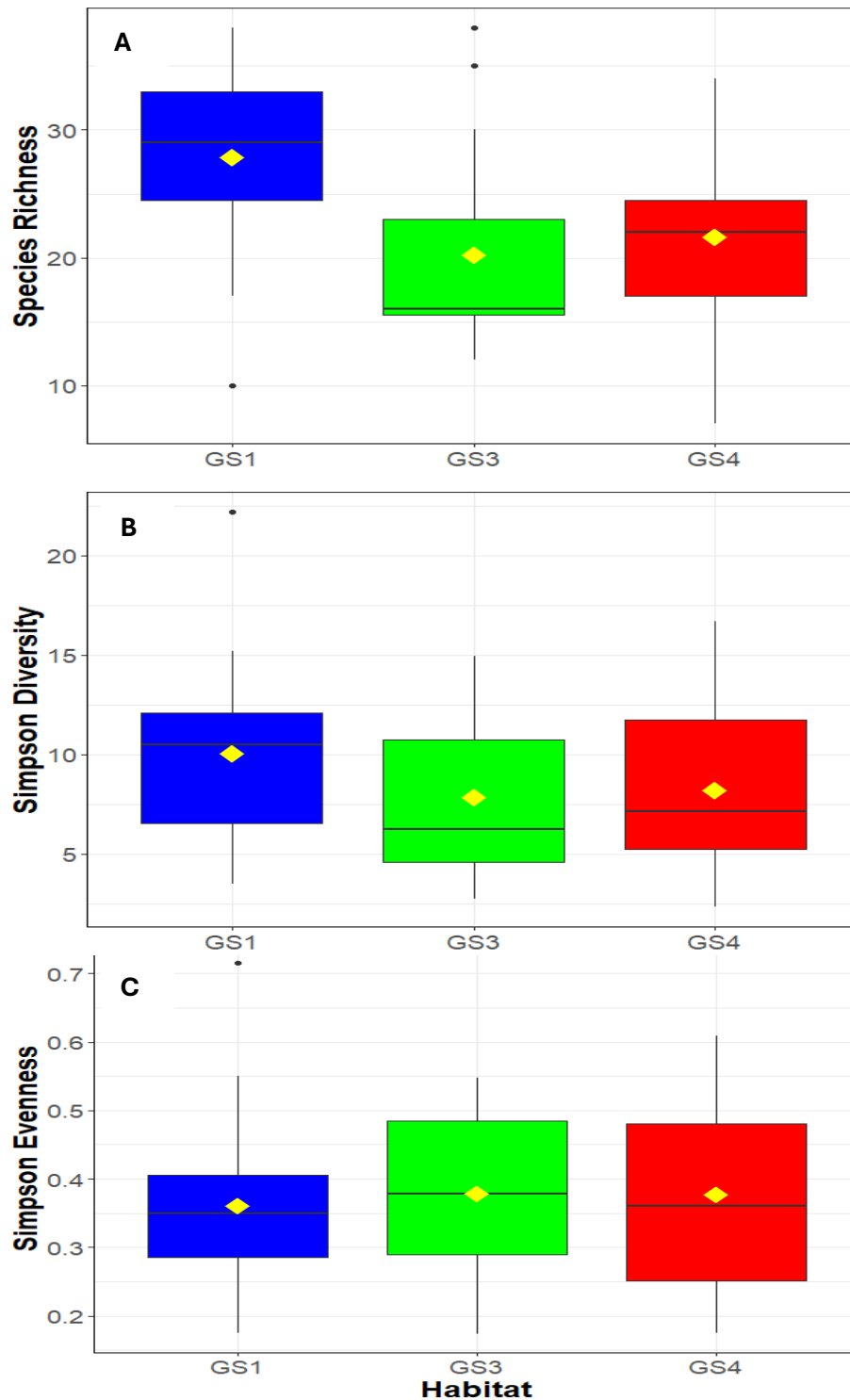


Figure 3.2: Boxplots showing the three diversity measures for relevés assigned to the three Fossitt habitats (SiteHab) (GS1: n = 20; GS3: n = 15; GS4: n = 19). A) Species Richness, B) Simpson's Diversity and C) Simpson's Evenness. Mean superimposed on figure as yellow diamonds. The boxes refer to the interquartile range (Top line = Q1, Centre line = Median, Bottom line = Q3). The whiskers refer to the Minimum (bottom) and Maximum (top). Outliers are represented by black dots. Statistical Tests: A: Kruskal-Wallis, $p = 0.003$; B = ANOVA, $p = 0.350$; C = Kruskal-Wallis, $p = 0.890$

The findings presented in Table 3.2 and Figure 3.2 suggests a similar diversity pattern to Section 3.2.1.1. This is regardless of whether the Relevé Habitat or Site Habitat is used for the analysis of differences across the habitat type. However, there are some exceptions. The Species Richness differed significantly (Kruskal-Wallis, $p = 0.003$) across the three habitat groups when grouped by the site habitat. Unlike with the relevé habitat as a grouping variable in 3.1.1.1, the Simpson's Diversity did not differ significantly across the Site Habitats (Kruskal-Wallis, $p = 0.350$). As with 3.2.1.1, no significant difference was found in the Simpson's Evenness between the three site habitats (ANOVA, $p = 0.890$).

3.2.2 Species diversity across the StableGrass Sites

The same diversity indices presented in section 3.2.1 were calculated as the means for each relevé collected within the 12 sites. Figure 3.3 is coloured by the Site Habitat to determine whether the diversity patterns in habitat presented in Section 3.2.1 were similar across the sites. The relevé habitat of each relevé within each site is not considered in this section. Table 3.3 presents summary statistics of these indices for sites. Across the sites, the Species Richness ranged from seven to 38, with a median Species Richness of 23.

Table 3.3: Summary statistics for the three diversity indices across the 12 Sites

			Species Richness			
Site	Site Habitat	No of relevés	Mean	Std. Dev	Min	Max
1	GS1	4	32.750	0.500	32	33
2	GS1	5	28.000	7.937	17	37
3	GS1	4	27.250	3.862	25	33
4	GS3	4	17.000	3.367	13	21
5	GS3	3	14.333	2.082	12	16
6	GS3	3	15.667	0.577	15	16
7	GS4	3	13.667	5.774	7	17
8	GS4	4	19.500	4.203	15	24
9	GS4	6	27.000	5.060	22	34
10	GS4	6	21.500	4.370	13	25
11	GS1	7	25.286	9.482	10	38
12	GS3	5	29.000	8.337	17	38
Site	Site Habitat	No of relevés	Simpson's Diversity			
1	GS1	4	12.470	1.111	11.255	13.727
2	GS1	5	12.300	6.088	5.671	22.184
3	GS1	4	9.793	2.173	7.027	11.531
4	GS3	4	5.714	1.605	3.640	7.476
5	GS3	3	3.065	0.505	2.727	3.645
6	GS3	3	7.554	1.323	6.030	8.412
7	GS4	3	3.326	0.895	2.322	4.039
8	GS4	4	8.515	2.725	5.758	12.043
9	GS4	6	8.031	4.430	3.859	16.683
10	GS4	6	10.521	3.269	4.686	14.023
11	GS1	7	7.121	3.886	3.493	15.179
12	GS3	5	12.587	3.757	5.996	14.910
Site	Site Habitat	No of relevés	Simpson's Evenness			
1	GS1	4	0.381	0.039	0.341	0.429

2	GS1	5	0.442	0.185	0.284	0.716
3	GS1	4	0.361	0.078	0.270	0.461
4	GS3	4	0.353	0.132	0.173	0.480
5	GS3	3	0.215	0.029	0.182	0.235
6	GS3	3	0.484	0.093	0.377	0.548
7	GS4	3	0.261	0.063	0.213	0.332
8	GS4	4	0.436	0.094	0.339	0.547
9	GS4	6	0.292	0.127	0.175	0.521
10	GS4	6	0.479	0.084	0.360	0.610
11	GS1	7	0.290	0.103	0.175	0.423
12	GS3	5	0.433	0.071	0.353	0.520

Species Richness varied across the twelve sites, with means ranging from 13.667 to 32.750 (Table 3.3, Figure 3.3A). The difference in Species Richness across the 12 sites was significant (Kruskal-Wallis, $p = 0.002$). Site 1 had the highest mean Species Richness (32.750 \pm 0.5), with a minimum of 32 species and a maximum of 33 species, showing a stable and high Species Richness at this site. In contrast, Site 7 had the lowest mean Species Richness (13.667 \pm 5.774) with a minimum of 7 species and a maximum of 17 species, showing a more variable and reduced Species Richness in comparison to other GS4 sites of higher diversity. As well as this, noticeable variation occurred in Sites 2, 11 and 12, with standard deviations of 7.937, 9.482, and 8.337, respectively. The Simpson's Diversity also varied across the 12 sites, but with a different pattern to that observed in the Species Richness. The mean Simpson's Diversity ranged from 3.065 to 12.587 (Table 3.3, Figure 3.3B). As with the Species Richness, the Simpson's Diversity differed significantly across the 12 sites (Kruskal-Wallis, $p = 0.003$). Site 12 had the highest Simpson Diversity values, and Site 5 had the lowest. The highest variability in the Simpson's Diversity across sites is observed in Site 2, with a standard deviation of 6.088. This site also has the highest Simpson's Diversity, as well as the largest range (Figure 3.3B). The Simpson's Evenness values showed some variation in the distribution of species abundances in the sites (Table 3.3, Figure 3.3C), and differed significantly across the 12 sites (ANOVA, $p = 0.005$). The

mean Simpson's Evenness ranged from 0.215 to 0.484, which suggests that across all 12 sites, only a moderate degree of evenness was encountered.

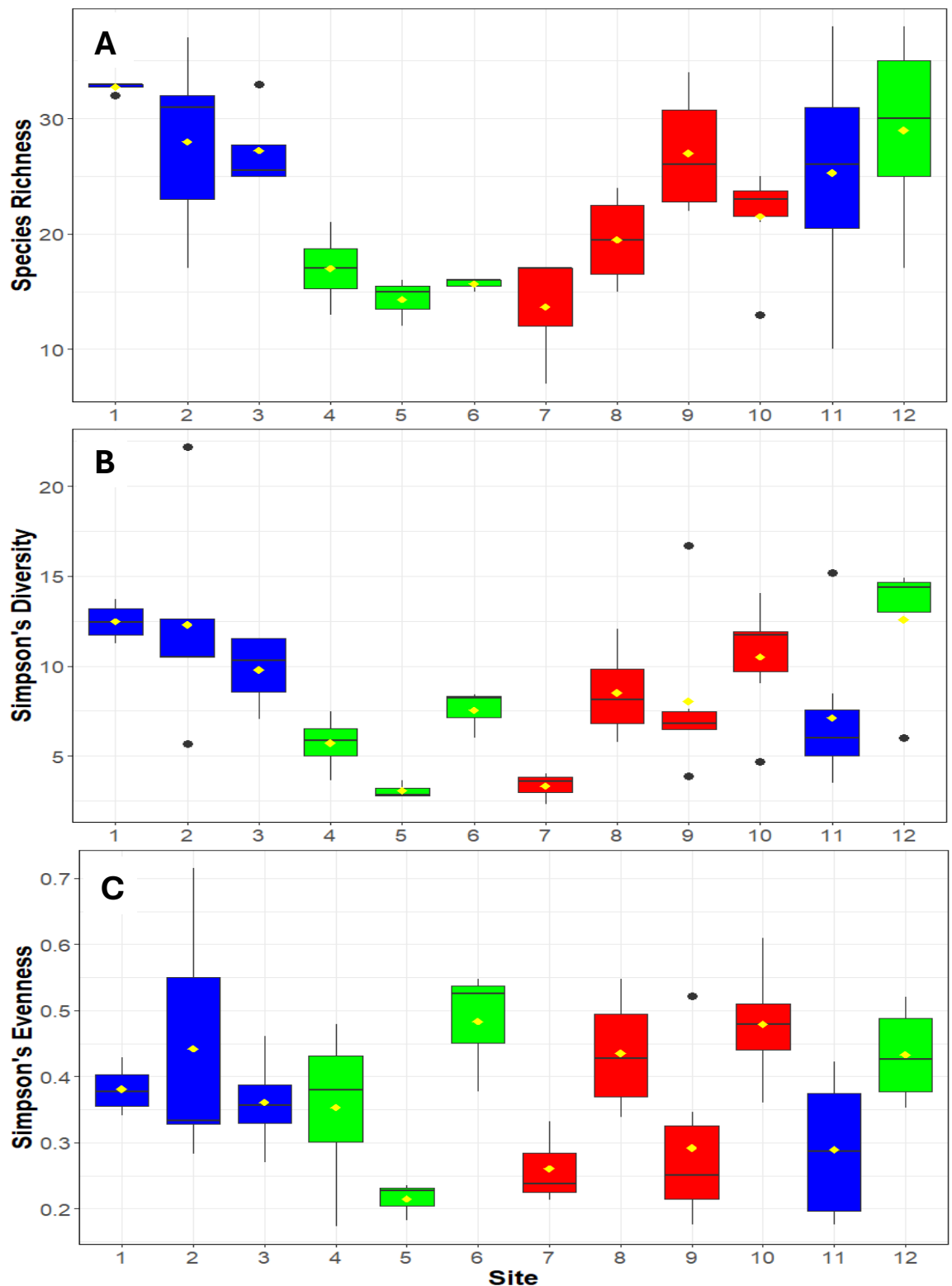


Figure 3.3: Boxplot showing the three diversity measures across the 12 study sites. Means for the relevés within the sites are presented. A) Species Richness, B) Simpson's Diversity and C) Simpson's Evenness. Mean superimposed on figure as yellow diamonds. The boxes refer to the interquartile range (Top line = Q1, Centre line = Median, Bottom line = Q3). The whiskers refer to the Minimum (bottom) and Maximum (top). Outliers are represented by black dots. Colours refer to the Site Habitat: Blue (GS1), Green (GS3), Red (GS4). Statistical Tests: A: Kruskal-Wallis, $p = 0.0015$; B = Kruskal-Wallis, $p = 0.0035$; C = ANOVA, $p = 0.0049$

Differences in diversity across the sites may be partly explained by site habitat (Figure 3.3). To test this, the four sites each per site habitat (GS1, GS3, GS4), were analysed separately, with the datasets being split by Site Habitat. Across each site habitat subset, the differences in diversity between the four sites were assessed. There was no significant difference found in the Species Richness, Simpson's Diversity, or Simpson's Evenness between the sites of the GS1 site habitat (Kruskal-Wallis $p = 0.367$, Kruskal-Wallis $p = 0.258$, and ANOVA $p = 0.0893$, respectively), suggesting the diversity did not differ across the GS1 sites. For the sites of the GS3 site habitat, the Species Richness and Simpson's Diversity did differ significantly (Kruskal-Wallis $p = 0.0496$ and ANOVA $p = 0.000$, respectively). The Simpson's Evenness was the only index that did not differ significantly between the GS3 sites (ANOVA, $p = 0.101$). Finally, there was no significant difference found in the Species Richness (Kruskal-Wallis, $p = 1.108$), Simpson's Diversity (Kruskal-Wallis, $p = 0.085$), or Simpson's Evenness (ANOVA, $p = 0.417$) for relevés within the GS4 habitat group.

3.2.3 Changes in diversity since ISGS

The differences that occurred in the diversity indices between the ISGS (2007-2012) and StableGrass (2023) surveys are shown in Table 3.4. As these analyses used paired statistical tests, only those relevés that were directly resurveyed from the ISGS ($n = 44$) are used and presented in the following analysis.

Table 3.4: Summary statistics for diversity between survey period (n = 44 for both StableGrass (SG) and ISGS surveys)

	Min		Max		Mean		Standard Deviation	
	ISGS	SG	ISGS	SG	ISGS	SG	ISGS	SG
Diversity Measure								
Species Richness	12	7	45	38	24.78	23.52	7.19	7.81
Simpson's Diversity	2.837	2.322	12.649	22.184	7.872	8.766	2.309	4.264
Simpson's Evenness	0.187	0.173	0.570	0.716	0.329	0.371	0.093	0.125

In total, 173 species were recorded in the StableGrass survey, compared to the 209 species recorded in the ISGS survey. Between the two surveys, a total of 239 species were recorded in the grassland sites. The differences in the species between the two surveys will be presented in more detail in Section 3.5. The mean Species Richness across the relevés was 24.78 ± 7.19 in the ISGS survey and 23.53 ± 7.81 in the StableGrass Survey (Table 3.4, Figure 3.4A). This slight decline in Species Richness between the two surveys was not significant (Wilcoxon signed rank test, $p = 0.116$). The mean Simpson's Diversity for the ISGS survey was 7.872 ± 2.309 and 8.766 ± 4.264 for the StableGrass survey. This slight increase in the Simpson's Diversity between the two surveys was not significant (Paired T-test, $p = 0.270$, Figure 3.4B). There was only a slight increase in the mean Simpson's Evenness in the StableGrass survey in comparison to the ISGS survey, but it was not significant (Paired T-test, $p = 0.127$, Figure 3.4C).

