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The effects of climate change on the  
competitive ability of *Impatiens glandulifera*  
relative to a native plant community

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Master of Science by Research

Durham University  
School of Biosciences

2022

## Abstract

*Impatiens glandulifera* is one of the most widespread invasive plant species in the UK. This thesis explores how invasive plants such as *I. glandulifera* utilise ‘plant-soil feedbacks’ to alter the biotic and abiotic components of soil in order to facilitate invasions, and how this phenomenon responds to climate change. Native plants commonly co-occurring with *I. glandulifera*, alongside the invader itself, were grown in two experiments simulating different effects of climate change. The first investigated the effects of water availability, with *I. glandulifera* and a native community grown in a range of watering treatments simulating different water availability scenarios under climate change. The second experiment explored the temperature effects of climate change, and consisted of two phases. In the first, exclusive communities of *I. glandulifera* or native plants were grown in two growth chambers simulating present-day and warmer future temperatures. In the second, *I. glandulifera* and a native community were grown in those same pots in the chambers, allowing the effects of invader plant-soil feedbacks to be observed. Plant physical parameters were recorded in both studies, with results confirming that 1) *I. glandulifera* consistently shows a greater competitive ability than native species, even under watering treatments that negatively affect its growth, and 2) that *I. glandulifera* exhibits a positive plant-soil feedback effect, and that this effect can complement the warming effects of climate change to negatively affect a native community. Finally, soil extracts from the temperature experiment had their DNA extracted and sequenced for metabarcoding of the soil bacterial and fungal communities, further investigating the drivers of invasive species plant-soil feedbacks. This analysis exhibited potential effects of *I. glandulifera* soil conditioning on microbial communities, as well as microbial responses to increased temperature under climate change. The findings of these three studies have important implications for future efforts to manage invasive species.

## Table of contents

Abstract .....	2
Table of contents .....	3
Acknowledgments .....	7
Statement of copyright .....	7

### **Chapter 1:** Literature Review – How will climate change affect the role of plant-soil feedbacks in plant invasions?

1.1 Introduction .....	9
1.2 Mechanisms of plant invasions .....	10
1.2.1 Enemy release .....	10
1.2.2 Mutualisms .....	11
1.2.3 Allelopathy .....	11
1.3 Plant-soil feedbacks (PSFs) .....	12
1.3.1 Invasional meltdown .....	13
1.3.2 PSFs and seedling establishment .....	13
1.3.3 PSFs in <i>Impatiens glandulifera</i> .....	14
1.3.4 Inconsistencies in PSFs .....	15
1.4 Impacts of climate change on plant invasions .....	16
1.4.1 Altered microbial community composition .....	18
1.4.2 Disturbance .....	19
1.4.3 Soil litter changes .....	21
1.4.4 Substituting space for time to predict invasions .....	22
1.5 Conclusion .....	23

**Chapter 2:** The effects of water surplus and deficit on the competitive ability of *Impatiens glandulifera* relative to a native plant community

Abstract .....	26
2.1 Introduction .....	27
2.2 Methodology .....	29
2.2.1 Site descriptions .....	29
2.2.2 Native species selection, seed and soil collection .....	29
2.2.3 Experimental design .....	30
2.2.4 Statistical analyses .....	32
2.2.5 Measuring soil water content.....	33
2.3 Results .....	33
2.3.1 Biomass .....	33
2.3.1.1 Aboveground biomass .....	33
2.3.1.2 Proportional <i>Impatiens glandulifera</i> aboveground biomass .....	38
2.3.2 Height .....	40
2.3.3 Soil volume water content .....	43
2.4 Discussion .....	46

**Chapter 3:** The effects of temperature and soil conditioning on the competitive ability of *Impatiens glandulifera* relative to a native plant community

Abstract .....	52
3.1 Introduction .....	53
3.2 Methodology .....	56
3.2.1 Site descriptions .....	56
3.2.2 Native species selection and seed collection .....	57
3.2.3 Soil collection .....	58
3.2.4 Experimental design .....	58
3.2.5 Soil nutrient content .....	61

