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# Heathland Restoration after Mineral Extraction

Lane, Mary Louise

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**HEATHLAND RESTORATION AFTER MINERAL EXTRACTION.**

By

**MARY LOUISE LANE**

A thesis submitted to the University of Plymouth in partial fulfilment for

the degree of

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# Author's declaration

At no time during the registration for the degree of *Doctor of Philosophy* has the author been registered for any other University award without prior agreement of the Doctoral College Quality Sub-Committee.

Work submitted for this research degree at the University of Plymouth has not formed part of any other degree either at the University of Plymouth or at another establishment.

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# Abstract

## Mary Louise Lane

## Heathland Restoration after mineral extraction

Restoration Ecology has been developed over the last 40 years in efforts to better understand how to restore, recreate or reclaim land damaged by human activities. Lowland heath is one of the more difficult habitats to recreate due to the complicated interactions of soil chemistry and plant physiology and structure. This study investigated the ecological restoration of heathlands at a china clay mineral extraction site in the UK. Assessment of historical restoration practices revealed that previous work carried out on the study site is not achieving Atlantic Lowland Heath (ALH) and that specific amendments might be necessary. Consequently, work carried out in the greenhouse assessed the effectiveness of commercial Ericoid Mycorrhizal Fungi (ErM, Rhodovit) and organic matter (compost) on the growth of *Calluna vulgaris.* The highest survival of plants was observed in the stored topsoil, followed by stored topsoil with commercial ErM. DNA analysis confirmed the presence of ErM fungi in the order Heliotales on root hairs of plants grown in the ErM treated soil. Chemical analyses of topsoil stored in soil berms for up to five years identified some changes, in particular, downward movement of some chemical elements. While the soil remained suitable for restoration use after prolonged storage, the results indicated that amendments to achieve a higher soil C:N ratio may improve ALH establishment. Using methods developed from analysis of historical restorations, a large-scale field trial evaluated the effect on successful heathland restoration of adding plant accessible nutrients, organic material or ErM, as well as combinations of these treatments. The results showed that the addition of seeds to stored topsoil resulted in the highest number of *C. vulgaris* plants germinating in the final field season. Unfortunately, none of the treatments successfully created ALH within three field seasons. The conclusions

reached from field trials and detailed analyses of stored topsoil point towards the importance of adding organic matter that specifically elevates the C:N ratio of the substrate to be restored. This merits further investigation. Overall, this work supports the work and the findings of previous studies detailing that the successful establishment of ALH is a complex undertaking, requiring minimal intervention with controlled nutrient addition.

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# General Introduction

## Biodiversity loss

It is reported that the world is entering a sixth mass extinction phase (Barnosky et al., 2011) due to anthropogenic pressures on the environment. The impact of the increasing human population and consequent loss of biodiversity has long been recognised and was the focus of the 1992 Earth Summit in Rio. The resulting Convention on Biological Diversity (CBD) now underpins the majority of global biodiversity legislation (Secretariat of the Convention on Biological Diversity, 2010) with the main conclusions highlighting the persistent and intensifying effects of habitat loss and degradation on biodiversity due to land-use change arising from food production, urban expansion, and mineral extraction. All of these anthropogenic activities require space and result in biodiversity loss and impact on ecosystem services (Rands et al., 2010). In 2010, following on from the CBD, the UN developed the Aichi targets to provide measurable time-bound targets to stop or reverse biodiversity loss. Accordingly, in 2013, the UK government took stock of the priority habitats listed in the EU guidance and created the Biodiversity 2020 targets. These aimed to achieve a favourable or recovering condition for 90% of priority habitats and 50% of Sites of Special Scientific Interest (SSSIs). Further development of these targets specified maintaining 95% of habitats in favourable or recovering condition by 2020 (Defra, 2019).

An unintended consequence of human activity is biodiversity loss; however, mitigation attempts are frequently hampered because conservation legislation is rarely made by those who live with the detrimental consequences of biodiversity loss (Rands et al., 2010; Secretariat of the Convention on Biological Diversity, 2010; Hayhow et al., 2019). To address this, the international community is working towards the inclusion of biodiversity mitigation as a necessary cost consideration when making commercial business decisions (Evison & Knight, 2010). One method of including biodiversity loss in business decisions is by means of biodiversity offsetting, which involves conservation activities to compensate for the loss of, or damage to existing sites. This method has existed since the 1970s but has become much more widespread in recent decades despite concerns about the effectiveness and use of the technique (Evans et al., 2015; Gordon et al., 2015). This has accelerated the development of new ideas incorporated into the remit of restoration ecology for endangered and damaged habitats.

## Restoration ecology

Restoration ecology seeks to apply ecological principles to enable the recovery of disrupted habitats to a more natural state (Hobbs & Norton, 1996; Hobbs & Harris, 2001; Clewell et al., 2004). In the 1970s, changes of land use were identified as a problem and the need for

restoration began to be understood (Bradshaw, 1977). Emerging in the 1980s, the field of restoration ecology also incorporated policy, management, and socioeconomic aspects in its wider framework, representing one of the first truly multidisciplinary ecological fields. The EU directive on habitats (92/43/EEC) further aimed to ensure member states maintained or restored protected habitats, promoting the maintenance of biodiversity and fulfilling obligations to the Bern Convention (1979) (Convention on the Conservation of European Wildlife and Natural Habitats). Since then, restoration ecology has become an important discipline within the wider context of biodiversity conservation.

By its very nature, restoration ecology requires a biological understanding of a wide range of taxa, including soil microbes, fungi and plants, in addition to community and population ecology, and spatial and invasive species ecology (Miller & Hobbs, 2007). Restoration ecology defines field restorative concepts to allow clarity for practitioners and researchers alike. The most important of these are the meaning of restoration: the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed, as opposed to rehabilitation, which emphasizes the reparation of ecosystem processes, productivity and services (Clewell et al., 2004).

Restoration ecology differs in relation to traditional conservation ecology, as the latter is concerned with the causes of small population size and various consequences, such as rarity, demographic, environmental, and genetic stochasticity (Dobson et al., 1997). In contrast, although some conservation requires work or management to return a habitat to its original state, restoration ecology focuses on application of principles to rehabilitate devastated environments (Bradshaw, 1997a; Perrow & Davy, 2002; Allison & Ausden, 2004; Brown, 2005; Barr et al., 2016).

Restoration ecology seeks to apply ecological principles to achieve government-imposed habitat standards, yet government policy tends to lag behind scientific advances. Within this wider context, policy-based factors that influence restoration success and stakeholder interests must be considered. Morris et al. (2006) and Morris (2011) highlight the conflict between the needs of businesses and the need to protect threatened habitats. Restoration policies consequently provide a framework for local councils and land managers to assess positive and negative impacts, whilst examining how the restoration process can be developed under planning guidance (Bullock et al., 2011). From a policy maker's perspective, ecosystem services and biodiversity are among the main drivers for ecological restoration, however, from a research perspective, empirical evidence supporting practices, such as biodiversity offsetting, are relatively scarce and such practices can even have negative effects (Gordon et al., 2015). It is accepted that ecosystem

services and biodiversity are intricately linked (Balvanera et al., 2013); it therefore follows that ecosystem services are facilitated by biodiversity processes and measures, such as determining richness of species. Consequently, to develop ecosystem services, the biodiversity of a habitat must be studied. Nevertheless, it is not certain that either will follow the planned trajectory (Lundholm & Richardson, 2010; Newby, 2010; DeJong et al., 2015). There may also be additional conflict between local and international restoration goals. At the international level, it may be, for example, important to target maximum carbon sequestration within habitats, yet this might not link in with local requirements. This can cause conflict and endanger restoration projects (Buckley & Crone (2008); Bullock et al., 2011).

To resolve potential conflicts, stakeholders need to be involved in restoration projects from the beginning. A feeling of ownership by all the stakeholders is essential to any project, especially when the project will continue after the initial restoration work has been completed (Cabin, 2007; Buckley & Crone, 2008). Ultimately, strong communication between project managers and stakeholders is critical for successful restoration (Brown, 2005). For example, post-restoration land use must be acceptable to the public as well as the farmer, landowner, and government stakeholders (Soltanmohammadi et al., 2010), once again reiterating the need for wider stakeholder consultation (Gann & Lamb, 2006). This is illustrated by the analysis of conflict within a large-scale US river restoration project that aimed to restore ecological processes

rather than particular habitat features (Buckley & Crone, 2008).

Buckley and Crone (2008) concluded that involving all interested parties and accepting compromise concerning the extent and end result of the restoration, was the best way to ensure the restoration was successful. In addition, their research concluded that people could understand conservation of a species better than restoration of an environment. In this instance, the restoration of heathland/wasteland next to arable farms increased the risk of weeds and pests contaminating the crops. Consequently, having neighbouring landowners consulted throughout the project minimised ill-feeling and increased the chances of restoration success (Buckley & Crone (2008).

Hobbs & Harris (2001) recommend more dialogue between theoretical and applied aspects of the field. Questions remain for restoration practitioners and theorists about the role of restoration ecology and whether it carries the authority to have sufficient pathways into policy for its efficient application (Hobbs & Harris, 2001; Cabin, 2007; Dickens & Suding, 2013). Therefore, theoretical restoration ecologists need to have a direct contribution to policy-making in order to support valued judgements by practitioners when deciding the feasibility of a restoration project (Anderson, 2014).

Outcomes from restoration ecology research will guide and support policymaking, such as those strategies arising from the Aichi

biodiversity targets. In turn, the structure of existing planning can be accommodated to support the biodiversity policies implemented as a result of the research. The UK government are bringing in a Net Gain process (Gov.uk, 2019) as a mandatory part of planning process to improve biodiversity. This means that any new development will need to provide a ten percent increase in biodiversity for any new planning permission (DEFRA, 2018).

## Passive restoration vs active restoration

There are two forms of restoration and both have passionate advocates (Zahawi, 2014; Legwaila et al., 2015; Prach &  del Moral, 2015). The first is passive restoration. This method allows nature 'to lead' the restoration with very little or no participation from restoration ecologists or stakeholders. This method is suggested to increase biodiversity in local areas where there is a smaller scale of disturbance and a large opportunity for seeds and other species to move into the disturbance area (Tropek et al., 2010; Tropek et al., 2014; Šebelíková et al., 2016). This has proved very effective in Czechia and other eastern European countries (Prach, 2001; Prach &  Hobbs, 2008; Prach et al., 2014).

Rewilding could fall into this category, although there is a difference between restoration and rewilding, with rewilding having its own definition and priorities. Rewilding is an adaptive approach to preserving ecological function and adaptation to shifts in climate as opposed to restoration, which looks to maintain an historical set point

despite changes such as climatic deviations (Perino et al., 2019).
Although the initial work could be active, such as translocating species,
the ecosystem is then left to achieve equilibrium (du Toit & Pettorelli,
2019). The more flexible rewilding requires large-scale areas to be
effective and would require a change in policy from the government
planning departments in order to be achieved by commercial
operations.

At the other end of the scale is active restoration. Active restoration
involves technical intervention and can range from full landscaping,
such as is required when restoring worked out mines and quarries
(Harris et al., 1996), to reclaiming unmanaged landscapes to the
successional point required (Walker et al., 2014). Active restoration can
be divided into two spheres, one with a focus on intervention in the soil
and the other with a focus on intervention in the plant communities.
The two different environments require different interventions, but both
must be understood and the implications on the other ought to be
assessed if only one is carried out (Allison & Ausden, 2004; Allison &
Ausden, 2006). Frequently, restoration studies focus only on one aspect
and a transdisciplinary approach would be beneficial to improve
restoration outcomes (Alday et al., 2012; Alday & Marrs, 2014). The
transdisciplinary approach, as demonstrated by Donnison et al. (2000),
Bardgett et al. (2005), Smith et al. (2008) and Van der Heijde et al.
(2008), enables restoration ecologists to develop biological, mycological
and soil conditions to kick start the ecosystems to develop in the

planned direction. The small-scale experiments prove the concept, enabling the large-scale restoration to deliver joined up landscape-scale improvements with the assistance of industry (Bate et al., 1998).

## Restoration and the mining industry

After the 1980s, with the introduction of the European Habitats Directive, restoration ecology began to be incorporated into UK local council planning and thus became a legal responsibility of mining companies. This process was and continues to be overseen by the ROMPS (Review of Old Mining Permissions), started in 1995, to review old mineral permissions set up during World War two (Devon MAP, 2017). New extraction operations have to provide a complete restoration scheme as part of the planning application. Reclamation of disused pits allows structured restoration to develop rare or missing habitats within the confines of the geography and geology of the area. Nevertheless, some factors cannot be changed, for example if the quarry has high faces, this cannot be altered without a lot of work, and as such, they are left as habitats for birds of prey that have sometimes settled and colonized the site whilst the quarry is in operation.

The '*Making Space for Nature*' report (Lawton, 2010) calls for restoration to be guided by four overarching principles of 'More, Bigger, Better, and Joined up'. This report provides the industry with guidance detailing

the ecosystem services priority habitats provide. The mineral industry works with NGO and academic partners in order to apply these principles to all restoration projects. The Lawton report builds a framework with a clarion call for legislation to set small-scale restoration projects into a larger view of whole landscape-scale projects, allowing the free movement of species across the landscape. Whilst Lawton's (2010) report 'Making Space for Nature' is a seminal work that has not only captured the imagination and willpower of conservationists, it has also made its way into policy, and business decisions. The State of Nature report was updated in 2019, highlighting the continued decline of most habitats and species identified with in it (Hayhow et al., 2019).

## Kaolinite extraction

Mineral extraction provides a 'blank canvas' for restoration, as no topsoil or seed banks remain at the site prior to restoration. This potential opportunity is tempered by the material used to restore the area. The majority of extraction operations store the material extracted prior to reaching the mineral deposits; this is known as overburden; the amount of stored material consequently depends on the depth of the extracted mineral. In mineral excavations, such as coal, there is a large amount of overburden to strip and store because the mineral deposit is deep. In contrast, kaolin can be as close to the surface as 5-10cm (Sheppard, 1977), meaning the soil is bulldozed off the surface and put

into a pile for the excavator to lift onto waiting dumper trucks to be
stored elsewhere as overburden.

Kaolin is formed by the decomposition of granite by hydrothermal
activity and as such is formed along seams where the water has forced
its way through the granite. This turns the granite into kaolinite and
feldspar with gravel and aggregates all held within the granite matrix.

The fact that the kaolinite is held within the matrix stops any contact
with other minerals (Sheppard, 1977). This lack of mixing with other
mineral gives kaolin its distinctive white colour and purity.

The kaolinite quarries involved in this study lie on the South East
boundary of the Dartmoor National Park just outside of Ivybridge,
Devon. The area lies along the granite massive of Dartmoor Forest,
which has been slowly decomposing in the last 10 million years (Floyd
et al., 1993). Shortly after kaolin was first discovered by William
Cookworthy in 1745 in Cornwall (Pounds, 1948), kaolin was discovered
on the South East edge of Dartmoor, and it has been extracted
commercially in this area since 1830 (Dyer et al., 2019).

The extraction method is water-based; the matrix is dug from test pits
and other areas of the quarry, and then moved to the quarry benches
where is it tipped off the floor to fall in front of high-pressured hoses
which turn the clay matrix into a suspension. The water clay
suspension is pumped up to settling tanks and hydrocyclones to

separate out the smaller clay particles from the larger sands and gravels. New processes and advances in technology mean that areas with less kaolin in the quarry can be utilised to obtain material that in the past would have been left (Dyer et al., 2019).

## Atlantic Lowland Heath

Created and maintained by light grazing and burning, heathlands account for 16% of the UK's priority habitats (Hobbs & Gimingham, 1987; Hulme et al., 2002; Defra, 2019). The Joint Nature Conservation Committee (JNCC) defines lowland heath as comprising mostly ericoid and gorse species (JNCC, 2004). It exists along the western Atlantic coast from Germany to Portugal and as far north as southern Scandinavia, forming on chemically poor acidic soils below altitudes of <300m (Symes & Day, 2003). The typical climate is one of cool moist summers and warm moist winters; the precipitation is <1000mm throughout the year, and the temperature ranges from 22$^{\circ}$C in the summer to 0$^{\circ}$C in the winter (Perrow & Davy, 2002). Webb (1986) has a tighter definition for lowland heath; the environment is at altitudes below 250 m, growing ericaceous dwarf-shrubs in acidic poor mineral nutrient soils, such as *Calluna vulgaris*, which grow to about 50 cm with four major stages of growth: pioneer, building, mature and dying. These four stages take approximately 30 years in total, providing different habitats throughout each stage for invertebrates such as Opiliones, Araneae, Collembola, Thysanoptera, Hemiptera, Coleoptera,

Diptera, Hymenoptera, Diplopoda, and Acarina. The highest diversity was found in the pioneer and dying phases as opposed to the building and mature phases (Watt, 1955; Gimingham, 1985).

Whichever definition is applied, lowland heaths remain a priority habitat in the UK, but current assessments show that the area and health of the identified heathlands have deteriorated and declined (Defra, 2019). The successful restoration of lowland heath has therefore become a high priority for restoration ecology. Unfortunately, lowland heath is a difficult habitat to restore (Putwain & Rae, 1988) and maintain post-restoration (Pywell et al., 1995) and it is not always straightforward to generalise from results of one restoration study to others (Pywell et al., 2011).

Heathland is the result of prehistoric land clearances, e.g. for grazing and peat turf cutting, creating the soil conditions for heath to flourish (Amesbury et al., 2008; Fyfe & Woodbridge, 2012). As the habitat is an arrested successional stage, there is a requirement for continued management by grazing and controlled burning to maintain the habitat (English Nature, 2002; Perrow & Davy, 2002). Since the mid-19th Century, there has been a global reduction in heathland. It is estimated that in England, only one sixth of original heathland remains (Perrow & Davy, 2002; JNCC, 2016). Its decline has resulted in it becoming classified as endangered with the need to preserve remaining heathland

alongside restoration of damaged areas and the creation of new areas. Devon holds approximately 14% of the South West's heathland; this is about 7% of the national total (Devon BAP, 2009). To flourish, heathland requires at least four factors: low soil pH, low soil nutrient content, dominant dwarf ericoid plant community, and human management to prevent succession to woodland (Usher & Thompson, 1993; Snow & Marrs, 1997; Box, 2003; Symes & Day, 2003; Martinez-Ruiz et al., 2007; Pywell et al., 2007; Kleijn et al., 2008; Newton et al., 2009).

## Factors affecting heathland restoration

Foremost, heathland restoration is expensive and labour intensive (£3120/ha$^{-1}$) (Davis et al., 2011) and throughout the years, several authors have provided guidance on the restoration of heathland after different disturbances (Putwain & Rae, 1988; Perrow & Davy, 2002; Symes & Day, 2003). Common restoration practices include providing seed or plant resources or soil amelioration to optimise the success of any colonising seeds. Providing seed or plant resources includes, but is not limited to, 'green haying', plug planting, turve translocation and hydroseeding (Pywell et al., 1996; Box & Hill, 1999; Box, 2003; Box et al., 2011). Green haying or brashing is the use and spreading of cuttings from a donor heath onto the restored area and spreading it at a maximum spread of 1 to 3 ratio on to the area to be restored. This technique is widely used within conservation work for small to medium

sized areas. It requires access to a donor heath, a way of cutting and collecting the cuttings, and a means of spreading the cuttings over the restoration area (Symes &  Day, 2003). Plug planting is the planting of small plants, usually *Calluna vulgaris* or *Erica tetralix or Erica cinerea,* within the area to be restored. This is labour intensive and again requires plants from a nursey or a donor site (Perrow &  Davy, 2002). The next method examined here using plants is turve translocation. This method uses chunks of heathland habitat instead of individual plants. As with the previous two methods, this method moves not only the plant but key parts of the associated soil ecosystem. This is important because the soil will likely contain ericoid mycorrhizal fungi, and any soil microbes within the lifted turves. This is a good method for use if an area of heathland is being lost due to land use change, for example housing estates, road building, or mineral extraction. It is not necessarily a good option or the 'go to' option, as new heathland is not being created, it is being moved. Nevertheless, it can be effective, but still does not guarantee a success (Box, 2003; Box et al., 2011). The final technique, well suited for the industrial scale, is industrial hydroseeding. The application of commercially bought seeds (or seeds collected and vernalised from a local donor heath); it carefully calculates percentages of different species of grass and ericoids. Seeds are then mixed with plant nutrients and a mulch to minimise the loss of seeds to erosion and provide a nurturing germinating substrate. The technique can be applied to ground covered in topsoil or on subsoils or mining waste. It is applied from a bowser in a manner similar to muck

spreading. It is an effective technique for producing a green area quickly but the effectiveness of creating a biodiverse habitat is less clear (Clemente et al., 2016). For the soil amelioration, Clarke (1997) agreed with older published studies that the use of heathland soil is the best route to restore heathland. The list of chemical and physical properties that need to be assessed prior to attempting restoration include soil pH, organic matter, exchangeable calcium, extractable phosphorus, and cation exchange capacity (Pywell et al., 1994; Manning et al., 2006). The ideal conditions within heathland are pH 2.8-3.9, with exchangeable calcium between 80-159 μm $Ca^{-1}$ and extractable phosphorus at 1mg/100 g soil. However, Clarke (1997) was comparing restoration to heathland from arable land, and not the restoration after-mineral extraction, therefore, the comparison of soil-forming material taken from overburden tips needs investigation to determine the full suite of nutrients required in the soil, as these will be different to the restoration from fertilised arable land. This is a pertinent point, as successful restoration of lowland heath requires an in-depth understanding of soil nutrient content, nutrient cycling, and depth of topsoil before building the soil horizons, as these are critical for the success of any treatment (Marrs et al., 1980; Clarke, 1997; Kleijn et al., 2008).

One judge of success is the JNCC framework for endangered habitats. This allows existing habitat to be monitored, establishing a benchmark for new habitat to ensure the quality of restoration. It is used by governments, businesses and ecologists to classify heathland

communities and their stages of development. The framework ensures restoration schemes can be qualified against a national standard and thus judged as successful or not (JNCC, 2004). It is based on the National Vegetation Classification developed by Rodwell (1998), Heathlands are caterogised as H1-22. This classification identifies different communities within different environments, soil types and dominant vegetation. The heaths aimed for in this project are H8 *Calluna vulgaris – Ulex gallii* heath.

## Why restore lowland heath?

Lowland heath is under the twin threats of urban expansion and increasing use for recreation (Symes &  Day, 2003; JNCC, 2004; Armsworth et al., 2010). Based on the UK Government's Biodiversity 2020 targets, lowland heath has deteriorated since the last assessment (Hayhow et al., 2019). Lowland heath has a natural heterogeneity, important for habitat provision. Several rare plants, such as Marsh Club Moss (*Lycopodiella inundata*) and notable invertebrates such as Scarce Blue-tailed Damselfly (*Ischnura pumilio*) and Fairy Shrimp (*Chirocephalus diaphanus),* need the disturbance of transitional landscapes to thrive. The local economy also needs heathland to provide commoners with grazing land (Hartley &  Mitchell, 2005; Mitchell et al., 2008). Other ecosystem services that heathlands provide are both climatic and anthropogenic. The development of soils and their capacity for carbon capture is being discovered and highlighted as much more

important than previously thought (Kopittke et al., 2013). The opportunity to expand the access to nature for local people for health benefits exists; potentially highlighting its use for cycling, walking, and horse riding, to support the maintenance of heterogeneity of the habitat. However, the potential for recreation needs to be discussed at a planning stage to ensure paths are made to minimise the erosion of soil by human interaction (Blaen et al., 2015; Wilker et al., 2016). Although heaths are an arrested successional stage and there are arguments for it to be left to return to Birch and Ash woodland (Caro, 2007; Bauer et al., 2009; Caro & Sherman, 2009), the soil chemistry and depth would make initial woodland restoration difficult to achieve (Mitchell et al., 1997), and woodland succession would cause a consequential change in biodiversity.

## Ericoid plants and associated fungi

Globally, Ericaceae are a diverse family, but in the UK; there are approximately 20 species. Examples are heathers (*Calluna vulgaris, Erica cinerea)* and bilberry (*Vaccinium myrtillus)* (Read, 1996; Bradshaw, 1997a; Pywell et al., 2007). Ericaceae are able to colonise low nutrient soils due in part to a symbiotic relationship with Ericoid mycorrhizal (ErM) fungi found on the hair roots of the majority of species (Read, 1996; Diaz et al., 2006; Hazard et al., 2014). This is a mutualistic relationship where the plant benefits from improved nutrition, which enables them to thrive on poor soils. The fungi, for example

*Rhizocyphus ericae,* receive up to 15% of the carbohydrate created by the plant during photosynthesis (Smith & Read, 2010). The plants obtain nutrients, in particular nitrogen, from sources that without ErM would be unavailable to them, such as proteins, amino acids and polypeptides, which the fungi break down using extracellular enzymes (Bending & Read, 1996a; Bending & Read, 1997; Read et al., 2005). This enables the ericoids to outcompete other species in the area, ensuring stronger vigorous growth, building the characteristic heathland (Diaz et al., 2006). The ErM fungi also provide protection from higher levels of toxic metals such as Al and Cu in the soil (Bradley et al., 1982). This protective aspect of the ErM would make it an ideal addition for restoration of mineral extraction sites if a commercial source proves to be as successful as natural inoculation of soils (Wubs et al., 2016; Wubs et al., 2018).

## Soils in lowland heath restoration and implications for management

Soil is a finite and fundamental resource for plant growth. Because of the importance of soil, there has been a long interest in its formation, development, improvement, and ecology. This early interest began in the 1880s in Russia with work on agricultural questions (Dokuchaev, 1883; Moon, 2018) and includes Darwin's work on the role of earthworms in pedogenesis (Darwin, 1881 ; Johnson & Schaetzl, 2015). Soil is a complex environment with physical, chemical and biological

characteristics. There are five main parameters; parent material, time, biological components, climate and topography, that all interact to produce the multitudes of different soils found in the world (Jenny, 1994). In addition to the main parameters, the physical part of soil, the size, shape, and compaction, of the grains of soil also varies. The chemical reactive nature of the grains, which includes the interstitial water, is vital for pH and the reactivity of ions. The biological part of soil, which includes microbes, fungi and micro and macro invertebrates, affects the soil's ability to maintain a sustainable chemical and physical environment, therefore all aspects of the soil environment, chemical, physical and biological, impact on soil creation (Anderson, 1988).

Temperate soils feature typical distinct horizontal sections called horizons, whose physical and chemical characteristics influence the soil's ability to support its biological communities and functions. The upper (surface) horizon is the litter layer (O Horizon), moving downwards there follows an organic-rich mineral layer (A Horizon). Dividing the A and B horizons in more mature developed soils is the E horizon, a layer that is created by leaching its mineral and organic content downwards, mainly leaving silicates. Subsoils are characterised by accumulated clay (B Horizon), unconsolidated weathered parent material (C Horizon), the Saprolite or weathered bedrock (D Horizon) and finally the bedrock (R Horizon) (McQueen & Scott, 2008).

Jenny (1994) suggests that whilst people have been dividing above ground and below ground restoration, the divide is arbitrary when moving down through the plant and litter layer. At what size does the decomposed litter become soil, and moving up, vice versa? This question has been carried on in the work by(Richter & Markewitz, 1995). This point is important when looking at restoring environments, especially after mining activities when the whole environment needs to be restored; so understanding the interaction of above ground, below ground, and mineral interactions, is important when attempting to achieve a specific environment by using an interdisciplinary approach (Marrs et al., 2007; Alday et al., 2011; Alday et al., 2012; Alday & Marrs, 2014; Walker et al., 2014; Marrs, 2016).

Soils typical of lowland heath are a product of the quantity and composition of water, photosynthetically-active radiation (PAR), ambient temperature, and the mineral nature of the bedrock (Clarke, 1997). The interaction between these factors results in the movement of complexes of nutrients, such as magnesium, calcium, potassium, phosphorus and nitrogen, down the soil profile into the sub soils, causing the upper soil levels of lowland heath to be acidic and nutrient impoverished. Typically, lowland heath soils around Dartmoor are sandy mineral soils; podsols formed due to a high level of leaching throughout the soil, rather than nutrient deposition by litter at the surface (Hawley et al., 2008). The vegetation and soil are a dynamic system and one may not accurately reflect the conditions within the other. Thus, understanding

the minutiae of soil nutrient combinations and the plants that these
interactions support, is important before starting any restoration
project (Smith et al., 2003).

