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Can anthropogenic biodiversity and geodiversity associated with legacy industrial waste help offset decreasing global biodiversity?

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1

Abstract

Anthropogenic substrates, including materials such as steel slag, paper mill sludge, oil shale spoil and colliery spoil, were frequently dumped and are still dumped today in many countries where such waste is produced. In some countries, dumping of such materials is restricted due to increased health and safety regulations and due to improved waste recycling streams. Such substrates have become byproducts and are used in various products and/or applications. Legacy dumps, those that have been around for many years, can theoretically be excavated for such useful byproducts, but this can be costly and time-consuming. Additionally, many legacy anthropogenic substrate sites have biodiverse and/or rare wildlife communities, as the characteristics of the substrate can support favourable or acceptable conditions for many species. Plant species that might easily be outcompeted on high-nutrient soils may benefit from reduced competition on lower-nutrient anthropogenic substrate, for example. Invertebrates on such sites can benefit from habitat mosaics, bare patches and varied topography, for example. Anthropogenic substrate sites can be grouped as a type of brownfield site, those that are associated with former industry and anthropogenic activities. Some biodiverse brownfield sites are recognised in legislation in multiple countries, for example, Open Mosaic Habitat on Previously Developed Land, or Calaminarian Grasslands. However, assessment and protection of wildlife on these sites is often lacking and they are often preferentially chosen for development by councils, governments, developers and so on. The very features of these sites which can be so advantageous to certain species are therefore vulnerable to being removed, covered or otherwise modified by people in the name of restoration, remediation or development, for example. In order to better understand the features of these sites that can be utilised by wildlife, it is important to recognise how the substrate heterogeneity and geodiversity of many anthropogenic substrates might influence or be associated with specific species, especially plants. This study addresses questions concerning the biodiversity and substrate associations of multiple plant species and communities of anthropogenic substrate sites. Substrate geochemistry and mineralogy was determined across a suite of case study sites with different types of anthropogenic substrate, in combination with plant surveys of the sites. Analyses were carried out to look for statistically significant associations between different substrate variables and the plant species and/or

communities recorded on the sites. Upon examination of the biodiversity levels and substrate associations of open plant communities and species on six case study sites, high biodiversity levels and/or rare and uncommon species were recorded in multiple plant communities across all six sites. It was found that the geochemistry on capped and uncapped areas of a partially remediated slag bank, while statistically significantly different, supported biodiversity levels which were not statistically significantly different, demonstrating that both remediated and unremediated parts of the slag bank contributed to the site's biodiversity. The sampling and analyses of overall geochemistry and plant communities and species present on two sites predominantly covered in blast furnace slag demonstrated multiple associations between decreased or elevated concentrations of certain elements and the presence of certain plant communities and species. Overall, many anthropogenic substrate sites can support high levels of biodiversity, but these can vary between substrate types. It was seen that in plant communities with lower biodiversity levels, there could be at least one rare or uncommon species present, so this study demonstrated that low biodiversity does not necessarily indicate the entire ecological value of the community. Additionally, biodiversity can be influenced by multiple factors, some of which were outside of the scope of this study due to time and sampling constraints. This study emphasises the need to assess anthropogenic substrate sites on a caseby-case basis, rather than treating them all with the same or similar management styles.

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Contents

5

Introduction

1.1 What are anthropogenic substrate sites and what habitats can they support?

Habitats which include anthropogenic substrate are, in many ways, markedly different from the vast majority of natural habitats (Bradshaw, 1977). The substrate present in such a habitat will have been generated by human's activities, typically industry (Bradshaw, 1977; Allan et al., 1997; Courtney et al., 2009; Butt & Briones, 2017; Gomes et al., 2020). Therefore, such substrates will have a surface texture, composition and geochemistry that is different from what is observed in natural substrates (Allan et al., 1997; Bradshaw, 1997; Di Carlo et al., 2019; Gomes et al., 2020). A wide variety of anthropogenic substrates are produced, some of these are acidic in nature (Bradshaw, 1977; Allan et al., 1997; Bradshaw 1997; Batty, 2005; Butt & Briones, 2017), some of these are predominantly alkaline (Courtney et al., 2009; Woods, 2012; Butt & Briones, 2017; Di Carlo et al., 2019; Gomes et al., 2020), some contain toxic trace metals (Allan et al., 1997; Bradshaw, 1997; Batty, 2005; Di Carlo et al., 2019; Affholder et al., 2020), or toxic levels of salt (Bradshaw, 1977; Bradshaw, 1997; Di Carlo et al., 2019) and some can produce leachates that can be harmful to human and ecosystem health (Allan et al., 1997; Mayes et al., 2006; Environment Agency, 2007; Mayes et al., 2008; Riley & Mayes, 2015).

Despite the harm that many of these anthropogenic substrates can confer through the toxic substances that they usually contain (Batty, 2005; Environment Agency, 2007; Gomes et al., 2020), much of the time, they can permit the (sometimes eventual) colonisation of plant and animal species (Ash, 1983; Ash et al., 1994; Gibson, 1998; Batty, 2005; Esfeld et al., 2008). Some plant species can tolerate the toxic trace metals and other substances present in the anthropogenic substrate (Allan et al., 1997; Bradshaw, 1997; Dobson et al., 1997; Batty, 2005; Affholder et al., 2020), meaning that, if plant propagules can access an anthropogenic substrate site (Ash et al., 1994; Bradshaw, 2000; van Diggelen & Marrs, 2003; Esfeld et al., 2008) they can potentially germinate and grow on-site (van Diggelen & Marrs, 2003; Batty, 2005; Woods, 2012; Chuman, 2015; Gomes et al., 2020). Plant communities on anthropogenic substrate are usually different from what is observed on most natural substrates, as nutrient deficiencies (particularly

nitrogen) (Bradshaw, 1977; Allan et al., 1997; Bradshaw, 1997; Wali, 1999; Di Carlo et al., 2019;) and high toxicity levels (Allan et al., 1997; Angold et al., 2006; Di Carlo et al., 2019), will commonly limit or otherwise alter plant community development (Allan et al., 1997; Wali, 1999; Macadam et al., 2013; Di Carlo et al., 2019; Holmes & Kuebbing, 2022). Despite the limitations on anthropogenic substrates, the communities that often form on anthropogenic substrates can demonstrate characteristics that other plant communities cannot. The mosaic habitat that often forms on anthropogenic substrates, where heterogeneous bare patches and stands of ruderal plants are interspersed throughout the habitat (Gibson 1998; Palmer, 2008; Riding et al., 2010; Macadam et al., 2013; Chuman, 2015), as well as other types of anthropogenic waste site vegetation, can support a high diversity of invertebrate species (Gibson, 1998; Batty, 2005; Angold et al., 2006; Cameron & Leather, 2012; Butt & Briones, 2017), which rely on bare patches for warmth and stands of ruderal plants for foraging, nesting and shelter (Palmer, 2008; Riding et al., 2010; Cameron & Leather, 2012; Macadam et al., 2013). Additionally, some plant species found on anthropogenic substrates may not be well-represented in natural habitats in the surrounding area, or even nationally, meaning that these anthropogenic substrates can provide a refuge for rare and/or unusual species of plants (Ash et al., 1994; Esfeld et al., 2008; Woods, 2012; Chuman, 2015; Gomes et al., 2020).

1.2 Details of previous relevant studies – what are some of the general research findings and what could be further investigated?

In some studies, links have been made between properties of the substrate and the plant and/or invertebrate species present, indicating that many plant and invertebrate species can be specialised for semi-natural or anthropogenic habitats due to the specific nature of the type of anthropogenic substrate in question (Gibson, 1998; Batty, 2005; Riding et al., 2010; Woods, 2012; Rainbow, 2018). Species that might be found in abundance on one anthropogenic waste site, for example, would not be found in abundance on a contrasting anthropogenic waste site, due to the specificities of the species in question (Bradshaw, 1997; Batty, 2005). Additionally, anthropogenic waste sites are sometimes less disturbed than many sites that are perceived to be more natural, including farmland and parkland (Gibson, 1998; Batty,

2005; Esfeld et al., 2008; Woods, 2012; Chuman, 2015). In recent years, frameworks have been developed for the identification of valuable brownfield sites, based on habitat characteristics, plant species presence and/or invertebrate species presence (Gibson, 1998). Open mosaic habitats, a particular type of anthropogenic substrate habitat, for example, have been designated as priority habitats, with many people now recognising and/or understanding their importance to biodiversity (Woods, 2012).

Considering the fact that, on many of these sites, soil is thin or non-existent, plant colonisation and growth is directly linked to the character of the anthropogenic substrate itself (Bradshaw, 1977; Allan et al., 1997; Hitchmough et al., 2001; Bonthoux et al., 2014; Gomes et al., 2020). While multiple studies have focussed on the species composition of a few/individual anthropogenic substrate sites (Ash et al., 1994; Cohn et al., 2001; Harvie, 2004; Butt & Briones, 2017; Affholder et al., 2020), there is currently a lack of understanding about the relationship between plants and substrate characteristics, particularly geochemistry and mineralogy, across varied anthropogenic substrate sites. There have been limited tests to ascertain whether there is a relationship between the chemistry of anthropogenic biodiversity at such sites. It would be beneficial to better understand this because: 1) with the current biodiversity crisis, better understanding of biodiversity on different habitats and substrates is very important (The Scottish Government, 2013; Grass et al., 2019); 2) better understanding of the relationships between plants and specific elements or minerals could lead to improved understanding of plant growth and/or community establishment, especially on contaminated substrates (Cohn et al., 2001; Harvie, 2004); and 3) better understanding of certain anthropogenic substrates and their contribution to biodiversity could lead to improvements in more targeted restoration, remediation, reclamation and/or similar (Maddock, 2010; Riding et al., 2010; Macadam & Bairnier, 2012). Such investigations on anthropogenic substrate sites globally would be a vast undertaking, but collecting suitable datasets for one country, to investigate multiple anthropogenic substrate sites with varying chemistries, is far more feasible and could lead to further understanding and scientific knowledge.

More than ever, people need to prioritise the protection, maintenance, environment and/or habitats of biodiversity (The Scottish Government, 2013). With ever-increasing habitat fragmentation (Esfeld et al., 2008; Grass et al., 2019), land use changes (Gibson, 1998; Esfeld et al., 2008) and shrinking of wildlife areas

(Grass et al., 2019), we need to continue to evaluate and recognise areas that are already valuable for wildlife, as well as those that are potentially valuable for wildlife in the near future (The Scottish Government, 2013). There are, however, many social barriers to the wider acceptance of anthropogenic substrate sites as being valuable for wildlife and being worthy of preservation and/or low-level maintenance (Gibson, 1998; Bonthoux et al., 2014). This is due to a number of factors, including: such sites are often associated with declining and derelict areas (Harrison & Davies, 2002; Ishimatsu & Ito, 2013; Bonthoux et al., 2014); anthropogenic substrate sites appear to be dirty and full of waste and rubbish to many people (Evans, 2002; Harrison & Davies, 2002); in many peoples' views, such sites demonstrate negligence on the part of urban planners concerning the relevant area's environmental services (Evans, 2002; Harrison & Davies, 2002; Bonthoux et al., 2014); some groups, including local authorities, prefer green areas with a manicured appearance, often using non-native plant species which are unsuitable for most native invertebrate species (Gibson, 1998; Hitchmough et al., 2001; Harrison & Davies, 2002; Washbourne, 2020); a large number of people see nature as being rural rather than urban and/or living on anthropogenic substrate, disregarding urban and anthropogenic substrate wildlife (Evans, 2002); and huge pressure exists to build over many anthropogenic substrate habitats, especially in large cities with expanding populations, such as London (Harrison & Davies, 2002). Therefore, studies that encourage supporting and/or maintaining the high biodiversity that anthropogenic substrate can host are important for prioritising the conservation of the most ecologically valuable anthropogenic substrate sites. Additionally, there need to be more dynamic approaches to promoting and conserving brownfield habitats including anthropogenic substrate habitats (Bonthoux et al., 2014) involving conservationists, ecologists, wildlife trusts, volunteer organisations (Hitchmough et al., 2001; Bonthoux et al., 2014), stakeholders (Woods, 2012; Bonthoux et al., 2014; Washbourne et al., 2020), private organisations (Harrison & Davies, 2002), industrial organisations (Woods, 2012), local enthusiasts, local natural historians (Harrison & Davies, 2002), and other interested people with different perspectives and experiences concerning brownfield site biodiversity (Evans, 2002; Harrison & Davies, 2002; Hitchmough et al., 2001; Woods, 2012; Washbourne et al., 2020), encouraging education (Gibson, 1998; Harrison & Davies, 2002) and compromising with planners (Gibson, 1998; Evans, 2002; Washbourne et al., 2020). Some

anthropogenic substrate sites have been recognised for their biodiversity importance and been designated as protected sites (Bradshaw, 1977; Gibson, 1998; Batty, 2005; Woods, 2012), but there is scope for protecting many poorly surveyed and underappreciated anthropogenic substrate sites (McCallum & Sardo, 2021).

1.3 How would further research on anthropogenic substrate sites support wider scientific knowledge, understanding and enthusiasm?

It is clear that anthropogenic substrates can support valuable biodiverse communities of plants (Allan et al., 1997; Esfeld, 2008; Palmer, 2008; Macadam et al., 2013; Butt & Briones, 2017). The research for this thesis provides the opportunity to study and better understand the relationships between these plants and the anthropogenic substrate chemistry. By better understanding the characteristics of anthropogenic material that can permit successful colonisation by important communities and species of plants, the relationships between the geodiversity and biodiversity in question can be better understood (Alahuhta et al., 2022). Investigations into the geochemistry and properties of the substrate on an anthropogenic waste site, as carried out across multiple sites in this study, can be combined with other practices that can help to protect and preserve the site's biodiversity, including: habitat surveys (Ash et al., 1994; Riding et al., 2010; Macadam et al., 2013); habitat management (Harrison & Davies, 2002; Bonthoux et al., 2014); designating protection for valuable sites (Bradshaw, 1977; Harrison & Davies, 2002; Batty, 2005; Woods, 2012); community engagement (Harrison & Davies, 2002; Bonthoux et al., 2014); nature outreach including events or volunteering (Harrison & Davies, 2002); and engagement with planners and developers (Harrison & Davies, 2002).

1.4 A summary of my thesis

The overall aim of my thesis is to determine the mineralogical and chemical characteristics that control, influence and/or are associated with species distribution, diversity and occurrence on legacy anthropogenic substrates. To achieve this aim, my objectives will involve case studies of plants on anthropogenic

substrate sites which include colliery spoil, oil shale spoil, blast furnace slag and/or steel slag, examining:

- 1) Various elements on parts of the study sites, in association with recorded plant communities and species;
- 2) pH levels on parts of the study sites, in association with recorded plants;
- 3) Minerals on sections of the study sites, recorded alongside the plants;
- 4) The species and communities on each site;
- 5) The biodiversity levels of different communities on each study site, represented by biodiversity indices calculated using various R packages (Kindt, 2018; Hsieh et al., 2022; Oksanen et al., 2022);
- 6) The relationships between different plant species and elements, as well as pH levels, recorded on the study sites, calculated using Canonical Correspondence Analyses (CCA) in the R package "vegan" (Oksanen et al., 2022);
- 7) The indicator plant species on each study site for specific minerals, calculated using a function in the R package "labdsv" (Roberts, 2023).

Additionally, more specific studies will be carried out on some of the

anthropogenic substrate sites, including:

- 1) Examination of spatial heterogeneity, mineralogy and chemistry across two ferrous slag sites. This will involve QGIS (Inverse Distance Weighted interpolation, IDW) to examine the spatial distribution of higher and lower concentrations of specific elements;
- 2) Tests to look for statistically significant differences between element concentrations and pH levels in uncapped and capped parts of a partially remediated slag bank.

The overall focusses and themes of the subsequent thesis chapters are as follows:

• Chapter 1: Literature Review – this chapter focusses on much of the body of literature that exists concerning anthropogenic substrate sites, brownfield sites more widely, species recorded on such sites, relevant restoration techniques and how these sites are currently perceived by and utilised by people. Many of the further sections provide information about the biodiversity and important species and communities on anthropogenic substrate sites, as well as examining the restoration strategies in a biodiversity context.

Therefore, there is some detail here about the various uses of and threats to biodiverse anthropogenic substrate sites and where mitigation might be able to preserve biodiversity where development is prioritised. Brownfield sites, including anthropogenic substrate sites, are discussed in a wide variety of different contexts, so it is important to understand and acknowledge these multiple perspectives in relation to the importance and relevance of the studies carried out in this thesis.

- Chapter 2: Materials and Methods This chapter includes methods which are relevant to multiple research chapters, including field methodology across the six study sites, substrate sample preparation and analyses and statistical analyses.
- Chapter 3: Could anthropogenic substrates be used for 'biodiversity offsetting' in Great Britain? – This chapter includes an overview on current knowledge about brownfield sites and anthropogenic substrate sites in different parts of Great Britain (as a case study) to assess whether such sites provide the potential for biodiversity offsetting.
- Chapter 4: "How the mineralogy and chemistry of anthropogenic substrates influence plant biodiversity" – This chapter focusses on the plant species and communities recorded in all six study sites, along with the associated substrate chemistry. The six different study sites are examined based on element concentrations, biodiversity indices for each community are calculated, indicator species throughout the six study sites are determined and associations with specific species and elements (as well as pH level) are examined.
- Chapter 5: "Slag substrate composition influences plant community distribution and biodiversity" – This chapter directly investigates two of the study sites, which both contain high proportions of blast furnace slag, as well as at least one other substrate type. Specifically, the spatial distribution of different plant communities on both sites, as well as the geochemistry throughout the two sites, is examined. Both literature analysis and quantitative analysis for the examined data lead to a nuanced understanding of the plant species and communities on these two sites. Specifically, the plant species and communities on these sites are similar to, but not exactly the same as,

what is seen on Calaminarian grassland, a priority habitat in the UK and the EU. Better understanding of the relationships between plant communities and specific elements on these sites may lead to further understanding about plants in similar plant communities, including those on anthropogenic substrate more widely.

- Chapter 6: "Substrate heterogeneity and high plant biodiversity on a partially clay-capped slag bank" – a case study for partial restoration" – This chapter examines the direct of effects of partial clay capping restoration on a slag bank, one of the study sites, where only some of the slag present on-site is directly accessible to plants at the surface. The differences between element concentrations on capped and uncapped parts of the substrate and the biodiversity levels for capped and uncapped communities are tested for statistically significant differences. This study addresses at least some of the general questions concerning the benefits of restoration and capping on anthropogenic substrates, with the situation being more nuanced and more complex than might be at first surmised.
- Chapter 7: Discussion This chapter summarises the findings of the thesis as a whole.
- Chapter 8: Conclusion This chapter concludes the thesis.
- The four Supplementary Information sections for the three research chapters.

To summarise, the main objectives for the thesis are as follows:

- 1. To determine whether biodiversity offsetting could be achieved or is already being achieved on anthropogenic substrate sites (using Great Britain as an example where some relevant data are publicly available).
- 2. To assess statistically significant differences between substrate characteristics (such as geochemistry and pH levels) on anthropogenic substrate sites.
- 3. To determine the biodiversity levels of anthropogenic substrate sites using various biodiversity indices.
- 4. To determine the elements and quantities of different elements that are statistically significantly associated with plant species and community presence throughout anthropogenic substrate sites.

Chapter 1: Literature Review

2.1 Introduction and definition of "anthropogenic substrate site".

Anthropogenic substrate sites are considered to be a type of brownfield site. This is because, as shall be further discussed, brownfield land, as a whole, encapsulates many of the features found on anthropogenic substrate sites more specifically. Further to this unsurprisingly, brownfield sites are more fully studied and surveyed than anthropogenic substrate sites. Brownfield land has been differently named and described in much literature, with synonymous/nearly synonymous terms including industrial land, vacant land, wasteland and derelict land (Richardson et al., 2010; Macadam & Bairnier, 2012; Mathey et al., 2018; Pueffel et al., 2018; Spiering et al., 2020). When studies mostly describe brownfield land in a negative light, terms such as 'wasteland', 'vacant' and 'derelict' are often used (Gunston, 1954), although studies can describe 'wasteland', 'vacant' or 'derelict' land in a positive way as well (e.g. highlighting their biodiversity) (El-Ghani et al., 2001; Macadam & Bairnier, 2012). The term 'brownfield' was first used by the British government in 1998 when a national target for housing development was set – for 60% of all new housing developments to be built on brownfield land (Macadam & Bairnier, 2012).

Contaminated land also falls under the definition of brownfield land in this study, with much contaminated land also possessing anthropogenic substrate. Under the Environmental Protection Act 1990 (amended by the Environment Act 1995 and subsequent regulations) in the United Kingdom defines contaminated land as "any land which appears to the local authority in whose area it is situated to be in such a condition, by reason of substances in, on or under the land, that significant harm is being caused or there is a significant possibility of such harm being caused" (Section 78A (2) (a)). In other words, contaminated land is defined based on assessments of measured contaminants and various non-numerical criteria relating to the risks that the land currently poses and/or could pose based on future use (Heathcote, 2018). Any land that has dangerously high concentrations of certain trace metals, for instance, could be designated as contaminated land, and such sites can possess contaminated anthropogenic substrate and/or be brownfield (Allan, 1997; Mayes et al., 2006; Mayes et al., 2008; Riley & Mayes, 2015; Riley et al., 2020).

2.2 Characteristics and Conservation value of brownfield land (including anthropogenic substrate sites).

Brownfield land has traditionally been seen and continues to be seen as unsightly, derelict wasteland with little to no purpose for human wellbeing or ecosystems (Gunston, 1954; Richardson et al., 2010; Mathey et al., 2018; Mills & McIntosh, 2020; McCallum & Sardo, 2021). However, many studies exist that demonstrate the importance of much brownfield land for biodiversity, with the potential to permit species conservation (Ash et al., 1994; Tropek et al., 2013; Włodarczyk-Marciniak et al., 2020; McCallum & Sardo, 2021; Macgregor et al., 2022), even if such land is highly altered by human activities (Allan et al., 1997; Cooke & Johnson, 2002; Lorimer, 2008; Lundholm & Richardson, 2010; Richardson et al., 2010). Geodiversity on brownfield sites occurs through the presence of anthropogenic substrates, especially in areas where the local substrates are very different in terms of geochemistry and mineralogy (Ash et al., 1994; Riley et al., 2020). It has been determined from multiple studies that anthropogenic substrate sites more specifically can support high levels of biodiversity and/or rare species and communities, with notable plant species (Ash, 1983), invertebrate species (Gibson, 1998) and bird species composition (Briggs, 1983; Palmer, 2008; Maddock, 2010; Watson, 2011; McCallum & Sardo, 2021), for example. Given the negative or neutral view of brownfield land, it is not too surprising that only very recently have certain types of brownfield habitat been 'officially' recognised for as being valuable for wildlife in the UK. Some rare species of plants and animals that have been recorded on brownfield land include, but are certainly not limited to: Linnet (*Linaria cannabina*) (Palmer, 2008); Ringed Plover (*Charadrius hiaticula*) (Briggs, 1983; Maddock, 2010; Watson, 2011); the Carabid beetle *Harpalus cupreus* (Gibson, 1998); Lesser Glowworm (*Phosphaenus hemipterus*) (Gibson, 1998); the moss *Buxbaumia aphylla* (Corner, 1967^a; Corner, 1967^b; Steven & Long, 1989; Porley & Hodgetts, 2005); and the Violet *Viola lutea* subsp. *calaminaria* (Antonovics, 1975; Batty, 2005).

2.3 Categorisations for brownfield land types – inconsistencies, overlaps and confusions.

Many pieces of land that are/were highly influenced by anthropogenic activities, especially previous development, are characterised and described in a variety of ways in the literature. For example, brownfield sites, as well as many types of urban habitat, are often viewed differently from 'urban green space' (Anderson & Minor 2017; Gallagher et al., 2011; Bottero et al., 2020). This can cause confusions when interpreting relevant literature, as in some studies, certain brownfield sites are considered as a type of 'urban green space' (El-Ghani et al., 2011; McPhearson et al., 2013; Botzat et al., 2016; Bretzel et al., 2016; Włodarczyk- Marciniak et al., 2020). Otherwise, these brownfield sites are viewed as being separate from and less desirable than urban green spaces (Gallagher et al., 2011; Sinnett et al., 2011; Anderson and Minor 2017; Bottero et al., 2020; Włodarczyk-Marciniak et al., 2020). Some studies look into ways in which local communities can better benefit from brownfield sites and/or be educated about their values, particularly their biodiversity value (McPhearson et al., 2013; Bretzel et al., 2016; Anderson & Minor, 2017; Pueffel et al., 2018; McCallum & Sardo, 2021) and their role in carbon sequestration and floodwater retention, for example (McPhearson et al., 2013; Kim et al., 2020; Włodarczyk-Marciniak et al., 2020). Authors in other articles discuss how best vacant land, usually referred to as a type of brownfield land (McEwan et al., 2020), could best be used as gardens, allotments, parking spaces, parks, or other functions (Sinnett et al., 2011; Fischer et al., 2013; Laprise et al., 2018; Simpson, 2019; Kim et al., 2020).

2.4 Recognition of biodiversity potential, realised or unrealised, on different brownfield land types

Biodiversity on brownfield sites can exhibit various positive characteristics, including: 1) brownfield site biodiversity can actually be significantly higher than on other types of urban green space, such as parks (Lorimer, 2008; Öckinger et al., 2009; El-Ghani et al., 2011; Macadam & Bairnier, 2012; Baldock, 2020); 2) a diversity of functional groups may be present on brownfield sites, fulfilling a variety of ecological functions (Robinson & Lundholm, 2012; Bretzel et al., 2016; Kollmann et al., 2016), including

soil or substrate formation, dynamics and availability, carbon sequestration and decomposition and nitrification (Kollmann et al., 2016); 3) brownfield sites may be less disturbed by humans than many other habitats, such as arable land (Strauss & Biedermann, 2006; Muratet et al., 2007; Buglife, 2009; Bretzel et al., 2016; Macadam & Bairnier, 2012), leading to higher biodiversity levels than what is seen on sites with increased human presence; 4) urban areas, including brownfield sites, usually have higher biodiversity than surrounding agricultural land (Strauss & Biedermann, 2006; Lorimer, 2008; Macadam & Bairnier, 2012; Auffret & Lindgren, 2020; Baldock, 2020); 5) green spaces (including brownfield sites) in urban areas, such as European cities, can support high levels of biodiversity (Baldock, 2020; Battisti, 2020); 6) in at least some countries, such as the United Kingdom, the countryside no longer provides the variety and abundance of habitats that are required by many native species, making brownfield biodiversity particularly important, providing refuges for many species (Millard, 2004; Dobson et al., 1997; Lorimer, 2008; Buglife, 2009; Macadam & Bairnier, 2012); 7) and habitat mosaics which support high levels of biodiversity, as well as semi-natural grasslands, were once common in the wider countryside, but in recent years, intensive farming has reduced the number of these (Millard, 2004; Buglife, 2009; Macadam & Bairnier, 2012; Bretzel et al., 2016; Rudolph et al., 2017). In the UK (and in many other countries), brownfield sites usually cluster around exindustrial areas, other urban areas and estuaries, which differ from the more isolated nature reserves, which are often also smaller in size (Angold et al., 2006; Muratet et al., 2007; Buglife, 2009; Macadam & Bairnier, 2012; Włodarczyk-Marciniak et al., 2020). While species in nature reserves run the risk of reducing their gene pools and population size through inbreeding and competition, species in brownfield sites, at least in urban areas, can sometimes fare better due to sizeable habitat mosaics and (often) better network of sites (Dobson et al., 1997; Millard, 2004; Angold et al., 2006; Buglife, 2009; Macadam & Bairnier, 2012). Brownfield sites in more rural areas can often be linked together by abandoned railway lines, which provide corridors for many different species (Allan et al., 1997).

2.5 Different designations and protections for different biodiverse brownfield land types

The biodiversity value of different types of brownfield sites as well as different types of ground has been recognised by different people in academic, amateur and professional settings. In the UK, recognition of the biodiversity value of much brownfield land has only recently been recognised by government Departments and other bodies, despite the legacy of literature on wildlife on brownfield sites (Thomas, 1930; Hind, 1956; Corner, 1967^a; Kelcey, 1975; Ash, 1983). The original UK Biodiversity Action Plan, first published in 1994 mentioned the importance of: bare ground for invertebrates; urban areas for uncommon early colonising species and ruderal species; disused land for specialised invertebrate species; and urban areas for nationally scarce species, although it did not specify any brownfield habitats as priority habitat (Department of the Environment, 1994). In August 2007, a more focussed and modified further document was published, being adopted by the Governments of the four UK administrations (Biodiversity Reporting and Information Group, 2007). This was the first full review of the UK BAP priority list, providing an opportunity to take account of new and continuing priorities for conservation action, conservation successes and the gathering of new information (Biodiversity Reporting and Information Group, 2007; Maddock, 2010). Two of the new Priority Habitat types in this document are restricted to and/or commonly associated with brownfield sites: Open Mosaic Habitats on Previously Developed Land, or OMHPDL; and Calaminarian Grassland (Biodiversity Reporting and Information Group, 2007). OMHPDL is made up of patchworks of previously disturbed bare ground and vegetated areas on a variety of different anthropogenic substrates and/or brownfield land more generally (Buglife, 2009; Maddock, 2010; Riding et al., 2010). In 2024, information about the different open mosaic habitats in England were provided by Natural England, which included data collected over many years of surveys and may provide further awareness of different open mosaic habitat sites (Natural England Open Data Geoportal, 2024). Calaminarian grassland has mostly been recorded on soils or substrates with elevated concentrations of heavy metals, including mine workings and spoil heaps in places such as North Wales and Northumberland (Palmer, 2008; Baker et al., 2010; Skelcher & Askew, 2014; Spalding, 2014; Rainbow, 2018). In the 2007 document (Biodiversity Reporting and Information

Group), several threats to OMHPDL were identified, such as: landfill; urban development, unsuitable restoration, reclamation or similar; lack of appropriate management; and natural succession. Calaminarian grassland was deemed in the 2007 document (Biodiversity Reporting and Information Group) to be of international importance, with threats including: rehabilitation of derelict land; mineral re-working; and landfill schemes. While these Priority Habitats, and other Priority Habitats, are not always strictly protected under UK laws, they can be sensitive to development and they ought to be considered during the determination of planning applications (Thomson Environmental Consultants, accessed 2020).

Both OMHPDL and Calaminarian grassland have features which are found in many biodiverse brownfield areas, but it is important to note that not all biodiverse brownfield sites fit into one of these two categories (Woods, 2012; Robins, 2013). Few specific plant species have been associated with OMHPDL; instead, the multiple habitat types, or micro-habitats, on OMHPDL, arranged in a complex pattern, comprising a variety of successional stages from ruderal communities to flower-rich grassland (Biodiversity Reporting and Information Group, 2007; Palmer, 2008; Maddock, 2010; Riding et al., 2010; Olds, 2019), are the primary features of the habitat. Calaminarian grassland, on the other hand, supports specific metallophyte and pseudometallophyte species such as *Viola lutea* subsp. *calaminaria,* Creeping Bent (*Agrostis stolonifera*) and Sheep's Fescue (*Festuca ovina*) (Antonovics, 1975; Batty, 2005; Preston, 2017; Rainbow, 2018). For both Calaminarian grassland and OMHPDL, many of the plant species and communities can be rare or not present at all in the wider landscape and outside of the Priority habitat (Biodiversity Reporting and Information Group, 2007; Baker et al., 2010; Maddock, 2010; Riding et al., 2010; Preston, 2017).

2.6 Arguments for the conservation of biodiverse or potentially biodiverse brownfield habitats

It will continue to be important to recognise that wildlife ought not to be solely conserved in natural environments (Maurer et al., 2000; Lorimer, 2008; Lundholm & Richardson, 2010; Richardson et al., 2010; Tropek et al., 2013). Brownfield land including anthropogenic substrate land is likely to be very different from and

sometimes more heterogeneous than surrounding natural land in terms of factors such as pH, nutrient concentration, organic matter, Ca levels, type of substrate, level of above-ground rock, decomposition rates and the level of compacted mixtures of anthropogenic materials (Allan et al., 1997; Strauss & Biedermann, 2006; Godefroid et al., 2007; Tropek et al., 2013; Tischew et al., 2014), helping to provide for higher levels of biodiversity than many intensively used substrates, such as agricultural land substrate (Dobson et al., 1997; Cooke & Johnson, 2002; Richardson et al., 2010; Buglife, 2012; Tropek et al., 2013).

It is important, during such a survey of a brownfield site, to take a holistic view of a habitat's value (Robins et al., 2013). Knowledge of the wider ecological networks present on a biodiverse brownfield site can help such sites be recognised for their positive impact on the wider environment (Buglife, 2009). Studying different aspects of the ecological connectivity of different brownfield sites, including those that connect to each other in urban areas, improve functional connectivity of ecosystems and allow for valuable increases in species pools and species matrices (Wolff et al., 2023). If biodiverse brownfield sites are assessed for their overall ecological value, it should be ensured that the most wildlife-rich brownfield sites would be protected from development, or else that any developments on the land would be carried out sympathetically, by minimising impacts with appropriate mitigation and compensation (Robins et al., 2013; McCallum & Sardo, 2021; Macgregor et al., 2022;). As further detailed in Section 2.10 titled "How can biodiverse brownfield sites benefit both wildlife and people?", mitigation measures such as the creation of green roofs with anthropogenic substrates as the primary plant growth substrate can, in some respects, replace an anthropogenic substrate site if the original site needs to be removed and/or developed (Lorimer, 2008; Brown & Lundholm, 2015; Krawczyk et al., 2021; Schröder & Kiehl, 2021). If a seed bank is already present in anthropogenic substrate directly transported from a site to a green roof, then much of the existing diversity may be able to persist, with reduced loss of biodiversity and ecological niches (Lorimer, 2008; Krawczyk et al., 2021; Schröder & Kiehl, 2021).

2.7 Characteristics of different biodiverse brownfield sites – what led to the creation of these sites?

Every biodiverse brownfield site is different because each site was used differently in varied, usually industrial contexts (Riding et al., 2010; Buglife, 2012; Macadam et al., 2013). Due to the industrial nature of many brownfield sites, including anthropogenic substrate sites, soils or substrate present on such land can be low in organic matter, and/or they are contaminated (Allan et al., 1997; Palmer, 2008; Riding et al., 2010; Akintola, 2020). Such substrate can exhibit extreme characteristics that can act as limiting factors when it comes to spontaneous succession of plant species and communities (Hind, 1956; Ash et al., 1994; Allan et al., 1997; Chapman, 2001; Lundholm & Richardson, 2010). For example, anthropogenic substrate may be dry or wet, very acidic to very alkaline, and may be deficient in nitrogen or (available) phosphate (Ash, 1983; Ash et al., 1994; Allan et al., 1997; Chapman, 2001; Maddock, 2010). Sites with extremes in pH, such as particularly alkaline conditions can also have deficiencies in nitrogen and available phosphate, including calcareous anthropogenic substrates such as blast furnace slag sites (Ash, 1983; Ash et al., 1994), Leblanc waste (produced during the chemical synthesis of sodium carbonate) (Hind, 1956; Greenwood & Gemmell, 1978; Butt & Briones, 2017), Solvay waste (the waste from the production of sodium carbonate) (Cohn et al., 2001); and calcareous quarry spoil (Hind, 1956; Greenwood & Gemmell, 1978; Maddock, 2010). Additionally, such characteristics demonstrated in brownfield sites, including anthropogenic substrate, OMHPDL and Calaminarian grassland, may only be suitable for specific suites of species (Palmer, 2008; Lundholm & Richardson, 2010; Maddock, 2010; Rainbow, 2018; Macgregor et al., 2022).

Industrial use of land can involve the removal of the organic layers of soil, disturbances of the soil horizon and changes in slope or drainage patterns (Hougen & Matlack, 2012). It can also involve the introduction of new substrates, including anthropogenically deposited substrates such as slag, fly ash and waste rock from mining (Hougen & Matlack, 2012; Tropek et al., 2013). Alternatively, urban land use can greatly alter its composition and chemical properties (Godefroid et al., 2007).

There is a continuum from highly urban to fully rural brownfield sites. Broadly, activities that occur outside of urban areas, that support city life, can be regarded as being part of the urbanisation process (Lehmann & Stahr, 2007). Thus, brownfield

land associated with mining, industry, infrastructure and building can contain anthropogenic substrate, including anthropogenic soils, whether they are within urban areas or not (Scottish Wildlife Trust, 1995; Lehmann & Stahr, 2007; Bonthoux et al., 2014).

Urban wildlife areas, for example allotments and gardens, can be similar to brownfield land in many respects. For example, they have been and are greatly influenced by human activity, either by regular disturbance and/or management, or else by the introduction of anthropogenic materials, including substrate (Godefroid et al., 2007; Lehmann & Stahr, 2007; Baldock, 2020). For example, soil in a city centre can be made up with a thick layer of filled earth alongside anthropogenic admixtures (see Table 1.2) (Godefroid et al., 2007). Many activities can lead to the generation and/or addition of these anthropogenic admixtures, such as trading, housing, disposal of waste, production of goods and traffic (Lehmann & Stahr, 2007). Anthropogenic urban substrates and/or soils are a common characteristic of cities and other populated urban spaces (Lehmann & Stahr, 2007).

Urban wildlife areas will exhibit additional abiotic factors that separate them from wildlife areas that are perceived as natural, as well as, potentially, other brownfield sites (especially more rural and/or remote brownfield sites). For example, urban wildlife areas can exhibit higher temperatures than nearby rural areas due to temperature excesses caused by urbanisation (Godefroid et al., 2007; Lehmann & Stahr, 2007; Baldock, 2020). Due to the variation in substrate throughout urban wildlife areas and/or less anthropogenic wildlife areas (as well as in many brownfield sites), factors such as the pH, mineral content and permeability of substrate and/or soil is varied (Godefroid et al., 2007; Bonthoux et al., 2014).

2.8 Plant species and plant growth on brownfield sites (including anthropogenic substrate sites)

Brownfield sites can sometimes support high levels of plant biodiversity (Hind, 1956; Allan et al., 1997; Ash et al., 1994; Riley et al., 2020; Macgregor et al., 2022), as well as providing areas for unusual plant communities (Thomas, 1930; Greenwood & Gemmell, 1978; Cohn et al., 2001; Harvie, 2004; Rahmonov et al., 2021). While some studies demonstrate that plant biodiversity is especially high on younger sites, where fewer species have had time to outcompete less competitive species (Angold et al., 2006; Maddock et al., 2010; Riding et al., 2010; Macgregor et al., 2022), some studies demonstrate the opposite, when later successional community stages have developed (Scottish Wildlife Trust, 1995; Rahmonov et al., 2020). Species assemblages can vary greatly on different brownfield sites (Thomas, 1930; Ash et al., 1994; Allan et al., 1997; Cohn et al., 2001; Harvie, 2004).

Table 1.1 details different plant species that have been recorded on brownfield land, including at least one type of anthropogenic substrate. Primarily western European species have been included in the list, although many interesting examples of species exist on brownfield land in other countries (Akintola, 2019). Other important non-plant species which have been recorded on anthropogenic substrate sites include: Field Dog-lichen (*Peltigera rufescens*)*,* which can grow on lime wastes and PFA; and the Pixie Cup *Cladonia pocillum* can be recorded on calcareous wastes – both of these species of lichen are declining in the wider countryside (Maddock, 2010).

In general, many plant species present on most biodiverse brownfield sites, including OMHPDL sites, are stress-tolerant (Baker, 1965; Lewontin, 1965; Mulligan, 1965; Maddock, 2010; Riding et al., 2010). Many initial colonisers of anthropogenic substrates are termed 'weeds'; these tend to be plants with propagules which can easily reach and grow in anthropogenic bare ground, waste land and disturbed land (Baker, 1965; Lewontin, 1965; Mulligan, 1965; Harvie, 2004). This commonly includes ruderal species (Harvie, 2004). Some brownfield communities that include drought-tolerant and nutrient-tolerant plants are reminiscent of plant communities found in coastal habitats (Ash et al., 1994; Steven, 2020). Many of the initial colonising plants of colliery spoil sites, such as highly plastic pioneer shrub species and 'weeds', for example, can have specific reproductive and physiological features

that can assist them in their colonisation of more extreme substrates, such as high seed production and specific root features (Rahmonov et al., 2020). Within-species variation can be exhibited on these sites as well. For example, some individuals of St John's Wort (*Hypericum perforatum*) have faster lifecycles and higher morphological plasticity than others, growing taller and forming stolons, flowers and seeds much more quickly than other St John's Wort (*H. perforatum*) individuals (Baker, 1965).

On OMHPDL, as well as other biodiverse brownfield sites, vegetation stands commonly comprise small and/or short patches and these may vary over relatively small areas, which reflect small-scale variation in topography and substrate (Maddock, 2010; Steven, 2020). As well as possible lichen or bryophyte species, early successional species in OMHPDL plant communities can include: annuals; ruderals; species that can cope with inundation; species typical of open grassland; flower-rich grassland species; and/or heathland species (Palmer, 2008; Maddock, 2010; Riding et al., 2010).

Many plants are adapted to grow on a wide variety of substrates, in a wide variety of habitats, although they may face stronger competition in more nutrient-rich ground, in which perennials can dominate and exclude other species (Baker, 1965; Ash et al., 1994), and in places where a full cover of plant species is already established (Baker, 1965). High nutrient levels created by nitrogen-fixing legumes including clovers (*Trifolium* spp.), and vetches (*Vicia* spp.), permit the incidence of scrub and/or promote the growth of fast-growing ruderal species on brownfield land that was previously less nutrient-rich, including: Common Nettle (*Urtica dioica*); Thistle (*Cirsium* spp.) (different species associated with different pH levels); and Rosebay Willowherb (*Chamaenerion angustifolium*), which can to become dominant in the overall plant community (Riding et al., 2010; Robins et al., 2013; Steven, 2020). As successional stages continue on any one site, the pioneer species present can create plant litter that, over time, will break down to form soil (Riding et al., 2010; Robins et al., 2013; Steven, 2020).

