Assessing pollinator diversity and sustainable plant control methods to inform biodiversity-focused management on public open spaces

A thesis submitted to Maynooth University
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"You are capable of more than you know. Choose a goal that seems right for you and strive to be the best, however hard the path. Aim high. Behave honourably. Prepare to be alone at times, and to endure failure. Persist! The world needs all you can give." ~ E. O. Wilson (1929-2021).

Declaration

This thesis has not been submitted in whole or part to this or any other university for any other degree and is, except where otherwise stated, the original work of the author.

Signed

Sophia Couchman.

18th March 2026

Date

'Action is the antidote to despair' – Joan Baez, 1941.

Abstract

Biodiversity loss is occurring due to the voracious depletion of natural stocks by an ever-increasing human population. Yet the more intact an ecosystem, the more resilient it will be in the face of climate change. It is therefore essential, that wherever possible, policies and practices to protect and promote biodiversity are enacted by those that manage and are responsible for our land. In Ireland significant areas of land are managed by county councils, who recently have adopted more sustainable, and biodiversity focused management approaches.

The aim of this thesis was to assess different management approaches on public open spaces as a basis for developing guidelines and policies. Using pollinators as proxies for biodiversity, surveys were conducted on several urban and agricultural sites, and roundabouts, where it was determined that species abundance and diversity of pollinators was significantly higher on low-intensity farmland and unmanaged ornamental wildflower roundabouts. An additional aim of this thesis was to determine the effectiveness of glyphosate-based herbicides and glyphosate alternatives to control vegetation and their effect on the soil microbiome. On a controlled greenfield site and a road verge the glyphosate treatment Roundup Flex and an alternative treatment, Hot Foam successfully reduced vegetation. The Hot Foam affected the abundance of the fungi; however, the abundance and diversity of the soil fungi and bacteria fluctuated in part due potentially due to abiotic factors. The key findings through this research suggests Hot Foam is a more sustainable alternative to glyphosate.

The results of this thesis have yielded practical and environmentally sustainable steps to improve biodiversity on public lands. Among these steps is the principle that the most effective means of enhancing biodiversity may simply derive from the acceptance that wild and unkempt often trumps neat and tidy. To convince the public to recognise this, our county councils will play a key role.

‘We are sawing through the branch of the tree on which we sit’ Andrea Cardini, 2023.

Chapter 1 The Inherent Value of Biodiversity

1.1 Introduction

The natural world is at a precipice, facing two inextricably linked crises in the form of climate change and biodiversity loss. Despite decades of warnings from scientists about climate change it is only recently, through the evidence of a world changing before our eyes, that society in general has accepted its significance. However, the same cannot be said about biodiversity loss that has occurred through the destruction of natural habitats and ecosystems, overexploitation of natural resources, the spread of invasive species, polluting the environment and climate change (IPBES, 2019; WWF, 2022). Even in the event that the crisis is accepted publicly, it may take years for an enhancement in environmental management due to the time lag, from a recognition of an environmental issue, to a suitably established and implemented intervention (Karlsson & Gilek, 2020; Hocherman *et al.*, 2025).

Biological diversity, or biodiversity at its broadest definition, relates to the diversity of life on earth and all their forms, plus the interactions between them (Alho, 2008; Dasgupta, 2021). Diversity includes both the physical and functional diversity at the levels of genes, populations, species and ecosystems (WWF, 2022), underpinning the ecosystems that provide us with natural and cultural services, and promoting resilience to climate change (Millennium Ecosystem Assessment, 2005; Demozzi *et al.*, 2024). Many of these contributions are irreplaceable (IPBES, 2019). Although the world's natural ecosystems are considered resilient to most external pressures, it is impossible to predict where exactly planetary boundaries and tipping points lie, and we may only become aware of these when a large part of the system collapses (Cardini, 2023). It is therefore clear that the health of our planet, global economies and possibly human existence itself, is reliant on protecting and promoting biodiversity and all efforts to do so, no matter how small, should be encouraged and supported.

As well as supporting humanity, numerous habitats are provided by ecosystems that support wildlife and some host specialised, dependant species. Healthy, dynamic, functioning ecosystems are more resilient to climate change due to plant and animal diversity aiding resilience against temperature change and sea-level rise, while wetlands, woodlands and natural grasslands can mitigate flood risk (Oliver *et al.*, 2015). However, the last 50 years has seen a swifter deterioration in biodiversity than at any other time in human history, with this trajectory set to persist into the future (Guénard *et al.*, 2025). The mass of 96% of the mammals on this Earth is comprised of us and our livestock, while 70% of bird species consist of poultry for our consumption (Dasgupta, 2021). According to the Living Planet Index (WWF, 2024), 73% percent of wildlife populations that were monitored between 1970 and 2020 have declined globally, leading many scientists to recognise a sixth mass extinction (EEA, 2019; EPA, 2024b; Bishop *et al.*, 2025). The International Union for Conservation of Nature (IUCN) Red List version 2025-1 found that of the 64,411 evaluated vertebrate species, 21% are threatened with extinction (Figure 1-1). For the majority of the invertebrates, plants, fungi and protists, there is insufficient data to assess the threat, but of the 18% described and evaluated plant species, 43% are threatened with extinction (IUCN, 2025).

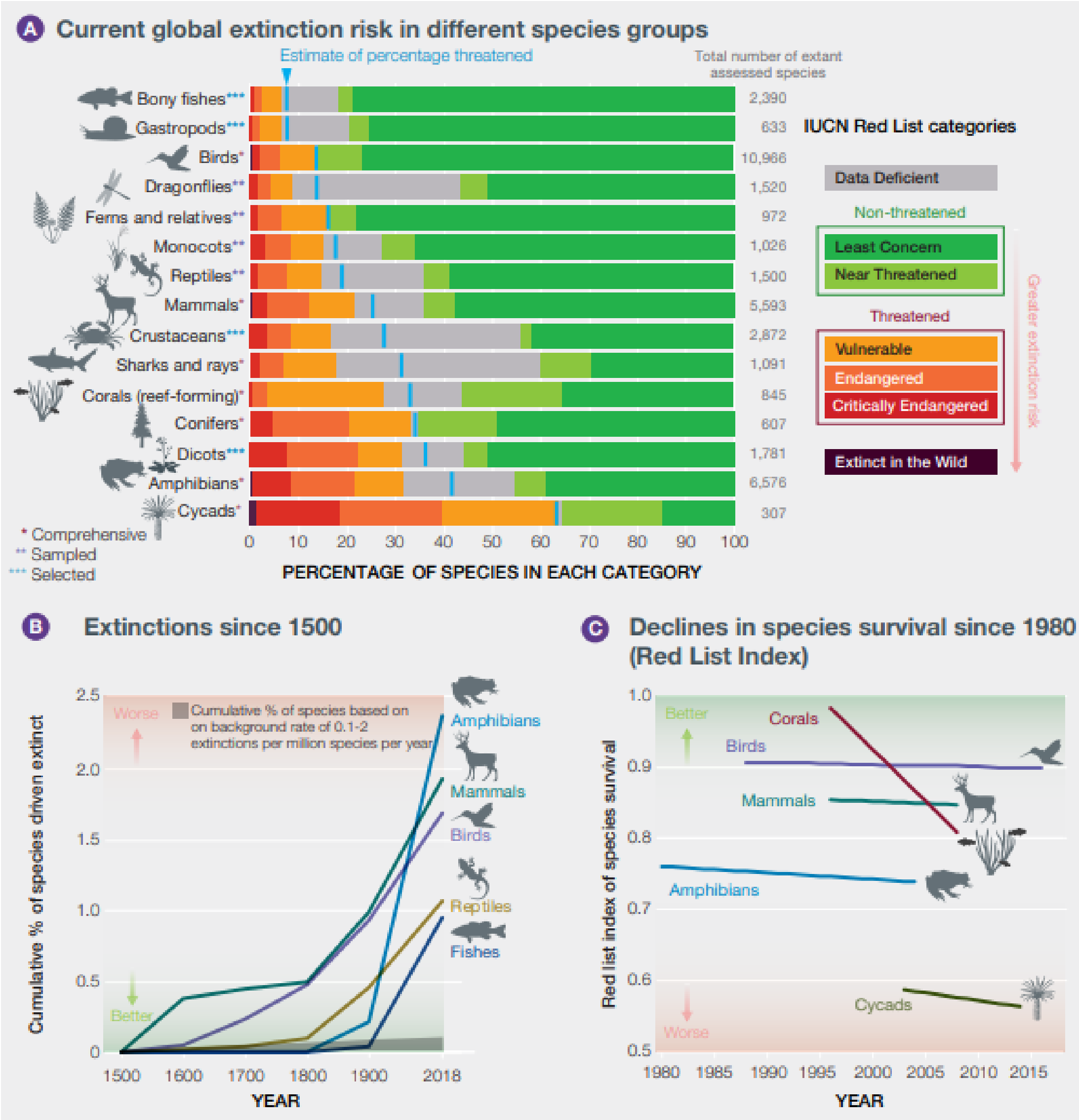


Figure 1-1 Extinction rates have increased and the overall trend in assessed species is declining. A- Taxonomic groups that have been assessed and the percentage of species threatened with extinction by the IUCN. B- Vertebrate groups with extinctions since the 1950s, though not all fish and reptiles have been assessed. C- The survival of taxonomic groups of Red List Index species and their survival. Least Concern is categorised as 1 and Extinct is categorized as 0 (after (IPBES, 2019).

1.2 Drivers of Biodiversity Loss

One of the greatest drivers of biodiversity loss is anthropogenic activity which ironically threatens nature and the vital contributions it delivers for people (Millennium Ecosystem Assessment, 2005; IPBES, 2019; Sockman, 2025). During the last five decades the human population has doubled, acting as a direct driver of both climate change and biodiversity loss through our rapacious consumption of natural resources, on land and sea, to the overexploitation of ecosystem goods and services, to the introduction of invasive species to natural ecosystems and pollution by increasing greenhouse gas emissions, industrial activities, and the quantities of untreated waste released into the environment (IPBES, 2019; Dasgupta, 2021; WWF, 2022). There are five major drivers of biodiversity loss: climate change, changes in land and sea use, pollution, invasive alien species and the overexploitation of all natural resources (WWF, 2022). These main drivers of biodiversity loss are further expanded below.

1.2.1 Climate change

It is estimated that over a thousand species have vanished due to climate change (WWF, 2022) brought about through global warming caused by human industry (IPBES, 2019; IPCC, 2023). This has triggered widespread changes to our atmosphere, oceans and terrestrial ecosystems resulting in damage and loss to the natural environment and humans (IPCC, 2023). There has also been an increase in the number and intensity of wildfires, heatwaves, rainfall events, and storms since the 1950s, causing irreversible losses to ecosystems and species (IPBES, 2019; IPCC, 2023). As greenhouse gas emissions continue to fuel global warming, due to its cumulative effects in the atmosphere, further intensity in weather events is expected (IPCC, 2023). Linked to increased temperatures, sea level rise is estimated to have risen 3mm in the last two decades. This will continue even with a reduction in greenhouse gas emissions, as ice-sheets and areas of frozen tundra continue to melt, all of which will have consequences for biodiversity through the alteration of certain habitats and ecosystems.

Climate change is leading to an adjustment in the timing of natural events, causing major alterations in the distribution of species, community composition within ecosystems and modifications within populations (Sharkey *et al.*, 2013; Bowgen *et al.*, 2022). A change in geographical range, phenology, genetics, abundance and communities have already been observed in many species (Sharkey *et al.*, 2013). Species either adapt to their new climate or they move where possible, but if neither is feasible, extinction becomes a distinct possibility (Sharkey *et al.*, 2013). Climate change impacts the life cycle of species in relation to the seasons (phenology) which may impact long term survival of certain species. Climate change is affecting the distribution of numerous species and this has manifested in a trend for species to move in a north easterly direction at approximately six kilometres per year (Sharkey *et al.*, 2013). The dispersal of species is highly dependent on the type of species, with birds having a high capacity to move whilst insects generally have a low capacity, exacerbating the impacts of climate change on less mobile species (Sharkey *et al.*, 2013). Generalist species are likely to thrive, while specialists may suffer due to a lack of suitable habitats leading to alterations within species composition of communities (Sharkey *et al.*, 2013). Climate change is already impacting humans, flora and fauna in Ireland (EPA, 2024b).

1.2.2 Changes in Land Use

Modern agricultural practices, particularly in the developed world, are contributing significantly to land use change and to biodiversity decline globally. The intensification of agricultural land has been driven by the ever increasing human population and demands for food (Redhead *et al.*, 2022). Agricultural intensification is known to impact soil health and biodiversity, which is significant given that a third of the land worldwide (FAO, 2025b), and an estimated half of land in Europe, is currently farmed (FAO et al., 2020; Phillips et al., 2024). 'Improved' grasslands and arable crops have caused an increase in erosion, lowered fertility from depleted nutrients, increased pollution through herbicide and fertilizer applications, and damaged soil structure through ploughing and compaction (Jeffery *et al.*, 2010). Over time, as terrestrial ecosystems are degraded, productivity will be reduced (IPBES, 2019).

It is estimated that habitat loss is set to increase by nearly 20% by 2050 globally (Sheridan et al., 2011). Agricultural intensification has led to increased field sizes at the cost of semi-natural habitats (Maskell *et al.*, 2019; Arnold *et al.*, 2025). Semi-natural habitats include, but are not limited to, hedgerows and treelines, scrub and native woodlands, grasslands, ponds, rivers, streams and wetlands. Species rich semi-natural grasslands have declined rapidly across Europe over the last century (Wagner *et al.*, 2020). We have lost over 85% of wetlands through drainage for farmland, forestry and infrastructure (IPBES, 2019). Overgrazing, under grazing, agricultural development and land abandonment have contributed to biodiversity loss nationally and internationally (EPA, 2024b; NPWS, 2024; Arnold *et al.*, 2025). Habitat removal has led to short-term gains for individuals but long term costs to society as a whole (Millennium Ecosystem Assessment, 2005).

1.2.3 Pollution

Pollution has increased due to an exponential rise in the use of pesticides and fertilisers, household chemicals, industrial pollutants and urban wastewater (Gunstone *et al.*, 2021). The continued use of most pesticides is unsustainable, leading to negative impacts to soil function and thereby ecosystem services and biodiversity (FAO, 2017). Almost half of our drinking water comes from ground water sources, with water treatment plants unable to remove pesticides effectively (Carretta *et al.*, 2022). Eutrophication in waterbodies from pesticides and fertilizers is ongoing, with water quality declining to such an extent that it has led to algal blooms, an increase in bacterial decomposers, and turbidity and hypoxia affecting organisms that require oxygen (Gioria, 2011). An overview of the literature conducted in 2021 found that 70% of studies found negative effects of pesticides on a variety of soil organisms and that these organisms were threatened by all forms of pesticide (Gunstone *et al.*, 2021). Many invertebrates from the terrestrial environment have declined and continue to do so, with pollution being cited as a major cause (Gunstone *et al.*, 2021). Bee colony fitness and pollination services are likely to be affected by pesticides, particularly the first brood in the spring when the queens are foraging and nest searching (Weidenmüller *et al.*, 2022).

1.2.4 Invasive Species

Invasive species are one of the main drivers of biodiversity loss on a global scale (Mitchell *et al.*, 2021). Often, the local native species are ill-adapted to predate on the exotic species leaving them free to proliferate (van Ham *et al.*, 2013). Invasive species have led to 60% of known extinctions, triggering global homogenisation of species and modified ecosystems (Roy *et al.*, 2024). Almost a quarter of native species abundance has reduced since 1900, mainly due to invasive species (IPBES, 2019). The global homogenization (Millennium Ecosystem Assessment, 2005) and loss of genetic diversity may potentially lead to reduced resilience to climate change (IPBES, 2019). Invasive species are generally spread by the movement of humans through travel and an increase in global trade via shipping and air travel, the trends of which are both showing an upward trajectory (Millennium Ecosystem Assessment, 2005; IPBES, 2019; Roy *et al.*, 2024). It has been estimated that invasive species cost the EU and

US €12.5 billion (van Ham *et al*., 2013) and $425 billion (Roy *et al*., 2024) respectively each year. In Ireland the cost has been estimated at €200 million (EPA, 2024b).

1.2.5 Overexploitation

Natural resources are currently being exploited faster than they are capable of being replenished, leading to biodiversity loss. Examples include water abstraction and peat extraction. A third of the European human population is affected by water scarcity, necessitating effective management of water abstraction for private and commercial use, to prevent unsustainable use (EC, 2022a). Overexploitation has seen water levels dropping in rivers and lakes drying out, negatively impacting fish and other aquatic organisms. Water abstraction has expanded due to technological advances and population growth within the last century (Rupérez-Moreno *et al.*, 2017). Peatlands cover 3-4% of earths terrestrial surface and hold a third of earths soil organic carbon, which is double the quantity of carbon held by the world's forests (Farrell *et al.*, 2024). The degradation of peatlands has reduced its capacity to sequester carbon and regulate water, thereby reducing the effects of flood mitigation and negatively impacting biodiversity, including rare species (Bullock *et al.*, 2012; Farrell *et al.*, 2024).

1.3 Addressing the Biodiversity Crisis

Globally, during the last three decades, there has been a 50% rise in the material footprint per head (UNEP, 2018). In the main, this consumption has led to environmental damage, with the chances of achieving ecological sustainability moving further and further out of reach (IPBES, 2019). Many policies designed to prevent further deterioration of nature have thus far been insufficient to stem the tide. The UN Convention on Biological Diversity aims to protect diversity within species, between species, and ecosystems. At the UN Convention on Biological Biodiversity in 2022, the Kunming Montreal Global Biodiversity Framework was adopted with goals to restore 30% of land and sea, reduce the spread of alien species and risk of pollution by 50%, and bring biodiversity into policy and practice. Such initiatives have led the United Nations to declare 2021 to 2030 as the Decade of Ecosystem Restoration with aims to prevent, halt and reverse the loss of nature (UNEP, 2019). Their strategy contains ten actions, one of which involves financing restoration through government bodies and international lenders. In an effort to protect and restore global biodiversity, the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) was established in 2012 by the United Nations Environment and Developmental Programmes. This intergovernmental organization aims to improve communication between science and policy on issues of biodiversity and ecosystem services.

1.3.1 European Policies

Outside the IPBES, a plethora of laws and policies have been adopted in the hopes of mitigating biodiversity loss, but so far most have been ineffectual. The European Environment Action Programme (EAP) is the EU's overarching framework for environmental policy (EC, 2022a). In the European EAP four of the priority objectives are:

(1) enhancing adaptive capacity, strengthening resilience and reducing vulnerability to climate change,

(2) pursuing a zero-pollution ambition, including for air, water and soil and protecting the health and well-being of Europeans,

(3) protecting, preserving and restoring biodiversity, and enhancing natural capital and

(4) reducing environmental and climate pressures related to production and consumption (particularly in the areas of energy, industry, buildings and infrastructure, mobility, tourism, international trade and the food system).'

The EU Biodiversity Strategy for 2030 is a long term plan to protect nature and reverse the degradation of ecosystems (EC, 2020). It aims to halt and reverse biodiversity loss in the EU and across the globe (EC, 2021a). The first pillar requires the protection of nature within the EU with 30% of the land to be legally protected, with 10% of this strictly protected. Pillar Two consists of a list of actions, to be facilitated by the EU Urban Greening Platform and the Urban Greening Plans (EC, 2021a), to restore nature and halt the deterioration of ecosystems, species and habitats. There are specific commitments, including one to prevent the further decline of pollinators. Pesticide and fertiliser use is to be reduced by 50%, 10% of farmland is to contain biodiverse rich habitats, and 25% of farmland is to become organic. In urban environments the use of pesticides is to be reduced or ideally eliminated. Soil ecosystems are to be restored with two strategies to be adopted: the EU Soil Thematic Strategy and the Zero Pollution Action Plan for Air, Water and Soil (EC, 2021a). The EU Soil Strategy and the proposed soil monitoring law are aimed at protecting soils (EPA, 2024b).

The Nature Restoration Law (EC, 2024b) implemented in 2024, sits within the EU Biodiversity Strategy and aims to restore ecosystems, habitats and species on 20% of the land and sea by 2030 with a requirement of 10% of these areas to be protected (Sundseth, 2022; EPA, 2024b). One of the specific targets is to increase pollinating insects. Other targets involve preserving all green spaces and trees within urban environments, increasing tree canopy cover in these environments by 10% and reversing the decline of butterflies and birds on farmland, and pollinating insect species in general (EC, 2022b; EPA, 2024b; Harpke *et al.*, 2025). Recommendations include ensuring that forest species composition and age are more diverse and farms are managed more extensively, by expanding hedgerows, reducing pesticides and fertilisers, planting trees, and creating ponds. In urban areas green spaces need to be enhanced by tree planting, introducing sustainable drainage systems, reducing pesticides and alien species. The cost of restoration measures for protected habitats in Europe is estimated at €154 billion which is dwarfed by the estimated projected costs of €1,700 billion for carrying on with 'business as usual' (Sundseth, 2022).

The EU Pollinators Initiative (EPI) was established in 2018 to improve efforts on the global conservation, and prevent further declines, of pollinators (NBDC, 2021; Vujić *et al.*, 2022). The aim of the EPI is to assess the status and trends of pollinators through improved monitoring and to raise awareness of pollinator conservation (Vujić *et al.*, 2022). The European Union is aiming to become the first climate neutral continent through the European Green Deal launched in 2019 (EC, 2024a) and the 2021 European Climate Law. The Green Deal aims to promote sustainable agriculture, allowing farming and nature to work side by side (Demozzi *et al.*, 2024). One of the major proposals is to reduce the loss of biodiversity of soil organisms, pollinators and economic losses via a decrease in the use and quantity of pesticides by half by 2050 (EC, 2022b).

It is clear that the EU is strongly committed to a fair transition to sustainable food systems, stating the need for a future that reconciles its food security, climate, biodiversity and socio-economic objectives (Demozzi *et al.*, 2024). However, the 6^{th} European Environment- State and Outlook 2020 report has identified serious gaps between the state of the environment and existing EU near- and long-term policy targets and declared that the message of urgency cannot be overstated in the face of environmental challenges. To achieve the EUs 2050 sustainability vision to become the worlds first climate-neutral continent will require the engagement of all stakeholders including citizens, businesses and authorities (EEA, 2019).

1.4 The Biodiversity Crisis and Ireland

Ireland is listed as the thirteenth lowest country for biodiversity worldwide (Mullan-Jensen *et al.*, 2024) as was found using the Biodiversity Intactness Index (Natural History Museum, 2019), and 20th out of 28 EU Member States (EPA, 2024b). Irelands greenhouse gas emissions are higher per person in comparison to the EU average (EPA, 2024b). The national long-term environmental objectives and targets are unlikely to be met, with our overall climate and water assessment rated as 'poor' and our overall nature assessment as 'very poor' (EPA, 2024b). As of 2025, habitats and species numbers and diversity are rapidly declining in Ireland with 90% of habitats in Unfavourable condition with 51% of these deteriorating, and assessed species by more than 18% (NPWS, 2025). Approximately 30% of grassland habitats, especially plants, have suffered in the last decade due to agricultural intensification or abandonment (NPWS, 2019). The latest Irish National Land Cover Map (EPA, 2025), released in 2023, shows a variety of habitats, but 'improved' grassland is the most dominant and covers an estimated 42% of Ireland with wet grassland at 10%. Hedgerows are estimated to cover 3.18% and treelines 1.04% (EPA, 2025). In 2022, 11.6% of Ireland was covered by forestry, however, this woodland was dominated by non-native conifer forestry that accounted for 69.4% (DAFM, 2023). Urban environments cover 1% of the total land cover in Ireland, while urban green space is estimated at less than half a percent (DAFM, 2023). Almost 95% of the terrestrial surface in Ireland has been modified by unsustainable development leading to species loss (Figure 1-2) (NPWS, 2024). The loss of 30% of ponds in the agricultural landscape due to drainage and land reclamation had occurred by 1986, with drainage still ongoing (Gioria, 2011). Freshwater species are most at risk, while over 50% of native plant species have declined or are threatened with extinction (NPWS, 2024).

In 2006, there were 102 native Irish bee species that lay under the IUCN regional categories, of which six were critically endangered and a third were threatened with extinction (FitzPatrick, 2006). In 2010, when the Red List of Irish Butterflies was updated, there were 33 resident and migrant species assessed, with 33% of these threatened (Regan *et al.*, 2010). As of 2010, no Irish insect species were listed on the IUCN red list, but the Marsh fritillary (*Euphydryas aurinia*) is listed in Annex II of the European Union Habitats Directive (Regan *et al.*, 2010). Ireland is relatively species poor for hoverflies (180 species) in comparison to other parts of Europe but on a Pan-European scale (892 recorded), 312 species have been assessed as threatened, excluding 40 species that are data deficient (Vujić *et al.*, 2022).

More must be done to enhance and restore biodiversity across Ireland on all ecosystem types. There are a number of state bodies and regulations in Ireland responsible for ensuring EU biodiversity targets are reached.

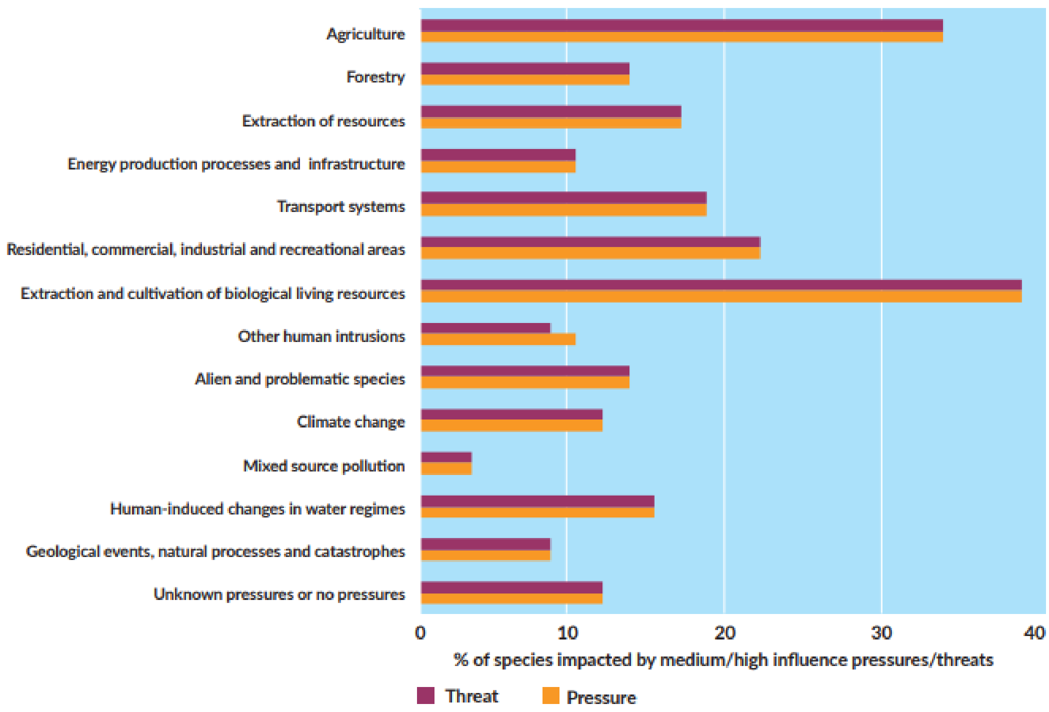


Figure 1-2 Percentage of species impacted by medium/high influence pressure (amber)/threats (red) in Ireland. (after (NPWS, 2025).

1.5 Irish State bodies and NGOs responsible for biodiversity

In a review of 186 global studies by Langhammer and colleagues (2024), it was found that almost 50% of the conservation and restoration efforts enacted over the past century have led to positive effects. In particular, measures to restore habitats and protected areas were significantly effective while extinctions have been prevented in areas where efforts to halt the decline of a species has been implemented (Langhammer *et al.*, 2024). It is therefore essential that countries have strong state, semi-state and NGO bodies in operation to measure, protect and enhance biodiversity nationally, as mandated by European policy.

1.5.1 National Parks and Wildlife Service

In Ireland, the National Parks and Wildlife Service (NPWS), part of the Department of Heritage, Local Government and Housing (DHLGH), provides a number of public services. It is tasked with delivering the EU Biodiversity Strategy and Nature Restoration Law and producing the National Biodiversity Action Plans (NPWS, 2023). Ireland's 4th National Biodiversity Action Plan 2020-2030 provides a framework to improve biodiversity in Ireland. The first objective aims to incorporate a 'Whole-of-Government and Whole-of-Society approach'. As the importance of biodiversity has been acknowledged, emphasis has been placed on increasing funding for a wide variety of projects that include local authorities, private landowners, businesses and communities (NPWS, 2024).

1.5.2 National Biodiversity Data Centre

The National Biodiversity Data Centre (NBDC) was established in 2006 by the Heritage Council to collect and manage biodiversity data in Ireland. Since then, its scope has expanded to incorporate the support of pollinator programmes, engage with citizen science projects and track invasive species. Its main strategic directive is to produce high quality scientific data to track the changes in Irelands biodiversity. This is facilitated by citizens across Ireland, who participate in an array of monitoring schemes aimed at gathering data on butterflies, bumblebees, rare plants, dragonflies and damselflies. The NBDC has functioned as a free public repository for Irelands biodiversity data for the last 15 years. The database contains over six million records and the NBDC trains and enables citizens to gather and record species (NBDC, 2025).

Ireland was one of the first countries in Europe to tackle the decline of pollinators through the highly successful, and widely recognised, publication of the All-Ireland Pollinator Plan (AIPP) 2015-2020 (NBDC, 2021). The AIPP was initiated to protect Irelands wild bee species with the main aim being to reverse the decline of pollinators through habitat restoration (FitzPatrick, 2020). This hugely successful framework is coordinated by the NBDC and brings together a variety of sectors across Ireland. The NBDC provides free resources to enable all sectors, from farmers to sports clubs to councils to local communities, with information on how to take steps to restore pollinator populations to sustainable levels. These include ways to create wildflower meadows, a list of top ten actions for increasing pollinators, pledging space in gardens for pollinators and adding these actions to an online map (NBDC, 2025).

1.5.3 Local Authorities

There are 31 local authorities in Ireland, each providing a plethora of services to a specific geographic area, to support local communities and businesses in their sustainable development. The role of local authorities was recognised in the National Biodiversity Plan (2002) to be pivotal in promoting and conserving biodiversity (KCC, 2008). To provide such a framework, the County Kildare Biodiversity Action Plan 2009-2014 was developed to ensure that national targets in biodiversity conservation were being achieved (KCC, 2008). The plan contains a number of practical actions, which include data collection, raising public awareness, promoting conservation and management, and informing policy on biodiversity issues (KCC, 2008). Local Biodiversity Action Funds provide grant funding to Local Authorities for local biodiversity initiatives, and in 2024, this amounted to €2.8 billion (DHLGH, 2024). The aim is to improve biodiversity at a local level by combining ecologists and local community groups to improve public awareness and provide essential education.

1.5.4 Irish Legislation

In Ireland there is legislation that provides the legal foundation for the protection of biodiversity. County councils are obliged to incorporate these legal frameworks locally by integrating them into County Development Plans. This thesis aligns with much of the legislation as it provides management approaches to support and enhance biodiversity, in particular pollinators, and to provide frameworks to reduce pesticide use. The Wildlife Act 1976 and the Wildlife (Amendment) Act 2000 are the principal pieces of legislation in Ireland for the protection of biodiversity. Natural Heritage Areas are the mechanism for protecting habitats at a national level in Ireland, while the Special Areas of Conservation (SAC) and Special Protection Areas (SPA) are the main designations under European Law (KCC, 2008). The Habitats and Species Directive (92/43/EEC) lists habitats and species in annexes under the Natura 2000 network which require legal protection. Ireland has designated habitats of high conservation importance (Annex I) and species of high conservation concern (Annex II), under SACs.

Article 4, Directive 2009/128/EC states that Ireland is legally required to develop a National Action Plan for the sustainable use of pesticides (DAFM, 2019b). There are a number of regulations with regard to pesticide legislation in Ireland: the Sustainable Use Directive (SUD) 2009/128/EC, Regulation (EC) No. 1185/2009 to collect statistics on pesticide use, the Water Framework Directive 2000/60/EC to achieve good qualitative and quantitative status of all water bodies, the Drinking Water Directive (EU) 2020/2184 to ensure we comply with chemical standards for drinking water (EPA, 2024a) and Regulation (EC) No. 1107/2009 that restricts the use of pesticides in areas used by the general public. EC and EU legislation requires that the effects of plant protection products on the behaviour of non-target species and the indirect effects on a potentially altered food web are taken into consideration (Ockleford *et al.*, 2017). However, the EU regulation on glyphosate use is currently approved until the 15th December 2033 (EC, 2023).

1.5.5 National Environmental Schemes

Kildare County Council leases farmland to local farmers for livestock and grazing. Over the years there have been a number of initiatives for farmers to improve biodiversity and lower pollution by facilitating low-intensity farming methods. In 1994 the Irish government brought in the Rural Environment Protection Scheme (REPS), which was followed by the Agri-Environment Options Scheme (AEOS) in 2010, and the Green Low-Carbon Scheme (GLAS) in 2015, in response to the EU's Agri-Environment Regulation 2078/92/EEC. The aim of these schemes was to enhance biodiversity and reduce pollution on farmland in Ireland, by compensating farmers for loss of earnings on land removed from intensive management (Tsakiridis *et al.*, 2022). These schemes have cost the state more than €3 billion, with little evidence of an improvement of biodiversity on agricultural land (Tsakiridis *et al.*, 2022).

The European Innovation Partnership Schemes (EIP), which falls under the Common Agricultural Policy Strategic Plan 2023-2027, furthers collaboration between scientists, farmers and public bodies to develop economically sustainable practices that are environmentally friendly. As of January 2025, this fund was worth €17.5 million (DAFM, 2024), and provides funding for projects involved in nature conservation such as the Agri-Climate Rural Environment Scheme (ACRES) and organic farming. ACRES aims to improve biodiversity and tackle pollution by giving direct payments to farmers as compensation for a loss of income from farming to enable conservation measures (NPWS, 2024). In this thesis low-intensity farmland was evaluated and does show how valuable these schemes may be for enhancing biodiversity.

1.6 Study Organisms

In order to recommend measures to improve current management practices on public open spaces for biodiversity there are two main research areas within this thesis. The first is to assess existing biodiversity on public open spaces using pollinators as proxies for biodiversity. Baseline data on existing flora and pollinators was gathered across a number of public open sites containing a wide variety of habitats and currently managed by Kildare County Council. Kildare County Council manages a variety of land types across County Kildare, from town parks containing juvenile woodlands, grass pitches and playgrounds, to road verges and roundabouts, to agricultural land.

The second section of this research investigates glyphosate and glyphosate alternatives for the control of plants in public open spaces. Glyphosate has been used, and is still being used, to clear vegetation by local authorities on public open spaces. The general public, however, is demanding alternatives, as awareness grows of the impact of glyphosate on biodiversity, and potentially, on public health. County councils in Ireland have been trialling alternatives, although to date there has been little research on their effects on the soil microbiome and their effectiveness at plant control in Ireland. This research was conducted to assess the effects of various alternative treatments being used in the real world on plants and soil fungi and bacteria.

To achieve these aims the biodiversity of three broad classes of organisms were focused on: pollinating insects and two soil microorganisms: fungi and bacteria.

1.6.1 Insect Pollinators

Insect pollinators can be important indicators of biodiversity (Van Swaay *et al.*, 2010). Fully functioning ecosystems are reliant on a large abundance of insects (Regan *et al.*, 2010) that deliver a wide range of ecosystem services, pollinating crops and wild plants, thereby enhancing food security (Potts *et al.*, 2016). Pollination services are indispensable to humans and farm animals, with 70% of cultivated crops, including forage for livestock, pollinated by animals and insects (Nieto *et al.*, 2014; Hopwood *et al.*, 2015; IPBES, 2019). The value of the pollination of plants by pollinators lies somewhere between $235 and 577 billion globally (Cullen *et al.*, 2019; Baldock, 2020; Benner *et al.*, 2023). In Europe it is estimated that up to €15 billion worth of the agricultural pollination services are provided by insects (Vujić *et al.*, 2022).

There are very few historical records on pollinator populations, which hinders assessment of the trends, but by using museum collections and recent monitoring efforts, reductions in species ranges have shown that some have gone extinct while many have declined (Potts *et al.*, 2016). Honeybees are estimated to have declined by 29% over the last 20 years due to disease, pesticides and parasites (Hopwood *et al.*, 2015). Agricultural crops dependent on pollinators have expanded over the last five years, while simultaneously exhibiting a decline in yield, that corresponds with a decline in pollinator abundance and richness (Potts *et al.*, 2016). The decline of pollinating insects therefore poses a direct threat to food security globally (Benner *et al.*, 2023).

1.6.1.1 Bees (Hymenoptera)

There are estimated to be around 20,000 described species of bee globally (Nieto *et al.*, 2014; Potts *et al.*, 2016). In the European Red List there are estimated to be 1,965 native bee species, whose ecology is based on food, nesting requirements and sociability (Nieto *et al.*, 2014). The pollen within flowers is used to feed the larvae and during pollen collection, pollination co-occurs. It has been estimated that between 0.9 to 4.5 inflorescences are required to provide for a single larva (Nieto *et al.*, 2014).

Spatial scales differ depending on the species of bee, with bumblebees capable of travelling a few kilometres while solitary bees are generally restricted to 200m (Hopwood *et al.*, 2015; Larkin & Stanley, 2021). Commercial hives containing honeybees are used worldwide for their pollination services on an estimated 50% of crops but wild bee species were shown to provide the majority of pollination services and increased yields (Nieto *et al.*, 2014; IPBES, 2016; Karbassioon *et al.*, 2023). Bumblebees have the ability to forage earlier in the year, and during colder weather, due to their hairiness (Hopwood *et al.*, 2015; Benner *et al.*, 2023).

However, according to criteria within the IUCN, 30% of bees worldwide are threatened with extinction. In Europe, over nine percent of assessed bee species are threatened, however, over a half of bees are unassessed as they are data deficient (Nieto *et al.*, 2014). In Ireland, of the 102 species of bee, over half of the 21 bumblebees and nearly half of 77 solitary bees are declining (FitzPatrick, 2006). The decline is suspected to be from pollution (pesticides and nutrients), climate change, invasive species and disease (Woodcock *et al.*, 2021; Barendregt *et al.*, 2022). Bumblebees face many threats from a lack of floral resources to habitat loss, to pathogens such as the Varroa mite (Potts *et al.*, 2016; Samuelson *et al.*, 2018).

1.6.1.2 Butterflies (*Lepidoptera*)

Europe has 482 species of butterflies of which a third are endemic (Van Swaay *et al.*, 2010). Butterflies have three life stages consisting of eggs that hatch into larvae (caterpillars) and then turn into a chrysalis through metamorphosis before becoming adults. Each life stage has a specific requirement for food and habitat, and these specific requirements, during the different life cycles make them especially sensitive to changes within their environment, making them excellent indicator species. Over a half of butterflies live in grasslands and a quarter have a preference for scrub and woodlands (Van Swaay *et al.*, 2010). So far there are few studies in this area, but it is suspected that butterflies aid in pollination and their contribution to the spread of pollen could be particularly high for species that fly long distances (Hopwood *et al.*, 2015). Butterflies are charismatic species, well-studied and important indicators of biodiversity and so are useful for monitoring ecosystem health (Regan *et al.*, 2010). Yet, almost 20% of butterflies are either threatened or near threatened according to the European Red List for Butterflies (Van Swaay *et al.*, 2010). In Ireland there are 33 resident and regularly migrating species of butterfly and a third of these are threatened, with one extinct species (Regan *et al.*, 2010). Across Europe their ranges and populations have declined with the main cause attributed to land use changes such as fragmentation and a move away from species rich grassland habitats towards agricultural intensification and land abandonment (Regan *et al.*, 2010; Van Swaay *et al.*, 2010; Harpke *et al.*, 2025).

1.6.1.3 Hoverflies (*Syrphidae*)

Hoverflies lie within the remarkably varied family of true flies (*Diptera*). There are almost 7,000 recognised species globally (with an estimated 10,000 species yet to be discovered) making up one of the principal families of true flies (Vujić *et al.*, 2022). There are four developmental stages: egg, larva, pupa and adult. The larval feeding stages are highly variable within hoverflies; some are predators of aphids or ants, some of living material, some of decaying material and wood, while others are sap suckers. The foraging habits of the adults are comparable with floral resources, their main requirement, though the floral species needed are diverse (Vujić *et al.*, 2022). Most hoverflies do not migrate and have small home ranges, but some species of hoverfly are known to migrate long distances, transporting pollen, providing pollination services and pest control, and redistributing nutrients such as nitrogen and phosphorous, with numbers migrating into the UK estimated between one and four billion per year (Wotton *et al.*, 2019). Hoverflies are ranked second in pollination services to bees (Vujić *et al.*, 2022). Estimates for hoverfly decline suggest a loss of 50% in the last decade, with 314 species of assessed hoverflies in Europe threatened, and 61 species classified as near threatened (Vujić *et al.*, 2022). There are frequently multiple threats to hoverfly species, which are usually complex due to the variety of niches they occupy during their life-cycles, but intensive agriculture and pesticides, urbanisation and pollution have taken their toll (Vujić *et al.*, 2022).

The majority of taxa receive very little funding support, with nearly a third of funding going towards species of 'least concern.' Much of the funding is directed towards 'charismatic' species rather than invertebrates and plants (Guénard *et al.*, 2025). In order to decide which species to fund the IUCN Red Lists are used in the decision making process, however, only 1.1 % of insects have been described due to a lack of knowledge (Guénard *et al.*, 2025). It is thought that by providing protection and enhancing habitats for charismatic species, that simultaneously other species would flourish under the 'umbrella' species (Guénard *et al.*, 2025). This is disputed as charismatic species are inferior substitutes for smaller threatened species (Guénard *et al.*, 2025). By improving conditions for insects, we thereby improve conditions for ourselves (Cardini, 2023).

1.6.2 Soil microorganisms

Soils are a key reservoir of global biodiversity, which ranges from microorganisms to flora and fauna (FAO, 2015). This realm is known as the 'factory of life' and is indispensable to humans and all life forms for our survival. The processes that occur within this medium are what drives ecosystem and global functions (Jeffery *et al.*, 2010). It has been estimated that approximately 59% of all species live in soil with an estimated five billion organisms present within any one handful of soil (FAO, 2020; Anthony *et al.*, 2023; Phillips *et al.*, 2024; Robinson *et al.*, 2024). Ecosystem services refers to the regulating and supporting services provided by soil free of charge to the human race and are worth tens of trillions of euro annually (Dasgupta, 2021). The numerous ecosystem services that soil and the rich diversity of soil organisms it contains include: carbon sequestration, nutrient cycling, mobilizing nutrients, maintenance of soil structure, providing a food source, water purification, pest and disease control, attenuation of pollutants, flood control, soil formation, genetic resources, aesthetic values and cultural biodiversity, and decomposing organic matter (Jeffery *et al.*, 2010; Sassi *et al.*, 2012; Creamer *et al.*, 2016; FAO, 2017; Ockleford *et al.*, 2017; FAO, 2020; Gunstone *et al.*, 2021; EPA, 2024b; Phillips *et al.*, 2024). It is vital to maintain soil biodiversity to safeguard these functions (FAO, 2015).

Soil consists of two abiotic characteristics, texture and structure. Textures (clay (<0.002 mm), silt (0.63 – 0.063 mm) and sand (0.063 -2.0 mm) impact the pores within the soils which affects oxygen and water flow, nutrient binding ability and creates habitats for soil organisms (Jeffery *et al.*, 2010). The structure relates to how these particles combine, or aggregate, within the soil creating a network of pore spaces that vary in size from micrometre films of water surrounding a particle, to air-pockets between 100µm to 2mm wide, and up to spaces that can contain the macro and megafauna (FAO, 2020). Plant roots enter the pores between these particles where they extract nutrients, water and oxygen (Orgiazzi *et al.*, 2016). It is within these pores that a vast array of communities exists. Soil structure is mainly formed by the organisms within it and this environment is extremely dynamic due to weather effects and the movement of organisms (Jeffery *et al.*, 2010). Soil communities are made up of four class sizes: microbes (bacteria and fungi- 20nm to 10µm), microfauna (nematodes- 10µm to 0.1mm), mesofauna (mites, springtails- 0.1mm to 2mm) and macrofauna (earthworms, ants, beetles - 2mm to 20mm) (Table 1-1) (Gunstone *et al.*, 2021; Phillips *et al.*, 2024). An increase in the abundance of these species correlates with the smaller their size (FAO, 2020). These organisms are indispensable to the functioning of ecosystems (Anthony *et al.*, 2023).

Table 1-1 Known and estimated number of species of soil organisms and plants organised according to size (largest to smallest). Based on Orgiazzi et al. (2016a) and Barrios (2007).

Group	Known species	Estimated species	% described
Vascular plants	350 700	400 000	88
Macrofauna			
Earthworms	7 000	30 000	23
Ants	14 000	25 000-30 000	60-50
Termites	2 700	3 100	87
Mesofauna			
Mites	40 000	100 000	55
Collembolans	8 500	50 000	17
Microfauna and microorganisms			
Nematodes	20 000-25 000	1 000 000-10 000 000	0.2-2.5
Protists	21 000	7 000 000-70 000 000	0.03-0.3
Fungi	97 000	1 500 000-5 100 000	1.9-6.5
Bacteria	15 000	> 1 000 000	< 1.5

Soil is our largest habitat (EPA, 2024b), however, our knowledge of the importance and function of soil biodiversity lags behind that of above ground ecosystems. At present, much focus is on high profile indicator species such as pollinators, but there is less attention on important functional groups of soil organisms. Therefore, the potential to damage the fragile interconnections that exist in soil, and hence increase biodiversity loss unwittingly, is greater. The sustainability of agriculture is crucially reliant on biological processes maintained by the soil biota, such as soil structure regulation, supply of nutrients and pest control (Barrios, 2007). However, there are enormous pressures on soil organisms from urbanisation and agricultural intensification due to an ever-increasing human global population (Orgiazzi *et al.*, 2016; Phillips *et al.*, 2024). Threats to soil include excess nutrients, compaction and biodiversity loss, and an estimated 60-70% of soils in the EU are unhealthy (EPA, 2024b). While biodiversity in the soil exceeds that of other terrestrial systems by orders of magnitude, particularly at the microbial scale, it remains remarkably undervalued (FAO, 2020). Research into soil biodiversity is sparse (Anthony *et al.*, 2023) and it is acknowledged that insufficient data is the main bottleneck on our knowledge on this realm (FAO, 2020).

1.6.2.1 Microfauna

The community of microbes below ground have been called the 'architects' of the soil and influence soil aggregation (Ockleford *et al.*, 2017). Soil microorganisms are composed of four groups: fungi, bacteria, protozoa and archaeans, measuring only in millimetres, with short generation times that run from hours to days (Ockleford *et al.*, 2017). Soil communities contain an array of decomposers within a food chain that create healthy soils (Hagner *et al.*, 2019). The organisms above and below ground are closely linked with underground communities driving growth above ground, and plants above ground driving species belowground (Wardle *et al.*, 2004). Organic carbon and resources are provided by plants for decomposers and soil organisms that rely on roots (Wardle *et al.*, 2004). The vast array of fungi and bacteria are known to be primary decomposers of organic matter, some regulate the availability and uptake of nutrients, while some are known to reduce pests and diseases, and detoxify soils (Jeffery *et al.*, 2010). The soil microorganisms are known to affect fertility and soil structure, and to fix nitrogen, potassium and phosphorous. Nutrient cycling is essential as it has direct effects on plant growth through the ability of bacteria to fix nitrogen (Barrios, 2007; Jeffery *et al.*, 2010), one of the most important plant nutrients.

Nitrogen occurs in the atmosphere as a gas but this gas form is unavailable to plants who require it as ammonia and nitrate for growth (Jeffery *et al.*, 2010; FAO, 2020).

In combination with bacteria, fungi decompose cellulose, organic material and pesticides, form soil organic matter, fix phosphorous, and aid in soil remediation and pest control (FAO, 2017). Through these abilities they enhance plant growth by reducing organisms that cause disease, detoxify added chemicals and provide plant hormones to improve plants immunity (Meena *et al.*, 2020). By breaking down organic items, nutrients are released into manageable forms which are taken back up by plants (Wardle *et al.*, 2004; Hagner *et al.*, 2019; Gunstone *et al.*, 2021). The rhizosphere is the area around the roots of plants known to be characterized by containing high quantities of nutrients and biological activity (Jeffery *et al.*, 2010). Fungi are known to control plant growth by facilitating exchanges of chemicals between the soil and roots (Orgiazzi *et al.*, 2016). It is through the mycorrhizae that nutrient cycling occurs once the saprophytic fungi have broken down the organic matter (FAO, 2020). The modification of soil structure is partly carried out by fungal hyphae which can bind soil aggregates together (Barrios, 2007; Jeffery *et al.*, 2010; FAO, 2020). This aggregation can reduce soil erosion through provision of porosity within soils to allow water flow (FAO, 2020). There are estimated to be between 0.8 and 3.8 million species of fungi (FAO, 2020).

The impacts of diseases and pests can be reduced by soil organisms like fungi and bacteria (Jeffery *et al.*, 2010). Four main ways in which beneficial microbes suppress pathogens are: nutrient competition, the production of enzymes and anti-biotics, predation and the elicitation of plant disease resistance (Ockleford *et al.*, 2017). For example, the bacteria *Burkholderia cepacian,* are useful at antagonizing nematodes while rhizosphere bacteria can protect plant roots from fungal diseases (Barrios, 2007). Some plant pathogens are known to be controlled by certain *Actinomycete* bacteria that exude antibiotics (FAO, 2020). The majority of the primary decomposer biomass are bacteria and fungi that are able to breakdown complex organic matter, including toxic compounds (Hagner *et al.*, 2019). Bacteria in soil is known to degrade pollutants (FAO, 2020) while fungi are known to enter, colonise and eventually kill insects (Ockleford *et al.*, 2017). However, some bacteria and fungi are well known to cause disease within plants (FAO, 2017) which can partly be controlled by mites and nematodes (Gunstone *et al.*, 2021). The composition of communities has been shown to be affected by abiotic and biotic factors, including plant species diversity above ground and agricultural activities (Zul *et al.*, 2007).

Bacteria are highly diverse, but they are hard to quantify (Anthony *et al.*, 2023). Due to the fact that the majority of bacteria are unknown, it makes it difficult to protect these at a population level (Ockleford *et al.*, 2017). The World Soil Charter has defined sustainable soil management as: 'Soil management is sustainable if the supporting, provisioning, regulating, and cultural services provided by soil are maintained or enhanced without significantly impairing either the soil functions that enable those services or biodiversity' (FAO, 2015). There is little protection for soil biodiversity with neither the European Habitats Directive or Natura 2000 even mentioning it, let alone Red Lists incorporating soil microorganisms or soil invertebrates (FAO, 2020).

1.7 Summary of Thesis Aims

Through the study of pollinators, vegetation and soil microorganisms the aims of this thesis will be addressed. The subsequent experimental chapters will contain specific aims and hypotheses in more detail, and these chapters can be grouped broadly into two sections. The first section comprises chapters 2 and 3 which explore the correlation between land management approaches and pollinator abundance and richness across twelve sites managed by Kildare County Council. The second section comprises chapters 4 and 5. Chapter 4 aims to assess the effects of glyphosate and glyphosate-alternatives on vegetation control and soil microbiome diversity on a greenfield site, whereas the aim of Chapter 5 is to assess the effects, practicalities and cost effectiveness of using glyphosate and alternative treatments, including thermal and mechanical, on the removal of vegetation on road verges, footpaths and cycle lanes.

The general aims of this study are therefore to compare and contrast different management approaches in public open spaces in different locations to determine which are better for biodiversity. The results of this research will inform local authority policies on ways to enhance and promote biodiversity on their lands. In addition, experiments on the effects of glyphosate and glyphosate alternatives on biodiversity were conducted specifically to demonstrate broad risks to biodiversity (if any) posed by glyphosate, but also to determine if similar risks are evident for products advertised as “less harmful to the environment.” It is hoped that the research conducted and presented in this thesis will result in meaningful recommendations and policies aimed at enhancing and protecting biodiversity and will be adopted by those who are entrusted with the stewardship of our public lands.

Chapter 2 A comparison of pollinator diversity and management approaches on peri-urban farmland and town park public open sites

2.1 Introduction- Pollinators in human-dominated landscapes

We rely on healthy ecosystems to support the pollination of 75% of the world's leading food crops globally (IPBES, 2016) and therefore pollination is important to food security (Gonzalez *et al.*, 2020; Demozzi *et al.*, 2024). Insect pollination is also essential for almost 90% for wild plants but this group is declining due to agricultural practices such as habitat loss and chemical use (IPBES, 2016; Larkin & Stanley, 2021; Maher *et al.*, 2024). A diverse range of pollinators are required for supporting food security through their ability to provide pollination services (Harrison *et al.*, 2014; Maher *et al.*, 2024).

2.1.1 Drivers of pollinator decline

Human activities are the main cause of pollinator decline (IPBES, 2016). Threats to pollinators include climate change, changes in land use leading to habitat loss from agricultural intensification and urbanisation, pollution from pesticides and fertilisers, disease and invasive species (Van Swaay *et al.*, 2010; Nieto *et al.*, 2014; Potts *et al.*, 2016; Harvey *et al.*, 2022; Vujić *et al.*, 2022; Frenzel *et al.*, 2024; Harpke *et al.*, 2025). Climate change is already affecting individuals and communities of insects as it aggravates the multiple human-induced threats (Harvey *et al.*, 2022). As temperatures increase, bumblebee abundance declines (Bottero *et al.*, 2023) and weather conditions affect bees' flying and foraging activity (Karbassioon *et al.*, 2023). Fragmented habitats often contain populations of butterflies that are susceptible to the effects of climate change such as drought and flooding (Van Swaay *et al.*, 2010). Invasive, non-native species can negatively affect the quality of habitats when they outcompete and reduce the diversity and abundance of native insects (Hopwood *et al.*, 2015). Hoverflies have been impacted by the introduced Harlequin ladybird (*Harmonia* *axyridis*) as it feeds on their larvae (Vujić *et al.*, 2022). The decline of both insect abundance and species richness on most taxa occurs across a multitude of habitats, including protected sites (Frenzel *et al.*, 2024).

Agriculture is crucial for human existence providing our food, maintaining our economy and determining our countryside, but modern, intensive agricultural systems have contributed to biodiversity loss and degradation of ecosystem health (Demozzi *et al.*, 2024). Agricultural farmland covers 40% of the terrestrial environment globally (Maskell *et al.*, 2019), and approximately 65% in Ireland (FAO, 2025a). There have been significant environmental impacts on soil, air and water due to the intensity of agricultural farming in Ireland with the knock-on effect of a decline in biodiversity and ecosystem services, including pollination (Rotches-Ribalta *et al.*, 2021; Redhead *et al.*, 2022). Agricultural intensification has led to land abandonment, habitat fragmentation and loss, in particular of semi-natural flower rich grasslands (FitzPatrick *et al.*, 2007; Regan *et al.*, 2010; Harpke *et al.*, 2025). Many pollinators rely on semi-natural habitats for much of their life-cycle but these have declined by 45% in Europe since the 1950s (EC, 2021b). Semi-natural habitats include hedgerows and treelines, veteran trees, ponds, ditches and banks, wetlands, semi-natural grasslands and abandoned areas, to name a few (Grashof-Bokdam & van Langevelde, 2005). The quality of a habitat is important, particularly the floral resources, for bee abundance and richness (Staley *et al.*, 2023). It has been estimated that over the last 100 years, Ireland has lost 17% of field boundaries which were found to be important semi-natural habitats alongside scrub and woodland (Sheridan *et al.*, 2011). Declines in pollinators are also attributed to pesticide usage, while the application of fertilisers is known to reduce plant species that prefer nutrient poor soils, which in turn leads to a decline in specialist bee species, hoverflies and butterflies (Nieto *et al.*, 2014;

Nichols *et al.*, 2022b; Vujić *et al.*, 2022; Karbassioon & Stanley, 2023; Harpke *et al.*, 2025). During pesticide application, bees are directly affected (Thompson *et al.*, 2022) and this exposure is known to reduce the time of development, survival rates, pupal weight and the size of wings, and these in turn impact the survival of a species (Hopwood *et al.*, 2015). Individual pesticides impact bees in a variety of ways from reducing foraging efforts to affecting the identity of nest sites, however, research into the synergistic effects of pesticides is lacking (Cullen *et al.*, 2019; Benner *et al.*, 2023; Karbassioon & Stanley, 2023; O'Reilly & Stanley, 2023). Crop yields have been shown to increase in conjunction with pollinator abundance and species richness (Woodcock *et al.*, 2013; NBDC, 2021). However, monocultures in agricultural landscapes lead to shorter flowering periods in crops leading to less foraging time for bees (Hall *et al.*, 2017).

Urbanisation is another driver of pollinator decline as it generally leads to infrastructural developments, pollution, and climate change that affect floral resources and influence species abundance, richness and the composition of communities. Impervious surfaces in urban areas reduces floral resources and nesting sites, especially for ground-nesting bees (Fenoglio *et al.*, 2021). The effect of urbanisation is species specific- some species will completely evade these landscapes, some will complete their whole life cycle in them, some species will move between urban and agricultural sites, while migratory species may use them as stopovers. The heat island effect in urban areas is also likely to effect interactions between plants and pollinators leading to the expansion of non-native, thermophilic plant species, while temperatures will affect bees sensitive to increases in temperature (Baldock, 2020). The heat island effect in cities can increase temperatures by 12°C and often it is the mobile, small, heat-tolerant generalists that are the dominant insects within urban environments

(Fenoglio *et al.*, 2021). The presence of a species in an urban environment does not mean that this is its preferred habitat and there may be a trade-off for species, for example, urban areas may contain a higher abundance of nesting facilities but less prey, so may lead to lower survival rates (Spotswood *et al.*, 2021). Also, some green island plant species may contain lower resources in exchange for visual displays and are therefore of little use to pollinators (Samuelson *et al.*, 2018).

2.1.2 Restoring landscapes for pollinators

Protection is not enough to alter the loss of biodiversity, and restoration is desperately required to not just sustain, but improve, enhance and expand biodiversity (Sundseth, 2022). Design and management of spaces for conserving pollinators is crucial to negate the effects of their decline (Tew *et al.*, 2022). Landscapes that fall under the remit of local authorities can be enhanced for biodiversity, including farmland and town parks.

2.1.2.1 Pollinators in agricultural landscapes

Biodiversity needs to be restored on farmland (Maskell *et al.*, 2019; Balfour & Ratnieks, 2022). Many solutions to biodiversity loss can be remedied through agricultural practices such as minimising fertilisers and pesticides, reducing livestock, and enhancing semi-natural habitats such as hedgerows and trees (Demozzi *et al.*, 2024). The intensification of agricultural landscapes has incurred a loss of semi-natural habitats but these act as refuges providing micro-climates for pollinators that provide low rates of disturbance, nesting sites and floral resources (Grashof-Bokdam & van Langevelde, 2005; Maskell *et al.*, 2019; Larkin & Stanley, 2021; Nichols *et al.*, 2022b; Biegerl *et al.*, 2025). Organic farming methods enhance local native plant species' ability to proliferate, simultaneously improving floral resources and diversity for pollinators, thereby increasing pollination (IPBES, 2016). Research undertaken in Germany found the richness of pollinators was improved significantly when local landscapes contained small, organic fields with increased connectivity between habitats (Biegerl *et al.*, 2025).

2.1.2.2 Pollinators in urban landscapes

Urban environments are often highly modified but by rehabilitating and restoring habitats within them, these areas can enhance wildlife conservation and human well-being (Sanderson & Huron, 2011). Urban areas can provide many unconventional habitats, from golf courses to cavities in infrastructure, and brownfield sites to town parks (Soanes *et al.*, 2019). Urban areas could be used to conserve synanthropic and urbanised species (Spotswood *et al.*, 2021) and may act as a refuge for pollinators with some species exploiting these types of habitats (Samuelson *et al.*, 2018; Baldock, 2020). For example, built environments have been found to give refuge to a range of bees from solitary and eusocial bees, generalists and specialists (Hall *et al.*, 2017). In the UK, the concentration of bumblebee nests were lower on farmland than urban habitats (Baldock, 2020). Some urban habitats are capable of supporting a high richness of bee species (Hicks *et al.*, 2016) and were discovered to be more successful at improving the fitness of *Bombus terrestris* than agricultural land (Samuelson *et al.*, 2018). Cities may be driving the morphological changes in bumblebees, with size potentially reducing due to increased temperatures, as smaller bees are less likely to overheat (Eggenberger *et al.*, 2019). In Europe, bumblebees' reproductive rates and nesting density increased in urban areas where there was more habitat heterogeneity and less exposure to chemicals than the surrounding intensively managed farms (Spotswood *et al.*, 2021). An increase in temperature due to the heat island effect may enhance the capabilities of some species to adapt to climate change (Spotswood *et al.*, 2021).

2.1.2.3 Restoring and enhancing habitats and floral resources for pollinators

There are three ways to enhance conditions for pollinators: providing corridors between habitats, improving grassland and improving floral resources. Connectivity has been hailed as one of the more effective ways to protect and restore ecological connections (WWF, 2022). Many insects rely on hedgerows for part or all of their life-cycle and it is estimated that presently hedgerows cover 1.5% in Ireland (Foulkes, 2013). Hedgerows and treelines act as corridors to allow dispersal for insects between fragmented habitats, especially for slow moving species such as plants and insects (Boyle *et al.*, 2015; Biffi *et al.*, 2023; Staley *et al.*, 2023). Other semi-natural habitats such as ponds can act as biodiversity hotspots within landscapes (Gioria, 2011), while veteran trees support insect communities of organisms that are vital in the process of decomposition (Alexander *et al.*, 2006; Wetherbee *et al.*, 2021). Creating corridors along road verges could link a multitude of these habitats together and provide connectivity.

It has been estimated that frequently mown grassland covers over 20% in urban environments in Europe (Norton *et al.*, 2019). In the UK this has been estimated at 75% and they are often amenity grassland used for sport and recreation. This type of grassland usually contains dandelions (*Taraxacum officinale*) and white clover (*Trifolium repens*), often used by pollinators if given time to flower. Research has shown that increasing the plant species richness, and allowing the sward height to increase by reduced mowing, has a positive impact on invertebrate abundance and richness (Garbuzov *et al.*, 2014; Norton *et al.*, 2019; Morrison *et al.*, 2025). Species richness increased by 81% and abundance doubled butterfly populations on semi-natural grasslands where fertiliser application was omitted from grasslands, entirely due to a reduction in biomass vegetation and an increase in species of forbs (Arnold *et al.*, 2025).

In rural areas arable crops tend to supply pollinators with temporal peaks of floral resources so supplies are often limited once crops have set seed, whereas in urban environments pollinators can disperse to other gardens where a nectar supply may be located (Nichols *et al.*, 2022a; Tew *et al.*, 2022). Cities often have relatively distinct assemblages of species, within a variety of habitats (Ives *et al.*, 2016; Soanes *et al.*, 2019). In one study on nine urban land use types (allotments, cemeteries, impermeable surfaces, nature reserves, parks, road verges, side walks, private gardens and other green spaces) across four UK cities, a high species abundance and richness of pollinators was found that was often comparable to rural and protected areas (Baldock *et al.*, 2019). Private gardens cover large areas in cities, while allotments may cover less area, but they both contain highly diverse floral resources, from native to ornamental plants, both important for provisioning pollinators (Baldock *et al.*, 2019; Griffiths-Lee *et al.*, 2022). Urban areas may provide floral resources by extending the foraging period for pollinators, particularly queen bees who emerge early and new queens foraging late before hibernation (Baldock, 2020; Spotswood *et al.*, 2021). Floral resources that provide pollinators throughout the season and include a good quality and quantity of resources, such as pollen and nectar, are vital to support a diversity and abundance of pollinators (Hicks *et al.*, 2016). Native plant species are considered important for insects due to their accessible floral resources and high nectar value. In Ireland the Noxious Weeds Act enacted in 1937 does not relate to specific native species but is interpreted to mean two species of thistle, spear (*Cirsium vulgare)* and creeping (*Cirsium arvense*), common ragwort (*Jacobaea vulgaris)* and two dock species, curly (*Rumex crispus)* and broad-leaved (*Rumex obtusifolius*). Noxious plants are judged to reduce yield in agricultural crops, however, they afford insects with food, shelter and oviposition sites for a multitude of species. Research in the UK highlighted that an estimated £10 million is spent on eradicating these species annually by public bodies while approximately £40 million is spent on enhancing pollinators through agri-environmental schemes (AES) schemes. Jarvis *et al*. (2025) found that double the abundance and diversity of pollinators visited the noxious weed species in contrast to the flower mixes used in AES schemes. They suggest that it may be advantageous to tolerate noxious weeds as a means of enhancing biodiversity rather than to plant short-lived, non-native species that may not offer the same resources as native species (Jarvis *et al.*, 2025). Native plants need little encouragement to grow and are widespread, with noxious plants supporting a wide variety of wildlife (Balfour & Ratnieks, 2022).

Evaluating biodiversity is extraordinarily complex leading to disputes on the value of indicator species. Pollinators are often used as bioindicators due to their sensitivity to changes in habitat (Harpke *et al.*, 2025). A better alternative may be to use semi-natural habitats as an indicator (Sheridan *et al.*, 2017). Semi-natural habitats support pollinators which increase crop yields, while biodiverse ecosystems become more resistant to extreme weather events (Redhead *et al.*, 2022). Nonetheless, enhancing sites for biodiversity needs to be considered at a landscape scale (Jarvis *et al.*, 2025).

2.1.3 The role of local authorities

To protect pollinators, we need action across all habitat types. Until recently much of the focus has been on agricultural systems and the role of policy for private actors (farmers, businesses and gardeners). However, public bodies in Ireland own and manage an estimated 8.5% of the land, covering a variety of habitats from farmland to heathlands, and forestry to wetlands (EPA, 2024b). Local authorities in Ireland are charged with managing much of this land and these areas could be utilised to enhance, conserve and restore biodiversity, and may have the potential to become reservoirs for rare species. This, therefore, means that local authorities are in a unique position to provide an assortment of habitats to support native Irish flora and fauna across Ireland (Figure 2-1). Ireland's 4th National Biodiversity Action Plan 2023-2030 recognised the fundamental role local authorities could achieve in meeting the urgent restoration and conservation of native species through a 'Whole of Government, Whole of Society' approach (NPWS, 2024). Local authorities need to be aware of the value of rural and urban environments for the conservation and restoration of species and habitats, including threatened species. The major advantage of urban environments is their dual ability to both conserve species and enhance the general public's engagement with wildlife (Soanes & Lentini, 2019). At the same time local authorities have the ability to contribute to provisioning services by providing spaces for orchards and allotments, thereby reducing food miles whilst enhancing food security, health and well-being within local communities (O'Sullivan *et al.*, 2017).

Evidence-based scientific advice is crucial to support nature restoration. Under the NBDC's Strategic Plan (2024-2028) one of their goals is to aid in more evidence-based actions using biodiversity data to support the development of policy documents (NBDC, 2024). This chapter provides evidence-based research on ways to improve biodiversity on public open spaces in County Kildare. Kildare County Council manage an estimated 171,000ha and of that, urban greenspace accounts for 5,800ha and sown pastures for 90,100ha (CSO, 2025). Using pollinating insects and plants as model species, it focuses on two land use types currently managed by Kildare County Council- town parks and peri-urban farmland.

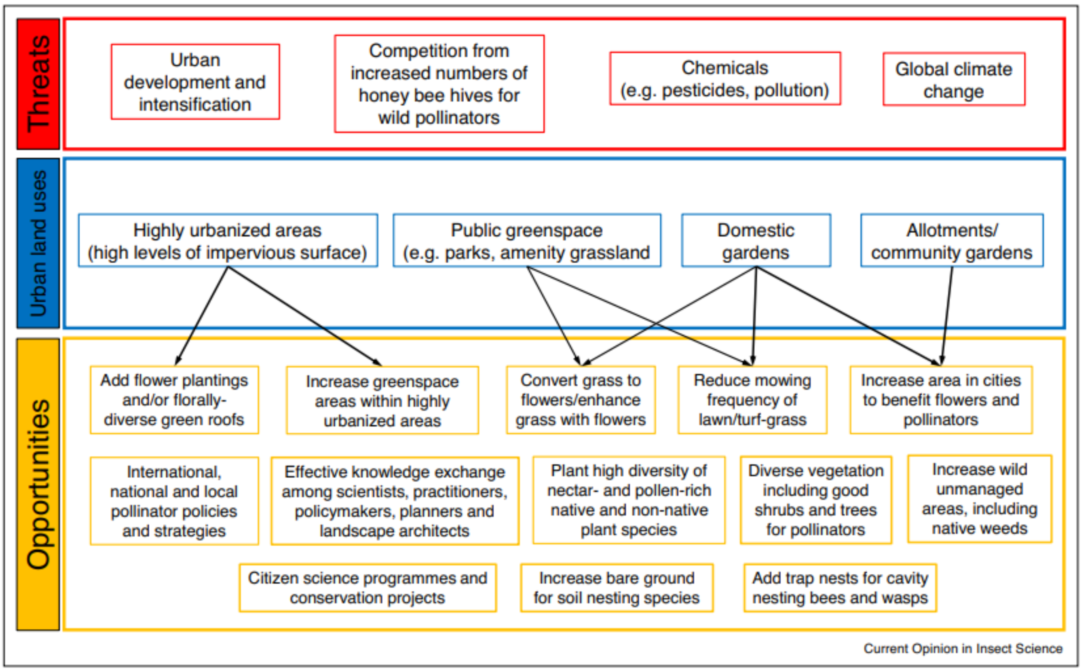


Figure 2-1 The threats, type of urban land use and opportunities that could be considered by local authorities for improving pollinators in urban areas (after Baldock, 2020).