The restoration of soil is more complex when there is no starting soil
structure. To restore soil for lowland health restoration after
agricultural use, the deep structure and parent materials are still
present from the previous land use, thus these can be used as a basis
for the restoration horizons. However, when mineral extraction sites are
restored, there are no parent materials, as these are the minerals that
were mined and thus removed. Therefore, whilst there may be
compaction and disruption to the surface layers of the soil and chemical
changes to the nature of the soils, there are no deep horizons left and
no surface to turn over, as the whole body of soil has gone. If
restoration is to succeed, then the soil needs to be built up using
whatever waste materials are suitable and the horizon structure must
be replaced. Where this is not possible, then pioneer plant species can
develop soils and nutrient cycling, such as *Ulex spp* and *Anthyllis
vulneraria;* these are useful to begin to build the ecosystem and the
soils that ALH relies on. This process to achieve ALH will take a much
longer time scale than the 5 years specified period of planned aftercare.
Roberts et al. (1981) found it could take up to 55 years to develop an
ALH habitat on waste kaolinite sands.

## Aims of study

The broad aim of this study is to investigate the large-scale restoration of Atlantic Lowland Heath (ALH) after china clay extraction. In particular, the thesis will address four key areas:

(1) Investigation of historical restoration practices and their impact on the community ecology of the site. This will also inform the design of experimental field trials (see below). Specifically, this chapter will investigate plant community composition in relation to time since restoration and differing restoration methods applied at the Sibelco Ltd. china clay extraction site in south Devon. This will provide insight into whether it is possible to reinstate ALH in the absence of active restoration.

(2) A greenhouse experiment to investigate the effect of different soil amendments –stored topsoil (i.e. overburden), addition of ericoid mycorrhizal fungi, and organic matter –on the growth of *Calluna* cuttings from local heathlands.

(3) Investigation of changes in soil chemistry during overburden storage. In particular, this chapter will assess how time and depth in storage affect the soil's key chemical and physical parameters and its suitability for use in heathland restoration.

(4) A large-scale investigation of different restoration treatments on vegetation community composition over three years in collaboration with the active participation of Sibelco Ltd UK using the china clay quarry on the South East edge of the Dartmoor National Park. The different restoration methods aim to address missing soil nutrients on areas requiring restoration post mineral extraction. It is hypothesized that addition of soil cations would increase the colonisation of kaolinite waste by dwarf ericoid shrubs. Furthermore, it is predicted that the addition of organic matter would enable acid grasses to act as nursery plants to restore ALH more rapidly than the sole addition of seeds onto spread stored topsoil.

# Chapter 1 Chronosequence of former kaolinite open cast mines suggests active intervention is required for the restoration of Atlantic heathland

## Introduction

The Atlantic lowland heaths (ALH) of NW Europe are a distinct habitat characterised by a dominant heather (*Calluna* and *Erica* species), shrubby Fabaceae (i.e. *Ulex* and other Genisteae species) and distinctive graminoid (e.g. *Molinia caerulea*) community growing on low nutrient, acid soils (Gimingham, 1972; Loidi et al., 2010). Most heathlands are the result of anthropogenic management imposed by periodic fire, grazing, or other disturbances, and as such, these habitats have an important cultural, as well as biodiversity and ecosystem service value (Mitchell et al., 2008; Pywell et al., 2011; Fagúndez, 2013). In the UK alone, 133 conservation priority (UK Post-2010 Biodiversity Framework) plant and animal species are associated with ALH, including 47 invertebrates with either a restricted, or very restricted UK distribution, which are also internationally rare or endangered (Webb et al., 2010). Globally, heathlands face a number of threats, and due to a combination of changing management, atmospheric N deposition, and

habitat loss associated with building development, the ALH habitats of western Europe, are particularly endangered (Fagúndez, 2013; Bähring et al., 2017). For example, only one sixth of the lowland heath present in England from the early 19th century remains, much of this lost to urbanisation and agricultural intensification (Perrow & Davy, 2002; Webb et al., 2010). This dramatic decline has prompted the European Union and individual member states to adopt and implement various protection and restoration strategies for the habitat (JNCC, 2004; Pywell et al., 2011).

ALH requires at least four elements to establish and persist. These include: (i) low soil pH (2.8-3.9, Clarke 1997), (ii) low soil nutrient content (exchangeable calcium 80-159 µg Ca $g^{-1}$, exchangeable phosphorus < 10 µg P $g^{-1}$ soil, Clarke 1997), (iii) propagule supply of dominant dwarf ericoid plants, and (iv) management to prevent succession to woodland (Martinez-Ruiz et al., 2007; Kleijn et al., 2008; Newton et al., 2009). On this basis, many different restoration techniques have been tested (Putwain & Rae, 1988; Pywell et al., 1994; Clemente et al., 2016) but these can be categorised into two classes: those involving soil amelioration (e.g. nutrient addition, overturning), and those involving the selective addition of plants or seeds (Allison & Ausden, 2004; Walker et al., 2004b; Pywell et al., 2007; Glen et al., 2017). The results of many previous studies indicate the most important factor for successful heathland restoration is prior land use, as the most successful restorations are situated on former heathland

(Walker et al., 2004a). Here, the removal of invasive scrub, coupled with the distribution of heather brash and native seeds (of local morphotype/genotype) cut and/or collected from local established adjacent heathland, have proved the most effective strategy (Walker et al., 2004a; Walker et al., 2007; Diaz et al., 2008). This approach not only provides the recipient community with necessary propagules and microbial symbionts, it also underscores the importance of soil biogeochemistry and the major contribution that the soil seed bank makes to heathland regeneration (Clarke, 1993; Pywell et al., 1996; Fagúndez, 2013; Nussbaumer et al., 2016). This is not, however, a sustainable or practical technique to cover large areas or where there has been significant land use change, such as quarrying operations over many decades.

In many cases, effective restoration of former open cast quarries may be achieved by 'passive restoration' (Prach, 2001; Tropek et al., 2010; Prach et al., 2013). This approach has many advantages, including relatively rapid colonisation by local ecotypes of well-adapted species, with minimal economic costs to the mine operator (Prach & Hobbs, 2008). Passive restoration seems to work best however, when the disturbed site is small, and surrounded by natural vegetation unaffected by the initial disturbance (Holl & Aide, 2011; Prach et al., 2014).

Many OCM (Open Cast Mining) sites in the UK are located in areas naturally-dominated by heathland habitats, where high concentrations of mineral deposits, such as cassiterite, ilmenite, and kaolinite occur beneath the low nutrient, highly acidic, soils. This pattern is generally true of European ALH, where the habitat is most commonly associated with soils originating over low nutrient, sand and gravel beds (e.g. Belgium and the Netherlands), or igneous, typically granitic, intrusions (Scandinavia, Western France & UK). In SW England, ALH frequently coincides with deposits of the aluminosilicate mineral, kaolinite, a product of in-situ alteration of the plagioclase feldspar component of the granite intrusions that surface throughout Cornwall and west Devon. As a result, there has been a 300 year history of OCM kaolinite extraction in the region, supporting over 5% of the global extent of ALH habitat (Devon BAP, 2009).

In general, the nature and success of any restoration depend on the planning conditions imposed and the suitability of post-OCM conditions for plant establishment (Cooke &  Johnson, 2002; Kuter, 2013). By its nature, OCM necessitates the removal of plant communities and underlying material to expose commercially-extractable minerals. The topsoil and underlying sediments (overburden) are mixed and stockpiled, destroying natural soil structure. Often, there is not enough original topsoil to cover the area left after extraction (Merino-Martín et al., 2017). Most problematic however, is the long period (between 1-20 years) of overburden storage, since over time, sub-surface soil layers in

storage berms develop sub- or anoxic conditions, causing changes in microbial communities and further deterioration of soil structure and quality (Golos et al., 2016; Merino-Martín et al., 2017). In the specific case of ALH restoration, the combination of stockpiling methods and age since removal means that the availability of donor soils containing seed of the community dominant, *Calluna vulgaris*, is often limited (Pywell et al., 2002). Even if seeds are present, the altered characteristics and microbial communities of donor soils degraded by storage can reduce subsequent *Calluna* seedling establishment (Bossuyt & Honnay, 2008). *C. vulgaris* can take 25-55 years to colonize mine spoil after cessation of mining operations (Roberts et al., 1982). In addition, the seeds of other component plant species, including many rare heathland specialists, are poorly represented in the overburden seed bank compared to their contribution in the natural ALH community (Bakker & Berendse, 1999). The decline of the seed bank and loss of soil structure and microbial community in stored overburden underscores why the most successful attempts to restore ALH have been on former heathland sites. In these cases, some vestige of pre-disturbance soil propagule availability, microbial community, and soil biogeochemistry remains (Pywell et al., 1996; Walker et al., 2004b; Walker et al., 2007; Diaz et al., 2008; Wubs et al., 2018).

The aim of this case-study was to determine whether, and how quickly, after kaolinite mining has ceased, plant communities' approach those of an undisturbed, target, ALH community. We also investigate temporal

changes in the establishment of plant communities to see how closely the vegetation of former kaolinite extraction sites followed observed changes in soil quality and how quickly a post-OCM site would converge with typical ALH. In doing so, we test the hypotheses that even without any active attempt to ameliorate overburden, given sufficient time, it is possible to re-instate ALH following kaolinite OCM.

## Methods

### *Study Sites*

Located on the periphery of the Dartmoor National Park, Devon, SW England, commercial OCM kaolin extraction has taken place at Headon China Clay Works (50.2510°N, 03.5930°W) since 1855. The quarry offers a sequential series of sites where kaolinite extraction ceased in 1868, 1990, 2013 and 2015. As described by De Palma et al. (2018), this provides a space-for-time substitution (where spatial comparisons are made to infer temporal change) under a Control-Impact model with the associated limitations. All locations were therefore at a similar altitude and experienced similar climate, although the 1990 and 2015 sites were north facing and the 2013 south facing slope. The 1868 site was also south facing but had a steeper slope angle (± 66%) compared to the younger sites (± 30%). While the 1868 site received no known post-OCM interventions, the 1990, 2013 and 2015 sites were covered in overburden stockpiled outside in large mounds approximately 6 m deep for 5 years, in order to help stabilise slopes. The nearby Trendlebere

Down Nature Reserve (50.3641°N, 03.4424°W) was selected as a typical ALH reference site, as it had no history of mining, but similar slope, aspect, altitude, and geomorphology to the commercial quarry prior to kaolinite extraction. At the time the soil and vegetation surveys were undertaken, these sites were 147, 27, 2 and 0 years old, respectively.

## *Soil sampling and analysis*

In summer 2015, ten sampling points at each restoration site were determined using the 'W-walk' method (JNCC, 2004). In each, a starting point was selected randomly and a quadrat (0.5 m × 0.5 m) placed to determine the first sampling position. The sample points were 20 m apart, and the total distance walked was 180 m. A 30 cm soil core was taken from the left corner of each quadrat using a manual soil corer (Soil Coring Kit 04.16, Eijkelkamp Soil & Water, Gisbeek, the Netherlands). The O horizon (~15 cm) was sampled from the cores and subsequently dried in a desiccator set to 65°C, disaggregated, sieved (2 mm mesh) and stored before analysis.

For pH analysis, 10 g of soil in 50 ml deionised water was mixed for 15 minutes with a magnetic stirrer, left to settle and determined using a Hanna 991001 pH and temperature probe (Jones Jr, 2001). As a proxy for organic matter, loss on ignition was used to quantify soil carbon content, with (~5 g) samples dried at 105°C for 1 hour, weighed and ashed at 400°C for 2 hours in a Gallenkamp hotspot furnace (Jones Jr, 2001). Mineral elements were extracted using the Mehlich 3 method

(Jones Jr, 2001), whereby an extraction solution (30 ml) was added to each soil sample (3 g) in centrifuge tubes and mixed on a reciprocating mechanical shaker at 200 rpm for 5 minutes. Samples were subsequently filtered through Whatman 42 filter papers, and the filtrate retained in the dark until analysis. The Na, K, Mg, Ca and P concentration of the extracted solution was analysed using a Thermo Scientific iCAP7400 ICP-OES instrument.

To assess soil nitrate/nitrite concentrations, 3 g samples were digested in 30 ml of 0.01 M calcium sulphate, shaken on a reciprocal shaker for 15 mins at 180 rpm, and filtered through a Whatman 42 filter paper, followed by a cadmium reduction reaction and quantification by colorimetry (HACH DR/890) (Jones Jr, 2001). Cation exchange capacity, a measure of soil ability to retain key nutrients in 'plant-available' form, was quantified using the sodium acetate method (Jones Jr, 2001). One-Way ANOVA was applied, with a Welch's correction for unequal variances, to explore how these key soil chemical parameters varied according to the factor 'time since restoration'.

### *Vegetation sampling and analysis*

Within each of the ten 0.5 m × 0.5 m quadrats positioned along the sample transect, species presence and absence was quantified (0 – absent, present – 1), and an nMDS using the Raup-Crick distance used to visualise variation in community patterns between sites (Clarke,

1993; Zuur et al., 2007). Analysis was performed in three dimensions using metaMDS and ordiellipse to highlight groupings in the 'vegan' (Oksanen, 2015) package in 'R' v.3.5.2. Once the communities were plotted onto an ordination plot, the physical characteristics of the soil were overlaid as vectors (for variables where P ≤0.001). Lines pointed in the same direction are positively correlated to each other (Zuur et al., 2007). This enabled interpretation of the significant physical factors and how they were aligned with the various communities. An ANOSIM was performed in the 'vegan' (Oksanen, 2015) package in 'R' v.3.5.2 to examine variation in plant community composition between restoration treatments.

# Results

## *Soil chemistry*

Little evidence was found to support the hypothesis that even several decades after OCM terminated, soils on former kaolinite sites would transition naturally towards soils favouring an ALH community. Even at the oldest (147-year-old) site, key aspects of soil chemistry were very different from the Trendlebere Down heathland (Table 1). Concentrations of major elements (Na, Ca, K and Mg) were generally an order of magnitude lower at the 1868 site, and soil P and $NO_3$ concentrations were 28% and 20% respectively of those in established heathland. With the exception of $NO_3$, the restored sites had lower major element levels; less than 25% of the reference site.

Established heathland soil was also more acidic (pH 3.8), and had

considerably higher organic matter content (67.5% OM) than all former

OCM sites (pH 4.5-4.9, <6% OM), showing that the addition of

stockpiled soil to the 1990, 2013, and 2015 sites had minimal beneficial

impact on soil chemistry (**Table 1**).

**Table 1**: Comparison of mean (±SE, n = 10) soil chemical conditions at an undisturbed Atlantic lowland heathland site (Trendlebere Down, Devon, UK – TBD) and former opencast kaolinite mine sites located in an adjacent commercial mine. The date when mining ceased at each site is given, and for the 1990, 2013 & 2015 sites, the termination of operations was followed by the replacement of stored overburden. The results of a one-way ANOVA of soil parameters are given, with different letters in superscript indicating significant difference in variance (P < 0.05) between site means, following Tukey's paired comparisons. CEC = Cation Exchange Capacity

| Site | | Na ($\mu g\ g^{-1}$) | Ca ($\mu g\ g^{-1}$) | K ($\mu g\ g^{-1}$) | Mg ($\mu g\ g^{-1}$) | P ($\mu g\ g^{-1}$) | $NO_3$ ($\mu g\ g^{-1}$) | pH | Organic Content (%) | CEC (*mEq /100g*) |
|---|---|---|---|---|---|---|---|---|---|---|
| TBD | Mean | $16.5^A$ | $73.2^A$ | $48.2^A$ | $56.4^A$ | $8.1^A$ | $2.0^A$ | $3.8^A$ | $67.5^A$ | $76.7^A$ |
| | (SE) | 1.2 | 5.8 | 4.3 | 4.7 | 0.9 | 0.4 | 0.0 | 5.8 | 3.7 |
| 1868 | Mean | $1.9^B$ | $7.3^B$ | $6.5^B$ | $4.3^B$ | $2.0^B$ | $0.4^B$ | $4.9^B$ | $3.4^B$ | $8.5^B$ |
| | (SE) | 0.3 | 1.7 | 1.1 | 0.7 | 1.2 | 0.1 | 0.1 | 0.6 | 0.6 |
| 1990 | Mean | $2.7^B$ | $7.2^B$ | $9.2^B$ | $3.9^B$ | $2.0^B$ | $1.8^A$ | $4.7^{BC}$ | $5.9^B$ | $17.6^B$ |
| | (SE) | 0.1 | 3.3 | 2.0 | 0.7 | 0.1 | 0.2 | 0.1 | 0.3 | 1.5 |
| 2013 | Mean | $1.5^B$ | $16.4^B$ | $4.1^B$ | $3.9^B$ | $2.0^B$ | $1.1^{AB}$ | $4.9^B$ | $3.8^B$ | $10.1^B$ |
| | (SE) | 0.1 | 4.1 | 0.5 | 0.6 | 0.1 | 0.3 | 0.1 | 0.5 | 1.5 |
| 2015 | Mean | $1.6^B$ | $9.9^B$ | $5.6^B$ | $3.5^B$ | $0.8^B$ | $1.4^{AB}$ | $4.5^C$ | $3.9^B$ | $11.4^B$ |
| | (SE) | 0.2 | 3.8 | 0.6 | 0.4 | 0.2 | 0.4 | 0.1 | 0.6 | 1.5 |
| ANOVA | $F_{(4,45)}$ | 120.1 | 32.16 | 65.5 | 103.5 | 21.36 | 4.85 | 38.19 | 168.6 | 118.5 |
| | *P* | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

## *Changes in vegetation community composition*

Multivariate analysis revealed considerable variation in the plant community characteristics between each site (global $R_{ANOSIM}$ = 0.496, P <0.001), (**Figure 1**). The reference ALH community at Trendlebere was tightly clustered around the major defining plant species for this habitat (i.e. *Calluna, Molinia*, and *Erica tetralix*), these species also being more abundant here than any other site. The former OCM sites were less tightly clustered around distinct species; the 1868 site in particular showed broad overlap across many different plants, most uncharacteristic of typical ALH communities (specifically, the graminoids *Deschampsia, Festuca,* and *Juncus*, and the forbs *Potentilla* and *Galium*; species more commonly associated with acid grasslands). Nonetheless, *Calluna* and *Molinia* at the 1868 site achieved the highest abundance recorded at any former kaolinite mine location.

The 1990 and 2013 sites were dominated by Poaceae species characteristic of acid and mesotrophic grasslands (e.g. *Deschampsia flexuosa* and *Festuca rubra*), although the position of the 1990 cluster in the nMDS reflects that the contribution of both *Calluna* and *Molinia* to the community was much greater here, than at the younger 2013 site. Also, of note is the fact that *Ulex europaeus* was considerably more abundant at the 1990 site than any other location (although the presence of this N-fixing legume appeared to have little impact on soil $NO_3$). The 1990 site had the tightest cluster of all the OCM restoration

sites. The 2015 site clustered around *Agrostis capillaris* (**Figure 1**), reflecting that quadrats here were dominated by bare ground and had no ALH-characteristic plants present. The most important environmental factors dictating plant community composition was the addition of overburden; K and P, and time (P <.001) (**Figure 1**).
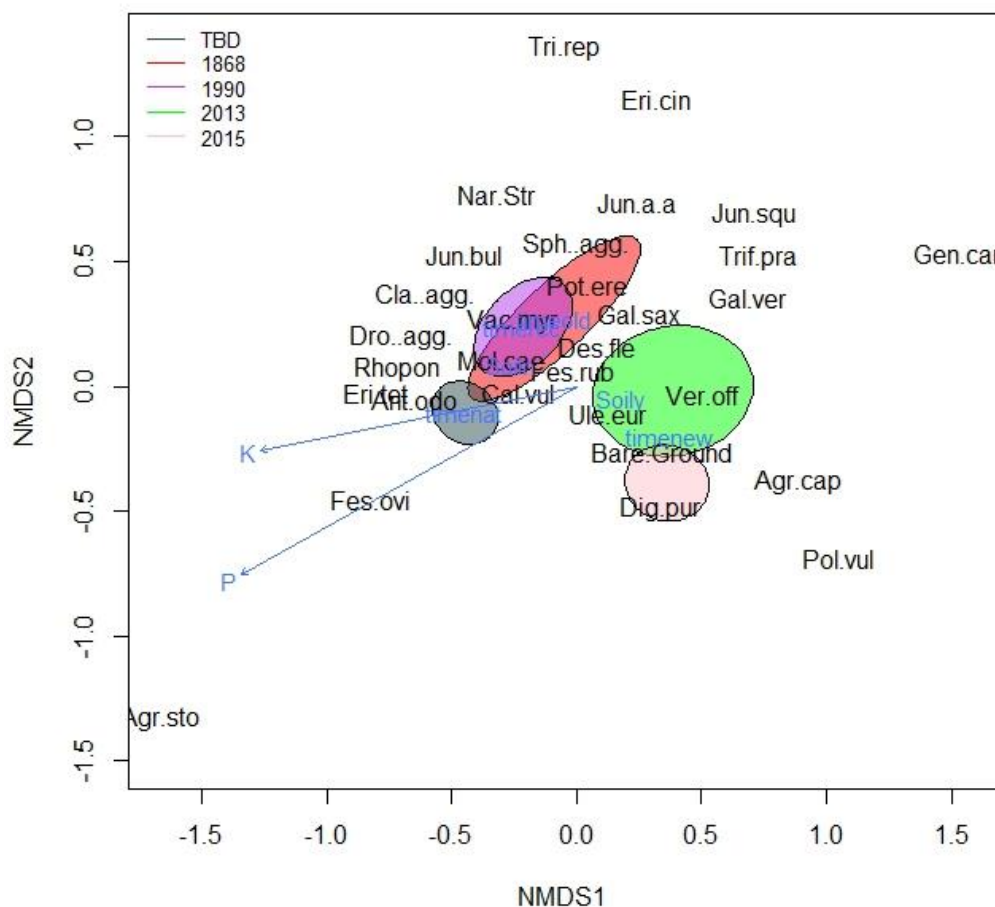
**Figure 1**: nMDS of the presence- absence data plant community of an undisturbed ALH site (TBD) and restored kaolinite mine sites (shown in 2 dimensions for ease of visualisation). The date labels denote the year when mining ceased. Stress = 0.11. Ordiellipse are present to show the overlap of the communities. The vectors are significant environmental factors (*P* < 0.001)

Key to environmental factors: Timenatural, TBD; Timeold, 1868; Timerecent, 1990; timenew, 2013,2015; Soiln, overburden not added; Soily, overburden added.

Key to plant species: Agr cap, *Agrostis capillaris*: Agr sto, *Agrostis stolonifera*: Ant odo, *Anthoxanthum odoraturm*: Des fle, *Deschampsia flexuosa*: Fes ovi, *Festuca ovina*: Fes rub, *Festuca rubra*: Mol cae, *Molinia caerulea:* Nar str, *Nardus stricta*: Cal vul, *Calluna vulgaris*: Eri tet, *Erica tetralix*: Eri cin, *Erica cinerea*: Rhopon, *Rhododendron ponticum* Vac myr, *Vaccinium myrtillus*: Ule eur, *Ulex europaeus*: Pot ere, *Potentilla erecta*: Ver off, *Veronica officinalis*: Gen cam, *Gentianella campestris*: Pol vul, *Polygala vulgaris*: Gal ver, *Galium verum*: Gal sax, *Galium saxatile*: Tri rep, *Trifolium repens*: Tri pra, *Trifolium pratense*: Dig pur, *Digitalis purpurea*: Dro agg*, Drosera* (agg): Jun a.a, *Juncus articulatus*: Jun bul, *Juncus bulbosus*: Jun squ, *Juncus squarrosus*: Sph agg*, Sphagnum* (agg): Cla agg*, Cladonia* (agg).

## Discussion

Although this case-study lacks true replication, results nevertheless corroborate the general view that effective ALH restoration is a long-term process with little or no guarantee of success (Miller et al., 2017). Indeed, even after nearly 150 years (albeit with minimal additional management; i.e. grazing by livestock), soil chemistry failed to approach the levels of acidity, organic content, CEC or key soil nutrients characteristic of, and important in, heathland soil (Clarke, 1993; Green et al., 2015). Similarly, although some species typical of established ALH were abundant in the 1868 site, the community was also characterised by species representative of acid or mesotrophic grasslands. There seems little potential therefore, to expect long-term, natural ALH recovery on the many kaolinite open cast mines located in regions where this habitat is most common, and especially where restoration occurs alongside active mining. Instead, and like many OCM sites globally, heathland restoration can likely only be facilitated by further interventions after mining operations cease (Holmes, 2001; Benigno et al., 2013; Clemente et al., 2016; Glen et al., 2017).

One commonly-applied approach is to reinstate stockpiled overburden onto former OCM sites, but the results suggest this practice did little to facilitate any improvement in key soil characteristics, or subsequent establishment of plant species typical of the target ALH community. Even on the 25-year-old (1990) site, organic content and pH of the

reinstated overburden had little in common with those in nearby natural ALH. In theory, the use of topsoil provides a source of native seed, mycorrhizal and bacterial symbionts with which to facilitate plant community restoration (Muñoz‑Rojas et al., 2016; Wubs et al., 2016). In practice, however, suitable topsoils are scarce and overburden (topsoil mixed with underlying mineral horizons) is stockpiled into large mounds to reduce footprint on the mine site, a procedure that diminishes key properties over relatively short periods (Golos et al., 2016). For the most part however, recent studies reporting the impact of soil stockpiling on restoration have focussed on (generally negative) changes in the soil seed bank (Dickie et al., 1988; Rokich et al., 2000) or soil microbial community (Harris et al., 1989; Poncelet et al., 2014). It may be the case however, that soil nutrients are less impacted by storage (Abdul-Kareem & McRae, 1984; Strohmayer, 1999).

A deficiency in the major macronutrients (NPK) required for plant establishment and growth in stored overburden and kaolinite mine waste is nonetheless well known (Marrs et al., 1981; Coppin, 1982). Phosphorus and potassium concentrations in the sites were considerably lower (i.e. less than 20%), even 25 years after overburden had been reinstated, than in the adjacent target community. Soil nitrate was, however, substantially higher in sites with overburden (1990, 2013, 2015) than in the 1868 site where no interventions were undertaken after OCM ceased. Other important heathland macronutrients, including Mg, Na and Ca (Clarke, 1993; Clarke, 1997),

were frequently present at concentrations less than one-tenth of those

seen in the adjacent ALH site. Removal and mixing of thin heathland

topsoils with the mineral soils that underlie them before mining

inevitably dilutes soil nutrients; subsequent storage and leaching from

a generally coarse-grained overburden, further diminishes fertility.

Reinstatement of a nutrient limited, mineral overburden where the

symbiotic soil microflora plants require to extract nutrients from low

fertility heathland soils are now absent, unsurprisingly limits

establishment of heathland specialists, even if propagules are available

(Diaz et al., 2006). To compound the problem, the low water retention

capacity of coarse-grained, low organic content mineral overburden

increases substantially the risk of plant mortality and reduced growth

during drought (de Miranda Machado et al., 2013; Bateman et al.,

2018). Although relatively uncommon in SW England, future climate

scenarios predict increased frequency of warm, dry summers, including

extreme heatwaves and drought (Guillod et al., 2018).

Unlike the majority of mine rehabilitation studies where the low pH

associated with overburden poses a major problem for plant community

restoration (Abdul-Kareem & McRae, 1984; Malik & Scullion, 1998),

none of the former kaolinite sites studied were as acidic as natural ALH.

Low soil pH is critical for the establishment of the ericoid shrubs that

characterise lowland heaths (Pywell et al., 1994; Marrs et al., 1998).

Moreover, low pH often results in loss of cations from soils; Green et al.

(2015) for example, reported a positive correlation between pH and

concentrations of extractable K, Ca & Mg, but a negative association with phosphate. In-turn, soil concentrations of many elements affects the bioavailability of other key nutrients and also influence greatly the growth of species that might otherwise outcompete the target heathland species. Green et al. (2015) describe how at higher pH (5 or above), the vegetation of restored heathland sites was dominated by *Agrostis capillaris* and note how control of this highly competitive species is key to *Calluna* and *Erica cinerea* establishment. Similarly, the results show how *Agrostis capillaris*, along with at least one other mesotrophic grass species, was dominant on the 1868, 1990 and 2013 sites where soil pH remained above 4.7.