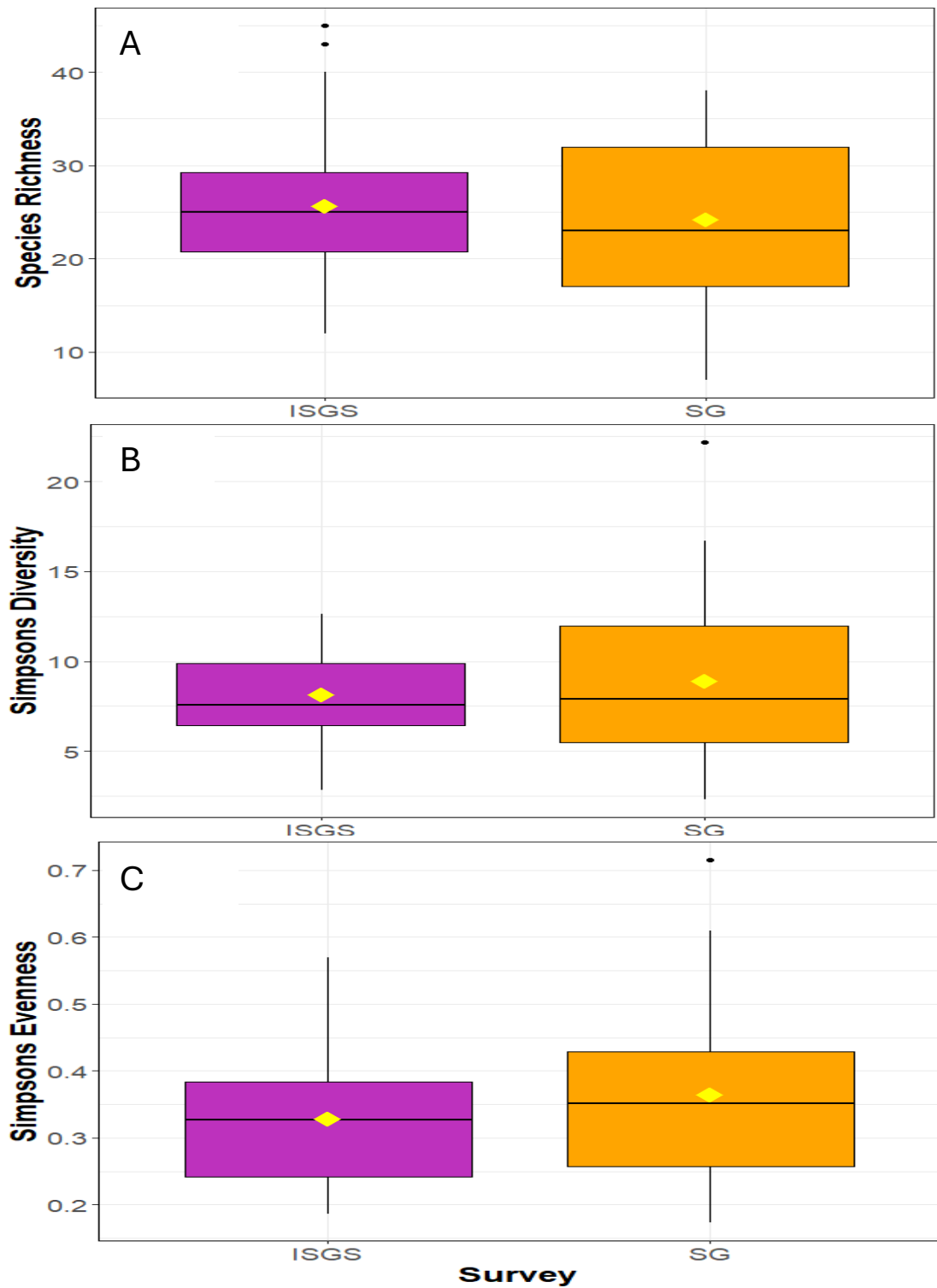


Figure 3.4: Boxplots showing the differences in three diversity measures between the ISGS (n = 44 relevés) and the StableGrass (n = 44 relevés) Surveys. A) Species Richness, B) Simpson's Diversity and C) Simpson's Evenness. Mean superimposed on figure as yellow diamonds. The boxes refer to the interquartile range (Top line = Q1, Centre line = Median, Bottom line = Q3). The whiskers refer to the Minimum (bottom) and Maximum (top). Outliers are represented by black dots. Statistical Tests: A: Wilcoxon signed-rank test, $p = 0.12$; B = Paired T-test, $p = 0.270$; C = Paired T-test, $p = 0.127$

The differences in diversity between the two survey periods varied. However, Site 7 (GS4 grassland, Portumna, Co. Galway) was the only site to have a statistically significant difference in the diversity measures between the ISGS and StableGrass surveys. This site had a 53% reduction in the Species Richness since the ISGS survey with a mean of 13.67 species in the StableGrass survey, and 29 species in the ISGS survey (Shapiro-Wilk test, $p = 0.253$. Paired T-test, $p = 0.02$, Appendix 9, Figure B1). The Simpson's Diversity index also differed significantly between the survey periods (Shapiro-Wilk test, $p = 0.051$. Paired T-test, $p = 0.032$). A 70% reduction in the Simpson's Diversity was observed with the mean for ISGS being 10.543, and the mean for StableGrass being 3.188. The Simpson's Evenness did not differ significantly between survey period for this site. Site 3 also had a 29% decrease in Species Richness since the ISGS (Appendix 9, Figure B1). However, the differences in diversity between survey periods for this site could not be statistically tested for significance due to only one relevé (data point) was collected for this site in the ISGS.

While the overall Species Richness and Simpson's Diversity and Evenness measures did not change significantly between the StableGrass and ISGS surveys, additional analysis was conducted to determine if changes in diversity occurred at the relevé habitat level (Table 3.5).

Table 3.5: Changes in the three diversity measures between the ISGS and StableGrass survey across the three different relevé habitats. Direction of change denoted by arrows, and significance denoted by an Asterisk (*)

Index	GS1	GS3	GS4
Species Richness	↑	↓	↓*
Simpson's Diversity	↑*	↑	↓
Simpson's Evenness	↑	↑	↑

Note: Changes are denoted by arrows: ↑ = Increase ↓ = Decrease.
A significant change is denoted by an Asterisk (*)
Simpson's Diversity – GS1 = Paired T-Test, p = 0.015
Species Richness – GS4 = Wilcoxon Rank-Signed Test, p = 0.043
All other tests and p values located in Table C1, Appendix 9

3.3 Vegetation Composition between surveys

3.3.1 Ordination of StableGrass and ISGS Plots

A Non-Metric Multidimensional Scaling (NMDS) plot was created for both the StableGrass and ISGS datasets separately to investigate the past and current vegetation composition. A combined analysis was also conducted to determine if there was overlap in the two datasets. The Bray-Curtis distance measure was used for the creation of these NMDS plots.

When the StableGrass relevés were plotted on an NMDS (Figure 3.5) the majority of relevés showed clear groupings and affinities to the habitats which have been assigned to those relevés (Relevé Habitat). However, some relevés had an overlap, with three GS1 relevés overlapping with the GS4 habitat, and three GS4 relevés overlapping with the GS1 habitat. Two GS4 relevés overlapped with the GS3 habitat. Despite the relevés sharing a similar ordination space (Scores -1 to +1), a significant difference was found in the site scores between habitats (PERMANOVA, p = 0.001). This demonstrates that, the habitat type has a significant influence on the species composition and explains the habitat groupings observed on the NMDS plot.

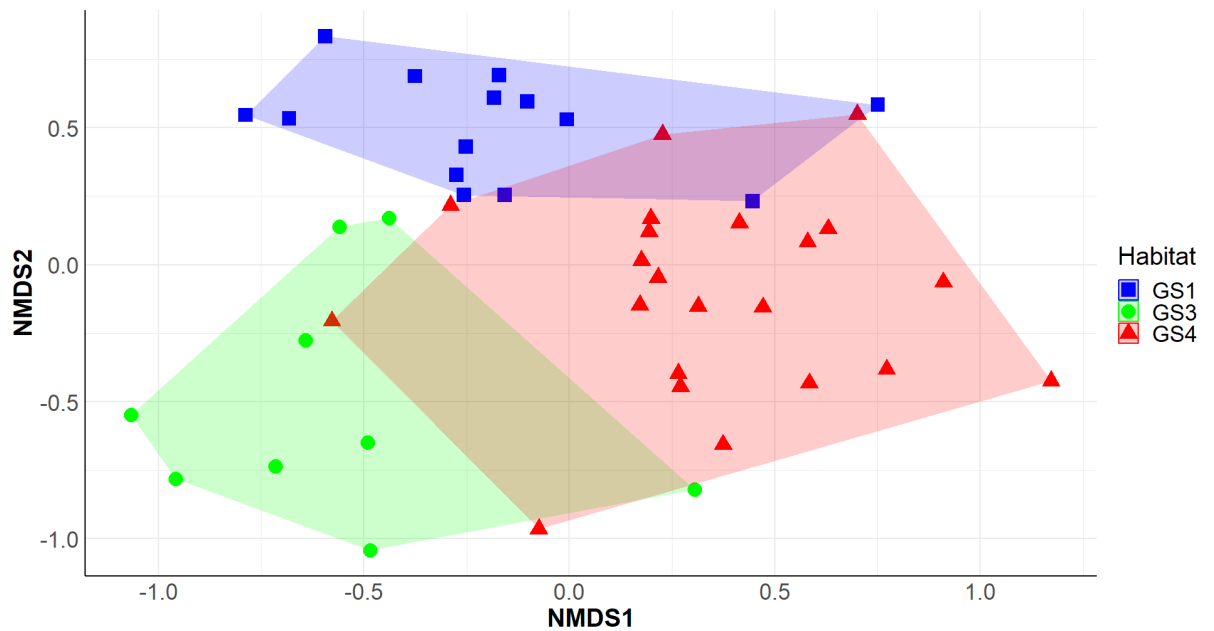


Figure 3.5: NMDS showing StableGrass relevés with points and convex hulls. These hulls are polygons graphed that encompasses all points for each habitat type on the ordination space. They are a means to give a visual representation of the habitat type. Blue (GS1, $n = 15$), Green (GS3, $n = 9$), and Red (GS4, $n = 22$). Habitat refers to the Fossitt habitat applied to each relevé. Only relevés that were included in both surveys formed part of this analysis. An NMDS with three dimensions ($k = 3$), was conducted as this produced the best stress values (Stress = 0.1509).

When the ISGS relevés were plotted on an NMDS (Figure 3.6), a similar but clearer grouping and habitat affinity was observed within the plots. While all relevés occupied a similar area on the plot, clear groupings by habitat are observed with no overlap. The shift in species composition, as indicated by the differences in ordination between Figure 3.5 and 3.6 was tested for a significant difference between the two surveys. This difference was significant (MRPP, $p = 0.002$), but had a very low effect size.

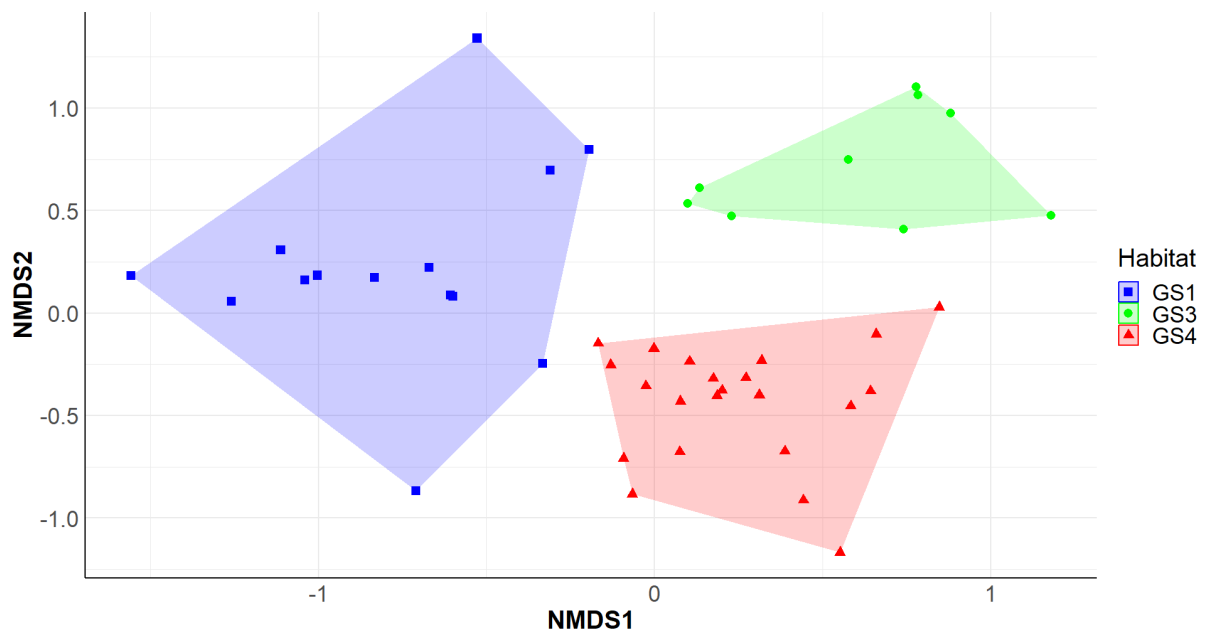


Figure 3.6: NMDS Showing Irish Semi-Natural Grassland Survey Relevés with points and convex Hulls representing the habitat type. Habitat refers to the Fossitt Habitat assigned to each relevé. Blue (GS1, n = 15), Green (GS3, n = 9), Red (GS4, n = 22). An NMDS with three dimensions (k = 3) was conducted as this produced the best stress values (Stress = 0.134)

3.3.2 Ordination of StableGrass vs ISGS Sites

For both the StableGrass and ISGS data, all 44 of the paired relevés have been summarised to site, for the 12 sites. This was done through the calculation of the mean abundances of all species recorded in the relevé to give a mean abundance of that species per site. This site-level dataset was used in this NMDS analysis, for each site comparing the StableGrass survey (Yellow) with the ISGS survey (Purple) (Figure 3.7). This allows for the identification of sites that may have changed in composition since the last survey. The Bray-Curtis distance measure was also used for this NMDS plot.

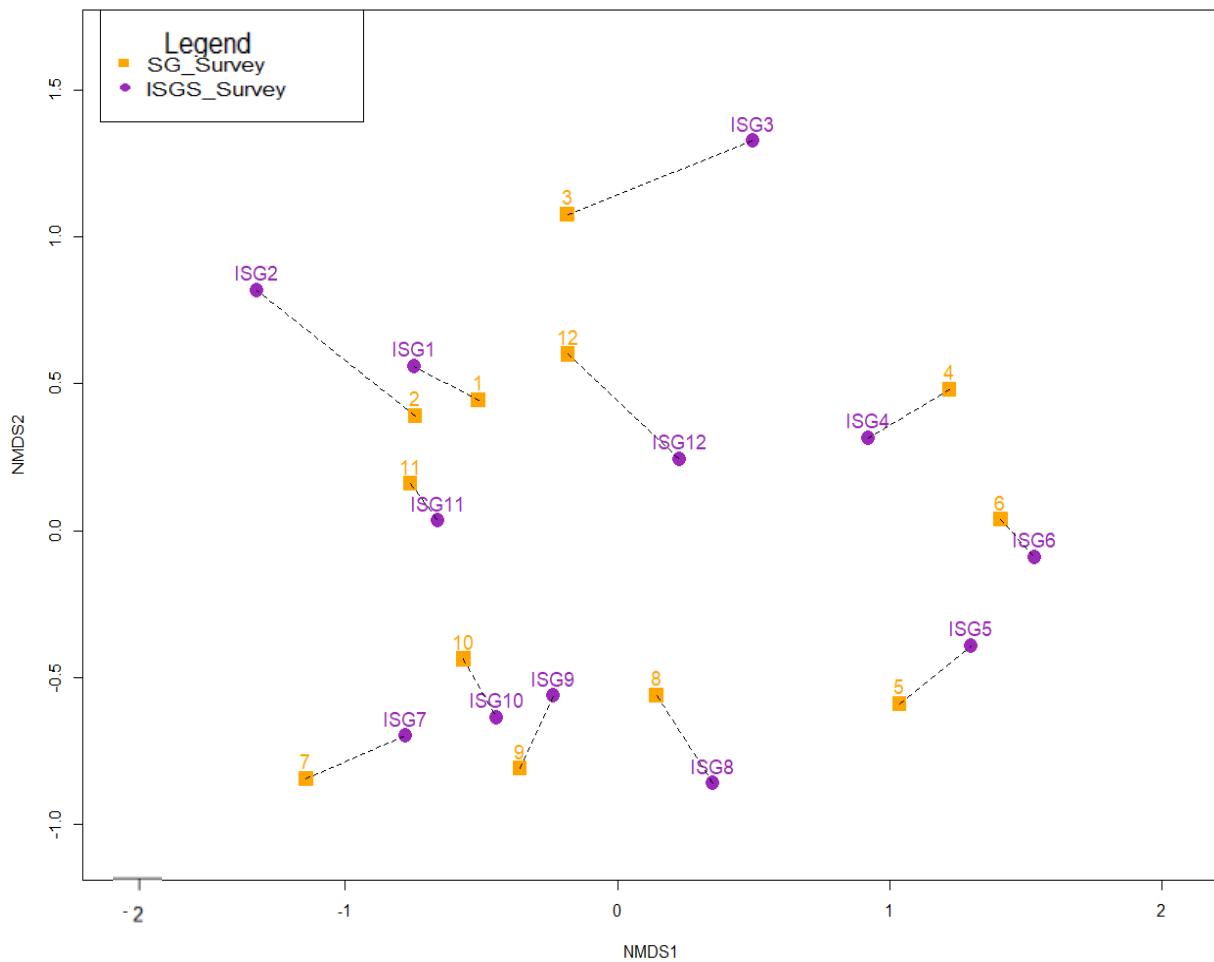


Figure 3.7: NMDS Showing the ISGS Sites (Yellow, $n = 12$), and the associated StableGrass Sites (Purple, $n = 12$). Each point for Site represents the mean abundance for all species recorded across the relevés within the sites. Dashed lines indicate the degree of separation that occur in the site between the two surveys.

