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Peatlands on the mend: Using plant-microbe interactions to restore peatland structure and function

by

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Thesis for the degree of Doctor of Philosophy

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Abstract

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Peatlands are important natural carbon stores. Peatlands are however experiencing widespread drainage, which increases vulnerability to wildfire and compromises their carbon sink function. Rewetting is a common technique used to restore disturbed peatlands. However, recent evidence suggests that even after decades of recovery, rewetted peatlands lag – taxonomically and functionally – behind their undisturbed counterparts, which could compromise peatland resilience to future climate change. Restoring the belowground microbial community is one method that could be used alongside rewetting to enhance the restoration of peatlands. Through utilising plant-microbe interactions, belowground microbial restoration action could be able to control secondary succession and expedite the recovery of key ecosystem functions, thus returning peatlands to their pre-disturbance state. However, at this moment there are few tests of the restoration of belowground microbial communities in enhancing peatland recovery.

In this thesis, I explore the use of plant-microbe interactions in restoring peatland structure and function. I largely focus on one key threat to peatlands – wildfire – to examine the factors controlling initial post-fire recovery and whether peat moss inoculation can be used to drive forward the recovery of key ecosystem functions and taxonomic compositions. I then use an established plant-removal experiment to test the resilience of peatland plant-microbe networks following long-term disruption. In doing so, I first identify multiple factors that limit peatland post-wildfire recovery. I then highlight the potential for plant-microbe interactions to enhance peatland restoration by expediting taxonomic and functional recovery. Finally, I demonstrate the peatland plant-microbe networks can reform following long-term disruption, but that they are largely restructured with new plant-microbe associations forming. Together, this thesis provides the first test for microbial restoration to enhance peatland post-fire recovery and helps develop a broader

understanding of the ecology surrounding peatland plant-microbe interactions that can be used to inform future research and guide management actions.

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Primary data used in chapter 2 can be found at: https://doi.org/10.5258/SOTON/D1767

Primary data used in chapter 3 can be found at: https://doi.org/10.5258/SOTON/D2276

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I declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

I confirm that:

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Abbreviations

ANOVA	Analysis of variance
AIC	Akaike information criterion
AICc	Akaike information criterion corrected
ASP	Actual species pool
ASV	Amplicon sequence variant
BC	Bray-Curtis
C	Carbon
CH_4	Methane
CN_n	Network Connectiveness
CO_2	Carbon dioxide
CWM	Community weighted mean
DNA	Deoxyribonucleic acid
ESP	Ecological species pool
EMV	Ellenberg moisture value
HSP	Habitat species pool
IE_n	Interaction evenness
ITS2	Internal transcribed spacer 2
LDMC	Leaf dry matter content
LMM	Linear mixed model
N	Nitrogen
NEE	Net ecosystem exchange
NMDS	Non-metric multidimensional scaling
PC1	Principal component axis 1
PCA	Principal component analysis
PERMANOVA	Permutational multivariate analysis of variance
PFG	Plant functional group

Abbreviations

PVC	Polyvinyl chloride
rRNA	ribosomal Ribonucleic acid
SD	Standard deviation
SE	Standard error
SES	Standardised effect size
SLA	Specific leaf area
UK	United Kingdom
USP	Upland species pool

Chapter 1 General introduction

In this section I seek to introduce the topic of this thesis by reviewing the relevant literature and placing this work in the context of current scientific research. I begin by highlighting the importance of peatlands with a focus on a selection of key ecosystem functions. I then introduce the topic of plant-microbe interactions and their role in peatlands. Following this, I describe the key factors currently threatening peatlands and the implications this can have on peatland function. Next, I discuss current peatland restoration techniques and the potential for plant-microbe interactions to expedite the recovery of disturbed peatlands. To end, I describe the research gap I am aiming to fill within this thesis, along with the research objectives and the contents of each of the subsequent chapters.

1.1 The importance of peatlands

Peatlands are wetland ecosystems that cover approximately 3% of the Earth's terrestrial surface (Xu et al., 2018). Peatlands occur where organic matter formed from living material has undergone incomplete decomposition, largely due to anoxic conditions driven by high water tables causing the build-up of carbon rich soils (i.e. peat) (Freeman et al., 2001; Rydin & Jeglum, 2006). Peatlands are often defined by high level of organic matter content in their soils (> 30% of total dry weight) and peat soil depth (> 40 cm) (Page & Baird, 2016). It is this high concentration of carbon that results in a key function of peatlands; the extraction of carbon from the atmosphere and subsequent long-term storage in their 'soil'. With estimates of approximately 500 Gt of carbon stored in Northern peatlands (Yu, 2012) and further 100 - 200 Gt stored in tropical peatlands (Page et al., 2022; Ribeiro et al., 2021), peatlands punch well above their weight in terms of their carbon sink function. To put this in perspective, these numbers are comparable to the total amount of carbon stored within the atmosphere (750 Gt; Grace, (2004)). Given therefore the current observed increase in global atmospheric carbon, peatlands represent a vital natural carbon store. Maintaining the carbon sink function is therefore a strong tool in the reduction or offsetting of anthropogenic-led emissions (Humpenöder et al., 2020; Leifeld & Menichetti, 2018; Tanneberger et al., 2021)

Peatlands play host to a unique array of species. Most notably a rich diversity of *Sphagnum* moss, a keystone peatland genus and ecosystem engineer essential for the maintenance of anoxic conditions and consequent peat accumulation (Rydin & Jeglum, 2006; van Breemen, 1995a). *Sphagnum* species (hereon *Sphagnum*) are highly specialised, with tissues that possess a large water-holding capacity allowing them to raise the water table and thus limit soil oxygen availability

(Hayward & Clymo, 1982; Rydin & Jeglum, 2006). In addition, they release exudates that lowers the pH of the soil and produce recalcitrant litter that together limits decomposition and produces highly stressful abiotic conditions (Clymo, 1984; Dorrepaal et al., 2005; van Breemen, 1995a). In doing so *Sphagnum* are able to engineer conditions that promotes their own persistence and suppresses the abundance of species with a lower stress-tolerance (Laine et al., 2021; van Breemen, 1995a). As a consequence, peatlands are dominated by a group of highly specialised species that are largely absent in other ecosystems, and they thus possess a unique biodiversity.

Alongside carbon storage and biodiversity, peatlands provide a range of additional functions and services (Bonn et al., 2016; Page & Baird, 2016). For example, peatlands can regulate water flow and quality. This can reduce flooding risks (Ikkala et al., 2021; Shuttleworth et al., 2019) and preventing nutrient leaching downstream that can harm freshwater ecosystems (Daniels et al., 2008; Martin-Ortega et al., 2014). Peatlands can also be used for agriculture (Rydin & Jeglum, 2006). This can have detrimental effects on peatland carbon storage (Leifeld et al., 2019). However, there is increasing interest in paludiculture, a sustainable way of harvesting peat-grown commodities such as *Sphagnum* whilst still maintaining the peat-forming function (Joosten, Gaudig, et al., 2016; Temmink et al., 2017). Additionally, peatlands act as historical artifacts: preserving pollen and testate amoebae in their soils as peat accumulates (Page & Baird, 2016). Through palaeoecological analysis of peat cores, peatlands can therefore be used to understand past climates (Wieder et al., 2009) and make predictions about future scenarios under anthropogenic climate change (Novenko et al., 2018), thus contributing to human knowledge. Together, the array of functions and services provided by peatlands highlights their importance that, despite the relatively low area covered, makes them relevant at a global scale.

1.2 Plant-microbe interactions and peatlands

Plant-microbe interactions is a term used to describe the relationship between aboveground plant communities and belowground organisms. Broadly speaking, the presence or absence of either aboveground (i.e. plant) or belowground microbial (i.e. fungi) taxa can positively or negatively affect the performance of the other, forming connections or interactions between the two communities (Bever, 1994; Klironomos, 2002; Wardle et al., 2004). These interactions can control community succession (Bauer et al., 2015b; Kardol et al., 2006), permit species co-existence (Crawford et al., 2019; in ‘t Zandt et al., 2022) and drive patterns of species diversity (Fukami & Nakajima, 2013). Importantly, plant-microbe interactions play a vital role in controlling the functioning of ecosystems (Bardgett & van der Putten, 2014; Crowther et al., 2019). As a recent example, Liu et al., (2022) demonstrated that greater fungal richness increased the stability of plant biomass over time, a key function of terrestrial ecosystems. As such, plant-microbe interactions are key drivers of terrestrial compositions and functioning.

Peatland structure and function, like other terrestrial ecosystems, is driven by an array of key plant-microbe interactions. As an example, *Sphagnum* litter and released metabolites contain structural polysaccharides and phenolic compounds that promote the presence or absence of specific micro-organisms (Bengtsson et al., 2018; Fudyma et al., 2019; Gilbert et al., 1998; Verhoeven & Liefveld, 1997). This includes sphagnan, a peptin-like carbohydrate polymer present in *Sphagnum* tissue that can induce reductions in local pH and consequently inhibit the growth of acid-sensitive bacteria species (Painter, 1991; Stalheim et al., 2009). This in turn helps *Sphagnum* maintain reduced decomposition rates (Bengtsson et al., 2018), contributing to the stability of carbon dynamics in peatlands (Turetsky et al., 2008). This also acts as a self-reinforcing mechanism that helps promote the sustained abundance of peat moss (Bragina et al., 2014; van Breemen, 1995a). Consequently, interactions between *Sphagnum* and belowground microbes ensure the maintenance of peatland structure and function.

Interactions between vascular plant composition and microbial communities can also control changes to peatland function. For example, the presence of different plant functional types in an ombrotrophic bog can drive changes in methane dynamics through alterations in fungal and bacterial compositions (Robroek et al., 2015b). Similarly, in a forested peatland, the release of phenolic compounds and recalcitrant carbon inputs by the shrub community produces conditions that increases dominance of slow-growing fungi in the soil microbial community, thus reducing decomposition (Wang et al., 2021). In addition, warming can stimulate shifts in the belowground fungi community of ombrotrophic peatlands, with a consequent increase in ericaceous cover (Bragazza et al., 2013). This can result in the release of labile carbon by the ericaceous species priming the system and stimulating the release of carbon degrading enzymes by the belowground microbial community (Bragazza et al., 2013). Together, these studies highlight the ability for the vascular plant and microbial community to interact with knock-on effects for peatland carbon dynamics.

Most studies on peatland plant-microbe interactions have focused on how a change in one aboveground or belowground microbial component impacts peatland function. For example, how different plant functional compositions shift belowground microbial communities and the consequences on carbon cycling (Robroek et al., 2015b). There is however increasing interest in how the strength and complexity of plant-microbe interactions can determine ecosystem function and resilience, and whether plant communities and belowground microbial communities show concurrent responses to environmental change (Jassey et al., 2018; Robroek et al., 2021). In grasslands for example, plants and microbes have been shown to exist in network of complex associations (Ramirez et al., 2018; Wardle et al., 2004), the strength of which can underpin the functioning and stability of the system (Morriën et al., 2017; Rudgers et al., 2020). Crucially, if these networks become disrupted, the reformation of species interactions can be slow, potentially limiting the rate at which key ecosystem functions such as carbon storage recover (Morriën et al., 2017). In peatlands, however, less is known about the complexity of plant-microbe networks and the consequent implications on ecosystem functions (Robroek et al., 2021). As such, there remains outstanding questions regarding the importance of plant-microbe networks in driving ecosystem function and the resilience of these associations across temporal and spatial gradients.

1.3 Threats to peatlands

Peatlands are increasingly threatened by a range of global change drivers. The primary threat to peatlands is drainage, which is often human induced for agricultural purposes, or peat extraction for fuel or horticulture (Lamers et al., 2015; Rydin & Jeglum, 2006). Temperate peatlands have for example experienced widespread and substantial drops in water table depth over recent decades, with one quarter of all European peatlands currently at their driest point in the last 2000 years (Swindles et al., 2019). Drainage can result in the loss of *Sphagnum* moss and characteristic vascular plant compositions (Baird et al., 2019; Talbot et al., 2010), enabling the colonisation of tree species (Talbot et al., 2010) and inducing an increase in dominance of shrubs (Weltzin et al., 2003). Importantly, a lowering of water tables can disrupt key hydrological feedbacks essential for peatland maintenance (Waddington et al., 2015). This can allow oxygenation in the surface peat, removing the conditions that prevent decomposition (Freeman et al., 2001). Together this can reduce carbon uptake and potentially switch peatlands from carbon-sink to source (Evans et al., 2021; Leifeld et al., 2019).

One major risk associated with peatland drainage is an increase in the frequency and severity of wildfires. Wildfires in undrained peatlands do naturally occur, albeit rarely. However, these are often superficial removing surface vegetation but avoiding all but the upper peat layer (Granath et al., 2016; Shetler et al., 2008). Drainage can increase peat flammability (Frandsen, 1997; Turetsky et al., 2011). This can exacerbate the risk of severe wildfire occurrence, thus compromising peatland carbon stores(Turetsky et al., 2002; Turetsky et al., 2015). This is due to drainage enabling wildfires to burn deeper into the peat and smoulder at temperatures up to 500°C, resulting in the immediate loss of previously locked up carbon (Granath et al., 2016; Rein, 2013; Rein & Huang, 2021; Turetsky et al., 2015). Along with this, wildfires can result in the complete removal of aboveground vegetation (Maltby et al., 1990) and the simultaneous loss or change in the soil microbial community (Andersen et al., 2013) Consequently, severe wildfires can lead to the loss of characteristic peatland vegetation, resulting in a resetting of peatland succession.

Severe wildfire can lead to drastic changes to the functioning of peatlands. Wildfire can reduce the hydraulic conductivity of peat soils (Holden et al., 2014) whilst increasing peat surface temperatures that raises evapotranspiration rates (Kettridge et al., 2012), potentially reducing the resilience of hydrological conditions to recover post-fire. Additionally, wildfire can lead to elevated nitrogen and phosphorus content in both the peat soil and the leachate (van Beest et al., 2019). These nutrient increases can last for multiple years post-fire (Kelly et al., 2018) with the potential to control the initial post-fire recovery of recolonising vegetation. It can also lead to the exposure of historic pollutants such as Pb that can leach into neighbouring ecosystems, there-by impacting the functioning of the overall ecological landscape (Rothwell et al., 2007). The high nutrient, oxygenated state of post-wildfire peat soils can consequently result in a shift towards fast-growing prokaryotes such as those from the *Proteobacteria* order, that are uncharacteristic of peatland microbial communities and could therefore lead to a change in microbial functioning and peatland nutrient cycling (Belova et al., 2014). Overall, by impacting on both the nutrient content, hydraulics and microbial community of peatlands, wildfire can lead to increases in CO_2 and CH_4 emissions, shifting peatlands to a carbon source (Grau-Andrés et al., 2019, but see also Gray et al., 2021). Perhaps most crucially, whilst peatland functions may recover over decadal timeframes, a combination of wildfire and drying events can allow the colonisation of non-peatland species post-fire that increase the flammability of the system (Kettridge et al., 2015). This can push the system into a self-reinforcing loop where wildfire occurrence increases resulting in the consequent long-term degradation of peatland carbon stocks and a loss of peatland function (Kettridge et al., 2015; Nelson et al., 2021). When such changes occur, they could prove difficult to reverse even with restoration action (Suding et al., 2004), compromising the long-term peatland functioning.

Alongside drainage and wildfires, there a range of other global change factors that also impact peatland composition and function. This includes the pollution of heavy metals (Rothwell et al., 2007) and the deposition and enrichment of nitrogen (Aerts & de Caluwe, 1999; Bragazza et al., 2013). Both rising temperatures and increasing drought periods, fingerprints of current anthropogenic climate change, can also cause changes in the structure and function of peatlands. For example, warming has been shown to decrease *Sphagnum* cover (Dieleman et al., 2015; Norby et al., 2019). This can result in the drying of peat soils allowing vegetation greater access to previously inaccessible carbon pools through an increase shrub fine-root growth (Malhotra et al., 2020). Consequently, higher temperatures and lower precipitation are predicted to reduce carbon storage in peatlands (Bragazza et al., 2016; Gallego-Sala et al., 2018). This could result in a destabilisation of current carbon stocks as greater variability in moisture conditions alters peatland processes (Barel et al., 2021; Fenner & Freeman, 2011).

1.4 The restoration of peatland structure and function

Ecosystem restoration has become a key topic in the prevention of biodiversity loss and mitigation against anthropogenic climate change. Peatlands have come under heavy focus due to their important ecosystem functions and widespread degradation making their restoration a key component to the United Nations 'Decade of Restoration' taking place between 2021 - 2030. Restoring peatlands can provide a range of benefits including reduced carbon emissions (Günther et al., 2020a; Strack et al., 2022), a lowered risk of wildfire (Baird et al., 2019; Granath et al., 2016), and a cooling benefit to the surrounding landscape (Worrall et al., 2022). In light of ongoing increases in atmospheric carbon and rising global temperatures, a quick and effective restoration of peatland structure and function is crucial (Nugent et al., 2019). This can allow peatlands a full realisation of functional potential and consequently mitigate against the impacts of anthropogenic climate change.

At present, most common peatland restoration techniques focus on two key aspects: returning desirable abiotic conditions and reintroducing keystone species. Thus, peatland restoration largely aims to alter environmental filters and overcome propagule limitation (Belyea & Lancaster, 1999) in order to facilitate the return of desirable vegetation compositions and consequent ecosystem functions. In particular: rewetting, the deliberate raising of previously lowered water tables, is a widely used peatland restoration technique (Chimner et al., 2017; Kreyling et al., 2021; Wilson et al., 2016). This alone can be successful in re-establishing *Sphagnum* (González et al., 2014 but see Granath et al., 2016) and lead to a reduction in peatland carbon emissions (Evans et al., 2021; Günther et al., 2020a; Renou-Wilson et al., 2019; Schaller et al., 2022; Schwieger et al., 2021; Wilson et al., 2016). Other techniques such as top-soil removal or the addition of straw mulch can also be used to manipulate resource availability and produce microclimates that promote the re-establishment of target vegetation (Harpenslager et al., 2015; Quinty & Rochefort, 2003; Rochefort et al., 2003a). Alternatively, techniques such as moss layer transfer and seeding that target propagule limitation can successfully aid the return of peat moss and peatland vegetation (González & Rochefort, 2014; Rochefort et al., 2016a; Tuittila et al., 2004). Given however the importance of hydrological controls on peatland processes (Waddington et al., 2015), the restoration of hydrology is often the starting point for the majority of peatland restorations (Chimner et al., 2017).

Despite many decades of research and advances in peatland restoration (Chimner et al., 2017), the taxonomic and functional recovery of restored peatlands can be slow and highly variable. For example, restoration of characteristic peatland vegetation and microbial communities is often dependent on initial starting conditions and the level of prior degradation (Emsens et al., 2020; Mälson et al., 2008). As a consequence, taxonomic compositions and biogeochemical functioning of restored peatlands lag behind undisturbed counterparts' often decades after management action (Kreyling et al., 2021; Moreno-Mateos et al., 2012). In particular, returning peatlands as a carbon sink can be slow, with restored peatlands in some instances still remaining a carbon source nearly 20 years after the start of restoration action (Schaller et al., 2022). Such slow recovery could have future implications for peatland resilience that are not currently understood (Blier-Langdeau et al., 2021; Hobbs et al., 2009; Kreyling et al., 2021) and limit the ability of restored peatlands to fully maximise their climate-change mitigating benefits. On this note, it has been suggested that focusing solely on individual restoration technique such as rewetting or top-soil removal could be inhibiting our ability to return peatlands to their prior functional properties (Granath et al., 2016; Klimkowska et al., 2019). As such, peatland restoration could benefit from new techniques, or combinations, that can be broadly applied and successfully fast-track recovery. This is essential to facilitate a quick return of the characteristic peatland vegetation and the carbon-sink function.

One aspect of peatland restoration that is often neglected is the re-establishment of biotic interactions. In other terrestrial systems, returning ecosystem complexity and multi-trophic interactions is seen as a key component in maximising restoration success (Holl et al., 2022; Moreno-Mateos et al., 2020) with plant-microbe interactions in particular highlighted as a vital component of effective taxonomic and functional recovery (Coban et al., 2022; Harris, 2009). In peatland restoration however, the recovery of the belowground microbial community is commonly seen as a consequence, not a driver, of peatland restoration (Ritson et al., 2021). Microbial recovery is likely to coincide with the restoration of aboveground biota and abiotic conditions (Emsens et al., 2020; Putkinen et al., 2018). However, there is potential that the slow reformation of plant-microbe interactions could be inhibiting peatland recovery success (Ritson et al., 2021). Restoring plant-microbe interactions could therefore enhance overall restoration success and help accelerate peatlands back towards their pre-disturbed taxonomic and functional compositions.

One technique in particular that could be used in peatland restoration is soil inoculation, the addition of a thin layer of soil or living matter from an intact target ecosystem. Notably, the single addition of intact soil inoculate can effectively steer the direction and recovery of abandoned agricultural fields (Wubs et al., 2016). In this study, the authors showed that the source location of the soil inoculate (either grassland or heathland) could determine the composition of the restored plant community and accelerate community formation (Wubs et al., 2016). Thus, through plant-microbe interactions they could catalyse and control the trajectory of recovery. If similar responses were observed in peatlands, then soil inoculation could be a useful tool in fast-tracking the recovery of damaged or degraded peatlands. However, whilst a number of studies on soil inoculation have looked at the role of microbial recovery in driving terrestrial restoration, very few, if none at all, have tested the technique on peatlands. Instead, many studies focus on grassland and heathland restoration (e.g. Emsens et al., 2022; Middleton & Bever, 2012; Neuenkamp et al., 2019; Radujković et al., 2020). Consequently, there is a need to understand the use of plant-microbe interactions in peatland restoration and the wider ability for plant-microbe networks to reform following sustained periods of disruption.

1.5 Research gaps, aims and objectives

This introductory chapter has discussed the role of plant-microbe interactions in peatlands and the threats to peatlands under current anthropogenic global change. In doing so, it has highlighted potential of plant-microbe interactions in enhancing peatland restoration. However, at current there is little research into this topic. Evidence-based restoration is crucial to maximising the success of management interventions (Cooke et al., 2018; Lamers et al., 2015)and therefore a prior understanding of the use of plant-microbe interactions and the mechanisms that might govern effective peatland restoration is key. As such, it is this gap in our knowledge that I will address in this thesis. As wildfire is a substantial threat to peatlands and their carbon stores (Turetsky et al., 2015) I will largely focus my attention on post-wildfire recovery.

In an attempt to fill this knowledge gap, I will focus on three main objectives:

Objective 1: Determine the drivers of post-fire recovery

Objective 2: Test the ability for microbial inoculation to enhance peatland recovery

Objective 3: Assess the resilience of peatland plant-microbe networks following sustained periods of disruption

Achieving each of these objectives will allow overarching assessment of the role of plant-microbe interactions in peatland restoration.

Determining the drivers of peatland post-wildfire recovery (Objective 1) will elucidate the limiting factors in peatland recovery. Restoration action can target primary limiting factors in order to enhance recovery (Catford, 2016). Importantly, if one factor is strongly limiting recovery (e.g. propagule availability, hydrology) then the impact of plant-microbe restoration may be diminished or absent, unless the primary limiting factor is simultaneously alleviated. Therefore, in order to effectively assess the role of plant-microbe interactions in peatland restoration a cohesive understanding of the factors limiting peatland post-fire recovery is required.

Testing the ability of reintroduced microbial communities to accelerate the recovery of peatland structure and function (Objective 2) will provide insight into the potential use for belowground microbial restoration in peatland recovery. This will be the first known trial of a technique that has proven successful in grassland and heathland restoration (Wubs et al., 2016). In doing so, this will provide a ‘proof-of-concept’ for the use of inoculation in peatlands that, depending on results, will set the basis that future work into restoring peatlands using plant-microbe interactions.

Assessing the resilience of peatland plant-microbe networks (Objective 3) will provide insight into the potential long-term mechanism in which plant-microbe interactions may help to restore peatland restoration. If, for example, plant-microbe networks are resilient and able to reform, then restoration action that targets this driver may only be able accelerate the rate of recovery. If however plant-microbe networks are unable to reform, then this provides an added potential for targeted belowground microbial restoration to steer peatland recovery back towards that of target reference sites. In addition to this, whilst not a direct test, the results of this objective will offer further insight into the factors limiting peatland post-fire recovery (Objective 1).

I will aim to achieve these objectives through a series of field and greenhouse experiments using field sites in the UK, Netherlands, and Sweden. In total this thesis will consist of three research chapters each with their own experimental setup and purpose that will provide together the information needed to address each of the three objectives. A conceptual diagram demonstrating the relationship between the objectives, chapters and the outcomes is provided below.

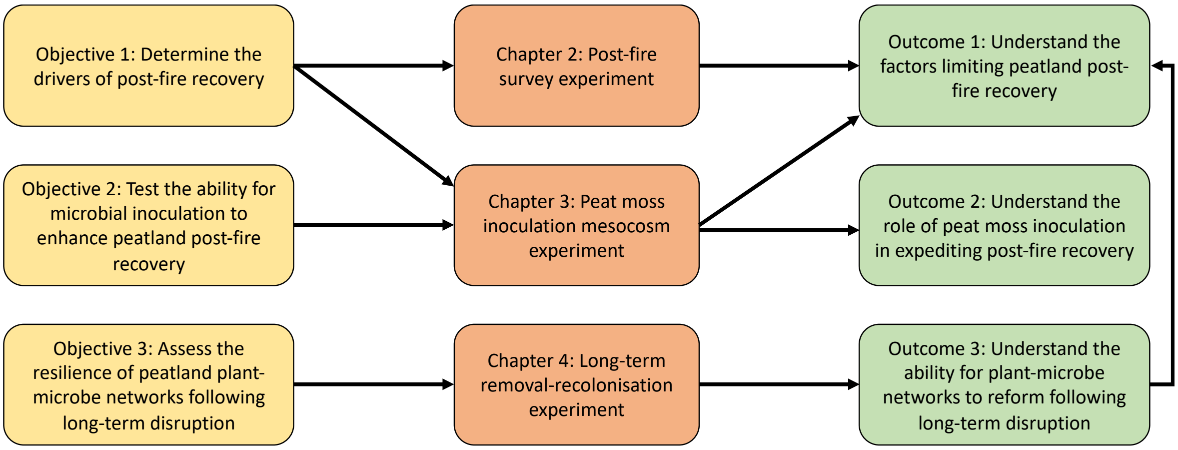

Figure 1.1: A conceptual diagram mapping out the main aims of this thesis, the chapter that will address each objective and the outcome provided.

1.6 Thesis structure and overview

This thesis has been prepared in paper format. Chapter 1 provides a general introduction to the research topic and will remain unpublished. Chapter 2-4 describe experimental results as a direct result of work throughout my PhD. Each of these chapters is prepared in a journal format and is prepared for submission to scientific journals. I represent the first author of each chapter and the relevant contributions of co-authors are provided below. Finally, chapter 5 provides a synthesis of the information provided by chapters 2-4 and is not prepared for publication. All references are provided in the reference sections of each chapter.

In chapter 2, I examine the factors that could drive the initial recovery of peatland plant community following a wildfire. I do this by applying a traditional community assembly framework adapted from Belyea & Lancaster (1999), and others, to vegetation data on the initial wildfire recovery of a UK blanket bog. I then apply a disturbance-colonisation framework that looks at the location of different source pools for recovery (e.g., seed bank, surrounding unburned vegetation) to provide a comprehensive assessment of the factors limiting post-fire recovery.

In chapter 3, I test the ability for microbial inoculation to accelerate both taxonomic and functional recovery of wildfire damaged peat. I do this using mesocosms collected from a wildfire-damaged Dutch bog. Peat moss is used as the inoculate due to the key role *Sphagnum* plays in shaping peatland ecosystems and the unique microbiome it possesses that is essential for peatland function (Lindo & Gonzalez, 2010; van Breemen, 1995a). I trial this across two different water table depths: low and high, representing drained and rewetted peatlands respectively. I then track both the recovery of the aboveground (vascular plant, *Sphagnum*) and belowground microbial (prokaryote) compositions along with several key peatland functions (e.g., net ecosystem exchange, methane emissions) across a four-month period.

In chapter 4, I test the ability for plant-microbe networks to reform following long-term periods of disruption. To do this, I use a field experiment in an ombrotrophic bog in Storre Moss national park, Sweden, where different plant functional groups (ericoids, graminoids, ericoids + graminoids) have been manually removed for nearly a decade. The plant community has then allowed to naturally recover over three years, offering a unique opportunity to look at the plant community and the soil microbial community that reforms and the connections between them.

In chapter 5, I provide an overview of the results from chapters 2-4. I highlight key results and the implication for this results in terms of achieving the objectives of this thesis. Finally, I suggest future avenues of research that can help build on the results of this thesis to advance our understanding of peatland plant-microbe interactions and enhancing peatland restoration.

1.7 Author contributions

Chapter 2 has appeared in publication as:

Shepherd HER, Catford JA, Steele MN, Dumont MG, Mills RTE, Hughes PDM & Robroek BJM (2021). Propagule availability drives post-wildfire recovery of peatland plant communities. *Applied Vegetation Science*, *24*(3). https://doi.org/10.1111/avsc.12608

Contributions: BJMR, PDMH, MGD and RTEM designed and established the experiment; HERS, BJMR and JAC conceived the idea for this work, with contributions from MNS; HERS and MNS collected the data, with contributions from BJMR, MGD and RTEM; HERS analysed the data, with contributions from BJMR and JAC; HERS wrote the first draft of the paper with contributions from BJMR and JAC; all authors contributed to revisions and subsequent drafts.

Chapter 3: is under consideration for publication, following initial submission

Shepherd HER, Martin I, Marin A, Cruijsen PMJM, Temmink RJM & Robroek BJM (2022) Post-fire peatland recovery by peat moss inoculation depends on water table depth.

Contributions: HERS and BJMR conceived the idea for the manuscript. AM, IM, RJMT, PMJMC & BJMR collected the mesocosms. AM, IM, and PMJMC carried out the experiment and data collection, with key guidance by HERS and BJMR. HERS led the analysis and wrote the first draft of the manuscript, with input from AM, RJMT and BJMR. All authors contributed to subsequent revisions.

Chapter 4: Manuscript being prepared for submission

Shepherd HER, Steele MN, Sahar N, Dumont, MG & Robroek BJM (2022) Plant-microbe networks restructure following long-term loss and recovery of plant functional groups.

Contributions: BJMR established and maintained the experiment. HERS and BJMR conceived the idea for the manuscript. HERS, MNS, NS & BJMR collected data for the experiment. HERS analysed the data with input from BJMR and MGD. HERS wrote the draft of the manuscript with contributions from BJMR.

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Chapter 2 Propagule availability drives post-wildfire recovery of peatland plant communities

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Abstract

Question: Northern peatlands are increasingly threatened by wildfire. Severe peatland wildfires can provide opportunities for new non-peatland species to colonise post-fire. Changes in plant colonisation could lead to longer-term shifts in community composition, compromising recovery of peatland structure and function. Understanding the process of post-fire recovery can thus inform restoration action and help restore peatland vascular plant communities. In this study, we ask: what drives initial vascular plant recovery following a peatland wildfire?

Location: Stalybridge moors, England (commonly referred to as the Saddleworth moors).

Methods: We used a series of vegetation surveys and seed germination experiments to identify the composition of vascular plant community one-year post-fire, along with potential propagule sources. We combined this with plant trait data and, using a series of null models, compared observed community trait values against random species assemblages.

Results: Our data suggests that plant species are able to arrive at the burned site through multiple non-exclusive recolonisation pathways. This includes colonisation through the soil seed bank, along with dispersal from surrounding unburned peatland and non-peatland vegetation. The composition and structure of the recolonised communities was largely determined by the ability of species to reach the post-fire site from these donor communities. This resulted in a post-fire community composed of species possessing lower seed masses relative to the wider pool of potential colonisers.

Conclusions: Our results highlight propagule availability as a driver of post-wildfire vascular plant recovery. This provides opportunities for new non-peatland species to colonise, potentially driving changes in the direction of vegetation recovery. Ensuring the availability of peatland species following a wildfire could therefore be key to the immediate recovery of these systems.