The failure of key soil characteristics or plant community composition throughout the chronosequence to trend towards those associated with the adjacent natural ALH strongly suggest that even where stored overburden is used, further manipulation is required. Benefits may accrue from reduction in overburden storage times and the depth of stockpiles (reducing compaction), and regular addition of organic material to retain soil meso-fauna and microbial populations and function, and water holding capacity during storage (Dickie et al., 1988; Rokich et al., 2000; Ngugi et al., 2018). Following reinstatement, further addition of organic matter to overburden is desirable for the same reasons (Smith & Read, 2010; Muñoz-Rojas et al., 2016) and the potential enhancement of nitrogen cycling rates (Van Vuuren et al., 1992), while fertilizer application can also encourage plant

establishment and growth, and concomitant benefits to soil biota (Ngugi et al., 2018). More specific to heathland restoration, soil pH is effectively reduced by the application of sulfur, with the additional benefit of increasing the bioavailability of phosphate without the need for fertilizer application (Green et al., 2015). Heather establishment is also strongly dependent on symbiotic interactions with ericoid mycorrhizal fungi (ErM) that do not respond well to long-term soil storage (Smith & Read, 2010). Consequently, the introduction of essential ErM to the soil may be essential to effective ALH restoration where former kaolinite OCMs are covered with overburden stored for long periods. Taken together therefore, we conclude that effective restoration of ALH communities on former kaolinite quarries requires multiple interventions that address the limiting effects of low soil fertility, relatively high soil pH, propagule limitation, and an absence of soil micro- and macro-biota. Time alone is insufficient to facilitate these changes.

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# Chapter 2 Effects of organic enrichment of stored topsoil and inoculation with commercial ericoid mycorrhizal fungi on survival and growth of *Calluna vulgaris* seedlings.

## Introduction

Current rates of species extinction suggest that anthropogenic impacts on the environment could lead to a sixth mass extinction (Barnosky et al., 2011). Against the background of this ongoing biodiversity crisis, the State of Nature Reports (Hayhow et al., 2016; 2019) emphasise the continued negative impact of anthropogenic activity on species' distribution and abundance including climate change, urbanisation, pollution, hydrological change, and agricultural management. Given these impacts, there is a need for a restorative approach to create more resilient natural environments (Lawton, 2010; DEFRA, 2018).

In this context, restoration ecology strives to restore, replace or reclaim habitats that have been impacted by humans (Hobbs & Norton, 1996; Clewell et al., 2004). In general, restoration aims to take a solutions-based approach to repair ecological damage not only to protect biodiversity, but to promote the delivery of ecosystem services and human health (McDonald et al., 2016). In order to do this, several methods have been reviewed (Hobbs & Cramer, 2008), starting with

passive restoration (no active intervention) and progressing to full technical restorations (landscaping, hydro- and geoengineering etc.) at a landscape level and involving multiple stakeholders (Coppin & Richards, 1990; Harris et al., 1996). The decisions on the scale of intervention need to be scientifically informed in order to clearly guide restoration schemes (Harris et al., 1996; Gilbert & Anderson, 1998; Williamson et al., 2003). However, in most cases, restoration decisions and implications are complex due to incomplete knowledge and little guidance (Hobbs & Cramer, 2008), therefore, larger-scale fundamental ecological research is needed, especially to connect experimental trials to field-scale restorations (Gellie et al., 2018).

Lowland heath is a priority habitat for conservation (English Nature, 2006) but it is a complex habitat to restore (Marrs & Bradshaw, 1980; Marrs et al., 1981; Roberts et al., 1981). Experimentation for lowland heath restoration started in 1975 with the work of Bradshaw et al. (1975) and English China Clays (today IMERYS) in Cornwall on the china clay sand tips. This early work on heathland restoration established the importance of nutrient cycling, especially the role of nitrogen, the accumulation of adequate levels and appropriate compartmentation into accessible pools throughout the complete nitrogen cycle (Marrs & Bradshaw, 1980; Marrs et al., 1981; Roberts et al., 1981; Skeffington & Bradshaw, 1981). Alongside the soil nutrient cycling work, Putwain & Gillham (1990) investigated seed bank viability

and established that *Calluna vulgaris* seeds can remain viable in the seed bank under conifer plantations for 40 years. Both sets of data informed the large-scale, long-term monitoring on the Dorset heathlands (Pywell et al., 2011). In addition to plant investigations, heathland soils have undergone intensive examination, especially regarding soil amelioration (Walker et al., 2004b; Coleman & Whitman, 2005; Wubs et al., 2016; Wubs et al., 2018). The results reported from the majority of studies recommend acidifying the soil by adding sulfur (Walker et al., 2004b; Diaz et al., 2008), removing nutrients by either deep ploughing (Pywell et al., 2002) or cropping of a nurse crop to remove nutrients from the soils (Pywell et al., 1994). Then introducing heather cuttings as a green hay or 'brash' (Pywell et al., 1996). Other important considerations for lowland heath restoration have highlighted the importance of location and finding that improved restoration outcomes were generated from land that had previously been heathland (Walker et al., 2004a). Restoration practices to restore previous heathland should also involve the removal of encroaching bracken and trees in order to create open space in the foliage to allow germination of established seed banks (Pywell et al., 2002).

A key characteristic of lowland heath is acidic, low nutrient soils. Ericoids are a key component of lowland heaths and they are one of the indicator families that define heathlands worldwide (Webb, 1986). Many ericoids have evolved an association with ericoid mycorrhizal fungi

(Read, 1991; Wubs et al., 2016) and this is an important factor allowing them to colonize such low nutrient habitats (Smith & Read, 2010). The fungal species most commonly associated with *Calluna* and *Erica* are *Rhizocyphus ericae* (formally *Hymenoscyphus ericae)* and *Oidiodendron maius,* in the Helotiales (Ascomycota), although these are not the only species that are capable of forming associations with the plants (Villarreal-Ruiz et al., 2004; Vrålstad, 2004; Vohník et al., 2012). The best studied of the ErM fungi known to colonize *Calluna vulgaris* (L.) is *Rhizocyphus ericae* (Bradley et al., 1982; Bending & Read, 1996b; Read et al., 2004; Villarreal-Ruiz et al., 2012).

ErM increase N and P uptake by the host plant (Smith & Read, 2010). In anxenic culture, *Rhizocyphus ericae* has been found to produced proteolytic enzymes which give the photobiont access to growth limiting nitrogen from the inaccessible sources of the organic soil nitrogen pool where in acidic condition nitrogen accumulated as proteins, polypeptides and amino acids in surface soil layers (Bending & Read, 1996b; Bending & Read, 1996a; Bending & Read, 1997). Previous work indicates that ErM assists in growth rather than storage of nutrients (Strandberg & Johansson, 1999) and ErM fungus can be found within the root system throughout all stages of the plant's life (Hobbs & Cramer, 2008; Wodika & Baer, 2015). The development of a commercial ErM inoculate was first tested in 1998 (Koron & Gogala, 1998); its effectiveness has been tested on blueberries, showing that

when inoculated, the plants perform and fruit optimally. Nevertheless, the high cost and difficulties of producing the ErM inoculate prevents the small market gardener from using it (Koron & Gogala, 1998; Vosatka et al., 2012). The commercial inoculator isolates ErM from the environment and grows it in liquid agar until the mycelium forms; it does not use spores (Symbiom, Czech Republic) (Albrechtova et al., 2012). Although the species provided commercially are the same as those found in the environment, this however does not allow for potential local adaptation between different source populations of ErM (Quoreshi, 2008). For example, Cairney & Meharg (2003) review and highlight the interaction between the ErM and ericoids and the ability to adapt and colonise anthropogenically polluted sites, such as acidic mine waste tips. These authors highlight the potential for a much greater diversity, possibly even endemic species, of ErM within local environments that have not been cultured yet. This ability to colonise anthropogenically polluted sites, such as acidic mine waste tips is due to the ErM having an important role in exclusion of uptake of harmful metals, such as aluminium, copper and zinc in shoots (Bradley et al., 1982; Read, 1983). Consequently, the use of mycorrhizal fungi in the restoration of lowland heath could be important (Diaz et al., 2006) although their use in the establishment of heathland after mining restoration projects is not widespread (Quoreshi, 2008). With lowland heath habitats, the application of mycorrhizal fungi should make the

plants better adapted to deal with alkaline soil and calcium salts (Quoreshi, 2008).

In addition to considering biotic interactions, the physical environment is also an important aspect of heathland restoration (Tischew et al., 2014; Henning et al., 2017). In particular, soil physical characteristics are important (Clarke, 1997) especially in restoration following mineral extraction because overburden (topsoil in the case of china clay extraction) is an expensive commodity thus mining companies do not use it immediately, and store it prior to restoration. This storage can affect the soil's potential restoration capabilities and is dependent on the storage conditions (see chapter 3). Abdul-Kareem & McRae (1984) and Strohmayer (1999) reported changes in chemical composition after storage, but when the soil berms are opened and the soil is spread, changes in the chemical composition was less significant for plant growth than loss of the soil's biological and physical characteristics (Harris et al., 1989; Harris et al., 1996; Bradshaw, 1997b; Harris, 2009).

The aim of this study was to assess if the addition of organic matter to stored topsoil, singularly or in combination with commercial ErM, increased the survival rate and growth parameters of *Calluna vulgaris*

cuttings. The rate of successful inoculation by *Calluna* plants with commercial ErM was also established.

## Method

### *Greenhouse Trial*

Two-hundred cuttings of *Calluna vulgaris* were taken from eleven plants within a 5 m radius at Trendlebere Down UK (50.36.141; 3.44.324). The chosen plants displayed new growth and were of a suitable size to withstand sampling. Softwood (new growth) cuttings were taken at the closest growth node, approximately 9 cm from shoot tip, and left in plastic bags with water to maintain humidity for 24 hours before planting (Morrison et al., 2000). The cuttings were placed in a 10 m x 3 m unheated greenhouse (east facing), in a 50:50 sand/peat mixture under a mist propagator at 95% humidity to maintain the shoots until root growth took place. The cuttings took 18 weeks to root and show new shoot growth. One-hundred and forty plants survived and were potted into four treatments: stored topsoil (control), stored topsoil with commercial ericoid mycorrhizal fungi (ErM), stored topsoil with organic matter and stored topsoil with organic matter and commercial ErM; creating 35 replicates per treatment. The organic matter/amendment consisted of a 12 week matured green waste compost from Viridor Ltd, an in vessel household waste composting facility based at Heathfield, nr Newton Abbot, Devon as described in Schofield et al. (2018). When compared with compost from garden waste, household waste compost

tends to be high in available N and P with a lower C:N ratio. The pH of compost generated from a mix of kitchen and garden waste typically has a neutral pH with low concentrations of lignin and tannins, and a lower C:N ratio (13 ± 3) (Schofield, 2015) which could be problematic for heathland soil restoration. The stored topsoil and organic matter were combined in a 2:1 v:v ratio. To inoculate with ErM, the root balls of the appropriate plants were dipped in the commercial ErM ('Rhodovit') as directed by the manufacturer, Symbiom (Albrechtova et al., 2012). Rhodovit contains mycelium fragments of three mycorrhizal fungal species *Oidiodendron maius, Hymenoscyphus ericae* strain 1, and *Hymenoscyphus ericae* strain 2. The pots were moved away from the mist propagator every week over eight weeks to acclimatise the plants to lower moisture levels to mimic heathland conditions. Following removal from the mist propagator the plants were watered daily by hand with rainwater (25 mL per day).

After nine month's growth, the number of surviving plants in each treatment was counted, and length of new growth, number of shoots, and number of flowering stalks were measured. The roots and shoots were separated at the first root growth. Two-way nested ANOVA tests were carried out in R to compare all non-destructive growth parameters across treatments and by original plant identity (to account for the genetic variability of individual plants). Association of number of surviving plants with treatment was tested using Chi-squared test.

The plants were then used in further experiments, first to analyse the ErM communities in their root system using DNA analysis and second, to characterise the infection rate by fungal staining. Roots were washed to remove as much soil from them prior to drying in an oven at 60°C until a constant dry mass was attained. After drying, a sub sample of the roots from the plants selected for molecular analysis was placed in 3 ml 1M acetic acid for 24 hours to clean and rehydrate the roots, and then 0.15 ml of Schaffers black ink was added to stain the fungal tissue. The roots were left in the stain for 12 hours. The roots were then washed in tap water to de-stain them and observed under a 400x magnification on an Olympus 672110 microscope using a blind trial to remove observer bias. The cells were then scored for infection. A percentage of root infection was then calculated for each treatment, percent values were arcsine transformed and an ANOVA test was carried out in R to compare infection rates across treatments.

### Cloning and sequencing

Three plants per treatment were selected to assess the fungal community using DNA isolation, ITS fungal barcoding, cloning and bacterial transformation. Using a magnifying glass, hair roots were selected and removed, then washed to remove soil. To extract DNA from the hair roots (0.5 g hair root sample per plant), each sample was

heated in 500 μL of Chelex (10%) and 7 μL of proteinase K for 1 hour at 55 ℃, vortexing every 15 minutes, followed by a final extension at 95 ℃ for 15 minutes to inactivate proteinase K. The samples were then spun in a centrifuge at 13.2 rpm for 30 s. The DNA extract was then stored in a fridge at 4 ℃ until needed.

Using the fungal specific primers ITS1-F (5'–CTT GGT CAT TTA GAG GAA GTA A–3') and ITS4-B (5'- TCC TCC GCT TAT TGA TAT GC -3'), the ITS region of the rDNA was amplified (Hazard et al., 2014). Polymerase chain reactions (PCR) were conducted in a volume of 25 μL containing 6.5 μL water, 2.5 μL BSA, 0.5 μL ITS1-F and 0.5 μL ITS4-B, 12.5 μL 2Xmaster mix and 2.5 μL template DNA. Amplification was performed using a Prime thermal cycler (Techne, Staffordshire) with the following cycle conditions: 94 ℃ for 2 minutes, 30 cycles of 94 ℃ for 1 minute, 55 ℃ for 1 minute, 72 ℃ for 1 minute 30 seconds, final extension took place at 72 ℃ for 9 minutes (Hazard et al., 2014).

Cloning was carried out using the Thermoscientific Clonejet PCR cloning kit following manufacturer's instructions. $CaCl_2$ bacterial transformation of *E. coli* strain B was then performed. For colony selection, duplicate samples of 100 μL and 10 μL were spread on ampicillin plates (50 μg ml$^{-1}$). All plates were incubated at 37 ℃ for 2 days. Colonies were then picked, and the fungal DNA extracted using

the hotshot lysis method and stored prior to using the Thermoscientific Clonejet PCR according to the manufacturer's instructions to run a PCR, creating a sample that was sent off for sequencing to Eurofins GATC Biotech GmbH (Konstanz, Germany).

Sequences were checked for quality by eye and trimmed in BioEdit v 7.0.5.3 (Hall, 1999). Sequences were aligned by Clustal W (Thompson et al., 1994). Trimmed sequences were BLAST searched in GenBank (nucleotide BLAST, nucleotide collection, organism = fungi, using blastn). Sequences were also BLAST searched against fungal records in the UNITE fungal database to identify closest matches (https://unite.ut.ee/analysis.php#) (blastn, Blast e value 1= default, dataset to include INSD Envir). Conspecific DNA sequences were also found in Fehrer et al. (2019) increasing the potential for a match from the samples. In order to place obtained species in their phylogenetic context, two types of tree were constructed using all sequences obtained (cloned sequences, Genbank and UNITE search results) in the programme MEGA 7. Neighbour-Joining (NJ) trees were based on the distance matrix of pairwise differences between the sequences and the Maximum Likelihood (ML) tree was also inferred. The best model of nucleotide substitution was selected using the model selection tool in MEGA using partial deletion and default options. Models with lowest Bayesian Information Criterion (BIC) were considered to describe the substitution pattern best, as recommended within MEGA. Tree-building

was done using Neighbour-Joining and Maximum Likelihood approaches. Both Neighbour-Joining and Maximum Likelihood trees were built using 1000 bootstraps with the selected model, partial deletion and otherwise default options.

## Results

### *Survival*

Little evidence was found to support the hypothesis that the addition of organic matter and ErM either separately or in combination will increase the growth of *C. vulgaris*. The highest survival rate of treatments was in the control group of stored topsoil (94%). The lowest survival rate was in the combined organic matter and combined ErM treatment (8%). In the ErM in stored topsoil treatment, there was an 85% survival rate and a 22% survival rate in the organic matter treatment (**Figure 2**). There was a significant association of survival rates and treatment ($X^2 = 37.5$, df = 3, p < 0.0001), however there was no significant difference between adding the commercial ErM and the plants growing in the stored topsoil.
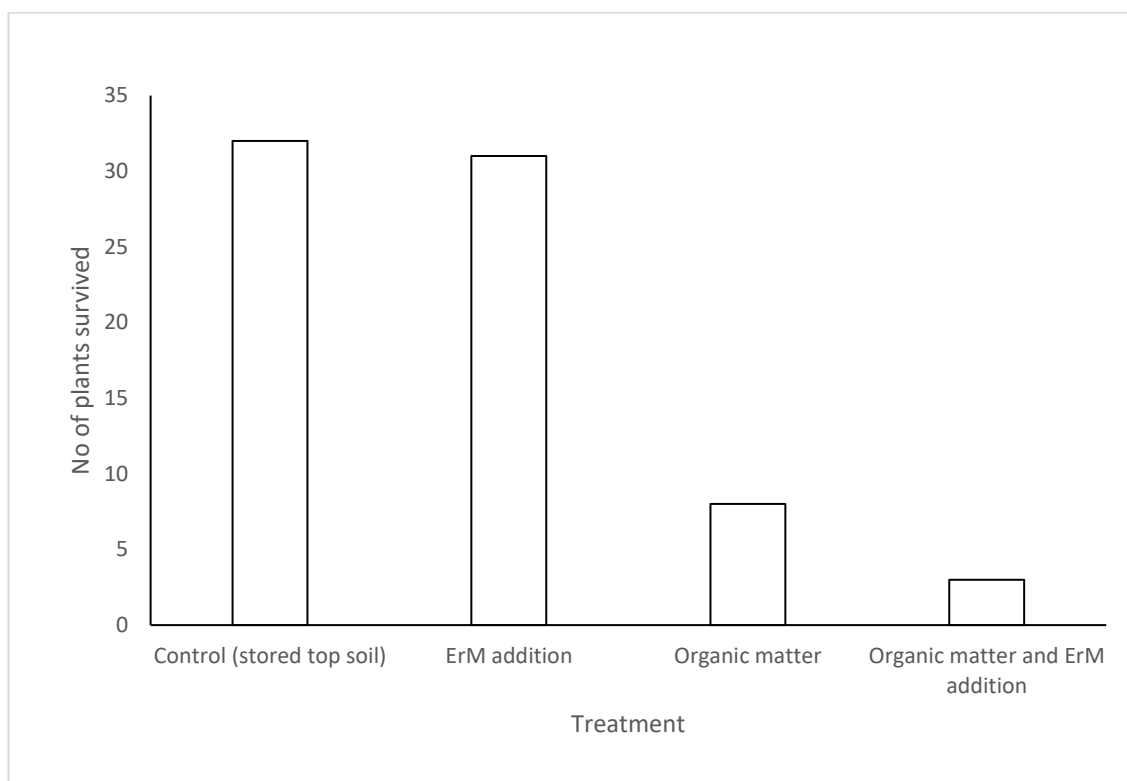
**Figure 2**: The survival of *C. vulgaris* plants grown in one of four soil treatments. The four treatments are control stored topsoil, ErM addition (commercial ErM innoculate added to stored topsoil), organic matter (50% volume green waste organic matter to stored topsoil), and organic matter and ErM addition (commercial ErM innoculate added to 50% volume green waste organic matter added to stored topsoil mix). Each treatment had 35 plants assigned to it.

## *Growth patterns*

There was no difference in shoot length between all treatments ($F_{(3,12)}$ = 1.418, p = 0.286) (**Figure 3**). There were no statistically significant differences ($F_{(3,12)}$ = *2.392*, p = 0.120) in the number of shoots growing in the different treatments (**Figure 4**). No significant difference ($F_{(3,12)}$ = 0.701 p = 0.5693) in the number of flowering spikes was observed (**Figure 5**). There was also no difference ($F_{(3,11)}$ = 0.106, p = 0.955) in any of the treatments of the dry weight (g) of the roots (**Figure 6**). However,

the shoot dry weight (g) of the treatments were significantly different

($F_{(3,11)}$ = 10.587, p < 0.01, **Figure 6**). Post hoc testing showed that for

dry weight, the organic matter added treatment and the organic matter

with commercial ErM treatment were significantly higher than the

commercial added ErM treatment and control. The plant identity factor

had no significant effect in any growth parameter.
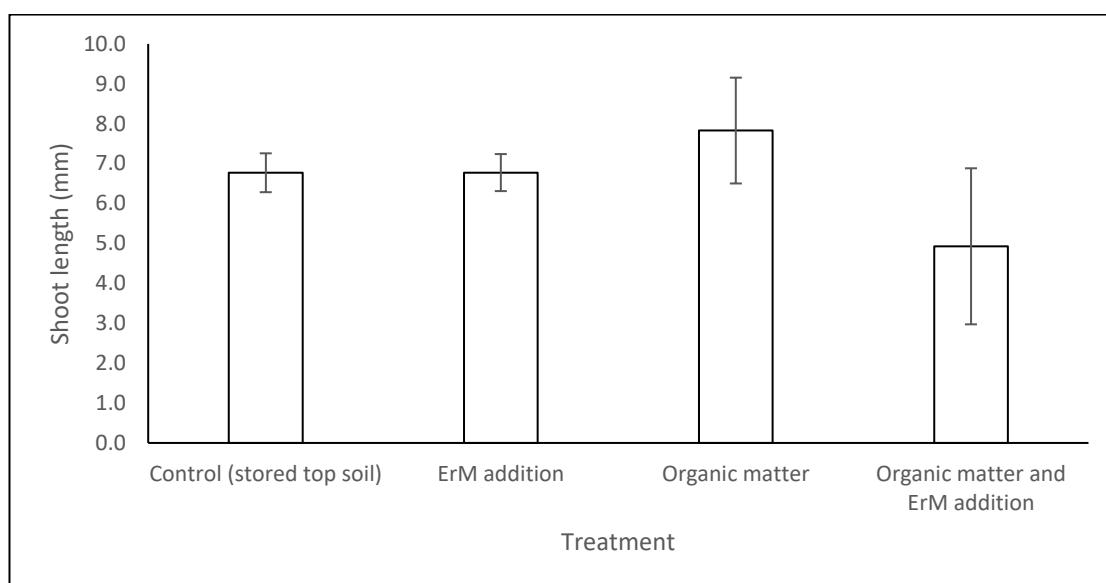


**Figure 3**: The average shoot length of *C. vulgaris* (mm), grown in one of four soil treatments. Bars indicate standard error. Plants that survived were measured 33 Control, 30 ErM addition, 8 Organic matter and 3 Organic matter and ErM addition. The four treatments are control stored topsoil, ErM addition (commercial ErM innoculate added to stored topsoil), organic matter (50% volume green waste organic matter to stored topsoil), and organic matter and ErM addition (commercial ErM innoculate added to 50% volume green waste organic matter added to stored topsoil mix).
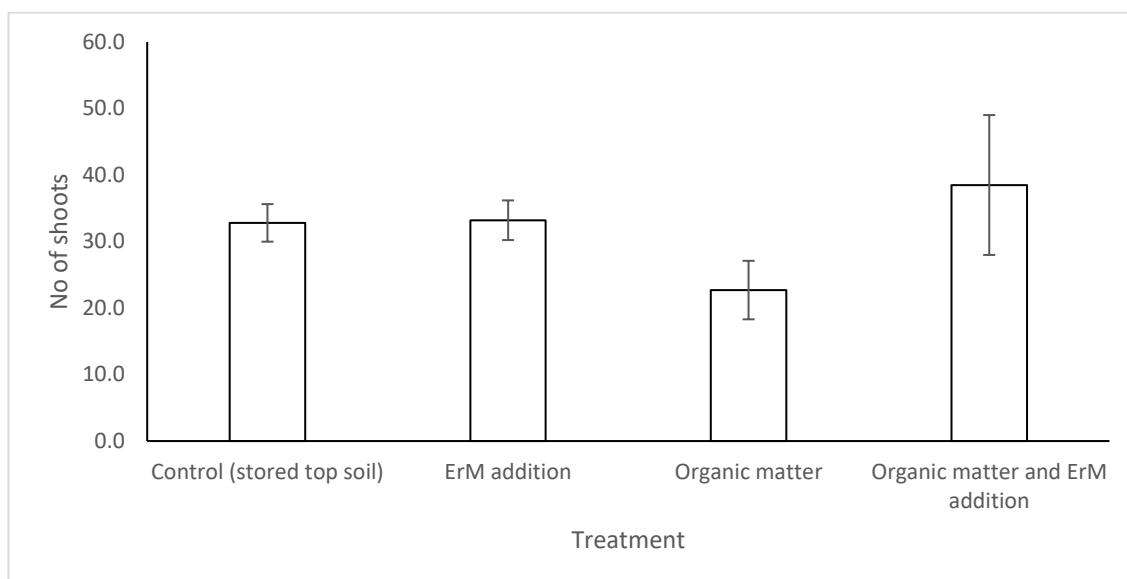
**Figure 4**: The average number of shoots of *C. vulgaris* (mm), grown in one of four soil treatments. Bars indicate standard error. The four treatments are control stored topsoil, ErM addition (commercial ErM innoculate added to stored topsoil), organic matter (50% volume green waste organic matter to stored topsoil), and organic matter and ErM addition (commercial ErM innoculate added to 50% volume green waste organic matter added to stored topsoil mix).
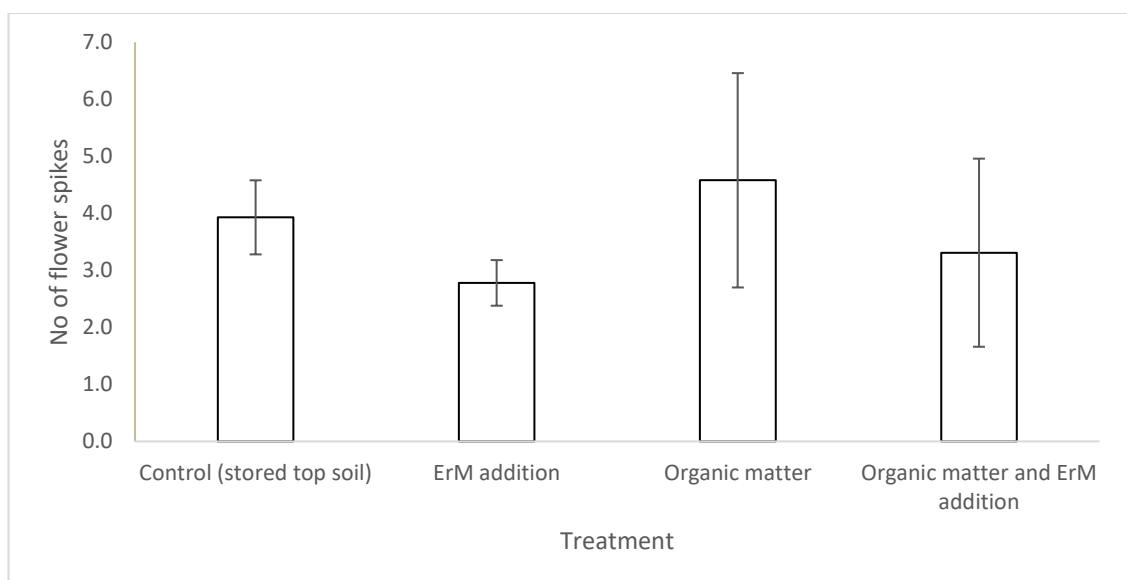


**Figure 5**: The average number of flowering spikes *C. vulgaris*, grown in one of four soil treatments. Bars indicate standard error. The four treatments are control stored topsoil, ErM addition (commercial ErM innoculate added to stored topsoil, organic matter (50% volume green waste organic matter to stored topsoil, and organic matter and ErM

addition (commercial ErM innoculate added to 50% volume green waste organic matter added to stored topsoil mix).
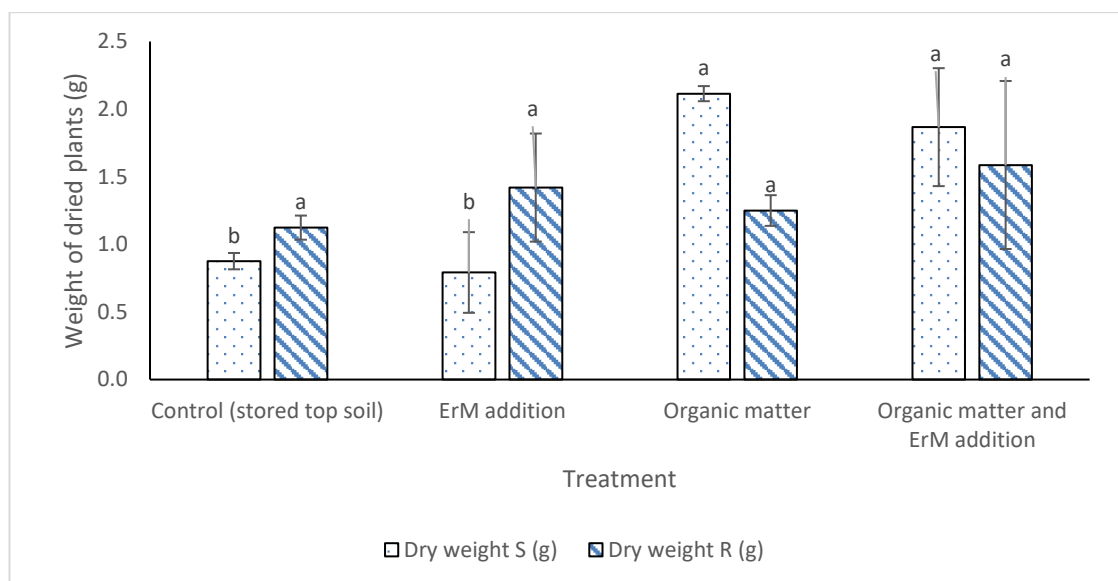


**Figure 6**: The average dry weight of shoots (S) and roots (R) of *C. vulgaris* (g), grown in one of four soil treatments. Bars indicate standard error. The letters above the bars indicate the groups created in post hoc testing. The four treatments are control stored topsoil, ErM addition (commercial ErM innoculate added to stored topsoil), organic matter (50% volume green waste organic matter to stored topsoil), and organic matter and ErM addition (commercial ErM innoculate added to 50% volume green waste organic matter added to stored topsoil mix).