2.2 Aims

The aim of this chapter was to assess the impact of two different management approaches on the abundance and diversity of four key pollinator groups (bumblebees, solitary bees, butterflies and hoverflies), in three low-intensity peri-urban farmland sites and three town parks (Plate 2-1), under the management of Kildare County Council. Vegetation surveys were used to categorise the various areas of land into habitats using Fossitts (2000). The results will be used to inform local authorities and land managers on best practices to enhance pollinator populations on council managed lands. Through this research the following hypotheses were tested:

1) Insect pollinator abundance and species richness varies between urban and peri-urban farmland.
2) Habitat type (grassland vs. woodland) drives differences in insect pollinator abundance and richness.
3) Pollinating insect community composition varies between urban and peri-urban farmland and is driven by habitat type and plant species richness.

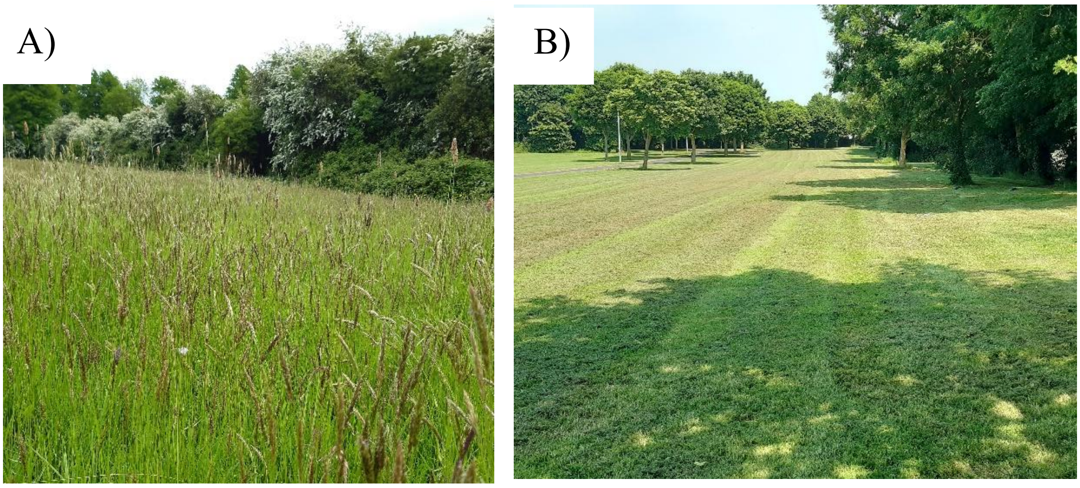


Plate 2-1 The peri-urban farmland and town park sites**.** A) Carton Avenue, one of the Peri-urban farmland sites and B) Monread Park, one of the town park sites

2.3 Methods

2.3.1 Study Region and sampling habitats.

The study region was located across six sites in County Kildare, Ireland (Figure 2-2) that were predicted to differ in pollinator communities as decided by their current management regimes. Initially, 23 sites across Kildare were surveyed to find sites to replicate, and sites were eventually chosen on their geographical location and the similarity of habitats. Three of the sites were categorized as peri-urban farmland and three as town parks. The peri-urban farmland in the context of this study is of low-intensity and is leased out to local farmers. The three sites all lay alongside towns and are requested by the council to be farmed organically with low fertiliser inputs and no pesticides. It was noted however, during surveys, that fertiliser had been used on one of the farmland sites. The hedgerows and treelines are not maintained, and this has allowed these semi-natural habitats to grow in height and width, creating high quality habitats for a wide variety of species providing shelter, nesting sites and food. The fields on these sites were classed as long-flowering meadows by Kildare County Council and were cut once a year for hay. A single site was grazed by cattle in the first year of surveys. There were a number of other semi-natural habitats within these sites such as drainage ditches, ponds, veteran trees, scrub and disused buildings (Plate 2-2).

In comparison, the three town parks were highly managed with the trees and hedgerows clipped and the majority of the grass mown, though the mowing regimes were varied ranging from two weeks to once a year in the autumn. Kildare County Council classify meadows as long flowering layered (some areas mown every two or three weeks, others once a year) or frequently mown playing pitches. There were a variety of features across these sites such as scattered immature trees, paved areas, grass verges, playgrounds, and obstacles such as benches and bins. Other habitats included immature and a riparian woodland, a river and occasionally, veteran trees (Plate 2-3). The study sites were located at least one kilometre apart to ensure independence between pollinator communities, although some pollinators are known to forage for several kilometres (Larkin & Stanley, 2021) and sites ranged in area from four to eighteen hectares in size (Table 2-1).

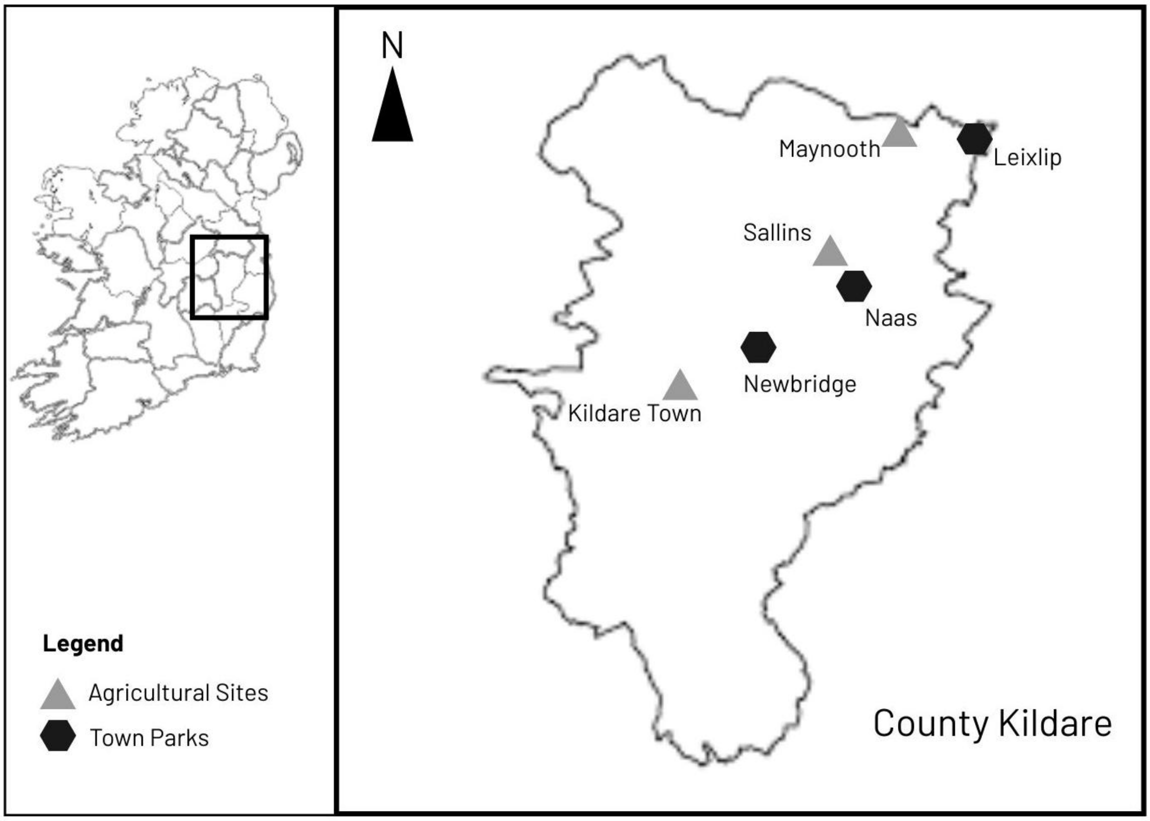


Figure 2-2 The distribution of the six study sites in County Kildare: three town parks and three peri-urban agricultural sites.

Initial habitat surveys were conducted during July and August 2021 on all sites and were classified according to Fossitt Level 3 (2000). ESRI ArcGIS pro (version 3.5.0) was used to digitise habitats using polygons and polylines according to each feature type and maps were created for all six sites (Appendix A Figure A- 1). Hedgerows and treelines were mapped as linear features while grasslands and woodlands were mapped as polygons. All habitats were mapped including features of note such as veteran trees and ponds. Further plant species were added to these habitats throughout the following two survey seasons in 2022 and 2023, as noted while conducting the pollinator surveys. Habitats were amalgamated into Fossitt classification level 1 (Fossitt, 2000) to allow for data analysis. The total site area in hectares, the area of each habitat and the overall length of linear habitats (metres) was calculated in ArcGIS (Table 2-1).

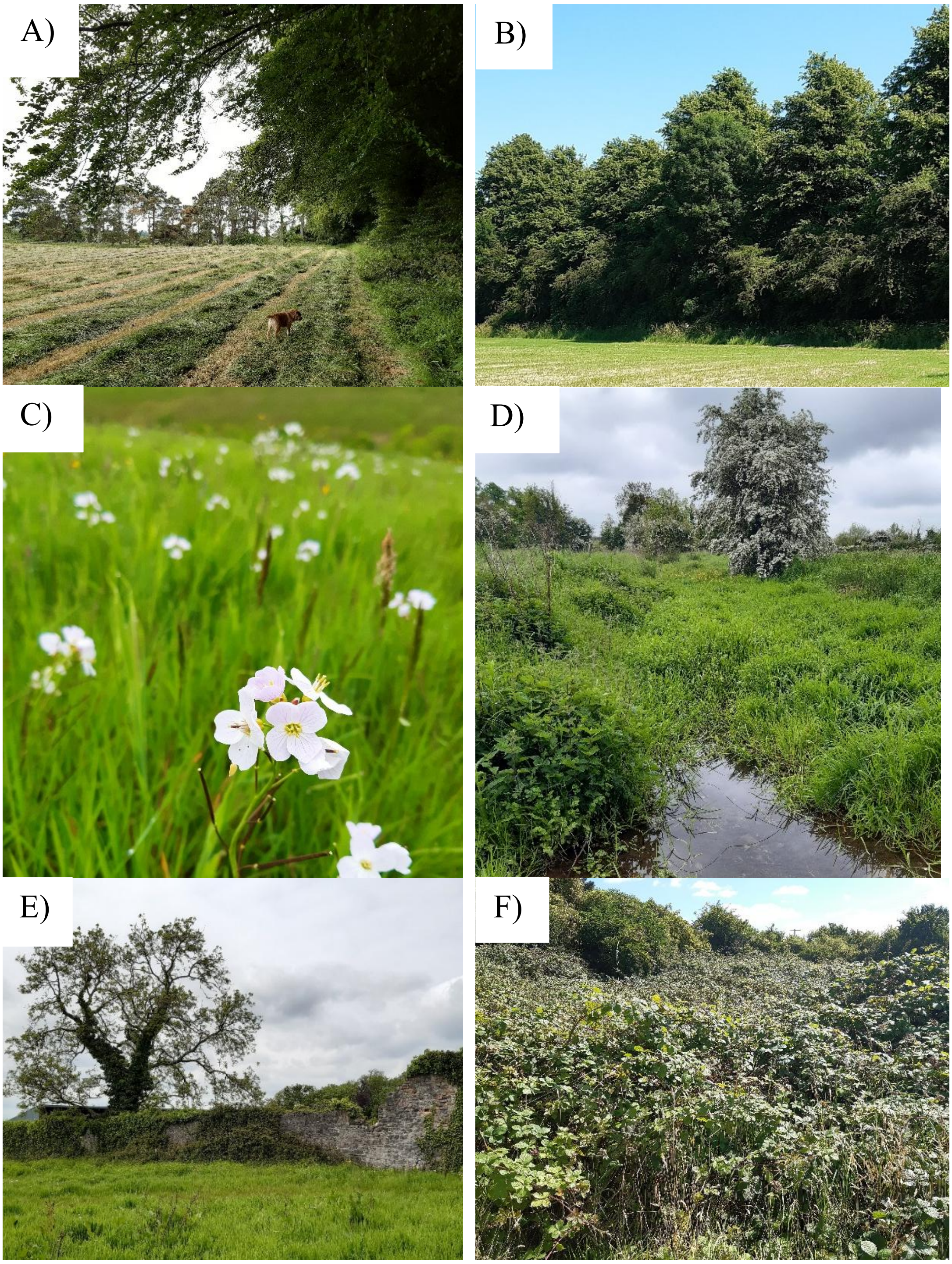


Plate 2-2 A few of the habitats that existed on the peri-urban farmland sites. A) A long-flowering meadow cut in late summer surrounded by mature treelines. B) A mature hedgerow. C) A long-flowering meadow with Cuckoo flower (*Cardamine pratensis*) D) A drainage ditch with flowering hawthorn (*Crataegus monogyna*) behind. E) A veteran tree with an old stone wall surrounding disused buildings. F) Scrub forming.

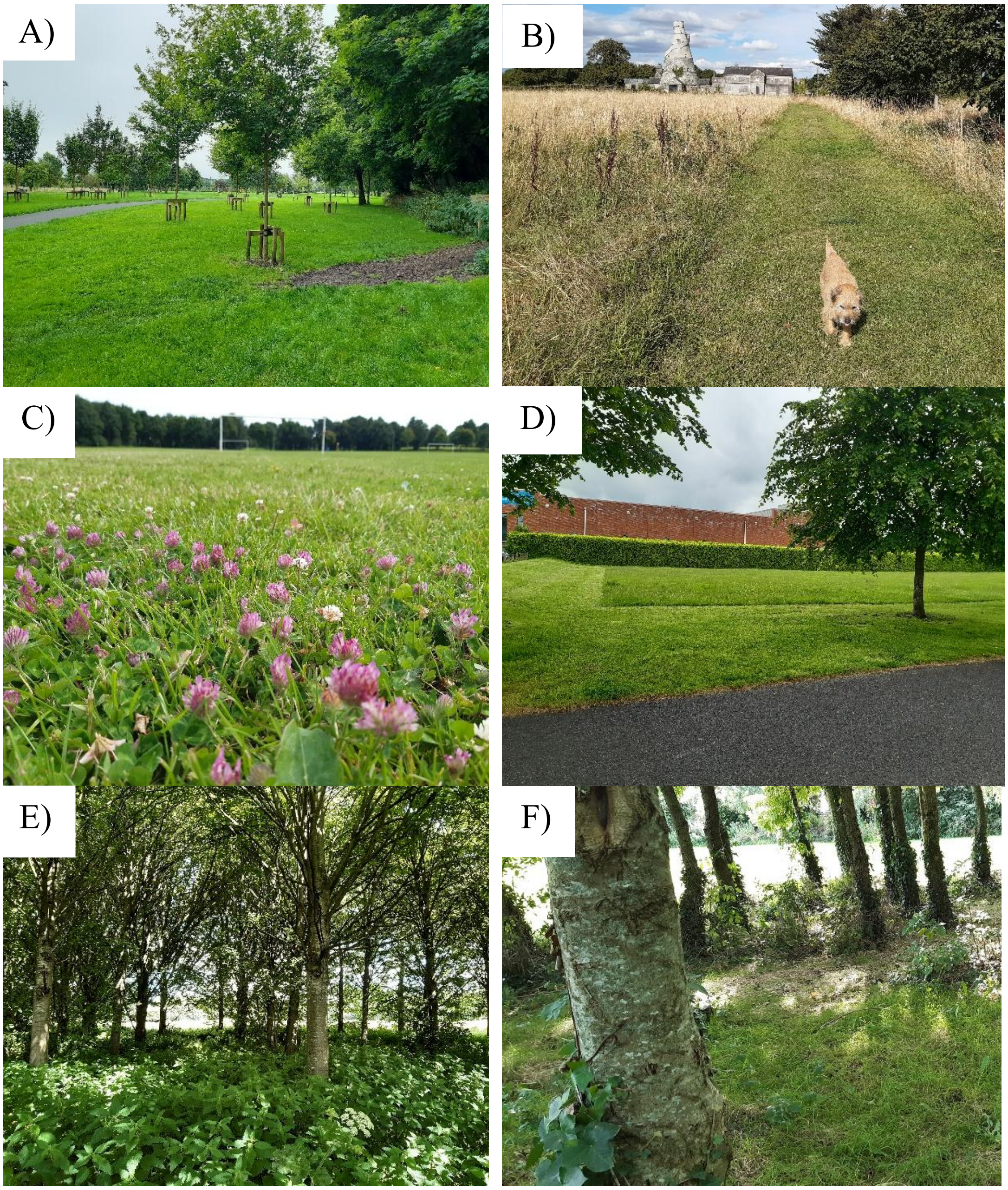


Plate 2-3 A few of the habitats that existed on the town park sites. A) Frequently mown grassland with scattered trees. B) A long-flowering meadow with mown footpath. C) A pitch mown less frequently to allow clover spp. (*Trifolium* spp.) to flower. D) A footpath running with frequently mown grassland and a highly maintained hedgerow in the background. E) and F) Immature woodland.

Table 2-1 The site type, the name, the area (hectares) and GPS location of the six sites surveyed. Habitat groups recorded on peri-urban farmland and town park sites during surveys at Levels 1 and 3 according to Fossitt (2000).

			Peri-urban farmland			**Town Park**		
Habitat Group Level 1	**Code**	**Habitat Type Level 3**	Carton Avenue, Maynooth	Cherry Avenue, Kildare	Sallins Amenity, Sallins	Liffey Linear Park, Newbridge	Monread park, Naas	Wonderful Barn, Leixlip
		GPS Location	53.384932, -6.579775	53.152799, -6.899376	53.247754, -6.676475	53.178185, -6.790258	53.232967, -6.654797	53.359622, -6.510397
Grassland and marsh	GA1	Improved agricultural grasslands	12.25	5.38	6.86			
	GA2	Amenity grassland	2.59	0.24	3.17	2.80	12.69	8.50
	GM1	Marsh				0.15		
	GS1	Dry calcareous and neutral grassland			2.66			
	GS4	Wet Grassland		0.95	1.67			
Woodland and scrub	WD1	(Mixed) broadleaved woodland			0.46		0.26	
	WD2	Mixed broadleaved/conifer woodland					0.05	
	WN5	Riparian woodland				0.36		
	WS1	Scrub		0.50				
	WS2	Immature woodland						2.57
	WL1	Hedgerow	0.97	0.36	0.31			0.03
	WL2	Treeline	2.39	0.74	1.16	0.45	2.24	0.86
Total area in hectares			18.2	8.17	16.30	3.76	15.24	11.96

The Irish National Landcover Map (EPA, 2025) was accessed to gather data on the land use within a 2km boundary of each site. The relative abundance of each of the four habitat types- developed, grassland, tree/wooded and water surfaces/bodies was calculated (Table 2-2). There was no significant difference between the relative percentage cover of any of the four surrounding land cover types between the peri-urban farmland and the town parks.

Table 2-2 The relative abundance (%), mean and standard error (SE) of Level 1 National Land Cover Classification System of the landscape within a 2 kilometre buffer of each of the six sites, peri-urban farmland and three town parks.

Site Type and Site Name	Artificial surfaces %	Grassland %	Woodland or scrub %	Waterbodies %
Peri-urban farmland				
Carton Avenue	22.02	59.11	16.60	2.27
Cherry Avenue	22.21	63.68	13.88	0.23
Sallins Amenity	17.65	69.53	12.05	0.77
Mean and SE	**20.63 ± 1.49**	**64.11 ± 3.01**	**14.18 ± 1.32**	**1.09 ± 0.61**
Town Park				
Liffey Linear Park	41.55	44.09	13.49	0.86
Monread Park	36.80	52.27	10.22	0.72
Wonderful Barn	30.10	47.56	16.86	5.47
Mean and SE	**36.15 ± 3.32**	**47.97 ± 2.37**	**13.52 ± 1.92**	**2.35 ±1.56**

2.3.2 Sampling pollinators

Insects were recorded over two consecutive summers from April through to September inclusively during 2022 and 2023. Surveys were undertaken between 10.00 and 16.00, when temperatures were above 13°C on dry days, with wind speeds at four or less on the Beaufort scale. It has been recommended that multiple sampling methods are used to assess insect pollinators (Griffiths-Lee *et al.*, 2023). Transects are the best method to detect the species abundance of insects while pan traps are better for collecting a broader diversity of bees. The small fast flying bees species are more likely to be detected in pan traps than transects where they may be missed (Potts *et al.*, 2024).

Pollinator monitoring was conducted using pan trap and transects surveys, and combining these two approaches permits the gathering of information on pollinator diversity and abundance in addition to floral visitations. Pan trap and transect locations were fixed and were sampled on all visits.

Pan trap surveys enable concurrent sampling at multiple locations and identification to species level under laboratory conditions. A total of 180 pan traps were deployed over the two seasons, with 15 pan trap events occurring per site per year. Pan traps were in place by 10:00 and removed after 16:00 with two pan traps positioned in an area of open grassland and one pan trap alongside a woodland/hedgerow/treeline habitat. Trials on pan trap duration suggest that six hours gathered as much data as 24 hours for analysis (Carvell *et al.*, 2016). A variety of coloured pan traps have been found to be best for monitoring bee species (Griffiths-Lee *et al.*, 2023). They provide passive sampling and enable an assessment of the diversity within communities of bees and hoverflies in a landscape (Gonzalez *et al.*, 2020). Pan trapping is important for collecting solitary bees that may not be collected/accurately identified on the wing during transects (Carvell *et al.*, 2016). Each pan trap consisted of three plastic bowls painted with UV paint in either blue, yellow or white, which were fastened to a post at the height of the surrounding vegetation. Water and a drop of Ecover fragrance-free washing up liquid was added to each bowl. Bees and hoverflies were identified to species level (using the books by Ball and Morris 2015 and Falk 2016) and all other species were identified to family level. All other species were omitted from further analysis, including honeybees, as these may often be managed and intentionally introduced into an area for honey production.

Transects were undertaken mid-afternoon between 1.30pm and 4pm as pollinators were shown to peak at this time during surveys in nearby County Carlow, Ireland

(Karbassioon & Stanley, 2023). Bumblebees are best monitored via transects (Griffiths-Lee *et al.*, 2023). Transect surveys were conducted along a one-kilometre length (Carvell *et al.*, 2016) with 500m along woodland margins (hedgerows, treelines and woodland) and 500m in open grassland. Transects were walked at a slow and steady pace with pollinators recorded, and their activity, within an imaginary 4m square box in front and to either side of me. Insects were identified to three groups: bees (*Apidae*), butterflies (*Lepidoptera*) and hoverflies (*Diptera: Syrphidae*), to species level where possible in the field. The white butterflies, (*Pieris napi, Pieris rapae* and *Pieris brassicae*) were recoded to genus level. as were the worker bumblebees unidentifiable without DNA analysis, specifically- *B. terrestris, Bombus cryptarum, Bombus lucorum* and *Bombus magnus* (Carolan *et al.*, 2012). All species were classified on their status within the European Red lists. Where identification in the field was not possible, a net was used to capture insects which were then retained for identification at a later time (using the books by Ball and Morris 2015 and Falk 2016). Where identification was not possible, the pollinator reference collection at the National Museum of Ireland's Storage facility was used and species were verified by entomological experts (see Acknowledgements). Taxa not identified to species level were not used in species richness analysis.

2.3.3 Data Analysis and model selection

Statistical analysis was conducted and figures were produced in R (RStudio Version: 2025.05.1). Response variables, abundance and richness, were pooled at the site level across sampling periods. Transect and pan trap data abundance were analysed separately to account for the different sampling methods. To standardise the pan trap data, the abundance in the woodland pan traps were doubled as two pan traps had been placed in an area of open grassland and only one by a linear feature, either a woodland, treeline or hedgerow. For insect richness analysis was conducted on pooled data by site, sampling period and years combined from amalgamated transect and pan trap data.

The effect of the type of site on pollinator abundance within the four groups: bumblebees (BB), butterflies (BF), hoverflies (HF) and solitary bees (SB) was examined. The response variables were either insect abundance or richness, site type and habitat type were used as fixed effects, while year and month were included as random effects to account for temporal independence. Mixed models were used using the lme4 package. All models were simplified by removing interactions that were not significant. To assess species diversity according to the type of site and habitat, the Shannon and Simpson Diversity was calculated for each insect group using the vegan package. To assess the differences in indices the 95% confidence intervals with non-parametric bootstrap with replacement (1000 times) was calculated. To examine the effect of floral richness recorded during quadrat surveys on pollinator richness the type of site was used as a fixed effect using a negative binomial GLM model in the vegan package.

QQ plots, histograms of the residuals and the DHARMa package were implemented to validate the models. Generalised Linear Mixed Models (GLMMs) with a negative binomial distribution were used to test abundance, as the data did not follow a Normal or a Poisson distribution as it was overdispersed. Poisson log transformed models were used to test richness. Where p values were significant the Tukey's post hoc tests were used in the multcomp package. To identify the potential drivers of the differences between community composition of each group and the type of sites, the area of each type of habitat on each site were analysed using Principal Component Analysis (PCA), to allow the identification of the key axes of variation. Prior to analysis the area variables were transformed using a Hellinger transformation for normality.

2.4 Results

One hundred and ninety pollinators were collected in pan traps: 55 bumblebees (seven species), one butterfly, 39 hoverflies (14 species) and 95 solitary bees (14 species) (Table 2-3). One thousand, eight hundred and thirty-three individual pollinators were recorded during transect surveys and represented 587 bumblebees (six species), 490 butterflies (15 species), 710 hoverflies (21 species) and 46 solitary bees (three species) (Table 2-3). Overall, 63 species were recorded, see Appendix Table A- 1. Peri-urban farmland supported the highest abundance and species richness overall.

Table 2-3 Total abundance and mean abundance of individual insects (±SE) of each pollinator group in pan traps and transects by the type of site (peri-urban farmland or town park).

	Pan Traps		**Transects**	
Peri-urban farmland	Total abundance	Mean abundance/site	Total abundance	Mean abundance/site
Bumblebee	28	9.33 ± 2.33	412	137.33 ± 18.41
Butterfly	1	0.33 ± na	415	138.33 ± 34.45
Hoverfly	27	9 ± 3.21	431	143.67 ± 29.17
Solitary bee	58	19.33 ± 7.69	36	12 ± 4.36
Town Park				
Bumblebee	27	9 ± 2.89	175	58.33 ± 8.41
Butterfly	0	0	75	25 ± 8.08
Hoverfly	12	4 ± 2	279	93 ± 22.65
Solitary bee	37	12.33 ± 9.87	10	3.33 ± 1.45

2.4.1 Pollinator abundance at the site scale

When comparing peri-urban farmland to town parks in the pan trap data (Plate 2-4), the abundance of all groups was marginally higher in peri-urban farmland, but not significantly so (Table 2-4**).** Butterflies were excluded from the analysis as only a single butterfly was recorded. In the transect dataset (Plate 2-4), peri-urban farmland contained significantly more individuals of all pollinator groups except hoverflies (Table 2-4**).**

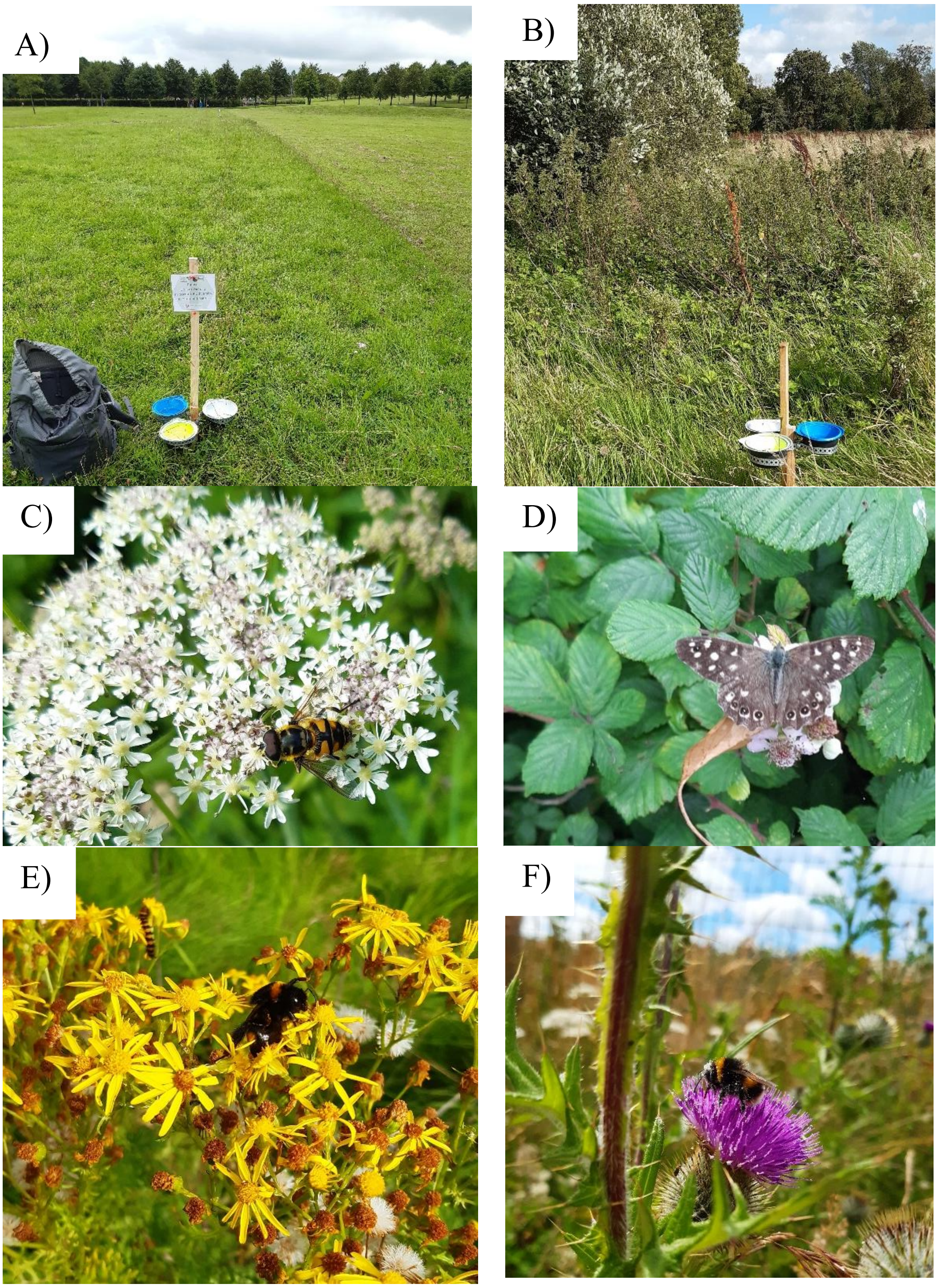


Plate 2-4 Pan trap surveys on A) a town park and B) a peri-urban farmland. Insect pollinators noted during transects C) A hoverfly (*Eristalis* sp.) foraging on hogweed (*Heracleum sphondylium*), D) Ringlet (*Aphantopus hyperantus*) butterfly foraging on bramble (*Rubus fructicosus* agg.), E) Bumblebee sp. foraging on ragwort (*J. vulgaris*) and F) thistle sp. (*Cirsium* sp.)

Table 2-4 Estimates, standard errors (SE) and 95% confidence intervals (95% CI) of the fixed effects included in the best-fitting model explaining the variation in pollinator abundance in pan traps and transects. Significant results are in bold.

Response	Predictors	Estimate	SE	df	t-statistic	p-value
Pan trap abundance						
Bumblebee	Site Type	0.0364	0.556	Inf	0.065	0.9478
Hoverfly	Site Type	0.4055	0.645	Inf	0.629	0.5294
Solitary bee	Site Type	0.4495	0.530	Inf	0.849	0.3959
Transect abundance						
Bumblebee	Site Type	0.856	0.264	Inf	3.246	**0.0012**
Butterfly	Site Type	1.711	0.278	Inf	6.158	**0.0001**
Hoverfly	Site Type	0.435	0.259	Inf	1.676	0.0937
Solitary bee	Site Type	1.281	0.435	Inf	2.945	**0.0032**

2.4.2 Pollinator abundance at the habitat scale

Two main habitat types were analysed:- grassland and woodland, on the peri-urban farmland versus the town park sites. The woodland includes the treelines and hedgerows representing the linear features. On average there was a greater area of grassland in the peri-urban farmland sites, but similar areas of woodland in both site types (Table 2-5). Butterflies were excluded from the analysis in the pan trap data as only a single butterfly was recorded. There were no significant differences in abundance in the pan traps between the remaining three pollinating groups on the woodland and grassland habitats between the peri-urban farmland and the town parks. However, during transects the abundance of all four pollinating groups were higher in the woodlands on the peri-urban farmland, significantly so for the bumblebees, butterflies and solitary bees (Table 2-6). And again, the grasslands held a higher abundance for all four groups, but only significantly so for the butterflies.

Table 2-5 Mean area per hectare (± SE) of habitat types, grassland and woodland (including the treelines and hedgerows), within the peri-urban farmland and town park sites.

Type of habitat	Peri-urban farmland	Town Parks
Grassland	11.93 ± 4.64	8.05 ± 4.89
Woodland	2.21 ± 0.91	2.24 ± 1.26

Table 2-6 Estimates, standard errors (SE) , t-statistic and p-values of the fixed effects included in the best-fitting model explaining the variation in pollinator abundance in pan traps and transects compared across the two broad habitat categories: woodland and grassland between the peri-urban farmland and town park sites. Significant results are in bold.

Response	Predictors	Estimate	SE	t-statistic	p-value
Pan trap abundance					
Bumblebee	Woodland	0.000	0.592	0.000	1.0000
	Grassland	-0.405	0.495	-0.818	0.4131
Hoverfly	Woodland	-0.539	0.731	-0.737	0.4612
	Grassland	0.080	0.696	0.115	0.9084
Solitary bee	Woodland	-0.137	0.529	-0.260	0.7952
	Grassland	0.780	0.460	1.697	0.0897
Transect abundance					
Bumblebee	Woodland	0.927	0.373	2.483	**0.0130**
	Grassland	0.574	0.407	1.411	0.1583
Butterfly	Woodland	1.569	0.386	4.069	**<.0001**
	Grassland	2.165	0.454	4.771	**<.0001**
Hoverfly	Woodland	0.417	0.369	1.130	0.2585
	Grassland	0.524	0.402	1.303	0.1925
Solitary bee	Woodland	1.322	0.536	2.466	**0.0137**
	Grassland	0.693	0.908	0.763	0.4453

2.4.3 Pollinator species richness at the site and habitat scale

Overall, there were more species found in peri-urban farmland than town parks (p=0.02) (Figure 2-3). There were no statistically significant differences for each of the four pollinating groups between peri-urban farmland and town park sites in either the Shannon or Simpson diversity indices (Appendix Table A- 2). However, the Shannon and Simpson Diversity indices were higher for all four pollinating groups in the peri-urban farmland (Table 2-7).

Two of the main habitat types were also analysed for pollinator richness, the grassland and woodland (including the treeline and hedgerow) habitats, on the peri-urban farmland versus the town park sites. There were no significant differences in the richness of either bumblebees or solitary bees in either the woodland or the grassland habitats, between the peri-urban farmland and the town parks, even though the diversity for both groups was higher on the peri-urban farmland (Table 2-8). There was a significant difference in the richness of butterflies in the grasslands with a higher species diversity on the peri-urban farmland. The hoverflies were significantly more diverse in the grassland and woodland habitats on the peri-urban farmland in comparison to the town parks (Table 2-8).

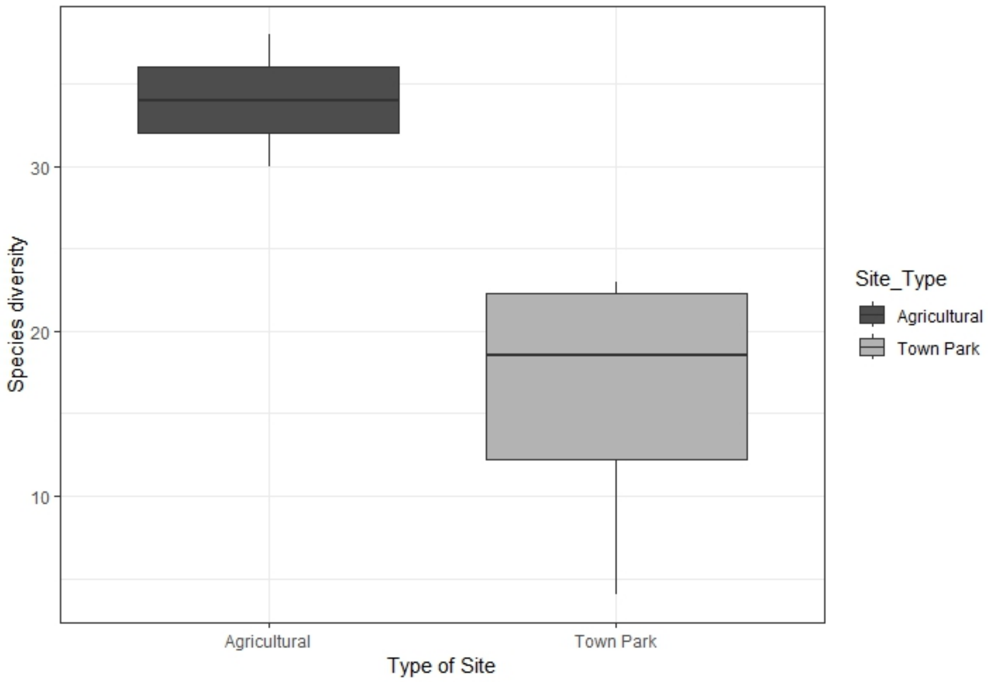


Figure 2-3 Diversity of all pollinator species in peri-urban farmland and town park sites. Each data point used to create the boxplot is the mean pollinator diversity measured at all six sites, three per type of site, over all twelve surveys. The solid bars represent the median, and the boxes represent the upper and lower quartiles. The whiskers represent the maximum or minimum value of the data.

Table 2-7 Pollinator richness in pan traps and transects , mean species richness ± SE, the mean Shannon Diversity ± SE, and the mean Simpson Diversity ± SE, across peri-urban farmland and town park sites.

	Total species richness	Mean species richness	Mean Shannon Diversity	Mean Simpson Diversity
Peri-urban farmland				
Bumblebee	6	5.67 ± 0.33	1.39 ± 0.10	0.71 ± 0.03
Butterfly	15	11.67 ± 0.33	1.71 ± 0.10	0.74 ± 0.04
Hoverfly	26	11.67 ± 0.88	1.93 ± 0.14	0.78 ± 0.04
Solitary bee	9	5 ± 1.53	1.10 ± 0.36	0.53 ± 0.15
Town Park				
Bumblebee	7	6 ± 0	1.27 ± 0.09	0.64 ± 0.03
Butterfly	9	5.67 ± 0.88	1.38 ± 0.10	0.69 ± 0.02
Hoverfly	12	5.33 ± 0.67	1.19 ± 0.26	0.58 ± 0.12
Solitary bee	9	3.67 ± 1.76	0.86 ± 0.45	0.45 ± 0.25

Table 2-8 Estimates, standard errors (SE), degrees of freedom (df), t-statistic and p-value of the fixed effects included in the best-fitting model explaining the variation in the diversity of each pollinator group in pan traps and transects compared across the two broad habitat categories: woodland (including hedgerows and treelines) and grassland between the peri-urban farmland and town park sites. Significant results are in bold.

Pan Trap and transect log richness						
Response	**Predictors**	**Estimate**	**SE**	**df**	**t-statistic**	**p-value**
Bumblebee	Site Type	0.158	0.347	17	0.456	0.6540
	Woodland	0.0608	0.424	15	0.143	0.8879
	Grassland	0.1649	0.373	17	0.442	0.6639
Butterfly	Site Type	0.748	0.370	17	2.019	0.0595
	Woodland	0.8162	0.424	15	1.925	0.0734
	Grassland	1.2365	0.399	17	3.102	**0.0065**
Hoverfly	Site Type	0.794	0.370	17	2.144	**0.0468**
	Woodland	0.9345	0.424	15	2.204	**0.0436**
	Grassland	0.9311	0.399	17	2.336	**0.0320**
Solitary bee	Site Type	0.462	0.370	17	1.247	0.2292
	Woodland	0.4406	0.474	15	0.929	0.3674
	Grassland	0.0393	0.399	17	0.099	0.9227

2.4.4 Community Composition

To assess what individual habitats at Fossitt level 3 may be driving the difference in community composition of the pollinating groups on the sites, PCAs were conducted. The bumblebee biplot (Figure 2-4) shows the first two principal components which together account for 80% of the variance in the data: PC1 explains 66% of the variation and PC2 explains an additional 16% of the variance. The bumblebee communities in the peri-urban farmland and town park sites cluster separately. *Bombus pascuorum* is more associated with the amenity grassland (GA2) while *B. lucorum agg.* is more associated with the treelines (WL2) and immature woodland habitats (WS2) on the town park sites. *Bombus lapidarius* is strongly associated with the peri-urban farmland, especially the grassland habitats (GS4 and GA2). *Bombus hortorum* and *Bombus pratorum* are not associated with either type of site but more with the woodland habitats (WD1 and WN5), while *Bombus jonellus* seems to be a generalist. There is no overlap in the community composition of the bumblebees.

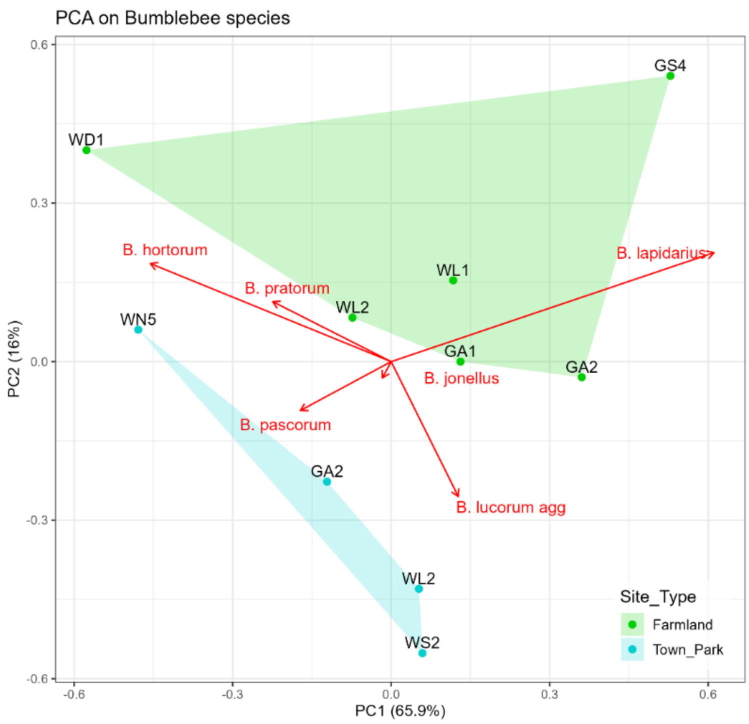


Figure 2-4 PCA plots showing the relationship between environmental variables and bumblebees after accounting for the area of habitats on peri-urban farmland and town parks. (WD1- (Mixed) broadleaved woodland, GS4-Wet grassland , GA2-Amenity grassland, GA1-Improved grassland, WL1-Hedgerow, WL2- Treeline, WN5- Riparian woodland and WS2- Immature woodland).

The butterfly biplot (Figure 2-5) shows the first two principal components which together account for 99% of the variance in the data. PC1 explains 62% of the variation and PC2 explains an additional 37% of the variance. The *Pieridae* butterflies are strongly influenced by the peri-urban farmland, in particular the hedgerows (WL1), treelines (WL2) and wet grassland (GS4) habitats. The *Nymphalidae* seem to be a more generalist family and are associated with the amenity grassland habitats (GA2) on both the peri-urban farmland and town park sites. The *Lycaenidae* are strongly correlated with the treelines (WL2) on the town parks sites. There is an overlap in the community composition of butterflies within the peri-urban farmland and the town parks.

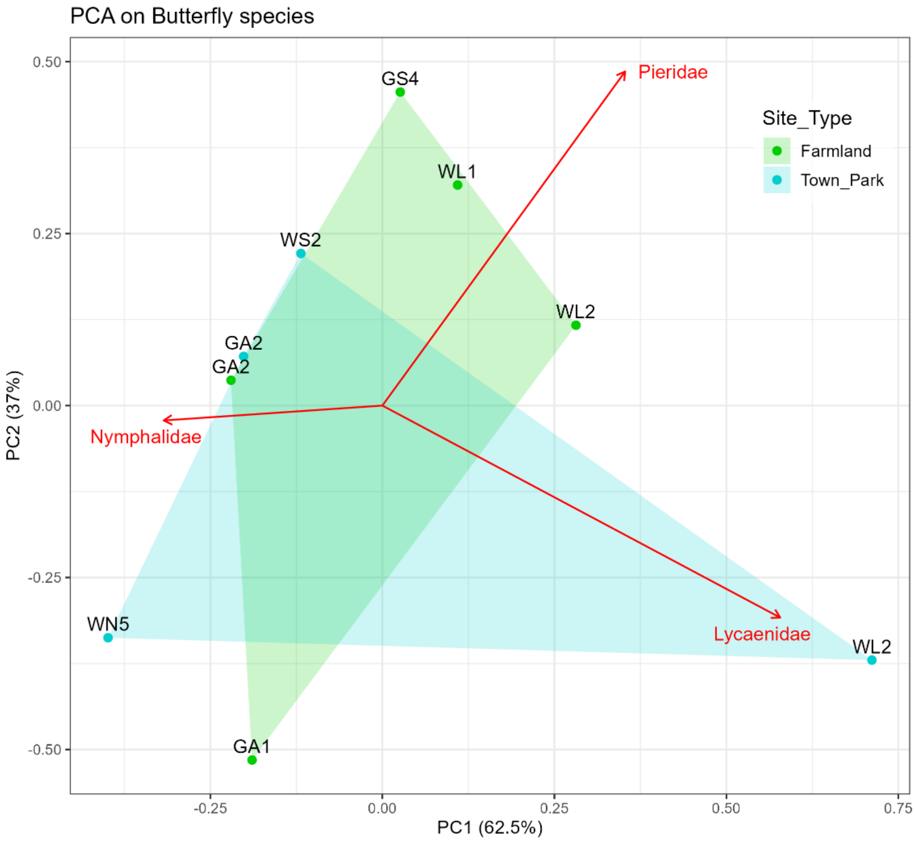


Figure 2-5 PCA plots showing the relationship between environmental variables and butterflies after accounting for the area of habitats on peri-urban farmland and town parks. (WD1- (Mixed) broadleaved woodland, GS4-Wet grassland , GA2- Amenity grassland, GA1- Improved grassland, WL1- Hedgerow, WL2- Treeline, WN5- Riparian woodland and WS2- Immature woodland).

The PCA biplot for hoverflies (Figure 2-6) shows the first two principal components which together account for 69% of the variance in the data. PC1 explains 51% of the variation and PC2 explains an additional 18% of the variance. The *Cheilosia* and *Eristalis* are influenced by the woodlands (WD1 and WN5) whereas both *Syritta* and *Episyrpus* are influenced by the grassland habitats (GA2) on both the farmland and town parks. The *Melanastoma* and *Syrphus* both respond to the treelines (WL2) on both site types, as do *Platycheirus* though weakly. There is an overlap in the community composition of hoverflies within the peri-urban farmland and the town parks.

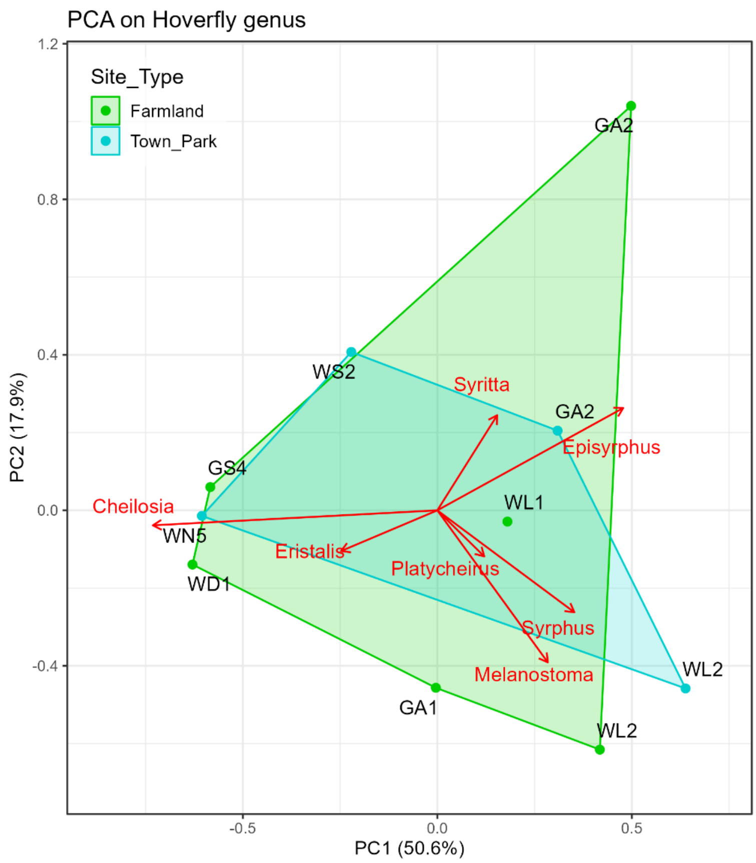


Figure 2-6 PCA plot showing the relationship between environmental variables and hoverflies after accounting for the area of habitats on peri-urban farmland and town parks. (WD1- (Mixed) broadleaved woodland, GS4-Wet grassland , GA2- Amenity grassland, GA1- Improved grassland, WL1- Hedgerow, WL2- Treeline, WN5- Riparian woodland and WS2- Immature woodland).

The biplot for the solitary bees (Figure 2-7) shows the first two principal components which together account for 73% of the variance in the data. PC1 explains 46% of the variation while PC2 explains an additional 27% of the variance. The genus *Hylaeus* are influenced by the agricultural woodland (WD1) and treeline habitats whereas the *Lasioglossum* genus seem to be associated with the town park grassland (GA2) and farmland wet grassland habitats (GS4). *Andrena* are associated with the grasslands (GA1, GA2 and GS4) on both types of site. *Halictus* are strongly associated with the agricultural grasslands (GA1) and hedgerows (WL1) while the *Megachiles, Sphecodes* and *Nomada* are shown to be generalists with no strong pattern of distribution. There is very little overlap in the community composition of the solitary bees.

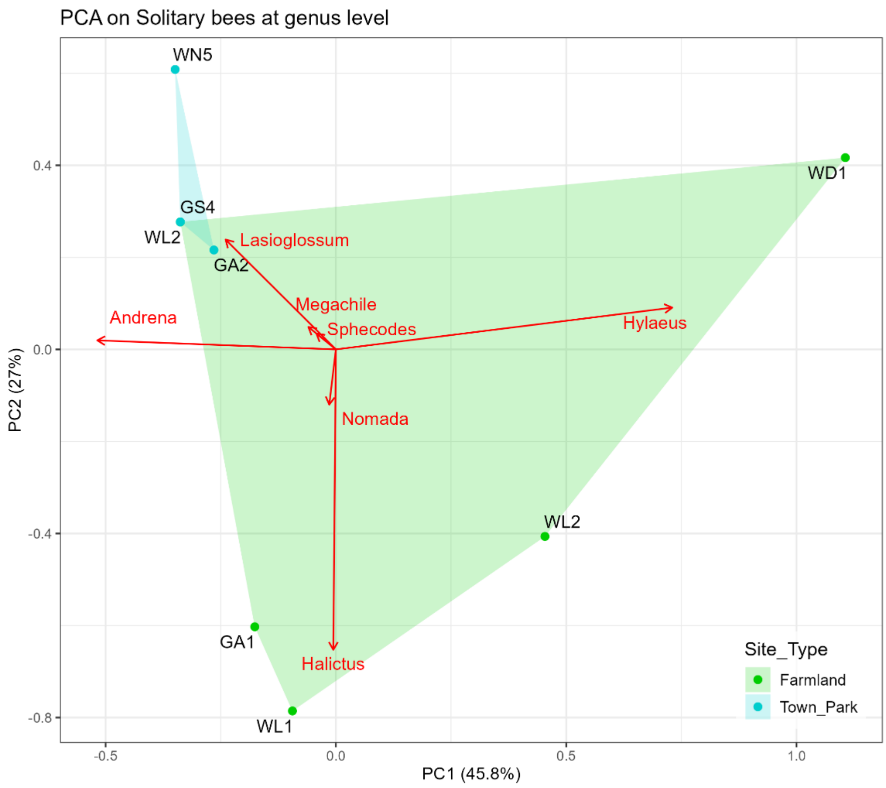


Figure 2-7 PCA plot showing the relationship between environmental variables and solitary bees after accounting for the area of habitats on peri-urban farmland and town parks. (WD1- (Mixed) broadleaved woodland, GS4-Wet grassland , GA2- Amenity grassland, GA1- Improved grassland, WL1- Hedgerow, WL2- Treeline, WN5- Riparian woodland and WS2- Immature woodland).

The differentiation between species highlights how the multiple habitats shape the patterns of species distribution. The community composition of the bumblebees and the solitary bees is strongly influenced by the environmental effect of peri-urban farmland while the butterfly and hoverfly communities are less affected by either type of site. The bumblebee community thrived in the treeline and grassland habitats, the butterfly community was influenced by the amenity grasslands, hedgerows and treelines, the hoverfly community seemed to be influenced by a variety of habitats, while the solitary bees associated with the treelines, grasslands and woodlands.

2.4.5 Plant species richness, floral visitations and floral richness.

The overall species richness of plants was not influenced by either type of site (p=0.78), or under the Shannon Diversity (p=0.56) or Simpson Diversity (p=0.56) indices (Table 2-9). For a full species list of plants found on the sites, see Appendix Table A- 3. During all transects, 673 floral visitations were observed on 38 varieties of plants, 515 by bumblebees, 58 by butterflies, 273 by hoverflies and 27 by solitary bees (Appendix Table A- 4). *Rubus fruticosus* agg. flowers were the most frequently visited (302 visits), followed by *J. vulgaris* (127 visits) and *Ranunculus* spp. (86 visits). Overall, there were 23 varieties of plants that were visited by bumblebees on peri-urban farmland and 19 on town parks. *R. fruticosus* agg. were most visited by bumblebees on both site types (52% on peri-urban farmland and 38% on town parks), with *T. repens* accounting for 23% of visits on town parks. Butterflies visited nine plant species on the peri-urban farmland and five on town parks, and again *R. fruticosus* agg. was important, particularly on the peri-urban farmland (52% of visits). *Cirsium dissectum* was the most visited in town parks at 46% followed *Heracleum sphondylium* with 23% of visits (Table 2-10). A similar finding for solitary bees was observed, with seven plants visited on peri-urban farmland and only two on town parks. *J. vulgaris* was the most visited on the peri-urban farmland while *Taraxacum* spp. received 75% of the solitary bee visitations on town parks. The hoverflies visited 19 plant varieties on peri-urban farmland, 31% on *J. vulgaris* and 11 on town parks, 22% on *Taraxacum* spp. and 18% on *H. sphondylium*. Using the quadrat data, the floral richness had no effect on pollinator richness (p= 0.64) as seen in Figure 2-8.

Table 2-9 Plant richness , mean species richness ± SE, the mean Shannon Diversity ± SE, and the mean Simpson Diversity ± SE, across peri-urban farmland and town park sites.

Site Type	Total species richness	Mean species richness	Mean Shannon Diversity	Mean Simpson Diversity
Peri-urban farmland	127	10.96 ± 2.62	4.28 ± 0.13	0.99 ± 0.002
Town Park	120	9.96 ± 2.56	4.17 ± 0.10	0.98 ± 0.001

Table 2-10 Total pollinator visits by percentage over the two year study to the top five most commonly visited plant species/taxon for each of the four groups on the peri-urban farmland and the town parks.

Plant species/taxon	Peri-urban farmland	Town Park	Plant species/taxon	Peri-urban farmland	Town Park
Bumblebee 			**Butterfly** 		
Rubus fruticosus agg.	52	38	*Rubus fruticosus* agg.	52	15
Jacobaea vulgaris	9	4	*Cirsium dissectum*	15	46
Cirsium vulgare	6		*Urtica dioca*	4	
Vicia sepium	6		*Jacobaea vulgaris*	4	8
Taraxacum spp.	4		*Ranunculus acris*	2	
Trifolium repens		23	*Heracleum sphondylium*		23
Heracleum sphondylium		6	*Taraxacum* spp.		8
Cirsium dissectum		5			
Hoverfly 			**Solitary bee** 		
Jacobaea vulgaris	31	15	*Jacobaea vulgaris*	43	
Taraxacum spp.	14	22	*Taraxacum* spp.	17	75
Rubus fruticosus agg.	10	6	*Heracleum sphondylium*	13	
Heracleum sphondylium	7	18	*Rubus fruticosus* agg.	9	
Ranunculus acris	5	6	*Geranium* spp.	9	
			Cirsium dissectum		25

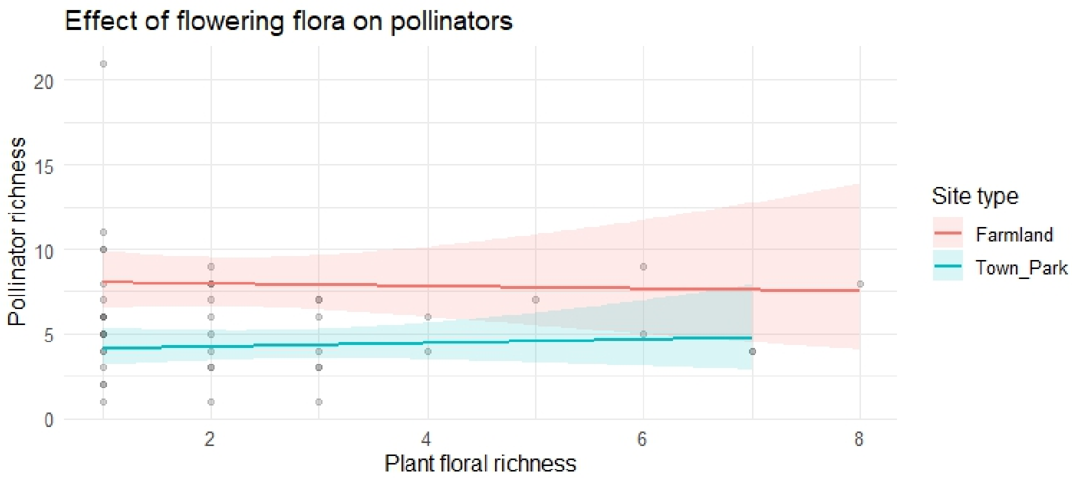


Figure 2-8 Scatterplot showing no significant relationship between floral richness and pollinator richness on peri-urban farmland (red) and town park (blue) sites. The light blue and light red around each of the lines indicates the 95% confidence interval of a linear relationship.

2.5 Discussion

There are notable differences between the pollinating groups of insects in the peri-urban farmland in comparison to the town parks, with a higher abundance and species richness of all four pollinating groups on the low-intensity farm sites. This is in contrast to Russo *et al.* (2022) who found peri-urban farmland contained lower species richness of pollinators in comparison to urban sites. One of the main reasons for this may be the management of the farmland in this study. These farms are composed of small field sizes, are not overgrazed by livestock and the boundaries that include the hedgerows, treelines and woodlands are unmanaged. This has led to more heterogenous landscapes with semi-natural habitats as boundaries and more sustainably managed farmland. Ecosystem function resilience can be improved by ensuring spatial heterogeneity offering a variety of resources and is known to support greater species richness and abundance (Oliver *et al.*, 2015). The grassland habitats in these sites are only mown once a year for hay. This contrasts starkly with the town parks where the woodlands and hedgerows are highly maintained and the grasslands are often frequently mown, though there are patches left unmown.

These environmental variables and habitats were found to produce differing responses between pollinator groups, as also noted by Maher *et al*. (2024). The abundance of bumblebees, butterflies and solitary bees was driven by the peri-urban farmland and the woodland habitats, but this was not the case for hoverflies. The diversity of all four groups was higher on the peri-urban farmland, in particular for the hoverflies, and the butterflies on the grassland habitats (Plate 2-5). Butterflies are known as reliable indicators of grassland habitats due to their dependence on them for much of their lifecycle (Harpke *et al.*, 2025). The hoverfly abundance findings differ from those of Alison *et al*. (2021) who found the opposite, but this may be attributed to their study occurring in Wales with different geographical and landscape context. The findings are in alignment with Stanley and Stout (2013) whose study was also located in Ireland.

It is well established that woodlands, hedgerows and treelines provide shelter, protection, micro-climates, nesting sites and food for a wide range of insect species. Hedgerows are thought to be important for bumblebees due to their relatively high floral resources and nesting site availability compared with other peri-urban farmland habitats (Maher *et al.*, 2024). An increase in bumblebee abundance has been noted in open areas in other studies, but Alison *et al.* (2021) found abundance was higher in woodland edge habitats. My findings corroborate this with abundance for most bumblebee species high in these woodland habitats. The community composition of bumblebees was highly influenced by three of the habitats in the peri-urban farmland, namely the hedgerows, treelines and grasslands. *B. lucorum agg.* was strongly associated with the treelines, while *B. lapidarius* was strongly associated with the grassland habitats. In a previous study, when the surrounding land consisted of organic farming it was shown to positively affect the abundance of bumblebees and butterflies, possibly due to less intensive applications of pesticides and fertilisers (Biegerl *et al.*, 2025).

The effects of eliminating fertilisers is known to improve plant species richness by reducing the tall grasses, in turn increasing the abundance and richness of butterflies (Biegerl *et al.*, 2025; Harpke *et al.*, 2025). While Biegerl *et al*. (2025) noted that an increase in the area of semi-natural grasslands and floral richness was found to increase the richness of butterflies. The grasslands on the peri-urban farmland did support a greater abundance and diversity of butterflies (Plate 2-5). This is likely influenced by the relaxation and passive restoration on the intensity of agricultural practices on these sites. The reduction in the mass of vegetation allows an increase in light and warmer conditions that allow butterfly larva to develop (Arnold *et al.*, 2025), while the edges of woodlands offer food resources for the larval stages of butterflies (Alison *et al.*, 2021). The community composition of the *Pieridae* family was driven by the hedgerows and wet grasslands on the peri-urban farmland. The *Lycaenidae* however, were strongly influenced by the treelines in the town parks.

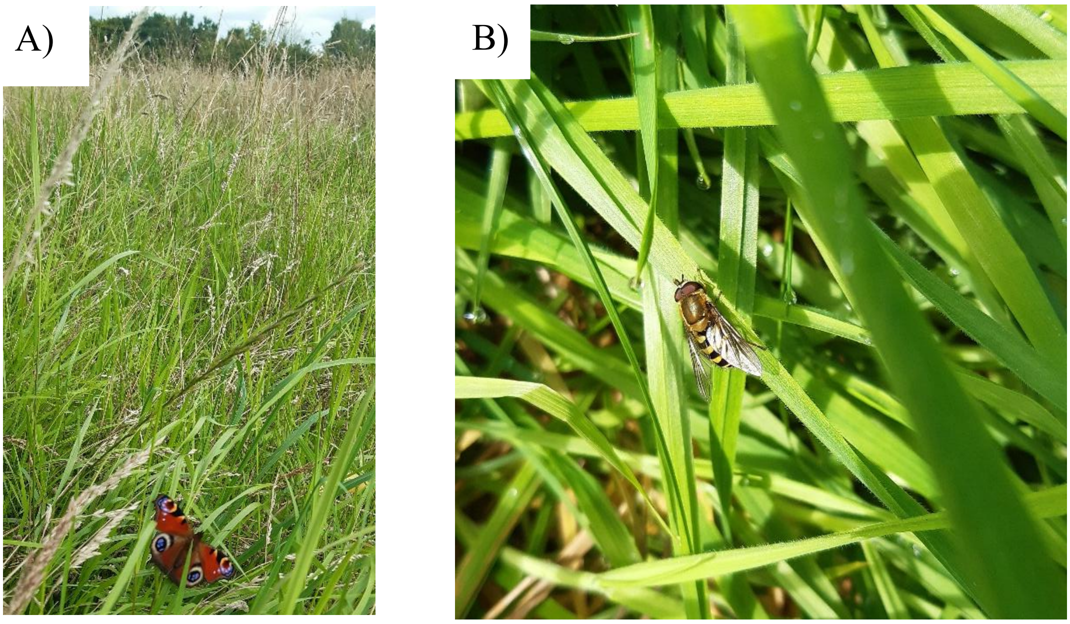


Plate 2-5 Pollinating insects in grasslands A) Peacock butterfly (*Aglais io*) and B) *Syrphus* sp.

In a recent German study, hoverflies were unaffected by any landscape variables (Biegerl *et al.*, 2025). Similar patterns were found in this study in terms of hoverfly abundance, but not richness of species. The diversity of hoverflies was lower in urban sites, potentially because of a lack of microhabitats for larval development (Grossmann *et al.*, 2022), or it could be due to human disturbance from recreation as this can be a powerful threat to hoverflies (Vujić *et al.*, 2022). Hoverfly diversity was positively correlated in the woodland and grassland habitats on the peri-urban farmland in this study. An explanation for this could be the veteran trees and the wetland habitats that were recorded on the peri-urban farmland with drainage ditches, ponds and water troughs for livestock available, providing decomposing material for hoverfly development. In other research, hoverfly richness and abundance was strongly associated with drainage ditches, a wet habitat required by saprophagous hoverfly larvae (Speight, 2024). Veteran trees, wetland habitats and sustainable agricultural practices are the three top recommendations for hoverfly conservation and restoration (Vujić *et al.*, 2022). The community composition of the hoverflies suggests the majority are generalists with three groups, the *Melanastoma, Eristalis* and *Syritta,* weakly associated with the peri-urban grasslands, the *Syrphus* and *Episyrphus* weakly associated with the town park grassland and hedgerow habitats.

The abundance of the solitary bees was strongly influenced by the peri-urban farmland and woodland habitats. These findings agree with those of Biegerl *et al*. (2025) who found that solitary bee abundance was positively correlated with smaller field size, potentially due to the existence of less intensively managed field margins. Ground-nesting solitary bees require suitable nesting sites like bare banks provided by drainage ditches, and bare earth often created by livestock (Maher *et al.*, 2024). One of the peri-urban farmlands did contain cattle and a *Halictus rubicundus* and *Andrena cineraria* nesting site was discovered on a south facing bank. This bank was being kept clear of vegetation by trampling from the cattle (Plate 2-6). The community composition of *Halictus* was strongly influenced by the peri-urban farmland grasslands and hedgerows while *Lasioglossum* positively correlated with the town parks habitats. The *Andrena* were negatively affected by the peri-urban farmland woodland and treeline habitats. There was no pattern noted for the *Megachile, Sphecodes* and *Nomada* suggesting they may be generalist species although this may be due to the low numbers recorded.

Plate 2-6 The bare bank created by cattle and used by solitary bees for nesting

To assess why the peri-urban farmland had a higher abundance and richness of pollinators, the plant species visited were considered. It may be the case that local floral resources are affecting the abundance as it was found that solitary bees were impacted in another study, potentially restricted by the distance of their short flight abilities (Maher *et al.*, 2024). An emphasis on increasing the diversity of floral resources to enhance the quality of habitats has been suggested for pollinators (Bishop *et al.*, 2025). There were four plant species that all four groups of pollinators utilised, and all four species occurred in the top five most commonly visited plants across both types of sites: *R. fruticosus* agg*., J. vulgaris, Taraxacum* spp**.,** and *H. sphondylium*. *Taraxacum* spp. are known to be an important species for the provision of pollen and nectar (Baldock, 2020). The pollinator communities responded strikingly to the peri-urban farmland possibly driven by a greater abundance of floral resources due to the lack of management of the field boundaries. There is evidence that floral richness positively impacts hoverfly abundance and richness (Biegerl *et al.*, 2025). *R. fruticosus* agg. was visited most by all pollinating groups (39%) across both types of site and was shown to be important as a floral resource in other studies (Alison *et al.*, 2021; Russo *et al.*, 2022). Some studies found that four floral species supported 81% of pollinators and another fourteen species supported almost 100% of pollinators (Griffiths-Lee *et al.*, 2022). In this study, six species provisioned 80% of species and an extra ten plants supported 97%.

The diversity of plant species visited by insects was higher on the peri-urban farmland suggesting that the provisioning of floral resources may be sustaining the greater abundance and richness of pollinators. This is corroborated by Salisbury *et al.* (2015) who found that by enhancing the plant biodiversity with native and near native species, pollinator abundance was improved, while the overall supply of floral resources improved visitations. The majority of the plant species in this study that provided floral resources were native species (Plate 2-7). Native plant species supported more bees and hoverflies than non-natives in a study specifically researching the effects of introducing non-native plant species (Salisbury *et al.*, 2015; Seitz *et al.*, 2020). Native, biodiverse, noxious ‘weeds’ are underappreciated in regards of their value to pollinators (Balfour & Ratnieks, 2022). *J. vulgaris* accommodated 16% of visitations across sites and was accessed by all four pollinating groups. Pollinator abundance is driven by floral resources, particularly for hoverflies (Salisbury *et al.*, 2015).

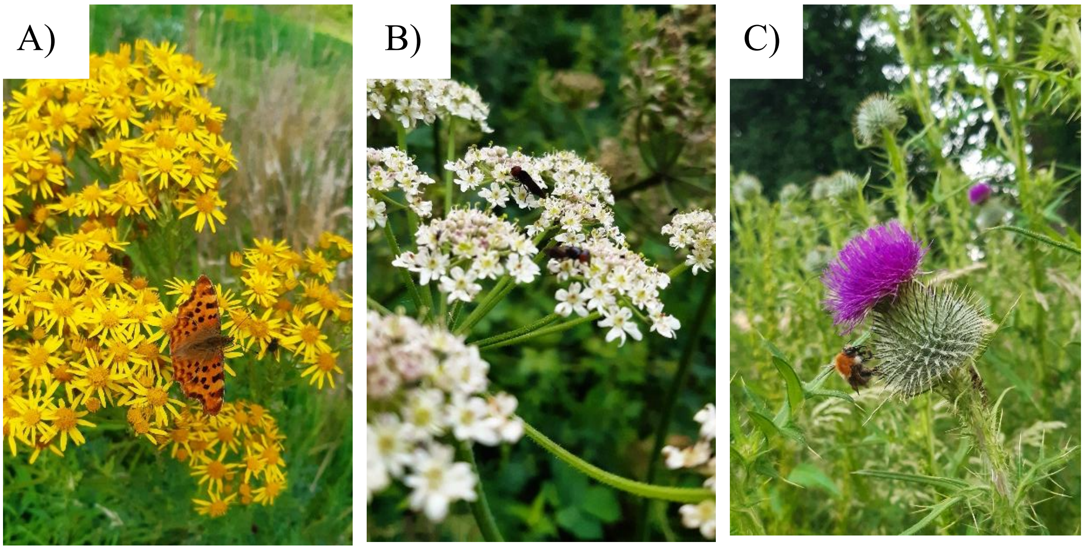


Plate 2-7 Pollinating insects foraging on native species. A) Comma butterfly (*Polygonia c-album*) on ragwort (*J. vulgaris),* B) Hoverfly sp. on hogweed (*Heracleum sphondylium*) and C) *Bombus pascorum* on thistle sp. (*Cirsium* sp.)