A clear shift in composition for the StableGrass sites has occurred since they were surveyed in the ISGS (Figure 3.7). Sites 1, 6, 9, 10 and 11 appear to have stable species composition between the two survey periods, while Sites 2, 3, 7, and 12 had the most notable shifts in species composition. After this, a Multi-response Permutational Procedure (MRPP) was conducted to determine if this shift was significant. First, MRPP analysis was conducted on the entire dataset containing both the StableGrass and ISGS relevés, using 'Survey' (SG; ISGS) as a grouping variable. While a significant difference in the species composition between Figures 3.4 and 3.5 (MRPP, $p = 0.002$) was identified, the same statistical test was not significant when used on the site-level dataset, using survey as the grouping variable, for which differences were assessed (MRPP, $p = 0.344$).

3.3.3 Ellenberg indicator values in the StableGrass Surveys

Ellenberg Indicator values (Light, Reaction (pH), Wetness, Nitrogen, and Salinity) were calculated for all relevés based on the weighted mean for all species following standard protocols using the IVC ERICA Web App (Section 2.3). These were correlated with the axes of the NMDS plots for the StableGrass data, using the Envfit function. This function calculated the regression of the listed Ellenberg values with the ordination axes, and the significance was tested through permutation tests.

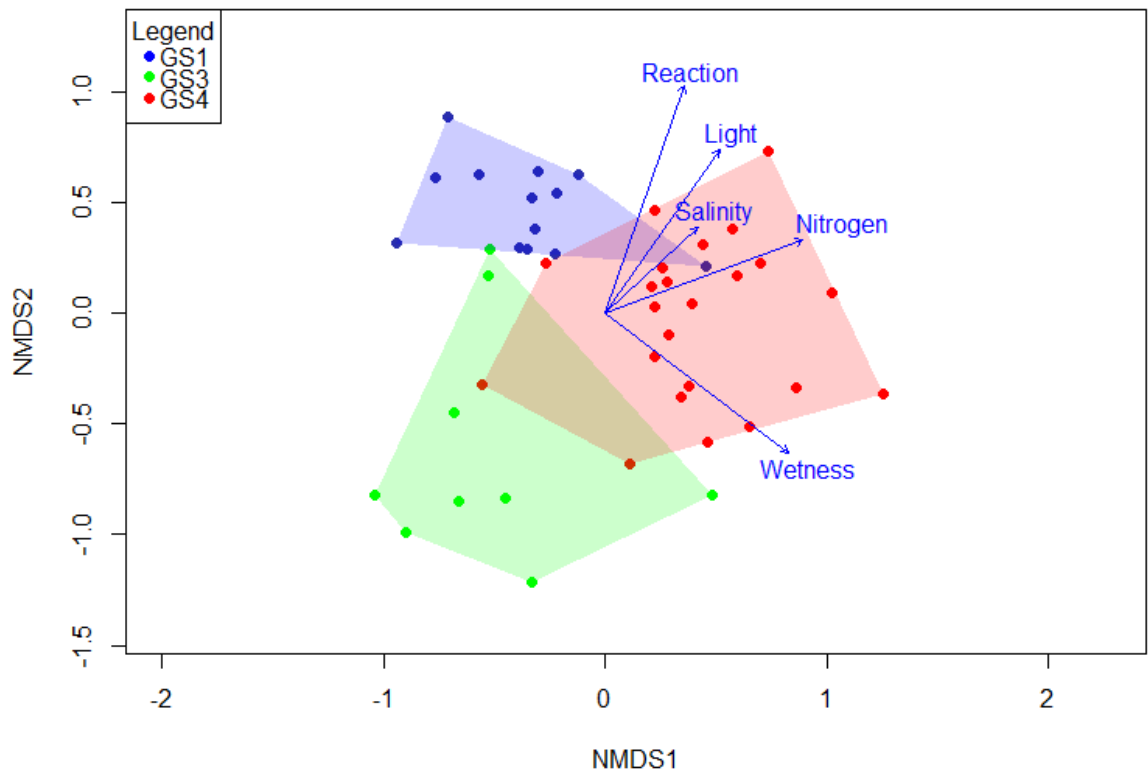


Figure 3.8: NMDS with StableGrass plots, grouped by habitat. Ellenberg values have been fitted. (GS1 = Blue, GS3 = Green, GS4 = Red). Only Ellenberg variables with a correlation of $p < 0.05$ are plotted. The direction of the arrows indicates the gradient where that variable has the highest influence, while the length of the arrow indicates the strength and the significance of the correlation.

All five Ellenberg Indicators were significantly correlated with the NMDS axes ($p < 0.05$). The NMDS plotted for StableGrass (Figure 3.8) indicated that the relevés of the GS1 habitat are associated with higher pH levels, as indicated by the Reaction arrow. However, some GS1 relevés were oriented away from the Reaction (pH) values. Some of the GS4 relevés may also be associated with this higher pH. GS3 plots are

associated with lower pH values, as indicated by the Reaction arrow pointing away from this grouping. From this NMDS, it would indicate that the GS3 relevés have lower Reaction, Light, and Nitrogen levels. This shows that they are more acidic environments, with more shading and have a low nutrient status. The GS3 relevés, due to the association with Wetness indicator, may indicate an intermediate moisture level. The GS4 relevés, as indicated by the Wetness, and Nutrient Ellenberg arrows, are more strongly associated with grasslands of a greater moisture and nutrient content.

Table 3.6: Correlations of the Ellenberg Indicator Values with the two NMDS axes for the StableGrass Survey

	NMDS1	NMDS2	R ²	p-Value
Light	0.574	0.819	0.394	0.001
Wetness	0.796	-0.605	0.526	0.001
Reaction	0.329	0.944	0.574	0.001
Nitrogen	0.938	0.348	0.437	0.001
Salinity	0.731	0.682	0.159	0.028

The Reaction, Wetness, Light, and Nitrogen were the Ellenberg values that had the strongest correlations, in order of decreasing R² values (Table 3.6), accounted for 57.43%, 52.61%, 43.74%, and 39.36% of the variation in the species composition, respectively. Axis 1 represents a gradient of Nitrogen (Fertility)/Wetness/Light/Reaction (pH). Points to the right of this axis represent relevés that are of higher fertility, with wetter conditions, higher light availability, and with less acidic soils. More specifically, Axis 2 further describes a gradient of Light/Reaction (pH)/Nitrogen (Fertility)/Wetness. Points to the top right of this axis represent relevés of higher light availability, with higher pH values, higher fertility, and drier conditions.

3.4 Soil properties across habitats and sites

3.4.1 Differences in soil properties by relevé habitat

The pH, percentage soil organic matter (SOM %) and SOM weight (g) varied between habitat type (in this case using the Relevé Habitat). The GS3 habitat was characterised by having the highest pH (5.57 – 6.82), followed by the GS1 habitat, with the GS4 habitat having the lowest values (Table 3.7, Figure 3.9A). The highest SOM (%) (12.76% – 86.35%) and SOM (g) (0.638 – 3.603) was observed in the GS3 habitat, followed by the GS1 habitat (Table 3.7, Figures 3.9B & 3.9C). The GS4 habitat had the lowest values of these two soil properties. The GS4 habitat had the lowest values for all three soil variables. The GS3 habitat, while having the highest values for the three soil variables, had the greatest variability in the soil properties, as indicated by the standard deviation (Table 3.7, Figure 3.9). The GS1 and GS4 habitats had similar variability in the three soil properties and were more stable in terms of the ranges and standard deviations. The pH, SOM (%), and SOM (g) did not differ significantly across habitat type (ANOVA, $p = 0.181$; ANOVA, $p = 0.481$; Kruskal-Wallis, $p = 0.780$, respectively).

Table 3.7: Summary statistics for Soil pH, % Soil Organic Matter (SOM), and SOM (g, amounts per 5 g sample) across Habitats (GS1 n = 5, GS3 n = 11, GS4 n = 17)

		GS1	GS3	GS4
pH	Mean	6.120	6.130	5.932
	Std. Dev	0.196	0.425	0.199
	Min	5.850	5.570	5.630
	Max	6.340	6.820	6.290
% SOM	Mean	35.30%	37.81%	30.14%
	Std. Dev	10.91%	25.78%	8.36%
	Min	22.95%	12.76%	17.74%
	Max	50.21%	86.35%	54.08%
SOM (g)	Mean	1.756	1.830	1.514
	Std. Dev	0.538	1.166	0.419
	Min	1.154	0.638	0.891
	Max	2.511	3.602	2.704

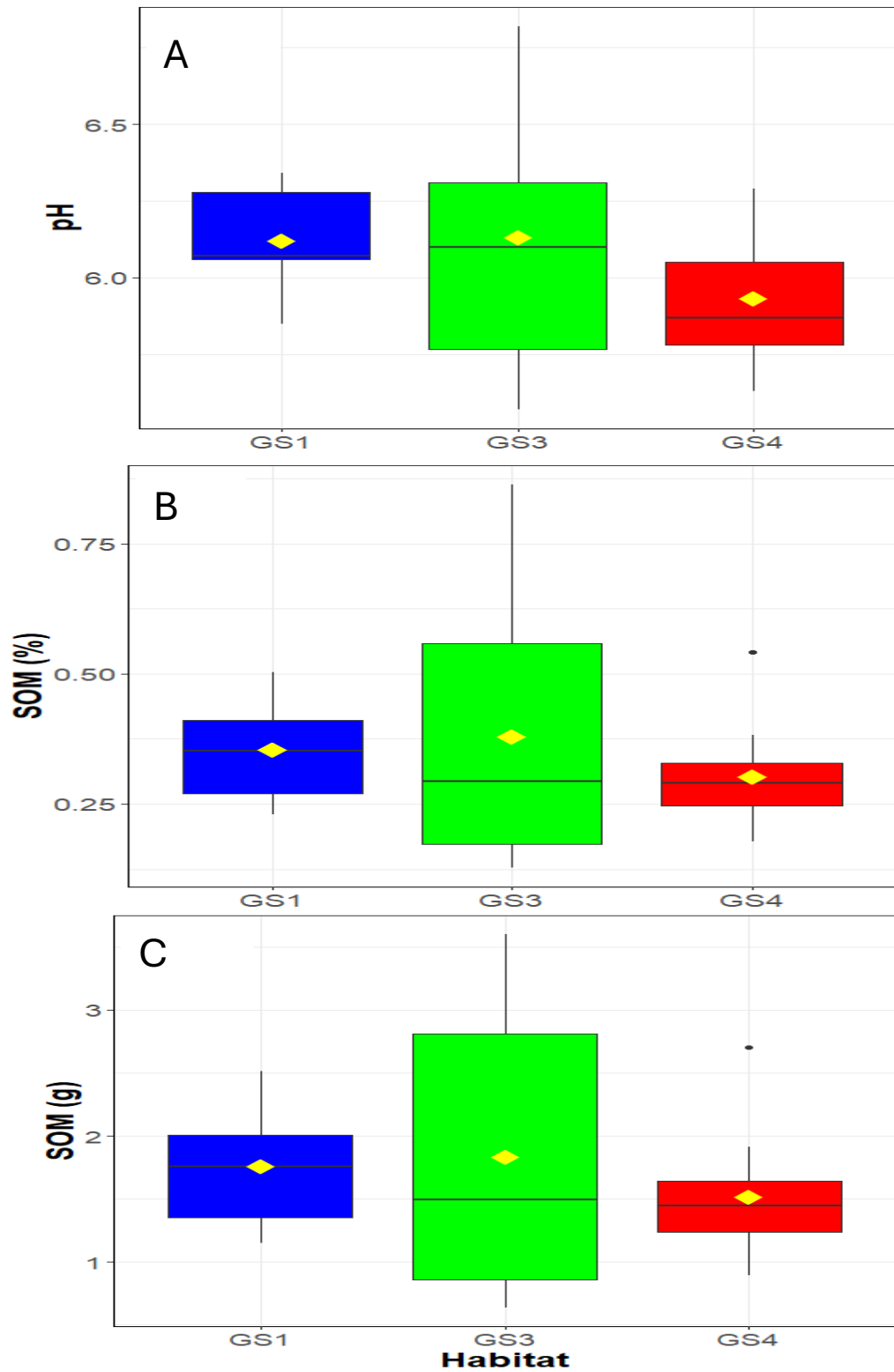


Figure 3.9: Boxplots showing three soil variables across relevé habitats (GS1 n = 5, GS3 n = 11, GS4 n = 17). A) Soil pH B) % SOM C) SOM (g). Mean superimposed on figure as yellow diamonds. The boxes refer to the interquartile range (Top line = Q1, Centre line = Median, Bottom line = Q3). The whiskers refer to the Minimum (bottom) and Maximum (top). Outliers are represented by black dots.

3.4.2 Differences in Soil Properties across Sites

The soil properties varied across sites. Site 4 had the lowest mean pH, while Site 12 had the highest mean pH (5.698 and 6.456, respectively) (Table 3.8, Figure 3.10A). Site 9 had the lowest variation in the soil pH, as indicated by the standard deviation (0.069), with Site 12 having the highest variability in the soil pH. The soil pH differed significantly across the seven sites that were assessed for soil properties (ANOVA, $p = 0.0426$). Site 4 had the highest mean % Soil Organic Matter (SOM %) (61%), which was also reflected by the weight of Soil Organic Matter (SOM (g)) (2.889 g). Site 12 had the lowest mean SOM % (20%) and SOM (g) (1.010 g) (Table 3.8, Figure 3.10B). Site 8 had the lowest standard deviation in the % SOM suggesting this parameter is stable across the relevés within the site, with Site 4 having the highest variability in % SOM across the relevés, as indicated by the standard deviation (Table 3.8, Figure 3.10C). The SOM (g) has similar interpretations to the soil carbon patterns described in the % SOM. Both the % SOM (Kruskal-Wallis, $p = 0.000$), and SOM (g) (ANOVA, $p = 0.048$) differed significantly across the seven sites.

Table 3.8: Summary statistics of Soil Organic Matter (%), Soil Organic Matter (g), and soil pH across Sites (n = 7 Sites)

			Mean	Std. Dev	Min	Max
Site	Habitat	n	Soil pH			
2	GS1	5	6.022	0.180	5.850	6.280
4	GS3	4	5.698	0.102	5.570	5.820
6	GS3	3	6.233	0.160	6.080	6.400
8	GS4	4	6.038	0.131	5.870	6.190
9	GS4	6	5.742	0.069	5.630	5.810
10	GS4	6	6.065	0.193	5.780	6.290
12	GS3	5	6.456	0.339	6.100	6.820
Site			Soil Organic Matter (%)			
2	GS1	5	42%	11%	27%	54%
4	GS3	4	61%	27%	24%	86%
6	GS3	3	31%	18%	13%	49%
8	GS4	4	27%	2%	24%	29%
9	GS4	6	30%	5%	24%	36%
10	GS4	6	29%	8%	18%	38%
12	GS3	5	20%	6%	13%	29%
Site			Soil Organic Matter (g)			
2	GS1	5	2.066	0.550	1.353	2.704
4	GS3	4	2.889	1.150	1.188	3.602
6	GS3	3	1.558	0.898	0.638	2.432
8	GS4	4	1.326	0.107	1.211	1.450
9	GS4	6	1.493	0.249	1.203	1.840
10	GS4	6	1.461	0.420	0.891	1.912
12	GS3	5	1.010	0.321	0.676	1.496

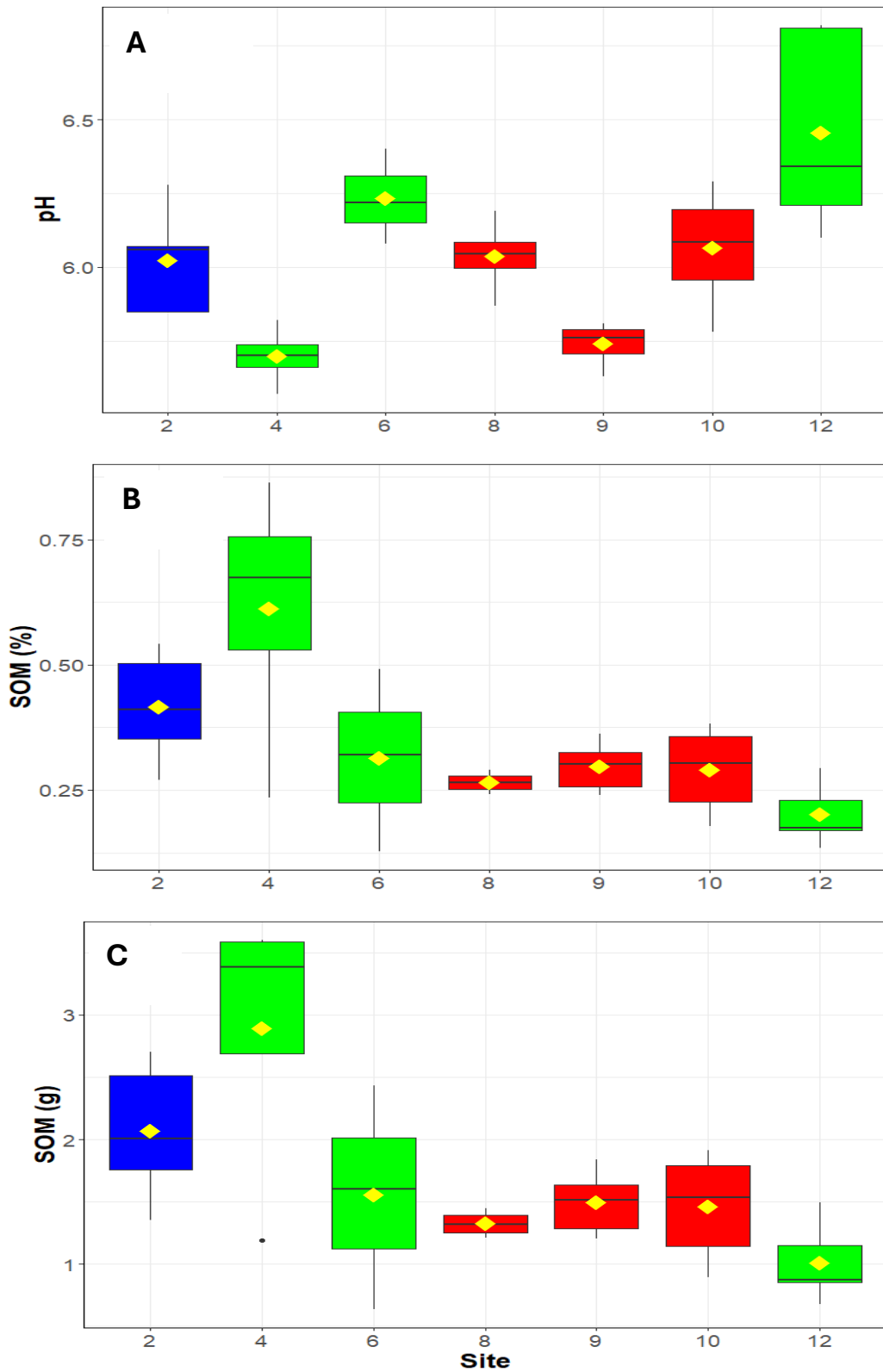


Figure 3.10: Boxplots showing A) pH, B) % Soil Organic Matter (SOM), and C) Soil Organic Matter (g) across 7 Sites. Sites are coloured by the site habitat (Blue = GS1, Green = GS3, Red = GS4). Mean superimposed on figure as yellow diamonds. The boxes refer to the interquartile range (Top line = Q1, Centre line = Median, Bottom line = Q3). The whiskers refer to the Minimum (bottom) and Maximum (top). Outliers are represented by black dots.