2.1 Introduction

Northern peatlands represent significant terrestrial carbon stores, holding an estimated 265 – 600 Pg of carbon (Yu 2012; Hugelius et al. 2020). This is equivalent to 80% of the carbon held within the atmosphere (Page & Baird 2016). However, peatlands are at increasing risk from severe wildfires (Turestsky et al., 2015). This is driven by widespread reductions in water table depths (Turestsky et al., 2011; Swindles et al. 2019) that can result in shifts towards highly flammable shrub-dominated plant communities deprived of their characteristic, water-holding peat moss layer (Talbot et al. 2010; Baird et al. 2019). When severe wildfires occur, they can provide opportunities for non-peatland species that were not present pre-fire to colonise. Kettridge et al. (2015) have, for example, reported the post-fire colonisation of non-peatland species to increase vegetation flammability, inducing positive feedback loops that leads to a further increase in wildfire regularity. If left unrestored, this could eventually shift a peatland from a carbon sink to carbon source (Kettridge et al., 2015). The restoration of peatland plant community composition could therefore be essential to the post-fire recovery of peatlands, with an overall aim of the preservation of peatland carbon stores.

Following a disturbance such as a peatland wildfire, initial vascular plant assembly is thought to be driven by propagule availability, abiotic conditions, and interactions with resident biota (Belyea & Lancaster 1999; Kraft et al. 2015). To examine the strength of these factors, community assembly is often conceptualised to constitute a series of filters, sequentially excluding species from the wider species pool and leading to the composition of the local community (e.g. Fig. 2.1a). The strength of each filter can be inferred through the traits of established communities (De Bello et al. 2013; Dawson et al. 2017). For example, strong dispersal and abiotic filters could result in trait convergence through the removal of species from the wider species pool with trait values that do not allow them to overcome one or more of these filters (Götzenberger et al. 2012). This results in trait values that allow a species to establish becoming highly abundant in the post-disturbance community. However, recovery also depends on the composition of donor communities that supply species to the disturbed site (Fig. 2.1b).

In order to colonise post-disturbance, a species may have to overcome a different set of ecological filters depending on the pathway it uses to arrive at a disturbed site (Fig. 2.1b). This could result in species with different trait values colonising through alternative pathways. For example, following a peatland wildfire, species that colonise through the seed bank must have been able to withstand the harsh abiotic conditions created by the fire (Rein, 2013; Fig. 2.1b). Species that disperse in from surrounding vegetation will not face this abiotic filter but may have to overcome stronger dispersal filters as a result. The pathways in which species recolonise will consequently affect the composition of the post-disturbance community. Therefore, integrating both a classical ecological filter framework (Fig. 2.1a) and a disturbance-colonisation framework (Fig. 2.1b) can provide insight into the drivers of peatland post-fire recovery. Using the insights from these frameworks can then be used to inform restoration by manipulating the recovery of the post-fire community.

Peatlands in the UK, which cover approximately 10% of the terrestrial area (Bain et al. 2011), are becoming increasingly vulnerable to wildfire (Bonn et al. 2008). UK peatlands therefore provide opportunities to examine the recovery of temperate peatland vegetation communities after wildfire. Maltby, Legg, & Proctor (1990) found that drought and a subsequent severe wildfire in a UK peatland resulted in large areas devoid of vegetation over a decade later. Recently, studies have focused on certain aspects of vascular plant recovery, such as soil seed banks (Kelly et al. 2016), the recolonization success of individual target species (Legg et al. 1992; Davies et al. 2010), or the relationship between vegetation restoration and ecosystem functional recovery (Worrall et al. 2011). However, studies that examine factors simultaneously affecting the recovery of temperate peatlands vegetation following wildfire are currently rare.

In this study, we examine the key factors of post-wildfire vascular plant recovery. Working in a temperate peatland that has recently experienced a severe wildfire – the Stalybridge Estate, part of the Stalybridge moors, UK – we aim to determine factors limiting vascular plant recovery one year after the fire. We test three hypotheses:

> Hypothesis 1: Wildfire will reduce the species richness and abundance of seeds that are able to successfully germinate from the soil seed bank (Kelly et al., 2016; Maltby et al., 1990; Fig. 2.1b).
>
> Hypothesis 2: The post-fire community will consist of species colonising through both the post-fire seed bank and surrounding peatland (or non-peatland) vegetation (Fig. 2.1b).

> Hypothesis 3: The composition of vegetation one year after burning will depend on the ability of species to reach the site in sufficient numbers and on local abiotic conditions that remain post-fire (Fig. 2.1a).

We assume that competition among vascular plant species will be negligible due to the complete removal of established vegetation following the fire. We therefore do not explicitly test for their role in the initial recovery. To test the hypotheses, we performed a series of vegetation surveys to establish the taxonomic compositions of the different vascular plant communities in Fig. 2.1b (Hypothesis 1, 2). We then combined taxonomic compositions with plant trait data and constructed a series of null models using species pools in Fig. 2.1a to detect trait filtering within our sampled communities (Hypothesis 3). In doing so, our work will provide insights into the drivers of initial temperate peatland wildfire recovery.

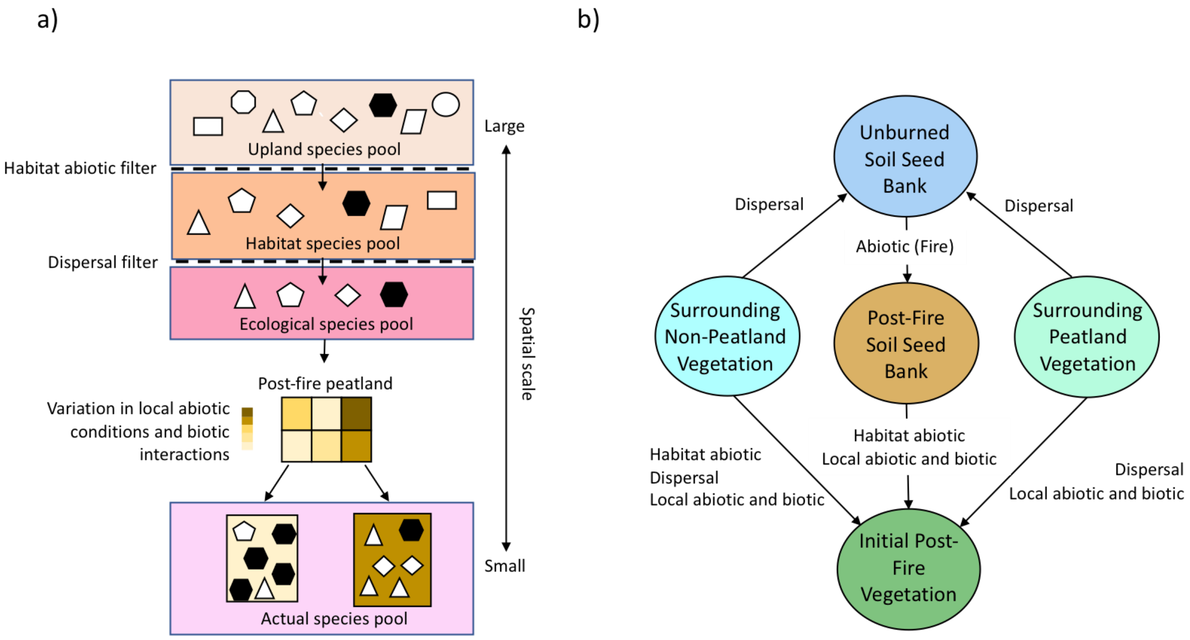

Figure 2.1: Two conceptual figures for the process of species recolonisation following a peatland wildfire. a) Classical ecological theory depicting the steps controlling the composition and structure of the recovering community. Black-filled shape represents a species not usually found within the undisturbed habitat that is able to colonise following the disturbance and, under certain post-fire conditions, dominate the established community. In this example new species are able to dominate the post-fire community when the strength of local abiotic and biotic interactions is weak. b) The multiple potential colonisation pathways from potential donor communities for a species to establish following a wildfire. The ecological filters that each species must overcome to progress to the next

community are labelled. Definitions and figures adapted from Belyea & Lancaster, (1999), Dawson et al., (2017) and Kraft et al., (2015); colours match the communities represented in Figs 2.2 - 2.5.

2.2 Methods

2.2.1 Study site

Blanket bogs are treeless peatlands that are limited to the higher latitudes (Moore 2002). The Stalybridge Estate, part of the Stalybridge Moor (often referred to as Saddleworth Moor), is a blanket bog near Manchester, UK (53°54’33 N, -01°95’58 E). Mean annual temperature is 8.2°C and mean annual rainfall for the area is 1160 mm yr^{-1} (Fick & Hijmans 2017). The vegetation is dominated by *Calluna vulgaris* (L.) Hull, *Eriophorum vaginatum* L. and *Eriophorum angustifolium* Honckeny. Moss cover is scarce on the site, consisting mainly of *Sphagnum* spp., *Pleurozium schreberi* (Brid.) Mitt. and *Hypnum jutlandicum* Holmen & Warncke. On 24 June 2018, during a drought, a wildfire broke out on Stalybridge Moor. The fire burned approximately 10 km^2 of peatland until it was extinguished on 18 July 2018, 24 days later.

2.2.2 Experimental design

We established ten 10 m × 10 m plots on the burned area of the Stalybridge estate in October 2018. We selected plot locations where aboveground vegetation was removed by the fire, but peat soil was still remaining. The plots were split into two groups (n = 5). Group 1 plots were situated at lower elevations, had steeper slopes and north-west facing, as compared to Group 2 which was south-west facing (Table S2.1, Fig. S2.1). Differences in local topography could alter burn severity (Davies et al., 2008) which can impact species recolonisation (Grau-Andrés et al. 2019). Using these plots along with the surrounding unburned peatland, we performed aboveground vegetation surveys and extracted soil for off-site seed germination experiments to determine the composition of the vascular plant communities outlined in Fig. 2.1b (excluding the surrounding non-peatland vegetation). The taxonomic identification of vascular plants followed Fitter and Peat (1994).

2.2.3 Soil seed bank surveys

To determine the post-fire viable soil seed bank composition, in October 2018 we collected and aggregated three soil samples (25 cm^2, 5 cm deep) in each of the ten burned plots. We repeated the methodology at five plots at an adjacent unburned site in order to determine the unburned soil seed bank. The unburned site was adjacent (< 1 km) to the burn site and had a similar vegetation composition to the site pre-burn. All samples were transported to the University of Southampton where they were subjected to a stratification treatment consisting of sequential storage of the soils at *c.* 5°C for 21 days and at -20°C for 48 hours. Soil samples were then weighed, mixed with approximately 1 L of nutrient-enriched sand and compost, and spread on germination trays in a greenhouse. Each tray had a soil layer of approximately three centimetres in depth. The soil was not sterilised before use. We added five extra trays consisting solely of the sand-compost mixture to test for between-tray propagule transfer or sand-compost mixture seed contamination. The soil samples were watered twice daily to ensure the seeds were exposed to optimal abiotic conditions for seed germination in peatlands.

The trays were examined at weekly intervals for germinating seeds after which their position in the greenhouse was re-arranged. Seedlings identified to the species level were then removed. The trays were left for twelve weeks, after which no new vascular plants were recorded, and the experiment was discontinued. Moss and liverwort cover were removed from the trays so as to not hinder the germination of remaining propagules. We did not disturb the soil to avoid damaging the seedlings and preventing species identification. We did not repeat the stratification period in order to best represent the conditions experienced by the seeds within the field at the point of this study (1-year post-fire).

We recorded nine vascular plant species. Singletons (*Holcus lanatus* L. and *Betula pubescens* Ehrh.) were removed before analysis to reduce the overrepresentation of rare species. To account for differences in initial soil weight the seedling abundance in each plot was multiplied as follows:

Seedling abundance = Number of recorded seedlings × 1000 / Original Soil Weight (g),

to make each soil sample equivalent to 1kg of soil. We observed minimal transfer of propagules between trays (Table S2.2). All species found in the control trays were species common to the site and likely to have contaminated the trays through lateral transfer from other trays and not from contamination in the sand-compost mixture or other experiments within the greenhouse.

2.2.4 Initial post-fire vegetation survey

We performed vegetation surveys in each of the ten burned plots in May 2019 to assess the vascular plant community that successfully re-colonised the site. We used a 1 m × 1 m pinpoint quadrat with 10 cm intervals to record species presence and abundance at two randomly placed points within each of the burned plots.

2.2.5 Surrounding peatland vegetation survey

To determine the community composition of surrounding peatland vegetation we obtained publicly available data (source: Natural England) from surveys performed at Dark Peak in summer 2018, a neighbouring site approximately 1 km from the burn site which has similar environmental conditions (Nisbet et al. 2017). In summary, the data was collected by surveying 50 2 m x 2 m plots, each split into 25 equally sized cells. The vegetation within each cell was recorded to the species level.

2.2.6 Plant trait data

We compiled trait data for seed mass (mg), specific leaf area (SLA) (mm^2 mg^{-1}), and Ellenberg moisture value (EMV) for all observed species. Seed mass can indicate dispersal capabilities (Weiher et al. 1999), and EMV and SLA can indicate abiotic filtering (Ellenberg et al. 1991; Weiher et al. 1999; Hedberg et al. 2014). We extracted the data from the databases LEDA (Kleyer et al. 2008) and Ecoflora (Fitter & Peat 1994), only using records measured in the UK. Seed mass was log-transformed, and all traits were standardised for analysis. Justification for the inclusion of these traits can be found in Table 2.1.

Table 2.1: The chosen indicator traits, the ecological filter they indicate and the justification for their inclusion.

Trait	**Indicated filter**	**Justification**	**References**
Seed mass	Dispersal	Smaller seed mass correlates with greater seed number and dispersal radius, increasing chance of post-disturbance colonisation and propagule availability.	Greene & Johnson (1993); Weiher et al. (1999); Kirmer et al. (2008); Tamme et al. (2014)
Specific leaf area (SLA)	Abiotic (Habitat and Local)	Increased nutrient availability post-disturbance favours fast growing species (high SLA). Stressful abiotic conditions favour slow growing species (low SLA).	Grime et al. (1997); Weiher et al. (1999); Reich (2014)
Ellenberg moisture value (EMV)	Abiotic (Habitat and Local)	Moisture content is a strong abiotic driver of vascular plant community assembly in temperate peatlands. Fire can decrease soil moisture content altering abiotic conditions.	Chambers et al. (2007); Sherwood et al. (2013); Gałka et al. (2015)

2.2.7 Null model construction

We produced a series of null models in order to test our observed species compositions against random community assemblages. Significant differences in the trait values of our observed communities compared with random (null) communities can indicate trait convergence (Götzenberger et al. 2012). To construct the null models, we first produced a series of species-abundance matrices based upon the framework in Fig. 2.1a. Species were included within each matrix if they were identified as being able to overcome the preceding filters in the framework. This allowed us to produce random assemblages from pools of species that varied in the filters they had passed. Once each species-abundance matrix was produced we input our observed species abundances into the matrices. The order of the columns (species identities) was then repeatedly randomised to produce 1000 null community matrices. We repeated this process for each of the sampled communities in Fig. 2.1b (all communities except the surrounding non-peatland vegetation). A full description of the null model constructions can be found in Appendix S2.1.

2.2.8 Statistical analyses

To test Hypothesis 1, we performed bootstrapped two-sample Kolmogorov-Smirnov tests comparing the seedling abundance of each observed species and the overall species richness in the unburned samples to the post-fire seed bank. We considered $p < 0.05$ to indicate a significant change in the seedling abundance of a species between the two seed banks.

To test Hypothesis 2, we used Non-Metric Multidimensional Scaling (NMDS) to visualise differences between the sampled seed bank (unburned and post-fire soil seed bank) and vegetation (surrounding peatland and initial post-fire vegetation) communities (Fig. 2.1b). Stress values were used to test the extent the NMDS plot explains the multidimensional differences in the community composition. We then used permutational multivariate analysis of variance (PERMANOVA, permutations = 999) to test for variation in centroid position of the four different vascular plant communities using community type (seed bank vs. vegetation) and wildfire history (unburned vs. post-fire) as interacting factors. We additionally included community type and wildfire history as single effects in the model. We consider $p < 0.05$ to indicate a significant effect of an explanatory variable on the vascular plant community composition.

To test Hypothesis 3, we calculated the community weighted mean (CWM) for the three traits described above for each sampled seed bank and vegetation community and their corresponding null communities. We then calculated standardised effect size (SES) values using Glass's delta $((\mathrm{CWM}_{\mathrm{observed}} - \mathrm{CWM}_{\mathrm{null}})/\mathrm{SD}_{\mathrm{nulll}})$ (Glass et al. 1981). When interpreting the SES values, we consider a response to be non-random if the 95% confidence limits do not overlap with zero.

As our study design had multiple groups nested within the post-fire site, we ran the analysis twice: once where each group was treated independently (n = 5) and once where post-fire sites were combined (n = 10). When treated independently, a large majority (86%; 20/24) of the null models tested resulted in the same response between the two groups, with the groups displaying either both a significant or non-significant difference compared with the null mean (Table S2.3). There was also little variation between the abundance of seedlings germinating from unburned and post-fire soil samples across groups (Figure S2.2). Additionally, previous research had shown fire severity to increase vascular plant beta diversity following a temperate peatland wildfire (Grau-Andrés et al. 2019). In our study, individual sampling plots were no more taxonomically dissimilar (no change in beta diversity) to plots in the other groups compared with plots within their own group (Figure S2.3). Given the largely similar response of the two groups, we present only data where the plots are combined to form one dataset (n = 10).

All analyses were conducted in R v.3.5.1 (R Core Team 2018). Bootstrapped Kolmogorov-Smirnov tests conducted using the package ‘Matching’ (Sekhon 2020). NMDS plots were produced and analysed using the package ‘vegan’ (Oksanen et al. 2018). CWM was calculated using the package ‘FD’ (Laliberté et al. 2014). SES calculations and confidence intervals were calculated using the package ‘effsize’ (Torchiano 2019).

2.3 Results

Wildfire had a limited effect on the abundance of successfully germinated seedlings in the soil seed bank; only two of seven species had lower seedling abundance in post-fire seed banks as compared with unburnt seed banks (Fig. 2.2). At the community level, the post-fire vegetation composition was distinct from the post-fire seed bank and surrounding peatland vegetation (Fig. 2.4), but shared species with both of these communities (Fig. 2.3). The initial post-fire vegetation was lower in CWM trait values for seed mass, SLA and EMV than predicted from random assemblages; however, this varied depending on the species included within the null models (Fig. 2.5).

2.3.1 Soil seed bank species richness and abundance

In the seed bank germination surveys, we found seven vascular plant species (Fig. 2.2; Table S2.4), all of which successfully germinated from both unburned and post-fire soil seed banks, except for *Chamerion angustifolium* (L.) Holub. (post-fire seed bank only) and *Vaccinium myrtillus* L. (unburned seed bank only). Species richness decreased from an average of four species per plot in the unburned soil seed bank, to 2.4 species in the post-fire soil seed bank (Bootstrapped two-sample Kolomorov-Smirnov test, $D = 0.8$, $p < 0.05$; Table S2.4), providing support for Hypothesis 1. However, only the abundance of *Festuca rubra* L. and *Juncus effusus* L. seedlings significantly reduced following the wildfire (Bootstrapped two-sample Kolmogorov-Smirnov test, *F. rubra*: $D = 0.8$, $p < 0.05$; *J. effusus*: $D = 0.6$, $p < 0.05$). There were no statistically significant changes in other species’ germinated seedling abundances (Fig. 2.2; Table S2.3).

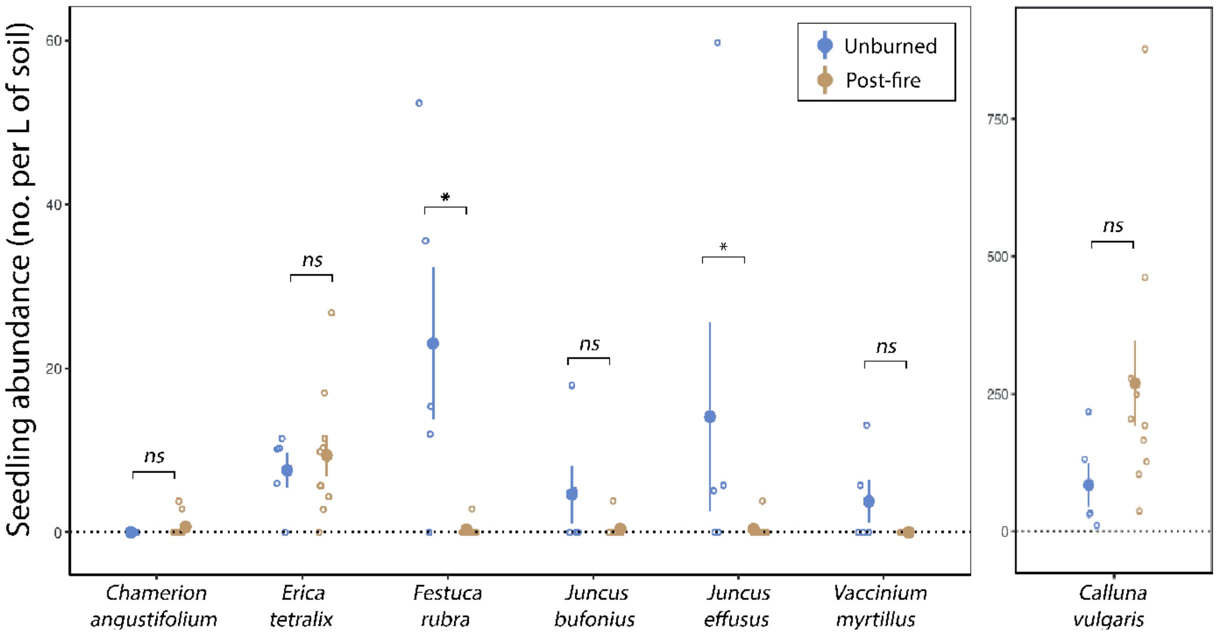

Figure 2.2: The effect of wildfire on the abundance of seedlings emerging from a temperate peatland soil seed bank. The density of seedlings per L of soil taken from a temperate peatland, part of which had experienced a wildfire approximately three months prior; full circles represent mean abundance values, open circles represent individual plot abundances, and error bars represent ± 1 standard error. The results of bootstrapped two-sample Kolmogorov-Smirnov tests are shown. *ns* = $p > 0.05$. * = $p < 0.05$. Colours represent communities in Fig. 2.1b. Note the y-axis scale for *Calluna vulgaris* is an order of magnitude larger.

2.3.2 Community composition of the soil seed bank and aboveground vegetation

We recorded five vascular plant species in the initial post-fire vegetation (Table S2.4). These were species that overlapped with either the post-fire seed bank (*C. angustifolium*, *F. rubra*), surrounding peatland vegetation (*E. vaginatum*), both of these communities (*C. vulgaris*) or neither (*Epilobium hirsutum* L.) (Fig. 2.3). Additionally, there were a number of species that were present in the donor communities but failed to recolonise the post-fire site (Fig. 2.3; Table S2.4). At a community level the post-fire vegetation was visually distinct from any other sampled community but had a greater similarity to each donor community (post-fire seed bank and surrounding peatland vegetation) than the donor communities had to each other (Fig. 2.4). Indeed, wildfire and community type (seed bank or vegetation) interacted to produce a different community composition (PERMANOVA, $F_{1,74}$= 20.2, p = 0.001). Taken together, these results are consistent with Hypothesis 2.

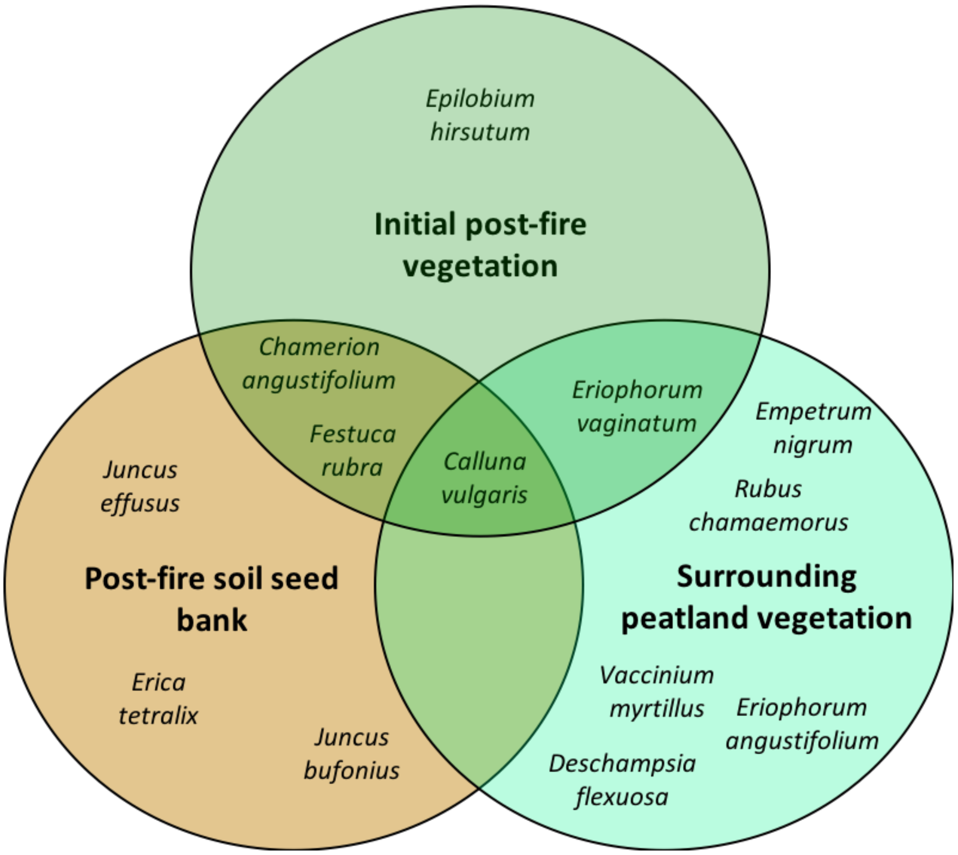

Figure 2.3: The shared species between the initial post-fire vegetation of a temperate peatland following a wildfire and two possible donor communities: the post-fire soil seed bank and surrounding non-peatland vegetation. Species that overlap multiple communities were present in both; colours represent communities in Fig. 2.1b.

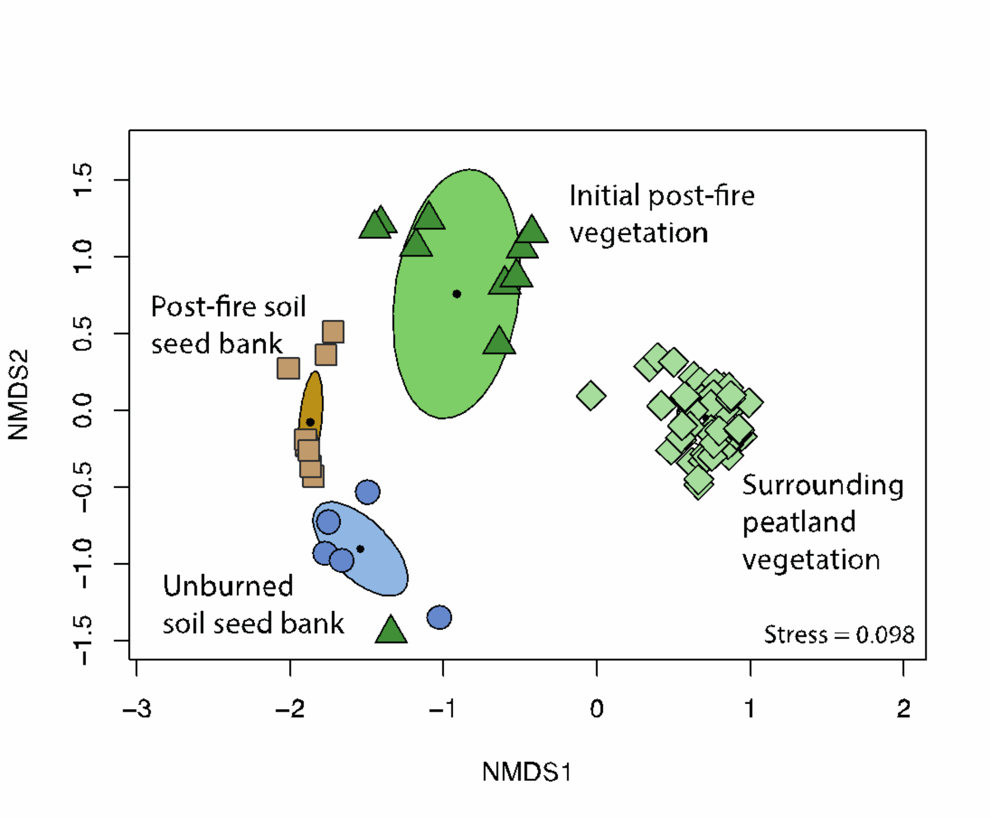

Figure 2.4: The effect of wildfire on the vascular plant community composition of a temperate peatland soil seed bank and intact vegetation. Non-Metric Multidimensional Scaling (NMDS) plot of the vascular plant community composition found in the soil seed bank and intact vegetation following a temperate peatland wildfire. These are compared with the composition of the surrounding peatland vegetation and the soil seed bank at unburned neighbouring sites. We did not survey the surrounding non-peatland vegetation so consequently it is not included within this figure. Shapes represent individual plots; black dots indicate centroid points for each community; envelopes = se around the centroid point; stress value is given; and colours represent communities in Fig. 2.1b.

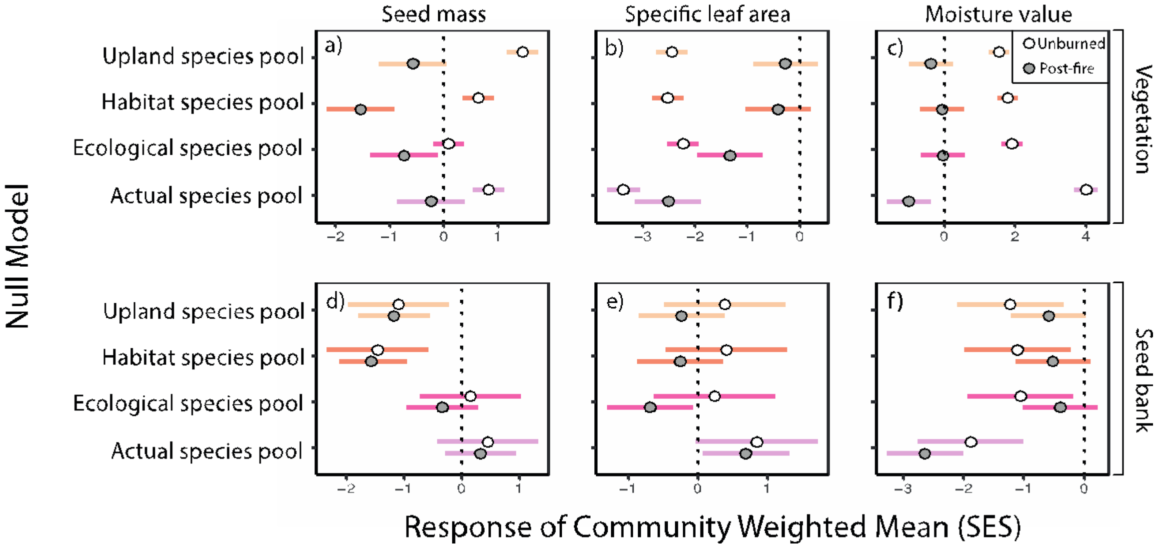

Figure 2.5: The response of trait community weighted means to wildfire in the seed bank and the vegetation vascular plant communities against randomly assembled null models. Response of community weighted means of seed mass (a, d), specific leaf area (b, e) and Ellenberg moisture index (c, f) of the intact vascular plant vegetation (a, b, c) and soil seed bank of a temperate peatland that has undergone a wildfire compared with surrounding unburned communities. Different null models are representative of varying species pools (Fig. 2.1b). Response represented as standardised effect size (SES) values (Glass's delta). Error bars represent 95% confidence errors.