## *Infection rates*

The infection rates showed a significant difference between the colonisation of root cells by mycorrhizal fungi between treatments and the control ($F_{(3,2)}$ = 33.561 p < 0.05). The lowest levels of infection occurred within the overburden (28.9%) followed by the organic matter (34.2%). Where a commercial mycobiont was added in combination with organic matter, the infection rate of root cells was 42.1% and with mycobiont added in just the overburden, the infection rate was 48%

(**Figure 7**). Any form of amelioration with the soil increased the infection although confirmation of this infection being ErM and not saprotrophic fungi was not possible using these techniques.
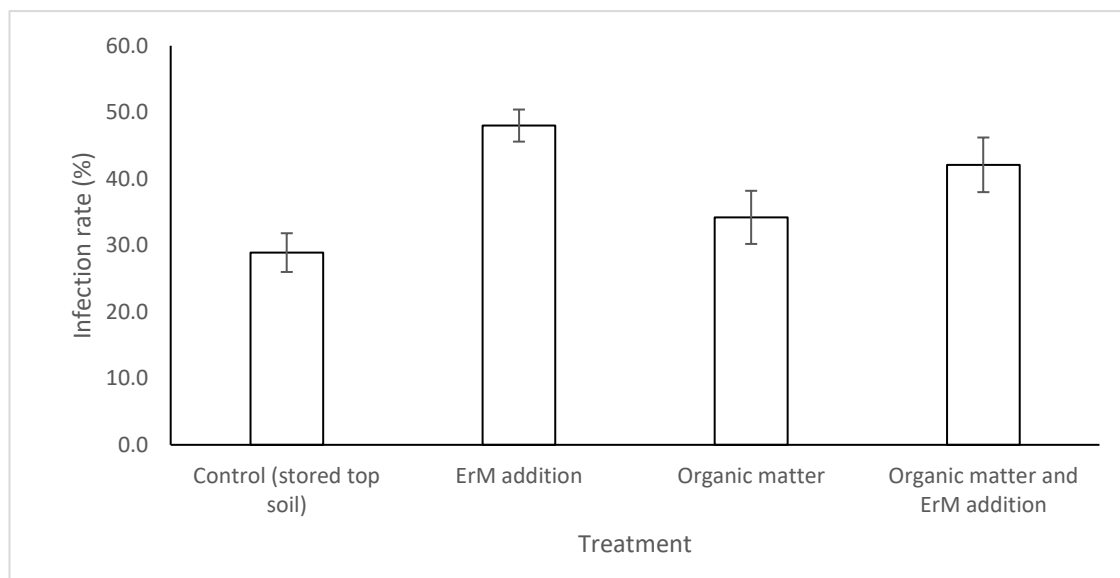


**Figure 7**: The percentage infection of ErM in *C. vulgaris* roots, grown in one of four soil treatments. The four treatments are control stored topsoil, ErM addition (commercial ErM innoculate added to stored topsoil), organic matter (50% volume green waste organic matter to stored topsoil), and organic matter and ErM addition (commercial ErM innoculate added to 50% volume green waste organic matter added to stored topsoil mix).

## *Molecular identification of fungi*

Unfortunately, only a small number of samples were successfully sequenced. Despite successful bacterial transformation of 236 colonies, clone PCRs were successful for only two samples. In order to improve success, a second round of cloning and bacterial transformation was undertaken (summer 2018), but this time, there was both a poor yield of transformants and a low PCR-success rate. Unfortunately, time and financial constraints meant there was no opportunity to troubleshoot

and resolve these issues a third time. However, samples were

successfully obtained from uninoculated (control) overburden samples.

The phylogenetic trees highlighted that both samples were related to the

known ErM, *R. ericae,* and were closely related to the ErM functional

group but neither fell within a clade of the *Rhizoscyphus ericae*

Aggregate (REA) as defined by Fehrer et al. (2019) (**Figure 8** & **Figure

9**). The samples were ericoid mycorrhizal fungi, potentially in the

Helotiales group.
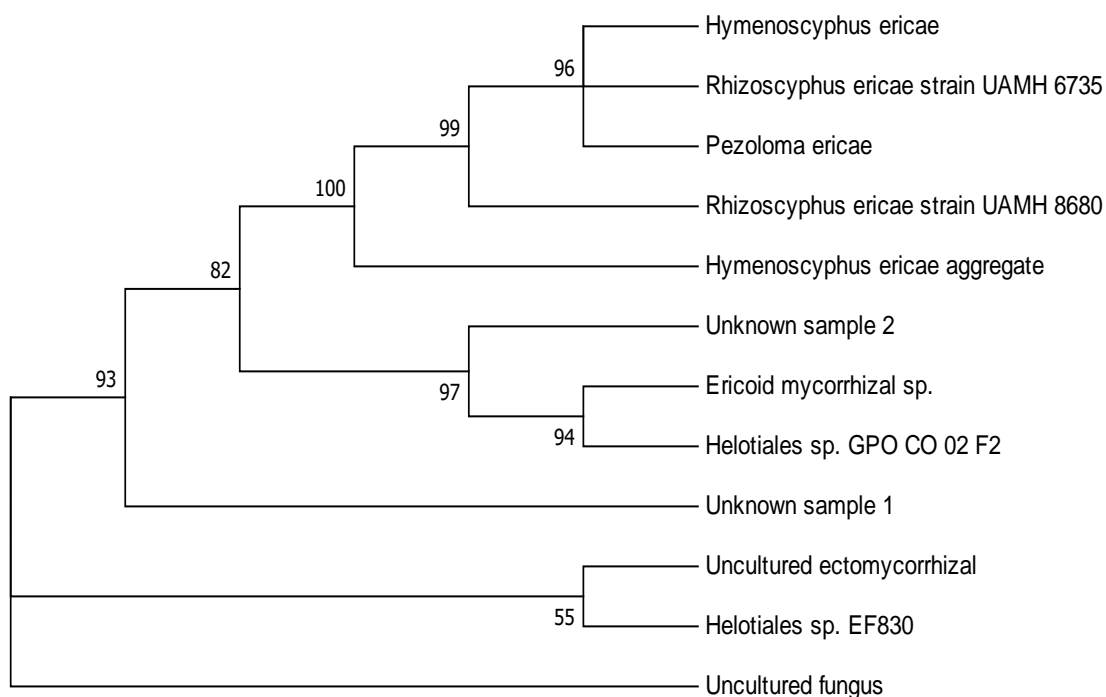
**Figure 8**: Molecular Phylogenetic analysis by Maximum Likelihood method The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980). The tree with the highest log likelihood (-1542.73) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.3732)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 12 nucleotide sequences. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 411 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016).
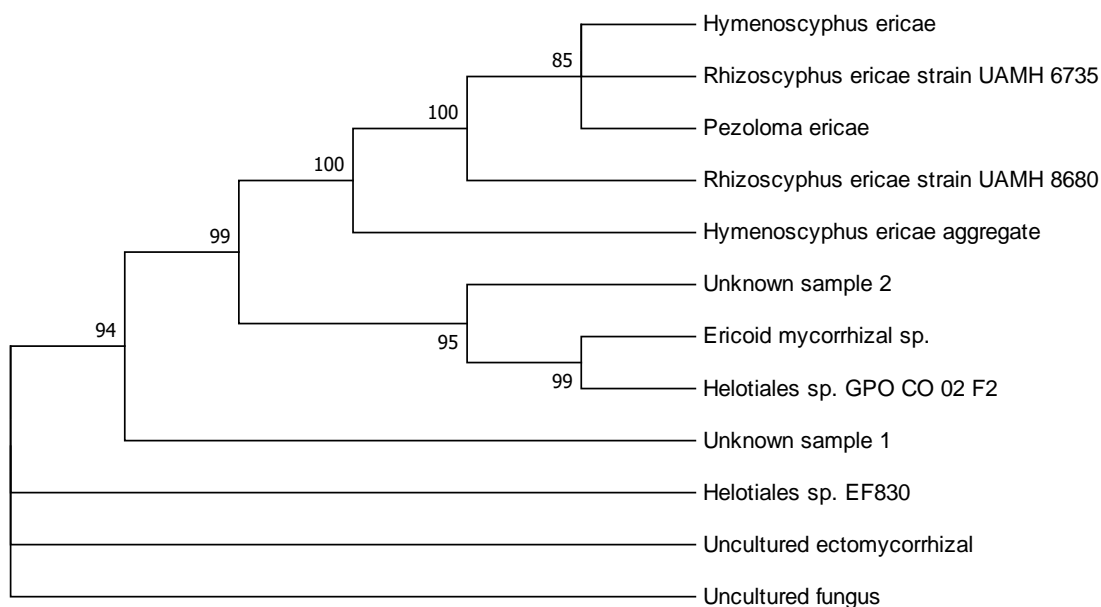
**Figure 9**: Evolutionary relationships of taxa The evolutionary history was inferred using the Neighbour-Joining method (Saitou & Nei, 1987). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analysed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and are in the units of the number of base substitutions per site. The rate variation among sites was modelled with a gamma distribution (shape parameter = 1). The analysis involved 12 nucleotide sequences. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 411 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016)

# Discussion

This study aimed to assess if the addition of organic matter to stored topsoil, singularly or in combination with commercial ErM, increased

the survival rate and growth parameters of *Calluna vulgaris* cuttings. The rate of successful inoculation by *Calluna* plants with commercial ErM was also investigated. The findings are that the highest survival was in unamended topsoil. Of the plants that survived, those grown with the addition of just organic matter had the highest shoot dry weight, however there were only 8 plants that survived in the organic matter addition treatment. There were no differences in shoot number or dry root weight under any treatment. Adding commercial ErM to unamended topsoil increased the infection rate of the roots but did not affect root growth. When sequenced, extracted DNA from hair roots of plants grown in uninoculated topsoil was found to be in the Heliotales group but did not match *R. ericae.*

All of the statistical tests performed on the parameters were affected by the survival of the plants in the initial cutting stages. Not all the cuttings rooted. As such, when dividing the new plants into treatments, although all treatments had at least one cutting from each plant, in certain cases this was only one cutting per plant per treatment. Nevertheless, at the outset, the design was balanced: cuttings from each parent plant were used in all treatments. By the end of the experiment, not all plants had survived in each treatment. While survival rates are a clear outcome of the relative benefit of the treatments *per se,* subsequent analyses of growth parameters are affected by the consequent unbalanced design and the only significant effect was

observed for shoot dry weight. Any lack of significance (or not) with regard to treatment is therefore compounded by the fact that plants of different genotypes were used in the different treatments and the lack of a significant effect of plant identity is affected by the fact that not all plants had cuttings represented in each treatment in the final analysis. However, for a restoration treatment to be effective, it ought to improve the rate of survival and growth of plants, regardless of their individual genotype. The results show significantly larger dry shoot weights in the organic amended treatments. These findings suggest that for the few plants that survived, the organic matter had a positive effect on shoot dry weight. Therefore, although the organic matter adjustment showed a reduced survival rate, the treatment increased growth in the remaining established seedlings. A side effect of organic matter amelioration is an increase in water holding capacity (Schofield et al., 2018) and therefore potential waterlogging, which in turn will lead to increases in seedling mortality; this would also apply to young cuttings beginning to establish in the treated soil. Furthermore, waterlogging could also potentially increase the probability of soil pathogen presence, which would also lead to poorer growth conditions and increasing seedling mortalities. The most likely outcome would be damping-off; a disease that causes seedling and cutting mortality in overwatered conditions. Damping-off abiotic conditions generally involve excessive soil moisture and excessive overhead misting, lower soil temperatures before emergence, higher soil temperatures after emergence, and

overcrowded flats or seedbeds (Lamichhane et al., 2017). Nevertheless, the findings strongly indicate that once established, the addition of organic matter aids seedling growth. Additionally, findings also indicate that the ErM was able to survive the consequential water logging, further highlighting it as a potential application for future commercial investigation alongside organic amendment to the soil.

The observation that the addition of ErM had no effect on any of the measured growth parameters and survival rates contradicts other findings in the literature, which indicate that ErM infection increases plant growth (Hobbs & Cramer, 2008; Wodika & Baer, 2015). Other experiments using turves and clippings of mainly *C. vulgaris* (L) show there is an increase in above and below ground growth in the plants with natural mycorrhizal infection over time (Diaz et al., 2006). However, as yet, commercial inoculates have not been used in large-scale restoration trials so their use in this context cannot be assessed, despite the increase in infection rates showing that commercial inoculates could support the restoration of ErM in the environment. A large-scale field trial will be required to assess if the results shown in pots are repeatable for outside restoration environment conditions.

The highest survival rate of the plants occurred in the overburden treatment. In free-draining soils, the addition of organic matter increases water holding capacity and the pool of organic nitrogen

(Schofield et al., 2018). However, observations from the pot-based study suggest that the waterlogging may have been partly responsible for lowering survival in the organic amended treatment. Many species of the Ericaceae are evolved for low nutrient stressed environments, (Read, 1983; Bajwa et al., 1985; Webb, 1986); in these nutrient stressed environments with low nitrogen availability, *Calluna* plants are able to compete with faster growing more nutrient- demanding perennial grass species (Friedrich et al., 2011).

A possible explanation for the results is that organic matter increased the water holding capacity of the overburden. This treatment combined with the watering system (misting) may have resulted in water logging and subsequent anaerobic conditions in the growing media although this was not noticeable when examining the plants during the experiment. Bannister (1964) indicated that waterlogging can release toxins in aerobic soils, which disturb the physiology of *C. vulgaris.* The roots of *C. vulgaris* die during waterlogging of 6 weeks or more (Bannister, 1964), therefore, in this study, in the organic addition treatments, prolonged periods of waterlogging were likely to have caused the increased mortality. Nevertheless, *C. vulgaris* has a wider range of tolerance to water availability and waterlogging than that of the other ericoids found on lowland heath (Williamson et al., 2003; Fagúndez, 2013). This could also account for the negative findings on the infection count of the combination treatment of organic matter and

ErM, as a plant in poor condition does not have the ErM association of plants in good health (Smith & Read, 2010).

Lane et al. (2020) reported that restoration required some form of organic soil amelioration, typically the addition of organic carbon. The results from this experiment indicate that restoration needs of soil are more complex than just adding organic matter to replace organic carbon and commercial isolates to replace the ErM that are removed during storage. Poor soil conditions, such as anoxic soils caused by excessive waterlogging, prevent associations (Bannister, 1964); the mycobiont is therefore unlikely to receive sufficient carbohydrate if the host plant is in a poor condition, leading to the breakdown of the mycorrhizal association (Smith & Read, 2010).

The results of this experiment suggest that the soil conditions need to be appropriate for short root growth and the subsequent development of mycorrhizal infections (Read, 1983; Lunt & Hedger, 2003; Marrs, 2016). This lack of survival of the combination of organic matter and ErM indicates that where the soil growth conditions are inadequate for the growth of *Calluna,* they are also likely to be inappropriate for the development of the mycorrhizal association. Therefore, before the addition of any form of commercial mycobiont, soil conditions will need to be optimal for *Calluna* survival and growth.

Since identification of the mycobiont was not possible using morphological features, the ability of the commercial ErM to assist with restoration is unable to be confirmed. Here, the aim was to use cloning and bacterial transformation to characterise the fungal community associated with all samples. Although we initially obtained 268 transformants, technical difficulties, which could not be resolved in the constraints of the project, meant that only two samples were sequenced. However, since these samples originated from plants grown in control overburden, it remains an interesting question to identify the fungi associated with hair roots. Results from these sequences suggest that roots were colonized with closely related species within the Heliotales order, but not *Rhizoscyphus ericae.* Unfortunately, neither GenBank nor UNITE databases were able to provide an exact match for these sequences. Nevertheless, it is an interesting result that plants grown in greenhouse trials, planted only in stored overburden, were colonized by Heliotales fungi. In general, the microbial composition of overburden, and its temporal nature over the storage period, is much understudied and this would merit further attention (Harris et al., 1989). Potential sources for the discovered Heliotales include the overburden (spore survival during storage), contamination from other plants in the experiment (spore transport), or incomplete removal of spores from the leaves of the original cuttings leading to contamination from the start despite best practise to maintain a sterile environment prior to planting.

If it were spore transport, then it would be expected to appear in Genbank as an exact match to *R. ericae* strains 1, 2 or *O. maie,* as these were the commercial ErM. As best practice was maintained throughout the experiment, the most likely source for the colonisation would be from the stored topsoil. In some of the pots where the *C. vulgaris* died, other plants, such as *Juncus effusus* and *Ulex europaeus* colonised, indicating a viable seed bank within the stored topsoil, thus it is possible that fungal spores could also survive (Birnbaum et al., 2017).

The increased infection rates using the commercial ErM on stored topsoil is an exciting development and supports the findings of natural soil inoculation by Wubs et al. (2018). A significant finding of the inoculation trial is that the ErM fungi did not need organic matter to survive. The results of this lab-based study will inform future studies (Chapter 4).

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# Chapter 3 Topsoil and restoration: depth in storage of stored overburden and potential effects on chemical and physical components needed for heathland restoration

## Introduction

Soils typical of Atlantic Lowland Heath (ALH) are a product of the mineral nature of the bedrock, the quantity and composition of water, photosynthetically-active radiation and ambient temperature combined with the action of a biological component i.e., microbes up to macro-arthropods (Clarke, 1997). The interaction between these factors results in the movement of complexes of nutrients, such as magnesium, calcium, potassium, phosphorus and nitrogen into the sub-surface soil profile, causing the upper levels of ALH soil to be acidic and nutrient impoverished (Brady et al., 2008). Typically, lowland heath soils on lower altitudes in and surrounding the Dartmoor National Park are sandy mineral soils, with podsols formed due to a high level of leaching of upper soil horizons, rather than soils characterised by nutrient deposition by litter at the surface (Hawley et al., 2008). In contrast, the higher altitude areas of Dartmoor experience higher rainfall (2330 mm per year) (Proctor, 2006) and high levels of litter and organic matter create peat. In the upland peats, compared to other temperate soils, there is a complex suite of processes for decomposition and movement of key soil components due to the low nutrient levels and the acidity of the soil (Webb, 1989). Temperate soils typically feature distinct

horizontal sections called horizons (**Figure 10**), whose physical and chemical characteristics influence the soil's ability to support its biological communities and functions. The upper (surface) horizon is the litter layer (O Horizon), moving downwards, there follows an organic-rich mineral layer (A Horizon). Dividing the A and B horizons in more mature developed soils is the E horizon, a layer that is created by leaching of mineral and organic content downwards, mainly leaving silicates. Subsoils are characterised by accumulated clay (B Horizon), unconsolidated weathered parent material (C Horizon), the Saprolite or weathered bedrock (D Horizon) and finally the bedrock (R Horizon). The dividing line drawn in soil studies is arbitrary, as not all of the horizons may be present or identifiable and the cut off points for what is bedrock or what is complete litter is subjective in most cases (Richter & Markewitz, 1995).
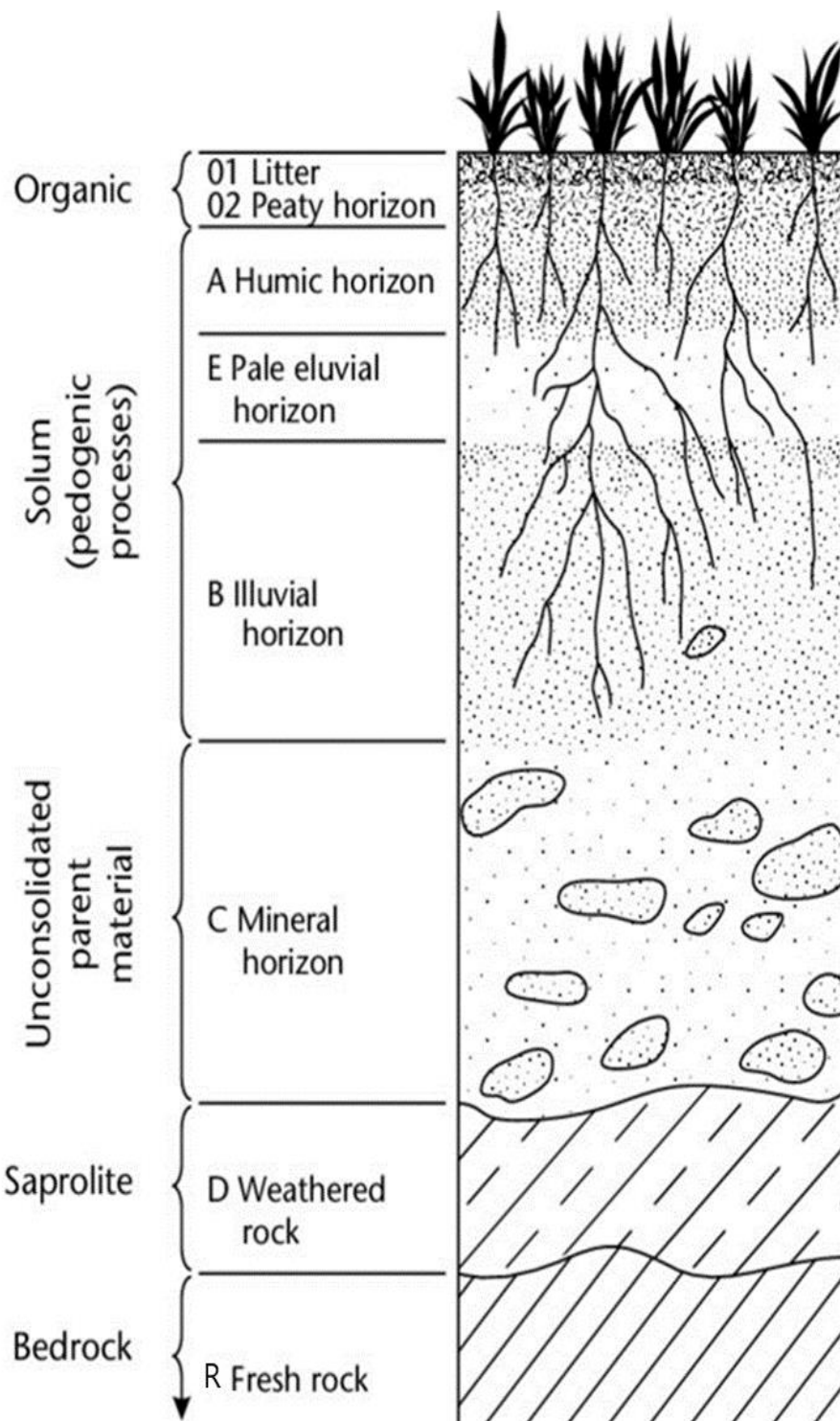
**Figure 10**: Typical soil horizons in temperate regions. Adapted from McQueen & Scott (2008)

For the purpose of restoration and mining, the horizons are separated during the mechanical stripping process. Hereby, the O and A Horizons are classed as 'topsoil' and stored for restoration. When mining clays, sands and gravels, B and C horizons are either processed for the desired mineral, or if the mineral content is low, are stored as 'subsoils or overburden' (Bradshaw, 1997a).

Topsoil is important in the restoration of worked out mines and it is paramount to ensure that the right topsoil is available to enhance the potential of achieving the desired restored habitat (Wubs et al., 2016). The mining industry uses soil stripped from previous areas within the quarries to minimise the cost, to minimise waste and to enable a local flora and fauna suite to recolonise after operations. Where possible, stripped soil is replaced onto areas undergoing restoration as soon as possible. Otherwise, the soil is stored in compacted tips of five to six metre depth (soil berms) for months or years, until required (Kundu & Ghose, 1997).

The list of key chemical and physical soil properties that support successful restoration of ALH need to be assessed prior to attempting restoration. These include soil pH, organic matter, exchangeable calcium, extractable phosphorus, and cation exchange capacity (Pywell et al., 1994; Manning et al., 2006). Notably, the ideal conditions for heathland soils are pH 2.8-3.9, with exchangeable calcium between 80-

159 µg Ca$^{-1}$ and extractable phosphorus of 1 mg/100 g soil (Clarke, 1997). Other important factors within the heathland soil for dwarf ericoid success are ratios of Al:Ca (0.6), N:P (23) and N:K (1.9), (with values in brackets recorded for a particular site at pH 4.2 and providing an indication of expected ideals (Roem & Berendse, 2000; von Oheimb et al., 2010)). These ratios may differ between locations and have an impact on micronutrient availability and metal toxicity, which is controlled by pH (Critchley et al., 2002; Critchley et al., 2004).

Understanding the minutiae of soil nutrient combination and how their interactions support the plant communities is important before starting any restoration project (Smith et al., 2003). To restore soil for ALH restoration after agricultural use, the deep structure and parent materials remain, so these can be used as a basis for the restoration of soil horizons for ALH development (Glen et al., 2016). For mining concerns however, the restoration of stored soils is complex as the natural soil structure has been substantially altered in the removal process and during the storage period. Surface layers have been removed prior to mining and thus disrupted, compacted and potentially changed chemically and biologically in storage. The parent materials are now absent as these are the minerals that were mined and thus removed. Therefore, whilst there may be compaction and disruption to the surface layers of the soil and chemical changes to the nature of the soils, there are no deep horizons left and no surface to turn over, as a

whole body of soil has been extracted. If restoration is to succeed, then the soil needs to be built up using whatever waste materials are suitable and the horizon structure must be replaced.

The use of heathland soil is the best route to restore heathland (Clarke, 1997). Where this is not possible, pioneer plant species, such as *Ulex spp.* and *Anthyllis vulneraria,* can support the development of soils, through the facilitation of nutrient cycling and beginning to build the ecosystem that allows ALH to colonize. Roberts et al. (1981) found it could take up to 55 years to develop an ALH habitat on waste kaolinite sands; the process to achieve ALH will therefore take a more extended period of time than the 5 years of planned aftercare specified by local government planning consents.

In addition to an extended restoration period, the vegetation and soil are a dynamic system and when not in equilibrium, one may not accurately reflect the conditions within the other. Therefore, whilst the structure of the soil is important, the functionality of the soil is more so (Doran & Zeiss, 2000). Soil functionality is defined as the capability of a soil to provide key services, such as nutrient cycling, physical stability to support plant growth, and biological productivity (Brussaard, 1997) and the ability of stored soils and restored soils to support successful revegetation and strong growth is a fundamental aspect of restoration. If

the restored soils and vegetation function naturally, then soil structure

can be developed by natural ecosystem engineers and physical

processes over decades (Cunningham et al., 2001; Muñoz-Rojas et al.,

2016; Ngugi et al., 2018).

Any topsoil that has been stripped is a source of propagules, microbes

and macroinvertebrates (Muñoz-Rojas et al., 2016), however once the

soil is stored, this biological resource can become denuded or altered by

sub or anoxic conditions, with the probability of greater changes

occurring, the longer the topsoil is stored (Waterhouse et al., 2014).

While fresh soils, used as soon as possible after stripping, have

demonstrated the greatest seedling emergence and the most diversity of

emerged species (Golos et al., 2016; Merino-Martín et al., 2017) stored

soils do have the ability to support revegetation of environments. For

example, research has shown that for species transplanted into fresh or

stockpiled soil, there is no significant difference in growth after four

months (Omari et al., 2018). There is also evidence that below conifer

plantations *Calluna vulgaris* seeds can remain viable for 40 years before

emerging when the plantation is cut down (Pywell et al., 2002).