3.4.3 Influence of measured soil variables on species composition

Soil samples were collected from a subset of 33 relevés across seven sites. The soil samples were analysed for pH, SOM (%), and the related SOM (g). The influence of these variables on the species composition was assessed (Figure 3.11). pH was the only one of these soil variables that was found to have a significant correlation with the species composition (Figure 3.11, $p = 0.013$). However, this variable only explained some of the variation (24.63%) in the observed species composition.

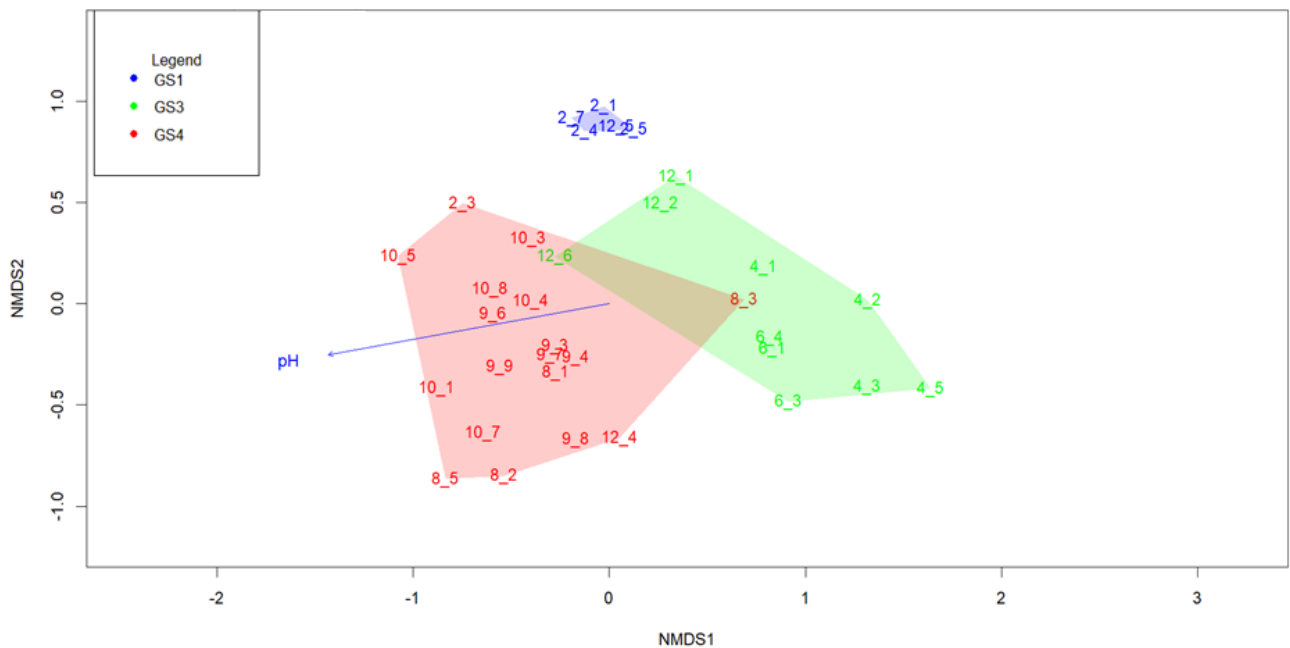


Figure 3.11: NMDS showing the species composition of 33 plots, from which soil samples were collected. The three habitats (according to Relevé Habitat) as convex hulls and are displayed as coloured text to denote the habitat of the relevé (Blue = GS1, Green = GS3, Red = GS4). The soil variables are overlaid ($p_{\max} = 0.05$) and are represented by blue arrows. The Bray-Curtis distance measure was used for this plot.

As observed in Figure 3.11, the pH is pointing in the direction of the relevés of the GS4 – wet grassland habitat, indicating a gradient of decreasing pH, as indicated by Figure 3.9, Table 3.7).

3.5 Changes in Species Composition and frequency patterns

3.5.1 Changes in Species frequency between surveys

As identified in section 3.3, there have been shifts in the species composition between the ISGS and StableGrass surveys. To investigate these species changes further, changes in species frequencies between the two surveys have been examined. Frequency refers to the number of times the species was recorded across Relevés and Sites. The relevé level frequency refers to the number of relevés (out of the total number of relevés collected) in which the species was recorded, and site level frequency refers to the number of sites (out of a total of 12) that the species was recorded in. This was calculated for both the StableGrass and ISGS surveys. This has enabled species that have gained, lost, or maintained the same frequency to be identified. A total of 173 species were recorded for the StableGrass surveys, while 209 species were recorded for the same sites during the ISGS surveys. Across both surveys, a total of 239 species were recorded. Between the two surveys, a total of 63 species have not been re-found in the StableGrass surveys (26%) (Table 3.9). A total of 31 species (13%) were recorded in the StableGrass survey that were not recorded in the ISGS survey (Table 3.10). Of these 31 new species eight were forbs, seven were graminoids, one was a pteridophyte, three were woody angiosperms, and 12 were bryophytes.

Table 3.9: Species that were recorded in the ISGS survey but not the StableGrass Survey. Species are grouped by functional group

Species	Group	Species	Group	Species	Group
<i>Alchemilla filicaulis</i>	Forb	<i>Viola riviniana</i>	Forb	<i>Entodon concinnus</i>	Bryophyte
<i>Anagallis tenella</i>	Forb	<i>Trifolium dubium</i>	Forb	<i>Frullania tamarisci</i>	Bryophyte
<i>Carlina vulgaris</i>	Forb	<i>Veronica persica</i>	Forb	<i>Hylocomium brevirostre</i>	Bryophyte
<i>Coeloglossum viride</i>	Forb	<i>Alopecurus pratensis</i>	Graminoid	<i>Hypnum cupressiforme</i>	Bryophyte
<i>Epilobium obscurum</i>	Forb	<i>Eriophorum angustifolium</i>	Graminoid	<i>Hypnum jutlandicum</i>	Bryophyte
<i>Geranium dissectum</i>	Forb	<i>Eriophorum vaginatum</i>	Graminoid	<i>Leucobryum glaucum</i>	Bryophyte
<i>Heracleum sphondylium</i>	Forb	<i>Festuca arundinacea</i>	Graminoid	<i>Lophozia ventricosa</i>	Bryophyte
<i>Lysimachia nemorum</i>	Forb	<i>Festuca pratensis</i>	Graminoid	<i>Marchantia polymorpha</i>	Bryophyte
<i>Odontites vernus</i>	Forb	<i>Juncus bulbosus</i>	Graminoid	<i>Neckera complanata</i>	Bryophyte
<i>Ophioglossum vulgatum</i>	Forb	<i>Ulex gallii</i>	Leguminous	<i>Pellia endiviifolia</i>	Bryophyte
<i>Parnassia palustris</i>	Forb	<i>Asplenium ruta-muraria</i>	Pteridophyte	<i>Pellia spp</i>	Bryophyte
<i>Plantago major</i>	Forb	<i>Huperzia selago</i>	Pteridophyte	<i>Peltigera spp</i>	Bryophyte
<i>Potentilla anglica</i>	Forb	<i>Phyllitis scolopendrium</i>	Pteridophyte	<i>Plagiothecium undulatum</i>	Bryophyte
<i>Pulicaria dysenterica</i>	Forb	<i>Selaginella selaginoides</i>	Pteridophyte	<i>Pohlia spp</i>	Bryophyte
<i>Rhinanthus minor</i>	Forb	<i>Erica cinerea</i>	Woody	<i>Racomitrium heterostichum</i>	Bryophyte
<i>Rumex crispus</i>	Forb	<i>Fraxinus excelsior</i>	Woody	<i>Rhynchostegium confertum</i>	Bryophyte
<i>Salix repens</i>	Forb	<i>Calliargon cordifolium</i>	Bryophyte	<i>Scapania gracilis</i>	Bryophyte
<i>Sonchus asper</i>	Forb	<i>Campylium stellatum</i>	Bryophyte	<i>Scapania spp</i>	Bryophyte
<i>Sonchus spp</i>	Forb	<i>Cephalozia bicuspidata</i>	Bryophyte	<i>Sphagnum denticulatum</i>	Bryophyte
<i>Stellaria graminea</i>	Forb	<i>Cinclidotus fontinaloides</i>	Bryophyte	<i>Thamnobryum alopecurum</i>	Bryophyte
<i>Teucrium scorodonia</i>	Forb	<i>Didymodon insulanus</i>	Bryophyte		
<i>Veronica montana</i>	Forb	<i>Diplophyllum albicans</i>	Bryophyte		

Table 3.10: Species recorded in the StableGrass survey, but not in the ISGS. Species are grouped by functional group

Species	Group	Species	Group
<i>Dactylorizha maculata</i>	Forb	<i>Erica tetralix</i>	Woody/Shrub
<i>Fragaria vesca</i>	Forb	<i>Rosa pimpinellifolia</i>	Woody/Shrub
<i>Geranium molle</i>	Forb	<i>Rubus fruticosus agg</i>	Woody/Shrub
<i>Myosotis scorpiodes</i>	Forb	<i>Brachythecium populeum</i>	Bryophyte
<i>Orchis mascula</i>	Forb	<i>Bryum pseudotriquetrum</i>	Bryophyte
<i>Rumex conglomeratus</i>	Forb	<i>Campylopus flexuosus</i>	Bryophyte
<i>Stellaria media</i>	Forb	<i>Didymodon fallax</i>	Bryophyte
<i>Veronica spp</i>	Forb	<i>Eurhynchium</i>	Bryophyte
<i>Carex demissa</i>	Graminoid	<i>Eurhynchium striatum</i>	Bryophyte
<i>Carex lasiocarpa</i>	Graminoid	<i>Fissidens taxifolius</i>	Bryophyte
<i>Eleocharis spp</i>	Graminoid	<i>Homalothecium lutescens</i>	Bryophyte
<i>Glyceria fluitans</i>	Graminoid	<i>Mnium hornum</i>	Bryophyte
<i>Phalaris arundinacea</i>	Graminoid	<i>Plagiomnium</i>	Bryophyte
<i>Schedolium loliaceum</i>	Graminoid	<i>Pleurozium schreberi</i>	Bryophyte
<i>Schedonorus pratensis</i>	Graminoid	<i>Trichostomum brachydontium</i>	Bryophyte
<i>Pteridium aquilinum</i>	Pteridophyte		

Frequency was calculated at both the Site and Relevé level. For both the Site and Relevé level frequencies, 111 species had decreased in frequency. At the Site and Relevé levels 61 and 53 species respectively, had not changed in frequency since the previous survey. Sixty-seven species had increased in frequency across Sites, while 74 species had increases in frequency across Relevés. See Table 3.11 for the breakdown of the number of species that decreased, increased, and remained stable in frequency across Relevés and Sites.

Table 3.11: Proportion of species that decreased, increased, and remained stable in frequency across the two survey periods.

Change	Site (n = 239 species)	Relevé (n = 239 species)
Decreased	46.443% (n = 111)	46.443% (n = 111)
Stable (No Change)	25.523% (n = 61)	22.176% (n = 53)
Increased	28.033% (n = 67)	30.962% (n = 74)

At the site level, the number of species that had no changes to frequency was greater than those that had no changes at the relevé level. The greatest number of species with increasing frequencies was recorded across the relevés, suggesting species were found in more relevés than in the ISGS survey. Similarly, at the site level, 67 species were also recorded in more sites in the StableGrass survey than at the time of the ISGS survey.

3.5.2 Changes in species frequency across functional group

Six different plant functional groups were described: Forbs, Graminoids (Grasses/Sedges/Rushes), Leguminous, Pteridophytes, Woody/Shrubs, and Bryophytes. Table 3.12 gives an overview of the numbers and frequencies of species by functional group. For this analysis, only frequency changes over Relevés are included because this captures the finer scale variation in the changes of frequency in the species.

Table 3.12: Changes in Frequency between the StableGrass and ISGS survey
according to functional group

Group	No. of Species	Decreased in Frequency	No Change	Increased in Frequency
Forbs	92	45	21	26
Graminoids	61	20	20	21
Legumes	6	2	1	3
Pteridophytes	6	5	0	1
Woody/Shrubs	9	4	1	4
Bryophytes	65	35	10	20

The forbs, bryophytes, and graminoid groups contained the highest number of species (38%, 27%, and 26%, respectively). The greatest percentage decrease in frequency was observed in the pteridophyte group (83%), with only one species (17% of pteridophytes) having an increased frequency between survey periods, followed by the bryophytes group (54%) (Table 3.12). Despite the high proportion of species decreasing in frequency, 31% of bryophyte species were encountered in higher frequencies. The forbs group experienced considerable declines in frequency between survey period with 45 out of 92 (49%) experiencing decreases in frequency across relevés (Table 3.12). Twenty-three percent of the forb species remained unchanged in frequency between the two surveys, while 28% of forbs had increased in frequency between the two survey periods. Graminoids, had similar numbers of species that decreased in frequency (33%), had no change in frequency (33%), and increased in frequency (34%). The Woody/Shrubs group had a 44% increase in the numbers of species that had an increased frequency.

3.6 Vegetation Classification

All relevés collected in the ISGS and StableGrass surveys have been assigned to an Irish Vegetation Classification and a Fossitt habitat. Similarities and differences between the vegetation recorded during both surveys were investigated to understand and describe the different types of vegetation communities that occurred

in StableGrass sites, and how these have changed since the ISGS. All relevés, from both the StableGrass and ISGS surveys have been classified to an IVC using the online ERICA system (Perrin, 2019). As with Sections 3.2.3, 3.3.1 and 3.3.3, only the relevés which are directly comparable between the ISGS and StableGrass survey are compared here for differences in the IVC (n = 44) (Section 3.6.2).

3.6.1 Irish Vegetation Communities recorded in StableGrass

For the StableGrass dataset (n = 54 relevés), a total of 19 different Irish Vegetation Communities (IVC) were recorded (Table 3.13). Forty-four relevés were given the status 'Assigned', meaning they were matched with confidence (>50% fidelity) to an IVC, and ten were assigned a 'Transitional' status (Plot membership of < 0.5, but falls within the scope of the current classification scheme, but may not represent the core definition of any community). A comparison to the Fossitt habitat for each corresponding IVCs is shown in Table 3.13. Quite a bit of variation occurred when investigating the IVC affinities to the Fossitt habitat of the IVCs. Only seven (~37%) of the IVCs had a definite affinity to one Fossitt habitat, with another seven (~37%) being representative of two Fossitt habitats, and five (26%) potentially being representative of three Fossitt habitats (Table 3.13). 36 out of 54 relevés had an IVC community that had some affinity or correspondence to the original Fossitt habitat that was assigned to that relevé while the remaining 18 relevés corresponded to Fossitt habitats that differed from the Fossitt applied to the relevé, or did not adequately represent a single Fossitt habitat.

Table 3.13: Irish Vegetation Communities and number of relevés assigned to each IVC. A comparison to the Fossitt classification scheme using the Fossitt Affinity provided by the IVC is provided.