Interestingly, in the context of a geographical location, the location of the sites did not seem to have an effect on pollinator species or richness, these were driven more by the management in this study. In Figure 2-2, it can be seen that there are roughly three pairs of sites, each accounting for one peri-urban farmland and one town park site, that lie almost in pairs running from the north-east of the county in a south westerly direction, with an approximate distance of 36 kilometers between the two most distant sites. The number of habitats and plant species were not statistically different between sites and so did not seem to account for the difference in pollinator richness and abundance either. The management of the various habitats did, however, as did the plant species providing the floral resources. This work contributes to the field through research into low intensity farmland in the east of Ireland. Most assessments are conducted on high nature value farms in the west, but this research shows that low intensity farmland is valuable and may act as refugia for insect pollinating species.

2.6 Conclusion

Improving the conditions of habitats leads to an improvement in pollinator richness and abundance. A mosaic of habitats produces considerable benefits for biodiversity and will optimise land for pollinators. Ensuring the provision of resources for all pollinators, as well as overwintering sites, nesting habitats and host plants for larvae are important. Urban environments could be improved for pollinators through less intensive management. By reducing mowing in open public spaces, floral resources can be improved by increasing the abundance and diversity of native plant species. Invertebrates can respond within months to changes in the alteration of plants species composition and habitat management, so planting areas with floral resources would be of considerable benefit. Establishing woodlands and orchards, creating allotments, allowing existing trees to mature and inputting water features have the ability to enhance pollinator diversity in urban environments. Conservation efforts within urban environments can improve local knowledge, support an interest on biodiversity issues in conjunction with improving biodiversity, leading to a win-win situation.

Chapter 3 A comparative analysis of pollinator biodiversity on ornamental wildflower and green island roundabouts

3.1 Introduction

Numerous environmental pressures have led to a decline in insect abundance and diversity (Ryalls *et al.*, 2022). In the UK alone, it is estimated that insect numbers reduced by 58.5% between 2004 and 2021 (Ball *et al.*, 2022). In Ireland, in 2023, half of the most common bumblebees were in decline (FitzPatrick & Judge, 2024) and a strong decline in butterfly populations was found between 2008 and 2024 (-66%) (Judge & Lysaght, 2025). Although several of the primary drivers of insect decline have been discussed in previous chapters, an additional contributor to reduced biodiversity is the construction and use of roads. There are an estimated 36 million kilometres (kms) of road networks globally and their attendant road verges are estimated to cover 270,000 km^2 (Phillips *et al.*, 2020b). The road verges consist of a variety of habitats including woodlands, hedgerows and grassy verges (Phillips *et al.*, 2020b). According to the Irish Central Statistics Office there were an estimated 94,000 kms of roadways in Ireland as of 2021 (CSO, 2021). An estimated 5,306 kms are primary and secondary roads consisting of motorways, dual carriageways and single roads with 134 kms of these in County Kildare (CSO, 2024). Road verges are typically 2-5m wide, cover large areas and are set to increase on a global scale (O'Sullivan *et al.*, 2017). Road verges and related infrastructure, including roundabouts, impact insects in a negative manner, but there are examples where it can have positive effects and some of these are explored further in this chapter.

Roads are generally built with productivity and economics in mind rather than their ecological consequences, however, they affect ecosystem dynamics, function and structure and have direct impacts on the composition of a variety of insect species (Coffin, 2007). Road related infrastructure can effect insect biodiversity in a number of ways; by establishing physical barriers, traffic causing disorientation and the impact of pollutants (Coffin, 2007). Infrastructural construction leads to habitat loss and fragmentation, habitat edges, disturbance, pollution, corridor effects and barriers, altering the suitability of habitats for terrestrial animals by interfering with ecosystems (Seiler, 2001; Hopwood *et al.*, 2015; Phillips *et al.*, 2021; Meinzen *et al.*, 2024) (Figure 3-1). The length and linear characteristic of roads exacerbate ecological edge effects. Edge effects alter available resources such as nutrients and water, increase wind and light intensity, and create micro-habitats affecting the composition of invertebrates and plant species (Seiler, 2001; Coffin, 2007). Higher rainfall run-off from hard surfaces may facilitate the growth of wetland vegetation, while on the other hand, as roads heat up, the soil and vegetation will become drier, potentially impacting vegetation and ground nesting species (Meinzen *et al.*, 2024). These combined effects, in conjunction with insect traits such as feeding requirements, size, and the ability to disperse, affect survival rates (Fenoglio *et al.*, 2021).

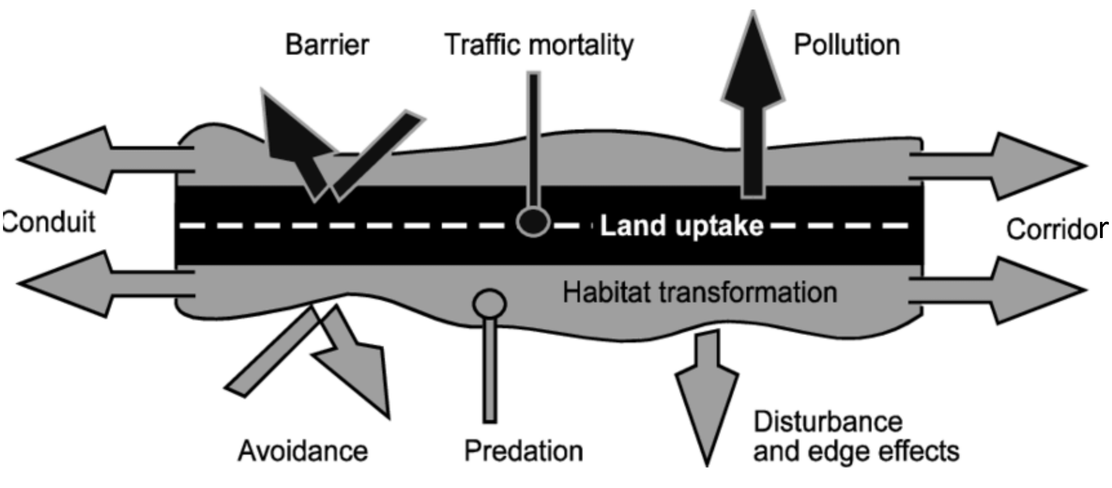


Figure 3-1 Five primary ecological effects of road infrastructure : Habitat transformation and loss, the barrier effect, traffic collisions and predation, and the corridor effect (after Seiler, 2001).

Although the barrier effects of roads can prevent the dispersion of insects, they can assist with navigation and dispersal (Lövei *et al.*, 1998; Dániel-Ferreira *et al.*, 2021).

The movement of insects depends on their flight range and the type of activity they are engaging in (Phillips *et al.*, 2020b). Some bumblebees and butterflies can fly across roads, while smaller sedentary insects with short distance flight ability can struggle. A study in New Zealand investigated the effects of road barriers on the movement of hoverflies and found that they did not disperse more than 20m from pollen resources or fly over non vegetated areas such as roads (Lövei *et al.*, 1998). Roadways have been found to create significant differences between the structure of the insect community on verges either side of a road (Phillips *et al.*, 2020b). Ultimately, these effects can lead to inbreeding and genetic isolation (Seiler, 2001).

3.1.1 The costs of road related infrastructure to biodiversity

Roads and related infrastructure change the abiotic conditions of associated habitats, and influence habitat use by insects. The soil and vegetation alongside road verges are contaminated by pollutants from traffic that include heavy metals, exhaust fumes, tyre particles and de-icing products (Coffin, 2007; Hopwood *et al.*, 2015). Turbulence and metals were avoided by insects, especially within 2m of a road (Phillips *et al.*, 2021). Pollinators seem to pause in their foraging activities due to turbulence, though this could be due to noise or vibration (Phillips *et al.*, 2021). Heavy metals in exhaust fumes increased the quantities of bumblebee brood mortality and impacted foraging by honeybees (Benner *et al.*, 2023). Dust contains heavy metals that accumulate on road side vegetation, including floral resources (Phillips *et al.*, 2021). Heavy metals are known to cause a decline in the abundance and diversity of bees and butterflies (Hopwood *et al.*, 2015; Meinzen *et al.*, 2024). Air pollution from vehicles produce volatile chemicals that include carbon monoxide, nitrogen oxides, volatile organic compounds (VOCs) and particulate matter (Coffin, 2007). Nitrogen oxides from diesel engine exhaust and ozone air pollutants are suspected to negatively impact insects by affecting floral aromas released by plants to attract pollinators (Hopwood *et al.*, 2015; Phillips *et al.*, 2020b; Ryalls *et al.*, 2022).

Mowing and herbicide use along road verges also impacts pollinators and vegetation (Meinzen *et al.*, 2024). Mowing can prove fatal to adult insects but also their egg and larval stages (Hopwood *et al.*, 2015). Butterfly mortality was high where frequent mowing occurred as they moved to find new habitats and collided with vehicles (Hopwood *et al.*, 2015). Herbicides create direct and indirect effects on insects. Direct exposure leads to a reduction in the time of development, survival rates, pupal weight and wing size while indirect exposure leads to persistent residues in flowers as insects forage, and ultimately, to a loss of floral resources (Hopwood *et al.*, 2015). The further from the road, the greater the density of pollinators with a tenth of the pollinator density found to be within a metre of the road (Phillips *et al.*, 2021).

Roadside habitats can have beneficial and detrimental effects for pollinators. There is much debate as to the costs and benefits of road verges for pollinators, with road verges often becoming ecological traps (Meinzen *et al.*, 2024). On the one hand they can provide refugia, floral resources and nesting habitats plus provide connectivity between habitats, on the other hand they can cause death by collisions with vehicles, exposure to herbicides and pollution, and act as barriers to their dispersal (Hopwood *et al.*, 2015). Infrastructure causes negative impacts to ecosystem services but the road verges may benefit some services (Phillips *et al.*, 2020a) (Figure 3-2).

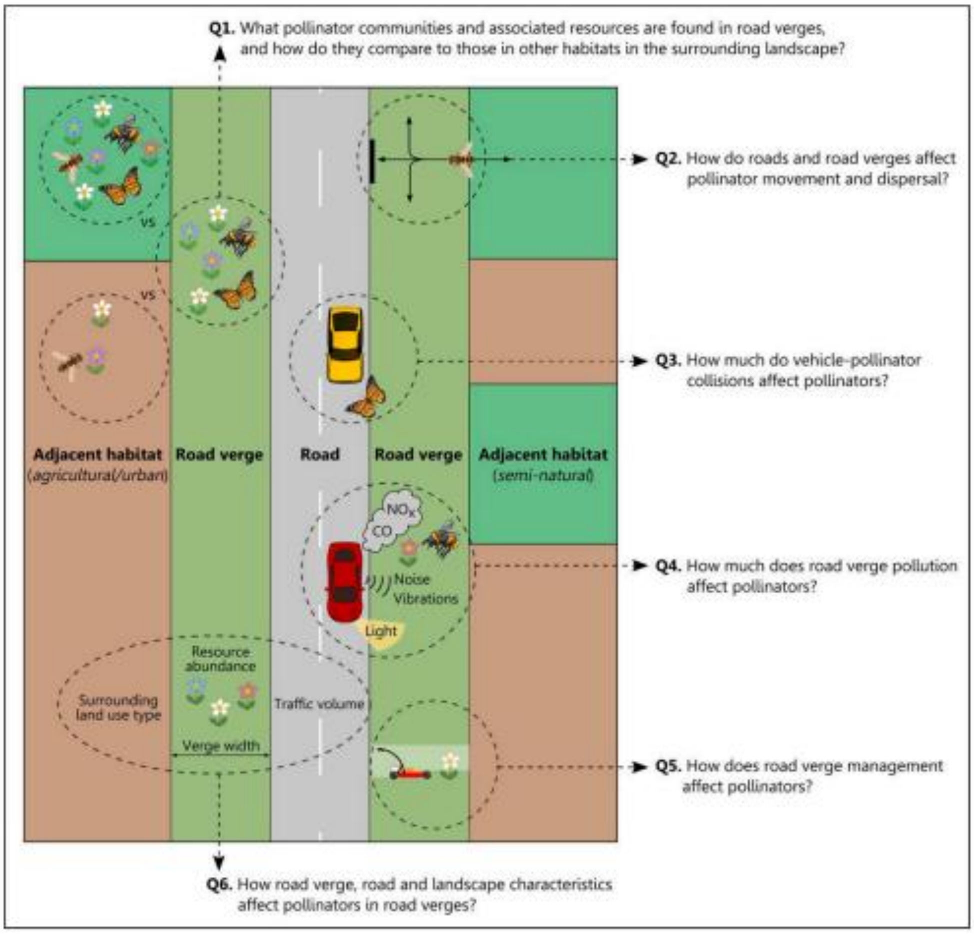


Figure 3-2 Research questions posed on the positive and negative effects of roads and road verges on pollinators addressed in a review by Phillips *et al*., (2020b)

3.1.2 The benefits of road related infrastructure to biodiversity

Although the detrimental effects of roads and urban infrastructure on biodiversity are generally self-evident (through turbulence, pollution, destruction of habitats, etc.) their importance for supporting biodiversity are becoming increasingly acknowledged. For instance, the verges along roads can vary considerably in width, in management intensity to plant and species diversity, and in plant species composition, hydrology, traffic density and surrounding landscape type (Phillips *et al.*, 2020a). These verge habitats can be utilised to enhance biodiversity, acting as stepping stones and corridors between green spaces and providing ecosystem services (O'Sullivan *et al.*, 2017). A study on a rare species of insect pollinated plant, *Fritillaria meleagris L.*, reliant on insect pollination for gene exchange, found that pollen grains were successfully transferred between small, isolated urban study sites that were used as stepping stones (Płaskonka *et al.*, 2024).

Regardless of size, the quality of a habitat has been shown to have more of an effect on butterflies than size, contradicting the species-area relationship hypothesis (Goodwin *et al.*, 2017). Road verges and roundabouts can contain significant varieties of habitat for delivering ecosystem services (Phillips *et al.*, 2020a). Trees have the ability to reduce pollution, store carbon, improve biodiversity by providing habitats for a range of species, and can reduce temperatures and soil erosion (O'Sullivan *et al.*, 2017; Phillips *et al.*, 2020a). Tree age and the type of species are important for supporting a wide range of butterfly species with tree and caterpillar abundance correlated on roundabouts, while the diversity in habitats afforded by the trees improved caterpillar species richness, possibly due to a variety of available niches (Goodwin *et al.*, 2017). Lepidopterans are important pollinators and at the same time, provide a valuable food source, in combination with caterpillars, for insectivorous birds and other animals (Goodwin *et al.*, 2017).

Other beneficial services include the removal of pollutants through filtration, thereby improving the health and well-being for local residents (Phillips *et al.*, 2020a). Pollutants on road verges and roundabouts may act as fertilisers but wildflower meadows may sequester pollutants better than grasslands, and some species like yarrow (*Achillea millefolium*), are excellent at phytoremediation (Skaldina *et al.*, 2024). Air quality improves with roadside vegetation, particularly when it contains plant species with hairy, rough and waxy leaves (O'Sullivan *et al.*, 2017). Verges can support diverse taxonomic groups and aid in their dispersal, as well as providing foraging and nesting habitats for a plethora of organisms (Seiler, 2001; Phillips *et al.*, 2020a). Usually it is the generalist species that thrive in these areas, though mortality is often high (Seiler, 2001). Fatalities were lower on roads with wider road verges and where they contained a higher diversity of plant species (Hopwood *et al.*, 2015). But it is well established that road verges can be hotspots for insects even though death from pollution and vehicle collisions occur (Phillips *et al.*, 2020b). Pollinator diversity has been found to be high with between 18% and 47% of native pollinators found on road verges (Seiler, 2001; Hopwood *et al.*, 2015). In a meta-analysis road verges had greater plant and pollinator species richness in road verges when compared to other types of habitats, except for semi-natural grasslands, and included numerous rare pollinators (Phillips *et al.*, 2020b). These habitats suit pollinators as many are highly mobile and so are able to take advantage of these areas, particularly isolated pockets like roundabouts (Phillips *et al.*, 2020b).

Road verges and roundabouts could be used for insect conservation by enhancing the habitats within them (Table 3-1). Insects have diverse requirements during their life cycles from nesting sites, larval host plants, floral resources, shelter and over-wintering habitats (Hopwood *et al.*, 2015; Phillips *et al.*, 2020b). Catering to the needs of the whole life-cycle for a species must be considered at the development stage of road construction, including water availability (Goodwin *et al.*, 2017). This could be achieved in conjunction with conserving and enhancing native plant species, as road verges are often biodiversity hotspots for native plants (Coffin, 2007; Mata *et al.*, 2023). Plant species pollinated by insects depend on the connectivity between plant populations to ensure their survival. Pollen transfer was successful between roundabouts even in instances where there was a lack of green corridors between these spaces (Płaskonka *et al.*, 2024). Allowing plants to colonise from the seed bank is cost efficient and allows local, environmentally adapted species to proliferate, simultaneously aiding local pollinators (Skaldina *et al.*, 2024). Native, locally occurring plants suited to such habitats are preferred as they require no fertilisers or pesticides, provide appropriate shelter and food requirements for native species and so can enhance local biodiversity (Hopwood *et al.*, 2015). In the US, it was found that some native plant species have extensive root systems and survive longer than non-native species and often entail less management (Campanelli, 2016). The more diverse the plant species, the more resilient the community and ecosystems will be to climate change, pests and disease (Hopwood *et al.*, 2015). Insect richness and occurrence increased after three years when a variety of native plants were grown in a small urban habitat in Australia. One year after planting, the insect species had increased by almost five times from the initial baseline survey, and after year three, this had further increased to over seven times (Mata *et al.*, 2023). In North America, a study found that native plant species doubled the quantity of bees on road verges, including ground-nesting bees (Hopwood *et al.*, 2015). Areas containing host plants for Lepidopteran specialists, however small, enable them to persist and can act as stepping stones (Goodwin *et al.*, 2017). Road verges in North America host milkweeds that are the larval hostplant for the monarch butterfly, a declining species (Phillips *et al.*, 2020b).

To enhance floral resources for pollinators and aesthetic purposes, the planting of wildflowers seed mixes has become popular, but the use of these mixes is controversial. Of twelve brands of wildflower seed mixes assessed, only 43% of the species were native to Ireland (Barry & Hodge, 2023). Another study found that 78% were considered native but worryingly 21% were from outside the European region (Smyth, 2023). The planting of exotic species may introduce competitive species and disrupt local ecological networks (Barry & Hodge, 2023). A better alternative may be to encourage native species. Grass cutting can improve floral resources when consideration is given to the local pollinator requirements. Mowing initially removes floral resources; however, the management of roadsides can be enhanced through strategic mowing to improve the diversity of plants. A variety of sward heights is important for pollinators creating micro-habitats, but often mowing creates a uniform habitat with a single height of vegetation (Hopwood *et al.*, 2015). To improve the communities of plants and invertebrates by mowing, removing the cuttings is essential (Phillips *et al.*, 2020a) to ensure nutrient enrichment does not occur and prevent nutrient loving species from proliferating. Costs can be saved through reduced mowing on these sites, whilst simultaneously enhancing biodiversity, reducing noise and enhancing aesthetics, thereby reducing stress in drivers (O'Sullivan *et al.*, 2017).

Table 3-1 General restoration goals to meet pollinator habitat requirements (after Hopwood *et al.*, 2015).

Pollinators	**Food**	**Shelter**	**Restoration goals**
Bees Bumblebees	Nectar for adults; nectar and pollen collected	Nest in small cavities, underground in abandoned rodent nests, under clumps of grass, or in hollow trees, birds' nests or walls	Increase density and diversity of native flowering plants. Provide native bunch grasses for bumble bee nesting habitat.
Ground-nesting	Nectar for adults; nectar and pollen collected	Nest in bare or partially vegetated, well-drained soil	Provide areas with partially vegetated well-drained soil.
Tunnel-nesting	Nectar for adults; nectar and pollen collected	Nest in narrow tunnels in dead standing trees or excavate nests in pith of stems and twigs. Some construct domed nests of mud, plant resins, saps, or gums on the surface of rocks or trees	Provide living and dead woody vegetation
Butterflies Caterpillar	Leaves of larval host plants	Host plants	

Pollinators	Food	Shelter	Restoration goals
Adult	Nectar: some males obtain nutrients, minerals, and salt from rotting fruit, tree sap, animal dung and urine, carrion, clay deposits, and mud puddles	Protected site such as a tree, bush, tall grass, or a pile of leaves, sticks or rocks	Increase density and diversity of native flowering plants. Include host plants. Provide refuge from burning and grazing during dormant
Hoverflies	Nectar and sometimes pollen as adults; insect prey such as aphids, scales, mites, thrips	Larvae found on plants near prey. Pupae and adults overwinter in soil and leaf litter	Increase density and diversity of native flowering plants. Provide refuge from burning and grazing during dormant season and early spring

3.1.3 Aims

The aim of this chapter is to assess the impact of different roundabout management approaches on four representative pollinator groups (bumblebees, butterflies, hoverflies and solitary bees). The two different management approaches can be classified as either ornamental wildflower or green island roundabouts (Plate 3-1). The green island roundabouts were highly maintained while the ornamental wildflower roundabouts were mown once a year in early autumn. The following hypotheses were tested in this chapter:

1) Insect pollinator abundance and species richness varies between green island and ornamental wildflower roundabouts.
2) Pollinating insect community composition differs between the green island and ornamental wildflower roundabouts.
3) Plant species and floral diversity drives the differences in insect pollinators between the green island and ornamental wildflower roundabouts.

Understanding these relationships is key to conserving and enhancing biodiversity on open public spaces.

Plate 3-1 The roundabouts A) Blackchurch 2, one of the Ornamental wildflower roundabouts and B) Greenhills, one of the Green island roundabouts

3.2 Methods

3.2.1 Study Region and sampling habitats.

The study region was located across six roundabouts northeast of Naas, County Kildare, Ireland, with three roundabouts lying to the north and three to the south of the N7 dual carriageway (Figure 3-3). All six roundabouts lay within a distance of one kilometre either side of the N7. They were predicted to differ in pollinator communities according to their current management regimes. Initially, in 2021, nine roundabouts were surveyed to find sites to replicate in County Kildare. Six roundabouts were selected; three roundabouts were categorized as green island and three as ornamental wildflower. The three green island roundabouts were highly maintained in comparison to the ornamental wildflower roundabouts that were maintained annually with a single autumn cut (Plate 3-2). The study sites ranged from

0.02 to 0.2 hectares in size (Table 3-2). The green island roundabouts consisted of occasional trees, shrubs, cobble stones and frequently mown grassland with short radiating rows of shrub and/or flower borders consisting of a single species. In the final year of surveys, areas of the grassland had been allowed to grow, and pyramidal orchids were recorded on one roundabout. The ornamental wildflower roundabouts contained wildflowers from a seed mix that were sown in 2014 and one of the roundabouts featured a non-native shrub at its centre (Plate 3-2). These roundabouts were cut once a year in the autumn. Initial habitat surveys were conducted during August 2021 on all sites. Plants were recorded to species level during walkover and pollinator surveys over the following two summers from April through to August inclusive. Poacaea were recorded to family level and have been excluded from further analysis. Due to the difficulty of the identification of *Taraxacum* and *Rumex* to species level, these were only identified to genus level. Further vegetation surveys were conducted using a 2m x 2m quadrat around all pollinator pan traps on all roundabouts from April through to August during 2022 and 2023. Flora were recorded using the Braun-Blanquet method.

The Irish National Landcover Map (EPA, 2025) was accessed to gather data on the land use within a two kilometre boundary of each roundabout. The relative abundance of each of the four habitat types- developed, grassland, tree/wooded and water surfaces/bodies was calculated (Table 3-3). There was no significant difference between the relative percentage cover of any of the four surrounding land cover types between either the ornamental wildflower or green island roundabout sites.

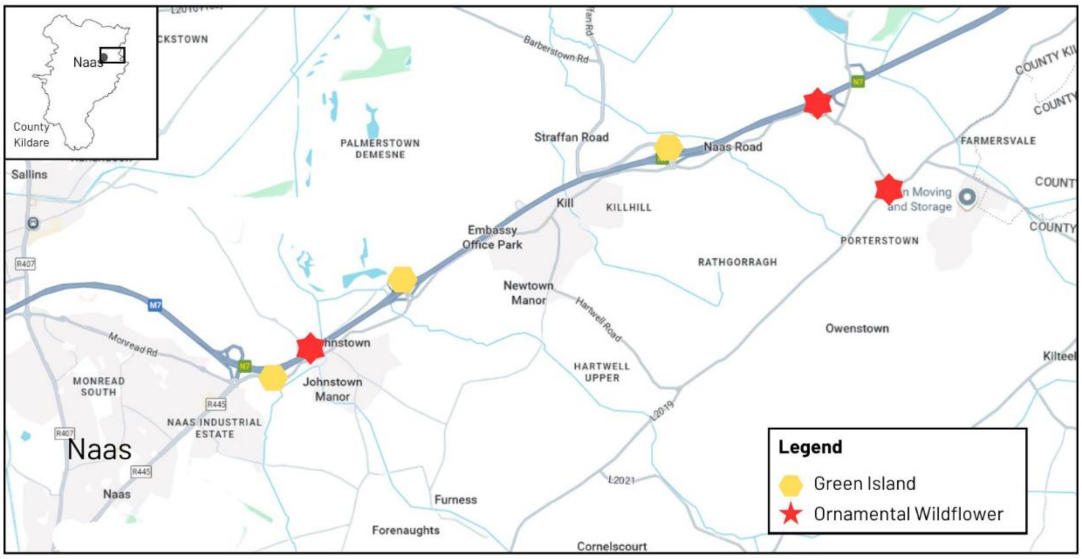


Figure 3-3 The distribution of the six study sites in County Kildare : three Green island roundabouts and three Ornamental wildflower roundabouts.

Table 3-2 The six sites surveyed- Green island or Ornamental wildflower with site type, the name, the area (hectares) and GPS location of the six sites surveyed.

Site Type	Site Name	GPS Location	Area (hectares)	Overall Area
Green island	Greenhills	53.242117, -6.613629	0.17	0.55
	Kill	53.253275, -6.576078	0.2	
	Naas to Johnstown	53.234258, -6.631246	0.18	
Ornamental wildflower	Blackchurch/ Palmerstown	53.256702, -6.555221	0.04	0.09
	Blackchurch 2	53.249847, -6.545029	0.03	
	Palmerstown	53.236685, -6.626289	0.02	

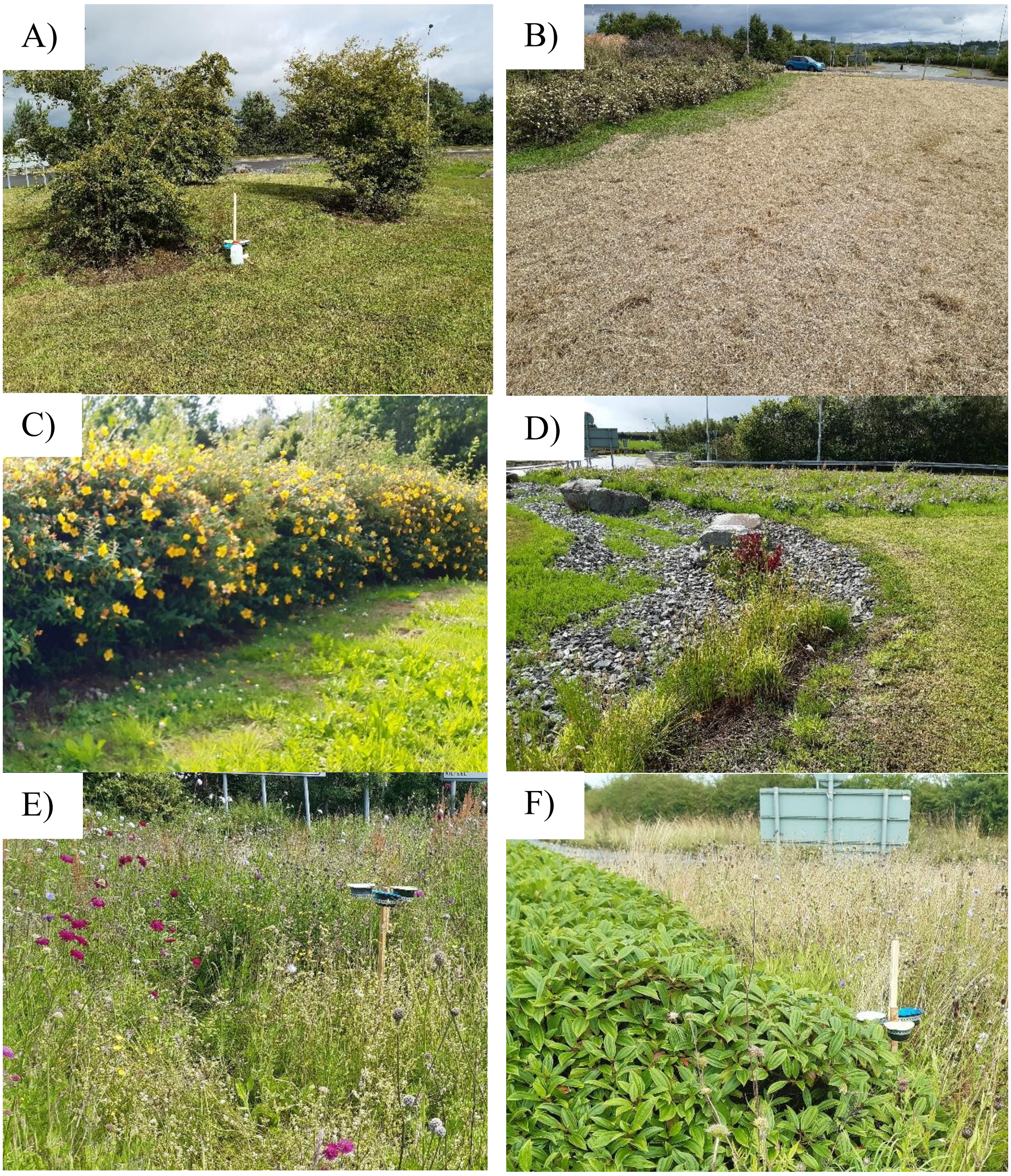


Plate 3-2 Examples of the roundabouts. A), B) and C) Shrubs and frequently mown grassland on green island roundabouts. D) Areas of cobble and flower beds with frequently mown grassland on green island roundabouts. E) An ornamental wildflower roundabout and F) the only ornamental wildflower roundabout with a single shrub in its centre.

Table 3-3 The relative abundance, mean and standard error (SE) of Level 1 National Land Cover Classification System of the landscape within a 2 kilometre buffer of each of the six roundabouts.

Site Type and Site Name	Artificial surfaces %	Grassland %	Woodland or scrub %	Waterbodies %
Green island				
Greenhills	15.78	65.58	17.37	1.28
Kill	13.72	71.48	14.61	0.19
Naas to Johnstown	22.81	60.93	15.35	0.91
Mean and SE	**17.44 ± 2.75**	**66 ± 3.05**	**15.78 ± 0.82**	**0.79 ± 0.32**
Ornamental wildflower				
Blackchurch/ Palmerstown	11.33	72.29	16.20	0.18
Blackchurch 2	9.76	71.00	19.10	0.14
Palmerstown	20.24	62.47	16.28	1.01
Mean and SE	**13.78 ± 3.26**	**68.59 ± 3.08**	**17.19 ± 0.95**	**0.44 ±0.28**

3.2.2 Pollinator sampling

I recorded insects over two consecutive summers from April to September inclusive, during 2022 and 2023. Surveys were undertaken between 10.00 and 16.00, when temperatures were above 13°C on dry days with wind speeds at four or less on the Beaufort scale. It has been recommended that multiple sampling methods are used to assess insect pollinators (Griffiths-Lee *et al.*, 2023). Transects are the best method to detect the species abundance of insects while pan traps are better for collecting a broader diversity of bees. The small fast flying bees species are more likely to be detected in pan traps than transects where they may be missed (Potts *et al.*, 2024). Pollinator monitoring was conducted using pan trap and transects surveys, and combining these two approaches permitted the gathering of information on pollinator diversity and abundance in addition to floral visitations. Pan trap and transect locations were fixed and were sampled on all visits.

Pan trap surveys were used as they allow for sampling at multiple locations and identification to species level under laboratory conditions (Stanley & Stout, 2013).

Pan traps were in place by 10am and removed after 4pm. Each pan trap consisted of three plastic bowls painted with UV paint in either blue, yellow or white which were fastened to a post at the height of the surrounding vegetation. Water and a drop of odourless Ecover washing up liquid was added to each bowl. One pan trap was erected in or near the centre of each roundabout. Bees and hoverflies were identified to species level (using Ball and Morris 2015 and Falk 2016), and all other species were identified to family level. All other species have been omitted from further analysis including honeybees as these are often managed honeybees brought into areas for honey production.

Transects were undertaken mid-afternoon between 1.30pm and 4pm as pollinators were shown to peak at this time during surveys in nearby County Carlow, Ireland (Karbassioon & Stanley, 2023). Bumblebees are best monitored via transects (Griffiths-Lee *et al.*, 2023). Transect surveys were conducted one metre in from the perimeter of each roundabout. Transects were walked at a slow and steady pace with pollinators recorded, and their activity, within an imaginary one metre square box in front and to either side of me. Insects were identified to three groups: bees (*Apidae*), butterflies (*Lepidoptera*) and hoverflies (*Diptera: Syrphidae*), to species level where possible in the field. The white butterflies, (*P. napi, P. rapae* and *P. brassicae*) were recorded to genus level as were the worker bumblebees unidentifiable without DNA analysis, specifically- *B. terrestris, B. cryptarum, B. lucorum* and *B. magnus* (Carolan *et al.*, 2012). All species were classified on their status within the European Red lists. Where identification in the field was not possible, a net was used to capture insects which were then retained for identification using Ball and Morris 2015 and Falk 2016. Where I was unable to identify species I used the pollinator reference collection at the National Museum of Ireland's Storage facility and species were verified by entomological experts (see Acknowledgements). Taxa not identified to species level were not used in species richness analysis.

3.2.3 Data Analysis and model selection

Statistical analysis and figures were conducted in R (RStudio Version: 2025.05.1). Response variables, abundance and richness, were pooled at the site level across sampling periods. Transect and pan trap data abundance were analysed separately to account for the different sampling methods. Insect richness analysis was conducted on amalgamated transect and pan trap data on pooled data by site, sampling period and years combined.

To examine the effect of the type of site on pollinator abundance within the four groups: bumblebees (BB), butterflies (BF), hoverflies (HF) and solitary bees (SB) separate models were created. Response variables were insect abundance and richness, site type was used as a fixed effect, while year and month were included as random effects to account for temporal independence. All models were simplified removing interactions that were not significant. To assess species diversity by the type of site the Shannon and Simpson Diversity was calculated for each group using the vegan package. To assess the differences in indices the 95% confidence intervals with non-parametric bootstrap with replacement (1000 times) was calculated. To examine the effect of floral richness recorded during quadrat surveys on pollinator richness the type of roundabout was used as a fixed effect using a negative binomial GLM model in the vegan package.

QQ plots, histograms of the residuals and the DHARMa package were implemented to validate the models. Generalised Linear Mixed Models (GLMMs) with a negative binomial distribution were used to test abundance, as the data were overdispersed and did not follow a Normal or a Poisson distribution. Poisson log transformed models were used to test richness. Where p values were significant the t.test/Tukey's post hoc tests were used in the multcomp package. To identify the potential drivers of the differences between community composition of each group, the type of site was analysed using Principal Component Analysis (PCA) to allow the identification of the key axes of variation. Prior to analysis, the area variables were transformed using a Hellinger transformation for normality.

3.3 Results

Sixty-eight pollinators were collected in pan traps: 26 bumblebees (four species), 16 hoverflies (eight species) and 26 solitary bees (six species) (Table 3-4). Four-hundred and forty-three individual pollinators were recorded during transect surveys and represented 372 bumblebees (seven species), six butterflies (four species), 63 hoverflies (ten species) and two solitary bees (one species) (Table 3-4). Overall, 31 species were recorded, see Appendix Table B- 1.

Table 3-4 Total abundance and mean abundance of individual insects (± SE) of each pollinator group in pan traps and transects by the type of site, green island or ornamental wildflower roundabout.

	Pan Traps		**Transects**	
Green island	Total abundance	Mean abundance/site	Total abundance	Mean abundance/site
Bumblebee	7	2.33 ± 0.88	56	18.67 ± 6.84
Butterfly	0	0	4	0.67 ± 0.67
Hoverfly	2	0.67 ± 0	22	7.33 ± 4.1
Solitary bee	11	3.67 ± 1.45	15	5 ± 1.15
Ornamental wildflower				
Bumblebee	19	6.33 ± 2.85	319	106.33 ± 50.63
Butterfly	0	0	2	1.33 ± 0.33
Hoverfly	14	4.67 ± 1.86	94	31.33 ± 16.75
Solitary bee	15	5 ± 2.08	7	2.33 ± 1.2

3.3.1 Pollinator abundance at the site scale

When comparing green island roundabouts to ornamental wildflower roundabouts using the pan trap data (Plate 3-3), there were too few observations to conduct statistical analysis on the hoverflies and butterflies. The abundance of bumblebees and solitary bees, though not significant, was marginally higher on the ornamental wildflower roundabouts (Table 3-5) From the transect data (Plate 3-3), the ornamental wildflower roundabouts had significantly more bumblebees and hoverflies, while there were more butterflies and solitary bees on ornamental wildflower roundabouts, though not significantly (Table 3-5).

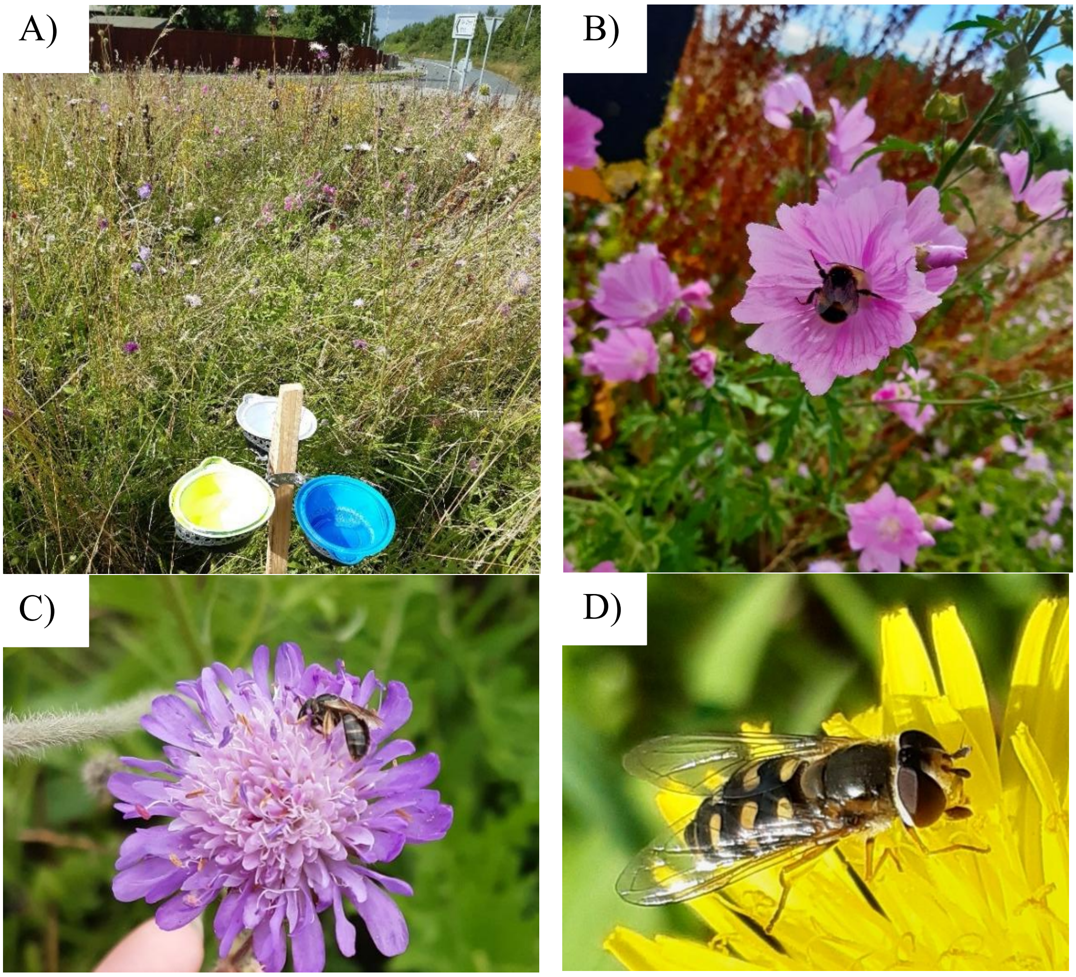


Plate 3-3 Pan trap surveys and transects on the roundabouts A) A pan trap on an ornamental wildflower roundabout. Insect pollinators noted during transects. B) A bumblebee, *Bombus* sp., foraging on a common mallow (*Malva sylvestris*) flower. C) A solitary bee foraging on a field scabious (*Knautia arvensis*) flower. D) A hoverfly, *Eupeodes* sp., foraging on a dandelion sp. (*Taraxacum* sp.).

Table 3-5 Differences in insect abundance on ornamental and wildflower roundabouts Estimates, standard errors (SE), t-statistic and p-value of the fixed effects included in the best-fitting model explaining the variation in pollinator abundance, in pan traps (bumblebees and solitary bees only) and transects (all pollinating taxa). Significant results are in bold.

Response	Predictors	Estimate	SE	t-statistic	p-value
Pan trap abundance					
Bumblebee	Site Type	-0.999	0.547	- 1.854	0.0681
Solitary bee	Site Type	-0.310	0.512	-0.606	0.5443
Transect abundance					
Bumblebee	Site Type	-1.740	0.598	-2.909	**0.0036**
Butterfly	Site Type	0.405	1.190	0.340	0.7340
Hoverfly	Site Type	-1.452	0.627	- 2.317	**0.0205**
Solitary bee	Site Type	0.357	0.794	0.449	0.6533

3.3.2 Pollinator species richness at the site scale

Overall, there were more species found on the ornamental wildflower roundabouts than on the green island roundabouts (Table 3-6 and Figure 3-4) though richness was not significant (p=0.29). The Simpson Diversity indices differed significantly for the hoverflies (p=0.05) only, see Appendix Table B- 2. There were three dominant species of hoverfly on the green island roundabouts whereas species were more evenly represented on the ornamental wildflower roundabouts (Table 3-7).

Table 3-6 Total pollinator richness in pan traps and transects pooled, mean species richness, the mean Shannon Diversity, ± SE, and the mean Simpson Diversity, ± SE, across green island and ornamental wildflower roundabouts.

	Total species richness	Mean species richness	Mean Shannon Diversity	Mean Simpson Diversity
Green island				
Bumblebee	5	3.67 ± 0.67	0.96 ± 0.27	0.52 ± 0.14
Butterfly	2	2 ± NA	0.69 ± NA	0.5 ± NA
Hoverfly	3	1.5 ± 0.58	0.23 ± 0.23	0.14 ± 0.14
Solitary bee	5	2.67 ± 0.88	0.79 ± 0.40	0.45 ± 0.23
Ornamental wildflower				
Bumblebee	6	5.33 ± 0.67	1.24 ± 0.14	0.62 ± 0.06
Butterfly	3	1.33 ± 0.33	0.23 ± 0.23	0.17 ± 0.17
Hoverfly	12	6.67 ± 2.91	1.57 ± 0.48	0.73 ± 0.12
Solitary bee	4	1.67 ± 0.33	0.37 ± 0.2	0.25 ± 0.14

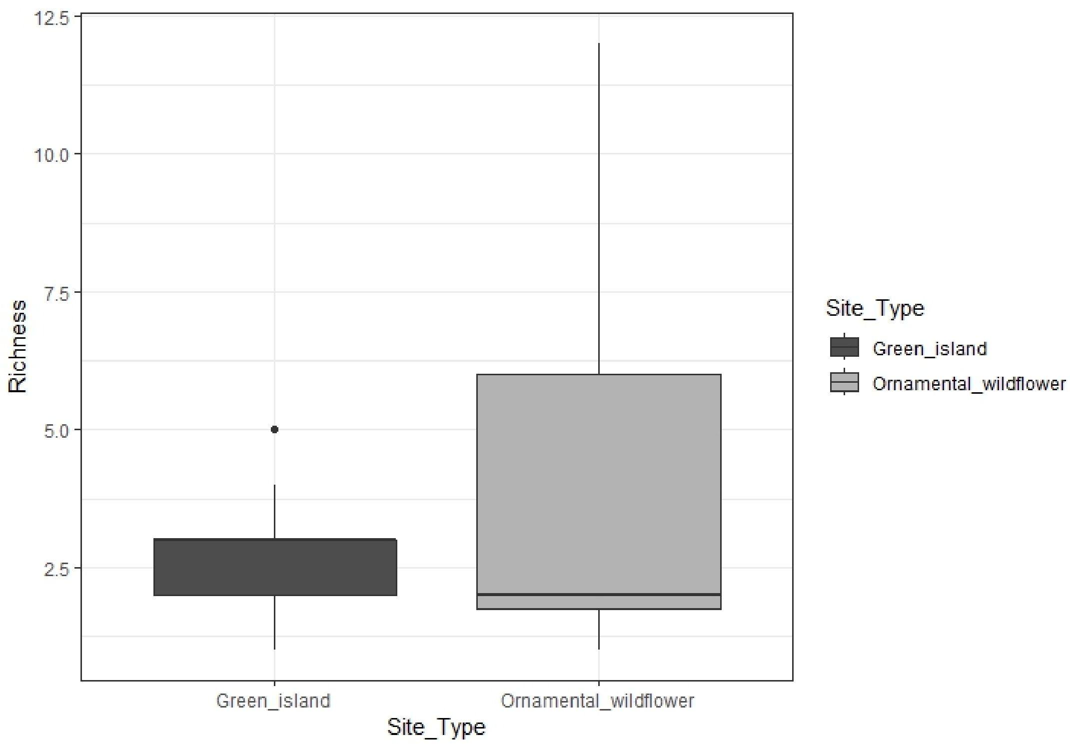


Figure 3-4 Species richness of all pollinator species on green island and ornamental wildflower roundabouts . Each data point used to create the boxplot is the mean pollinator richness measured at all six sites, three per type of site, over all twelve surveys. The solid bars represent the median, and the boxes represent the upper and lower quartiles. The whiskers represent the maximum or minimum value of the data.

Table 3-7 Estimates, standard errors (SE), t-statistic and p-value of the fixed effects included in the best-fitting model explaining the variation in pollinator richness in pan traps and transects on roundabouts. Significant results are in bold.

Pan trap and transect richness					
Response	**Predictor**	**Estimate**	**SE**	**t-statistic**	**p-value**
Bumblebee	Site Type	-0.460	0.369	- 1.246	0.2127
Butterfly	Site Type	0.405	0.866	0.468	0.6397
Hoverfly	Site Type	-1.492	0.619	-2.409	**0.0160**
Solitary bee	Site Type	0.470	0.570	0.824	0.4097

3.3.3 Community Composition

The bumblebee biplot (Figure 3-5) shows the first two principal components which together account for 81% of the variance in the data. The two types of roundabout cluster separately. *B. lapidarius* are strongly associated with the ornamental wildflower roundabouts while *B. terrestris*, *B. muscorum and B. pratorum* are weakly associated those roundabouts. The green island roundabouts influence *B. hortorum* and *B. pascuorum.* The bumblebee community associated with the ornamental wildflower roundabouts are more diverse than the green island roundabouts.

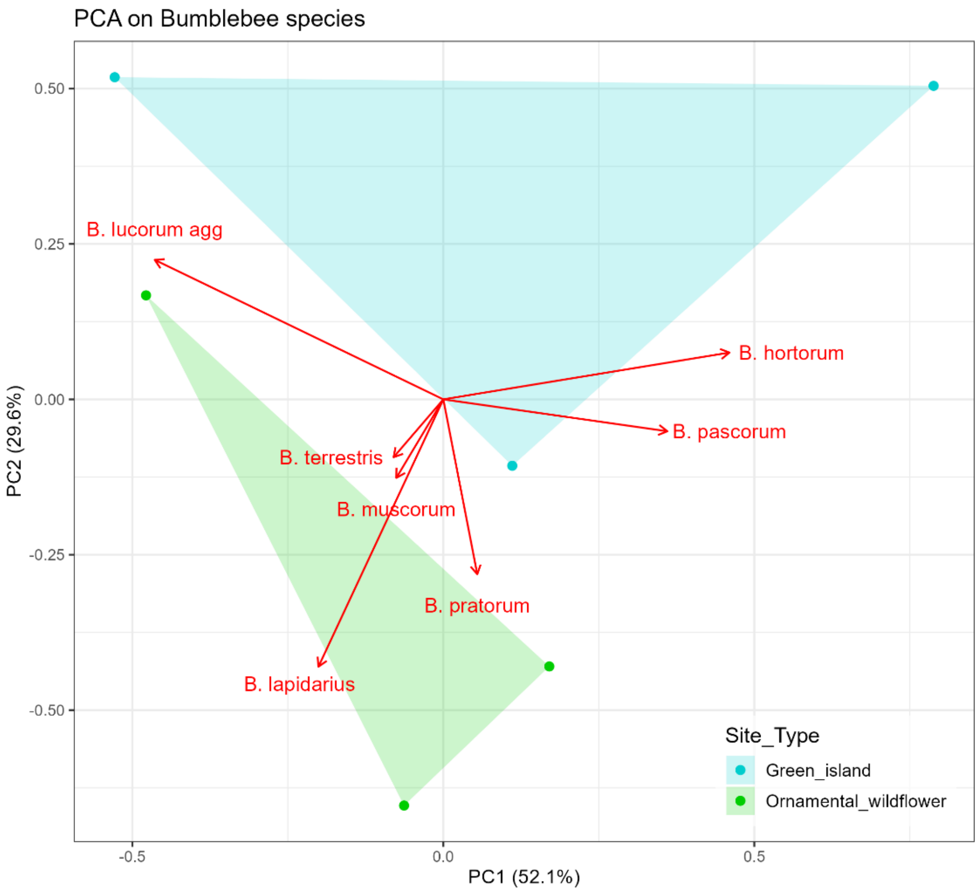


Figure 3-5 Principal Components Analysis (PCA) plots for bumblebees showing the relationship between the bumblebees and the green island and ornamental wildflower roundabouts.

The butterfly biplot (Figure 3-6) shows the first two principal components which together account for 89% of the variance in the data. The butterfly communities cluster separately on the green island and ornamental wildflower roundabouts. Three species of butterfly were associated with the ornamental wildflower roundabouts- namely *Polyommatus icarus, Aglais urticae* and *P. brassica*. On the other hand, *Vanessa atalanta* is associated with the green island roundabouts. The butterfly community associated with the ornamental wildflower roundabouts are more diverse than the green island roundabouts.

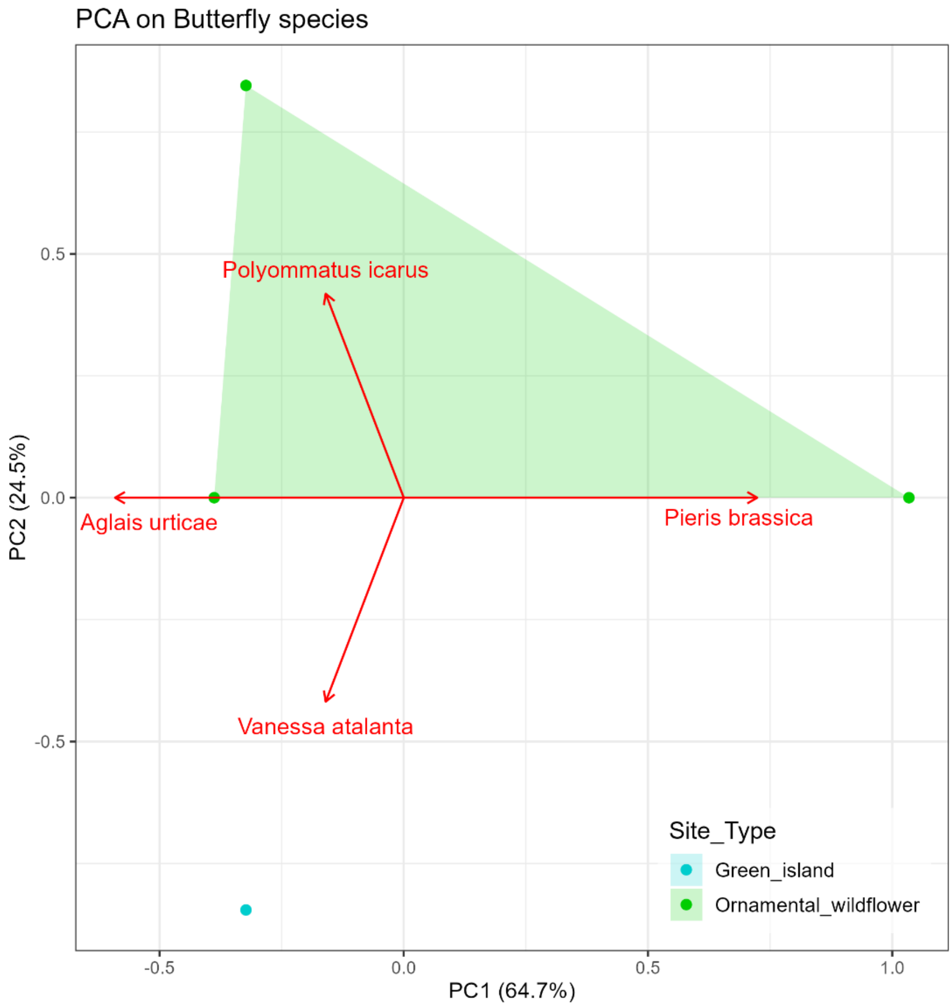


Figure 3-6 Principal Components Analysis (PCA) plots for butterfly species showing the relationship between butterfly species and green island and ornamental wildflower roundabouts. Only one butterfly was recorded on a green island roundabout, *Vanessa atalanta*.

The hoverfly biplot (Figure 3-7) shows the first two principal components which together account for 78% of the variance in the data. The hoverfly communities cluster separately between the green island and ornamental wildflower roundabouts. The majority of hoverflies were generalists and were very weakly associated with the ornamental wildflower roundabouts. On the other hand, the *Sphaerophoria* and *Orthonovera* species were strongly associated with two of the green island roundabouts. The hoverfly community associated with the ornamental wildflower roundabouts were more diverse than the green island roundabouts.

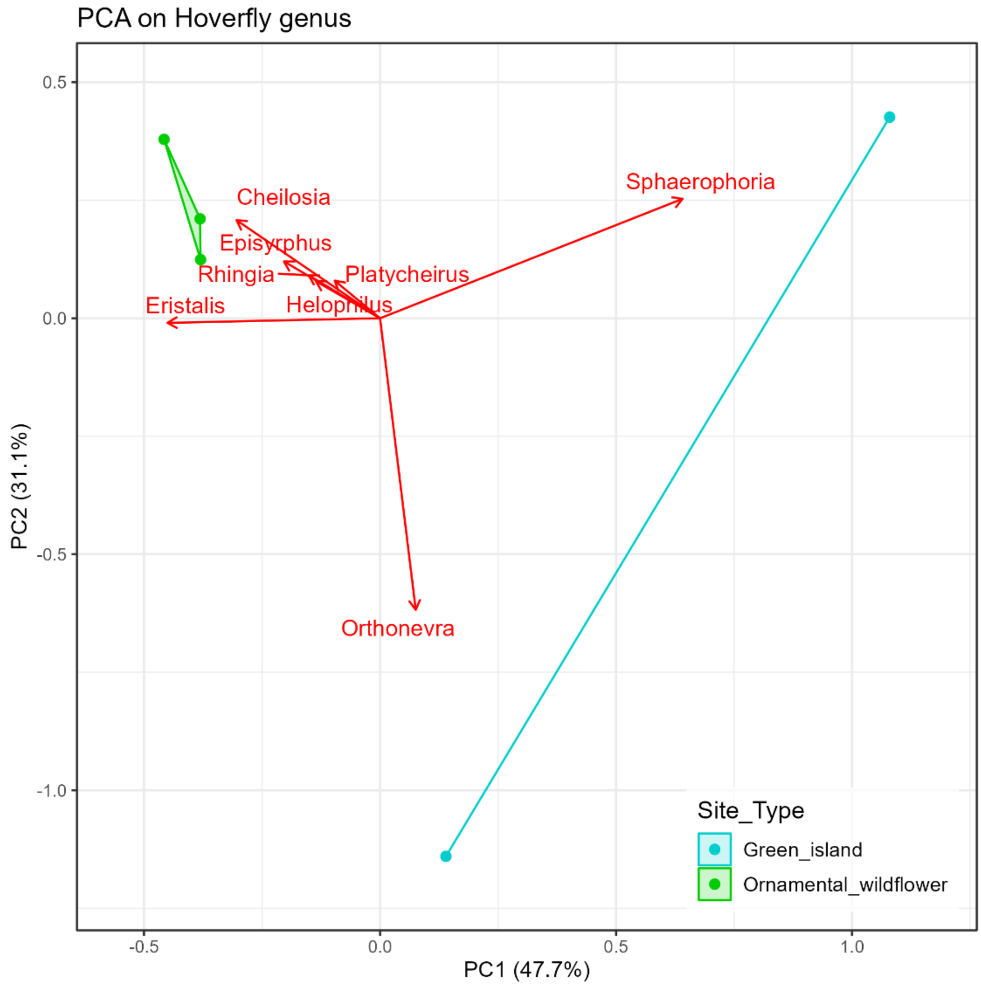


Figure 3-7 Principal Components Analysis (PCA) plots for hoverfly species showing the relationship between the hoverflies and green island and ornamental wildflower roundabouts.

The solitary bee biplot (Figure 3-8) shows the first two principal components which together account for 73% of the variance in the data. The solitary bee communities cluster separately on the green island and ornamental wildflower roundabouts. The green island roundabouts influence the *Lasioglossum albipes* while the ornamental wildflower roundabouts influence two species of solitary bee: *Osmia bicornis* and *Andrena haemorrhoa. Lasioglossum leucopus* was associated with one roundabout of each type. *Andrena praecox* and *Halictus tumulorum* seem to be generalists with no strong preference for either type of roundabout. The solitary bee community associated with the ornamental wildflower roundabouts are slightly more diverse than the green island roundabouts.

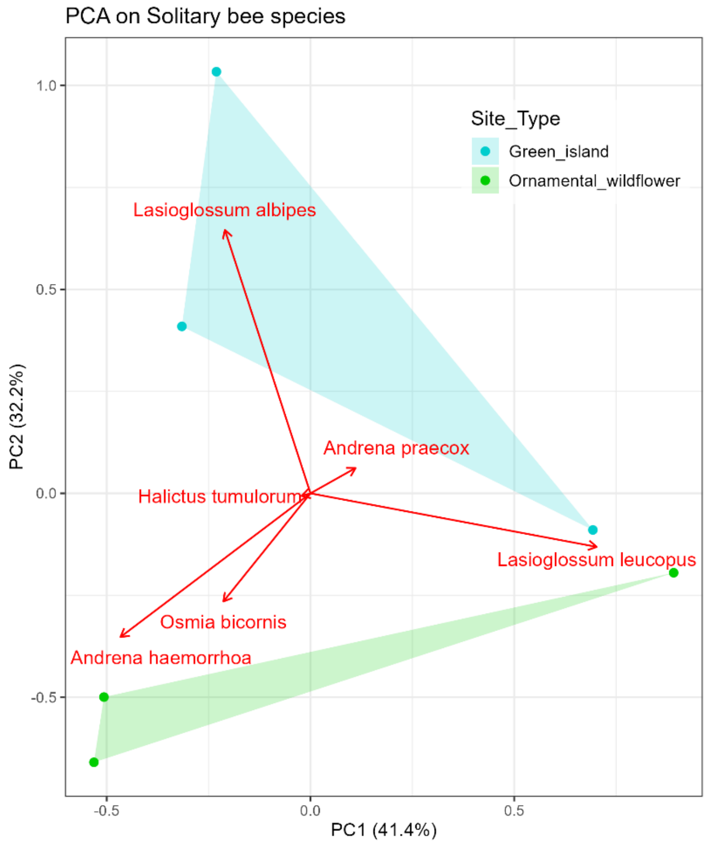


Figure 3-8 Principal Components Analysis (PCA) plots for solitary bee species showing the relationship between the solitary bees and the green island and ornamental wildflower roundabouts.

3.3.4 Plant species richness, floral visitations and floral richness.

More plant species were recorded on ornamental wildflower roundabouts, but this difference was not significant (p=0.07) (Figure 3-9). There was no significance difference either for the Shannon Diversity (p=0.08) or Simpson Diversity (p=0.08) (Table 3-8). For a full species list of plants found on the sites see (Appendix Table B-3). During transects 453 floral visitations were observed on 21 varieties of plants, 356 by bumblebees, one by butterflies, 74 by hoverflies and 22 by solitary bees (Appendix Table B- 4). *Centaurea nigra* was the most frequently visited (191 visits), followed by *Knautia arvensis* (101 visits) and *Taraxacum* spp**.** (34 visits). Overall, there were eight varieties of plants that were visited by bumblebees on green island roundabouts and 13 on ornamental wildflower roundabouts. *C. nigra* were most visited by bumblebees, 56%, on ornamental wildflower roundabouts with *Hebe* sp. accounting for 60% of visits on green island roundabouts (Table 3-9). A single butterfly foraged on *Buddleja davidii* on a green island roundabout. *Taraxacum* spp. received 100% of the solitary bee visitations on green island roundabouts and 71% on ornamental wildflower roundabouts, with *K. arvensis* supporting the other 29%. The hoverflies visited 13 plant varieties on both sites, 43% on *Ranunculus acris* on green island roundabouts and 33% on *C. nigra* on ornamental wildflower roundabouts. *Taraxacum* spp**.** was the single plant species visited by all pollinating groups barring butterflies. *R. acris* and *T. repens* were the only species visited by both bumblebees and hoverflies on green island and ornamental wildflower roundabouts. Using the quadrat data the floral richness had a highly significant positive effect on pollinator richness (p=0.003) with the ornamental wildflower roundabouts impacting the richness of pollinators significantly, as seen in Figure 3-10.

Table 3-8 Plant richness across green island and ornamental wildflower roundabouts with the mean species richness, the mean Shannon Diversity, ± SE, and the mean Simpson Diversity, ± SE.

Site Type	Total species richness (mean)	Mean Shannon Diversity	Mean Simpson Diversity
Green island	44 (20)	2.97 ± 0.04	0.95 ± 0.001
Ornamental wildflower	46 (27)	3.21 ± 0.09	0.96 ± 0.004

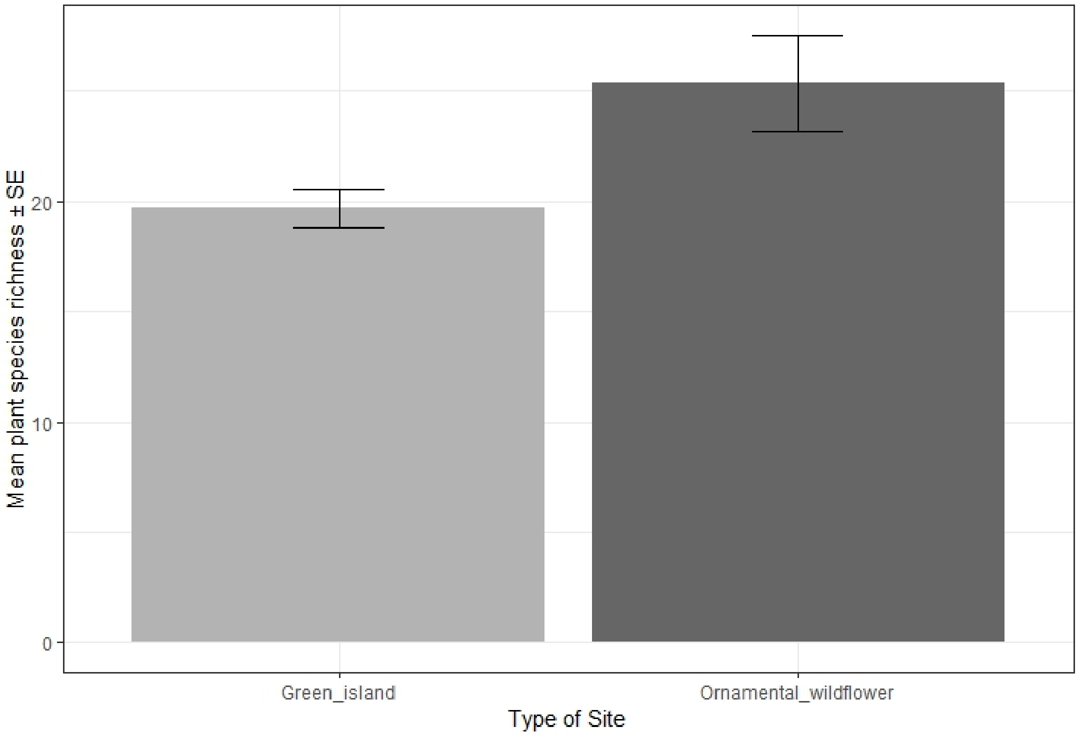


Figure 3-9 Mean number of all plant species on green island and ornamental wildflower roundabouts . Each data point used to create the boxplot is the mean plant diversity measured at all six sites, three per type of site, over all twelve surveys. The solid bars represent the median, and the boxes represent the upper and lower quartiles. The whiskers represent the maximum or minimum value of the data.

Table 3-9 Total pollinator visits by percentage over the two year study to the top five most commonly visited plant species for each of the four groups on the green island and ornamental wildflower roundabouts.

Bumblebee			**Hoverfly**		
Plant species	**Green island %**	**Ornamental wildflower %**	**Plant species**	**Green island %**	**Ornamental wildflower %**
Hebe sp.	60		*Ranunculus acris*	43	12
Hosta sp.	13		*Taraxacum* spp.	29	10
Potentilla fruticosa	11		*Potentilla fruticosa*	14	
Taraxacum spp.	6		*Primula veris*	7	
Trifolium repens	4	1	*Hebe sp.*	7	
Centaurea nigra		56	*Centaurea nigra*		33
Knautia arvensis		27	*Knautia arvensis*		28
Vicia sepium		6	*Malva sylvestris*		5
Malva sylvestris		6			
Butterfly			**Solitary bee**		
Buddleja davidii	100		*Knautia arvensis*		29
			Taraxacum spp.	100	71

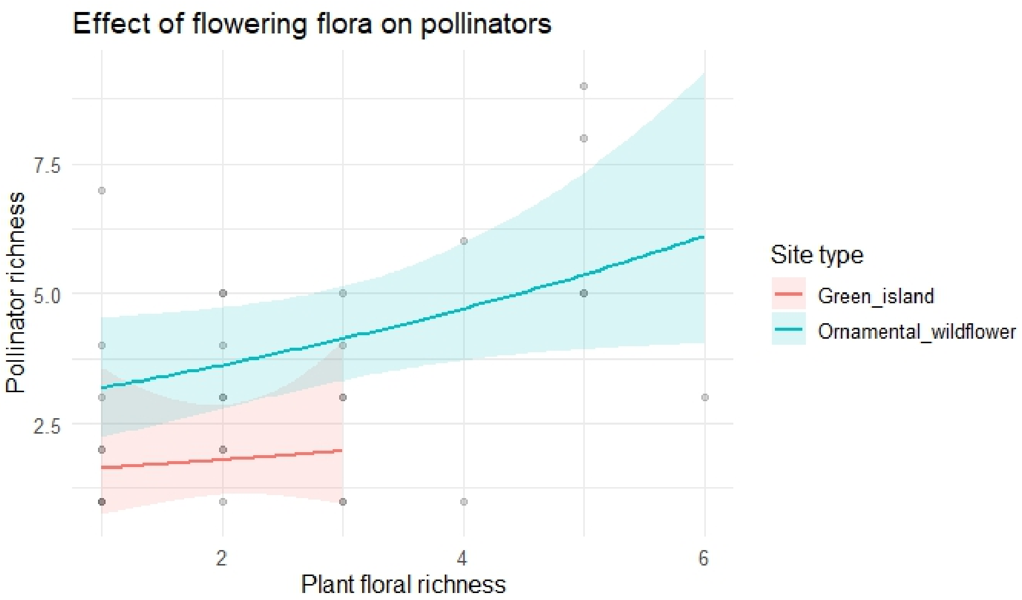


Figure 3-10 Scatterplot showing the significant relationship between floral richness and pollinator richness on green island (red) and ornamental wildflower (blue) roundabouts. The light blue and light red around each of the lines indicates the 95% confidence interval of a linear relationship.