2.3.3 Plant trait responses

We observed a significant reduction in the post-fire vegetation community trait values for all of our chosen traits against one or more of our null models (Fig. 2.5). Depending on the trait, the greatest reductions occurred following different ecological filters (Fig. 2.1a), with seed mass displaying the greatest reduction against the habitat species pool null model (SES = -1.53, CI = 0.63, Fig. 5a), and SLA and EMV against the actual species pool null model (SLA, SES = -2.51, CI = 0.63, Fig. 5b; EMV, SES = -1.0, CI = 0.63, Fig. 5c). Additionally, we did observe similar results in the seed bank for seed mass (habitat species pool: SES = -1.57, CI = 0.56, Fig. 5d) and EMV (actual species pool; SES = -3.28, CI = 0.63, Fig. 5f). Whilst SLA trait responses varied considerably between the post-fire seed bank and vegetation, we observed strong convergence for reduced SLA values in the surrounding peatland vegetation (Fig. 2.5e).

2.4 Discussion

Using a combination of seed bank analysis and field surveys, we have assessed the drivers of initial post-fire recovery of peatland vegetation. Notably, our work provides insights into the immediate recovery trajectory of peatlands after wildfire and the sources for plant recolonisation. Our study shows that wildfire reduces the overall species richness of the soil seed bank, along with the overall viability of two species (seven species total) (Fig. 2.2), providing partial support for Hypothesis 1. This effect was sufficient to cause differences between the composition of the soil seed bank in the burnt and unburnt areas (Fig. 2.4). The composition of standing vegetation one year after fire differed from both the post-fire soil seed bank and the surrounding peatland vegetation (Fig. 2.4). This suggests that vegetation recovery post-fire depends on colonists from the surviving seed bank and surrounding (but not necessarily peatland) vegetation (Hypothesis 2.2; Fig. 2.1b; Fig. 2.3). Finally, we identified lighter seed mass and lower SLA and EMV values in the post-fire vegetation compared with randomly assembled communities (Fig. 2.5a-c). However, similar trait responses were observed in the potential donor communities (Fig. 2.5). Combining the trait evidence with taxonomic observations, our results suggest propagule availability largely determines the post-fire vegetation community composition (Hypothesis 3; Fig. 2.1a).

2.4.1 Drivers of initial vascular plant recovery

We combined two related frameworks, a classical ecological filter framework (Fig. 2.1a) and a disturbance-colonisation framework (Fig. 2.1b) to determine the drivers of vascular plant recovery one-year post-fire. Our results suggest mixed evidence for abiotic filtering via wildfire to reduce seedling abundance of individual species within the peat soil seed bank (Fig. 2.2). These findings potentially contradict earlier observations from other temperate peatland wildfire studies which showed post-fire reductions in seedling abundances (Maltby et al. 1990; Kelly et al. 2016); however, these studies largely considered seedling abundance at a community level rather than species level. In line with earlier results, wildfire did result in a reduction in overall species richness and changes to the vegetation composition (Kelly et al. 2016), producing seed bank communities with a higher relative abundance of ericaceous species (Hypothesis 1; Fig. 2.4; Table S2.4). Notably, Kelly et al., (2016) found only approximately half of the species within their post-fire seed bank established in the recovering vegetation, similar to the results of our study (Fig. 2.3). Taken together, our results indicate species arrival through multiple colonisation pathways drives the composition and structure of the initial post-fire vegetation (Hypothesis 2; Fig. 2.1b). Additionally, wildfire can act as an abiotic filter that influences the overall composition of the soil seed bank.

Using our assembly framework (Fig. 2.1), we compiled a series of null models to test for non-random convergence of traits within our initial post-fire vegetation. Our results provide evidence of trait convergence for all three of our indicator traits (Table 2.1). However, this does not necessarily confirm our earlier predictions (Hypothesis 3) that propagule availability and abiotic conditions will drive the composition of the post-fire vegetation community. If this were true, then trait convergence (i.e. the difference between the trait CWM of the observed community compared with a random assemblage) should be reduced once the species that are unable to overcome the corresponding filter(s) have been removed from the null matrices. Such a response was observed in the case of seed mass with a reduced effect size (i.e. weaker trait convergence), observed when comparing between species pools either side of the dispersal filter. However, the opposite effect was observed for SLA and EMV, with the removal of species unable to overcome the local abiotic conditions resulting in increased trait convergence. This unexpected result could instead be driven by the composition of the donor communities (Fig. 2.1b), which displayed similar trait patterns to the post-fire vegetation. Trait convergence within these communities could therefore skew the availability of species that are able to colonise the post-fire site. Consequently, our study only provides evidence for propagule availability, and not post-fire abiotic conditions, driving the initial post-fire composition of a temperate peatland vascular plant community.

2.4.2 Implications for temperate peatlands

Initial species colonisation can drive the trajectory of taxonomic and functional recovery in a wildfire-damaged peatland (Kettridge et al. 2015). Following the wildfire, we observed species that were not found within the surrounding peatland vegetation. These were non-peatland species known for their abilities to exploit disturbances such as fire (e.g. *Chamerion angustifolium*; Moss, 1936). Additionally, their establishment success has previously been shown to increase with the removal of bryophyte layers (e.g. *Festuca rubra*; Otsus & Zobel, 2004), a widespread feature of this site. Given the short time frame of this study, it is unclear whether these species will disappear during natural succession and become replaced by a community that is more representative for peatlands. Nevertheless, in the short term the early colonisation of new non-peatland species increases the risk of inducing novel fire regimes (Kettridge et al. 2015), altering nutrient cycles (Gogo et al. 2011) and reductions in water table levels (Ohlson et al. 2003) that could compromise the recovery of this system.

Ecological restoration can target drivers of plant community assembly, manipulating them in order to produce a composition that meets their restoration aims (Temperton et al. 2004; Catford 2016; Johnson et al. 2018). Our results show that the initial natural recovery of temperate peatlands following a wildfire is driven by the availability of propagules. The importance of propagule availability for the restoration of pre-disturbance plant communities has previously been observed in grasslands (Johnson et al. 2018) and wetlands (Kettenring & Galatowitsch 2011). Conventional peatland restoration methods that target this driver (e.g. seeding; Quinty & Rochefort 2003; Schumann & Joosten 2008; Mitchell et al. 2009) may therefore be effective in manipulating the recovery of temperate peatlands post-fire by ensuring that desirable species are able to reach the post-fire site. Additionally, the presence of non-peatland species may require further actions such as the manual removal of individuals (Quinty & Rochefort 2003), reducing the availability of undesirable species on site. Our results do not mean that restoration techniques targeting alternate drivers will be ineffective. However, they do suggest that propagule availability should be considered when planning the potential restoration of a wildfire-damaged peatland.

2.4.3 Study considerations

This study combines classical community assembly theory with an additional disturbance-colonisation framework (Fig. 2.1) to determine the drivers of peatland wildfire recovery. By using a single time point, our study represents a snapshot of the drivers of recolonisation one-year post-fire. However, drivers of plant community assembly can vary throughout succession (Chu et al. 2007; Maire et al. 2012) and become more complex and interlinked as communities re-establish (Tilman 2004). Stochastic elements, including initial species arrival times (e.g. priority effects; Fukami 2015; Weidlich et al. 2017), along with soil microbial compositions (Wubs et al. 2016), can alter the direction of post-disturbance plant community composition adding temporal and spatial variability to the recovery process. In peatlands, this could drive observed patterns of species turnover and compositional changes to the vegetation community in the decade following a fire (Maltby et al. 1990; Noble et al. 2019). Future work could therefore focus on the relative role of ecological community assembly drivers across a fire-recovery gradient, in order to maximise the effectiveness of restoration action given the time point and direction of post-fire recovery.

2.4.4 Conclusions

Severe peatland wildfires threaten the taxonomic composition and functioning of peatlands (Maltby et al. 1990; Kettridge et al. 2015). Consistent with findings from other ecosystems (Morzaria-Luna & Zedler 2007; Kettenring & Garatowitsch 2011; Johnson et al. 2018), our study highlights that propagule availability drives the initial recovery of temperate peatlands post-fire. Additionally, we show that species are able to colonise through multiple colonisation pathways. This in turn provides opportunities for new non-peatland species to colonise, which could compromise the recovery of these systems (Kettridge et al. 2015). Taken together, these results suggest ensuring the availability of peatland species following a wildfire could be key to the immediate recovery of these systems.

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2.7 Supplementary materials

Table S2.1: Burn site plot characteristics

Plot Code	Group	Elevation (m)	Slope (degrees)
G1_1	1	390.2	9.42
G1_2	1	399.3	7.49
G1_3	1	392.3	6.44
G1_4	1	399.4	4.59
G1_5	1	393.8	5.41
G2_1	2	414.9	2.28
G2_2	2	414.9	2.28
G2_3	2	413	2.11
G2_4	2	409	3.16
G2_5	2	406.1	4.45

Table S2.2: Number of individual seedlings observed in the five control trays. Species found in the non-control trays that were not found in the control trays are also included.

Control Tray	*Calluna vulgaris*	*Erica tetralix*	*Juncus bufonius*	*Juncus effusus*	*Epilobium angustifolium*	*Festuca rubra*	*Vaccinium myrtillus*
1	4	0	1	1	0	0	0
2	1	0	1	1	0	0	0
3	1	0	0	1	0	0	0
4	1	0	0	0	0	0	0
5	0	1	0	0	0	0	0

Table S2.3: The effect of wildfire on seedling abundance within the soil seed bank of a UK temperate peatland. Summary of results from bootstrapped two-sample Kolmogorov-Smirnov test. Number of bootstraps = 1000. Significant results are highlighted in bold. Data is even involving all post-fire plots (Combined) or individual plot locations (Group 1 and Group 2).

Species	**Combined**		**Group 1**		**Group 2**	
	D -value	*P* -value	*D* -value	*P* -value	*D* -value	*P* -value
Calluna vulgaris	0.6	0.13	0.6	0.26	0.6	0.26
Erica tetralix	0.3	0.85	0.2	1.00	0.4	0.75
Juncus bufonius	0.4	0.15	0.4	0.37	0.4	0.27
Juncus effusus	**0.6**	**< 0.05**	0.6	0.14	0.6	0.11
Epilobium angustifolium	0.2	0.52	0.2	0.55	0.2	0.52
Festuca rubra	**0.8**	**< 0.05**	**0.8**	**< 0.05**	**0.8**	**< 0.05**
Vaccinium myrtillus	0.4	0.07	0.4	0.26	0.4	0.28

Table S2.4: Species recorded abundances in the soil seed bank (unburned and post-fire) surveys and the post-fire vegetation.

Community	**Plot**	**CV**	**FR**	**ET**	**EV**	**EA**	**EH**	**JB**	**JE**	**VM**
Unburned SB	1x	32	52	0	0	0	0	0	0	13
Unburned SB	2x	10	15	10	0	0	0	5	0	0
Unburned SB	3x	30	35	10	0	0	0	0	5	0
Unburned SB	4x	217	0	11	0	0	0	0	5	5
Unburned SB	5x	131	11	5	0	0	0	17	59	0
Post-fire SB	1a	126	0	26	0	0	0	0	3	0
Post-fire SB	2a	278	0	11	0	0	0	3	0	0
Post-fire SB	3a	168	0	0	0	0	0	0	0	0
Post-fire SB	4a	36	0	5	0	3	0	0	0	0
Post-fire SB	5a	248	0	9	0	0	0	0	0	0
Post-fire SB	1b	462	0	5	0	3	0	0	0	0
Post-fire SB	2b	204	0	10	0	0	0	0	0	0
Post-fire SB	3b	192	0	4	0	0	0	0	0	0
Post-fire SB	4b	103	0	2	0	0	0	0	0	0
Post-fire SB	5b	877	2	17	0	0	0	0	0	0
Post-fire Veg	1a	56	4	0	2	6	3	0	0	0
Post-fire Veg	2a	48	0	0	20	2	10	0	0	0
Post-fire Veg	3a	35	0	0	42	1	3	0	0	0
Post-fire Veg	4a	28	0	0	0	2	3	0	0	0
Post-fire Veg	5a	7	0	0	13	3	4	0	0	0
Post-fire Veg	1b	26	0	0	30	0	7	0	0	0
Post-fire Veg	2b	59	0	0	0	6	2	0	0	0
Post-fire Veg	3b	30	0	0	1	3	4	0	0	0
Post-fire Veg	4b	23	0	0	128	0	0	0	0	0
Post-fire Veg	5b	18	135	0	0	0	0	0	0	0

Community abbreviations: SB = Seed bank; Veg = Vegetation. Species abbreviations: CV = *Calluna vulgaris*; FR = *Festuca rubra*; ET = *Erica tetralix*; EV = *Eriophorum vaginatum*; EA = *Epilobium angustifolium*; EH = *Epilobium hirsutum*; JB = *Juncus bufonius*; JE = *Juncus effusus*; VM = *Vaccinium myrtillus*. Plots refer to the replicate number (1-5) and the sampling group (Group 1 = a; Group 2 = b). See **Methods** for details.

Appendix S2.1: Supplementary Methods on the construction of null models.

Null model construction.

We compared the observed species abundances from our four sampled communities (initial post-fire vegetation, surrounding peatland vegetation, unburned seed bank, post-fire seed bank) against four different null models. These null models were constructed based on the framework in Fig. 2.1. This involves two steps: the production of species-abundance matrices and the construction of the null models.

Step 1: Production of species-abundance matrices.

To produce the species-abundance matrices, we first needed to classify each species based on its ability to overcome the different ecological filters in Fig. 2.1b. In doing so, we could produce four species abundance matrices consisting of the upland species pool (USP), habitat species pool (HSP), ecological species pool (ESP) and actual species pool (ASP). As the order in which a species faces each filter depends on the pathway in which it takes to recolonise (Fig. 2.1a) the matrices constructed to compare against the vegetation communities (initial post-fire vegetation and surrounding non-peatland vegetation) and observed seed bank communities (unburned seed bank, post-fire seed bank) were produced differently.

<u>Vegetation species-abundance matrices</u>:

USP: Consisted of all species observed within the study, in either of the vegetation or seed bank communities.

HSP: We removed all species from the USP matrix that were not observed within either the surrounding peatland vegetation or the initial post-fire vegetation. These were species constrained to the seed bank and therefore were assumed to be unable to overcome the wider abiotic conditions and germinate.

ESP: We produced separate ESP matrices for the initial post-fire vegetation and the surrounding peatland vegetation. Within each matrix, individuals were removed if they were not present within that vegetation community. These species were assumed to be absent due to a lack of propagule availability.

ASP: We produced individual ASP matrices for each plot that we sampled. Species were removed from the ESP if they were not present within a plot. These species were assumed to be absent due to local abiotic/biotic interactions.

Seed bank species abundance matrices:

The construction of the USP and the HSP for the seed bank communities was the same as for the vegetation communities.

ESP and ASP: In both of these matrices we did not remove species absent from the vegetation communities (those unable to overcome wider environmental conditions). This is as presence within the soil seed bank is driven by dispersal limitation and fire tolerance, not the ability to overcome abiotic conditions (Fig. 2.1a).

Step 2: Null model construction.

To produce the null models, we inputted our observed species abundances from plot level surveys into the matrices. The order of the columns (species identities) were then repeatedly randomised to produce 1000 null community matrices. Therefore, within each null matrix the relative abundances of species were maintained relative to the plot level observations, but the species identities were randomised.

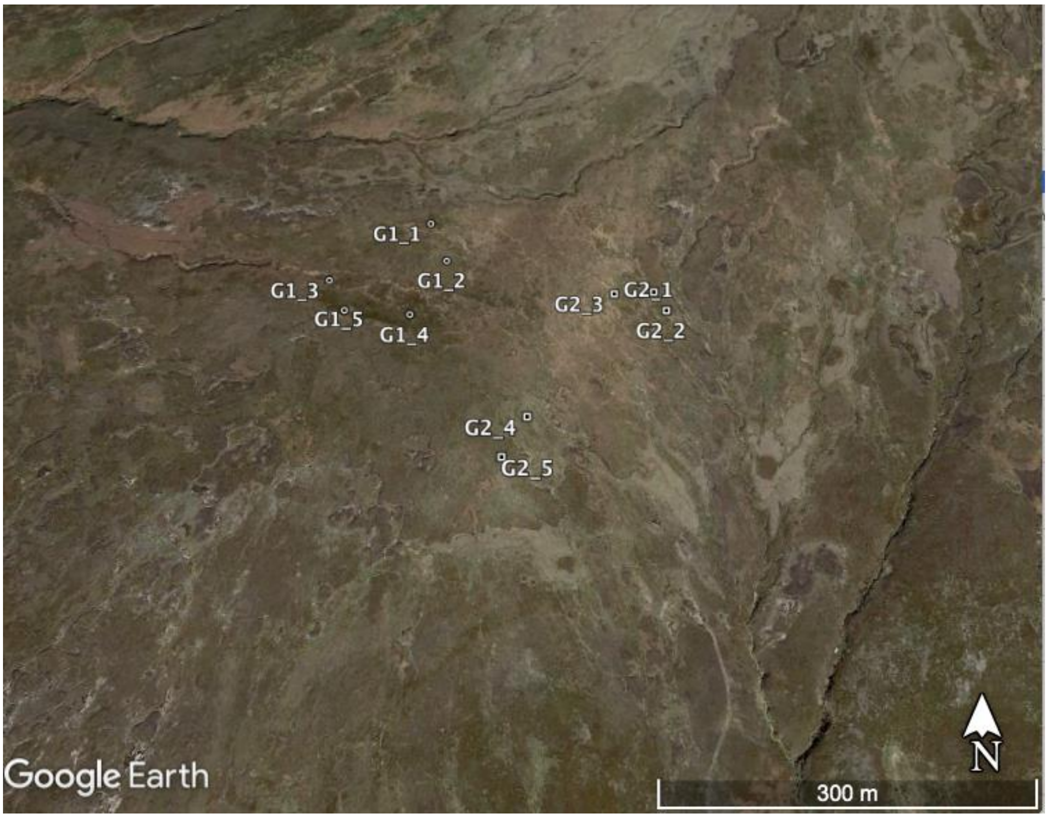

Figure S2.1: Burn site plot locations. Square points refer to individual plot locations. Plot IDs refer to the sampling group of the plot (G1= Group 1, G2 = Group 2) and the replicate number. Image source: Google Earth

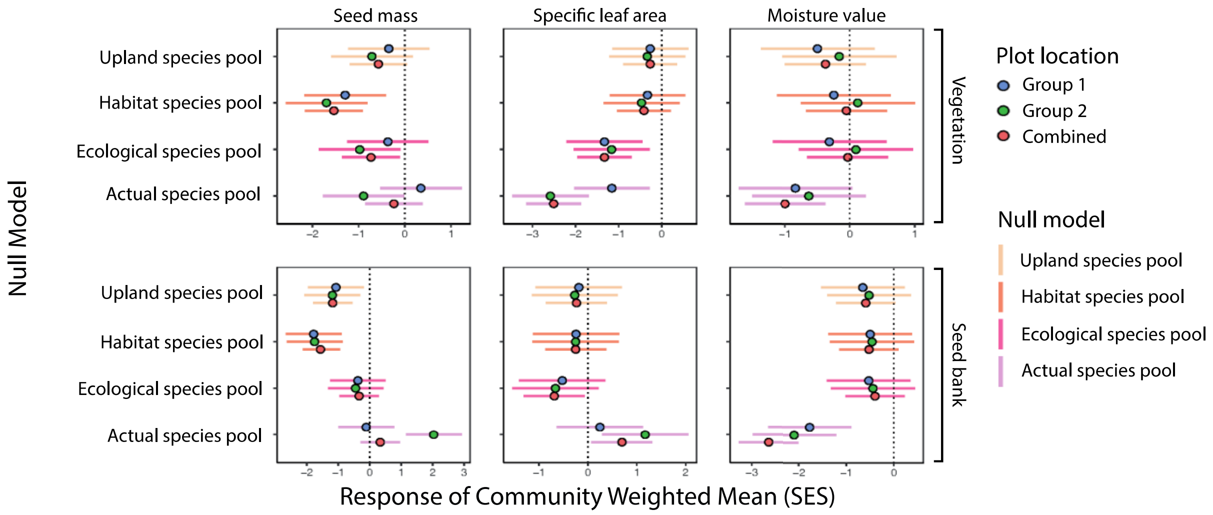

Figure S2.2: The response of trait community weighted means post-fire seed bank and the vegetation vascular plant communities against randomly assembly null models, grouped by plot location. Response of the seed mass (a, d), specific leaf area (b, e) and Ellenberg moisture index (c, f) community weighted means of the intact vascular plant vegetation (a, b, c) and soil seed bank of a temperate peatland that has undergone a wildfire compared. The responses are either split into the two plot locations (group 1 or group 2) or combined to form one dataset. Different null models are representative of varying species pools (Fig. 1b). Response represented as standardised effect size (SES) values (Glass's delta). Error bars represent 95% confidence errors. Error bar colours represent the community in Fig 2.1a.

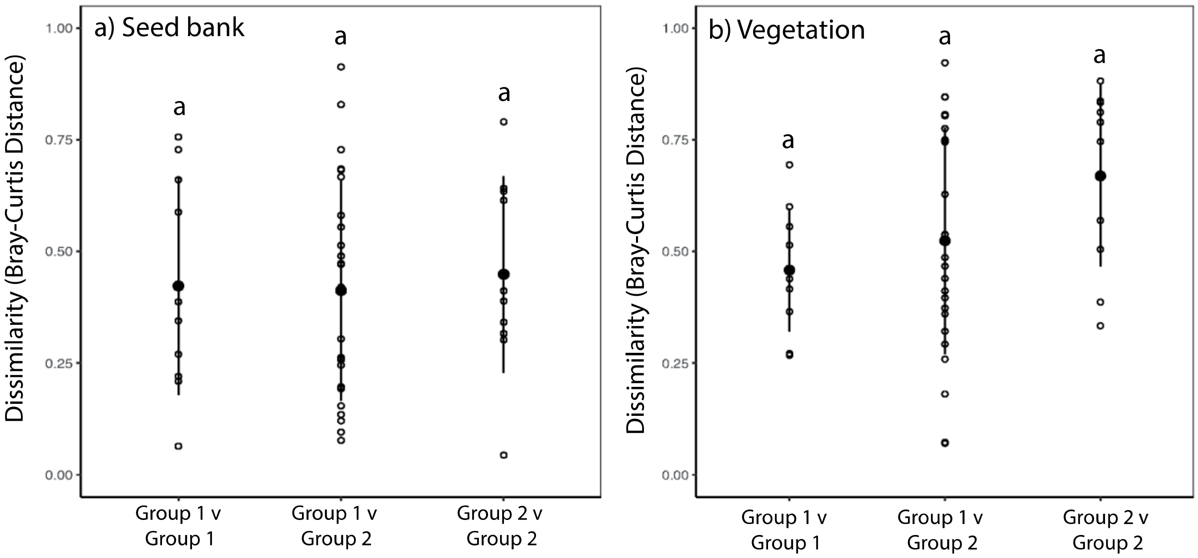

Figure S2.3: Taxonomic dissimilarity (Bray-Curtis distance) between the community compositions of post-fire (a) seed bank and (b) vegetation between plots within the same plot group and between different groups. Letters denote results of two-sample Kolmogorov-Smirnov tests ($p < 0.05$). Solid circles represent dissimilarity mean. Error bars represent the standard deviation. Open circles represent individual pairwise dissimilarities.

Chapter 3 Post-fire peatland recovery by peat moss inoculation depends on water table depth

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Abstract

Peatland restoration is essential to preserve biodiversity and carbon stored in peat soils. Restoration techniques such as rewetting do not always result in the full recovery of peatland taxonomic and functional properties, threatening the resilience of restored peatlands and their carbon stores. To expedite taxonomic and functional restoration, soil inoculation – the addition of a small amount of soil or living matter– has been proven successful in other ecosystems. Here, we study the use of peat moss inoculates in stimulating the short-term taxonomic and functional recovery of a wildfire-impacted peatland using mesocosms at high and low water table depth. Our results show that inoculation altered both the vascular plant and prokaryote composition. Importantly, peat mosses – the keystone genus in these peatlands – only established in inoculated mesocosms. These results indicate that inoculation can be used to accelerate the establishment of peatland-specific species. Furthermore, soil inoculation resulted in an increase in CO_2 uptake by approximately 17 g m^{-2} day^{-1}. This increase was dependent on high water tables, suggesting the potential to combine peat moss inoculation and hydrological restoration to accelerate the uptake of carbon back into the system post-fire. Overall, our results highlight the use of peat moss inoculation in controlling the initial taxonomic and functional recovery of a wildfire-damaged peatland. This offers a basis for future work exploring the long-term use of inoculation in peatlands to return disturbed peatlands to their pre-degraded state, and a wider application of soil inoculation as a mechanism for functional restoration.

3.1 Introduction

Peatlands are key global carbon stores, holding up to at least 500 Gt of carbon in their soils (Yu 2012). Peatland carbon storage is driven largely by high water table depths that provide anaerobic conditions which hamper decomposition. Consequently carbon-rich peat can accumulate in the form of slowly accumulated non-decomposed plant material (S. E. Page & Baird, 2016). Recent centuries have reported widespread degradation of peatlands (Tanneberger et al., 2021), which was mostly driven by drainage (Swindles et al., 2019), nutrient enrichment (Aerts & de Caluwe, 1999) and wildfires (Turetsky et al., 2015). These environmental impacts can shift peatlands away from their characteristic peat-forming vegetation compositions (Bellamy et al., 2012; Kettridge et al., 2015; Noble et al., 2019b). Importantly, peatland degradation results in the loss of key ecosystem functions, including the release of large quantities of carbon into the atmosphere (Joosten, Sirin, et al., 2016; Juutinen et al., 2018; Kettridge et al., 2015; Larmola et al., 2013; Leifeld et al., 2019), switching these ecosystems from carbon-sinks to sources. Peatland conservation and restoration is therefore essential to ensure peatlands remain carbon stores and help to mitigate the current rise in global atmospheric carbon (Leifeld & Menichetti, 2018).

Peatland restoration commonly involves rewetting – the raising of previously lowered water tables, which can be an effective approach in returning peatlands to their original state as carbon sequestering ecosystems and to restore peatland-specific biodiversity (Günther et al., 2020b; Schwieger et al., 2021). Additionally, rewetting of peatlands reduces the risk of severe wildfires (Turetsky et al., 2011). However, recently it has been shown that after hydrological restoration, the recovery of peatland plant communities and functional properties lag behind their undisturbed counterparts; even decades after restoration action (Kreyling et al., 2021). Such slow biotic and functional recovery could have knock-on effects on peatland resilience to future disturbances that are not currently understood (Hobbs et al., 2009). In the light of ongoing climatic warming, a rapid return of typical peatland vegetation is crucial (Nugent et al., 2019). Consequently, additional restoration action may be necessary along with rewetting to push restored peatlands towards pre-disturbed states and ensure the long-term stability of restored peatlands (Granath et al., 2016; Huth et al., 2022).

Alongside rewetting, additional peatland restoration action commonly aims to achieve two aspects: the re-introduction of lost vegetation and the re-establishment of desirable abiotic conditions (Rochefort et al., 2003b). For example, techniques such as litter spreading and moss layer transfer can provide desirable propagules to peatlands, which can stimulate vegetation recovery (Quinty & Rochefort, 2003; Rochefort et al., 2016b). Alternatively, techniques such as top-soil removal and liming can control nutrient availability and reduce pH, producing abiotic conditions that can benefit the re-establishment of desirable vegetation (Huth et al., 2022; Quinty & Rochefort, 2003). A potentially neglected aspect of peatland recovery is the restoration of microbial communities, which has been increasingly recognised as a powerful tool in the restoration of terrestrial ecosystems (Coban et al., 2022; Harris, 2009). Soil microbial communities can promote or inhibit the performance of plant species, controlling overall compositions and consequent directions in vegetation succession (Bauer et al., 2015b; van der Putten et al., 2013) as well as ecosystem functions (Bardgett & van der Putten, 2014). Ecosystem degradation can alter the soil microbiome, which hampers the natural recovery of systems back to their pre-disturbance state (Harris, 2009). Whilst many peatland restoration techniques are likely to alter the microbial community alongside their main restoration aims(Kreyling et al., 2021; Putkinen et al., 2018), the focus is often on the aboveground, not belowground, recovery. Yet, focusing action on restoring peatland soil microbiomes, either alone or in conjunction with the aboveground community, could help steer succession towards desired taxonomic compositions (Wubs et al., 2016) and restore lost or degraded functions (Coban et al., 2022).

Soil inoculation - the addition of a small amount of soil or living matter from an intact target ecosystem - is one method used to restore degraded microbial communities (Wubs et al., 2016). Soil inoculation can provide a disturbed ecosystem with an intact microbial community, accelerating the course of recovery and steering it towards a target aboveground plant community (Wubs et al., 2016). In addition, inoculation can also provide propagules that help plant species to overcome dispersal filters and delayed arrival times that can provide advantages to early colonisers (Weidlich et al., 2017). However, despite a number of promising studies on the use of soil inoculation, evidence is often focused on i) grasslands and heathlands and on ii) taxonomic, rather than functional recovery (Emsens et al., 2022; Middleton & Bever, 2012; Neuenkamp et al., 2019; van der Bij et al., 2018; Wubs et al., 2016). Consequently, questions still remain as to the wider effectiveness of soil inoculation across a range of terrestrial systems, and in recovering key functions lost through ecosystem degradation.

In this study, we assess the use of inoculation in enhancing the post-fire recovery of peatland plant and microbial communities. We test this method using peat from a recently burned bog, the Deurnsche Peel, in the Netherlands. A wildfire damaged peatland was selected due to recovery being potentially limited by both propagule availability (Shepherd et al., 2021) and microbial composition (Andersen et al., 2013). Due to the key role of *Sphagnum* in shaping peatland ecosystems (van Breemen, 1995b), we tested the use of blended *Sphagnum* as an inoculate. We hypothesise that the addition of *Sphagnum* inoculate will provide i) a new microbial community, ii) vascular plant propagules, and iii) a source of peat moss that together can steer the taxonomic and functional recovery of a peatland following a wildfire. As rewetting is a commonly used peatland restoration technique (Kreyling et al., 2021), we trial the use of peat moss inoculate in stimulating the short-term taxonomic and functional recovery of a wildfire-damaged peatland using mesocosms at two water table depths: high (5 cm below surface level) and low (25 cm below surface level), simulating the hydrological conditions of rewetted and drained peatlands respectively. In doing so, our work will provide insights into the use of *Sphagnum* inoculate as a tool for peatland restoration, and in the general restoration of lost or diminished ecosystem functions.

3.2 Methods

3.2.1 Study site and experimental design

The Deurnsche Peel is a raised bog-remnant located in the Netherlands (51°24'59.3"N 5°52'37.2"E), which is part of a larger peatland complex – *De Verheven Peel* – which covers an area of roughly 6000 ha. t. The mean annual temperature is 9.7°C and the mean annual precipitation is 773 mm yr^1 (Fick & Hijmans, 2017). In April 2020, the Deurnsche Peel suffered a wildfire that burned for over 2 months and affected *c.* 800 ha. The fire was mainly superficial but left large areas of peatland void of vegetation. Pre-fire vegetation was dominated by *Molinia caerulae*, *Sphagnum cuspidatum* and *S. fallax*, with sparse cover of *Betula pubescens*, *Calluna vulgaris* and *Erica tetralix* (R. Bakker, 2018). In November 2020, five months after the fire, the fire-affected area was heavily dominated by *Molinea caerulea* and was accompanied by young *Betula pubescens* and *Populus nigra.* Areas where the peat smouldered for a longer time remained barren.

In November 2020 (i.e., seven months after the fire), 20 intact soil cores (mesocosms; 40 cm diameter, 30 cm deep) were collected from barren locations in the post-fire peatland (Fig. 3.1a). We selected five locations that were at least 25 m apart. At every location, four cores were taken and placed in tight fitting PVC buckets with small holes in the bottom. At the same time as the mesocosm collection, *Sphagnum* moss – mainly *Sphagnum fallax* and *S. cuspidatum* – was collected from an unburned location at the same site.