IVC Community	Fossitt Affinity	No. of Relevés
FE3B	FL6/CD5/PF1	1
GL1A	GM1/GS4	3
GL1B	GS4	2
GL1C	FL6/GM1/GS4	7
GL1D	FL6/GS4	3
GL1E	GS4	2
GL2A	FL6/GM1/GS4	1
GL2B	GS4	2
GL2C	GA1/GS4	1
GL2D	GS4	1
GL3A	GS1	5
GL3B	GA1/GS1	1
GL3C	GS1/GS2	2
GL3D	GA1/GS1	10
GL4A	GS1/GS3/GS4	3
GL4B	GS3	7
GL4C	GS1/GS3/GS4	1
GL4D	GS3/GS4	1
HE4D	HH3	1

The three most frequently encountered IVC in the StableGrass survey were the GL3D (*Cynosurus cristatus* – *Trifolium pratense* grassland), GL1C (*Molinia caerulea* – *Succisa pratensis* grassland), and GL4B (*Nardus stricta* – *Potentilla erecta* grassland) (Table 3.13; Figure 3.12). These three most frequent IVCs are described in more detail below. Two relevés of non-grassland vegetation communities FE3B (*Carex nigra* – *Potentilla anserina* fen) and HE4D (*Molinia caerulea* – *Calluna vulgaris* – *Erica tetralix* heath)) were recorded. The FE3B relevé was classed as ‘Transitional’ while the HE4D relevé was classed as ‘Assigned’. The remaining communities were recorded in low

frequencies (≤ 5 relevés) and describe a wide range of varying vegetation within the sites.

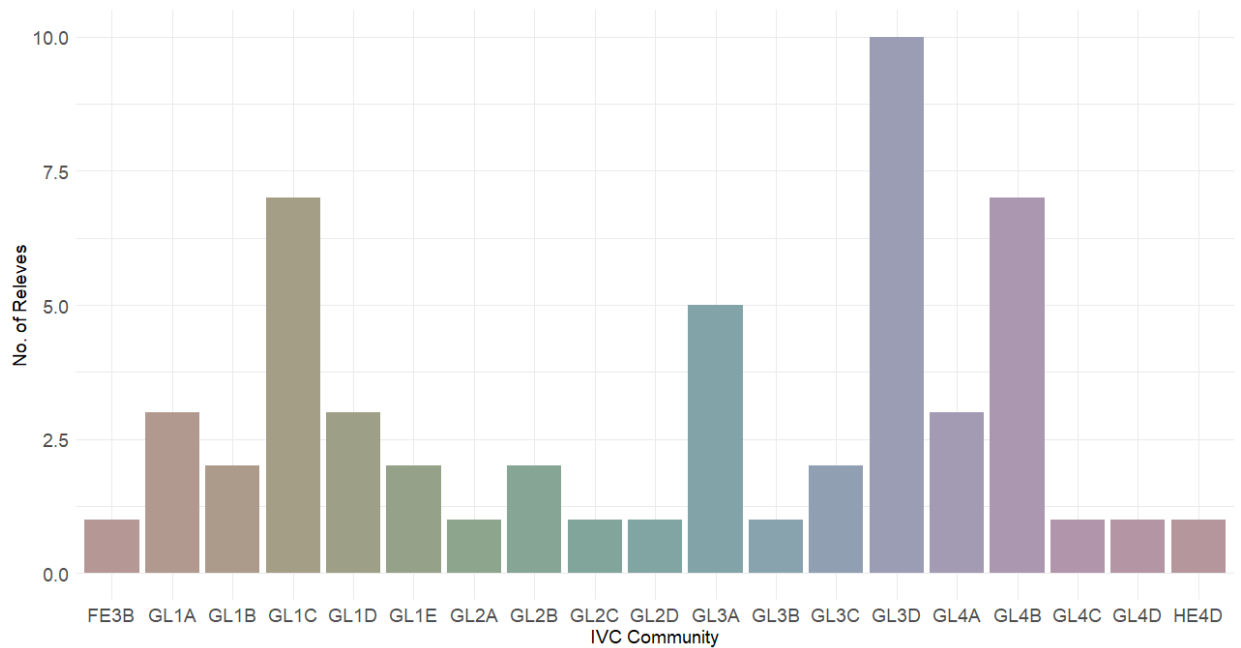


Figure 3.12: Frequency of IVCs across Relevés in the StableGrass surveys 2023 (N = 54 relevés)

The most common species that were identified in the GL3D (*Cynosurus cristatus* – *Trifolium pratense* grassland) community in the StableGrass survey were *Anthoxanthum odoratum*, *Holcus lanatus*, *Trifolium repens*, *Festuca rubra*, *Ranunculus acris*, *Ranunculus repens*, *Rhytidiadelphus squarrosus*, *Agrostis stolonifera*, *Calliergonella cuspidata*, *Cynosurus cristatus*, *Lotus corniculatus* and *Potentilla erecta*. This community was typically recorded in the lowland GS1 sites, except for one relevé (Site 11, Relevé 1), which was on an upland site. Another exception to the majority of relevés of the GL3D being recorded in GS1 sites occurred at Site 12, which was a GS3 site.

The next most frequent vegetation community, GL1C (*Molinia caerulea* – *Succisa pratensis* grassland) is largely defined by having *Festuca ovina*, *Agrostis capillaris*, *Anthoxanthum odoratum*, *Galium saxatile*, *Hylocomium splendens*, *Potentilla erecta*, *Rhytidiadelphus squarrosus*, *R. loreus*, *Luzula campestris*, *Scleropodium purum*, and *Thuidium tamarascinum* as the most frequently occurring species, in order of most frequent to lowest (to ~50%). This community was recorded in lowland, gently sloping

wet grasslands (GS4), which were poorly drained, as indicated by standing water in the areas around the relevés recorded in these sites. The seven relevés containing this vegetation community were on gleys, or basin peat soils.

The GL4B (*Nardus stricta* – *Potentilla erecta* grassland) community was the third most frequently recorded grassland vegetation community with *Anthoxanthum odoratum*, *Rhynchospora squarrosus*, *Calliergonella cuspidata*, *Cynosurus cristatus*, *Trifolium repens*, *Agrostis capillaris*, *Carex flacca*, *Festuca rubra*, *Holcus lanatus*, *Potentilla erecta*, *Scleropodium purum*, *Plagiomnium undulatum*, and *Plantago lanceolata* were the most recorded species in order of decreasing cover (to ~50%). This vegetation community was found in the upland and mountainous grasslands (i.e. Sites 4 and 6).

3.6.2 Changes in IVC between the ISGS and StableGrass Surveys

As with the StableGrass relevés, the relevé data extracted from the ISGS were classified to an IVC. This enabled a comparison to be conducted between the IVC of the relevés in the ISGS survey, and the same relevés during the StableGrass survey. In the ISGS survey, a total of 16 IVCs were recorded, while 19 IVCs were recorded in the StableGrass survey (Table 3.14). Four communities (GL2A, GL3B, HE4D, FE3B) were recorded in the StableGrass survey that were not previously identified in the ISGS survey. One community that has been identified in the ISGS surveys (GL4D), was not identified in the StableGrass surveys. For the StableGrass surveys, the most frequently recorded IVC were GL1C (16%), GL3D (13%), GL3A (11%), and GL4B (9%). The remaining IVCs were recorded in low frequencies across the remaining 23 relevés (51%). In the ISGS survey, the most frequently recorded IVC were GL1C (20%), GL1E (9%), GL3A (9%), GL3C (9%), GL4B (9%), and GL4C (9%). The remaining IVCs were recorded in low frequencies across the remaining 14 relevés (total of 31%). Out of the 45 comparable relevés between the ISGS and StableGrass surveys, six were given transitional status when assigned to an IVC in the ISGS, while 10 received this status in the StableGrass survey.

Table 3.14: Comparison of the frequency of IVC communities assigned per relevé for both StableGrass (SG, n = 45) and ISGS Surveys (n = 45). Note: StableGrass relevés without an ISGS counterpart were not included in this analysis. Asterisk (*) refers to an IVC that has not been encountered in the ISGS survey

Community	ISGS	SG
GL1A	2	3
GL1B	1	2
GL1C	9	7
GL1D	1	3
GL1E	4	2
GL2A*	0	1
GL2B	1	1
GL2C	2	1
GL2D	1	1
GL3A	4	5
GL3B*	0	1
GL3C	4	1
GL3D	2	6
GL4A	3	3
GL4B	4	4
GL4C	4	1
GL4D	2	1
HE1A	1	0
HE4D*	0	1
FE3B*	0	1

As the IVCs are assigned to a hierarchical structure (Division (i.e. Grassland), Group (e.g. GL3), and Community (e.g. GL3A)), comparisons can be made between the vegetation communities recorded in the ISGS and StableGrass surveys and change according to this hierarchy can be determined. A total of twenty relevés (44%) were found to have IVCs that differed from the IVC of the same relevé collected in the ISGS survey. Twenty-five relevés (56%) retained the same IVC that was assigned to that

relevé in the ISGS survey. Of the twenty relevés that changed community, the largest changes that occurred was at the 'Group' level of the IVC classification, with eight (40%) relevés having a different grassland group in comparison to the ISGS survey. Ten of these changed relevés (50%) had different 'Communities' to those assigned in the ISGS survey. Two of these relevés (10%) were assigned a different 'Division' to those assigned to the relevés of the ISGS survey. These changes indicate that 44% of the relevés recorded in the StableGrass survey either underwent subtle compositional changes (in the case of Communities) or more substantial composition changes (in the case of Groups and Division).

3.6.3 Fossitt Classification

The original Fossitt Classification System (Fossitt, 2000) was used in the ISGS survey that applied a Fossitt habitat to each relevé collected in the survey. These relevés with their associated Fossitt habitat were repeated in StableGrass (Relevé Habitat). In the StableGrass survey, the GS4 habitat (n = 24 relevés) was the most well represented, followed by GS1 (n = 17 relevés), and with GS3 (n = 13 relevés) having the least number of relevés. As described in Table 3.1, the GS1 relevés were the most species rich on average, followed by the GS4 relevés, with the GS3 relevés having the lowest Species Richness. Described below is each Fossitt habitat, including constant species (>50% frequency) that have been identified for those habitats in the current survey.

The GS1 relevés had a species composition that represented both neutral to calcicolous species, among other generalist plant species that can occur in other grassland habitats. In the StableGrass survey, the constant species (species with a cover/frequency >50%) of this habitat, in order of decreasing frequency included *Trifolium repens*, *Anthoxanthum odoratum*, *Cynosurus cristatus*, *Plantago lanceolata*, *Rhynchospora squarrosus*, *Festuca rubra*, *Dactylis glomerata*, *Galium verum*, *Holcus lanatus*, *Achillea millefolium*, *Ctenidium molluscum*, *Lotus corniculatus*, *Calliergonella cuspidata*, *Cerastium fontanum*, *Arrhenatherum elatius*, *Bellis perennis*, *Hypochaeris radicata*, *Lolium perenne*, *Prunella vulgaris*, *Rumex acetosa*, and *Veronica chamaedrys*. The GS1 relevés were more diverse than those of the other two habitat types and varied from intermediate diversity to high species diversity. The GS1 relevés

were typical of lowland sites, which were well drained, and either on deeper calcareous soils, or on shallow soils with areas of exposed limestone. The more diverse relevés of this habitat were consistent with the EU Annex I Festuco-Brometalia (6210).

The GS3 relevés were characterised by communities of calcifugous species, which were short in stature. Other plant species that are common to other habitats, were also present. In order of decreasing frequency (to the top $\geq 50\%$) the composition of this habitat, in the StableGrass survey was described by *Agrostis capillaris*, *Anthoxanthum odoratum*, *Potentilla erecta*, *Festuca ovina*, *Galium saxatile*, *Hylocomium splendens*, *Rhytidiadelphus squarrosus*, *Thuidium tamariscinum*, and *Holcus lanatus*. The majority of these relevés are associated with upland, and steeply sloping sites (Sites 4, 5, 6). However, three GS3 relevés were recorded in Site 12, which was a lowland environment over calcareous substrates, which contrasts with the standard description of the GS3 habitat (Figure 4.1).

The GS4 habitat was the most varied habitat in species composition surveyed during the StableGrass surveys. They ranged from low diversity, *Juncus* dominated swards, to diverse swards, which are more akin to the EU Annex 1 *Molinia* meadows [6410] habitat. The species composition of the recorded GS4 relevés consisted of *Anthoxanthum odoratum*, *Holcus lanatus*, *Potentilla erecta*, *Trifolium repens*, *Ranunculus repens*, *Agrostis stolonifera*, *Calliergonella cuspidata*, *Carex panicea*, *Juncus acutiflorus*, *Ranunculus acris*, *Festuca rubra*, *Ranunculus flammula*, and *Rhytidiadelphus squarrosus*. In most cases, GS4 relevés were typically recorded in lowland, gently sloping, and poorly drained sites (Sites 8, 9 and 10), or in mosaics in sites of differing habitat types (e.g. Sites 2, 11 and 12, where the dominant habitat is GS1, GS1, and GS3, respectively). One exception to this, is Site 11, which is a GS1 site of moderate altitude (380 m). Three relevés in this site are recorded as GS4 and were recorded on a gently sloping and poorly drained plateau one at the base of the hill.

3.6.4 Co-occurrence of species in the StableGrass survey

Analysis was conducted to identify the species that were found to be unique to the three habitat types in the StableGrass survey. For this analysis, the Relevé Habitat was used. A total of 38 species were recorded as occurring in all three habitat types (Table 3.15). Ninety species were specific to one habitat and the remaining forty-five species occur in two habitat types. The GS1 habitat had 36 unique species, with the GS3 habitat having 18 unique species, and the GS4 habitat had 34 unique species (Table 3.16).

Table 3.15: Species that were recorded in all three habitats.

<i>Agrostis capillaris</i>	<i>Trifolium repens</i>
<i>Anthoxanthum odoratum</i>	<i>Calliergonella cuspidata</i>
<i>Carex flacca</i>	<i>Danthonia decumbens</i>
<i>Cirsium palustre</i>	<i>Kindbergia praelonga</i>
<i>Climacium dendroides</i>	<i>Rhytidiadelphus loreus</i>
<i>Cynosurus cristatus</i>	<i>Scleropodium purum</i>
<i>Festuca ovina</i>	<i>Cerastium fontanum</i>
<i>Festuca rubra</i>	<i>Luzula campestris</i>
<i>Galium verum</i>	<i>Ranunculus acris</i>
<i>Holcus lanatus</i>	<i>Rumex acetosa</i>
<i>Hylocomium splendens</i>	<i>Agrostis stolonifera</i>
<i>Hypochaeris radicata</i>	<i>Agrostis canina</i>
<i>Lotus corniculatus</i>	<i>Carex pulicaris</i>
<i>Plantago lanceolata</i>	<i>Poa trivialis</i>
<i>Potentilla erecta</i>	<i>Carex panicea</i>
<i>Prunella vulgaris</i>	<i>Ranunculus repens</i>
<i>Rhytidiadelphus squarrosus</i>	<i>Carex nigra</i>
<i>Thuidium tamariscinum</i>	<i>Eurhynchium striatum</i>
<i>Trifolium pratense</i>	<i>Potentilla reptans</i>

Table 3.16: Species that were unique to GS1, GS3, and GS4 grasslands

GS1	GS3	GS4
<i>Avenula pubescens</i>	<i>Calluna vulgaris</i>	<i>Achillea ptarmica</i>
<i>Bellis perennis</i>	<i>Carex binervis</i>	<i>Ajuga reptans</i>
<i>Brachythecium populeum</i>	<i>Carex hirta</i>	<i>Alopecurus geniculatus</i>
<i>Breutelia chrysocoma</i>	<i>Carex hostiana</i>	<i>Carex demissa</i>
<i>Bromus hordaceus</i>	<i>Carex lasiocarpa</i>	<i>Carex disticha</i>
<i>Bryum pseudotriquetrum</i>	<i>Eleocharis spp</i>	<i>Carex echinata</i>
<i>Campanula rotundiflora</i>	<i>Erica tetralix</i>	<i>Cirsium dissectum</i>
<i>Campylopus flexuosus</i>	<i>Fissidens adianthoides</i>	<i>Dactylorhiza fuchsii</i>
<i>Cirsium arvense</i>	<i>Leontodon hispidus</i>	<i>Dactylorhiza maculata</i>
<i>Conopodium majus</i>	<i>Leontodon taraxacoides</i>	<i>Deschampsia cespitosa</i>
<i>Didymodon fallax</i>	<i>Luzula sylvatica</i>	<i>Equisetum arvense</i>
<i>Fissidens dubius</i>	<i>Polytrichum commune</i>	<i>Eurhynchium</i>
<i>Fissidens taxifolius</i>	<i>Prunus spinosa</i>	<i>Filipendula ulmaria</i>
<i>Fragaria vesca</i>	<i>Racomitrium lanuginosum</i>	<i>Glyceria fluitans</i>
<i>Geranium molle</i>	<i>Sagina procumbens</i>	<i>Hydrocotyle vulgaris</i>
<i>Hieraceum spp</i>	<i>Sphagnum subnitens</i>	<i>Iris pseudacorus</i>
<i>Homalothecium lutescens</i>	<i>Tortella tortuosa</i>	<i>Juncus conglomeratus</i>
<i>Hypericum pulchrum</i>	<i>Vaccinium myrtillus</i>	<i>Juncus effusus</i>
<i>Hypnum lacunosum</i>		<i>Juncus inflexus</i>
<i>Koeleria macrantha</i>		<i>Lophocolea bidentata</i>
<i>Leucanthemum vulgare</i>		<i>Lotus pedunculatus</i>
<i>Neckera crispa</i>		<i>Lychnis flos-cuculi</i>
<i>Orchis mascula</i>		<i>Lythrum salicaria</i>
<i>Polygala vulgaris</i>		<i>Mentha aquatica</i>
<i>Primula vulgaris</i>		<i>Myosotis scorpiodes</i>
<i>Ranunculus bulbosus</i>		<i>Phalaris arundinacea</i>
<i>Rhytidiadelphus triquetrus</i>		<i>Plagiomnium</i>
<i>Rosa pimpinellifolia</i>		<i>Pleurozium schreberi</i>
<i>Schedolium loliaceum</i>		<i>Ranunculus flammula</i>
<i>Sesleria caerulea</i>		<i>Rubus fruticosus agg</i>
<i>Stellaria media</i>		<i>Rumex conglomeratus</i>
<i>Tortula muralis</i>		<i>Senecio aquaticus</i>
<i>Trichostomum brachydontium</i>		<i>Viccia cracca</i>
<i>Trisetum flavescens</i>		<i>Viola palustris</i>
<i>Veronica chamaedrys</i>		
<i>Veronica officinalis</i>		

Chapter 4: Discussion

4.1 Overview

The diversity and species composition across semi-natural grasslands have undergone significant changes in the past century (Bullock et al., 2011; Ridding, Redhead and Pywell, 2015; Stroh et al., 2020). In Ireland, 1,192 semi-natural grassland sites were identified and surveyed in the period between 2007–2012 for the Irish Semi-natural Grassland Survey (ISGS). Threats such as agricultural intensification and abandonment were cited in the ISGS (O’Neill et al., 2013) and the BSBI Plant Atlas 2020 as being responsible for the large loss in numbers of Irish grassland flora (Stroh et al., 2020). The primary objective of this thesis was to determine if changes in the plant diversity and species composition has occurred in a selection of these ISGS sites across three different habitat types, as defined by the Fossitt system.