The extreme and sometimes toxic conditions of anthropogenic substrates can lead to physiological or genetic problems for many plant species. For example, historical, including recent historical paper mills, have produced many harmful pollutants, many of which end up in waterways and can have multiple adverse

effects on plant species such as Royal Fern (*Osmunda regalis*), including harmful chromosome mutations (Klekowski, 1976; Klekowski & Berger, 1976). On particularly acidic anthropogenic substrates, including colliery spoil, increases in the concentration of H⁺ ions in soil or substrate can significantly affect the physiology and growth of plant species in said soil or substrate (Lodhi, 1982). Among the effects of H⁺ ions are: the increased mobilisation of Ca and Mg to deeper soil profiles, meaning that Ca and Mg become less accessible for plant roots (Lodhi, 1982); and the reduction of nitrifying bacteria (Lodhi, 1982). The variation between anthropogenic substrate sites for their contribution to plant biodiversity highlights the necessity of surveying and evaluating each site on a case-by-case basis, to better understand the use of different anthropogenic substrate sites by plants. Alongside such surveys, the risks of toxins and other risks posed by such substrate sites need to be assessed with care and consideration.

Table 1.1: Plant species recorded on brownfield sites, primarily in Western Europe. Names checked and edited if appropriate using GBIF (tracheophytes), Tropicos (bryophytes) and World Flora Online (bryophtyes).

2.9 Bioavailability of contaminants or excess concentrations of different elements – bearing in mind how this could affect the plants present on biodiverse brownfield sites.

Only some of the elements present on brownfield substrate will directly influence the presence, growth and/or health of individual plant and plant communities present. Trace elements present within reach of plants, for example, will not all be accessible to said plants, as many of these are in chemical forms which cannot be taken up directly (Giller et al., 1998). Additionally, in terrestrial soils and substrates, substrate or soil pH will have a significant effect on the bioavailability of present metals (Smith & Huyck, 1999; Zhao & Masaihiko, 2007; Qiu et al., 2012; Lynch et al., 2014), as well as their mobility in the substrate or soil (Smith & Huyck, 1999; Lynch et al., 2014). For example, with exposure to water, metal Fe and Mn ions from sites impacted by metal mining can undergo reduction and be converted to forms which are increasingly bioavailable to plants, risking aquatic plants and terrestrial plants also affected by mine-polluted water to toxic Fe and Mn levels (Lynch et al., 2014).

When assessing the bioavailability of certain elements for different plant species, the absorption of such elements can be examined in the plant organs at the point of absorption, such as the roots or the leaves, or else the different plant tissues can be examined if such elements were translocated and accumulated (Smith & Huyck, 1999). Certain elements are much more bioavailable to plants than others, as has been established by Kabata-Pendias (2010), with specific measures of bioavailability, including Biological Absorption Coefficient, Index of Bioaccumulation and Transfer Factors. These measures demonstrate that certain trace elements are more likely to be taken up by plants than others, even when such trace elements may be present in relatively low concentrations in soil or substrate. The most bioavailable trace elements are, according to Kabata-Pendias, in order: Cd, B, Br, Cs, Rb, Zn, Mo, Hg, Cu, Pb, Sr, Ag, Te, Ge, As and Co. Toxic levels of different particularly bioavailable elements in soil or substrate can have varying different effects on plants. Different stages of photosynthesis are negatively affected by toxic Cd and Zn levels, for example (Dias et al., 2012). Phloem mobility can be significantly reduced in plants that have accumulated high levels of B (Brdar-Jokanović, 2020). Shoot biomass can be reduced by toxic Zn levels (Kaur & Garg,

2021). While physiological responses can vary between species (Brdar-Jokanović, 2020; Kaur & Garg, 2021), generally, toxic levels of different elements will negatively impact individual plants. Having said that, deficiencies of many of these elements, at least those utilised by the plants, will likewise have negative consequences on plant growth and health (Brdar-Jokanović, 2020; Kaur & Garg, 2021).

While at least one investigation has been made into the relationship between plant biodiversity and bioavailability of various elements (Hernández & Pastor, 2008), studies into plant bioavailability have focussed primarily on topics such as phytoremediation, contamination of ground, including pollution and potential toxicity of agricultural plants that have grown on contaminated or polluted land. Phytoremediation involves the use of plants to remove toxins (including metalloids) from a contaminated site (Zhuang et al., 2007; Haller & Jonsson, 2020; Baragaño et al., 2020; Jaskulak et al., 2020). Phytoextraction, for example, is primarily associated with the uptake and tolerance of specific metals and metalloids by specific plant species, to permit soil decontamination (Haller & Jonsson, 2020; Baragaño et al., 2020, Fernández et al. 2017).

Hyperaccumulator plant species are characterised by heavy metal concentrations that are 10-100 times higher than expected for plant species in general, specifically, in the above-ground vegetative organs (Fernández et al. 2017; Haller & Jonsson, 2020). These plant species include those that are endemic to metalliferous soils and in Europe, a large proportion of these are members of Brassicaceae (Fernández et al. 2017). It is not known how many species of hyperaccumalator plants that are suitable for phytoremediation exist worldwide, but at least 500 appropriate species could exist (Haller & Jonsson, 2020). Various factors, in general, affect the efficiency of trace element uptake in plants, including the substrate or soil pH, the clay content and the amount of organic matter present (Jaskulak et al., 2020). These factors influence trace element uptake to the tissues of plants, via adsorption-desorption mechanisms (Jaskulak et al., 2020).

Phytoremediation research has identified several different species that take up trace elements more readily than other species. Herbaceous species have been the primary focus for most phytoremediation studies, but some attention has also been paid to woody species (Zhuang et al., 2007; Haller & Jonsson, 2020). Such species include Crack Willow (*Salix fragilis*) which can take up Cd, Cr, Cu, Ni, Pb and Zn at much higher levels than most other woody species (Zhuang et al., 2007; Haller & Jonsson, 2020). Cu and Zn can be taken up easily by species such as *Betula* (Haller & Jonsson, 2020). Woody legumes can be particularly useful for phytoextraction in some sites, especially those in which contaminants are present well below the soil surface (Zhang et al., 2001). Few hyperaccumulator plants are grasses or grass-like (Haller & Jonsson, 2020), although *Festuca arundinacea* is an examples of a grass that can accumulate Zn and Mn during phytoextraction (Bogatu et al., 2007).

Conversely, many plants that can be used during phytoremediation are involved in phytoexclusion (Haller & Jonsson, 2020; Jaskulak et al., 2020). Such plants exclude contaminants, including heavy metals, by retaining them at the root surface, or else accumulating contaminants in the root cortex (Haller & Jonsson, 2020).

Another type of phytoremediation is phytostabilisation (Haller & Jonsson, 2020). This involves plants and associated and microorganisms for a long-term trace metal and metalloid, or TMM, contaminant containment and immobilisation, through the reduction of solubility and transport (Haller & Jonsson, 2020, 1.4.3, Fernández et al. 2017). As well as angiosperms, including grasses and legumes (Fernández et al. 2017), at least one fern species, including *Pteridium aquilinum*, has been demonstrated to be potentially useful for phytostabilisation (Fernández et al. 2017).

Phytoremediation, while a promising solution for contamination in many brownfield sites, is not a widely used method, although phytostabilisation in particular may be more promising in certain instances, as this reduces the bioavailability of harmful trace elements to other, more vulnerable plants, or plants that are being produced for agricultural purposes (Megharaj & Naidu, 2017). Different types of anthropogenic substrate and plant growth could potentially reduce bioavailability of certain trace elements as part of a phytostabilisation method or similar. The bioavailability of elements in certain anthropogenic substrates has been studied by several authors and varies considerably between different types of substrate (Ruyters et al., 2011; Qiu et al., 2012). In steel slag, for example, high alkalinity levels can immobilise metals that are present, such as Cd, reducing their

bioavailability for plants (Qiu et al., 2012; He et al., 2020). However, steel slag can increase the bioavailability of certain elements, such as As, to harmful or potentially harmful levels (He et al., 2020). Steel slag, in combination with at least one other substrate and a certain suite of native plant species could, potentially (based on our current understanding), reduce bioavailability and phytostabilise sites that have particularly acidic contaminated substrates (Qiu et al., 2020; Kim et al., 2021; Radziemska et al., 2021; Yang et al., 2021). Using any highly alkaline anthropogenic substrates as a soil amendment before testing how they influence bioavailability is inadvisable, particularly as certain anthropogenic substrates have particularly harmful effects in excess levels. While bauxite residue (also known as red mud), for instance, may reduce bioavailability of Cd and Pb, it has exceedingly high concentrations of Na that can harm plants (Ruyters et al., 2011).

2.10 How can biodiverse brownfield sites benefit both wildlife and people?

As well as providing important habitat for many unusual and/or rare species, brownfield sites (and urban natural areas in general) can provide important services to people (Lorimer, 2008; Mathey et al., 2018; Pueffel et al., 2018; Anderson and Minor 2017; Faulkner, 2023), which could help to motivate action to conserve such habitats (Maurer et al., 2000; Botzat et al., 2016; Lumber et al., 2017; Pueffel et al., 2018; Faulkner, 2023). Importantly, brownfield sites and other urban green spaces allow people to engage with biodiversity and conservation, which is especially important in urban environments where interaction with nature can be limited (Godefroid et al., 2007; Hartig et al., 2014; Botzat et al., 2016; Guo et al., 2018; Włodarczyk-Marciniak et al., 2020).

Overall, if brownfield sites are managed in such a way as to actively encourage and support biodiversity, such sites can provide a great benefit in terms of ecosystem services (Tischew et al., 2014; Anderson & Minor, 2017; Pueffel et al., 2018; Faulkner, 2023). Ecosystem services are the benefits humans derive from the various processes and functions of ecosystems (Battisti, 2020). These include the decomposition, plant matter production, nutrient cycling, stormwater retention, increased pollination and bio/phytoremediation of contaminated sites (Macadam &

Bairnier, 2012; Robinson & Lundholm, 2012; Anderson & Minor, 2017; Battisti, 2020; Faulkner, 2023). Often, more invertebrate pollinators will exist on a brownfield site than on nearby lawn and forest sites (Robinson & Lundholm, 2012). Species that are at risk of extinction can benefit greatly from certain brownfield sites, for example (Lundholm & Richardson, 2010; Macadam & Bairnier, 2012; Buglife, 2012; Anderson & Minor, 2017). Having said that, there is limited knowledge and literature concerning the biodiversity potential of many individual brownfield sites (Lundholm & Richardson, 2010), again, emphasising the importance of careful and thorough biodiversity surveys prior to potential development on such sites (Bickers, 2017; Battisti, 2020).

High quality green infrastructure can incorporate biodiverse brownfield sites, benefitting both people and wildlife (Buglife, 2009; McPhearson et al., 2013; Mathey et al., 2018; Palliwoda et al., 2020; Seddon et al., 2020). Such green infrastructure can include: planned and/or managed natural and semi-natural landscapes (Auffret & Lindgren, 2020); (perhaps urban) agricultural areas (Kim et al., 2020); allotment gardens; smaller green areas; and brownfield areas (Kim et al., 2020; Palliwoda et al., 2020; Faulkner, 2023). Urban green spaces in general, including wetlands, can reduce flood risks in urban areas (Kim et al., 2020; Seddon et al., 2020; Faulkner, 2023). Brownfield sites can have amenity value as well as biodiversity value and this should be taken into account both when treating land as important, informal urban greenspace (Botzat et al., 2016), as well as when planning green infrastructure in development areas (Buglife, 2009; McPhearson et al., 2013; Guo et al., 2018; Pueffel et al., 2018; Faulkner, 2023). Green infrastructure can have many additional positive effects, such as combating pollution and ameliorating the local microclimate (Millard, 2004; Grilli et al., 2020). Parks and lawns, in particular, which are often biodiversity-poor, could greatly benefit from targeted modifications and planting to encourage habitats that are similar in nature to many biodiverse brownfield habitats (Threlfall et al., 2017; Guo et al., 2018; Baldock, 2020). Native plants and permeable surfaces are well-utilised in biodiverse and sustainable brownfield and/or urban green space (Fiorelli, 2020; Faulkner, 2023). Green infrastructure can also provide for improved micro-climate regulation, noise reduction and air filtration (Grilli et al., 2020; Palliwoda et al., 2020). Opportunities for urban green infrastructure, or other types of nature-based solutions to problems in urban areas, may often be missed due to a lack of knowledge or planning (Frantzeskaki et al., 2020).

Unfortunately, many biodiverse urban brownfields also provide spaces for people that can lead to activities that can pose social problems, including danger or a loss of safety to multiple group of people, such as: the sale of illegal drugs; assault; robbery; vandalism; sex work; and various petty crimes (Draus et al., 2020; Frantzeskaki et al., 2020; Palliwoda et al., 2020; Włodarczyk-Marciniak et al., 2020). Helping to make these spaces more accessible to people, while also maintaining and/or gently managing for wildlife can be important for the wellbeing of people in the local area (Palliwoda et al., 2020; Włodarczyk-Marciniak et al., 2020). While in many instances biodiverse brownfield sites should be left undeveloped for the benefit of wildlife (Guo et al., 2018), if accessibility and safety of these sites can be improved, through such measures as providing benches and good-quality paths on-site (Pueffel et al., 2018; Grilli et al., 2020; Kim et al., 2020; Sun & Shao, 2020; Włodarczyk-Marciniak et al., 2020), and/or if they can be incorporated well into development areas, they can provide valuable, sometimes aesthetic (Millard, 2004; Sun & Shao, 2020; Włodarczyk-Marciniak et al., 2020) landscapes and nature areas for communities to engage in various educational, physical and/or social activities (Macadam & Bairnier, 2012; Mathey et al., 2018; Pueffel et al., 2018; Fiorelli, 2020; Grilli et al., 2020). Therefore, such sites can provide multiple social and environmental benefits (Buglife, 2009; McPhearson et al., 2013; Palliwoda et al., 2020; Sun & Shao, 2020; Faulkner, 2023). As many brownfield sites are situated near less-advantaged and marginalised groups with limited access to the countryside, improving accessibility and safety of these sites can give valuable nature spaces to people from these groups (Lorimer, 2008; Faulkner, 2023). Brownfield site information and interpretation for visitors and local people can help to educate and provide people with information concerning the biodiversity value and wildlife of such sites (Buglife, 2009; Buglife, 2012; Bretzel et al., 2016). If wildlife experts in the local brownfield area highlight specific species and/or wildlife communities, this can often be a good starting point to garner interest for local brownfield site conservation (Lorimer, 2008). Green and brown space provides welldocumented benefits to human wellbeing, including recreation, health and access to wildlife (Lumber et al., 2017; Simpson, 2019; Kim et al., 2020; Osawa et al., 2020; Seddon et al., 2020). Recreation, in particular, is important for many people who regularly visit brownfield sites (Maurer et al., 2000; Richardson et al., 2015; Lumber et al., 2017; Butt & Quigg, 2020; Włodarczyk-Marciniak et al., 2020), which includes

outdoor play for children (Mathey et al., 2018) and a space for people to spend time outdoors in an area that can be considered, in an urban or suburban context, as wild and undisturbed (Pueffel et al., 2018; Palliwoda et al., 2020). As biodiversity increases, the psychological benefits associated with natural spaces can also increase (Botzat et al., 2016; Anderson & Minor, 2017; Fiorelli, 2020; McEwan et al., 2020; Osawa et al., 2020). Likewise, a diversity of habitats can also have positive effects on human wellbeing (Osawa et al., 2020). In particular, wildflower meadows, which can be generated on brownfield sites, including urban sites, can have much amenity value and be aesthetically pleasing to many people, as well as catering well for biodiversity (Macadam & Bairnier, 2012; Bretzel et al., 2016).

Brownfield areas within and/or associated with communities can provide opportunities for community involvement and/or management (Anderson & Minor, 2017; Fiorelli, 2020; Kim et al., 2020; Marshall et al., 2020; Mathey et al., 2018). In general, gentle and low-level restoration and/or habitat management provides good opportunities for community and/or volunteer engagement (Hobbs & Cramer 2008; Threlfall et al., 2017; Mathey et al., 2018; Simpson, 2019; Kim et al., 2020). More specifically, communities can be involved with brownfield site management, such as mowing or cutting grass, to improve access and to demonstrate that such sites are being cared for (Hobbs & Cramer 2008; Buglife, 2009; Marshall et al., 2020; Włodarczyk-Marciniak et al., 2020), as this type of management is particularly visually appealing to many people (Marshall et al., 2020). People are willing to accept the more 'wild' nature of brownfield sites (or urban green spaces in general) if signs of collective or individual care are visible (Botzat et al., 2016; Fiorelli, 2020; Marshall et al., 2020). In order for effective community participation for the management of biodiverse brownfield land, diverse groups and organisations ought to work together, solving often complex community problems and resolving any conflicts that may arise (Mathey et al., 2018; Kim et al., 2020). Much of this can be achieved through effective communication and education between and to various organisations, as well as the general public (Mathey et al., 2018; Kim et al., 2020).

One new type of urban greenery which can be botanically similar, in many ways, to less regulated brownfield, derelict and/or vacant land, are green roofs. Green roofs, including those which utilise anthropogenic substrate, can provide urban greenspaces where there is little or no room on the ground in certain cities and/or areas of cities (Nagase & Koyama 2020; Hitti et al., 2021). Green roofs can utilise anthropogenic substrate such as blast furnace slag to 1) reuse industrial waste and 2) provide an effective growth substrate for plants (Hitti et al., 2021).

If wildlife on brownfield sites is to be effectively preserved, maintained and managed for the present and the future, it is important to make these sites safer and more appealing to members of the local community, so that they can continue to encourage interest in wildlife and conservation (Faulkner, 2023; Lorimer, 2008; Macadam & Bairnier, 2012; Mathey et al., 2018; Palliwoda et al., 2020).

2.11 Previous and current management of brownfield land – how restoration can impact the wildlife on biodiverse brownfield sites

Ecological restoration is often used loosely in the literature, but is here utilised as such: Restoration implies that a damaged, degraded and/or destroyed habitat, or similar, is being reinstated, or restored, to some former state (Anderson, 1995; Bradshaw, 1995; Cooke & Johnson, 2002; Hobbs & Cramer 2008; Waller et al., 2017). Many terms can be used to describe this process, including (but not limited to): rehabilitation, remediation, reclamation and revegetation (Bradshaw, 1995; Wozniak & Kompala, 2001; Cooke & Johnson, 2002; Hobbs & Cramer 2008). Habitat creation, conversely, involves the creation of new habitat (Anderson, 1995; Allan et al., 1997; Pakeman et al., 2002; Waller et al., 2017). It is often beneficial to consider the benefits and costs of habitat restoration or creation for a particular site (Anderson, 1995; Bradshaw, 1995; Hobbs & Cramer 2008), as different sites benefit from different habitat management techniques (Anderson, 1995; Bradshaw, 1995; Hobbs & Cramer 2008; Smart et al., 2016). Throughout the literature, these terms have been used to describe the various processes of altering brownfield land, including anthropogenic substrate sites, to a state that can be vastly different from their condition just prior (Anderson, 1995; Cooke & Johnson, 2002; Singh et al., 2002; Gorman, 2009; Stalmachova & Sierka, 2014). Usually, the primary purposes of brownfield site remediation are as follows: making the land useful from an economic standpoint (Allan et al., 1997); accelerating economic regeneration by creating new landforms for specific new developments; enhancing the quality of the local environment (Ash, 1983); making the land usable (Allan et al., 1997); and making the land safe (Allan et al., 1997).

People tasked with carrying out restoration on brownfield sites used to primarily focus on revegetation (Gunston, 1954; Smyth, 1997; Pakeman et al., 2002; Schmidt, 2002; Baasch et al., 2012), but later other people focussed on establishing communities that had a high conservation interest (Anderson, 1995; Bradshaw, 2000). Many traditional restoration techniques were based on out-dated, at least partially incorrect ideas about plant communities, that were primarily based on misunderstandings about the time it took for certain successional stages to occur or for certain plant communities to establish (Gunston, 1954; Tausch et al., 1993; Wozniak & Kampala, 2005). Traditionally, it was often recommended during restoration and/or habitat creation to plant propagules of the desired species in edges or lines, although from an ecological perspective, clusters of propagules may now be recommended, as these tend to better resemble more natural plant dispersal and growth (Robinson & Handel, 2000; Stalmachova & Sierka, 2014). Mixed species stands may also be recommended, as these confer more benefits to natural ecosystems and may better provide ecosystems services (such as soil water retention and nutrient cycling) than mono-specific stands (Stalmachova & Sierka, 2014). Due to the fact that many brownfield sites have immigration barriers for plant species, including those sites which are far away from those with analogous natural substrates, many authors and specialists have recommended that, on brownfield sites that have not yet reached their biodiversity potential, plant propagules of species suited to the brownfield substrate should be sown and spread (Ash et al., 1994; Robinson & Handel, 2000; Zhang et al., 2001; González-Alday et al., 2008; Kirmer et al., 2012). Additionally, it has been recognised that management practices that work for certain wildlife species, such as late vegetation cutting (Bretzel et al., 2016; Auffret & Lindgren, 2020), would be detrimental to other species, such as invertebrates, ground-nesting birds and small mammals (Anderson, 1995). Where pollution is a major problem on brownfield sites, understandably, dealing with this pollution will be priority for human and wider environmental health (Fiorelli, 2020).

Many different restoration methods have been used over the years to manage wildlife sites in general (Singh et al., 2002; Hobbs & Cramer 2008), with only some of these management techniques being directly appropriate for biodiverse brownfield sites (Anderson, 1995; Allan et al., 1997; Robins et al., 2013). In such cases, the term 'restoration' is not entirely appropriate, at least in its strict definition (Lundholm & Richardson, 2010; Richardson et al., 2010; Anderson & Minor, 2017; Walmsley et

al., 2017). As already discussed, the wildlife present and/or the potential wildlife present on a brownfield site (with OMHPDL or similar) is unlikely to be similar to what was present in the past (Lundholm & Richardson, 2010; Richardson et al., 2010; Stalmachova & Sierka, 2014; Anderson & Minor, 2017; Walmsley et al., 2017). 'Restoration', in these cases, could aim for a similar level of biodiversity (if not a higher level (Ash, 1983; Wozniak & Kompala, 2001; Lundholm & Richardson, 2010; Walmsley et al., 2017)), rather than trying to recreate previous conditions (Cooke & Johnson, 2002; Lundholm & Richardson, 2010; Richardson et al., 2010; Stalmachova & Sierka, 2014), which may be much more difficult, time-consuming and costly (Anderson, 1995; Wozniak & Kompala, 2001; Hobbs & Cramer 2008; Richardson et al., 2010; Stalmachova & Sierka, 2014). Many brownfield sites undergo landscaping, such as reshaping, as a sort of restoration and/or management, often to make such sites safer and more stable, such as in the case of colliery spoil bings (Allan et al., 1997).

There are differing opinions when it comes to restoration of certain areas – for example, whilst spontaneous succession is often the best way for biodiverse habitat to develop on a brownfield site (including species that are not easily commercially available, including bryophytes, ferns, lichens and fungi (Anderson, 1995)), on certain brownfield sites, particularly mine waste sites, such methods may not be perceived as being acceptable, as active restoration is strongly encouraged and/or enforced (Ash, 1983; Allan et al., 1997; Cooke & Johnson, 2002). This is especially pertinent on sites with at least some level of contamination, including metal contamination (Affholder et al., 2020).

Sometimes, spontaneous succession of brownfield land can lead to a mixture of negative and positive outcomes. For example, invasive species may spontaneously colonise brownfield sites (Bodsworth et al., 2005; Maurel et al., 2010; Albrecht et al., 2011; Stalmachova & Sierka, 2014; Young & Kettenring, 2020) and many argue that such species can spread because of declines in native species, through direct or indirect competition (García-Palacios et al., 2010; Maurel et al., 2010; Trentanovi et al., 2013; Doizy et al., 2018; Young & Kettenring, 2020). For example, invasive species such as Japanese Knotweed (*Reynoutria japonica*) can alter the soil that they grow in at the expense of native species, helping to promote dominant spread and growth of their own species (Maurel et al., 2010). Many who manage habitats argue for the removal of many invasive species (Anderson, 1995;

Hobbs & Cramer 2008; Maurel et al., 2010; Kollmann et al., 2016; Young & Kettenring, 2020), but there is a great deal of discussion as to how and why invasive species should be removed from brownfield sites (Anderson, 1995; Bodsworth et al., 2005; Young & Kettenring, 2020), as some of these (along with less obvious introduced species) can potentially benefit brownfield biodiversity, including plant species (Anderson, 1995; Stalmachova & Sierka, 2014) and host invertebrate species (Bodsworth et al., 2005). Some brownfield sites support certain invasive/introduced species particularly well (Angold et al., 2006; Muratet et al., 2007). Buddleja (*Buddleja davidii*), for example, is successful along many railway lines (Wittig, 2012). Some urban wasteland species such as Michaelmas Daisy (*Symphyotrichum novi-belgii*) and Lupin (*Lupinus* spp.) have been used in at least one grassland enhancement scheme (Anderson, 1995). Having said that, particularly dominant invasive species may have much more of a negative impact on the wider ecosystem than previously realised (Doizy et al., 2018), so it will still be important to assess the presence of each invasive species on brownfield sites on a case-by-case basis.

Prior to restoration, landscaping and/or habitat creation, for existing biodiversity, site surveys should be undertaken to establish what plants and animals have already naturally colonised the site (Anderson, 1995; Wozniak & Kompala, 2001; Hobbs & Cramer 2008; Macadam & Bairnier, 2012; Battisti, 2020). Such surveys can allow for greater understanding about what type and level of management a site requires, potentially saving time and money in the long-term if a site does not need to undergo intensive restoration and/or habitat creation (Anderson, 1995; Wozniak & Kompala, 2001; Hobbs & Kramer 2008; Macadam & Bairnier, 2012). In many cases, it is desirable to retain native plant species that are already present on the site (Threlfall et al., 2017; Wozniak & Kompala, 2001). After this stage, as well as in cases where future restoration is being planned (such as restoration following mining activities), restoration objectives should be decided upon so that sites can be restored appropriately and efficiently (Cooke & Johnson, 2002; Wozniak & Kompala, 2001).

When such brownfield substrates have been changed, or where spontaneous succession has been prioritised over more intensive restoration methods, a few things will need to be borne in mind for future restoration and/or management (Hobbs & Cramer 2008; Buglife, 2009; Lundholm & Richardson, 2010; Baasch et al., 2012; Buglife, 2012). As a general rule, brownfield sites that exhibit biodiversity value, including those categorised as OMHPDL, should be managed on a regular basis, but not in a way that removes and/or greatly changes the current overall character of the habitat (Buglife, 2009; Buglife, 2012; Baasch et al., 2012; Macadam & Bairnier, 2012). Because bare substrate will often characterise brownfield sites, successional dynamics of plant species need to be taken into consideration, as some plant species are more suited to certain successional stages than others (Martínez-Ruiz & Fernández-Santos, 2005; Hobbs & Cramer 2008; Albrecht et al., 2011; Stalmachova & Sierka, 2014). During both restoration and habitat creation, other modifications to urban and/or brownfield habitats may be beneficial to increase biodiversity potential, such as increasing surface heterogeneity (to benefit water and nutrient flows, for example) (Hobbs & Cramer 2008). On many biodiverse brownfield sites, management should seek to maintain and encourage an open mosaic of different habitats (Olds, 2019). This may require, for example, the removal of scrub, so the scrub and tree cover can be limited for a given site (Anderson, 1995). This is because this vegetation can shade bare ground areas and other brownfield microhabitats, thus affecting biodiversity and reducing the mosaics of habitats (Buglife, 2012). Additionally, scrub can limit the presence of species characteristic of threatened habitats such as dune grassland or calcareous grassland (Anderson, 1995). Such management techniques can help to ensure that those brownfield sites which exhibit a complex mosaic, such as OMHPDL, are structurally diverse and include a diversity of plant species as well as bare ground for invertebrate diversity (Olds, 2019).

Ecosystem analogues can be used as restoration targets, for example, a brownfield site with calcareous, alkaline waste could support an ecosystem that is analogous to those found in calcareous limestone or chalk grassland, for example (Lundholm, 2006; Lundholm & Richardson, 2010; Robinson & Lundholm, 2012; Tischew et al., 2014). Likewise, a metal-contaminated brownfield site may mimic natural ecosystems where high concentrations of metals are present, such as serpentines, metal outcrops and soils and/or substrates associated with weathered mineral deposits (Lundholm & Richardson, 2010; Richardson et al., 2010; Gallagher et al., 2011), meaning that metal-tolerant plant species that have evolved in such natural environments may grow well on contaminated brownfield sites (Lundholm & Richardson, 2010). Rubble and sand on certain urban and/or brownfield sites are

particularly good for biodiverse plant communities (Godefroid et al., 2007; Buglife, 2012), promoting varied vegetation structure (Buglife, 2012) and they limit the spread and growth of less desirable plant species (Godefroid et al., 2007; Buglife, 2012), so such substrates can be valuable in brownfield conservation and management (Godefroid et al., 2007; Buglife, 2012).

Due to the differing nature of brownfield site substrates, succession stages on different sites will occur at varying rates, with some sites supporting herbaceous grasslands or meadows for decades and other sites being dominated by shrubs after a similar period of time, for example (Spiering et al., 2020). There is still much to be done regarding the study of substrates and how they are related to living things within an ecosystem, as studies which only study substrate are more prevalent than those which look at both abiotic and biotic aspects of an ecosystem (Kollmann, et al., 2016).

2.12 Differences in understanding and perception of biodiverse brownfield sites – potential methods for use of anthropogenic substrate for future wildlife conservation

Much of the conflict surrounding brownfield land arises from the fact that while many people are inherently focussed on their biodiversity value, others are focussed on their physical benefits, or perceived lack of (Gunston, 1956; Richardson, 1957; Allan et al., 1997; Osawa et al., 2020). Many authors have agreed that brownfield and/or urban environments are inherently different from natural environments (Millard, 2004; Strauss & Biedermann, 2006; Muratet et al., 2007; Lundholm & Richardson, 2010; Fischer et al., 2013; Baldock, 2020) and that, therefore, ecology and ecosystems of these environments differ (Millard, 2004; Muratet et al., 2007; Lundholm & Richardson, 2010; Fischer et al., 2013). It is generally believed that anthropogenic ecosystems (those found in brownfield and/or urban wildlife areas) are ecologically novel, because they are highly altered and influenced by conditions that were not present before anthropogenic activities began, e.g, certain climatic conditions, soils, toxins and hydrology (Millard, 2004; García-Palacios et al., 2010; Lundholm & Richardson, 2010; Fischer et al., 2013; Tischew et al., 2014; Bretzel et al., 2016; Baldock, 2020; Battisti, 2020; Fiorelli, 2020). Ecosystems present in brownfield areas (or urban wildlife areas in general) are often seen to be largely distinct in function and structure from the natural ecosystems they replaced (Allan et al., 1997; Cooke &

Johnson, 2002; Lundholm, 2006; García-Palacios et al., 2010; Lundholm & Richardson, 2010; Richardson et al., 2010; Tischew et al., 2014; Battisti, 2020). However, in cases where human activity has resulted in habitats that have natural analogues, including stone walls that resemble natural cliffs, urban environments with hard surfaces that resemble natural rock outrcrops, or brownfield grassland that is similar to chalk grassland, these anthropogenic habitats are ecologically analogous to certain natural habitats (Lundholm, 2006; Lundholm & Richardson, 2010; Richardson et al., 2010; Robinson & Lundholm, 2012; Tropek et al., 2013).

Those who manage and/or use brownfield land do not always hold its biodiversity value in high regard (Allan et al., 1997; Buglife, 2009; Anderson & Minor, 2017; Laprise et al 2018; McCallum & Sardo, 2021). While some people seem to have little understanding of the potential high biodiversity value of brownfield land (Wozniak & Kompala, 2001; Muratet et al., 2007; Lorimer, 2008; Tropek et al., 2013; McCallum & Sardo, 2021), others appear to be inherently biased against brownfield land (Allan et al., 1997; Dobson et al., 1997; Smyth, 1997; Laprise et al., 2018; Oppio et al., 2020), often encouraging dramatic changes in brownfield land (Allan et al., 1997; Vojkovská et al., 2013; Bates et al., 2015; Abdullahi & Pradhan 2016), including restoration that involves complete changes in substrate and vegetation cover (Allan et al., 1997; Smyth, 1997; Wozniak & Kompala, 2001; Singh et al., 2002; Fischer et al., 2013), as well as preferentially building on brownfield land over greenfield land (Lorimer, 2008; Buglife, 2009; Pueffel et al., 2018; Bottero et al., 2020; Fiorelli, 2020). Brownfield land appears to many people to be neglected (Gunston, 1956; Allan et al., 1997; Buglife, 2009; Robins et al., 2013; Botzat et al., 2016), and apparently biodiversity-poor (Maurer et al., 2000; Singh et al., 2002; Lorimer, 2008; Buglife, 2009; Robins et al., 2013), partly because it looks so different from land that is traditionally viewed as being good for biodiversity (Singh et al., 2002; Lorimer, 2008). The plants present on brownfield land can also be viewed negatively, with a potentially prevailing lack of knowledge of their biodiversity value (Parraga-Aguado et al., 2013; Anderson & Minor, 2017). Many native plant species in brownfield habitats, including ruderal species, are often perceived to have low aesthetic value, leading to a lack of awareness of their biodiversity value (Guo et al., 2018). The apparent brown colour and lack of complete vegetation cover on many brownfield sites is unattractive to many (Allan et al., 1997), while green, more

'complete' vegetation cover (which can be less biodiverse) is usually preferred from an aesthetic standpoint (Lorimer, 2008).

It should be borne in mind that over time, if the nature of the substrate of a brownfield site is particularly amenable to plant growth, then OMHPDL/habitat that could be classified as OMHPDL/similar may be lost over time (usually after 15-20 years), due to thick, successful growth of plant species, with a loss of heterogeneity and/or bare patches, as plant communities go through different stages of succession (Chapman, 2001; Strauss & Biedermann, 2006; Muratet et al., 2007; Buglife, 2012; Spiering et al., 2020). When this occurs, the site will need to be managed to achieve previous conditions if biodiversity levels are to return to desirably high levels (Chapman, 2001; Strauss & Biedermann, 2006; Albrecht et al., 2011; Buglife, 2012; Muratet et al., 2017). Otherwise, brownfield sites may be created in other places on a regular basis, so sometimes sites will not need to be maintained and/or restored, as they are, in a sense, replaced (Macadam & Bairnier, 2012). Traditionally, these has been done with techniques such as grazing with livestock or haymaking (Chapman, 2001; Millard, 2004; Baasch et al., 2012; Bretzel et al., 2016). Many invertebrates, for example, rely on certain vegetation structures and patches of bare ground so if these are lost in a habitat, their populations within that habitat will suffer as a result (Strauss & Biedermann, 2006; Buglife, 2012; Macadam & Bairnier, 2012; Robins et al., 2013).

If brownfield sites themselves cannot be directly protected from development, then some measures can be put in place to help to mitigate for the habitat lost (Lorimer, 2008; Buglife, 2009; Buglife, 2012; Robins et al., 2013; Bates et al., 2015). It is very important that up-to-date environmental and ecological information on the brownfield site/s in question is available to better inform the mitigation strategy (Robins et al., 2013; Buglife, 2009). The phasing of mitigation should be decided upon carefully so that, when the habitat on the brownfield site is destroyed and/or removed by development, such actions have as minimal an impact as possible on the local ecosystems (Buglife, 2009). For example, brownfield habitat replacing the original site habitat could be in place before the original area is impacted, so that any necessary translocations can be carried out efficiently and with as little disturbance as possible to the species concerned (Buglife, 2009). In some circumstances, as previously discussed, the original brownfield substrate could be moved to a green roof, moving the seed bank and continuing to provide a similar habitat for much of

the wildlife that would have previously lived on the substrate in its original site (Lorimer, 2008; Robins et al., 2013; Bates et al., 2015; Threlfall et al., 2017; Fulton, 2020).

There are many differences between restoration that is planned with a longterm goal such as biodiversity increases (Wozniak & Kompala, 2001) from restoration that is planned with short-term goals such as erosion control (García-Palacios et al., 2010; Beesley et al., 2014) or aesthetic revegetation. Ultimately, if brownfield land is to continue to provide for biodiversity, it will need to be effectively and carefully managed to maximise its biodiversity potential and/or to continue to preserve rare species and communities (Thomas, 1930; Hind, 1956; Greenwood & Gemmell, 1978; Ash et al., 1994; Harvie, 2004).

2.13 Conclusion

With habitat loss, climate change and other anthropogenic pressures being placed upon species in different environments, it is vital to recognise the importance of anthropogenically influenced land for biodiversity. While the urban heat island effect is increasingly problematic for species in more urbanised brownfield areas (Villalobos-Jiménez & Hassall, 2017), different species will take advantage of conditions that suit them and develop ecosystems that, if given the best opportunities, will grow increasingly resilient and adaptable to further anthropogenic changes. Acknowledging how best to manage sites for biodiversity, as well as allowing places to be 'taken over' by nature, could allow for a healthy balance in areas that have undergone development and will likely not return to a completely natural state (at least not in the short-term).

Chapter 2: Materials and Methods

As most of the methodologies referred to throughout the research chapters of this thesis are the same, the details of these methodologies are provided here. Each section will be cited in the relevant research chapters throughout the thesis for clarity.

3.1 Field Methodology

Between March-August 2021, plant species, including bryophytes and vascular plants, were surveyed and recorded on open plant communities on six anthropogenic substrate field sites. Bryophytes are (small) plants which lack flowers and reproduce by means of asexual gemmae or tubers and/or spores (Atherton et al., 2010) while vascular plants, including angiosperms (flowering plants) are those that have lignified tissues, including xylem, to conduct minerals and water (Simpson, 2010). Stratified random sampling (Roleček et al., 2007) was used to sample open plant communities on each study site. Plant communities in the study were identified based on dominant species (if any) and overall species composition (See Tables S1.1 and S1.3, S2.1 and S2.4). Because plant communities on brownfield sites, including anthropogenic substrate sites can often not be easily classified according to standard delineations, such as NVC (National Vegetation Classification) (Cohn et al., 2001; Harvie, 2004; Maddock, 2010; Lush et al., 2013; Dennis, 2014), studyspecific plant communities were defined. It is important to note that the communities studied on the field sites were not defined using strict phytosociological principles, such as those used in the NVC, for example.

Five or more open plant communities were identified on each field site. One random sample, using a quadrat frame (1 x 1 m) was used to record both the abundance and frequencies of species in each community. The 1 x 1 m quadrat was subdivided into 16 cells for ease of recording, with total abundance counts from each sample being used for further analyses. Bryophytes were surveyed on three of the study sites (Addiewell Bing, Fallin Bing and South Band Wood (Penicuik)) in March 2021 and in three of the study sites (the Barrow-in-Furness slag bank, the Warton slag bank and the Hodbarrow RSPB Reserve) in July 2021. Vascular plants,

including angiosperms, were surveyed on three of the study sites (Addiewell Bing, Fallin Bing and South Band Wood (Penicuik)) in May 2021 and in three of the study sites (the Barrow-in-Furness slag bank, the Warton slag bank and the Hodbarrow RSPB Reserve) in July 2021. March and May surveys were not carried out in the English sites (the Barrow-in-Furness slag bank, the Warton slag bank and the Hodbarrow RSPB Reserve) due to time and sampling constraints and Covid-19 restrictions.

Species were recorded with reference to Rose & O'Reilly (2006), Hubbard & Hubbard (1992), Price (2016) Fitter & Fitter (1984), Atherton et al (2010) and Smith (2004), as well as with the assistance of other recorders. Species names have been updated in the text (e.g in Tables and Figures) to reflect recent changes in taxonomy using the websites GBIF (vascular plants), Tropicos (bryophytes) and World Flora Online (bryophytes). Species lists and species .csv files have been generated so that names can be checked and updated at any time in the future using the 'species matching' function on GBIF (further information in S2, S3 and S4).

In July 2021, after plant recording took place, substrate samples were taken from within the community sample spaces recorded within the quadrat frame, so as to be directly associated with the plants recorded. Substrate samples were between 0.75 and 2 kg of material and were collected with a trowel (with lower levels of material generally being collected in cases where substrate consisted mostly of smaller particles, such as soil). Substrate samples where bare ground was mostly present were taken at a depth of approximately 10 cm, while substrate samples associated with more well-developed plant cover were taken at least 10 cm below most of the associated plant cover, between 10 cm and 50 cm below the surface. During substrate sample collection, plant cover disturbance was kept to a minimum, with larger plants, such as tall grasses and flowers, being left mostly undisturbed.

3.2 Substrate preparation and analyses

For the mineralogical, geochemical and pH analyses, material was taken from the collected substrate samples and ground into powder. 41 samples were collected associated with plant quadrat records: 5 from Addiewell Bing; 5 from Fallin Bing; 5 from South Bank Wood (Penicuik); 11 from the Barrow-in-Furness slag bank; 8 from the Warton slag bank; and 7 from the Hodbarrow RSPB Reserve. Grid samples were taken from the Warton slag bank and the RSPB Hodbarrow Reserve to create geochemical maps using spatial analyses. 27 samples were collected from the Warton slag bank, while 24 were collected from the Hodbarrow RSPB Reserve.

Material from the substrate samples was crushed using either a pestle and mortar or a jawcrusher. Crushed and fine-grained sample material underwent pH analysis, with pH 4.01, 7.00 and 9.21 buffers used to calibrate the pH meter within a minimum of 7 days before use. For X-Ray Diffraction analyses, crushed and finegrained sample material was milled using a Retsch MM400 ball mill at the University of Glasgow (UoG). The ball mill containers used were stainless steel and 50 ml, with stainless steel balls 2 cm in diameter. Substrate sample X-Ray Diffraction (XRD) analyses, for mineralogy. All of this work was carried out at the University of Glasgow. Further details of substrate sample methodology are provided in the Supplementary Information (S2.2).