3.4 Discussion

There are costs and benefits for insects surviving on road verges and roundabouts, and whether these habitats function as sinks or not, needs to be considered. The costs include mortality events through collisions with road traffic plus pollution from exhaust fumes, while the benefits include the provision of floral resources, nesting habitats and micro-climates for shelter. The comparison between the green island and ornamental wildflower roundabouts provides evidence that wildflower seed mixes can enhance pollinator abundance and richness in certain situations. In this study, the comparison was made with green island roundabouts where large areas were frequently mown, and only eight plants provisioned pollinating insects, in contrast to thirteen on the ornamental wildflower roundabouts. The major disadvantage of the green island roundabouts is that they contained a limited number of shrub species and so incurred temporal gaps with few floral resources for pollinating insects. On the other hand, the ornamental wildflower roundabouts provided a wide variety of floral resources that contained pollen, nectar and larval hosts throughout the survey period, as is advocated by Hopwood *et al*. (2015). However, the ornamental wildflower roundabouts, due to the small area they covered (six times less than the green island roundabouts), did not have the space for structural diversity. Enhancing the floral resources on the green island roundabouts has the ability to positively impact the local pollinator population, especially considering the effect the large area of the ornamental roundabouts may have for providing floral resources. As seen in this study, the floral richness positively impacts pollinator richness as was also found by Biegerl *et al*. (2025).

A variety in the structural diversity of the vegetation is recommended to provide nesting, egg laying and overwintering opportunities (Hopwood *et al.*, 2015), which may be provided by the surrounding hedgerows, fields and road verges in this situation. The green island roundabouts in this study did contain an occasional *Quercus* spp. tree or *Crataegus monogyna*, bare banks, rows of shrubs, areas of cobble stones and some areas of long grass creating diversity within the landscape. On the other hand, the ornamental wildflower roundabouts contained no other micro-habitats other than a single roundabout that supported a single non-native shrub. Mining bees require a variety of materials from wood, mud, leaves and tree resin (Hopwood *et al.*, 2015). In this study two species of mining bee were recorded: namely, *A. praecox* and *A. haemorrhoa*. From the community composition, *A. praecox* showed no preference for either type of roundabout, whereas *A. haemorrhoa* was associated with the ornamental wildflower roundabouts. The ornamental wildflower roundabouts would have provided floral resources to both species but no bare banks for nesting or other materials. Ideally, all the requirements would be contained in one habitat, though this may not be possible on roundabouts due to size restrictions (Hopwood *et al.*, 2015).

The decision on which plant species to provide for improving biodiversity does depend on the local ecological conditions. Roadside features vary considerably, physically and ecologically, due to soil pH, aspect, slope and surrounding habitat so there is no one-size-fits-all when it comes to decisions on plant composition (Hopwood *et al.*, 2015; Campanelli, 2016). Enhancing and restoring roundabouts can have a positive impact on pollinators and be an opportunity to create habitats to conserve native species. In this study, the overall abundance and diversity of pollinators was higher on the ornamental wildflower roundabouts, and both types of roundabouts contained native and non-native species of plants. There were three species of butterfly recorded on ornamental wildflower roundabouts however only a single butterfly was foraging, and this was on the invasive *B. davidii* on a green island roundabout. Bumblebees, hoverflies and solitary bees have been negatively affected by the plant species planted on the green island roundabouts. Sixty percent of the bumblebee visits were on *Hebe* sp. and the long-tongued bumblebee, *B. hortorum*, was the only species to visit the *Hosta* sp. Twenty-one percent of foraging by hoverflies occurred on non-native species on the green island roundabouts but the majority of visits took place on the naturally occurring native *R. acris* and *Taraxacum* spp**.** The hoverfly, *Orthonovera nobilis*, was noted on green island roundabouts foraging on *R. acris,* a species found by Hicks *et al*. (2016) to contain low quantities of pollen and nectar. On green island roundabouts no solitary bees were found foraging on exotic species with 100% of the foraging on *Taraxacum* spp**.,** as was found by Nichols *et al*. (2022a). In a U.K study the native *Taraxacum* spp**.** species provided high resources of both pollen and nectar (Hicks *et al.*, 2016). The lack of floral diversity due to the limited number of plant species on green island roundabouts has negatively influenced all pollinating groups, barring the butterflies. However, the frequent mowing did allow for the proliferation of *Taraxacum* spp**.** which provided the floral resources for the solitary bees on the green island roundabouts. These roundabouts also feature areas of grass that needs to be allowed to grow to ensure there are nesting habitats for bumblebees and larval host plants for butterfly species (Hopwood *et al.*, 2015).

Research in the U.K. found that some of the species that contributed the highest quantities of nectar were cornflower (*C. cyanus*) and common knapweed (*C. nigra*), while higher pollen counts were found in the common poppy (*Papaver rhoeas*) and musk mallow (*Malva moschata*) (Hicks *et al.*, 2016). The ornamental wildflower roundabouts in this study contained similar plant species, such as common knapweed (*C. nigra*) and common mallow (*Malva sylvestris*), that were subjected to high rates of visitations by bumblebees, in particular, and hoverflies. Field scabious (*K.*

arvensis) was the second most visited plant species. This species is known to be a good source of nectar and pollen for a wide spectrum of insects (Varga *et al.*, 2022). In this study bush vetch (*Vicia sepium*) received 21 visitations but in a previous study by Hicks *et al*. (2016) the similar species, tufted vetch (*Vicia cracca*), was found to contain low counts of both pollen and nectar. Oriental poppies (*Papaver orientale*) were on the ornamental wildflower roundabouts but even though Hicks *et al*. (2016) found high quantities of pollen, no pollinators were recorded visiting them here.

The use of ornamental wildflower seed mixes for promoting pollinators in rural areas is controversial, has produced mixed results and needs careful consideration. A diversity of plant species with a variety of colours, heights and habits will encourage a diversity of insects, while floral resources are known to increase the abundance of butterflies on road verges (Hopwood *et al.*, 2015; Goodwin *et al.*, 2017; Darst *et al.*, 2024). It has been suggested that by increasing the quantity and quality of floral resources in road verges, butterflies are less likely to cross roads whereas bumblebees prefer not to cross roads at all (Meinzen *et al.*, 2024). Wildflower seeds have been advocated to support pollinators but are controversial as they may contain non-native, and potentially invasive plant varieties (Barry & Hodge, 2023; Smyth, 2023). Caution is needed in the use of wildflower seed mixes as they may have contributed to the rise of one invasive species in Ireland, black grass (*Alopecurus myosuroides*), a pernicious weed in agricultural settings. In relation to pollinators, wildflower seed mixes have produced differing results. In one study, the sowing of wildflower seeds did have a positive impact on pollinator richness and abundance. Natural regeneration of plant species in vineyards in the UK increased solitary bee richness while a wild bee seed mix had a significant effect on the higher richness of overall pollinators. Two seed mixes were found to increase the abundance of bumblebees and solitary bees two years post planting when compared to a control with no seed mix. However, no difference was found between sites for hoverfly abundance (Griffiths-Lee *et al.*, 2022).

Nichols *et al*. (2022) discovered that one of the seed mixes prescribed by DEFRA to farmers had little effect on insects and bees, losing flowers early as grass species dominated the area. However, the sowing of seed mixes has been shown to improve floral resources for insects, increasing both their abundance and richness, as was also found in this study (Alison *et al.*, 2021; Griffiths-Lee *et al.*, 2022; Darst *et al.*, 2024). Creating seed mixes based on scientific research resulted in the greatest number of visitations by insects, along with the greatest abundance and species richness of bees (Nichols *et al.*, 2022a). Bumblebees preferred the sown species especially common birds-foot trefoil (*Lotus corniculatus*) and common kidney vetch (*Anthyllis vulneraria*). In this situation, as the sown species were planted in 2014, wild species had self-seeded, and it was not possible to identify whether species such as *C. nigra* and *K. arvensis* had been planted or had self-seeded.

Pollen and nectar resources are important to consider when choosing seed mixes (Hicks *et al.*, 2016). Planting these areas with perennial native seed mixes instead of annuals enhances pollen and nectar resources for pollinators and may also provide floral resources earlier (Hicks *et al.*, 2016; Baldock, 2020). It must be ensured that seed mixes contain flowering species that provide floral resources from late spring to late summer, and it has been suggested that seed mixes are resown every five years to reduce homogenisation (Nichols *et al*., 2022). However, results from this study suggest that the plant species had not become homogenised even after eight years post sowing. If anything, the species best adapted to the local environmental conditions had proliferated, and native species had also colonised and thrived, creating a beneficial floral resource for the bumblebee and hoverfly groups.

The use of non-native species, especially when closely related species are used, is not less significant for flower-visiting species as many are generalists (Alexander *et al.*, 2006). This study has shown that this is the case, especially on the ornamental wildflower roundabouts. The optimal management for enhancing pollinators may be to have a heterogeneity of plant species, with exotic species utilised to extend the resources (Salisbury *et al.*, 2015). Non-native plants can increase pollinator abundance but may change the community assemblage (Seitz *et al.*, 2020). As there is no baseline data on pollinators for these sites this statement cannot be corroborated.

There is a plethora of research into ideal mowing regimes, however, so much depends on the species of interest. The research shows that there is no ideal time to mow, and no clear recommendations for the number of cuts per year. Some suggest once a year, some twice, but often it depends on the species being studied, the location, the climate and the vegetation under consideration (Hopwood *et al.*, 2015). The use of flushing bars when mowing is recommended to reduce mortality (Meinzen *et al.*, 2024). Mowing frequencies will affect larval foodplants, and the eggs and larva of insects (Phillips *et al.*, 2020b). In this study there were only six butterflies recorded and only one was foraging. The mowing regime was altered in 2024 on the green island roundabouts to allow areas to become long grass and two pyramidal orchids (*Anacamptis pyramidalis*) appeared. The change in management may lead to an increase in the abundance of butterflies and other pollinators over time.

Road verges and roundabouts have the potential to become hotspots for floral resources and pollinators. There is evidence that suggests that road verges enhance pollinator diversity and density and these benefits outweigh the costs, though further research is required (Phillips *et al.*, 2020b). Research into the threshold levels of traffic densities, pollutants and mowing is needed bearing in mind that the effects will be species specific (Meinzen *et al.*, 2024). Further investigation is needed into the effects of light, exhaust fumes, noise and vibrations from road traffic on pollinators, individually and synergistically (Phillips *et al.*, 2021). The effects of heavy metals from pollution is also understudied in pollinators (Hopwood *et al.*, 2015). There are relatively few studies looking into the heights at which insects fly. One species of butterfly was estimated to fly at only a meter, some bee species no higher than two meters (Hopwood *et al.*, 2015). The question of whether road verges are sinks or sources of pollinators needs to be answered (Meinzen *et al.*, 2024).

However, roundabouts have the potential to provide valuable ecosystem services as they are widely dispersed, and management can be adaptable to the local situation. They should be seen as an asset to the improvement of biodiversity (Phillips *et al.*, 2020a). There are negative impacts from roads but mitigation measures to enhance roundabouts could lead to connectivity between habitats where they can act as stepping stones offering food and shelter. Many of the procedures needed to improve habitats for biodiversity are often free and could potentially lead to savings. The consequences of all of these measures need effective monitoring to understand whether these measures provide long-term benefits to pollinator populations (IPBES, 2016). The benefits could entail floral resources, nesting sites, hibernating sites, and corridors while the costs may include pollution, collision with traffic and ecological traps (Phillips *et al.*, 2020b).

The surrounding landscape will impact the insect pollinators in this study as the study sites are so small. All six roundabouts lie within a kilometre and alongside the N7 dual carriageway that runs in a south-west to northeast direction with three roundabouts lying on the north side and three on the south side. The surrounding landscape did not vary significantly between the relative abundance of each of the four broad landcover types assessed- artificial surfaces, grassland, woodland and scrub, and waterbodies. Two of the roundabouts, one of each type, did have a higher percentage of artificial surfaces, as one green island roundabout lay on the outskirts of Naas town, while one ornamental wildflower roundabout lay on the outskirts of the village of Kill. The area of the ornamental wildflower roundabouts was also considerably smaller than the green island roundabouts, so it seems that it is the type of management on the ornamental wildflower roundabouts that is enhancing the richness and abundance of pollinators, and not the surrounding landscape.

This study does accept the hypothesis that there was a significant relationship between the abundance and species diversity of the four pollinating groups and the type of roundabout surveyed. It also accepts the hypothesis that the abundance, richness and community composition was positively associated with the ornamental wildflower roundabouts. The diversity of the plant species providing floral resources to pollinators did drive a difference, particularly for the bumblebees and hoverflies, who were positively influenced by the ornamental wildflower roundabouts. It is important to note that the solitary bees were driven by *Taraxacum* spp**.** and so shows the importance of mown areas thereby allowing this plant to flower and thrive without having to outcompete taller native plants like grasses.

3.5 Conclusion

A wildflower seed mix has enhanced the biodiversity of pollinating insects in comparison to the green island roundabouts that provided few floral resources but potentially offered more micro-habitats for shelter and nesting sites. The size of a roundabout may need to be taken into consideration during the design phase. Traffic generally travels slower around a smaller roundabout potentially reducing collisions with insects. This could allow for these small spaces to be sown with wildflowers enhancing resources for pollinators in conjunction with providing road users with pleasing aesthetics. The road verges could then be used to furnish pollinating insects with their other requirements such as ditches or swales for water, fruit trees for nesting sites and a variety of plants to afford shelter, larval hosts and structural diversity. To expand structural diversity mowing should be conducted in parts of the landscape to allow *Taraxacum* spp**.** to flourish, especially for solitary bees. Providing seed mixes containing plants with pollen and nectar resources, and that do not contain invasive species, may augment pollinating insects with the supplies they need for their conservation.

The costs and benefits of using roundabouts must be weighed up. However, these small islands may function as stepping stones aiding in the diffusion of genetic isolation and inbreeding for both pollinating insects and plants species.

Chapter 4 Assessing the impacts of glyphosate and its alternatives on the soil microbiome

4.1 Introduction

For almost a century, pesticides including herbicides, fungicides, bactericides, insecticides and rodenticides have been used ubiquitously worldwide, to control or kill unwanted organisms (Aydinalp & Porca, 2004). Heralded as silver bullets for controlling pests and pathogens, many are now irretrievably integrated into our agricultural and landscaping practices. It is estimated that 85% of pesticides are used in agriculture with the rest used to control plants and insects in private residences and open public spaces (Kim *et al*., 2017). However, there are growing concerns that many of these chemicals are being overused, contaminating our food chains and waterways, and having significant detrimental effects on organisms beyond those they target. It is therefore essential that where possible evidence for the risks these chemicals pose is gathered, and alternative and more environmentally sustainable approaches are found. As far back as the 1960s, Professor Rachel Carson, in her seminal book- Silent Spring (Carson, 1962), raised concerns about pesticides and their impact on the environment and human health. Carson advocated the precautionary principle which cautions against using chemicals in our environment until a better understanding of their risk is known. However, despite the regulatory requirements regarding pesticide use both nationally and internationally, global pesticide use has doubled from two million to four million tonnes between 1990 to 2023. In Ireland alone during the same time period it has increased from 2,014 tonnes to 2,274 tonnes (FAO, 2025c), and to date in the EU, plant protection products contain approximately 450 different active ingredients that are approved for use (DAFM, 2019a).

One of the single biggest issues with the widespread application of chemical pesticides, which is often indiscriminate and unfocussed, is the impact on organisms other than those targeted. The vast majority of pesticides end up as environmental waste (van der Werf, 1996; Meena *et al.*, 2020) and it has been estimated that only 1% of an applied pesticide reaches its intended organism (Arias-Estévez *et al.*, 2008; Vickneswaran *et al.*, 2023). Even when precisely applied, many pesticides eventually disperse through the environment and inevitably end up in the aquatic and terrestrial environments, where they may impact microbial communities (van der Werf, 1996; Kim *et al.*, 2017; Zioga *et al.*, 2022). Dust drift and the use of treated seeds also result in the unintended spread of a pesticide (Ockleford *et al.*, 2017).

Of all the pesticide classes, herbicides are the most ubiquitously used, accounting for about half of all pesticide use globally. In fact, seventy-five percent of all plant protection products used in Ireland in 2023 were herbicides (CSO, 2023). Although difficult to categorically demonstrate and with ambiguous or often contradictory results observed (Gunstone *et al*., 2021), it is becoming increasingly clear that the non-target effects of herbicide use and their potential for impacting environmental, and even human health, need to be more fully understood. In particular this relates to glyphosate which, without doubt is becoming one of the most controversial herbicides available on the market to date.

4.1.1 Glyphosate

In 1974 Roundup, of which glyphosate is the main ingredient, was specifically marketed to farmers and it has been the most widely used herbicide ever produced (Myers *et al.*, 2016; Duke, 2018). Globally, sales and the use of glyphosate is increasing annually (FAO, 2017). In the last ten years in the US roughly two thirds of the total amount of glyphosate sprayed since 1974 has been applied (Myers *et al.*, 2016; Beckie *et al.*, 2020; EC, 2023). There are three main reasons why there has been such an increase in the amount of glyphosate used. The first being that over time plants have built up resistance to it (Myers *et al.*, 2016; Xu *et al.*, 2019). It was estimated in 2017 that there were 38 species of plant that had become glyphosate-resistant, with a further two becoming immune each year (Duke, 2018; Meftaul *et al.*, 2020). The second reason is the use of glyphosate for the desiccation of crops prior to harvesting and the third is due to its use on genetically modified crops (Myers *et al.*, 2016). The introduction of genetically modified crops that are immune to the effects of Roundup has led to the dramatic increase in the use of glyphosate in the last few years, with an estimated 90% of genetically modified crops now glyphosate-resistant (Duke, 2018; Zhang *et al.*, 2019).

As mentioned above, many pesticides affect non-target organisms, many of which may be found far from the site of application. This has led to the levels of glyphosate and its main breakdown product, aminomethyl phosphonic acid (AMPA), increasing across environments (Socha *et al.*, 2021). Glyphosate has been found in wastewater treatment plants, rainfall, water and seawater, and even in the atmosphere (Van Bruggen *et al.*, 2018; Matozzo *et al.*, 2020). Chronic low doses of glyphosate and AMPA can build up over time in the environment, and they have both been found to accumulate in topsoil over repeated application (Van Bruggen *et al.*, 2018; Zioga *et al.*, 2022). Herbicides also have a variety of direct and indirect impacts on non-target species (van der Werf, 1996; Arias-Estévez *et al.*, 2008; PAN Europe, 2018; Van Bruggen *et al.*, 2018). Glyphosate is non-selective and so affects non-target organisms (Kanissery *et al.*, 2019; Pochron *et al.*, 2019) and is known to impact non-target plants for up to one month post spraying (Zioga *et al.*, 2022).

4.1.1.1 Mode of action and metabolism

Glyphosate is an organophosphate herbicide that affects plant meristematic tissues (Duke, 2018; Connolly *et al.*, 2019) by suppressing the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the shikimate pathway (Myers *et al.*, 2016; Hagner *et al.*, 2019). This enzyme is involved in the synthesis of aromatic amino acids, which when inhibited, leads to the death of the plant (Hagner *et al.*, 2019). These amino acids also play a role in plant defences through their metabolism into antimicrobial phytolexins, the absence of which makes the plant more susceptible to pathogen attack (Van Bruggen *et al.*, 2018). The degradation of glyphosate and its main breakdown product, AMPA, is dependent on a number of environmental factors but there seems to be little consensus. It was initially presumed that glyphosate degraded quickly (a matter of days to months) but recent research has indicated a much longer half-life, in some cases years, depending on the soil type (Myers *et al.*, 2016; Hagner *et al.*, 2019). Half-life estimates range from between 1.7 days to several months, and approximately 30 days in temperate zones (Kepler *et al.*, 2020; Matozzo *et al.*, 2020; Meftaul *et al.*, 2020). Aminomethyl phosphonic acid with a supposed half-life of 23-958 days, is known to persist in the environment longer than glyphosate (Meftaul *et al.*, 2020). Glyphosate accumulates predominantly in top soils due to its capacity to bind to particles of soil which limits its movement (Kanissery *et al.*, 2019). Due to its molecular size, glyphosate is also easily taken up by soil minerals (Van Bruggen *et al.*, 2018; Meftaul *et al.*, 2020). Soil, known for its glyphosate adsorbing capacity, can also be subjected to leaching, which results in glyphosate contamination of groundwater (Carretta *et al.*, 2022). It is now widely recognised that glyphosate contaminates water courses in high concentrations (Myers *et al.*, 2016; Matozzo *et al.*, 2020) and that this contamination is directly linked to farming practices, weather conditions and the timing of the application of pesticides (Carretta *et al.*, 2022). Glyphosate washes into the aquatic environment via soil leaching, run-off and when sprayed directly onto aquatic plants, as glyphosate is used on invasive species, including those within watercourses (Van Bruggen *et al.*, 2018).

4.1.1.2 The effects of glyphosate on non-target organisms

Testing the effects of pesticides on organisms is complicated as every pesticide and every species will react differently, leading to an infinite number of experimental permutations (Gunstone *et al.*, 2021). There are three main routes of exposure of pesticides to organisms: through food, through air, or via water (Ockleford *et al.*, 2017). As animals lack the shikimate pathway, it has been assumed that glyphosate is non-harmful or of low risk to animals (Socha *et al.*, 2021). However, more and more evidence of the detrimental impact of glyphosate on animals (including humans) is being discovered (Myers *et al.*, 2016). Several studies have shown, for example, the link between glyphosate and tumour growth, and kidney and liver damage in rodents (Myers *et al.*, 2016; Van Bruggen *et al.*, 2018) and a reduction in fertility levels in chickens post injection of their eggs with Roundup (Fathi *et al.*, 2019). Pesticides have been responsible for fish kills but the chronic effects of glyphosate and AMPA in the aquatic environment needs more research (van der Werf, 1996; Arias-Estévez *et al.*, 2008; Matozzo *et al.*, 2020).

There is also an increasing body of evidence of the impacts of glyphosate exposure on human health, particularly in those that work with glyphosate or live close to areas where glyphosate is frequently used (e.g. farms, managed parks, golf courses, etc.) (Connolly *et al.*, 2018; Gerken *et al.*, 2024). Pesticide risk to humans is hard to quantify due to many variables involved from the level of exposure to the variety of toxins and their persistence (Kim *et al.*, 2017). In 2015, the International Agency for Research on Cancer categorized glyphosate as 'probably carcinogenic to humans' which has led to many studies (Connolly *et al.*, 2022). The European Chemical Agency does not classify glyphosate as carcinogenic, while the Agricultural Health Study found no statistically significant links between them (Connolly *et al.*, 2022). However, in a meta-analysis review, a compelling link was found between glyphosate-based herbicides and non-Hodgkin's lymphoma in humans (Zhang *et al.*, 2019). The increased incidence rates of asthma, cancer, issues with reproduction, attention and behavioural issues in children, have all been linked to exposure to pesticides (Hyland *et al.*, 2019). In Brazil, a statistically significant relationship has been found between the expansion and intensification of soy production leading to increased use of pesticides (up to 70% higher than other countries) and an increase in the deaths of children from leukaemia through downstream water consumption (Skidmore *et al.*, 2023).

It is becoming increasing clear that the widespread use of glyphosate and the potential detrimental impacts it may have on our environment and human health require new approaches to food production and land management that are less reliant on this chemical.

4.1.1.3 The effects of glyphosate on the soil microbiome

The overall effects of pesticides on the soil microbiome are thought to be minor. To date, much of the research conducted involves evaluating the activity, community structure and abundance of bacteria and biomass for fungi (FAO, 2017). It is extremely difficult to predict the impact of pesticides on above ground vegetation and the consequences to the soil microorganisms and vice versa (Wardle *et al.*, 2004). The nutritional quality of soils may be affected by a disruption to the soil microbial community that can then have a knock-on effect on plant growth (Arora & Sahni, 2016; Meena *et al.*, 2020). Fungi breakdown soil organic matter and cycle nutrients through complex substrates (Sassi *et al.*, 2012). Alterations in the quantity of fungi and bacteria may effect the functional abilities of the soil ecosystem (Sassi *et al.*, 2012). Pesticide chronic toxicity testing on soil organisms in Europe is overseen by the European Food Safety Authority, who require testing on earthworms, springtails and mites, plus measures of nitrogen transformation from microbial activity (Gunstone *et al.*, 2021). The results from experiments on the impacts of pesticides on taxa varies greatly on the chemical composition of the pesticide used and the type of taxa tested (Gunstone *et al.*, 2021).

Certain pesticides have been banned (e.g. DDT) after using the soil ecotoxicological approach (FAO, 2017). This approach tests species in the lab first and if an acceptable risk is found, further tests are undertaken in realistic outdoor spaces (FAO, 2017). A decrease in numbers and the time it has taken for abundance to recover has been used to assess the impacts of pesticides on soil microorganisms (FAO, 2017). A study conducted by the FAO in 2017, found that pesticides do have significant effects on the soil microbiome through fluctuations in biomass, enzyme activity and alterations of species (FAO, 2017). Depending on the type of herbicide used, there can be a negative impact on the population of the microbes within 7 to 30 days post application (Meena *et al.*, 2020). However, recovery post pesticide application has been shown to be quick, with reports of between 28 to 114 days (Ockleford *et al.*, 2017), though it has been suggested that it may take as long as fifteen years for soil communities to recover (Gunstone *et al.*, 2021). Soil microorganisms are known to have differing sensitivities to glyphosate that lead to a change in the microbial composition (Van Bruggen *et al.*, 2018).

The negative effects of glyphosate on bacteria and fungi have been shown in several studies with some reporting the reduction in arbuscular mycorrhizal fungi by as much as 40% (Meena *et al.*, 2020). Sensitive organisms are reported to have died after heavy doses of herbicide, while glyphosate reduced the growth and activity of important nitrogen fixing bacteria, *Azotobacter* (Meena *et al.*, 2020). However, there are a few prokaryotic and fungal species that are capable of metabolising glyphosate (Van Bruggen *et al.*, 2018; Kepler *et al.*, 2020; Matozzo *et al.*, 2020). Microbes are known to degrade chemical compounds with their high abundance and diversity, and rapid growth rates, allowing them to evolve rapidly to altered conditions (Ockleford *et al.*, 2017). Bacterial-mediated actions are the main way glyphosate is broken down (Kanissery *et al.*, 2019). Bacteria are well known for their ability to break down pollutants, whereas fungi can alter chemicals to enable bacteria to break them down and then transport and redistribute them through their hyphal networks (Ockleford *et al.*, 2017). The microbiome is known to breakdown glyphosate while AMPA, is adsorbed and so accumulates in soils (Hagner *et al.*, 2019; Carretta *et al.*, 2022).

Glyphosate is released into soil indirectly as plants die off and decay, and is exuded by roots into the rhizosphere (Kanissery *et al.*, 2019). There are some positive indirect effects from glyphosate use such as an increase in carbon surrounding the rhizosphere after vegetation above ground had been depleted (Wardle *et al.*, 2004). The effects of glyphosate are highly dependent on the quantity of glyphosate used, with higher quantities leading to an increase in biomass and respiration levels by providing carbon, nitrogen and phosphorous as sources for metabolization (FAO, 2017). However, negative effects persist and include a rise in root disease in soil where glyphosate has accumulated, possibly explained by the removal of beneficial microorganisms, leaving plant and animal pathogens free to increase in abundance (Van Bruggen *et al.*, 2018). Glyphosate could potentially be altering the soil microorganisms that affect nutrient availability, thus reducing their overall ability to resist disease (Kanissery *et al.*, 2019). In a meta-analysis it was concluded that pesticides do disrupt food webs (Zhao *et al.*, 2013).

4.2 Alternatives to Glyphosate

There has been growing public demand to find alternative ways to remove and control unwanted plants. Glyphosate has been an incredibly useful means of controlling unwanted plants, but the increasing knowledge of the effects of glyphosate on public health and the environment are leading land managers and some farmers to explore potential alternatives. Three countries in Europe have banned the household use of glyphosate: France, the Netherlands and Belgium, while Germany has managed to ban it in public spaces (Anon, 2023). County councils in Ireland have begun to search for alternative ways to control plants within their public open spaces. Alternatives to glyphosate are also relevant to many other land managers of places such as railways, universities and golf courses, with the number of alternatives being used in public open spaces increasing (Hudek *et al.*, 2021).

4.2.1 Aims

The aim of this study was to measure the impact of six plant control treatments on i), their effectiveness at controlling vegetation and ii), their impact on the abundance and diversity of the soil microbial community and composition. The treatments chosen were based on their availability, suitability for use in urban environments and those currently used by Irish county councils. The treatments included a control, two glyphosate-based treatments: Roundup Flex and Nomix Dual, and three alternatives: New Way (acetic acid), Mowing and Hot Foam (thermal).

Roundup Flex and Nomix Dual are herbicides that are absorbed by the foliage of plants and subsequently translocates to the roots, rhizomes and stolons. Both suppress the 5-enolpyruvylshikimate-3-phosphate synthase, the enzyme that is essential to plant growth, which when inhibited leads to the death of a plant (Myers *et al.*, 2016; Hagner *et al.*, 2019). Both products also contain a blend of surfactants that enhances the ability of glyphosate to penetrate a plants surface through its cuticles. Nomix Dual contains sulfosulfuron that is both foliar and soil acting, and prevents seeds from germinating (Nomix Enviro, 2016). The advantages of the Nomix Dual are that no mixing is required, it can be used on a variety of areas, it is fast-acting, and the effects last for six months. It is apparently non-hazardous to operators, while the droplet control applicator means that there is little spray drift, unlike the Roundup Flex. New Way is biodegradable with bioaccumulation being unlikely. It is a non-selective herbicide with acetic acid as its active ingredient. The Hot Foam thermal process combines hot water at ~ 98°C and biodegradable natural plant oils to create a foam. Proteins found within plants break down above a specific temperature over a period of time. The foam creates an insulating blanket, retaining heat for 19 seconds to ensure it reaches the root of the plant. The system kills, or severely damages, a plant by the heat penetrating the leaves' waxy outer layer and travelling down the stem. The longer a plant is kept at temperatures above 57° Celsius, the more likely it is to die (Weedingtech, 2022). The foam also sterilises any seeds and spores in the immediate vicinity of the foam.

The following hypotheses were tested in this chapter:

- Hypothesis 1: There is a relationship between the surface vegetation cover and treatments.
- Hypothesis 2: Nutrient levels and soil physio-chemical parameters will correlate with differences in the microbial abundance and diversity of bacteria and fungi between treatments.
- Hypothesis 3: Differences in bacterial and fungal microbial diversity and community composition are affected by alternatives to glyphosate and glyphosate-based herbicides.

4.3 Methods

All experiments were conducted at a Teagasc greenfield site in Kinsealy, County Dublin (GPS Coordinates: 53.422025, -6.178068). Teagasc are the Agricultural and Food Development Authority in Ireland and they provide integrated research to agricultural and food industrial bodies. Vegetation surveys and soil samples were collected one week prior to the application of treatments, and one month and four months post application.

4.3.1 Treatments

Roundup Flex and the New Way were diluted in water to the correct concentrations as per the manufacturer's directions. A pre-prepared working solution of Nomix Dual was obtained, and a commercial machine was used for the application of the Hot Foam. A lawnmower was used to mow. Roundup Flex is a soluble concentrate containing 480g/l glyphosate which is present in the potassium salt of glyphosate at 588g/l. The potassium salt keeps the solution highly concentrated yet at a low viscosity. The recommended maximum individual dose, litres of product per hectare, is 4.5 for permanent grassland. The knapsack sprayed the recommended delivery range of 80-300l/ha. The correct dosage of Roundup Flex (200ml) was added to a knapsack spray tank of clean water (15l). The spray was applied at a walking speed of one m/second for each plot of one metre width as per instructions. Nomix Dual is an oil emulsion that contains 162g/l (16.8% w/w) isopropylamine salt of glyphosate (equivalent to 120g/l glyphosate) plus 2.22g/l (0.23%w/w) sulfosulfuron. The Nomix Dual pack was shaken and applied using the droplet control applicator at a rate of nine litres per hectare. New Way contains acetic acid, alcohol ethoxylate and C13. A mixture of 1-part New Way to 3 parts clean water was used in a knapsack sprayer. Hot Foam is an organic biocide that uses high temperatures and low-pressure. A Foamstream L12 was used with a water flow rate of 12 l/minute at a temperature of 98° Celsius to apply the Hot Foam. The oil was made up of the organic ingredient rapeseed oil at a ratio of 0.005% of oils to water.

4.3.2 Experimental Design

Each treatment type, including an untreated control, was replicated five times across three blocks, each measuring 16m x 13m. The six treatments to be used were randomized across the 90 plots using Minitab 20. Within each block five separate $1m^2$ quadrats separated by two metres were used as technical replicates and each block represented one biological sample (Figure 4-1). Each treatment was assigned a letter and was colour coded for ease of application for the contractors: A- Control, B- Roundup Flex, C- Nomix Dual, D- Mow, E- New Way and F- Hot Foam (Table 4-1). All six treatments were applied on the 21st of April 2022 (Plate 4-1).

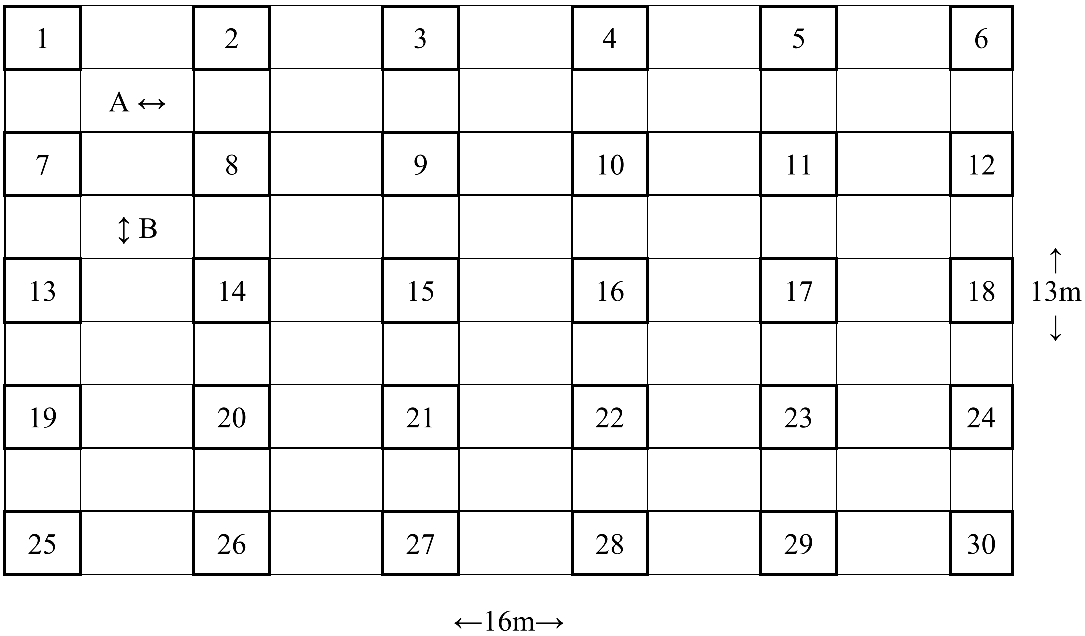


Figure 4-1 An overview of the experimental design to enable assessment of the effects of different plant management approaches. Each of the treatments including the control was assigned five replicate $1m^2$ quadrats in each block. A and B represent the 2m gap between plots.

Table 4-1 Block 1, 2 and 3 with the randomised treatments entered into each row, labelled and colour coded. A- Control, B- Roundup Flex, C- Nomix Dual, D- Mow, E- New Way and F- Hot Foam.

Block 1						
Row 1	B	D	F	C	A	E
Row 2	E	F	C	A	D	B
Row 3	C	D	B	A	F	E
Row 4	B	E	A	F	C	D
Row 5	F	B	D	C	E	A
Block 2						
Row 6	B	A	F	D	E	C
Row 7	C	D	B	A	E	F
Row 8	E	F	B	D	A	C
Row 9	E	A	B	F	D	C
Row 10	C	F	B	E	A	D
Block 3						
Row 11	D	F	C	B	E	A
Row 12	C	F	E	A	D	B
Row 13	F	D	B	C	E	A
Row 14	B	D	C	F	A	E
Row 15	F	A	C	E	D	B

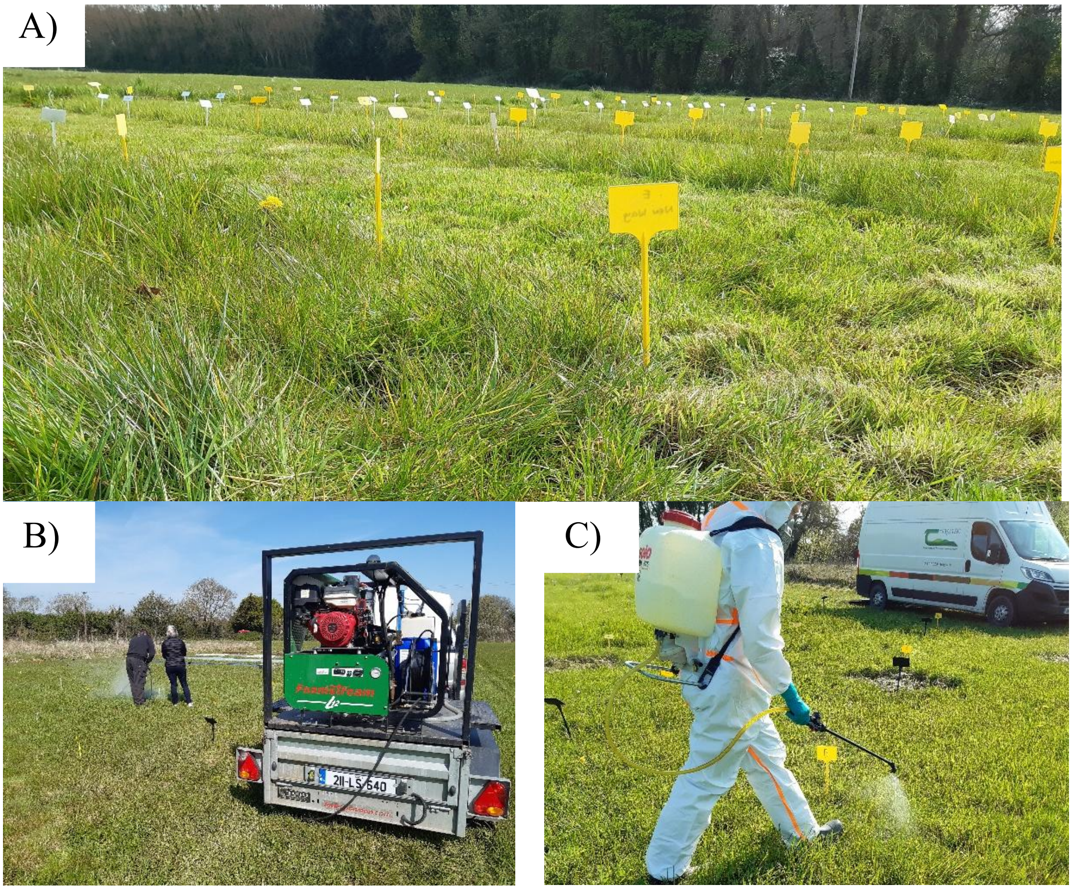


Plate 4-1 Soil experiments conducted at Kinsealy. A) Individual plots measured out and labelled according to treatment. B) The Hot Foam being applied and C) A glyphosate treatment being applied.

Three main experiments were conducted to assess the effectiveness of the treatments. The first measured the impact of the six treatments in conjunction with the soil physiochemical characteristics and nutrients, the second on the effectiveness of reducing vegetation cover and the third assessed the impact of the treatments on the bacteria and fungi within the soil microbiome. The mowing treatment was removed from further investigation due to financial restrictions.

4.3.2.1 Soil samples

Soil samples were removed once pre-treatment and twice post-treatment, at one month and four months. The soil core samples were taken from each plot and were extracted from the organic horizon, the upmost layer of soil, at a depth of 15cm and were pooled at the treatment and block level (Plate 4-2). These samples were taken to the laboratory and stored at 4° C overnight. Soil samples were sieved to 2mm and divided into six tubes (two 50ml and four 25ml). All tubes were labelled with the date, relevant block number and treatment letter (Plate 4-2). Two 25ml tubes were stored at -80° C for DNA extractions. Two 50 ml and two 25 ml tubes were stored in a fridge at 4° C for physiochemical characterisation.

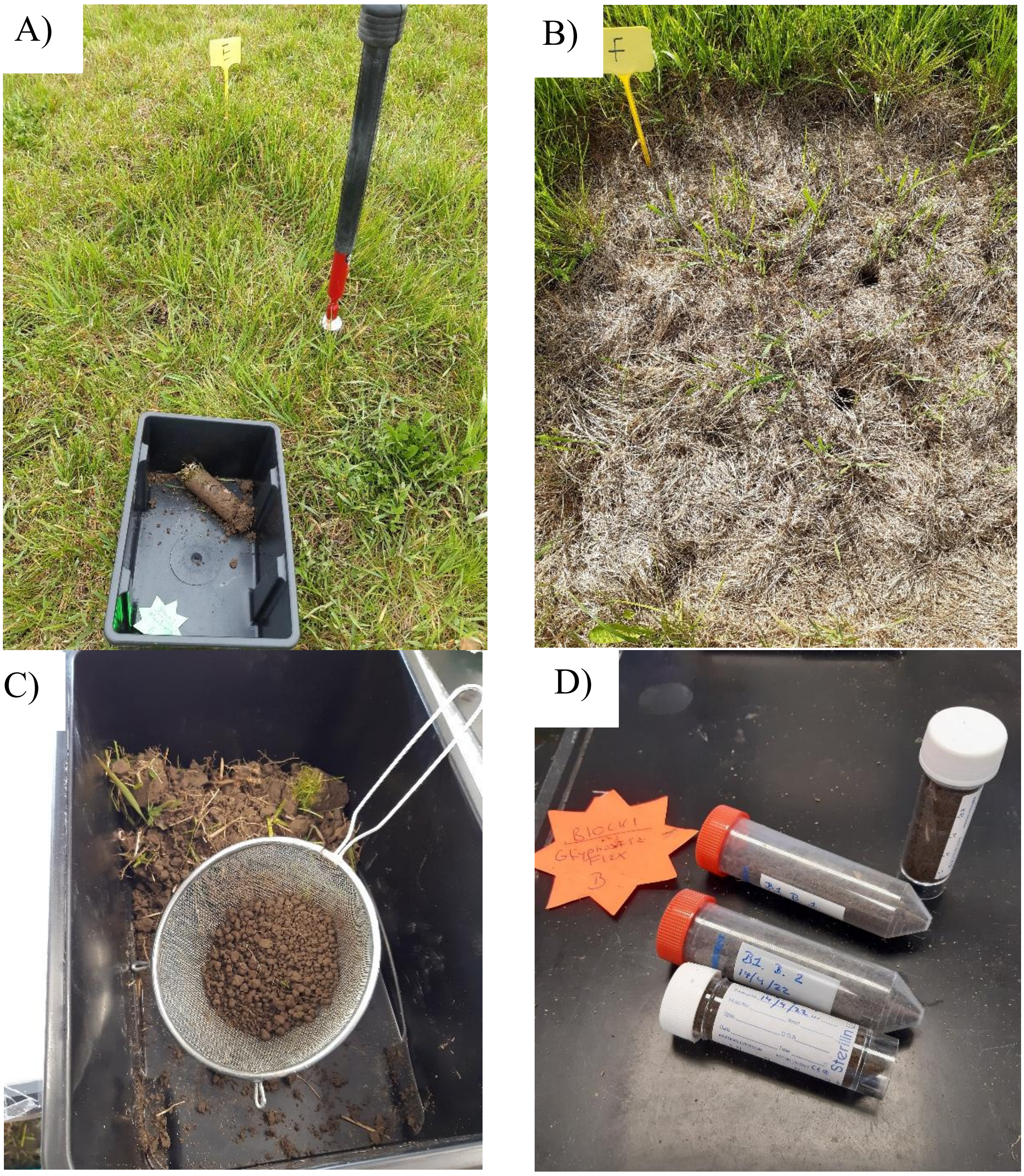


Plate 4-2 Soil samples being removed, sieved and stored. A) Soil sample placed in a box for transport to the laboratory and B) a plot showing the location of where the soil sample was removed. C) Soil sample being sieved in the laboratory and D) soil samples in labelled tubes ready for storage.

4.3.3 Soil Physio-chemical Properties

The physical and chemical properties were conducted on amalgamated soil samples from each of the three blocks at each timepoint- once pre-treatment and twice post-treatment (Table 4-2 and Plate 4-3). To measure moisture content (MC), 150g of fresh soil from each of the blocks was weighed into tinfoil, placed in an oven and dried for three days at 105° C. The difference between the initial and final weight of the soil was calculated. The Loss on Ignition method was used to calculate the soil organic matter (SOM). 20g of soil was weighed into a crucible which was inserted into a furnace at 550° C for 4 hours, the furnace was turned off, and the crucibles were left overnight. The difference in weight before and after was calculated. The water holding capacity (WHC) of the soil was measured by adding 100ml of water to 50g of air-dried soil in a closed funnel. After three hours the funnel was opened, and the excess water was measured. To measure the soil pH (pH), 5g of air-dried soil was mixed with 20ml calcium chloride and 15ml of water, shaken for one hour and left overnight before an electrode was used to measure the pH. The soil texture was measured using a standard ASTM hydrometer after 50g of air-dried soil had been dispersed in sodium hexametaphosphate ($50gL^{-1}$).

Table 4-2 The mean (± SE) of the physiochemical properties of the soils at each timepoint- once pre-treatment and twice post-treatment at one month and four months.

Properties analysed	**Pre-treatment**	**Post-treatment One month**	**Post-treatment Four months**
Physio-chemical properties	**Mean (± SE)**	**Mean (± SE)**	**Mean (± SE)**
Clay (%)	16.21 (± 1.03)	18.21 (± 1.13)	18.04 (± 0.98)
Silt (%)	9.5 (± 2.33)	12.67 (± 3.16)	12.5 (± 3.36)
Sand (%)	66.43 (± 5.51)	61.1 (± 6.65)	61.6 (± 6.75)
pH (CaCl2)	5.91 (± 0.05)	5.94 (± 0.02)	5.97 (± 0.01)
Soil Organic Matter (%)	6.12 (± 0.01)	6.45 (± 0.02)	6.57 (± 0.02)
Moisture Content (%)	21.2 (± 0.48)	20.80 (± 1.22)	13.28 (± 0.5)
Water Holding Capacity (%)	33 (± 0.58)	31.67 (± 1.33)	37.33 (± 1.33)

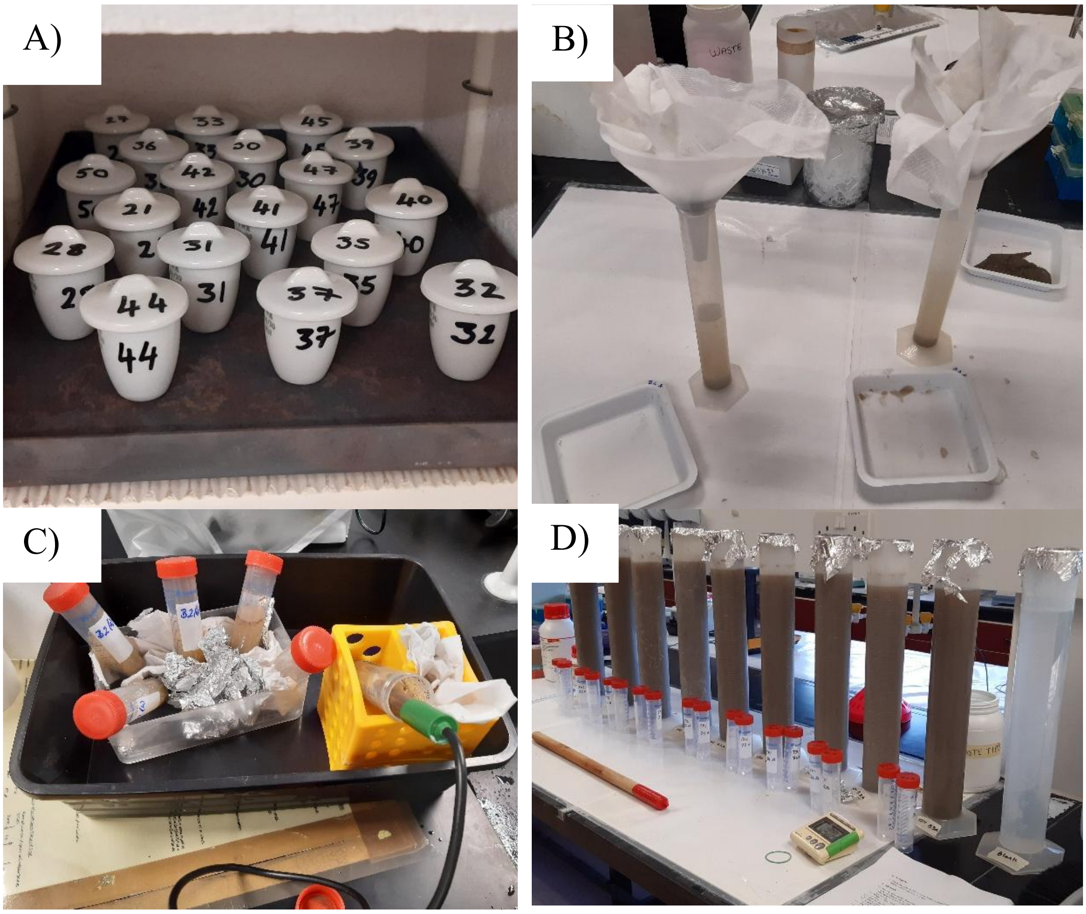


Plate 4-3 Soil-physio-chemical experiments. A) Soil in crucibles for the Loss on Ignition method for measuring the soil organic matter. B) Soil in funnels to measure the water holding capacity. C) Soil pH measured using an electrode. D) Soil texture measurements using a standard ASTM hydrometer.

4.3.4 Soil Nutrients

The quantification of nutrient levels was conducted on soil samples from the five treatment types from each of the three blocks at each timepoint, once pre-treatment and twice post-treatment, resulting in a total of 45 samples. The relationship between soil nutrients and plant control treatments was conducted as nutrient levels such as organic carbon, phosphorous and nitrogen have been found to fluctuate as vegetation above ground dies off. This has been partly attributed to an alteration in the abundance and diversity of soil bacteria and fungi that pesticides have prevented from functioning normally, for example, reducing nitrogen-fixation (Arora & Sahni, 2016). These soil organisms are known to provide the fixation and mineralisation of nutrients that stimulate plant growth and activate responses to stress and resistance to disease (Kanissery *et al.*, 2019; Meena *et al.*, 2020). Glyphosate has been found to negatively impact the uptake of nutrients in plants (Kanissery *et al.*, 2019).

4.3.4.1 % Organic Carbon

The organic carbon % was measured using a 0.34M potassium dichromate solution and a 0.0117M of sucrose solution. Standards were prepared according to Table 4-3 in 15ml tubes.

Table 4-3 Sucrose standards used for % organic carbon determination . All were made up to a final volume of 15ml.

Sucrose Standard to be added (ml)	Distilled water to be added (ml)	Final Organic carbon mass (mg)
0.00	1.000	0
0.125	0.875	0.5
0.250	0.750	1
0.375	0.625	1.5
0.500	0.500	2
0.625	0.375	2.5
0.750	0.250	3
0.875	0.125	3.5
1.00	0.00	4

All 45 soil samples were air dried separately for 10 days at 25° C (Plate 4-4). For each dried soil sample 0.25g was weighed and transferred into a 15ml tube. Under a fume hood, 1ml of 10% v/w potassium dichromate solution was added to each sample. Tubes were swirled to ensure soil was fully mixed with the reagent. To each tube 2.5ml of concentrated sulfuric acid was added whereupon an exothermic reaction occurred. The tubes were left to stand and cool for 30 mins. All tubes were brought to a final volume of 13.5ml with distilled water and left to stand overnight. In triplicate 200µl of each sample was transferred to a 96 well plate (Plate 4-4) and read at an absorbance level of 600nm in a UV-Vis spectrophotometer reflecting the turbidity caused by suspended organic matter. The average absorbance of each sample was calculated.

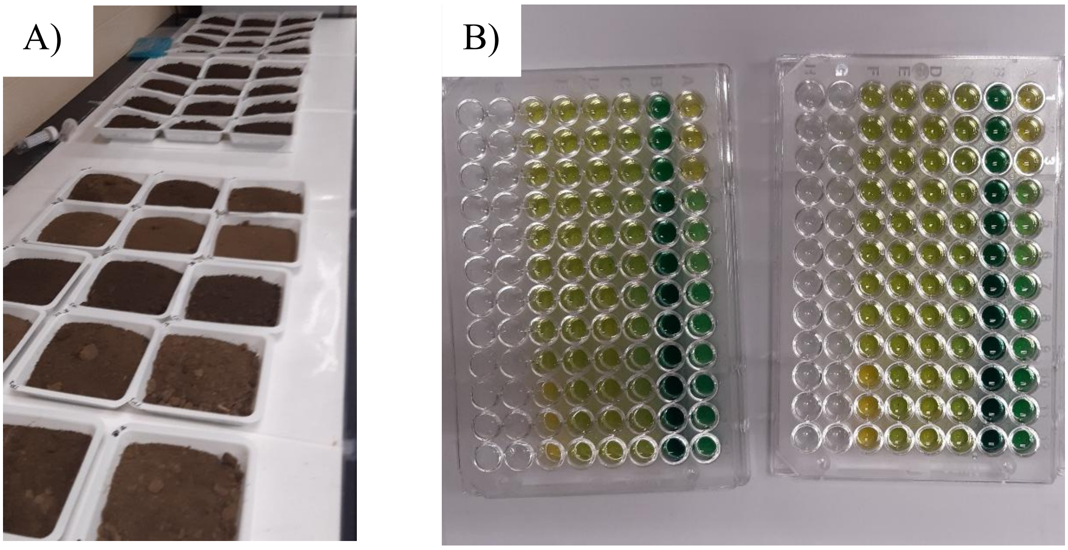


Plate 4-4 Soil organic carbon experiments. A) Air drying all 45 soil samples and B) Samples in 96 well plates having been left overnight.

4.3.4.2 Phosphorous

A 0.0194M solution of ammonium molybdate and a 0.003M solution of antimony potassium tartrate solutions were combined and brought to 500ml with distilled water. A 3.44M of sodium hydroxide reagent was prepared under a fume hood and on the day of the experiments, a 0.1M of l-ascorbic acid reagent was prepared and then wrapped in tin foil. A Stock P of 371µM of sodium dihydrogen orthophosphate was also prepared.

For the acid digest, 50mg of soil from each sample was weighed into boiling tubes. Under a fume hood, 1ml of concentrated sulfuric acid was added slowly to avoid boiling and was then mixed. A blank with no soil was prepared, with only 1ml sulfuric acid. The tubes were boiled at 350° C for 15 minutes, then removed from the heat and allowed to cool for one minute. 100µl of hydrogen peroxide (10%) was slowly added to each tube except the blank. The tubes were returned to the heat for one minute and then removed and allowed to cool. This step was repeated until the sample was transparent, though not colourless: a further 100µl of hydrogen peroxide (10%) was slowly added to each tube, returned to the heat for one minute, removed and cooled. Distilled water was used to bring the final volume of all tubes up to 10mls. Standards were then prepared according to (Table 4-4) in labelled cuvettes.

Table 4-4 Stock P standards used for phosphorous determination . All were made up to a final volume of 10mls.

Stock P to be added (ml)	Blank to be added (ml)	Distilled water to be added (ml)	Final conc. PPM
0.000	0.5	2.600	0.00
0.025	0.5	2.575	0.50
0.050	0.5	2.550	1.00
0.075	0.5	2.525	1.50
0.100	0.5	2.500	2.00
0.150	0.5	2.450	3.00
0.200	0.5	2.400	4.00
0.250	0.5	2.350	5.00

Half a millilitre of each acid digested sample was placed in labelled cuvettes in duplicate and 2.6ml of distilled water was added. To all standards and samples 0.5ml of the ammonium molybdate+antimony potassium tartrate reagent was added followed by 0.2ml of 0.1M l-ascorbic acid. These were left to develop for 45 mins. If no colour developed, 0.5ml of 3.44M sodium hydroxide was added to the cuvettes to neutralise the acid. The optical density was measured at 882nm using a spectrophotometer and the average absorbance was calculated for each sample.

4.3.4.3 Nitrogen

4.3.4.3.1 Soil extraction and clarification

First, the soil was extracted for clarification. A 2M potassium chloride was prepared while 1.5g (±0.05g) of air-dried soil was weighed into a 50ml centrifuge tube. To each tube, 15ml of the prepared 2M potassium chloride solution was added which was placed horizontally on a shaker for 30 minutes at 180 oscillations per minute. The samples were removed from the shaker and inverted several times to consolidate the soil. These were centrifuged at 3000g for 10 minutes. Grade 1 filter paper was rinsed with 2M potassium chloride, and the supernatant was filtered from the samples through this filter paper into fresh 20ml tubes. The clarified samples were quantified within 48 hours.

4.3.4.3.2 Ammonium

A 7.14mM of ammonium-N stock solution was prepared. The standards were then prepared according to Table 4-5.

Table 4-5 2M Potassium chloride, Ammonium-N stock standards used for ammonium determination.

2M Potassium chloride to be added (ml)	Ammonium-N stock to be added	Final concentration (ppm)
20.0	0	0
19.8	0.2	1
19.6	0.4	2
19.4	0.6	3
19.2	0.8	4
18.8	1.2	6
18.4	1.6	8
18.0	2.0	10

A 0.647M of ammonia salicylate and a 0.697M of ammonia cyanurate were prepared and shaken until they had completely dissolved. A multichannel pipette was used to load 100µl of the clarified samples and prepared standards in duplicate to a 96 well plate. The ammonia salicylate reagent was poured into a clean petri dish. Using a multichannel pipette, 40µl of the reagent was loaded into each well. Three minutes later the ammonia cyanurate reagent was poured into a clean petri dish. A multichannel pipette was used to load 40µl of the reagent to each well. The plate was covered and placed in the dark for 10-20 minutes before being read on the spectrometer at 630nm. The average absorbance of each sample was calculated.

4.3.4.3.3 Nitrate

A Nitrate-N stock solution (100ppm) solution in 2M potassium chloride with a molarity of 0.00714M KNO_3 was prepared. A 400mL vanadium chloride reagent was prepared under the fume hood and mixed thoroughly before being wrapped in tin foil to avoid degradation. A multichannel pipette was used to load 10µl of the clarified samples and prepared standards in duplicate to a 96 well plate. The vanadium chloride reagent was poured into a clean petri dish, and a multichannel pipette was used to load 200µl the reagent to each well. The plate was left in the dark for five hours before being read on the spectrometer at 480nm. The average absorbance was calculated for each sample.

4.3.5 Vegetation surveys

Vegetation surveys were conducted once pre-treatment and twice post-treatment, at one month and four months. The Braun-Blanquet abundance cover survey method was used for the vegetation surveys plus the overall percentage of live versus dead vegetation was gathered for each plot. Pre-treatment vegetation surveys included one random 1m x 1m plot in each row of each block giving a total of eighteen plots surveyed across the three blocks. Every plot was surveyed during both post-treatment site visits. All species were listed and the percentage cover of each species within a 50cm x 50cm quadrat was recorded. The scale of quadrat was reduced to ensure the survey incorporated an equal area of treated plot.

4.3.6 DNA Extraction

DNeasy PowerSoil Pro Kits (Qiagen) were used for total genomic DNA extractions prior to the assessment of the fungal and bacterial diversity. This extraction method is used to isolate microbial genomic DNA from soil, and the procedure entailed a number of steps. All 45 soil samples from the five treatments in each of the three blocks were assessed; n=15 pre-treatment, n=15 one-month post-treatment and n=15 four months post-treatment, see Appendix Table C- 1 for results.

An 800µl solution of CD1 was added to a previously spun PowerBead Pro tube with 250mgs of soil (Plate 4-5). Tubes were attached horizontally to an IKA Vibrax machine for 10 minutes at maximum speed. This step aids in soil dispersal and dissolution of the humic acids using chemicals and mechanical agitation, while protecting the nucleic acids from degradation, and is critical for homogenisation and cell lysis. Tubes were then placed in a centrifuge (Plate 4-5) for one minute at 15,000 x g and the resultant supernatant was transferred to a clean 2ml Microcentrifuge tube. To remove any leftover soil fragments the tubes were microcentrifuged at 15,000 x g for a minute with the CD2 solution containing the reagent IRT that precipitates non-DNA material. The pellet was avoided to extract 700µl of the supernatant to a clean 2ml microcentrifuge tube.

Six hundred microlitres of CD3 was added and was vortexed for five seconds. This solution contains a high concentration of salt as DNA binds tightly to silica and the filter membrane of the MB Spin Column. This is used to ensure the loss of the last of the non-DNA organic and inorganic material. The flow through was discarded and this last step was repeated to ensure all the lysate was removed. The MB Spin Column was placed in a clean 2ml Collection Tube and 500µl of Solution EA (Plate 4-5) was added.

This was centrifuged at 15,000 x g for one minute to remove proteins and contaminants from the filter membrane. The flow through was discarded and the MB Spin Column was placed back into the same 2ml Collection Tube.

Five hundred microlitres of solution C5 was added to the MB Spin Column and was centrifuged at 15,000 x g for a minute. This solution washes residual salt and any contaminants while retaining the DNA on the silica membrane. The flow through was discarded and the MB Spin Column was placed into a new 2ml Collection Tube and centrifuged at 16,000 x g for two minutes. This spin ensures that all the Solution C5 is removed as it can interfere with further DNA applications. The MB Spin Column was put into a 1.5ml Elution Tube and 100µl of Solution C6 was placed in the centre of the small white membrane. The Solution C6 releases the DNA from the silica membrane as it passes through it. The MB Spin Column and the Elution Tube was then centrifuged at 15,000 x g for 1 minute, the MB was removed and the Elution Tube with the extracted DNA was stored at -80° C.

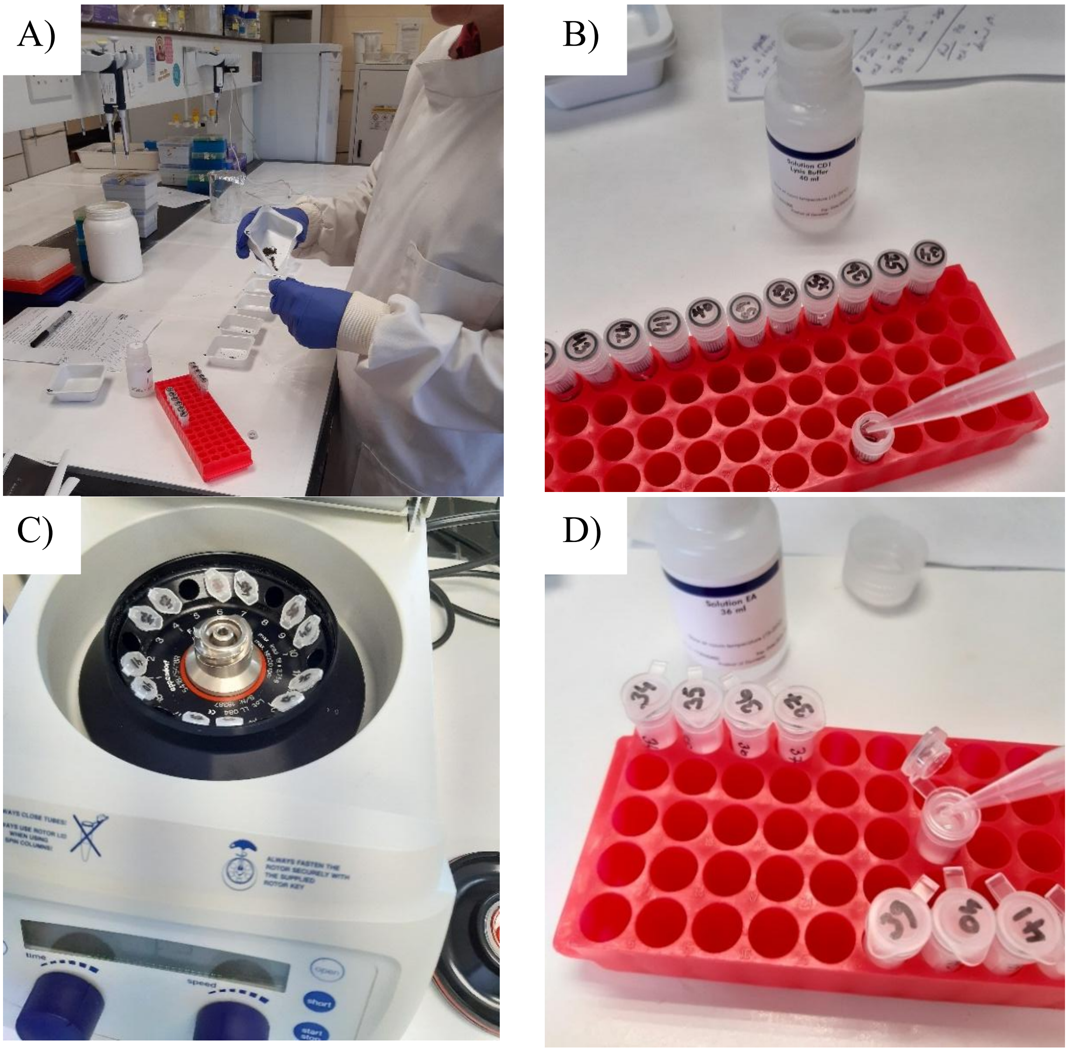


Plate 4-5 DNA extraction experiments. A) Transferring 250mgs of soil into the PowerBead Pro tube. B) Pipetting 800µl solution of CD1 to the PowerBead Pro tube to facilitate the separation of soil from DNA. C) Tubes in the microcentrifuge for spinning. D) Pipetting the 500µl of Solution EA to remove proteins and contaminants.

4.3.6.1 DNA Quality and Concentrations

To confirm and visualize the presence of DNA, an Agarose Gel Electrophoresis was conducted using a PowerPac Basic and a SynGene box. 1.2g of Agarose was placed into a conical flask and filled with 100ml of TAE buffer. The conical flask was put into the microwave for 2 minutes till the agarose had melted, whereupon 10µl of Syber Safe was added. This solution was poured into the prepared casting stand rigg, and air bubbles were removed. The combs were inserted, and this was then left for 40 minutes to set. The rigg was then filled with TAE buffer to the correct level, the combs were removed and 6µl of HyperLadder 1kb was placed in the first well. A 20µl droplet of x 5 DNA Loading Buffer Blue was placed on a piece of paraffin. An 8µl of DNA sample was mixed with the buffer using a pipette and placed in the next well. Once all wells were full, the PowerPack Basic was left running for 40 minutes at 110v of electrical current. To check that DNA fragments were present, the gel was carefully transferred to a SynGene gene box where UV light was passed through the gel and the image was captured. High concentrations of DNA produced brighter bands. The concentration and purity of the DNA was assessed using a NanoDrop 1000 Spectophotometer and WD Programme. A 1.2µl of water was used to check the system. A 1.2µl of the C6 Solution was used as a blank to measure the DNA samples against it. For each soil sample, 1.2µl was measured and the quantity of nano per ng/1µl was recorded along with the 260/280 ratio to assess the purity of the DNA.

4.3.6.2 Novogene Sequencing Methods

Next generation sequencing of the fungal Internal Transcribed Spacer (ITS) regions and the bacterial 16S rRNA was conducted by Novogene. The experiments generated bacterial and archaeal 16S rRNA gene profiles and fungal nuclear ribosomal ITS profiles. These were used to identify and compare species across the soil samples. Different loci are used within the fungal and bacterial genome for sequencing. Within fungi the most widely sequenced DNA region is the ITS region and in bacteria the 16S rRNA region is used as they contain variable regions. These variable regions are implemented as they undergo rapid evolution which leads to a high degree of variation between species. Firstly, the DNA/rRNA is prepared for sequencing using Polymerase Chain Reaction (PCR) that amplifies specific regions. Primers (sections of DNA/ rRNA) are used that contain barcodes (unique tags used as metadata). Gel electrophoresis is utilized to check the length of the DNA/rRNA, and similar lengths are pooled. The DNA library is checked and measured using Qubit and real-time PCR to measure the quantity of DNA present. The Dirty Data (low-quality) is removed using Qiime to produce Clean Data. Similar DNA/rRNA sequences were then clustered with 97% identity according to Operational Taxonomic Units (OTUs) prior to taxonomic annotation to obtain the corresponding taxa information and abundance distribution. To identify the organisms to different taxonomic levels, sequences are compared using reference databases; UNITE for fungi and SILVA for bacteria and the chimeras (false sequences) are removed.

4.3.7 Data Analysis and Statistical Methods

All data were processed using Excel and R Studio. The significance level for all tests was set at $p < 0.05$. GLMs were used to assess the effects of time and treatments on nutrient levels and their interaction. Data was imported into R Studio for statistical analysis and for plotting. Probability plots were created to test for normal distribution. Analysis of variance (ANOVA) tests and Dunnett's Multiple Comparisons of Means test was implemented to determine significant differences of the means in contrast to the control for percentage plant coverage.

The DHARMa package was implemented to check residuals, a GLM and ANCOVA negative binomial distribution model was chosen as the data was overdispersed, to assess the effects of nutrient levels, type of treatment and their interactions on microbial abundance. To visualise the beta-diversity compositional differences and similarities among treatments and over time Non-Metric Multidimensional Scaling (NMDS) plots were constructed. To test community composition between treatments and timepoints, plus the variance, PERMANOVA and PERMDISP were used.

4.4 Results

4.4.1 Soil Nutrient Levels

The nutrient levels of phosphorous, ammonium nitrate and the % of organic carbon varied within the soil samples (Table 4-6 and Figure 4-2). The phosphorous levels did not change significantly over time and neither did ammonium nitrate, but the % of organic carbon had risen significantly after four months, see Appendix Table C- 2. A significant interaction was noted as the levels of phosphorous had increased under the Hot Foam after four months (Figure 4-3). The levels of ammonium in the soil fell significantly under the Nomix Dual treatment one month and four months post application (Figure 4-4). No significant interactions occurred between any of the treatments and the % of organic carbon (Figure 4-5).

Table 4-6 The mean and standard error (SE) of nutrient levels in the soil samples taken from under each treatment at each of the three timepoints- Pre-treatment and two post-treatment at one month and four months.