4.2 Vegetation Diversity

4.2.1 Vegetation diversity of the StableGrass survey

Using the Fossitt habitats (GS1, GS3, or GS4) (Fossitt, 2000) that were initially applied to the relevés during the ISGS survey, the differences in diversity between these habitats recorded during the StableGrass surveys were assessed. The Species Richness and Simpson’s Diversity values differed significantly between the three different habitats (Figure 3.1); however, because the sample sizes differed between the habitat types this may not be an entirely robust result (Table 4.1).

Table 4.1: Number of relevés collected per Fossitt habitat type. This refers to the number of relevés assigned to each habitat type, rather than the number of relevés collected per site of each different habitat type

Code	Habitat	No. of Relevés
GS1	Dry calcareous & neutral grassland	17
GS3	Dry-humid acid grassland	13
GS4	Wet grassland	24

The GS1 (calcareous grassland) relevés had the highest mean Species Richness, followed by the GS4 (wet grassland) relevés, and then the GS3 (acid grassland) relevés (Figure 3.1A). Similarly Diekmann et al., (2019) found European calcareous grasslands to be generally more diverse than wet grasslands. Diekmann et al., (2019) also found that some wet grassland relevés had high species richness, despite the overall finding of the calcareous grassland being the most species rich. In the current study, some GS4 relevés had Species Richness values similar to the GS1 relevés. For example, Site 9 (GS4) has mean Species Richness values that are similar to Sites 2 & 3, which are GS1 sites (Appendix 9, Figure A1). Pairwise analysis showed a significant difference in Species Richness between the GS1 and GS4 habitats, reflecting the pattern of greater Species Richness in GS1, despite occasionally high richness in some GS4 relevés. The same analysis showed no significant difference in richness between the GS3 and GS4 habitats. For example, Sites 4–6 (GS3 sites) showed similar Species Richness values to Sites 7 and 8 (GS4), while Sites 9 and 10 (GS4) had similar values to Site 12 (GS3). However, Site 12 is a GS3 site that contrasts the finding of the GS3 habitat having the lowest mean Species Richness and Simpson’s Diversity. Despite the predominant habitat type (Site Habitat) being GS3, with the species composition demonstrating this, Site 12 was located over limestone bedrock (Figure 4.1). This calcareous influence resulted in a vegetation composition that was more complex and diverse than what is typical for GS3 sites, and as a result, relevés of Site 12 were often not assigned an IVC with confidence (Transitional) through ERICA. This intergradation of the habitats is due to periodic flooding at this site that results in mineral leaching (O’Neill et al., 2013) and allows for a diverse mix of species with differing pH preferences to occur.



Figure 4.1: Site 12 (GS3 site, ISGS ID 1854), Relevé 1. GS3 Relevé with characteristic species and vegetation of the GS3 habitat, but with obvious calcareous influence with exposed limestone rock

The highest Species Richness and Simpson's Diversity values were recorded at Sites 1 (GS1), 2 (GS1) and 12 (GS3), with the lowest recorded at Site 5 (GS3) (Figure 3.3, Figure A1, Appendix 9). The variation in diversity across the sites may be explained by the site habitat, and in turn the differences observed across the relevé habitat (GS1 > GS4 > GS3). In contrast to the other two diversity indices Simpson's Evenness was highest in the GS3 relevés, followed by GS4 and then the GS1 relevés. These differences could also be observed at the site level. Site 6 (GS3) has the highest mean Simpson's Evenness, followed by Site 10 (GS4), and then Site 2 (GS1) (Figure 3.3). These differences could be explained by differences in management across the sites, as discussed further in Section 4.5.

The diversity indices may be explained by the vegetation structure observed in the relevés across the three habitats. The intermediate Species Richness values of the GS4 relevés and low Simpson's Evenness values (Figure 3.1A & C) suggest that these relevés have increased species dominance in comparison to other habitats. This

increased species dominance can suppress the Species Richness of these relevés (Sasaki and Lauenroth, 2011; Niu et al., 2018) through competitive exclusion (Grime, 1973; Mariotte et al., 2013; Czarniecka-Wiera et al., 2019). While lower Species Richness does not always imply there is increased species dominance, there are examples in the data where this is the case. For example, the relevés of the GS4 habitat were quite varied and represented swards that were wet and rush (*Juncus spp*) dominated to species rich meadows (*Molinia* meadows) that were similar to those found at the Shannon Callows (Fossitt, 2000; O' Neill et al., 2013). The relevés that were rush or *Filipendula ulmaria* dominated (i.e. GS4 relevés of Sites 7, 8, 11 and 12) were those that have been discussed above and have increased species dominance with observable impacts on the Species Richness values. These relevés account for the lower end of range observed in Simpson's Evenness in Figure 3.1C. However, as noted, the species-rich *Molinia* meadow grasslands (Sites 9 & 10) had diversity values that were in some cases as high as the GS1 relevés. These diverse GS4 relevés account for the higher end of the range in the Simpson's Evenness values.

4.2.2 Changes in diversity since the ISGS 2007–2012

This study aimed to compare the vegetation diversity between the StableGrass and the ISGS surveys to determine if any changes have occurred. There was no significant difference found in the three diversity measures (Species Richness, Simpson's Diversity and Simpson's Evenness) between the ISGS and StableGrass surveys. This contradicts findings of other studies that have reported significant differences in diversity over time (Duprè et al., 2010; Newton et al., 2012; Hülber et al., 2017; Mitchell et al., 2017; Bauer and Albrecht, 2020; Ridding et al., 2020; Klinkovská et al., 2024). Despite this lack of an overall change, 44 (52%) of the StableGrass relevés had reductions in Species Richness values while 14 (32%) of the relevés had gains in Species Richness since the ISGS. Seven (16%) of the relevés had Species Richness values that have remained the same since the ISGS. The mean loss of species in the dataset when compared to the ISGS was six, with losses and gains ranging from -18 to +18 species. Many different factors could have influenced the lack of a significant difference in the diversity measures since the ISGS. The length of time (~11–15 years)

since the ISGS may not have been sufficient time for significant changes to have occurred. The studies that have reported significant changes in diversity over time, typically studied longer time periods than this study, ~40–100 years (Mitchell et al., 2017; Diekmann et al., 2019; Bauer and Albrecht, 2020). Measures such as Species Richness can be slow to respond to changes because the grassland plant species may have long life cycles with slow intrinsic dynamics (Eriksson, 1996; Fischer and Stöcklin, 1997). Grassland plants have been shown to persist and occur despite threats such as fragmentation (Maurer et al., 2003). High species turnover may also explain the lack of a significant difference, especially if species lost to a site are matched by species new to the site. In diverse grasslands, co-existence of many species not be in an equilibrium (Gigon and Leutert, 1996), meaning temporal changes and stochasticity can influence the change or lack of a change in diversity being detected.

While it was expected that Species Richness, among the other diversity measures, would have decreased or changed over time, this is not always the case as seen in other studies (Berlin et al., 2000; Mitchell et al., 2017; Diekmann et al., 2019). For example, Diekmann et al., (2014) observed slight positive to no change in the Species Richness of calcareous grassland plots that were assessed over the 70-year study period. These changes were irrespective of difference in species composition and turnover. Other studies into temporal changes in grassland vegetation reported increases in Species Richness (e.g. Newton et al., 2012; Phoenix et al., 2012; Mitchell et al., 2017). In the StableGrass survey, similar variations in the changes that occurred in Species Richness since the ISGS survey can be observed at the relevé-level (plot level). For example, despite no overall significant change in Species richness, 14 relevés (32%) did show an increase in Species Richness. This shows that the detection of changes in the diversity of semi-natural grasslands over time is complex, especially when assessing changes at the relevé-level.

While no overall changes in the species diversity were detected, site-by-site analysis identified one StableGrass site that experienced considerable changes in diversity since the ISGS. Site 7 (ISGS ID 2344), a GS4 site located in Portumna, Co. Galway, had a 50% reduction in the mean species richness since the ISGS, which could be explained by abandonment (Section 4.5). However, these changes were not

statistically significant, likely due to a small sample size to compare the diversity of Site 7 between the StableGrass survey (n = 3 relevés) and ISGS survey (n = 3 relevés). Site 3 (ISGS ID 1624, GS1, Burren, Co. Clare) also had similar decreases in mean Species Richness but between survey differences could not be statistically tested because only one relevé was taken in the ISGS. Changes in management at this site, as well as other subtle changes to management that may explain the diversity variation across relevés, sites and habitats are discussed in Section 4.5.

4.2.3 Irish Vegetation Communities and changes since ISGS

The diversity of grasslands can also include the number and types of different vegetation communities that may occur. The classification of both the ISGS and StableGrass relevés to the Irish Vegetation Classification (IVC) system has allowed for a comparison to be made and to monitor the changes that may occur in the grasslands at the community level. There was no significant difference found in the frequencies of the IVCs between the two survey periods (Fisher Test, $p = 0.869$). However, there are some notable changes that have occurred in the IVCs between the two surveys. Twenty-one relevés were assigned to a different IVC during the StableGrass survey when compared to the IVCs applied to the same relevés but using the ISGS data. Out of the 21 relevés that changed community, eight changed at the group level (i.e. from GL2 to GL3 for example). Four communities were recorded in the StableGrass survey that were not encountered in the ISGS survey (Table 3.14). IVCs are classified based on the species composition and abundance, so these changes support the shift in species composition observed between Figures 3.5 and 3.6. Additionally, 10 relevés were assigned to a 'transitional' IVC status in the StableGrass survey, compared to six in the ISGS. Considering the changes in community, this may be evidence for homogenisation of the vegetation (Section 4.3). Changes in vegetation community are consistent with other UK semi-natural grasslands, with shifts from species-rich NVC communities to mesotrophic communities that are typified by competitive species (Smart et al., 2003; Bennie et al., 2006). However, other studies found no shift in vegetation community, despite observing a shift in species composition (Ridding et al., 2021). The changes in the

IVCs, as well as the diversity and species composition since the ISGS could be explained by subtle changes in the management since the ISGS (Section 4.5). Changes in the vegetation community may be interpreted as movement along a gradient of changing land-use intensity and other environmental factors, e.g. hydrological regime and fertility, can result in changes to the vegetation communities over time (Smart et al., 2003; Houlihan et al., 2006; Wesche et al., 2012). Changes in the IVCs can have implications for the conservation value of the grassland vegetation. Out of the 21 relevés that changed IVC, eight were described as having a lower conservation value (based on synopses provided for each community in the IVC) (Perrin, 2015), with four being described as having a greater conservation value than the IVC assigned to that relevé during the ISGS.

4.2.4 Comparison of IVC communities to Fossitt habitat classification

The Fossitt habitat classification system was used throughout this study from the initial site selection process to the analysis of differences of species diversity and composition across the sites. Discussed here is a comparison between the Fossitt and the IVC. It must be noted that the Fossitt system is a habitat classification system, while the IVC is a vegetation classification system, which have their differences. Habitat classification refers to the living environment on a whole that supports a variety of species, while vegetation classification focuses specifically on assigning discrete patches of area to a designation based on the species composition and abundance. Contained in the synopsis of all IVCs is the Fossitt habitat to which that IVC may have an affinity. Ten relevés had IVCs assigned to them that had a Fossitt affinity that was different to the Fossitt habitat that was assigned to the relevé by the ISGS. In some cases, relevés (n = 7 relevés) were assigned an IVC that was poorly represented by a single Fossitt habitat. This resulted in two to three different Fossitt habitats that could potentially support that vegetation community. This demonstrates that the IVC has greater resolution in describing the vegetation community and composition in comparison to the Fossitt system.

The seven relevés that had an IVC community that was assigned to more than one Fossitt habitat could indicate a movement towards a semi-improved community. This

means management intensity may have increased but that the grassland still is not improved enough to be considered an agriculturally improved grassland (GA1). However, while this semi-improved habitat still has some conservation importance (Sullivan et al., 2010), it is a more degraded version of the initial habitat. Two relevés were assigned to IVCs (GL2C and GL3B) that have a Fossitt affinity to the habitats GA1/GS4 or GS1/GA1, that can indicate semi-improvement of these relevés. Three other relevés were assigned to communities that are described by the IVC as being representative of a semi-improved grassland. This increase in the number of semi-improved grasslands is consistent with other studies, and is recognised as a sign of degradation (Fuller, 1987; Blackstock et al., 1999; Ridding et al., 2015). Two relevés also changed to peatland habitats, namely fen or flush and heathland habitats in the IVC (Table 3.14).

4.2.5 Site selection may have influenced the lack of a significant change in diversity

It must also be noted that the grassland study sites were subjected to various selection criteria. As a result, only those sites that were large in area (>9 hectares) and had large areas of continuous grassland habitat (the site was not divided up into discrete land compartments) and most importantly, did not undergo significant land-use change since the previous survey were considered. Land-use changes, for example afforestation or agricultural intensification, and fragmentation can be attributed to considerable declines in area and diversity of semi-natural grasslands in Europe (Fischer and Stöcklin, 1997; Lindborg and Eriksson, 2004; Cousins and Eriksson, 2008; McGovern et al., 2011; Hülber et al., 2017; Aune et al., 2018). As a result, it could be argued that there was a bias towards the better quality ISGS sites during the site selection process. Hence the lack of a significant difference in the diversity of these sites could be expected considering that they may not have experienced significant land-use change or conversion and had a relatively large patch size which would reduce the effects of fragmentation.

4.3 Vegetation homogenisation over time

4.3.1 What is homogenisation?

Homogenisation is the process where vegetation communities become more like each other over time. Homogenisation is a threat globally to grassland biodiversity, with moderate increases in land-use intensity, as well as abandonment (Section 4.5) resulting in homogenisation across microbial, animal and plant taxa (Gossner et al., 2016; Schrama et al., 2023). Heterogeneity, which is an important component for promoting a high species diversity (Adler et al., 2001; Rook et al., 2004; Marion et al., 2010; Reisch and Hartig, 2021), is reduced when homogenisation occurs. The increased overlap of habitats and relevés in Figure 3.5 evidences the view of increasing homogenisation in the semi-natural grassland sites. An increasing trend towards homogenisation is consistent with other studies into the changing composition of grasslands (e.g. Halada et al., 2008; Pakeman and Fielding, 2021; Vidaller et al., 2022).

4.3.2 Evidence of increased homogenisation

While no significant differences were found in the species diversity between the ISGS and StableGrass surveys, changes to the species composition have been observed (Figures 3.5 & 3.6) (Section 3.3). This shift in the species composition of the StableGrass survey is evidenced by a greater overlap in the habitat types when compared to the ISGS. A shift in species composition despite no changes in species diversity is consistent with Diekmann et al. (2014). Changes in grassland species composition over time is consistent with other European grassland studies (e.g. Köhler et al., 2005; Bennie et al., 2006; Bauer et al., 2009; Mitchell et al., 2017; Diekmann et al., 2019; Ridding et al., 2020). The change identified in the StableGrass survey is likely due to homogenisation of the grassland habitats. This is consistent with Ridding et al. (2020) who undertook a long-term study into grassland change and showed a similar change in composition. While a significant difference occurred in the species composition between the ISGS and StableGrass occurred at the relevé level (MRPP conducted on Figs 3.5 & 3.6), the effect size was weak. The survey

differences at the site level (Figure 3.7) were non-significant. Despite this, a trend towards homogenisation was identified in the ordination.