Bulk sample geochemical analysis for the majority of the samples was conducted by ALS Global Laboratories (UK) Limited in Galway, Ireland. Major elements were determined by fusion decomposition followed by ICP-AES (Inductively Coupled Plasma Atomic Emission Spectroscopy) measurement, while trace elements were determined by aqua regia digestion with ICP-AES analyses. Measured values for standards were within 10% of accepted values for all elements, apart from Bi, Cd, La, Li and Sc (within 20%), Sb (within 30%) and Hg (within 60%). This means that for most elements, the maximum error of concentration was less than 10%, ensuring that measurements made were as accurate as realistically possible. Two quadrat samples and five grid samples which had particularly high native metal concentrations which could not be analysed by these methods were analysed by X-Ray Fluorescence (XRF) at the Materials Processing Institute in Middlesbrough. For these XRF analyses, 32 Certified Reference Materials, with a range of elements covering the analysis for this material were measured – elemental analysis was within 3% of standard values. Full analytical details and data quality control of the substrate analyses is given in the Supplementary Information (S2.2).

3.3 Statistical analyses

This section details the various statistical analyses carried out for the data, the results for which are reported in Chapters 5, 6 and 7.

In summary, biodiversity analyses were carried out using the iNEXT package and the vegan package (version 2.6-4) to generate Hill numbers (representing species richness), species richness, Simpson's Diversity, Shannon Diversity and species evenness values for each quadrat on each study site, using the "iNEXT" function in iNEXT and the "specnumber" function and equation in vegan (Hsieh et al., 2022; Oksanen, 2022; Oksanen et al., 2022).

Biodiversity can be calculated in many different ways and multiple authors suggest different indices, analyses and measurements of biodiversity, according to the perceived usefulness, interpretation and so on (Gaines et al., 1999; Magurran et al., 2013; Morris, 2014; Hsieh et al., 2016; Roswell et al., 2021). To represent different aspects of the plant biodiversity on the six study sites, a variety of different biodiversity indices were calculated using multiple R packages. Some analyses were carried out using the iNEXT package (version 3.0.0) (Hsieh et al., 2022), to 1) calculate sample coverage, which is deemed by some authors to represent community diversity well, accounting for limitations of typical biological sampling and recording (Roswell et al., 2021) and 2) generate Hill numbers, which represent species richness (including in the context of species coverage), $q = 0$, modified Shannon Diversity, *q* = 1 and modified Simpson Diversity *q*= 2 (Hsieh et al., 2016; Roswell et al., 2021; Hsieh et al., 2022). The Hill numbers are defined in Chao et al. 2014 (and referenced in Hseih et al., 2016) as follows:

$$
{}^qD = \left(\sum_{i=1}^S p_i^q\right)^{1/(1-q)}
$$

The equation taken from Chao, 2014 represents the generation of a Hill number, where *S* is the number of species in the assemblage and the *i*th species has a relative abundance of p_i , $i = 1, 2,..., S$. The parameter q relates to the relative frequencies, determining the sensitivity of the measure. For example, when $q = 0$, the abundances of individual species do not contribute to the sum in the equation. Rather, presences are counted, so that ⁰D is merely species richness. The Shannon index, a traditional biodiversity index (Van Dyke & Lamb, 2020) can be replicated by having the *q* parameter as 1, referred to as Shannon diversity (equation taken from Chao, 2014):

$$
{}^{1}D = \lim_{q \to 1} {}^{q}D = \exp\left(-\sum_{i=1}^{S} p_i \log p_i\right).
$$

When *q* = 2, meanwhile, Simpson's diversity is yielded (another traditionally used biodiversity index (Van Dyke & Lamb, 2020)), the inverse of the Simpson concentration is represented in this equation from Chao, 2014:

$$
^2D = 1 \bigg| \sum_{i=1}^S p_i^2
$$

Simpson's Diversity represents the probability of two randomly sampled individuals in a community or population belonging to different species, with higher values showing high levels of Simpson's Diversity (He & Hu, 2005). The inverse of the Simpson concentration places more weight on abundant species frequencies and discounts rare species compared with the equations generated using $q = 0$ and *q* = 1. Chao, 2014 and Hsieh et al., 2016 emphasise the importance of reporting all of these q numbers, so that, in summary, diversity of all species $(q = 0)$, the diversity of "typical" species $(q = 1)$ and dominant species $(q = 2)$ are represented.

The vegan package (version 2.6-4) was used to generate species evenness values for each quadrat on each study site, using the "specnumber" function and equation in vegan (Oksanen, 2022; Oksanen et al., 2022). Renyi diversity values were obtained in BiodiversityR, using the function "renyiresult" (BiodiversityRGUI for Windows (Kindt, 2018)) (Kindt & Coe, 2005; Kindt, 2023), to calculate diversity values based on species richness in the context of the concentrations of geochemical variables present on the different study sites (Kindt & Coe, 2005; Kindt, 2023). The geochemical variables chosen for the Renyi analyses are the most bioavailable trace elements that were recorded by ALS and MPI (Kabata-Pendias, 2010), these are elements that are most likely (out of the ones recorded) to have been readily available to and (negatively or positively) influence the plant species on the different study sites.

Alpha diversity for the six study sites is represented by species richness and Pielou's species evenness (Table 3.6) (Van Dyke & Lamb, 2020). Species richness, as reported in Table 3.6, is merely the number of species recorded, meanwhile species evenness, a value between 0 and 1 (with 1 indicating complete evenness), demonstrates how many species exist in the same or similar numbers in a

community. *q*0, a measure of Hill Diversity, as recorded in Table 3.6, also represents species richness. Further values provided in Table 3.6 were calculated using a modified version of Shannon's index (*q* = 1) and Simpson's Reciprocal Index (*q* = 2), where 1 indicates no diversity (Hsieh et al., 2016; Roswell et al., 2021).

Indval analyses were carried out using the "indval" function in the labdsv package, version 2.1-0 (Roberts, 2023), to assess whether any of the recorded species were commonly associated with specific minerals recorded throughout the Barrow slag bank. The calculation involved uses the relative average abundances in specific clusters to calculate an indicator value of 'd', as represented in the equation from Roberts 2023:

$$
f_{ic} = \frac{\sum_{j \in c} p_{ij}}{n_c}
$$

$$
a_{ic} = \frac{\sum_{j \in c} x_{ij}/n_c}{\sum_{k=1}^{K} (\sum_{j \in k} x_{ij}/n_k)}
$$

$$
d_{ic} = f_{ic} \times a_{ic}
$$

In the equation, Pij = presence/absence (1/0) of species *i* in sample j ; xij = the abundance of species *i* in sample j ; n_c = the number of samples in cluster c; and for cluster $c \in K$. Indval is calculated on a 'stride' – the function calculates the indicator values for each of the separate partitions in the stride (Roberts, 2023).

Canonical correspondence analysis (CCA) is a constrained unimodal ordination analysis technique commonly used by ecologists to assess associations between abiotic variables and plant species presence (Lepš & Šmilauer, 2003; ter Braak & Verdonschot, 1995). The generated CCA graphs have arrows demonstrating the strength of the association between, in this case, plants species and abiotic factors/variables. The longer the arrow, the more statistically significant the association. There are numerous discussions and interpretations in the literature about the use of CCA and similar ordination techniques (Minchin, 1987; ter Braak & Verdonschot, 1995; Lepš & Šmilauer, 2003; Oksanen et al., 2022). One alternative to CCA in to represent species and communities is non-metric multidimensional scaling (NMDS) (Minchin, 1987; Oksanen et al., 2022; Ramette, 2007). NMDS analyses use a matrix of resemblances (similarities or dissimilarities) to visually represent data, such as biodiversity data (Minchin, 1987; Ramette, 2007). The NMDS constructs a configuration of data points in a specified number of dimensions,

visually, this means that patterns of species and similarities between species on a site are better identified, with the proximity between objects corresponding to their similarity (Minchin, 1987; Ramette, 2007). It was decided that while CCAs would be generated for both the Warton and Hodbarrow datasets, NMDS may be more appropriate should the CCA not be statistically robust. To determine the statistical robustness and/or significance for CCAs or NMDS, statistically significant differences between plant species in different concentrations of elements both throughout the study sites and in each individual study site were tested for with Analyses of Similarities (ANOSIM), using the "anosim" function on the vegan package (version 2.6-4) (Oksanen et al., 2022) (see S2.7.3, S.3.7.2 and S.4.5.3). This was done to better assess the effect of element variability on the plant species present, alongside using the CCAs. Additionally, to determine which chemical variables were significantly associated with plant species, many CCAs were performed on the appropriate datasets, changing the chemical variables in the analysis each time. The most statistically robust CCAs were chosen based on 1) the lengths of the axes on the relevant CCA plots and 2) p-values of 0.001 from anova tests for the analyses using the "anova.cca" function in the R package vegan (version 2.6-4) (Oksanen et al., 2022); and F statistic of 1.3 or higher ("anova.cca", vegan, version 2.6-4, Oksanen et al., 2022).

Chapter 3: Could anthropogenic substrates be used for 'biodiversity offsetting' in Great Britain?

4.1 Introduction

Biodiversity offsetting schemes involve methods, for example, to offset negative impacts of development on biodiversity by enhancing habitats and biodiversity, including providing priority habitat and/or restoring sites for wildlife (Department for Environment, Food and Rural Affairs, 2012; Department for Environment, Food and Rural Affairs, 2023). In the context of Biodiversity Net Gain (BNG), developers must make sure that they deliver a BNG of 10%, calculating the biodiversity units of different habitat based on qualities such as size, quality, location and type, either onsite or off-site. On-site BNG delivery on a biodiverse brownfield site might be, for example, the provision of green roofs with substrate from the site (Lorimer, 2008; Brown & Lundholm, 2015; Krawczyk et al., 2021; Schröder & Kiehl, 2021) or provision of equivalent habitat niches for vulnerable species in the area.

Anthropogenic substrate sites could be used as locations for biodiversity offsetting, ideally in the context of off-site BNG delivery, especially when developing anthropogenic substrate sites for other uses is difficult and/or undesirable from a practical and financial point of view. In the context of on-site BNG delivery, ideally, the anthropogenic substrate site would be sympathetically incorporated into the new development to reduce impact on wildlife as much as possible (the creation of green roofs in these cases may well be appropriate).

It is difficult to quantify the potential for biodiversity offsetting on anthropogenic substrate sites, especially given a paucity of data and a lack of relevant surveys. There is no single database for anthropogenic substrate sites or brownfield more widely, but there is much brownfield land throughout Great Britain. Having said that, data for certain anthropogenic substrate sites and brownfield sites more widely are available and can be used as examples of areas where biodiversity offsetting could occur.

Using available data concerning brownfield sites and, more specifically, anthropogenic substrate sites, the question: "Can we work out how biodiversity might be offset by the presence of biodiverse brownfield sites?" might be answered, at least partially.

4.2 Methods

Various datasets and reports exist detailing the spatial extent of different types of anthropogenic substrate or brownfield sites more generally (although within a specific category, rather than more broadly) (Table 2.1). Open mosaic habitats (OMHPDL) in England have been listed in a recent dataset which includes spatial data (Natural England Open Data Geoportal, 2024). For Calaminarian grasslands, which include those with mine spoil as the primary growth substrate, Simkin (2017) estimated that between $1.09 - 100$ km² exist in the UK, if the overall area of metalliferous vegetation is taken into consideration (although the spatial area is closer to 2.29 km² when Calaminarian grassland is defined more strictly). Historic landfill data are freely available on the relevant UK government website (Department for Environment, Food and Rural Affairs, 2024). Limitations of this data for this analysis are that many historic landfills have undergone various restoration, remediation and reclamation strategies (many of which were explored in further detail in different sections of the Review Chapter), so only a small number of these will be biodiverse anthropogenic substrate sites. Ferrous slag site information was available in supplementary information provided by Riley et al. (2020). Spatial information is partially accessible online on the relevant Welsh government websites (DataMapWales, 2024), accessed 6th November 2024. OMHPDL data (Natural England Open Data Geoportal, 2024) were selected to specifically select sites that were more likely to contain anthropogenic substrate than others, although this by no means eliminated or included any or all relevant sites (further information available in S1: Data calculations).

Data on the location of SSSIs were used to provide equivalent data for land that has been set aside for natural biodiversity (as well as geodiversity). SSSI data were obtained and examined from the relevant websites, accessed $6th$ November 2024 (Wales) and 12th November 2024 (Scotland). While some anthropogenic substrate sites are located within SSSIs (Skelcher & Askew, 2014), the vast majority could not be classed as anthropogenic substrate sites or brownfield sites.

Data, including the OMHPDL data (Natural England Open Data Geoportal, 2024) were accessed from different sources and various calculations were made in QGIS (version 3.38.3, Grenoble) and Microsoft Excel to determine the spatial extent (in km²) of various types of brownfield land (mostly limiting the analysis to anthropogenic substrate). These results are summarised in Tables 2.1 and 2.2 and further detailed in S1.1.

Plant species were identified that could benefit from the inclusion of anthropogenic substrates in biodiversity offsetting programs, based on their inclusion in different recent Red Lists (see Table 2.3).

4.3 Results

It is difficult to obtain a true estimate of the extent of anthropogenic substrate sites in the UK. While several data sets exist, they include different land uses and focus on a variety of spatial scales (local-national). Through examination of the various datasets include in this study, the spatial extent of such sites and the number of such sites demonstrate the presence that they have in the landscape (Table 2.1), with thousands of sites throughout different parts of the country (Great Britain). While the smallest amount of land calculated is taken up by Calaminarian grassland (Table 2.1), historic landfills, for example, are more numerous and take up a much greater area.

In comparison to anthropogenic substrate sites shown in Table 2.1, SSSIs are much more abundant across the UK, taking up a particularly large areas of Scotland and Wales and much of England (Table 2.2). These sites cover a wide range of habitats and so whilst the difference in land area is large, the habitats, substrate types, soil types and so on are much more numerous, while those provided by anthropogenic substrate sites are (at least somewhat) more specific to certain species and wildlife communities (Ash et al., 1994; Maddock, 2010; Riding et al., 2010; Lush et al., 2013; Riley et al., 2020).

Table 2.3 shows a selection of species that have the potential to benefit from biodiversity offsetting on anthropogenic substrate sites. These included five vascular plant species and two bryophyte species. These species thrive across different types of anthropogenic substrates, with some, such as the Carline Thistle (*Carlina vulgaris*) and Autumn Gentian (*Gentianella amarella*) favouring calcareous, dry substrates, and others more commonly found on metalliferous and potentially more acidic substrates, including Spring Sandwort (*Sabulina verna* subsp. *verna*) and *Ditrichum plumbicola.*

Table 2.1: Areas (in km²) of brownfield sites where data are available and percentage cover within England, Wales and/or Scotland or more than one of these as specified. Additional information is also included.

Table 2.2: Areas (in km²) of Sites of Special Scientific Interest and percentage cover within England, Wales and/or Scotland and Great Britain overall. Additional information is also included.

Table 2.3: Near-threatened, vulnerable and/or endangered species in Great Britain that utilise anthropogenic substrate sites, and could benefit from being conserved in biodiversity offsetting schemes on such sites.

4.4 Discussion and Conclusion

It is clear from examination of the data summarised in Tables 2.1 and 2.2 that the potential area for biodiverse brownfield is much less than that of SSSIs in Great Britain. Therefore, compared with protected natural sites, anthropogenic substrate sites are much less likely to provide ample space for biodiversity offsetting in the current biodiversity and climate crisis (Ares et al., 2024). The potential for inclusion of brownfield sites would lead to only a minimal increase in total protected land area but could have disproportionate benefits. As demonstrated in Table 2.3, rare plant species are currently growing on anthropogenic substrate sites (Steven & Long, 1989; Ash et al., 1994; Atherton et al., 2020; Buglife, 2012; Riley et al., 2020) and conservation for these species could be improved to ensure the persistence and increase in such populations. This could be done in specified off-site BNG delivery, for example, or for on-site delivery where development is proposed on a site where these species are present. Where species have been lost due to development of anthropogenic substrate sites, it is important to limit such developments as much as possible in the future to protect the anthropogenic substrate sites where these species currently exist. The presence of plant species and communities on anthropogenic substrate sites is not just important in the wider national context, but in the local context as well. In South Wales, a high number of bings (spoil heaps) are present in a small geographical area, providing for nationally scarce and rare species at different trophic levels, such as plant, fungi and invertebrate species (Olds, 2019). In fact, ninety-nine South Wales bings are within SSSIs and a further thirty-two are registered as special areas of conservation (SACs) (Welsh Government, 2023; Lee, 2023). It has been argued (Olds, 2019) that additional bings, which are currently unprotected, should be protected and conserved for their contribution to biodiversity. If these are not available for on-site or off-site BNG delivery, for example, then carrying out offsetting schemes on other anthropogenic substrate sites that are similarly biodiverse should be considered. In cases such as this where sites are fairly close together in a limited geographical area, dispersal of species to and from areas of similar substrate can be fairly straightforward, especially for ruderal plant species which have small, light-weight seeds and can colonise bare substrates reasonably quickly (Baker, 1965; Rahmonov et al., 2020).

Commented [AJ1]: Sure - but in offsetting terms the key question then would become - is it OK to destroy/develop an area of species rich habitat that these species already occur in if we enhance a post industrial site where some of them occur. You are using a very broad brush here because I don't think you've got to grips with the purpose or technical functioning of offsetting and biodiversity net gain in the introduction.

Even from the small number of examples shown here and from the limited spatial data available, it is clear that biodiverse anthropogenic substrate sites are valuable for local biodiversity and for the conservation of rare species, including the provision of niches for specialist species that can be rare in natural habitats. It is likely that anthropogenic substrate sites in Great Britain can make a small but valuable contribution in terms of biodiversity offsetting, and further surveying and data gathering is recommended to further assess their positive contributions towards biodiversity.
Chapter 4: How the mineralogy and chemistry of anthropogenic substrates influence plant biodiversity

5.1 Abstract

Anthropogenic substrate sites are common in areas characterised by previous industries, such as those occurring during the Industrial Revolution and the early 20th century. Historically, waste materials, anthropogenic substrates, were dumped on land nearby or next to industry buildings, such as furnaces, processing plants, mills and so on. Such wastes can include blast furnace and steel slag, paper mill sludge, oil shale spoil and many, many more. Plant species communities can establish on various anthropogenic substrate sites, many of these communities can be otherwise uncommon or rare in the local area, due to various factors such as geochemistry, topography, soil characteristics and pH levels. Many previous studies have focussed on plants growing on narrow types of anthropogenic substrate sites, limiting the findings and interpretations that could be made about plants growing on anthropogenic substrate sites more widely. This study aims to assess the biodiversity levels and relationships between plants and substrate on multiple different types of anthropogenic substrate, many with highly heterogeneous geochemistries, mineralogies and pH levels, on six sites in the north of the United Kingdom, to attempt to address this knowledge gap. Biodiversity indices indicated that biodiversity levels can vary on different types of anthropogenic substrate, with much of the variation existing within sites rather than between sites. Many of the species identified are rare in their local geographical area and/or nationwide. Canonical correspondence analyses indicated pH level, and the concentration of aluminium, and vanadium, for example, are directly (positively) statistically significantly associated with the presence of plant species on anthropogenic substrate sites, with varying responses to trace elements between sites indicated by Renyi biodiversity analyses. Anthropogenic substrate can support high levels of plant biodiversity, and, in light of the Anthropocene and the current biodiversity crisis, much of this needs to be better studied and understood to fully ensure the

conservation and survival of rare species and unusual plant communities that are often present.

5.2 Introduction

Post-industrial waste sites, or anthropogenic substrate sites, such as blast furnace slag banks, paper mill sludge hills and oil shale bings, exhibit much of the floral diversity and plant specialists that are indicative of many brownfield sites more generally (Thomas, 1930; Ash et al., 1994; Allan et al., 1997; Cohn et al., 2001; Harvie, 2004). While many people often view brownfield sites, including anthropogenic substrate sites, as being unsightly wastelands with little to no value in their current state (Allan et al., 1997; Bickers, 2017; Buglife, 2009; McCallum & Sardo, 2021; Macgregor et al., 2022), various studies and surveys have demonstrated the biodiversity, including the novel and interesting ecosystems, that can be found on such land (Albrecht et al., 2011; Ash et al., 1994; Greenwood & Gemmell, 1978; Palmer, 2008; Rahmonov et al., 2021). Many of the plants that can be found on certain anthropogenic substrate sites are limited in the wider geographical area, due to the chemistry and properties of the anthropogenic substrate being very different from that of local natural substrates (Hind, 1956; Greenwood & Gemmell, 1978; Ash et al., 1994; Allan et al., 1997; Harvie, 2004). Commonly, these post-industrial sites are isolated and less frequently visited or disturbed by people, giving plants and other organisms more opportunities and space to establish themselves (Kelcey, 1975; Lorimer, 2008; Rahmonov et al., 2020; Macgregor et al., 2022). Brownfield sites, including anthropogenic substrates sites, can act as refugia for species which are less common than they used to be (Hind, 1956; Kelcey, 1975; Greenwood & Gemmell, 1978; Lorimer, 2008; Macgregor et al., 2022). Even when the chemistry and properties of anthropogenic substrates are similar to that of surrounding land, anthropogenic substrate sites can provide additional spaces and/or wildlife corridors for many different plant species, especially in areas with much dense urban space or intensive agricultural land (Kelcey, 1975; Muratet et al., 2007; Buglife, 2009; Macadam & Bairner, 2012; Macgregor et al., 2022). Despite the findings of many scientists in regards to the high biodiversity levels and species of conservation concern found on many anthropogenic substrate

sites, these sites are often undervalued by planners, councillors, developers and the general public (Allan et al., 1997; Lorimer, 2008; Buglife, 2009; Bickers, 2017; Macgregor et al., 2022). Therefore, these sites are increasingly being altered in such a way that they lose their inherent benefits for specific species of plants, such as during redevelopment, for example (Allan et al., 1997; Palmer, 2008; Riding et al., 2010; Harvie, 2012; Riley et al., 2020). In the current biodiversity crisis, it is more important than ever that biodiversity-rich and ecologically important anthropogenic substrate sites are recognised and that species are actively or passively conserved on these sites (Bickers, 2017; Lorimer, 2008; Riding et al., 2010; Macadam & Bairner, 2012; Riley et al., 2020).

5.2.1 Conservation priorities on anthropogenic substrate sites

It is worth acknowledging that some limited management is necessary for specific important species on some anthropogenic substrate sites, along with other brownfield sites. This is because anthropogenic substrate sites often exhibit early successional stages of plant communities and the noteworthy and/or rare species in these communities can be outcompeted or otherwise replaced by plants through later successional growth stages (Greenwood & Gemmell, 1978; Ash et al., 1994; Callaghan, 2022; Macgregor et al., 2022). Anthropogenic substrate site management should prioritise species conservation and/or preservation, rather than being carried out in a more traditional mindset that can prioritise, say, 'aesthetic attractiveness', as well as landscaping to make the anthropogenic substrate site fit better into the overall landscape (Greenwood & Gemmell, 1978; Ash et al., 1994; Riding et al., 2010; Macadam & Bairner, 2012; Rahmonov et al., 2020). As well as management, anthropogenic substrate sites need to be better studied and surveyed to 1) determine the species pool that is present on the site 2) study and/or identify the different communities that are present on the site and 3) assess appropriate actions to be taken to benefit different species and/or increase biodiversity (Greenwood & Gemmell, 1978; Buglife, 2009; Bickers, 2017).

Many of the features peculiar to anthropogenic substrate sites include novel presences and concentrations of certain minerals and elements (Tilley, 1944; Piatak et al., 2015). Steel and blast furnace slags, for example, (Greenwood & Gemmell,

1978; Ash et al., 1994; Pullin et al., 2019; Riley et al., 2020), are highly heterogeneous in chemical composition due to a number of factors, such as: the raw materials fed into iron and steel furnaces (Yildirim & Prezzi, 2011; Piatak et al., 2015); the method of cooling the slag once it leaves the furnace (Yildirim & Prezzi, 2011; Piatak et al., 2015); and what kind of furnace is used, which influences the iron or steel produced (Yildirim & Prezzi, 2011; Piatak et al., 2015). In countries with industrial history, there exists a large variety of different types of anthropogenic substrate sites, produced primarily particularly in the late 1800's and the first half of the 1900's (Thomas, 1930; Barrett, 1992; Blignaut & Milton, 2005; Pullin et al., 2019; Riley et al., 2020).

These anthropogenic substrates can be very different from natural substrates in the surrounding area in terms of mineral composition, pH level and elemental composition (Ash et al., 1994; Lundholm, 2006). Ecosystems that can be found on anthropogenic substrates may be very different from anything that might have existed were anthropogenic substrate not to exist (Lundholm 2006), although there are parallels where there are at least some overlaps in mineralogy and chemistry (Lundholm & Richardson, 2010). There is limited research on the relationship between plants and chemistry on multiple anthropogenic substrate sites with varied chemistries, topographies and site histories, with studies tending to focus on one type of anthropogenic substrate (Cohn et al., 2001; Harvie, 2004). These studies only give a glimpse into the relationships that plants have with anthropogenic substrate, with the interactions between plants and substrate being little understood on many substrates and on many anthropogenic substrate sites throughout the world.

This study aims to investigate the relationships between plants and substrate properties on multiple anthropogenic substrate sites with differing industrial histories and types of waste and/or substrate present. What these sites all have in common is that they replaced previously natural sites or, at least, areas with natural substrate. The overall aim was addressed through the following research questions:

1. What plant communities exist on the six study sites?

- 2. What are the biodiversity levels present on the six study sites? Do plant communities differ in their biodiversity levels within sites as well as between sites?
- 3. Which, if any, elements, minerals and/or pH levels, are statistically significantly associated with plant species presence and distribution on the six study sites?
- 4. Do higher and lower levels of particularly bioavailable trace elements influence plant biodiversity levels within and between sites, and can any trends be seen with increasing and decreasing concentrations of these elements?

5.3 Materials and Methods

5.3.1 Field methodology

Fieldwork took place between the $20th$ of March and the 31st of July 2021 following the methodology provided in Chapter 2: Materials and Methods (section 3.1 Field methodology).

5.3.2 Study Sites

Plant species and communities were recorded on six study sites, all with differing amounts and types of anthropogenic substrates, in the spring (May) and/or summer of 2021: the Barrow-in-Furness slag bank, Cumbria, made up of blast furnace slag, steel slag and a clay capping cover (Figure 1.1); the Warton slag bank, Lancashire, consisting primarily of blast furnace slag with a small proportion of steel slag (Figure 1.2); the RSPB Hodbarrow Nature Reserve, made up of blast furnace slag and iron sands from former mining activity (Figure 1.3); Fallin Bing, Stirlingshire, a colliery spoil bing (a term for a waste heap (Allan et al., 1997; Harvie, 2004) (Figure 1.4); Addiewell Bing, West Lothian, an oil shale spoil bing (Figure 1.5); and South Bank Wood, Penicuik, Midlothian, made up primarily of paper mill sludge from paper mill processes (Figure 1.6). Further details on these six study sites are available in S2.1. Further clarification and information concerning both the chemistries of the different types of anthropogenic substrates studied, as well as plant species commonly

recorded on these anthropogenic substrate types, are provided in Tables S2.1 and S2.2.

Figure 1.1: Aerial view of the Barrow slag bank, with the black outline representing the study area and with the different quadrat sample locations labelled – each of these represents one open plant community on the site. The red marker in the smaller inset map shows the location of the site in the wider geographical area.

Figure 1.2: Aerial view of the Warton slag bank, with the black outline representing the study area and with the different quadrat sample locations labelled – each of these represents one open plant community on the site. The red marker in the smaller inset map shows the location of the site in the wider geographical area.

Figure 1.3: Aerial view of the RSPB Hodbarrow Nature Reserve, with the black outline representing the study area and with the different quadrat sample locations labelled – each of these represents one open plant community on the site. The red marker in the smaller inset map shows the location of the site in the wider geographical area.

Figure 1.4: Aerial view of Fallin Bing, with the black outline representing the study area and with the different quadrat sample locations labelled – each of these represents one open plant community on the site. The red marker in the smaller inset map shows the location of the site in the wider geographical area.

Figure 1.5: Aerial view of (North) Addiewell Bing, with the black outline representing the study area and with the different quadrat sample locations labelled – each of these represents one open plant community on the site. The red marker in the smaller inset map shows the location of the site in the wider geographical area.

Figure 1.6: Aerial view of South Bank Wood, with the black outline representing the study area and with the different quadrat sample locations labelled – each of these represents one open plant community on the site. The red marker in the smaller inset map shows the location of the site in the wider geographical area.

5.3.3 Substrate preparation and analyses

The 41 substrate samples from the 41 plant communities across the six study sites were utilised according to the methods described in Chapter 2: Materials and Methods (section 3.2 Substrate preparation and analyses).

5.3.4 Statistical analyses

Details about the biodiversity analysis, canonical correspondence analysis (CCAs) and Indval analysis methodologies are provided in Chapter 2: Materials and Methods (Section 3.3: Statistical analyses). Supplementary information related to these analyses can be found in: S2.2, S2.3, S2.5; and S2.7.

Principal Component Analyses (PCAs) were carried out in Python for the anthropogenic substrate geochemistry data to visually represent the geochemical data as well as to attempt to differentiate between the different study sites and samples on a geochemical basis. PCA analyses were carried out in Python, using Scikit-learn (Pedregosa et al., 2011), various visual representations for the data, including a heatmap and matrix loadings were generated using Matplotlib (Hunter, 2007) and seaborn (Waskom, 2021) was used to visually represent the PCA loadings that best represented the significant differences between element concentrations between samples. Data management on Python was handled using pandas (The pandas development team, 2020) and NumPy (Harris et al., 2020).

A correlation matrix was generated for Fe and P recorded throughout the six study sites, to see whether there was a significant correlation between these two elements on the study sites, R packages Hmisc (Harrell Jr, 2024) and corrplot (Wei 7 Simko, 2024) were used to carry out the analysis and visualise the correlation matrix (see S2.7 for further information).

5.4 Results

42 open plant communities were recorded across the six study sites, with 5 each in Fallin Bing, Addiewell Bing and South Bank Wood, 7 on the Hodbarrow RSPB Reserve, 8 on the Warton slag bank and 11 on the Barrow-in-Furness slag bank (see more in S2.4). While there were similarities between communities within sites (in terms of species overlap), each one differed in terms of overall species composition and there were at least some differences in terms of shelter, exposure and topography, including within sites. Additionally, some sites are coastal, meanwhile others are more inland and/or closer to freshwater sources. Many of the study sites had plant communities that could not be included either due to lack of accessibility or because of the fact that they consisted primarily of forest, woodland

and/or shrubland, so were not considered to be open plant communities for the purposes of the study. A total species lists is provided in Table 3.1, while a list of locally/nationally uncommon/rare species on the sites is provided in Table 3.2.

Table 3.1: Plant species recorded on the six study sites during fieldwork in 2021. B = the Barrow-in-Furness slag bank, W = the Warton slag bank, H = the Hodbarrow RSPB Nature Reserve, F = Fallin Bing, A = Addiewell Bing and S = South Bank Wood. * = species that have been recorded in a large pH range on anthropogenic substrate sites, with a preference for acidic substrates (Harvie, 2004). ** = species that were recorded in a fairly large pH range on Scottish oil shale bings by Harvie (2004) and preferentially occurred on neutral or slightly alkaline substrates.

85

86

Table 3.2: Species recorded in this study that are uncommon or rare locally and/or more widely (Blockeel, 2014^a; Blockeel, 2014^b; Porley & Blockeel, 2014^a; **Porley & Blockeel, 2014^b ; Porter & Foley, 2023^b ; Rich, 2023; Smith & Blockeel, 2014; Stroh et al., 2014; Dines & Walker, 2023; Dixon & Dines, 2023^b ; Pearman, 2023^b ; Perring & Pearman, 2023; Porley & Stroh, 2023; Stroh & Wilmore, 2023; Wilmore, 2023). Additionally, Wild Strawberry (***Fragaria vesca***), Wood-sorrel (***Oxalis acetosella***) and Common Speedwell (***Veronica officinalis***) were recorded in Scottish sites, these are both Nearly Threatened species in England (Stroh et al., 2014).**

Geochemical data, reported in mg/kg in most cases (apart from Loss on Ignition, which is reported in %), are provided in Tables 3.3 and 3.4. Varying concentrations of elements were recorded on the anthropogenic substrate sites. Concentrations of many elements, especially Si and Ca, varied considerably within the study sites as well as between study sites, highlighting the geodiversity and geochemical heterogeneity of these locations.

Table 3.3: Substrate geochemistry across the study sites – elements reported in oxides and LOI (loss on ignition). All

Table 3.4: Substrate geochemistry across the study sites – trace elements. All values are reported in mg/kg.

Figures 1.7, S1.1 and S1.2 (S2.7.1) represent results of PCA analyses in Python of all of the substrate data. To best represent the different principal components in the dataset, the data were scaled and then PCA analyses were carried out in scikit-learn (see S2.7.1 for further context and information). 4 principal components were needed to explain most of the variance (99.832%) in the data Figures 1.7, S1.1 and S2.7.1. As seen in Figure 1.7, within the dataset, $SiO₂$, Al₂O₃, CaO and Fe₂O₃ statistically significantly varied between different plant communities, demonstrating differing concentrations of these elements between sites and explaining most of the variance shown in the data, and further emphasising the heterogeneity of the substrate data as seen in Tables 3.3 and 3.4. Barrow Community 9, in particular, had a significantly different CaO concentration from any other plant community, including within the Barrow site itself (Figure 1.7 and S2.7.1). Figures 1.7C and S1.1 visually represent different principal components plotted together for the different study sites. It appears that at least some of the sites cluster together in terms of overall similarity in geochemistry – Addiewell, Fallin and South Bank Wood (Penicuik) tend to cluster close together (although South Bank Wood less so), while the more heterogeneous ferrous slag sites, labelled Barrow, Carnforth (Warton) and Hodbarrow, tend to separate out more, but show some clustering (Figure S1.1).

Figure 1.7: A. The PCA loadings matrix for the substrate element data. This matrix shows each original geochemical dimension, represented in Principal Components (PC) 1, 2, 3 and 4. These four PCs, out of the original forty-one PCs, represent almost all of the variation in the data. CaO influences almost all of PC 1, while PC 3, for example, shows positive correlations with Fe2O³ and SiO² and a negative correlation with Al2O3. B. The PCA scores matrix, which represents a dimensionality reduction for the substrate element data. This matrix describes the positive and/or negative correlations each sample has with each principal component. It can be seen, for example, that CaO in Barrow Community 9 sample (in reference to the loadings matrix in Figure

2.7A) has a significant influence on the substrate data for the community sample as a whole. C. A visualisation of the scores matrix as a scatter plot, with different study sites represented by different symbols for clarity. Note the heterogeneity within sites as well as between sites, for example, some of the Warton points are separate from the main 'Warton cluster.'

As further detailed in S2.7.1, the correlation matrix made in R using Hmisc (Harrell Jr, 2024) did not demonstrate a statistically significant correlation between Fe and P on the study sites (cor value = 0.252 , p value = 0.112). The relationship between these two elements is particularly evident in acidic soils, as Fe is particularly active in acidic soils (Yang et al., 2024), while most of the soils sampled are alkaline in nature (Table 3.6).

A wide range of minerals were recorded throughout the different study sites (Table 3.5), with some being commonly associated with anthropogenic substrates (Guang-hua et al., 2008; Jung & Sohn, 2014; Sun et al., 2014; Piatak et al., 2015; Ding et al., 2021). Gehlenite, akermanite and mullite, for example, are commonly associated with blast furnace and steel slag (Guang-hua et al., 2008; Jung & Sohn, 2014; Sun et al., 2014; Piatak et al., 2015; Ding et al., 2021). Some of the minerals found in some of the samples are associated with slag that underwent a specific cooling rate upon leaving the furnace, such as cuspidine, pigeonite and augite (Guang-hua et al., 2008; Jung & Sohn, 2014; Ding et al., 2021). Other minerals recorded (Table 2.2) most likely come from iron sands, in the case of Hodbarrow (Table 2, column "H") or from clay capping, from the remediated areas of the Barrowin-Furness slag bank (Table 2.2, column "H"). This explains the presence of some minerals that would not be commonly associated with anthropogenic substrates, such as aluminium-oxide-hydroxide and microcline.

As shown in Table 3.6, pH values for the study locations, for the most part, represented what is commonly found on other sites with similar types of waste (Barrett, 1992; Ash et al., 1994; Allan et al., 1997; Harvie, 2004; Cherian & Siddiqua, 2019), with most of the study sites possessing alkaline substrate.

Table 3.5: Minerals identified in samples from each study site by XRD. B = the Barrow-in-Furness slag bank, W = the Warton slag bank, H = the Hodbarrow RSPB Nature Reserve, F = Fallin Bing, A = Addiewell Bing and S = South Bank Wood. Starred minerals were those recorded solely on capped parts of the partially remediated Barrow-in-Furness slag bank and so may or may not come from anthropogenic substrate.

Table 3.6: Minimum, maximum and mean pH values for the six study sites, with values reported to 3 decimal places (as results were originally reported on the pH meter). B = the Barrow-in-Furness slag bank, W = the Warton slag bank, H = the Hodbarrow RSPB Nature Reserve, F = Fallin Bing, A = Addiewell Bing and S = South Bank Wood.

Table 3.7 shows a number of biodiversity indices for each community on the six study sites, represented by their quadrat sample. The community with the highest species richness and q0 was the community represented by 'Quadrat 3' in Addiewell Bing. The highest Shannon's Diversity and Simpson's Diversity results, however, were for the 'Quadrat 5' community in Fallin Bing and the highest species evenness was in the 'Quadrat 2' community on the Barrow-in-Furness slag bank. Sample coverage was high for the field sites, being particularly high at the Warton slag bank and at Fallin Bing (Table 3.7), which indicates that these two sites, along with the other field sites, were sampled thoroughly and that a high proportion of the species were represented by the sampling method used for each community (Roswell, 2021).

Table 3.8 represents findings based on Renyi diversity analyses using the renyiresult function in the BiodiversityRGUI (Kindt, 2018; Kindt, 2023). The results (see S2.7.2) demonstrate some possible influence of decreased or increased concentrations of certain trace elements on higher or lower Renyi biodiversity values, although trends or relationships were not evident in most cases. Possibly the most clear trend was that shown in the Hodbarrow RSPB Reserve - the three highest Renyi values corresponded with the three highest Cu concentrations, while the three lowest Renyi values were associated with the three lowest Cu concentrations recorded on the site.

Table 3.7: Biodiversity indices for each quadrat, representing each plant community, on the six study sites, calculated in R. Values are reported to 3 decimal places. Hill Number *q* **= 0 was calculated using the "iNEXT" function in iNEXT, Hill Number** *q* **= 1 was calculated using the "iNEXT" function in iNEXT, Hill Number** *q* **= 2 was calculated using the "iNEXT" function in iNEXT, sample coverage was calculated using the "iNEXT" function in iNEXT (Hsieh et al., 2022), Pielou's Species evenness was calculated using the "specnumber" function in vegan (Oksanen et al., 2022).**

Table 3.8: Interpretation of Renyi diversity results calculated using the renyiresult function in the BiodiversityRGUI (Kindt, 2018; Kindt, 2023) (S2.7.2) for communities growing on differing concentrations of the most commonly recorded elements. Further context and geochemistry measurements, including maximum, minimum, mean and median measurements, with standard deviations (reported to 3 decimal places), are provided. Figures and information on bioavailability in soils more widely provided in Kabatas-Pendias (2010), with additional citations provided in the text of the Table.

Indval analyses were carried out to assess indicator plant species for the minerals on the six study sites; the results with the highest indicator values are reported in Table 3.9 (see S2.5). Higher indicator values indicate higher importance of a species in relation to that mineral, with a maximum value of 1. Indicator plant species with a value of 1 were only recorded in one community and in association with a specific mineral throughout the six study sites. Therefore, interpretation of these results may be limited to the sites studied.

Table 3.9: Indicator species with the highest Indval scores throughout the six study sites, based on calculations done using the function "indval" in the

labdsv R package, version 2.1-0 (Roberts, 2023). Indicator values and p values are reported to 3 decimal places.

After carrying out several CCAs (canonical correspondence analyses) on the substrate and plant data for the slag bank (S2.7), one CCA was chosen based on its high F statistic (1.4809) and low p value (0.001) from its test anova. Figure 1.8 shows this CCA plot representing the plant data along with the substrate variables which best accounted for much of the plant species' presence and number, with longer arrows on the graph indicating a stronger or more significant association with plant species occurrence and/or number.

Figure 1.8: Plot for a canonical correspondence analysis for plant data for all six field sites, including the most relevant variables (Al2O3, K2O, P2O5, SiO2, pH level and V), anova F statistic = 1.4809, anova p = 0.001). White circles represent plant communities and red crosses show plant species, some selected species are labelled to represent variation between species and substrate chemistries.

5.5 Discussion

5.5.1 Findings from the substrate-specific analyses

Substrate heterogeneity was statistically significantly demonstrated in the PCA analyses carried out for this study, highlighting the variation between the study sites in terms of elemental composition (Figures 1.7 and S1.1 and S2.7.1). As would be expected based on overall chemical composition, Addiewell Bing and Fallin Bing tended to cluster together, South Bank Wood somewhat clustered with the two bings and the ferrous slag sites exhibited the highest amount of heterogeneity, while still at least somewhat clustering together (Figure S1.1). Ca was demonstrated to vary more within sites than was expected, with a particularly high Ca measurement for Community 9 in Barrow-in-Furness, accounting for most of PC1 (Figure 1.7A), this was reported as 44.3% Ca (S2.6). This shows great contrast with much lower Ca concentrations in other samples taken throughout the study, such as: 0.34% and

0.52% Ca for Communities 2 and 4 in Fallin Bing (S2.6.4). Blast furnace slag and steel slag are particularly heterogeneous substrates – their chemical composition differs depending on: raw materials fed into the blast furnace or steel furnace; furnace type; and cooling rate upon leaving the furnace (Yildirim & Prezzi, 2011; Piatak et al., 2015). It would be beneficial to carry out similar analyses for elemental composition of further anthropogenic substrate sites to better understand the variation between these sites and which elements are particularly high on specific parts of a site.