3.2.6 Statistical analyses .....	62
3.2.7 Soil volume water content .....	62
3.3 Results .....	63
3.3.1 Soil nutrient content .....	63
3.3.2 Biomass .....	65
3.3.2.1 Total biomass .....	65
3.3.2.2 Proportional <i>Impatiens glandulifera</i> total biomass .....	67
3.3.2.3 Aboveground biomass .....	68
3.3.2.4 Proportional <i>Impatiens glandulifera</i> aboveground biomass .....	70
3.3.2.5 Belowground biomass .....	71
3.3.2.6 Proportional <i>Impatiens glandulifera</i> belowground biomass .....	73
3.3.2.7 Root mass fraction .....	74
3.3.2 Height .....	75
3.3.3 Soil volume water content .....	77
3.4 Discussion .....	78

**Chapter 4:** The effects of temperature and on the soil microbial community associated with *Impatiens glandulifera* and a native plant community

Abstract .....	83
4.1 Introduction .....	84
4.2 Methodology .....	86
4.2.1 Sampling .....	86
4.2.2 DNA extraction and amplicon sequencing .....	88
4.2.3 Microbial community analyses .....	91
4.3 Results .....	93
4.3.1 Bacteria .....	93
4.3.1.1 Alpha diversity .....	93
4.3.1.2 Beta diversity .....	97

4.3.1.3 Taxonomic summary .....	99
4.3.2 Fungi .....	103
4.3.2.1 Alpha diversity .....	103
4.3.2.2 Beta diversity .....	106
4.3.2.3 Taxonomic summary .....	108
4.4 Discussion .....	111

**Chapter 5: Final conclusions**

References .....	120
Appendices .....	134

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## **Chapter 1:**

Literature Review: How will climate change affect the role of plant-soil feedback in plant invasions?

## 1.1 INTRODUCTION

One major feature signalling the start of the ‘Anthropocene’ epoch is the mass introduction of species by humans to areas outside their native ranges, either deliberately or accidentally (van Kleunen *et al.*, 2015). These species can become invasive, outcompeting native species and spreading rapidly throughout their new territory. Invasive plants can reduce the diversity of and displace native species, as well as alter the activity and structure of soil microbial and fungal communities (Moroń *et al.*, 2009; Hejda *et al.*, 2009; Batten *et al.*, 2006; Pattison *et al.*, 2016). These alterations have the potential to affect larger ecosystem-wide processes such as nitrogen cycling and litter decomposition (Wang *et al.*, 2015; Liao *et al.*, 2008), although the extent of these effects depends on the individual species (Jo *et al.*, 2015). Some of the most widely-dispersed invasive species in Europe, such as *Impatiens glandulifera*, the focus of this study, can homogenise soil conditions in invaded landscapes, resulting in novel niche construction; this has the potential to facilitate further invasion by both the same and other invasive species (Dassonville *et al.*, 2011). This ‘legacy effect’ can persist after the removal of the invader, increasing the challenge and cost of restoring invaded sites to their uninvaded state (Stefanowicz *et al.*, 2017).

Research on the factors influencing biological invasions has been a priority in ecology for the past decade (Sol *et al.*, 2012). Many studies have identified plant traits (Blossey & Notzold, 1995; van Kleunen *et al.*, 2010), novel functional attributes (Shea & Chesson, 2002), plant-soil feedback (PSF) (Kulmatiski *et al.*, 2008), and even genome size (Pysek *et al.*, 2018) as important predictors of whether a plant has the capability to become invasive. However, the extent to which these factors will transform under the effects of future climate change remains unclear. Several studies have predicted that alterations in the environment under future climate change will increase the occurrence and severity of plant invasions (Dukes & Mooney, 1999; Thullier *et al.*, 2007; Dawson *et al.*, 2011; Robinson *et al.*, 2020). This is a result of the high phenotypic plasticity and tolerance to a wide array of environmental conditions common to invasive species, which in turn facilitates comparatively greater survival than natives under altered environmental conditions as a result of climate change (Davidson *et al.*, 2011). Invasive plants also take greater advantage of CO<sub>2</sub> enrichment than natives, possibly as a result of the intrinsically high growth rate found in many invasive plant species (Marushia *et al.*, 2010; Dawson *et al.*, 2011; Liu *et al.*,