		Pre-treatment	**Post-treatment One month**	**Post-treatment Four months**
	Treatment	**Mean (± SE)**	**Mean (± SE)**	**Mean (± SE)**
Phosphorous ppm	Control	4.56 (± 0.66)	4.27 (±0.88)	2.95 (±1.08)
	Roundup Flex	4.69 (±1.37)	6.39 (±1.02)	5.40 (±0.58)
	Nomix Dual	5.15 (±1.49)	5.16 (±1.86)	5.04 (±0.90)
	New Way	5.27 (±1.58)	4.25 (±0.81)	4.41 (±0.74)
	Hot Foam	5.21 (±1.20)	3.68 (±0.90)	6.06 (±0.69)
Ammonium ppm	Control	3.07 (±0.16)	3.59 (±0.23)	3.52 (±0.42)
	Roundup Flex	3.43 (±0.40)	3.01 (±0.50)	4.04 (±0.11)
	Nomix Dual	4.71 (±0.23)	2.97 (±0.18)	3.15 (±0.31)
	New Way	3.52 (±0.48)	2.94 (±0.23)	3.58 (±0.29)
	Hot Foam	3.13 (±0.46)	3.17 (±0.20)	3.47 (±0.34)
% Organic Carbon	Control	7.06 (±0.48)	6.95 (±0.30)	8.61 (±0.21)
	Roundup Flex	7.44 (±0.58)	7.78 (±0.49)	9.00 (±1.26)
	Nomix Dual	7.17 (±0.42)	7.78 (±0.25)	7.98 (±0.57)
	New Way	7.35 (±0.13)	8.03 (±0.20)	8.61 (±0.40)
	Hot Foam	7.17 (±0.27)	8.46 (±0.11)	8.02 (±0.28)

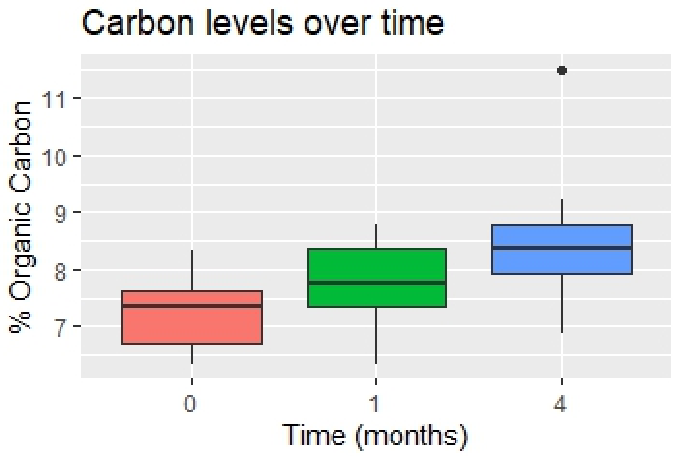


Figure 4-2 % of organic carbon over time at 0 months pre-treatment, one month and four months post-treatment.

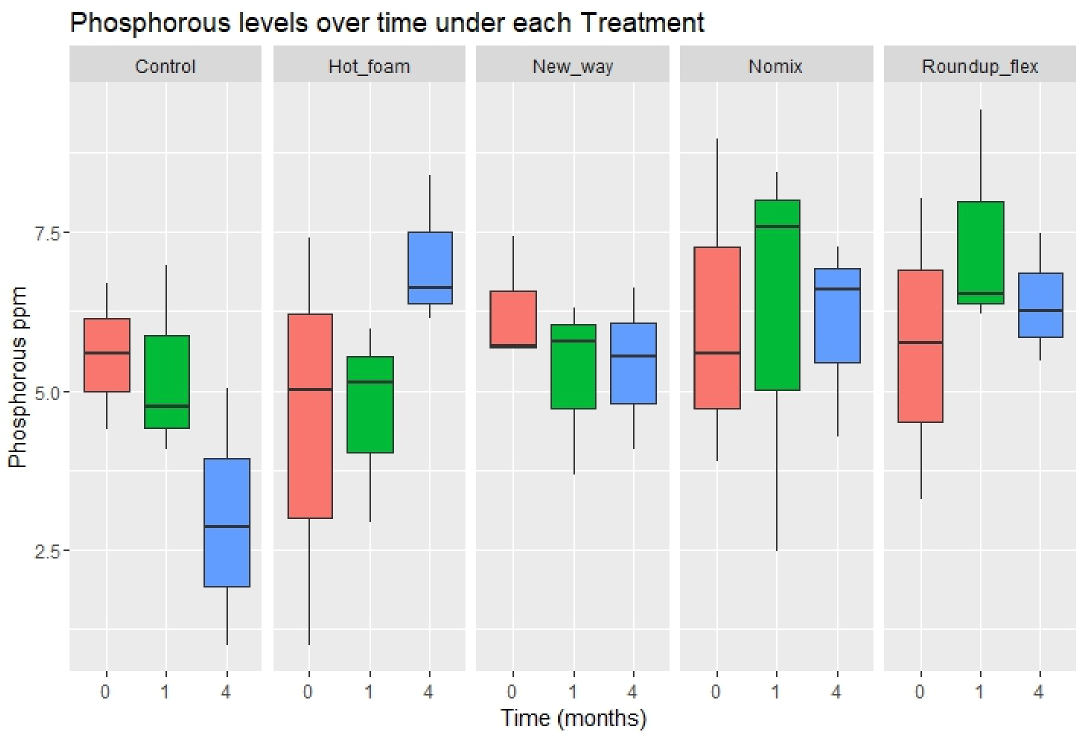


Figure 4-3 The levels of phosphorous ppm over the time under each treatment.

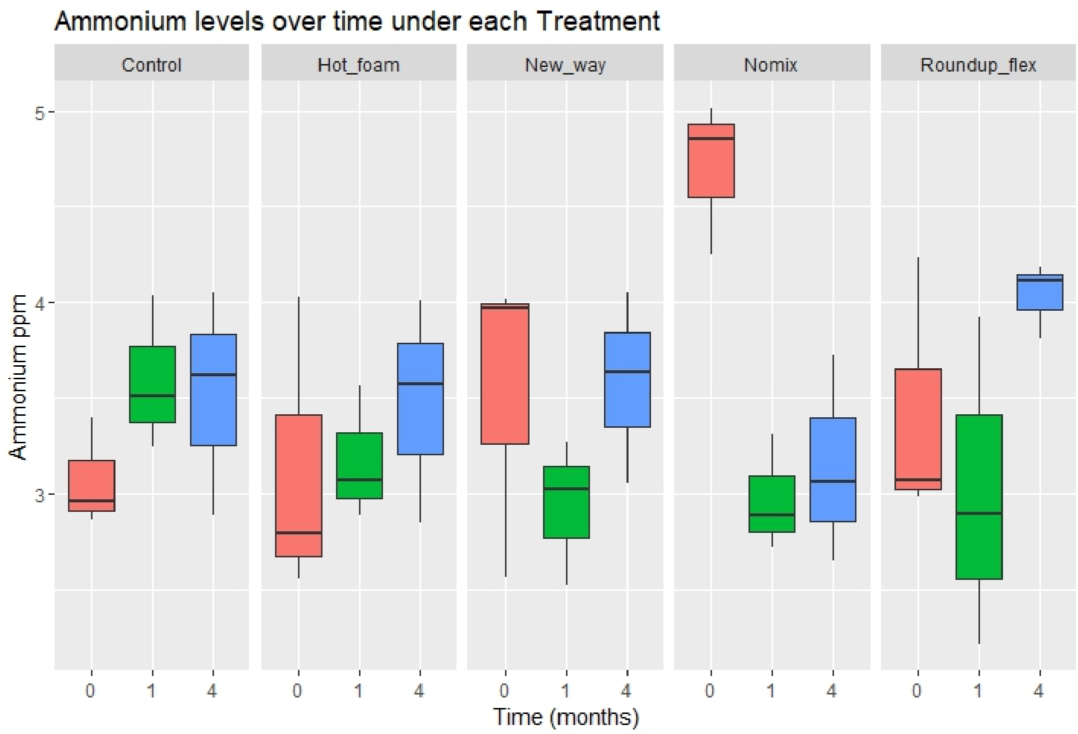


Figure 4-4 The levels of ammonium ppm over the time under each treatment.

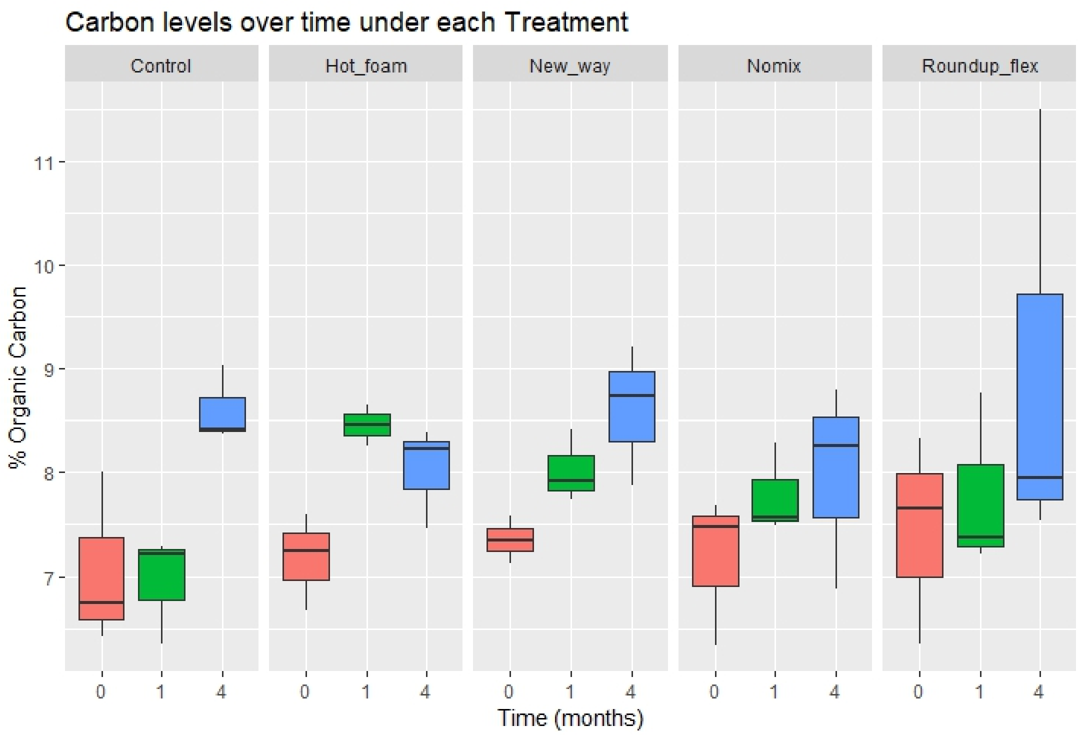


Figure 4-5 The % of organic carbon over the time under each treatment.

4.4.2 Vegetation Surveys

The effect that each of the five treatments had on the overall percentage cover of live vegetation for each 50cm x 50cm quadrat was assessed at one month and four months post-treatment. In contrast to the control, after one month the Hot Foam, Nomix Dual and Roundup Flex all had significant effects on the vegetation, while the New Way treatment had little impact (Table 4-7 and Figure 4-6). After four months there were no significant differences between the control and the treated plots due to the regrowth of vegetation (Table 4-8) and (Figure 4-6).

Table 4-7 Effects of each treatment on the mean percentage cover of live vegetation after one month in contrast to the control. Significant results are in bold.

Treatment	Estimate	Standard Error	t value	p-value
Hot Foam	-90.400	2.914	-31.028	**0.0001**
New Way	-2.667	2.914	-0.915	0.769
Nomix Dual	-95.000	2.914	-32.607	**0.0001**
Roundup Flex	-76.600	2.914	-26.291	**0.0001**

Table 4-8 Effects of each treatment on the mean percentage cover of live vegetation after four months in contrast to the control.

Treatment	Estimate	Standard Error	t value	p-value
Hot Foam	-5.267	7.321	-0.719	0.8827
New Way	-1.067	7.321	-0.146	0.9997
Nomix Dual	-16.467	7.321	-2.249	0.0897
Roundup Flex	17.133	7.321	2.340	0.0730

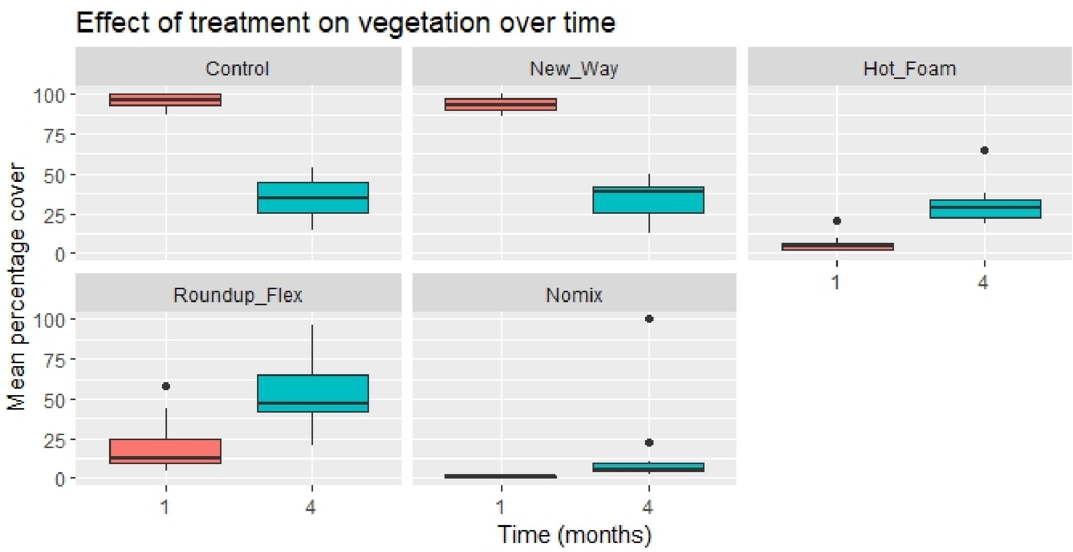


Figure 4-6 The effects of each treatment on the mean percentage cover of live vegetation after one month and four months.

4.4.2.1 Plant species

The improved grassland site contained twelve plant taxa: Bryophyta spp. (Moss spp.), *Cerastium* spp. (Chickweed spp.), *Cirsium* spp. (Thistle spp.), *Hieracium* spp. (Hawkweed spp.), *Persicaria maculosa* (Redshank), Poaceae spp. (Grass spp.), *Ranunculus* spp. (Buttercup spp.), *Rumex* spp. (Dock spp.), *J. vulgaris* (Ragwort), *Taraxacum* spp**.** (Dandelion spp.), *Trifolium* spp. (Clover spp.) and *Veronica* spp. (Speedwell spp.). Nine plant taxa were excluded from further analysis due to low percentage cover across plots at one month and four months respectively: Bryophyta spp. (0.13% and 1.85%), *Cerastium* spp. (0.07% and 0.00%), *Cirsium* spp. (0.07% and 0.75%), *Hieracium* spp. (0.05% and 0.00%), *Persicaria maculosa* (0.00% and 0.03%), *Ranunculus* spp. (0.33% and 0.27%), *Rumex* spp. (0.76% and 0.08%), *J. vulgaris* (0.00% and 0.08%) and *Veronica* spp. (0.00% and 0.05%), see Appendix Table C- 3 and Table C- 4. The following three plant taxa were assessed, as between the three of them they accounted for 48% of the overall coverage at one month and 29.5% at four months, with the percentage coverage for each type as follows: Poaceae spp. (35.12% and 15.59%, *Taraxacum* spp**.** (0.97% and 4.25%) and *Trifolium* spp. (12.31% and 9.41%).

Hot Foam was successful at significantly reducing two of the plant taxa, Poaceae spp. and *Trifolium* spp. at one month, however, after four months Poaceae spp. (Figure 4-7) had recovered (Table 4-9 and Table 4-10). *Trifolium* spp. were still negatively impacted after four months (Figure 4-7). Interestingly, the Hot Foam seemed to have no impact on *Taraxacum* spp., with this species increasing over time (Figure 4-7). New Way had no effect on any of the three plant taxa at either one or four months post-treatment. At one month post-treatment, Roundup Flex significantly reduced Poaceae spp. and *Trifolium* spp., but not *Taraxacum* spp**.** However, at four months the effects of the treatment had worn off for both plant groups. Overall, the Nomix Dual performed well and had succeeded in reducing all three types of plant taxa by both surveys. This treatment significantly reduced both Poaceae spp. and *Trifolium* spp. at one month and four months post-treatment, but not *Taraxacum* spp**.** at either time.

Table 4-9 Effect of each treatment on plant taxa after one month using the control as the comparison . Significant results are in bold.

Plant Species	Treatment	Mean	Standard Error	t value	p-value
Poaceae spp.					
	Hot Foam	-81.267	3.473	-23.402	**<0.001**
	New Way	-4.667	3.473	-1.344	0.473
	Nomix Dual	-83.733	3.473	-24.113	**<0.001**
	Roundup Flex	-73.400	3.473	-21.137	**<0.001**
***Trifolium* spp.**					
	Hot Foam	-28.933	5.419	-5.339	**<0.001**
	New Way	-2.400	5.419	-0.443	0.977
	Nomix Dual	-28.133	5.419	-5.191	**<0.001**
	Roundup Flex	-23.633	5.419	-4.361	**<0.001**
***Taraxacum* spp.**					
	Hot Foam	1.933	0.817	2.366	0.069
	New Way	1.000	0.817	1.224	0.555
	Nomix Dual	-0.600	0.817	-0.734	0.875
	Roundup Flex	-0.467	0.817	-0.571	0.944

Table 4-10 Effect of treatment on plant taxa after four months using the control as the comparison . Significant results are in bold.

Plant Species	Treatment	Mean	Standard Error	t value	p-value
Poaceae spp.					
	Hot Foam	-5.733	3.131	-1.831	0.212
	New Way	-0.000	3.131	0.000	1.000
	Nomix Dual	-19.40	3.131	-6.196	**<0.001**
	Roundup Flex	3.067	3.131	0.979	0.726
***Trifolium* spp.**					
	Hot Foam	6.867	2.589	-2.652	**0.034**
	New Way	-0.533	2.589	-0.206	0.999
	Nomix Dual	-6.933	2.589	-2.677	**0.032**
	Roundup Flex	5.733	2.589	2.214	0.097
***Taraxacum* spp.**					
	Hot Foam	10.400	1.711	6.078	**<0.001**
	New Way	0.133	1.711	0.078	1.000
	Nomix Dual	-2.200	1.711	-1.286	0.512
	Roundup Flex	1.267	1.711	0.740	0.872

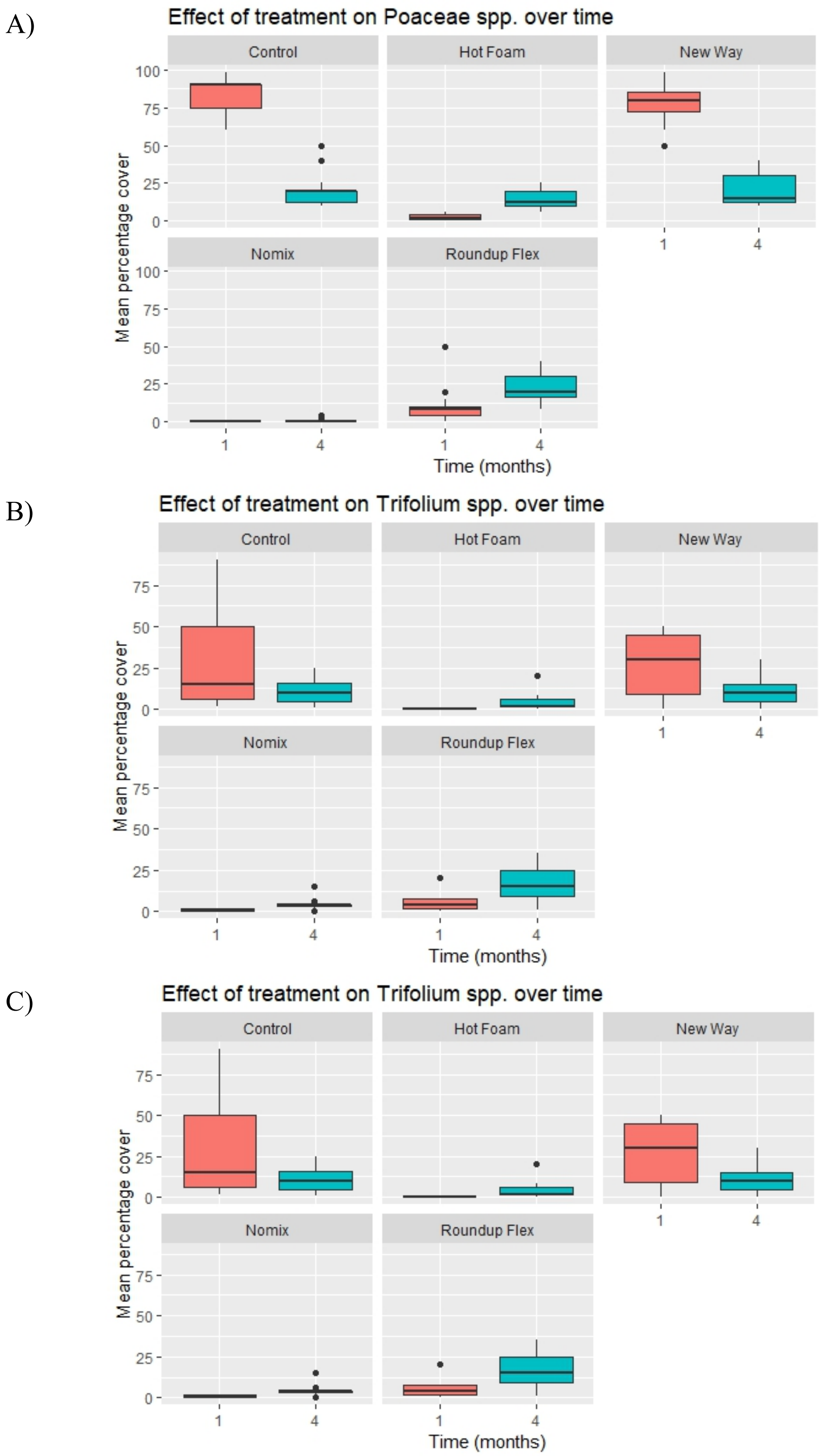


Figure 4-7 Effect of treatments on vegetation over time . Effect of each treatment on Poaceae spp. at one month and four months post-treatment (A). Effect of each treatment on *Trifolium* spp. at one month and four months post-treatment (B). Effect of each treatment on *Taraxacum* spp. at one month and four months post-treatment (C).

4.4.3 DNA analysis of the soil microbiome

DNA analysis was conducted on bacterial 16S rRNA and fungal ITS OTUs. The effect of the five treatments (Control, Hot Foam, New Way, Nomix Dual and Roundup Flex) at each of the three timepoints (0- initial sample pre-treatment, 1- one month post-treatment and 4- four months post-treatment) were assessed. The effect of each treatment was also analysed in conjunction with the soil physio-chemicals (moisture content, soil organic matter, water holding capacity, pH and texture- % clay, silt and sand) and nutrients (phosphorous ppm, ammonium nitrate ppm and % organic carbon).

4.4.3.1 Bacteria

Sequencing of total 16S rRNA soil samples resulted in 4,161,871 bacterial species counted representing 47 phyla. When the abundance of bacterial phyla were assessed against the control at each timepoint no significant differences were found (Appendix Table C- 5). However, when the bacteria were assessed over the three timepoints, a significant decrease in abundance was found under the Hot Foam treatment between the initial soil sample at zero months and the final sample at four months (Table 4-11 and Figure 4-8). Upon further investigation, the top ten phyla accounted for 90% of bacteria so these groups were analysed further (Appendix Table C- 6) for relative abundances. Four phyla decreased significantly under the Hot Foam treatment after four months; namely: Acidobacteriota, Firmicutes, Planctomycetota and Verrucomicrobiota while Crenarchaeota were significantly affected at one month and four months (Figure 4-9 and Appendix Table C- 7). Crenarchaeota are a major phylum within the Archaea.

Table 4-11 The results of bacterial abundance for each treatment against each timepoint . 0- initial sample at 0 months, 1- one month and 4- four months. Significant findings are in bold.

Treatment=Control:				
Contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	0.028	0.056	0.488	0.877
Timepoint 0-Timepoint 4	0.078	0.056	1.392	0.345
Timepoint 1-Timepoint 4	0.051	0.056	0.904	0.638
Treatment=Hot Foam:				
Contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	0.042	0.056	0.74	0.739
Timepoint 0-Timepoint 4	0.158	0.056	2.81	**0.014**
Timepoint 1-Timepoint 4	0.117	0.056	2.07	0.096
Treatment=New Way:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	-0.053	0.056	-0.934	0.618
Timepoint 0-Timepoint 4	-0.027	0.056	-0.485	0.879
Timepoint 1-Timepoint 4	0.025	0.056	0.449	0.895
Treatment=Nomix Dual:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	-0.008	0.056	-0.135	0.989
Timepoint 0-Timepoint 4	0.053	0.056	0.934	0.619
Timepoint 1-Timepoint 4	0.060	0.056	1.07	0.533
Treatment=Roundup Flex:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	0.0176	0.056	0.312	0.948
Timepoint 0-Timepoint 4	0.0482	0.056	0.855	0.669
Timepoint 1-Timepoint 4	0.0306	0.056	0.543	0.85

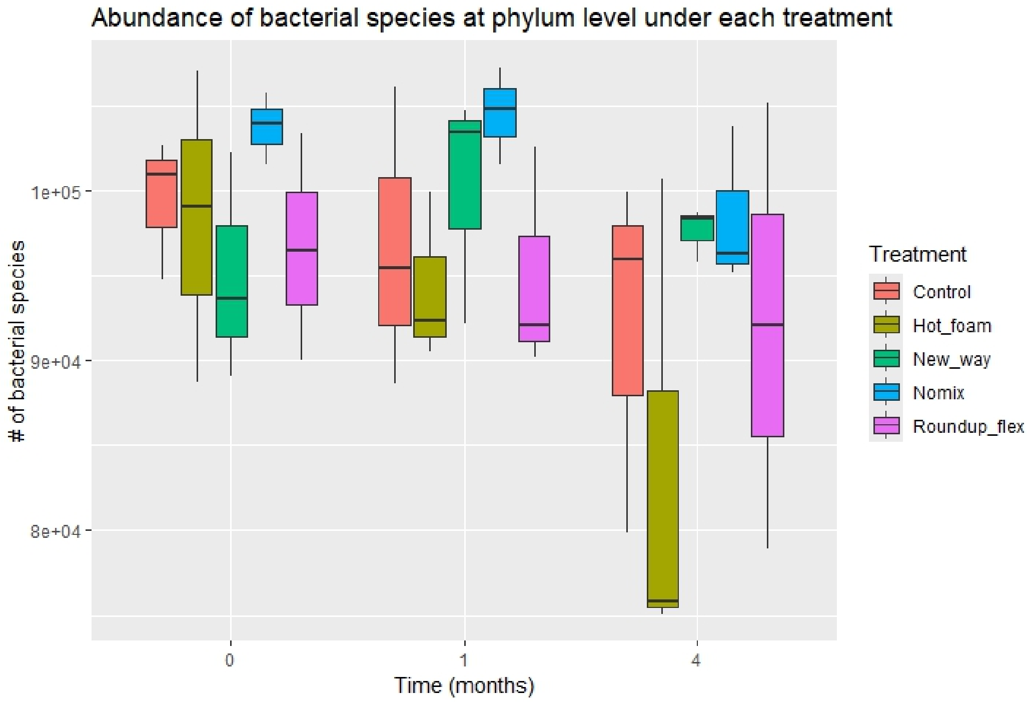


Figure 4-8 Abundance of bacterial species under each treatment at phyla level at each timepoint - 0- initial sample pre-treatment, 1- one month post-treatment and 4- four months post-treatment.

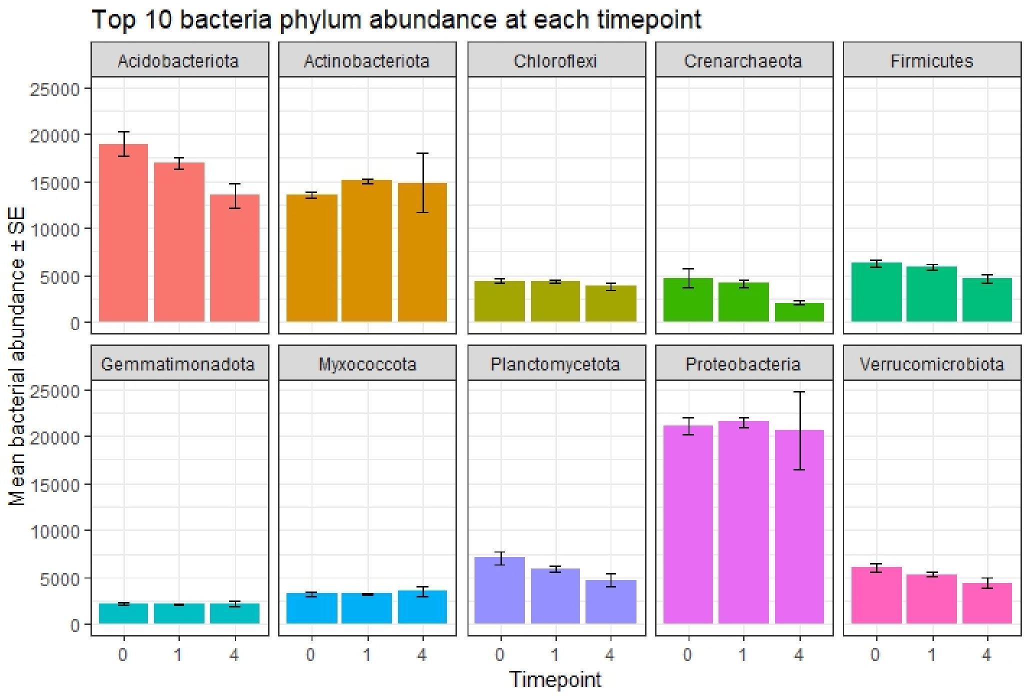


Figure 4-9 The effect of Hot Foam on the top ten most abundant soil bacteria at phylum level over time; 0- pre-treatment, 1- one month and 4- four months**.**

4.4.3.2 Bacteria and soil physio-chemical properties

The majority of the physio-chemicals in the soil in combination with each of the treatments had no effect on the abundance of bacteria (Appendix Table C- 8 and Figure C- 1). However, a significant interaction was observed between phosphorous and the Hot Foam treatment suggesting that the effect of Hot Foam may increase bacterial abundance in conjunction with a rise phosphorous (Table 4-12 and Figure 4-10).

Table 4-12 The effects of phosphorous levels in combination with treatment on the abundance of bacteria . Significant results are in bold.

Phosphorous ppm	Estimate	SE	z value	p-value
Hot Foam-Control==0	-0.31122	0.12244	-2.542	**0.0385**
New Way-Control==0	-0.05028	0.13716	-0.367	0.9883
Nomix Dual-Control==0	-0.02909	0.10855	-0.268	0.9964
Roundup Flex-Control==0	0.01190	0.12305	0.097	0.9999

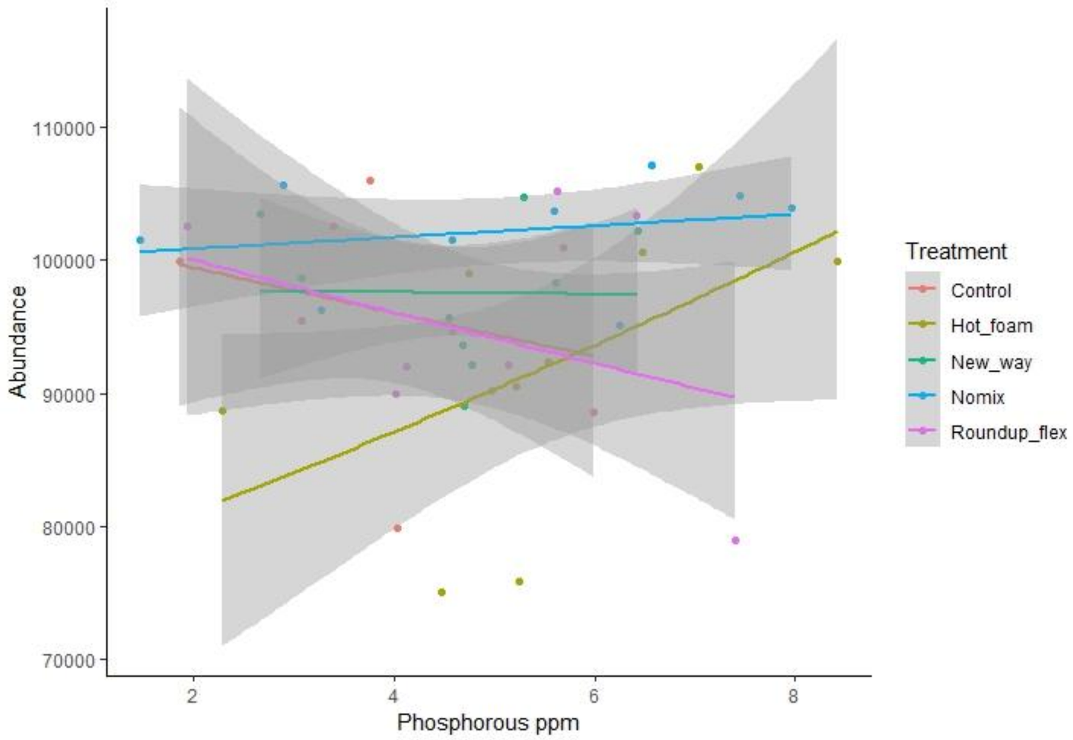


Figure 4-10 The levels of phosphorous in the soil under each treatment and the abundance of bacteria.

4.4.3.3 Bacterial community at species level

The species richness of the bacterial community did not differ significantly by treatment (p=0.109) or over time (p=0.055) in the Shannon and Simpson indices (Table 4-13 and Figure 4-11). The Shannon diversity measures both the species diversity and evenness in a sample, the higher the value the higher the diversity. Simpson diversity measures the dominance of species with higher values relating to lower diversity.

The environmental variables were assessed over time and by treatment using correlations between the environmental variables. Nine of the environmental variables were significant; namely, % clay content, % silt content, water holding capacity, pH, soil organic matter, % organic carbon, ammonium nitrate, % sand content and moisture content (Table 4-14). They show how the species richness of the bacteria is structured in relation to time (Figure 4-12). In relation to time, there is a stronger correlation between the water holding capacity, pH and soil organic matter after four months than the previous two timepoints showing how the species composition changed over time. The % of sand lies in the opposite direction to clay and silt showing an inverse relationship in regard to soil texture. In relation to treatment there is little difference between the species composition as the ellipses mainly overlap. Soil organic matter in conjunction with the Roundup Flex treatment may slightly influence species richness. The NMDS ordination revealed little distinction between the clustering of treatments suggesting that the soil properties do not mediate effects on the community structure of the bacteria.

However, even though the Shannon and Simpson diversity indices showed no significant differences in bacterial diversity under treatments or over time, PERMANOVA revealed significant shifts in the composition of bacterial species structure under the Nomix Dual treatment in comparison to the control and between the initial time and four months later (Table 4-15). This indicates that the bacterial community structure changed over time and in response to Nomix Dual even though the overall diversity remained stable.

Table 4-13 Mean diversity of bacterial species over time and by treatment with Shannon and Simpson diversity indices.

Time (months)	Treatment	Mean ± SE	Shannon ± SE	Simpson ± SE
0	Control	173.00 ± 5.03	0.40 ± 0.01	0.10 ± 0.00
0	Hot Foam	172.33 ± 4.18	0.41 ± 0.01	0.11 ± 0.00
0	New Way	177.00 ± 6.51	0.42 ± 0.01	0.11 ± 0.00
0	Nomix Dual	170.67 ± 9.91	0.41 ± 0.04	0.11 ± 0.01
0	Roundup Flex	176.67 ± 4.48	0.41 ± 0.01	0.11 ± 0.00
1	Control	172.67 ± 2.96	0.43 ± 0.01	0.11 ± 0.00
1	Hot Foam	175.00 ± 5.86	0.43 ± 0.01	0.11 ± 0.00
1	New Way	179.67 ± 5.49	0.43 ± 0.03	0.11 ± 0.01
1	Nomix Dual	173.67 ± 1.45	0.39 ± 0.02	0.10 ± 0.01
1	Roundup Flex	163.00 ± 7.00	0.40 ± 0.03	0.10 ± 0.01
4	Control	178.00 ± 3.21	0.48 ± 0.01	0.13 ± 0.00
4	Hot Foam	166.33 ± 5.17	0.42 ± 0.02	0.11 ± 0.01
4	New Way	177.00 ± 4.36	0.44 ± 0.01	0.12 ± 0.00
4	Nomix Dual	177.33 ± 5.78	0.44 ± 0.00	0.11 ± 0.00
4	Roundup Flex	178.00 ± 7.21	0.43 ± 0.02	0.12 ± 0.01

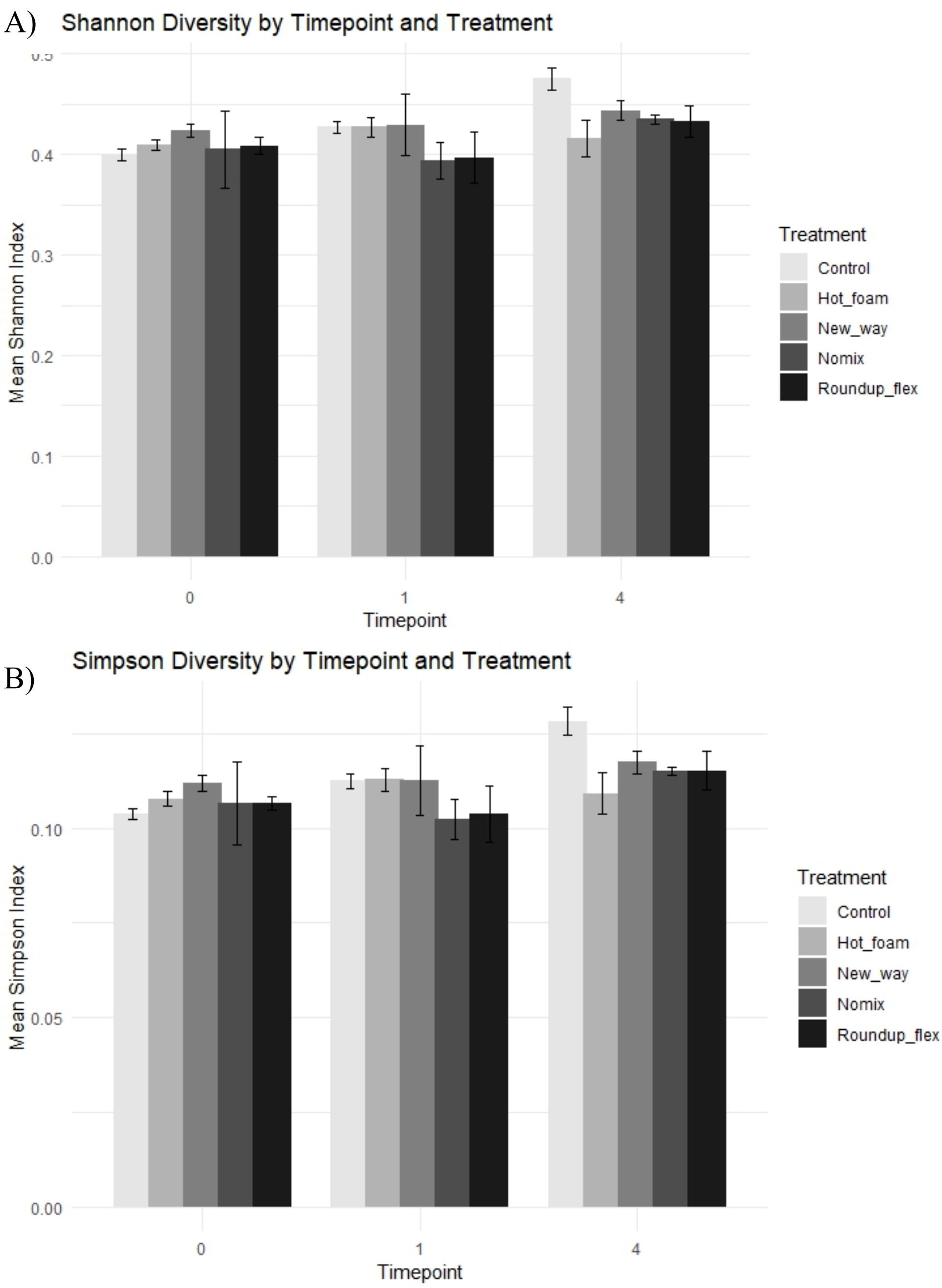
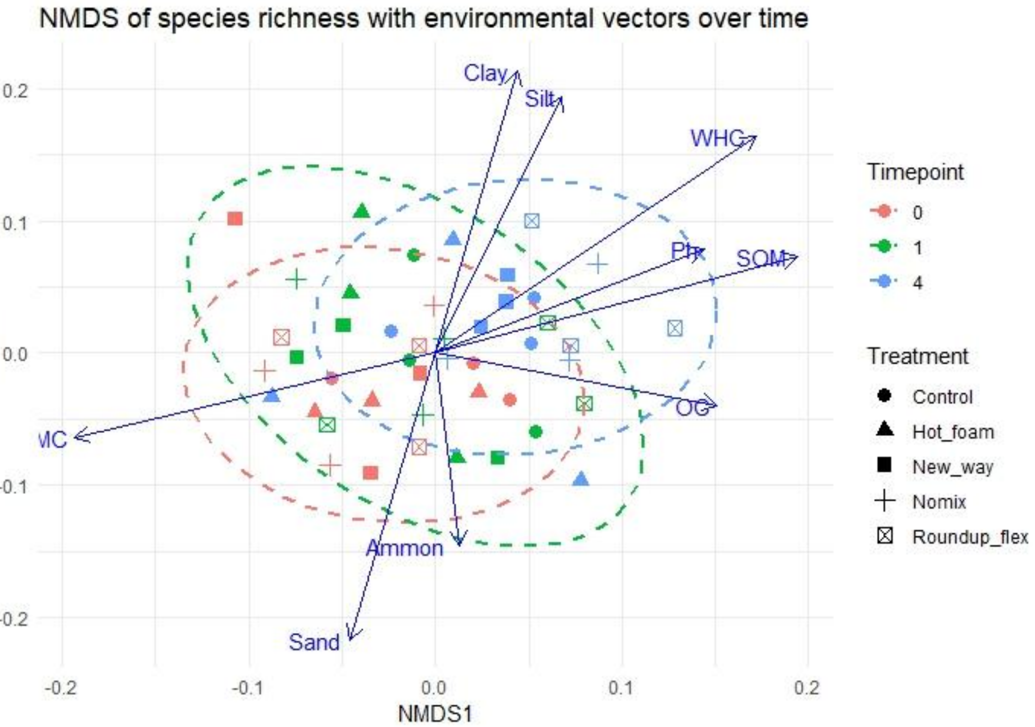


Figure 4-11 Bacterial diversity . The Shannon diversity (A) and the Simpson diversity (B) over time and treatment with standard error.

Table 4-14 The significance of environmental variables and NMDS ordination axes testing each variable independently. Significant results are in bold.

Variable	**R^2**	**P-value**	**Variable**	**R^2**	**P-value**
Phosphorous	0.016	0.73	Water holding capacity	0.366	**0.001**
Ammonium nitrate	0.110	0.083	pH	0.167	**0.021**
% Organic carbon	0.095	0.113	Clay	0.191	**0.012**
Moisture content	0.249	**0.003**	Silt	0.226	**0.006**
Organic matter	0.252	**0.002**	Sand	0.222	**0.007**

A)



B)

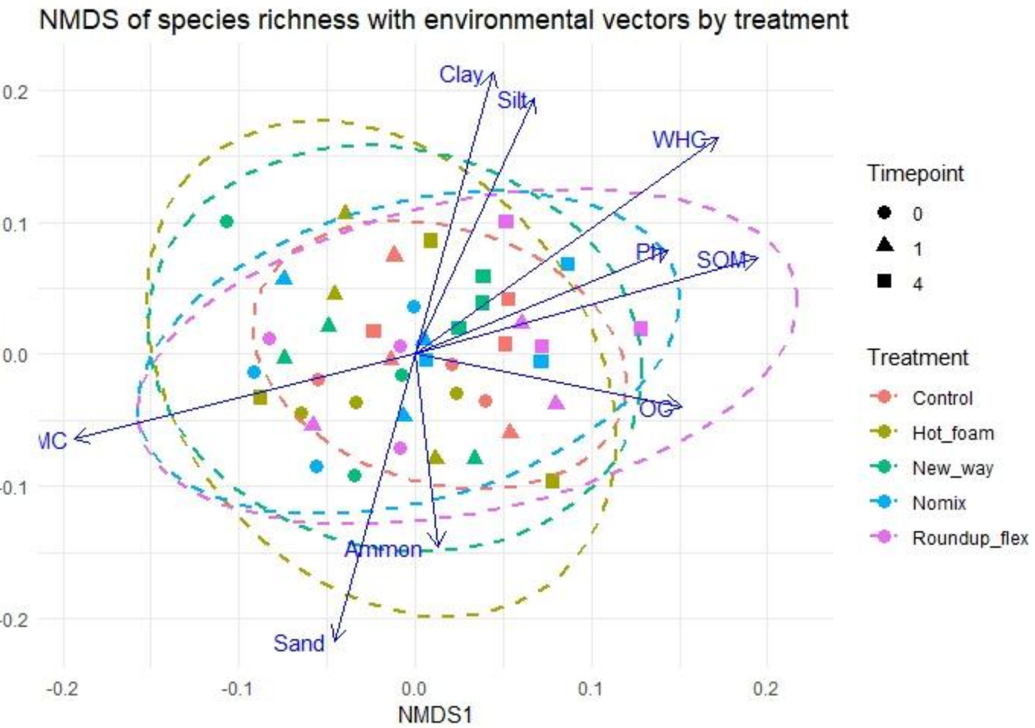


Figure 4-12 Bacterial species richness over each of the three timepoints (A) and species richness over each of the five treatments (B) with the significant environmental variables (Clay- % clay content, Silt- % Silt content, WHC- Water holding capacity, Ph- pH, SOM- Soil organic matter, OC- % organic carbon, Ammon- Ammonium nitrate, Sand- % sand content and MC- moisture content).

Table 4-15 Pairwise PERMANOVA comparisons between each of the treatments and each of the timepoints on bacterial species diversity . Significant differences are in bold.

Comparisons- Treatment	F-statistic	R^2	p-value
Control vs Hot Foam	0.750	0.040	0.410
Control vs Nomix Dual	3.910	0.200	**0.040**
Control vs New Way	0.260	0.020	0.730
Control vs Roundup Flex	0.160	0.010	0.810
Comparisons- Time	**F-statistic**	**R^2**	**p-value**
0 vs 1 month	0.320	0.010	0.640
0 vs 4 months	4.040	0.130	**0.050**
1 vs 4 months	3.080	0.100	0.080

4.4.3.4 Fungi

The results revealed a rich fungal diversity with 13 phyla, 42 classes, 96 orders, 190 families, 350 genera and 464 species identified in the soil samples. When fungal abundance was assessed in contrast to the control at each timepoint, the fungal abundance had significantly increased under the Hot Foam at four months (Figure 4-13 and Appendix Table C- 9). The fungi were then assessed under each treatment over time and a significant decrease in abundance was found under the Control and Roundup Flex treatments between the second sampling at one month and the third sampling at four months. Fungal abundance significantly decreased under the Nomix Dual treatment between the initial sampling event and four months later (Table 4-16 and Figure 4-13).

The dominant four phyla accounted for 98% of fungi so these were further investigated (Appendix Table C- 10). The abundance of the four phyla namely: Ascomycota, Basidiomycota, Mortierellomycota and Others (as yet unnamed) varied under each of the treatments at one month and four months post-treatment (Figure 4-14). The abundance of Ascomycota fungi grew significantly under the Control in the first month post the application of treatments before falling significantly by the fourth month (Appendix Table C- 11). Under the Hot Foam Ascomycota increased steadily over the four months. The abundance of these phyla rose in the first month but by the third sampling date had decreased significantly under the Roundup Flex treatment.

Basidiomycota increased significantly under the Hot Foam in the first month, before decreasing in abundance. Under the Nomix Dual treatment the Basidiomycota abundance rose during the initial month but had decreased significantly by the fourth month. Mortierellomycota decreased under the Control significantly between month one and four, while the abundance significantly decreased over the four months under the Nomix Dual. The Others decreased significantly over the four months under the Control, Hot Foam and Nomix Dual treatments while under the New Way and Roundup Flex, they increased initially during the first month before falling significantly by the fourth month.

Table 4-16 Fungal abundance for each treatment against each timepoint . 0- initial sample at 0 month, 1- one month and 4- four months. Significant findings are in bold.

Treatment=Control:				
Contrast	Estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	-0.0897	0.162	-0.552	0.8452
Timepoint 0-Timepoint 4	0.2894	0.162	1.783	0.1754
Timepoint 1-Timepoint 4	0.3791	0.162	2.335	**0.0511**
Treatment=Hot Foam:				
Contrast	Estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	-0.1692	0.162	-1.042	0.5502
Timepoint 0-Timepoint 4	-0.2461	0.162	-1.516	0.2832
Timepoint 1-Timepoint 4	-0.077	0.162	-0.474	0.8835
Treatment=New Way:				
Contrast	Estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	-0.097	0.162	-0.598	0.8214
Timepoint 0-Timepoint 4	0.0126	0.162	0.078	0.9967
Timepoint 1-Timepoint 4	0.1096	0.162	0.675	0.778
Treatment=Nomix Dual:				
Contrast	Estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	0.2599	0.162	1.601	0.2452
Timepoint 0-Timepoint 4	0.5398	0.162	3.325	**0.0025**
Timepoint 1-Timepoint 4	0.2799	0.162	1.724	0.196
Treatment=Roundup Flex:				
Contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	-0.3155	0.162	-1.944	0.1266
Timepoint 0-Timepoint 4	0.2351	0.162	1.448	0.3163
Timepoint 1-Timepoint 4	0.5506	0.162	3.392	**0.002**

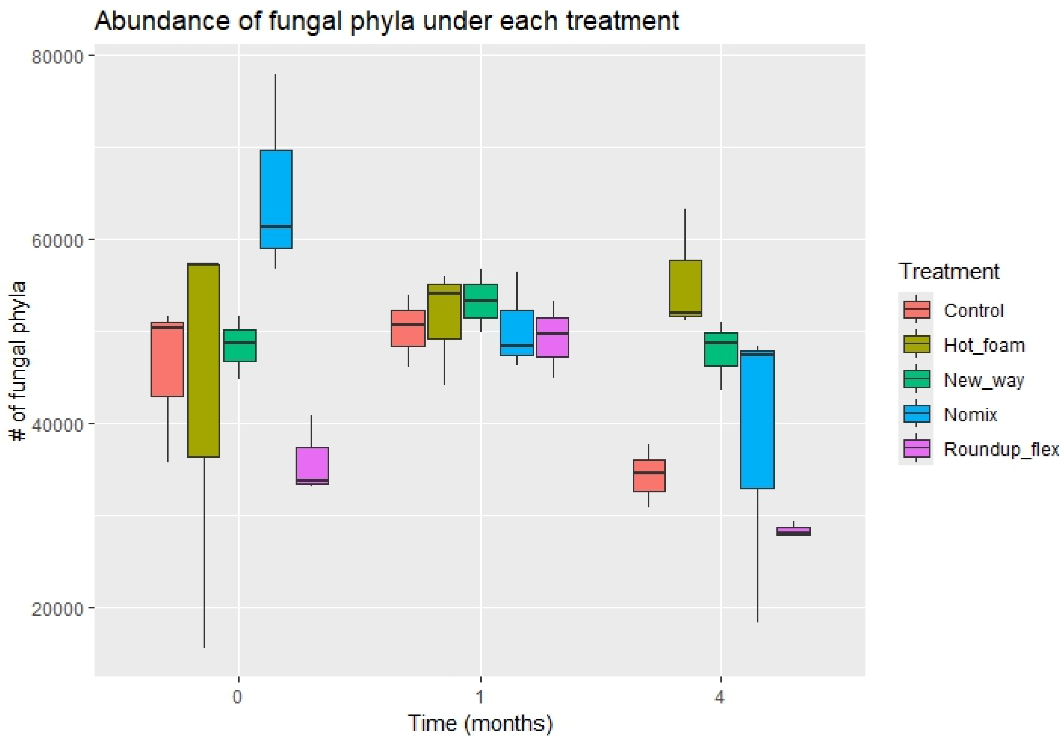


Figure 4-13 Abundance of fungi under each treatment at phyla level at each timepoint - 0- initial sample pre-treatment, 1- one month post-treatment and 4- four months post-treatment.

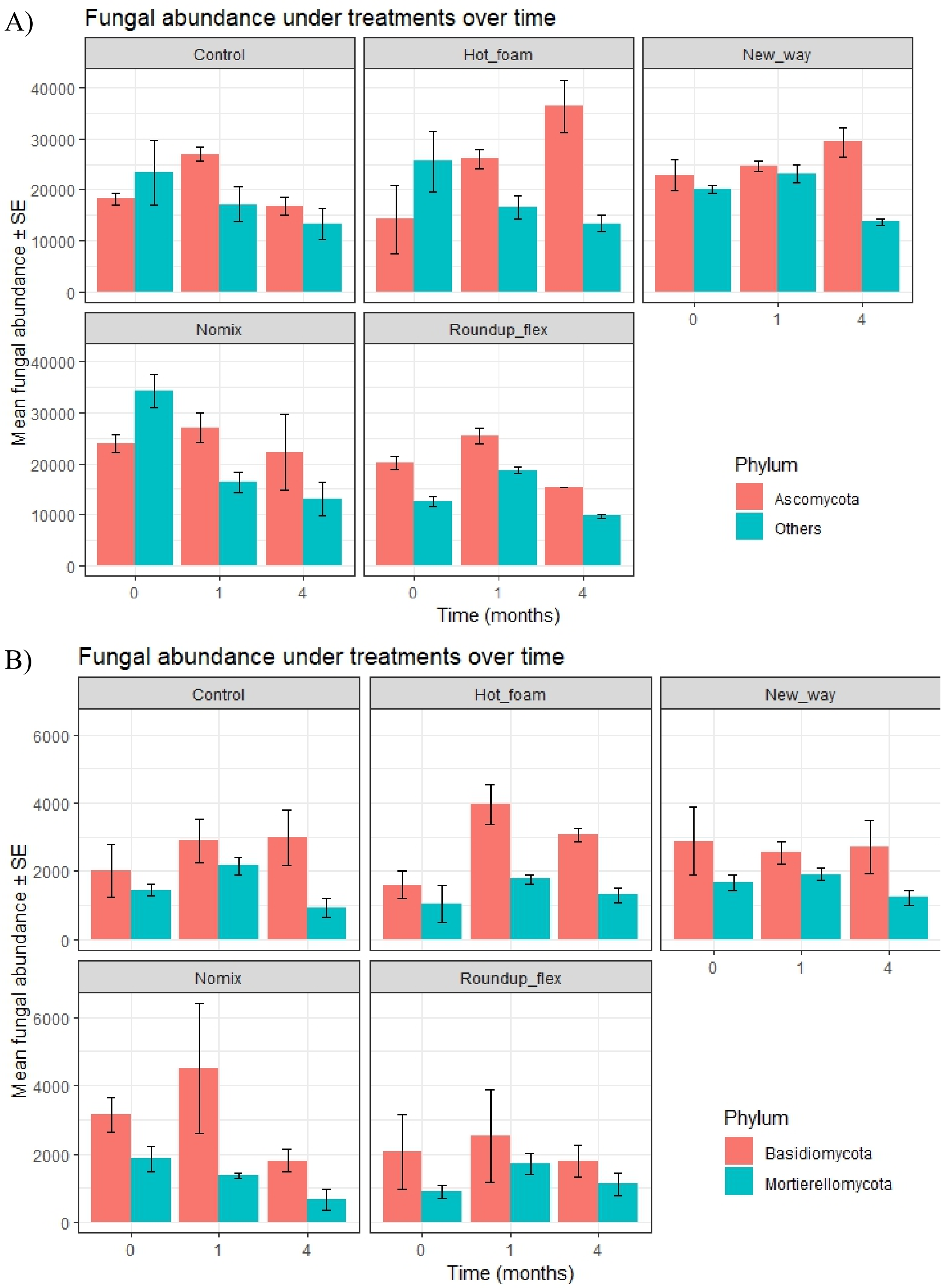


Figure 4-14 Abundance of Ascomycota and "Others" over time (A) abundance over time under each treatment at the initial pre-treatment time- 0 months, one month and four months post-treatment. Basidiomycota and Mortierellomycota (B) abundance over time under each treatment at the initial pre-treatment time- 0-month, one month and four months post-treatment.

4.4.3.5 Fungi and soil physio-chemical

Modelling revealed a significant interaction between pH and the Hot Foam treatment suggesting that the effect of Hot Foam with an increase in pH may increase fungal abundance (Table 4-17 and Figure 4-15). No other soil physio-chemicals in conjunction with a treatment impacted fungal abundance (Appendix Table C- 12 and Figure C- 2).

Table 4-17 The effects of pH levels on the abundance of fungi under all treatments . Significant results are in bold**.**

pH	Estimate	SE	z value	p-value
Hot Foam-Control==0	-59.475	12.126	-4.905	**0.0004**
New Way-Control==0	-12.425	12.125	-1.025	0.693
Nomix Dual-Control==0	-1.551	12.124	-0.128	1
Roundup Flex-Control==0	-13.823	12.125	-1.14	0.61

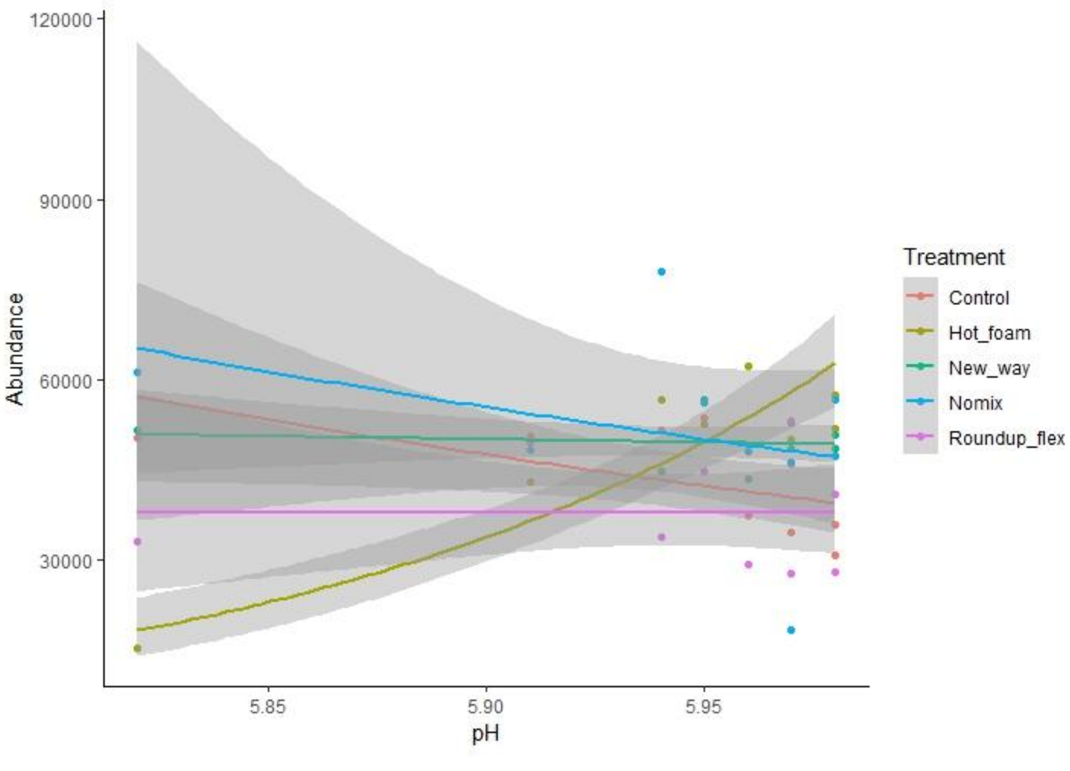


Figure 4-15 The levels of pH in the soil under each treatment and the abundance of fungi phyla.

4.4.3.6 Fungal community at species level

The species richness of the fungal community did differ significantly over time in the Shannon and Simpson indices: after one month (p=0.0003) and after four months (p=0.022) (Table 4-18 and Figure 4-16). The environmental variables were assessed over time and by treatment using correlations between the environmental variables, with four found to be significant; namely, % organic carbon, moisture content, soil organic matter and pH (Table 4-19). They show how the species richness of the fungi are structured in relation to time (Figure 4-17). In relation to time, the moisture content shows a stronger correlation with the initial timepoint pre-treatment. There is a stronger relationship between pH, soil organic matter and the % organic carbon after four months than the previous two timepoints. The clear clustering over time suggests that the temporal variation may mediate the effects of the fungal community structure. The NMDS ordination revealed little distinction between the clustering of treatments, suggesting little effect of either treatment or environmental variables, on the community structure of the fungi.

PERMANOVA revealed significant shifts in the composition of fungal species structure over time (Table 4-20). It did not however, find any significant differences in species composition under any of the treatments. This indicates that the fungal community structure changed over time but not necessarily because of treatment.

Table 4-18 Mean diversity of fungal species over time and by treatment with Shannon and Simpson diversity indices.

Time (months)	Treatment	Mean ± SE	Shannon ± SE	Simpson ± SE
0	Control	142.67 ±17.40	1.11 ± 0.18	0.36 ± 0.06
0	Hot Foam	109.33 ±34.16	0.74 ± 0.23	0.23 ± 0.07
0	New Way	164.33 ±2.33	1.30 ± 0.03	0.43 ± 0.02
0	Nomix Dual	162.33 ±4.10	1.17 ±0.05	0.37 ± 0.02
0	Roundup Flex	134.67 ±8.69	1.22 ±0.10	0.41 ± 0.04
1	Control	200.67 ±2.33	1.60 ± 0.17	0.52 ± 0.06
1	Hot Foam	193.67 ±11.46	1.61 ±0.09	0.50 ± 0.04
1	New Way	180.33 ± 7.86	1.30 ± 0.08	0.41 ± 0.02
1	Nomix Dual	195.67 ±6.67	1.46 ± 0.09	0.44 ± 0.02
1	Roundup Flex	183.67 ± 14.99	1.39 ± 0.15	0.44 ± 0.04
4	Control	132.00 ±6.24	1.17 ± 0.07	0.38 ± 0.03
4	Hot Foam	197.67 ±8.41	1.62 ± 0.06	0.59 ± 0.04
4	New Way	182.67 ±15.86	1.46 ± 0.07	0.45 ± 0.02
4	Nomix Dual	144.67 ±45.29	1.13 ± 0.24	0.36 ± 0.06
4	Roundup Flex	137.67 ±5.46	1.33 ± 0.08	0.42 ± 0.03

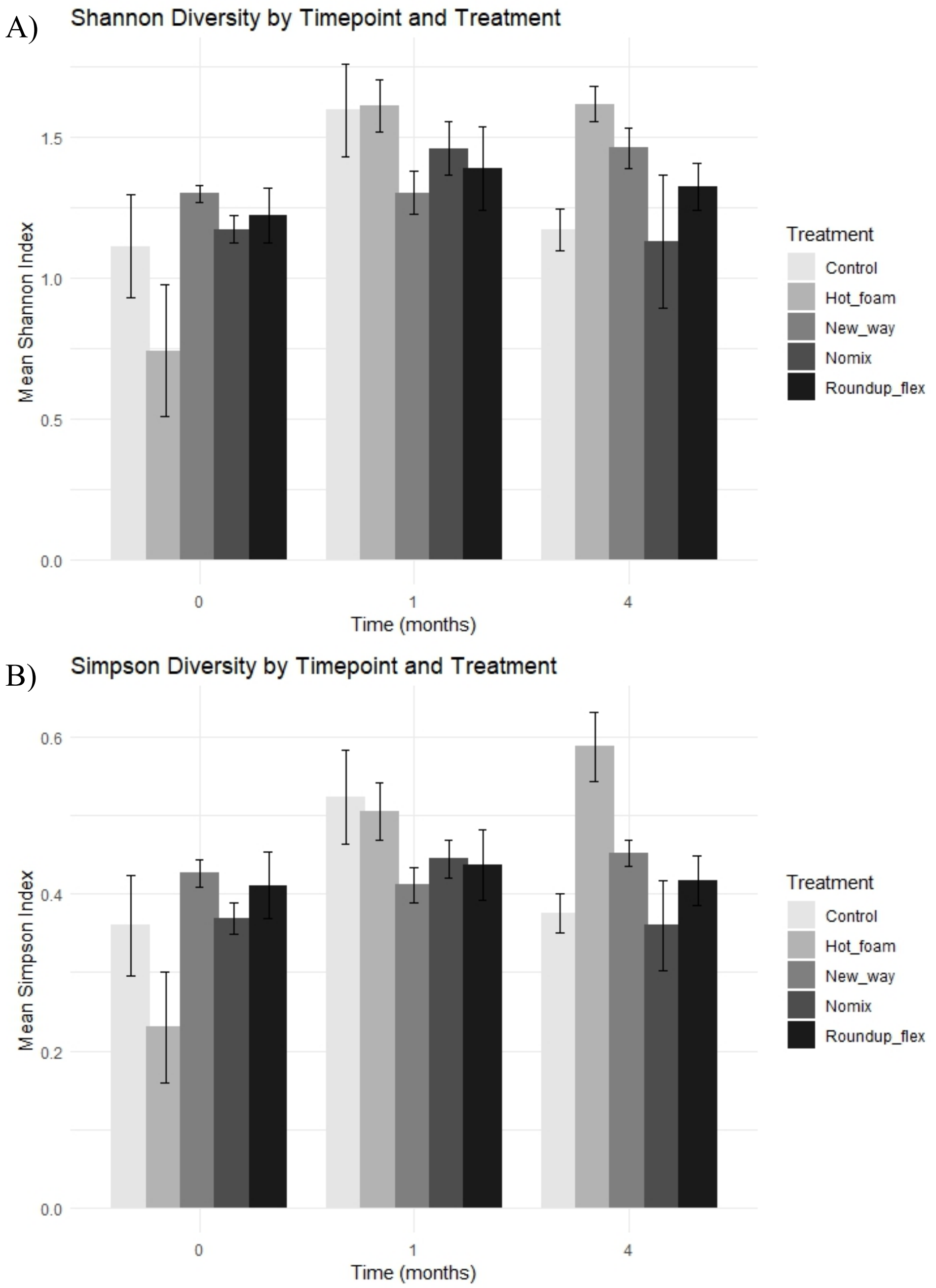


Figure 4-16 Fungal diversity under each treatment over time. The Shannon diversity (A) and the Simpson diversity (B) over time and treatment with standard error.

Table 4-19 The significance of environmental variables and NMDS ordination axes testing each variable independently . Significant results are in bold.

Variable	R^2	P-value	Variable	R^2	P-value
Phosphorous	0.006	0.887	Water holding capacity	0.077	0.194
Ammonium nitrate	0.030	0.549	pH	0.303	**0.001**
% Organic carbon	0.245	**0.003**	Clay	0.031	0.514
Moisture content	0.274	**0.003**	Silt	0.054	0.302
Organic matter	0.259	**0.002**	Sand	0.024	0.592

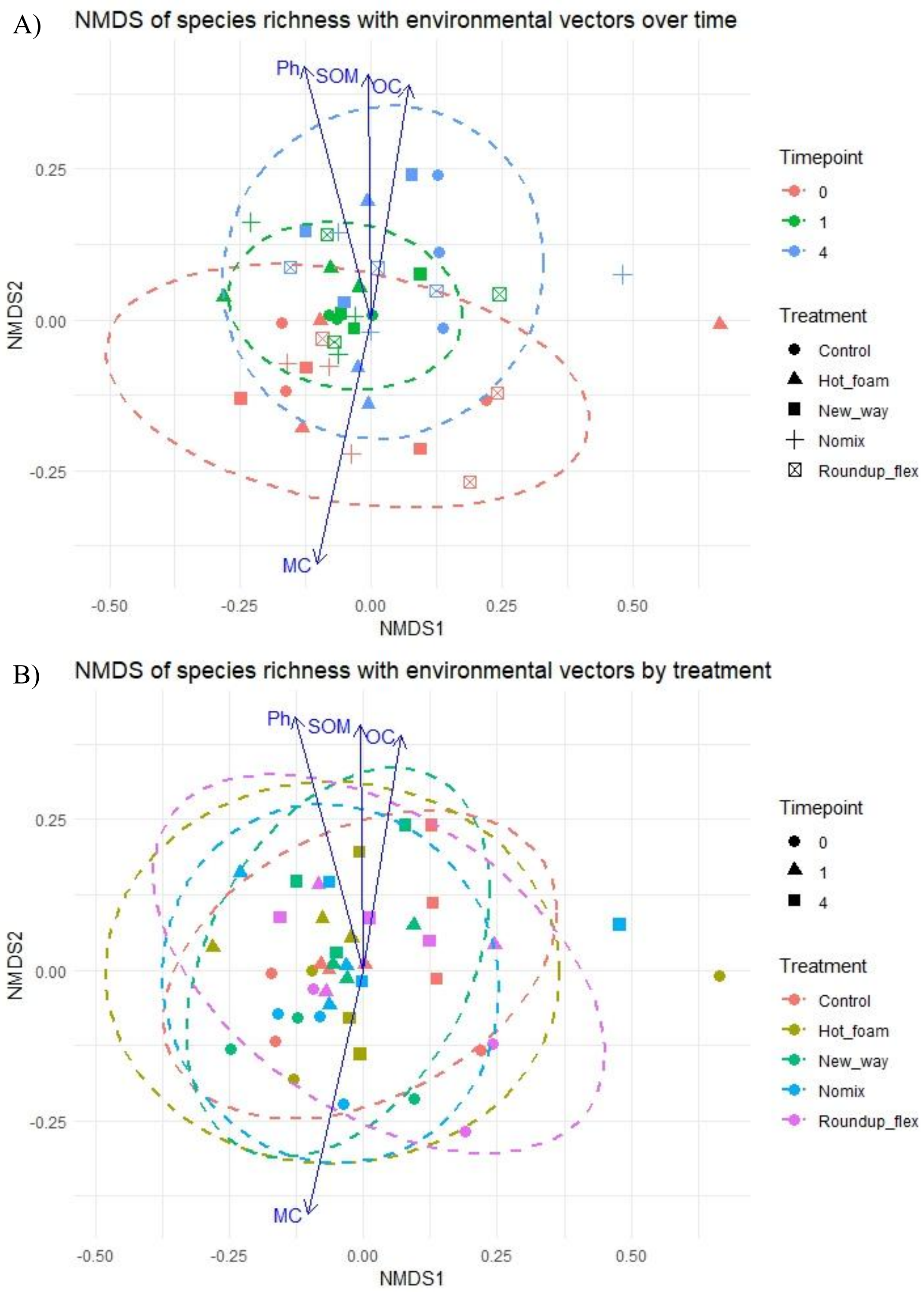


Figure 4-17 Fungal species richness over each of the three timepoints (A) and over each of the five treatments (B) with significant environmental variables (Ph- pH, SOM- Soil organic matter, OC- % organic carbon and MC- moisture content).