The mean pH value for the Barrow-in-Furness slag bank was perhaps higher than what might have been expected, as while high pH levels are recorded in blast furnace and steel slag (Yildirim & Prezzi, 2011; Piatak et al., 2015) clay capping is present on much of the site, which would normally be expected to have a neutral or close to a neutral pH (Melchior, 2001) (Table 3.6). The minimum value for Addiewell Bing also demonstrates that at least parts of the site are more acidic than what is normally reported for Scottish oil shale bings, as Harvie (2004) reported a minimum of 5.72 on a chemistry and plant survey of multiple spent shale bings.

It is at least somewhat surprising that the correlation matrix analysis carried out for the Fe and P recorded on-site (see S2.7.1) did not show a statistically significant correlation between the two elements. Seeing as the two elements are interconnected in growth substrate such as soil, with soil phosphate naturally associating with ferric oxides, for example, one would expect increases or decreases in one would lead to respective increases or decreases in the other (Nussaume & Desnos, 2022; Yang et al., 2024). It may be the case that there are deficiencies of iron and/or phosphorous in one or more of the plant communities leading to dissimilar values (Nussaume & Desnos, 2022), which ought to be explored further in future studies, including examinations of bioavailable Fe and P in anthropogenic substrates.

5.5.2 Findings regarding biodiversity – does biodiversity differ between study sites and do highly bioavailable trace elements influence biodiversity levels?

Despite the high biodiversity levels for many of the plant communities studied including in Addiewell Bing and Fallin Bing, others have lower, or at least comparatively lower, biodiversity values (Table 3.7), including communities on the Barrow-in-Furness slag bank, the Warton slag bank, the RSPB Hodbarrow Reserve and South Bank Wood (Table 3.7). For some of these communities, the lack of soil and the extreme levels of pH and certain elements, such as Ca, likely had a negative influence on the number of individuals and species present. For example, the akermanite and/or gehlenite in blast furnace and/or steel slag, when present, releases OH⁻ ions which increase pH levels (Haynes et al., 2013). Additionally, blast furnace slag and steel slag have high levels of Si (Haynes et al., 2013) and Ca (Ash et al., 1994; Haynes et al., 2013). This means that only certain plant species can grow directly on steel or blast furnace slag, as many plants species cannot tolerate these aspects of slag chemistry (Ash et al., 1994; Palmer, 2008). As demonstrated by the Renyi Biodiversity analyses (Table 3.8), in many cases, the higher the concentration of a certain element in a substrate sample, the lower the biodiversity recorded where that substrate sample was collected. Elevated concentrations of any element, particularly trace metals, have the potential to limit and/or impair individual plant growth and/or limit the presence of plant species. The bioavailability of metals such as Cd, Zn, Cu and Co can increase their risk to plant growth and health when they are present in elevated concentrations (Kabata-Pendias, 2010). In particular, high levels of elements such as Co, Pb and Zn that were recorded during the study could have negatively impacted the growth of some of the plant species although, conversely, they may have reduced competition for species that tend to be more dominant on less metalliferous soils. As can be seen in Table 3.1, many of the recorded species were limited to one study site and/or anthropogenic substrate, which does emphasise the importance of multiple types of anthropogenic substrate with different chemistries in the wider conversation and discussion concerning wildlife conservation and biodiversity on such substrates. The Indval analyses (Table 3.9 and S2.5) demonstrated the association of certain plant species with certain minerals, many of which were present in elevated quantities on anthropogenic

substrate sites (Guang-hua et al., 2008; Jung & Sohn, 2014; Sun et al., 2014; Piatak et al., 2015; Ding et al., 2021). The associations between specific plant species and specific minerals on the study sites, such as Wild Carrot (*Daucus carota*) with aragonite and both Meadow Foxtail (*Alopecurus pratensis*) and Meadow Oat-grass (*Helictochloa pratensis*) with merwinite merit further study on additional anthropogenic substrate sites, to assess whether such associations are observed elsewhere. Based on the results from the various biodiversity analyses, it is worth bearing in mind that no plant species can grow and proliferate on all anthropogenic substrate types. Even in natural, less disturbed settings, all plant species will grow better on certain substrates than others (Ash et al., 1994).

5.5.3 Which geochemical variables were most closely associated with recorded plant species throughout the six study sites?

The CCA for the entire plant and substrate dataset indicated some statistically significant associations between plants and substrate. Al appears to have had a significant effect on plant species presence and distribution on the anthropogenic substrate sites studied (Figure 1.8). Al ions in soil vary depending on pH levels, the most mobile fractions occur in pH levels of less than 5.5, so Al solubility on most of the plant communities (Table 3.6) would have been low (Pavlovkin et al., 2009; Kabata-Pendias, 2010). Plants can benefit from low levels of Al, with this element helping to activate some enzymes and helping to dictate the physical properties of membrane and plasma permeability (Kabata-Pendias, 2010). As most of the plant communities grew on highly alkaline soils, increasing levels of Al (with only a limited amount being available) may have been particularly beneficial for individual plants and different plant species in most of the communities recorded. However, while some plant species, such as the Tea plant (*Camella sinensis*), can accumulate high levels of Al (Mossor-Pietraszewska, 2001), certain levels of Al are toxic to most species of plants (Gigon & Rorison, 1972; Ash, 1983; Mossor-Pietraszewska, 2001; Pavlovkin et al., 2009), with toxic effects including inhibition of $O₂$ uptake by root apices through disruption of ATP production (Pavlovkin et al., 2009). High Al content of the colliery spoil on Fallin Bing and the oil shale spoil on Addiewell Bing (Table

3.3) may have had more negative effects on the plants growing there, especially on the exposed spoil on parts of the site, but this ought to be studied in more detail.

Figure 1.8 demonstrated that V was statistically significantly associated with plant species presence. In low doses, V can be beneficial for plants, assisting with the developments of certain amino acids, sugars and chlorophyll (Chen et al., 2021^b). Having said that, V can be harmful to most plant species in high doses, inhibiting metabolism (Chen et al., 2021^b). It can be observed that none of the quadrat substrate samples had excessive concentrations of V, in fact, with the highest concentrations of V being 100 ppm (Table 3.3). Much of this, but not all, would have been relatively bioavailable for plants on the site, with V being the 20th 'most bioavailable' trace element according to Kabatas-Pendias (2010), although it is more bioavailable in acidic than in alkaline soils. The average concentration of V in global soils is 129 mg/kg, with a natural range of 69-320 mg/kg (Kabata-Pendias, 2010), so the V concentrations in the study samples are actually much lower than what is normally recorded, meaning that the higher levels available, particularly in the alkaline substrates recorded in most of the plant communities, would likely have been increasingly beneficial for the plants.

K is a necessary element in plants, and in its limited bioavailable forms (Yadav & Sidhu, 2016), it can be used, for example, for or as: an activator of multiple important plant enzymes (Ericsson & Kähr, 1993; Xu et al., 2020); cell growth (Xu et al., 2020); osmoregulation (Ericsson & Kähr, 1993) and plant growth (Xu et al., 2020). K had a significant association with plants on the anthropogenic field sites studied, as demonstrated in Figure 1.8.

P is a major and necessary element for plant growth and survival, being a key component of energy metabolism and the synthesis of cell membranes and nucleic acids (Raghothama, 2005). P deficiencies are common among plants due to its low availability in many substrates, such deficiencies can lead to, for example, decreased plant growth, decreased hydration and an increased root to shoot ratio (Atkinson, 1973; Raghothama, 2005). P was directly significantly associated with plant species presence on the study sites, which is shown in Figure 1.8.

Si is an important element for many plant species (Epstein, 1999; Kim et al., 2017; Katz et al., 2021; Meena et al., 2021) and was statistically significantly

associated with the plant species present on the RSPB Hodbarrow Nature Reserve (Figure 4.13). Si is most readily bioavailable in the form of dissolved silicate and is usually most mobile between pH levels of 7 and 9, being more likely to form insoluble precipitates in acidic soils (Kabatas-Pendias, 2010). Si is involved in various aspects of plant physiology, including: use in increasing photosynthetic pigments (Meena et al., 2021); use in physiological defences against pests and high concentrations of trace metals (Epstein, 1999; Katz et al., 2021; Meena et al., 2021); use in responses to stress, such as drought stress (Kim et al., 2017), high salinity levels (Kim et al., 2017) and decreasing oxidative stress (Meena et al., 2021).

5.5.4 Importance of anthropogenic substrate sites for plants

Various different mechanisms can explain the establishment of different species on a waste site, such as: wind dispersal (which is common for many species with small seeds, spores and other propagules) (Darlington, 1969; Ash et al., 1994); footwear (direct spread by humans of certain propagules) (Darlington, 1969); run-off (for example, from water courses) (Darlington, 1969) and dispersal by vehicles (Darlington, 1969). Many initial colonisers of anthropogenic substrates are termed 'weeds', these tend to be plants with propagules that can easily reach and grow in anthropogenic bare ground, waste land and disturbed land (Baker, 1965; Lewontin, 1965; Mulligan, 1965; Harvie, 2004), commonly including ruderal species (Harvie, 2004). These plants can exhibit high levels of plasticity compared with 'non-weedy' species, especially in the context of primary succession (Harvie, 2004). Many of the initial colonising plants of colliery spoil sites, such as highly plastic pioneer shrub species and 'weeds', for example, can have specific reproductive and physiological features that can assist them in their colonisation of more extreme substrates, such as high seed production and specific root features (Rahmonov et al., 2020). Physiological variations can be exhibited within species as well, for example, some individuals of St John's Wort (*H. perforatum*) are more 'weedy' in their growth and physiology than others, growing taller and forming stolons, flowers and seeds much more quickly than 'non-weedy' St John's Wort (*H. perforatum*) individuals (Baker, 1965). Plants termed as 'weeds' can often cause problems, or at least perceived problems, in many urban areas. It may often be the case that on some field sites,

including the sites in this study, that many of the so-called 'weeds', or other colonising plants, vigour and wide environmental tolerance may perhaps be more or just as important than the ability to set seed in terms of colonisation and establishment (Baker, 1965; Harvie, 2004). Many plants, including those which are common on the field sites studied, are adapted to grow on a wide variety of substrates, in a wide variety of habitats, although they may face stronger competition in more nutrient-rich ground, in which perennials can dominate and exclude other species (Baker, 1965; Ash et al., 1994), and in places where a rich cover of plant species is already established (Baker, 1965).

Some plant species are solely found in soils or substrates with a particular chemistry (Barth, 2020). For example, calcicoles only grow successfully on calcareous substrates (Barth, 2020). Calcicolous plants are able to control absorption of Ca more easily than non-calcicolous species. Rough Hawkbit (*Leontodon hispidus*), for example, can exhibit an increase in stomatal closure with increases in Ca, decreasing the likelihood that excess Ca levels negatively impact their stomatal guard cells (De Silva & Mansfield, 1994; De Silva et al., 2001). Calcicoles grow preferentially on soils with poor nutrients and high levels of Ca (Barth, 2020), including the steel and blast furnace slag sites studied (Gomes, 2020) and other alkaline waste sites such as Solvay process waste tips (Cohn et al., 2001). The high number of calcicoles recorded on one or more of the field sites, including species such as Kidney Vetch (*Anthyllis vulneraria*)*,* Carline Thistle (*Carlina vulgaris*), Yellow Wort (*Blackstonia perfoliata*) and Salad Burnet (*Poterium sanguisorba*) (Rose & O'Reilly, 2006), relate to the high Ca levels on some of the field sites studied, including, especially, the slag banks and slag sites studied – the Barrow-in-Furness slag bank, the Warton slag bank and the RSPB Hodbarrow Reserve.

Many plant species on Scottish oil shale spoil bings were carefully recorded in terms of pH level by Harvie (2004). Species with one asterisk in Table 3.1 were recorded by Harvie (2004) as growing in a large or fairly large range of pH compared with some species of plants, and typically favoured acidic substrates. On Addiewell Bing, these plant species were found growing in pH ranges between 5.046 and 6.227 (see S2.3 for further details), which is in line with what Harvie recorded in the 2004 study. Additionally, Harvie (2004) found that some species, including those with
two asterisks in Table 3.1, grew in a fairly large pH range on Scottish oil shale bings and preferentially occurred on neutral or slightly alkaline substrates. Somewhat contrarily, some of these species, including, especially, Broad-leaved Willowherb (*Epilobium montanum*), grew in numbers on oil shale spoil that was between 5-6 in pH, including between 5.01-5.10 in pH, indicating that some of these species might be more tolerant of low pH levels on oil shale spoil than was originally surmised (see S2.3). It has been shown that *Epilobium montanum* can successfully germinate in solutions with pH levels between 4 and 5 (Myesrcough & Whitehead, 1965). *Hypnum cupressiforme* grew on a wide range of pH levels and *Polytrichum commune* was only reliably found in acidic substrates in Harvie's 2004 study, which is in line with what was found on at multiple anthropogenic substrate site communities, not just on the oil shale spoil (S2.3).

Many of the anthropogenic substrate sites studied have high and/or varying levels of certain metals. Some species of plants can only be reliably found on substrates or soil with a high metal content, as these plants have adapted to metalliferous ground so much that they do not grow so well and/or are outcompeted on ground with a reduced concentration of certain metals (Shaw, 1987; Affholder et al., 2020). These plants, which are often referred to as metallophytes in the literature (Rainbow, 2018; Affholder et al., 2020) can be much more common on contaminated land, including certain anthropogenic substrate sites, than on non-anthropogenic substrates (Affholder et al., 2020). The growth of Wild Thyme (*Thymus praecox* subsp. *polytrichus*) on (almost exclusively) blast furnace slag on the Warton slag bank and the RSPB Hodbarrow Nature Reserve is indicative of what is found on Calaminarian grassland, a habitat type which is categorised separately from OMHPDL and has mostly been recorded on metal-rich substrates such as mine workings and spoil heaps in places such as north Wales and Northumberland, although it is also present on natural serpentine deposits (Palmer, 2008; Baker et al., 2010; Skelcher & Askew, 2014; Rainbow, 2018). Other species commonly associated with Calaminarian grassland that were also recorded on the Warton slag bank and the RSPB Hodbarrow Reserve included: Common Bird's-foot-trefoil (*Lotus corniculatus*), Fairy Flax (*Linum catharticum*), and Sheep's Fescue (*F. ovina*) (Rainbow, 2018)*.* The first two of these, along with Wild Thyme (*T. praecox* subsp. *polytrichus*) are facultative metallophytes, while they do not grow exclusively on metalliferous substrates or soils, they can grow well on them, especially compared

with most plant species (Baker et al., 2010; Rainbow, 2018). Having a mixture of Calaminarian grassland and other habitat types on both the Warton slag bank and the RSPB Hodbarrow Reserve in an area where Calaminarian grassland is limited (Rainbow, 2018) greatly increases the local importance of these sites for wildlife, including specialist species.

It is worth bearing in mind the effects of different elements on plants on different anthropogenic field sites. Cohn et al. (2001) reported the following for Solvay process waste tips in Poland: Carline Thistle (*C. vulgaris*) and Salad Burnet (*P. sanguisorba*) were significantly associated with Ba; Black Medick (*Medicago lupulina*) was significantly associated with pH (Cohn et al., 2001); and Yellow Rattle (*Rhinanthus minor*) was significantly associated with Na (Cohn et al., 2001). The pH of a Solvay process waste tip was recorded as being a little alkaline, with pH values between 7.5 and 7.9, which was in line with the pH values recorded on Addiewell Bing and parts of the South Bank Wood paper sludge site.

Many plant species growing on anthropogenic substrate sites can act as indicators for various aspects of their growth substrates. Carline Thistle (*C. vulgaris*)*,* while also being a calcicole indicative of calcareous substrates (Rose & O'Reilly, 2006; Perring & Walker, 2023), such as blast furnace and steel slag in this study, has been recorded growing on remediation landfill caps subject to heavy mechanical pressure (Darlington, 1969; Perring & Walker, 2023). Field Horsetail (*Equisetum arvense*), which was recorded on both iron sands and some blast furnace slag at Hodbarrow, and was also growing on part of the former landslip area at South Bank Wood (see S2.3) has been recorded growing on old landfill tips (Darlington, 1969). Annual Meadow-grass (*Poa annua*), Dandelion (*Taraxacum* agg.), Buck's-horn Plantain (*Plantago coronopus*), Ribwort Plantain (*Plantago lanceolata*) and Daisy (*Bellis perennis*) can grow on trampled paths and other types of disturbed ground on waste sites (Darlington, 1969; Rose & O'Reilly, 2006; Leach & Pearman, 2023^c), these species were all recorded on at least two of the study sites. In this study, various indicator species were found for different minerals, it is worth carrying out further studies of these indicator species to further assess their presence and growth in anthropogenic and/or natural substrates containing those specific minerals. These include Garden Lady's-mantle (*Alchemilla mollis*) in Anhydrite, Downy Birch (*Betula pubescens*) in Birnessite, and the mosses *Weissia controversa* and *Zygodon stirtonii* growing in Linnaeite on two separate study sites, Fallin Bing and the Hodbarrow RSPB Reserve respectively.

Despite the significant associations of certain geochemical variables with certain plant species on the study sites and previously studied anthropogenic substrate sites, many of the plant species recorded are generalists and will grow in a particularly wide variety of habitats and substrates. For example, on oil shale spoil bings, as recorded by Harvie (2004), Yorkshire-fog (*Holcus lanatus*), Knapweed (*Centaurea nigra*) and Ribwort Plantain (*P. lanceolata*) are generalists on Scottish oil shale bings and are not strongly associated with any one chemistry variable. While some plant species are reliably found on sites with certain geochemical characteristics (Rose & O'Reilly, 2006), it can be surmised that trying to work out every link between substrate element and plant species may well turn out to be ignoring and/or overlooking the plasticity of many of those plant species (Baker, 1965; Harvie, 2004).

5.5.5 Concerns regarding anthropogenic substrate sites – their limitations and how their histories relate to their current conditions

When the legacy sites studied were originally created, when dumping first began, the anthropogenic substrates present were completely devoid of plant life, with spontaneous colonisation and the establishment of pioneer communities being necessary for any plant communities to grow (Allan et al., 1997; Ash et al., 1994; Harvie, 2004). All of the field sites studied consist of material that was dumped many years ago, with the oldest anthropogenic substrate site studied being South Bank Wood, where waste was first dumped in the 1700s (Wilson, 1891; Watson, 1987). The chemistry of anthropogenic substrates on legacy sites can change greatly over time due to processes such as weathering and leaching (Cherian & Siddiqua, 2019; Riley et al., 2020). For example, the alkalinity of anthropogenic substrates on many waste sites may well decrease over time with weathering and the formation of soil layers (Gemmell & Gemmell, 1978).

The 'soil' that may be present on anthropogenic substrate sites, including landfill sites, can form in very different ways from soil on natural substrate (Darlington, 1969). The shallow nature of soils on many anthropogenic substrates sites can limit the size of plant species present (Darlington, 1969), only permitting growth for species with shallow root systems (Darlington, 1969). However, this is not the case on sites such as Addiewell Bing, where soil layers were able to form enough over the course of 100 years to allow for planting of large trees such as (Lodgepole Pine (*Pinus contorta*) and European Larch (*Larix decidua*)) as well as multiple broadleaf trees (Scottish Wildlife Trust, 1995; West Lothian District Council, 1995).

Various physical characteristics of many anthropogenic substrate sites can make it difficult for many plant species to establish themselves, including, on some sites: a lack of nutrients for optimal plant growth (Harvie, 2004; McCallum & Sardo, 2010) and lack of organic matter, especially on newer sites (Barrett et al., 1992; Harvie, 2004; Palmer, 2008; Riding et al., 2010; Rahmonov et al., 2021). Waste dumping in the first few years of major industrial production was far more indiscriminate than modern waste dumping, with little to no regulation, leading to potential negative health effects including, for example, toxic leachates (Pullin et al., 2019; Scattolin et al., 2021).

Despite some of the limitations on many anthropogenic substrate sites, including toxic concentrations of bioavailable trace elements (Kabata-Pendias, 2010), extreme pH levels (Barrett, 1992; Allan et al., 1997; Scattolin et al., 2021), compaction (Barrett, 1992; Allan et al., 1997), pyrite toxicity (a particular problem on many colliery spoil sites) (Barrett, 1992) and/or contamination (Barrett et al., 1992; Rahmonov et al., 2021), it is worth remembering and understanding the differences between many anthropogenic substrate sites, including how so many of them, including the sites studied, already support much plantlife (Hind, 1956; Kelcey, 1975; Greenwood & Gemmell, 1978; Ash et al., 1994; Allan et al., 1997). Low-nutrient substrates, whilst restricting colonisation and establishment of certain plant species, can favour the colonisation of wildflowers which are often outcompeted by fastgrowing plants including grasses, Bindweed (*Convolvulus* spp. and *Calystegia* spp.) and Hawthorn (*Crataegus monogyna*) on nutrient-rich substrates (McCallum &Sardo, 2010). In blast furnace slag and different types of steel slag there are differences in the leaching of different elements – such differences can both positively or negatively impact the plant species present and may have more of an influence on legacy slag heaps than is currently realised or understood (O'Connor et al., 2021). For example,

blast furnace slag can increase the availabilities of P, Ca and Si in soil, while electric arc furnace slag has high leaching rates of Ca, Fe, Si, Al, Mn and Mg, and releases P slowly, which can help with the long-term growth of plants (O'Connor et al., 2001; Kong & Nurulakmal, 2018; Wang et al., 2018). Electric arc furnace slag is present on the Barrow-in-Furness slag bank (Henderson & Royal, 2015), so it is possible that on the Barrow-in-Furness slag bank, the P availability varies compared with the other slag sites studied (the Warton slag bank and the Hodbarrow RSPB Reserve), as those sites have much higher ratios of blast furnace slag (see S2.1 for relevant historical and geochemical context). These differences likely have a large impact on plants throughout the different slag study sites, as well as on legacy heaps throughout the UK with differing levels of different types of slag.

5.5.6 The importance of anthropogenic substrate habitats in the wider context of wildlife habitats and local and national conservation

It seems that the variety of plant species and communities on anthropogenic substrate sites cannot be underestimated, with this heterogeneity not just being valuable in terms of plant species, but also for such ecological functions as: increased habitat for invertebrates; increased opportunities for use by people; and increased habitats for various vertebrates such as birds and mammals (Palmer, 2008; Riding et al., 2010; Macadam & Bairner, 2012; Mathey & Rink, 2012; Palliwoda et al., 2020). Rahmonov et al., (2020) recorded that, on a study site with a mixture of non-reclaimed and reclaimed anthropogenic substrate, plant biodiversity was higher on the non-reclaimed colliery spoil area.

Multiple authors, surveyors and/or botanists have expressed concern about the future careful conservation and management of many anthropogenic substrate communities. For example, it has been noticed that communities of floristic interest on alkaline waste heaps and banks in the UK are, have been or could be in danger of disappearing with further successional stages in plant community composition, as these more unusual communities are replaced with more 'typical' plant communities (Greenwood & Gemell, 1978; Callaghan, 2022). This contrasts with what is found on Open Mosaic Habitats on Previously Developed Land, or OMHPDL, which was defined in the UKBAP (Biodiversity Action Programme) as a Priority Habitat of conservation importance (Maddock, 2010; Riding et al., 2010; Woods, 2012). This Priority Habitat is defined by and made up of patchworks of previously disturbed bare ground and vegetated areas (Buglife, 2009; Maddock, 2010; Riding et al., 2010). The multiple habitat types on OMHPDL, are arranged in a complex pattern, comprising a variety of successional stages, from ruderal communities (those which are associated with wasteland and similar habitats) to flower-rich grassland (Palmer, 2008; Riding et al., 2010; Olds, 2019), this is not too dissimilar from what is seen on some of the study sites, particularly Addiewell Bing, Fallin Bing and the Hodbarrow RSPB Reserve.

Habitat mosaics which are or are similar to those found on OMHPDL, as well as semi-natural grasslands (which can also be part of OMHPDL), were once common in the wider countryside, but in recent years, intensive farming has reduced the number of these a great deal (Millard, 2004; Bretzel et al., 2016; Lorimer, 2008; Buglife, 2009; Macadam & Bairner, 2012). Today, OMHPDL wildlife communities can be rare or absent in the wider landscape and often include rare, scarce and/or specialist invertebrates and plant species, including species from the Red Data Book/List (Buglife, 2009; Riding et al., 2010; Woods, 2012; Robins et al., 2013). While Priority Habitats are not always strictly protected under UK laws, OMHPDL sites can be sensitive to development and they ought to be considered during the determination of planning applications (Maddock, 2010; Riding et al., 2010). In addition to being described in the UK BAP, OMHPDL was also included as a habitat of principal importance in the Natural Environment and Rural Communities Act (NERC Act), 2006; (Woods, 2012; Robins et al., 2013).

Because anthropogenic substrate sites may be colonised more slowly than sites with natural soil or substrate, any plans for species conservation and/or potential species value for new anthropogenic substrate sites, as well as those that have recently been quarried or disturbed, have to be very much for the long-term (Ash et al., 1994; Lorimer, 2008; Maddock, 2010; Riding, 2010). Sowing an area with appropriate plant propagules, rather than relying on a more expensive method of reclamation, restoration or remediation can be more beneficial from an ecological perspective, but having an awareness of which plants are most appropriate for certain sites is important (Ash et al., 1994). Sowing native species as part of nature conservation is also particularly important (Ash et al., 1994).

If development has to be carried out that would fundamentally change the chemistry, properties, topography, plant species and other species on anthropogenic substrate, then appropriate mitigation measures ought to take place (Riding et al., 2010; Bickers, 2017), such as: removal and deposition of anthropogenic substrate in a more appropriate area local to the development, the advantage of this is that a seed bank is already present; use of the anthropogenic substrate in the creation of green or brown roofs on the site or on top of other buildings, such as city office buildings (Lorimer, 2008; Krawczyk et al., 2021; Schröder & Kiehl, 2021); and habitat creation that would cater well for at least some specific species on-site, such as a pond, a wildflower meadow or a rockery/shrubbery. Ideally, mitigation strategies for all brownfield sites that undergo development should include multiple phases and actions (Lorimer, 2008; Riding et al., 2010; Bickers, 2017).

5.5.7 Implications of the findings

The results for this study demonstrate the high geodiversity and biodiversity of the six study sites, emphasising the scientific interest in such sites, as well as serving as a reminder as to the importance of the protection, conservation and management of such places. Some of the plant species recorded, including those recorded in Table 3.2, are rare, uncommon and/or declining in the local area and/or in the UK more widely, emphasising the importance of their presence as part of their plant communities. While it could be argued that these wastes should never have been dumped in the first place and involved the destruction of the original natural habitat, it is unfeasible from a practical and economical standpoint to completely remove all legacy anthropogenic substrate in the UK and return the locations to their former state (Bradshaw, 1996; Bradshaw, 1997). It is noteworthy that these anthropogenic substrate sites are often valued from a heritage and cultural point of view, often they are the only physical reminders left of the industries that once existed and provided employment for local communities (Harvie, 2004; Henderson & Royal, 2015). All in all, in the modern context, it is worthwhile to assess these sites for their biodiversity, as well as their cultural, historical geodiversity and recreational value.

5.6 Conclusion

Plant species and substrate chemistry on six anthropogenic substrate sites with varying substrate mineralogy and geochemistry were studied. Statistically significant differences were found in elements within and between the study sites, particularly in regards to Ca, Si, Al and Fe, satisfying Objective 2 (To assess statistically significant differences between substrate characteristics (such as geochemistry and pH levels) on anthropogenic substrate sites).

While there was at least some overlap between plant species on different sites, each study site had unique species and communities that demonstrated biological variation and substrate specificities. Biodiversity levels were high in many of the communities sampled, indicating the importance of all of the study sites for biodiversity and wildlife, particularly sites such as Addiewell Bing and Fallin Bing. Overall, the communities that have been recorded on the six study anthropogenic substrate sites, as well as those that have been recorded on anthropogenic substrate sites previously show the variation of novel, or at least unusual, plant assemblages that can be found on such sites, including at different successional stages. Biodiversity levels were positively correlated with increasing levels of Cu on Hodbarrow and, in most cases, higher levels of bioavailable elements contributed to relatively low biodiversity levels on all six study sites, although this was not the case in every instance. These investigations satisfied Objective 3: To determine the biodiversity levels of anthropogenic substrate sites using various biodiversity indices.

Certain elements, such as Ca, Al and V, were statistically significantly associated with the species present throughout the different study sites, with the arrows on the CCA demonstrating that plant numbers increased with increasing levels of these certain elements. On individual study sites, many plant species were at least somewhat associated with specific minerals, and many plant species were frequently recorded in comparable substrate chemistries. These findings satisfy Objective 4: To determine the elements and quantities of different elements that are statistically significantly associated with plant species and community presence throughout anthropogenic substrate sites.

It is clear from this study that while each individual anthropogenic substrate site is unique and should be surveyed and studied individually, the potential importance of anthropogenic substrate sites for post-industrial ecology, with often high

biodiversity and geodiversity, should not be ignored. Such sites should factor into conservation strategies and biodiversity studies so people can better understand, study and conserve nationwide biodiversity, especially as biodiversity is drastically decreasing worldwide.

Chapter 5: Slag substrate composition influences plant community distribution and biodiversity

6.1 Abstract

Legacy ferrous slag deposits are present in many industrial landscapes, with modern slag deposits being created in many countries where waste streams and regulations are not in line to efficiently recycle and reuse such waste. Despite the harsh environment of many slag deposits, with high pH levels, a lack of topsoil and potential high concentrations and low concentrations of certain elements, including metals, plant communities can establish themselves on many slag deposits. While many studies have been carried out on plant biodiversity on anthropogenic substrate sites, including some ferrous slag sites, studies are limited as to the relationship between the chemistry of the ferrous slag substrate and the plant species and communities present. In order to investigate the relationship between chemical substrate variables within a slag site and the variation in plant species or communities on the site, two legacy slag deposits – Warton, Lancashire and the Hodbarrow RSPB Reserve, Cumbria - were chosen for intensive plant species and community study, with plants across different taxonomic groups being recorded throughout. Substrate samples were also taken, including samples directly associated with plant community quadrat samples and samples taken in a grid-like manner across both sites. Grid sample mapping and community mapping showed that numerous elements had direct influences on some of the plant communities present, whether it was due to their lower or higher concentration. Such elements included Al, As, K, Mn and multiple others. Biodiversity indices calculated for the two study sites demonstrated that there is variation across both study sites. Species records highlighted the presence of some locally and/or nationally rare species, such as Fairy Flax (*Linum catharticum*)*,* Lesser Centaury (*Centaurium pulchellum*) and Carline Thistle (*Carlina vulgaris*). Renyi biodiversity analyses revealed a positive trend between Renyi biodiversity values and Cu levels on the Hodbarrow RSPB Reserve. The most statistically significant Canonical Correspondence Analysis (CCA) for Hodbarrow demonstrated that Ba, Be, Cr, Si, and Ti were all statistically

significantly associated with plant species presence on the Hodbarrow RSPB Reserve. With the possible presence of the priority Calaminarian grassland habitat on both sites, some unusual and interesting geodiversity and some high biodiversity levels and uncommon species presence, these legacy slag deposits demonstrate the significance of such sites for a variety of biological, chemical and historical reasons.

6.2 Introduction

Legacy slag sites, including slag banks and slag heaps, primarily made up of blast furnace and/or steel slag, are a predominant feature of many industrial landscapes (Josephson et al., 1949; Ash et al., 1994; Riley et al., 2020). Steel slag is still being generated in vast quantities today - more than 300 million tonnes of steel and more than 800 million tonnes of steel slag were produced in 2021 and 2022 alone (World Steel Association 2021; World Steel Association 2022; World Steel Association 2023). While most steel slag in many countries is reused in a variety of different applications (Riley et al., 2020), such as the production of cement (Gomes et al., 2020; Liu et al., 2020; Shipa, 2020; Song et al., 2020; Chen et al., 2021^a), wastewater treatment (Bowden et al., 2009; Gomes et al., 2020; Shipa, 2020; Li et al., 2021; Vu et al., 2021), carbon sequestration (Liu et al., 2020; Riley et al., 2020; Chen et al., 2021^a; Gomes et al., 2021) and thermal energy storage (Agalit et al., 2020; Gomes et al., 2020; Haunstetter et al., 2020; Kocak et al., 2021), much steel slag is dumped in industrial areas near to the furnaces in which they were produced (Riley, 2020). Much steel and blast furnace slag is dumped in countries today where efficient recycling and waste streams have not been as fully developed (Kambole et al., 2019; Schoeman et al., 2021). In countries such as the United Kingdom, legacy slag deposits can be found in the form of heaps or banks, for example, with many of these banks being situated along coastlines (Riley, 2020). In the United States, slag was dumped in a variety of different ways, for example, in heaps (Josephson et al., 1949), but also in depressions in marshes and in lakes or along lake-sides (Colten, 1986).

Blast furnace slag is produced in a blast furnace during the creation of pig iron. Blast furnace slag can be produced in a variety of different processes, depending on 1) the raw materials fed into the furnace: fluxes (usually limestone or dolomite); silica sand;

and a reductant (usually coke or coal) (Piatak, 2018) and 2) the specific method of cooling the blast furnace slag, which affects its physical properties (Piatak et al., 2015). Blast furnace slag is highly heterogenous in composition, being mostly composed of 27 – 61% Si (45.2% average), >0-62% Fe (16.1% average) and 1-41% Ca (15.2% average) (Piatak, 2018) (additional details in Table S4.1). Steel slag can be produced and classified in a variety of ways. Bessemer furnace steel slag is produced during the Bessemer steel-making process, developed by Henry Bessemer in 1856 (Lancaster & Wattleworth, 1977; Arnold, 2012), an acid process that uses ores with a high iron content and a near absence of phosphorous (Marshall & Davies-Shiel, 1969; Lancaster & Wattleworth, 1977; Jacobs & Daroub, 2001) (further information in Table S3.1). Basic oxygen furnace steel slag is created when (typically) 10-20% steel scrap and 80-90% molten iron, along with a flux (such as limestone or dolomite) and fuel (such as coke), are charged in a basic oxygen furnace (Yildirim & Prezzi, 2011; Piatak et al., 2015). Basic oxygen furnace slag is primarily made up of 30-60% Ca, 3-38% Fe and 7-16% Si (Yildirim & Prezzi, 2011) (further information in Table S3.1). Electric arc furnace steel slag is formed from a high percentage of steel scrap and a lower percentage of pig iron compared with basic oxygen furnaces (Yildirim & Prezzi, 2011; Piatak et al., 2015) with a flux, usually limestone or dolomite (Yildirim & Prezzi, 2011; Piatak et al., 2015). The steel slag which comes from this process is mostly made up of 23-60 % Ca, 5-35 % Fe and 9-20 % Si (Yildirim & Prezzi, 2011) (further information in Table S3.1). Both blast furnace slag and steel slag can be highly heterogeneous in terms of their overall elemental composition, meaning that, while there are broad similarities across different slag deposits (Ash et al., 1994; Riley et al., 2020), it should not be assumed that every slag deposit has consistent geochemistry and pH levels across its surface. Additionally, many slag deposits contain more than one type of ferrous slag, as many industrial sites have and had many different types of furnace, producing pig iron and steel simultaneously or at different times in history, as well as in the present day (Price, 1983; Mourholme Local History Society Book Group, 2009; Henderson & Royal, 2015; Grosse, 2017).

Plants have successfully colonised and grown on some legacy blast furnace and/or steel slag sites (Ash et al., 1994; Kay et al., 1997; Merwin et al., 2020; Riley et al., 2020; Holmes & Kuebbing, 2022). The high pH levels, lack of organic matter and

poor water retention of ferrous slag deposits cause plant communities to develop slowly compared with communities on natural substrate (Ash et al., 1994; Zou et al., 2019). On some slag deposits, pH levels and concentrations of certain elements can be so extreme that little to no plants will grow at all (Scattolin et al., 2021) In cases where the pH levels of the slag substrate is not too alkaline for plant growth, those plants that can grow well in substrates with a high calcium content, including specific habitat specialists, calcicoles, rare species and generalists, can colonise said slag substrate (see further information in Table S3.2) (Ash et al., 1994; Woods, 2012; Raper, 2015).

Different plant species and communities have been observed on multiple legacy slag banks and heaps (Ash et al., 1994; Raper et al., 2015; Riley et al., 2020). Often, these communities and species can vary within a single site. Due to the heterogeneity of blast furnace slag and the different types of steel slag even within a site, it can be argued that the plant species and communities may perhaps vary accordingly. Plant species are strongly influenced by their growth substrate and are strongly influenced by numerous substrate characteristics, including nutrient availability, absence or presence of toxic levels of trace metals and pH levels (Clarkson & Hanson, 1980; White & Broadley, 2003; Raper et al., 2015; Barth, 2020). There has been much interest in the unusual plant communities and species found on alkaline anthropogenic substrates (Hind, 1956; Kelcey, 1975; Greenwood & Gemmell, 1978; Ash, 1983; Cohn et al., 2001), but studies that examine the relationships between plants and abiotic properties of alkaline anthropogenic substrates are limited (Cohn et al., 2001). This study aims to address this deficit by examining substrate heterogeneity – any and all variations in element composition, minerals and pH levels - and how these relate to plant community distribution, plant species presence and plant biodiversity levels. Various techniques such as element mapping, multivariate analyses and the calculation of a few biodiversity indices will be used for this.

6.3 Materials and Methods

6.3.1 Field methodology

Fieldwork was carried out between the $10th$ and the $20th$ of July 2021 following the methodology provided in Chapter 2: Materials and Methods (section 3.1 Field methodology). In addition to the methodology detailed in Chapter 2: Materials and Methods, mapping of the distribution of the identified communities on each site were generated during fieldwork with QGIS (see Figures 4.1 and 4.2) using field observation and photographs. Additional substrate samples were collected across the Warton slag bank and the RSPB Hodbarrow Reserve, at approximately regular intervals in an approximate grid pattern, allowing for variability in slag bank topography (see Figures 4.3 and 4.4), to represent geodiversity and geochemical variation across the sites.

6.3.2 Warton slag bank, Warton, Lancashire

The Warton slag bank (Figure 2.1) is made up of waste blast furnace and steel slag (from the iron and steel industries), with a high proportion of blast furnace slag, due to the fact that the local steelworks closed down after less than two years of limited operations (Mourholme Local History Society Book Group, 2009). In the Warton area, slag was dumped between 1865 and 1929 in two sites near the river Keer (Price, 1983; Mourholme Local History Society Book Group, 2009; Grosse, 2017). The iron and steel waste was transported from the Carnforth ironworks by rail to the developing slag tips and bank in industrial tank locomotives (Grosse, 2017). Initially, slag was dumped at Keer Marsh, closer to Carnforth, on the north side of the Keer estuary. However, this area was extended - the Warton slag bank along Morecambe Bay (the study site) was created from about the early 1880s onwards, not just for the purposes of industrial waste dumping, but also: to reclaim sand flats in the area, with the slag bank acting as a sea wall (Skelcher & Askew, 2014; Grosse, 2017; Thompson & Poole, 2019; Riley et al., 2020); and for a planned alkali works which would use the slag as a feeder material, although this did not occur (Grosse, 2017). Ultimately, this slag bank did not end up enclosing the sand flats, but it does help to shelter saltmarsh today (Skelcher & Askew, 2014; Thompson & Poole, 2019; Riley et al., 2020).

Calaminarian grassland has been identified by Graeme Skelcher and AONB Officer David Askew (2014) on the site. Plants in Calaminarian grassland communities are found on soils or substrates with elevated concentrations of heavy metals compared with most substrates. Mine spoil commonly supports Calaminarian grassland, but plants in these communities can be found on a range of both anthropogenic and natural substrates (Palmer, 2008; Spalding, 2014). Other authors have not identified any plant community or communities here as Calaminarian grassland, taking note instead of the saltmarsh that borders and makes up part of the slag bank site (Thompson & Poole, 2019).

Figure 2.1 Aerial view of the Warton slag bank, with the black outline representing the study area and with the different quadrat sample locations labelled – each of these represents one open plant community on the site. The red marker in the smaller inset map shows the location of the site in the wider geographical area.

6.3.3 Hodbarrow RSPB Reserve, Hodbarrow, Cumbria

The Hodbarrow RSPB Reserve (Figure 2.2) is on the site of the former Hodbarrow iron ore mines. Iron ore was first extracted from the Hodbarrow mines in 1856 (Bidwell, 1906), with much of it being sent to the former blast furnace in nearby Millom, where blast furnace slag was created (Marshall & Davies-Shiel, 1969). While most of the local dumped blast furnace slag is found in a large slag bank in Millom to the north-west of the Reserve, much slag was brought down to the Hodbarrow mines before and after the closure of the mines, as part of sea wall developments (Bidwell, 1906; Marshall & Davies-Shiel, 1969). Construction for the first Hodbarrow mines sea wall originally began between 1889 and 1890 (Marshall & Davies-Shiel, 1969), but this wall and subsequent walls/portions of walls subsided and/or became unfit for purpose multiple times due to ingress of water and structural instability (Bidwell, 1906; Beaver, 1944; Marshall & Davies-Shiel, 1969). In about 1900, an outer barrier began to be constructed, with cement blocks and slag from the ironworks used as primary materials (Marshall & Davies-Shiel, 1969; Riley et al., 2020). Following the closure of the mines in 1968 (Marshall & Davies-Shiel, 1969), the decision was made to completely flood most of the formerly mined area, creating today's Hodbarrow Lagoon (Radford, 1995; Palmer, 2008). Today, the Hodbarrow RSPB Nature Reserve consists not just of blast furnace slag from Millom but also great quantities of iron ore sands that were brought up along with the iron ore during the mining process (Palmer, 2008). The RSPB Hodbarrow Nature Reserve is now an internationally important area for numerous species of seabirds and wading birds, with common species on-site including Ringed Plover (*Charadrius hiaticula*), Little Tern (*Sternula albifrons*) and Oystercatcher (*Haematopus ostralegus*) (Radford, 1995; Palmer, 2008).

Figure 2.2: Aerial view of the RSPB Hodbarrow Nature Reserve, with the black outline representing the study area and with the different quadrat sample locations labelled – each of these represents one open plant community on the site. The red marker in the smaller inset map shows the location of the site in the wider geographical area.