2017). A meta-analysis by Liu *et al* (2017) found that elevated temperature, atmospheric CO<sub>2</sub> concentrations, and N deposition favour the performance of invasive plants over native plants, although decreased precipitation may negatively affect invasive species more than natives. Nevertheless, the results suggest that a number of factors associated with climate change may further promote the activity of invasive plant species (Liu *et al.*, 2017).

Climate is intimately linked with the dispersal and establishment of invasive species; as a result, areas particularly susceptible to climate change are also at risk of plant invasions (Flanagan *et al.*, 2015). Water flow facilitates the spread of invasive plant propagules, such as those from *I. glandulifera*, across long distances, enabling invasions far from the source population (Leuven *et al.*, 2009). Riparian wetlands also act as sinks for debris, sediments, nutrients, and plant propagules (Flanagan *et al.*, 2015). Unfortunately, the frequent habitat disturbances in these areas provides prime opportunities for the establishment of non-native species, and thus can result in high levels of invasion (Zedler and Kercher, 2005).

In addition, higher temperatures as a result of global climate change are predicted to increase the frequency and intensity of precipitation events (Easterling *et al.*, 2000). Alterations in temperature and hydrology are potential factors that may increase the frequency of riparian invasions (Hellmann *et al.*, 2008; Flanagan *et al.*, 2015). Changes in soil moisture often occur alongside alterations in temperature, which in turn can affect soil microbial responses to climate change (Classen *et al.*, 2015). For example, drought amplifies the temperature sensitivity of bacteria and fungi, perhaps mediating the effects of PSF (Briones *et al.*, 2014). Future climate change will likely reduce the competitiveness of native plants in riparian areas, whilst increasing the competitiveness of invaders with greater nutrient and sediment content, as well as increased disturbance and changes in PSF (Flanagan *et al.*, 2015). *I. glandulifera*, as a successful invasive species around our area of study and throughout Europe, is an appropriate choice to investigate the multifaceted interactions between climate, the soil microbiome, and plant invasions (Dassonville *et al.*, 2011).

## 1.2 MECHANISMS OF PLANT INVASIONS

### 1.2.1 ENEMY RELEASE

One potential explanation for the success of invasive plant species in their invaded range is the ‘enemy release’ hypothesis. This is the prediction that an exotic plant species will be especially successful in a new habitat devoid of its former enemies, such as parasites or pathogens (Keane & Crawley, 2002). If these alien plants escape the negative effects of their native enemies, they will gain a competitive advantage native species in the community, which continue to suffer from their own native enemies. This has the potential to grant alien plant species a competitive advantage, facilitating their growth and survival, and making them invasive rather than simply exotic (Mlynarek, 2015).

The invasive species *Impatiens glandulifera* shows some support for the enemy-release hypothesis. In its invaded range, *I. glandulifera* has an impoverished endophyte community with low diversity, dissimilar to that of its native habitat (Coakley & Petti, 2021). This is indicative of the invader being ‘released’ from its usual soil pathogens, perhaps enabling it to grow better and establish in its invaded range. However, *I. glandulifera* has also been shown to associate with novel microbes in its invaded community: three common endophytes in the UK that improve plant resistance to the rust fungus *Puccinia komarovii* var. *glandulifera* have been found in the soil community of *I. glandulifera*. This rust fungus was purposely released as a method of biological control to inhibit the spread of *I. glandulifera* in the UK, however its association with native endophytes have led to resistance in some populations of *I. glandulifera* (Currie *et al.*, 2019; Coakley & Petti, 2021). This is but one of the conflicting examples of enemy release, the presence of which is still under some debate (Currie *et al.*, 2019; Coakley & Petti, 2021).