Consequently, it is possible that the long-term storage of soil may not

cause damage to prospective seedling emergence, although this has not

been tested in the context of ALH restoration after mining. The use of

stored soil with an added seed mixture may therefore increase the

success of ALH restoration, as certain seeds will have not survived in the soil storage berm.

This chapter investigates how time and depth in storage affect key chemical and physical parameters of overburden and its suitability for use in heathland restoration accounting for the original heterogeneity of the original soil prior to translocation into the soil berm.

## Method

### *Soil berm and sampling*

The topsoil storage berm under investigation is located on top of the sand waste tip in the North of Headon China Clay works (**Figure 11**) and was created over a period of five years. It is six metres deep, wider at the bottom and has a flat top of 11.52 m width and 90.4 m length. The berm was created by tipping soil by A40 Volvo dumper trucks and pushing the individual loads into a pile by a Caterpillar D8 bulldozer. The soil was stripped in phases in dry weather during summer campaigns (approximately six weeks), during which ground is prepared for mining. This created an annual vertical structure to the berm. Once each campaign was completed, a bulldozer drove over the top to compact the soil to minimise water ingress destabilising the soil berm.

In October 2017, two samples per soil storage time period were taken from the soil berm to investigate the effects of prolonged storage on the characteristics of soil. For health and safety considerations, aiming not to destabilise the tip, samples were taken from the outer edges of its flattened top (**Figure 12**) with a Komatsu Geo600 Drill Rig fitted with a coring system. The cores were retrieved in 750 mm sections and assembled in core boxes (**Figure 13**). A subsample was taken off the bottom of each section for moisture testing. The remaining sample was air dried and each section separately sieved (2 mm aperture) and homogenised. This gave 29 samples over five time periods: 1 month and 2, 3, 4 and 5 years in storage.

**Figure 11**: Location of topsoil storage berm at Headon China Clay works. Photograph taken 2016 copyright of Sibelco.

**Figure 12**: Location of coring samples (yellow circles) on soil tip. Photograph taken Oct 2017 copyright of Sibelco.



**Figure 13**: Example of drill core sample no 5 (second yr 2 sample) in two sections: bottom section right to left 0 to 3.75 meters, top section right to left 3.75 to 5.25 m deep.

## Sample treatment and analysis

Moisture content was analysed by drying each sample to a constant

weight in an oven at 105°C. The subsample taken from the bottom of

each core section was placed in the oven within 3 hours of extraction. The delay was due to health and safety of access to the cores. They had to be brought to the laboratory by the drill team; they did this twice a day to minimise alteration before sampling for moisture. Unless stated otherwise, soil analysis was carried out on air dried, sieved and homogenised samples.

Soil pH was tested in a 1:5 (w/v) ratio (10 g soil in 50 mL deionised water), mixed for 15 minutes. After leaving the mixture to settle, pH was determined with a portable instrument (Hanna 991001) in the supernatant (Jones Jr, 2001).

The available extractable mineral elements of the soil were extracted using the Mehlich III method from (Jones Jr, 2001). Mehlich III extraction solution (5.5 L of 0.2 M $CH_3COOH$ (BDH, AnalaR, 100001CU), 0.25 M $NH_4NO_3$ (BDH, AnalaR, 100303S), 0.015 M $NH_4F$ (FISHER, Analytical Reagent Grade, A/4960/53), 0.013 M $HNO_3$ (FISHER, Certified Analytical Reagent Grade, N/2300/PB17), 0.001M EDTA (BDH, Laboratory Reagent, 28021) were prepared in MilliQ water (Millipore, Milli-Q (Organex –Q) system <18 M$\Omega$ cm$^{-1}$) using the procedure from Jones (2001), adapted for 3 g of soil. To each soil sample (3 g), 30 mL of extraction solution was added in centrifuge tubes and mixed on a reciprocating mechanical shaker at 200 rpm for 5

minutes. Following the filtration of the sample through Fisher scientific 11455198 filter papers (125 mm), 20 mL of filtrate was stored in plastic tubes (Thomson scientific 25 mL Sterilin tubes) in the dark at room temperature until analysis. The concentration of Mg, Ca, K, Cu, Mn, Al, P and S in the extracted solution were analysed by inductively coupled plasma – optical emission spectroscopy (Thermo Scientific iCAP7400 ICP-OES). Standards of 0, 50, 100, 200 and 400 mg/L were used to calibrate the instrument and to check for instrumental drift throughout each analytical run.

Soil C, H and N concentrations were analysed using an elemental microanalysis EA1110 CHN analyser. Soil samples (~10 mg) and Peat Standard Soil (~3 mg) were weighed into 6 x 4 mm high purity tin sample pots. These were gently crushed to exclude atmospheric nitrogen. The samples were flash combusted in an oxygen-rich environment and the oxidation products measured by a thermal conductivity detector in a column maintained at 65°C.

The cation exchange capacity was measured following Jones Jr (2001). Sodium acetate (1 M, 30 mL) was added to 5 g soil samples in centrifuge tubes, mixed for 5 min at 180 rpm in a reciprocal mixer, then centrifuged for 2 min at 3500 rpm. The supernatant was carefully discarded. This process was repeated once with sodium acetate, then

twice again with industrial methylated spirit (IMS). Ammonium acetate (1 M, 30 mL) was then added to the soil and mixed for 15 min at 180 rpm in a reciprocal mixer. The sample was centrifuged, and 1 mL of the supernatant diluted with 100 mL of MilliQ water (Millipore, Milli-Q (Organex –Q) system <18 MΩ cm$^{-1}$) in a 100 mL flat bottomed volumetric flask and analysed in a flame photometer. Cation exchange capacity was calculated using the following equation.

*CEC (mEq  100 g$^{-1}$) = Photometer reading x 300000/At. Wt. Na 5 x 1000 x wt. soil (where 'at.wt.' is atomic weight and 'wt. soil' is weight of the soil in grams).*

Particle size distribution (PSD) between 0.02 and 2000 μm was determined by laser light scattering (Mastersizer 2000e, Malvern, UK). The instrument used red light (wavelength 633 nm) in samples dispersed in sodium hexametaphospate ($[NaPO_3]_6$).

## Thermodynamic calculations and statistical analysis

In order to investigate possible processes occurring within the soil berm during storage, a series of calculations were carried out using the thermodynamic equilibrium speciation software Visual MINTEQ, version 3.1 (Gustafsson, 2011).

The calculations were carried out for the soil solution (without sorption), which is relevant for the assumption that the processes that determine

the mobility of elements within the soil berm occur at the solid/solution interface and that the concentrations determined by Mehlich III extraction (pH 2.5) are the maximum present. The processes determining the mobility of elements in the soil solution under investigation to relate to changes in redox conditions, which could not be determined experimentally. Precipitation of solids was allowed, while no sorption interactions were included in the calculations. Different scenarios were run, all at 25°C, for a surface (0-0.75 m) and depth (3.75-4.5 m) sample of the 1460 day core, with contrasting composition, pH and redox conditions imposed. The data input for components is provided in **Table 2**. The two different scenarios are described here:

1) Mimicking soil solution based on element concentrations extracted by Mehlich III extracted concentrations, rainwater and literature, without the Mehlich extraction solution present. The reactions are open to atmosphere ($CO_2$ at 0.00042 atm pp), the composition as per **Table 2**, $Fe^{2+}/Fe^{3+}$ redox couple specified with default parameters, pH fixed at value determined experimentally (pH 5.8).

2) Eh sweep from -200 mV to +500 mV under conditions for 1).

**Table 2**: Data input for thermodynamic equilibrium speciation calculations with Visual MINTEQ. Extractable aluminium, calcium, copper, iron, potassium, magnesium, manganese, sodium, phosphorus and sulfur (mM, Mehlich III extraction), in samples taken from cores of the storage soil berm at Headon China Clay works. Data sources: Me III ex – Mehlich III extractable concentration, Rain – typical rainwater composition (Bearman et al., 2007), Literature – value based on 2 µg g$^{-1}$ extractable N in typical Atlantic heathland soils (Antisari & Sequi, 1988). PHC – place holder concentration to allow redox reactions.

| Concentrations | 0 – 0.75 m (mM) | 3.75 – 4.5 m (mM) | Data source |
|---|---|---|---|
| $Al^{3+}$ | 4.0 | 9.1 | Me III ex |
| $Ca^{2+}$ | 0.24 | 0.32 | Me III ex |
| $Cl^-$ | 0.11 | 0.11 | Rain |
| $Cu^{2+}$ | 0.014 | 0.03 | Me III ex |
| $HN_4^+$ | 0.1E-6 | 0.1E-6 | PHC |
| $Fe^{3+}$ | 0.85 | 1.98 | Me III ex |
| $K^+$ | 0.16 | 0.14 | Me III ex |
| $Mg^{2+}$ | 0.22 | 0.11 | Me III ex |
| Mn | 0.01 | 0.03 | Me III ex |
| $Na^+$ | 0.078 | 0.078 | Rain |
| $NO_3^-$ | 1.4E$^{-3}$ | 1.4E$^{-3}$ | Literature |
| $PO_4^{3-}$ | 0.036 | 0.004 | Me III ex |
| $SO_4^{2-}$ | 0.061 | 0.060 | Me III ex |

To test for significant differences in parameters with varying depth and time, the permutational multivariate analysis of variance (PERMANOVA), an analog of MANOVA for partitioning distance matrices among various sources of variation was performed (Anderson, 2001).

The null hypothesis of this test is that the distance metric centroid, (the central point of the cluster of data points), does not differ between groups (within the factors depth and time) (Anderson & Walsh, 2013). PERMANOVA was calculated with the adonis function in the vegan package (http://cran.r-project.org/package=vegan) (Oksanen, 2019). Since this test is sensitive to data dispersion (Anderson, 2001), an analysis of multivariate homogeneity (PERMDISP) was performed (Anderson et al., 2006) with the betadisper function to test if groups differed in their dispersion. The null hypothesis of this test is that the average within-group dispersion is the same in all groups (Anderson & Walsh, 2013). In each of these two tests, the number of permutations was set to 9999.

## Results

Depth profiles illustrating the physicochemical and chemical composition of the cores obtained from the soil storage berm show large variations in some parameters with time in storage and/or with depth (e.g. pH, **Figure 15** and P**, Figure 16**). The multivariate PERMANOVA for the whole data set confirms significant differences in soil characteristics within the cores (depth) ($F_{(6,86)}$ = 59.8734, $p < 0.01$) and between the cores (length of time in storage) ($F_{(4,86)}$ = 8.0705 , $p < 0.01$). However, for the factor time, the betadisper analysis revealed significant differences in dispersion between groups (*betadisper* $F_{(4,82)}$ = 14.134, p <

0.01, **Figure 14**) so this result should be interpreted cautiously. For

depth, the test of dispersion was not significant (*betadisper* $F_{(6,80)}$ =

1.2365, p=0.303 **Figure 14**).
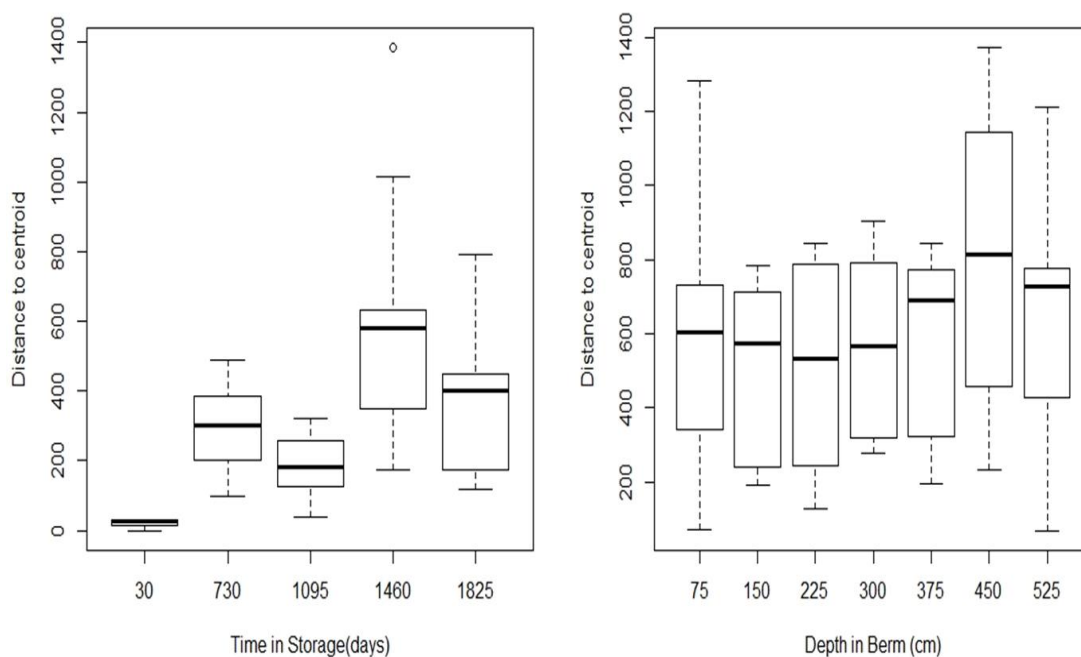


**Figure 14:** Box plots of the beta distribution of variability between central centroids of all measured variables grouped by time (left graph, days in storage) and depth (right graph, depth in cm) in samples from the stored soil berm at Headon China Clay works. There is a significant difference in the variability of the central centroids of the grouped by time variables (*betadisper* $F_{(4,82)}$ = 14.134, p < 0.01), However when the same variables are grouped by depth there is no significant difference ($F_{(6,80)}$ = 1.2365, p = 0.303).

Soil composition and potential processes that effect changes over time

and with depth are investigated in more detail in the following sections.

## *Physiochemical soil characteristics*

Soil pH was between 3.41 (75 cm, 730 days in storage) and 6.11 (525 cm, 1460 days in storage), mostly above the typical range for acidic heathland soils (optimum range pH 2.8-3.9 (Clarke, 1997)). The oldest and youngest cores feature the most acidic soils of the berm (**Figure 15**). With more than two orders of magnitude difference, the pH profiles highlight the heterogeneity of the soil collected around the site throughout the years, clearly relating to the difference with time indicated by the PERMANOVA. All cores exhibited pH ≥5.4 in bottom samples, with three cores showing increasing values down core (1.4 to 2 pH units), while the 1460 day core remained relatively stable down core (0.4 pH unit decrease). This indicates processes occurred at depth that affect pH, which will be considered further in the discussion.

The dominant grainsize fractions in all sampled cores were silt (46-68%) and sand (32-54%), with clay making up less than 1% of the soils (**Table 3**). The cation exchange capacity (CEC) of the berm was between 8.6 mEq / 100 g (300 cm, 730 days in storage) and 20 mEq / 100 g (375 cm, 1825 days in storage) (**Table 3**), lower than seen for other heathland soils (Pywell et al., 1994). The moisture content of the cores ranged between 13% (150 cm, 730 days in storage) and 27% (525 cm, 1460 days in storage). With the exception of pH, none of the physical characteristics in the soil berm showed any discernible patterns.
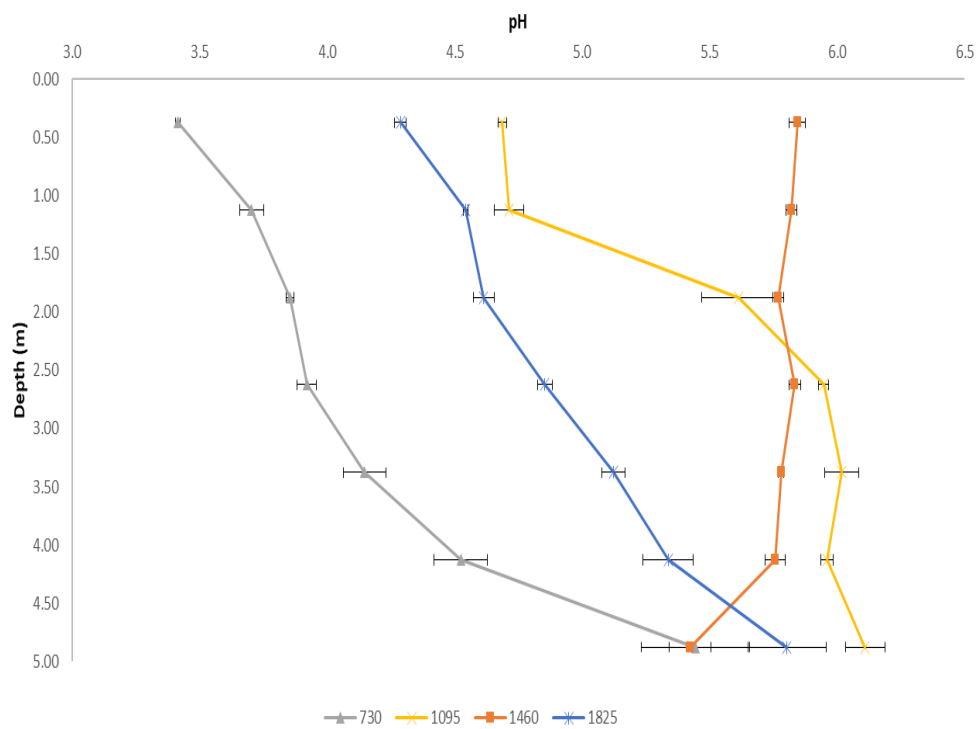
**Figure 15:** pH depth profiles in soil berm cores of 730 to 1825 days in storage at Headon China Clay works. Multiple extractions of the same sample were measured to calculate SE error bars.

**Table 3:** Maxima and minima of recorded soil characteristics of the storage soil berm at Headon China Clay works showing changes with depth of sample and time in storage.

|        | Time (Days) | Sand (%) | Silt (%) | Clay (%) | CEC (mEq /100 g) | MC (%) | pH |
|--------|-------------|----------|----------|----------|------------------|--------|-----|
| **Max** | 30 | 42.0 | 58.1 | 0.55 | 22.4 | 12 | 4.8 |
| **Min** |    | 41.4 | 57.5 | 0.52 | 21.7 | 11 | 4.6 |
| **Max** | 730 | 47.4 | 64.1 | 1.06 | 17.8 | 17 | 5.4 |
| **Min** |    | 35.9 | 52.6 | 0.55 | 8.61 | 13 | 3.4 |
| **Max** | 1095 | 48.8 | 56.8 | 0.58 | 19.4 | 27 | 6.1 |
| **Min** |    | 43.2 | 51.2 | 0.34 | 11.9 | 18 | 4.7 |
| **Max** | 1460 | 53.8 | 67.7 | 0.78 | 16.7 | 27 | 5.8 |
| **Min** |    | 32.3 | 46.2 | 0.45 | 10.1 | 15 | 5.4 |
| **Max** | 1825 | 54.2 | 64.8 | 0.92 | 20.3 | 25 | 5.8 |
| **Min** |    | 35.2 | 45.8 | 0.38 | 8.87 | 20 | 4.3 |

## *Macronutrients*

The macronutrients (NPK: nitrogen, phosphorus, potassium) in soil samples showed some variability, ranging from 0.04% to 0.29% (345-2878 µg g$^{-1}$) for total N, 1.10-22.6 µg g$^{-1}$ for extractable P and 31.1-120 µg g$^{-1}$ for extractable K (**Table 4**). The values of total N for the soil berm range from very low to twice values reported in other studies of lowland heath soils (1300-1500µg g$^{-1}$) (Walker et al., 2004a). P and K concentrations were within the optimum range (1.3-2.0 µg g$^{-1}$ P, acetic acid extraction; 28.0-49.3 µg g$^{-1}$ K, ammonium acetate extraction (Pywell et al., 1994)) in some samples and somewhat elevated (up to factor 17 for P and 2.4 for K) in others. Total nitrogen concentrations determined using the CHN analyser yielded results that are comparable

to those obtained with the Kjeldahl method and provides no information on organic N speciation (Antisari & Sequi, 1988). However, as ericoid mycorrhizal fungi are capable of using all forms of N, reporting total N is more relevant than bioavailable N for this study (Bending & Read, 1996b; Bending & Read, 1996a; Bending & Read, 1997; Bárcenas-Moreno et al., 2011).

The depth profiles of the macronutrients differed between elements (**Figure 16**). For total N, any pattern is absent and these results are more likely representative of the result of the N content of the original soils prior to stockpiling and atmospheric deposition. Extractable P concentrations are similar at the top and bottom, with large changes occurring in the middle of the cores. K concentrations show the least variability with a tendency to have a maximum in the middle of the core or just slightly below. This is a similar pattern to the micronutrients discussed later.

The carbon to nitrogen ratio shows some variability within cores, and the highest and lowest C:N ratio occurred in the core stored longest (34.1, 18.4, 1825 days), while the ratio of other cores is less variable (24 ± 4) (**Table 4**). The C:N ratio is an important factor in determining and regulating the soil microbial community (Wan et al., 2015), which is an important component of successful restoration (Marrs, 2016). Although

the C:N ratio changes with latitude, soil formation and carbon content

(Cleveland & Liptzin, 2007), von Oheimb et al. (2010) reports values of

the C:N in the O horizon of *C. vulgaris* heathlands ranging from 22.1-

27.7. These reported values are similar to those found within the berm

in this study and undisturbed heathland (**Figure 17**).

**Table 4:** Ranges of total nitrogen (%, CHN analyser), extractable phosphorus and potassium (µg g$^{-1}$, Mehlich III extraction), total carbon (%, CHN analyser) and the total carbon to total nitrogen ratio in samples taken from cores of the storage soil berm at Headon China Clay works, in relation to time in storage.

|  | Time (Days) | N (%) | P (µg g$^{-1}$) | K (µg g$^{-1}$) | C (%) | C:N |
|---|---|---|---|---|---|---|
| Max | 30 | 0.31 | 4.8 | 47.3 | 6.58 | 23.1 |
| Min | | 0.15 | 4.6 | 43.1 | 3.51 | 21.0 |
| Max | 730 | 0.19 | 17.3 | 91.3 | 4.20 | 28.1 |
| Min | | 0.04 | 7.10 | 28.5 | 1.01 | 21.9 |
| Max | 1095 | 0.23 | 22.6 | 120 | 5.69 | 27.6 |
| Min | | 0.11 | 7.30 | 41.3 | 2.85 | 22.8 |
| Max | 1460 | 0.22 | 15.0 | 75.5 | 5.39 | 25.3 |
| Min | | 0.13 | 1.10 | 39.4 | 2.61 | 20.1 |
| Max | 1825 | 0.29 | 17.3 | 115 | 5.57 | 34.1 |
| Min | | 0.05 | 5.60 | 31.1 | 1.62 | 18.4 |
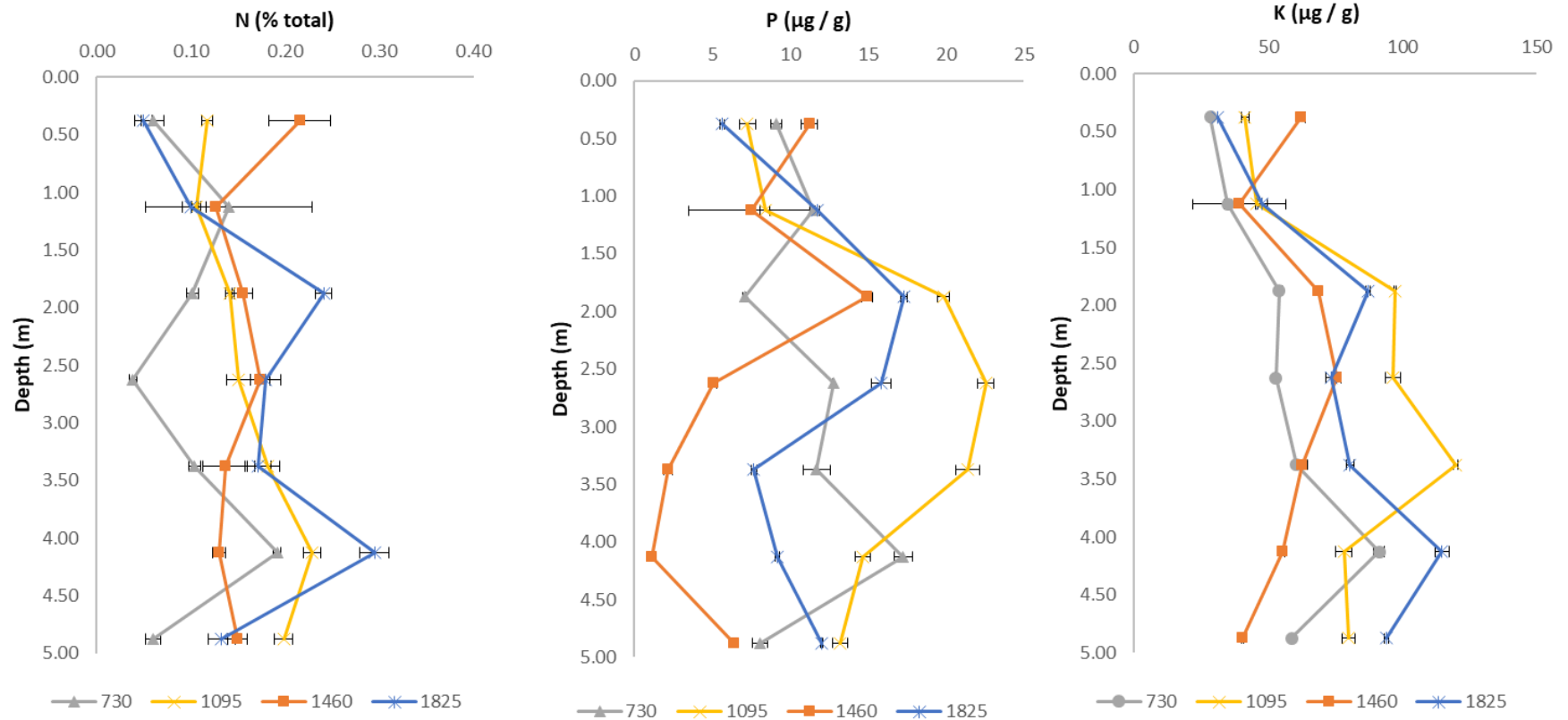
**Figure 16:** Profiles of total nitrogen (%, CHN analyser), extractable phosphorus and potassium (µg g$^{-1}$, Mehlich III extraction) of the storage soil berm at Headon China Clay works in relation to time in storage (730 – 1825 days). Multiple extractions of the same sample were measured to calculate SE error bars.
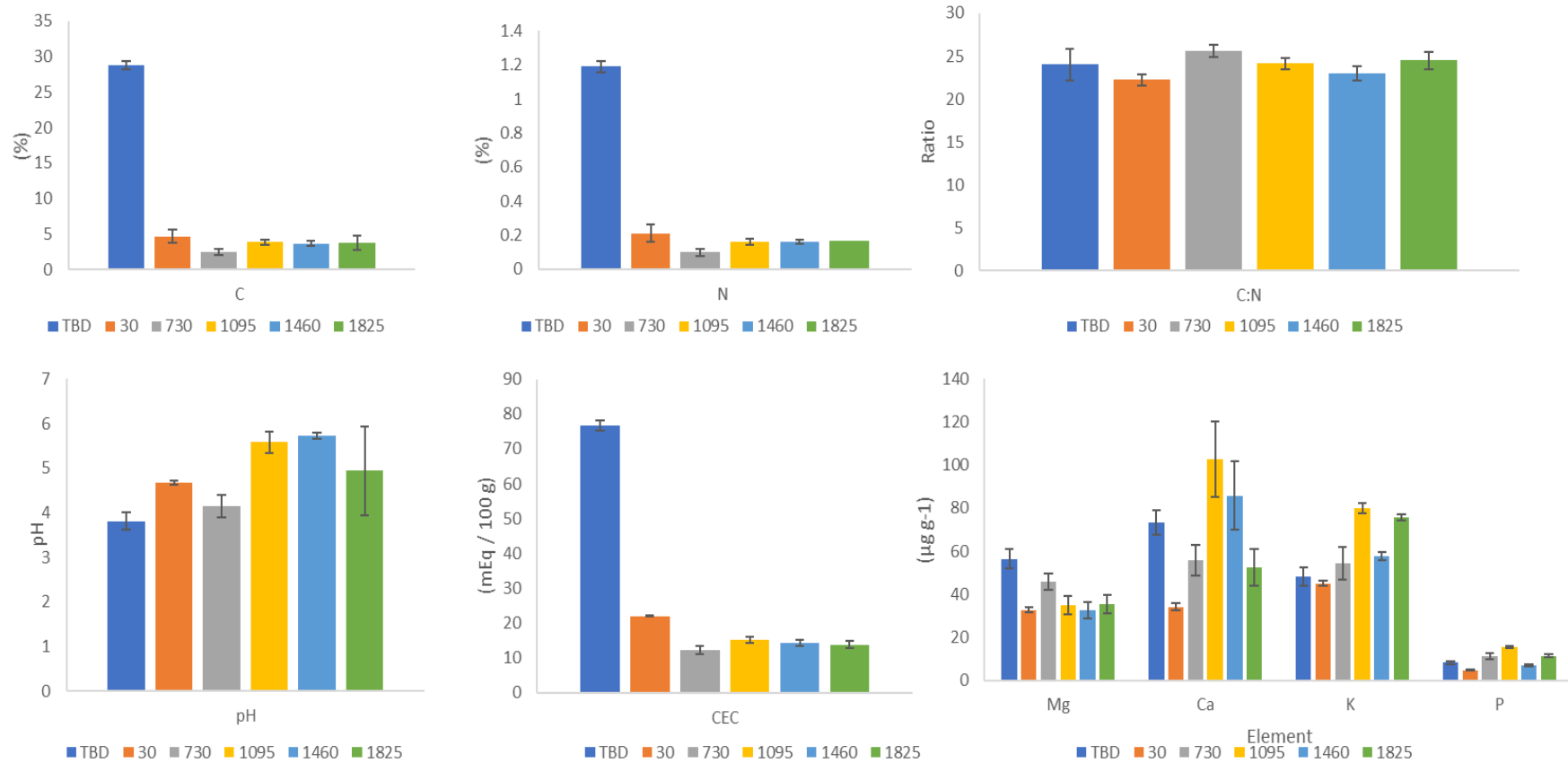
**Figure 17**: The biologically important components of the stored top soil when compared with undisturbed heathland soil from Trendlebere down on Dartmoor.