6.3.4 Substrate preparation and analyses

The 15 substrate samples from the 15 plant communities across the two study sites and the 51 grid samples across the two study sites were utilised according to the methods described in Chapter 2: Materials and Methods (section 3.2 Substrate preparation and analyses), including ICP-AES analyses carried out by ALS Global Laboratories (UK) Limited in Galway, Ireland and Materials Processing Institute in Middlesbrough.

6.3.5 Statistical analyses

Details about the biodiversity analysis, canonical correspondence analysis (CCAs) and Indval analysis methodologies are provided in Chapter 2: Materials and Methods (Section 3.3: Statistical analyses). Supplementary information related to these analyses can be found in: S3.2; S3.3; S3.7 and S3.7.3.

To examine the mapping of element concentrations and plant communities throughout the two study sites, the following processes were carried out in the mapping software QGIS, version 3.38, Grenoble: 1) Inverse Distance Weighted interpolation (IDW interpolation) analyses were carried out for most elements to visually represent variable concentrations of elements across each site; 2) the images of the IDW interpolations were altered so that community maps and interpolations could be viewed on QGIS at the same time (this involved changing the transparency of the IDW image to easily view it overlaid on the relevant community map); and 3) observations were made to assess any associations between relatively high or low concentrations of different elements and the presence of specific plant communities (see S3.6 for further details).

6.4 Results

8 open plant communities were recorded on the Warton slag bank and 7 open plant communities were recorded on the Hodbarrow RSPB Reserve, with 59 species being recorded in total (Table 4.1). Rare and uncommon species on the two sites are shown in Table 4.2. While some of the plant communities had similarities with each other and at least some overlap of species (Table 4.1), they all demonstrated species variation, as well as similarities and differences in ecological niches (Table S2.3) throughout the two study sites.

Table 4.1: Plant species recorded on the study sites during fieldwork in 2021. W = the Warton slag bank, H = the Hodbarrow RSPB Nature Reserve. Species marked with an * are uncommon and/or rare in the local area and/or in the UK

more widely (Dines & Walker, 2023; Dixon & Dines^b , 2023; Porley & Blockeel, 2014; Porter & Foley, 2023^b ; Smith & Blockeel, 2014; Rich, 2023; Stroh &

Wilmore, 2023).

Table 4.2: Species recorded in this study that are uncommon or rare locally and/or more widely (Porley & Blockeel, 2014^a ; Porter & Foley, 2023^b ; Rich, 2023; Smith & Blockeel, 2014; Stroh et al., 2014; Dines & Walker, 2023; Dixon & Dines, 2023^b ; Pearman, 2023^b ; Stroh & Wilmore, 2023; Wilmore, 2023).

Table 4.3: Warton slag bank and Hodbarrow RSPB Reserve plant communities, their species and the frequency of species in the quadrat sample representing each community. Frequency was recorded based on percentage of within-

quadrat square presence, i.e 100% for presence in all 16 squares of the

quadrat, 6.25% for presence in just 1 square of the quadrat, and so on.

Geochemical data for the quadrat (community) samples, reported in mg/kg in most cases (apart from Loss on Ignition, which is reported in %), are provided in Tables 4.4 and 4.5. Further to these plant community samples, 51 additional grid substrate samples across the two study sites (27 for Warton and 24 for Hodbarrow), data for which are provided in Tables 4.6 – 4.9. The substrate samples taken provided a detailed overview of the geochemistry, pH levels and mineralogy throughout the study sites surveyed, which could be further examined in the context of the plant communities present. Figures 2.3 and 2.4 demonstrate the locations of the grid samples on the Warton slag bank and the Hodbarrow RSPB Reserve respectively. Figures 2.5 and 2.6 show community maps of the two study sites, which were made during and shortly after fieldwork (the labels correspond to the community names used throughout the study).

Table 4.5: Substrate geochemistry across the Warton (Carnforth) and Hodbarrow sites – trace elements. All values are

reported in mg/kg.

Sample Location	Si _{O2}	Al2O3	Fe203	CaO	MgO	Na20	K20	Cr203	TiO ₂	MnO	P ₂₀₅	SrO	BaO	LOI
Carnforth 3	158460.4	54777.41	3077.472	241565.5	8924.931	1261.157	1826.325	10.26304	1857.963	1548.915	199.9742	253.6784	806.0862	19.25
Carnforth 4	140230.5	47314.98	6574.6	241565.5	8080.681	1112.785		2407.429 10.26304	2577.174	3097.83	199.9742	253.6784	1253.912	22.9
Carnforth 5	148176.9	55835.91	5805.232	260147.4	10251.61	1335.342	3652.65	20.52607	6113.298	2555.71	199.9742	338.2378	1074.782	16.15
Carnforth 6	154721	45356.75	11050.92	249427.1	10251.61	1483.714	3569.635	13.68405	2996.714	1703.806	199.9742	338.2378	806.0862	19.4
Carnforth 7	150981.5	48003.01	7623.738	249427.1	8080.681	1335.342		2822.502 13.68405	4435.137	1858.698	99.98709	253.6784	1164.347	19.35
lCarnforth 8	150514	56100.54	5945.117	239421.4	9286.753	1632.085	2573.458	13.68405	2697.043	1394.023	199.9742	253.6784	626.956	18.9
Carnforth 9	176690.4	52766.26	6434.715	213692.5	11940.11	2967.428	4980.887	61.57822	3416.255	1703.806	99.98709	253.6784	985.2165	17.8
lCarnforth 10	155655.8	48955.66	6085.002		235133.3 11397.38	1186.971	2324.414 10.26304		1498.357	697.0117	199.9742	253.6784	537.3908	19.25
Carnforth 11	228902.9	64621.47	35390.93	192609.2	9889.789		3041.613 8135.448	68.42024	3775.86	3794.842	999.8709	338.2378	1164.347	16.33
Carnforth 12	172950.9	56100.54	9372.302	220124.8	8321.895	1854.642	4233.754	20.52607	3416.255	1858.698	299.9613	253.6784	985.2165	16.1
Carnforth 13	244001	54777.41	12589.66	140436.7	8321.895	3783.47	8467.507	34.21012	3775.86	2710.601	699.9096	253.6784	806.0862	10.55
Carnforth_14	192115.8	53983.53	13149.2	210119.1	7839.467	2448.128	5894.049	20.52607	2876.846	2478.264	299.9613	253.6784	895.6514	13.45
lCarnforth 15	186974	51284.36	10911.04	197254.7	7176.127	2522.313	5645.005	27.3681	2756.977	1858.698	499.9354	253.6784	716.5211	17
lCarnforth 16	201698.2	58693.86	35740.64	240922.3	9226.449		2151.385 8716.552 68.42024		3296.386	4027.179	699.9096	338.2378	806.0862	15.99
Carnforth 17	198052.2	57000.26	22311.67	260790.7	10070.7	1780.457	5312.946		4854.677	2865.493	699.9096	338.2378	985.2165	13.14
Carnforth 18	189778.6	50331.71	9092.532	187963.7	8683.717	2893.242	6558.167	27.3681	4075.532	3407.613	499.9354	253.6784	716.5211	16.7
lCarnforth 19	183701.9	47156.21	13428.97	184390.2	8804.324	2225.571	5478.975	20.52607	3356.32	1703.806	599.9225	253.6784	806.0862	18.85
Carnforth 20	164537.1	46150.63	8113.336	247997.7	8683.717	1483.714	3403.606	13.68405	3655.992	1316.578	299.9613	253.6784	806.0862	17.85
Carnforth 21	201931.9	51390.21	18954.43	161520.1	7236.431	2373.942	7637.36	34.21012	3356.32	1858.698	799.8967	169.1189	1074.782	16.4
Carnforth 22	142100.2	45833.08	4336.438	268009	9527.967	890.2283		2241.399 10.26304	3116.583	1471.469	199.9742	253.6784	895.6514	19.15
Carnforth 23	153786.1	52078.23	5735.289	242994.9	8563.11	1335.342	2905.517	10.26304	2697.043	1084.24	399.9484	338.2378	806.0862	19.1
Carnforth 24	185571.7	48161.78	12170	194395.9	7115.824	1928.828	4814.857	20.52607	3056.649	2633.155	699.9096	253.6784	1164.347	18.8
Carnforth 25	152383.8	43292.68	5875.174	237992	7960.074	1335.342	2822.502	13.68405	2517.24	1316.578	299.9613	253.6784	895.6514	20.6
Carnforth 26	147242	42922.2	4686.151	243709.6	11095.86	1409.528	2324.414	13.68405	2876.846	1239.132	99.98709	253.6784	895.6514	21.1
Carnforth 27	139763	45092.13	6224.887	258718.1	9950.092	1632.085	2075.369	13.68405	4854.677	1084.24	99.98709	253.6784	806.0862	20.3
Carnforth 28	164537.1	40593.5	5665.347	231559.8	12121.02	1780.457	3071.547	13.68405	2756.977	1316.578	299.9613	253.6784	895.6514	20.4
Carnforth 29	200997	40805.2	7064.198	190822.4		10492.82 3560.913 5645.005 20.52607			2457.306	1239.132	199.9742		253.6784 1164.347	16.35

Table 4.6: Grid sample substrate geochemistry across the Warton (Carnforth) slag bank – major elements and LOI (loss on ignition). All values are reported in mg/kg.

Table 4.7: Grid sample substrate geochemistry across the Warton (Carnforth) slag bank – trace elements. All values are

reported in mg/kg.

Table 4.8: Grid sample substrate geochemistry across the Hodbarrow RSPB Reserve – major elements and LOI. All values

(apart from LOI) are reported in mg/kg.

Table 4.9: Grid sample substrate geochemistry across the Hodbarrow RSPB Reserve – trace elements. All values (apart from LOI) are reported in mg/kg.

After IDW interpolation analyses were carried out for most of the elements recorded on each site, the visual data for these were examined with the plant community data. Some of the analyses did not result in an easily interpretable image, as some of the elements were recorded in similar concentrations across the site. For other elements, areas of lower concentration showed as being darker in tone, while elements of higher concentration were shown as being lighter in tone. Interpretations of the IDW interpolation maps of most of the elements are reported in Table 4.10 (Warton) and Table 4.11 (Hodbarrow). Additionally, information about plant communities in relation to element concentration are reported for each of these elements – it appears that many of the plant communities on both study sites were associated with higher or lower concentrations of specific elements. For some of the clearest element and plant community associations, representative Figures are provided for Warton (Figures 2.7, 2.8) and Hodbarrow (Figures 2.9, 2.10) respectively.

Figure 2.3: Grid sample and plant community quadrat location map for the Warton slag bank. All grid samples were taken in the study area (see Fig 2.1) at approximately regular intervals in an approximate grid pattern, allowing for variability in slag bank topography. The quadrat substrate samples are labelled "CQ1", "CQ2" etc.

Figure 2.4: Grid sample and plant community quadrat map for the Hodbarrow RSPB Reserve. All grid samples were taken in the study area (see Fig 2.2) at approximately regular intervals in an approximate grid pattern, allowing for variability in slag bank topography. The quadrat substrate samples for the plant communities are labelled "HQ1", "HQ2" etc.

Figure 2.5: Plant community map for Warton slag bank. This map was generated in the field using general observations and QGIS. The legend on the bottom left of the map corresponds to the numbers of the plant communities used throughout the study (e.g 1 in the Figure for Community 1, etc) (see Table 4.1 for species information).

Figure 2.6: Community map for the Hodbarrow RSPB Reserve. This map was generated in the field using general observations and QGIS. The legend at the bottom of the Figure corresponds to the numbers of the plant communities used throughout the study (e.g 1 in the Figure for Community 1, etc) (see Table 4.1 for species information) as well as a label for the track and areas of more intense human disturbance on-site.

Table 4.10: Substrate assessment for elements which exhibited at least some variation in concentration throughout the Warton slag bank, along with assessments of plant communities in relation to those elements throughout the site, determined from IDW analyses in QGIS (see methodology in S3.6). Further information on bioavailability in is mostly provided in Kabatas-Pendias (2010), with additional citations provided in the text of the Table.

with plant growth in certain quantities.

Figure 2.7: IDW interpolation map for Al on the Warton slag bank, made in QGIS (see methodology in S3.6) and set to 70% transparency. Darker areas indicate decreased levels of Al, vice versa for light areas. Community mapping is shown in the layer below the IDW interpolation map. It was found that relatively higher Al concentrations were associated with the presence of Communities 1, 2 and 7, while relatively lower Al concentrations were associated with the presence of Communities 6 and 8.

Figure 2.8: IDW interpolation map for P on the Warton slag bank, made in QGIS (see methodology in S3.6) and set to 70% transparency. Community mapping is shown in the layer below the IDW interpolation map. Darker areas indicate decreased levels of P, vice versa for light areas. It was found that relatively higher concentrations of P associated with the presence of Community 3.

Table 4.11: Substrate assessment for elements which exhibited at least some variation in concentration throughout the Hodbarrow RSPB Reserve, along with assessments of plant communities in relation to those elements throughout the site, determined using IDW analyses in QGIS (see methodology in S3.6). Further information is provided in Kabatas-Pendias (2010), with additional citations provided in the text of the Table.

Figure 2.9: IDW interpolation map for Be on the Hodbarrow RSPB Reserve, made in QGIS (see methodology in S3.6) and set to 75% transparency. Community mapping is shown in the layer below the IDW interpolation map. Darker areas indicate decreased levels of Be, vice versa for light areas. It was found that relatively higher concentrations of Be were associated with the presence of Community 1.

Figure 2.10: IDW interpolation map for Sr on the Hodbarrow RSPB Reserve, made in QGIS (see methodology in S3.6) and set to 70% transparency. Community mapping is shown in the layer below the IDW interpolation map. Darker areas indicate decreased levels of Sr, vice versa for light areas. It was found that relatively higher concentrations of Sr were associated with the presence of Community 1, while decreased concentrations of Sr were associated with Communities 2 and 3.

Many different minerals were recorded on both the Warton slag bank and on the Hodbarrow RSPB Reserve (Table 4.12), some of which are typical of steel and/or blast furnace slag, such as calcite, akermanite and gehlenite (Ding et al., 2021; Guang-hua et al., 2008; Jung & Sohn, 2014; Piatak et al., 2015; Sun et al., 2014). Some of the minerals found in some of the samples are associated with slag that underwent a specific cooling rate upon leaving the furnace, such as cuspidine, pigeonite and augite (Ding et al., 2021; Guang-hua et al., 2008; Jung & Sohn, 2014). Quartz was particularly common in areas characterised by the iron-rich sands in Hodbarrow, although this mineral was recorded in other substrate samples in Hodbarrow, as well as in Warton.

Table 4.12: Minerals identified in samples from each study site by XRD. W = the Warton slag bank, H = the Hodbarrow RSPB Nature Reserve.

Further to the IDW interpolation analyses for the elements, mineral maps were made for the study sites to assess any associations between the presence of certain minerals and the presence of plant communities. Akermanite appeared to be at least somewhat associated with Community 5 in Warton and it was recorded throughout Community 5 in Hodbarrow, although this mineral was recorded throughout much of the rest of the site. While the presence or absence of Calcite had no obvious association with individual plant communities in Warton, this was not the case in Hodbarrow. Community 4 and Community 2 in Hodbarrow had a lack of Calcite, whilst Calcite was consistently recorded in the RSPB Reserve's Communities 1, 5, 6 and 7 - this is consistent with the fact that both Communities 2 and 4 grew in increased levels of iron sands, while the other communities grew on increased

amounts of blast furnace slag. Gehlenite was most commonly recorded on Community 1 in Warton, but the presence or absence of this mineral did not seem to be associated with any of the communities in Hodbarrow. In Hodbarrow, Linnaeite was only recorded in Community 6, Linnaeite may have been associated with Community 6, while this mineral did not appear to have any association with the Warton plant communities. Nitratine has at least some association with Community 8 in Warton and Quartz was at least somewhat associated with Community 1, but neither of these minerals, in elevated or decreased concentrations, appear to have had any specific association with plant communities in Hodbarrow.

Minimum, maximum and mean pH values for the two study sites are provided in Table 4.13. Typically, pH levels were high, demonstrating the alkaline nature of blast furnace and/or steel slag. In fact, there were no values below 7, meaning that acidic substrates were non-existent or extremely limited on the sites studied.

Table 4.13: Minimum, maximum and mean pH values for the study sites, with values reported to 3 decimal places. W = the Warton slag bank, H = the Hodbarrow RSPB Nature Reserve.

Table 4.14 shows a number of biodiversity indices for each community on the two study sites, represented by their quadrat sample. The community with the highest species richness, $q = 0$ (Hill number), $q = 1$ (modified Shannon Diversity) and q = 2 (modified Simpson's Diversity) was Community 5 in Hodbarrow, represented by 'Quadrat 5'. Other biodiverse communities recorded on-site included Warton Community 1, Hodbarrow Community 4 and Hodbarrow Community 7, which had the highest Pielou's species evenness value (Table 4.7) Sample coverage was high for the field sites, being particularly high at the Warton slag bank (Table 4.7), which indicates that the two sites were sampled thoroughly and that a high proportion of

the species were represented by the sampling method used for each community (Roswell, 2021).

Table 4.11 includes interpretation of Renyi biodiversity analyses, including the biodiversity values obtained with the commaned "renyiresult" (BiodiversityRGUI (Kindt, 2018; Kindt, 2023)). The elements analysed are the most bioavailable trace elements to plants (Kabata-Pendias, 2010) and so will likely influence the presence, growth and health of plants on a given growth substrate. For the most part, higher or lower Renyi biodiversity values were not associated with higher or lower levels of specific trace elements. One exception to this was a trend observed on the Hodbarrow RSPB Reserve - the three highest Renyi values corresponded with the three highest Cu concentrations, while the three lowest Renyi values were associated with the three lowest Cu concentrations recorded on the site.

Table 4.14: Biodiversity indices for each quadrat, representing each plant community, on the Warton slag bank and the Hodbarrow RSPB Reserve, calculated in R. Values are reported to 3 decimal places. Hill Number q = 0 was calculated using the 154stimated function in iNEXT, Hill Number q = 1 was calculated using the iNEXT function in iNEXT (Hsieh et al., 2022), Hill Number q = 2 was calculated using the iNEXT function in iNEXT, sample coverage was calculated using the estimateD function in iNEXT, Pielou's Species evenness was calculated using the specnumber function in vegan (Oksanen et al., 2022).

Table 4.15: Interpretation of Renyi diversity results calculated using the renyiresult function in the BiodiversityRGUI (Kindt, 2018; Kindt, 2023) for communities growing on differing concentrations of the most commonly recorded elements. Further context and geochemistry measurements, including maximum, minimum, mean and median measurements, with standard deviations (reported to 3 decimal places), are provided. Figures and information on bioavailability in soils more widely provided in Kabatas-Pendias (2010), with additional citations provided in the text of the Table.

Indval analyses were carried out to assess indicator plant species for the minerals on both of the study sites, results for the successful analyses are provided in Table 4.9. Higher indicator values indicate higher importance of species in relation to a mineral, with a maximum indicator value of 1. With an indicator value of 0.860 and a p value of 0.009, Mouse-ear Hawkweed (*P. officinarum*) was reported as the most statistically significant indicator species, for Nitratine. This species was also recorded as the indicator species for Periclase. It may be of benefit to further investigate relationships between plants and specific minerals on anthropogenic substrate sites more widely, as the findings are limited in terms of samples and data.

Table 4.16: Indicator species with the highest Indval scores on the Warton slag bank and the Hodbarrow RSPB Reserve, based on calculations done using the function "indval" in the labdsv R package, version 2.1-0 (Roberts, 2023). Indicator values and p values are reported to 3 decimal places.

Upon carrying out multiple ANOSIMs (Analyses of Variance) for the Warton and Hodbarrow geochemical and plant species data ("anosim" function, vegan package (version 2.6-4) (Oksanen et al., 2022)), it was found that 1) none of the ANOSIMs for the Warton data showed statistically significant differences between plant species growing on different concentrations of specific elements and 2) several of the ANOSIMs for the Hodbarrow data demonstrated statistically significant differences between species growing on different concentrations of specific elements (S3.7.2). This meant that NMDS analyses may be more statistically appropriate for the Warton

plant data, while CCAs would be more statistically appropriate for the Hodbarrow plant and substrate data. While multiple CCAs were carried out for both Warton and Hodbarrow, and NMDS analyses were done for the Warton data ("metaDMS" function in vegan, vegan package (version 2.6-4) (Oksanen et al., 2022)), only the most statistically robust results are shared here (determined using relevant ANOSIMs and anova analyses (Oksanen et al., 2022) as detailed in the Statistical Methods section of Chapter 2: Materials and Methods section 3.3, also see S3.7.2).

Figures 2.12 visually represent NMDS analysis results for the plant species and communities on the Warton slag bank. There appeared to be some variation between communities and species, as shown best in Figure 2.11. Figure 2.12 shows many of the species recorded on the Warton slag bank. While some species were clustered close together, including Yorkshire-fog (*H. lanatus*) and *Calliergonella cuspidata*, others, such as *Fissidens exilis*, were not as closely associated with other species. This demonstrates some of the species on the site that show differences in terms of Community and number, along with others which were more similar to each other.

Figure 2.11: NMDS plot for the Warton slag bank plant data. Circles represent plant communities and red crosses represent species.

After multiple CCAs were carried out for the Hodbarrow data, combining different substrate variables with the plant data, the most statistically significant (with the highest F statistic and the lowest p value) was chosen to be the most appropriate and robust CCA for the Hodbarrow plant and substrate data, seen in Figure 2.13. It can be seen in this CCA that Ba, Be, Cr, Si and Ti are statistically significantly associated with the plant species present on the Hodbarrow RSPB Reserve.

Figure 2.13: Plot for a canonical correspondence analysis for plant data for the Hodbarrow RSPB Reserve, including the most relevant variables (BaO, Be, Cr2O3, SiO2, TiO2), anova F statistic = 6.148, anova p = 0.001). The numbers represent the different plant communities on the site. Some selected species are labelled to represent variation between species and substrate chemistries.

6.5 Discussion

6.5.1 Findings from the substrate-specific analyses, spatial analyses and ordination analyses - Which geochemical variables were most closely associated with recorded plant species throughout the two study sites?

Substrate characteristics vary somewhat between the two sites, although both sites have highly alkaline and predominantely calcareous substrate (Tables 4.4-4.9, 4.12 and 4.13). Various minerals characteristic of anthropogenic substrate, including

akermanite and gehlenite (Ding et al., 2021; Guang-hua et al., 2008; Jung & Sohn, 2014; Piatak et al., 2015; Sun et al., 2014) were recorded in multiple samples from the two sites (Table 4.12). Both quadrat samples (associated with specific plant communities) and grid samples (taken across the site in a grid-like pattern) (Tables 4.5, 4.7 and 4.9) demonstrated high levels of trace elements that can pose risks in terms contamination, although this does not have such a detrimental effect on plants as to exclude them entirely or reduce biodiversity levels drastically (Tables 4.14 and 4.15).

It is clear from the spatial analyses for this study that, on both the Warton slag bank and the Hodbarrow RSPB Reserve, elevated or decreased concentrations of many elements were associated with the presence of multiple open plant communities. Specific communities on both/either Warton or Hodbarrow were associated with elevated concentrations of Al, Be, P and Sr, for example (Tables 4.10 and 4.11 and Figures 2.7, 2.8, 2.9 and 2.10). Conversely, many plant communities were restricted to or grew primarily in areas of lower concentrations of elements such as Al, Ca and Sr (Tables 4.11 and 4.7 and Figure 2.7). There were also clear differences between some plant communities within sites, with at least one plant community growing on substrate with relatively lower concentrations of one element, while at least one other community grew on relatively higher concentrations of that same element (Tables 4.3 and 4.4 and Figures 2.7-2.10).

It is demonstrated in both the IDW interpolation analyses (Tables 4.10 and 4.11) and CCA for Hodbarrow (Figure 4.13) that Be was significantly associated with plant species and at least one plant community on-site. This element's influence on plant growth and physiology is much less well-studied than that of other elements (Tanveer & Wang, 2019), but it is known from previous studies that Be can have deleterious effects on plants, although such deleterious effects are reduced in soils with high pH levels (Romney & Childress, 1965; Shah et al., 2016; Tanveer & Wang, 2019). Negative effects of elevated Be concentrations can include plant growth and seed germination reduction and, potentially, reduced Mg uptake (Romney & Childress, 1965; Shah et al., 2016; Tanveer & Wang, 2019). Considering the fact that pH levels throughout the Hodbarrow RSPB Reserve are high, it may be surmised that Be would not have such deleterious effects on the plants present as on neutral or acidic soils. However, it is not known whether Be is an essential

element for plants (Tanveer & Wang, 2019), although there are at least some studies indicating that limited levels of Be can be beneficial for P uptake in at least one plant species in the early stages of the plants' growth (Romney & Childress, 1965).

It appears that Cr levels on the Hodbarrow RSPB Reserve were significantly associated with the plant species present (Fig 4.13). This is somewhat unexpected considering the fact that Cr is not a particularly bioavailable element (Kabata-Pendias, 2010). Cr is a non-essential element for plants and therefore it can be extremely toxic to plants in high concentrations (Shanker et al., 2005; Singh et al., 2013). The toxicity of Cr affects many aspects of plant physiology and cellular processes, including: leaf and root growth (Shanker et al., 2005; Singh et al., 2013); induction of leaf chlorosis and necrosis (Singh et al., 2013); enzyme inhibition (Shanker et al., 2005). Some plants, such as those in the Brassicaceae family, can safely accumulate higher levels of Cr than other plants (Singh et al., 2013).

Si is an important element for many plant species (Epstein, 1999; Kim et al., 2017; Katz et al., 2021; Meena et al., 2021) and was significantly associated with the plant species present on the RSPB Hodbarrow Nature Reserve (Figure 4.13). Si is most readily bioavailable in the form of dissolved silicate and is usually most mobile between pH levels of 7 and 9 (Kabatas-Pendias, 2010). Si is involved in various aspects of plant physiology, including: use in increasing photosynthetic pigments (Meena et al., 2021); use in physiological defences against pests and high concentrations of trace metals (Epstein, 1999; Katz et al., 2021; Meena et al., 2021); use in responses to stress, such as drought stress (Kim et al., 2017), high salinity levels (Kim et al., 2017) and decreasing oxidative stress (Meena et al., 2021).

Ti was found to be significantly associated with plant species on the Hodbarrow RSPB Reserve (Fig 4.13). Seeing as Ti is relatively unavailable for plant uptake (Kabata-Pendias, 2010), this result is somewhat unexpected. This element can be beneficial for plants, with various positive effects for different plant species in different quantities, such as: increases in yield (Carvajal & Alcaraz, 1998); involvement in the symbiotic fixation of N in the nodules of legumes and in other species (Carvajal & Alcaraz, 1998); increased nutrient uptake (Dumon & Ernst, 1988; Carvajal & Alcaraz, 1998; Bacilieri et al., 2017); stimulation of chlorophyll content

(Dumon & Ernst, 1988; Bacilieri et al., 2017) and increased nitrate reductase, catalase and peroxidase activity (Carvajal & Alcaraz, 1998; Bacilieri et al., 2017).

Ba appeared to be statistically significantly associated with plant species presence on the Hodbarrow RSPB Reserve (Figure 4.13). Ba is not particularly bioavailable (Kabata-Pendias, 2010), nor is it an essential element in plants but it can cause deleterious effects on plant tissues and growth (Lamb et al., 2013), it would be worthwhile studying potential Ba toxicity levels for species on anthropogenic substrate sites in the future to assess this link between plant presence and Ba further.

The NMDS analyses for the Warton slag bank plant species data gave some indication as to similarities and differences between species on the site (Figures 4.11 and 4.12). Both species of the *Fissdens* moss, *F. exilis* and *F. dubius* exhibit differentiation throughout the site, demonstrating the differing ecological niches of these two closely related species (Atherton et al., 2010) (Figure 4.12). Some species were at least somewhat clustered together, such as Ribwort Plantain (*P. lanceolata*), Common Restharrow (*Ononis spinosa* subsp. *procurrens*) and White (Dutch) Clover (*Trifolium repens*), indicating similarities at both the species and community level (although many species throughout the site were not restricted to just one community, see Table 4.2).

Findings regarding biodiversity – does biodiversity differ between the two sites and do highly bioavailable trace elements influence biodiversity levels?

A variety of biodiversity indices were reported for both study sites, with some communities being particularly biodiverse, including Hodbarrow Community 5 and Warton Community 1 (Table 4.7). While the biodiversity levels of some communities on the two sites were not as high as in others, a number of the recorded species (Table 4.2) are rare, uncommon and/or declining in the local area and/or in the UK more widely, emphasising the importance of their presence as part of their plant communities, even in communities that have fewer species and reduced species evenness.

Renyi diversity analyses (Table 4.15) demonstrated that Renyi biodiversity levels were differently affected by the most bioavailable trace elements recorded, with some high biodiversity levels recorded in high concentrations of some trace elements, with the opposite being true in other cases. No clear or positive or negative trends were observed except for Cu in Hodbarrow - the three highest Renyi biodiversity values were associated with the three highest Cu concentrations, while the three lowest Renyi values were associated with the three lowest Cu concentrations.

The Indval analyses carried out for the study (see Table 4.9) demonstrate the associations between certain minerals and plants on both the Warton and Hodbarrow slag banks. It is worth interpreting these results with caution as, due to the sample sizes involved, as well as the qualitative mineral data, the Indval results (see Table 4.9) may be site-specific, with no or little relevance to other sites.

Does previous literature on plants on metal-rich substrates contextualise the Warton and Hodbarrow findings?

Comparing and contrasting the relationships between plants and substrate chemistry variables on different anthropogenic substrate sites can better inform our understanding of the colonisation and subsequent growth of plants on these sites (Clarkson & Hanson, 1980; Epstein, 1988; Dumon & Ernst, 1999; Raper et al., 2015; Bacilieri et al., 2017) as well as the biodiversity levels and different appropriate management and/or restoration practices (Bradshaw, 2000; Palmer, 2008; Maddock, 2010; Riding et al., 2010; Walmsley et al., 2017). Cohn et al. (2001) reported that on Solvay process waste tips in Poland: Carline Thistle (*C. vulgaris*) was significantly associated with Ba; and Black Medick (*M. lupulina*) was significantly influenced associated with pH level (Cohn et al., 2001). The pH of a Solvay process waste tip was recorded as being mildly alkaline (Cohn et al., 2001), with pH values between 7.5 and 7.9, which is much less than the typical values recorded for both sites (Table 4.13). Having said that, there are multiple shared characteristics across numerous alkaline anthropogenic substrate sites, such as presence of high Ca levels, large areas of bare ground (compared with sites with neutral natural soil) and reduced competition among certain species which increases the likelihood of the presence of

less competitive and also calcicolous species (Hind, 1956; Kelcey, 1975; Greenwood & Gemmell, 1978; Ash, 1983; Cohn et al., 2001).

It is already widely understood that the presence of certain elements promotes and/or limits the colonisation and persistence of certain plant species (Clarkson & Hanson, 1980; White & Broadley, 2003; Raper et al., 2015; Barth, 2020). Some plant species are solely found in soils or substrates with a particular chemistry (Barth, 2020). For example, calcicoles only grow successfully on calcareous substrates (Barth, 2020). Calcicolous plants are able to control their Ca absorption and will grow preferentially on soils with poor nutrients and high Ca levels (Barth, 2020). Some calcicoles which were recorded on the study sites, including species such as Carline Thistle (*C. vulgaris*) (Rose & O'Reilly, 2006), are restricted almost entirely to substrates with high Ca concentrations. It is therefore not surprising that differing levels of Ca affected different plant communities on both Warton and Hodbarrow (Tables 4.3 and 4.4). Soils rich in Ca tend to have lower concentrations of Ba than soils that are less rich in Ca (Bowen & Dymond, 1955).

While the assessment of species and communities is specific to the study, the current knowledge and understanding of plant growth on blast furnace and steel slag sites, as well as other alkaline anthropogenic substrate sites, informs much of the conservation, preservation and management of species and communities on these sites (Hunter et al., 1980; Ash et al., 1994; Woods, 2012; Skelcher & Askew, 2014; Holmes & Kuebbing, 2022). Skelcher & Askew (2014), for example, categorised the habitat on the Warton slag bank as Calaminarian grassland, a community that can be found on metalliferous soils and substrates (Holyoak, 2009; Maddock, 2010; Baumbach, 2012; Spalding, 2014; Jędrzejczyk-Korycińska & Szarek-Łukaszewska, 2020).

What is worth noting here, though, is that Calaminarian grassland is almost exclusively recorded on serpentine and river gravels as well as former mine sites, including those influenced by ore processing and mining spoil (Holyoak, 2009; Maddock, 2010; Baumbach, 2012; Spalding, 2014; Jędrzejczyk-Korycińska & Szarek-Łukaszewska, 2020). This habitat type is not usually recorded on blast furnace and/or steel slag. Calaminarian grassland is considered to be a priority by many organisations, policy-makers and others for conservation and preservation

(Holyoak, 2009; Maddock, 2010; Baumbach, 2012; Martin et al., 2013; Jędrzejczyk-Korycińska & Szarek-Łukaszewska, 2020).

The metal content of substrates supporting Calaminarian grassland was reported in studies by Baumbach (2012), Spalding (2014) and Preston (2017) and some of the element concentrations reported in these studies are similar to those on Warton and Hodbarrow: Cr levels tended to be in line with Spalding's (2014) measurements, if not higher; Mn levels were actually much higher on the two study sites than for those recorded in Baumbach (2012); Some high Pb levels were recorded on the Warton slag bank that were similar to values recorded in Spalding (2014), although most of the Pb concentrations in Baumbach (2012) and Preston (2017) were much higher again; Ni concentrations in parts of both sites were similar to those reported in Spalding (2014) and Baumbach (2012), although concentrations were mostly elevated in Preston (2017); and Zn levels on parts of both Warton and Hodbarrow were similarly high to those reported in both Baumbach's (2012) and Spalding's (2014) study, although Zn levels tended to be higher on Preston's (2017) sites (Tables 4.5, 4.7 and 4.9). However, both As and Cu levels on Warton and Hodbarrow were lower than almost all of the As and Cu concentrations reported (Baumbach, 2012; Preston, 2017; Spalding, 2014) and Co and Cd levels were lower on the study sites than almost all of those reported in Baumbach (2012) and Preston (2017).

It would seem that, based on the concentration of selected metals on both the Warton slag bank and the Hodbarrow RSPB Reserve, the substrates on both of these sites are comparable to those found on areas where Calaminarian grassland is present and has been previously recorded. However, labelling all of the communities on the Warton slag bank and the Hodbarrow RSPB Reserve as 'strict' Calaminarian grassland would be inappropriate, due to the variety of species and the differences between communities on both sites (Tables S2.2 and S2.3), particularly as some of the communities on-site are more similar to saltmarsh communities and/or dune communities (Table S2.3) than they are to Calaminarian grassland. Additionally, many of the species commonly recorded on Calaminarian grassland were not recorded on either of the two study sites (Baumbach, 2012; Holyoak, 2009; Jędrzejczyk-Korycińska & Szarek-Łukaszewska, 2020; Spalding, 2014), although the

fact that such species were not recorded does not necessarily mean that all of them were or are absent.

Species of plants that can only be reliably found on substrates or soil with a high metal content, not growing so well and likely outcompeted on ground with a reduced concentration of certain metals (Shaw, 1987; Holyoak, 2009; Holyoak & Lockhart, 2009; Baumbach, 2012; Preston, 2017). They are often referred to as metallophytes in the literature (Baumbach, 2012; Preston, 2017; Rainbow, 2018; Affholder et al., 2020). These species can be much more common on contaminated land, including certain anthropogenic substrate sites, than on non-anthropogenic substrates (Holyoak & Lockhart, 2009; Preston, 2017; Affholder et al., 2020; Jędrzejczyk-Korycińska & Szarek-Łukaszewska, 2020). While no obligate metallophytes were recorded on either of the two field sites, a few species of facultative metallophytes or pseudometallophytes were recorded on both study sites. While these species do not grow exclusively on metalliferous substrates or soils, they can grow well on them, especially compared with most plant species (Baker et al., 2010; Baumbach, 2012; Spalding, 2014; Rainbow, 2018). Species that are commonly associated with Calaminarian grassland that were recorded on the Warton slag bank and/or the RSPB Hodbarrow Reserve included: Harebell (*Campanula rotundifolia*); Wild Thyme (*T. praecox* subsp. *polytrichus*); Common Bird's-foot-trefoil (*L. corniculatus*); Fairy Flax (*L. catharticum*); White (Dutch) Clover (*T. repens*); Eyebright (*Euphrasia* agg.); Ribwort Plantain (*P. lanceolata*); Colt's Foot (*Tussilago farfara*); Kidney Vetch (*A. vulneraria*); and Sheep's Fescue (*F. ovina*) (Baumbach, 2012; Preston, 2017; Rainbow, 2018; Jędrzejczyk-Korycińska & Szarek-Łukaszewska, 2020)*.* The first six of these are facultative metallophytes or pseudometallophytes (Rainbow, 2018). For the most part, these species were found in varying numbers in plant communities across the two study sites, with some of them being absent from many or most of the plant communities, demonstrating spatial and community variation in both Warton and Hodbarrow (S2.3 and S2.4). Calaminarian grassland is a rare habitat type in both Lancashire and Cumbria (Palmer, 2018; Rainbow, 2018), so having this habitat, or something that is certainly at least similar to this habitat, on both study sites enhances local habitat heterogeneity and local biodiversity. The presence of legumes, including Common Bird's-foot-trefoil (*L. corniculatus*) and White (Dutch) Clover *Trifolium repens* can,

importantly, help to increase nutrient levels and improve nutrient cycling on both the Warton slag bank and the Hodbarrow RSPB Reserve (Rose & O'Reilly, 2006; Preston, 2017).

Some of the bryophytes recorded on the Warton slag bank and the Hodbarrow RSPB Reserve are beneficial for a number of reasons. Many of the species recorded on the two study sites are valuable indicators of medium or high-level nutrient status. According to Simmel et al. (2020) (who developed Ellenberg values specifically for bryophyte species, with higher values representing increased reliance of certain nutrient levels) three of the Warton species had a bryophyte Ellenberg value of 4, two of them had a value of 5 and two of them have a value of 6; and 1 one of the Hodbarrow species had a bryophyte Ellenberg value of 4, one of them had a value of 5 and one of them had a value of 6. This demonstrates that nutrient levels on both sites are sufficiently high for bryophytes, which, crucially, rely on nutrients from precipitation water, aerosols and airborne dust, as well as their growth substrate (Simmel et al., 2021). The bryophyte species recorded have a number of different ecological niches and habitat/substrate preferences. *Zygodon stirtonii*, recorded on the Hodbarrow RSPB Reserve, for example, has recently been recognised as a separate species of *Zygodon* (Stebel & Żarnowiec, 2017) and is typically recorded on bare rocks or on trees, being primarily restricted to coastal areas (*Proceedings, Transactions of the British Bryological Society*, 1963; Stebel & Żarnowiec, 2017). The *Ptychostomum* species recorded on Community 4 of the Warton slag bank could not be determined to species level, due to the absence of indicative physiological characteristics such as sporophytes (only present at certain times of year in most species of moss) but there is a chance that this could be *Ptychostomum salinum* (based on personal observation and reference to Holyoak, 2021). This is a species that is rare and specialist in the UK and other parts of Europe on saltmarshes, as well as in areas regularly grazed by sheep (Nyholm & Crundwell, 1958; Adam, 1976; Holyoak, 2021). Considering the fact that the Warton slag bank exists alongside and as part of a saltmarsh and is grazed by sheep (Skelcher & Askew, 2014), this could be a suitable habitat for *P. salinum* and further searches for this species at a more suitable time of year are worth consideration.

Despite the biodiversity and plant community value of both the Warton slag bank and the Hodbarrow RSPB Reserve, not all anthropogenic substrate sites

globally are able to support plant species. On some blast furnace and steel slag heaps, no plant growth has been observed (Scattolin et al., 2021). Chlorosis has been observed in plants growing on a blast furnace slag site, likely due to the low iron and high Ca contents in the growth substrate (Ash et al., 1994). Al(OH)-⁴, CrO₄²⁻ , MoO₄²⁻ and WO₄²⁻ have been negatively correlated with plant growth on steel slag, while Mg²⁺, Mn²⁺, Ni²⁺, HPO₄²⁻, SO₄²⁻ and Zn²⁺ positively correlated with plant growth (Scattolin et al., 2021). Plants can accumulate toxic levels of elements such as cadmium and lead in their leaves whilst growing on steel slag (dos Santos et al., 2021). Al concentration in leaves increases with increasing levels of pH in a plant's growth substrate (Scattolin et al., 2021), which could be an issue on multiple anthropogenic substrate sites. Additionally, Cr is accumulated far more in leaves with increases in substrate or soil pH (Scattolin et al., 2021).

While ferrous slag itself can potentially have high levels of certain elements (including bioavailable forms), it can also immobilize and/or reduce metal ions in substrates that have even higher concentrations of Cu, Cr, Pb and Zn, such as contaminated soils from mining processes (Kim et al., 2021; Radziemska et la., 2021; Yang et al., 2021). The ameliorating effect of slag in the presence of more acidic anthropogenic substrate can potentially provide continued future benefits to many contaminated environments. When blast furnace slag and/or steel slag is mixed with other substrates, and/or when it is used as a fertiliser, it can be beneficial for plant growth, especially in regards to the provision of Si and Ca (Wardani et al., 2021; Díaz-Piloneta et al., 2022; Lim, et al., 2022). Some plant species, such as rice, seem to grow particularly well whilst utilising various elements from blast furnace slag (Lim, et al., 2022; Wardani et al., 2021), highlighting the usefulness of this anthropogenic substrate in certain contexts. More specifically, blast furnace slag can increase the availabilities of P, Ca and Si in soil, while electric arc furnace slag has high leaching rates of Ca, Fe, Si, Al, Mn and Mg, and releases P slowly, which can help with the long-term growth of plants (O'Connor, 2001; Kong & Nurulakmal, 2018; Wang et al., 2018). These differences likely have a large impact on plants throughout the different slag study sites, as well as on legacy heaps throughout the UK with differing levels of different types of slag. It is worth understanding the nuances of ferrous slag in relation to plant growth in a variety of different contexts, to better relate the fundamentals of slag chemistry, such as pH level, to effective management, conservation, remediation, restoration and rehabilitation, for example.