The mesocosms (i.e. buckets with burned peat soil) were transported to Radboud University (Nijmegen) where they were stored in the experimental garden for eleven days to acclimatise. Next, the mesocosms were brought into the experimental glasshouse for a further twelve day-acclimation period. To control the water table in the mesocosm, they were placed in larger PVC buckets (50 cm diameter) that drained at the mesocosm specific water table (see below). To prepare the inoculate for each mesocosm, 650 g of fresh peat moss was mixed with 0.9 L of demineralised water to produce 0.09 L of inoculate per mesocosm (n = 10). The inoculate was homogenised using an immersion blender, to produce a slurry with small (c. 0.5 cm) diaspores of peat moss.

In the experimental glasshouse, all mesocosms were placed in a full-factorial randomised block design, keeping the 5 × 4 collection set-up (5 locations, 4 mesocosms per location). In each block, all mesocosms were randomly assigned an inoculation and water table treatment. The homogenised inoculate was applied to the surface of half of the mesocosms (n = 10), while the other half were left non-inoculated to serve as controls. Simultaneously, half of the inoculum and control mesocosm treatments received a water table treatment: high (water table of 5 cm below surface level) and low (water tables of 25 cm below surface level). This resulted in four treatments: 1) inoculation, high water table; 2) inoculation, low water table; 3) control, high water table; 4) control, low water table (Fig. 3.1b, c). To maintain the required water tables and simulate rainfall, mesocosms were watered twice a week using collected rainwater. Following a 120-day period, we terminated the experiment in April 2021.

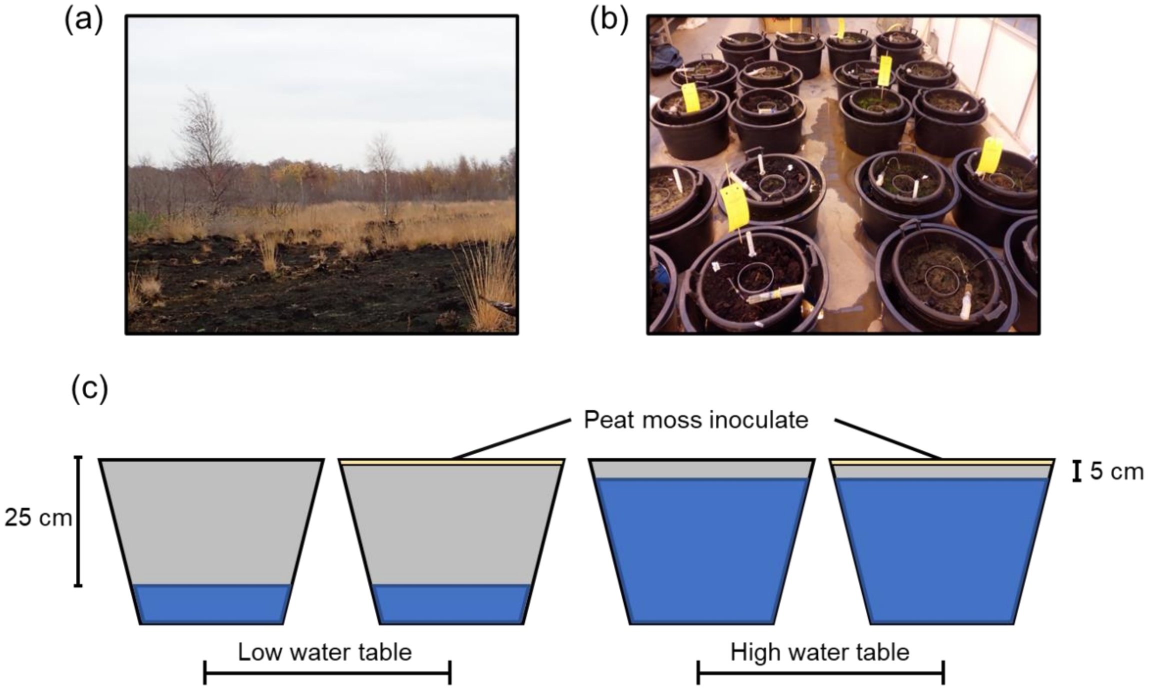

Figure 3.1: Study site and experimental set-up. a) Burn site location where mesocosms were collected from (Credit: Ralph Temmink). b) Mesocosm setup in Radboud university experimental greenhouse (Credit: Isa Martin). c) Mesocosm setup outlining the four different experimental treatments.

3.2.2 Plant (functional) and microbial community composition estimations

In each mesocosm, vascular plant and bryophyte covers (%) – at species level if possible – were estimated 4 months post-inoculation. Total vascular plant cover, due to community structure, can exceed 100%, while maximum bryophyte cover, which is based on surface cover, could not exceed 100%. To determine aboveground biomass (g m^{-2}) at the end of the experiment, the aboveground vascular plants were harvested, and oven dried for 7 days at 70ºC.

We compiled trait data for five plant traits: seed mass (mg), specific leaf area (SLA; mm^2 mg^{-1}), leaf dry matter content (LDMC; mg g^{-1}); plant height (m) and Ellenberg moisture value (EMV). Traits selected were core plant traits (Díaz et al., 2016) and/or have previously been shown to be selected for within recolonising peatland vascular plant communities (Shepherd et al., 2021). Trait values for each species were extracted from in LEDA (Kleyer et al., 2008) except EMV which were taken from Ecoflora (Fitter & Peat, 1994). For each species, we extracted all available trait data then produced a species average. If species level data was unavailable, genus level means were used.

To assess the composition of the prokaryote communities and its trajectory of recovery in the mesocosms, we extracted peat samples (2.5 cm diameter; 5 cm deep) at three time points (10, 35 and 112 days) in the recovery period (i.e. post-inoculation). From all samples, DNA was extracted using the QIAGEN DNeasy powersoil pro kit following standard manual specifications. Extracted DNA was sequenced using the 515F/806R primers that target the V4 regions of the 16S rRNA gene. Prokaryote sequences were checked for quality and ASVs assembled through the DADA2 pipeline (Callahan et al., 2016) before further analysis. The SILVA database (https://www.arb-silva.de) was used as a reference to match ASVs to their taxonomic identities.

3.2.3 Greenhouse gas measurements and peat soil edaphics

Carbon dioxide (CO_2) and methane (CH_4) fluxes at the mesocosm level were measured using plexiglass transparent PVC chamber (20 cm diameter, 40 cm high) equipped with a fan and connected to a LI-COR LI-7810 CH_4/CO_2/H_2O Trace Gas Analyzer (*LI-COR* Biosciences, Nebraska, US). The chamber had a rubber seal on the bottom and a capped hole on the top which remained open when the chamber was placed on the PVC collars that were pre-inserted in all mesocosm to a depth of 5 cm. The measurements began 6 weeks post-inoculation, when the vegetation started to emerge. All flux measurements were carried out around midday local time, ensuring optimal conditions for both *Sphagnum* and vascular plant photosynthesis. Each measurement lasted 2 minutes by which we avoided excessive heat and humidity causing condensation inside the chamber. CO_2 and CH_4 fluxes were calculated as a linear change in concentration (ppm), considering ambient atmospheric pressure and gas temperature. In this study, positive NEE represents a net CO_2 uptake by the mesocosms.

Pore water samples were collected in the first 10 cm below the peat surface using Rhizon soil moisture samplers (type MOM, pore size 0.2 µm, Eijkelkamp, Giesbeek, NL). Samples were taken from the mid-point between the outer mesocosm wall and the PVC collar, 7, 32, 64 and 98 days post-inoculation, and stored in collection syringes before being transported to the lab. Following pore water extraction, pH was measured using a titrator (Metrohm 877 Titrino plus). All samples were analysed spectrophotometrically for NO_3-N, NH_4-N and PO_4-P and Cl^- concentrations using an AutoAnalyser (Bran+Luebbe GmbH, Nordestedt, Germany). Na^+ and K^+ were measured using a Sherwood 420 flame-photometer.

The peat soil C to N ratio was assessed at the end of the experiment from 10 cm deep peat soil cores (2.5 cm diameter) taken at the centre of each mesocosm. Each core was dried for at least 48 h at 70ºC, ground and then subsampled (0.25 - 0.35 mg per sample). Carbon and nitrogen content was then measured in a CHNO element analyser (EA NA1500 - EA 1110 device, Carlo Erba/Thermo Fisher Scientific).

3.2.4 Statistical analyses

The effects of soil inoculation and water table depth on community compositions of the vascular plants and bryophytes was examined using non-metric multidimensional scaling (NMDS). To test the impact of the experimental treatments on each community composition, we performed permutational multivariate analysis of variance (PERMANOVA, permutations = 999). Species that were rarely present within the mesocosms (< 25%) and when present, occurred at low relative abundances (< 5% cover) were removed to reduce the impact of rare species on the multivariate analysis. Species abundance matrices were standardised before inclusions using Hellinger transformations. Inoculation and water table depth were included as single factors and as two-way interactions for each PERMANOVA. As variables are sequentially added in PERMANOVA construction, we trialled modifying the order of variable specification to check if this altered our interpretation of the results.

To examine the functional composition of the aboveground plant community, we first calculated community weighted means (CWMs) for each of the five selected traits (see *2.3*) in each mesocosm. We then performed a principal component analysis (PCA) to visualise the functional composition of each plant community. To examine the effect of inoculation and water table depth on *Sphagnum* cover, we performed bootstrapped two-sample Kolomogorov-Smirnov (KS) tests (nboot = 1000). We performed this test only on mesocosms where inoculation had occurred, as no *Sphagnum* was observed in uninoculated plots (Fig. S3.1).

Prokaryote composition was visualised using NMDS and the effect of each treatment tested through PERMANOVA analysis, following the procedure described above. As composition was measured across multiple time points, time (days) since inoculation was included in the analysis as an additional single factor along with the experimental treatments (inoculation and water table depth), with all three variables also included as two- and three-way interactions. To account for the repeated measurements within the same mesocosm, we constrained permutations using mesocosm identity.

To further examine the effect of inoculation on the prokaryote community, we used linear mixed models (LMMs) to determine changes in the relative proportions of the ten most abundant prokaryote ASVs. In each LMM, we included inoculation, water table depth and time (days) since inoculation as individual variables, and as two- and three-way interactions. In each model mesocosm identity was included as a random intercept. For each LMM we then performed a backwards model selection process to identify the best performing model (see Appendix S3.1 for details). For each best performing model, qq-plots were examined visually, and residual diagnosis was performed using the R packages *DHARMa* and *performance* to ensure the assumptions of linear models were met. Pseudo-R^2 values for the entire model (conditional) and only fixed effects (marginal) were then estimated either from the single best model or across the range of models from which model estimates were averaged from Nakagawa and Schielzeth (2013).

To assess the effect of the treatments (inoculation and water table depth) on functional recovery over time, we performed LMMs on the effect of the inoculation and water table treatments on CO_2 fluxes (NEE), CH_4 fluxes and pore water biogeochemical composition. In each model we included water table depth, inoculation and days since inoculation as single factors, as two-way interactions and as a three-way interaction. Porewater biogeochemical composition was determined by Principal Components Analysis (PCA) using the concentrations of NO_3^-, NH_4^+, PO_4^-, Cl^-, K^+ and Na^+ along with pH as explanatory variables. The first axis of the PCA was then extracted and used as a response variable in the LMM. In each model, mesocosm identity was included as a random intercept. Model performance was assessed by the same protocol as for the prokaryote phylum models (Appendix S2). Finally, measures of ecosystem functions – taken at the end of the experiment (aboveground biomass, peat C to N ratio) – were examined using two-way ANOVA, with inoculation and water table depth as single and two-way interactions. Assumptions of ANOVA (e.g., normality of residuals, homogeneity of variance) were examined visually using the R package *performance*, with homogeneity of variance examined additionally using Levene's test for homogeneity. CN ratio was consequently log-transformed to ensure adherence to these assumptions.

We considered $p < 0.05$ to indicate a significant effect of an experimental treatment. All statistical analyses were conducted in R v. 4.1.2 (R Core Team, 2021). A list of all packages used in the analyses is provided in Appendix S3.1.

3.3 Results

3.3.1 Aboveground community composition

Inoculation caused a shift in the overall composition in both the vascular plant and bryophyte communities (Fig. 3.2a, c; Table S3.1). Under natural, non-inoculated, conditions the aboveground vegetation became dominated by *Molinea caerula* (17.9 ± 5.8%; average ± SE) and *Bryum* spp. (39 ± 10.0%) (Table S3.1). Inoculation resulted in the vegetation to be dominated by *Juncus bulbosus* (43.5 ± 7.6%), *Sphagnum* spp. (21.6 ± 8.3%) and *Hypnum cupressiforme* (15.3 ± 7.0%) (Table S3.1). Functionally, it led to a vascular plant community that possessed lower leaf dry matter content, plant height and seed mass, and higher EMVs compared to uninoculated communities (Fig. 3.2b; Fig. S3.2). *Sphagnum* was only found in inoculated plots (Fig. 3.2d). Despite its establishment in double the amount of mesocosms when the water table was raised (80% in high water table depths, 40% in low water table depths), we did not find evidence that wetter conditions increased overall *Sphagnum* cover (Two-sample bootstrapped Kolmogorov-Smirnov test, $D = 0.6$, $p = 0.24$). Neither did the water table result in an overall shift in the bryophyte composition (Fig. 3.2c; Table S3.1) or vascular plant community (Fig. 3.2a; Table S3.1). However, we did observe *Sphagnum* species differences across water table depths, with *S. cuspidatum* in greater abundance in higher water tables (Two-sample bootstrapped Kolmogorov-Smirnov test, $D = 0.8$, $p = 0.04$, Fig. S3.1), whereas *S. fallax* was unaffected by the water table depth (Two-sample bootstrapped Kolmogorov-Smirnov test, $D = 0.2$, $p = 0.793$, Fig. S3.1). Overall, the use of inoculation was the strongest driver of aboveground community composition (Fig. 3.2, Table S3.1).

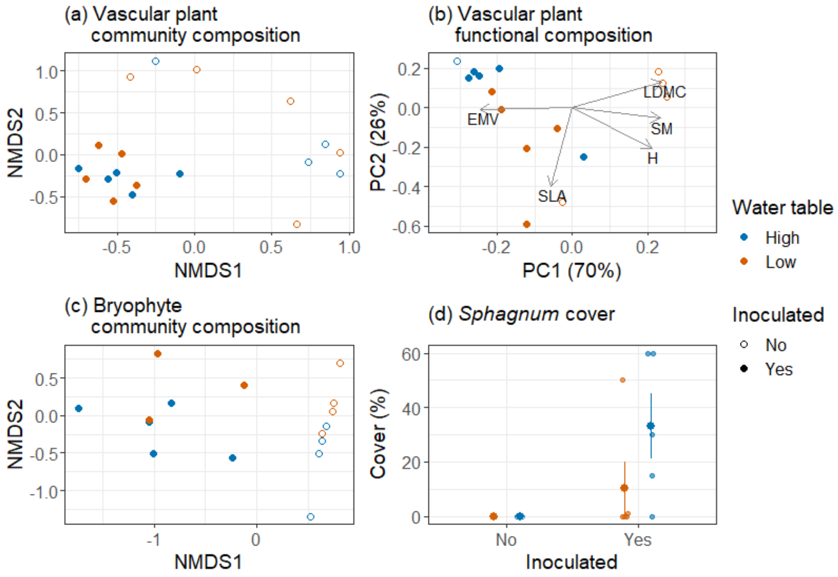

Figure 3.2: Effect of inoculation and water table depth on peatland aboveground taxonomic and functional composition. a) Vascular plant communities (non-metric multidimensional scaling; *stress* = 0.14); b) Functional composition of recovered vascular plant communities (principal component analysis); c) Bryophyte community composition (non-metric multidimensional scaling; *stress* = 0.05); d) *Sphagnum* cover. EMV = Ellenberg moisture value, SLA = Specific leaf area; LDMC = Leaf dry matter content; Plots with high water table and uninoculated were functionally identical so appear layered in b. Smaller circles in d represent individual plot values Error bars in d represent ± SE. All surveys were recorded 15 weeks following the initial inoculation.

3.3.2 Prokaryote composition

Time (days) since inoculation was the strongest driver of prokaryote composition throughout the course of the recovery (Fig 3.3a-c; Table S3.1). Additionally, both inoculation and water table depth drove changes in the overall composition (Fig 3.3a-c; Table S3.1), with an interaction observed between the two experimental treatments (Table S3.1). There was also an interaction observed between water table depth and days since inoculation (Figure 3a-c; Table S3.1). At the phylum level, the communities were dominated by Actinobacteria, Acidobacteria and Proteobacteria, constituting up to 50% of the entire prokaryote community (Fig. 3.2d-g). Time (days) since inoculation appeared a significant predictor in nine out of the ten most abundant prokaryote phyla best performing models (all except for WPS-2; Table S3.2). The effects were more variable with certain phyla responding to the treatments, and others showing minimal effect, with inoculation present in four of the 10 top phylum models, but only demonstrating a significant effect in one (Cyanobacteria, estimate = -0.023, p = 0.007; Table S3.2). Water table depth was present in four of the 10 top models, but only showed a significant effect in two (Cyanobacteria, estimate = -0.019, p = 0.039; Verrucomicrobia, estimate = -0.020, p = 0.001, Table S3.2). Interactions between water table depth and inoculation were included within three of the top models (Bacteroidetes, Cyanobacteria, Verrucomicrobia) but the effects of these interactions were not statistically significant.

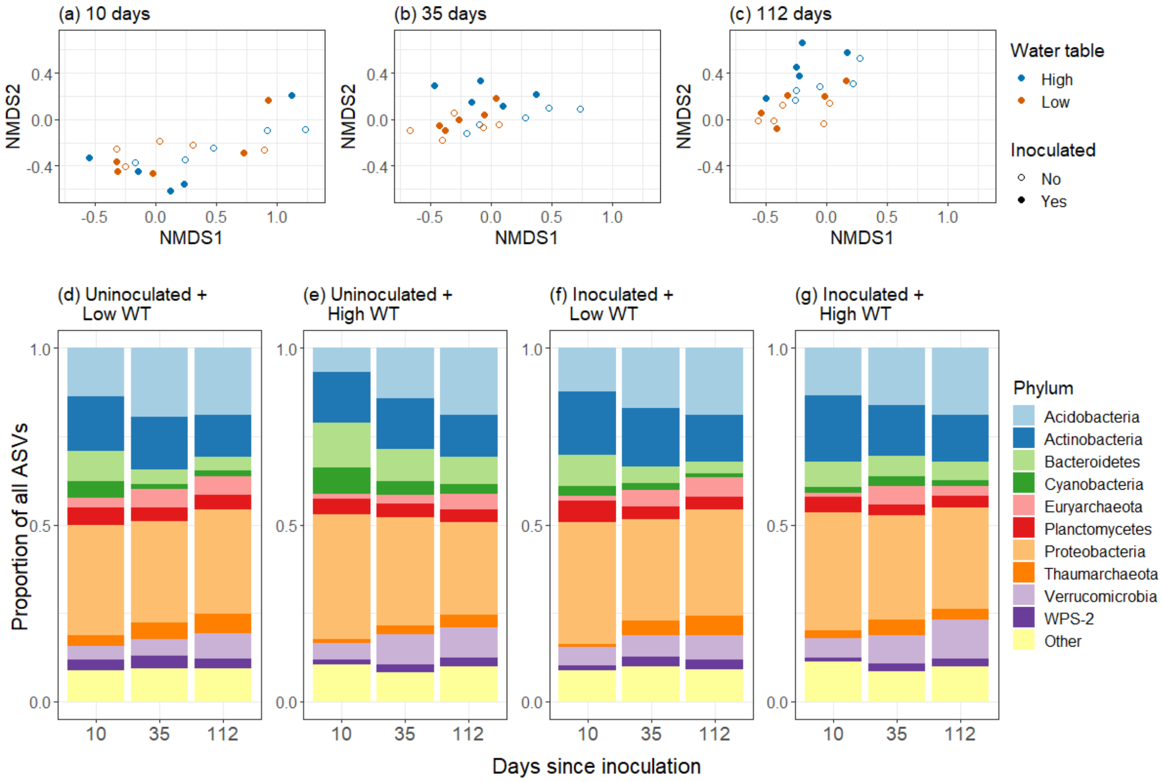

Figure 3.3: Effect of inoculation and water table depth on the belowground bacterial composition during the initial wildfire recovery. a-c: non-metric multidimensional scaling (NMDS) of the initial post-fire community across three different time points throughout the initial recovery (*stress* = 0.18). Each panel is representative of one time point, but is part of the same NMDS, split in order to aid interpretability. d-g) total proportion of ASVs for each of the top 10 most observed phylum across each of the four experimental treatment combinations at three separate time points.

3.3.3 Ecosystem functions

We observed an interaction between inoculation and water table depth on net ecosystem exchange, highlighting that inoculation resulted in increased CO_2 uptake over time when water tables are high (Fig. 3.4a; Table S3.3). This led to an average CO_2 uptake of ~19 g m^{-2} day^{-1} 120 days post-inoculation under a high water table, compared to just ~2.2 g m^{-2} day^{-1} when water tables were lowered (Fig. 3.4a). However, CH_4 production was driven by water table depth and time since inoculation (Fig. 3.4b; Table S3.3), with higher water tables generally resulting in a net release of CH_4 (day 120 average: 0.83 mg m^{-2} day^{-1}) compared to lower water tables which resulted in a net uptake of CH_4 (day 120 average: -0.81 mg m^{-2} day^{-1}). Inoculation did result in changes in pore water composition over the course of the initial mesocosm recovery by increasing the PC1 score of inoculated plots, with a two-way interaction between inoculation and days since inoculation (Fig. 3.4c; Table S3.3). The pore water PC1 itself explained ~54% of the total variation with a higher PC1 correlating with the reduction in concentration of PO_4^-, K^+, and Cl^- among other nutrients (Fig. S3,3). Inoculation also led to higher C:N ratios in the peat soil (ANOVA; $F_{1,19} = 4.6$ $p = 0.047$; Fig. 3.4d), corresponding with reductions in peat N content (Fig S3.4). Finally, higher water tables reduced aboveground biomass (ANOVA; $F_{1,19} = 22.2$, $p < 0.001$; Fig. 3.4e).

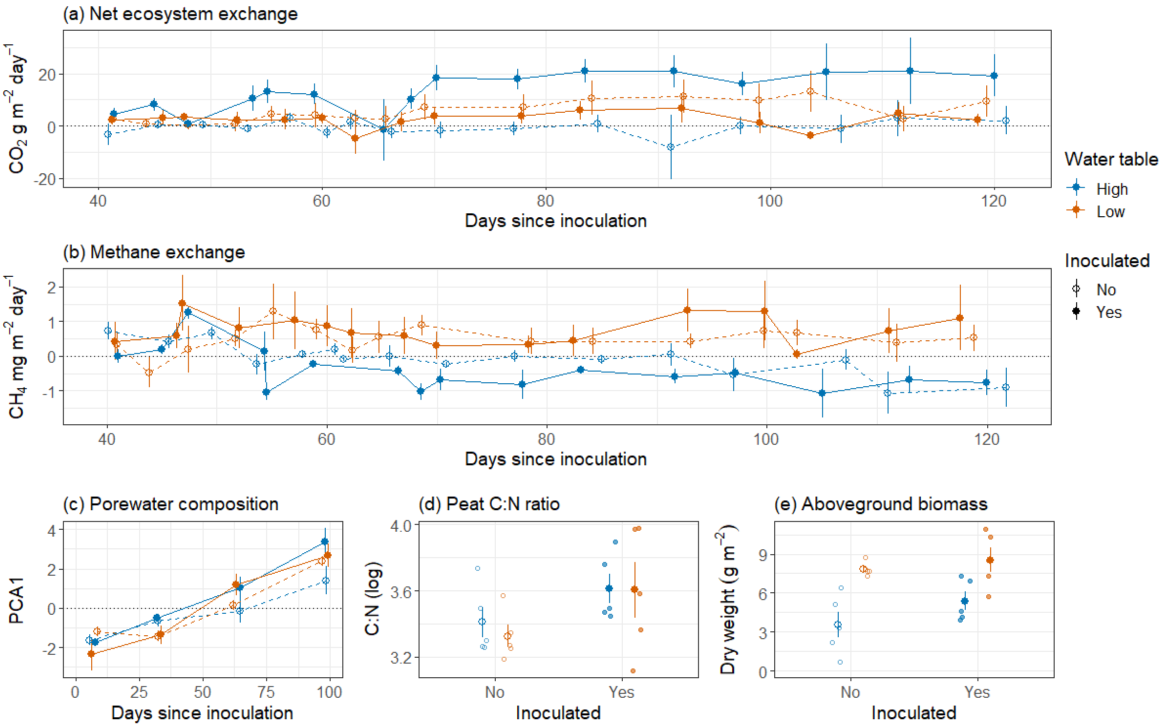

Figure 3.4: Effect of inoculation and water table depth on ecosystem functioning during the initial post-fire recovery. a-c: change over time (days) since inoculation of a) net ecosystem exchange, b) methane release and c) pore water composition. d,e: 18 weeks post-inoculation d) peat C:N ratio and e) aboveground harvested dried biomass (g m^{-2}). Larger circles represent treatment means; intervals represent ± SE. Smaller circles in d, e, represent individual plot values. Data is jittered to aid

interpretation. Pore water PC1 explained 54% of pore water variation (Fig S3.3). Net ecosystem exchange is transformed such that a positive value represents a net uptake of CO_2. Note that days since inoculation in a-c are not aligned.

3.4 Discussion

Soil inoculation has become increasingly recognised as a potential tool for ecosystem restoration (Wubs et al., 2016). Yet, previous studies have largely focused on a select few systems including grasslands and heathlands and only on taxonomic recovery (Emsens et al., 2022; Wubs et al., 2016). Peatland restoration is one example where soil inoculation could enhance recovery but there is limited evidence testing the use of soil inoculation in controlling peatland recovery. Our results show that inoculation can alter the initial community composition of vascular plants, bryophytes, and the prokaryote community, leading to differences in the functioning of the recovered peat, including reductions in pore water nutrient concentrations and increasing C:N ratios of the peat. Notably, inoculation and raising water table depth interacted to control both aboveground (*Sphagnum* establishment) and belowground (prokaryote) compositions, and the initial carbon dynamics by increasing the amount of CO_2 taken up. Therefore, we argue that inoculation could be a useful tool alongside hydrological restoration to enhance the recovery trajectory of degraded peatlands. Moreover, inoculation – as our work shows – aids in the initial taxonomic and functional recovery of a previously unexplored ecosystem type (i.e. peatlands) and may thus be suitable to increase restoration success of other vital ecosystems such as fresh and saltwater wetlands.

3.4.1 Initial taxonomic and functional recovery

Inoculation had a strong effect on the aboveground community composition, altering both the taxonomic and functional composition of vascular plants and bryophytes (Fig. 3.2). This included the presence of common peatland vascular plants such as *Juncus bulbosus*, and *Sphagnum* moss that were both absent without the use of inoculation. Interestingly, we found no effect of raising the water table on the vascular plant community. This is despite the use of inoculation producing a vascular plant community that preferred wetter conditions (Fig. 3.2b). Providing a greater functional breadth of vascular plant colonists could however allow abiotic factors such as water table depth to increasingly shape the community as recovery continues with a greater pool of species to ‘filter’ from (Belyea & Lancaster, 1999). This suggests an initial overriding effect of dispersal limitation on initial vascular plant composition post-fire (Shepherd et al., 2021).

Sphagnum establishment success was doubled when water tables were raised, resulting in a change in bryophyte composition (Fig. 3.2), which suggests a potential interaction between inoculation and water table depth in re-establishing lost peat moss communities. This agrees with previous work which shows that raising water table depths can increase *Sphagnum* recolonisation (Ferland & Rochefort, 1997; Robroek et al., 2009). However, it is important to note that the effects of water table on *Sphagnum* recolonisation were driven largely by *S. cuspidatum*, which prefers wetter conditions (Andrus et al., 1983) and only established in our mesocosms at high water table depths. *Sphagnum* establishment can vary depending on individual species microhabitat preferences (Robroek et al., 2009) and our results suggest that *Sphagnum* establishment may therefore depend on both inoculation and the species chosen for use in the inoculate. Thus, selecting different *Sphagnum* species for inoculation may have resulted in a different vegetation composition. Regardless, inoculation showed clear changes to the taxonomic and functional composition of the initial post-fire aboveground community.

Inoculation did drive a change in the prokaryote composition, but the effect was less pronounced than in the aboveground community (Fig. 3.3, Table S3.1). The driver of these changes remains unclear and could be due to the direct effect of adding a new prokaryote community through inoculation or as an indirect consequence of differences in establishing vegetation that promotes the presence of certain phylum (e.g., increased vegetation cover; Elliott et al., 2015). However, evidence for phylum level effects from inoculation was limited (Table S3.2). The lack of interaction between time and inoculation suggests this could be driven by the direct addition of a new microbial community (Table S3.2). Yet, when focusing on single time points, we did not find strong evidence of an initial change in community composition following inoculation (Fig. S3.5). Consequently, the mechanism behind altered prokaryote communities upon *Sphagnum* inoculation remains unclear. Along with this, water table depth also altered the composition (Fig. 3.3, Table S3.1). However, the strongest driver of prokaryote composition was time, suggesting the initial direction of belowground microbial recovery is largely dictated by the process of post-fire recovery. Abiotic and biotic controls result instead in more subtle changes in community structures, at least in the early post-fire recovery.

Inoculation led to changes in the overall functioning of the mesocosms, reducing the concentration of nutrients (e.g., PO_4^-) in the pore water composition (correlated to increasing PCA axis) and increasing the C:N ratio through reductions in peat N concentration (Fig. 3.4, Fig S3.4). Together, along with raising water table depths, it led to an increase in the uptake of CO_2 (net ecosystem exchange) across the course of the recovery. The drivers of these changes in function are likely to be the shift in composition of the plant community and the belowground microbial communities that together drive peatland functions (Robroek et al., 2015c; Wang et al., 2021). The mechanism behind these changes is largely beyond the scope of this study due to the lack of data on other important drivers of peatland function such as fungi communities (Juan-Ovejero et al., 2020). It does demonstrate that through steering the plant communities and belowground microbial compositions, inoculation can result in changes to ecosystem functions during initial peatland recovery.

3.4.2 Implications for peatland restoration

Rewetting is not always successful in the short term in returning degraded peatlands to their pre-disturbance state (Granath et al., 2016; Kreyling et al., 2021). This could be due to a number of limiting variables, including propagule limitations, and altered microbial communities (Emsens et al., 2022; Shepherd et al., 2021). Consequently, additional restoration action may be required. Additional peatland restoration techniques that are often introduced along with rewetting include removing the top layer of the surface peat, seeding target species and moss layer transfer (Chimner et al., 2017; Huth et al., 2022; Quinty & Rochefort, 2003). This can be with the aim of returning degraded peatlands to their pre-disturbed state, or for use in paludiculture (e.g. *Sphagnum* farming, Temmink et al., 2017). *Sphagnum* inoculation is one potential restoration method that could also be used to enhance the restoration of peatlands by introducing new microbial communities and propagules that can steer peatland recovery back towards pre-disturbance conditions. Our results indeed show that the initial recovery can be directed by the introduction of *Sphagnum* inoculate, resulting in changes to the taxonomic composition and functioning of the recovered community. High water tables also showed a significant role in determining the initial recovery, interacting with *Sphagnum* inoculation, and suggesting that rewetting and inoculation in combination are a promising technique to steer peatland recovery. Notably, our results show that *Sphagnum* inoculation can result in the successful re-colonisation of *Sphagnum* onto wildfire-damaged peat, which is an important component of peatland restoration (Huth et al., 2022; Rochefort, 2000). Consequently, this raises a number of follow up questions that future research should address to determine the effectiveness of soil inoculation in peatland restoration: how long do the effects of *Sphagnum* inoculation on taxonomic and functions last in peatlands? Does inoculation increase taxonomic and functional similarities between restored and baseline (undisturbed) sites? Do the effects of inoculation vary across peatland gradients (e.g., fen-to-bog transitions)? Does inoculate origin (e.g., species, environmental conditions) drive target species establishment? Addressing these questions will allow an increased understanding of the potential role of *Sphagnum* inoculation in peatland restoration. For now, this study provides a stepping stone for further exploring the role in *Sphagnum* inoculation in steering and expediting the initial taxonomic and functional recovery of degraded peatlands.