## *Thermodynamic calculations*

The results of the inorganic thermodynamic equilibrium speciation calculations presented here are for the core 1460 days in storage at the determined soil calculations without the Mehlich extraction solution present. The pH was set at the determined soil value (5.8); for all the reactions the system was open to the atmosphere and the $Fe^{2+}/Fe^{3+}$ redox couple specified with default parameters ($CO_2$ at 0.00042 atm pp).

In the first scenario at the soil surface, the ionic strength was calculated to I = 0.002 M, Eh = 542 mV and the charge difference between anions and cations was 1.8%, indicating a well-balanced calculation (**Table 2**). The species saturation index indicated an apparent equilibrium for diaspore, hematite and $MnHPO_4$, with calculation results showing precipitation of 100% Al, 100% $Fe^{3+}$, 99% Mn and 27% PO4, while Cu(II) and Fe(II) were mainly present as free ions in solution (>97%). This indicates that Cu remained relatively mobile, while Fe (mainly present as Fe(III)), Al and Mn were likely to be largely precipitated. The depth (3.75-4.5 m) sample results were similar, at the same high redox potential, but with a higher charge imbalance, indicating a lack of cations, or excess of anions, in the composition in the calculations. Again, precipitation of Al, Fe(III) and Mn was at or near 100% of the concentration, and the dissolved species distribution suggested high mobility for Cu.

In the second scenario, for the sample extracted at 3.75-4.5 m depth, the Eh was altered in steps of 50 mV, performing an Eh sweep from -200 mV to +500 mV, otherwise calculations were carried out under identical conditions as in the first scenario. The results at the 3.75-4.5 m depth showed the total dissolved iron concentration was highest at the lowest redox potential (Eh = -200 mV) simulated in this sweep, dropping by one order of magnitude for each 100 mV Eh increase up to around Eh = +350 mV. This is a consequence of precipitating Fe solids (as seen in the first scenario above) brought about by the oxidation of more soluble Fe(II) to less soluble Fe(III) with increasing Eh. The dissolved iron speciation was dominated by Fe(II) (~100%) at Eh = -200 mV through to Eh= +250 mV, and then changed to 75% Fe(II) at Eh = +400 mV and to 30% Fe(II) at Eh = +450 mV and to 6% Fe(II) at Eh = +500 mV. The results indicate that at any redox potential below +350 mV, it is likely the main Fe speciation will become the reduced and mobile form $Fe^{2+}$ within the soil berm, showing potential for movement and leaching. Fe can be leached from within the soil as Fe(II).

## *Micronutrients*

No pattern of minimum values over depth or time was discerned for any of the micronutrients (Mg, Ca, Al, Fe, S, Cu and Mn), but the maxima occurred mostly in the older, deeper samples (**Figure 18**). This was particularly so for Fe and Mn, which, in all cores, exhibited higher

concentrations in the mid layer samples (300 to 425 cm), irrespective of

the concentrations, which varied between storage time.

Sulfur concentrations show little variation between samples, ranging

from 11.4 µg g$^{-1}$ (150 cm, 1460 days in storage) to 22.9 µg g$^{-1}$ (450 cm,

1825 days in storage). The calculated Al:Ca is far higher than ALH soils

reported in the literature (**Table 5**) (Roem &  Berendse, 2000).
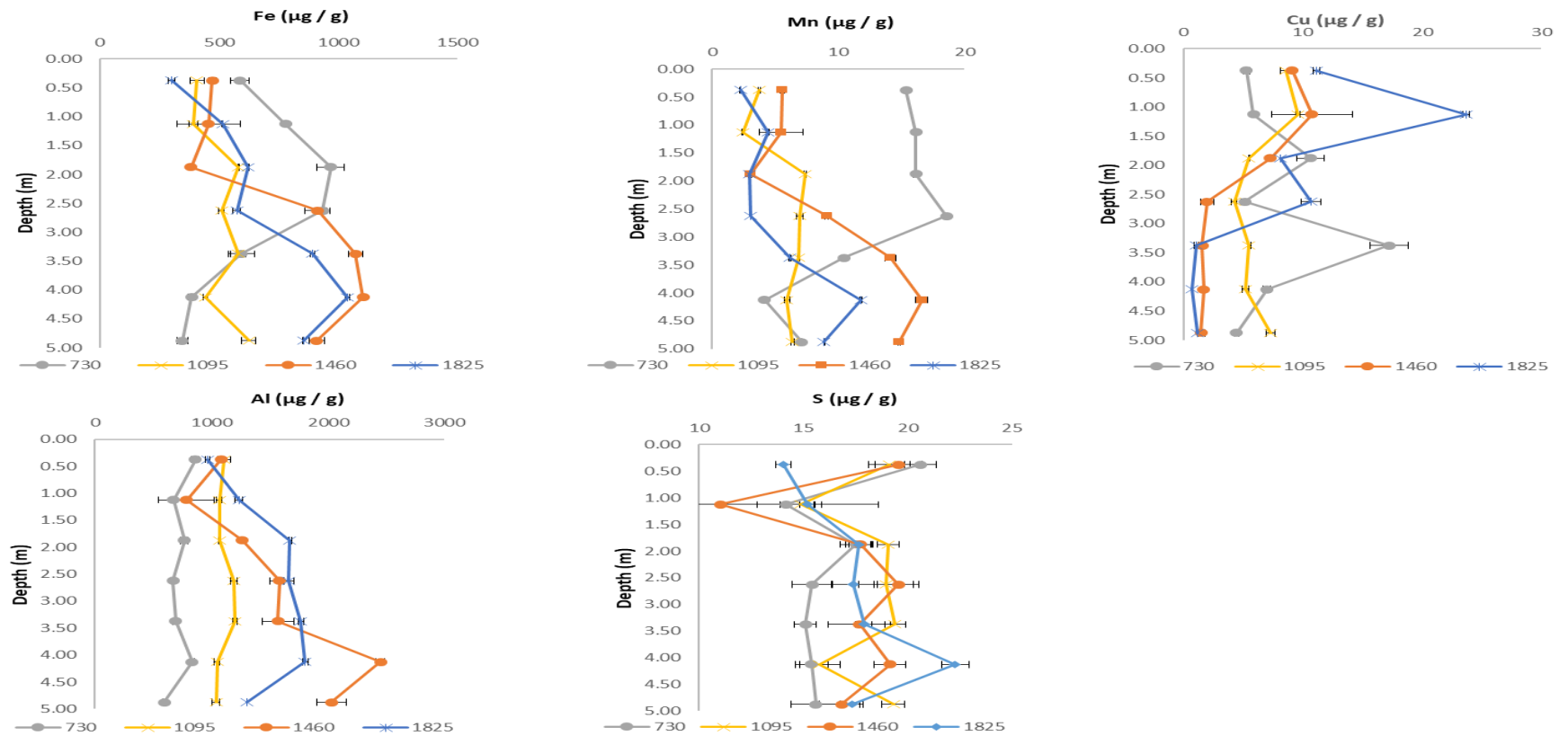
**Figure 18:** Profiles of extractable iron, manganese, aluminium, copper and sulfur (µg g$^{-1}$, Mehlich III extraction) in samples from storage soil berm cores at Headon China Clay works, in relation to time in storage (730–1825 days). Multiple extractions of the same sample were measured to calculate SE error bars.

**Table 5:** Range of extractable magnesium, calcium, aluminium, sulfur, iron, copper and manganese (µg g$^{-1}$, Mehlich III extraction) and the aluminium to calcium ratio of samples from storage soil berm cores at Headon China Clay works in relation to time in storage (730–1825 days).

| | Time (Days) | Mg (µg g$^{-1}$) | Ca (µg g$^{-1}$) | Al (µg g$^{-1}$) | Al:Ca | S (µg g$^{-1}$) | Fe (µg g$^{-1}$) | Cu (µg g$^{-1}$) | Mn (µg g$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| Max | 30 | 34.8 | 37.4 | 1768 | 47.2 | 24.2 | 561 | 0.78 | 0.93 |
| Min | | 31.6 | 31.2 | 1717 | 54.1 | 23.9 | 548 | 0.73 | 0.8 |
| Max | 730 | 61.3 | 75.5 | 860 | 26.9 | 20.6 | 968 | 17.2 | 18.6 |
| Min | | 30.1 | 22 | 592 | 10 | 14.2 | 345 | 4.5 | 4.2 |
| Max | 1095 | 49.6 | 152 | 1204 | 31 | 19.4 | 625 | 9.6 | 7.4 |
| Min | | 20.3 | 35.9 | 1040 | 6.9 | 14.9 | 390 | 4.3 | 2.4 |
| Max | 1460 | 52.6 | 155.1 | 2453 | 27.5 | 19.6 | 1103 | 10.8 | 16.6 |
| Min | | 24.2 | 44.9 | 785 | 11.2 | 11 | 382 | 1.5 | 3 |
| Max | 1825 | 53 | 91.5 | 1807 | 65.6 | 22.3 | 1037 | 23.7 | 11.8 |
| Min | | 24 | 25.4 | 970 | 19.7 | 14.1 | 301 | 0.8 | 2.2 |

# Discussion

Overall, there was a significant effect of time and depth on the whole chemical profile of the overburden samples. However, the betadisper tests showed significant differences in dispersion for the factor time, but not depth (**Figure 14**). Results for the time analysis should therefore be interpreted with caution (although the test is relatively robust to differences in dispersion for balanced designs, this can be a problem for unbalanced designs, Anderson, 2001). Individual parameters within individual cores revealed complex patterns with both time and depth and merit further discussion.

Depth profiles provided no evidence of differences in the physical structure of the soil, which could not be explained by the heterogeneity of the original soil. There is no indication of a major net loss of N, P, K within the soil berm over time. Equally, there was no net loss of extractable metals and cations in the cores, although for some elements there is an indication of downward movement within cores (**Figure 18**). The potential processes causing the downward movement are discussed below. The lack of evidence from the particle size distribution (PSD) analyses for sorting indicates that the majority of parameters in the soil berm are dominated by the character of the original soil (Omari et al., 2018). The soil is a sandy loam or a sandy silty loam; unlike the undisturbed soil at Trendlebere Down which has a much higher organic content of a peat character, which also enhances cation binding and hence mineral retention. (Brady et al., 2008).

The pH depth profiles indicate hydrogen ion consumption processes, many of which involve redox reactions. In this study, an increase of pH with depth was observed in cores that had relatively low values at surface (pH <4.8). However, a direct relationship between pH and Eh cannot be inferred and many processes, both biotic and abiotic, may influence the redox potential in soil (Husson, 2013). In most soils, redox processes are related to the amount of oxygen present, with those that decrease the amount of oxygen, and thus decrease redox potentials, needing a source of decomposable organic material. In the case of the

berm, plant material included in the soil storage by stripping processes is a likely source. A population of microbes exists within the stripped soil, although it remains uncertain how long the original community survives within the berm, given that oxygen diffusion and temporary water logging at depth may create conditions that favour facultative over aerobic bacteria (DeLaune & Reddy, 2005). In addition, microbes are not uniformly distributed across the environment and this heterogenic dispersal may also contribute to heterogenic intensity or rate of redox reactions throughout the berm. Redox conditions were not determined in this study because the available methodologies did not meet the rigorous standards (standardised, reproducible) suggested by Husson et al. (2016). Nevertheless, one process, which may be related to the observed increase in pH with increasing depth (**Figure 15**) and the concomitant decrease in redox potential, is the reduction of Fe(III)( $Fe(OH)_3 + CH_2O + 8H^+ = 4Fe^{2+} + CO_2 + 11H_2O$). This reduction of $Fe(OH)_3$ and oxidation of organic matter, yields the more mobile $Fe^{2+}$, with carbon dioxide and water as by products. The net consumption of $H^+$ ions during reduction leads to an increase in pH within the soil solution. The biotic reduction of $Fe^{3+}$ from $Fe^{2+}$ is an anaerobic respiration pathway coupling the oxidation of carbon with the reduction of iron. Microbially, the reduction of Fe can also be coupled to $NH_4$ oxidation, potentially creating nitrogen gas that may escape from the soil. The limited oxygen diffusion throughout the berm at depth, in combination with Fe reduction and potential oxidation of both, organic

carbon and/or ammonium, is likely to favour progressively more reducing conditions in the soil berm. This could facilitate the mobilisation of some soil elements, including C, N, Cu and Mn. The apparent downward movement of Mn and Fe was more distinct in the soils that had been stored for the longest period of time, possibly indicating the beginning of the formation of podzolic soils (Cunningham et al., 2001).

Podzolic soils are acidic soils and are often found underneath ALH habitat (Lundström et al., 2000). Typically, they have high C:N ratios (recorded soil range for heathland soils are 22.1-28.6 (Forgeard & Frenot, 1996; von Oheimb et al., 2010)) and low plant nutrient concentrations (80-159 ug/g Ca, 1 ug/g P (Clarke, 1997)), with medium to coarse grain size (Lundström et al., 2000). The development of a full podzolic soil profile could take between 350-10,000 years (Lundström et al., 2000). However, Cunningham et al. (2001) reported a shift to podzolalisation and iron pan formation could begin within decades under conditions where anthropogenic activities have affected the soil.

Podzolic soils form when macro- and micro-nutrients are leached down through the soil profile, generally due to water ingress, facilitating the mobilization of $Fe^{2+}$. Anderson et al. (1982) postulated three stages of podzol formation: 1) Al and Fe moved from the A horizon to the B

horizon, subsequently illuviated as silicate complexes without organic matter. 2) Migration of organic matter and precipitation on the silicate complexes. 3) Leaching of ferrous Fe from the B horizon caused by impeded percolation of organic complexes to form a thin pan (Buurman & Van Reeuwijk, 1984). In soils, the mobility of Fe and Al is enhanced by complexation with simple organic acids (for example: citric, lactic, oxalic), which facilitates the transfer of Al and Fe from the A horizon to the B horizon (Buurman & Van Reeuwijk, 1984). Thermodynamic equilibrium calculations of the soil solution in the 1460 day old core indicate that Al and Fe (hydr)oxides (haematite, diaspore and, at low Eh, magnetite) precipitation may occur at the concentrations extracted from the core at depth. This supports the theory that over time, some elements are leached from surface layers and that metal (hydr)oxides may precipitate and accumulate, or at least, are not depleted at depth, providing a mechanism for the capture of other metals, such as Mn and Cu by hydroxides (McBride, 1989) within deeper layers. The soil berm is exposed to weathering and has a wetting-drying cycle throughout the seasons, whereby the redox potential may oscillate around the low Eh conditions (~350 mV), that separates Fe oxidation and reduction, hence Fe (oxy)hydroxide precipitation and dissolution, indicated by thermodynamic calculations. It is important to note that soils are dynamic systems with changing redox conditions in both directions (increase and decrease), and that the rates of precipitation and dissolution reactions differ, thus, equilibrium may not be reached. This

limits the interpretation of the speciation calculations. Conditions that favour such alterations include exposure to high rainfall and infiltration (no vegetation cover), high permeability (low organic matter, clay content and compaction), and redox and pH values that facilitate dissolution and mobility of metals. All these conditions exist within the site berm.

The topsoil removed and collected was from areas previously mined (from 15th Century onwards) within the quarry boundaries (radius 1 mile). Some unplanned revegetation growth on the berm and soil development within it will have taken place during this process and its basic characteristics after storage have been found to be different from those of typical heathland soil with respect to pH, organic matter, cation exchange capacity and C:N ratio. The total N ranges from far too low to double the literature values (**Figure 17**, **Table 6**). Understanding whether these differences make the berm unusable for the restoration of ALH after mining is an important investigation of this chapter. If the soil is unsuitable or unusable for ALH then it must be used to develop other habitats or ameliorated until suitable.

The median CEC is low compared to other heathland soil, limiting ion retention and this may have implications for the survival of rarer plant species. Kleijn et al. (2008) report that larger numbers of rare heathland

plants are associated with the higher CEC. The carbon content of the soil appeared to be slightly lower than some values reported in the literature results of other heathlands (3.5% -18% (Marrs, 1993; Pywell et al., 1994; Clarke, 1997; Mitchell et al., 1999)). The most common carbon replacement strategy within the industry is to add compost, which also enhances water retention, maintaining concentrations of other macronutrients (N and P) as a consequence. Other potential carbon enhancing strategies being developed include using green waste compost, as detailed in this thesis, and the use of paper mill crumb or biosolids, a by-product of human waste (Dudeney et al., 2004; Palmer & Davies, 2014). A further option potentially suitable for ALH restoration is using lignite, a product of mine waste with a high C content and a N content analogous to ALH soils (Rumpel et al., 1998).

Nitrogen levels need to be low to maintain a heathland community as increased nitrogen can push *C. vulgaris* communities towards non heathland species, such as *Pteridium aquilinum* and bryophytes (Måren et al., 2008). Clarke (1997) states that the N is not as important a factor as the other elements, such as exchangeable P and extractable Ca. However, some authors argue that the most important aspect of heathland restoration is the $NH_4$ and $NO_3$ content of the soil (Marrs & Bradshaw, 1980; Kleijn et al., 2008). As the median value of the soil berm total N is 1440 $\mu g\ g^{-1}$, with complete mixing during restoration the soil berm N is likely to be suitable for ALH development (**Table 6:** The

results from the values of the stored soil berm fit within the literature

and how that affects the usefulness of the soil for restoration to ALH.

Key to references a: (Pywell et al., 1994), b: (Walker et al., 2004a) c:

(Mitchell et al., 1999), d: (Strandberg et al., 2018) e: (von Oheimb et al.,

2010) f: (Forgeard & Frenot, 1996). 0 neutral reaction).

The C:N ratio in the soil berm is much lower than in other ALH soils.

This may result in the development of a non-ALH microbe community,

with bacteria outcompeting the fungal components necessary for ericoid

plants to outcompete grasses and survive the low nutrient environment

(Strandberg et al., 2018). The ability of N to leach out of the soil is

shown to be lessened by having a higher C:N ratio (Rowe et al., 2006).

The availability of N and C within the soil are important as not only a

source of plant nutrients but also redox reactants as discussed above.

The median C:N ratio in this study (22.5) suggests that almost half of

the samples analysed indicated values that put heathland at risk

(Strandberg et al., 2018), as C:N < 22 could promote the colonisation of

ruderal grasses (Hawley et al., 2008). Furthermore, low C:N ratios could

result in a change of bacterial to fungal ratio in the soils, favouring

bacterial growth, and consequentially minimise the development of ALH

habitats and promoting succession to bush or scrub (von Oheimb et al.,

2010). Nevertheless, adding C is a potentially feasible and sustainable

option and as N concentrations are within the optimum range, carbon

additions would optimise the C:N ratio, which has the added advantage of improving soil status with respect to total C concentrations and CEC.

P, K and the micronutrient elements are within the very wide ranges shown in the literature (Pywell et al., 1994; Clarke, 1997; Critchley et al., 2002; Walker et al., 2004a). Whilst P is at the higher end of reported ideals (Clarke, 1997), K, Mg and Ca are all at the lower end of the literature ranges. These could become limiting factors for any restoration project. Whilst the movement and reduction of Fe, Al, Cu and Mn have been discussed in depth earlier, during the process of restoration the stored soil is mixed and spread.

For some parameters (P, K, Mg, Ca and C:N), the stored soil shares similar results with the undisturbed soil from Trendlebere down (**Figure 17**). These results are similar to Trendlebere's undisturbed soil and support findings from the literature (**Table 6**). The C:N ratio for the undisturbed soil is 24, which is a low value for ALH but not an undesirable value. When comparing pH values, the stored soil pH was, on average, one pH unit higher than the pH at the undisturbed site (3.81), which is an optimal value for ALH.

Further comparisons indicate that carbon, nitrogen and CEC are higher in Trendlebere than in the stored soil and values shown in the literature (Forgeard & Frenot, 1996; Mitchell et al., 1999; Walker et al., 2004a).

The C content is over 28%. The N is at 11900 µg g$^{-1}$. The high C and N values could be due to the high levels of partially decomposed litter in the samples compared to the more mineralised samples of the soil berm. Furthermore, the plant communities and soil conditions are linked through litter deposition and mycorrhizae fungal activities (Van Vuuren et al., 1992; Berendse, 1998; Roem & Berendse, 2000), highlighting that these results implicate that maintaining 'ideal' soil conditions is difficult in established ALH.

Without amendment, the plants that can exist in this soil are the hardier grasses, such as *Festuca, Agrostis*, and the leguminous *Ulex*, as some of the major plant nutrients (K, P, C and N) are low. However, the majority of nutrients cycling in this type of habitat occurs in the litter layer (Pywell et al., 2002). The litter layer will take time to develop during restoration projects, so soil amelioration may be needed to kick start this process in soil to be utilised for ALH habitat creation.

The work here shows that the storage of soil has an impact on its physical structure and some elements of its chemical structure, with movements of some elements within the soil berm towards the bottom of the berm. A thin layer of stored topsoil enhanced with organic matter to provide 'litter' for nutrient cycling, as well as carbon and CEC, spread over kaolinite sands may be the best option for using the stored soil.

The literature specifies the importance of soil conditions for the

reinstatement and rehabilitation of heathland vegetation (Bradshaw,

1997b; Clarke, 1997; Allison & Ausden, 2004; Gasch et al., 2014).

**Table 6:** The results from the values of the stored soil berm fit within the literature and how that affects the usefulness of the soil for restoration to ALH. Key to references a: (Pywell et al., 1994), b: (Walker et al., 2004a) c: (Mitchell et al., 1999), d: (Strandberg et al., 2018) e: (von Oheimb et al., 2010) f: (Forgeard & Frenot, 1996). 0 neutral reaction

| Parameter | Min | Max | Median | Literature values | Ref | Median comparison with literature | Supportive | Hindrance | Consequence | Action |
|---|---|---|---|---|---|---|---|---|---|---|
| pH | 3.4 | 6.1 | 4.8 | 3.63-4.06 | a | within literature range | 0 | | Grass invaded as pH above 5 | Lower pH by spreading small amounts over acidic sands |
| N ($\mu$g g$^{-1}$) | 345.0 | 2878.0 | 1440.0 | 1300-1500 | b | within literature range | 0 | | Higher values likely to increase grass recruitment | Ensure through mixing prior to restoration |
| P ($\mu$g g$^{-1}$) | 1.1 | 22.6 | 11.4 | 0.5-20.8 | a | within literature range | 0 | | | Do not add anything extra to the restored soil |
| K ($\mu$g g$^{-1}$) | 28.5 | 120.0 | 61.3 | 7.8-508.8 | a | within literature range | ** | | | Assess individual areas before |
| C (%) | 1.0 | 5.7 | 1.7 | 2.5-10.3 | c | too low | | *** | Low water retention | Careful addition checking C:N for microbiome shifts |
| C:N | 18.4 | 34.1 | 22.5 | 10-46 | d,e | within literature range but almost half of the samples may put heathland at risk | | **** | C:N ratios below 22 indicate a heathland at risk | Low C:N indicate change in fungal bacterial ratio and could put heathland at risk |
| CEC (mEq/100g) | 8.6 | 19.4 | 14.1 | 28.6 | f | too low | | **** | Implications for nutrient retention | |
| Mg ($\mu$g g$^{-1}$) | 20.3 | 61.3 | 34.7 | 12.0-65.0 | c | within literature range | 0 | | | |
| Ca ($\mu$g g$^{-1}$) | 22.0 | 155.1 | 61.4 | 12.4-1167.3 | c | within literature range | 0 | | | |

The conclusions from this chapter are that storage in a 5 m high soil berm exposed to weathering for not greater than 5 years does not change the chemical characteristics and physical structure of the stripped soil, beyond identifying the original heterogeneity. There are processes acting upon the soil during this time that enable the movement of metal ions and indicate the embryonic development of podzolic soils. Despite the stored soils not conforming to ideals identified from the literature ideal in some aspects, the soil is likely to be suitable for heathland restoration (Harris et al., 1989; Critchley et al., 2002; Kneller et al., 2018). The success of heathland restoration with this soil may be enhanced by adjusting CEC, C content, moisture retention and C:N ratio through addition of organic material that does not result in enhanced nitrogen availability to plants.

For general guidance, the recommendations that can be made from this work are (a) as short a time stockpiling as possible and (b) a thorough mixing of materials from the whole height of the berm, so that any movement of elements within the berm are reversed prior to use and (c) the addition of a low N carbon source.

# Chapter 4 Influence of ericoid mycorrhizae and soil nutrient addition on heathland restoration

## Introduction

Atlantic Lowland Heath (ALH) is defined as areas of ericaceous dwarf-shrubs with acidic nutrient-poor mineral soils, growing at low altitudes below 250m (Webb, 1986). Created and maintained by light grazing and burning, heathlands have continued to deteriorate since the mid-19th century (Hawley et al. 2008) thus ALH was identified as a continuing priority habitat in the UK in the implementation of the 2010 Biodiversity Framework (2012-2019) (Defra, 2019).

The declining extent and condition of ALH has made its successful restoration a high priority for restoration ecologists (Hayhow et al., 2019). Since the 1970s, restoration techniques in general, and reestablishment of ALH in particular, have been investigated to mitigate anthropogenically caused disturbances of this habitat (Bradshaw et al., 1975; Pywell et al., 1995; Miller & Hobbs, 2007; Box et al., 2011; Pywell et al., 2011; Vogels et al., 2020). Unfortunately, ALH habitats are difficult and unpredictable to successfully restore and maintain, with initial restoration a challenging problem (Marrs & Bradshaw, 1980;

Marrs et al., 1980; Marrs et al., 1998; Marrs, 2016) and long-term,

natural recolonization unlikely (Lane et al., 2020).

The search for the most effective restoration practices continues and is

especially relevant for the kaolinite waste sand tips of Devon and

Cornwall in SW England. Early attempts to restore ALH highlighted

nutrient cycling, principally the establishment and maintenance of a

functioning nitrogen cycle (Marrs & Bradshaw, 1980; Marrs et al.,

1981; Roberts et al., 1981; Skeffington & Bradshaw, 1981) as crucial

for successful restoration. Studies also report improved restoration

outcomes through soil acidification via sulfur addition, combined with

nutrient removal by either deep ploughing or cropping (growing a crop

and cutting and removal of vegetation to stop nutrients being recycled),

followed by introducing heather cuttings as a green hay or 'brash'

(Allison & Ausden, 2004; Walker et al., 2004b; Pywell et al., 2007; Glen

et al., 2017). Other important factors for ALH restoration are location

and land use; historically the least successful restorations have been

attempted on former agricultural land (Walker et al., 2004a) while

abandoned or reclaimed heathland tend to give rise to the most

biodiverse outcomes, followed by conifer plantations (Pywell et al.,

2002). The most successful outcomes have been observed when former

heathland is restored by removing the encroaching bracken and trees to

create an open space in the foliage to allow the seed bank to germinate

(Allison & Ausden, 2004; Walker et al., 2004b; Pywell et al., 2007; Glen

et al., 2017). By drawing on the evidence-based strategies as recommended by past and recent contemporary research, heathland restoration practice continues to implement best practice (Putwain & Rae, 1988; Symes & Day, 2003; White & Gilbert, 2003; Anderson, 2014).