Conclusion

Plant and substrate surveys carried out on the Warton slag bank and the Hodbarrow RSPB Nature Reserve in 2021 demonstrated a variety of different species and habitats, as well as geodiversity in the form of anthropogenic and mixed substrates, such as blast furnace slag, steel slag, iron sands and saltmarsh substrate. Substrate grid sample mapping and community mapping showed that varying concentrations of many of the recorded elements, such as Al, Ca, Fe, Ni, P, Sc and Si had statistically significant associations with at least some of the communities present. While the relationships between specific elements and species could not be determined for the Warton slag bank, Ba, Be, Cr, Si, and Ti were all significantly associated with plant species on the Hodbarrow RSPB Reserve. Collectively, these investigations satisfied both Objective 2: To assess statistically significant differences between substrate characteristics (such as geochemistry and pH levels) on anthropogenic substrate sites and Objective 4: To determine the elements and quantities of different elements that are statistically significantly associated with plant species and community presence throughout anthropogenic substrate sites.

Biodiversity levels varied across both study sites, but even in communities with lower biodiversity indices many locally and/or nationally rare species were recorded, such as *Fissidens exilis* on the Warton slag bank and Variegated Horsetail (*Equisetum variegatum*) on the Hodbarrow RSPB Reserve. Cu levels were positively correlated with biodiversity levels on the Hodbarrow RSPB Reserve. This investigation satisfies Objective 3: To determine the biodiversity levels of anthropogenic substrate sites using various biodiversity indices.

The relationship between plants and ferrous slag substrate deserves further investigation on multiple field sites, especially considering the current biodiversity crisis and the changes in community composition across anthropogenic substrate sites with successive vegetation communities. With the further understanding and context from this study, conservation and management of plant species and communities on ferrous slag sites can, hopefully, be much better informed and directed.

Chapter 6: Substrate heterogeneity and high plant biodiversity on a partially clay-capped slag bank

7.1 Abstract

Unremediated slag from the iron and steel industries can support uncommon and rare (Red Data list) plant species and communities. Deposits of anthropogenic substrate at brownfield sites are typically either completely capped as part of restoration efforts, or else they are not capped at all. The Barrow-in-Furness slag bank, made up of a mixture of blast furnace slag and steel slag, is unusual in that it exhibits partial clay capping, where a one metre thick clay cover was placed over part of the site. This situation presents an ideal opportunity to study the effect of capping and substrate heterogeneity on plant biodiversity. In order to test for significant differences between plant biodiversity levels on capped and uncapped parts of the site, plant biodiversity was recored in specific locations throughout the site according to plant community composition and substrate samples were collected and geochemically analysed from the same locations. The geochemical compositions of the uncapped and capped parts of the slag bank were statistically significantly different, particularly for Si, Ca, Al and V. While the biodiversity levels of capped and uncapped parts of the site were not statistically significantly different, high biodiversity levels were recorded on both capped and uncapped parts of the site, in the eleven distinct open plant communities that were studied and recorded. pH levels, as well as Al, Ba, Cd, K, Ni and Calcite were significantly statistically associated with the plant species recorded on-site. Si, Fe and Na were present in higher quantities in capped areas, for example, whilst Ca, Sr and Ba were present in higher quantities in the slag. Some species, such as Lady's Bedstraw (*Galium verum*) were almost entirely present on capped areas, whilst others, such as Mouseear-hawkweed (*Pilosella officinarum*) and Sheep's Fescue (*Festuca ovina*) were only present on the slag substrate. The substrate heterogeneity and geodiversity of the capped and uncapped parts of the slag bank provide valuable habitat for unusual plant communities and some locally uncommon and rare species of plants, including the Yellow Wort (*Blackstonia perfoliata*). This study demonstrates the importance of careful management and restoration of sites, to increase the number and diversity of

plant communities while also increasing opportunities for amenity and community use.

7.2 Introduction

Clay capping, the covering of a (usually) contaminated area with a layer of clay soil, has occurred on many sites as part of a restoration, rehabilitation, reclamation and/or remediation strategy (Melchior, 2001; Viswandham & Jessberger, 2001; Gorman, 2009; Wuana & Okieimen, 2011; Cruz & Rodrigues, 2020). Clay capping has multiple functions, which vary somewhat depending on the site in question, such as: covering of a low-nutrient substrate to permit increased plant growth (Melchior, 2001; Gorman, 2009; EPA, 2012); covering of a contaminated substrate to limit the spread and/or leaching of contaminants from the substrate (with limited success depending on various factors such as topography and permeability) (Melchior, 2001; Wuana & Okieimen, 2011; EPA, 2012; Pullin et al., 2019; Cruz & Rodrigues, 2020); and making areas of bare substrate more visually appealing in the longer term (Richards et al., 1993; Gorman, 2009; EPA, 2012; Ritchey, 2020). Clay capping is one of many types of restoration (Bradshaw, 1996; Cooke & Johnson, 2002; Hobbs and Cramer 2008; Wuana & Okieimen, 2011). Ecological restoration, revegetation, reclamation and remediation are terms that can be used rather loosely in the literature (Hobbs & Cramer, 2008), but can be defined as thus according to some papers: Restoration implies that a damaged, degraded and/or destroyed habitat, or similar, is being reinstated, or restored, to some former state (Anderson, 1995; Bradshaw, 1996; Cooke & Johnson, 2002; Hobbs and Cramer 2008; Smart et al., 2016); remediation is the process of alleviating and/or minimising the impact of harmful substances (O'Connor et al., 2021); rehabilitation can involve the modification of a terrain and/or landscape to fulfil a specific purpose, such as agriculture or forestry (Lewis et al., 2022); reclamation's purpose is to 'reclaim' land for a former purpose prior to the anthropogenic altering of such land, such as agriculture (Richards et al., 1993); and revegetation simply involves planting and/or sowing propagules on a bare or poorly vegetated site (Bradshaw, 1997). Clay capping can be used as part of any of these forms of restoration.

Some authors argue that clay capping can be a type of habitat creation (Anderson, 1995; Allan et al., 1997; Pakeman et al., 2002; Waller et al., 2017). This is because the topography of the site being capped is often very different from the original land prior to dumping or quarrying. Additionally, the clay cover may be at least somewhat different from the pre-existing natural soil that existed on the site, leading to a different plant assemblage and, therefore, a different habitat, from what was there before.

7.2.1 Different contexts of clay capping

In the UK, there are many waste sites, also known as anthropogenic substrate sites, which exist today with different levels, stages and types of restoration, remediation, reclamation, rehabilitation and revegetation (Barrett, 1992; Riley et al., 2020). Anthropogenic substrate usually consists of waste that is at least somewhat analogous to natural rock, such as colliery spoil, steel slag and oil shale spoil, which constitute natural rock that has undergone anthropogenic processes (Allan et al., 1997; Riley et al., 2020). However, because some anthropogenic substrate sites contain a mixture of many different types of waste, including old industrial equipment, plastics and ashes, along with many other materials, these additional anthropogenic materials can be part of anthropogenic substrate (Darlington, 1969; Browne & Golledge, 2007). It has been understood since the mid-late 20th century that dumping waste substrates in the environment can have negative environmental consequences which can pose threats to human health and wildlife, including: leaching of toxic metals (Barrett, 1992; Wuana & Okieimen, 2011; Scattolin et al., 2021); wind dispersal of eroded waste, including metallic dust (Barrett, 1992; Wuana & Okieimen, 2011; Cruz & Rodrigues, 2020; Scattolin et al., 2021); and contamination of groundwater with high levels of metals (Barrett, 1992; Wuana & Okieimen, 2011; Firpo et al., 2021). Various different techniques have been used to cap different types of anthropogenic substrates, depending on 1) the type of waste being capped 2) the purpose of capping 3) the current capping technologies including materials and 4) cost and feasibility of capping.

While some contaminated sites in recent years have undergone complex capping strategies (Melchior, 2001; Viswandham & Jessberger, 2001; Wuana & Okieimen, 2011; Firpo et al., 2021) with a variety of different materials including geomembranes (Firpo et al., 2021); liquid sodium silicate (Melchior, 2001), paper mill sludge (Cherian & Siddiqua, 2019), polymers (Melchior, 2001), asphalt (Viswandham & Jessberger, 2001) and granular water glass (Melchior, 2001), others have much more simple cappings with primarily or only natural materials. Clay capping can include a mixture of clay, silt, sand and gravel and can come from different areas depending on suitability of local material (Melchior, 2001; Firpo et al., 2021).

7.2.2 Blast furnace and steel slag waste sites

Anthropogenic substrate sites vary greatly in terms of toxicity, chemistry and stability (Allan et al., 1997; Riley et al., 2020). Blast furnace slag and steel slag heaps and banks have been very common in Great Britain, with many still existing today in former and current industrial areas such as South Wales, North Lincolnshire, North Lanarkshire and Cumbria (Riley et al., 2020). Blast furnace slag and steel slag are the waste products from the (crude or pig) iron and steel industries (Yildirim & Prezzi, 2011; Piatak et al., 2015; Riley et al., 2020), having a generally grey appearance and being rock-like in shape, size and texture. Both blast furnace slag and steel slag are primarily made up of CaO, SiO2, Al2O³ and MgO, but the ratios of these differ between blast furnace and steel slag $-$ SiO₂ is much more predominant in blast furnace slag than steel slag and CaO tends to make up a larger part of steel slag than blast furnace slag (Piatak et al., 2015). These variations are primarily due to differences in the raw materials fed into iron and steel furnaces (Yildirim & Prezzi, 2011; Piatak et al., 2015).

Many blast furnace and steel slag sites have undergone clay capping and other types of remediation, reclamation and restoration (Gorman et al., 2009; Riley et al., 2020). These sites can pose some risk to human health, specifically in the context of leachates (Gomes et al., 2020; Riley et al., 2020). Steel and blast furnace slag, being highly alkaline in nature, can, when in contact with water, can produce drainage water with pH values in excess of 10 (significantly increased alkalinity when compared with most levels recorded in nature) (Mayes et al., 2006; Mayes et al., 2008; Riley & Mayes, 2015; Gomes et al., 2020) and with elevated levels of metals such as Al, Cr, Cd, Pb, Cu and Ni (Mayes et al., 2008; Riley & Mayes, 2015; Gomes

et al., 2020). Blast furnace and steel slag can also contain potentially toxic concentrations of trace elements such as Ca, Fe, Mg and Si (Riley & Mayes, 2015; Scattolin et al., 2021).

Where small particles of slag are present in great quantities, dust may become an issue, especially as such dust can be harmful if inhaled (Gomes et al., 2020; Riley et al., 2020). One might argue that the capping of many steel slag sites is not required if they are not within direct contact of groundwater or freshwater, especially as these sites can have a positive impact on the capture of carbon dioxide, through the carbonation of the surfaces of pieces and/or particles of slag (Pullin et al., 2019; Gomes et al., 2020; Riley et al., 2020; Macdonald et al., 2022). However, the carbon capture potential of steel slag has only been recognised recently (Gomes et al., 2020; Riley et al., 2020; Macdonald et al., 2022) and there have been other motivations for the capping of slag banks and heaps, including both the desire to make the banks/heaps more aesthetically in keeping with the surrounding landscape and to increase plant cover on the heaps/banks (Gorman, 2009). It is also worth bearing in mind that coastal legacy slag banks and heaps likely have been and are causing issues for aquatic environments, again, due to the effects of leachates (Riley et al., 2020).

Given the various arguments for and against capping of anthropogenic substrate sites summarised, this study uses the partially capped Barrow-in-Furness slag bank in Cumbria, UK, as a case study site to investigate whether or not there are significant differences between biodiversity levels on capped and uncapped parts of the site, as well as how and if any substrate variables are statistically significantly associated with the plant species present.

7.3 Materials and Methods

7.3.1 Site background and history

The Barrow-in-Furness slag bank, made up of blast furnace and steel slag, is situated by and makes up part of the site of former steel and blast furnaces in Hindpool, Barrow-in-Furness, Cumbria (Figure 3.1 and 3.2). This was at one time the longest slag bank in Europe (Henderson & Royal, 2015). The land associated with

and near to the Barrow-in-Furness slag bank was bought by the Furness Railway Company in the 1850s (Henderson & Royal, 2015; Arnold, 2016). The first two blast furnaces in Barrow-in-Furness were constructed in 1857 and put to work in 1859 (Bainbridge, 1939; Henderson & Royal, 2015), with all ironworks on the site closing in 1963 (Henderson & Royal, 2015; Jepson, 2017). Steel production first began at Barrow-in-Furness in 1865 (Bainbridge, 1939) and continued until 1983 (Henderson & Royal, 2015; Jepson, 2017). The Barrow ironworks and steelworks, situated by the Furness railway line, were supplied with: iron ore from various mines, including the Park Iron Ore mine and the Hodbarrow mine (Bainbridge, 1939; Arnold, 2012); coke from South Durham and later from Lancashire (While, 1901; Pollard, 1955; Arnold, 2012; Henderson & Royal, 2015); and limestone from nearby quarries (While, 1901). By 1870, the Barrow Haematite Steel Works was home to fourteen blast furnaces, making iron, and eighteen Bessemer converters, making steel (Henderson & Royal, 2015). In the early 1870s, the Barrow steelworks was the largest and most efficient in the world (Pillard, 1955; Arnold, 2012). The Bessemer steel-making process, which had been recently developed in by Henry Bessemer in 1856 (Lancaster & Wattleworth, 1977; Arnold, 2012), relied on ore with a high iron content and a near absence of phosphorous (Marshall & Davies-Shiel, 1969; Lancaster & Wattleworth, 1977) (unusual in iron ore worldwide (Arnold, 2012)), which was supplied in abundance by local mines (Arnold, 2012). Siemens-Martin steel was also produced at Barrow-in-Furness from 1880 (While, 1901; Henderson & Royal, 2015), with four Siemens furnaces situated on-site in the early 1900's (While, 1901). Additionally, an electric arc furnace for steel-making was installed and was producing steel in 1952 (Henderson & Royal, 2015). The variety of furnaces on the Barrow-in-Furness slag bank led to the creation of many different types of blast furnace and steel slag, adding to the heterogeneity of the geodiversity of the Barrow-in-Furness slag bank.

Over the lifetime of the Barrow-in-Furness ironmaking and steel industries, millions of tons of blast furnace and steel slag were generated (Henderson & Royal, 2015). This slag waste was run from the furnaces into self-tipping bogies (While, 1901) and slag bank steam locomotives (Henderson & Royal, 2015) and dumped on the coastline, both on existing slag and into the sea beside the site from narrowgauge locomotives on tramways (Van Nostrand's Engineering Magazine, 1879; While, 1901). The slag bank, as it grew, continuously supplied new land for the

ironworks and steelworks (Van Nostrand's Engineering Magazine, 1879). Many changes have been made to the slag bank, including its vegetation, over time – before 1980, Common Spotted Orchid (*Dactylorhiza maculata* subsp. *fuchsii*) was well-distributed across the slag bank, but these populations had to be translocated due to redevelopment by the British Steel Corporation (who owned the site at the time) (Hunter et al., 1980). In recent years, some of the plants that have been recorded on the Barrow slag bank and on the nearby dock area include Dittander (*Lepidium latifolium*), Sickle Medick (*Medicago sativa*), Common Broomrape (*Orobanche minor*) and French Sorrel (*Rumex scutatus*) (Halliday, 1997).

On most field sites where clay capping occurs, a cap is placed over the entire site or the majority of the site in question, whether it be a bank or a heap of waste. Having said that, partial capping can occur, with the Barrow-in-Furness slag bank in Cumbria being a very interesting example. After the closure of the last ironworks on the site in 1983 (Henderson & Royal, 2015), the site has been: quarried multiple times, including in 1987; and partially remediated with clay capping in the early 2010s, which involved adding a 1 m-high layer of soil to sections of the site, including most of the southern half of the site and different large sections of the northern half of the site. (Gorman, 2009). Plant cover on the capped areas is much more than that of the uncapped areas of the slag bank, which appear to be more bare and exposed, with more limited plant cover (Figures 3.1 and 3.2). While plant cover on the uncapped areas of the slag bank may seem to be insufficient and perhaps aesthetically unsatisfactory (Ash et al., 1994; Allan et al., 1997; Lorimer, 2008; Bickers, 2017; McCallum & Sardo, 2021), there is much literature demonstrating the floristic importance of exposed/more exposed blast furnace, steel slag and other alkaline wastes (Hind, 1956; Greenwood & Gemmell, 1978; Ash et al., 1994; Cohn et al., 2001), as 1) the chemistry of blast furnace and steel slag can greatly differ from that on surrounding natural substrates, providing the potential for more specialist plant species to colonise which otherwise struggle to colonise local natural substrate (Ash et al., 1994; Skelcher & Askew, 2014; Gomes et al., 2020) and 2) the heterogeneity of slag substrate could provide for increased biodiversity and heterogeneity of species within the local area (Gomes et al., 2020). With some claycapped areas and some uncapped areas, the Barrow-in-Furness slag bank provides an ideal case study for examination of plant species, plant communities and

biodiversity levels on both capped and uncapped slag, where plant propagules are free to colonise both capped and uncapped parts of the slag bank with relative ease, in comparison to separate capped and uncapped sites.

Figure 3.1: Capped and uncapped areas of the Barrow-in-Furness slag bank, taken by Savanna van Mesdag during 2021 fieldwork. A: north-facing view (foreground horizontal field of view ~3 m) of uncapped part of the slag bank, on the western side of the top of the bank. See corresponding arrow in Figure 3.2 for direction of the view. B: north-facing view (foreground horizontal field of view ~3 m) of capped part of the slag bank, on the western side of the top of the bank. See corresponding arrow in Figure 3.2 for direction of the view. C: south-facing view (foreground horizontal field of view ~2 m) of uncapped part of the slag bank, on the eastern side of the bank. See corresponding arrow in Figure 3.2 for direction of the view. D: south-facing view (foreground horizontal field of view ~3 m) of capped part of the slag bank, on the western side of the top of the bank. See corresponding arrow in Figure 3.2 for direction of the view.

Figure 3.2: An aerial view of the Barrow-in-Furness slag bank, with the shaded area indicating the approximate areas of clay cover and the numbered points showing the sample locations, one for each plant community. The A arrow shows the direction of view in Figure 3.2A, the B arrow shows the direction of view of Figure 3.2B, etc.
7.3.2 Field methodology

Fieldwork was carried out between the 15th and 21st of July 2021 following the methodology provided in Chapter 2: Materials and Methods (section 3.1 Field methodology). Eleven plant communities were defined and recorded on the Barrowin-Furness slag bank.

7.3.3 Substrate preparation and analyses

The 11 substrate samples from the 11 plant communities across the six study sites were utilised according to the methods described in Chapter 2: Materials and Methods (section 3.2 Substrate preparation and analyses), including ICP-AES analyses carried out by ALS Global Laboratories (UK) Limited in Galway, Ireland.

7.3.4 Statistical analyses

Details about the biodiversity analysis, canonical correspondence analysis (CCAs) and Indval analysis methodologies are provided in Chapter 2: Materials and Methods (Section 3.3: Statistical analyses). Supplementary information related to these analyses can be found in: S4.2; S4.3; S.4 and S4.5.4).

To investigate the potential differences between substrate properties (pH values, major and trace element concentrations) of capped and uncapped parts of the slag bank, two-sample t-tests and Wilcoxon Rank Sum tests were conducted in the R statistical environment (Posit team, 2023)) for most of the substrate data (except for those elements where concentrations were the same or too similar in all substrate samples). Normality of data were tested using a combination of randomisation tests, observations of histograms and observations of means for the data sampled. Most data were non-normal so Wilcoxon Rank Sum tests were done for most of the substrate samples rather than two sample t-tests, which are only appropriate for normally distributed data. Further details can be found in S4.

7.4 Results

55 plant species (Table 5.1) were recorded in 11 open plant communities (Table 5.4) across the Barrow-in-Furness slag bank. 7 of these plant communities occurred on capped ground on the site, the other 4 grew on uncapped, exposed steel and blast furnace slag. The slag bank had plant communities that could not be included in sampling either due to lack of accessibility or due to the fact that they consisted primarily of shrubland, which were not considered to be open plant communities for the purposes of the study. Table 5.2 shows species on the site that are uncommon and/or rare locally or more nationally.

Table 5.1: Plant species recorded on the Barrow-in-Furness slag bank during the fieldwork period in July 2021, with information about presence in uncapped and/or capped substrates.

Table 5.2: Species recorded in this study that are uncommon or rare locally and/or more widely (Stroh et al., 2014; Pearman, 2023b; Porley & Stroh, 2023).

The pie charts in Figure 3.3 show the species numbers and total abundance counts in both capped and uncapped parts of the site. It can be seen that uncapped species numbers and total abundance are lower than those of capped of substrates.

Figure 3.3: Pie charts representing the total species numbers and total abundances of plants on the uncapped and capped parts of the Barrow-in-Furness slag bank.

Geochemical data, reported in mg/kg in most cases (apart from Loss on Ignition, which is reported in %), is provided in Tables 4.3 and 4.4.

All values reported in mg/kg.

Table 5.4: Substrate geochemistry across the Barrow-in-Furness slag bank – trace elements. All values reported in mg/kg.

Table 5.5: Commonly recorded plant communities for those species recorded

on the Barrow-in-Furness slag bank.

Quadrat 11 community This community had some similarities with the Quadrat 9 community, with many bare patches of slag and mostly scattered plants Red Fescue (*F. rubra*) was the most common species, other common plants included Oxeye Daisy (*Leucanthemum vulgare*), Perforate St John's-wort (*Hypericum perforatum*) (exclusive to this community) and Common Bird's-foot-trefoil (*L. corniculatus*). While Blue Fleabane (*E. acer*) was also present in this community, it was less common than in the Quadrat 9 community.

Recorded on uncapped blast furnace and/or steel slag.

A variety of different minerals were recorded on the Barrow slag bank (Table 5.6), some of which are typical of steel and/or blast furnace slag, such as calcite, akermanite and gehlenite (Ding et al., 2021; Guang-hua et al., 2008; Jung & Sohn, 2014; Piatak et al., 2015; Sun et al., 2014). Some of the minerals found in some of the samples are associated with slag that underwent a specific cooling rate upon leaving the furnace, such as cuspidine, pigeonite and augite (Ding et al., 2021; Guang-hua et al., 2008; Jung & Sohn, 2014). Others, such as kaolinite and muscovite, are more characteristic of natural substrate, due to being recorded from the capped soil.

Table 5.6: Minerals present in for each plant community (labelled as "C1", "C2" etc) on the Barrow slag bank substrate as determined by X-ray Diffraction. Asterisks denote plant communities growing on capped substrates.

pH levels indicated that the pH throughout the site was alkaline (Table 5.7), including in the clay capping substrate. While pH levels on the capped and uncapped parts of the site were not statistically significantly different ($W = 5$, $p = 0.1091$), the two highest pH levels were recorded from uncapped, partially vegetated areas (Table 3.4 – Quadrats 9 and 11, further context in Figure 3.3). Figure 3.4 shows the mean values and standard deviations of pH on the uncapped and capped parts of the slag bank.

Table 5.7: pH levels of the Barrow slag bank substrate for each of the plant communities. pH values are reported to 2 decimal places. pH values were determined by calculating the mean of 2 matching samples (from the same plant community sample) (see further sample methodology in S4.2).

Mean of uncapped = 9.964 , SD = 1.058 Mean of capped = 8.864 , SD = 0.917

Figure 3.4: Means and standard deviations of pH measurements on the uncapped and capped parts of the Barrow-in-Furness slag bank. Values reported to 3 decimal places.

A range of different elements was recorded on the Barrow slag bank (Tables 5.3 and 5.4). Elemental concentrations varied due to the presence of clay substrate on the site (Table 5.5). There were significant differences between capped and uncapped areas in terms of geochemistry (Table 5.8). In particular, levels of Na, V, Ca, Si, Fe and Be were statistically significantly different between the capped and uncapped parts of the slag bank (Table 5.8). These results demonstrate key

differences between the different parts of the site in terms of geochemistry, highlighting the geodiversity and site heterogeneity.

Table 5.8: Results from t-tests and Wilcoxon Rank Sum Tests comparing element concentrations of capped and uncapped areas of the Barrow slag bank, with the geochemistry results (mg/kg) presented to 2 dp and the p values presented to 3 dp.

Table 5.9 shows multiple biodiversity indices, demonstrating the variation in biodiversity across the site between the different plant communities. Sample coverage for the site is high, indicating that a high proportion of the species present in the community were recorded during sampling. While species richness ($q = 0$ in Table 3.6) was highest for the Quadrat 5 community, one of the 'slag communities', species evenness was highest for the Quadrat 2 community (Pielou's species evenness in Table 3.6), which was on a capped part of the slag bank (Van Dyke & Lamb, 2020). Shannon Diversity in Table 3.6 (q = 1) increased as the number of species became more equal in abundance, explaining why Shannon Diversity was highest in the Quadrat 2 community (Van Dyke & Lamb, 2020). Simpson's Diversity $(q = 2)$ was also highest for the Quadrat 2 community. It can be surmised that while Species Richness was highest in the Quadrat $5 (q = 0$ values in Table 5.9), biodiversity was in fact highest in the Quadrat 2 community.

There were no significant differences observed between biodiversity indices in Capped and Uncapped sites on the Barrow slag bank (mean of qD and Species diversity capped = 13.351 (SD = 2.786), uncapped = 14.5 (SD = 4.214), $W = 10$, P = 0.501; mean of Shannon diversity capped = 6.299 (SD = 1.726), uncapped = 6.779 $(SD = 2.07)$, W = 12, P = 0.788; mean of Simpson diversity capped = 4.837 (SD = 1.632), uncapped = 4.532 (SD = 1.813), t = 0.278, P = 0.791; mean of species evenness capped = 0.704 (SD = 0.8), uncapped = 0.714 (SD = 0.125), t = -0.137 , P = 0.897) (Figure 3.4 A, B, C and D). It is worth noting that the community with the highest diversity was on an uncapped part of the slag bank and that species richness was slightly higher on uncapped parts of the slag bank overall (see $q = 0$ values in Table 3.6).

Table 5.9: Biodiversity indices for the different communities on the Barrow slag bank, calculated in R. Values are reported to 3 decimal places. Hill Number q = 0 was calculated using the "iNEXT" function in iNEXT (Hsieh et al., **2022), Hill Number q = 1 was calculated using the "iNEXT" function in iNEXT, Hill Number q = 2 was calculated using the "iNEXT" function in iNEXT, sample coverage was calculated using the "iNEXT" function in iNEXT, Pielou's Species evenness was calculated using the "specnumber" function in vegan (Oksanen et al., 2022).**

Figure 3.5: Means and standard deviations of the different biodiversity indices calculated using functions in iNEXT (Hsieh et al., 2022 and vegan (Oksanen et al., 2022).

Table 5.10 shows results of Renyi diversity analyses (using renyiresult in the BiodiversityRGUI (Kindt, 2018; Kindt, 2023)) for the Barrow-in-Furness slag bank communities growing on both the lowest and highest concentrations of the most

bioavailable trace elements recorded (Kabatas-Pendias,2010). There were no clear positive or negative trends associated with Renyi biodiversity levels and concentrations of the trace elements. The lowest Renyi values were associated with the highest concentrations of certain elements – Hg, Pb and Sr. Meanwhile, the highest Renyi values were associated with the highest concentration of Mo. See further information and context in S4.5.2.

Table 5.10: Interpretation of Renyi diversity results calculated using the renyiresult function in the BiodiversityRGUI (Kindt, 2018; Kindt, 2023) for communities growing on differing concentrations of the most commonly recorded elements. Further context and geochemistry measurements, including maximum, minimum, mean and median measurements, with standard deviations (reported to 3 decimal places), are provided. Figures and information on bioavailability in soils more widely provided in Kabatas-Pendias (2010), with additional citations provided in the text of the Table.

Results and Interpretation

Indval analyses were successfully carried out for some of the minerals on the Barrow-in-Furness slag bank (see S4.5.4), shown in Table 5.11. The closer the indicator value was to 1, the more it demonstrated the association between the specific plant species and the specific mineral. Such findings may be specific to the Barrow-in-Furness slag bank, considering the sample size and the qualitative mineral data generated.

Table 5.11: Indicator species for commonly recorded minerals on the Barrowin-Furness slag bank, based on calculations done using the function "indval" in the labdsv R package (Roberts, 2023). Indicator values are reported to 3 decimal places.

After carrying out several CCAs on the substrate and plant data for the slag bank (see S4.5), one CCA was chosen based on its high F statistic (1.6234) and low p value (0.001) from its test anova, the plot for this CCA is shown in Figure 3.6. It can be seen from Figure 3.3 that many of the plant communities and species were directly statistically significantly associated with specific substrate chemical variables, with clusters in the CCA plot demonstrating that some plant species and communities were more associated with specific variables than others.

Figure 3.6: Plot for a canonical correspondence analysis demonstrating the associations of the most statistically significant variables (Al2O3, BaO, Cd, K2O, Ni, Calcite and pH level), (anova F statistic = 1.6234, anova p value = 0.001) with plant species presence on the Barrow slag bank. The numbers throughout the graph represent different communities (Table .2), while selected species are represented on the graph to demonstrate differences between species and substrate throughout the site.

7.5 Discussion

7.5.2 Findings from substrate-specific analyses.

T-tests and Wilcoxon tests (Table 5.8) demonstrated that the capped and uncapped substrates on the Barrow-in-Furness slag bank are statistically significantly different in terms of most of the recorded elements, including Si, Ca and Ni. This demonstrates the substrate heterogeneity of the site provided by the mixture of clay capping and uncapped ferrous slag substrate. Minerals and pH levels also varied across the site, demonstrating further differences in chemistry, although pH levels were not statistically significantly different across the slag bank. Clay capping put down as part of remediation usually has pH levels closer to neutral (Melchior, 2001), so the alkaline clay capping happens to be closer to the typical ferrous slag pH than would otherwise be expected.

7.5.2 Findings regarding biodiversity – does biodiversity differ between uncapped and capped parts of the Barrow-in-Furness slag bank and do highly bioavailable trace elements influence biodiversity levels?

Biodiversity indices calculated using the plant data (Table 5.9) indicate that plant biodiversity on the Barrow slag bank varied throughout the site, representing the diverse communities and somewhat less diverse communities on both capped and uncapped parts of the slag bank. Interpretation of the Renyi biodiversity analyses (Table 5.10) indicated varying concentrations of bioavailable trace elements associated with differing biodiversity levels throughout the site. The highest levels of Hg, Pb, Sr were associated with the lowest biodiversity levels, while the highest level of Mo was associated with the highest biodiversity level on the slag bank. Indval analyses (Table 3.8) indicated that certain plant species were closely associated with certain minerals on the Barrow-in-Furness slag bank. This merits further study to inform our understanding about the mineral preferences of certain plant species on other anthropogenic substrate sites. The fact that biodiversity was not significantly lower on capped or uncapped parts of the site demonstrates the value of the substrate heterogeneity and geodiversity present for plant species on the site. Such substrate heterogeneity is important from a geodiversity point of view (Alahuhta et al., 2022), particularly in regards to the fact that blast furnace and steel slags have high carbon dioxide capture potential, with the mineralisation of the outer surfaces of slag pieces and particles (Pullin et al., 2019; Gomes et al., 2020; Riley et al., 2020; Macdonald et al., 2022).

The plant species recorded have demonstrated a range of substrate and habitat preferences (Table 3.2 and S4.1) and demonstrated some taxonomic diversity (Table 3.1). Some of the plant species recorded have scattered distribution or else are uncommon in Cumbria, such as Yellow Wort (*B. perfoliata*) (Porley & Stroh, 2023)*,* Carline Thistle (*C. vulgaris*) (Perring & Walker, 2023), Parsnip (*Pastinaca sativa*) (Southam & Mountford, 2023), Salad Burnet (*P. sanguisorba*) (Pescott, 2023) and Lesser Chickweed (*Stellaria apetala*) (Watts & Lusby, 2023^b)

and so are worth protecting and preserving from a local conservation point of view (Halliday, 1997). The *Helictotochloa* record is likely to represent Annual Meadowgrass (*Helictochloa pratensis*), which is rare in most of Cumbria (Halliday, 1997; Perring & Pearman, 2023), making this species particularly important for the Barrowin-Furness slag bank. Yellow Wort (*B. perfoliata*) is a rare species in the UK, so its presence on a capped part of the slag bank and its possible presence on other parts of the slag bank, including uncapped sections, reinforces the floristic importance of the site (Gomes, 2020; Porley & Stroh, 2023).

7.5.3 Which geochemical variables were most closely associated with recorded plant species throughout the six study sites?

Multiple elements were statistically significantly associated with plant species presence throughout the Barrow-in-Furness slag bank, as demonstrated in Figure 3.3. Al appears was statistically significantly associated with plant species presence on the Barrow-in-Furness slag bank (Figure 3.6). Al ions in soil vary depending on pH levels, the most mobile fractions occur in pH levels of less than 5.5, so Al solubility would have been low for all of the plant communities (Pavlovkin et al., 2009; Kabata-Pendias, 2010). Plants can benefit from low Al concentrations, with this element helping to activate some enzymes and help to dictate the physical properties of membrane and plasma permeability (Kabata-Pendias, 2010). As the plant communities all grew on alkaline soils, increasing levels of Al (with only a limited amount being available) may have been particularly beneficial for individual plants and different plant species in most of the communities recorded.

Calcite was statistically significantly associated with plants on the slag bank (Figure 3.6). Ca from calcite is utilised by plants for multiple cellular and physiological purposes - for example, it is needed in cell walls and membranes as part of the overall structure and is also used as an intracellular messenger (White & Broadley, 2003). Despite this, Ca can be present in excess concentrations in soil or substrate for many plant species (White & Broadley, 2003; Barth, 2020). Calcicoles, plants that are adapted to high levels of Ca in soils, seemed to be particularly wellsuited to the high-calcium substrates on the site (Table 5.1, Figure 3.5 and S4.1), particularly on the exposed slag (see CaO Wilcoxon Rank Sum test results and

mean values for capped and uncapped in Table 3.5), while other plant species which were absent on the site may prefer a decreased level of Ca (White & Broadley, 2003; Barth, 2020). High Ca concentrations in soil also increase the pH levels, the alkalinity produced by high Ca levels also favours calcicoles and excludes species which are not adapted to such levels. pH levels were also statistically significantly associated with plants on the Barrow-in-Furness slag bank (Figure 3.6).

K is a necessary element in plants, and in its limited bioavailable forms (Yadav & Sidhu, 2016), it can be used, for example, for or as: an activator of multiple important plant enzymes (Ericsson & Kähr, 1993; Xu et al., 2020); osmoregulation (Ericsson & Kähr, 1993); cell growth; and plant growth (Xu et al., 2020). Differences between levels of K on different parts of the Barrow-in-Furness slag bank (Table 5.3) may have led to certain levels of K being statistically significantly associated with plant species presence across the site (Figure 3.6).

Ba appeared to be significantly associated with plant species presence and distribution on the Barrow-in-Furness slag bank (Figure 3.6). This is perhaps unexpected as Ba is not particularly bioavailable compared with many other elements (Kabata-Pendias, 2010). It is not an essential element in plants but it can cause deleterious effects on plant tissues and growth (Lamb et al., 2013). It would be worthwhile studying potential Ba bioavailability on anthropogenic substrate sites in the future to assess this finding further (Bowen & Dymond, 1955).

Pb is not an essential element for plants and toxic levels of this metal can lead to problems such as decreases in plant growth and, potentially, decreases in chlorophyll content (Zeng et al., 2007; Collin et al., 2022). It is one of the most bioavailable trace elements, with some of the levels recorded on-site highlighting potential Pb contamination (Table 5.4 and Table 5.10) (Kabata-Pendias, 2010). At lower levels, Pb can stimulate microorganisms in soil, leading to benefits for plants such as an increase in chlorophyll levels (Zeng et al., 2007; Collin et al., 2022). It might be surmised that Pb had mixed positive effects on some of the plants on at least one of the plant communities, as it is statistically significantly associated with plants on the site (Figure 3.6).

Ni, readily bioavailable to plants (Kabata-Pendias, 2010), is an essential element for many plant species (Brown et al., 1987; Seregin & Kozhevnikova, 2006): it is a component of the enzyme urease, which breaks down excess urea in, for example, the tips of leaves in plants (Brown et al., 1987; Seregin & Kozhevnikova, 2006) and it is needed during embryo development in seeds in at least some plant species (Brown et al., 1987; Seregin & Kozhevnikova, 2006). Lasota et al., (2020) found that on anthropogenically modified land, higher soil Ni concentrations were associated with increased urease activity, benefitting plants. It is demonstrated in Figure 3.6 that Ni was significantly associated with the presence of plant species on the Barrow-in-Furness slag bank.

The fact that Cd levels were statistically significantly associated with plants on the slag bank (Fig 3.6) is surprising, seeing as Cd is a non-essential element to plants and is also the most bioavailable trace element (Kabata-Pendias, 2010). Some of the Cd levels recorded on the slag bank were not at levels that pose contamination risks for plants (Table 5.5 and Table 5.10), although Cd is less bioavailable to the plant communities in the study than those recorded in neutral pH levels (Martin-Garin et al., 2002; Kabata-Pendias, 2010). Cd levels, in fact, pose an advantage for individual plants in regards to herbivory and disease. Levels of herbivory and pathogens are reduced in plants with higher than typical Cd levels (Boyd, 2007; Wang et al., 2022), which could have helped the plants on the Barrowin-Furness slag bank.

7.5.4 How does substrate heterogeneity promote biodiversity?

Many of the plant species recorded are specialists of certain types of substrate, so are able to utilise the various chemical aspects of the exposed and uncapped areas of the slag bank, as well as the capped areas of the slag bank (Hind, 1956; Ash et al., 1994; Halliday, 1997; Cohn et al., 2001). For example, multiple species on the Barrow-in-Furness slag bank, including Carline Thistle (*C. vulgaris*) and Yellow Wort (*B. perfoliata*) are calcicoles, meaning these plants are able to control absorption of calcium and will grow preferentially on soils with poor nutrients and high levels of calcium (Barth, 2020), including the bare slag areas on the Barrow slag bank and on other slag banks (Gomes et al., 2020). It is statistically significantly supported by the CCA for this study (Figure 3.6) that pH levels are statistically significantly associated with plant species presence on the Barrow-in-Furness slag bank. This is in line with

many previous studies which have demonstrated the influence of pH levels on plant species on various types of anthropogenic substrates with varying pH levels (Thomas, 1930; Greenwood & Gemmell, 1978; Allan et al., 1997; Ash et al., 1994; Harvie, 2004).

Species which have previously been recorded on blast furnace slag and were also recorded on the Barrow-in-Furness slag bank, some of which are calcicoles, include: Common Bird's-foot-trefoil (*L. corniculatus*); Yellow Wort (*B. perfoliata*)*;* False Oat-grass (*Arrhenatherum elatius*), Common Mouse-ear (*Cerastium fontanum*); Red Fescue (*Festuca rubra*); Yorkshire-fog (*H. lanatus);* Black Medick (*M. lupulina*); Cat's-Ear (*Hypochaeris radicata*), Ribwort Plantain (*P. lanceolata*), Common Ragwort (*Senecio jacobaea*); Glaucous Sedge (*Carex flacca*); Common Centaury (*Centaurea erythraea*); Dandelion (*Taraxacum* agg.), Mouse-earhawkweed (*P. officinarum*); Wild Mignonette (*Reseda lutea*); Red Clover (*Trifolium pratense*); and White Clover (*Trifolium repens*) (Ash et al., 1994). Other plant species which, to the authors' knowledge, have not been previously recorded on blast furnace or steel slag, have grown on substrates which are similar in chemical composition. For example, Blue Fleabane (*Erigeron acer*), an introduced plant species in the UK recorded on the Barrow-in-Furness slag bank, is commonly associated with limestone dumps in the north of England, meaning it specialises on highly alkaline substrate, at least in the UK (Hind, 1956). Many of the calcicolous species recorded on the Barrow slag bank have been previously recorded on alkaline waste heaps and banks (Hind, 1956; Gemmell, 1978; Cohn et al., 2001; Greenwood & Gomes et al., 2020). In Europe, the establishment of calcareous grassland on many alkaline waste heaps has permitted this floral assemblage to grow in some areas where they would otherwise been unable or less likely to establish (Ash et al., 1994; Gomes, 2020).

The Barrow-in-Furness slag bank plant communities were dissimilar to calcareous grassland communities in other parts of Cumbria, which are often characterised by different dominant species (Palmer, 2008). It can be surmised that the species assemblages on-site are unusual in the context of the local area and the county as a whole (Palmer, 2008) and, with some locally important species, the communities existing on the slag bank deserve careful management and conservation. The reason that management is worthwhile is because, as many

authors, surveyors and/or botanists have noted, communities of floristic interest on alkaline waste heaps and banks in the UK are, have been, or could be in danger of disappearing with further successional stages in plant community composition, as these more unusual communities are replaced with more 'typical' plant communities (Hind, 1956; Greenwood & Gemell, 1978; Ash et al., 1994; Cohn et al., 2001; Palmer, 2008). The Barrow slag bank itself has also undergone quarrying in the past (Gorman, 2009), which would be undesirable and unwise from a current biodiversity standpoint.

7.5.5 Limitations of anthropogenic substrates as growth substrates for plants

While the Barrow-in-Furness slag bank hosted many species on both the capped and uncapped parts of the site, including locally important species, not all anthropogenic substrate sites globally are able to support plant species. On some blast furnace and steel slag heaps, no plant growth has been observed (Scattolin et al., 2021), while this is, of course, not the case on many other slag heaps and banks (Hunter et al., 1980; Ash et al., 1994; Skelcher & Askew, 2014). It is possible that the chemistry of some sites is just too 'extreme' for the growth of plants without amendment of the substrate, meanwhile the chemistry of some slags, including those on legacy heaps, was and is less prohibitive for plant growth, even if the plant communities that still exist on those banks or heaps are at a lower stage of succession than plant communities on many natural substrates. Plants can accumulate toxic levels of elements such as Cd and Pb in their leaves whilst growing on steel slag (dos Santos et al., 2021). Al(OH)⁻⁴, CrO₄²⁻, MoO₄²⁻ and WO₄²⁻ have been negatively correlated with plant growth on steel slag, while Mg^{2+} , Mn²⁺, Ni²⁺, $HPO₄²$, SO₄² and Zn²⁺ positively correlated with plant growth (Scattolin et al., 2021). Chlorosis has been observed in plants growing on a blast furnace slag site, likely due to the low iron and high calcium contents in the growth substrate (Ash et al., 1994). While the physiology and growth of individual plants was not recorded or directly investigated during this study, the statistically significant differences between certain elements on capped and uncapped parts of the slag bank, such as Cd and Ni (Table 5.8) will most likely have direct effects on the physiology and growth of individual plants.