4.3.3 Evidence of increased homogenisation shown at the species level

4.3.3.1 Loss and gains of species since the ISGS

A total of 239 species were recorded between the ISGS and StableGrass surveys collectively. In the ISGS 209 species were recorded across the 12 sites, while 173 were recorded in the StableGrass survey (11–16 years later, site dependent). The most notable changes at the species level were the loss of 66 species (Table 3.9) and the gain of 31 species since the ISGS (Table 3.10). This is evidence for increasing homogenisation of the grasslands especially since ten of the species lost were specialist species (Schrama et al., 2023), and species gained were more indicative of intensive management. Among the species lost was *Coeloglossum viride* (Frog Orchid), a grassland specialist and classed as Near Threatened on the Irish Vascular Plant Red List (Wyse Jackson et al., 2016) that has been recorded in less than 100 of Ireland's hectads (10 km x 10 km distribution squares) (Stroh et al., 2020). *Ophioglossum vulgatum* (Adder's Tongue) is a small fern that has only been recorded in 166 hectads, and *Parnassia palustris* (Grass-of-parnassus) has been recorded in only 191 hectads were both also absent from StableGrass surveys. Both species are specialists of calcareous wetlands and bordering grasslands. The loss of grassland specialists and species of conservation concern is consistent with studies into changes that have occurred in other European grasslands (Fischer and Stöcklin, 1997; Diekmann et al., 2019; Bauer and Albrecht, 2020).

Some notable species were gained since the ISGS. Gains in species can include species that may be positive for the grassland condition or those that are of a 'cosmopolitan' nature and indicate agricultural intensification. *Dactylorhiza maculata* and *Orchis mascula*, two orchid species, were recorded during StableGrass surveys but were not recorded in the ISGS. While these are common orchids (644 and 486 hectads respectively (Stroh et al., 2020)), any orchid species is considered a High Quality indicator for the 6410 and 6210 Annex I grassland habitats (O' Neill et al., 2013), meaning the gain of these species are positives for the conservation of these semi-

natural grasslands. Another example of a gained species that would indicate good quality calcareous grassland is *Rosa pimpinellifolia* (Burnet Rose) (Stroh et al., 2020). This species has been only recorded in 276 hectads in Ireland and is characteristic of sand dune grasslands or limestone grassland (Stroh et al., 2020). While this could be interpreted as woody succession, the cover was less than 25%, which meets the criteria for 6210 Annex calcareous grassland (O' Neill et al., 2013) and may suggest that in this instance, it is not a sign of succession. However, some species that could be indicative of succession were observed. *Pteridium aquilinum* (Bracken) was found in the StableGrass survey and not the ISGS. This species is likely to have increased due to more intensive sheep grazing of upland grasslands (Stroh et al., 2020), which is consistent with the field observations in the 2023 StableGrass survey (e.g. 2429). *Rubus fruticosus* agg. (Bramble) was another woody species that was gained since the ISGS. This species is competitive and has responded to a decline in grazing of semi-natural habitats (Marrs et al., 2010; Stroh et al., 2020), indicating succession in relevés where this species was recorded. *Schedonorus pratensis* (Meadow Fescue) was recorded in the current survey and is described in the BSBI Plant Atlas as being a species of neutral, usually fertile soils (Stroh et al., 2020). This suggests that the relevés that contained this species may have had a trajectory of increasing fertility. However, this implication should be taken with caution as these inferences are based on one species. With more time, and more detailed analysis into species traits, especially of those that are changing in frequency, more definitive conclusions on changes to nutrient status may be determined.

4.3.3.2 Changes in species frequency since the ISGS

Across both the site and relevé levels, considerable changes in species frequency occurred since the ISGS, with 111 species (46% of the total species pool) declining in frequency between surveys (Table 3.11). The frequency of specialist species is expected to be higher in old grasslands with minimal management (McCook, 1994; Schmid et al., 2017). The reduction of the frequency of these species in the StableGrass study is further evidence for homogenisation due to increased

management intensities. The implications of these frequency changes in relation to management are discussed further in Section 4.5.

It must be noted that factors other than changes in management intensity and homogenisation may have resulted in the changes observed in the species frequency. The climate variability in the intervening period between the ISGS and the StableGrass surveys could have resulted in differences in species composition from year-to-year (Adler et al., 2006). Dostálek and Frantík (2011) observed increased dominance and richness of graminoid species in dry grasslands after wetter conditions in the Winter. Matesanz et al. (2009) observed that long-term increases and high interannual variability between temperature and precipitation had strong relationships with the trends and interannual variation observed in the plant communities.

4.3.4 Homogenisation and extinction debt

The increase in homogenisation observed in StableGrass could have negative impacts on grassland conservation. The findings of the StableGrass survey suggest that an extinction debt could be building in these semi-natural grasslands. Extinction debt refers to the persistence of species in a habitat after the conditions have become unsuitable, but may ultimately go extinct after a given time (Helm et al., 2006; Krauss et al., 2010; Otsu et al., 2017) Extinction debts can take a long time to become apparent, especially since perennial grassland species are often slow to respond to change (Helm et al., 2006). This has been demonstrated by Krauss et al. (2010), Cousins and Vanhoenacker (2011), Culbert et al. (2017) and Otsu et al. (2017), which have shown Species Richness and composition are better explained by the past landscape patterns (often up to 100 years prior) than current management practices. An example of such may be the significant losses of semi-natural grasslands from the 1970s in Ireland due to agricultural intensification (O' Neill et al., 2013). As a result, many of Ireland's semi-natural grasslands are in the form of discrete patches, fragmented by an intensified agricultural landscape and by coniferous plantations (Martin et al., 2008). Fragmentation can increase the likelihood of an extinction debt being present in grasslands (Krauss et al., 2010). Helm et al. (2006) calculated an

extinction debt of 43% in Estonian grasslands that have reduced in area from 3.64 km² in the 1930s to 0.21 km² at the time of its publication. The current study lacks data into the past landscape, the amount of fragmentation that may have occurred, and as a result the extinction debt. However, considering the evidence provided in this study an extinction debt is likely occurring. The identified trend towards homogenisation and as a result, extinction debt could be more significant in the future.

4.4 Environmental impacts on species diversity

4.4.1 Influences of soil pH and Ellenberg indicators on the vegetation diversity

The pH of the soil or grassland environment can have a considerable influence on the plant species diversity and composition (Blackstock et al., 1998; Critchley et al., 2002a; Chytrý et al., 2003; Pärtel et al., 2004; Raatikainen et al., 2007). In the StableGrass study, contaminated water used in the pH measurement process resulted in soil pH values that were inaccurate. Instead, this discussion will focus on the Ellenberg Reaction values calculated for the relevés, which will act as a proxy for the soil pH. The Ellenberg Reaction indicator was significantly correlated with the Species Richness with a moderate positive relationship between the two (Appendix 9, Figure D1). This supports the findings of multiple studies (Blackstock et al., 1998; Critchley et al., 2002a; Chytrý et al., 2003; Pärtel et al., 2004; Raatikainen et al., 2007), showing a positive effect of soil pH on plant diversity. Examples of where increased pH could have resulted in higher Species Richness and other diversity values would be the GS1 – calcareous grassland sites (Appendix 9, Figure A1). Some of these sites (e.g. Sites 2 & 3) had large areas of exposed limestone bedrock, which meant that there was a direct influence of this calcareous geology with the soil and subsequently the species composition. On the other hand, the upland GS3 sites (e.g. 4, 5 and 6) were located on sandstone bedrock and peat soils, which can explain the low pH of these habitats. As a result, and in line with the positive relationship between pH and species diversity (Roem and Berendse, 2000; Critchley et al., 2002b; Pärtel et al., 2004), the Species Richness values of these GS3 sites were the lowest out of the 12 sites on average.

As well as explaining the species diversity, pH can also influence the grassland species composition. As the soil pH values were unusable, the Ellenberg Reaction values are used for this discussion. This indicator accounted for 52.6% (Figure 3.8) of the variation in the species composition, respectively, and is consistent with the literature (e.g. Critchley et al., 2002b; 2002a; Pärtel et al., 2004; Duprè et al., 2010; Riesch et al., 2018). As the Reaction is oriented away from the GS3 habitat, and towards the GS1, and to some extent the GS4 relevés in Figure 3.8 this shows that the GS3 relevés typically had low pH values. This is consistent with the expected pH values of these acidic grassland habitats (Fossitt, 2000; Dorland et al., 2013). As described above, the opposite to this is the GS1 relevés that have a high pH with the Ellenberg Reaction values pointing towards this habitat. The Ellenberg Reaction indicator is also orientating towards some GS4 relevés (Sites 7, 9 and 10) indicating these relevés have higher pH values. These relevés are located over limestone bedrock, which would increase the pH of these grasslands. This results in these relevés having species that are of more calcareous conditions and as a result bared some similarities to the species composition of some GS1 relevés.

As well as pH, other environmental factors such as moisture, light and nitrogen can influence the diversity of semi-natural grasslands. Ellenberg Indicator Values for Wetness, Light and Nitrogen, respectively, can be used as proxies for these conditions. Notably, Light was found to have a moderate, positive and significant correlation with Species Richness (Appendix 9, Figure E1). This shows that relevés that had greater Ellenberg Light values had greater Species Richness values. This could be due to increased light availability that reduces light competition and promotes greater Species Richness (Borer et al., 2014; Eskelinen et al., 2022). The StableGrass study found a strong negative significant relationship between Ellenberg Wetness and Species Richness (Appendix 9, Figure F1). This shows that relevés with higher Wetness values had lower Species Richness values. This is consistent with Moeslund et al. (2013) who reported increased Species Richness values in drier grasslands. This relationship is expected to be hump-backed since it is expected that conditions that are too dry and too wet would reduce species richness. Examples of this in the StableGrass study are the GS1 relevés that had the lowest Ellenberg Wetness values

(Appendix 6, Sheet 4) but had the highest diversity values. Some of these relevés were located on areas of shallow soils over exposed limestone bedrock and had reduced water availability compared to other grassland habitats (e.g. Sites 2, 3 and relevé 5 of Site 2). The relationship between Wetness and Species Richness is driven by the influence of soil moisture on the soil nutrient availability (Giesler et al., 1998; Rodriguez-Iturbe et al., 1999; Loiseau et al., 2005). In contrast, the GS4 relevés that had the highest Wetness values (Appendix 6, Sheet 4) had reduced species diversity. With increasing moisture, biomass has been shown to increase (Maestre and Reynolds, 2007; Deng et al., 2016), which is reflected in the vegetation height of the relevés of the GS4 grasslands (Appendix 6, Sheet 3). The higher vegetation height is reflected by the increased dominance of fast growing and competitive species in these conditions (Grime, 1973; Borer et al., 2014), namely *Juncus* species and *Filipendula ulmaria*. However, this expectation of reduced species diversity due to increased moisture availability, and potentially nutrient availability was not always the case. Relevés of the GS4 habitat in Sites 9, 10 and 11 had moderate levels of Species Richness and Simpson's Diversity and is likely due to the calcareous influence at these sites.

4.4.2 Environmental changes since ISGS through Ellenberg values

As the Ellenberg Indicator Values reflect the long-term responses of plants to their environment (Krecek et al., 2010), changes in these values since the ISGS can indicate a changing environment over time. A significant difference was found in the Wetness indicator between the ISGS and StableGrass surveys, with a mean difference of 0.183 units since the ISGS (Paired T-test, $p = 0.011$). This indicates an increase in the moisture conditions over time. This shift towards greater Ellenberg Wetness is indicated by the increased frequency of species such as *Myosotis scorpioides*, *Glyceria fluitans*, and *Phalaris arundinacea*. These are species typical of wet, often inundated environments (Stroh et al., 2020). The greatest changes in Wetness were detected in relevés of Sites 5 (GS1), 6 (GS3), 7 (GS4), 8 (GS4), and 11 (GS1) (Appendix 9, G).

There was no significant difference in the Ellenberg Light indicator between the ISGS and StableGrass surveys (Appendix 9, G). This was unexpected considering the ongoing abandonment that was observed at Site 7 (Section 4.5). Overall, this does not support the view of increased management intensity discussed in Section 4.5. There was also no significant difference found in the Ellenberg Nitrogen between the ISGS and StableGrass surveys, which shows no evidence of increased Nitrogen in the period between the two surveys. This result is unexpected in an European and global context with studies finding increases in the Ellenberg Nitrogen over time across a variety of ecosystems (e.g. Duprè et al., 2010; Delgado and Ederra, 2013). While increases have been reported, some studies reported decreases in Ellenberg Nitrogen values over time (e.g. Ridding et al., 2020). The Ellenberg Nitrogen values may not be directly related to the soil nitrogen availability, and more so with factors that are correlated with the soil nitrogen (Chytrý et al., 2009; Löfgren et al., 2020). This means that the Ellenberg Nitrogen may not accurately reflect the nutrient status at the study sites, and whether this has changed over time.

4.4.3 Influence of species diversity and composition on carbon storage

The literature has shown that increased Species Richness of grassland improves the carbon storage (e.g. Ostle et al., 2009; Chen et al., 2018; Xu et al., 2020; Spohn et al., 2023). A positive correlation would be expected between the Species Richness and the Soil Organic Matter (SOM). However, in the case of this study, a significant but negative correlation was observed between the SOM and Species Richness (Appendix 9, Figure G1). The SOM values were highest in the soils of the GS3 relevés, in particular Sites 4, 5 and 6 (upland GS3 sites) (Table 3.8, Figure 3.10) that also had the lowest mean Species Richness values. Conversely, the relevés that had high Species Richness values (typically of the GS1 grassland sites) had lower SOM values. Most of the studies that have reported positive relationships between Species Richness and soil carbon often only assessed species richness increases to approximately 8–20 species (e.g. Cong et al., 2014; Chen et al., 2018; Xu et al., 2020). However as with the StableGrass survey, many relevés had Species Richness values greater than those assessed in the above studies. This could be explained by a hump-backed relationship

between Species Richness and soil carbon as demonstrated by Anacker et al. (2021). This may explain why the relevés that had high Species Richness values (>20 species) had low SOM values and high SOM values in the relevés with ~15 or less species (in the case of Sites 4, 5 and 6).

Despite the influence of diversity on carbon storage, some authors argue that species composition may have a greater influence on carbon storage in semi-natural grasslands (e.g. Fornara and Tilman, 2008; Conti and Díaz, 2013; Roscher et al., 2019). However, this study did not find any significant correlation between SOM and the species composition. Other factors may influence this relationship and have not been detected in this study; hence no significant relationship was detected. These may include climate, SOM quality, disturbance and presence of different functional groups (e.g. Anacker et al., 2021; Spohn et al., 2023). It is expected that there are numerous below ground factors that may have influenced the carbon storage in these grasslands (Jobbágy and Jackson, 2000; Poeplau et al., 2018; Xu et al., 2020) that were not detected in the StableGrass study. This finding has implications for the StableGrass project that could suggest a negative relationship with plant diversity and carbon storage occurs in Irish semi-natural grasslands.

4.4.4 Landscape factors that may explain the observed species diversity

While the soil conditions described above, as well as management (Section 4.5) can influence the species diversity across the sites, environmental factors such as geology and climate (Section 4.4) may influence observed diversity. Despite selecting four sites of each habitat type, they did not have the same landscape characteristics. These differing characteristics include elevation, topology, aspect, soil and geology. Differences in these factors resulted in variation across and within the 12 sites. Data on these confounding factors were not assessed in this study. While the sites were all located on the West coast of Ireland, there were differences in the climate based on the elevation, slope and aspect, which influenced localised temperature and precipitation patterns (Bennie et al., 2006; Diekmann et al., 2014; Reitalu et al., 2014; Kuhn et al., 2021; Deng et al., 2022). These have been cited as important determinants of plant diversity and species composition (Moreno et al., 1990;

Duckworth et al., 2000; Zelnik and Čarni, 2008; Wehn et al., 2017; Raduła et al., 2022). Sites that had an elevation or topography that would have influenced the climatic conditions were Sites 4, 5, 6 (GS3) and 11 (GS1). These grasslands were located on upland and sloped areas, with the remaining grasslands having a low lying and flat to gently sloping topology.

Other factors that influence semi-natural grassland diversity at the landscape level are both the habitats of the surrounding landscape, and the landscape history, often back to ~1800s. These factors have not been determined in the current study, but it is important to appreciate the potential influences that these broad scale factors may have on the diversity of the StableGrass sites. It is expected that the increasing numbers of semi-natural grasslands in the immediate vicinity (4 km² radius) also increases the species diversity of the site in question (Janišová et al., 2014). The positive effect of the presence of semi-natural grasslands in the surrounding landscape on diversity could be related to fragmentation. Increased fragmentation leads to reduced species diversity over time and an increased risk of extinction debt (Helm et al., 2006; Cousins, 2009; Noda et al., 2022). Hence, this impact of fragmentation would be reduced with the presence of other areas of semi-natural habitats in the vicinity.

However, in the current study the surrounding landscape did not appear to explain the diversity observed in the StableGrass sites. Sites 1 and 2 had the highest mean diversity values despite being surrounded by habitats that were predominantly intensively managed. Also, the GS3 sites (except for Site 12) were surrounded by similar acid grassland habitats or heathland habitats. However, the mean diversity of these sites was quite low despite the high connectivity with the surrounding landscape. These habitats typically have low Species Richness values in comparison to the other two grassland habitats. However, as these habitats typically have reduced Species Richness, the diversity surrounding the site could be like that recorded within the site. The historic land use and cover patterns have also been found to determine the current species richness and composition of these grasslands (e.g. Reitalu et al., 2012; Kuhn et al., 2021). Hence, determining the influence of the

landscape on the individual StableGrass sites is complicated and would need more study.