Soil condition is a major part of ALH restoration. Indeed, Clarke (1997) advised maintaining or creating the soil conditions found in undisturbed heathland, specifically a pH range of 2.8-3.9, and extractable calcium of 80-159 μg Ca g$^{-1}$, with extractable phosphorus of less than 1 mg/100 g. Lane et al. (2020) discovered that even after 25 years of restoration with topsoil, certain cations and plant nutrients were at levels below the ideal conditions reported above. The lack of these cations (Mg, Ca and Na) were described in the literature as being important alone and in combination with each other (Clarke, 1997; Allison & Ausden, 2004). The concept of inoculating soil with 'indigenous' soil from the environment is an innovative way of replacing all of the missing nutrients in a bioavailable form, as well as the biological aspects of soil that are under-represented in soils prior to restoration (Bashan et al., 2014; Wubs et al., 2016). This technique still requires a donor site but it could potentially be a more attractive solution for large-scale restoration depending on quantities required for inoculation. Whilst research to understand the effects of storage on soil (Chapter 3) and the potential use of either donor soils or commercially

available inoculants continues, the idea that stored soil could be inoculated to enable more effective restoration outcomes remains unexplored.

More recently, attention has switched from soil and plant seed bank environmental interventions to investigating how the soil microbe community can be included in restoration practice; highlighting especially the role of mycorrhizal fungi in habitat restoration (Genney et al., 2001; Collier & Bidartondo, 2009; Hazard et al., 2014; Wubs et al., 2016; Wubs et al., 2018). However, the implications of translocating soil microbes has not developed from conservation theory to full commercial restoration practice. As discussed in Chapter 2, ericoids have an association with heliotales soil fungi, essential to ericoid colonisation and establishment (Smith & Read, 2010). During restoration, the use of stored soil means that the potential for the association to develop is lessened due to a reduction in viable fungal spores (Birnbaum et al., 2017). Whilst it has been speculated that colonizing seeds may already carry ericoid mycorrhizal fungal (ErM) spores, there is no direct evidence to support this possibility (Smith & Read, 2010). Instead, the translocation or inoculation of fungal spores using a commercial application of sterile mycelium of mycorrhizae may be essential to restoration success (Bashan et al., 2014).

Some strategies for fungal inoculation, such as plug planting or turve transplants, are problematic and challenging to implement, and lack widespread commercial backing due to high costs and the necessity for an existing donor heath ( £3120 /ha$^{-1}$) (Davis et al., 2011). Therefore the introduction of commercial ErM in combination with soil amelioration and seeding techniques has yet to be trialled within the industry in large-scale restoration projects.

The aims of this Chapter were to assess the impact of different soil amelioration methods on a commercial-scale, post-mineral (kaolinite) extraction site. Specifically the following objectives were addressed: (1) assessment of soil chemistry following addition of organic matter, ErM, missing important cations and combination treatments (in comparison with untreated controls); (2) assessment of colonization by ericoid plants and (3) establishment of the wider community (shrubs, forbs and graminoids).

## Method

### *Study site*

At the Headon China Clay Works site (full description in Chapter 1), a 166 m long by 12 m wide SW facing (~30% gradient) slope was selected. This site is located on a quartz sand waste tip, one of the largest areas scheduled for restoration at the site at the time. The slope was initially prepared using a Caterpillar D8 Bulldozer to repair water damage and

to spread stored topsoil evenly to a depth of around 10 cm (topsoil had

been stored in the site tip for 5 years (see chapter 3)). This area was

then divided into 99 (4 m x 3 m) treatment plots arranged in an 11 x 9

grid pattern, with a 1 m boundary surrounding each plot. From these,

eleven replicates of nine different treatments were assigned in a

stratified random pattern, such that one replicate treatment was

allocated to each row of nine plots (**Figure 19**).
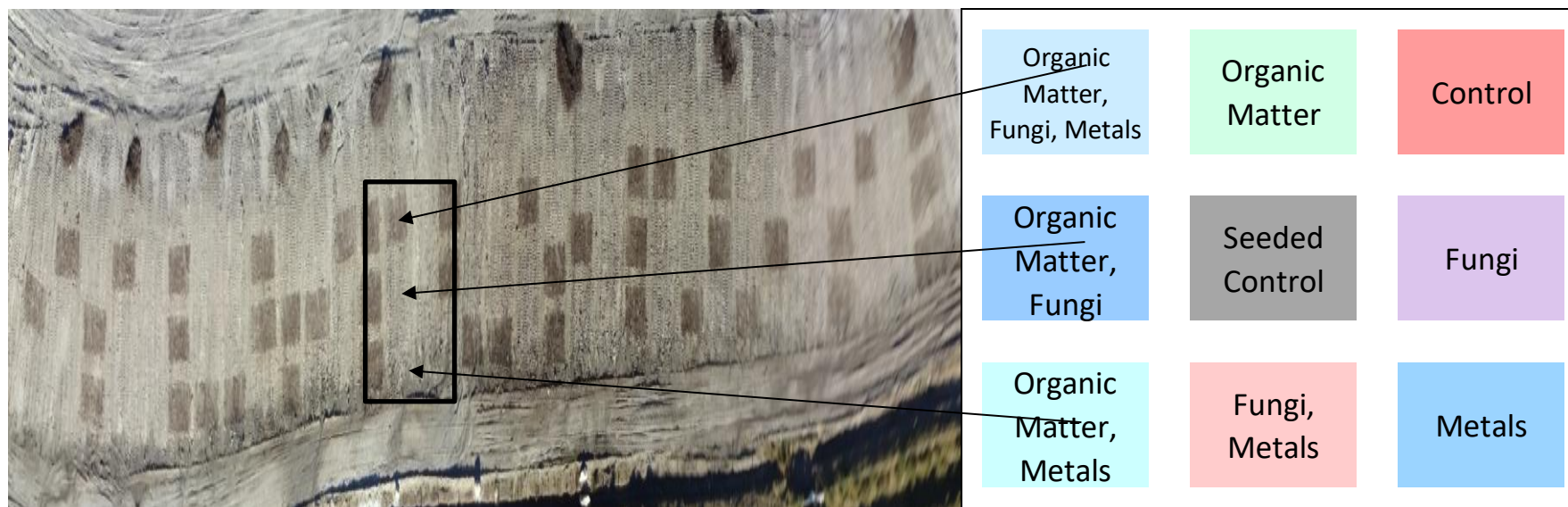
**Figure 19:** Aerial image above trial area with the treatment plots and location of treatments with the gridded areas shown. Each plot is 4 m wide and 3 m high. Picture taken autumn 2016 copyright of Sibelco.

## Treatments

In October 2016, eight of the nine-treatment groups were seeded with a 173 g mixture of heathland seeds added to each plot comprised of 34 g each of *Calluna vulgaris, Erica cinerea, Erica tetralix* and *Festuca rubra,* a further 17 g each of *Molinia caerulea* and *Festuca ovina,* and ~3 g of *Deschampsia flexuosa* (William Eyre, Bradwell, UK). The proportion of seeds was selected based on commercially available seed mixes. The plots were broadcast hand sown to ensure as even a spread of seeds as possible. In addition to the treatment (i) '**Seed Control'**, which received no further intervention, the following single factor treatments were included:

(ii)   '**Fungi'** – 35 mL Rhodovit commercial ErM was added to the centre 1 m$^2$ of fungal treatment plots. Rhodovit was selected as (at the time of the experiment in 2016) it was the only commercially available inoculation (i.e. to utilise in sufficiently large quantities) for practical post-mine restoration. Although subsequent analysis of the vegetation focused on the treated centre 1 m$^2$ area, it was anticipated that successful inoculation would facilitate wider spread of mycelia beyond this limit.

(iii)   '**Metal'** –Key cations were added at the following amounts per plot; sodium 123 g (13.50 mg kg$^{-1}$), calcium – 111 g (12.5 mg kg$^{-1}$), potassium 324 g (35.40 mg kg$^{-1}$), and magnesium 449 g

(49.03 mg kg$^{-1}$). All cation ions were bought from Thompson and Morgan, Suffolk (commercial availability). Application concentrations were based on increasing the quantity of elements in the soil (Lane et al., 2020) to levels within the ideal values as recorded by Clarke (1997). As all the plant nutrients came in pellet form, the pellets were mixed and broadcast by hand to ensure an even spread.

(iv) **Organic matter -** 25% of the stored topsoil was replaced with compost to increase the available organic matter. Having first removed the top 2 cm (~150 kg) of topsoil from each plot, 150 kg of Viridor green waste compost was incorporated throughout the depth of the spread topsoil (~10 cm). The organic matter/amendment consisted of a 12 week matured green waste compost from Viridor, Heathfield's in-vessel household waste composting facility (full description in Chapter 2). Viridor could supply the amount of compost required and this material has been used in previous work on artificial soils (Schofield et al., 2018).

In addition, the following mixed treatment combinations were employed:

(v) **Fungi & metals**, (vi) **Organic matter & fungi,** (vii) **Organic matter & metals, and** (viii) **Organic matter, fungi & metals**.

The ninth treatment was an unseeded, untreated **Control** where the plots were exposed only to colonisation by windblown or soil-derived propagules.

## *Soil sampling and analysis*

In summer 2017, a 10 cm soil core (Eijkelkamp Soil & Water, Gisbeek, The Netherlands) sample was taken from the south-west corner of each plot. The sample was subsequently dried in a desiccator at 60 °C, disaggregated, sieved (2 mm mesh) and stored prior to analysis. For pH, 10 g of soil in 50 mL deionised water was mixed for 15 minutes with a magnetic stirrer. It was left to settle and quantified using a Hanna 991001 pH and temperature probe (Jones Jr, 2001). Mineral elements were extracted using the Mehlich III method (Jones Jr, 2001), whereby an extraction solution (30 mL) was added to each soil sample (3 g) in centrifuge tubes and mixed on a reciprocating mechanical shaker at 200 rpm for 5 minutes. Samples were subsequently filtered through Whatman 42 filter paper, and the filtrate retained in the dark until analysis. The Na, K, Mg, Ca and P concentration of the extracted solution was analysed using a Thermo Scientific iCAP7400 ICP-OES instrument. C, H and N were analysed using an elemental microanalysis EA1110 CHN analyser. For the following tests, three sub samples from bulked treatment samples were analysed due to cost. The soil samples (~10 mg) and Peat Standard Soil (~3 mg), were weighed

into 6 x 4 mm high purity tin sample pots. These were gently crushed to exclude atmospheric nitrogen. The samples were flash combusted in an oxygen-rich environment and the oxidation products measured by a thermal conductivity detector in a column maintained at 65 $^0$C. To measure cation exchange capacity, 30 mL 1M sodium acetate was added to 5 g soil samples. These were mixed for 5 mins at 180 rpm in a reciprocal mixer, then centrifuged for 2 mins at 3500 rpm. The solution was then discarded. This process was repeated once with sodium acetate, then twice more with IMS. Thirty millilitres of 1M ammonium acetate was then added to the soil and mixed for 15 mins at 180 rpm in a reciprocal mixer. The sample was centrifuged, and the supernatant diluted and analysed in a flame photometer. Cation exchange capacity was calculated using the following equation (Jones Jr, 2001): -

*CEC (mEq  100 g$^{-1}$) = Photometer reading x 300000/At. Wt. Na 5 x 1000 x wt. soil (where 'at.wt.' is atomic weight and 'wt. soil' is weight of the soil in grams).*

### *Vegetation sampling and analysis*

In June 2019, plant cover data for all species was collected from the entire (4 × 3 m) plot area. The number of individual dwarf ericoids were counted in the 1 m$^2$ centre portion for 2017, 2018 and 2019. Unfortunately, several squares were lost due to commercial operations

in 2019 and only eight replicates per treatment were included in the final analysis.

## Statistics

Analysis of cover data for 2019 was performed in three dimensions using metaMDS and ordiellipse to highlight groupings in the 'vegan' (Oksanen, 2015) package in 'R' v.3.5.2. Once the communities were plotted onto an ordination plot, the physical characteristics of the soil were overlaid as vectors (for variables where $P \leq 0.05$) to facilitate identification of how physical factors varied with, and influenced development of, the various communities. An ANOSIM was performed in the 'vegan' (Oksanen, 2015) package in 'R' v.3.5.2 to examine variation in plant community composition between restoration treatments. The centre metre square plant counts were analysed using a one way ANOVA for individual species ericoid plants. Mean differences in soil characteristics across treatments were analysed using one way ANOVA.

# Results

## Soil Chemistry

The control soil (i.e. lacking additional compost, nutrients, or seeds), was more acidic (pH 4.8), than any of the treatment sites with organic matter added (pH 5.2-5.6, **Table 7**). The pH of plots where organic matter was added singly or in combination were significantly higher when compared to all other soil intervention treatments (**Table 7**). The

pH of seeded control plots was most similar to plots where organic matter was added singly or in combination with both fungi and metals (**Table 7**). The control plots had significantly lower total carbon content (1.8% C) than where organic matter was added singularly (3.8%), with a smaller range of total carbon between the other treatments (2.3- 2.9 % C, **Table 7**). The total nitrogen concentration ranged from 0.06% (control) to 0.17% (organic matter); the only significant difference was between the control and organic matter alone (**Table 7**). However, in the organic matter and fungi plots the total N % was the same as the highest non-organic matter added treatments (0.1%). The other organic matter enrichment treatments rose to a total N % of 0.17% in organic matter added singularly. The other macronutrients (P, K) showed a similar pattern to each other with the lowest vales being recorded in the addition of commercial ErM treatment and the largest where organic matter, commercial ErM and plant nutrients were added (**Table 7**). The carbon: nitrogen ratio showed a wide range from 20.5 in the organic matter and metals treatment through to 31.6 in the control. Concentrations of micronutrients (Na, Ca, Mg) were generally an order of magnitude higher in the sites where either they or compost was added. Na was 9-fold higher in the treatments that combined fungi and metals than in the control, and Ca was three times higher where organic matter had been added singularly. The addition of organic matter had the largest effect on the retention of Ca, with significantly higher mean concentration following the addition of compost (**Table 7**).

The cation exchange capacity and pH were also positively correlated to the cations, as was the total carbon content and total nitrogen content (**Figure 20**).

**Table 7**: Comparison of mean (±SE, n=11) chemical conditions across treatment plots recorded in 2017 after 1 year of lowland heath restoration experiment on sand tip at kaolinite mine using seed addition and soil amelioration. ErM fungi, major plant nutrient cations (identified as metals in the table) and organic matter were added singly and in combination. The results of a one-way ANOVA of soil parameters are given, with different letters in superscript indicating significant difference (P<0.05) between site means, following Tukey's paired comparisons. Different df values relate to different numbers of samples as described in the method. CEC = Cation Exchange Capacity, CN = C:N ratio. ANOVA was performed on concentration of $H^+$ ions but presented as pH.

| Treatment | | pH | C (%) | N (%) | P (µg g⁻¹) | K (µg g⁻¹) | CN | Na (µg g⁻¹) | Mg (µg g⁻¹) | Ca (µg g⁻¹) | CEC (mEq /100g) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | Mean | 4.8 [d] | 1.8 [b] | 0.06 [c] | 18.4 [c] | 63.8 [b,c] | 31.6 [a] | 5.9 [c] | 51.2 [b,c] | 202.0 [b] | 8.4 [f] |
| | (SE) | (0.07) | (0.06) | (0.00) | (3.53) | (5.30) | (2.15) | (4.67) | (6.69) | (55.09) | (0.17) |
| Seeded Control | Mean | 4.9 [b,c,d] | 2.4 [a,b] | 0.09 [a,b,c] | 12.3 [c] | 38.6 [c] | 26.1 [a,b,c,d] | 48.2 [a,b] | 40.5 [c] | 116.7 [b] | 11.2 [c,d] |
| | (SE) | (0.07) | (0.07) | (0.01) | (2.19) | (3.39) | (1.04) | (3.21) | (3.58) | (13.94) | (0.15) |
| Fungi | Mean | 4.8 [c,d] | 2.3 [a,b] | 0.09 [b,c] | 8.6 [c] | 28.6 [c] | 26.0 [a,b,c,d] | 41.4 [a,b] | 36.7 [c] | 97.2 [b] | 9.6 [e,f] |
| | (SE) | (0.04) | (0.08) | (0.01) | (1.11) | (1.93) | (0.87) | (2.89) | (4.44) | (9.53) | (0.17) |
| Fungi, Metals | Mean | 4.8 [d] | 2.9 [a,b] | 0.10 [a,b,c] | 10.2 [c] | 52.5 [b,c] | 29.2 [a,b] | 58.4 [a] | 47.4 [c] | 108.6 [b] | 10.3 [d,e] |
| | (SE) | (0.07) | (0.36) | (0.01) | (1.48) | (7.00) | (0.91) | (2.73) | (4.32) | (8.80) | (0.23) |
| Metals | Mean | 4.8 [d] | 2.3 [b] | 0.08 [b,c] | 23.4 [c] | 94.0 [b] | 26.7 [a,b,c] | 15.2 [c] | 90.7 [a,b] | 181.0 [b] | 10.6 [d,e] |
| | (SE) | (0.09) | (0.22) | (0.01) | (3.35) | (8.20) | (0.84) | (2.70) | (12.88) | (26.50) | (0.17) |
| Organic Matter | Mean | 5.2 [a,b] | 3.8 [a] | 0.17 [a] | 71.7 [a] | 163.8 [a] | 22.4 [c,d] | 12.5 [c] | 113.1 [a] | 644.3 [a] | 13.0 [a,b] |
| | (SE) | (0.09) | (0.73) | (0.04) | (7.30) | (14.01) | (0.86) | (4.02) | (7.49) | (63.65) | (0.23) |
| Organic Matter, Fungi | Mean | 5.4 [a] | 2.5 [a,b] | 0.10 [a,b,c] | 33.3 [b,c] | 66.2 [b,c] | 23.9 [b,c,d] | 41.8 [a,b] | 57.1 [b,c] | 323.3 [a,b] | 11.9 [b,c,d] |
| | (SE) | (0.13) | (0.07) | (0.01) | (7.37) | (3.78) | (1.23) | (4.42) | (5.60) | (65.53) | (0.35) |
| Organic Matter, Fungi, Metals | Mean | 5.2 [a,b,c] | 2.8 [a,b] | 0.12 [a,b,c] | 75.8 [a] | 193.7 [a] | 22.9 [c,d] | 20.6 [c] | 120.6 [a] | 647.6 [a] | 13.6 [a] |
| | (SE) | (0.07) | (0.23) | (0.01) | (13.56) | (19.48) | (1.10) | (5.95) | (13.90) | (121.14) | (0.78) |
| Organic Matter, Metals | Mean | 5.6 [a] | 2.8 [a,b] | 0.14 [a,b] | 70.0 [a,b] | 90.8 [b] | 20.5 [d] | 40.1 [b] | 109.1 [a] | 675.9 [a] | 12.5 [a,b,c] |
| | (SE) | (0.15) | (0.23) | (0.02) | (17.91) | (7.46) | (1.53) | (3.00) | (15.74) | (172.61) | (0.15) |
| Anova | $F_{(8,90)}$ | 12.26 | | | 11.31 | 35.26 | | 22.22 | 13.41 | 10.01 | |
| | $F_{(8,18)}$ | | 3.349 | 4.224 | | | 7.87 | | | | 25.74 |
| | P | 0.001 | 0.05 | 0.001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |

## *Plant Community Establishment*

Multivariate analysis highlighted major differences between plots where organic matter was added when compared to all other treatment groups ($R_{ANOSIM}$ = 0.2531, *P* < 0.001). Specifically, all organic matter treatments were clustered ('top right' in the nMDS plot) and separate from the other treatment groups. Organic matter appeared to favour establishment of at least two (non-target) grass species, evidenced by clustering around *Agrostis stolonifera* and *Festuca ovina*, and the shrub, *Ulex europaeus*. The control treatment was clustered around *Juncus effusus* and *Agrostis curtisii.* All other treatments were clustered around bare ground, demonstrating only moderate plant establishment, even 32 months after plot establishment (**Figure 20**).

The amount of bare ground is striking, and there is a marked contrast between treatments with and without the addition of organic matter. In the absence of organic matter, bare ground ranged from 27.7% coverage in the control to 33.2% in the metals treatment, but bare ground cover was negligible where organic matter was added (**Table 8**).

The most common plant species across all plots was *Ulex europaeus. Ulex* was most abundant in the untreated control plots (55%), dropping to 34% in the metal treatment. There was no obvious treatment specific effect on the cover of *Ulex.* The second most common plant species was *Juncus effusus,* however this was observed at much smaller proportions than the *Ulex. Juncus* was most abundant in the organic matter and

metal treatment plots (15.6%) and lowest in the organic matter, fungi and metal treatment plots (6.6%). There was no treatment specific effect on *Juncus* cover.

Even after three years, recruitment of target ericoid species remained poor, and by 2019, the highest abundance (percentage cover) of ericoids achieved only 1.5% in the 'seeded control' treatment, with zero recruitment in the organic matter and fungi treatment. Focussing analysis on the central square metre, however, ANOVA revealed significant variation in the number of ericoid plants ($F_{(8,90)}$ = 3.427, $P < 0.001$) with the seeded control having more (Mean 4.3 ± SE 0.98) plants m$^{-2}$ than the organic matter and fungi treatment (0.6 m$^{-2}$, SE = 0.31). The major component of ericoid recruits was *C. vulgaris*, followed by *E. tetralix* (**Figure 21**). *C. vulgaris* dominated plots where organic matter was added in combination with other treatments and plots with no organic matter, (92.3% (F) to 73.2% (FM), this dropped to 87.5% were organic matter was added singularly. The control sites by contrast supported *C. vulgaris*, *E. tetralix* and *E. cinerea* in more equal proportions compared to the other plots, although unlike all other treatments, *E. tetralix* was the most abundant (41.2%, 47.2%, 11.8%, respectively).

The total grass cover ranged from 12.9 % (control) to 59.1% (organic matter) with the most dominant species being *Festuca ovina* in five of the nine treatments (6.8% F, 18.6% OMM). *Agrostis curtisii* dominated the seeded control (6.8%) and control treatments (9.1%), and *Agrostis stolonifera* the organic matter and fungi (19.1%) and metal treatments (10.6%). The organic matter fungi treatment had most (i.e. seven) grass species present, dominated by *Agrostis stolenifera (*19.1%) and *Festuca ovina* (13.6%). All other species were below 10% cover. The seeded control treatment contained (on average) six (co-dominant) grass species, but notably did not include *Molinia caerula. Agrostis curtisii* colonised the entire area between the 2018 and 2019 surveys. There were remarkably few forbs present in any plot (**Table 8**). The most common forb present was *Rumex acetosella* (control, and metal plots). *Potentilla erecta* was recorded in the seeded control and the metals, *Trifolium pratense* in the fungi treatment, and *Galium saxatile* in the metals treatments. Other notable species included *Juncus bufonius* appearing in metals, organic matter and organic matter and metals. The forbs had a larger than expected effect on the nMDS.
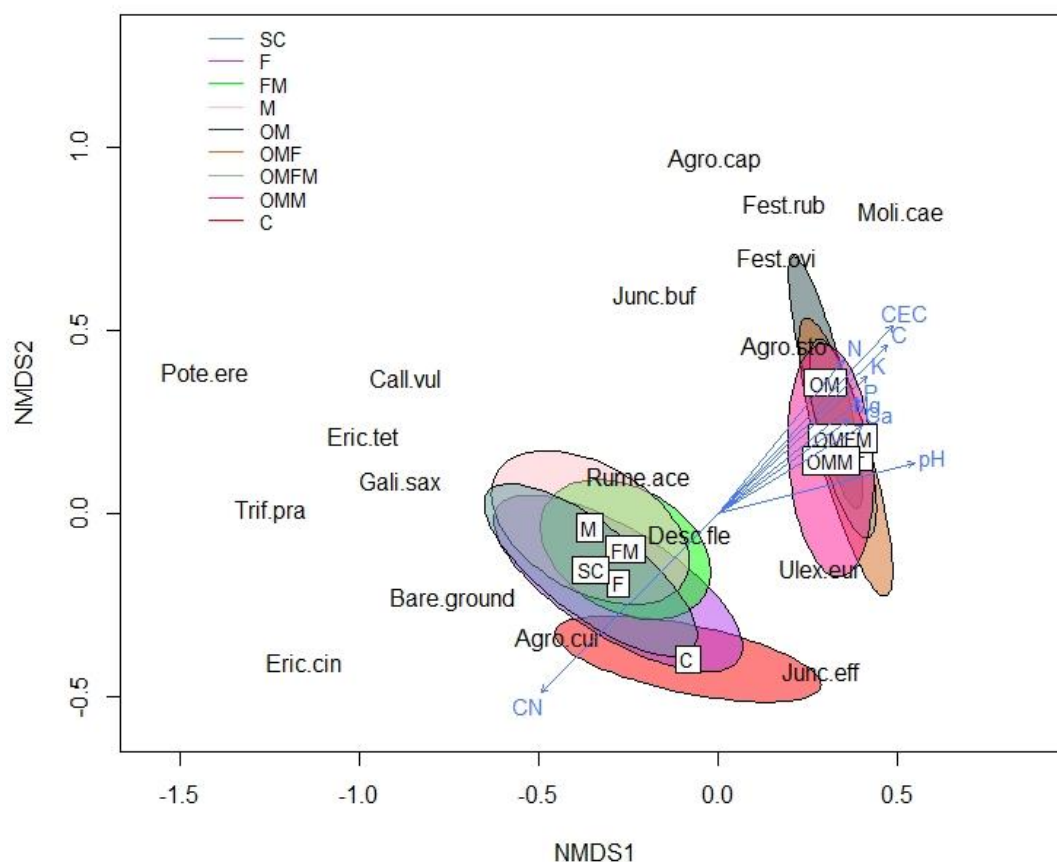
**Figure 20:** nMDS of the percentage cover data of the plant community across treatment plots recorded in 2019 after three years of lowland heath restoration experiment on sand tip at kaolinite mine using seed addition and soil amelioration. ErM fungi, major plant nutrient cations (identified as metals in the table) and organic matter were added singly and in combination (shown in 2 dimensions for ease of visualisation). The letters denote the treatment of soil. Stress = 0.09. Ordiellipse are present to show the overlap of the communities. The vectors are significant environmental factors ($p < 0.001$). Key to treatments: C Control, SC seeded Control, F Fungi, M Metal, OM Organic matter, FM Fungi and metals, OMF Organic matter with fungi, OMM Organic matter with metals, OMMF Organic matter with both fungi and metals

Key to plant species: Agro cap, *Agrostis capillaris*: Agro sto, *Agrostis stolonifera*: Agro cur, *Agrostis curtisii*: Desc fle, *Deschampsia flexuosa*: Fest ovi, *Festuca ovina*: Fest rub, *Festuca rubra*: Moli cae, *Molinia caerulea:* Call vul, *Calluna vulgaris*: Eric tet, *Erica tetralix*: Eric cin, *Erica cinerea*: Ulex eur, *Ulex europaeus*: Rume ace, *Rumex acetosella:* Pote ere, *Potentilla erecta*: Gali sax, *Galium saxatile*: Trif pra, *Trifolium pratense*: Junc buf, *Juncus bufonius*: Junc eff, *Juncus effusus*:
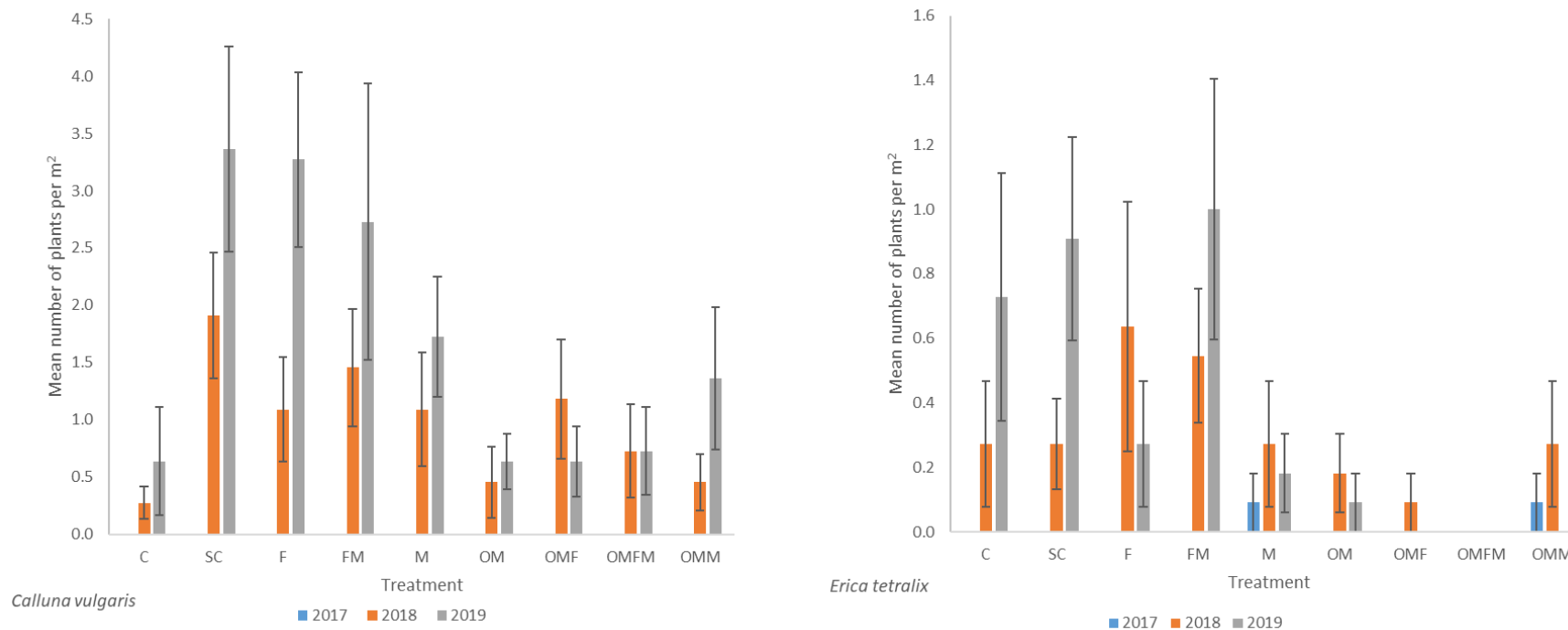
**Figure 21**: Mean number (±SE) of dwarf ericoids (by species) counted in centre 1m$^2$ of treatment plots recorded over 3 years of lowland heath restoration experiment on sand tip at kaolinite mine using seed addition and soil amelioration. ErM fungi, major plant nutrient cations and organic matter were added singly and in combination every year by species. Key to graphs C = control, SC = seeded control, F = fungi, M = metals (plant nutrients) OM = organic matter, OMFM = organic matter, fungi, metal, OMM = organic matter.