200

Despite the difficulties that many plants have with growing directly on blast furnace and/or steel slag, there are some plant species that in fact are adapted to tolerate high concentrations of certain elements that would otherwise be toxic to other plant species. Some generalist plant species, such as Ribwort Plantain (*P. lanceolata*) will tolerate a wide range of substrate and soil conditions and so can reliably be found on many natural and anthropogenic habitats (Sagar & Harper, 1964). Some species of plants can only be reliably found on substrates or soil with a high metal content, as these plants have adapted to metalliferous ground so much that they do not grow so well and/or are outcompeted on ground with a reduced concentration of certain metals (Shaw, 1987; Affholder et al., 2020). These plants, which are often referred to as metallophytes in the literature (Affholder et al., 2020) can be much more common on contaminated land, including certain anthropogenic substrate sites, than on non-anthropogenic substrates (Affholder et al., 2020). While no strict metallophytes were recorded on the Barrow slag bank in this study, facultative metallophytes (those that are not restricted to substrates with high metal concentrations) have been recorded on other blast furnace and/or steel slag banks (S4.1) and, potentially, individual plants that are adapted to high metal concentrations grow on the Barrow-in-Furness slag bank, although demonstrating this conclusively was outside the scope of this study.

7.5.6 Relative advantages of the uncapped and capped areas on the Barrow-in-Furness slag bank

While slag itself can potentially have high levels of certain elements, it can also immobilize and/or reduce metal ions in substrates that have even higher concentrations of Cu, Cr, Pb and Zn, such as contaminated soils from mining processes (Kim et al., 2021; Radziemska et al., 2021; Yang et al., 2021). The ameliorating effect of slag in the presence of more acidic anthropogenic substrate can potentially provide future benefits to many contaminated environments in the future. When blast furnace slag and/or steel slag is mixed with other substrates, and/or when it is used as a fertiliser, it can be beneficial for plant growth, especially in regards to the provision of Si and Ca (Wardani et al., 2021; Díaz-Piloneta et al., 2022; Lim et al., 2022). Some plant species, such as rice, seem to benefit

particularly well from various elements from blast furnace slag (Wardani et al., 2021; Lim et al., 2022). All of this demonstrates that the utilisation of ferrous slag is nuanced and provides different services in different contexts, whether it is used directly on land or in other applications.

While industries such as the iron and steel industries in Barrow-in-Furness have caused large amounts of pollution, health risks and ecological damage (Bradshaw, 1995; Bradshaw, 1997; Henderson & Royal, 2015), it is not feasible, both from a practical and an economical point of view to try and return every single post-industrial site to exactly what it was before the industries began (Bradshaw, 1995). Considering this, it is worthwhile to assess the value of such sites in the modern day, both for wildlife and for people. It is clear from this study that the Barrow-in-Furness slag bank provides a large area for: multiple plant communities which differ from plant communities on natural substrates; locally uncommon and rare plant species; and habitat and substrate specialists. From a 'people point of view', the Barrow slag bank is now used primarily for recreation – there are many tarmac and other well-established paths throughout the site, with a good portion of these being suitable for wheel-chair users (Gorman, 2009). Concerning other wildlife on the Barrow-in-Furness slag bank – an uncommon species of butterfly in the UK, multiple Grayling (*Hipparchia semele*), was observed on the site (personal observation), demonstrating the value of anthropogenic substrate, including the areas of bare, uncapped slag, for this invertebrate species (Tropek et al., 2017). Overall, it is clear that the Barrow-in-Furness slag bank is a valuable site for plant species and communities, especially with its substrate heterogeneity in both the capped and uncapped areas. The preservation, conservation and gentle, ecologically-minded management of the Barrow-in-Furness slag bank for future generations is justified by both the findings of the study and the findings of previous studies.

7.6 Conclusion

The Barrow-in-Furness slag bank (Gorman, 2009) was used as a case study to investigate whether or not the substrate heterogeneity on-site leads to statistically significantly different biodiversity levels on capped and uncapped substrate. Eleven open plant communities were identified which exhibited variation in terms of overall species composition throughout the site, with each of these communities being associated with capped or uncapped substrate. In terms of substrate properties, there were statistically significant differences between uncapped and capped parts of the site, with Si, Ca and Ni being just some of the elements that significantly differed in concentration in clay capping substrate and uncapped slag. This satisfies Objective 2: To assess statistically significant differences between substrate characteristics (such as geochemistry and pH levels) on (an) anthropogenic substrate site.

While biodiversity levels, and geochemical and mineralogical properties, were not statistically significantly different between capped and uncapped parts of the site, the Barrow-in-Furness slag bank demonstrates how a mixture of clay capping remediation and bare, partially vegetated exposed slag, can provide an area of substrate heterogeneity and biodiversity. Renyi biodiversity analyses did not find any negative or positive trends associated with Renyi values and bioavailable trace element concentrations, but the highest or lowest concentrations of some elements were associated with the highest or lowest biodiversity values in some cases. These findings satisfy Objective 3: To determine the biodiversity levels of anthropogenic substrate sites using various biodiversity indices.

Some species were recorded as indicators for certain minerals on the slag bank, including Perennial Ryegrass (*Lolium perenne*), which was recorded as an indicator species for calcite - it must be borne in mind that due to limited sampling, this may be site-specific. Across the site, some substrate properties were statistically significantly associated with the presence of plant species and community composition, including pH level, the presence of the mineral calcite, and the concentration of the elements Al, Cd, Ni and Ba. These results satisfy Objective 4: To determine the elements and quantities of different elements that are statistically significantly associated with plant species and community presence throughout anthropogenic substrate sites.

All of the study's findings help to demonstrate that the Barrow-in-Furness slag bank provides opportunities for conservation and recreation, as well as carbon dioxide capture (Pullin et al., 2019; Gomes et al., 2020; Riley et al., 2020; Macdonald et al., 2022).

Chapter 7: Thesis Discussion

8.1 Findings related to Objective 1: To determine whether biodiversity offsetting could be achieved or is already being achieved on anthropogenic substrate sites (using Great Britain as an example where some relevant data are publicly available).

Calculation of spatial extent of various brownfield sites (focussing primarily on anthropogenic field sites as much as possible) revealed that only a small proportion of land in Great Britain is taken up by such land. Having said that, more locally, different anthropogenic substrate sites, such as colliery spoil bings in Wales, can have a much bigger role to play in local biodiversity, especially where rare species have already been recorded. After additional examination of Red List species which can be found on various anthropogenic field sites, it can be determined that there is much potential for further prioritising conservation of such species, using techniques such as or similar to Biodiversity Net Gain (BNG).

8.2 Findings related to Objective 2: To assess statistically significant differences between substrate characteristics (such as geochemistry and pH levels) on anthropogenic substrate sites.

Various analyses and calculations regarding the substrate data collected throughout the study revealed statistically significant differences between and within study site substrates in regards to: 1) mineralogy; 2) pH levels and 3) elemental composition. Ca levels were particularly differentiated throughout the sites, although there was a general pattern of high Ca levels on the ferrous slag sites and lower Ca levels on the colliery spoil site (Fallin Bing), the oil shale spoil site (Addiewell Bing) and the paper mill sludge site (South Bank Wood). pH levels were mostly alkaline throughout the study sites, although some sites had closer to neutral or acidic pH, with no recorded pH levels below 5. Some of the trace elements were recorded in unusually high levels on some of the plant communities, which is in line with what can be found in anthropogenic substrates more generally. High substrate heterogeneity on both the Warton slag bank and the Hodbarrow RSPB Reserve was thoroughly recorded and

represented by the quadrat and grid data collected for the two sites, emphasising the geodiversity of these two sites.

8.3 Findings related to Objective 3: To determine the biodiversity levels of anthropogenic substrate sites using various biodiversity indices.

Biodiversity levels varied both within and between sites, with higher biodiversity levels on sites such as Addiewell Bing and lower biodiversity levels on sites such as the Warton slag bank, although there was much variation. Communities that did not possess high numbers of plant individuals and/or species often still possessed at least one specialist species and/or at least one uncommon/Red List species, highlighting the value of more sparse communities alongside more 'busy' communities.

Biodiversity levels did not necessarily show clear trends with increasing or decreasing levels of particularly bioavailable elements, this may be due to various factors such as: 1) different bioavailability levels for the elements in different plant communities due to factors such as pH level, rhizosphere properties and 2) different plants throughout the plant communities recorded had differing responses to bioavailable trace elements.

Plant communities on the Warton slag bank and Hodbarrow RSPB Reserve were associated with varying elements in decreasing or increasing concentrations. For example plant communities on the Warton slag bank were recorded in elevated levels Al, Fe and K compared with much of the rest of the site.

Several indicator species for specific minerals throughout all six study sites were reported, such as Garden Lady's-mantle (*A. mollis*) with anhydrite and Lesser Chickweed (*Stellaria apetala*) with microcline. These are worth exploring further to assess how site-specific these indicator species are.

8.4 Findings related to Objective 4: To determine the elements and quantities of different elements that are statistically significantly associated with plant species and community presence throughout anthropogenic substrate sites.

This research shows that specific elements and minerals can be associated with the growth of plants, including specific species or communities. For example, Be was found to be an important element when considering the presence of plant species on the Hodbarrow RSPB Reserve, while V was statistically significantly associated with the presence of plant species across all six study sites studied during the course of the PhD. Future studies could further ascertain the relationships between specific plant species and communities with specific elements. While substrate has much influence on plant growth, other abiotic and many biotic factors likely influenced the presence and number of certain plant species on the study sites.

Chapter 8: Conclusion

There is much scope for further biological and geochemical research of anthropogenic substrate sites. The direct effects of anthropogenic substrates on plant growth have been highlighted in other studies, but this could be further explored in laboratory conditions and with additional plant species which are likely to grow on anthropogenic substrate sites. Bryophytes have been investigated on some anthropogenic substrate sites (Corner, 1967^a; Corner, 1967^b; Porley & Hodgetts, 2005; Riding et al., 2010; Steven & Long, 1989), but proliferation and growth of these species on many types of anthropogenic substrate remains to be explored in great detail. While bryophytes are significantly influenced by air and water particulates, unlike angiosperms and other vascular plant species (Simmel et al., 2020), the growth substrate of bryophytes does have great influence on their establishment and growth and this is poorly understood in relation to certain anthropogenic materials. Mycorrhizae have been studied on some brownfield sites (Balacco et al., 2022; Moorman & Reeves, 1979 Visconti et al., 2022), but as the importance of these microorganisms, along with other microorganisms in the rhizosphere, has become more fully understood, there is much scope for further examination in this area of research. It is not currently fully understood how remediation techniques for many anthropogenic substrate sites, such as those that utilise polymer linings (Viswandham & Jessberger, 2001) will last hundreds, and thousands of years into the future. Investigations into how such anthropogenically modified land could influence such factors as biodiversity, land stability, erosion and topsoil properties would be beneficial for further understanding the long-lasting effects of these restoration techniques, especially on contaminated land. It is clear from multiple studies that anthropogenic substrate sites can cater for high invertebrate biodiversity levels, as well as multiple rare and endangered species of invertebrates. The invertebrate biodiversity of many anthropogenic substrate sites has only been recently investigated (Olds, 2019), and this is an important area of study to continue, especially on sites that have not yet been surveyed and have the potential for varied and important invertebrate microhabitats.

While many anthropogenic substrate sites are considered differently (Allan et al., 1997; Harvie, 2004; Angold et al., 2006; Palmer, 2008; Riding et al., 2010), they are often considered together as 'brownfield sites' by many councils, governments,

developers, planners and others. While the term 'brownfield' is useful to ascertain the previous anthropogenic use and state of the land in question, it does nothing to delineate the vast variety of sites that are included. While many anthropogenic substrate sites seem to be almost indistinguishable from the surrounding natural landscape, leading to much confusion about their anthropogenic origins (Browne & Golledge, 2007), others are more conspicuous in the overall landscape (Gunston, 1956; Allan et al., 1997; Ash, 1983; Harvie, 2012; Riley et al., 2020), especially when they contribute to local pollution and contamination (Riley et al., 2020). The overall perception of brownfield sites has led, in many cases, to a disregard and paucity of research into their biodiversity and wildlife, with much of this only being highlighted in recent years (Riding et al., 2010; Harvie, 2012; Macadam & Bairner, 2012; Olds, 2019; Macgregor et al., 2022), or, at least, multiple years after the creation of the sites (Hind, 1956; Greenwood & Gemmell, 1978). Not all anthropogenic substrate sites will support high biodiversity, but it is these differences between biodiversity levels which emphasises the importance of careful study, survey and research of individual sites as much as possible.

This study, as well as the research of related literature, can allow one to come to a general conclusion about the biodiversity of anthropogenic substrate sites. While, of course, not all anthropogenic substrate sites will cater for high levels of biodiversity, many of them do certainly have a role to play in maintaining or increasing biodiversity levels, as well as providing wildlife habitat and wildlife conservation. It needs to be better appreciated and understood that legacy anthropogenic substrate sites can support high levels of biodiversity and have an important role to play in species preservation and conservation going forward. Further study of anthropogenic substrate sites more widely, including newer sites, can better evaluate the potential for newer sites, especially in developing countries, for wildlife and biodiverse habitats.

S1: How the mineralogy and chemistry of anthropogenic substrates influence plant biodiversity

S1.1 Data calculations

The data calculations performed for this chapter are available in the zipped folder titled "Biodiversity_Offsetting_Calculations" on the University of Glasgow Enlighten thesis repository: [http://dx.doi.org/10.5525/gla.researchdata.1575.](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdx.doi.org%2F10.5525%2Fgla.researchdata.1575&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cd55bdec9fca3408c912808dc180eac4b%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638411698487295719%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=AAly5BQCzn%2FQ%2BDMfPC%2B47UYb4FyMvc1Y%2Bcs7vVN9nPE%3D&reserved=0) Please refer to this file for further context and information.

S2: How the mineralogy and chemistry of anthropogenic substrates influence plant biodiversity

S2.1 Site Backgrounds and Further Information

S2.1.1 Barrow-in-Furness slag bank, Barrow-in-Furness, Cumbria

The Barrow-in-Furness slag bank is situated on and by the site of former blast and steel furnaces in Hindpool, Barrow-in-Furness, Cumbria. It is made primarily of blast furnace and steel slag waste from iron-making and steel-making industries between the late 1850s and the early 1980s (Henderson & Royal, 2015).

By 1870, the Barrow Haematite Steel Works was home to fourteen blast furnaces, making iron, and eighteen Bessemer converters, making steel (Henderson & Royal, 2015). In the early 1870s, the Barrow steelworks was the largest and most efficient in the world (Arnold, 2012). The Bessemer steel-making process, which had been recently developed in 1856 (Arnold, 2012), relied on ore with a high iron content and an unusually low phosphorous content (Marshall & Shiel-Davies, 1969; Lancaster & Wattleworth, 1977; Arnold, 2012), which was supplied in abundance by local mines (Arnold, 2012). Siemens-Martin steel was also produced at Barrow-in-Furness from 1880 (While, 1901; Henderson & Royal, 2015), with four Siemens furnaces situated on-site in the early 1900's (While, 1901). Additionally, an electric arc furnace for steel-making was installed and was producing steel in 1952 (Henderson & Royal, 2015). Over the lifetime of the Barrow-in-Furness ironmaking and steel industries, millions of tons of blast furnace and steel slag were generated (Henderson & Royal, 2015). This slag waste was run from the furnaces into selftipping bogies (While, 1901) and slag bank steam locomotives (Henderson & Royal, 2015) and dumped on the coastline, both on existing slag and into the sea beside the site from narrow-gauge locomotives on tramways (Van Nostrand's Engineering Magazine, 1879; While, 1901). The slag bank, as it grew, continuously permitted for

new land for the ironworks and steelworks (Van Nostrand's Engineering Magazine, 1879).

The slag bank, including its vegetation, has been modified on multiple occasions – before 1980, Common Spotted Orchid (*Dactylorhiza maculata* subsp. *fuchsii*) was well-distributed across the slag bank, but these populations had to be translocated due to redevelopment by the British Steel Corporation (who owned the site at the time) (Hunter et al., 2015). After the closure of the last ironworks on the site in 1983 (Henderson & Royal, 2015), the site has been: partially quarried multiple times, including in 1987; and partially remediated with clay capping in the 1990s and early 2010s, which involved adding a 1 metre high layer of soil to much of the site (Gorman, 2009).

S2.1.2 Warton slag bank, Warton, Lancashire

Similar to the Barrow-in-Furness slag bank, the Warton slag bank is made up of waste blast furnace and steel slag from the iron and steel industries. However, the Warton slag bank has a far higher proportion of blast furnace slag, due to the fact that the local ironworks, in nearby Carnforth, were much more prominent and successful than the local steelworks, which closed down after only about a year of limited operations (Mourholme Local History Society Book Group, 2009). In Warton, slag was dumped between 1865 and 1929 in two sites near the river Keer (Price, 1983; Mourholme Local History Society Book Group, 2009; Grosse, 2017). The iron and steel waste was transported from the ironworks by rail to the developing slag tips and bank in industrial tank locomotives (Grosse, 2017). Slag was originally dumped at Keer Marsh, close to Carnforth, on the north side of the Keer estuary. However, this area was extended - the Warton slag bank along Morecambe Bay (the study site) was created from about the early 1880s onwards, not just for the purposes of continuing to dump slag from the ironworks, but also: to reclaim sand flats in the area, with the slag bank acting as a sea wall (Skelcher & Askew, 2014; Grosse, 2017; Thompson & Poole, 2019; Riley et al., 2020); and for a planned alkali works which would use the slag as a feeder material, although this did not come to realisation (Grosse, 2017). Ultimately, this slag bank did not end up enclosing the

sand flats, but it does help to shelter much saltmarsh today (Skelcher & Askew, 2014; Thompson & Poole, 2019; Riley et al., 2020).

Calaminarian grassland has been identified by Graeme Skelcher and AONB Officer David Askew (2014) on the site. Plants in Calaminarian grassland communities are found on soils or substrates with elevated concentrations of heavy metals compared with most substrates. Mine spoil commonly supports Calaminarian grassland, but plants in these communities can be found on a range of both anthropogenic and natural substrates (Palmer, 2008). Other authors have not identified any plant community or communities here as Calaminarian grassland, taking note instead of the saltmarsh that borders and makes up part of the slag bank site (Thompson & Poole, 2019).

S2.1.3 Hodbarrow RSPB Reserve, Hodbarrow, Cumbria

The Hodbarrow RSPB Reserve exists on the site of the former Hodbarrow iron ore mines. Iron ore was first extracted from the mines here in 1856 (Bidwell, 1906), with much of it being sent to the former local blast furnace in Millom, where blast furnace slag that is now present on the site was created (Marshall & Davies-Shiel, 1969). While most of the local dumped blast furnace slag is found in a large slag bank in Millom to the north-west of the Reserve, much slag was brought down to the Hodbarrow mines before and after the closure of the mines, as part of sea wall developments (Bidwell, 1906; Marshall & Davies-Shiel, 1969). Construction for the first sea wall on the site originally began between 1889 and 1890 (Marshall & Davies-Shiel, 1969), but this wall and subsequent walls/portions of walls subsided and/or became unfit for purpose multiple times due to ingress of water and structural instability (Bidwell, 1906; Beaver, 1944; Marshall & Davies-Shiel, 1969). In about 1900, an outer barrier began to be constructed, with cement blocks and slag from the ironworks used as some of the primary materials (Marshall & Davies-Shiel, 1969; Riley et al., 2020). Following the closure of the mines in 1968 (Marshall & Davies-Shiel, 1969), the decision was made to completely flood most of the formerly mined area, creating today's Hodbarrow Lagoon (Radford, 1995; Palmer, 2008). Today, the Hodbarrow RSPB Nature Reserve consists not just of blast furnace slag from Millom but also much iron ore sands that were brought up along with the iron ore during the

mining process (Palmer, 2008). The RSPB Hodbarrow Nature Reserve is now an internationally important area for multiple species of seabirds and wading birds, with common species on-site including Ringed Plover *Charadrius hiaticula*, Little Tern *Sternula albifrons* and Oystercatcher *Haematopus ostralegus* (Radford, 1995; Palmer, 2008).

S2.1.4 Fallin Bing, Fallin, Stirlingshire

Fallin Bing is a colliery spoil heap associated with the former Polmaise Colliery that began to be worked in 1904 and was closed in 1987 (McGrail, 1986; Oglethorpe, 2008). While the Polmaise colliery was in operation, the colliery spoil waste was dumped in a large bing on land associated with the site (National Mining Museum Scotland F/C/2/3/16/11). Like other typical bings of the 1900s, Fallin Bing was initially very steep and had potential stability issues (Allan et al., 1997). Stirling Council took possession of the bing in 1991, then reprofiling the bing due to concerns about stability, local health and welfare (Currie, 2013; Dennis, 2014; Mills & McIntosh, 2021). This reprofiling was followed by Silver Birch *Betula pendula* and Alder *Alnus glutinosa* planting in patches of the site, along with a grassland reseeding and re-establishment programme, both instigated by the Stirling Council (Currie, 2013; Mills & McIntosh, 2021). Since 2004, a management plan for Fallin Bing, produced by Stirling Council, has involved long-term conservation management for all of the habitats within the sites, bearing public access and amenity requirements in mind (Currie, 2013). Buglife did much work on the site in 2014 and 2015, primarily to increase the amount of habitat for certain invertebrate species, especially pollinator insect species (Inner Forth Landscape Initiative, 2017), by planting wildflower meadows and removing scrub on much of the site (Inner Forth Landscape Initiative, 2017).

S2.1.5 Addiewell Bing, Addiewell, West Lothian

The West Lothian oil shale spoil bings make up vast heaps, resulting from the oil shale industry that began in the mid to late 1800s (Carruthers et al., 1927; Harvie, 2004). These large bings are noticeable in the landscape, making up a large share of the anthropogenic substrate sites in Central Scotland and are often the only physical reminders of the oil shale industry (Harvie, 2004). Addiewell Bing was created when oil-bearing shale was processed at the Addiewell works, set up in 1863 and later known as Young's Paraffin Light and Mineral Oil Company (Redwood, 1897). This oil shale was processed for the extraction of various substances such as paraffin oil that were primarily used in the mid to late 1800s and the first half of the 1900s (Carruthers et al., 1927; Louw & Addison, 1985). Spent shale, or oil shale spoil as it often called today (Carruthers et al., 1927; Harvie, 2004), was discharged from the Addiewell works into large self-tipping bogies which were transported, by rail, to empty out waste onto the bing (Louw & Addison, 1985). It is believed that the last oil shale spoil was dumped on the bing in 1932, but the Addiewell works were not closed until 1960 (Scottish Wildlife Trust, 1995). From 1978 until the late 1980s, the Regional Council ordered for various restoration work to be carried out on the site, which included 1) reshaping of the bing to reduce pollution from the shale spoil into the Breich Water on the northern side of the site and 2) the planting of approximately 50,000 trees (Lodgepole Pine (*Pinus contorta*) and European Larch (*Larix decidua*)) and 16,000 broadleaf trees and shrubs for stabilisation and amenity purposes (Scottish Wildlife Trust, 1995; West Lothian District Council, 1995). Today, Addiewell Bing is separated into a north and south section, with the north section managed and conserved by the Scottish Wildlife Trust since 1987 (Scottish Wildlife Trust, 1995; West Lothian District Council, 1995). Some of the Scottish Wildlife Trust's restoration work has included the removal of much of the Lodgepole Pine (*Pinus contorta*) and European Larch (*Larix decidua*) planted on-site to provide room for specific plant communities, including orchid communities on parts of the site (Scottish Wildlife Trust, 1995).

S2.1.6 South Bank Wood, Penicuik, Midlothian

The town of Penicuik, near Edinburgh, was known throughout much of the 1700s and 1800s as the centre of the papermaking industry (Wilson, 1891). Papermaking raw materials in Penicuik initially included linen and then cotton rags in the 1700s (Wilson, 1891; Watson, 1987). Up until 1861, with the abolishment of the paper duty, rags had been, by far, the cheapest raw material for paper production (Wilson,

1891). With the abolishment of the paper duty, raw materials primarily consisted of Esparto grass and then, much later, wood pulp (Wilson, 1891; Watson, 1987; Browne & Golledge, 2007). Much solid paper mill sludge waste was produced in the paper-making process, which was dumped onto the banks of the River Esk – in 1930, at least some of the waste was still being dumped by horses conveying bogeys full of the waste (Watson, 1987). The River Esk was also, at the time, contaminated with waste effluent from the paper mills (Wilson, 1891; Watson, 1987). The largest and most successful of the paper mills on the River Esk was the Valleyfield mill, producing the majority of the paper mill waste, although other mills included Polton, Springfield and Dalmore (Wilson, 1891; Watson, 1987). After the first paper mill in Penicuik opened in the early 1700s (Wilson, 1891), Valleyfield Mill, which produced by far the highest amount of paper mill sludge waste, closed down in 1975 (Midlothian Department of Planning and Building Control, 1985).

In the early months of 2007, a landslip occurred in a large part of South Bank Wood, exposing paper mill sludge and other waste materials that had long been well-vegetated. This landslip caused much of the paper mill waste to flow directly in the River North Esk, which included much toxic material. The waste material exposed included: paper waste, wood and/or paper pulp, chimney soot, boiler ash, bricks, plastic sheets and large rubber bands (Browne & Golledge, 2007). In 2021, during study of the site, this area is almost completely revegetated, with improved surface stability due to successful plant growth.
Table S1.1: Details from previous literature about the plant species recorded on certain anthropogenic substrates which were surveyed during the study (species names checked on the websites GBIF (for higher plants), Tropicos and World Flora Online (both for bryophytes) so up-to-date taxonomy could be included). Information obtained from: Thomas, 1930; Ash et al., 1994; Scottish Wildlife Trust, 1995; Halliday, 1997; Harvie, 2004; Rahmonov et al., 2020; and Riley et al., 2020.

Table S1.2: Details from previous literature about the production, pH levels and geochemistry of anthropogenic substrates that were studied and analysed

waste can be much higher in CaO, with MgO also making up a large volume, and with smaller levels of silica, sand, iron, aluminium and SO₃ (Thomas, and)
1930). Paper mill waste has an average pH of 11, with a range of 8 to 13 (Thomas, 1930; Cherian & Siddiqua, 2019). The high alkalinity usually results from the lime present in the waste (Thomas, 1930; Cherian & Siddiqua, 2019). Common minerals in modern and legacy paper mill waste include quartz, calcite, lime, portlandite, tricalcium silicate and tricalcium aluminate (Thomas, 1930; Cherian & Siddiqua, 2019).

S2.2 Materials and Methods – further details

S2.2.1 Initial substrate sample preparation

One substrate sample was collected from the field for every plant community, within the space that the plant quadrat sample occupied. These samples were partially prepared, which involved the use of a pestle and mortar as well as a jawcrusher. Two sieves, one 0.5 mm and the other 90 μm, were used to separate sample material into 0.5 mm fractions and 90 μm fractions. Lab surfaces, the sieves and the jawcrusher, including jawcrusher equipment, were thoroughly cleaned between sample preparations, to reduce the risk of contamination, following lab protocol. The two different fractions were used for different analyses.

S2.2.2 Ball mill use and XRD analyses

Sample material between 90 μm and 0.5 mm in diameter was ground down into fine powder for mineral analysis, more specifically, X-ray Diffraction (XRD) analysis. This was done using a Retsch MM400 ball mill and stainless steel containers, with stainless steel balls 2 cm in diameter. Samples were milled at a minimum of 30 seconds each at a frequency of 1/s = 30, but some samples needed additional milling time depending on powder consistency (this was always checked before material was stored and later used). Ball mall containers and lab surfaces were thoroughly cleaned between the preparation of different samples to reduce the risk of contamination, following lab protocol. Powder samples were analysed using a Rigaku Miniflex powder diffractometer. The results were then viewed on the SmartLab Studio II program. In this software, the following was carried out for each sample:

- In the peak evaluation stage, peaks were examined and specified or deleted as needed.
- In the phase identification stage, some elements were set to unknown, while, to refine the phase identification results, the following elements were set to "Not Included": Be; Fr; Ra; Y; Nb: Tc: Ru; Rh; Pd; Ag; In; Te; I; Hf; Ta; W; Re; Os; Ir; Pt; Au; Hg; Tl; Bi; Po; At; He; Ne; Ar; Kr; Xe; Rn; La; Ce; Pr; Nd; Pm; Sm; Eu; Gd; Tb; Dy; Ho; Er; Tm; Yb; Lu; Ac; Th; Pa; U; Np; Pu; Am; Cm; Bk; Cf; Es; Fm; Md; No; and Lr. Additionally, the option to automatically include phases was not selected – this was done so the results could be carefully and more easily examined.
- Many of the phases that were generated in the phase identification run were not identified as minerals present in the sample. Minerals that were identified as being present in the sample had at least one substantial influence on at least one of the large peaks present in the sample.

S2.2.3 pH analysis methodology

pH analysis methodology for the substrate samples was devised with technical staff in the School of Geographical and Earth Sciences at the University of Glasgow. 2 sets of 10 mg of material were prepared with 25 ml of distilled water for each sample. These 2 sets of material were then shaken on a shaking table for 30 minutes. Following this shaking period, the 2 samples were left to stand for another 30 minutes. After this, pH measurements would be taken using a pH meter. The mean measurement of the two samples would be recorded as the representative pH reading for each substrate sample. The pH meter would be regularly calibrated, so that pH readings were taken at a maximum of 1 week after the previous calibration. Mettler Toledo pH 4.01, 9.21 and 7.00 buffer solutions were used to calibrate the pH meter.

S2.2.4 Substrate sample analysis by ALS Global

Geochemistry analyses for most of the samples were done by ALS Global. Samples were ground to <90 μm diameter particle size and screened for high concentrations

of iron or steel blebs. Samples that had a particularly high concentration of these were instead sent to the Materials Processing Institute (see next section). Samples underwent ICP-AES analyses following fused bead acid digestion analysis for major elements and aqua regia digestion for minor elements. 5 reference standards were used. Measured values for standards were within 10% of accepted values for all elements, apart from Bi, Cd, La, Li and Sc (within 20%), Sb (within 30%) and Hg (with 60%). See further details in the Excel file titled

""ALS_QC_Results_and_Calculations.xlsx" on the zipped folder titled "Data_for_six_study_sites" on the University of Glasgow Enlighten thesis repository: [http://dx.doi.org/10.5525/gla.researchdata.1575.](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdx.doi.org%2F10.5525%2Fgla.researchdata.1575&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cd55bdec9fca3408c912808dc180eac4b%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638411698487295719%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=AAly5BQCzn%2FQ%2BDMfPC%2B47UYb4FyMvc1Y%2Bcs7vVN9nPE%3D&reserved=0)

Prior to statistical analyses, all of the geochemistry data provided in oxide form were transformed into mg/kg form, while the data provided in ppm form were already in mg/kg form, as ppm=mg/kg. This was done so that geochemistry measurements could be standardised, rather than having values in different forms, for statistical analyses in R and QGIS. Details, methods and metadata concerning the calculations required to transform the oxide data are provided in the file titled "Six_Study_Sites_Oxide_Geochemistry_Data_and_Metadata.xlsx", also provided in the zipped folder titled "Data_for_six_study_sites" in the Enlighten thesis repository: [http://dx.doi.org/10.5525/gla.researchdata.1575.](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdx.doi.org%2F10.5525%2Fgla.researchdata.1575&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cd55bdec9fca3408c912808dc180eac4b%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638411698487295719%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=AAly5BQCzn%2FQ%2BDMfPC%2B47UYb4FyMvc1Y%2Bcs7vVN9nPE%3D&reserved=0) If you have any issues accessing the data repository and/or the data file, please email Savanna van Mesdag at savannankvm@gmail.com or message her on her Researchgate profile.

S2.2.5 Substrate sample analysis by Materials Processing Institute

While most of the geochemistry analyses for substrate samples were carried out by ALS Global, the Materials Processing Institute (MPI) in Middlesbrough carried out equivalent analyses for samples that had a particularly high metal concentration, as their equipment would not be damaged by such high metal concentrations. XRF analyses were carried out for some samples, using 90 μm sample material. For these analyses, an in-house quantitative application that has been developed using a Fluxana Raw calibration sample set was used. 32 Certified Reference Materials, with a range of elements covering the analysis for this material were measured – elemental analysis was within 3% of standard values.

Prior to statistical analyses, all of the geochemistry data provided in oxide form were transformed into mg/kg form, while the data provided in ppm form were already in mg/kg form, as ppm=mg/kg. This was done so that geochemistry measurements could be standardised, rather than having values in different forms, for statistical analyses in R and QGIS. Details, methods and metadata concerning the calculations required to transform the oxide data are provided in the file titled "Six_Study_Sites_Oxide_Geochemistry_Data_and_Metadata.xlsx", provided in the zipped folder titled "Data_for_six_study_sites" in the Enlighten thesis repository: [http://dx.doi.org/10.5525/gla.researchdata.1575.](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdx.doi.org%2F10.5525%2Fgla.researchdata.1575&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cd55bdec9fca3408c912808dc180eac4b%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638411698487295719%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=AAly5BQCzn%2FQ%2BDMfPC%2B47UYb4FyMvc1Y%2Bcs7vVN9nPE%3D&reserved=0)

S2.3 Species records

Details about the species records data files are provided below. All of the following species records files are available in the zipped folder "Data_for_six_study_sites" on Savanna van Mesdag's Enlighten thesis repository: [http://dx.doi.org/10.5525/gla.researchdata.1575.](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdx.doi.org%2F10.5525%2Fgla.researchdata.1575&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cd55bdec9fca3408c912808dc180eac4b%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638411698487295719%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=AAly5BQCzn%2FQ%2BDMfPC%2B47UYb4FyMvc1Y%2Bcs7vVN9nPE%3D&reserved=0)

Please note that the input "Species_List_for_GBIF_vo3.csv", which was made for the "Species matching" function in GBIF, to update the taxonomic names ([https://www.gbif.org/tools/species-lookup\)](https://www.gbif.org/tools/species-lookup) and the output .csv file from the "Species matching" function, named "Species_List_GBIF_generated.csv" include species records for all six study sites. Bryophyte names were additionally checked using Tropicos and World Flora Online, preference was given to accepted names on these websites for bryophytes over those on GBIF based on recommendations by Dr Neil Bell.

If you have any issues accessing the data repository and/or the data, please email Savanna van Mesdag at savannankvm@gmail.com or message her on her Researchgate profile.

S2.3.1 Addiewell Bing plant data

Data files titled "Addiewell_Angiosperm_Records.xlsx", "Addiewell_Bing_Bryophytes.xlsx", "Plant_Species_Total.csv" and "Plant Species with Site Names_Total.csv", contain information relating to the angiosperms and bryophytes records on Addiewell Bing made during the 2019

fieldwork period. The first two of these files contain the original, untransformed data with metadata such as quadrat locations, while the third and fourth of these files contain the data (along with the data for the other recorded species in the study) for analyses in programs such as RStudio.

S2.3.2 Barrow-in-Furness slag bank plant data

Data files titled "Barrow Angiosperm_Records.xlsx", "Barrow_Bryophytes.xlsx", "Plant Species Total.csv" and "Plant Species with Site Names Total.csv", contain information relating to the angiosperms and bryophytes records on the Barrow-in-Furness slag bank made during the 2019 fieldwork period. The first two of these files contain the original, untransformed data with metadata such as quadrat locations, while the third and fourth of these files contain the data (along with the data for the other recorded species in the study) for analyses in programs such as RStudio.

S2.3.3 Warton slag bank plant data

Data files titled "Carnforth_Angiosperm_Records.xlsx", "Carnforth_Bryophytes.xlsx", "Plant Species Total.csv" and "Plant Species with Site Names Total.csv", contain information relating to the angiosperms and bryophytes records on the Warton (Carnforth) slag bank made during the 2019 fieldwork period. The first two of these files contain the original, untransformed data with metadata such as quadrat locations, while the third and fourth of these files contain the data (along with the data for the other recorded species in the study) for analyses in programs such as RStudio.

S2.3.4 Fallin Bing plant data

Data files titled "Fallin_Bing_Angiosperm_Records.xlsx", "Fallin_Bing_Bryophytes.xlsx", "Plant_Species_Total.csv" and "Plant_Species_with_Site_Names_Total.csv", contain information relating to the angiosperms and bryophytes records on Fallin Bing made during the 2019 fieldwork period. The first two of these files contain the original, untransformed data with metadata such as quadrat locations, while the third and fourth of these files contain the data (along with the data for the other recorded species in the study) for analyses in programs such as RStudio.

S2.3.5 Hodbarrow RSPB Reserve plant data

Data files titled "Hodbarrow Angiosperm Records.xlsx", "Hodbarrow_Bryophytes.xlsx", "Plant_Species_Total.csv" and "Plant_Species_with_Site_Names_Total.csv", contain information relating to the angiosperms and bryophytes records at the Hodbarrow RSPB Reserve made during the 2019 fieldwork period. The first two of these files contain the original, untransformed data with metadata such as quadrat locations, while the third and fourth of these files contain the data (along with the data for the other recorded species in the study) for analyses in programs such as RStudio.

S2.3.6 South Bank Wood plant data

Data files titled "Penicuik_Angiosperm_Records.xlsx", "Penicuik_Bryophytes.xlsx", "Plant_Species_Total.csv" and "Plant_Species_with_Site_Names_Total.csv", contain information relating to the angiosperms and bryophytes records at South Bank Wood (in Penicuik) made during the 2019 fieldwork period. The first two of these files contain the original, untransformed data with metadata such as quadrat locations, while the third and fourth of these files contain the data (along with the data for the other recorded species in the study) for analyses in programs such as RStudio.

S2.4 Plant community information

Table S1.3: Common plant communities for species recorded in different open plant communities during the PhD study (Information obtained from: Paton, 1969; Gardiner, 1981; Hubbard & Hubbard, 1984; Ellenberg, 1988; Hill, 1988; Rodwell, 1991; Bishop & Davy, 1994; Bates, 1995; Zhaouhui, 1996; Müller-Schärer & Fischer, 2001; Stern, 2001; Jiménez et al., 2002; Rose & O'Reilly, 2006; Atherton et al., 2010; Gilbert, 2012; Kelcey & Müller, 2011; Blockeel, 2014^a ; Blockeel, 2014^b ; Hodgetts & Blockeel, 2014; Porley & Blockeel, 2014; Preston & Blockeel, 2014; Wigginton & Hodgetts, 2014; Wigginton & Hodgetts, 2014; Price, 2018; Rainbow, 2018;

S2.5 Indval analysis results and statistical analyses

Table S1.4 includes Indval analysis results for every mineral recorded throughout the six study sites. The full code and results for the Indval analyses are available on the University of Glasgow thesis repository:

[http://dx.doi.org/10.5525/gla.researchdata.1575,](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdx.doi.org%2F10.5525%2Fgla.researchdata.1575&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cd55bdec9fca3408c912808dc180eac4b%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638411698487295719%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=AAly5BQCzn%2FQ%2BDMfPC%2B47UYb4FyMvc1Y%2Bcs7vVN9nPE%3D&reserved=0) in the zipped folder titled "Indval_Analyses_for_Data_for_six_study_sites". The relevant files are "S_van_Mesdag_Indval_Analyses_for_Six_Study_Sites.html" and "S_van_Mesdag_Indval_Analyses_for_Six_Study_Sites.Rmd". This data analysis file in is an .rmd file, there is a corresponding .html file. The .rmd file for the study can be used in RStudio or a similar program. Please adjust any code as necessary to, for example, install R packages and read in the data files into RStudio from your computer. The corresponding .html file may be easier to read and interpret for those who are unfamiliar with R coding. If you have any issues accessing the data repository and/or the files in the repository, please email Savanna van Mesdag at savannankvm@gmail.com or message her on her Researchgate profile.

Table S1.4: Indicator species for all minerals throughout the six study sites, based on calculations done using the function "indval" in the labdsv R package, version 2.1-0 (Roberts, 2023). Indicator values and p values are reported to 3 decimal places.

S2.6 Substrate data

See below for details about the availability of substrate data for each study site, accessible in the zipped folder titled "PhD-Data-for-six-study-sites" on the University of Glasgow Enlighten thesis repository:

[http://dx.doi.org/10.5525/gla.researchdata.1552.](http://dx.doi.org/10.5525/gla.researchdata.1552) If you have any issues accessing/viewing the data repository and/or the data, please email Savanna van Mesdag at savannankvm@gmail.com or message her on her Researchgate profile.

S2.6.1 Addiewell Bing substrate data

Substrate data for this study site in formats for data analysis are available in rows 7- 11 of "Plant_CHEMISTRY_MAJOR_AND_TRACE_ELEMENTS_WITH_SITE_NAMES_M G_KG.csv" and in "Plant Chemistry_with_site_names.csv". Raw data and transformed data for Addiewell Bing are also provided in the files titled "Six_Study_Sites_Oxide_Geochemistry_Data_and_Metadata.xlsx" and "ALS_Quadrat_Results_spreadsheet_A_FB_SB.csv".

S2.6.2 Barrow-in-Furness slag bank substrate data

Data for the Barrow-in-Furness slag bank are available in rows 25-35 of "Plant_CHEMISTRY_MAJOR_AND_TRACE_ELEMENTS_WITH_SITE_NAMES_M G_KG.csv" and in "Plant_Chemistry_with_site_names.csv". Raw data and transformed data for the Barrow-in-Furness slag bank are also provided in the files titled "Six_Study_Sites_Oxide_Geochemistry_Data_and_Metadata.xlsx" and "ALS_Quadrat_Results_spreadsheet_B_C_HB.csv".