### 1.2.2 MUTUALISMS

Many alien plant species utilise mutualisms in their novel range to establish and potentially become invasive (Richardson *et al.*, 2000). These include the formation of mutualisms, such as those with mycorrhizal fungi to increase nutrient uptake, as well as the disruption of established mutualisms, which negatively affects native plant species (Bowles *et al.*, 2017). Hayward *et al.*

(2015) found that a single ectomycorrhizal fungal species, *Suillus luteus*, was the largest driving force behind a pine invasion in Chile, with many of the invasive trees having their nutrient uptake supported by this fungus alone. This effect has been observed multiple times, with “many of the world’s worst invasive alien species only invading after the introduction of symbionts” (Richardson *et al.*, 2000).

Invasive species can also negatively affect established mutualisms of native plant species. Bowles *et al.* (2017) found that the increased root surface area and subsequent nutrient uptake offered by arbuscular mycorrhizal fungi (AMF) to their host plants increased resistance to drought stress. As some invasive plant species have shown the ability to decrease AMF colonisation and density in soil, their presence may affect AMF-regulated drought stress tolerance in natives, lending a competitive advantage to the invaders (Zybeck *et al.*, 2016). The invasive plant *I. glandulifera* is only weakly dependent on AMF for growth, with corresponding invaded soil only showing sparse colonisation by AMF (Tanner & Gange, 2013).

### **1.2.3 ALLELOPATHY**

Allelopathy is the chemical interaction between plants or plants and microbes in their immediate community, and can have both positive or negative effects (Rice, 1984). However, in the context of plant invasions allelopathy is generally considered a negative effect, decreasing neighbour plant performance and altering the structure of the surrounding community (Kalisz *et al.*, 2020; Zhang *et al.*, 2020). A recent meta-analysis by Zhang *et al.* (2020) found that overall, allelopathy reduced plant performance by 25%. However, this effect was highly variable, and depended on the type of allelopathy (Zhang *et al.*, 2020). These involve four pathways: “leaching from plants by rain, litter decomposition, root exudates, and volatilization” (Zhang *et al.*, 2020). Litter decomposition and root exudates are particularly well studied in the context of plant invasions (Zhang *et al.*, 2020; Thorpe *et al.*, 2009; Helsen *et al.*, 2018). Whilst invasive species may employ different methods of allelopathy, their eventual effect favours the invasive plant over the native community, potentially facilitating invasions (Coakley & Petti, 2021).

The invasive plant *I. glandulifera* has been shown to produce 1,4-naphthoquinone (2-MNQ), a potential allelochemical capable of inhibiting the growth of nearby native plants or disrupting their soil microbial community (Perglová *et al.*, 2009; Coakley & Petti, 2021). 2-MNQ has the capability to affect soil microbial communities, potentially to the benefit of the invader and detriment of native species (Gaggini *et al.*, 2018; Cipollini *et al.*, 2012; Coakley & Petti, 2021). In-vitro studies support this hypothesis, with extracts of 2-MNQ from *I. glandulifera* negatively impacting native species from the invaded range of *I. glandulifera*, such as the AMF-associating *Dactylis glomerata* and *Urtica dioica* (Bieberich *et al.*, 2018; Coakley & Petti, 2021).