3.4.3 Soil inoculation as a general tool for ecosystem restoration

Soil inoculation has been shown as a powerful tool to steer ecosystem recovery towards target community compositions (e.g., Wubs et al., 2016). However, much of our understanding in the use of soil inoculation has come from studies focused on a select few systems (e.g., grasslands and heathlands) and on taxonomic recovery. Key carbon-storing ecosystems such as wetlands have received little, if any, attention. Restoration is however increasingly focused on restoring functional properties of ecosystems (Kollmann et al., 2016). If inoculation can be effective in returning key functions lost across a variety of degraded ecosystems - e.g., carbon storage, ecosystem productivity; it could be useful to reach restoration goals set by the United Nations (i.e., UN Decade on Ecosystem Restoration, 2021 - 2030). Our study provides evidence that inoculation can lead to changes in the direction of recovery of peatlands in the short term and that this can impact the functioning of the system. Our work sets the basis for future work to explore the use of soil (or peat moss) inoculate to steer the functional recovery of degraded ecosystems.

The success of restoration action is often limited by numerous different constraints, such as post-disturbance abiotic conditions and dispersal limitations (Pywell et al., 2006). Consequently, despite repeated success in steering ecosystem recovery, soil inoculation may not always be an effective restoration tool (Emsens et al., 2022). Understanding when and where soil inoculation is effective, is key to the techniques widespread use in restoration projects. Our results suggest that abiotic conditions (i.e., water table depth) could mediate the use of inoculation in peatlands. This is unsurprising given the key role of water table depth in determining peatland composition and function (Waddington et al., 2015). In grasslands, soil pH has been observed to mediate the effectiveness of soil inoculation (Emsens et al., 2022). However, inoculation has also been shown to override abiotic conditions (Radujković et al., 2020). Consequently, the relationship between soil inoculation and factors that may mediate its effectiveness remain unclear. Expanding research into soil inoculation across a wider range of ecosystems, such as peatlands, could provide broader insight into the use of the technique. In turn, this may offer greater opportunities for generalities to be drawn and consequently helping to establish soil inoculation as a general tool for ecosystem restoration.

3.4.4 Conclusions

Soil inoculation has been shown as an effective method to steer the recovery of grassland communities following disturbance (Emsens et al., 2022; Wubs et al., 2016), but more research is needed to understand its potential for use in other ecosystems and in restoring lost or diminished ecosystem functions. In this study, we show that soil inoculation can steer both the initial short-term taxonomic composition and functional properties of a wildfire-damaged peatland. In addition, we highlight the importance of abiotic conditions (water table depth) in controlling the post-fire recovery, suggesting rewetting and soil inoculation could be used in conjunction to control the recovery of peatlands. As such, our work provides the stepping-stone in which scaling up inoculation with peat material – ideally from paludiculture – could be explored in the restoration of damaged or degraded peatlands and in the wider recovery of degraded ecosystem functions.

3.5 Acknowledgements

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3.7 Supplementary materials

Table S3.1: PERMANOVA results for the composition of the vascular plant, bryophyte, and prokaryote communities. *p* values < 0.05 are marked in bold.

Community	**Variables**	**Df**	**Sum Sq**	**Pseudo-F**	**R^2**	***p value***
Vascular plants	Inoculation	1	3.624	11.257	0.392	**0.001**
	Water table	1	0.314	0.975	0.034	0.389
	Inoculation: Water table	1	0.157	0.488	0.017	0.701
	Residuals	16	5.151		0.557	
	Total	19	9.245		1.000	
Bryophyte	Inoculation	1	5.174	18.249	0.522	**0.001**
	Water table	1	0.526	1.855	0.053	0.144
	Inoculation: Water table	1	0.242	2.424	0.024	0.419
	Residuals	14	3.969		0.400	
	Total	17	9.912		1.000	
Prokaryote	Inoculation	1	0.517	2.026	0.030	**0.001**
	Water table	1	1.014	3.975	0.059	**0.001**
	Days since inoculation	1	1.304	5.110	0.076	**0.001**
	Inoculation: Water table	1	0.377	1.479	0.022	**0.001**
	Inoculation: Days since inoculation	1	0.205	0.802	0.012	0.342
	Water table: Days since inoculation	1	0.335	1.307	0.019	**0.048**
	Inoculation: Water table: Days since inoculation	1	0.223	0.875	0.130	0.248
	Residuals	52	13.269		0.770	
	Total	59	17.242		1.000	

Table S3.2: Linear mixed model results for each of the top 10 prokaryote phylum observed in the mesocosms. Models where averaging has occurred (see **Methods 3.2**) have a range of R^2 values, ranging from the worst to the best performing model included in the top model subset. Columns 4 and 5 vary depending on whether averaging has occurred, with df and t-values given for single models and adjusted se and z-values given when averages have occurred. p values < 0.05 are highlighted in bold.

	Value	**Se**	**df/adjusted se**	***t*-value/ *z*-value**	***p*-value**
Acidobacteria (Observations = 60, AICc = -198.0, Marginal R^2 = 0.23, Conditional R^2 = 0.52)					
(Intercept)	0.16	0.01	39	19.67	**< 0.001**
Days since inoculation	0.02	0.00	39	5.17	**< 0.001**
Actinobacteria (Observations = 60, AICc = -215.2, Marginal R^2 = 0.16-0.23, Conditional R^2 = 0.33-0.34)					
(Intercept)	0.14	0.01	0.01	15.57	**< 0.001**
Days since inoculation	-0.02	0.00	0.00	3.57	**< 0.001**
Inoculation (Yes)	0.01	0.01	0.01	0.99	0.32
Bacteroidetes (Observations = 60, AICc = -227.6, Marginal R^2 = 0.22-0.34, Conditional R^2 = 0.40 - 0.41)					
(Intercept)	0.085	0.013	0.013	6.374	**< 0.001**
Days since inoculation	-0.016	0.004	0.004	3.793	**< 0.001**
Inoculation (Yes)	-0.022	0.018	0.019	1.181	0.24
Water table (low)	-0.024	0.018	0.019	1.242	0.21
Inoculation (Yes): Water table (low)	0.017	0.023	0.023	0.718	0.47
Cyanobacteria (Observations = 60, AICc = -290.5, Marginal R^2 = 0.26 - 0.38, Conditional R^2 = 0.91 - 0.92)					
(Intercept)	0.046	0.006	0.006	7.831	**< 0.001**
Days since inoculation	-0.011	0.004	0.004	2.638	**0.008**
Inoculation (Yes)	-0.023	0.008	0.009	2.696	**0.007**
Water table (low)	-0.019	0.009	0.009	2.069	**0.039**
Days since inoculation: Inoculation (Yes)	0.007	0.006	0.006	1.090	0.276
Inoculation (Yes): Water table (low)	0.016	0.013	0.013	1.197	0.231
Euryarchaeota (Observations = 60, AICc = -250.8, Marginal R^2 = 0.09, Conditional R^2 = 0.35)					
(Intercept)	0.036	0.005	39	7.460	**< 0.001**

Days since inoculation	0.009	0.003	39	2.749	**0.009**
Planctomycetes (Observations = 60, AICc = -205.0, Marginal R^2 = 0.04, Conditional R^2 = 0.21)					
(Intercept)	0.194	0.006	39	30.956	**< 0.001**
Days since inoculation	-0.009	0.005	39	-1.739	**0.090**
Proteobacteria (Observations = 60, AICc = -193.7, Marginal R^2 = 0.09, Conditional R^2 = 0.37)					
(Intercept)	0.306	0.008	39	39.522	**< 0.001**
Days since inoculation	-0.015	0.005	39	-2.905	**0.006**
Thaumarchaeota (Observations = 60, AICc = -279.3, Marginal R^2 = 0.13, Conditional R^2 = 0.38)					
(Intercept)	0.034	0.004	39	9.107	**< 0.001**
Days since inoculation	0.009	0.003	39	3.448	**0.0014**
Verrucomicrobia (Observations = 60, AICc = -285.4, Marginal R^2 = 0.41-0.44, Conditional R^2 = 0.45 - 0.49)					
(Intercept)	0.075	0.005	0.005	15.24	**< 0.001**
Days since inoculation	0.016	0.004	0.004	4.157	**< 0.002**
Water table (low)	-0.020	0.006	0.006	3.209	**0.001**
Days since inoculation: Water table (low)	-0.003	0.005	0.005	0.599	0.549
Inoculation (Yes)	0.003	0.005	0.005	0.528	0.597
WPS-2 (Observations = 60, AICc = -191.7, Marginal R^2 = 0 - 0.11, Conditional R^2 = 0.42 - 0.46)					
(Intercept)	0.021	0.004	0.003	5.818	**< 0.001**
Days since inoculation	0.001	0.002	0.002	0.823	0.411
Water table (low)	0.004	0.005	0.005	0.811	0.417

Table S3.3: Linear model results for mesocosm carbon fluxes (CO_2 and CH_4) and pore water composition. *p* values < 0.05 are highlighted in bold.

	Estimate	**SE**	**df**	***t*-value**	***p*-value**
CO_2 flux (Observations = 319, AIC = 2287.80, Marginal R^2 = 0.255, Conditional R^2 = 0.478)					
(Intercept)	-0.597	2.758	287	-0.22	0.829
Days since inoculation	0.353	0.992	287	0.36	0.722
Inoculation (Yes)	13.932	3.900	16	3.57	**0.003**
Water level (low)	5.615	3.918	16	1.43	0.171
Days since inoculation: Inoculation (Yes)	5.052	1.402	287	3.60	**< 0.001**
Inoculation (Yes): Water level (low)	-16.575	5.529	16	-3.00	**0.009**
Days since inoculation: Water level (low)	2.149	1.420	287	1.51	0.131
Inoculation (Yes): Days since inoculation: Water level (low)	-7.506	2.001	287	-3.75	**< 0.001**
CH_4 flux (Observations = 304, AIC = 763.06, Marginal R^2 = 0.218, Conditional R^2 = 0.488)					
(Intercept)	0.255	0.189	282	1.35	0.179
Days since inoculation	0.343	0.063	282	5.48	**< 0.001**
Water level (low)	-0.876	0.269	18	-3.26	**0.004**
Days since inoculation: Water level (low)	-0.409	0.090	282	-4.57	**< 0.001**
Pore water composition (Observations = 75, AICc = 228.4, Marginal R^2 = 0.721, Conditional R^2 = 0.752)					
(Intercept)	-0.196	0.197	53	-0.99	0.325
Inoculation (Yes)	0.369	0.278	18	1.33	0.2
Days since inoculation	1.238	0.164	53	7.56	**< 0.001**
Inoculation (Yes): Days since inoculation	0.718	0.231	53	3.11	**0.003**

Appendix S3.1: Supplementary methods outlining the modelling process and the R packages used for this studies analysis.

Model selection process. On each full linear mixed effects ‘global model’ we performed a full backwards model selection, trialling all combinations of the model variables in order to produce a subset of models within 6 AICc of the best performing model (Richards 2005). The subset was then further refined to exclude those that have better performing (lower AIC/AICc) simpler models ‘nested’ within them (Burnham and Anderson, 2002; Richards, 2008). We then either took the remaining single model or, in cases where multiple models were still present, took an average model value. In this case, we calculated a 'zero' average as we were interested in the relative importance of different explanatory variables in explaining our response variable (Nagawaki and Freckleton 2011). We followed this procedure for all linear mixed effects models conducted within this study.

R packages required. NMDS plots for vascular were produced and PERMANOVA performed using the package *vegan* (Oksanen et al., 2020). Bacterial community compositions were analysed using the package *phyloseq* (McMurdie & Holmes, 2013). Linear mixed effects models were performed using the package *nlme* (Pinheiro et al., 2022), with *lme4* (Bates et al., 2015) used only for model simulation in *DHARMa* (Hartig 2022). Model selection and averaging was performed using the package *MuMIn* (Barton 2020). CWMs were calculated and principal component analysis (PCA) was performed using the package *FD* (Laliberté, Legendre & Shipley, 2014). Bootstrapped Kolomogorov-Smirnov tests were conducted using the package *Matching* (Sekhon, 2011). All visualisations were produced using *ggplot2* (Wickham 2016).

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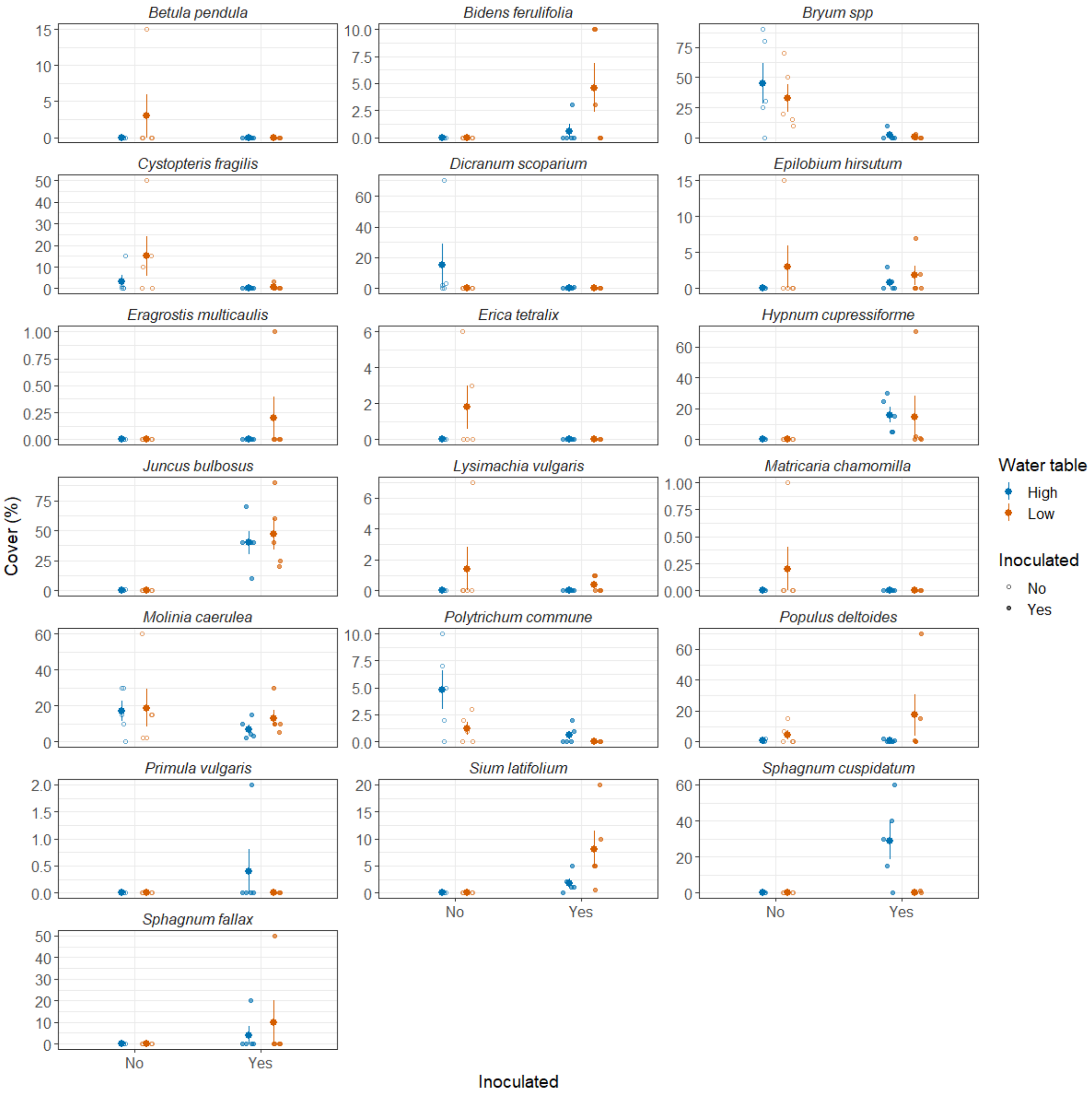

Figure S3.1: Vascular plant and bryophyte species cover (%). Error bars represent ± 1 SE. Points represent individual mesocosms.

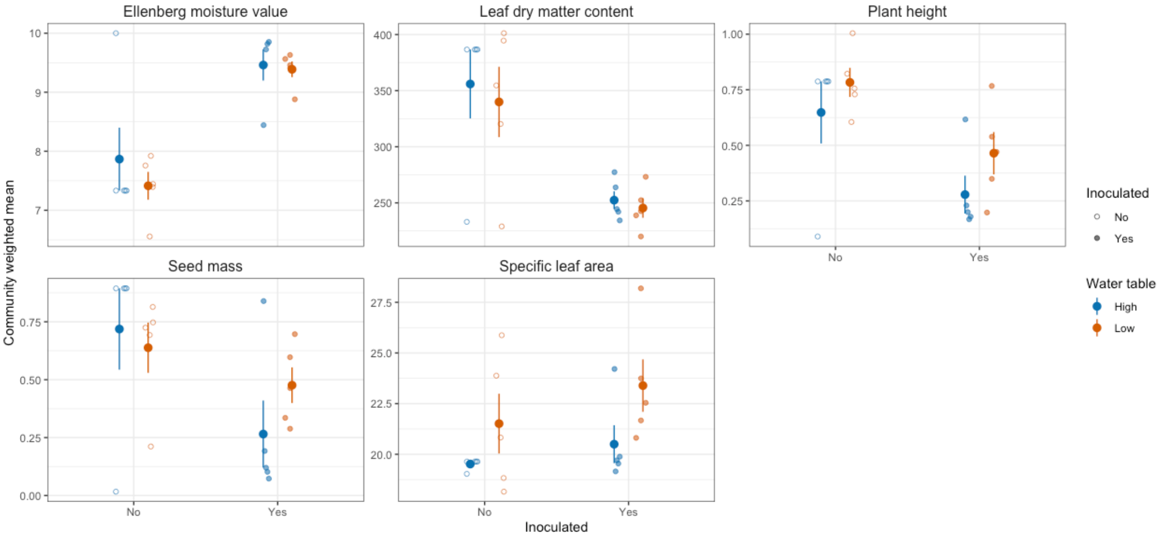

Figure S3.2: Community weighted mean (CWM) trait values for the five selected plant traits. Error bars represent ± 1 SE.

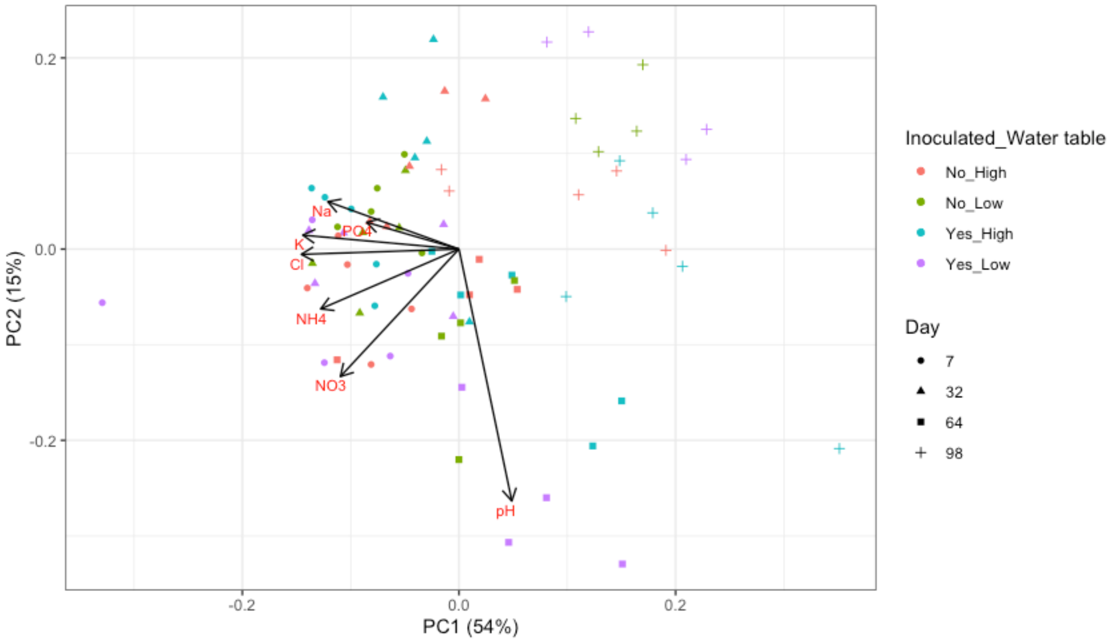

Figure S3.3: Principal component analysis (PCA) of the composition of porewater across 4 different time points representing days since inoculation. Colours represent individual treatments.

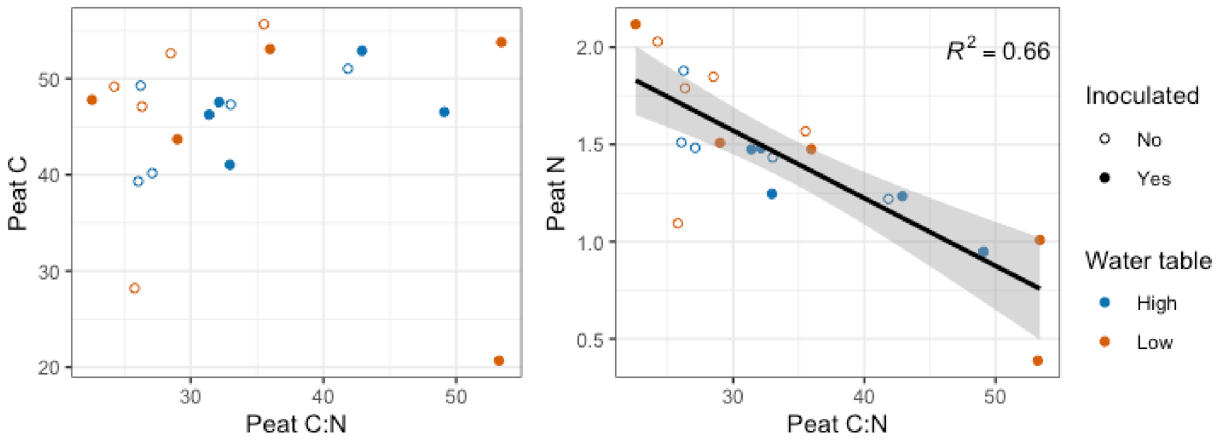

Figure S3.4: Relationship between peat CN ratio and the amount of carbon and nitrogen in the peat soil. Significant linear relationship ($p < 0.05$) and R^2 value are shown on the figure. Envelopes represent 95% confidence intervals.

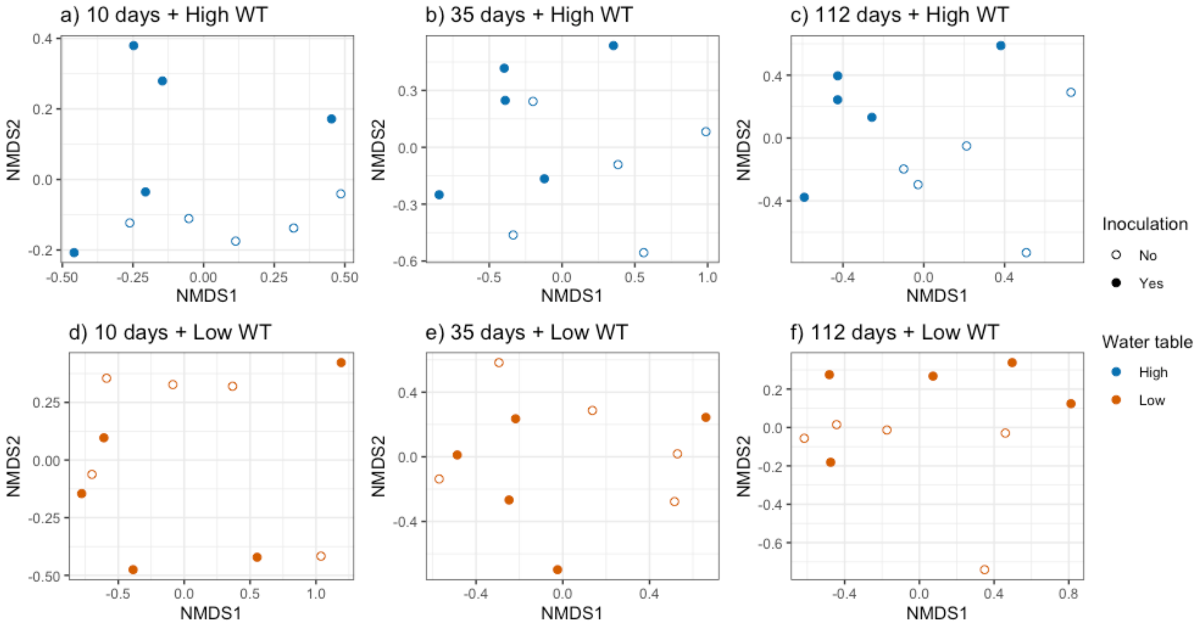

Figure S3.5: Individual non-metric multidimensional scaling (NMDS) for each time period (days since inoculation) in each water table depth. a) 10 days, high water table; b) 35 days, high water table; c) 112 days, high water table; d) 10 days; low water table; e) 35 days, low water table; f) 112 days, low water table

Chapter 4 Plant-microbe networks restructure following long-term loss and recovery of plant functional groups

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Abstract

Plant-microbe networks can become disrupted during species loss. As ecosystems recover and vegetation recolonises, the resilience of plant-microbe networks to reform could have implications for the trajectory of ecosystem recovery. However, at present, it is not clear how resilient plant-microbe networks are following long-term disruption. In this study, we use a plant removal experiment conducted in an ombrotrophic bog to test the ability for plant-prokaryote and plant-fungi co-occurrence networks to recover as different plant functional groups (ericoids, graminoids or both) recolonise, following almost a decade of manual removal. We show that as the aboveground community recovers, the plant-microbe links that form are largely new associations not observed in surrounding undisturbed peatland communities. Plant-fungi networks showed faster signs of recovery than plant-prokaryote networks, displaying both increases in the evenness of interactions and overall connectance of the plant-microbe communities. However, the slow and varied recovery of plant-prokaryote interactions could be the legacy of a drought that occurred during the experiment, potentially disrupting network associations. Overall, our results show a restructuring of plant-microbe networks that occurs as the vegetation recolonises following long-term disruption, highlighting potential implications for the recovery of disturbed or degraded ecosystems.

4.1 Introduction

Interactions between plants and soil microorganisms are key drivers of terrestrial ecosystem functioning (Bardgett & van der Putten, 2014). Plants can modify soil microbial communities through litter inputs and root exudates along with providing microhabitats in root nodules, that can promote changes in soil microbial abundances (van der Putten et al., 2013; Wardle et al., 2004). Together, this can result in a complex web of associations between aboveground and belowground organisms (Ramirez et al., 2018; Wardle et al., 2004) that underpin the performance of ecosystem functions such as soil carbon storage (Lindo et al., 2013; Morriën et al., 2017). However, human-led disturbances such as nutrient enrichment and land-use change can induce shifts in aboveground plant and belowground microbial compositions, including species loss (Adair et al., 2019). This can destabilise ecological networks (Nuwagaba et al., 2017), leading to a weakening of plant-microbe associations (Huang et al., 2019; Lau & Suwa, 2016). If plant-microbe networks are slow or unable to recover following such disruption, then it could inhibit post-disturbance recovery (Moreno-Mateos et al. 2020; Holl et al. 2022), potentially limiting the return of lost or diminished ecosystem functions. Consequently, there is need to understand the resilience of plant-microbe networks to reform following sustained periods of disruption.

Shifts in plant community composition can result in a concurrent change in belowground microbial composition that leads to a restructuring of aboveground-belowground microbial associations (de Vries et al., 2018; Fanin et al., 2019; Hagedorn et al., 2019). Take as an example a plant community consisting of six species, three species of which come from one of two functional groups: ericoids and graminoids (Fig 4.1a). In natural conditions this community has an array of positive and negative associations between plant species and soil microbes (Robroek et al., 2021). Under the influence of human-mediated global change, it is foreseeable that a shift in vegetation is observed, with one functional group becoming locally extinct or rare and the other becoming dominant in the overall community composition (Fig 4.1b). This could be representative of shrubification observed in peat bogs following drainage or managed burn regimes (Noble et al., 2019a; Talbot et al., 2010), increased grass dominance following nitrogen enrichment in temperate grasslands (Song et al., 2012) or livestock grazing promoting the abundance of short annual plants in marshes (Jones et al., 2011). Plant species that remain are now able to create new plant-microbe associations, including with soil microbes previously associated with the now-absent biota (e.g., graminoids, Fig 4.1b). As disturbance events can last long periods of time (> 10 years), this could allow the formation of strong plant-microbe associations between the new resident plant composition and soil organisms (Morriën et al., 2017).

Following the cessation of a human-mediated disturbance, a return to prior conditions could facilitate the return of previously lost species (but see Isbell et al. 2013). Species that were previously removed from the local community due to environmental filters or competitive exclusion could now recolonise, either naturally through propagule dispersal from undisturbed plant communities or through targeted restoration action such as seeding. When they arrive, they must establish among the new conditions modified by the established plant community (i.e. niche modification, Fukami 2015). This includes shifts in belowground microbial compositions and changes in resource availability (Debray et al., 2022; Kardol et al., 2013). Together, this can alter both the pool of potential microbes from which associations could form and modulate the dependence of plant-microbe symbiosis and thus the relative strength of specific plant-microbe associations (Frater et al., 2018). Given the change in biotic and abiotic conditions there are a number of potential scenarios upon species recolonisation. Firstly, it is possible that returning species could successfully retain their old network associations (Fig 4.1c). Alternatively, it could result in the formation new plant-microbe links with microbes not previously associated with plant species (Fig 4.1d). It is also possible that during the process of species loss and recolonisation network associations could become rewired (Kaiser-Bunbury et al. 2010; Robroek et al. 2021; Fig 4.1e) whereby soil microbes may have continued to associate with the individual species within the vegetation community, but the taxonomic identity of the plant has switched (Fig 4.1e). Importantly, if during the network restructuring (Fig 4.1b) the retained vegetation has itself 'rewired' onto the microbes previously associated to the lost vegetation, then in order to re-establish that association the recolonising species must 'invade' into the restructured network (Fig 4.1c) which could itself limit the ability for intact associations to reform. Depending on the ability of recolonising plants to reform or restructure their plant-microbe associations during recolonisation could determine the resilience of plant-microbe networks following long-term disruption and have knock-on effects for the trajectory and functioning of the recovering ecosystem.

In this study we use a long-term plant removal experiment to test the ability for plant-microbe networks to reform following long-periods of disruption and shifts in plant functional compositions. The experiment, conducted in an ombrotrophic bog in Storre Moss national park, Sweden, involved the maintained removal of either graminoid, ericoid or both plant functional groups (PFGs) for nine years (Robroek et al., 2015a). This allowed a restructuring of the soil microbial communities driven by the presence, or absence, of specific PFGs and their interspecific associations. These treatments were then stopped, allowing the recovery of previously removed species through natural recolonisation. Through this process we tracked the aboveground (plant) and belowground microbial compositional recovery and their network interactions with two key peatland microbial groups: prokaryotes and fungi, over the course of three years. In doing so, our work will provide insight into the resilience of plant-microbe networks following long-term disruption.