S2.6.3 Warton slag bank (Carnforth) substrate data

Data for this slag bank are available in rows 17-24 of "Plant_CHEMISTRY_MAJOR_AND_TRACE_ELEMENTS_WITH_SITE_NAMES_M G_KG.csv" and in "Plant_Chemistry_with_site_names.csv". Raw data and transformed data for the Warton slag bank are also provided in the files titled "Six_Study_Sites_Oxide_Geochemistry_Data_and_Metadata.xlsx" and "ALS_Quadrat_Results_spreadsheet_B_C_HB.csv".

S2.6.4 Fallin Bing substrate data

Data for Fallin Bing are available in rows 2-6 of "Plant_CHEMISTRY_MAJOR_AND_TRACE_ELEMENTS_WITH_SITE_NAMES_M G_KG.csv" and in "Plant_Chemistry_with_site_names.csv". Raw data and

transformed data for Fallin Bing are also provided in the files titled "Six_Study_Sites_Oxide_Geochemistry_Data_and_Metadata.xlsx" and "ALS_Quadrat_Results_spreadsheet_A_FB_SB.csv".

S2.6.5 Hodbarrow RSPB Reserve substrate data

Data for this site are available in rows 36-42 of "Plant_CHEMISTRY_MAJOR_AND_TRACE_ELEMENTS_WITH_SITE_NAMES_M G_KG.csv" and in "Plant_Chemistry_with_site_names.csv". Raw data and transformed data for the Hodbarrow RSPB Reserve are also provided in the files titled "Six_Study_Sites_Oxide_Geochemistry_Data_and_Metadata.xlsx" and "ALS_Quadrat_Results_spreadsheet_B_C_HB.csv".

S2.6.6 South Bank Wood substrate data

Data for South Bank Wood are available in rows 12-16 of "Plant_CHEMISTRY_MAJOR_AND_TRACE_ELEMENTS_WITH_SITE_NAMES_M G_KG.csv" and in "Plant_Chemistry_with_site_names.csv". Raw data and transformed data for South Bank Wood are also provided in the files titled "Six_Study_Sites_Oxide_Geochemistry_Data_and_Metadata.xlsx" and "ALS_Quadrat_Results_spreadsheet_A_FB_SB.csv".

S2.7 Statistical analyses

All of the analyses carried out for this study, as well as the relevant datasets, are available in different files on Savanna van Mesdag's Enlighten thesis repository: [http://dx.doi.org/10.5525/gla.researchdata.1575.](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdx.doi.org%2F10.5525%2Fgla.researchdata.1575&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cd55bdec9fca3408c912808dc180eac4b%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638411698487295719%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=AAly5BQCzn%2FQ%2BDMfPC%2B47UYb4FyMvc1Y%2Bcs7vVN9nPE%3D&reserved=0) Please read further for relevant information and details regarding the various links and files for different datasets and different analyses.

S2.7.1 Principal Component Analyses and correlation matrix.

The PCA analyses for this study were carried out by Matt Divers at the University of Glasgow, using substrate data generated during the study. All of the files associated with these analyses are available on the Github repository in the zipped folder titled "Principal_Component_Analyses_for_PhD_Data_for_six_study_sites", on the University of Glasgow Enlighten thesis repository:

[http://dx.doi.org/10.5525/gla.researchdata.1575/.](http://dx.doi.org/10.5525/gla.researchdata.1575/) In order to access the files and look into the data analysis further, please follow the following instructions (as provided by Matt Divers):

- 1. a. Install anaconda: [https://www.anaconda.com/download/success](https://eur03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.anaconda.com%2Fdownload%2Fsuccess&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cde2a239636244034eb0c08dcd72aa7dc%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638621825403052485%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C0%7C%7C%7C&sdata=kTb4IITAxrLknDa1HJaGkHVZBZPvKCtKv8SnjEneyoE%3D&reserved=0) b. Follow all the steps.
- 2. a. Open "Anaconda prompt" (this is a terminal)

b. Check where the base path location is. Example

(base) C:\Users\Matt>

- 3. Download the "geochem.yml" file in the subfolder "Principal-Component-Analyses-for-PhD-Data-for-six-study-sites" and paste into the base path location (as shown in image above).
- 4. Run the following commands: Conda env create -f geochem.yml activate Geochem
- 5. Now you can launch jupyter notebook by typing the following into anaconda: jupyter notebook

This will then create an exact copy of your environment in jupyter notebook, online. Assuming you have downloaded the "Principal-Component-Analyses-for-PhD-Datafor-six-study-sites" folder and extracted the files as needed on a specific and easily navigable location on your computer, you can then access the relevant folders in the subfolder labelled "mg-kg". To directly access the code to see the analyses, click on the file labelled "Geochem_PCA_mg-kg.ipynb. The file format is a Jupyter notebook type format. If you have any issues accessing any of the files using this anaconda and Jupyter notebook method, please contact Savanna van Mesdag at her personal

email address, [savannankvm@gmail.com,](mailto:savannankvm@gmail.com) by sending her a private message on her Researchgate profile, or by sending her an email to her current work email address.

Figure S1.1: A. Plots of different PCs against each other, with samples for the different study sites represented by different symbols for clarity. While there was some clustering of points for specific sites, there was little consistent overlap between study sites throughout each graph. This shows the differences between the samples of each study site and the heterogeneity of the anthropogenic substrate overall. B. A plot with PC2 as the x axis and PC1 as the y axis, with samples for the different study sites represented by different symbols for clarity.

S1.2: A visualisation of the scores matrix as a scatter plot, showing clustering of some points and separation of others.

A correlation matrix was made for Fe and P throughout the study sites. The code for the analyses, along with input data, results and visualisations is available in the zipped folder titled: "Additional_Calculations_for_the_Six_Study_Sites" in the University of Glasgow repository: [http://dx.doi.org/10.5525/gla.researchdata.1575.](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdx.doi.org%2F10.5525%2Fgla.researchdata.1575&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cd55bdec9fca3408c912808dc180eac4b%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638411698487295719%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=AAly5BQCzn%2FQ%2BDMfPC%2B47UYb4FyMvc1Y%2Bcs7vVN9nPE%3D&reserved=0) Please note that the .rmd file and the .html file (the latter of which is easier to view) are titled "Additional_Calculations_Six_Study_Sites" and the relevant data file is "PLANT_CHEMISTRY_Fe2O3_P2O5_MG_KG.csv". If you have any issues

accessing the data repository/files, please email Savanna van Mesdag at savannankvm@gmail.com or message her on her Researchgate profile.

S2.7.2 Biodiversity analyses

The relevant files are available in the zipped folder titled "Biodiversity-analyses-forsix-study-sites" on the University of Glasgow Enlighten thesis repository: [http://dx.doi.org/10.5525/gla.researchdata.1575.](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdx.doi.org%2F10.5525%2Fgla.researchdata.1575&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cd55bdec9fca3408c912808dc180eac4b%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638411698487295719%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=AAly5BQCzn%2FQ%2BDMfPC%2B47UYb4FyMvc1Y%2Bcs7vVN9nPE%3D&reserved=0) If you have any issues accessing this data repository, please email Savanna van Mesdag at savannankvm@gmail.com or message her on her Researchgate profile.

The data analysis file for most of the biodiversity analyses is an .rmd file, with a corresponding .html file. The .rmd file for most of the biodiversity analyses for the study can be used in RStudio or a similar program. Please adjust any code as necessary to, for example, install R packages. (The corresponding .html file may be easier to read and interpret for those who are unfamiliar with R coding.) Please note, however, that the .rmd file for the Renyi biodiversity analyses, titled "SvM_Renyi_Biodiversity_Analyses_1.Rmd", is meant to be run in specifically in a GUI, rather than in, say, RStudio. The instructions for how to open the GUI and run the .rmd file are provided in a PDF titled:

"S_van_Mesdag_Renyi_Biodiversity_Analyses_vo1.pdf".

S2.7.3 Canonical Correspondence analyses and Non-metric multidimensional scaling analysis results

(All files described in this section are available in the Enlighten thesis repository: [http://dx.doi.org/10.5525/gla.researchdata.1575,](http://dx.doi.org/10.5525/gla.researchdata.1575) zipped folder "Canonical_Correspondence_analyses_six_study_sites")

The files relate to: analyses of similarities; canonical correspondence analyses and the relevant anovas; non-metric multidimensional scaling analyses; and relevant data (used in the analyses for the six study sites).

This data analysis file is an .rmd file, there is a corresponding .html file. The .rmd file for the study can be used in RStudio or a similar program. Please adjust any code as necessary to, for example, install R packages. The corresponding .html file may be

easier to read and interpret for those who are unfamiliar with R coding. The various data files can be used to look at the data run for the analyses and can be used to rerun the analyses. If you have any issues accessing the data repository/files, please email Savanna van Mesdag at savannankvm@gmail.com or message her on her Researchgate profile.

S2.8 Further calculations

Further data calculations are provided in the

[http://dx.doi.org/10.5525/gla.researchdata.1575,](http://dx.doi.org/10.5525/gla.researchdata.1575) in the zipped folder titled ": "Additional Calculations for the Six Study Sites". Please note that the .rmd file and the .html file (the latter of which is easier to view) are titled "Additional_Calculations_Six_Study_Sites" and the calculation data file is titled "PLANT_CHEMISTRY_MAJOR_AND_TRACE_ELEMENTS_MG_KG.csv". If you have any issues accessing the data repository/files, please email Savanna van Mesdag at savannankvm@gmail.com or message her on her Researchgate profile.

S3: Slag substrate composition influences plant community distribution and biodiversity

S3.1 Further Information

Table S2.1: Details from previous literature about the production, pH levels and geochemistry of the anthropogenic substrates that were studied and analysed

Raw materials, chemistry and mineralogy of substrate as previously recorded in the literature Blast furnace and/or steel slag Both blast furnace slag and steel slag are primarily made up of CaO, SiO₂, Al2O³ and MgO, but the ratios of these differ between blast furnace and steel slag – SiO₂ is much more predominant in blast furnace slag than steel slag
and CaO tends to make up a larger part of steel slag than blast furnace slag (Piatak et al., 2015). Blast furnace slag contains many Ca-Al silicates, along with calcium carbonate and calcium hydroxide (Ash et al., 1994). Blast furnace and steel slag are highly heterogeneous in composition, with different papers
reporting multiple proportions of various different elements and minerals,
including Fe, Mg, Mn, Ti, K, Na and BaO (Ash et al., 1994; Piatak et Riley et al., 2020). These variations are primarily due to differences in the raw materials fed into iron and steel furnaces, as well as the cooli

Table S2.2: Details from previous literature about the plant species recorded on the anthropogenic substrates which were surveyed during the study

S3.2 Materials and Methods – further details

S3.2.1 Initial substrate sample preparation

One substrate sample was collected from the field for every plant community, within the space that the plant quadrat sample occupied. These samples were partially prepared, which involved the use of a pestle and mortar as well as a jawcrusher. Two sieves, one 0.5 mm and the other 90 μm, were used to separate sample material into 0.5 mm fractions and 90 μm fractions. Lab surfaces, the sieves and the jawcrusher, including jawcrusher equipment, were thoroughly cleaned between sample preparations, to reduce the risk of contamination, following lab protocol. The two different fractions were used for different analyses.

S3.2.2 Ball mill use and XRD analyses

Sample material between 90 μm and 0.5 mm in diameter was ground down into fine powder for mineral analysis, more specifically, X-ray Diffraction (XRD) analysis. This was done using a Retsch MM400 ball mill and stainless steel containers, with stainless steel balls 2 cm in diameter. Samples were milled at a minimum of 30 seconds each at a frequency of 1/s = 30, but some samples needed additional milling time depending on powder consistency (this was always checked before material was stored and later used). Ball mall containers and lab surfaces were thoroughly cleaned between the preparation of different samples to reduce the risk of contamination, following lab protocol. Powder samples were analysed using a Rigaku Miniflex powder diffractometer. The results were then viewed on the SmartLab Studio II program. In this program, the following was carried out for each sample:

- In the peak evaluation stage, peaks were examined and specified or deleted as necessary
- In the phase identification stage, most elements were set to unknown, while, to refine the phase identification results, the following elements were set to "Not Included": Be; Fr; Ra; Y; Nb: Tc: Ru; Rh; Pd; Ag; In; Te; I; Hf; Ta; W; Re; Os; Ir; Pt; Au; Hg; Tl; Bi; Po; At; He; Ne; Ar; Kr; Xe; Rn; La; Ce; Pr; Nd; Pm; Sm; Eu; Gd; Tb; Dy; Ho; Er; Tm; Yb; Lu; Ac; Th; Pa; U; Np; Pu; Am; Cm; Bk;

Cf; Es; Fm; Md; No; and Lr. Additionally, the option to automatically include phases was not selected.

• Many of the phases that were generated in the phase identification run were not identified as minerals that were actually present in the sample. Minerals that were identified as being present in the sample had at least one substantial influence on at least one of the large peaks present.

S3.2.3 pH analysis methodology

pH analysis methodology for the substrate samples was devised with technical staff in the School of Geographical and Earth Sciences at the University of Glasgow. 2 sets of 10 mg of material were prepared with 25 ml of distilled water for each sample. These 2 sets of material were then shaken on a shaking table for 30 minutes. Following this shaking period, the 2 samples were left to stand for another 30 minutes. After this, pH measurements would be taken using a pH meter. The mean measurement of the two samples would be recorded as the representative pH reading for each substrate sample. The pH meter would be regularly calibrated, so that pH readings were taken at a maximum of 1 week after the previous calibration. Mettler Toledo pH 4.01, 9.21 and 7.00 buffer solutions were used to calibrate the pH meter.

S3.2.4 Substrate sample analysis by ALS Global

Geochemistry analyses for most of the samples were done by ALS Global. Samples were ground to <90 μm diameter particle size and screened for high concentrations of iron or steel blebs. Samples that had a particularly high concentration of these were instead sent to the Materials Processing Institute (see next section). Samples underwent ICP-AES analyses following fused bead acid digestion analysis for major elements and aqua regia digestion for minor elements. 5 reference standards were used. Measured values for standards were within 10% of accepted values for all elements, apart from Cr₂O₃, La, Li, MnO, Sc, (within 20%), SrO (within 30%) and Bi (within 130%). Geochemistry analyses for most of the samples were done by ALS Global. Samples were ground to <90 μm diameter particle size and screened for

high concentrations of iron or steel blebs. Samples that had a particularly high concentration of these were instead sent to the Materials Processing Institute (see next section). Samples underwent ICP-AES analyses following fused bead acid digestion analysis for major elements and aqua regia digestion for minor elements. 5 reference standards were used. Measured values for standards were within 10% of accepted values for all elements, apart from Bi, Cd, La, Li and Sc (within 20%), Sb (within 30%) and Hg (with 60%). See further details in the Excel file titled ""ALS_QC_Results_and_Calculations.xlsx" on Savanna van Mesdag's zipped folder titled "Data_and_QGIS_data_for_Warton_and_Hodbarrow" on the University of Glasgow Enlighten thesis repository:

[http://dx.doi.org/10.5525/gla.researchdata.1575.](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdx.doi.org%2F10.5525%2Fgla.researchdata.1575&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cd55bdec9fca3408c912808dc180eac4b%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638411698487295719%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=AAly5BQCzn%2FQ%2BDMfPC%2B47UYb4FyMvc1Y%2Bcs7vVN9nPE%3D&reserved=0)

Prior to statistical analyses, all of the geochemistry data provided in oxide form were transformed into mg/kg form, while the data provided in ppm form were already in mg/kg form, as ppm=mg/kg. This was done so that geochemistry measurements could be standardised, rather than having values in different forms, for statistical analyses in R and QGIS. Details, methods and metadata concerning the calculations required to transform the oxide data are provided in the file titled "W+H_Geochemistry_Data_and_Metadata_Quadrats.xlsx", also provided in the zipped folder titled "Data_and_QGIS_data_for_Warton_and_Hodbarrow" in the Enlighten thesis repository: [http://dx.doi.org/10.5525/gla.researchdata.1575.](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdx.doi.org%2F10.5525%2Fgla.researchdata.1575&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cd55bdec9fca3408c912808dc180eac4b%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638411698487295719%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=AAly5BQCzn%2FQ%2BDMfPC%2B47UYb4FyMvc1Y%2Bcs7vVN9nPE%3D&reserved=0) If you have any issues accessing the data repository and/or the data file, please email Savanna van Mesdag at savannankvm@gmail.com or message her on her Researchgate profile, (Savanna van Mesdag).

S3.2.5 Substrate sample analysis by Materials Processing Institute

While most of the geochemistry analyses for substrate samples were carried out by ALS Global, the Materials Processing Institute (MPI) in Middlesbrough carried out equivalent analyses for samples that had a particularly high metal concentration, as their equipment would not be damaged by such high metal concentrations. XRF analyses were carried out for some samples, using 90 μm sample material. For these analyses, an in-house quantitative application that has been developed using a Fluxana Raw calibration sample set was used. 32 Certified Reference Materials,

with a range of elements covering the analysis for this material were measured – elemental analysis was within 3% of standard values.

Prior to statistical analyses, all of the geochemistry data provided in oxide form were transformed into mg/kg form, while the data provided in ppm form were already in mg/kg form, as ppm=mg/kg. This was done so that geochemistry measurements could be standardised, rather than having values in different forms, for statistical analyses in R and QGIS. Details, methods and metadata concerning the calculations required to transform the oxide data are provided in the file titled "W+H_Geochemistry_Data_and_Metadata_Quadrats.xlsx", also provided in the zipped folder titled "Data_and_QGIS_data_for_Warton_and_Hodbarrow" in the Enlighten thesis repository: [http://dx.doi.org/10.5525/gla.researchdata.1575.](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdx.doi.org%2F10.5525%2Fgla.researchdata.1575&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cd55bdec9fca3408c912808dc180eac4b%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638411698487295719%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=AAly5BQCzn%2FQ%2BDMfPC%2B47UYb4FyMvc1Y%2Bcs7vVN9nPE%3D&reserved=0) If you have any issues accessing the data repository and/or the data file, please email Savanna van Mesdag at savannankvm@gmail.com or message her on her Researchgate profile, (Savanna van Mesdag).

S3.3 Species records

Details about the species records data files are provided below. All of the following species records files are available in the zipped folder "Data_and_QGIS_data_for_Warton_and_Hodbarrow" on Savanna van Mesdag's Enlighten thesis repository: [http://dx.doi.org/10.5525/gla.researchdata.1575.](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdx.doi.org%2F10.5525%2Fgla.researchdata.1575&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cd55bdec9fca3408c912808dc180eac4b%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638411698487295719%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=AAly5BQCzn%2FQ%2BDMfPC%2B47UYb4FyMvc1Y%2Bcs7vVN9nPE%3D&reserved=0)

Please note that the input "Species_List_W+H.csv", which was made for the "Species matching" function in GBIF, to update the taxonomic names ([https://www.gbif.org/tools/species-lookup\)](https://www.gbif.org/tools/species-lookup) and the output .csv file from the "Species matching" function, named "Species_List_C+H_GBIF_Generated.csv" include species records for the two study sites. Bryophyte names were additionally checked using Tropicos and World Flora Online, preference was given to accepted names on these websites for bryophytes over those on GBIF based on recommendations by Dr Neil Bell.

If you have any issues accessing the data repository and/or the data, please email Savanna van Mesdag at savannankvm@gmail.com or message her on her Researchgate profile.

S3.3.1 Warton slag bank plant data

Data files titled "Carnforth_Angiosperm_Records.xlsx" and "Carnforth_Bryophyte_Records.xlsx" contain information relating to the angiosperms and bryophytes records on the Warton (Carnforth) slag bank made during the 2019 fieldwork period. The first two of these files contain the original, untransformed data with metadata such as quadrat locations, while the third and fourth of these files contain the data (along with the data for the other recorded species in the study) for analyses in programs such as RStudio.

S3.3.1 Hodbarrow RSPB Reserve plant data

Data files titled "Hodbarrow_Angiosperm_Records.xlsx" and "Hodbarrow_Bryophyte_Records.xlsx" contain information relating to the angiosperms and bryophyte records on the Hodbarrow RSPB Reserve made during the 2019 fieldwork period. The first two of these files contain the original, untransformed data with metadata such as quadrat locations, while the third and fourth of these files contain the data (along with the data for the other recorded species in the study) for analyses in programs such as RStudio.

S3.4 Plant community information

Table S2.3: Common habitats of species recorded in each plant community on both the Warton slag bank and the Hodbarrow RSPB Reserve.

S3.5 Substrate data

See below for details about the availability of substrate data for each the study sites, available in the zipped folder "Data_and_QGIS_data_for_Warton_and_Hodbarrow" on the University of Glasgow Enlighten thesis repository: [http://dx.doi.org/10.5525/gla.researchdata.1575.](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdx.doi.org%2F10.5525%2Fgla.researchdata.1575&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cd55bdec9fca3408c912808dc180eac4b%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638411698487295719%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=AAly5BQCzn%2FQ%2BDMfPC%2B47UYb4FyMvc1Y%2Bcs7vVN9nPE%3D&reserved=0) If you have any issues accessing either data repository, please email Savanna van Mesdag at

savannankvm@gmail.com or message her on her Researchgate profile.

S3.5.1 Warton slag bank substrate data

Data for the Warton/Carnforth slag bank are available in "CARNFORTH_PLANT_CHEMISTRY_MG_KG.csv", "MAP_GRID_AND_GRID_SAMPLES_CARNFORTH.csv" and "CARNFORTH_AND_HODBARROW_CHEMISTRY_WITH_SITE_NAMES_MG_KG" . Raw data and transformed data for the Warton slag bank are also provided in the files titled "W+H_Geochemistry_Data_and_Metadata_Quadrats.xlsx".

S3.5.2 Hodbarrow RSPB Reserve substrate data

Data for the Hodbarrow RSPB Reserve are available in "HODBARROW_PLANT_CHEMISTRY_MG_KG.csv", "MAP_GRID_AND_GRID_SAMPLES_ HODBARROW.csv" and "CARNFORTH_AND_HODBARROW_CHEMISTRY_WITH_SITE_NAMES_MG_KG" . Raw data and transformed data for the Reserve are also provided in the files titled "W+H_Geochemistry_Data_and_Metadata_Quadrats.xlsx".

S3.6 QGIS mapping data and analyses

QGIS Desktop version 3.32.0 Lima was used to create maps and Inverse Distance Weight interpolation analyses for the study. The data for the QGIS mapping data and analyses are provided in the zipped folder titled

"Data_and_QGIS_data_for_Warton_and_Hodbarrow" on the University of Glasgow Enlighten thesis repository: [http://dx.doi.org/10.5525/gla.researchdata.1575.](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdx.doi.org%2F10.5525%2Fgla.researchdata.1575&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cd55bdec9fca3408c912808dc180eac4b%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638411698487295719%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=AAly5BQCzn%2FQ%2BDMfPC%2B47UYb4FyMvc1Y%2Bcs7vVN9nPE%3D&reserved=0) If you have any issues accessing the data repository/data, please email Savanna van Mesdag at savannankvm@gmail.com or message her on her Researchgate profile.

In order to view the data as it was viewed during the study:

• You will 1) have to download the zipped folder, saving it in a sensible location if need be and 2) open the relevant .qgz file/s from the relevant subfolder/s in

the latest version of QGIS - there is one .qgz for Warton (Carnforth) and one for Hodbarrow.

After opening the relevant .qgz files, you will then need to add the relevant layers to each/either file, so that different data can be accessed and viewed on the map as needed:

• For each .qgz file, add the following files in the same folder (using Data Source Manager or a similar function on QGIS is recommended for efficiency and ease of use).

If there are issues with missing layers/similar, please contact Savanna van Mesdag at savannankvm@gmail.com , s.van-mesdag.1@research.gla.ac.uk or via a private message on her Researchgate profile.

Once all of the relevant layers have been put into the QGIS .qgz file, including the community maps and the geochemistry data, IDW interpolation analyses can be carried out. To carry out the IDW interpolation analyses:

- Select "Processing" from the top toolbar.
- Select "Toolbox".
- A Toolbox window should now appear on the QGIS window, possibly on the right-hand side of the window.
- In the search bar for the Toolbox window, type "IDW".
- Select "IDW interpolation" from the search results.
- You should now see a window which looks similar to this:

- Make sure the vector layer selected is the grid samples (for either Carnforth or Hodbarrow, file name in the picture may not match the file name provided in the relevant subfolder).
- Select the chosen element for your "Interpolation Attribute", such as $SiO₂$, Al2O³ or CaO, for example.
- Select the green plus symbol to add this element to the Vector layer table.

- Make sure the "Distance coefficient P" is set to 2.
- To select the extent, press on the small arrow to the right of the (blank) Extent box. Select the 'site_boundary_for_analysis' or similar (depending on the file) and then select "Calculate from Layer."

• Select the "..." and arrow to the right of the "Interpolated" (blank) box to save the file (as a .tif file) after the analysis is completed. Be aware that every IDW analysis generates a fair amount of data, hence why none of the analyses are provided on the Github repository.

Once the analysis has run, you may need to wait for a few seconds for the layer to display properly on the map. It should show as a rectangular black and white image with at least some areas of lighter and darker shades (sometimes showing as black and white spots on a grey background). Areas that are lighter in tone show higher concentrations of an element, while darker areas show lower concentrations of that same element. To view this on top of the plant community layer: 1) make sure that this IDW layer is above the plant community layer (this layer can be dragged in the layer panel to be put above the community layer if need be); 2) Right click on the IDW layer and select "Properties"; 3) Go to the "Transparency" section in "Properties"; and 3) Adjust the Transparency of the layer by dragging the Transparency bar to the desired transparency level. Around 70% transparency is recommended to look at both the IDW layer and the Community layer at the same time.

Again, if there are any issues with opening these files in QGIS and/or using QGIS, please contact Savanna van Mesdag by email [\(savannankvm@gmail.com](mailto:savannankvm@gmail.com) or [s.van](mailto:s.van-mesdag.1@research.gla.ac.uk)[mesdag.1@research.gla.ac.uk\)](mailto:s.van-mesdag.1@research.gla.ac.uk) or over Researchgate.

S3.7 Statistical analyses

All of the additional statistical analyses carried out for this study, as well as the relevant datasets, are available in different files on Savanna van Mesdag's Github or Enlighten thesis repository. Please read further for relevant information and details regarding the various links and files for different datasets and different analyses.

S3.7.1 Biodiversity analyses

The relevant files are available in the zipped folder titled

"Biodiversity_analyses_for_Warton_and_Hodbarrow" on the University of Glasgow Enlighten thesis repository: [http://dx.doi.org/10.5525/gla.researchdata.1575.](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdx.doi.org%2F10.5525%2Fgla.researchdata.1575&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cd55bdec9fca3408c912808dc180eac4b%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638411698487295719%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=AAly5BQCzn%2FQ%2BDMfPC%2B47UYb4FyMvc1Y%2Bcs7vVN9nPE%3D&reserved=0) If you have any issues accessing this data repository, please email Savanna van Mesdag at savannankvm@gmail.com or message her on her Researchgate profile.

The data analysis file for most of the biodiversity analyses is an .rmd file, with a corresponding .html file. The .rmd file for most of the biodiversity analyses for the study can be used in RStudio or a similar program. Please adjust any code as necessary to, for example, install R packages. (The corresponding .html file may be easier to read and interpret for those who are unfamiliar with R coding.) Please note, however, that the .rmd file for the Renyi biodiversity analyses, titled "SvM_Warton+Hodbarrw_Renyi_Biodiversity_Analyses.Rmd", is meant to be run in specifically in a GUI, rather than in, say, RStudio. The instructions for how to open the GUI and run the .rmd file are provided in a PDF titled: "S_van_Mesdag_Renyi_Biodiversity_Analyses_Warton+Hodbarrow.pdf".

S3.7.2 Canonical Correspondence analyses and Non-metric multidimensional scaling analysis results

(All files described in this section are available in the Enlighten thesis repository: [http://dx.doi.org/10.5525/gla.researchdata.1575,](http://dx.doi.org/10.5525/gla.researchdata.1575) zipped folder "Canonical_Correspondence_analyses_Warton_and_Hodbarrow")

The files relate to: analyses of similarities; canonical correspondence analyses and the relevant anovas; non-metric multidimensional scaling analyses; and relevant data (used in the analyses for the six study sites).

This data analysis file is an .rmd file, there is a corresponding .html file. The .rmd file for the study can be used in RStudio or a similar program. Please adjust any code as necessary to, for example, install R packages. The corresponding .html file may be easier to read and interpret for those who are unfamiliar with R coding. The various data files can be used to look at the data run for the analyses and can be used to rerun the analyses. If you have any issues accessing the data repository/files, please email Savanna van Mesdag at savannankvm@gmail.com or message her on her Researchgate profile.

S3.7.3 Indval analyses

The relevant files are available in the zipped folder titled "Indval_Analyses_for_Warton_and_Hodbarrow" on the University of Glasgow Enlighten thesis repository: [http://dx.doi.org/10.5525/gla.researchdata.1575.](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdx.doi.org%2F10.5525%2Fgla.researchdata.1575&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cd55bdec9fca3408c912808dc180eac4b%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638411698487295719%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=AAly5BQCzn%2FQ%2BDMfPC%2B47UYb4FyMvc1Y%2Bcs7vVN9nPE%3D&reserved=0) This data analysis file in this repository is an .rmd file, there is a corresponding .html file. The .rmd file for the study can be used in RStudio or a similar program. Please adjust any code as necessary to, for example, install R packages. The corresponding .html file may be easier to read and interpret for those who are unfamiliar with R coding. If you have any issues accessing the data repository/files, please email Savanna van Mesdag at savannankvm@gmail.com or message her on her Researchgate profile.

S4: Substrate heterogeneity and high plant biodiversity on a partially clay-capped slag bank

S4.1 Further information on plant communities

Table S3.1: Commonly recorded substrates and habitats for those species recorded on the Barrow-in-Furness slag bank

S4.2 Materials and Methods – further details

S4.2.1 Initial substrate sample preparation

One substrate sample was collected from the Barrow slag bank for every plant community, within the space that the plant quadrat sample occupied. These samples were partially prepared, which involved the use of a pestle and mortar as well as a jawcrusher. Two sieves, one 0.5 mm and the other 90 μm, were used to separate sample material into 0.5 mm fractions and 90 μm fractions. The jawcrusher, jawcrusher equipment, pestle and mortar and lab surfaces were thoroughly cleaned between sample preparations, to reduce the risk of contamination, following lab protocol. The 0.5 mm and 90 μm fractions were used for different analyses.

S4.2.2 Ball mill use and XRD analyses

Sample material between 90 μm and 0.5 mm in diameter was ground down into fine powder for mineral analysis, more specifically, X-ray Diffraction (XRD) analysis. This was done using a Retsch MM400 ball mill and stainless steel containers, with stainless steel balls 2 cm in diameter. Most samples were milled for 30 seconds each at a frequency of 1/s = 30, although some samples needed more time to be milled depending on powder consistency (this was always checked before material was stored and later used). Ball mall containers and lab surfaces were thoroughly cleaned between the preparation of different samples to minimise the risk of contamination, following lab protocol. Powder samples were analysed using a Rigaku Miniflex powder diffractometer. The results were then viewed on the SmartLab Studio II program. In this software, the following was carried out for each sample:

- In the peak evaluation stage, peaks were examined and specified or deleted as necessary
- In the phase identification stage, some elements were set to unknown, while, to refine the phase identification results, the following elements were set to "Not Included": Be; Fr; Ra; Y; Nb: Tc: Ru; Rh; Pd; Ag; In; Te; I; Hf; Ta; W; Re; Os; Ir; Pt; Au; Hg; Tl; Bi; Po; At; He; Ne; Ar; Kr; Xe; Rn; La; Ce; Pr; Nd; Pm; Sm; Eu; Gd; Tb; Dy; Ho; Er; Tm; Yb; Lu; Ac; Th; Pa; U; Np; Pu; Am; Cm; Bk; Cf; Es; Fm; Md; No; and Lr. Additionally, the option to automatically include phases was not selected.
- Many of the phases that were generated in the phase identification run were not identified as being minerals actually present in the sample. Minerals that were identified as being present in the sample had at least one substantial influence on at least one of the large peaks present in the sample.

S4.2.3 pH analysis methodology

pH analysis methodology for the Barrow-in-Furness slag bank substrate samples was developed by Lab Technician Charlotte Slaymark. 2 sets of 10 mg of material for each sample were prepared with 25 ml of distilled water. These 2 sets of material were then left on a shaking table for 30 minutes. Following this shaking period, the 2

samples stood for another 30 minutes. After this, pH measurements would be taken using a pH meter. The mean measurement of the two samples would be recorded as the representative pH reading for each substrate sample. The pH meter would be regularly calibrated, so that pH readings were taken at a maximum of 1 week after the previous calibration. Mettler Toledo pH 4.01, 9.21 and 7.00 buffer solutions were used to calibrate the pH meter.

S4.2.4 Substrate sample analysis by ALS Global

Geochemistry analyses for most of the samples were done by ALS Global. Samples were ground to <90 μm diameter particle size and screened for high concentrations of iron or steel blebs. Samples that had a particularly high concentration of these were instead sent to the Materials Processing Institute (see next section). Samples underwent ICP-AES analyses following fused bead acid digestion analysis for major elements and aqua regia digestion for minor elements. 5 reference standards were used. Measured values for standards were within 10% of accepted values for all elements, apart from Bi (within 95%), Cr₂O₃ (within 30%) and Bi, Cd, La, Li, Sb and Sc (within 20 See further details in the Excel file titled

""ALS_QC_Results_and_Calculations.xlsx" on Savanna van Mesdag's zipped folder titled "Data_for_the_Barrow_in_Furness_slag_bank_study" on the University of Glasgow Enlighten thesis repository:

[http://dx.doi.org/10.5525/gla.researchdata.1575.](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdx.doi.org%2F10.5525%2Fgla.researchdata.1575&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cd55bdec9fca3408c912808dc180eac4b%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638411698487295719%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=AAly5BQCzn%2FQ%2BDMfPC%2B47UYb4FyMvc1Y%2Bcs7vVN9nPE%3D&reserved=0)

Prior to statistical analyses, all of the geochemistry data provided in oxide form were transformed into mg/kg form, while the data provided in ppm form were already in mg/kg form, as ppm=mg/kg. This was done so that geochemistry measurements could be standardised, rather than having values in different forms, for statistical analyses in R and QGIS. Details, methods and metadata concerning the calculations required to transform the oxide data are provided in the file titled "Barrow_Geochemistry_Data_and_Metadata.xlsx", also provided in the zipped folder titled "Data_for_the_Barrow_in_Furness_slag_bank_study" in the Enlighten thesis repository: [http://dx.doi.org/10.5525/gla.researchdata.1575.](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdx.doi.org%2F10.5525%2Fgla.researchdata.1575&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cd55bdec9fca3408c912808dc180eac4b%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638411698487295719%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=AAly5BQCzn%2FQ%2BDMfPC%2B47UYb4FyMvc1Y%2Bcs7vVN9nPE%3D&reserved=0) If you have any issues accessing the data repository and/or the data file, please email Savanna van Mesdag at savannankvm@gmail.com or message her on her Researchgate profile.

S4.3 Species records

Details about the species records data files are provided below. All of the following species records files are available in the zipped folder "Data for the Barrow in Furness slag bank study" on Savanna van Mesdag's Enlighten thesis repository: [http://dx.doi.org/10.5525/gla.researchdata.1575.](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdx.doi.org%2F10.5525%2Fgla.researchdata.1575&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cd55bdec9fca3408c912808dc180eac4b%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638411698487295719%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=AAly5BQCzn%2FQ%2BDMfPC%2B47UYb4FyMvc1Y%2Bcs7vVN9nPE%3D&reserved=0)

The input "Species_List_Barrow.csv", which was made for the "Species matching" function in GBIF, to update the taxonomic names (

[https://www.gbif.org/tools/species-lookup\)](https://www.gbif.org/tools/species-lookup) and the output .csv file from the "Species matching" function, named "Barrow_Species_GBIF_generated.csv" include species from across the study site. Bryophyte names were additionally checked using Tropicos and World Flora Online, preference was given to accepted names on these websites for bryophytes over those on GBIF based on recommendations by Dr Neil Bell.

Additional files include: "Barrow_Angiosperm_Records.xlsx", "Barrow Bryophyte records.xlsx" (both containing raw plant data and associated metadata, including spatial information), "BarrowPlantSpecies.csv" and

"BarrowPlantSpecies_with_site_names.csv" (both in formats for data analyses in a program such as RStudio).

If you have any issues accessing the data repository and/or the data, please email Savanna van Mesdag at savannankvm@gmail.com or message her on her Researchgate profile.

S4.4 Substrate data

Substrate data for the Barrow-in-Furness slag bank are available in the zipped folder ""Data_for_the_Barrow_in_Furness_slag_bank_study" on the University of Glasgow Enlighten thesis repository: [http://dx.doi.org/10.5525/gla.researchdata.1575.](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdx.doi.org%2F10.5525%2Fgla.researchdata.1575&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cd55bdec9fca3408c912808dc180eac4b%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638411698487295719%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=AAly5BQCzn%2FQ%2BDMfPC%2B47UYb4FyMvc1Y%2Bcs7vVN9nPE%3D&reserved=0) The names of the relevant files are: "BARROW_PLANT_CHEMISTRY_MG_KG.csv" and "BARROW_PLANT_CHEMISTRY_WITH_SITE_NAMES_MG_KG.csv" If you have any issues accessing the data repository/files, please email Savanna van Mesdag at savannankvm@gmail.com or message her on her Researchgate profile.

S4.5 Statistical analyses

S4.5.1 T tests and Wilcoxon Rank Sum tests

The relevant files are in the zipped folder titled

"T_tests_and_Wilcoxon_rank_sum_tests_Barrow" on the University of Glasgow Enlighten thesis repository: [http://dx.doi.org/10.5525/gla.researchdata.1575.](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdx.doi.org%2F10.5525%2Fgla.researchdata.1575&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cd55bdec9fca3408c912808dc180eac4b%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638411698487295719%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=AAly5BQCzn%2FQ%2BDMfPC%2B47UYb4FyMvc1Y%2Bcs7vVN9nPE%3D&reserved=0)

The data analysis file is an .rmd file, there is a corresponding .html file. The .rmd file can be used in RStudio or a similar program. Please adjust any code as necessary to, for example, install R packages. The corresponding .html file may be easier to read and interpret for those who are unfamiliar with R coding.

If you have any issues accessing either data repository, please email Savanna van Mesdag at savannankvm@gmail.com or message her on her Researchgate profile.

S4.5.2 Biodiversity analyses

The relevant files are available in the zipped folder titled "Biodiversity-analyses-for-Barrow Data" on the University of Glasgow Enlighten thesis repository: [http://dx.doi.org/10.5525/gla.researchdata.1575.](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdx.doi.org%2F10.5525%2Fgla.researchdata.1575&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cd55bdec9fca3408c912808dc180eac4b%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638411698487295719%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=AAly5BQCzn%2FQ%2BDMfPC%2B47UYb4FyMvc1Y%2Bcs7vVN9nPE%3D&reserved=0) If you have any issues accessing this data repository, please email Savanna van Mesdag at savannankvm@gmail.com or message her on her Researchgate profile.

The data analysis file for most of the biodiversity analyses is an .rmd file, with a corresponding .html file. The .rmd file for most of the biodiversity analyses for the study can be used in RStudio or a similar program. Please adjust any code as necessary to, for example, install R packages. (The corresponding .html file may be easier to read and interpret for those who are unfamiliar with R coding.) Please note, however, that the .rmd file for the Renyi biodiversity analyses, titled

"SvM_Barrow_Renyi_Biodiversity_Analyses.Rmd", is meant to be run in specifically in a GUI, rather than in, say, RStudio. The instructions for how to open the GUI and run the .rmd file are provided in a PDF titled:

"S_van_Mesdag_Renyi_Biodiversity_Analyses_Barrow.pdf".

S4.5.3 Canonical Correspondence analyses and Non-metric multidimensional scaling analysis results

(All files described in this section are available in the Enlighten thesis repository: [http://dx.doi.org/10.5525/gla.researchdata.1575,](http://dx.doi.org/10.5525/gla.researchdata.1575) zipped folder "Canonical_Correspondence_analyses_for_Barrow_Data")

The files relate to: analyses of similarities; canonical correspondence analyses and the relevant anovas; non-metric multidimensional scaling analyses; and relevant data (used in the analyses for the six study sites).

This data analysis file is an .rmd file, there is a corresponding .html file. The .rmd file for the study can be used in RStudio or a similar program. Please adjust any code as necessary to, for example, install R packages. The corresponding .html file may be easier to read and interpret for those who are unfamiliar with R coding. The various data files can be used to look at the data run for the analyses and can be used to rerun the analyses. If you have any issues accessing the data repository/files, please email Savanna van Mesdag at savannankvm@gmail.com or message her on her Researchgate profile.

S4.5.4 Indval analyses

The relevant files are available in the zipped folder titled "Indval_Analyses_for_Barrow_Plant_and_Substrate_Data" on the University of Glasgow Enlighten thesis repository:

[http://dx.doi.org/10.5525/gla.researchdata.1575.](https://eur03.safelinks.protection.outlook.com/?url=http%3A%2F%2Fdx.doi.org%2F10.5525%2Fgla.researchdata.1575&data=05%7C02%7Cs.van-mesdag.1%40research.gla.ac.uk%7Cd55bdec9fca3408c912808dc180eac4b%7C6e725c29763a4f5081f22e254f0133c8%7C1%7C0%7C638411698487295719%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=AAly5BQCzn%2FQ%2BDMfPC%2B47UYb4FyMvc1Y%2Bcs7vVN9nPE%3D&reserved=0) This data analysis file in this repository is an .rmd file, there is a corresponding .html file. The .rmd file for the study can be used in RStudio or a similar program. Please adjust any code as necessary to, for example, install R packages. The corresponding .html file may be easier to read and interpret for those who are unfamiliar with R coding. If you have any issues accessing the data repository/files, please email Savanna van Mesdag at savannankvm@gmail.com or message her on her Researchgate profile.

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