4.5 Implications of management on conservation of Irish grasslands

4.5.1 Management and influences on diversity across habitats and sites

Management of semi-natural grasslands is a major determinant of the plant species diversity and composition (e.g. Čop et al., 2009; Tälle et al., 2015; 2018; Bonari et al., 2017; Török et al., 2018; Wehn et al., 2018; Kuhn et al., 2021). While management data have not been collected in this study, differences in management across the 12 sites would explain some of the variation in the diversity observed. The positive effects of management on diversity were observed at the GS1 sites (Sites 1, 2, 3 and 11) and the following GS4 sites (Sites 8, 9 and 10). These sites had some of the highest mean diversity values. Both habitats were observed to be under extensive cattle grazing (which promotes a heterogenous sward), with GS4 sites sometimes also being mown in Autumn during the StableGrass survey. The GS4 sites that were mown (e.g. Sites 9 and 10) later in the year had the highest diversity values out of the four GS4 sites. This positive benefit of mowing is in agreement with Güsewell et al. (1998) and Tälle et al. (2016). Grazing in this study was the most common management for the grassland sites throughout the year. This demonstrates the importance of this management in these semi-natural grassland sites to maintain the species diversity.

In contrast, there were instances observed during the StableGrass site surveys where the management intensity negatively impacted the plant species diversity and composition. In Sites 4, 5 and 6 (upland GS3 sites) overgrazing by sheep was observed. The low species diversity, in particular richness of forb species is consistent with Tóth et al. (2018) who observed reduced forb richness in sheep grazed grasslands. Other studies also reported increases in graminoid cover at the expense of specialist grassland forbs in overgrazed grasslands (e.g. Grime, 1973; Dumont et al., 2011; Varga et al., 2021; 2024; Hempson et al., 2022). For example, in these upland GS3 sites *Galium saxatile*, *Potentilla erecta* and *Euphrasia officinalis agg* were often the only herbaceous species found in these relevés. In contrast to cattle, which promote a heterogenous sward structure, sheep are selective grazers, meaning that

they graze the more palatable species, leaving behind the unpalatable (Stewart and Pullin, 2008; Socher et al., 2012; Lyons et al., 2022). This increases the dominance of these species, for example *Nardus stricta* in the upland GS3 sites. An abundance of this species is indicative of overgrazing (Grant et al., 1996; Bullock et al., 2011). Other evidence of overgrazing in the vegetation structure would be the median sward height, which was predominantly short at these sites (Appendix 6, Sheet 3). Signs of overgrazing may also be demonstrated in the diversity measures. For example, the GS3 relevés that are described above as being overgrazed, had some of the highest Simpson's Evenness values. This shows that the sward structure of this habitat was more uniform, likely due to sheep grazing. This is consistent with Török et al. (2018) where increased grazing intensity resulted in greater Simpson's Evenness values. Increased disturbance frequency (i.e. increased grazing intensity) has been shown to reduce species richness, and increase the species evenness of ecosystems (Svensson et al., 2012; Yeboah et al., 2016). Overgrazing is a considerable threat to the diversity of semi-natural grasslands (Török et al., 2018; Varga et al., 2024).

When management of semi-natural grasslands ceases, species diversity is often reduced followed by a change in the species composition (Baur et al., 2006; Vassilev et al., 2011; Valkó et al., 2018; Johansen et al., 2019a; McKeon et al., 2022; Prangel et al., 2023). In the StableGrass survey Site 7 (GS4, Portumna, Co. Galway) had 52%, 68% and a 29% loss in the mean Species Richness, Simpson's Diversity and Simpson's Evenness measures, respectively between surveys. This loss in diversity is due to abandonment. The losses in diversity at this site was the only statistically significant change detected out of the 12 sites. During the ISGS no grazing animals, or at least a low number of grazers were observed at this site, with areas of grassland becoming rank (O' Neill et al., 2013). This initial abandonment was observed to be at a more advanced stage during the StableGrass survey. The South-west side of the site had been used as a landfill making the use of machinery for management impossible (Pers., Comm., Galway County Council). The abandonment and reduction in diversity were observed in two relevés (7_1 and 7_3) that were previously recorded as Annex I *Molinia* meadow grassland. These relevés in the StableGrass survey were beginning to become dominated by *Molinia caerulea*. The second relevé (7_2) was dominated

by *Filipendula ulmaria*, with only seven species recorded in this relevé, mostly with low abundances, compared to 18 species in the ISGS. Areas surrounding these relevés were dominated by *Arrhenatherum elatius*, a grass species that thrives in abandoned areas (Stroh et al., 2020). The sward heights of the three relevés have increased two-fold since the ISGS (Paired t-test, $p = 0.016$, Appendix 9, Table I2), consistent with the abandonment. This increase in tall and competitive plant species leads to a reduction in diversity due to increased light competition and litter accumulation (Hamre et al., 2010; Loydi et al., 2012; Kelemen et al., 2014). These act as barriers to the regeneration of plant species by seed (Pakeman and Small, 2005; Kahmen and Poschlod, 2008; Kapás et al., 2020). Abandonment is one of the greatest threats to semi-natural grasslands after agricultural intensification (Section 1.6).

4.5.2 Changed management and homogenisation

The trend towards homogenisation discussed in Section 4.3 could be due to an increase in management intensity (Gossner et al., 2016; Hilpold et al., 2018; Kuhn et al., 2021). Most sites were under continuous management since the ISGS and maintained species' diversity. However, subtle changes to this management over time may explain the observed changes to the species composition in Figure 3.5. These changes could be a result of increased management intensity that is evidenced by a short sward height in many of the grasslands (Appendix 6, Sheet 3). Other evidence supporting increased management intensity can be seen in the bryophyte cover. The bryophyte cover of the StableGrass survey is significantly lower than in the ISGS (Paired T-test, $p = 0.000$, Appendix 9, J). As bryophytes are sensitive to land-use intensification (Löbel et al., 2006) it makes them a good indicator for management intensification. Boch et al. (2018) has demonstrated an increased bryophyte cover in grasslands that are under low-intensity management, with Müller et al. (2012) showing a two-fold decrease when intensification of management occurs.

Even slight changes in management can result in changed species composition (Peppler-Lisbach et al., 2020). Kuhn et al. (2021) found that both the current management practices and the land-use history had a significant influence on the species composition. From observations made during the field visits, changes in

management are likely to be increased grazing intensities towards overgrazing. GS3 sites appear to be homogenising because of intensive grazing. In Wales, overgrazing was found to significantly alter the species composition of upland acid grasslands towards an increase in grass cover, and reduced forb richness (McGovern et al., 2011). As with McGovern et al. (2011) and Pakeman and Fielding, (2021) the upland GS3 sites in the StableGrass study appear to be showing a more homogenous species composition likely driven by overgrazing.

The impact of increased management intensity and resultant homogenisation of the grassland sites is further supported by the changes observed in the frequencies of the different plant functional groups. Over 48% of the forb species, 83% of the pteridophyte species and 54% of bryophyte species had declining frequencies since the ISGS (Table 3.12). These species are sensitive to changes in management intensity and can be sensitive to increased grazing intensity or nutrient addition. Supporting this, 34% of graminoid species experienced increases in frequency, which could also support the view of increased management intensity. This is consistent with changes that have been identified in other European semi-natural grasslands. For example, Berlin et al., (2000) showed increased proportions of graminoid species with associated decreases in proportions of forb species. An increase in graminoid species was also observed over time in Swedish semi-natural grasslands (Linusson et al., 1998).

4.5.3 Conservation implications

As there was a lack of an overall significant difference in the diversity measures (except for Site 7) between the ISGS and the StableGrass survey (Section 4.2.2), the diversity of the grassland sites has remained stable. This shows that the management at the sites for the most part maintained the diversity of these sites since the ISGS. Sites 9 and 10 contained Annex I grassland and continue to support this habitat in the StableGrass survey. This is due to the continued grazing and mowing at these sites. These management practices are positive for these Annex I habitats (O' Neill et al., 2013; Martin et al., 2018), with this study showing this benefit. In the StableGrass study, the GS1 habitat had the highest number of relevés that maintained a similar

Species Richness to the previous survey. This shows that the management since the ISGS is largely continuing to promote the diversity at these sites. However, there are indications of increased management intensity across other relevés that experienced reductions in the diversity values and changes in species composition.

The abandonment observed at Site 7 has implications for the conservation status of this site and in other grasslands that may be undergoing abandonment. An area of this site was identified as the Annex I *Molinia* meadow [6410] in the ISGS (O' Neill et al., 2013). Whether it still qualifies as an Annex sites is debatable due to the degradation at this site. Abandonment has been identified as one of the most frequently encountered threats of the 6410 habitat (NPWS, 2013; 2019b; Martin et al., 2018). As the effects of abandonment are harder to reverse with increasing time (Öckinger et al., 2006; Reis et al., 2022), the restoration of management at this site is urgently required. Joyce (2014) found that abandoned grasslands may be restored to the representative semi-natural grassland before abandonment, if management is restored within 20 years. Although it can take some time before the abandoned grassland is restored (Pykala, 2003), it may still be possible to reverse the abandonment observed at this site.

4.6 Limitations and Future Work

4.6.1 Limitations

A few limitations exist within this study. A large limitation lies with the number of time periods that are available for comparison. Only two time periods exist in this study. Other studies into changes in semi-natural grasslands also cited difficulties with limited temporal coverage (Hédl et al., 2017). Short-term studies may only provide limited perspective on the drivers of change in grassland sites and vegetation communities (Hédl et al., 2017). Multiple observations over a long period of time is recommended for comparison and for recognising trends in the changes to diversity and species composition (Ridding et al., 2020). This study is further limited by the lack of management data in the intervening period making it difficult to determine what is driving the observed changes and give quantitative evidence for the discussed homogenisation. The next limitation lies with the relocation of the ISGS relevés in the

selected sites during the repeat survey. Relocation and observer biases can occur during vegetation re-surveys (e.g. Kapfer et al., 2017; Verheyen et al., 2018). The ISGS did not use permanently marked relevés, which means the relevés had to be relocated using a GPS unit. This introduced a degree of inaccuracy. This was not expected to be a major problem in the current study because most relevés were within 1–9 metres of the original relevé and located within similar stands of vegetation. This is much less than the average discrepancies reported in other resurvey studies e.g. in Verheyen et al. (2018), the average discrepancy with plot relocations was 24 metres.

Observer errors may result in errors due to the misidentification or difference in the relocation of species in the repeated plots. However, the StableGrass surveys were carried out by the student and an independent botanical consultant, which reduced this potential error considerably. Finally, seasonal errors could have arisen due to differences in the time of year that the relevés were surveyed in the ISGS and StableGrass surveys. This can result in either the lack of detection of some species or the abundances of species to be over or under estimated in comparison to the ISGS (change due to timing rather than actual change) (Vymazalová et al., 2012). The fieldwork was conducted between June and August, it was expected that there were no species from the ISGS missed due to timing and that an absence would have reflected a genuine loss. Vegetative identification using Poland and Clement (2020) and the assistance of the independent botanist ensured any species that were not flowering were still identified and recorded with confidence. The only species that the author was unable to differentiate between due to the survey timing was *Viola riviniana* and *V. reichenbachiana*. These are early flowering species (~March–June (Stroh et al., 2020)) and are practically impossible to differentiate when not in flower.

4.6.2 Future work

While this StableGrass study did not find a significant change in the species diversity and composition of the semi-natural grassland sites, there is evidence that the sites could experience significant changes in the future. Additional re-surveys of the sites and relevés in the future, especially in another ten years' time, would be beneficial.

The findings of this study could promote the re-survey of a wider number of the ISGS sites that have not been re-surveyed to determine if the same changes are occurring. The potential drivers of the homogenisation identified in this study and more detailed trends could be identified in a more definite way. The collection of more detailed management data as well as soil data in the future re-surveys could also assist with these interpretations.

Compounding factors such as climatic conditions over time can also influence the changes described above. More analysis could be conducted, incorporating historical weather and climate data to determine whether these factors and the interannual variation may have accounted for the patterns detected in this study, or whether they interact with the management and drivers of change. This could either be conducted in retrospect on the current data and on any future monitoring programmes.

Additional analysis could include modelling to predict changes in the species richness and diversity of the semi-natural grasslands, considering an overall (but not significant) negative trend was becoming apparent in the current analysis. This modelling may also be used to extrapolate the findings from these twelve study sites to encompass more of the semi-natural grassland sites in Ireland to determine whether the trends observed in the StableGrass sites are being observed elsewhere. Such models could include time series models, regression models, and more complex, but powerful methods, through machine learning. These models could be further enhanced with the collection of management data and coupled with the weather data detailed above to simulate changes in the species composition and diversity of these grasslands over time in Ireland. Similar modelling approaches have been taken in the literature that have assessed factors influencing the diversity and composition of semi-natural grasslands (e.g. Reitalu et al., 2014; Weiss et al., 2014; Giarrizzo et al., 2017; Etzold et al., 2020). While environmental variables and management have been discussed to influence the vegetation diversity and composition in Sections 4.4 and 4.5, modelling could provide more powerful and statistically detectable influences of these factors on the diversity at the sites.

Chapter 5: Conclusions

This study has provided valuable insights into the diversity and species composition of 12 grassland sites since the 2007–2012 Irish Semi-natural Grassland Survey (ISGS). In this thesis the plant diversity and species composition has been determined across three Fossitt habitats: 1) GS1 – Dry calcareous and neutral grassland; 2) GS3 – Dry-humid acid grassland; 3) GS4 – Wet grassland for four sites from each habitat category. The GS1 grasslands were most diverse in terms of Species Richness and Simpson's Diversity, followed by the GS4 habitat. The GS3 habitat had the lowest mean diversity values. Consistent with the literature and like European semi-natural grasslands, the differences in diversity across the 12 sites were explained by a range of factors, such as environmental and management factors. This has important conservation implications, namely in selecting a suitable management practice for each site given these influencing factors. For example, sites which have a more productive environment would be expected to need a greater management intensity to maintain diversity.

The changes in the species diversity since the ISGS were also determined. Despite the literature showing significant losses of semi-natural grassland diversity across the UK and European semi-natural grasslands, this study shows that this was not the case for the StableGrass semi-natural grassland sites. However, when changes in diversity were assessed at the habitat level, GS1 had a significant increase in Simpson's Diversity, while GS4 had significant declines in Species Richness. This decline is pronounced at Site 7, a GS4 grassland in Portumna, Co. Galway, where approximately 50–68% losses in the diversity values (Species Richness and Simpson's Diversity, respectively) since the ISGS survey. These losses were likely due to grassland abandonment, a recognised threat of Irish and European semi-natural grasslands.

An analysis of the StableGrass species composition revealed that the relevés are becoming increasingly compositionally like one another, whereas in the ISGS, the relevés were more distinctly grouped by habitat. This indicated homogenisation in this study. While this compositional shift was not statistically significant, a trend towards increasing homogenisation was identified. Homogenisation has consequences for the conservation of grassland plant diversity as it reduces the area

of quality habitat and number of different niches that different plant species could occupy. At a landscape level, this impact can be even more considerable.

Impacts of the identified homogenisation were observed at the species level. The StableGrass study identified declines in the numbers of species observed in the grassland sites. There was a 17% reduction in the total number of species encountered across the 12 sites in comparison to the ISGS survey. Additionally, 66 species that were recorded in the ISGS were not found during the StableGrass survey. These lost species included rarer species, e.g. *Coeloglossum viride* (Orchid) and *Ophioglossum vulgatum* (Fern), and specialist species, e.g. *Carlina vulgaris* and *Rhinanthus minor* (Herbs of diverse GS1 grasslands). Forty-six percent of the species pool had declined in frequency at both the Site and Relevé levels. The loss of these species can be related to changes in management intensity. These changes in management have been indicated by declines in frequency of forbs (herbaceous species) and gains in the frequency of graminoid species.

While this study lacked direct measurements for many of the environmental influences on species diversity and composition, e.g. soil pH, moisture content and nutrient content, Ellenberg Indicator Values have given some insights on these aspects. This study found a positive relationship between Species Richness and Ellenberg Reaction (pH), which is consistent with the literature. The Ellenberg Reaction, Wetness, Nitrogen, Light and Salinity (in order of decreasing amount of variation explained) were significantly correlated with the species composition in the StableGrass survey. This highlighted the importance of local environmental conditions in determining the species diversity and composition in these semi-natural grasslands.

The overall StableGrass project aimed to determine the influence of Species Richness on carbon storage. The literature has demonstrated that increased numbers of species results in greater carbon storage in semi-natural grasslands. However, this study has revealed a negative relationship between Species Richness and the measured Soil Organic Matter (SOM). This can be explained by the GS3 relevés typically having the lowest Species Richness values, but often had the highest SOM values. This means that there is a trade-off in these Irish semi-natural grasslands

between increasing plant diversity and increasing the carbon storage potential of Irish grasslands.

This study has identified some important implications for the future conservation of these grassland sites. While this study only assessed 12 of the ISGS sites, it is not unlikely that the trends identified in this study are also occurring in other semi-natural grasslands nationally. The lack of a significant difference in the diversity measures and composition overall since the ISGS suggest that the diversity is being maintained over time in Irish semi-natural grasslands, to the extent that the changes are not statistically detectable. However, the trend of increasing homogenisation is slowly resulting in changes to the vegetation structure and composition is concerning as is the reduction in overall species richness and declining frequency of many species. There may still be time to reverse this homogenisation through subtle changes in management. Studies have shown that restoration of semi-natural grasslands to reference conditions is possible but successful restoration diminishes with increasing time. Future re-surveys of these grassland sites are recommended to better identify trends.

Chapter 6: References

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