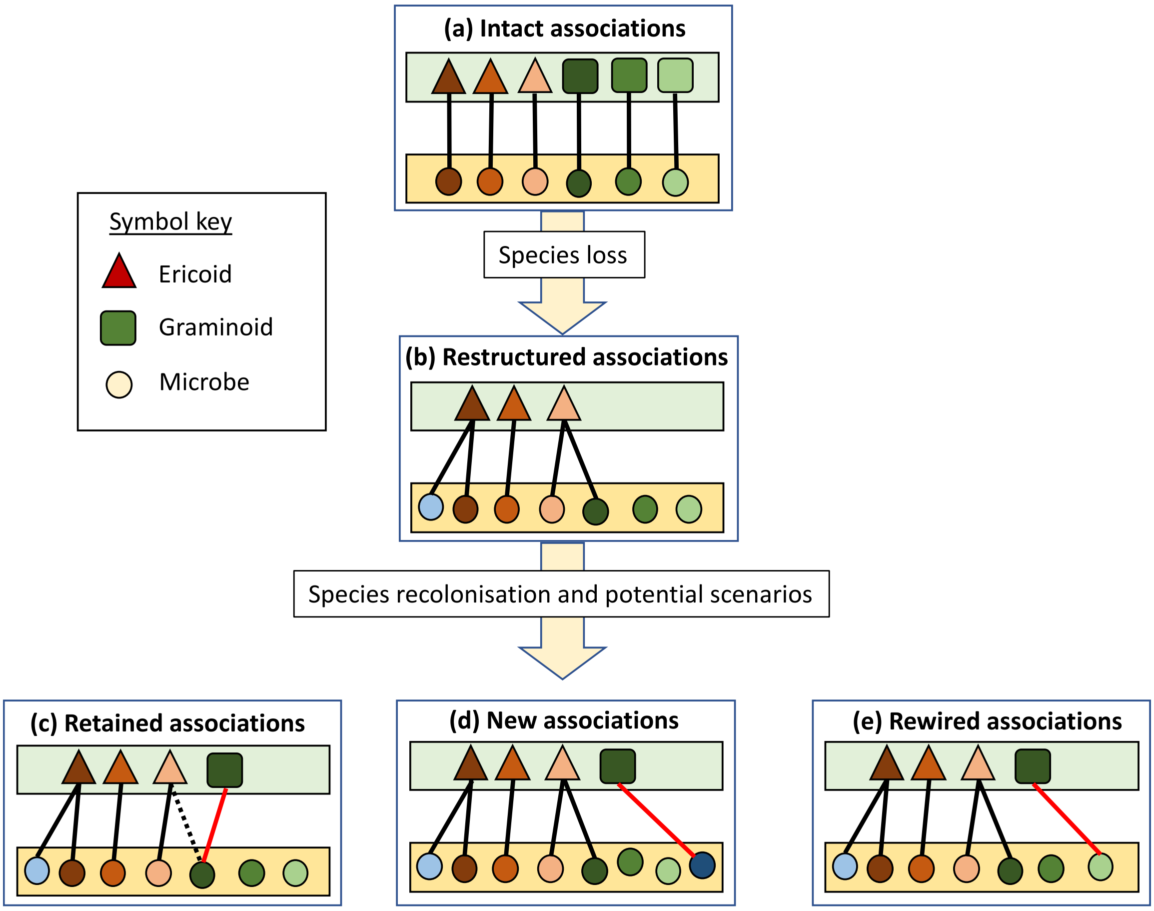

Figure 4.1: Conceptual figure outlining the potential bipartite network associations that could reform following the recolonisation and recovery of previously lost vegetation. The figure depicts a hypothetical plant community consisting of three ericaceous species and three graminoid species. (a) These six species form an array of associations with belowground microbes prior to disturbance. (b) Following the loss of graminoid species due to human activity there is a restructuring of network associations, with remaining biota forming new associations either with new microbes or with those previously associated with the lost graminoid species. As the plant community is reformed through species recolonisation, network associations can either (c) reform as they had previously; (d) form new associations or (e) rewire into a new array of plant-microbe associations. For simplicity, only one graminoid species is shown to recolonise, and the nature of the association (either positive or negative) is not indicated. Red lines highlight

the association that has formed between the recolonising graminoid species and soil microbes. Dotted line represents an association that has been ‘invaded’ by the recolonising species in order to reform their own intact association. Figure adapted from Robroek et al. (2021).

4.2 Methods

4.2.1 Experimental site and setup

In summer 2009, 40 experimental plots were established in a *Sphagnum*-dominated ombrotrophic bog in Store Mosse national park, Sweden (57°17°54 N, 14°00°39 E) (Robroek et al., 2015). These plots were split into two microhabitats characteristic of *Sphagnum*-dominated bogs: lawns (*n*=20) and hummocks (*n*=20). Lawns constitute wet depressions in the local environment with water table close to the peat moss surface (approx. 0 - 5 cm; Rydin and Jeglum (2006)). These microhabitats at the site are dominated by *Sphagnum cuspidatum*, *Rhynchospora alba*, *Trichophorum cespitosum* and *Vaccinium oxycoccos*. Hummocks sit higher above the water table (approx. 20 – 50 cm; Rydin and Jeglum (2006)) and are dominated by *Sphagnum rubellum*, *S. magellanicum*, *Calluna vulgarus* and *Eriophorum vaginatum*. Other species on-site include *Drosera* sp. that are protected within the national park and consequently were not removed from the plots.

Each plot was subjected to one of four experimental treatments (*n* = 5). Plant functional composition was either allowed to remain in its natural state or manipulated with the removal of vegetation. This was either the removal of ericaceous species, graminoid species or both ericoid and graminoid species. Vegetation was initially removed by clipping the aboveground components of the target vegetation at the surface (moss) level during the first summer establishing the treatments. Following on from this, plant removal was maintained by pulling newly established seedlings and their belowground components (e.g. roots) from the plots. Plant regrowth was removed three times a year (once each season excluding winter) and was maintained continuously for 9 years. Following a final removal in July 2018 natural recolonisation and recovery was allowed to proceed.

4.2.2 Plant and soil microbial community recovery

We tracked the recovery of the aboveground plant and belowground microbial communities across the first three years following the cessation of the removal treatments. In the summers of 2019 and 2021 (one and three years of recolonisation respectively) we performed vegetation surveys in all

40 experimental plots. The 2019 survey was performed during the first week of July whereas the 2021 survey was performed during the first week of September. For each survey we used a 1 m x 1 m pinpoint quadrat with 10 cm intervals to measure the composition of the vegetation communities. All surveys were completed by, or under the supervision of the same two observers (HS and MS), to ensure consistent species identification. Vascular plants and *Sphagnum* mosses were identified to the species level. Non-*Sphagnum* bryophytes were identified to genus level. Individual vascular plant species could be measured multiple times per pin measurement and as such cover could exceed 100%. Moss cover was taken at surface level and as such each species was bounded by 100%. The plant coverage from the inside 80 cm x 80 cm was used in analysis, with the outer 10 cm edge of the quadrat excluded to prevent species that are established outside the plot but occur due to lateral growth being included in later analysis.

To determine the belowground prokaryote and fungal community compositions we collected a peat sample from the centre of each plot during each survey year, taken within the same week as the vegetation surveys. Peat was collected from 5 cm below the surface, constituting the acrotolm where plant matter is largely living and consequently is the likely to define the point at which the plant and microbial community has the strongest interaction (Lamit et al., 2021). Extracted peat was stored at 4°C following collection and transported to the laboratory where samples were stored at least at -20°C prior to the extraction of DNA.

4.2.3 DNA extraction, sequencing, and ASV compilation

DNA was extracted from 0.25g of peat using the QIAGEN DNeasy Powersoil kit as per manufacturer’s instructions. Quality and concentration of the DNA was determined using the QIAGEN QIAxpert and Qubit fluorometer for the 2019 and 2021 samples respectively. Samples were then packaged with dry ice and shipped overnight to Novagene Co., Ltd (Cambridge, UK; https://en.novogene.com) for DNA amplification and sequencing. Amplification was focused on two distinct sections of the genome. For prokaryotes, the V4 region of the 16S rRNA gene was targeted for amplification using the 515F/806R primers. For fungi, the Internal Transcribed Spacer 2 (ITS2) region was targeted for amplification using the ITS3 and ITS4 primers. Amplicons were then sequenced on Illumina paired-end platform, generating 250 base pair reads. Once sequenced, we used the DADA2 pipeline (Callahan et al., 2016) to remove chimeric reads, assess the quantity and quality of our sequencing reads and produce an Amplicon Sequence Variant (ASV) table for the microbial sequences. Taxonomies were then assigned to the ASVs using the SILVA (httos://www.arb-silva.de) and UNITE (Nilsson et al., 2019) databases for prokaryotes and fungi taxa respectively.

4.2.4 Plant and soil microbial community recovery

To assess the rate of recovery of each of the vascular plant, *Sphagnum*, prokaryote, and fungi communities we calculated Bray-Curtis distances between each experimental treatment plot and the controls. This was contained between individual years and microhabitats. As there is likely to be a base level of dissimilarity between intact peatland vegetation, we calculated the internal dissimilarity between each pair of control plots.

4.2.5 Plant-microbial bipartite networks

We used bipartite networks to examine the strength of associations between individual plant species and microbial ASVs. We constructed one network for each treatment across each survey year. As such, both microhabitats were pooled together to ensure a range of vegetation covers and to maximise replication. Microbial ASVs and vascular plant species that appeared in less than 40% of all plots within individual treatments per year were removed to avoid the inclusion of rare species. Vegetation and microbial species-abundance matrices were then Hellinger transformed. To produce each network, we calculated Spearman's rank correlations between plant and ASV abundance. We selected correlations that were ≥ 0.7 or ≤ -0.7 and had a *p*-value < 0.05. Each species level co-occurance was then transformed to a binary (0, 1) matrix to indicate the presence or absence of an association between plant species and microbial ASV.

To determine the nature of the associations between recovering plant species and microbial ASVs we classified each included network association into one of three categories (Fig 4.1c-e). Retained associations were those that appeared in both control and removal plots during the same year, thus the association remained despite the shift in PFG composition (Fig 4.1c). New associations were those with ASVs that did not have associations in the control plots thus the association was new following PFG removal and recovery (Fig 4.1d). Rewired associations were those were associated with plant species in the control plots and had associations with different species in the treatments, thus that association had become 'rewired' (Fig 4.1e). We also considered that associations could become reversed (same plant-ASV association, different sign) however we observed no occurrence of this. We classified the associations of both established non-removed plant species and recolonising plant species in this way.

To further assess the recovery of the networks over the course of species recolonisation we calculated network interaction evenness (IE_n) and connectance (CN_n) for each network. Interaction evenness is the relative spread of interactions species, with a higher value indicating a more even spread of interactions across species included within the network (Tylianakis et al., 2007), indicative of a more robust ecological network (Kaiser-Bunbury & Blüthgen, 2015). Connectance is the percentage of associations relative to all possible plant-microbe associations that could form and can indicate how tightly connected components of aboveground-belowground microbial networks are (Morriën et al., 2017).

All networks were constructed, and IE_n calculated, using the R packages *bipartite* (Dormann et al., 2009)and *igraph* (Csardi & Nepusz, 2006).

4.2.6 Statistical analysis

We used linear mixed-effects models (LMMs) to assess the recovery of the vascular plant, *Sphagnum*, prokaryote, and fungi communities over the three years of recolonisation. LMMs were constructed using the R package *lme4* (Bates et al., 2015). In each model we considered Bray-Curtis (BC) distance from each control plot as a function of year, microhabitat, and recovering vegetation treatment (none, ericoid only, graminoid only, ericoid + graminoid) along with their two and three-way interactions. Individual removal plots and the comparative control plot were treated as random intercepts to account for plot level variations in both the recovering plots and controls. All potential models were then ranked by Akaike information criterion (corrected) (AICc), with those within 6 AICc of the best performing model retained. Within this subset, models were then removed if they contained a nested better performing model (Richards, 2007). We then assessed the remaining model subset for alternative models. As all non-best performing models were subsets of the best performing model, we selected the best performing model for further analysis. ANOVAs were then used to assess the difference in BC means for the different explanatory variables within the best performing model. Model performance and assumptions (normality or residuals, homogeneity of variance) were determined using the R packages *performance* (Lüdecke et al., 2021) and *DHARMa* (Hartig, 2021). Finally, as we were largely focused on the recovery of the vascular plant community, and the best performing vascular plant model involved a 3-way interaction between year, microhabitat, and the identity of recolonising vegetation, we produced a linear model for each microhabitat and year with vascular plant dissimilarity a function of recolonising species identity. Subsequent least square means post-hoc tests of each vascular plant dissimilarity models were performed using the R package *emmeans* (Lenth, 2022) with pairwise comparisons through R package *multicomp* (Hothorn et al., 2008).

To additionally assess the differences in community composition between the treatments for each community we constructed PERMANOVAs (permutational multivariate analysis of variance) using the R package *vegan* (Oksanen et al., 2018). In each PERMANOVA: year of recovery, microhabitat and recolonising vegetation were considered as single variables and as two and three-way interactions. Permutations were constrained using plot identity to account for repeat measurements and plot-level variation.

All analysis was completed using R version 4.1.2 (R Core Team, 2021). We consider $p < 0.05$ to represent a significant of an explanatory variable on the response of an aboveground or belowground microbial community.

4.3 Results

4.3.1 Plant and belowground microbial community recovery

As previously removed vascular plant functional groups (PFGs) were allowed to recolonise, the vascular plant community reduced in their dissimilarity from the control plots relative to the internal control plot dissimilarity (Fig. 4.2a; Table S4.1). In the first year of recovery all the removal plots showed greater dissimilarity to the controls than the internal control dissimilarity (Fig. 4.2a; Table S4.1). The only exception to this was in the lawn microhabitats where ericoids alone were recovering (Fig. 4.2a; Table S4.1). However, in the third year of recovery only plots that had both PFGs (ericoids + graminoids) recovering showed a greater dissimilarity (Fig 4.2a; Table S4.1). Recovering plots also increased in plant cover over the three years of PFG recovery, with graminoids generally showing greater increase than ericoids over the recovery period in the plots they had been previously excluded from (Fig. S4.1). However, this did vary at the species level (Fig. S4.2). For example, out of the three abundant graminoids *Rhynchospora alba* and *Eriophorum vaginatum* increased in cover more than *Trichophorum cuspitiosum* (Fig. S4.2). Across the course of the three years, we observed signs of successful species recolonisation and an overall recovery of the vascular plant community (Fig. 4.2).

Sphagnum dissimilarity and overall composition was largely driven by microhabitat (Fig. 4.2b, S4.3b; Table S4.2, S4.3). However, plots with recovering vegetation did generally possess greater overall *Sphagnum* cover than intact peatland communities (control plots), a response which was consistent across both surveyed years (Fig. S4.1). In the prokaryote and fungi communities' dissimilarity from the control plots was driven by annual variation and microhabitat (Fig. 4.2c, d; Table S4.3) with the identity of recovering vegetation also having a small but significant effect on prokaryote dissimilarity. In general, the prokaryote community was dominated by Acidobacteria (35%) and Proteobacteria (30%) (Fig. S4.4) and showed a shift in composition between the two survey years (Table S4.2; Fig. S4.3). The fungi community was driven largely by microhabitat (Table S4.2) and was dominated by Ascomycota (52%). However, we were unable to identify 43% of all fungi ASVs so the overall composition of the fungi community remains uncertain (Fig. S4.5).

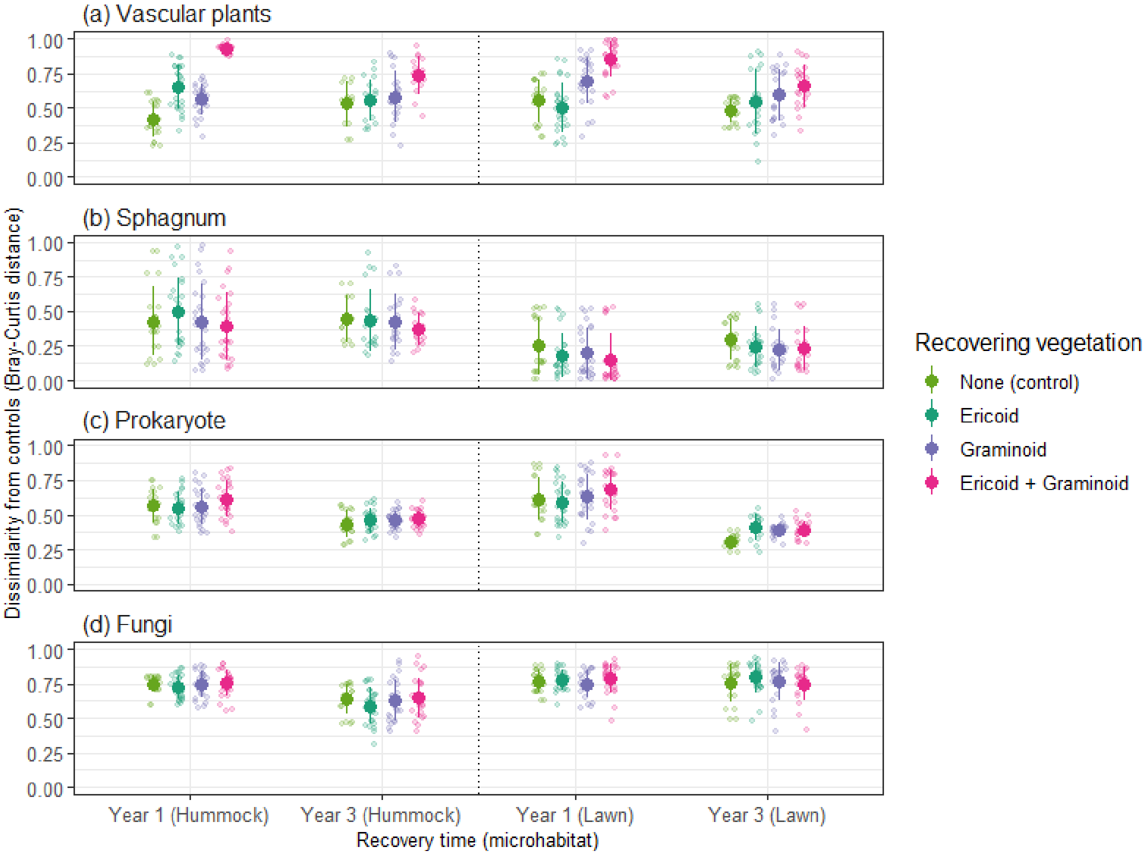

Figure 4.2: Community dissimilarity from control plots. Bray-Curtis distance between the each of the control plots and the other control plots along with plots where vegetation was removed. Hummocks and lawns are compared separately. Small circles represent individual pairwise Bray-Curtis distances. Large circles represent treatment mean. Error bars represent ± SD. Results of post-hoc least squares tests are provided for vascular plant dissimilarities.

4.3.2 Plant-microbe network recovery

Recovering plant-microbe network associations largely consisted of new associations (Prokaryote: 80%; Fungi: 69%; Fig. 4.3-4.4). Rewired associations made approximately one fifth of the association (Prokaryote: 17%; Fungi: 25%; Fig. 4.3 - 4.4). Retained associations were however largely rare in the recovering plant-microbe networks, making up just 3% and 6% of the total associations in prokaryotes and fungi respectively (Fig. 4.3 - 4.4). Despite evidence of vascular plant recovery over time, this was largely consistent between the first and third year of recovery and between plant-prokaryotes and plant-fungi networks (Fig. 4.3 - 4.4). There were a few exceptions where retained and rewired associations contributed to a greater proportion of the overall total (e.g., 45% combined, Fig 4.4f) however new associations always consisted of the greatest proportion of total associations. Of the observed associations, we observed a similar proportion of positive and negative links (Prokaryote: 1707 positive; 1506 negative; Fungi: 280 positive; 284 negative) however this did differ between both plant species identities, year, and removal treatment (Fig. S4.6 -S4.9). Overall, we observed a restructuring of plant-prokaryote (Fig. 4.3) and plant-fungi (Fig. 4.4) networks following PFG loss and subsequent recovery.

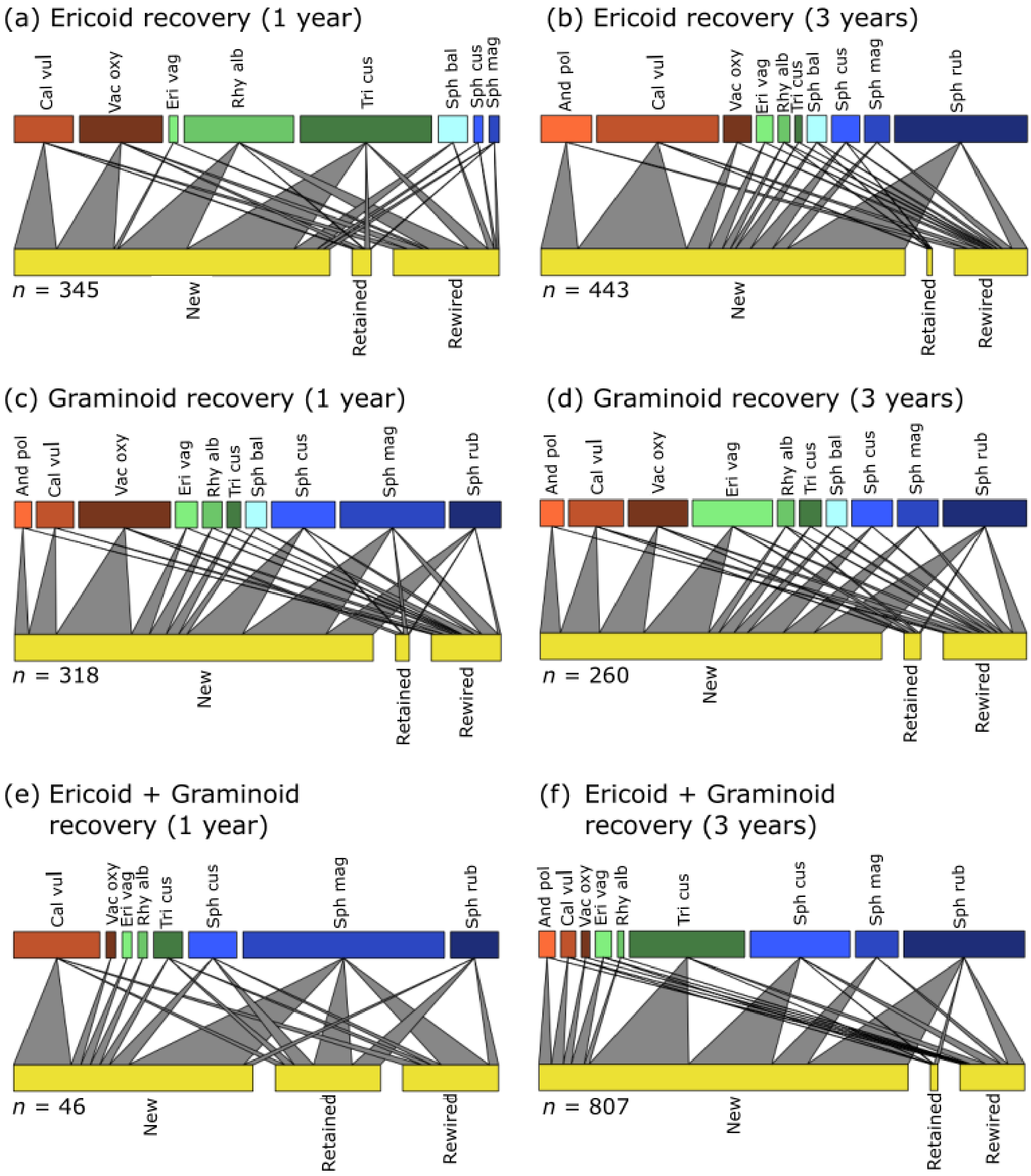

Figure 4.3: Bipartite network visualisation between vegetation and prokaryote amplicon sequence variants (ASVs) in manipulated plant removal plots across three years of species recolonisation and recovery. Network associations are grouped depending on their relationship to control plots, as described in Fig. 4.1. Plants and prokaryotes are visualised on the top and bottom bar of the network respectively. The total number of associations is provided on each network. The size of the bar relates to the number of network associations. Full uncategorised networks are provided in Fig. S4.10. Plant species with the same colour, but different shading, refer to species from the same functional group (orange = ericoid; green = graminoid; blue = *Sphagnum*).

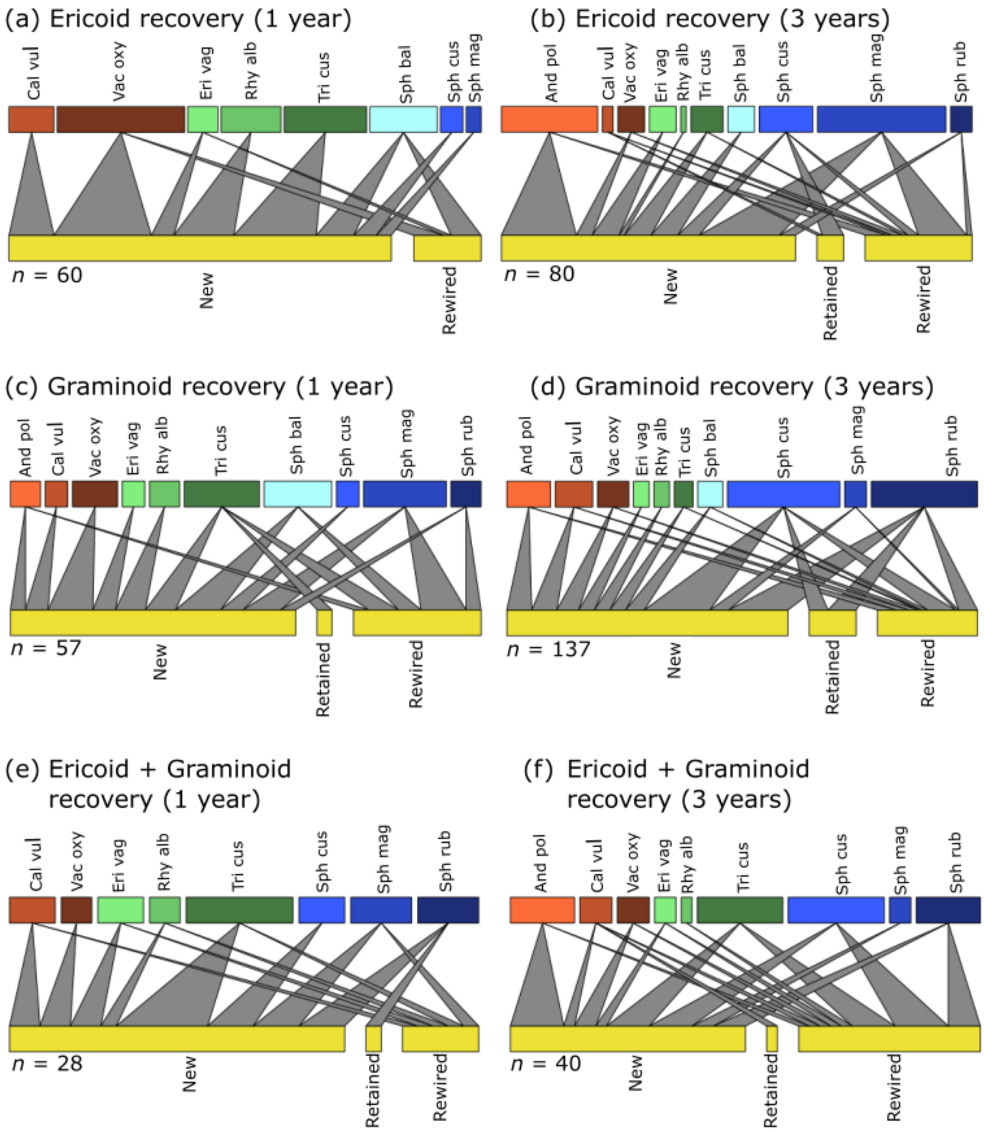

Figure 4.4: Bipartite network visualisation between vegetation and fungi amplicon sequence variants (ASVs) in manipulated plant removal plots across multiple years of species recolonisation. Network associations are grouped depending on their relationship to control plots, as described in Fig. 4.1. Plants and fungi are visualised on the top and bottom bar of the network respectively. The total number of associations is provided on each network. The size of the bar relates to the number of network associations. Full uncategorised networks are provided in Fig. S4.11. Plant species with the same colour, but different shading, refer to species from the same functional group (orange = ericoid; green = graminoid; blue = *Sphagnum*).

Table 4.1: Network properties of each plant-microbe network. Plant-prokaryote and plant-fungi networks are visualised in Fig. S4.10 and S4.11 respectively.

Microbe	**Recovering vegetation**	**Years of recovery**	**Network interaction evenness (IE_n)**	**Network connectivity (CN_n) (%)**
Prokaryote	None (control)	1	0.79	7.2
		3	0.72	1.2
	Ericoid	1	0.76	3.8
		3	0.77	5.2
	Graminoid	1	0.74	3.3
		3	0.73	2.4
	Ericoid + Graminoid	1	0.66	0.6
		3	0.80	9.0
Fungi	None (control)	1	0.70	3.5
		3	0.70	3.9
	Ericoid	1	0.67	2.8
		3	0.69	4.4
	Graminoid	1	0.67	2.0
		3	0.74	8.2
	Ericoid + Graminoid	1	0.64	1.4
		3	0.69	4.2

Over the three years of recovery, the recovering plant-fungi networks all increased both in the number of associations (Fig. 4.4) the overall connectiveness of the networks (CN_n) and the evenness of interactions (IE_n) (Table 4.1). This varied from the control fungi network which showed a decrease in associations (Fig. S4.11) and no change in IE_n (Table 4.1). The plant-prokaryote networks showed greater variation in their response (Fig. 4.3; Fig. S4.10). In plots where ericoids were recovering (ericoids only and ericoids alongside graminoids) we observed similar responses across the three years with IE_n and CN_n all increasing between the first and third years, albeit only marginally when only ericoids were recovering (Table 4.1). However, when just graminoids were recovering, we observed a decrease in IE_n and CN_n (Table 4.1). Overall, we observed differences in the metric response of recovering plant-prokaryote and plant-fungi networks.

4.4 Discussion

Using a decade-long plant removal experiment, we examined the ability of plant-microbe networks to reform following the long-term loss and subsequent recovery of different plant functional groups (PFGs). We show that as vascular plant communities recover towards an intact, undisturbed composition (Fig. 4.2) the plant-microbe associations formed are largely new and not those present in intact undisturbed peatland communities (Fig. 4.3, 4.4). In addition, plant-fungi networks showed greater signs of recovery than plant-prokaryote networks, with increased number of associations, interaction evenness and connectance developing over the course of PFG recovery (Table 4.1). Taken together, our results highlight a restructuring of plant-microbe networks during ecosystem recovery, and potential variation in the rate and direction of recovery of networks dependent on the identity of the microbial taxa.

4.4.1 Plant-microbe networks are restructured during ecosystem recovery

We used peatlands as a model system to test the ability of plant-microbe networks to reform following the long-term loss, and subsequent recolonisation, of different PFGs. To do this, so we set-out three potential occurrences in which plant-microbe associations could reform as the community recovers (Fig. 4.1). New associations were the largest component of the observed links in each plant-microbe network and, with a few exceptions, consistently made up approximately three quarters of all associations (Fig. 4.3, 4.4). Rewired interactions made up the majority of remaining interactions with retained interactions rare in the recovering network compositions (Fig. 4.3, 4.4). There are a number of potential explanations for the high proportion of new associations that formed. Firstly, plant removal could result in changes to the local environment, such as increased warming on the peat surface as a result of reduced plant cover (Fig. S4.1; Grace and Marks 1978). It is possible therefore that the high proportion of new associations is in part driven by changing abiotic conditions that facilitates shifts in the abundance of different microbial groups (Andersen et al., 2013; Birnbaum et al., 2022). Indeed, changing environmental conditions has been previously shown to lead to a turnover in peatland plant-microbe network composition and result in a restructuring of aboveground-belowground associations (Robroek et al., 2021). Additionally, changes in vascular plant compositions could modify species interspecific interactions, such as the interactions between root exudates that influence microbial abundances (Hamard et al., 2019). Driven by one or both of these factors, we did observe differences in both prokaryote and fungi community compositions within the different plant removal treatments (Table S4.2; Robroek et al. (2015)). As such, the new suite of associations could therefore be a legacy effect driven by a changing pool of potential microbes in which new plant-microbe associations can form. It is important to note that the associations in this study are based purely on correlative relationships and as such, are not themselves evidence of an interaction (Blanchet et al., 2020). Regardless, the large switch from intact to new associations suggests that even though plant-microbe networks recover, they are not bound by specific associations. Whether this is important in the context of ecosystem functional recovery remains undetermined. For now, this result shows plant-microbe networks become restructured during PFG loss and subsequent recovery.