### **1.3 PLANT-SOIL FEEDBACKS (PSFs)**

There is a large repository of evidence demonstrating that plant species are able to alter the biotic and abiotic components of soil, resulting in feedback chains that subsequently affect the performance of plants within the surrounding community (Kulmatiski *et al.*, 2008; Beals *et al.*, 2020). These plant-soil feedbacks (PSFs) can result in increased growth and survival when positive; however when negative can have the opposite effect (Bennett & Klironomos, 2018; Beals *et al.*, 2020). PSFs are often positive for invasive plant species in their invaded range yet negative between native and exotic plants in these communities; this can result in increased growth and survival of invasive species, facilitating further invasions and often resulting in monotypic stands at the expense of natives (Kulmatiski *et al.*, 2006; van der Putten *et al.*, 2013). The expansion and soil-conditioning effects of alien plants can cause losses not only to the structure and function of invaded ecosystems, but also on a social and economic level (Linders *et al.*, 2019; Diagne *et al.*, 2021).

#### **1.3.1 INVASIONAL MELTDOWN**

Accentuating the issue of altered PSF is the finding that, in many cases, alien plant species appear to favour other aliens over natives (Kulmatiski *et al.*, 2006): an ‘invasional meltdown’ (Simberloff & Von Holle, 1999). As a result, invasive species frequently aggregate with other non-native species in species-poor, high biomass communities in their invaded range (Stotz *et al.*, 2019). Zhang *et al.* (2020) found that on non-conditioned soil, alien and native plants

produced similar biomasses, indicating no underlying competitive advantage possessed by alien species. However, on soil conditioned by aliens, other aliens produced more biomass than natives, thus outcompeting them. This phenomenon appears to be a case of invaders affecting the growth of other invasive plants less negatively than natives, possibly due to differences between their fungal endophyte communities, or through PSFs (Gaggini *et al.*, 2018; Coakley & Petti, 2021). The impact of soil conditioning by one plant species on another became less negative as their fungal endophyte communities became less similar, with less similar fungal endophyte communities between two aliens than between natives and aliens, or between two natives (Zhang *et al.*, 2020). This effect was mainly driven by pathogenic endophytes, whereby endophytes remaining in the soil following conditioning by an alien are more likely to negatively affect the more-similar native species than the less-similar alien (Zhang *et al.*, 2020).

### **1.3.2 PSFs AND SEEDLING ESTABLISHMENT**

PSFs appear to be especially important in seedling establishment. Aldorfova *et al.* (2019) showed that invasive species have more positive PSFs for seedling establishment than aliens that fail to become invasive. PSF for seedling establishment was a better predictor of invasiveness than other commonly-considered species characteristics, such as propagule shape, genome size, or ploidy level (Aldorfova *et al.*, 2019). This finding suggests an importance of early plant stages for the successful transition from naturalised to invasive. Similar to those described previously, these positive effects may arise from phylogenetic novelty of invasive species in their invaded range, freeing them from the negative PSFs associated with their host-specific enemies that would normally occur in the native range (Aldorfova *et al.*, 2019). In a similar fashion, invasive species have been shown to generate more positive or less negative intra-specific plant-soil feedbacks than native species, as well as experiencing less negative intraspecific plant-soil feedbacks in their invaded range due to enemy release (Callaway *et al.*, 2004; Zhang *et al.*, 2019; Aldorfova *et al.*, 2019; Zhang *et al.*, 2020).

### **1.3.3 PSFs IN *Impatiens glandulifera***

Other drivers of positive PSFs in invasive species exist. *Impatiens glandulifera* (also known as Himalayan balsam) is one of the most widespread invasive plant species in the UK, and has been shown to alter soil chemistry and microbial communities (Pattison *et al.*, 2016). One aspect of these effects is the depletion of arbuscular mycorrhizal fungi (AMF). AMF form a mutualistic symbiosis with many plant species in native habitats, providing enhanced nutrient uptake through the creation of a mycelial network (Hartley & Gange, 2009). However, *I. glandulifera* has little dependence on AMF; this may lead to decreased biological diversity in belowground communities, potentially reducing the fitness of native plants in the vicinity of invasive *I. glandulifera* (Tanner & Gange, 2013). Pattison *et al.* (2015) showed that when grown in *I. glandulifera*-conditioned soil, *I. glandulifera* was taller, produced more leaves, grew faster, and had higher biomass than in non-conditioned soil; this suggests a positive PSF. AMF root colonisation in conditioned soil was half that of control soil, whilst bacterial biomass increased almost two-fold in conditioned versus control soil (Pattison *et al.*, 2016). As discussed previously, the positive PSF conditioning effect may have arisen as a result of the decrease in AMF, which normally facilitates P uptake in plants at the cost of C from the host plant (Richardson *et al.*, 2009). As *I. glandulifera* has been shown to be only weakly dependent on AMF for P uptake, high AMF colonisation may decrease the mutualistic benefits granted; thus, a decrease in AMF colonisation may reduce the C-cost imposed on *I. glandulifera*, and allow for greater growth (Pattison *et al.*, 2016). This reduced colonisation by AMF in conditioned soil can negatively affect native species that depend more on AMF for nutrient uptake, whilst facilitating further invasions (Ruckli *et al.*, 2014; Pattison *et al.*, 2016).