Table 4-20 Pairwise PERMANOVA comparisons between each of the treatments and each of the timepoints on fungal species diversity . Significant differences are in bold.

Comparisons- Treatment	F-statistic	R^2	p-value
Control vs Hot Foam	0.932	0.055	0.462
Control vs Nomix Dual	0.513	0.031	0.671
Control vs New Way	1.536	0.088	0.222
Control vs Roundup Flex	0.850	0.050	0.461
Comparisons- Time	**F-statistic**	**R^2**	**p-value**
0 vs 1 month	4.313	0.133	**0.007**
0 vs 4 months	5.785	0.171	**0.002**
3 vs 4 months	7.243	0.206	**0.003**

4.5 Discussion

Bacterial and fungal microorganisms are known to react swiftly to changes in their environment, are essential in the carbon and nitrogen cycles, and are part of the network of other plants and organisms within the soil ecosystem. Numerous studies have suggested that these communities are influenced by soil nutrients and physio-chemical properties (Liu *et al.*, 2023). It is also known that these microbial communities are impacted by chemical inputs in the form of fertilisers and plant protection products such as pesticides (Walters & Martiny, 2020). Several experiments have been conducted on the effects of glyphosate on the soil microbiome, but results can be contradictory. For example, Meftaul *et al*. (2020) have suggested that there is low toxicity to microorganisms and Van Bruggen *et al*. (2018) observed that in short experiments with glyphosate, the microorganism community recovered with little effect. In an experiment against ten alternative treatments, glyphosate was the most effective at reducing vegetation with no discerned effects on soil microbiota and physiochemical properties (Hudek *et al.*, 2021). On the other hand, negative effects of glyphosate on bacteria and fungi have been shown in several studies, with some reporting the reduction in arbuscular mycorrhizal fungi by as much as 40% (Meena *et* *al.*, 2020). The effect of glyphosate is highly dependent on the quantity of glyphosate used, with higher quantities leading to an increase in biomass and respiration levels by providing C, N and P as sources for metabolisation (FAO, 2017). Studies on the use of glyphosate and its impact on the soil microbiome have shown conflicting results with location often determining the structure of the soil microbial community rather than glyphosate (Kepler *et al.*, 2020). Standard protocols to test and analyse the hazards of pesticides are limited when it comes to their effects on microorganisms within the soil ecosystem.

To determine the effects of the widely used herbicide, glyphosate, and some potential alternatives on key indicators such as vegetation cover and microbial community composition and function, experiments were conducted on a greenfield site previously used as improved grassland. The specific aims of these experiments were to determine i), the effects of glyphosate on overall soil health and ii), whether products and approaches deemed more "environmentally friendly" have similar plant control effectiveness but with less impact on soil health. It is hoped that through this investigation, recommendations can be made to our county councils that result in the phasing out of glyphosate and replacing it with viable and sustainable alternatives.

Vegetation cover and composition initially decreased significantly under three treatments: the two glyphosate treatments- Nomix Dual and Roundup Flex, and one alternative treatment, the Hot Foam, after one month, but by the final survey four months later, the overall vegetation cover had recovered. The same three treatments affected Poaceae spp. and *Trifolium* spp. after a month but interestingly had had no effect on *Taraxacum* spp. Four months post-treatment the effects of the Roundup Flex had dissipated, Nomix Dual still had a significant effect on the Poaceae spp. and *Trifolium* spp., whereas the Hot Foam still affected *Trifolium* spp. but not Poaceae spp.

Of particular interest, *Taraxacum* spp. flourished under the Hot Foam treatment at four months. This plant is notoriously difficult to control due to its long tap root. Steam treatment in another study was found to have had similar effects, reducing vegetation initially, though its efficacy has been attributed to the length of exposure, the type of plant and its growth stage (Hudek *et al.*, 2021). Overall, both the glyphosate treatments, Nomix Dual and Roundup Flex performed well, successfully reducing vegetation after one month. The Nomix Dual continued to suppress the vegetation for a further four months. Of the two alternatives, only the Hot Foam produced comparable results to the glyphosate treatments. These findings are corroborated by research conducted in Greece (Antonopoulos *et al.*, 2023). The New Way treatment, constituting acetic acid, failed to have any impact on either the vegetation, bacteria or fungi as has been found in other studies using acetic acid products (Hudek *et al.*, 2021; Antonopoulos *et al.*, 2023).

The impact on bacterial and fungal abundance and diversity was investigated in relation to treatments, soil nutrients and physio-chemical properties. Of the nutrients assessed, the overall percentage of organic carbon in the soil increased over time, potentially due to the decomposition of decaying plant material as the vegetation above ground died off, and carbon was released into the rhizosphere. This was noted as a positive indirect effect of glyphosate use by Wardle *et al*. (2004). The levels of phosphorus increased under the Hot Foam, and this may be attributed to the high temperatures created while applying this treatment. It is known that an increase in temperature can cause mineral transformations to occur that lead to the release of phosphorous into the soil (Croat *et al.*, 2020). The ammonium nitrate decreased under the glyphosate treatment, Nomix Dual, but this may have been an anomaly, as the initial levels of ammonium nitrate were high pre-spraying, in comparison to the other treatments. Herbicides are known to alter the composition of the soil microbiome and effect the nitrogen cycle (Brochado *et al.*, 2023). In other studies nitrogen increased and this was thought to be due to a lack of plants using the mineralized nitrogen and its production by the decaying plant material and soil microorganisms (Hudek *et al.*, 2021).

In comparison to the control, the abundance of bacteria did not decrease significantly under any of the treatments at any timepoint, either one month or four months post-treatment application. This could be due to an increase in the microbial biomass of glyphosate-resistant bacterial communities as non-resistant species declined. The bacteria in the greenfield site used in this experiment may be composed of species with the ability to tolerate and break down glyphosate as glyphosate was used in previous years on this site. Over the course of the experiments, the bacterial abundance rose in conjunction with phosphorous levels under the Hot Foam treatment. The abundance and diversity of Firmicutes were found to correlate with physio-chemical parameters such as phosphorous concentrations and soil texture (Ramos *et al.*, 2019). Thermal exposure is also known to alter the abundance and composition of bacteria and fungi in soil, as was found in this study (Ali *et al.*, 2024). Thermal exposure to Hot Foam seems to have affected four bacterial phyla, namely; Acidobacteriota, Firmicutes, Plantomycetota and Verrucomycetota and these are some of the most dominant phyla globally (Steger *et al.*, 2019). Most bacteria are understudied and so the understanding of their ecological roles is limited but Acidobacteria are known to be pervasive and widely distributed across numerous ecosystems. They are keystone taxa and are described as ecosystem engineers and associated with decomposing soil organic matter, hence their potentially important role in the carbon cycle (Kalam *et al.*, 2020). Acidobacteria are known to colonise the rhizosphere and are crucial in nitrogen cycling, respond positively to nitrates, and are known to produce growth hormones that stimulate the growth of plant roots. Firmicutes usually occur in less abundance than other phyla like Proteobacteria and Acidobacteria but are ubiquitous in microbial communities, as was found in this study.

The relative abundance of Planctomycetota found under the Hot Foam was 6.4% which correlates with other findings of between 0 and 10% where the diversity was thought to be driven by the soil and sediment type (Klimek *et al.*, 2025). This phylum is known to decompose biomass and for their ability to cycle nutrients. Planctomycetota often occurs in oil and metal contaminated environments demonstrating their potential for use in bioremediation. They are slow growing but are known to utilize nitrates and ammonium as sources of nitrogen (Vitorino & Lage, 2022). The nitrogen cycle is vital for food production, but herbicides have the ability to alter the activity of nitrogen fixing bacteria reducing their capacity to convert the atmospheric nitrogen into forms that plants can utilize (Brochado *et al.*, 2023). This would account for the abundance of these bacteria in this greenfield site as fertilisers were used previously on this improved grassland. Verrucomycetota are oligotrophic and have the capacity to tolerate low levels of carbon (Steger *et al.*, 2019). The results here showed an increase in carbon levels over time which may have led to the reduction in the abundance of these bacteria. The richness of the bacteria did not alter, either over time or between treatments. However, the community composition of bacteria did change considerably in this study over time, but this change may have been mediated by the environmental gradients, pH, soil organic matter, moisture content, water holding capacity and soil textures- clay, silt and sand.

The abundance of fungi was significantly reduced under the Hot Foam treatment after four months, in comparison to the control at that time. Over the duration of the study the abundance did decrease significantly under the Nomix Dual treatment, while between one month and four months the abundance reduced under both the control and Roundup Flex treatments. Ascomycota accounted for 50% of the fungi in this study and this group increased significantly over the first month, before decreasing under the control. The abundance declined under the Hot Foam treatment over the four months while under the Roundup Flex the decline was significant between the first and fourth month. The Others accounted for 39% of fungal abundance and consist of either novel organisms or rare taxa. Under all treatments their decline was significant over time. The Basidiomycota increased under the Hot Foam in the first month while under the Nomix Dual they declined in the final three months. The Mortierellomycota (3%) decreased over the four months under the Nomix Dual treatment and in the latter timeframe under the control. Soil function and health is often attributed to the activities of enzymes, with Ascomycota known to have a broader enzymatic profile than the Basidiomycota, that includes an enzyme for phosphorous (Manici *et al.*, 2024). These two are known to dominate the upper layer in soil and are highly effective at aggregating soil particles, nutrient uptake and at decomposing plant material (Manici *et al.*, 2024). There were no clear trends between any of the treatments and fungal abundance, though overall their abundance declined over the four months of the study, except for the Ascomycota, whose numbers increased under the Hot Foam. Ascomycota is adapted to stress which may indicate that their increase under the Hot Foam treatment was related to increased stress levels (Zhu *et al.*, 2022).

The impact of time was significant on fungal diversity and the NMDS plots clearly show these alterations. The significant environmental variables: pH, soil organic matter, organic carbon and moisture content may have influenced some of these changes. Organic carbon and phosphorous are important environmental factors for both bacterial and fungal communities (Zhu *et al.*, 2022). Despite the higher levels of organic carbon, this did not correlate with fungal diversity. Liu *et al*. (2023) found a negative correlation between pH and fungal diversity. The diversity of fungi at the initial timepoint seems to have been driven by the moisture content, while over time the changes in the community composition may have been driven by pH, soil organic matter and organic carbon, although it is challenging to determine whether the differences occur due to treatment, the varying re-growth above ground or temporal changes. There is a relationship between above-ground vegetation and the soil microbial fungi where plants and their roots can alter the rhizosphere and fungi, which are known to be extremely sensitive to changes in the surrounding environment (Liu *et al.*, 2023). The initial reduction in vegetation may have led to the increase of fungal decomposers as the roots decomposed, as seen under the Hot Foam treatment in this study. Ascomycota and Basidiomycota are decomposers known to compete for resources, but Basidiomycota may appear after the Ascomycota and are efficient at decomposing obstinate organic matter (Bai *et al.*, 2024).

Although the growth of microorganisms is generally driven by soil physio-chemical factors, in this study the findings are unresolved. Several other environmental factors and ecological interactions may have influenced the soil microorganisms including rainfall, temperature, weather conditions, soil type and above ground plant cover (Kepler *et al.*, 2020). It is also challenging to establish whether the discerned alterations in abundance, richness and communities of bacteria and fungi were as a result of the treatments or the reduction in vegetation, as it is thought that they influence each other (Liu *et al.*, 2023). It has been proposed that abiotic factors have a greater influence over the diversity of soil bacteria rather than plants (Walters & Martiny, 2020). One inhibitor that may have driven the decrease in the bacterial abundance is the reduction in soil water content that did decline during the experiment and may have led to water stress. Reduced water availability can lead to decreased enzyme activity of microbial cells and therefore may inhibit the microbial activity (Chen *et al.*, 2023). Studies have shown that small changes in pH can impact soil microbes and also that it is the soil properties, rather than land management, that effects the succession within the microbial community (Kuramae *et al.*, 2012). Seasonal changes are known to directly alter populations of soil microbes, more so than treatment, and this study supports those findings for both the abundance and community composition (Hudek *et al.*, 2021). The changes in abundance, diversity and community composition of fungi between timepoints were likely driven by seasonal changes, rather than the effects of treatment, whose influence seems to be minimal. These findings agree with those of Zhu *et al*. (2022) who observed that on aerated drip irrigation there was an increased fungal, but not bacterial, diversity.

There was also some random variation between soil samples within the study site that indicates soil microbial plasticity within the same greenfield site. There were considerable differences in the abundance and diversity of bacteria and fungi before the treatments were even applied across all three blocks. This variation continued across the controls over the subsequent four months throughout the experiment. Biotic and abiotic factors are likely to have contributed to this dissimilarity. Some abiotic factors include the disparity in the moisture content which changed over time as temperatures increased over the summer months, and lower rainfall led to drier soil conditions. Biotic discrepancies are likely to comprise a difference in soil microbes such as earthworms, nematodes and mites that may have altered their communities under some of the treatments. Their movements, or lack thereof, may have also shaped the bacterial and fungal communities.

4.6 Conclusion

A variation in biotic and abiotic factors seem to have collectively driven the dynamics of the fungal and bacterial communities investigated in this study. This has made it difficult, with no clear picture of the impact the treatments may solely have caused, to provide precise conclusions. However, the vegetation was successfully suppressed by both an alternative treatment, Hot Foam and both glyphosate treatments, Roundup Flex and Nomix Dual. The Hot Foam treatment may have negatively impacted the bacterial abundance whereas the Nomix Dual had no impact, though this may be due to those bacterial species that did occur, having the ability to metabolise glyphosate through historic use of this product on the site. The fungal abundance rose and fell under all treatments except for the Ascomycota, that rose significantly over the summer months under the Hot Foam. The fungal diversity shifted considerably over time, plus there was clear clustering of both the fungal and bacterial community compositions over time too with no clear effect of treatment. This shift in the community composition over the duration of the study is likely caused by seasonal changes rather than attributable to any treatment. Overall, the Hot Foam and Nomix Dual performed best at reducing the vegetation, and if, as suspected, the microbiome has become tolerant to the effects of glyphosate, in this scenario Nomix Dual may be the least hazardous to the soil microbiome. The high temperatures generated by the Hot Foam machine may have had an impact on the bacterial abundance, but this is likely to be the first time the bacteria on this greenfield site had encountered such high temperatures.

Chapter 5 A comparative analysis of mechanical and chemical alternatives to glyphosate for vegetation control on roadside verges and hard surfaces.

5.1 Introduction

Pavements, cycle ways and roadside verges are extremely common in urban environments and can be highly prone to colonisation by plants, especially when there is a suitable substrate, low footfall and appropriate weather conditions (Kempenaar *et al.*, 2006). The control of weeds (defined here as wild plants growing where unintended in urban areas) is required, particularly on pavements and streets, to avoid structural damage to hard surfaces, remove potential trip hazards for the public, avoid blockages to drains and water flow, and to maintain the aesthetics of the urban environment (Peruzzi *et al.*, 2010; DEFRA, 2015; Parks for London and Amenity Forum, 2025). Plants can also make hard surfaces slippery, create a substrate for further seed establishment, reduce driver visibility and are often perceived as indicating an area in decline (Rask, 2012). Plants decrease the lifetime of hard surfaces and curtail their function incurring a cost to society (Hansson *et al.*, 2006; Melander *et al.*, 2009; Fagot *et al.*, 2011), and in Ireland, as in most countries, it is the local authorities that have a legal requirement to maintain these surfaces. Councils have a duty of care to the public as plants can pose as a trip hazard, degrade pavements, and interfere with drains impeding water flow (DEFRA, 2015; Parks for London and Amenity Forum, 2025).

Traditionally herbicides have been the most common method for controlling weeds but as plants become more resistant to glyphosate their use is likely to decline (Duke, 2018; Beckie *et al.*, 2020). Often the roots are only partially affected by herbicide use leading to the plants re-growth, in addition to which they rarely prevent seeds from germinating (Kempenaar & Spijker, 2004). In Ireland, the quantities of herbicide use by local authorities is not known publicly, although records of use are kept. Transport Infrastructure Ireland estimated that 45,000 litres and 800kg of pesticides were used over a five-year period between 2017 and 2022 on national roads. These figures did not include the 12,200 weedkiller 'eco' plugs that are placed into tree stumps to control vegetation (Lacchia, 2022). Glyphosate-based herbicides are extremely cost-effective in comparison to other plant control methods and usually represent the method requiring the lowest cost in terms of labour (Rask, 2012; De Cauwer *et al.*, 2014). However, as discussed in previous chapters, growing public health and well-being concerns have resulted in greater awareness about the potential risks of using herbicides.

The hard surfaces in urban areas are designed to ensure a rapid run-off to prevent flooding, leading to the contamination of watercourses from pesticides and potentially ground water, and thus drinking water (Rask, 2012). In Sweden, glyphosate residues have been discovered in surface and ground water so local authorities have stopped its use in public areas due to the high risk of contamination (Hansson *et al.*, 2006). There are further risks associated with herbicide use such as storage, handling and their disposal (Moretto & Di Domenico, 2017).

Where pesticide use is being discussed, the precautionary principle should be applied for guidance. This principle has been used as the basis in environmental law in Europe and it states: "When an activity raises threats of harm to human health or the environment, precautionary measures should be taken, even if some cause-and-effect relationships are not fully established scientifically" (Hayes, 2005). Despite this, and the growing body of evidence for the risks of over-use of glyphosate based herbicides, only eight European member states have, or are, in the process of banning pesticides in sensitive public areas such as parks and playgrounds (PAN Europe, 2022). In the last 10 years, over a million signatures have been collected demanding that pesticides are phased out (PAN Europe, 2022). The EU Sustainable Use of Pesticide Directive, Article 12, specifies that member states need to ensure that there is minimal use of pesticides and that it is prohibited in specified areas. These range from protected sites to gardens, schools and sports grounds, and recreation areas (PAN Europe, 2022). PAN Europe (2022) has recommended that no-spray buffer zones are put in place, and have suggested that beside private and public properties, watercourses, paths and roads, a 50m buffer zone is required (PAN Europe, 2022). Around the world there has been a growth in negative publicity around pesticide use, and there are now more people looking to see it banned within urban areas. A recent poll undertaken by Kildare County Council in 2020 showed that 98% of the public wanted to see herbicides reduced. This change is due to a concern over the safety of chemical use and the safety of members of the public as well as other species, particularly pollinators. Other European towns and cities have been pesticide free, for example Ghent, which went pesticide free 20 years ago, so it has and can be done (PAN UK, 2017).

The advantages of going pesticide-free include improving habitats for wildlife and thus biodiversity, through decreasing water and air pollution. There are also health benefits to consider, for the contractors and council workers that apply pesticides, in addition to the members of the public that may be exposed to the chemicals during, or just after, spraying. However, viable alternatives to glyphosate are few, and those that are available are not as effective and generally cost more due to the acquisition of specialised equipment (Beckie *et al.*, 2020). The use of non-chemical treatments often relies on mechanical and thermal treatments and frequently requires more applications, especially on plants with long tap roots (DEFRA, 2015). In general, non-chemical treatments need repeated application, thereby increasing the cost (Fagot *et al.*, 2011; Rask, 2012). Initial investments for alternative equipment can be costly but will diminish once the new products and equipment have been acquired, and further savings can be made if equipment is shared with other bodies.

Hard surfaces come in many forms, from tarmac or concrete surfaces to asphalt, paving stones or areas of crushed material (Rask & Kristoffersen, 2007). Hard surfaces bestow harsh conditions on plants, with limited soil availability for germination, warmer conditions that lead to frequent desiccation and human footfall to contend with for survival (Rask, 2012). Plants can grow in cracks in pavements and small fissures and a knowledge of the ontogeny of plants is essential to know how best to treat them. There are characteristics associated with these plants that include the quantity of seeds they produce and seed dormancy, or whether they reproduce vegetatively (Parks for London and Amenity Forum, 2025). Generally, plants found on hard surfaces have their meristems near the surface to cope with constant trampling, and if they have rhizomes under the hard surface, both of these factors make them harder to remove permanently, as the surface affords the roots protection (Rask, 2012). Whether or not plant growth on hard surfaces is deemed acceptable depends on function, use and location of the surface and the expectations of the public and local authorities (Kortenhoff *et al.*, 2001).

Non-chemical treatments usually only affect the above-ground vegetation, unlike chemical treatments that can kill the entire plant. The frequency of treatments depends on the plant species, the methods used and the type of surface (Rask & Kristoffersen, 2007). Research in the Netherlands suggests that through the assessment of pavements and the size of plants, their treatments can be adjusted, leading to between 11% and 66% reduction in the quantity of herbicides used (Kempenaar *et al.*, 2007). There are four main approaches to deal with plants in urban environments, namely, chemical, mechanical, thermal, and physical.

5.1.1 Chemical

Research in the Netherlands found that herbicide use on pavements was negatively impacting water quality and therefore drinking water (Kempenaar *et al.*, 2006). They discovered that five elements affected run-off: the herbicide dose, rainfall, the type of hard surface, spraying technique and distance to the watercourses (Kempenaar *et al.*, 2006). The highest concentrations of glyphosate and AMPA were detected during and soon after a rainfall event. To combat this a framework called the Sustainable WEEd control on Pavements (SWEEP) was produced for weed control on hard surfaces that aimed to reduce the run-off of herbicides to surface waters. Within this framework, no herbicides were allowed to be used in areas within 10kms upstream of a watercourse used for abstracting drinking water, on surfaces bordering watercourses, or when rain was forecast and best practice guidelines had to be followed (Kempenaar *et al.*, 2006). By following the SWEEP guidelines, the run-off of herbicides was reduced by 90%.

Kempenaar and Saft (2006) then produced a framework to identify and evaluate the environmental impact of materials from start to finish, the Environmental Life Cycle Assessment. This assessed all the resources and wastes that were used/produced during the life time of five plant removal methods, namely; brushing and herbicide, flaming, hot water, herbicides 50%, and two sweeping levels: Sweep 25% and Sweep 3%, dependent on the quantity of herbicide used in relation to watercourses (Kempenaar & Saft, 2006). Herbicide use impacted the environmental score significantly and was due to direct and indirect emissions on aquatic ecotoxicity. The brushing score was high as it was used in conjunction with herbicides and because of the high fuel consumption. The results from this survey have subsequently been used by policy decision makers (Figure 5-1).

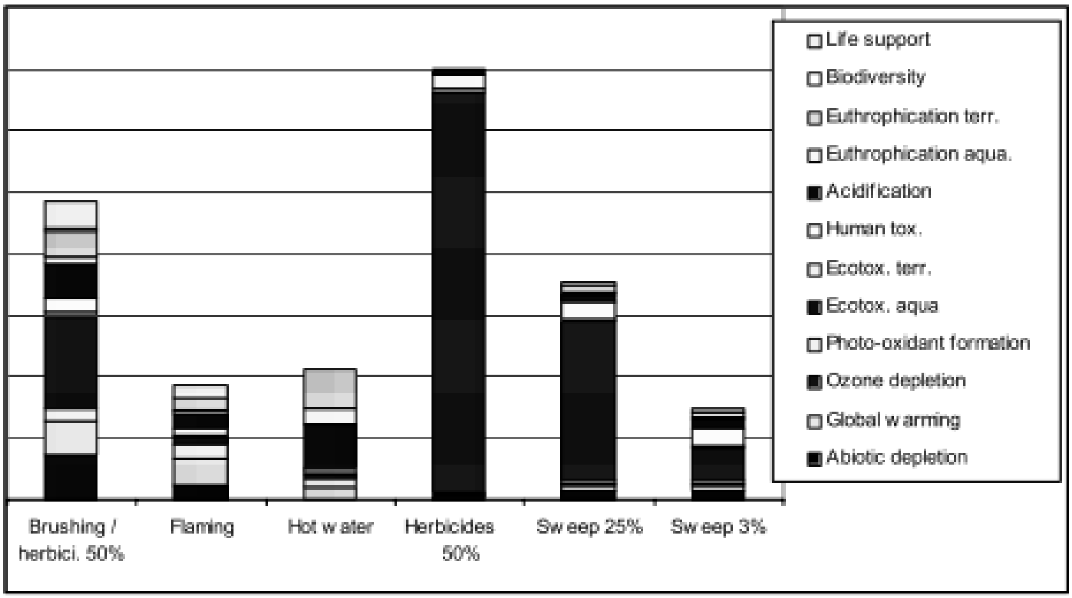


Figure 5-1 Impact scores from the Life Cycle Assessment scores on weed control on pavements . The higher the bar chart the higher the potential impact on ecosystems and human health. Sweep 25% used a higher herbicide quantity in the proximity of watercourses whereas Sweep 3% followed the framework to ensure herbicide use followed the SWEEP framework (Kempenaar & Saft, 2006).

Acetic acid is a chemical that has been trialled for the suppression of plants. Trials in Sweden with acetic acid were successful but large quantities were needed with an average 0.25 litres of 12% per m^2 required. However, it did not result in long lasting effects (Hansson *et al.*, 2006). The acetic acid also affected the pH levels but the levels did recover after two days (Hansson *et al.*, 2006). Complaints were received due to the smell of the acid and, as per other alternative treatments, it was not as economically viable as glyphosate (Hansson *et al.*, 2006). Another study found that acetic acid treatment was fully effective when applied thrice during the growing season, and on seedlings, but it failed to kill the roots of established plants (Campanelli, 2016). As an alternative to glyphosate, acetic acid is biodegradable but not nearly as effective.

5.1.2 Mechanical

Large, abandoned areas with older plant species are ideal for mechanical treatment but these methods may cause damage to hard surfaces (Rask & Kristoffersen, 2007). Mechanical methods are suitable on flat surfaces and provide good results with reduced energy inputs (Kempenaar & Spijker, 2004). Mechanical tractor-driven weeders, with connections for wire/plastic brushes spinning at high speeds, rip out the weeds and also act as a preventative, as it keeps detritus clear. Mechanical brushing can be used on a number of surfaces from paving to tarmac to concrete, but do have the potential to cause damage to surfaces, especially the wire brushes (PAN UK, 2021). Often though, only the above ground vegetation is removed, and the resulting wear and tear on hard surfaces, plus the noise, can affect local residents (Kortenhoff *et al.*, 2001). Regular sweeping is recommended to reduce weed growth by removing the debris and soil, and so limits a build-up of organic matter that should inhibit seeds from germinating (Kortenhoff *et al.*, 2001; De Cauwer *et al.*, 2014). However, mechanical methods are often labour-intensive and come at a high environmental cost (Barker & Prostak, 2008), using large quantities of fuel leading to greenhouse gas emissions (De Cauwer *et al.*, 2014). Some mechanical equipment is unsuitable for certain areas so their use will be limited (Rask & Kristoffersen, 2007). Mowing and strimming are good for use around obstacles and for moderate-to-heavy vegetation (Kortenhoff *et al.*, 2001).

5.1.3 Thermal

Thermal methods are effective on areas with uneven surfaces (Kempenaar & Spijker, 2004). Excess heat has been shown to be successful at killing plants and includes the use of infrared, boiling water, hot water/foam, steaming, burning/flaming, hot air and electrocution. Hot Foam is non-toxic so it is safe to use near watercourses and there is therefore no need for a licence (Martelloni *et al.*, 2020). Hot Foam uses vegetable oils as this holds the heat for longer, but it does require repeated treatments. The advantages of Hot Foam is that it can be used in all weather conditions, plus it is capable of removing moss and algae, and also chewing gum and graffiti, from pavements (PAN UK, 2021). Thermal control does, however, require large quantities of fuel, water and time (Barker & Prostak, 2008). It is expensive to run but the costs can be shared with other contractors and councils. Hot Foam has been successfully used to treat railways in Sweden (Martelloni *et al.*, 2020) while hot water was found to control young and annual plants, although older perennial plants could not be controlled efficiently (Rask & Kristoffersen, 2007). In general these treatments only affect the surface vegetation and regrowth occurs (Kortenhoff *et al.*, 2001)

5.1.4 Physical

Plants can also be removed physically, but this method is expensive at a large scale due to labour costs, though it may be possible on small sites like town parks. Basic manual tools used in gardens, like hoes, can be implemented to aid plant removal while other tools, such as rakes and weeding hooks, work well on areas of gravel. Hoeing though can cause damage to the roots of the existing plants that were purposefully planted (Looker, 1996). Steel brushes are excellent for removing light plants and plants could be taken out by hand in small areas like playgrounds, and these are both cost-effective methods (Rask & Kristoffersen, 2007).

5.1.5 An integrated approach

This approach utilizes all available methods with the aim of being sustainable and holistic. It does implement chemical pesticides but reduces the level of use to be justifiable both ecologically and economically, thereby lowering the risks to human health and biodiversity (Parks for London and Amenity Forum, 2025). Using this method plants can be monitored and then either suppressed or prevented, but the method does need to be structured around seasonal requirements (Parks for London and Amenity Forum, 2025). Prevention needs to be prioritized to inhibit plants from becoming established. This measure includes the design stage to lessen areas that are difficult to maintain, such as intersections on pathways to decrease bare ground, and by reducing sharp angles in pavements. Monitoring to assess plant growth to deduce whether to tolerate or manage plants is second, with intervention only occurring if acceptable tolerance levels are exceeded. Physical control should be selected, where practical, with chemical methods only applied if strictly necessary. All areas need to be constantly monitored and reviewed using an integrated approach (Figure 5-2) (Parks for London and Amenity Forum, 2025).

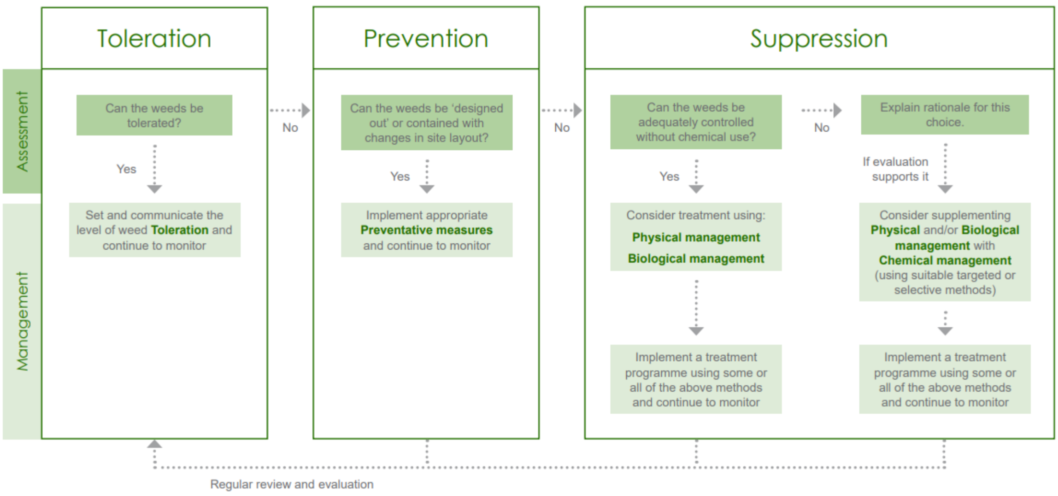


Figure 5-2 Framework for an Integrated Weed Management approach setting out a structured decision making process to support an integrated approach to plant management. This framework ensures that the actions are proportionate, targeted and environmentally responsible and that regular reviewing is pivotal to decision making (Parks for London and Amenity Forum, 2025).

There are advantages and disadvantages to the various methods of plant removal (Table 5-1). An integrated approach is an alternative, particularly when the non-chemical treatments are ineffective at eliminating species with long tap roots, and the rotation of treatments will subdue the likelihood of herb resistance (DEFRA, 2015). The effectiveness of treatments is related to the species of plants and their growth stage (Fagot *et al.*, 2011). For all alternative methods the control of post-emergence vegetation is required (Barker & Prostak, 2008). Different treatment measures will lead to a mix of selective pressures on plants (De Cauwer *et al.*, 2014). However, it should be noted that there will be an alteration of plant species due to the repeated use of a single treatment method (Fagot *et al.*, 2011).

Table 5-1 Advantages and disadvantages of three approaches to plant control on hard surfaces (DEFRA, 2015).

	Advantages	Disadvantages
Chemical	Quick Efficient Cost effective Fixed number of treatments per season Reduction in greenhouse gas emissions	Pesticide pollution Resistance to herbicides Public perception
Integrated	Less pesticide risk Less PPE needed	Increased fuel consumption= pollution Monitoring needed Increased costs (x2) More treatments needed (2-6)
Non-chemical	No pesticide risk No PPE needed	Greater pollution from fuel consumption Greater costs (x8) Persistent perennial weeds More treatments needed (3-6)

5.1.6 Aims

The primary aim of this study was to evaluate the potential for improving the management of road verges for biodiversity and ecosystem services, whilst accounting for economic feasibility and practical limitations. This chapter aims to assess the effects, practicalities and cost-effectiveness of using glyphosate and alternative treatments, including mechanical, on the removal of vegetation on a road verge currently managed by Kildare County Council. There are two distinct sections of the road verge: the grass verge and the intersection. There were seven treatments assessed on the grass verge, namely, a control, three chemical (New Way, Nomix Dual, Roundup Flex), one thermal (Hot Foam), and two mechanical (Multihogg and WeedHex machines). For the intersection the same chemical and thermal treatments were examined against the control.

The following hypotheses were tested using the experiments and data generated during this thesis:

- Hypothesis 1: There is a difference in the effectiveness of glyphosate and alternative treatments on the complete removal of vegetation and soil from a grass verge.
- Hypothesis 2: There is a difference in the cost of completely removing soil and vegetation from a grass verge using glyphosate and alternative treatments.
- Hypothesis 3: Differences between the effectiveness of alternative plant control treatments to glyphosate will correlate with the reduction of naturally occurring plant species *in situ* experiments on a narrow intersection, excluding mechanical.

5.2 Methodology

The Millennium Link Road, Naas, County Kildare was the site chosen to conduct the cost-effectiveness of treatments on plant removal along a road verge. The experiments were conducted along a two and a half kilometre stretch of the Millennium Link Road (between GPS Coordinates: 53.228097, -6.685809 and 53.211745, -6.699951) during

2022, 2023 and 2024. The Millennium Link Road is a regional road that runs northeast to southwest and is located west of Naas, County Kildare. The road has a homogenous road verge on either side that is a consistent width measuring approximately 2m. The road verge is comprised of a grassy verge (A) on either side of a cycle lane/footpath that contains a narrow intersection (B) (Plate 5-1). Vegetation and soil were gradually encroaching across the hard surface from the grass verges on either side, up to 40cms from the original pavement edge, and had accumulated up to 10cms of soil and organic matter facilitating the growth of plants. The intersection measured approximately 2cms and in sections was becoming overgrown.

The two principal areas requiring plant control were:

A. the grass verge either side of the cycle lane and footpath, and
B. the narrow intersection between the cycle lane and footpath.

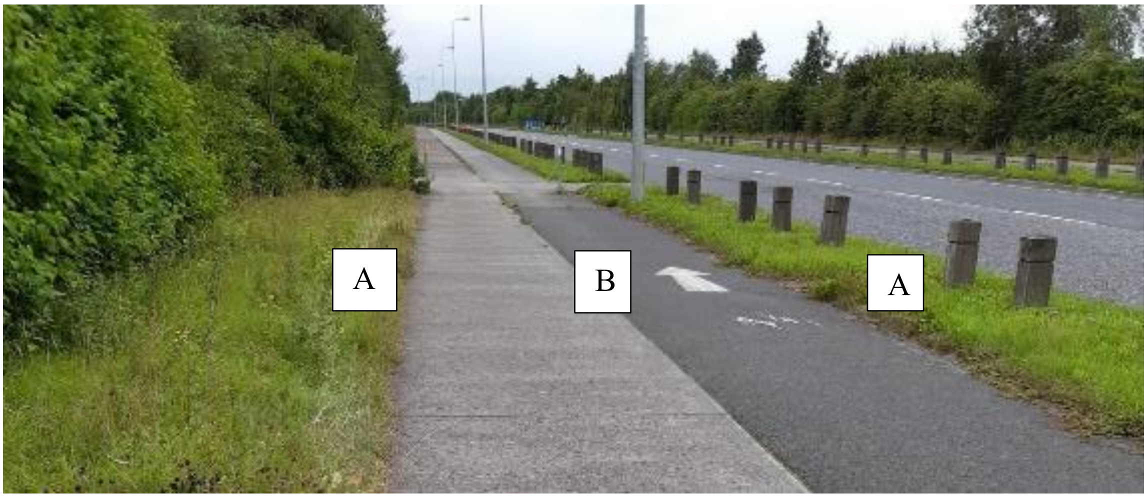


Plate 5-1 The road verge on the Millenium Link Road with a footpath and cycle way. 'A' indicates the encroaching road verge on either side and 'B' indicates the intersection, some sections of which are vegetated.

Experiment 1 was conducted in 2022 on both the grass verge and the intersection on the northern half of the Millennium Link Road and included mechanical, thermal and chemical treatments. These experiments were of a qualitative and practical nature, rather than quantitative. The local authorities need to find cost-effective, practical methods to remove the build-up of deep soil and vegetation to use in this scenario. Experiment 2 was conducted over the winter of 2023 and 2024 on the intersection alone, on the southern section of the Millennium Link Road and only thermal and chemical treatments were applied. Qualitative results from the first experiment include the grass verges and exclude the intersection, as sections of the intersection were frequently sparse or bare of vegetation, so were incomparable (Plate 5-1). Also, as this experiment was conducted during the summer months much of the vegetation in the intersection reduced over time due to desiccation.

5.2.1 Experiment 1- the grass verge

In April 2022, 50m sections were measured out along the cycle lane/footpath. Nine treatment types were chosen for this experiment, and each treatment was allocated a designated number (1-9) and was colour-coded. Each treatment was assigned 150m sections, and these were further subdivided and randomised into 50m lengths and allocated a letter- A, B or C to give a Site Code:

1. Control (1A, 1B and 1C),
2. Hot Foam (2A, 2B and 2C) (Plate 5-2),
3. Roundup Flex (3A, 3B and 3C),
4. Nomix Dual (4A, 4B and 4C),
5. Strim/Mow (5A, 5B and 5C),
6. New Way (6A, 6B and 6C),
7. WeedHex Machine (7A, 7B and 7C) (Plate 5-2),
8. Multihogg Machine (8A, 8B and 8C) (Plate 5-2) and

9. Mechanical burner (9A, 9B and 9C).

Each section was labelled with spray paint with the relevant Site Code at the start and end of each section and a 20m gap was left between each section to reduce the effects of spray drift. A map was created of the layout that included the colour coded Site Code for each treatment. Each contractor was supplied with a map and a survey sheet for recording the time taken to apply their treatment and the total cost per hour (Appendix Figure D- 1 and Table D- 1).

5.2.1.1 Treatments

Nine treatments were applied in this experiment and consisted of a control, three chemical treatments: two glyphosate (Nomix Dual and Roundup Flex) and New Way (acetic acid), two thermal treatments: Hot Foam and flame and three mechanical treatments; namely, strimming/mowing machine, a WeedHex machine and a Multihogg machine. The details on the chemical treatments are the same as in the previous chapter, Chapter 4. The strim/mow and flame treatments have both been removed from hereon in as the strim/mow experiment used two methods to remove vegetation and information was not gathered in relation to cost or time spent on either. The flame treatment ran out of gas after only one section of road was treated.

The AS 30 WeedHex (Plate 5-2) machine weighs 35kg and works at rates of up to 700m^2/h. With a low brush speed of 600rpm, it reduces the likelihood of dislodging stones and dust creation. It has been designed to work on even and uneven surfaces like cobblestones, around curbs and along gravel roads. For edges there is a lateral offset between the brush and the chassis, and the wheels are height-adjustable for depressions. There are two types of brushes available depending on the surface:- a plate brush nylon (gentle cutting force) and a plate brush steel (hard cutting force).

The compact Multihogg CV350 muti-purpose sweeper (Plate 5-2) has one of the cleanest engines on the market boasting Tier 4 Final/Stage V emissions. Two independently controlled sweeper brushes were used with a sweeping width of 1200-2350mm.

All treatments were applied in April, and surveys were conducted at one, two, five and eleven months post-application.

Plate 5-2 Mechanical and thermal experiments on the Millenium Link Road. The WeedHex machine (A), Hot Foam (B) and the Multihogg machine (C) treating the grass verge along the Millennium Link Road.

5.2.2 Experiment 2- The intersections

These experiments were conducted on the southern section of the Millennium Link Road over the winter of 2023 and 2024 to reduce the effects of desiccation. Vegetation surveys were conducted on the 7th of October prior to treatment to ensure suitable lengths of intersection were located, and to guarantee comparisons on the effectiveness of treatments could be analysed robustly. All 10m sections were measured out, marked and further subdivided into 1m lengths for surveying (Plate 5-3). A map was created to enable a visual picture of the layout for the contractors that included the colour coded Site Codes for each treatment (Appendix Figure D- 2 Experiment 2: The map layout of the site along the Millennium Link Road with the colour coded Site Codes denoting the location of each treatment. Five treatments were chosen for these experiments, and each treatment was assigned a designated number (1-5) and a colour. Each treatment was allocated a randomised 40m stretch of intersection. These were further subdivided by four into 10m lengths and allocated a letter:- A, B, C and D, and a Site Code:

1. Control (1A, 1B, 1C and 1D),
2. Hot Foam (2A, 2B, 2C and 2D),
3. Roundup Flex (3A, 3B, 3C and 3D),
4. Nomix Dual (4A, 4B, 4C and 4D) and
5. New Way (5A, 5B, 5C and 5D).

All treatments were applied in October and vegetation surveys were conducted at one, two, five and eleven months post-application. Vegetation surveys were conducted on the intersection using the Braun-Blanquet survey method adjusted for a linear feature for each one metre section, see Appendix Table D- 2 for survey sheets. Often species were too small to identify to species level, due to the time of year or trampling from pedestrians. Where possible, plants were identified to family or genus level, while unidentified species were classified as unknown.

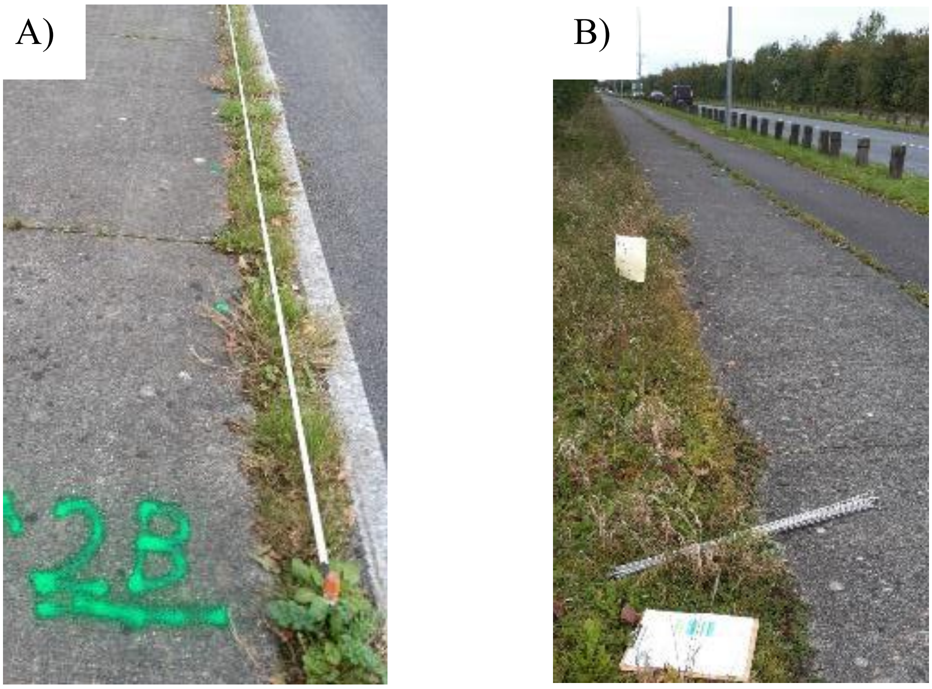


Plate 5-3 Ten metre sections marked out on the Millenium Link Road prior to the application of treatments (A and B).

5.2.3 Data Analysis and Statistical Methods

All data were processed using Excel and R Studio. The significance level for all tests was set at $p < 0.05$. Data was imported into R Studio for statistical analysis and for plotting. AIC values were implemented to find the best fit. Probability plots were created to test for normal distribution, and the DHARMa package was implemented to check residuals. GLMs were used to assess the effects of treatments on vegetation cover. Analysis of variance (ANOVA) tests and Dunnett's Multiple Comparisons of Means test was implemented to determine significant differences of the means in contrast to the control, for percentage plant coverage.

5.3 Results

5.3.1 Experiment 1- the grass verge and cost effectiveness

The qualitative results on the grass verge were clear:- none of the chemical treatments or the strim/mow had any impact apart from reducing the vegetation initially, but due to the existing bed of soil and organic matter, the vegetation had recovered after eleven months (Plate 5-4). The WeedHex machine and the Multihogg (Plate 5-4) however, having cleared all the soil and vegetation, were all still clear of both, eleven months later. The results from the intersection are excluded here as the sections containing vegetation varied considerably. Experiment 2 addresses the intersection further.

The cost of treatments varied considerably. Of the chemical treatments, the Nomix Dual was the cheapest and quickest treatment to apply, followed by the New Way and Roundup Flex. The Hot Foam was more expensive and took the longer time to apply. The mechanical treatments were far more expensive, particularly the Multihogg machine, and took longer to apply (Table 5-2).

Table 5-2 The time (minutes) taken to apply treatments over 150m sections, the cost per hour and the total cost over 150m in 2022.

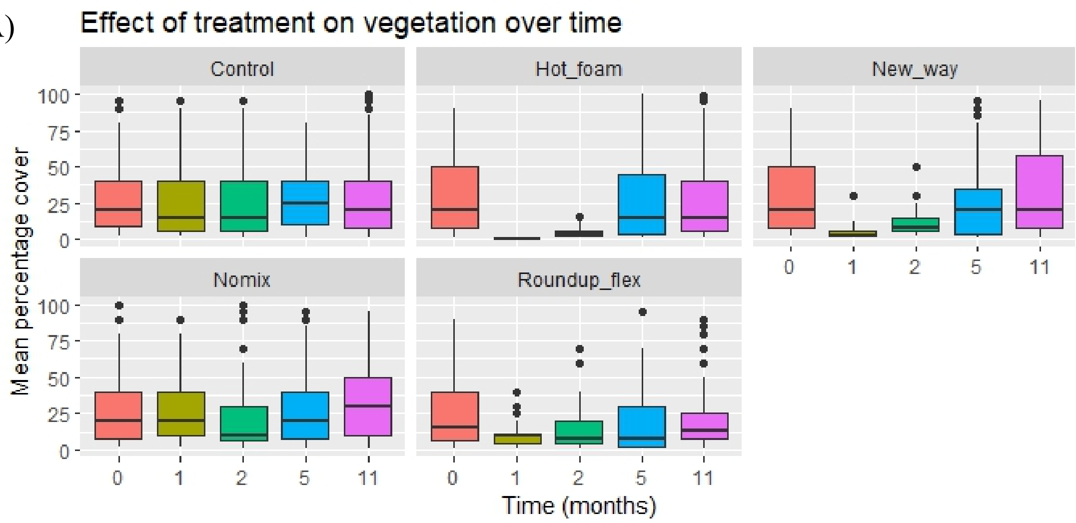
Treatment	Total Time (minutes) over 150m	Cost per hour	Total Cost over 150m
1- Control	0	€0	€0
2- Hot Foam	82	€20	€27.33
3- Roundup Flex	17	€38	€10.77
4- Nomix Dual	9	€38	€5.70
5- Strim/Mow	80	€38	€50.67
6- New Way	16	€38	€10.13
7- WeedHex Machine	150	€34	€85.00
8- Multihogg	125	€60	€125.00

Plate 5-4 The effect of treatments along the grass verge. Control (A), Hot Foam (B), Roundup Flex (C), Nomix Dual (D), Strim/Mow (E) and New Way (F) had no impact on the grass verge eleven months post-treatment. The WeedHex machine (G) and Multihogg machine (H) had little impact on the intersection but cleared the grass verge completely. The grass verge was still cleared of soil after both mechanical treatments, but vegetation was beginning to encroach from the grass verges, by the final survey eleven months later.

5.3.2 Experiment 2- the intersection

Three families accounted for 83% of the flora recorded during all surveys, namely, Poacaea spp. (32%), *Taraxacum* spp. (26%) and Bryophytes (25%) (Appendix Table D- 3). Overall, the Roundup Flex was the most effective at reducing the vegetation cover along the intersection, significantly diminishing the vegetation over all surveys (Figure 5-3 and Appendix Table D- 4). The Hot Foam and New Way successfully reduced the plants after one and two months but after this time their effectiveness had worn off. The Poacaea spp. were adversely affected by the Hot Foam, New Way and Roundup Flex up until the five-month survey, with Roundup Flex the only treatment still effective after eleven months (Figure 5-3 and Appendix Table D- 5). The *Taraxacum* spp. were negatively affected by the Hot Foam and New Way after one month but recovered after two months (Figure 5-4 and Appendix Table D- 6). After five months this species had decreased under the Roundup Flex treatment. Two treatments were highly effective at reducing the Bryophytes: the Hot Foam and the New Way, for up to five months. By the final survey the Hot Foam treatment was the only one still affecting this plant. Interestingly, the Roundup Flex had no effect on the Bryophytes (Figure 5-4 and Appendix Table D- 7).

A)



B)

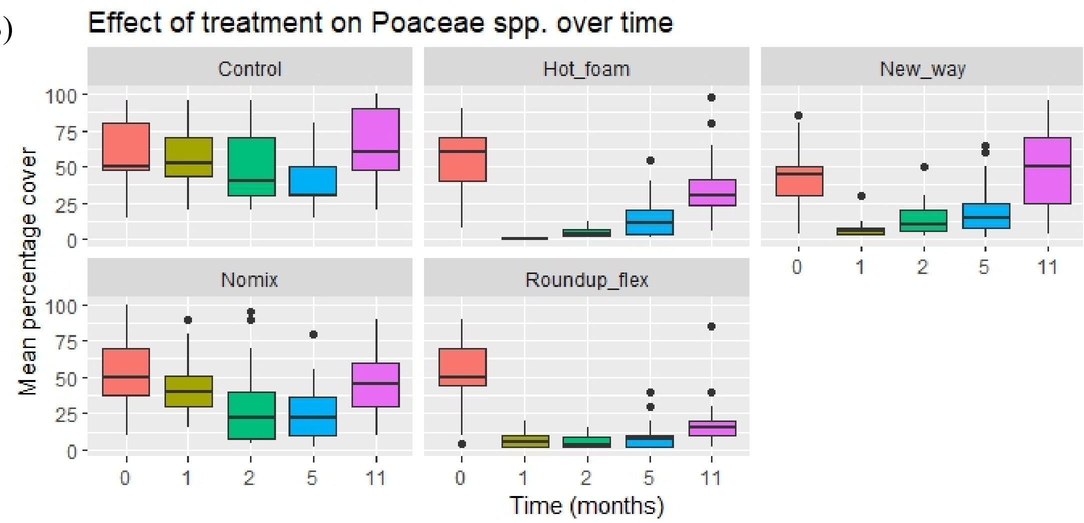


Figure 5-3 Overall vegetation cover in the intersection (A) and Poacaea spp. cover (B) along the intersections of the Millennium Link Road at zero, one, two, five and eleven months under each treatment. Zero month is prior to the application of treatments.

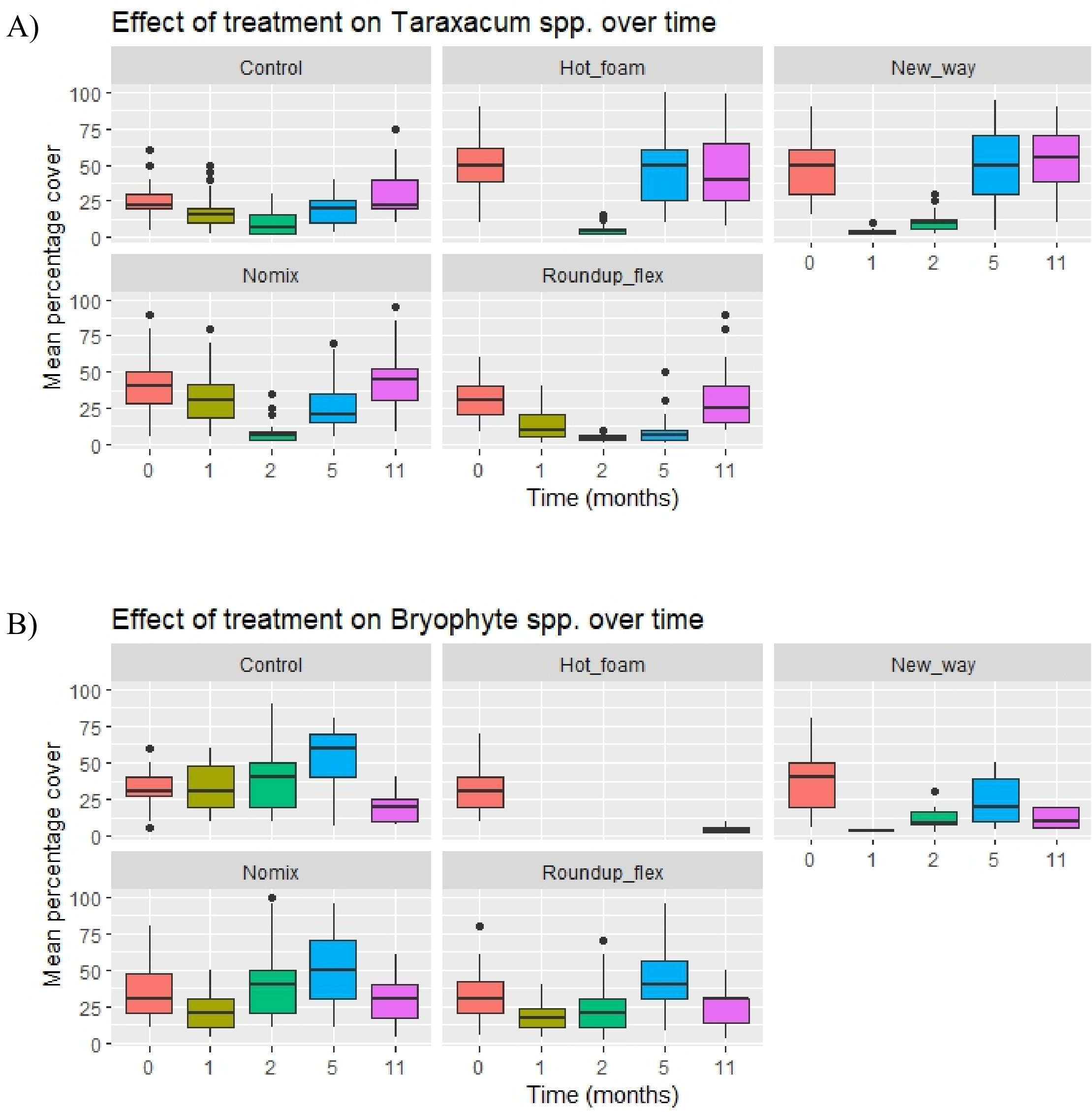


Figure 5-4 *Taraxacum* spp. (A) and Bryophyte spp. (B) cover along the intersections of the Millennium Link Road at zero, one, two, five and eleven months under each treatment. Zero month is prior to the application of treatments.

5.4 Discussion

Hard surfaces in urban environments are extremely common and in certain circumstances the local authorities need to ensure they are kept clear of vegetation. Vegetation can damage hard surfaces over time, become trip or slip hazards, and are unsightly to some members of the public. The grass verges, and the foot and cycle paths of the Millennium Link Road, outside the town of Naas, County Kildare, Ireland is one such place that needs to be kept clear for cyclists and pedestrians and is an area where vegetation has slowly encroached. The road verge is a harsh environment for plants to exist due to the hard surfaces, consisting of concrete and tarmac, that ensure rapid water loss after rainfall events. Desiccation is likely to be high due to the lack of soil to hold moisture. Salt spreading on the roads in icy conditions will also influence the survival of plant species that cannot tolerate high salt levels. However, despite these seemingly harsh conditions, plants do persist in these environments and local authorities are obliged to remove them.

5.4.1 Mechanical Treatments

The mechanical removal of vegetation is necessary in situations where soil and organic matter have led to the colonisation of vegetation and as seen in this study, is highly effective. To reduce the number of times chemical and thermal treatments are required, the ongoing removal of soil and organic matter is essential to prevent seeds from germinating. In this study there were two treatments used for the mechanical removal of plants and the soil, a Multihogg and a WeedHex machine. The Multihogg and the WeedHex machines both performed well in this experiment, though there was a considerable difference in cost, with the Multihogg almost double that of the WeedHex machine. Once the thick vegetation and soil has been removed from an area using one of these machines, a brushing machine may be enough to prevent the build-up of soil and so prevent vegetation from gaining a foothold. Deep cleaning from spring through to the autumn has been highly successful in trials in the UK. Cornwall Council for example, treated their sites with hot air thermal lances followed by mechanical brushing to remove the debris. By starting in the spring and using these methods, they only needed to resort to a herbicide once during the season (Parks for London and Amenity Forum, 2025).

5.4.2 Glyphosate treatments- Roundup Flex and Nomix Dual

Glyphosate-based herbicide treatment has been the preferred plant control method for the past 50 years, despite our growing knowledge of their environmental impacts, potentially reducing drinking water quality and affecting non-target flora and fauna (Kortenhoff *et al.*, 2001; Parks for London and Amenity Forum, 2025). It should be noted that rainfall can wash glyphosate from plant surfaces for as much as 4-6 hours after treatment, reducing the effect. The movement of pesticides and their break down products through food chains, ending and bioaccumulating within humans, is well documented (Arias-Estévez *et al.*, 2008). However, herbicides are the most effective as application is easy and provides long lasting control (Kortenhoff *et al.*, 2001). In one study glyphosate (Roundup Pro) only needed to be applied once in a growing season in the U.S. to control plants (Barker & Prostak, 2008). In another study, spot spraying was noted to be the most effective treatment as only one or two treatments were required, especially when hard surfaces had been brushed prior to winter (Vermeulen *et al.*, 2006). It has been recommended that chemicals such as the spot treatment Nomix Dual could be deployed on difficult to remove perennials (Rask & Kristoffersen, 2007). Nevertheless, certain species may not be affected by glyphosate as was found in this study, where one glyphosate treatment, the Nomix Dual, seemed to have no effect on the vegetation overall, or the Poacaea spp. and Bryophytes in the intersection. The Roundup Flex, however, was most effective at reducing the overall vegetation in the intersection, including the *Poacaea* spp. and the *Taraxacum* spp., but failed to have an impact on the Bryophytes.

5.4.3 Alternatives to glyphosate- New Way and Hot Foam

In general, alternatives to glyphosate are not cost effective due to the need for repeated treatments and so are usually more labour-intensive. The results from a similar study to this one, trialing alternatives that included acetic acid found it to be fast acting, but a minimum of three applications to control perennial weeds was required (Barker & Prostak, 2008). Another acid, Nanonoic acid, had no effect on controlling plants (Martelloni *et al.*, 2020). New Way was effective in this study on the overall vegetation for two months, and reduced the growth of Poacaea spp., *Taraxacum* spp. and the bryophytes for five months.

Research evaluating flaming, steaming, mechanical brushing and sweeping methods found that steam was comparable to glyphosate, but the initial outlay was a disadvantage due to the substantial cost of thermal machines (Rask & Kristoffersen, 2007). Another study investigating hot air was also effective but the energy consumption was high (Rask & Kristoffersen, 2007). As noted in this study, the Hot Foam machine needs to move slowly to be effective at controlling weeds, but the cost incurred is therefore higher and includes the excessive use of water and fuel. The Hot Foam did perform effectively on the vegetation in this experiment, in particular on the Bryophytes. The advantage of the Hot Foam is that it is chemical-free, using only vegetable oil, plus it can be used in all weather conditions (Parks for London and Amenity Forum, 2025). In an Italian study, flame weeding was the most effective at weed control in two cities, on hard and gravel surfaces (Peruzzi *et al.*, 2010). Hot Foam and flaming were found to be most effective in another study in Italy (Martelloni *et al.*, 2020). A third study in the U.K. found that a foamstream machine and glyphosate took a similar length of time to apply and the regrowth was roughly the same (PAN UK, 2021). The Hot Foam was removed as a method in the U.K. due to high costs and energy requirements, and was not found to produce proportional results to the mechanical sweepers (Parks for London and Amenity Forum, 2025). Other studies discovered that the intervals between thermal treatments varied with flame treatments recommended at intervals of between two and five weeks, while others suggested between 11 and 12 weeks were necessary (Vermeulen *et al.*, 2006; Rask & Kristoffersen, 2007). Thermal treatments need to be applied when plants are young, so their roots have not stored resources, and their seeds have not yet developed. As technology improves, the emissions and carbon footprint may continue to reduce for alternative thermal treatments, therefore making them more viable options.

5.4.4 Plant species and treatments

Plants generally accumulate on soil and organic material, and species best adapted to an environmental niche will proliferate (Kortenhoff *et al.*, 2001). However, the species composition was found to alter over time in plots treated with a single method (De Cauwer *et al.*, 2014). Plants are highly adaptable and are capable of becoming resistant to treatments, therefore a mixture of methods may be required (Rask, 2012). The most common species in a study in Belgium were *Poa annua* L. (90.8%) and *T. officinale* (63.8%) (Fagot *et al.*, 2011). This study found that Poacaea spp. accounted for 35%, *Taraxacum* spp. for 29% and mosses for 24% of the plant taxa. In another study conducted across Europe, *P. annua* was the most recurrent species noted, followed by mosses (Melander *et al.*, 2009). *P. annua* is known to grow all year round, they have the ability to cope with trampling, the seeds do not lie dormant and they germinate rapidly (Melander *et al.*, 2009; Fagot *et al.*, 2011). Along with *T. officinale* they are adept at coping with compacted and less well aerated soils, such as those found around hard surfaces (Melander *et al.*, 2009; Fagot *et al.*, 2011). Research discovered that *P. annua* was hard to control by thermal treatment potentially due to its protected meristems (Fagot *et al.*, 2011). This was the opposite to the findings in this study, where Hot Foam was highly effective for up to five months post-treatment on Poacaea spp. Experiments using just flame found that six sufficiently high treatments a year were required to suppress *Lolium perenne* L. effectively (Rask, 2012).

T. officinale is often noted on hard surfaces and is known to be difficult to control with thermal treatment (Rask, 2012), most likely due to its deep taproot (Fagot *et al.*, 2011; De Cauwer *et al.*, 2014). Here, the Hot Foam and New Way did initially reduce this species in the first couple of months but by the fifth and sixth survey it had fully recovered and had significantly increased. The Nomix Dual reduced the *Taraxacum* spp. initially in the first month but had no impact from then on. *Sphagnum* spp. have been found to resist glyphosate treatment, and have the ability to establish rapidly post glyphosate treatment and become established prior to the application of the next treatment (Fagot *et al.*, 2011). Here, the findings were similar for the mosses with neither glyphosate treatment having any effect, whereas both the New Way and Hot Foam were effective for five months, and the Hot Foam continued to suppress the moss spp. for eleven months. Mosses are known to be excellent pioneer species with spores capable of germinating easily on any suitable bare patches of soil (Fagot *et al.*, 2011). Bryophytes are not thought to create much damage to pavements (Melander *et al.*, 2009). However, they may act as a seed bed for the establishment of other species such as *P. annua*.

5.4.5 Recommendations

Ideally, for the efficient control of plants, prevention is better than cure and therefore must be considered at the design stage. Local authorities need to consider long-term strategic planning for new projects (Rask & Kristoffersen, 2007) and provide surfaces that reduce cracks and crevices to prevent seeds from germinating in the first place. This will result in a reduced requirement for plant control in the future (Looker, 1996; De Cauwer *et al.*, 2014). Permeable paving leads to a reduction of runoff after heavy rainfall, thereby mitigating flooding in conjunction with reducing the water availability to plants (De Cauwer *et al.*, 2014).

Where plants have taken hold, the local authorities need to think strategically, by ranking the location of hard surfaces in order of priority, while regular monitoring is imperative so that progress can be reviewed (DEFRA, 2015). A framework needs to be developed to include a preparation phase to include the type of hard surfaces, their extent, and the methods to be implemented (Kempenaar & Spijker, 2004). The next decision relates to tolerance levels from a complete lack of plants to being completely covered in vegetation (Kempenaar & Spijker, 2004). There are good reasons to 'do nothing': it saves money and the native plants are good for insects and biodiversity (Kehlenbeck *et al.*, 2016; PAN UK, 2021). For instance, in this study many pyramidal and bee orchids were observed along the Millennium Link Road in the areas where spraying and mowing had been reduced.

The use of brushes should be considered early in the growing season to remove soil to inhibit seeds from germinating (Rask, 2012). Systematic sweeping is highly recommended to keep plants under control. One study found that sweeping between seven and ten times a year controlled plants and did no damage to road surfaces, but ongoing monitoring was essential (Rask & Kristoffersen, 2007). Gutters in pavements should always be brushed rather than sprayed with herbicide to reduce pollution to water courses (Kortenhoff *et al.*, 2001). The application of alternative treatments needs to be sufficiently high to kill off all the above ground vegetation, thereby reducing the number of treatments necessary throughout the year (Rask, 2012). Treatment needs to be conducted at the earliest developmental stage and in dry conditions to reduce energy costs (Rask & Kristoffersen, 2007). The cost using sophisticated equipment needs weighing up as these methods are often slower to apply, and are energy and labour intensive (Rask & Kristoffersen, 2007). Where chemical treatment is necessary the dose needs to be adapted and guidelines strictly followed (Rask & Kristoffersen, 2007).

From the information gathered from the experiments conducted on the grass verge of the Millennium Link Road, the mechanical treatments were the most expensive but once the soil and organic matter has been removed, ongoing brushing should be sufficient to maintain hard surfaces. As for the chemical and thermal treatments on plants in the intersection, the Hot Foam was by far the most expensive and took the longest to apply. It was, however, effective at plant control on the intersection and is less toxic to aquatic environments. The Nomix Dual treatment was not successful at reducing the vegetation on the Millennium Link Road, though it was cheapest and took the least amount of time to apply. The New Way and Roundup Flex both cost the same while the Roundup Flex performed well at reducing vegetation along the intersection.

There is no silver bullet when it comes to controlling plants and a one-size-fits-all approach will not work for local authorities when it comes to plant removal. We need to re-examine our outlook of plants (Looker, 1996) and learn to tolerate rather than automatically eradicate them. An integrated approach may be the best way to manage plants in urban environments. To reduce the quantities of glyphosate being used, a combination of glyphosate and alternative treatments may be effective for plant control but does entail more effort and higher costs (Kempenaar *et al.*, 2006). A transparent approach is also required to inform and educate members of the public to manage their expectations on the aesthetics of public open spaces.

5.5 Conclusion

We need to become more tolerant of plants in urban environments. This has a double bonus for enhancing biodiversity while also reducing the risk of pesticide use on human and environmental health. Where it is necessary for local authorities to ensure plants do not become established, prevention is better than cure, so designing areas with no bare soil should be a priority. Hard surfaces, however, incur knock-on effects such as rapid run-off of rainwater potentially leading to flooding. To prevent plants from becoming established on existing hard surfaces the accumulation of soil and organic matter must be averted by brushing. In a situation where plants have become established, a framework is required to agree on the best approach to deal with them with the initial decision being the tolerance level, followed by an integrated approach. Alternative treatments take longer, and in the case of thermal treatments are energy heavy, so are not always cost-effective. Treatment with glyphosate should be used sparingly and only in situations where alternative treatments are not practical. The appropriate measures must be taken to prevent the release of glyphosate to the aquatic environment.

Chapter 6 General Discussion

6.1 Summary

Biodiversity loss is occurring at an alarming rate due to the voracious depletion of natural stocks by an ever-expanding human population. Our current global economy is so reliant on healthy and functioning ecosystems that every effort, however small, must be made to reduce negative human impacts and enhance biodiversity in order to promote and preserve these ecosystems (NPWS, 2024). The more biodiverse an ecosystem, the more resilient it will be in the face of future anthropogenic challenges, and principally, climate change. There is considerable responsibility on those that manage our rural environments, national parks, farms etc., as the stewardship of our land must prioritise biodiversity, reduce environmental damage and recognise that the health of our environment and ourselves are inextricably linked (EPA, 2024b). One such group that bears this responsibility are our local authorities who are in a unique position to enhance and restore biodiversity as they manage substantial areas of land and biodiversity-rich habitats. These public open spaces can be treated as refugia for biodiversity while the roads, and more specifically their verges, should serve as corridors between these refugia, and indeed, other vital habitats. My PhD research was borne from a determination to understand the value of public open spaces for biodiversity and to assess how best to manage them. As a contribution to these aims four specific research questions were asked to assess i) the value of urban town parks for pollinators, in comparison with neighbouring peri-urban farmland landscapes consisting of low-intensity farmland, ii) how the management of road infrastructure, specifically roundabouts, affects pollinator distributions, iii) how weed control practices involving glyphosate and glyphosate alternatives effect vegetation, and soil fungal and bacterial communities, and iv) whether non-glyphosate based plant-treatments represented viable alternatives for the management of roadside verges. I now provide here a synthesis and discussion of the main findings of my thesis, explore the limitations and provide recommendations for future work and research.