4.4.2 Network disruption and recovery was dependent on the magnitude of species loss and the identity of microbial taxa

Species that persist during a disturbance could have a competitive advantage over recolonising species through priority effects (Fukami, 2015). This could limit the ability of recovering plant species to reform their own plant-microbe networks and provide established species a chance to capitalise on the loss of competition and form increasingly complex networks of their own (Fig. 4.1). Our results however did not provide evidence for an inhibitory effect of late arrival on network reformation. Instead, in all cases recolonising PFGs were able to re-establish network associations in the first year of recolonisation and continued to increase their total number of associations by year three (Fig. 4.3, 4.4). Alternatively, the biggest impact on network recovery was the number of species (functional groups) lost. Previous research has suggested the impact of species loss can vary depending on the functional identity of the lost species (Schleuning et al., 2016). Our results however suggest that, in the context of plant-microbe networks, the identity of lost species is not as important as the magnitude of loss. Notably regardless of the species that were lost, *Sphagnum* that remained throughout did not appear to capitalise on reduced competition and instead the loss of PFG had a negative impact on its network associations. These results potentially conflict predictions that reduced resource availability could increase network connectivity (Morriën et al., 2017), with resources including oxygen availability likely to become limiting as vascular plants are removed (Robroek et al., 2016). Instead, this response could be as a result of a loss of complex belowground interactions that exist between the different peatland PFGs (Chiapusio et al., 2018) along with the greater spatial heterogeneity provided by increasing functional diversity (Chroňáková et al., 2019). Rather than the potentially inhibitory effect of species loss on network recovery conceptualised in Fig. 4.1, our results suggest that the return of interspecific interactions between PFGs could drive the recovery of complex plant-microbe networks.

We did however observe differences in the recovery of the plant-prokaryote and plant-fungi networks. Whilst recovering plant-fungi networks increased in network connectance and interaction evenness, plant-prokaryote networks were more variable in their overall recovery. One potential explanation for this is a drought that occurred in Storre Moss during July 2021. The legacy effect of reduced precipitation was distinct in the prokaryote compositions, with phyla such as Verrucomicrobia that prefer wetter conditions rare in the 2021 communities and Firmicutes, which have the ability to withstand periods of drought and rewetting, showing large increases in relative abundance as a result (Fig. S4.4; Barnard et al. 2013; Chodak et al. 2015). Indeed, recent evidence has shown prokaryotes to have a greater sensitivity to changes in water availability in peatlands than fungi, which instead respond stronger to plant functional composition (Lamit et al., 2021). Consequently, the difference in response could be due to prokaryote turnover driven by changing water availability that results in a decoupling of plant-prokaryote associations. This could also explain the large increase in network plant-prokaryote associations when both PFGs are recovering, where the loss of vascular plant vegetation combined with reduced precipitation increases the importance of *Sphagnum* in controlling local environmental conditions and thus the high number of *Sphagnum*-prokaryote associations that form. Consistent with this, in grasslands, prokaryote networks have been shown to be more sensitive to disruption by drought compared to fungi networks (de Vries et al., 2018). As such, our work is in line with the response of soil microbial communities observed in other ecosystems. Together, this highlights that plant-microbe network recovery may vary depending microbial identities along with the environmental conditions experienced during ecosystem recovery.

4.4.3 Plant-microbe network recovery across ecosystems: where next?

The result of this study offers insight into the resilience of plant-microbe networks following long-term disruption. However, there should be some caution when extrapolating out to naturally disturbed or recovering ecosystems. The experimental setup involved targeted removal of PFGs, which is likely to be a rare occurrence in nature. Instead, species loss is likely to coincide with one or multiple ecological stressors that impact various facets of the ecosystem, for example soil properties (e.g., hydrophobicity) alongside species abundances (Rillig et al., 2019). Consequently, even after the cessation of a stressor there is likely to be legacy effects, such as persistent changes in resource availability (Isbell et al., 2013), that could impact the trajectory of recovery and the consequent reformation of plant-microbe networks. As species are removed through human-mediated disturbance it is also possible that new species will colonise that were previously absent, including invasive species, further complicating network recovery (Parra-Tabla & Arceo-Gómez, 2021; Wainright et al., 2021). Thus, there is likely additional context-dependence in the recovery of plant-microbe networks dependent on the nature of prior disruption. Consequently, exploring the ability for plant-microbe networks to reform following a range of different disturbance could be one future avenue of research to further our understanding on the resilience of plant-microbe networks to long-term disruption.

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4.7 Supplementary materials

Table S4.1: Least square means post-hoc test results with pairwise-comparison sidak test.

Recovering vegetation	LS mean	SE	df	Lower CI	Upper CI	group
Year 1 (Hummock)						
None (control)	0.42	0.03	44.00	0.34	0.49	a
Graminoid	0.56	0.03	33.52	0.49	0.63	b
Ericoid	0.65	0.03	33.52	0.58	0.72	c
Ericoid + Graminoid)	0.93	0.02	33.52	0.86	1.00	d
Year 3 (Hummock)						
None (control)	0.53	0.05	52.51	0.41	0.65	a
Ericoid	0.56	0.04	30.51	0.46	0.65	a
Graminoid	0.58	0.04	30.51	0.49	0.67	a
Ericoid + Graminoid	0.74	0.04	40.26	0.63	0.84	b

Year 1 (Lawn)						
Ericoid	0.50	0.04	16.16	0.38	0.62	a
None (control)	0.55	0.05	20.25	0.43	0.68	a
Graminoid	0.70	0.04	16.16	0.58	0.82	b
Ericoid + Graminoid	0.85	0.04	16.16	0.73	0.98	c
Year 3 (Lawn)						
None (control)	0.48	0.05	20.76	0.35	0.61	a
Ericoid	0.55	0.05	20.76	0.42	0.68	ab
Graminoid	0.60	0.05	20.76	0.47	0.73	ab
Ericoid + Graminoid	0.66	0.05	20.76	0.53	0.79	b

Table S4.2: PERMANOVA results for the composition of the vascular plant, *Sphagnum*, prokaryote, and fungi communities. *p* values < 0.05 are marked in bold. Treatment refers to the different plant removal treatments applied prior to the start of the experiment.

	Df	**Sum Sq**	**Pseudo-F**	**R^2**	***p* value**
Vascular plant					
Treatment	3	5.66	12.31	0.29	**0.001**
Year	1	1.04	6.82	0.05	**0.001**
Microhabitat	1	1.10	7.15	0.06	**0.001**
Treatment: Year	3	1.06	2.31	0.05	**0.007**
Treatment: Microhabitat	3	0.90	1.95	0.05	**0.001**
Year: Microhabitat	1	0.14	0.90	0.01	0.384
Treatment: Year: Microhabitat	3	0.45	0.980	0.02	0.353

Residuals	59	9.04		0.47	
Total	74	19.40			
Sphagnum					
Treatment	3	0.47	2.30	0.03	0.079
Year	1	0.03	0.51	0.00	0.215
Microhabitat	1	13.02	191.41	0.72	0.081
Treatment: Year	3	0.02	0.09	0.00	0.899
Treatment: Microhabitat	3	0.38	1.85	0.02	0.084
Year: Microhabitat	1	0.15	2.15	0.01	**0.001**
Treatment: Year: Microhabitat	3	0.04	0.19	0.00	0.681
Residuals	59	4.01		0.22	
Total	74	18.12			
Prokaryote					
Treatment	3	0.59	1.36	0.04	**0.001**
Year	1	2.18	15.18	0.14	**0.001**
Microhabitat	1	2.37	16.45	0.15	**0.001**
Treatment: Year	3	0.37	0.87	0.02	0.757
Treatment: Microhabitat	3	0.49	1.13	0.03	**0.001**
Year: Microhabitat	1	0.51	3.56	0.03	**0.007**
Treatment: Year: Microhabitat	3	0.31	0.72	0.02	0.916
Residuals	61	8.77		0.22	
Total	76	15.59			
Fungi					
Treatment	3	0.98	1.23	0.04	**0.001**
Year	1	1.96	7.39	0.08	**0.001**
Microhabitat	1	3.01	11.36	0.12	**0.001**
Treatment: Year	3	0.62	0.78	0.02	0.717
Treatment: Microhabitat	3	1.01	1.27	0.04	**0.001**
Year: Microhabitat	1	0.52	1.95	0.02	**0.017**
Treatment: Year: Microhabitat	3	0.63	0.79	0.03	0.736
Residuals	62	16.42		0.65	
Total	77	25.13			

Table S4.3: ANOVA results from the best performing linear model for the dissimilarity (Bray-Curtis distance) of the experimental plots vs the control plots, along with control-control internal dissimilarity. Treatment refers to the different plant removal treatments applied prior to the start of the experiment.

Explanatory variable	**Sum Sq**	**Mean Sq**	**Df**	**DenDF**	***F*-value**	***p*-value**
Vascular plants						
Microhabitat	0.00	0.00	1	30.50	0.04	0.844
Year	0.08	0.08	1	19.20	4.44	**0.048**
Treatment	1.52	0.51	3	40.18	29.93	**< 0.001**
Microhabitat: Year	0.01	0.01	1	19.20	0.49	0.494
Year: Treatment	0.52	0.17	3	294.15	10.29	**< 0.001**
Microhabitat: Treatment	0.15	0.05	3	40.18	3.04	**0.040**
Microhabitat: Year: Treatment	0.34	0.11	3	294.15	6.59	**< 0.001**
Sphagnum						

Microhabitat	0.28	0.28	1	27.45	12.25	**0.002**
Prokaryote						
Microhabitat	0.00	0.00	1	26.42	0.36	0.553
Year	1.11	1.11	1	20.63	107.86	**< 0.001**
Treatment	0.09	0.03	3	39.54	3.05	**0.040**
Microhabitat: Year	0.16	0.16	1	20.34	15.58	**< 0.001**
Year: Treatment	0.09	0.03	3	314.52	3.00	**0.031**
Fungi						
Microhabitat	0.067	0.07	1	31.05	8.98	**0.005**
Year	0.04	0.04	1	20.01	5.93	**0.024**
Microhabitat: Year	0.01	0.04	1	20.01	4.79	**0.041**

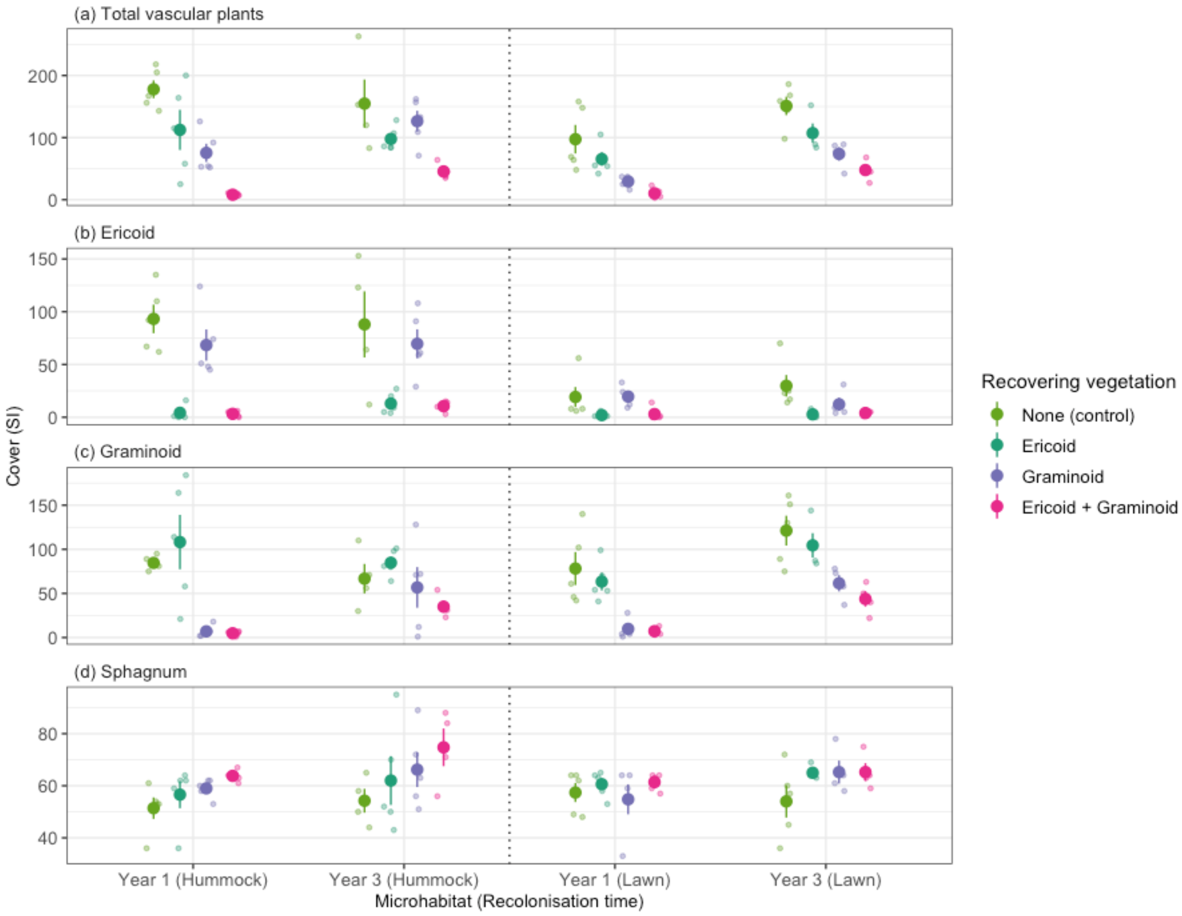


Figure S4.1: Plant cover over the first three years of species recolonisation and recovery. Recorded cover of total vascular plants (a, d), ericoids (b, e) and graminoids (c, f) over the course of three years, following the end of the vegetation removal treatments and the recovery of previously lost vegetation. The vegetation covers are split into hummocks and lawns. Error bars represent ± SE. Cover was measured using a pinpoint quadrat and therefore is a standardised index of cover (SI).

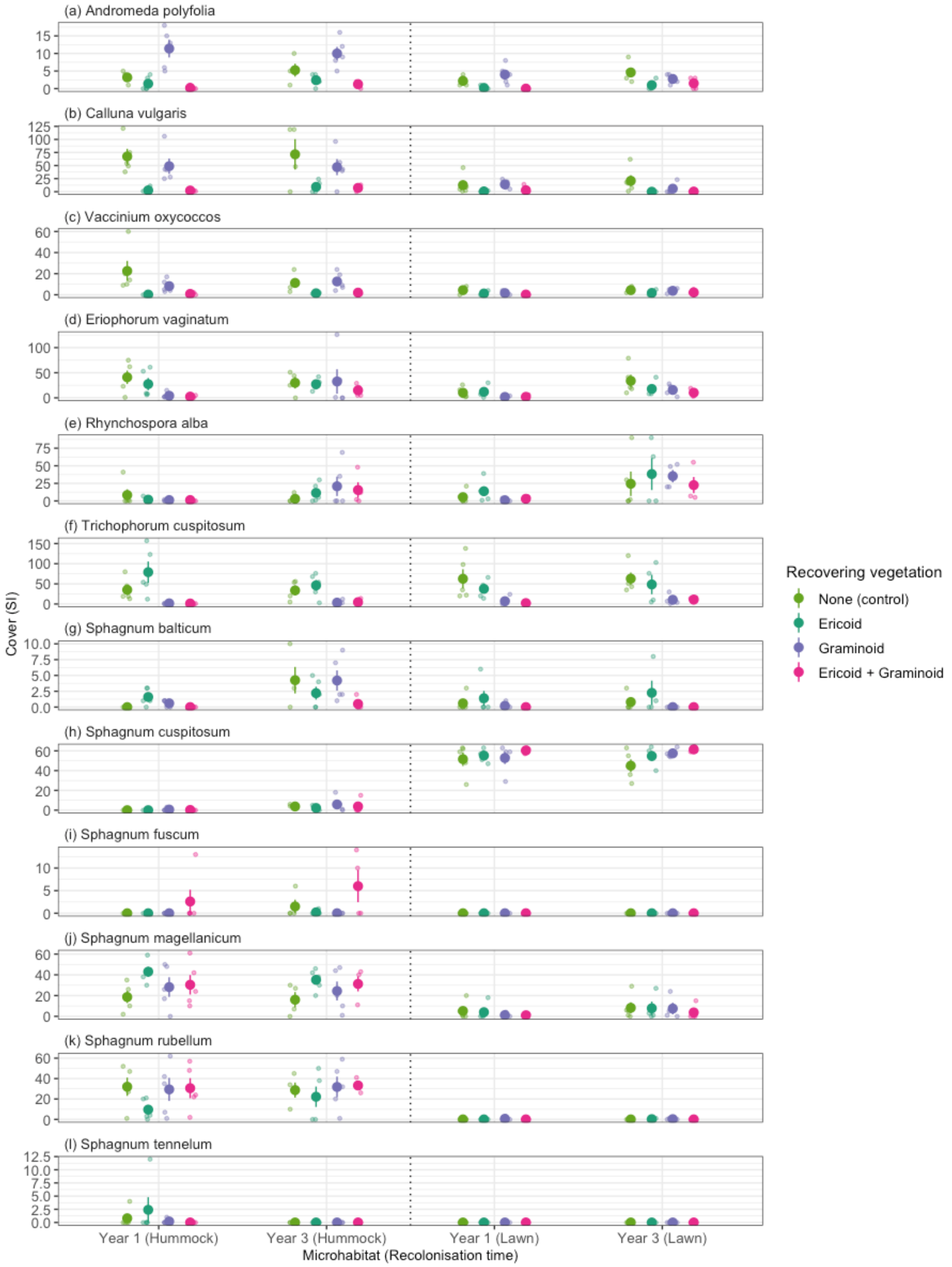


Figure S4.2: Plant species cover over the first three years of recolonisation. Recorded cover of ericoid species (a-c), graminoid species (d-f) and *Sphagnum* (g-l) following the end of the vegetation removal treatments. The vegetation covers are split into hummocks and lawns. Error bars represent ± SE. Cover was measured using a pinpoint quadrat and therefore is a standardised index of cover (SI).

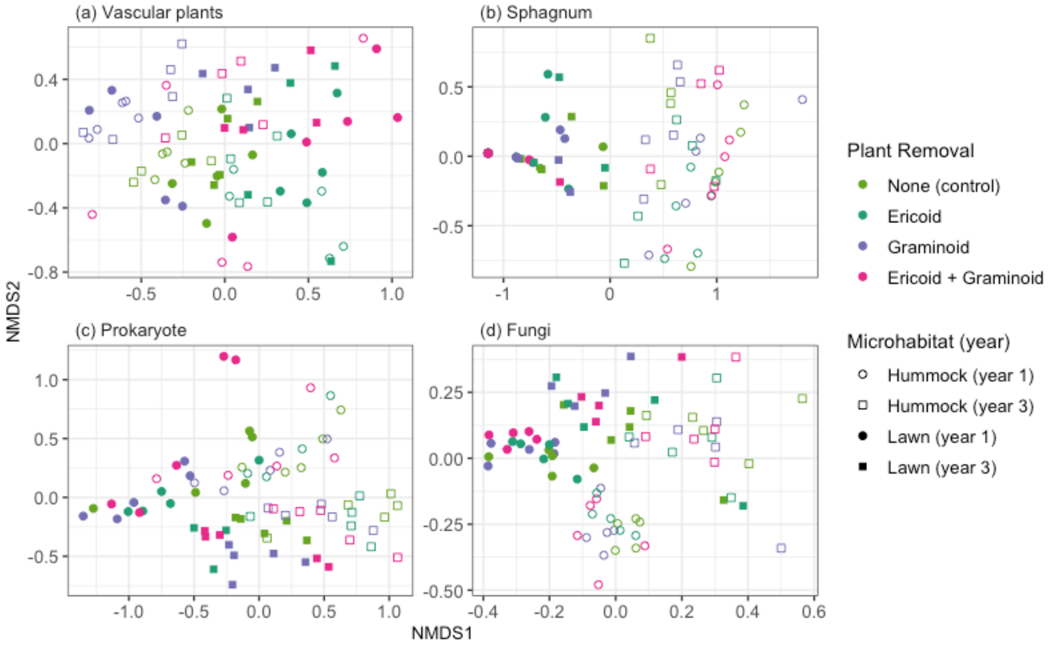


Figure S4.3: Plant and soil microbial community composition over the course of species recolonisation. Each panel represents an individual non-metric multidimensional scaling (NMDS) of the community composition of the aboveground vascular plant (a) and *Sphagnum* (b) communities and belowground prokaryote (c) and fungi (d).

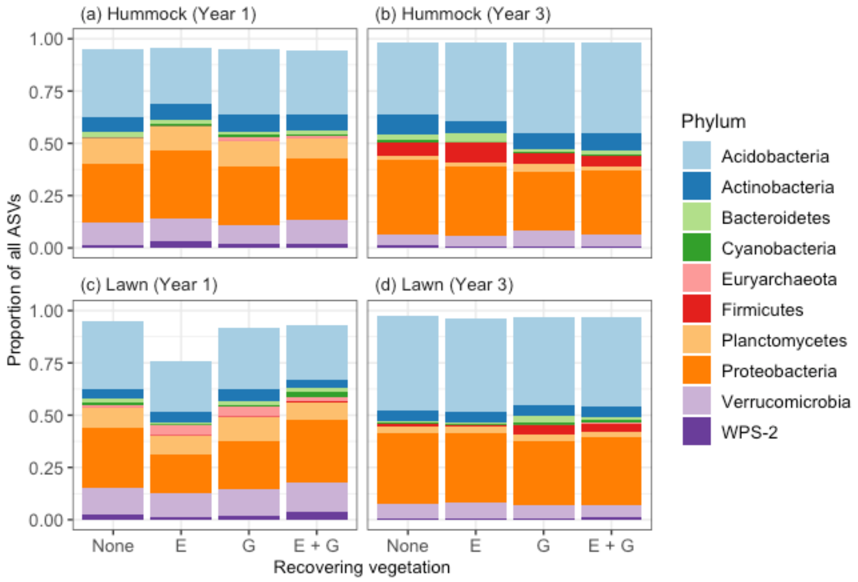


Figure S4.4: Proportional abundance of the 10 most abundant prokaryote phylum observed over the course of species recolonisation.

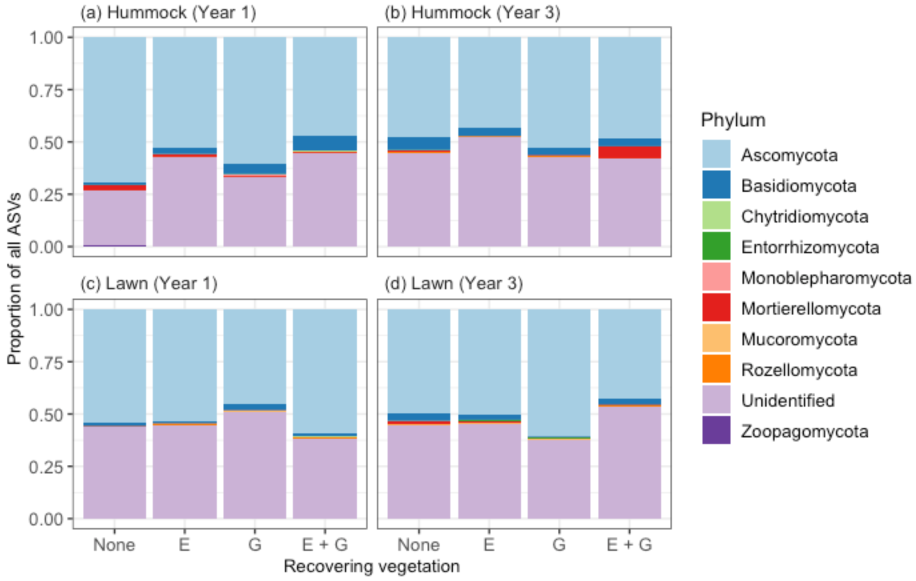


Figure S4.5: Proportional abundance of the 10 most abundant fungi phylum overserved over the course of species recolonisation. Unidentified amplicon sequence variants (ASVs) are included as a 'phylum' to highlight the high abundance of unidentifiable ASVs.

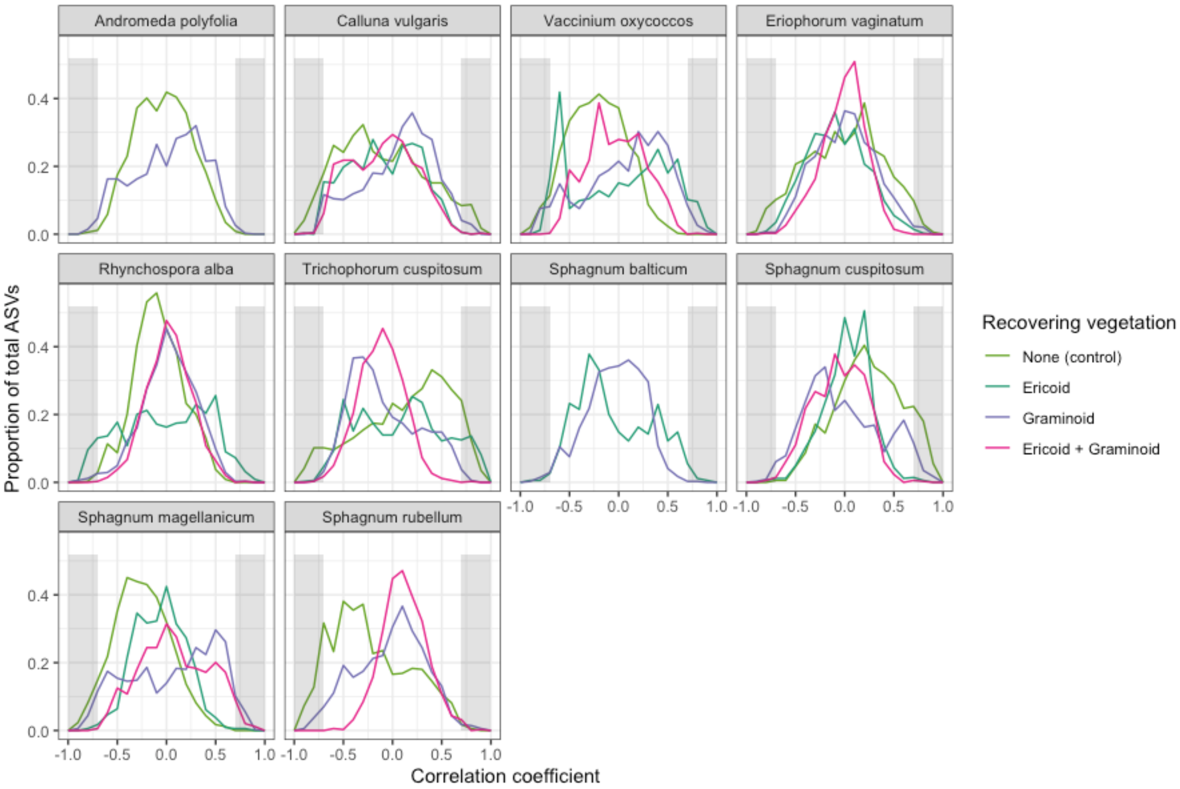


Figure S4.6: Frequency distributions of correlations in prokaryote networks in the first year of species recolonisation. Shaded areas represent those correlations considered for inclusion as an association between plant and microbe. Proportion is a measure of the total amount of ASVs observed within this year summed across all the experimental treatments.

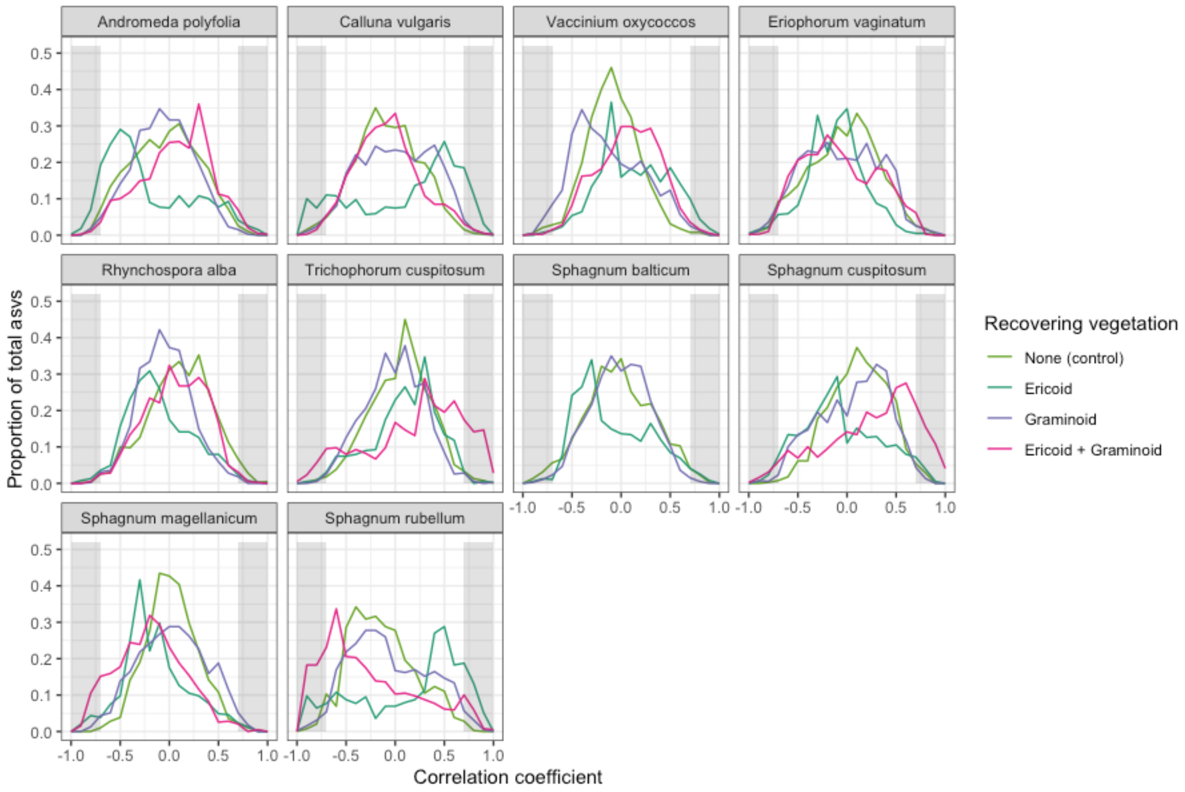


Figure S4.7: Frequency distributions of correlations in prokaryote networks in the third year of species recolonisation. Shaded areas represent those correlations considered for inclusion as an association between plant and microbe. Proportion is a measure of the total amount of ASVs observed within this year summed across all the experimental treatments.