### **1.3.4 INCONSISTENCIES IN PSFs**

The role of PSFs in plant invasions is not consistent, however. A recent meta-analysis of 52 studies (42 invasive plant species and 46 native species) by Zhang *et al.* (2019) found that invasive plants may affect soil biota variably through two pathways: litter effects through changes in detritus inputs, and rhizosphere effects through modifications in root exudation and root-biota interactions (Zhang *et al.*, 2019; Wolfe & Klironomos, 2005). Many successful invaders produce more and faster-decomposing litter than native plants, which in turn provides

greater resources for decomposers in the soil, resulting in modifications of soil moisture, pH, and composition (Arthur *et al.*, 2012; Prescott & Zekker, 2016). Increased levels of soil nutrients and greater release of C in the system may then facilitate further invasions in the future, as many invasive species have been shown to better-utilise these resources than natives (Ehrenfeld *et al.*, 2001; Liu *et al.*, 2017). In turn, rhizosphere effects such as the release of allelopathic compounds, as well as alterations in mycorrhizal fungi and bacterial communities, may also further facilitate later invasions (Thiebaut *et al.*, 2019; Ruckli *et al.*, 2014; Pattison *et al.*, 2016).

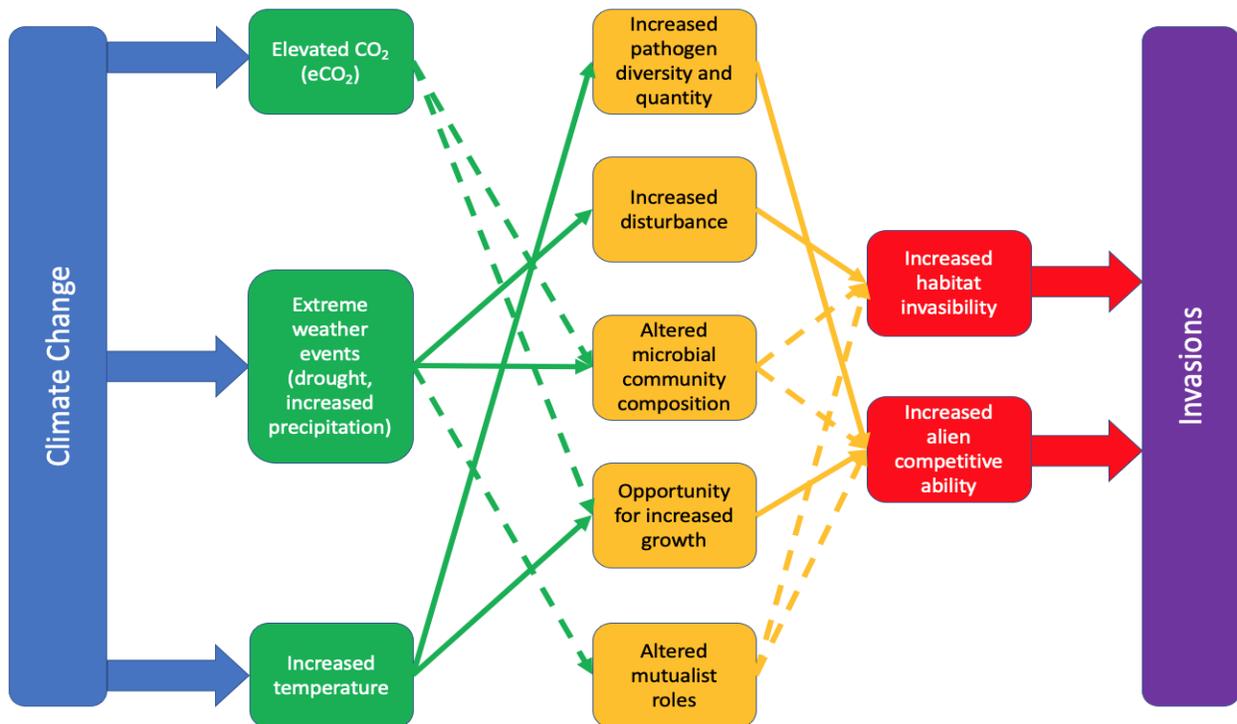
However, the extent to which allelopathy plays a role in plant invasions is debated (Fabbro *et al.*, 2014). Zhang *et al.