6.2 Major findings

The overarching findings show that public open spaces could be utilised as a major national asset for the protection and restoration of biodiversity. The land currently managed by local authorities represents a significant opportunity to highlight the effectiveness of enhancing and restoring habitats through low-intensity management approaches, and as a consequence provide pollinating insects (Chapters 2 and 3) and soil microorganisms (Chapters 4 and 5) with the resources they require to thrive. This is particularly true when the local authorities and those responsible for managing our public lands recognise the importance of biodiversity and the environment and realise their responsibility in averting the biodiversity and climate crises. In Kildare County Council this is the prevailing view, and the county is benefitting from it. From the findings presented here, it is clear that the most direct way to improve biodiversity is to simply take a more hands-off approach to land management and learn to tolerate what is perceived to be untidy or unkempt. This does not have to come at the expense of public safety, and inputs and efforts are still required in certain circumstances. The main findings of my thesis, answering essential questions on ways to improve biodiversity on public open spaces, are summarised and further discussed below.

6.2.1 Management approaches effect the abundance and richness of pollinating insects on public open spaces.

The findings in this study indicate that the management approaches do effect pollinating insects. The results imply that a significant relationship exists between insect pollinator abundance and richness, and the type of management (Biegerl *et al.*, 2025). Those sites classified as low-intensity peri-urban farmland, despite not being in High Nature Value areas, tended to have the highest levels of pollinator abundance and diversity (Chapter 2). A similar finding was presented in Chapter 3, in that the roundabouts that were managed less intensively and had a more varied plant species composition, had greater pollinating insect abundance and richness. Combined, it seems that the maintenance of the semi-natural habitats on farmland (Chapter 2) and the provisioning of floral resources from spring to late summer on ornamental wildflower roundabouts (Chapter 3), provides enhanced habitats for insect pollinators (Baldock *et al.*, 2019; Spotswood *et al.*, 2021; Griffiths-Lee *et al.*, 2022; Tew *et al.*, 2022). The management of grasslands and woodlands (including hedgerows and treelines) drove a difference between the urban town parks and the peri-urban farmland sites (Chapter 2), with a higher abundance for all pollinator groups, though not statistically so for the hoverflies, on the less intensively managed peri-urban farmland sites (Bishop *et al.*, 2025). The unmanaged boundaries on the peri-urban farmland contained a higher diversity of all four groups, especially the hoverflies, with a better structural diversity providing them with good shelter, floral resources, nesting sites, wet habitats and micro-habitats, while the grasslands were left unmown until late summer/early autumn, enhancing the richness of all groups, particularly the hoverflies and butterflies (Maher *et al.*, 2024).

There was also a difference in the community composition of pollinators between the town parks and peri-urban farmland sites, and the green island and ornamental wildflower roundabouts. Bumblebees and solitary bees were associated with the peri-urban farmland while there was more of an overlap in the community composition of the butterflies and hoverflies between the peri-urban farmland and urban sites. All four pollinating groups were clustered separately between the ornamental wildflower and green island roundabouts, with more diversity in general on the ornamental wildflower roundabouts. The constant supply of floral resources on the ornamental wildflower roundabouts together with the plant species that had either been purposefully sown, or the naturally regenerated native species, are likely to have caused these effects.

The results suggest that the diversity of habitats and plants did not drive the difference in pollinating groups between urban and farmland sites, as the diversity in neither habitats nor plants was significant between the type of sites. This was also the case on the roundabouts where there was no difference between the plant diversity. These findings indeed confirm that the specific approaches to management have a major effect on insect pollinator abundance and richness on public open spaces.

6.2.2 Is there a relationship between soil microbial communities, soil physio-chemicals and treatments?

The results from this study did find differences between glyphosate and glyphosate alternative treatments and the soil fungi and bacteria, but that soil physio-chemical properties including nutrients and weather conditions also play a role in influencing soil microbiota (Chapter 4). Under the Hot Foam treatment, a positive correlation was found between the abundance of bacteria and phosphorous levels in the soil, whereas a negative association was observed between these variables over time, potentially due to the increase in temperature (Ali *et al.*, 2024). The Nomix Dual treatment was determined to have altered the community composition of the bacteria, in part due to associated changes in the pH, water holding capacity and soil organic matter content. The bacterial community structure did change over four months even though the overall diversity remained stable.

The abundance of the fungi fluctuated under the control, Hot Foam, Nomix Dual and Roundup Flex treatments over time. Overall, there was an increase in fungal abundance in the one month post-treatment application, before a decrease over the final months. There was a rise in fungal abundance under the Hot Foam in conjunction with pH. The results here suggest that the fungal species richness changed over time but not due to treatment (Kepler *et al.*, 2020; Zhu *et al.*, 2022). The community composition clearly changed over the course of the experiment. The environmental variables- organic carbon, moisture content, soil organic matter and pH, may have influenced the change in fungal abundance, diversity and community composition.

The findings here suggest that seasonal effects may have had more of an impact on the abundance of the soil bacteria and fungi, and the richness of fungi, than any of the treatments. The richness of the bacteria remained stable. The effect of treatments was not clear in this study, with random variation potentially due to plasticity in response to environmental variables, though the Hot Foam may have negatively impacted the bacterial abundance. These micro-organisms are known to react swiftly to change, and the alterations could have been due to any number of other influences such as temporal variations and water stress (Ockleford *et al.*, 2017; Liu *et al.*, 2023). For both communities there were clear shifts in the composition over time and are in all likelihood due to seasonality.

6.2.3 Is there a difference in the effect of treatments on plant species in *in situ* experiments on a greenfield site and a road verge?

The results from this research found a difference between the effectiveness of treatments on a greenfield site (Chapter 4) and the road verge experiments (Chapter 5). One month after treatment the Hot Foam, Nomix Dual and Roundup Flex applications resulted in significantly reduced vegetation on the greenfield site (Chapter 4) whereas on the road verge (Chapter 5) the Hot Foam, New Way and Roundup Flex reduced the vegetation after one month, while only the Roundup Flex succeeded in controlling the vegetation five months post-treatment. As for the individual species, Poaceae spp. were negatively impacted by the Hot Foam, Nomix Dual and Roundup Flex one month post-application on the green field site, whereas on the road verge, the Hot Foam, New Way and Roundup Flex were still effective two months post-treatment. Nomix Dual had no effect on vegetation on the intersection of the road verge while the New Way failed to reduce the vegetation significantly on the greenfield site. *Taraxacum* spp**.** on the road verge were unaffected by any treatments at two months but after five months the Roundup Flex was the only treatment that reduced their abundance. Hot Foam was the only treatment where *Taraxacum* spp. increased at four months on the greenfield site and five months on the road verge. However, Hot Foam negatively impacted the bryophytes, pioneer species along the road verge, throughout the duration of the experiment.

6.2.4 Are there differences in the cost effectiveness between treatments?

As regards the cost-effectiveness, the findings from this study observed differences in vegetation control between the greenfield site (Chapter 4) and the road verge (Chapter 5) and the glyphosate and glyphosate alternatives on vegetation. The Roundup Flex was the most effective along the intersection of the road verge, followed by the Hot Foam, with both treatments being equally effective on the greenfield site. Although the Hot Foam is almost three times the cost of Roundup Flex, it is potentially less damaging to the environment. On the other hand, the Roundup Flex was the only treatment with the ability to moderate the *Taraxacum* spp**.** after five months, so if all the guidelines are followed to lessen the potentially damaging effects of this glyphosate treatment, it may be the best solution for keeping this species under control.

Chapter 5 also assessed the cost effectiveness of treatments plus the efficacy of alternative treatments that included the use of machinery, to remove vegetation in conjunction with soil from the road verge. The WeedHex machine and Multihogg machines both successfully removed the vegetation and soil, however, the WeedHex machine was the most cost-effective.

6.3 Significance of findings

Biodiversity loss is occurring at an unprecedented rate, with almost 73% of globally monitored wildlife populations diminished over the last five decades (WWF, 2024). The EU has strict regulations concerning nature restoration with the EU biodiversity strategy 2030. In light of the state of biodiversity in Ireland, the findings of this PhD contribute significantly towards finding practical measures to improve and re-establish a significant area of land in Ireland that lies under the remit of local authorities. Through my thesis, I provide a holistic approach to enhance and restore state-owned land. My field work reveals the environmental condition of peri-urban farmland, town parks and roundabouts under the various management approaches of Kildare County Council. I provide insights into the reasons why some management approaches enhance insect pollinator, and how others effect vegetation and soil biodiversity, and finally I provide recommendations for restoring and enhancing biodiversity on public open spaces.

There are a number of EU regulations that explicitly require the monitoring and improved conditions for pollinators and soil organisms. The Nature Restoration Regulation, a key element of the EU Biodiversity Strategy, aims to restore degraded ecosystems by increasing biodiversity in conjunction with building up our resilience to climate change (EC, 2024b). The main objective is to restore ecosystems, habitats and species through specific targets, such as reversing the decline of pollinators by 2030, increasing urban green spaces and enhancing butterflies on farmland. The EU Pollinators Initiative was established to prevent further declines of pollinators, through improved monitoring, management of lands for pollinators and by encouraging organic methods (NBDC, 2021; Vujić *et al.*, 2022). The Soil Strategy for 2030 is aimed at prioritising soil health for biodiversity and climate resilience to ensure they continue to provide essential ecosystem services. Two of the objectives include reducing soil pollution and sustainable management practices (EC, 2021c). Member States are required to ensure the safe use of pesticides, including glyphosate, under the Sustainable Use of Pesticides Directive (EU, 2019).

Six percent of the land cover in Ireland is under state management, with county councils managing 1.2%, of which 0.3% is managed by Kildare County Council (Land Development Agency, 2023). The National Biodiversity Action Plan (2002) recognises the role of local authorities as pivotal to the promotion and conservation of biodiversity while the County Kildare Biodiversity Action Plan 2009-2014 promotes conservation and management to inform policies on biodiversity issues (KCC, 2008). The EU Pollinators Initiative commits Europe to discover the causes, confront those causes and activate society to reverse pollinator decline by 2030 (EC, 2022c). My findings show that county councils in Ireland are in an exceptional position to achieve all of the above. Considering that this council land is spread over a variety of habitats across the island of Ireland, county councils and state bodies are in a unique position to enhance and restore biodiversity. The findings in this research show the negative impacts of high intensity management approaches, be they on urban town parks or roundabouts. Through the findings in this thesis, I provide a number of practical measures below that county councils can take to ensure the state assets managed by them are improved for biodiversity. Some of the outcomes of this study show how three specific Nature Restoration Regulation targets can be achieved through lowering the management intensity on sites, namely, increasing pollinators, and by improving both agricultural and urban ecosystems. Member States need to have 'due regard' for biodiversity in areas such as public parks and sports grounds (EU, 2019).

6.4 Limitations and opportunities for further research

The main limitations of this work are related to external factors that were beyond my control such as weather conditions, financial restrictions and feasibility restraints. However, these can all be seen as opportunities for further research. More specifically:

1. Sampling pollinators in the field is highly weather dependant, with narrow criteria required in order to ensure as many insects as possible are surveyed. If the weather is wet or too windy, pollinators, especially the smaller species, are likely to take shelter rather than being out foraging, reducing their likelihood of being recorded. Ensuring weather conditions are perfect during surveys is not always possible in an Irish climate. Hoverfly identification was an issue, particularly in the first season, due to unfamiliarity with species, especially during transects where I was learning in the field. Having specialists in the field, although incurring increased costs, may enhance the variety of species recorded. As the only surveyor, the number of sites I could sample each month was restricted.
2. The soil experiments were limited by the quantities of soil used in the experiments and a lack of funds to expand the number of experiments further.

This would be an ideal opportunity for further research to enhance our understanding of soil biodiversity, which is lacking in this crucial ecosystem, through further field and laboratory experiments. Laboratory experiments would enable an assessment of the changes within the soil in a controlled environment.

3. Biotic and abiotic factors are likely to have influenced differences between microbial communities. It was impossible to control for other environmental variables such as weather conditions, nutrients and physio-chemical variables in this field experiment. The movement of other soil organisms such as earthworms and nematodes may also have influenced the soil bacteria and fungi. Future research in this area on both field (recording weather conditions) and laboratory experiments is highly recommended.
4. Both the soil and road verge experiments were conducted where glyphosate had previously been sprayed, and this may have affected the existing species composition and the glyphosate resistance of some of the plant species. Whether the bacteria and fungi had become inured to the effects of glyphosate, may have hindered the results of this treatment on these microorganisms. There are many other alternative treatments being used globally by local authorities and members of the public, that could also have been trialled in this experiment. Further research into other alternatives could be highly beneficial.

6.5 Practical recommendations and policy implications

Fully functioning ecosystems provide us with services such as pollination and clean water, are more resilient to climate change, but are irreplaceable due to the scale and expense of recreating ecosystems (IPBES, 2019; Demozzi *et al.*, 2024). A change in land use on public open spaces does improve conditions for pollinators and soil microbes, as agricultural intensification and urbanisation are key drivers of decline in both pollinators (Regan *et al.*, 2010; Baldock *et al.*, 2019; Harpke *et al.*, 2025) and soil biodiversity (Orgiazzi *et al.*, 2016; EPA, 2024b; Phillips *et al.*, 2024). The following seven practical recommendations will ensure that public open spaces are enhanced for biodiversity and reduce the impacts of ongoing biodiversity loss.

1. **Reducing maintenance**

My highest recommendation is that local authorities reduce maintenance where practical on all public open spaces. My research did find that an increase in spatial heterogeneity on the low-intensity peri-urban farmland supported a greater abundance and species richness of insect pollinators, than the highly maintained town parks. The woodland edge habitats (incorporating the hedgerows, treelines and wooded areas) did sustain greater abundances of bumblebees, butterflies and solitary bees and a greater diversity of all four pollinator groups, including hoverflies. These habitats may be particularly important for bumblebees by providing nesting sites and floral resources (Alison *et al.*, 2021; Maher *et al.*, 2024). Local authorities can reduce the impact of climate change on pollinators by reducing the maintenance approaches on their land. Climate change is expected to reduce pollinator diversity (Karbassioon *et al.*, 2023). However, structural diversity is known to provide protection, shelter, food and nesting sites, and an improvement of the semi-natural habitats, such as the hedgerows and trees (Hopwood *et al.*, 2015; Maher *et al.*, 2024), is acknowledged to support higher species richness and abundance by providing resources, thereby enhancing ecosystem resilience (Oliver *et al.*, 2015). Ensuring the trees and shrubs are allowed to flourish will also reduce the heat island effect in urban environments, the negative effects of which on bees and floral resources are well documented (Fenoglio *et al.*, 2021).

2. **Reduce mowing, herbicide and fertiliser use.**

I observed a higher abundance and diversity of butterflies on the peri-urban farmland in the grassland habitats where management is of low intensity and the grass was mown late in the year. Based on my findings the grassland areas need to be managed organically and sustainably, by preventing the use of fertilisers and curtailing the number of cuts per year. Fertiliser use results in thick swathes of grass thereby reducing the quality of the habitat for butterflies (Van Swaay *et al.*, 2010; Arnold *et al.*, 2025). Butterflies depend on these habitats for a large part of their life cycle (Harpke *et al.*, 2025). The bumblebee diversity was greater in the grasslands of the peri-urban farmland too. The cutting of silage earlier in the season rather than traditional hay making which is cut later, is thought to have led to a decline in late-emerging grassland nesting bumblebees (FitzPatrick *et al.*, 2007). Of note, the frequent mowing of the grass on the green island roundabouts in this study did allow *Taraxacum* spp**.** to flower and was the only plant that solitary bees visited during surveys of these roundabouts. Allowing organic livestock on council land, instead of mowing, is recommended to keep bare banks open, but over grazing must be prevented. Solitary bees need bare banks, which can be provided by the livestock and drainage ditches (Maher *et al.*, 2024) on the peri-urban farmland. There is no one-size-fits-all and a variety of sward heights will encourage a variety of plant species. This is where livestock is invaluable as a range of sward heights occur.

3. Incorporate water bodies.

I found the diversity of hoverflies was greater on the peri-urban farmland, potentially due to the variety of extra microhabitats that existed on this land, particularly the drainage ditches, ponds and water troughs that the saprophagous hoverflies require. Many species of hoverfly need wetland habitats, and dead wood in veteran trees, for their larvae (Vujić *et al.*, 2022). Incorporating water features, however small, will enhance conditions for a multitude of species, not just pollinators (Gibbons *et al.*, 2023). None of the urban sites had a single still water body, therefore, to enhance biodiversity I highly recommend incorporating a water feature, however small.

4. Provide floral resources.

The results from the ornamental wildflower roundabouts proved the importance of floral resources as the abundance and diversity of insect pollinators were highest on these unmanaged sites, in comparison to the green island roundabouts. Ensuring council land contains sufficient floral resources throughout the season for pollinators is critical, as a lack of flowers is known to be a limiting factor (Nieto *et al.*, 2014; Hopwood *et al.*, 2015; Salisbury *et al.*, 2015; Hicks *et al.*, 2016; Goodwin *et al.*, 2017; Baldock *et al.*, 2019; Griffiths-Lee *et al.*, 2022; Biegerl *et al.*, 2025; Bishop *et al.*, 2025). The floral resources were limited by one or two shrub species on the highly maintained green island roundabouts that expanded the temporal gaps, reducing the supply of nectar and pollen. *R. fruticosus* agg., an important native species, provided floral resources for 39% of the pollinator visitations across both farmland and town park sites (Alison *et al.*, 2021; Russo *et al.*, 2022). Native species, including those on the Noxious Weeds Act, 1936, list should be allowed to flourish where practical. Native plant species supported more pollinator visitations than non-native plants in this study, on both the farmland and urban sites, and included *J. vulgaris*, *Cirsium* spp., *Ranunculus* spp. and *Taraxacum* spp**.** On the roundabouts*, C. nigra, K. arvensis* and *Taraxacum* spp**.** were the most frequently visited plants and accounted for 68% of visits by pollinators. All of these species naturally occur in Ireland. Both *J. vulgaris* and *Cirsium* spp. are on the Noxious Weeds Act and local authorities are responsible for controlling these species on land they maintain. However, this study shows their importance as a floral resource for insect pollinators. Natural regeneration of plants should be prioritised over seed mixes to ensure invasive species are not introduced, as species best adapted to local environmental conditions will flourish, as seen on the ornamental wildflower roundabouts. However, seed mixes need not be ruled out as long as native, local species are sown, and this could be accomplished through green hay transfer (Wagner *et al.*, 2020). Most species are of value to wildlife, irrespective of whether or not they are native and the more biodiverse the plant species, the more biodiverse the animals (Alexander *et al.*, 2006). Furthermore, incorporating orchards and in particular allotments, are known to significantly enhance plant-pollinator interactions through their provision of highly diverse floral resources (Baldock *et al.*, 2019).

5. Develop roundabouts and road verges for biodiversity.

I highly recommend that roundabouts and road verges should be developed for pollinators. The ornamental wildflower roundabouts did support a higher abundance and diversity of pollinating insects. Even though there is the question as to whether these sites may act as sources or sinks, they may aid some species by acting as refugia. The roundabouts could be used as stepping stones between habitats, while the road verges can be utilised as corridors to other habitats, thereby reducing potential genetic bottlenecks. With climate change, pollinators and other smaller species could use the corridors managed by local authorities to move to more suitable environmental niches. The fragmentation of habitats has caused issues for poor dispersers (Vujić *et al.*, 2022) but the road verges could be implemented to connect habitats for these species. International and national monitoring of pollinators is essential to gather data on species trends (Potts *et al.*, 2016). Future research needs to compare the nesting, reproduction and overwintering success of pollinators in road verges and roundabouts (Phillips *et al.*, 2020b), and future government funding needs to prioritise threatened species, particularly the arthropods, of which only 1.1% have been described (Guénard *et al.*, 2025). Also, standardised and systematic monitoring of pollinator species does need to be established across Europe to assess trends. Insects generally have short life cycles, so react quickly to environmental changes providing excellent species to study impacts, both positive and negative (Regan *et al.*, 2010).

6. **Tolerate plants in public open spaces thereby reducing the use of glyphosate.**

To prevent the further deterioration of biodiversity it is recommended that a more tolerant attitude is required towards plants that grow along road verges and in other public open spaces. To achieve this, the importance of plant species for biodiversity and the negative impacts of herbicide use, needs to be conveyed to the general public through public campaigns and education, upskilling landscapers and maintenance contractors, and changes in policy. Fifteen percent of pesticides are used in private residences and public open spaces (Kim *et al.*, 2017) but there are many reasons to reduce our reliance on pesticides. Soil is one of the key reservoirs of biodiversity (FAO, 2015), but this ecosystem is severely threatened by agricultural intensification, including pesticides, and human expansion (Orgiazzi *et al.*, 2016; Phillips *et al.*, 2024). This realm provides a plethora of ecosystem services from water purification, formation of organic matter, nutrient cycling, flood control, carbon sequestration, and food provision to name a few (Jeffery *et al.*, 2010; Creamer *et al.*, 2016; FAO, 2017; Ockleford *et al.*, 2017; Gunstone *et al.*, 2021). However, due in part to pollution from pesticides, this ecosystem is deteriorating. The majority of pesticides (99%) used are wasted and contaminate our soil and waterways (van der Werf, 1996; Arias-Estévez *et al.*, 2008; Meena *et al.*, 2020; Vickneswaran *et al.*, 2023). These pesticides and their breakdown products often accumulate in soils (Van Bruggen *et al.*, 2018; Zioga *et al.*, 2022) and have been found in drinking water supplies (Horth, 2009; Carretta *et al.*, 2022). Experiments have shown the effects of glyphosate on organisms, from tumours and kidney damage in rats (Myers *et al.*, 2016; Van Bruggen *et al.*, 2018), to a decrease by 66% in the amount of chicks hatching (Fathi *et al.*, 2019). Direct and indirect effects of pesticides on pollinators include a lack of floral resources and impacts on foraging behaviour, both resulting in reduced colony fitness (Cullen *et al.*, 2019; Benner *et al.*, 2023; Karbassioon & Stanley, 2023). The effect of glyphosate on humans is unknown as we are exposed to a cocktail of chemicals (Gerken *et al.*, 2024) through our drinking water, food, inhalation and skin (van der Werf, 1996; Kim *et al.*, 2017). Through biomonitoring of urine, blood and breast milk we do know that humans contain glyphosate (Camiccia *et al.*, 2022; Connolly *et al.*, 2022), however, it is very hard to prove there is a link between glyphosate and human disease (Myers *et al.*, 2016; Kim *et al.*, 2017).

The effects of glyphosate on the soil microbiome are inconclusive and vary greatly depending on the pesticide and species being tested (Gunstone *et al.*, 2021), as my results showed. Recovery has purportedly been fast (Ockleford *et al.*, 2017), though there may be a change in the composition due to the differing sensitivities of species (Van Bruggen *et al.*, 2018). The community composition of the bacteria did change under the Nomix Dual treatment, but I found no change in the fungal composition under either of the two glyphosate treatments. Some experiments have found little impact on soil microorganisms (Van Bruggen *et al.*, 2018; Meftaul *et al.*, 2020; Hudek *et al.*, 2021). Others have found negative effects such as a reduction in mycorrhizal fungi by 40%, or lethal effects on sensitive organisms (Meena *et al.*, 2020), another found the activities of the microbes were curbed (Yousaf *et al.*, 2013). But some species have the ability to metabolise glyphosate (Van Bruggen *et al.*, 2018; Kepler *et al.*, 2020; Matozzo *et al.*, 2020) through their fast growth rates and their ability to evolve rapidly to changing conditions (Ockleford *et al.*, 2017).

There is no doubt that glyphosate is highly effective at killing off vegetation as was shown in my results. One of the glyphosate-based products, Roundup Flex, performed well on both the greenfield and road verge experiments, as did the thermal alternative treatment, Hot Foam. However, my results found that the abundance of the bacteria did fall significantly under the Hot Foam. The Hot Foam treatment implements high temperatures which have been found to alter the abundance and composition of both bacteria and fungi (Ali *et al.*, 2024). The Nomix Dual glyphosate treatment shifted the composition of the bacterial species and although the diversity of the bacterial species did not change over time, the community composition did. The fungal abundance fluctuated significantly under all treatments throughout the duration of the experiments. Fungal diversity and community composition altered over time; however, no treatments were deemed to have caused significant changes. The impacts of treatments on the soil bacteria and fungi may have been more influenced by the soil physio-chemicals, nutrients and weather conditions that could have caused fluctuations in both the abundance and diversity of these soil microorganisms, as has been noted in other studies (Liu *et al.*, 2023). Over the course of the experiment the abundance, species richness and the community composition of both the bacteria and fungi did alter over time, potentially due to seasonal changes, soil moisture in particular, in the local environment. Chen *et al*. (2023) and Zhu *et al*. (2022) noted that reduced water availability led to an inhibition in microbial activity. Also to note, glyphosate may not have impacted the bacterial and fungal diversity to the same extent as the Hot Foam due to the historic use of this herbicide on the greenfield site.

To minimise the risk of glyphosate to human health, biodiversity and aquatic environments, this study recommends a more holistic approach to plant control through the use of integrated management practices. The precautionary principal should be applied where possible as, until we have definitive proof, we must assume that these pesticides pose a potential threat to human health and biodiversity. In fact, the vast majority of research already indicates this, but by adopting practices that are based on reduced pesticide use, many of these risks will be mitigated. The results of this thesis also highlight the importance of including preventative measures for vegetation control in the planning design phase, a position also recommended by Rask and Kristoffersen (2007). Ensuring narrow intersections on road verges are sealed initially will prevent plants gaining a foothold. In regard to the maintenance of hard surfaces, it seems that much of the vegetation that requires control could be avoided if soil and organic matter build-up is avoided in the gaps between paths and verges. The frequent brushing of these junctions can prevent seed germination and through the research carried out in this thesis, it seems that mechanical brushing would present the best option. The WeedHex machine was highly effective at removing vegetation and the deep build up of soil along the road verge, is very cost effective and it looks neat and tidy. Brushing may also prevent young plants from gaining a foothold if applied early in the season.

Poacaea spp., *Taraxacum* spp**.** and mosses were the most prolific along the intersection of the road verge but, except for the *Taraxacum* spp**.,** the Hot Foam was successful at reducing these two. My results have shown that overall, the Hot Foam was effective at reducing the vegetation on both the greenfield site and the road verges. The Hot Foam treatment, though costly and involving high energy and water use, may be a suitable alternative especially as it was effective on the mosses, a known pioneer species tolerant to glyphosate. By reducing mosses, the germination of other plants may be inhibited through the prevention of their establishment due to the lack of a suitable seed bed. The advantage of Hot Foam treatment is that it uses no chemicals and can be implemented in all weather conditions. The use of glyphosate may be required on species such as *Taraxacum* spp. as, because of its long tap root, the likelihood of alternative treatments effectively killing these species is low.

7. Mandatory reporting of herbicides

I recommend the mandatory reporting of herbicide use in public open spaces of pesticide treated areas by local authorities. At present farmers are required to report the herbicides and the quantities used, and these can be found on the DAFM website. However, trying to find the quantities and location of herbicide use by local authorities was not possible for this thesis.

6.6 Conclusion

Although actions are being taken by local communities, local authorities and governments to reduce biodiversity loss (Millennium Ecosystem Assessment, 2005), it is clear that we need to urgently expand efforts to help monitor, restore and conserve our precious biodiversity. Local authorities have a statutory obligation to protect our environment by improving water quality and protecting our biodiversity (EPA, 2024b). Through the efforts of groups such as the National Biodiversity Data Centre and the All-Ireland Pollinator Plan, we are provided with guidance and measures that are proven to enhance biodiversity. These measures can be highly effective, and indeed, the rates of effectiveness are improving over time (Langhammer *et al.*, 2024). However, in order to tackle the biodiversity crisis, these conservation and restoration efforts need to be expanded rapidly and on a global scale. The implementation of global measures to prevent further biodiversity loss through conservation was estimated at between $178 billion and $524 billion per year, but these figures are miniscule in comparison to the value of ecosystem services we receive. Restoration and conservation measures need to be seen as investments that yield high returns (Langhammer *et al.*, 2024).

There has been progress in adaptation measures in urban planning with varying degrees of success, such as urban greening and nature-based solutions, and it is clear that these measures are also effective at reducing flooding and urban heat effects (IPCC, 2023). Government bodies and Local Authorities should know that there is no silver bullet to tackle the biodiversity crisis (Demozzi *et al.*, 2024) but they can however reduce biodiversity loss by implementing some of the extremely easy measures noted above, mainly by lowering their management approaches and learning a more tolerant approach to plants that thrive in the ‘wrong’ places. Through this research I have deduced that we must learn to take a hands-off approach and allow nature to proliferate where practical. The ‘do nothing’ approach is cost-effective, and improves biodiversity (Kehlenbeck *et al.*, 2016). Nature-based solutions provide multiple benefits by reducing biodiversity loss and the impact of climate change, improve health and well-being, and are cost effective (Dasgupta, 2021). ‘We all have a role to play in the protection and restoration of nature’ (NPWS, 2024).

"Look closely at nature. Every species is a masterpiece, exquisitely adapted to the particular environment in which it has survived. Who are we to destroy or even diminish biodiversity?" ~ E. O. Wilson (1929-2021).

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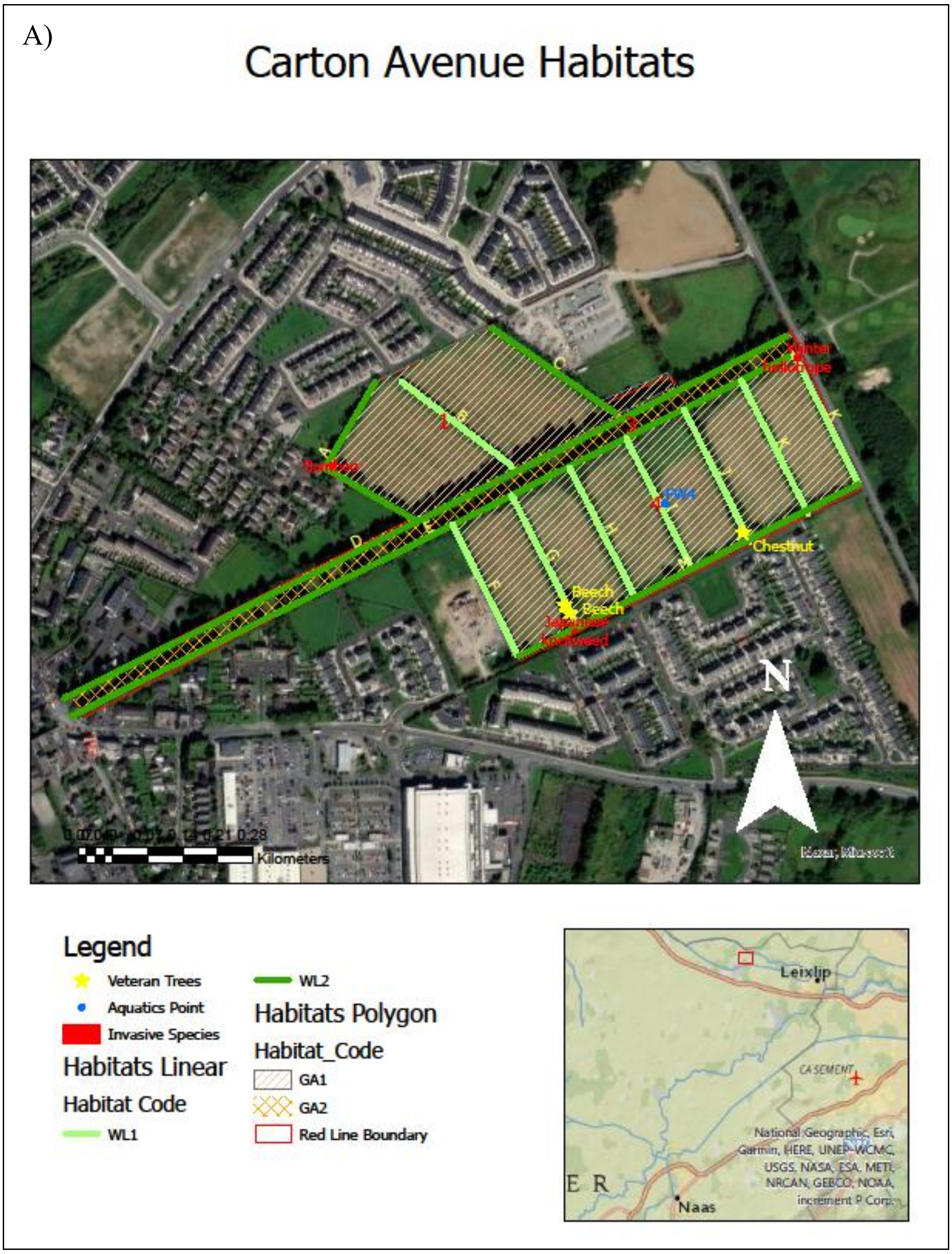
Appendices

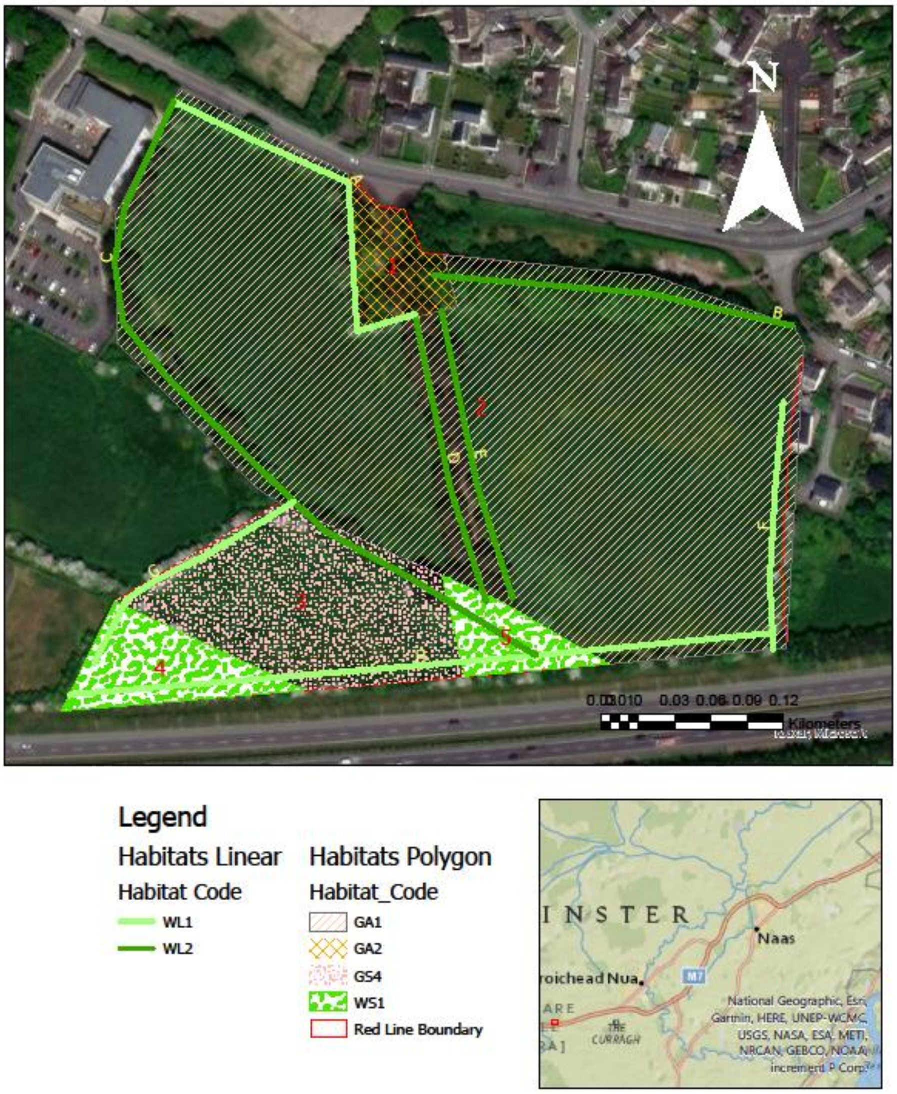
Appendix A Supplementary Data for Chapter 2

Figure A- 1 Maps of the six sites with their habitats- Agricultural: Carton Avenue (A), Cherry Avenue (B), Sallins (C) and Town Parks: Liffey Linear Park (D), Monread Park (E) and the Wonderful Barn (F).



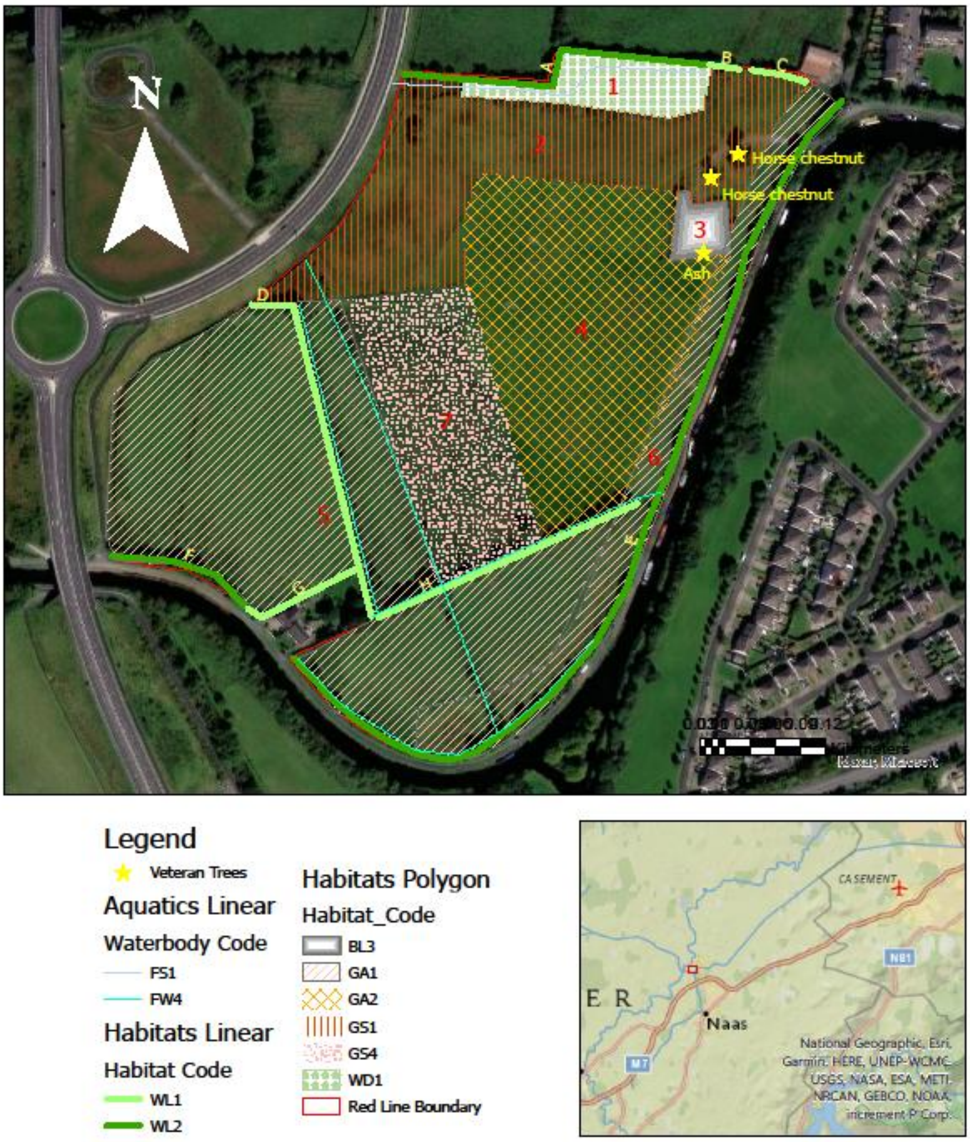
B)

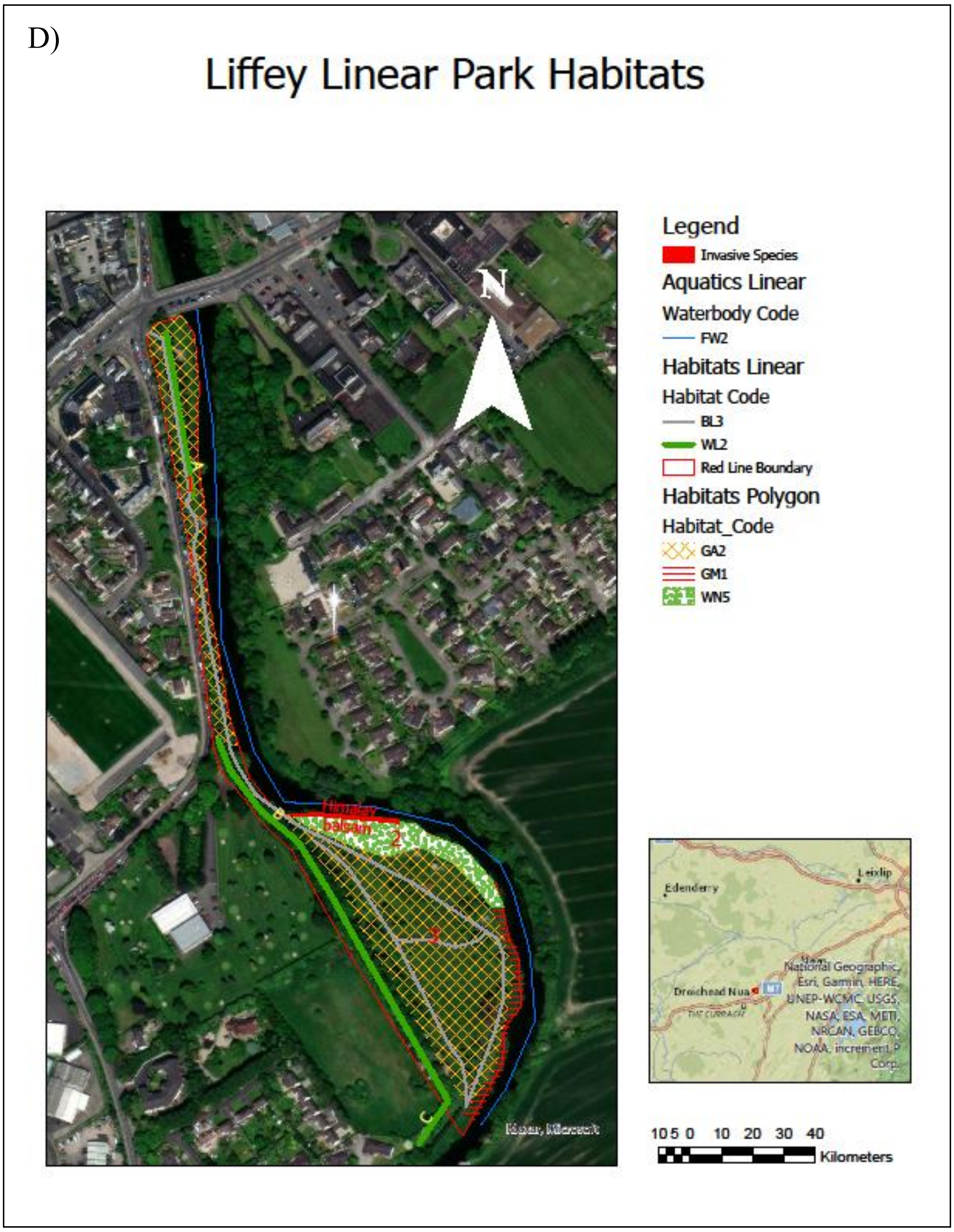
Cherry Avenue Habitats

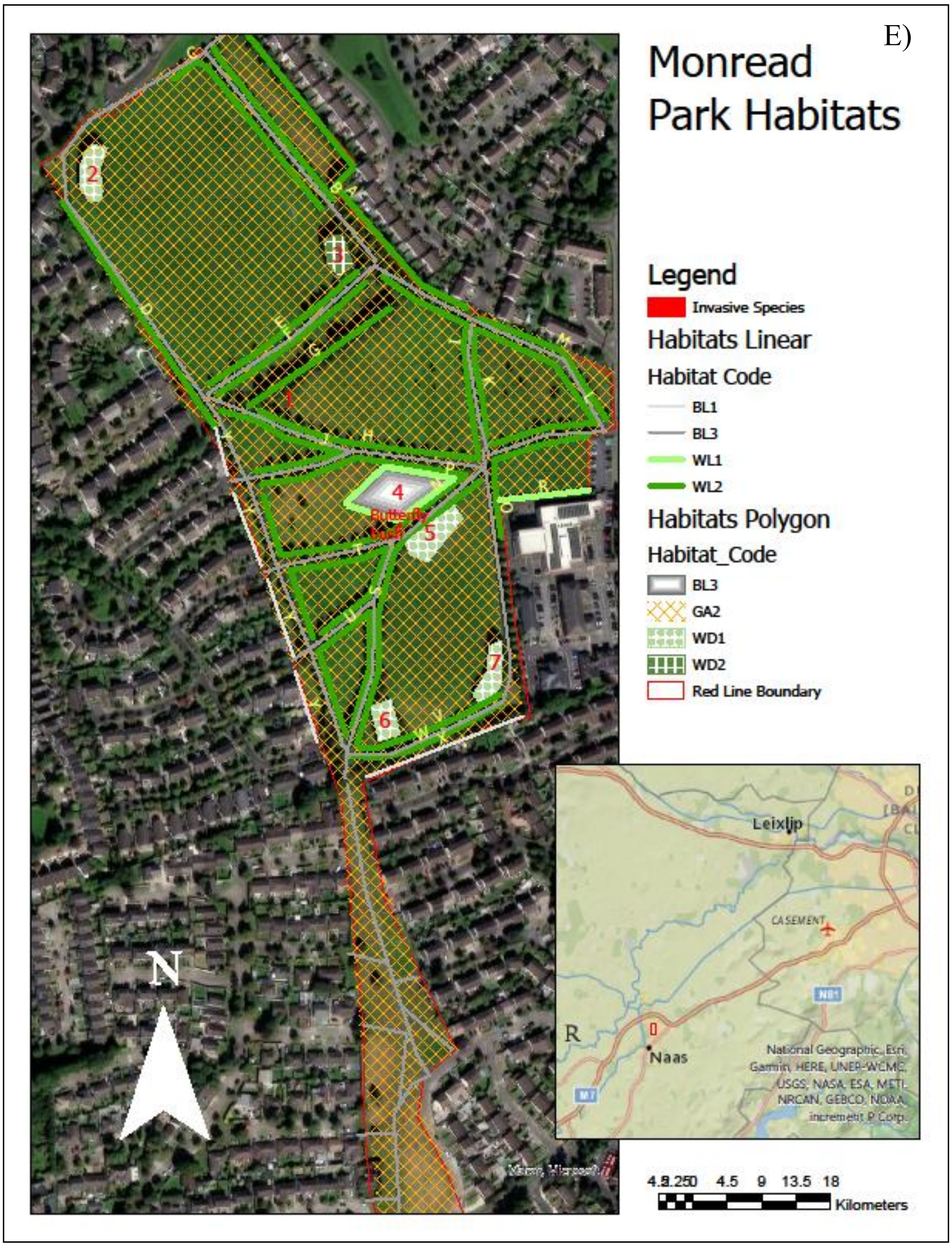


C)

Sallins Habitats




D)
Liffey Linear Park Habitats
N
Legend
Invasive Species
Aquatics Linear
Waterbody Code
FW2
Habitats Linear
Habitat Code
BL3
WL2
Red Line Boundary
Habitats Polygon
Habitat_Code
GA2
GM1
WN5
Edenderry
Leixlip
Droichead Nua
National Geographic, Esri, Garmin, HERE, UNEP-WCMC, USGS, NASA, ESA, METI, NRCAN, GEBCO, NOAA, increment P Corp.
10 5 0 10 20 30 40
Kilometers


E)
Monread Park Habitats
Legend
Invasive Species
Habitats Linear
Habitat Code
BL1
BL3
WL1
WL2
Habitats Polygon
Habitat_Code
BL3
GA2
WD1
WD2
Red Line Boundary
Leixlip
CASEMENT
N81
Naas
M7
National Geographic, Esri, Garmin, HERE, UNEP-WCMC, USGS, NASA, ESA, METI, NRCAN, GEBCO, NOAA, increment P Corp.
4.5 2.25 0 4.5 9 13.5 18
Kilometers
N

F)

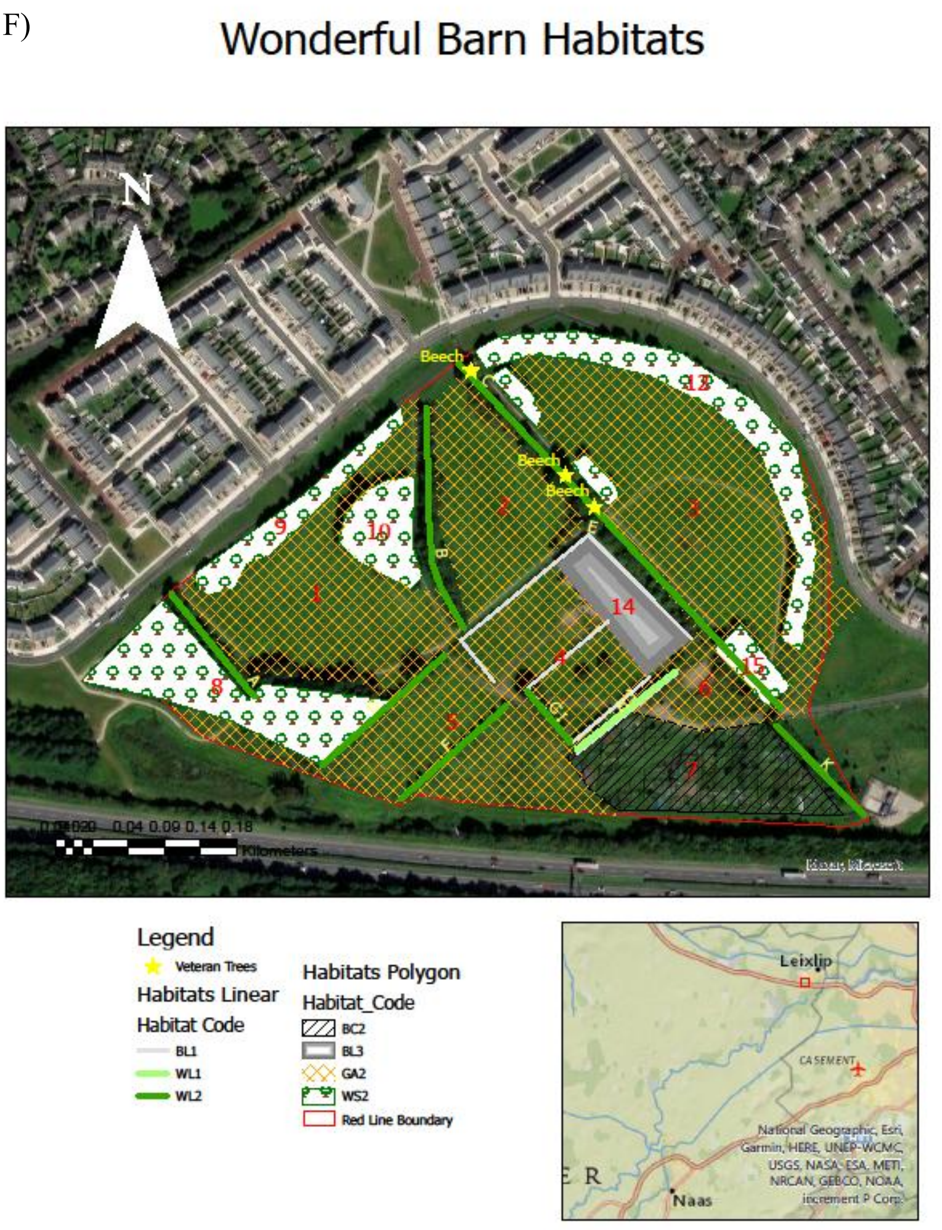

Wonderful Barn Habitats
N
Beech
Beech
Beech
1
2
3
4
5
6
7
8
9
10
12
14
15
Kilometers
Legend
Veteran Trees
Habitats Linear
Habitat Code
BL1
WL1
WL2
Habitats Polygon
Habitat_Code
BC2
BL3
GA2
WS2
Red Line Boundary
Leixlip
CASEMENT
Naas
National Geographic, Esri, Garmin, HERE, UNEP-WCMC, USGS, NASA, ESA, METI, NRCAN, GEBCO, NOAA, increment P Corp.

Table A- 1 List of pollinator species recorded during pan trap and transect surveys.

Guild and Species	Agricultural	Town Park	IUCN Red List Category
Apidae (Bumblebees)			
Bombus hortorum	X	X	LC
Bombus jonellus		X	LC
Bombus lapidarius	X	X	LC
Bombus lucorum agg	X	X	LC
Bombus pascorum	X	X	LC
Bombus pratorum	X	X	LC
Bombus terrestris	X	X	LC
Apidae (Solitary bees)			
Andrena bicolor	X	X	LC
Andrena haemorrhoa	X	X	LC
Andrena praecox		X	LC
Andrena subopaca	X		LC
Halictus rubicundus	X	X	LC
Halictus tumulorum	X		LC
Hylaeus communis	X		LC
Hylaeus confusus	X		LC
Lasioglossum albipes		X	LC
Lasioglossum leucopes	X	X	LC
Megachile versicolor		X	DD
Megachile willughbiella		X	LC
Nomada marshamella	X		LC
Sphecodes ephippus		X	LC
Lepidoptera (Butterflies)			
Aglais io	X	X	LC
Aglais urticae	X	X	LC
Anthocharis cardamines	X		LC
Aphantopus hyperantus	X	X	LC
Celastrina argiolus	X	X	LC
Cupido minimus	X		EN
Lycaena phlaeas	X		LC
Maniola jurtina	X		LC
Pararge aegeria	X	X	LC
Pieris brassica	X	X	LC
Pieris napi	X		LC
Pieris rapae	X	X	LC
Polygonia c-album	X		LC
Polyommatus icarus	X	X	LC
Vanessa atalanta	X	X	LC
Syrphidae (Hoverflies)			
Cheilosia albitarsus	X	X	LC
Cheilosia pagana	X	X	LC
Cheilosia scutellata	X		LC

Guild and Species	Agricultural	Town Park	IUCN Red List Category
Episyrphus balteatus	X	X	LC
Eristalinus sepulchralis	X		LC
Eristalis arbustorum	X	X	LC
Eristalis pertinax	X		LC
Eristalis tenax	X	X	LC
Eupeodes corollae	X		LC
Eupeodes latifasciatus	X		LC
Eupeodes luniger	X		LC
Ferdinandea cuprea		X	LC
Helophilus hybridus	X		LC
Helophilus pendulus	X		LC
Melanastoma scalare	X	X	LC
Melanostoma mellinum	X		LC
Myathropa florea	X	X	LC
Platycheirus albimanus	X	X	LC
Platycheirus granditarsus	X		NT
Riponnensia splendens	X		LC
Sphaerophoria interrupta	X		LC
Sphaerophoria scripta		X	LC
Syritta pipiens	X		LC
Syrphus ribesii	X	X	LC
Syrphus vitipennis	X	X	LC
Volucella bombylans	X		LC
Volucella pellucens	X		LC

Table A- 2 Shannon and Simpson Diversity Results of pollinators between agricultural and town park sites

	Df	Sum Sq.	Mean Sq.	F value	p-value
Bumblebee					
Shannon	1	0.02	0.02	0.78	0.43
Simpson	1	0.01	0.01	1.86	0.24
Butterfly					
Shannon	1	0.17	0.17	5.48	0.08
Simpson	1	0.00	0.00	1.10	0.35
Hoverfly					
Shannon	1	0.81	0.81	6.07	0.07
Simpson	1	0.06	0.06	2.55	0.19
Solitary bee					
Shannon	1	0.08	0.08	0.17	0.70
Simpson	1	0.01	0.01	0.09	0.78

Table A- 3 List of plant species or genus and their taxonomy (Royal Botanic Gardens Kew, 2026), their presence or absence at each type of site, and their invasive species impact according to the NBDC.

Taxonomy	Scientific name	Agricultural	Town Park	Invasive impact
Species	*Acer pseudoplatanus* L.	X	X	Medium
Species	*Achillea millefolium* L.	X	X	
Species	*Adiantum capillus-veneris* L.		X	
Species	*Aegopodium podagraria* L.		X	
Species	*Aesculus hippocastanum* L.	X		
Species	*Alliaria petiolata* (M.Bieb)	X	X	
Species	*Allium triquetrum* L.	X		Medium
Species	*Allium ursinum* L.		X	
Species	*Alnus glutinosa* (L.) Gaertn.	X	X	
Species	*Anacamptis pyramidalis* (L.) Rich.		X	
Species	*Angelica sylvestris* L.		X	
Species	*Anthriscus sylvestris* (L.) Hoffm.	X	X	
Species	*Arctium lappa* L.	X	X	
Species	*Artemisia vulgaris* L.	X		
Species	*Asplenium scolopendrium* L.	X	X	
Genus	*Aster* L.	X	X	
Species	*Avena fatua* L.		X	
Species	*Bellis perennis* L.	X	X	
Species	*Betula pendula* Roth	X	X	
Genus	*Brassica* L.	X		
Species	*Buddleja davidii* Franch.		X	Medium
Species	*Buxus sempervirens* L.	X		
Species	*Cardamine hirsuta* L.	X		
Species	*Cardamine pratensis* L.	X	X	
Species	*Carex pendula* Huds.	X		
Species	*Carpinus betulus* L.	X	X	
Species	*Castanea sativa* Mill.		X	
Species	*Centaurea nigra* L.		X	
Species	*Centaurium erythraea* Rafn	X		
Species	*Cerastium fontanum* Baumg.	X	X	
Species	*Chelidonium majus* L.	X	X	
Species	*Cicuta virosa* L.		X	
Species	*Cirsium acaulon* (L.) Scop.	X	X	
Species	*Cirsium dissectum* (L.) Hill	X	X	
Species	*Cirsium vulgare* (Savi) Ten.	X	X	
Species	*Convolvulus arvensis* L.	X	X	
Species	*Cornus sanguinea* L.	X	X	
Species	*Corylus avellana* L.	X		
Genus	*Cotoneaster* Medik.	X		
Species	*Crataegus monogyna* Jacq.	X	X	
Species	*Crepis biennis* L.		X	

Taxonomy	Scientific name	Agricultural	Town Park	Invasive impact
Species	*Crepis capillaris* (L.) Wallr.	X		
Species	*Crepis vesicaria* L.	X		
Species	*Cymbalaria muralis* G. Gaertn., B.Mey. & Scherb.		X	
Species	*Dactylorhiza maculata* subsp. *maculata*		X	
Species	*Daucus carota* L.	X		
Species	*Digitalis purpurea* L.		X	
Species	*Epilobium angustifolium* L.	X	X	
Species	*Epilobium hirsutum* L.	X	X	
Species	*Equisetum arvense* L.	X	X	
Species	*Erythranthe guttata* (DC.) G.L. Nesom		X	
Species	*Euphorbia hyberna* L.		X	
Species	*Euphrasia officinalis* L.	X	X	
Species	*Fagus sylvatica* f. *purpurea*	X		
Species	*Fagus sylvatica* L.	X	X	
Species	*Filipendula ulmaria* (L.) Maxim.	X	X	
Species	*Fragaria vesca* L.	X		
Species	*Fraxinus excelsior* L.	X	X	
Genus	*Fuchsia* Plum. ex L.	X		
Species	*Fumaria muralis* Sond. ex W.D.J.Kock	X	X	
Species	*Galium aparine* L.	X	X	
Species	*Galium verum* L.	X	X	
Species	*Geranium dissectum* L.	X	X	
Species	*Geranium pyrenaicum* Burm.f.	X	X	
Species	*Geranium robertianum* L.	X	X	
Species	*Geranium sanguineum* L.	X		
Species	*Geum urbanum* L.	X	X	
Species	*Ginkgo biloba* L.		X	
Species	*Glechoma hederacea* L.	X	X	
Species	*Heracleum sphondylium* L.	X	X	
Species	*Hyacinthoides hispanica* (Mill.) Rothm.	X	X	Invasive
Genus	*Hylotelephium* H. Ohba		X	
Genus	*Hypericum* Tourn. ex L.	X		
Species	*Ilex aquifolium* L.	X	X	
Species	*Impatiens glandulifera* Royle		X	High
Species	*Iris pseudacorus* L.	X		
Species	*Jacobaea vulgaris* Gaertn.	X	X	
Species	*Juncus conglomeratus* L.	X		
Species	*Juncus effusus* L.	X		
Species	*Juncus inflexus* L.	X		
Species	*Knautia arvensis* (L.) Coult.		X	
Species	*Lamium album* L.		X	

Taxonomy	Scientific name	Agricultural	Town Park	Invasive impact
Species	*Lamium galeobdolon* (L.) L.	X		
Species	*Lamium purpureum* L.		X	
Species	*Lapsana communis* L.		X	
Species	*Larix decidua* Mill.	X		
Species	*Lathyrus pratensis* L.	X		
Species	*Leontodon saxatilis* Lam.	X		
Species	*Ligustrum vulgare* L.	X	X	
Species	*Lonicera periclymenum* L.	X	X	
Species	*Lotus corniculatus* L.	X		
Species	*Luzula campestris* (L.) DC.	X	X	
Species	*Lysimachia arvensis* (L.) U. Manns & Anderb.	X		
Species	*Malus sylvestris* (L.) Mill.	X	X	
Species	*Medicago lupulina* L.	X	X	
Species	*Mentha aquatica* L.	X	X	
Species	*Mutarda arvensis* (L.) D.A. German		X	
Species	*Myosotis sylvatica* Ehrh. ex Hoffm.	X	X	
Species	*Nasturtium officinale* W.T. Aiton	X		
Species	*Odontites vernus* (Bellardi) Dumort.	X	X	
Species	*Ononis repens* L.		X	
Species	*Origanum vulgare* L.		X	
Species	*Papaver rhoeas* L.	X	X	
Species	*Parthenocissus quinquefolia* (L.) Planch.		X	Medium
Species	*Petasites pyrenaicus* (Loefl.) G. Lopez	X		
Species	*Phalaris arundinacea* L.	X	X	
Species	*Pimpinella saxifraga* L.		X	
Species	*Pinus sylvestris* L.	X	X	
Species	*Plantago lanceolata* L.	X	X	
Species	*Plantago major* L.	X	X	
Species	*Potentilla anserina* (L.) Rydb.	X	X	
Species	*Potentilla reptans* l.	X	X	
Species	*Primula veris* L.	X	X	
Species	*Prunella vulgaris* L.	X	X	
Species	*Prunus avium* (L.) L.	X	X	
Species	*Prunus laurocerasus* L.	X	X	High
Species	*Prunus spinosa* L.	X	X	
Species	*Pteridium aquilinum* L.	X		
Species	*Quercus ilex* L.		X	
Genus	*Quercus* L.	X	X	
Species	*Ranunculus acris* L.	X	X	
Species	*Ranunculus repens* L.	X	X	
Species	*Reseda luteola* L.	X		

Taxonomy	Scientific name	Agricultural	Town Park	Invasive impact
Species	*Rhinanthus minor* L.		X	
Genus	*Ribes* L.	X		
Species	*Rosa canina* L.	X	X	
Species	*Rubus fruticosus* agg. L.	X	X	
Species	*Rubus idaeus* L.	X		
Species	*Rumex acetosa* L.	X		
Species	*Rumex obtusifolius* L.	X	X	
Genus	*Salix* L.	X	X	
Species	*Sambucus nigra* L.	X	X	
Species	*Scrophularia auriculata* L.	X	X	
Species	*Silene dioica* (L.) Clairv.		X	
Species	*Silene vulgaris* (Moench) Garcke	X	X	
Species	*Sisymbrium officinale* (L.) Scop.	X	X	
Species	*Solanum dulcamara L.*	X		
Species	*Solanum nigrum* L.		X	
Species	*Sonchus asper* (L.) Hill	X		
Species	*Sonchus oleraceus* L.		X	
Species	*Sorbus aucuparia* L.		X	
Species	*Stachys sylvatica* L.	X	X	
Species	*Stellaria media* (L.) Vill.	X		
Species	*Symphoricarpos albus* (L.)	X		
Genus	*Taraxacum* spp. F.H.Wigg	X	X	
Species	*Tilia × europaea* L.	X	X	
Species	*Torilis japonica* (Houtt.) DC	X		
Species	*Trifolium pratense* L.	X	X	
Species	*Trifolium repens* L.	X	X	
Species	*Tussilago farfara* L.	X	X	
Species	*Typha latifolia* L.	X		
Genus	*Ulmus* L.	X	X	
Species	*Urtica dioica* L.	X	X	
Species	*Valeriana officinalis* L.	X		
Species	*Veronica chamaedrys* L.	X	X	
Species	*Veronica serpyllifolia* L.	X	X	
Species	*Vicia cracca* L.	X	X	
Species	*Vicia sativa* L.	X		
Species	*Vicia sepium* L.	X	X	
Genus	*Viola* L.	X		
Species	*x Cuprocyparis leylandii*	X	X	

Table A- 4 The plant species visited by the four groups of pollinating insects- bumblebees, butterflies, hoverflies and solitary bees.

Pollinator guild	Agricultural		Agricultural Total	Town Park		Town Park Total	Grand Total
Plant species	**Grassland**	**Woodland**		**Grassland**	**Woodland**		
Apidae (Bumblebee)	**60**	**312**	**372**	**34**	**109**	**143**	**515**
Buddleja davidii Franch.				1		1	1
Centaurea nigra L.					1	1	1
Centaurea spp. L.					2	2	2
Cerastium fontanum Baumg.	1		1				1
Cirsium dissectum (L.) Hill	5	1	6	1	6	7	13
Cirsium vulgare (Savi) Ten.	5	18	23		4	4	27
Cretaegus monogyna Jacq.		5	5				5
Epilobium hirsutum L.		2	2				2
Filipendula ulmaria (L.) Maxim.		4	4				4
Geranium robertianum L.		1	1		1	1	2
Heracleum sphondylium L.		1	1		9	9	10
Impatiens glandulifera Royle				1		1	1
Jacobaea vulgaris Gaertn.	3	30	33	1	5	6	39
Lamium album L.					2	2	2
Odontites vernus (Bellardi) Dumort.				1	1	2	2
Ranunculus repens L.	7		7				7
Ranunculus spp. L.	20	4	24	1	3	4	28
Rosa canina L.					2	2	2
Rubus fruticosus agg. L.	7	187	194	8	47	55	249
Scrophularia auriculata L.		1	1				1
Stellaria media (L.) Vill.	1		1				1
Symphoricarpos albus (L.)		7	7				7
Taraxacum spp**.** F.H.Wigg	4	11	15	2	4	6	21
Tilia × europaea L.		2	2				2

Pollinator guild	Agricultural		Agricultural Total	Town Park		Town Park Total	Grand Total
Plant species	**Grassland**	**Woodland**		**Grassland**	**Woodland**		
Trifolium pratense L.	2		2	1		1	3
Trifolium repens L.	5	4	9	14	19	33	42
Vicia cracca L.		7	7		1	1	8
Vicia sativa L.		2	2				2
Vicia sepium L.		22	22	3	2	5	27
Vicia spp. L.		3	3				3
Lepidoptera (Butterfly)	**19**	**26**	**45**	**4**	**9**	**13**	**58**
Cirsium dissectum (L.) Hill	7		7	1	5	6	13
Cirsium spp. L.	1		1				1
Heracleum sphondylium L.					3	3	3
Jacobaea vulgaris Gaertn.	1	1	2		1	1	3
Ranunculus acris L.	1		1				1
Ranunculus spp. L.	6		6				6
Rubus fruticosus agg. L.	1	23	24	2		2	26
Symphoricarpos albus		1	1				1
Taraxacum spp**.** F.H.Wigg				1		1	1
Trifolium repens L.		1	1				1
Urtica dioca L.	2		2				2
Syrphidae (Hoverfly)	**53**	**156**	**209**	**24**	**40**	**64**	**273**
Anthriscus sylvestris (L.) Hoffm.		5	5		1	1	6
Cerastium fontanum Baumg.	1		1				1
Chelidonium spp. L.					2	2	2
Cirsium dissectum (L.) Hill	1		1				1
Cirsium vulgare (Savi) Ten.		2	2	1	2	3	5
Crepis spp. L.		3	3				3
Cretaegus monogyna Jacq.		2	2				2
Filipendula ulmaria (L.) Maxim.		2	2				2

Pollinator guild	Agricultural		Agricultural Total	Town Park		Town Park Total	Grand Total
Plant species	**Grassland**	**Woodland**		**Grassland**	**Woodland**		
Geranium robertianum L.		1	1	1		1	2
Heracleum sphondylium L.		14	14	3	9	12	26
Jacobaea vulgaris	15	50	65	4	6	10	75
Nasturtium officinale W.T. Aiton	1		1				1
Ranunculus acris L.	8	3	11	1	3	4	15
Ranunculus repens L.	6	1	7	1	1	2	9
Ranunculus spp. L.	19	22	41	6	5	11	52
Rosa canina L.		1	1				1
Rubus fruticosus agg. L.		21	21	1	3	4	25
Taraxacum spp**.** L.	2	27	29	6	8	14	43
Vicia cracca L.		1	1				1
Vicia spp. L.		1	1				1
Apidae (Solitary bee)	**5**	**18**	**23**	**1**	**3**	**4**	**27**
Cirsium dissectum (L.) Hill					1	1	1
Geranium spp. L.		2	2				2
Heracleum sphondylium L.		3	3				3
Jacobaea vulgaris Gaertn.	1	9	10				10
Ranunculus acris L.	1		1				1
Rubus fruticosus agg. L.		2	2				2
Taraxacum spp. L.	3	1	4	1	2	3	7
Trifolium repens L.		1	1				1
Grand Total	**137**	**512**	**649**	**63**	**161**	**224**	**873**

Appendix B Supplementary Data for Chapter 3

Table B- 1 List of pollinator species recorded during pan trap and transect surveys.