**Table 8:** Effect of different rehabilitation treatments on mean (±SE) abundance (percentage cover) of the most common plants recorded in plots three years after the start of a lowland heath restoration experiment on a kaolinite mine site in Dartmoor, SW England. Key to plant species: Agr sto, *Agrostis stolonifera*: Agr cur, *Agrostis curtisii*: Des fle, *Deschampsia flexuosa*: Fest spp include *Festuca ovina* and *Festuca rubra*: Ule eur, *Ulex europaeus*: Jun eff, *Juncus effusus*: Forbs identified in text

| Treatment (2019) | Graminoids | | | | | | | Shrubs | Forbs | Bare Ground |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Agr cap* | *Agr cur* | *Agr sto* | *Des fle* | *Fest spp* | *Jun eff* | **Total** | *Ule eur* | | |
| **Control (SEM)** | 0 (0) | 9.1 (2.2) | 1.5 (1) | 0 (0) | 2.4 (1.9) | 13.2 (3.9) | **26.1** | 55 (6.7) | **0.5** | 27.7 (7.7) |
| **Seeded control (SEM)** | 0.5 (0.5) | 6.8 (1.5) | 6.4 (1.7) | 1.8 (0.8) | 8.2 (3.8) | 8.8 (2.9) | **32.4** | 37.7 (6.2) | **0.1** | 30.9 (4.6) |
| **Fungi (SEM)** | 0 (0) | 4.2 (1.9) | 4.5 (1.6) | 1.4 (0.7) | 9.1 (4) | 12.9 (3.7) | **32.1** | 40.5 (6.9) | **0.1** | 29.5 (5.2) |
| **Fungi Metal (SEM)** | 2.3 (1.6) | 6.4 (1.5) | 10.6 (4.4) | 0.9 (0.6) | 12.8 (4.1) | 11.6 (3.2) | **44.5** | 40.8 (6.5) | **0** | 29.5 (4.8) |
| **Metal (SEM)** | 0 (0) | 4.3 (1.4) | 6.4 (1.5) | 0.9 (0.6) | 14.6 (4.6) | 12.6 (6) | **38.8** | 33.6 (5.2) | **0.8** | 33.2 (5.6) |
| **Organic matter (SEM)** | 1.4 (1) | 4.1 (2) | 15.5 (3.5) | 0.9 (0.6) | 37.3 (8.7) | 8.4 (2.9) | **67.5** | 44.1 (7.5) | **0** | 0 (0) |
| **Organic matter, fungi (SEM)** | 0.5 (0.5) | 2.3 (1.2) | 19.1 (6.2) | 1.8 (1) | 23.1 (6.5) | 11.8 (4.7) | **58.7** | 50.9 (8.5) | **0** | 0 (0) |
| **Organic matter, fungi, Matter (SEM)** | 1.8 (1.8) | 1.4 (1) | 13.6 (2.5) | 0.9 (0.9) | 28.7 (6.2) | 6.6 (2.8) | **53** | 53.6 (4.9) | **0** | 0 (0) |
| **Organic matter, metal (SEM)** | 0.9 (0.9) | 2 (1.2) | 14.5 (3.7) | 0 (0) | 25.4 (6) | 15.6 (7.1) | **58.5** | 47.7 (5.2) | **0** | 0.5 (0.5) |

## Discussion

The study aimed to establish whether commonly applied mine restoration techniques combined with microbial symbiont and plant nutrient augmentation would stimulate dwarf ericoid establishment towards restoring a target ALH community. The fact that the seeded control treatment had the highest density of dwarf ericoid plants, strongly suggests that in this case, interventions such as the addition of organic matter, ErM and cation addition singularly or in combination have no significant benefit in ALH reestablishment.

Natural ALH is a species-poor habitat, in some cases dominated by *C. vulgaris* (Webb, 1986). Restored habitat often follows that pattern with low numbers of species and high monocultures of ericoids. Our results showing a habitat dominated by *Ulex* and *Juncus* did not match the successful restoration habitat shown by other authors (Putwain & Rae, 1988; Pywell et al., 2011). The only striking result indicating the habitats created within the treatment plots could become ALH, was the amount of bare ground on plots where no organic matter had been added. The natural heterogeneity and mosaic nature of ALH includes patches of bare earth, which provide habitat for a range of flora and fauna e.g. sunning spots for vertebrates (Symes & Day, 2003). The bare space is available for germination, however, dwarf ericoids are difficult to germinate; survival of seedlings is reduced on slopes, as they can be

washed away by heavy rainfall (Symes & Day, 2003). Nevertheless, the low nutrient value of the stored soil may inhibit the germination and growth of grass seedlings allowing the ericoids time to establish (Canals & Sebastià, 2002). Once ericoids are established, ErM and ericoid roots are able to inhibit grass root colonization in the organic soil layer, increasing the success of ericoid seedlings and thus the targeted restoration (Genney et al., 2000).

Plant communities associated with organic material enrichment were mostly dominated by graminoids, followed by *Ulex.* The treatments without organic enrichment were dominated by *Ulex europaeus.* The dominance of the *Ulex* and *Juncus* are potentially caused by the long-lasted seeds of the *Ulex* within the seed bank and the wind-blown colonisation of *Juncus* (Ervin & Wetzel, 2001; Hill et al., 2001). There is *Juncus* around the site, so the potential for windblown colonisation is high. The ericoids appear to benefit from an association with *Ulex*, demonstrated by larger ericoids found in the trial sites growing underneath or through the *Ulex* bushes. Although there is no evidence of associations of dwarf ericoids and *Ulex* as a nurse species, there is evidence of bushes being used in this way by other species in similarly harsh environments (Gómez-Aparicio et al., 2004; Padilla & Pugnaire, 2006; Alday et al., 2014). Due to its N-fixing abilities, *Ulex* may be providing a secondary service to ericoids by increasing the amount of bio-accessible N-compounds in the soil. Interestingly, where *Ulex* cover

was highest (control treatments), the total N content was the lowest recorded, although this may simply be an artifact of sampling in areas of bare soil away from *Ulex* root systems where N-levels are likely to be highest (Magesan et al., 2012).

Enhanced colonisation of mesotrophic grasses, specifically *Agrostis curtisii* and *Agrostis stolonifera,* occurred in the higher soil pH of the organic enrichment treatments. These highly competitive grasses outcompete *E. cinera* and *C. vulgaris* when the pH is above 5 (Green et al., 2015; Lane et al., 2020). Based on pH values alone, this meant that in four of the nine treatments, *Agrostis* species of grasses had a competitive advantage over the ericoids. When combined with the elevated levels of other heathland macronutrients, including Mg, Ca and Na, mesotrophic grasslands resulted with few surviving ericoids. Despite the ericoid ability to inhibit grass root growth, once grass swards dominate, it is difficult for ericoids to establish, especially as *Ulex* inputs N into the system (Alonso & Hartley, 1998).

Whilst the macronutrients (N, P, K) present were within ranges found for ALH soils (Chapter 3,**Table 6**), other important heathland micronutrients (Mg, Ca and Na) were substantially lower than the concentrations seen in the ameliorated soils. Soil organic matter was consistently much lower but similar to undisturbed heathland (Lane et al., 2020) even for the ameliorated soils (OM) (3.5%-18% (Clarke, 1997)).

In the organic matter ameliorated soils the combination of higher pH values, higher concentration of available cations, higher organic matter content, and therefore water retention, provided almost perfect conditions for *Agrostis* and other mesotrophic grasses to outcompete the heathland species (Critchley et al., 2002). Even the low C:N ratio, which indicates a high bacterial to fungal ratio, meant fewer ErM were available to associate with the ericoid hair roots, making colonisation more difficult (Strandberg et al., 2018). Combined with the low macro and micro nutrients, the C:N ratio of all plots, except the control, was below the values reported in a healthy heathland soil (30) (Strandberg et al., 2018). This, combined with either too much water retention within the organic matter plots or too little in the other plots, could have resulted in low ericoid germination and seedling success (Bannister, 1964). All of the soil interventions trialled within this study did not create ALH suitable soil. With the findings of Chapter 3 regarding the use of a high C, low N organic supplement, this could potentially promote a more suitable habitat within the soil for ErM and therefore increase ericoid colonisation.

Lane et al. (2020) previously suggested that long-term failure of natural ALH establishment in post-mining sites highlighted the need for environmental amelioration to facilitate successful restoration. However, in this extensive, and perhaps unprecedented in scale, field trial, minimal intervention (i.e. – the addition of seeds) corresponded with the

most successful (even if limited) dwarf ericoid establishment. All of the treatments trialed resulted in unfavorable conditions in one parameter or another (**Table 7**, **Table 8**) and were associated at best, with the development of either mesotrophic or acidic grassland rather than ALH. Nonetheless, ALH may develop over time using the grasslands as a nursey area and the *Ulex* as a nursey plant. Progressive changes in soil chemistry post restoration occur (Roem & Berendse, 2000), and although results presented here only give a snapshot, further work exploring how ameliorated soils change would be interesting. This could be combined with community studies evidencing restored sites functioning similarly to natural sites; demonstrating alterations of soil and microbe communities evolving with plant succession. The succession of plants toward ALH was discussed by Roberts et al. (1981) who noted that on kaolinite sand tips not covered with top soil there was a lag recorded between the end of mining and the natural colonisation of 25-55 years by *C. vulgaris* and *Ulex europaeus*. Roberts et al. (1981) work is on the pure kaolinite waste sand. However, Lane et al. (2020) noted colonisation of ericoids on top soiled tips within 2 years, so the addition of stored top soil has shortened the colonisation process and the first ericoids established on this study within six months on the ameliorated soils.

The trialled soil interventions failed to create a soil environment suitable for sustainable ALH. This was demonstrated by the control experiment

findings, which resulted in the greatest density of dwarf ericoid plants when compared with the other treatments that received additions. These actions aimed to mitigate the shortfalls theorised to prevent ALH restoration. Nonetheless, the goal of a successful ALH environment is to stimulate dwarf ericoid establishment to enable heathland community restoration. Therefore, a healthy heathland soil must be targeted for the initial step to promote habitat restoration by aiming for high monocultures of ericoids with low numbers of additional ALH species. Additions of organic matter, ErM and cations had no significant benefit; only plots without organic matter created an ALH-type habitat. It is likewise noteworthy that dwarf ericoids are notoriously difficult to germinate; the small seedlings are vulnerable and survival on slopes is difficult especially with environmental and weather conditions that batter exposed seedlings, which without shielding from larger species, can be washed away by precipitation. In conclusion, observations highlighted that ericoids profited from the presence of *Ulex*, which acts as a buffer, nurturing the vulnerable ericoid seedlings and benefiting their growth through bio-accessible N-fixing abilities. Of the tested restoration strategies the most effective to achieve the establishment of dwarf ericoids for ALH restoration on this commercial china clay mineral extraction setting was the minimal intervention in the form of spreading stored topsoil with seeds. Potentially further work with high C low N additions (e.g. lignite or wood chip) could enhance the success of ALH restoration.

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# General Discussion

This study investigated the large-scale restoration of Atlantic Lowland Heath (ALH) after china clay mineral extraction with a focus on whether adding commercial ErM to established restoration techniques (additions of soil minerals, metals, and organic matter) could provide a cost effective and reliable restoration technique post mineral extraction. Additionally, the study also assessed soil chemistry during the long-term storage of overburden. The rationale for this direction was to ascertain whether the process of storage would significantly alter the soil and change its heathland characteristics, rendering it unsuitable for large-scale restoration. Notwithstanding the scientific aims, an important applied research goal was to assess cost effective solutions for creating biodiverse heathland within a 5-year aftercare period. This is the time usually given to mineral extraction companies by UK planning authorities.

Specifically, this large-scale research was divided into four work packages: (1) Investigation of vegetation community composition with different restoration methods and restoration periods to identify lessons to be learned from historical restoration projects. (2) A pot-based greenhouse trial to gain an understanding of the effectiveness of commercially available ErM for improving *Calluna* growth. ErM was

blended with stored topsoil and also combined with organic matter in stored topsoil. (3) An investigation of topsoil chemical changes during storage (both time and depth conditions). (4) A large-scale field trial to assess the effectiveness of the restoration techniques using results from package 1, 2, and 3. It must be noted that due to delays in the first three work packages, work package 4 was started before full analysis was completed. Current best practice for commercial extraction industry was used in combination with academic literature and initial results from the earlier chapters.

At the time of this study, research combining the multiple treatments outlined above in a large-scale field study had been missing from literature. The findings from this study therefore represent a novel approach to commercial restoration research using grand scale experiments, which can be applied to china clay mineral extraction mitigation projects to restore ALH habitats. However, there remains a paucity of research on how commercial ErM impacts *C. vulgaris* growth during active and passive restoration of ALH. Despite this, the mineral extraction industry has a legal obligation to restore used land back to a specific state as required by planning conditions. Achieving Atlantic lowland heath habitat is a challenging and expensive requirement, thus this study aimed to achieve a scientific evidence-based answer to whether more effective and shorter ways were available to achieving

ALH restoration by using higher levels of soil amelioration on kaolinite sands.

The assessment of vegetation community composition after historical restoration efforts showed that time alone was insufficient to facilitate recovery and none of the active interventions studied sped the development of ALH. Results in Chapter 2 showed that the plants grew better when organic matter had been added, whereas results in the field trials reported in Chapter 4 indicated that plots treated with organic matter became dominated by fast growing acidic grasses. This has also been observed previously (Aerts & Heil, 1993; Bakker & Berendse, 1999). The field trial and pot trials were not comparable due to the extreme weather conditions experienced during the field trial period. This resulted in long periods of dry soil interspersed with high rainfall periods, whereas the pot trial was conducted under controlled conditions including maintenance of soil moisture. The limiting factors to ALH restoration suggested by the initial survey of historical restoration in this study and reviews of existing literature included soil fertility, soil pH and changes to the soil microbial component (Gough & Marrs, 1990; Marrs, 1993; Alday et al., 2012; Marrs, 2016). This last aspect was addressed by conducting work package 2 through ErM growth trials in the greenhouse. The higher ErM infection rates in pots trials however did not equate to greater survival rates in either the pot-based study or the field trial, which was surprising. The most

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encouraging literature results for soil inoculation (Marrs, 2016; Wubs et al., 2016; Wubs et al., 2018) occurred where the whole soil was used from existing habitats. This would therefore be the best way to inoculate the soil, as potentially the whole community of soil microbiota is transferred. It also indicated that the storage of soil removed from ALH alters the microbiota in such a way that it does not provide effective inoculation for ericoids. Future work could address the inoculation of soil with commercial ErM in comparison with locally adapted fungal strains from locally stored topsoil (overburden) compared to control soils. Prior to such experimental work, it would be interesting to obtain more data regarding the fungal community associated with root hairs of local populations of *Calluna vulgaris* and *Erica* spp. The staining of root hair cells here specifically confirms the presence/absence of fungi associated with hair roots and not fungal identity. The cloning work undertaken was unfortunately largely unsuccessful, although the presence of unknown Heliotales fungi was confirmed. Larger scale sequencing projects using DNA barcoding of fungi isolated from hair roots and known samples would facilitate the characterisation of both the identity of fungal species and provide information regarding intraspecific variation amongst ErM fungi (Hazard et al., 2014). Due to the low numbers of dwarf ericoids found within the treatment plots where commercial ErM was added, the infection rate was unable to be assessed. Consequently, the increase of root infection found in Chapter 2 was not confirmed in the field study. As an initial trial of commercial

ErM, it is interesting that there was no apparent effect on growth of ericoids. The commercial inoculant is a sterile mix of mycelium that has been grown from collected strains rather than grown from spores ('Rhodovit', Symbiom) (Albrechtova et al., 2012; Vosatka et al., 2012). These interesting results require further trials. There are several lines of further work with the commercial ErM, for example, varying the amount of ErM and plug planting with commercial increased ErM root infection. Other areas could include looking into local adaption phenotype and morphotype combined with some form of soil inoculation as discussed in (Wubs et al., 2018). Having said that, it is unlikely that local strains of ErM would be straightforward to culture (Smith & Read, 2010).

As well as the microbial properties of stored overburden, the physical and chemical characteristics of soil berms are also fundamental to restoration. In Chapter 3, these properties were examined to assess changes in key soil parameters during storage. The results indicate that whilst there is movement within the berm and processing action on the soil to start podzolisation, there is no major loss of ions, and whilst the soil does not meet ideal ALH soil conditions in some key characteristics (C, C:N and CEC), the physical and chemical components of the soil are within ranges shown from other studies and therefore the soil ought to have the ability to function as a heathland soil. One of the conclusions drawn would be to trial the use of lignite as a low N carbon source. This would increase the CEC, and C:N without affecting the N availability

and thus may suppress the colonisation by grasses while favouring the establishment of an ALH community. The soil was taken from the berm to build the large experiment bank (Chapter 4) and therefore it was expected that ALH would develop. Unfortunately, the bank was built before the results indicated the need to add a source of carbon without increasing the nitrogen concentration in the soil, so green waste compost was identified and used in the large scale field trial.

The experimental field trial examined all these aspects in an industrial setting. The most important finding within this part of the study was that with the addition of appropriate seeds, the use of local soil, even after storage, provided the highest number per metre$^2$ of ericoids, within 36 months. Where soil amelioration was trialled the soil did not match ALH soil in one or another parameter and accordingly, the plant communities reflected those differences. Although in seeded controls there was competition from grasses such as *Agrostis curtisii,* the ericoids established and contributed significantly to the percentage cover.

Overall, there is some contradiction in the results: Chapter 1 showed the need for soil amendment as time only was not sufficient to restore heathland, Chapter 3 suggested that the viability of the stored topsoil for ALH establishment could be enhanced with respect to CEC and C:N ratio by adding organic matter with high carbon and low nitrogen content. The field trials of Chapter 4, however, showed some of the best

outcomes in unamended or seeded control soils. Nevertheless, the need for amendment of the soil to improve fertility identified in Chapter 1 and 3 is consistent with earlier research. In the 1980s, research established that on the kaolinite sand waste remaining from kaolin extraction, there was a low and slow building of an ecosystem, requiring the initial build-up of a working nitrogen cycle throughout, above, and below ground interactions (Roberts et al., 1982). However, as mentioned, although the survey of historical restoration here showed that some intervention is required (Chapter 1), large-scale field trials demonstrated that the use of stored topsoil along with seeds has the highest colonisation of dwarf ericoids. Also, the only plot that developed *E. cinerea* was the untreated control site. This supports the argument that minimal intervention of the soil with seed addition creates maximum colonization potential for diverse species, as demonstrated in eastern Europe (Prach & Hobbs, 2008; Romana et al., 2010; Šebelíková et al., 2016).

Technical and active restoration also includes the earth moving and landscaping activities prior to any addition of biologically active components, such as top soil, plants and the translocation of fauna. This results in an active set of actions in mining restoration to return land heights to set points agreed within the planning permission (Gov.uk, 2019). Consequently, mining restoration cannot be truly called passive, as there is the initial active restoration described above. However, passive and active can also refer to the extent of biological or

ecological intervention. In this context, this study highlights potential advantages of both passive and active restoration processes. On the one hand, local unamended soils demonstrated that best outcome for ericoids, the addition of plant nutrients (Ca, Mg, Na, K) results in the maximum diversity of species. This indicates that the addition of these elements is necessary because of the low CEC. From a commercial and stake holder point of view the addition of organic matter resulted in a grass sward within six months, reducing visual impact and increasing slope stability. Perhaps a mixture of local stored soils, local fungal strains combined with stakeholder understanding that ALH takes time will improve future restoration outcomes.

This thesis sits alongside a wider body of research investigating ALH restoration. Putwain & Rae (1988) focused on techniques for restoring ALH and found that there could be a large seed bank within soils and that a low-density sward of slow growing grasses often enhance seedling establishment. Since then a large body of work has developed with the aim of establishing the most effective restoration techniques (Diaz et al., 2006; Diaz et al., 2008; Walker et al., 2014; van der Bij et al., 2018; Wubs et al., 2018; Radujkovic et al., 2020). The results presented in this thesis supports the literature in that soil conditions have the largest effect on the plant communities found and that the inoculation of soil microbes may be the most effective way when combined with local seeds to achieve a diverse heathland. The advantage of using local

seeds is there may be genetic adaptations that enable local flora to succeed in the low nutrient soil and local climatic conditions (Oliveira et al., 2013; Oliveira et al., 2014). Again, local soil inoculation will allow locally adapted strains of fungi to colonise and assist the flora in soils to which they are well adapted. Additionally, ongoing work in the Netherlands looking at large scale restorations and removal of soil P, showed that the removal of sods removed too many nutrients and as such, traditional methods (deep ploughing, cropping) need to be employed (Vogels et al., 2020). The best guidance for industry to arise from Vogels et al., (2020) work is to use local topsoil and a local seed bank, again concurring with the findings of this study, along with the guidance to remove grazing for a minimum of 5 years to give ALH the best chance to regenerate (Putwain & Rae, 1988).

Due to planning requirements, a specific habitat is decided upon when producing the restoration plan after mineral extraction. This is created with guidance from local government (Devon MAP, 2017). Whether there is a restoration to an environmental process (e.g. river flows, assembling missing guilds of animals in dynamic habitats) or a classified habitat (e.g. ALH) depends on the discussion with the local government. In certain circumstances and certain areas, a specific ecosystem functionality could be developed with caveats about success (Lei et al., 2016). Maybe in the future rewilding could be included into local government planning portfolio allowing natural regeneration (Fyfe &

Woodbridge, 2012). Allowing 'nature to decide' what is appropriate rather than restoring landscapes to a particular historical baseline is a part of modern rewilding philosophy (Corlett, 2016). As heathland is a mid-successional stage habitat, some re-wilding priorities will cause it to be lost as a habitat. Proponents of rewilding argue that by allowing natural processes to occur biodiverse habitats will arise, regardless of whether this restores a historical baseline or not. If rewilding is to become a routine part of post-mineral extraction biodiversity plans, then adjustment to planning requirements will need to take place to allow for the dynamic nature of this natural regeneration.

An additional problem with the rewilding agenda with regard to restoration in the UK is space. Rewilding was developed in the US as a way to return large swathes of the country to a pre-Colombian point (Bauer et al., 2009). Rewilding in Europe is made difficult since large areas have been altered and managed for millennia (Corlett, 2016). The idea of this type of restoration is romantic and an easy concept for the public. However, rewilding is complex and the majority of restoration is not at this scale and in locations where free roaming large herbivores would be inappropriate or dangerous (Bauer et al., 2009; Caro & Sherman, 2009; Ockendon et al., 2018; du Toit & Pettorelli, 2019; Perino et al., 2019) (without which human intervention would be needed to mimic natural processes affecting plant communities). The EU habitats and Aichi targets (which is what the majority of restoration

guidelines are built on) are built for specific species or constructed

priority habitats. This supports endangered populations allowing

specific achievable goals that stakeholders can buy into with end points

that can be judged as successful or not. Rewilding and specific habitat

creation are not juxtapostioned and can support each other. Perhaps

the ideal scenario for mining companies is to complete the restoration

and then leave nature 'to it'. Nevertheless, the work carried out here

suggests that active and passive restoration must be combined in order

to achieve the closest approximation of ALH within 5 years.


As well as addressing debate about the merits of rewilding versus

achieving particular conservation outcomes, modern restoration ecology

needs to be 'future-proofed' to encompass the effects of climate change,

as the cost implications are extensive and expensive if incorrect

restoration has to be readdressed and corrected. The Aichi Biodiversity

targets (2010) consequently provide a framework to improve the

scientific argument, policy development, and the business case for

science-based restoration. However, the possibility of assisted

colonization (translocating species outside their current distribution) in

certain conditions may need to be discussed (Ricciardi & Simberloff,

2009). This controversial approach to restoration not only requires more

research, it also requires conversations with government and other

stakeholders, as the natural movement of species or invasion of more

southerly species is being noticed in many habitats and could also

occur in lowland heath. ALH exists from southern Spain and Portugal although the ericoids species there are more likely *Erica lusitanica, Erica arborea, Erica andevalensis* (known to tolerate high levels of toxic chemicals). These Mediterranean species are likely to more adapted to the predicted climate change. Increased awareness will enable trials to take place to study various impacts on native flora and faunal species, leading to necessary mitigation action (Rose et al., 2000). The distribution of ALH will change as the climate changes, but disagreement regarding future climate models and their inherent uncertainty mean that it is hard to predict changes in biological communities. Potentially, the south-west UK will have wetter and windier winters and drier summers (Guillod et al., 2018; Murphy et al., 2019), so looking at the use of more southerly species and using assisted colonisation of ericoids could maintain the habitat. However, the effect of this process on the fauna is untested and literature indicates this is not ideal due to unknown impacts on composition development and functioning of existing ecosystems (Ricciardi & Simberloff, 2009).

In general, mineral extraction companies have the responsibility and the experience to carry out large-scale earth movements on their land holdings that require reinstatement. This occurs after the temporary use of the land for mineral extraction, but companies lack access to the most recent science, so an important necessity for restoration

practitioners is ensuring the science is applicable, and correctly applied (Bate et al., 1998; Armsworth et al., 2010; Salgueiro et al., 2020). The majority of large-scale restoration projects within the UK are carried out in partnerships with NGOs, such as the Wildlife Trusts and the RSPB with multiple aims; for example, flood alleviation, access to nature for the public, ecosystem services, and biodiversity (White & Gilbert, 2003; Davies, 2006; Jones et al., 2018). Consequently, an important aim of this research was to generate realistic restoration applications that can be applied at the industrial scale.

Recommendations for business and commercial enterprise include managing stakeholders' expectations, especially with regard to time. ALH takes time to develop and requires management to maintain the initial phases, even with additional seeds. This is an important phase that enables the ericoids and pioneer species to colonize. The slow growth of dwarf ericoids and the low nutrient content of the soil prevents colonization of large quantities of grasses. An area of further study would be to extend the research to investigate the role of soil microbes and the timeline for their colonization in restored lowland heath (Marrs, 2016). The work carried out here suggests that active and passive restoration must be combined in order to achieve the closest approximation of ALH within 5 years.

The study emphasises the complexity of restoring heathland, especially highlighting that interventions, whether passive or active, are unlikely

to result in heathland habitats in a short period to time; indeed, time and patience are underlined as crucial factors in the post-extraction establishment of ALH. However, avenues of extended study appear promising, especially investigating plant-fungal interactions alongside biochemical relationships involving plant physiology, microbial ecology and complex specific heathland soil chemistry exchanges. Importantly, the situation involving the role of organic matter for ALH restoration needs to be made clearer in order to establish ALH rather than encourage the establishment of mesotrophic grasslands.

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