* (2019) showed through their meta-analysis that invasive plants “increased bacterial biomass by 16%, detritivore abundance by 119% and herbivore abundance by 89%” via the litter pathway, whilst in the rhizosphere, “invasive plants reduced bacterial biomass by 12%, herbivore abundance by 55% and predator abundance by 52%, but increased AM fungal biomass by 36%”. In addition, invaded soils showed higher CO<sub>2</sub> efflux, N-mineralisation, and enzyme activities than native soils. Interestingly, the 12% decrease in rhizosphere bacterial biomass, as well as the 36% increase in AM fungal biomass, are the opposite effects to those displayed by the invasive *I. glandulifera* in Pattinson *et al.* (2016). This demonstrates that whilst many invasive plants appear to alter PSFs, the pathways taken to achieve these effects may differ between species. Further study is needed to differentiate the effects of the litter and rhizosphere pathways, as well as to determine how these effects may be altered under future climate change.

## **1.4 IMPACTS OF CLIMATE CHANGE ON PLANT INVASIONS**

Plant-soil feedback has the potential to react differently to climate change than other factors underpinning biological invasions, as belowground communities are buffered to changes in temperature, precipitation, and even extreme climate events to a certain degree (Duran *et al.*, 2014). Thus, the direct environmental pressures of global climate change on plants may differ from those of their associated soil community (Classen *et al.*, 2015). Furthermore, some invasive species can selectively alter the soil community, creating a plant-soil feedback that facilitates their own growth (Kulmatiski *et al.*, 2008). These changes may occur through nutrient changes in the soil due to leaf litter and microbial decomposers, as well as root exudates and the