Order and Species	Green island	Ornamental wildflower	IUCN Red List Category
Apidae (Bumblebees)			
Bombus hortorum	X	X	LC
Bombus lapidarius	X	X	LC
Bombus lucorum agg	X	X	LC
Bombus muscorum		X	VU
Bombus pascorum	X	X	LC
Bombus pratorum	X	X	LC
Bombus terrestris	X	X	LC
Apidae (Solitary bees)			
Andrena haemorrhoa	X	X	LC
Andrena praecox	X		LC
Halictus tumulorum	X	X	LC
Lasioglossum albipes	X		LC
Lasioglossum leucopus	X	X	LC
Osmia bicornis		X	LC
Lepidoptera (Butterflies)			
Aglais urticae	X	X	LC
Pieris brassica		X	LC
Polyommatus icarus		X	LC
Vanessa atalanta	X		LC
Syrphidae (Hoverflies)			
Cheilosia albitarsus		X	LC
Cheilosia impressa		X	LC
Episyrphus balteatus		X	LC
Eristalis arbustorum		X	LC
Eristalis horticola		X	LC
Eristalis nemorum		X	LC
Eristalis pertinax		X	LC
Eristalis tenax	X	X	LC
Helophilus pendulus		X	LC
Orthonevra nobilis	X		LC
Platycheirus clypeatus		X	LC
Rhingia campestris		X	LC
Scaeva pyrastri		X	LC
Sphaerophoria scripta	X		LC

Table B- 2 Shannon and Simpson Diversity of pollinators between green island and ornamental wildflower roundabouts.

	Df	Sum Sq.	Mean Sq.	f value	p value
Bumblebee					
Shannon	1	0.11	0.11	0.78	0.43
Simpson	1	0.01	0.01	0.39	0.57
Butterfly					
Shannon	1	0.160	0.160	1	0.423
Simpson	1	0.083	0.083	1	0.423
Hoverfly					
Shannon	1	2.16	2.16	4.42	0.13
Simpson	1	0.42	0.42	10.30	**0.05**
Solitary bee					
Shannon	1	0.26	0.26	0.87	0.40
Simpson	1	0.06	0.06	0.58	0.49

Table B- 3 The list of plant species recorded during surveys of the green island and ornamental wildflower roundabouts.

List of plant species taxonomy, their presence or absence at each type of site, and their invasive species impact according to the NBDC.		
Scientific name	**Green island**	**Ornamental wildflower**
Achillea millefolium L.		X
Anacamptis pyramidalis (L.) Rich.	X	
Astilbe japonica (C. Morren & Decne.) A.Gray	X	
Bellis perennis L.	X	
Buddleja davidii Franch.	X	
Camassia spp. Lindl.	X	
Catananche caerulea L.		X
Centaurea cyanus L.		X
Centaurea nigra L.		X
Centaurea scabiosa L.		X
Cerastium fontanum Baumg.	X	X
Cirsium spp. L.		X
Convolvulus arvensis L.	X	
Crepis biennis L.	X	
Cretaegus monogyna Jacq.	X	
Daucus carota L.		X
Epibolium angustifolium L.	X	X
Equisetum arvense L.	X	X
Galium erectum Huds.		X
Galium verum L.		X
Geranium dissectum L.	X	X
Geranium pratense L.		X
Geranium robertianum L.	X	
Glechoma hederacea L.	X	
Hebe Comm. Ex. Juss.	X	
Heracleum sphondylium L.	X	
Hieracium spp. L.		X
Hosta spp. Tratt.	X	
Hypericum perforatum L	X	X
Jacobaea vulgaris Gaertn.	X	X
Juncus conglomeratus L.		X
Knautia arvensis (L.) Coult.		X
Lotus corniculatus L.	X	X
Lysimachia arvensis (L.) U. Manns & Anderb.	X	
Lythrum salicaria L.		X
Malva sylvestris (L.) Mill.		X
Medicago lupulina L.	X	X
Oenothera biennis L.		X
Origanum vulgare L.		X
Papaver orientale L.		X

List of plant species taxonomy, their presence or absence at each type of site, and their invasive species impact according to the NBDC.

Scientific name	**Green island**	**Ornamental wildflower**
Plantago lanceolata L.	X	X
Plantago major L.	X	
Potentilla fruticosa L.	X	
Potentilla reptans L.		X
Potentilla spp. L.	X	
Primula veris L.	X	X
Primula vulgaris L.		X
Prunella vulgaris L.	X	X
Ranunculus acris L.	X	X
Ranunculus repens L.	X	X
Ranunculus spp. L.	X	
Rosa canina L.	X	
Rubus fruticosus agg. L.	X	
Rumex spp. L.	X	X
Sanguisorba officinalis L.		X
Sonchus palustris L.	X	X
Sonchus oleraceus L.		X
Sonchus spp. L.	X	
Taraxacum spp. F.H.Wigg	X	X
Trifolium pratense L.	X	X
Trifolium repens L.	X	X
Trifolium spp. L.	X	X
Veronica chamaedrys L.	X	X
Viburnum spp. L.	X	X
Vicia cracca L.	X	X
Vicia sativa L.	X	X
Vicia sepium L.		X

Table B- 4 The plant species visited by the four pollinating groups of insects- bumblebees, butterflies, hoverflies and solitary bees.

Order and Species	**Green island**	**Ornamental wildflower**	**Grand Total**
Apidae (Bumblebee)	**53**	**303**	**356**
Centaurea cyanus L.		2	2
Centaurea nigra L.		171	171
Cirsium spp. L.		2	2
Hebe Comm. Ex. Juss.	32		32
Hosta spp. Tratt.	7		7
Jacobaea vulgaris Gaertn.		1	1
Knautia arvensis (L.) Coult.		82	82
Lotus corniculatus L.		1	1
Malva sylvestris (L.) Mill.		18	18
Origanum vulgare L.		2	2
Potentilla fruticosa L.	6		6
Potentilla reptans L.	1		1
Primula veris L.	1		1
Ranunculus acris L.		1	1
Sonchus oleraceus L.		1	1
Taraxacum spp. F.H.Wigg	3	1	4
Trifolium repens L.	2	3	5
Vicia sepium L.	1	18	19
Lepidoptera (Butterfly)	**1**		**1**
Buddleja davidii Franch.	1		1
Syrphidae (Solitary bee)	**14**	**60**	**74**
Centaurea cyanus L.		2	2
Centaurea scabiosa L.		20	20
Cerastium fontanum Baumg.		1	1
Galium verum L.		1	1
Hebe Comm. Ex. Juss.	1		1
Knautia arvensis (L.) Coult.		17	17
Malva sylvestris (L.) Mill.		3	3
Origanum vulgare L.		1	1
Potentilla fruticosa L.	2		2
Primula veris L.	1		1
Ranunculus acris L.	6	7	13
Taraxacum spp. F.H.Wigg.	4	6	10
Vicia sepium		2	2
Apidae (Solitary bee)	**15**	**7**	**22**
Knautia arvensis L.		2	2
Taraxacum spp. F.H.Wigg**.**	15	5	20
Grand Total	**83**	**370**	**453**

Appendix C Supplementary Data for Chapter 4

Table C- 1 Results from the NanoDrop Sequence

ID	Sample ID	Sample	Date Soil Samples Taken	Sequence ID	Quantity of DNA per Nano ng/µl	260/280
1	SC1	B1 A5	28/07/2022	TP3 C1	59.6	1.88
2	SC2	B3 A5	28/07/2022	TP3 C3	86	1.82
3	SC3	B1 B5	28/07/2022	TP3 RF1	51.8	1.87
4	SC4	B2 A5	28/07/2022	TP3 C2	94.1	1.86
5	SC5	B2 B5	28/07/2022	TP3 RF2	55.2	1.83
6	SC6	B3 B5	28/07/2022	TP3 RF3	50.4	1.81
7	SC7	B1 C5	28/07/2022	TP3 NM1	61.5	1.86
8	SC8	B2 C5	28/07/2022	TP3 NM2	79	1.82
9	SC9	B3 C5	28/07/2022	TP3 NM3	80.4	1.84
10	SC10	B1 E5	28/07/2022	TP3 NW1	73.4	1.88
11	SC11	B2 E5	28/07/2022	TP3 NW2	76.5	1.83
12	SC12	B3 E5	28/07/2022	TP3 NW3	87.8	1.88
13	SC13	B1 F5	28/07/2022	TP3 HF1	57.5	1.84
14	SC14	B2 F5	28/07/2022	TP3 HF2	58.5	1.83
15	SC15	B3 F5	28/07/2022	TP3 HF3	78.5	1.9
16	SC16	B1 A5	11/05/2022	TP2 C1	82.6	1.88
17	SC17	B2 A5	11/05/2022	TP2 C2	80.3	1.85
18	SC18	B3 A5	11/05/2022	TP2 C3	83.2	1.86
19	SC19	B1 B5	11/05/2022	TP2 RF1	48.7	1.79
20	SC20	B2 B5	11/05/2022	TP2 RF2	85	1.86
21	SC21	B3 B5	11/05/2022	TP2 RF3	90.3	1.89
22	SC22	B1 C5	11/05/2022	TP2 NM1	75.5	1.91
23	SC23	B2 C5	11/05/2022	TP2 NM2	67.6	1.81
24	SC24	B3 C5	11/05/2022	TP2 NM3	103.6	1.9
25	SC25	B1 E5	11/05/2022	TP2 NW1	74.4	1.85
26	SC26	B2 E5	11/05/2022	TP2 NW2	57.9	1.87
27	SC27	B3 E5	11/05/2022	TP2 NW3	77.4	1.88
28	SC28	B1 F5	11/05/2022	TP2 HF1	74.5	1.88
29	SC29	B2 F5	11/05/2022	TP2 HF2	73.7	1.85
30	SC30	B3 F5	11/05/2022	TP2 HF3	103.2	1.83
31	SC31	B1 A5	13/04/2022	TP1 C1	48.9	1.82
32	SC32	B2 A5	13/04/2022	TP1 C2	49.7	1.9
33	SC33	B3 A5	13/04/2022	TP1 C3	93.8	1.84
34	SC34	B1 B5	13/04/2022	TP1 RF1	68	1.86
35	SC35	B2 B5	13/04/2022	TP1 RF2	61.6	1.9
36	SC36	B3 B5	13/04/2022	TP1 RF3	80.3	1.88
37	SC37	B1 C5	13/04/2022	TP1 NM1	66.6	1.82
38	SC38	B2 C5	13/04/2022	TP1 NM2	56.5	1.84
39	SC39	B3 C5	13/04/2022	TP1 NM3	56.8	1.85
40	SC40	B1 E5	13/04/2022	TP1 NW1	112	1.87

ID	Sample ID	Sample	Date Soil Samples Taken	Sequence ID	Quantity of DNA per Nano ng/µl	260/280
41	SC41	B2 E5	13/04/2022	TP1 NW2	102.8	1.9
42	SC42	B3 E5	13/04/2022	TP1 NW3	91.5	1.86
43	SC43	B1 F5	13/04/2022	TP1 HF1	78	1.9
44	SC44	B2 F5	13/04/2022	TP1 HF2	67.5	1.79
45	SC45	B3 F5	13/04/2022	TP1 HF3	74.13	1.86

Table C- 2 Nutrient levels in soils after one month and four months post-treatment, and by treatment

Phosphorous	**Estimate**	**SE**	**t-value**	**p-value**
(Intercept)	5.5559	1.1143	4.986	**2.42E-05**
Timepoint 1	-0.2821	1.5758	-0.179	0.8591
Timepoint 4	-2.5914	1.5758	-1.645	0.1105
Treatment Hot Foam	-1.0815	1.5758	-0.686	0.4978
Treatment New Way	0.7158	1.5758	0.454	0.6529
Treatment Nomix Dual	0.5904	1.5758	0.375	0.7106
Treatment Roundup Flex	0.1358	1.5758	0.086	0.9319
Timepoint 1:Treatment Hot Foam	0.4859	2.2285	0.218	0.8289
Timepoint 4:Treatment Hot Foam	5.1724	2.2285	2.321	**0.0273**
Timepoint 1:TreatmentNew Way	-0.7419	2.2285	-0.333	0.7415
Timepoint 4:TreatmentNew Way	1.7294	2.2285	0.776	0.4438
Timepoint 1:TreatmentNomix Dual	0.2978	2.2285	0.134	0.8946
Timepoint 4:TreatmentNomix Dual	2.4869	2.2285	1.116	0.2733
Timepoint 1:TreatmentRoundup Flex	1.9801	2.2285	0.889	0.3813
Timepoint 4:TreatmentRoundup Flex	3.2968	2.2285	1.479	0.1495
Ammonium	**Estimate**	**SE**	**t-value**	**p-value**
(Intercept)	3.07383	0.31891	9.639	**1.07E-10**
Timepoint 1	0.52016	0.451	1.153	0.25787
Timepoint 4	0.44772	0.451	0.993	0.32878
Treatment Hot Foam	0.0521	0.451	0.116	0.9088
Treatment New Way	0.44155	0.451	0.979	0.33538
Treatment Nomix Dual	1.63283	0.451	3.62	**0.00107**
Treatment Roundup Flex	0.35547	0.451	0.788	0.43677
Timepoint 1:Treatment Hot Foam	-0.47286	0.63781	-0.741	0.46423
Timepoint 4:Treatment Hot Foam	-0.09948	0.63781	-0.156	0.87711
Timepoint 1:TreatmentNew Way	-1.09907	0.63781	-1.723	0.09515
Timepoint 4:TreatmentNew Way	-0.38286	0.63781	-0.6	0.55283
Timepoint 1:TreatmentNomix Dual	-2.25526	0.63781	-3.536	**0.00134**
Timepoint 4:TreatmentNomix Dual	-2.00754	0.63781	-3.148	**0.00371**
Timepoint 1:TreatmentRoundup Flex	-0.93619	0.63781	-1.468	0.15256
Timepoint 4:TreatmentRoundup Flex	0.1583	0.63781	0.248	0.80568

% Organic carbon	Estimate	SE	t-value	p-value
(Intercept)	7.057751	0.481734	14.651	3.27E-15
Timepoint 1	-0.10855	0.681274	-0.159	0.8745
Timepoint 4	1.550563	0.681274	2.276	**0.0302**
Treatment Hot Foam	0.114174	0.681274	0.168	0.868
Treatment New Way	0.292663	0.681274	0.43	0.6706
Treatment Nomix Dual	0.108269	0.681274	0.159	0.8748
Treatment Roundup Flex	0.383975	0.681274	0.564	0.5772
Timepoint 1:Treatment Hot Foam	1.396581	0.963467	1.45	0.1576
Timepoint 4:Treatment Hot Foam	-0.7021	0.963467	-0.729	0.4718
Timepoint 1:TreatmentNew Way	0.786501	0.963467	0.816	0.4208
Timepoint 4:TreatmentNew Way	-0.29367	0.963467	-0.305	0.7626
Timepoint 1:TreatmentNomix Dual	0.725644	0.963467	0.753	0.4572
Timepoint 4:TreatmentNomix Dual	-0.73816	0.963467	-0.766	0.4496
Timepoint 1:TreatmentRoundup Flex	0.451681	0.963467	0.469	0.6426
Timepoint 4:TreatmentRoundup Flex	0.003167	0.963467	0.003	0.9974

Table C- 3 The mean percentage cover of vegetation at one month post-treatment for each treatment.

One month post-treatment						
Plant /Family/Species	**Control**	**Hot Foam**	**New Way**	**Nomix Dual**	**Roundup Flex**	**Mean percentage cover**
Bryophyta spp.	0.00	0.00	0.00	0.00	0.67	0.13
Cerastium fontanum Baumg.	0.13	0.00	0.20	0.00	0.00	0.07
Cirsium spp. L.	0.00	0.00	0.13	0.00	0.00	0.03
Hieracium spp. L.	0.27	0.00	0.00	0.00	0.00	0.05
Jacobaea vulgaris Gaertn.	0.00	0.00	0.00	0.00	0.00	0.00
Persicaria maculosa Gray	0.00	0.00	0.00	0.00	0.00	0.00
Poaceae spp. Barnhart.	83.73	2.47	79.07	0.00	10.33	35.12
Ranunculus spp. L.	0.00	0.00	1.40	0.00	0.27	0.33
Rumex spp. L.	1.93	0.67	0.93	0.00	0.27	0.76
Taraxacum spp. F.H.Wigg	0.60	2.53	1.60	0.00	0.13	0.97
Trifolium spp. Tourn. Ex L..	28.93	0.00	26.53	0.80	5.30	12.31
Veronica spp. L.	0.00	0.00	0.00	0.00	0.00	0.00
Total percentage cover	**115.60**	**5.67**	**109.87**	**0.80**	**16.97**	**49.78**

Table C- 4 The mean percentage cover of vegetation at four months post-treatment for each treatment

Four months post-treatment						
Plant /Family/Species	**Control**	**Hot Foam**	**New Way**	**Nomix Dual**	**Roundup Flex**	**Mean percentage cover**
Bryophyta spp.	2.73	0.00	3.20	0.67	2.67	1.85
Cerastium fontanum Baumg.	0.00	0.00	0.00	0.00	0.00	0.00
Cirsium spp. L.	0.40	2.20	0.00	0.40	0.73	0.75
Hieracium spp. L.	0.00	0.00	0.00	0.00	0.00	0.00
Jacobaea vulgaris Gaertn.	0.00	0.13	0.00	0.13	0.13	0.08
Persicaria maculosa Gray	0.00	0.13	0.00	0.00	0.00	0.03
Poaceae spp. Barnhart.	20.00	14.27	20.00	0.60	23.07	15.59
Ranunculus spp. L.	0.07	0.13	0.40	0.13	0.60	0.27
Rumex spp. L.	0.27	0.13	0.00	0.00	0.00	0.08
Trifolium spp. Tourn. Ex L..	2.33	12.73	2.47	0.13	3.60	4.25
Veronica spp. L.	11.13	4.27	10.60	4.20	16.87	9.41
Bryophyta spp.	0.00	0.27	0.00	0.00	0.00	0.05
Total percentage cover	**36.93**	**34.27**	**36.67**	**6.27**	**47.67**	**32.36**

Table C- 5 Bacterial phyla level abundance at the initial sampling at 0 months, one month and four months post-treatment. No significant differences in contrast to the control at any time.

0 months- initial sample pre-treatment				
contrast	estimate	SE	z.ratio	p.value
Hot Foam-Control	-0.0117	0.0429	-0.272	0.9837
New Way-Control	-0.0458	0.0429	-1.067	0.641
Nomix Dual-Control	0.0424	0.0429	0.988	0.6911
Roundup Flex-Control	-0.0284	0.0429	-0.662	0.8691
One month- post-treatment				
contrast	estimate	SE	z.ratio	p.value
Hot Foam-Control	-0.0259	0.0436	-0.594	0.8975
New Way-Control	0.0344	0.0436	0.79	0.8063
Nomix Dual-Control	0.0775	0.0436	1.779	0.2296
Roundup Flex-Control	-0.0184	0.0436	-0.423	0.9535
Four months- post-treatment				
contrast	estimate	SE	z.ratio	p.value
Hot Foam-Control	-0.0916	0.0762	-1.203	0.5533
New Way-Control	0.0601	0.0762	0.789	0.807
Nomix Dual-Control	0.0681	0.0762	0.895	0.7476
Roundup Flex-Control	0.0019	0.0762	0.025	0.9999

Table C- 6 Relative abundance of bacteria at phylum level under the Hot Foam treatment.

Bacteria Phylum	Relative abundance	Bacteria Phylum	Relative abundance
Proteobacteria	22.92	Fibrobacterota	0.07
Acidobacteriota	17.90	Patescibacteria	0.03
Actinobacteriota	15.76	Dependentiae	0.04
Crenarchaeota	3.94	Sumerlaeota	0.02
Verrucomicrobiota	5.70	SAR324_clade(Marine_group_B)	0.01
Planctomycetota	6.40	Spirochaetota	0.01
Firmicutes	6.07	FCPU426	0.01
Chloroflexi	4.52	Hydrogenedentes	0.00
Myxococcota	3.60	Deferrisomatota	0.00
Gemmatimonadota	2.39	Halanaerobiaeota	0.00
Others	2.24	GAL15	0.00
Bacteroidota	1.75	WS4	0.00
Desulfobacterota	1.50	Zixibacteria	0.00
Latescibacterota	1.33	Halobacterota	0.00
Methylomirabilota	1.07	Thermoplasmatota	0.00
Nitrospirota	0.91	Campilobacterota	0.00
NB1-j	0.50	WS2	0.00
Entotheonellaeota	0.38	Abditibacteriota	0.00
RCP2-54	0.24	Dadabacteria	0.00
Bdellovibrionota	0.22	Nitrospinota	0.00
Cyanobacteria	0.13	Nanoarchaeota	0.00
Armatimonadota	0.18	Deferribacterota	0.00
MBNT15	0.09	Synergistota	0.00
Elusimicrobiota	0.06		

Table C- 7 Top ten bacterial phyla affected by Hot Foam.

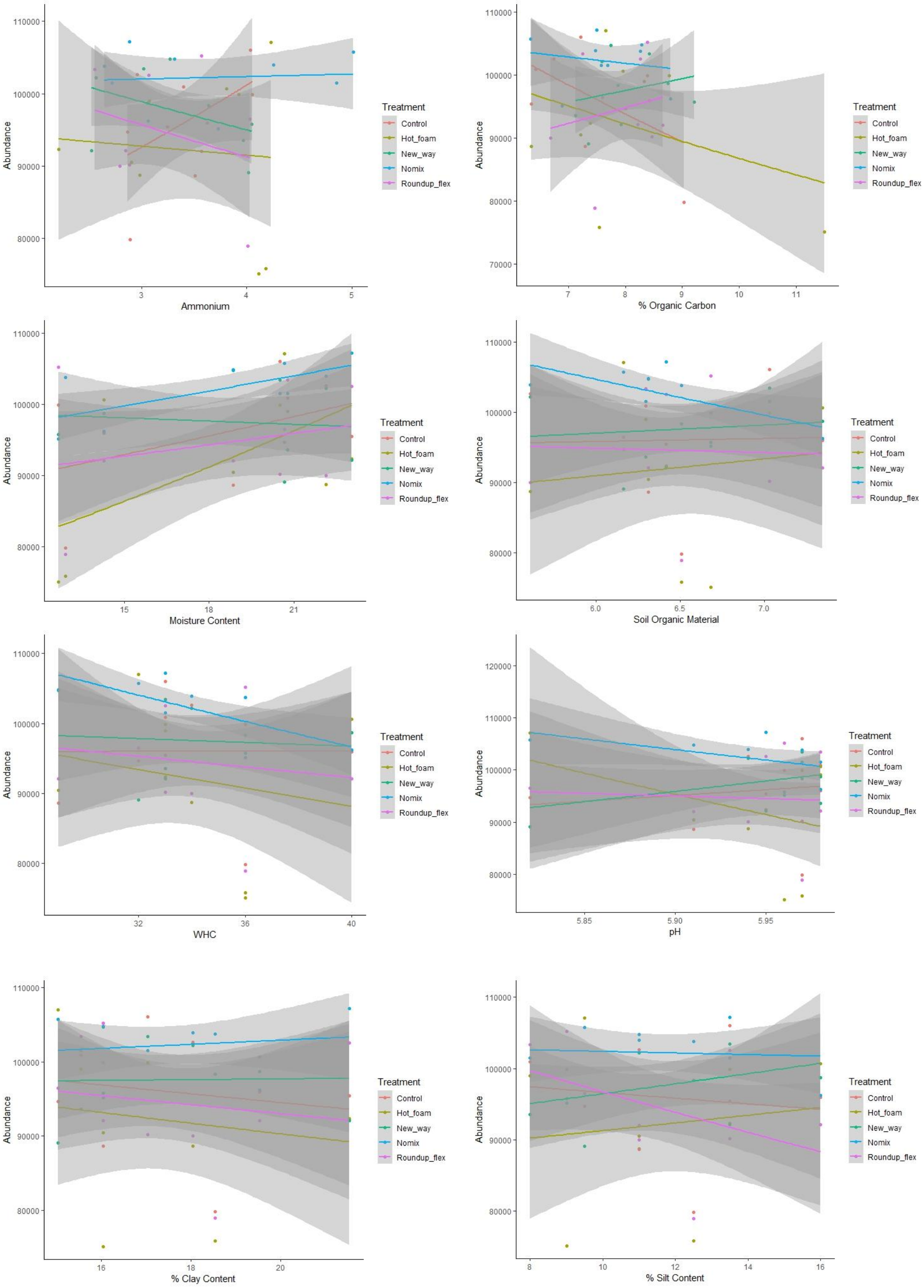
Phylum Acidobacteriota:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	0.11227	0.114	0.981	0.5889
Timepoint 0-Timepoint 4	0.34047	0.114	2.975	**0.0083**
Timepoint 1-Timepoint 4	0.2282	0.114	1.994	0.1138
Phylum Actinobacteriota:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	-0.10278	0.114	-0.898	0.6417
Timepoint 0-Timepoint 4	-0.08926	0.114	-0.78	0.7155
Timepoint 1-Timepoint 4	0.01352	0.114	0.118	0.9923
Phylum Chloroflexi:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	0.01489	0.115	0.13	0.9908
Timepoint 0-Timepoint 4	0.14263	0.115	1.24	0.4293
Timepoint 1-Timepoint 4	0.12774	0.115	1.111	0.5073
Phylum Crenarchaeota:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	0.13692	0.115	1.191	0.4584
Timepoint 0-Timepoint 4	0.81043	0.115	7.03	**<.0001**
Timepoint 1-Timepoint 4	0.67352	0.115	5.84	**<.0001**
Phylum Firmicutes:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	0.06532	0.115	0.569	0.8365
Timepoint 0-Timepoint 4	0.31837	0.115	2.773	**0.0154**
Timepoint 1-Timepoint 4	0.25305	0.115	2.204	0.0706
Phylum Gemmatimonadota:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	0.06108	0.116	0.528	0.8574
Timepoint 0-Timepoint 4	0.01129	0.116	0.098	0.9947
Timepoint 1-Timepoint 4	-0.04978	0.116	-0.431	0.9028
Phylum Myxococcota:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	-0.00882	0.115	-0.077	0.9968
Timepoint 0-Timepoint 4	-0.09308	0.115	-0.808	0.6978
Timepoint 1-Timepoint 4	-0.08426	0.115	-0.732	0.7446
Phylum Planctomycetota:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	0.18345	0.115	1.599	0.246
Timepoint 0-Timepoint 4	0.40151	0.115	3.498	**0.0014**
Timepoint 1-Timepoint 4	0.21806	0.115	1.899	0.1389
Phylum Proteobacteria:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	-0.01723	0.114	-0.151	0.9876
Timepoint 0-Timepoint 4	0.02409	0.114	0.211	0.9759

Timepoint 1-Timepoint 4	0.04132	0.114	0.361	0.9306
Phylum Verrucomicrobiota:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	0.13332	0.115	1.161	0.4763
Timepoint 0-Timepoint 4	0.31867	0.115	2.775	**0.0153**
Timepoint 1-Timepoint 4	0.18535	0.115	1.613	0.2399

Table C- 8 Effect of treatments and soil physio-chemicals on bacteria.

Ammonium	Estimate	SE	z value	p-value
Hot Foam-Control==0	0.3083	0.2397	1.286	0.44
New Way-Control==0	0.4524	0.2501	1.809	0.181
Nomix Dual-Control==0	0.3525	0.2278	1.547	0.292
Roundup Flex-Control==0	0.4411	0.2562	1.722	0.213
% Organic Carbon	**Estimate**	**SE**	**z value**	**p-value**
Hot Foam-Control==0	-0.1565	0.2522	-0.62	0.936
New Way-Control==0	-0.4968	0.3707	-1.34	0.495
Nomix Dual-Control==0	-0.2225	0.3369	-0.661	0.922
Roundup Flex-Control==0	-0.5854	0.3701	-1.582	0.341
Moisture Content	**Estimate**	**SE**	**z value**	**p-value**
Hot Foam-Control==0	-0.20452	0.16096	-1.271	0.517
New Way-Control==0	0.21487	0.16095	1.335	0.473
Nomix Dual-Control==0	0.1054	0.16095	0.655	0.913
Roundup Flex-Control==0	0.05136	0.16096	0.319	0.993
Soil Organic Matter	**Estimate**	**SE**	**z value**	**p-value**
Hot Foam-Control==0	-0.18107	0.50254	-0.36	0.989
New Way-Control==0	-0.03211	0.50253	-0.064	1
Nomix Dual-Control==0	0.4137	0.50252	0.823	0.827
Roundup Flex-Control==0	0.05481	0.50254	0.109	1
WHC	**Estimate**	**SE**	**z value**	**p-value**
Hot Foam-Control==0	0.20448	0.42595	0.48	0.97
New Way-Control==0	0.06536	0.42594	0.153	1
Nomix Dual-Control==0	0.37609	0.42593	0.883	0.79
Roundup Flex-Control==0	0.12372	0.42594	0.29	0.995
pH	**Estimate**	**SE**	**z value**	**p-value**
Hot Foam-Control==0	6.274	4.391	1.429	0.412
New Way-Control==0	-1.049	4.391	-0.239	0.998
Nomix Dual-Control==0	3.755	4.391	0.855	0.807
Roundup Flex-Control==0	1.985	4.391	0.452	0.975
% Clay	**Estimate**	**SE**	**z value**	**p-value**
Hot Foam-Control==0	-0.0125	0.3219	-0.039	1
New Way-Control==0	-0.10124	0.32189	-0.315	0.994
Nomix Dual-Control==0	-0.09268	0.32188	-0.288	0.995
Roundup Flex-Control==0	-0.01059	0.3219	-0.033	1
% Silt	**Estimate**	**SE**	**z value**	**p-value**
Hot Foam-Control==0	-0.15632	0.17572	-0.89	0.785
New Way-Control==0	-0.11532	0.17572	-0.656	0.913
Nomix Dual-Control==0	0.02741	0.17571	0.156	1
Roundup Flex-Control==0	0.11106	0.17572	0.632	0.923
% Sand	**Estimate**	**SE**	**z value**	**p-value**
Hot Foam-Control==0	0.1819	0.4988	0.365	0.989
New Way-Control==0	0.2786	0.4988	0.558	0.949
Nomix Dual-Control==0	0.2665	0.4988	0.534	0.956
Roundup Flex-Control==0	-0.2248	0.4988	-0.451	0.976

Figure C- 1 The abundance of bacteria and levels of soil physio-chemicals under each treatment.



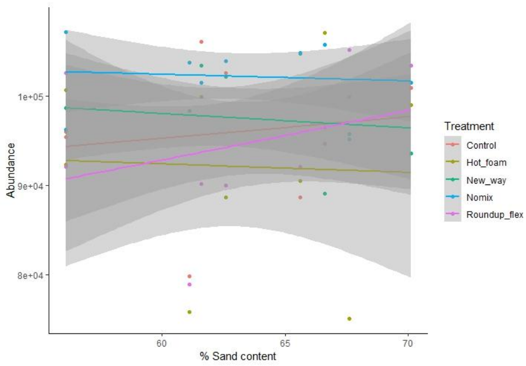


Table C- 9 Fungal phyla level abundance at the initial sampling at zero months, one month and four months post-treatment using the control to contrast.

0 months- initial sample pre-treatment				
contrast	estimate	SE	z.ratio	p.value
Hot Foam-Control	-0.0564	0.221	-0.256	0.9859
New Way-Control	0.0522	0.221	0.236	0.9883
Nomix Dual-Control	0.3524	0.221	1.596	0.3169
Roundup Flex-Control	-0.2438	0.221	-1.104	0.617
1 months – post-treatment				
contrast	estimate	SE	z.ratio	p.value
Hot Foam-Control	0.02307	0.063	0.366	0.967
New Way-Control	0.0595	0.063	0.944	0.718
Nomix Dual-Control	0.00287	0.063	0.046	0.9998
Roundup Flex-Control	-0.018	0.063	-0.286	0.9817
4 months – post-treatment				
contrast	estimate	SE	z.ratio	p.value
Hot Foam-Control	0.479	0.162	2.965	**0.0113**
New Way-Control	0.329	0.162	2.036	0.1364
Nomix Dual-Control	0.102	0.162	0.631	0.8825
Roundup Flex-Control	-0.19	0.162	-1.173	0.5729

Table C- 10 Relative abundance of fungi at phylum level.

Taxonomy	**Relative abundance**	**Taxonomy**	**Relative abundance**
Ascomycota	50.0589	Chytridiomycota	0.2510
Others	38.7756	Rozellomycota	0.2205
Basidiomycota	5.8014	Aphelidiomycota	0.0309
Mortierellomycota	3.0160	Kickxellomycota	0.0295
Glomeromycota	0.8819	Olpidiomycota	0.0066
Mucoromycota	0.6016	Basidiobolomycota	0.0042
Zoopagomycota	0.3215	Monoblepharomycota	0.0005

Table C- 11 The top four fungal phyla over time and under each treatment

Phylum: Ascomycota				
Treatment=Control:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	-0.3866	0.151	-2.56	**0.0282**
Timepoint 0-Timepoint 4	0.0844	0.151	0.559	0.8419
Timepoint 1-Timepoint 4	0.471	0.151	3.119	**0.0052**
Treatment=Hot Foam:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	-0.2178	0.169	-1.29	0.4009
Timepoint 0-Timepoint 4	-0.5523	0.169	-3.272	**0.0031**
Timepoint 1-Timepoint 4	-0.3346	0.151	-2.216	0.0685
Treatment=New Way:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	-0.0667	0.151	-0.442	0.8979
Timepoint 0-Timepoint 4	-0.2441	0.151	-1.617	0.2384
Timepoint 1-Timepoint 4	-0.1774	0.151	-1.175	0.4682
Treatment=Nomix Dual:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	-0.1249	0.151	-0.827	0.686
Timepoint 0-Timepoint 4	0.0737	0.151	0.488	0.8769
Timepoint 1-Timepoint 4	0.1987	0.151	1.316	0.3864
Treatment=Roundup Flex:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	-0.2394	0.151	-1.586	0.2518
Timepoint 0-Timepoint 4	0.2628	0.151	1.74	0.1902
Timepoint 1-Timepoint 4	0.5023	0.151	3.326	**0.0025**
Phylum: Basidiomycota				
Treatment=Control:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	-0.3692	0.362	-1.019	0.5646
Timepoint 0-Timepoint 4	-0.398	0.362	-1.099	0.5147
Timepoint 1-Timepoint 4	-0.0289	0.362	-0.08	0.9965
Treatment= Hot Foam:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	-0.8995	0.362	-2.484	**0.0347**
Timepoint 0-Timepoint 4	-0.642	0.362	-1.772	0.1789
Timepoint 1-Timepoint 4	0.2575	0.362	0.711	0.7568
Treatment= New Way:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	0.1256	0.362	0.347	0.9359
Timepoint 0-Timepoint 4	0.0617	0.362	0.17	0.9841
Timepoint 1-Timepoint 4	-0.0638	0.362	-0.176	0.983
Treatment=Nomix Dual:				
contrast	estimate	SE	z.ratio	p.value

Timepoint 0-Timepoint 1	-0.3603	0.362	-0.995	0.5799
Timepoint 0-Timepoint 4	0.5593	0.362	1.544	0.2703
Timepoint 1-Timepoint 4	0.9196	0.362	2.539	**0.0299**
Treatment= Roundup Flex:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	-0.2092	0.362	-0.578	0.8321
Timepoint 0-Timepoint 4	0.1275	0.362	0.352	0.934
Timepoint 1-Timepoint 4	0.3367	0.362	0.929	0.6216
Phylum: Others				
Treatment=Control:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	0.311	0.182	1.707	0.2024
Timepoint 0-Timepoint 4	0.561	0.182	3.078	**0.0059**
Timepoint 1-Timepoint 4	0.25	0.182	1.371	0.3561
Treatment= Hot Foam:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	0.437	0.182	2.397	**0.0436**
Timepoint 0-Timepoint 4	0.644	0.182	3.534	**0.0012**
Timepoint 1-Timepoint 4	0.207	0.182	1.137	0.4914
Treatment= New Way:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	-0.145	0.182	-0.796	0.7058
Timepoint 0-Timepoint 4	0.376	0.182	2.065	0.0972
Timepoint 1-Timepoint 4	0.521	0.182	2.861	**0.0118**
Treatment=Nomix Dual:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	0.728	0.182	3.995	**0.0002**
Timepoint 0-Timepoint 4	0.964	0.182	5.289	**<.0001**
Timepoint 1-Timepoint 4	0.236	0.182	1.294	0.3984
Treatment= Roundup Flex:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	-0.403	0.182	-2.21	0.0695
Timepoint 0-Timepoint 4	0.245	0.182	1.347	0.3693
Timepoint 1-Timepoint 4	0.648	0.182	3.557	**0.0011**
Phylum: Mortierellomycota				
Treatment=Control:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	-0.405	0.326	-1.243	0.4278
Timepoint 0-Timepoint 4	0.423	0.327	1.296	0.3975
Timepoint 1-Timepoint 4	0.829	0.326	2.539	**0.0299**
Treatment= Hot Foam:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	-0.122	0.365	-0.335	0.9401
Timepoint 0-Timepoint 4	0.183	0.365	0.502	0.8704
Timepoint 1-Timepoint 4	0.305	0.326	0.935	0.6179

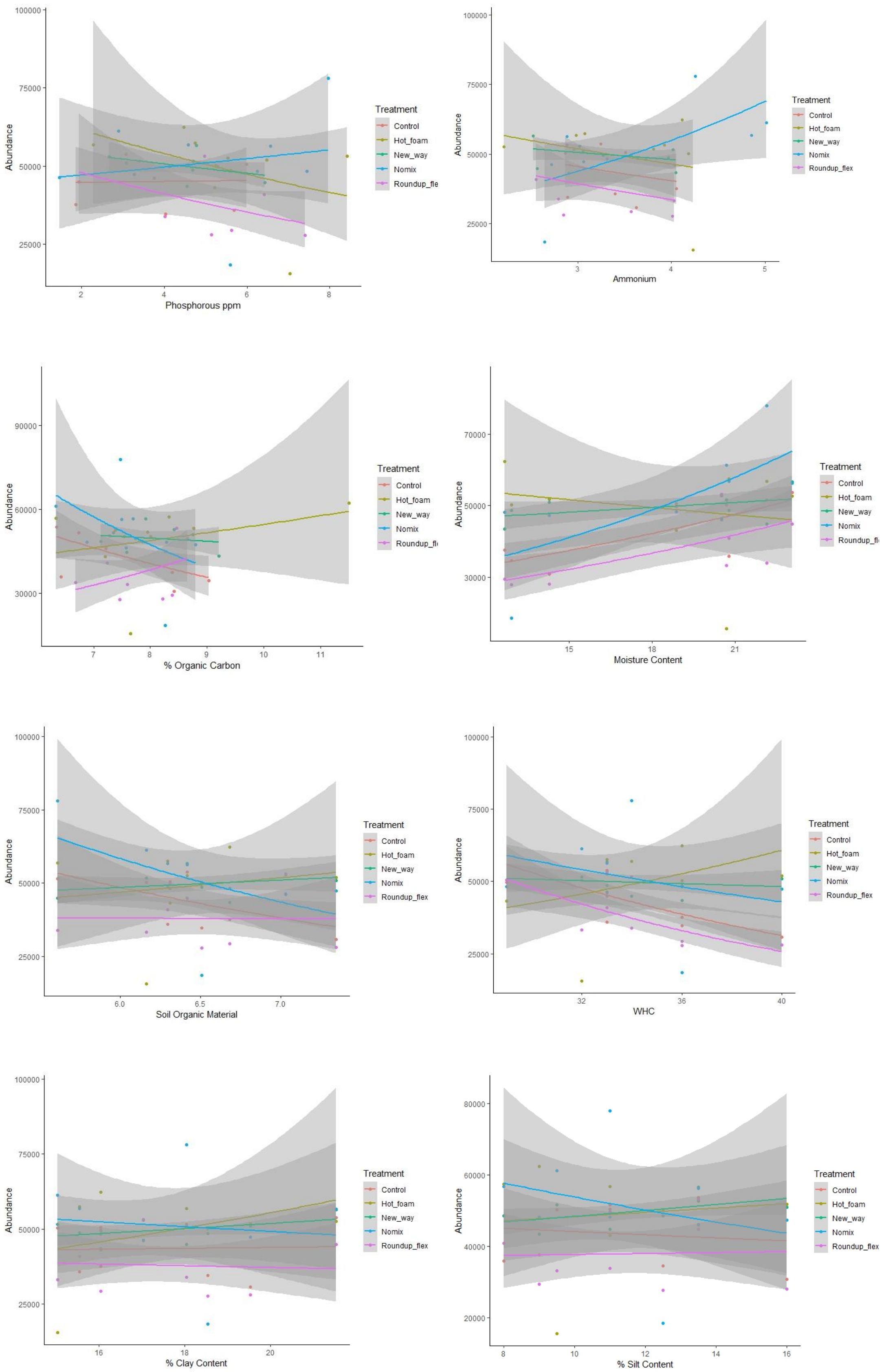
Treatment= New Way:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	-0.136	0.326	-0.416	0.9088
Timepoint 0-Timepoint 4	0.309	0.326	0.947	0.6108
Timepoint 1-Timepoint 4	0.445	0.326	1.363	0.3605
Treatment=Nomix Dual:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	0.31	0.326	0.95	0.6088
Timepoint 0-Timepoint 4	1.051	0.327	3.218	**0.0037**
Timepoint 1-Timepoint 4	0.741	0.327	2.269	0.0603
Treatment= Roundup Flex:				
contrast	estimate	SE	z.ratio	p.value
Timepoint 0-Timepoint 1	-0.661	0.326	-2.024	0.1064
Timepoint 0-Timepoint 4	-0.23	0.327	-0.704	0.7612
Timepoint 1-Timepoint 4	0.431	0.326	1.32	0.3837

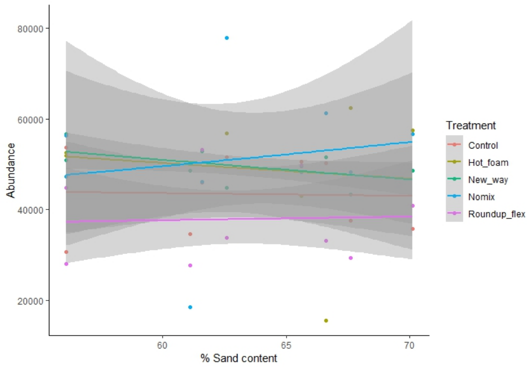
Table C- 12 Effect of treatments and soil physio-chemicals on fungi using Dunnett Contrasts Multiple Comparisons of Means Test

Phosphorous	**Estimate**	**SE**	**z value**	**p-value**
Hot Foam-Control==0	0.46491	0.40994	1.134	0.612
New Way-Control==0	0.25702	0.45923	0.56	0.947
Nomix Dual-Control==0	0.00156	0.36346	0.004	1
Roundup Flex-Control==0	0.22543	0.41201	0.547	0.951
Ammonium	**Estimate**	**SE**	**z value**	**p-value**
Hot Foam-Control==0	0.1724	0.75719	0.228	0.997
New Way-Control==0	-0.09278	0.7901	-0.117	1
Nomix Dual-Control==0	-1.06943	0.71977	-1.486	0.323
Roundup Flex-Control==0	-0.02737	0.80931	-0.034	1
% Organic Carbon	**Estimate**	**SE**	**z value**	**p-value**
Hot Foam-Control==0	-1.2732	0.8217	-1.55	0.359
New Way-Control==0	-0.6752	1.2078	-0.559	0.955
Nomix Dual-Control==0	0.6316	1.0976	0.575	0.951
Roundup Flex-Control==0	-2.3311	1.206	-1.933	0.175
Moisture content	**Estimate**	**SE**	**z value**	**p-value**
Hot Foam-Control==0	1.1223	0.5056	2.22	0.0869
New Way-Control==0	0.7116	0.5056	1.407	0.4253
Nomix Dual-Control==0	-0.1684	0.5056	-0.333	0.992
Roundup Flex-Control==0	-0.2075	0.5056	-0.41	0.9827
Soil Organic Matter	**Estimate**	**SE**	**z value**	**p-value**
Hot Foam-Control==0	-2.0708	1.5734	-1.316	0.486
New Way-Control==0	-1.7646	1.5734	-1.122	0.624

Nomix Dual-Control==0	0.4919	1.5734	0.313	0.994
Roundup Flex-Control==0	-1.6996	1.5734	-1.08	0.654
WHC	**Estimate**	**SE**	**z value**	**p-value**
Hot Foam-Control==0	-2.7979	1.2744	-2.195	0.092
New Way-Control==0	-1.4524	1.2744	-1.14	0.611
Nomix Dual-Control==0	-0.625	1.2744	-0.49	0.967
Roundup Flex-Control==0	0.1568	1.2744	0.123	1
% Clay	**Estimate**	**SE**	**z value**	**p-value**
Hot Foam-Control==0	-0.73744	1.04581	-0.705	0.89
New Way-Control==0	-0.0857	1.04582	-0.082	1
Nomix Dual-Control==0	0.51249	1.04582	0.49	0.967
Roundup Flex-Control==0	0.06267	1.04584	0.06	1
% Silt	**Estimate**	**SE**	**z value**	**p-value**
Hot Foam-Control==0	-0.1423	0.5866	-0.243	0.998
New Way-Control==0	-0.1702	0.5866	-0.29	0.995
Nomix Dual-Control==0	0.4491	0.5866	0.766	0.859
Roundup Flex-Control==0	-0.2946	0.5866	-0.502	0.964
% Sand	**Estimate**	**SE**	**z value**	**p-value**
Hot Foam-Control==0	0.6027	1.6487	0.366	0.989
New Way-Control==0	0.584	1.6487	0.354	0.99
Nomix Dual-Control==0	-0.5926	1.6487	-0.359	0.989
Roundup Flex-Control==0	-0.3734	1.6487	-0.227	0.998

Figure C- 2 The abundance of fungi and the levels of soil physio-chemicals under each treatment



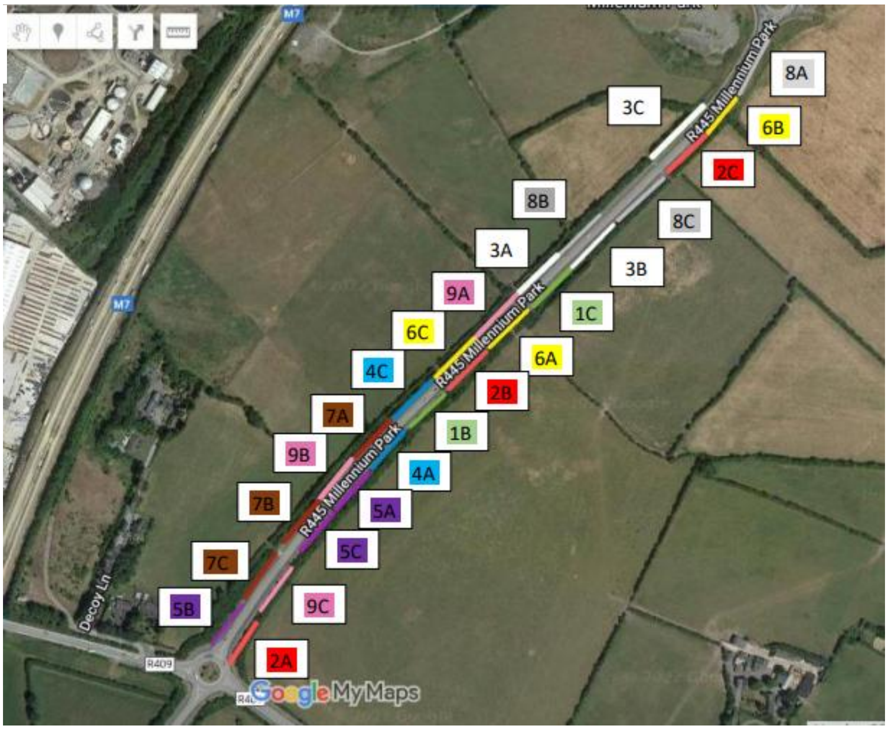


80000
60000
40000
20000
Abundance
60
65
70
% Sand content
Treatment
Control
Hot_foam
New_way
Nomix
Roundup_flex

Appendix D Supplementary Data for Chapter 5

Figure D- 1 Experiment 1: The map layout of the site along the Millennium Link Road with the colour coded Site Codes denoting the location of each treatment (A and B). Two experimental sites were moved further south (B) due to previous use of a trial mechanical treatment.

A)



B)

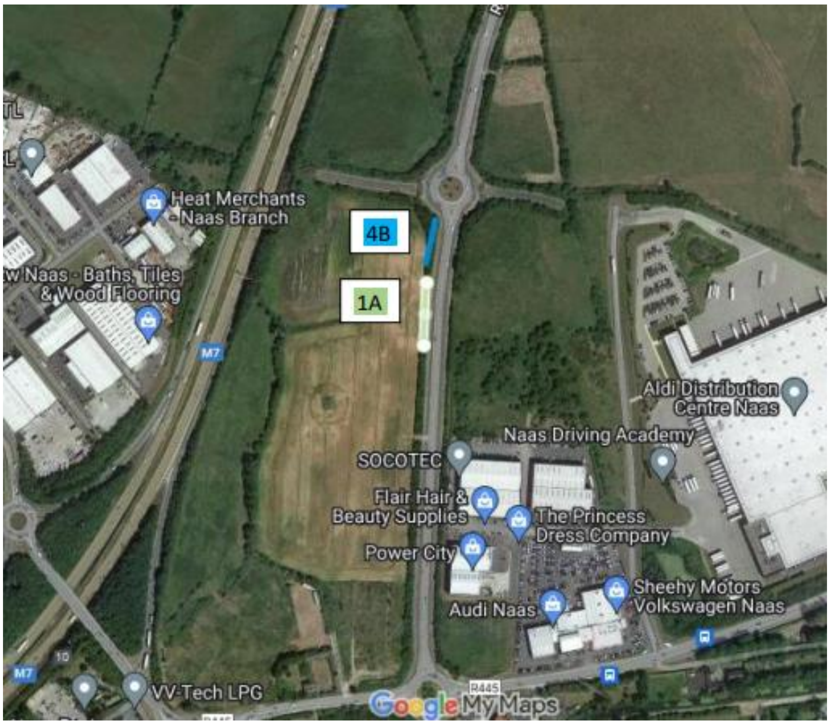


Table D- 1 Recording sheets for the contractors. Each treatment has three 50m sections (A, B and C) of the Millennium Link Road.

Treatment	Treatment A- Time Started	Treatment A-Time Ended	Treatment B- Time Started	Treatment B-Time Ended	Treatment C- Time Started	Treatment C-Time Ended	Total Cost of Treatment per Hour
1. Control							
2. Hot foam							
3. Roundup flex (glyphosate)							
4. Nomix Dual (glyphosate)							
5. Strim/Mow							
6. New Way (acetic acid)							
7. WeedHex Machine-cycle path and footpath intersection and verge only							
8. Multihogg Machine-cycle path and footpath intersection and verge only							

Figure D- 2 Experiment 2: The map layout of the site along the Millennium Link Road with the colour coded Site Codes denoting the location of each treatment.

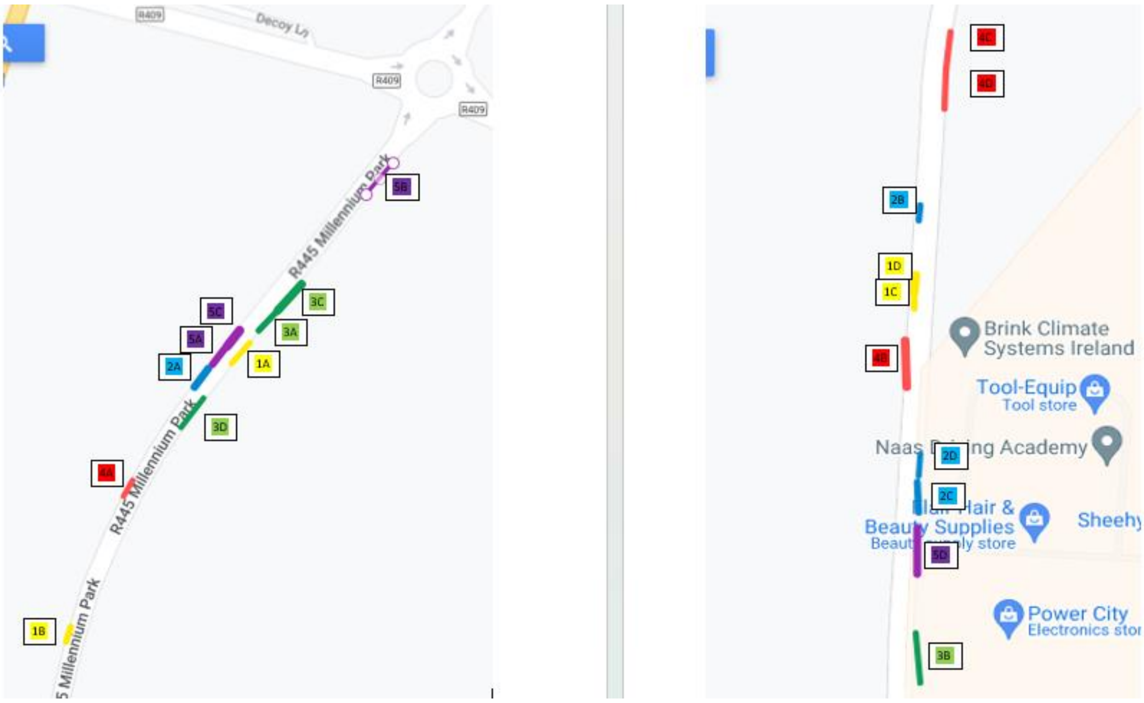


Table D- 2 Survey sheets for the vegetation surveys on the Millennium Link Road for experiment 2.

Millennium Link Road	**Date**	**% Cover**							
Section	**1st m**	**2nd m**	**3rd**	**4th**	**5th**	**6th**	**7th**	**8th**	**9th**	**10th**
Grass										
Dandelion										
Moss										
Mouseear										
Ragwort										
Bare										
Willowherb										
Clover										
Self heal										
Black medick										
Creeping Buttercup										
Greater plantain										
Vetch sp.										

Table D- 3 The list of flora and percentage cover recorded by month and treatment on the Millennium Link Road during surveys.

Flora and Treatment	0 Month	1 Month	2 Months	5 Months	11 Months	Grand Total
Achillea millefolium	**0.40**				**0.40**	**0.40**
Control	0.40				0.40	0.40
Anthriscus sylvestris	**0.73**	**0.85**	**1.40**	**2.00**	**1.85**	**1.34**
New Way	1.00	0.20	1.40	2.00	1.60	1.24
Nomix Dual	0.20				1.20	0.70
Roundup Flex	1.00	1.50			2.30	1.78
Asteraceae	**0.99**	**1.90**	**1.40**	**0.80**	**1.00**	**1.13**
Control	1.80	3.40	1.40	0.80	2.40	1.96
Hot Foam	0.50				0.60	0.55
New Way	0.90					0.90
Nomix Dual	1.15	0.40			0.40	0.78
Bellis perennis	**1.32**	**0.90**	**1.00**	**0.30**	**1.10**	**1.03**
Control			1.00			1.00
Hot Foam	1.90				1.10	1.63
New Way	0.80			0.40		0.60
Nomix Dual				0.20		0.20
Roundup Flex	1.10	0.90				1.02
Bryophyte	**31.40**	**18.64**	**26.31**	**42.86**	**9.09**	**25.27**
Control	33.38	27.63	39.63	55.93	12.15	33.74
Hot Foam	29.00				1.08	15.04
New Way	28.88	0.30	6.45	18.23	2.55	12.91
Nomix Dual	30.13	18.40	38.75	50.05	19.88	31.44
Roundup Flex	35.63	13.10	20.40	41.08	9.80	24.57
Cardamine hirsuta				**0.43**		**0.43**
Control				0.30		0.30
Hot Foam				0.23		0.23
Nomix Dual				0.20		0.20
Roundup Flex				0.90		0.90
Cerastium fontanum	**5.37**	**1.91**	**2.75**	**0.58**	**5.21**	**3.77**
Control	5.58	1.90	2.53	0.70	3.00	2.96
Hot Foam	4.05				3.25	3.78
New Way	5.63	1.05	1.50	0.65	2.77	2.86
Nomix Dual	4.25	3.23	3.60	0.20	1.75	3.20
Roundup Flex	7.33	0.73			13.47	7.19
***Cyperaceae* sp.**	**0.80**	**0.87**	**0.47**		**0.60**	**0.67**
Control	0.80	1.40	1.00		0.60	0.88
Hot Foam			0.20			0.20
New Way		0.20	0.20			0.20
Nomix Dual		1.00				1.00
***Epilobium* spp.**	**2.42**	**1.92**	**1.63**	**0.75**	**2.58**	**1.96**
Control	2.05	1.90	1.70	1.47	1.50	1.74
Hot Foam	1.13			0.60	0.80	0.90

Flora and Treatment	0 Month	1 Month	2 Months	5 Months	11 Months	Grand Total
New Way	2.63	0.75	2.20	0.60	1.98	1.79
Nomix Dual	1.67	1.40	1.07	0.53	0.67	1.07
Roundup Flex	4.10	2.93	1.85	0.48	6.48	3.32
Galium aparine				**0.20**		**0.20**
Roundup Flex				0.20		0.20
Geranium dissectum	**0.60**					**0.60**
Nomix Dual	0.20					0.20
Roundup Flex	1.00					1.00
Geranium robertianum					**0.85**	**0.85**
Hot Foam					0.20	0.20
Roundup Flex					1.50	1.50
Heracleum sphondylium	**1.95**	**0.20**		**1.10**	**0.65**	**0.99**
New Way	1.95	0.20		1.10	0.80	1.13
Roundup Flex					0.50	0.50
***Hieracium* spp.**	**0.25**				**2.60**	**1.66**
Control					0.90	0.90
Nomix Dual	0.30					0.30
Roundup Flex	0.20				6.00	3.10
Luzula campestris				**0.23**		**0.23**
Control				0.25		0.25
Nomix Dual				0.20		0.20
Medicago lupulina	**3.96**				**2.57**	**3.54**
Control	2.50					2.50
Hot Foam	1.40					1.40
New Way	1.40				0.30	1.03
Nomix Dual	16.50					16.50
Roundup Flex	2.00				3.70	3.13
Myosotis arvensis	**0.40**					**0.40**
Control	0.40					0.40
Plantago major	**1.13**	**1.13**	**0.60**		**5.70**	**2.62**
Control	1.30	1.90	0.60		3.40	1.80
Hot Foam					0.40	0.40
New Way	1.00				3.00	1.67
Nomix Dual	0.60	1.00				0.80
Roundup Flex	1.45	0.50			10.85	5.02
Poaceae spp.	**52.36**	**22.19**	**21.79**	**21.82**	**41.32**	**32.43**
Control	58.13	57.25	48.25	37.13	62.15	52.58
Hot Foam	54.20	0.00	3.43	14.03	33.63	21.06
New Way	43.98	5.83	11.88	21.07	47.50	26.31
Nomix Dual	51.63	43.63	28.58	26.00	47.50	39.47
Roundup Flex	53.85	4.23	2.00	7.00	15.80	19.91
Prunella vulgaris	**1.67**	**1.54**	**1.20**	**0.87**	**1.81**	**1.56**
Control	1.40	0.80	0.40		3.30	1.77

Flora and Treatment	0 Month	1 Month	2 Months	5 Months	11 Months	Grand Total
Hot Foam	1.87				0.40	1.28
New Way	1.20	0.30		0.40	0.77	0.77
Nomix Dual	2.43	2.30	1.87	1.10	1.77	1.92
Roundup Flex	1.33	1.65	0.80		2.83	1.63
Ranunculus repens	**0.84**	**0.85**	**0.71**	**0.68**	**0.87**	**0.79**
Control	0.93	0.93	1.03	1.00	1.00	0.97
Hot Foam	0.88		0.10			0.62
Nomix Dual	1.17	1.20	0.87	0.57	0.80	0.91
Roundup Flex	0.50	0.35	0.20			0.41
Sapling	**3.40**	**0.65**	**0.80**		**0.50**	**1.41**
Control	6.00	1.00	0.80		0.60	2.10
Hot Foam					0.40	0.40
Nomix Dual	0.80	0.30				0.55
Jacobaea vulgaris	**3.47**	**2.66**	**1.93**	**1.58**	**2.82**	**2.62**
Control	2.33	2.68	1.80	1.80	1.90	2.10
Hot Foam	4.17			0.40	2.95	2.74
New Way	5.00			1.90	5.23	4.31
Nomix Dual	2.55	3.85	2.05	2.10	3.33	2.78
Roundup Flex	3.88	1.07		0.30	1.35	2.03
Senecio vulgaris	**0.34**	**0.40**	**0.15**			**0.30**
Control			0.20			0.20
Hot Foam	0.60					0.60
New Way	0.30					0.30
Nomix Dual	0.40	0.40	0.10			0.30
Roundup Flex	0.20					0.20
***Taraxacum* spp.**	**38.53**	**15.80**	**5.59**	**28.68**	**40.63**	**26.26**
Control	26.33	15.65	5.60	18.10	27.60	18.66
Hot Foam	49.25		4.30	47.75	46.25	36.89
New Way	46.50	2.03	10.33	48.85	53.48	32.24
Nomix Dual	39.78	32.50	5.98	23.73	44.70	29.34
Roundup Flex	30.78	13.03	1.73	4.98	31.10	16.32
***Trifolium* spp.**	**3.57**	**5.38**	**5.70**	**3.03**	**1.83**	**4.02**
Control	4.30	5.18	7.23	3.68	2.40	4.79
Hot Foam	1.00			0.50		0.75
New Way	12.60		0.60			6.60
Nomix Dual	1.05	5.80	11.70	3.00	2.80	4.28
Roundup Flex	2.13		0.20		0.30	1.38
Unknown	**1.13**	**0.95**	**0.20**	**2.14**	**4.23**	**2.15**
Control	1.00	1.27		0.50	0.90	0.95
Hot Foam	0.60			0.40		0.50
New Way		0.47	0.20	2.20		0.76
Nomix Dual				3.50	1.40	2.45
Roundup Flex	1.80	1.20		3.05	6.60	3.89
***Veronica* spp.**	**4.30**	**1.00**	**1.00**		**0.60**	**1.73**

Flora and Treatment	0 Month	1 Month	2 Months	5 Months	11 Months	Grand Total
Control		1.00	1.00		0.60	0.87
New Way	4.30					4.30
***Vicia* spp.**	**0.99**	**1.05**	**0.98**	**0.63**	**0.38**	**0.81**
Control			0.80	0.20	0.40	0.47
Hot Foam	1.10				0.40	0.75
Nomix Dual	1.10	1.05	1.03	0.77	0.30	0.93
Roundup Flex	0.80					0.80
Grand Total	**13.28**	**8.03**	**8.42**	**13.28**	**12.16**	**11.35**

Table D- 4 The overall results from the live vegetation at each month of surveys in contrast to the control on the Millennium Link Road.

Contrast to Control	estimate	SE	df	t.ratio	p.value
Month = 0:					
Hot Foam - Control	1.8807	3.4 7	5	0.553	0.9133
New Way - Control	1.4085	3.4 7	5	0.414	0.9558
Nomix Dual - Control	1.3156	3.4 7	5	0.387	0.9624
Roundup Flex - Control	-3.2056	3.4	75	-0.943	0.7207
Month = 1:					
Hot Foam - Control	-25.255	3.4	75	-7.426	**<.0001**
New Way - Control	-20.9918	3.4	75	-6.172	**<.0001**
Nomix Dual - Control	0.2336	3.4	75	0.069	0.9994
Roundup Flex - Control	-15.5433	3.4	75	-4.57	**0.0001**
Month = 2:					
Hot Foam - Control	-20.2142	3.4	75	-5.944	**<.0001**
New Way - Control	-13.2672	3.4	75	-3.901	**0.0008**
Nomix Dual - Control	-5.0327	3.4	75	-1.48	0.3883
Roundup Flex - Control	-11.611	3.4	75	-3.414	**0.0039**
Month = 5:					
Hot Foam - Control	-2.2219	3.4	75	-0.653	0.8732
New Way - Control	-3.4836	3.4	75	-1.024	0.6707
Nomix Dual - Control	-1.8549	3.4	75	-0.545	0.916
Roundup Flex - Control	-10.6034	3.4	75	-3.118	**0.0096**
Month = 11:					
Hot Foam - Control	0.0831	3.4	75	0.024	0.9999
New Way - Control	5.6869	3.4	75	1.672	0.2869
Nomix Dual - Control	4.0313	3.4	75	1.185	0.5686
Roundup Flex - Control	-9.0314	3.4	75	-2.656	**0.0344**

Table D- 5 The results on Poacaea spp. at each month of surveys in contrast to the control on the Millennium Link Road.

Contrast to control	**estimate**	**SE**	**df**	**t.ratio**	**p.value**
Month = 0:					
Hot Foam - Control	-0.0523	0.289	71	-0.181	0.9938
New Way - Control	-0.2437	0.289	71	-0.844	0.7778
Nomix Dual - Control	-0.1135	0.289	71	-0.393	0.961
Roundup Flex - Control	-0.0517	0.289	71	-0.179	0.994
Month = 1:					
Hot Foam - Control	-4.0411	0.289	71	-13.998	**<.0001**
New Way - Control	-2.1112	0.289	71	-7.313	**<.0001**
Nomix Dual - Control	-0.2849	0.289	71	-0.987	0.6939
Roundup Flex - Control	-2.0915	0.289	71	-7.245	**<.0001**
Month = 2:					
Hot Foam - Control	-2.1925	0.289	71	-7.595	**<.0001**
New Way - Control	-1.1611	0.289	71	-4.022	**0.0006**
Nomix Dual - Control	-0.6522	0.289	71	-2.259	0.0904
Roundup Flex - Control	-1.8025	0.456	71	-3.949	**0.0007**
Month = 5:					
Hot Foam - Control	-1.0785	0.289	71	-3.736	**0.0014**
New Way - Control	-0.7661	0.289	71	-2.654	**0.0349**
Nomix Dual - Control	-0.4512	0.289	71	-1.563	0.3429
Roundup Flex - Control	-1.5028	0.312	71	-4.819	**<.0001**
Month = 11:					
Hot Foam - Control	-0.6024	0.289	71	-2.087	0.1314
New Way - Control	-0.2877	0.289	71	-0.997	0.6879
Nomix Dual - Control	-0.2603	0.289	71	-0.902	0.745
Roundup Flex - Control	-1.1873	0.289	71	-4.113	**0.0004**

Table D- 6 The results on *Taraxacum* spp. at each month of surveys in contrast to the control on the Millennium Link Road.

Contrast to Control	estimate	SE	df	t.ratio	p.value
Month = 0:					
Hot Foam - Control	0.5862	0.25	72	2.342	0.0747
New Way - Control	0.5613	0.25	72	2.242	0.0937
Nomix Dual - Control	0.3794	0.25	72	1.516	0.3684
Roundup Flex - Control	0.1412	0.25	72	0.564	0.9093
Month = 1:					
Hot Foam - Control	nonEst	0.25	72	NA	**<.0001**
New Way - Control	-1.5378	0.25	72	-6.144	**<.0001**
Nomix Dual - Control	0.617	0.25	72	2.465	**0.0439**
Roundup Flex - Control	-0.2812	0.25	72	-1.123	0.5364
Month = 2:					
Hot Foam - Control	-0.502	0.25	72	-2.005	0.1552
New Way - Control	0.1771	0.25	72	0.707	0.8485
Nomix Dual - Control	-0.1469	0.25	72	-0.587	0.9006
Roundup Flex - Control	-0.4692	0.25	72	-1.875	0.2002
Month = 5:					
Hot Foam - Control	0.8701	0.25	72	3.476	**0.0033**
New Way - Control	0.9386	0.25	72	3.75	**0.0014**
Nomix Dual - Control	0.3046	0.25	72	1.217	0.5487
Roundup Flex - Control	-0.9338	0.25	72	-3.731	**0.0015**
Month = 11:					
Hot Foam - Control	0.4213	0.25	72	1.683	0.2821
New Way - Control	0.6652	0.25	72	2.658	**0.0344**
Nomix Dual - Control	0.4498	0.25	72	1.797	0.2312
Roundup Flex - Control	0.0598	0.25	72	0.239	0.988

Table D- 7 The results on Bryophytes at each month of surveys in contrast to the control on the Millennium Link Road.

Contrast to Control	**estimate**	**SE**	**df**	**t.ratio**	**p.value**
Month = 0:					
Hot Foam - Control	-0.145	0.321	61	-0.452	0.9458
New Way - Control	-0.0985	0.321	61	-0.307	0.9782
Nomix Dual - Control	-0.1334	0.321	61	-0.416	0.9554
Roundup Flex - Control	0.0488	0.321	61	0.152	0.9959
Month = 1:					
Hot Foam - Control	nonEst	NA	NA	NA	**<.0001**
New Way - Control	-2.0992	0.507	61	-4.139	**0.0003**
Nomix Dual - Control	-0.398	0.321	61	-1.241	0.4642
Roundup Flex - Control	-0.5461	0.346	61	-1.576	0.282
Month = 2:					
Hot Foam - Control	nonEst	NA	NA	NA	**<.0001**
New Way - Control	-1.5091	0.321	61	-4.705	**<.0001**
Nomix Dual - Control	-0.0207	0.321	61	-0.065	0.9984
Roundup Flex - Control	-0.6476	0.321	61	-2.019	0.1232
Month = 5:					
Hot Foam - Control	nonEst	NA	NA	NA	**<.0001**
New Way - Control	-0.8646	0.346	61	-2.496	**0.0417**
Nomix Dual - Control	-0.1399	0.321	61	-0.436	0.9212
Roundup Flex - Control	-0.371	0.321	61	-1.157	0.516
Month = 11:					
Hot Foam - Control	-1.1934	0.321	61	-3.72	**0.0017**
New Way - Control	-0.714	0.321	61	-2.226	0.0988
Nomix Dual - Control	0.2942	0.321	61	0.917	0.7361
Roundup Flex - Control	-0.2799	0.321	61	-0.873	0.762