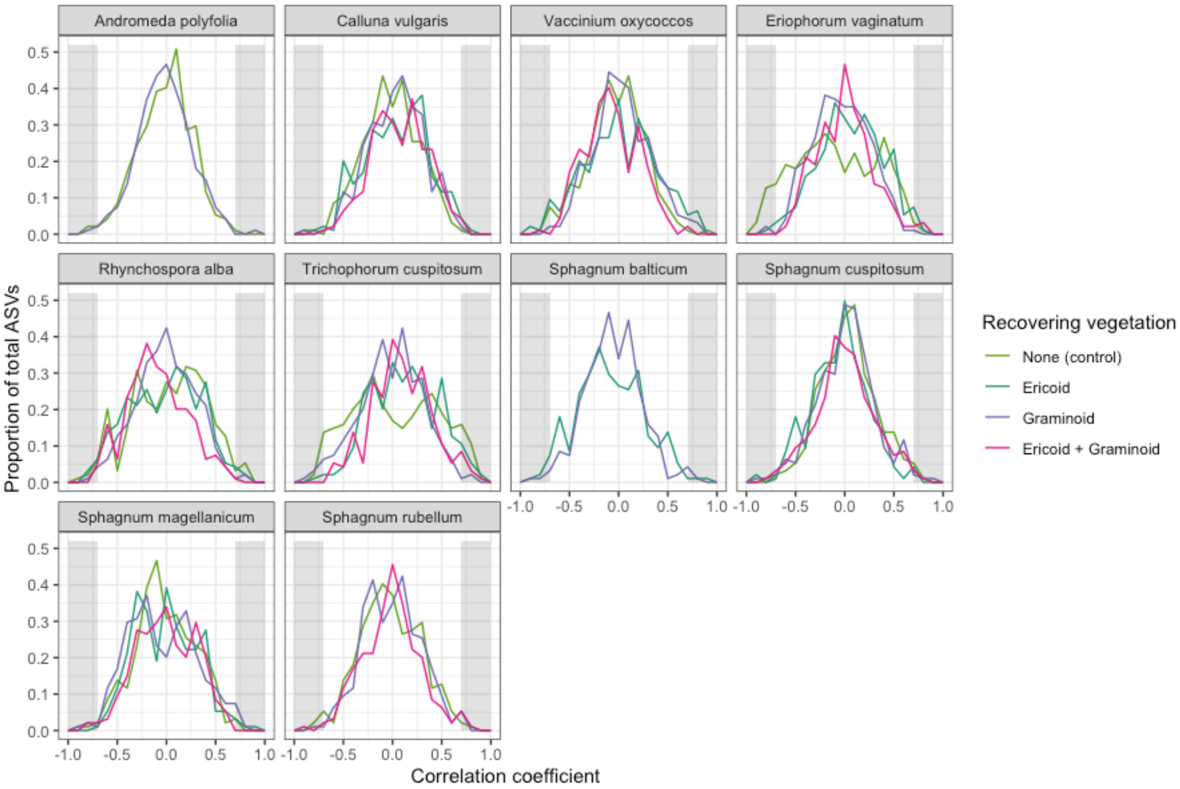


Figure S4.8: Frequency distributions of correlations in fungi networks in the first year of species recolonisation. Shaded areas represent those correlations considered for inclusion as an association between plant and microbe. Proportion is a measure of the total amount of ASVs observed within this year summed across all the experimental treatments.

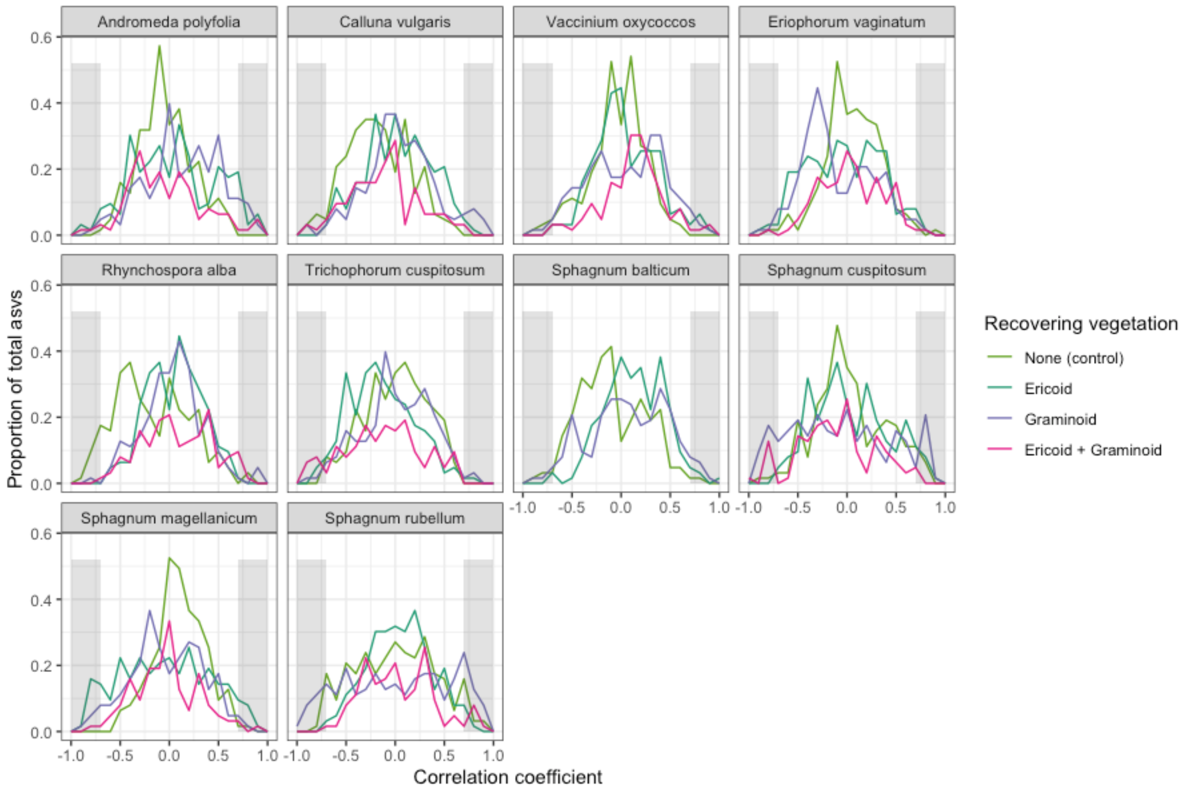
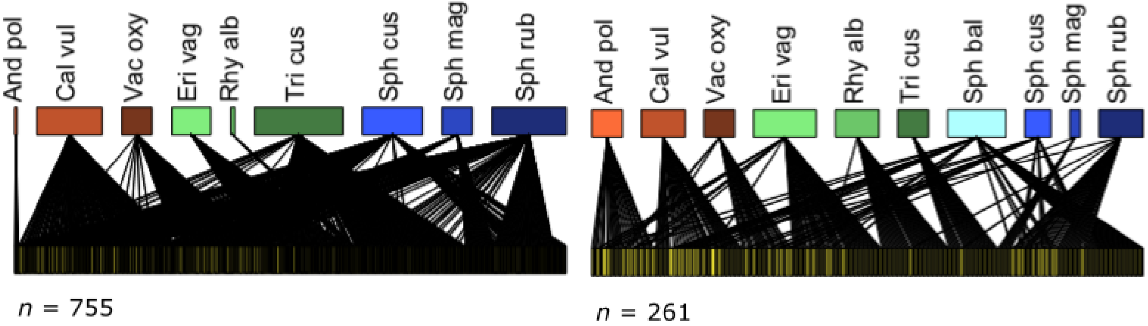
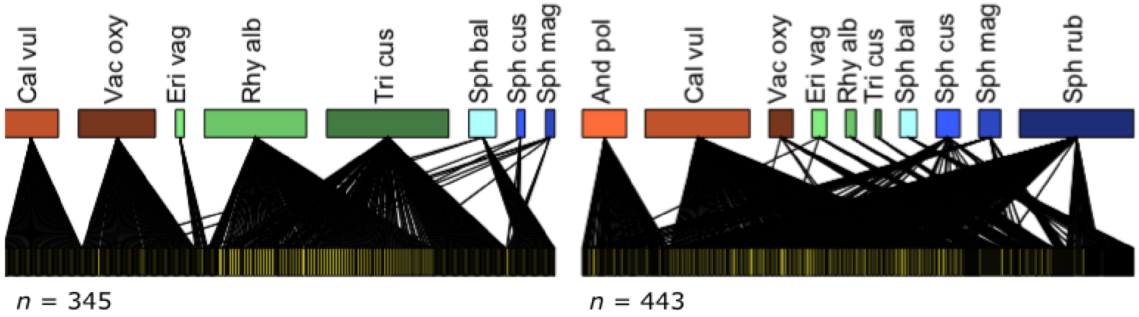


Figure S4.9: Frequency distributions of correlations in fungi networks in the third year of species recolonisation. Shaded areas represent those correlations considered for inclusion as an association between plant and microbe. Proportion is a measure of the total amount of ASVs observed within this year summed across all the experimental treatments.



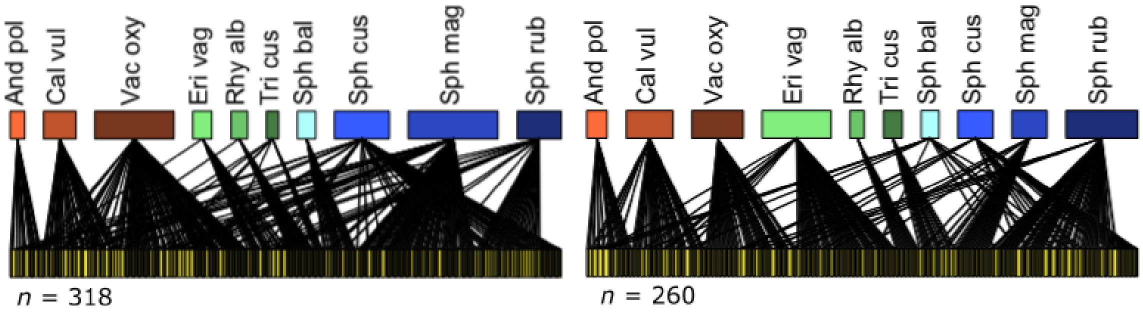
(a) Control (Year 1)

(b) Control (Year 3)



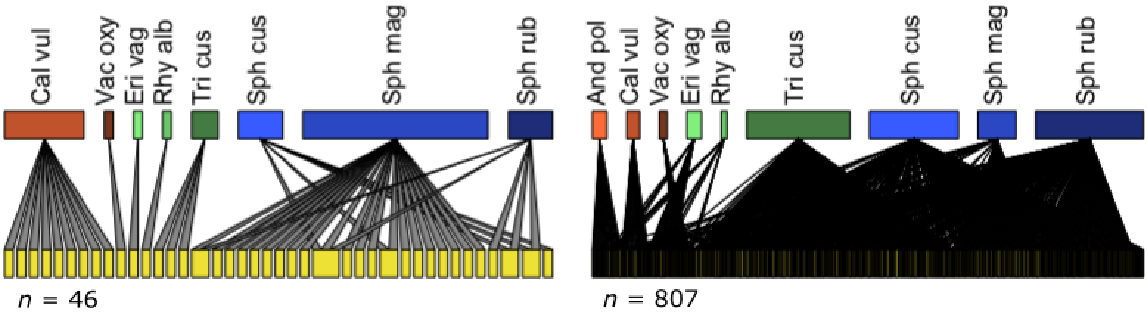
(c) Ericoid recovery (Year 1)

(d) Ericoid recovery (Year 3)



(e) Graminoid recovery (Year 1)

(f) Graminoid recovery (Year 3)



(g) Ericoid + Graminoid recovery

(h) Ericoid + Graminoid recovery

Figure S4.10: Bipartite network visualisation between vegetation and prokaryote amplicon sequence variants (ASVs) in manipulated plant removal plots across multiple years of species recolonisation. Plants and prokaryote are visualised on the top and bottom bar of the network respectively. The total number of associations is provided on each network. The size of the bar relates to the number of network associations. Matching colours refer to species from the same functional group. Each line refers to the association between one plant species and one prokaryote ASV. (orange = ericoid; green = graminoid; blue = Sphagnum).

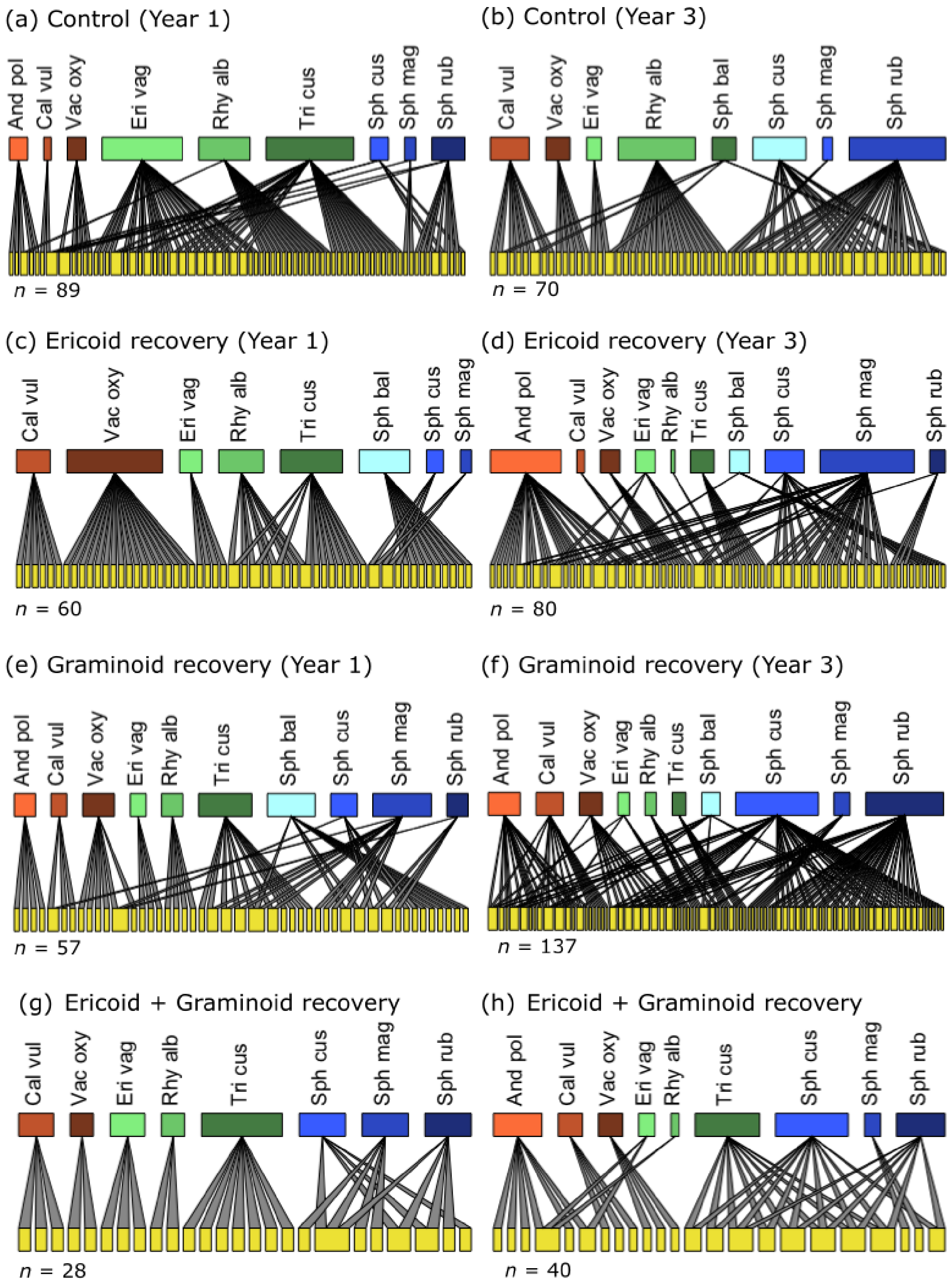


Figure S4.11: Bipartite network visualisation between vegetation and fungi amplicon sequence variants (ASVs) in manipulated plant removal plots across multiple years of species recolonisation. Plants and fungi are visualised on the top and bottom bar of the network respectively. The total number of associations is provided on each network. The size of the bar relates to the number of network associations. Matching colours refer to species from the same functional group. Each line refers to the association between one plant species and one fungi ASV. (orange = ericoid; green = graminoid; blue = *Sphagnum*).

Chapter 5 General discussion and synthesis

In this thesis I have examined the initial drivers of peatland wildfire recovery (Chapter 2). I then trialed the use of plant-microbe interactions to control peatland post-fire taxonomic and functional recovery (Chapter 3). Finally, I tested the ability of plant-microbe networks to recover following long-term disruption (Chapter 4). In this section, I begin by taking the outcomes from Fig. 1.1 and providing a summary in relation to the results from each chapter and the general conclusions that can be drawn. Following this, I then provide a general synthesis of the broad themes that are drawn from the contents of this thesis. Finally, I suggest three future avenues of research that can build on the work of this thesis. To outline this further, an adaption of the thesis outline in Chapter 1 (Fig. 1.1) is provided below including the key conclusions and implications.

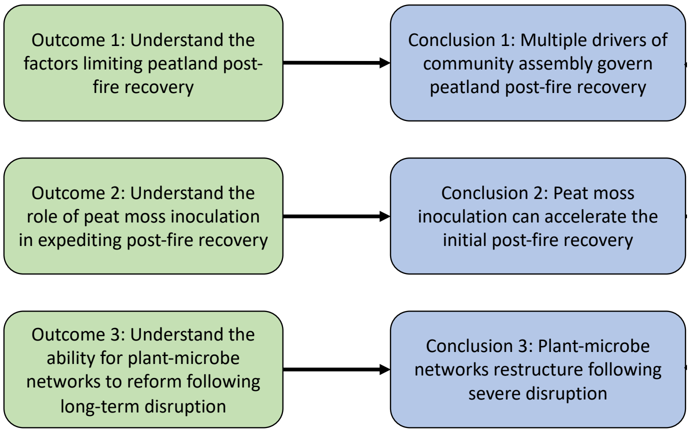


Figure 5.1: An outline of the conclusions drawn from each research outcome established in Fig. 1.1.

5.1 Thesis conclusions

In this section, I summarise the three main conclusions drawn from the chapter outcomes set out in section 1.5 of this thesis and shown again in Fig. 5.1.

5.1.1 Conclusion 1: Multiple drivers of community assembly govern peatland post-fire recovery

The assembly of ecological communities can be determined by propagule availability, abiotic conditions, and species interactions (Belyea & Lancaster, 1999; Kraft et al., 2015). Peatland vascular plant recovery following disturbance can be limited by the availability of desirable propagules (Klimkowska et al., 2019) a phenomenon also observed in the initial post-fire recovery (Chapter 2). Inoculation consequently stimulated a change in post-fire vascular plant composition (Chapter 3). The result from Chapter 2 suggests this is therefore likely through the addition of seeds contained within the inoculate alongside any role from introducing an intact microbial community (i.e., species interactions). However, the relative importance of these two components is difficult to untangle within the experimental set-up.

Inoculation also drove changes to the composition of both *Sphagnum* and belowground prokaryote communities. However, unlike in the vascular plant community an interaction with water table depth was also observed (Chapter 3). As such, at least in the short-term recovery, this suggests bryophyte and prokaryote compositions are co-limited by both propagule availability. In the long-term there is potential for a restructuring of plant-microbe associations to hinder ecosystem recovery (Chapter 4). However, the impact of this is not clear given the artificial nature of the disturbance in the Storre Moss experiment and uncertain role of plant-microbe networks in peatland functioning. Together, the results of this thesis suggest peatland recovery is driven by multiple drivers of community assembly that vary across functional groups.

5.1.2 Conclusion 2: Peat moss inoculation can accelerate the initial post-fire recovery

The restoration of plant-microbe interactions could be an important component of ecosystem recovery (Harris, 2009; Kardol & Wardle, 2010; van der Bij et al., 2018). The restoration of peatland belowground microbial communities has however received little attention, despite the potential for soil microbes to have a disproportionately large role in controlling peatland functions compared to other terrestrial ecosystems (Ritson et al., 2021). Peat moss inoculate expedited the initial recovery of wildfire-damaged peat (Chapter 3) including aiding the re-establishment of characteristic peatland vegetation (*Sphagnum*; Fig 2.2d) and a greater initial uptake of carbon (net ecosystem exchange; Fig 2.4a). Questions remain about the relative importance of soil microbes in directing the recovery, but for now this provides evidence that the use of peat moss inoculate can accelerate the initial post-wildfire recovery.

5.1.3 Conclusion 3: Plant-microbe networks become restructured following severe disruption

Species loss can lead to a destabilisation of ecological networks (Schleuning et al., 2016; Weiner et al., 2014), a response partially observed in chapter 4 (Fig. 4.3, 4.4). As the vegetation compositions recovered and became increasingly similar to the undisturbed plots (i.e. controls) we witnessed an increase in network stability (interaction evenness) and connectiveness, in particular in plant-fungi networks, suggesting that as in other systems peatland plant-microbe networks increase in strength during ecosystem recovery (Morriën et al., 2017). However, this largely consisted of new network associations not present in undisturbed peatlands and as such, the plant-microbe networks became restructured during ecosystem recovery.

5.2 General discussion and synthesis

In this section, I draw on the wider themes that are discussed throughout this thesis with an aim of putting the findings into a broader ecological context.

5.2.1 Functional recovery in ecological restoration

Across this thesis, the focus on the research has not just been on the taxonomic compositions of the communities that are forming post-disturbance, but on the function of the ecosystem. A focus on ecosystem functioning in restoration is not a new idea (Ruiz-Jaen & Mitchell Aide, 2005). Yet despite a functional approach taking center-stage in across many areas of ecology over the last few decades (e.g. Violle et al., 2007), a large proportion of studies on ecosystem restoration either do not explicitly measure functions, or only measure a select few (Kollmann et al., 2016), overlooking others that are less prominently studied or harder to measure (Bliege Bird & Nimmo, 2018). The approach used in chapter 3 specifically, following ecosystem recovery from concurring taxonomic and functional change, helped to offer greater insight into the mechanisms driving post-disturbance recovery than solely examining taxonomic compositions or single functions. This is one way to bring together both taxonomic and functional restoration together, particularly when the link between the two can be uncertain (Cortina et al., 2006), and can aid comparison between studies that focused solely on taxonomic recovery, hereby helping to build on past evidence of restoration success or failure.

Moving forward, a function-centric focus to restoration target-setting may provide more insight into management success with the identity of species present of less importance (Dufour & Piégay, 2009). Historically, taxonomic compositions have been proposed as measures of restoration success (Bakker et al., 2000). However, baselining restoration using historic compositions of species, whilst useful to quantify the impact of restorative action (Stoddard et al., 2006; Suding, 2011; Wortley et al., 2013), only provides measures of past or present conditions and may itself not represent viable taxonomic compositions as global change drivers alter the dynamics of ecological communities (van Dyke et al., 2022). Although it is possible this is less true in peatlands than other ecosystems given the strong link between specific taxa (e.g. *Sphagnum*) and peatland functioning (Rydin & Jeglum, 2006). One complication with using ecosystem functions as baseline measures is ecosystem functions may possess non-linear responses to combinations of stressors (Flory et al., 2022; Rillig et al., 2019; Speißer et al., 2022) complicating our ability to predict functional responses and therefore set reasonable targets or baselines for restoration projects. Chapter 3 did examine the interaction of two global change factors (drainage and wildfire) with the results of this chapter suggesting that interactions between global change factors may mediate peatland responses and recovery. However, there are wide range of potentially interacting factors that can impact terrestrial ecosystems (Rillig et al., 2021) and more work is needed to understand how ecosystems may function under different global change drivers.

5.2.2 Temporal scales in ecological restoration

One of the challenges in this thesis, and across the field of ecological restoration, is understanding the temporal scale in which recovery occurs (Suding, 2011). All three of the chapters examined ecosystem recovery over different timescales (120 days – 3 years) and reconciling the results of studies across different time-periods can make drawing collective conclusions difficult. However, in all cases, these timescales are small compared to realistic periods of ecosystem recovery. In terrestrial ecosystems recovery can for example take decades and even centuries to return to pre-disturbed conditions (Isbell et al., 2019; Nerlekar & Veldman, 2020; Schaller et al., 2022; Schmid et al., 2020). In addition, it is also well recognized that terrestrial ecosystem recovery is often non-linear, with sudden shifts in composition and function (Frouz et al., 2008; Maltby et al., 1990) meaning that initially slow-recovering communities could show rapid acceleration in recovery, overtaking those that may have shown initial positive responses to management for example. To add additional complications, initial positive recovery trajectory does not assure long-term success from management interventions. Indeed, studies on historical restoration action have found in areas that were initially shown to have positive restoration impacts can lose such effects over longer time-periods (Kendall et al., 2021; Matthews & Spyreas, 2010), highlighting the caution that should be applied to results such as those observed in chapter 3. Conversely, a recent study has shown that effects of microbial inoculation of arbuscular mycorrhizal (AM) fungi on grassland diversity to strengthen over a 4-year period (Koziol et al., 2022), suggesting the full impact of inoculation, as an example, may not be abundantly clear within the time frames contained within this thesis. Whilst the results of this thesis can offer insight into both the processes of peatland post-disturbance recovery and the impact of management action on peatland recovery, a consideration of the temporal scale that ecosystems processes recover at and potential non-linearity in responses is essential.

One additional complication with understanding the temporal aspect of ecosystem recovery is how the processes that control post-disturbance recovery may also change through time. Recent evidence has shown both colonisation and extinction within ecological communities to be increasing (Dornelas et al., 2019), resulting in greater turnovers of species. Both chapters 2 and 4 were centered around plant colonisation and therefore depending on how colonisation patterns change over time could alter the processes identified in each chapter, hereby changing the recovery of the ecosystem. For example, increasing rates of colonisation could weaken the dispersal filter observed in chapter 2 if all potentially colonising species arrive at the wildfire damaged site at greater rates, making dispersal no longer limiting. Alternatively, if only certain species increase their rate of colonisation, as is predicted (Dornelas et al., 2019), this could strengthen the role of dispersal filters. Alongside this, increasing extinction and colonisation rates could accelerate the restructuring of plant-microbe networks observed in chapter 4 as species turnover increases. Higher rates of colonisation and extinction would likely weaken the relationships between the plant and soil microbial communities (chapter 4) threatening the resilience of these networks and reducing the complexity of the recovering ecosystems, a crucial component to ecosystem restoration (Bullock et al., 2022; Moreno-Mateos et al., 2020). Changing assembly patterns, largely driven by anthropogenic activity, could therefore alter the processes identified in this thesis and, in doing so, complicate the recovery of disturbed ecosystems over time.

5.2.3 Synthesis of restoration ecology knowledge across ecosystems

Restoration ecology is rapidly building an extensive body of evidence that can inform and enhance our understanding of ecosystem restoration through large-scale data collation and synthesis, allowing generalities amongst management practices to be drawn and to explain apparent context-dependence in the success of restoration projects (Ladouceur & Shackelford, 2021; Wortley et al., 2013). This has led to calls to increase the availability of data and the sharing of ideas across restoration ecology (Ladouceur et al., 2022). In agreement of this call, the results of this thesis highlight the importance of drawing of evidence from across different ecosystems to better inform the restoration-based ecological studies in order to advance current management practices.

Each research chapter in this thesis is built upon evidence-based research from non-peatland systems. Chapter 2 leans heavily on an assembly filter framework by Belyea & Lancaster (1999). Whilst this and other comparable frameworks are by no-means absent from peatland literature (e.g. Belyea, (2004); Robroek et al., (2017)), much of our understanding of the assembly processes of ecological communities has been developed in other study systems. Chapter 3 and 4 lean heavily on evidence taken from non-peatland systems such as grasslands (see chapters for details). By drawing on information from outside peatland literature, this allowed the input of new ideas to peatland contexts and experimental designs adapted from sub-sections of ecology where our overall understanding of the specific processes (such as microbial-driven restoration) is at a more advanced stage. Conversely, whilst this thesis provides examples where peatland ecology could learn from other systems, the same could be said for other systems of peatland ecology. For example, peatland and wetland studies make up the second-most studied ecosystems in restoration ecology (Temperton et al., 2019) and are more likely to consider functions when assessing ecosystem restoration success than studies conducted in any other system (Kollmann et al., 2016). Consequently, peatland restoration studies may possess a greater breadth in understanding of ecosystem functional recovery, information which could help inform the practices and study designs in other systems. Indeed, the results of this study, whilst peatland focused, are based on general ecological principles, and could be applied to other systems as evidence for post-disturbance assembly and ecosystem processes. This is not to say that cross-system sharing of information does not already occur to varying extents, or that the results of this thesis would be directly replicated in other systems. This thesis does however highlight the importance of shared knowledge in ecosystem restoration spanning across a broad range of ecosystems, helping to avoid redundancy through experimental replication and to thus accelerate the advancement of the scientific field.

5.3 Future avenues of research

In the context of the conclusions drawn in this thesis and the above wider synthesis, I propose several avenues that future research could follow to advance our understanding of peatland plant-microbe interactions and to pave the way for enhanced ecological restoration, both in peatlands and across the wider terrestrial area.

5.3.1 Avenue 1: A wider test of peat moss inoculation

Obtaining a full understanding of the use of microbial inoculation to enhance ecosystem recovery requires field trials over relevant time frames and across a range of ecological contexts including disturbance types and environmental conditions (Emsens et al., 2022; Wubs et al., 2016). The results from Chapter 3 demonstrated that peat moss inoculation could accelerate the taxonomic and functional recovery of a wildfire-damaged peatland. However, the study lasted 4 months and took place in controlled greenhouse conditions. As such the study lacked natural variability in environmental conditions and was removed from the full scope of assembly processes that govern post-disturbance recovery (Kraft et al., 2015; Temperton et al., 2004). Consequently, the next step would be to scale this up in terms of both temporal scale and in field locations at disturbed peatlands. Whilst this technique was trialed in an ombrotrophic bogs, fens often show higher variability and lower success in restoration (Chimner et al., 2017). Therefore, expanding trials across different types of peatlands could greater potential to enhance recovery. Importantly, comparisons with undisturbed and unrestored sites are vital in monitoring the performance of this technique and providing a baseline in which restoration can be compared to (Suding, 2011).

5.3.2 Avenue 2: The importance of plant-microbe networks in peatlands

Ecological communities exist in a web of species interactions, forming complex networks of species-species associations (Bascompte, 2009). Recently, plant-microbe networks have received increasing attention (Montesinos-Navarro et al., 2012; Morriën et al., 2017; Ramirez et al., 2018). In peatlands, plant-microbe network analysis is limited (but see Robroek et al., (2021); Chapter 4). The primary outstanding question in peatlands is how important they are aboveground-belowground networks in determining ecosystem function? Addressing this question would provide greater context to the results from Chapter 4. In addition, establishing parallels between the role of plant-microbe networks in peatlands and grasslands for example can allow generalities to be drawn and thus provide (i) a greater understanding of peatland plant-microbe networks and (ii) an ability to draw information across from non-peatland studies to inform future peatland research and management interventions.

5.3.3 Avenue 3: Restoring species interactions in peatland post-disturbance recovery

A focus on returning species interactions rather than solely taxonomic compositions or individual functions could enhance terrestrial ecosystem restoration (Moreno-Mateos et al., 2020). This thesis has focused on plant-microbe interactions in peatland restoration; however, this is just a small component of a complex multi-trophic network of species containing a broad array of diversity including spiders, mites, and nematodes (Rydin & Jeglum, 2006) that may fail to recover following degradation (Elo et al., 2015). The success of peatland restoration can unsurprisingly be focused on the return of the carbon-storage function and accumulation of peat (Lucchese et al., 2010). A greater focus on the return of multi-trophic species interactions, and the restorative techniques that can bring about their return, could help enhance the future resilience of peatlands and maximise the functional recovery of this ecosystem.

5.4 Concluding remarks

The aim of this thesis was to test the use of plant-microbe interactions in the restoration of peatland structure and function along with developing a broader understanding of the mechanisms the govern peatland post-wildfire recovery. The use of techniques such as soil inoculation have been shown to enhance the restoration of terrestrial ecosystems – notably heathlands and grasslands (Emsens et al., 2022; Wubs et al., 2016)– but in this aspect peatland restoration has previously been neglected. In this thesis, I demonstrated that peat moss inoculation could expedite the return of unique peatland biodiversity and drive forward the recovery of key ecosystem functions (Chapter 3). Alongside this, I highlighted the multiple factors controlling initial post-fire recovery (Chapter 2; Chapter 3) and the variable ability for plant-microbe networks to reform following sustained disruption (Chapter 4). Together, this provides the first test for microbial restoration to enhance peatland post-fire recovery and a broader understanding of the ecology surrounding peatland plant-microbe interactions that can be used to inform future research and guide management actions. Most notably, this thesis highlights the potential for plant-microbe interactions to accelerate the recovery of disturbed or degraded peatlands. Consequently, plant-microbe interactions could yet be a useful tool in the restoration of peatland structure and function.

5.5 References

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