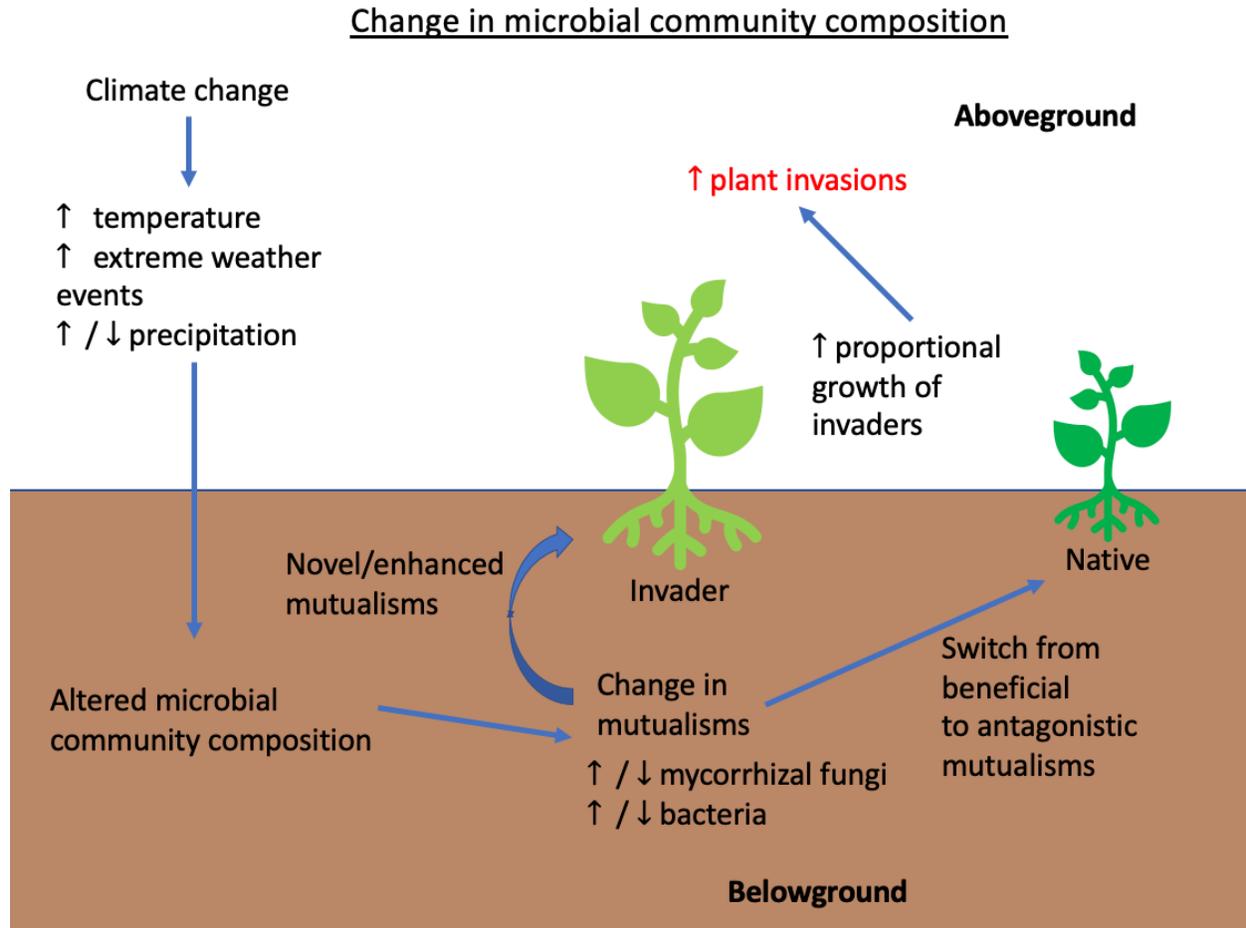
accumulation of local pathogens (Zhang *et al.*, 2019). This alteration of the soil community can be detrimental to native plant species (Ehrenfield, 2010). What effect climate change may have on these plant-soil feedback activities of invasive plants, if any, is an area requiring more research.

Climate change as the result of elevated CO<sub>2</sub> levels has the potential to increase temperature, alter precipitation patterns, and lead to more frequent extreme weather events, among others (Harley *et al.*, 2011; Parasiewicz *et al.*, 2019). These have the potential to combine and impact plant invasions via varied, interconnected pathways, resulting in either increased habitat invasibility, greater alien plant competitive ability, or a combination of the two. Figure 1 is a simplified model of the interactions between climate change and plant invasions, with areas that have been the subject of less research highlighted. Sections 1.4.1 to 1.4.3 demonstrate three potential scenarios of PSFs under the effects of climate change, all of which result in increased invasive plant competitiveness compared to native species.



**Fig 1.** Possible impacts of climate change on plant invasions. Dotted lines represent less-studied areas of research.

### 1.4.1 ALTERED MICROBIAL COMMUNITY COMPOSITION

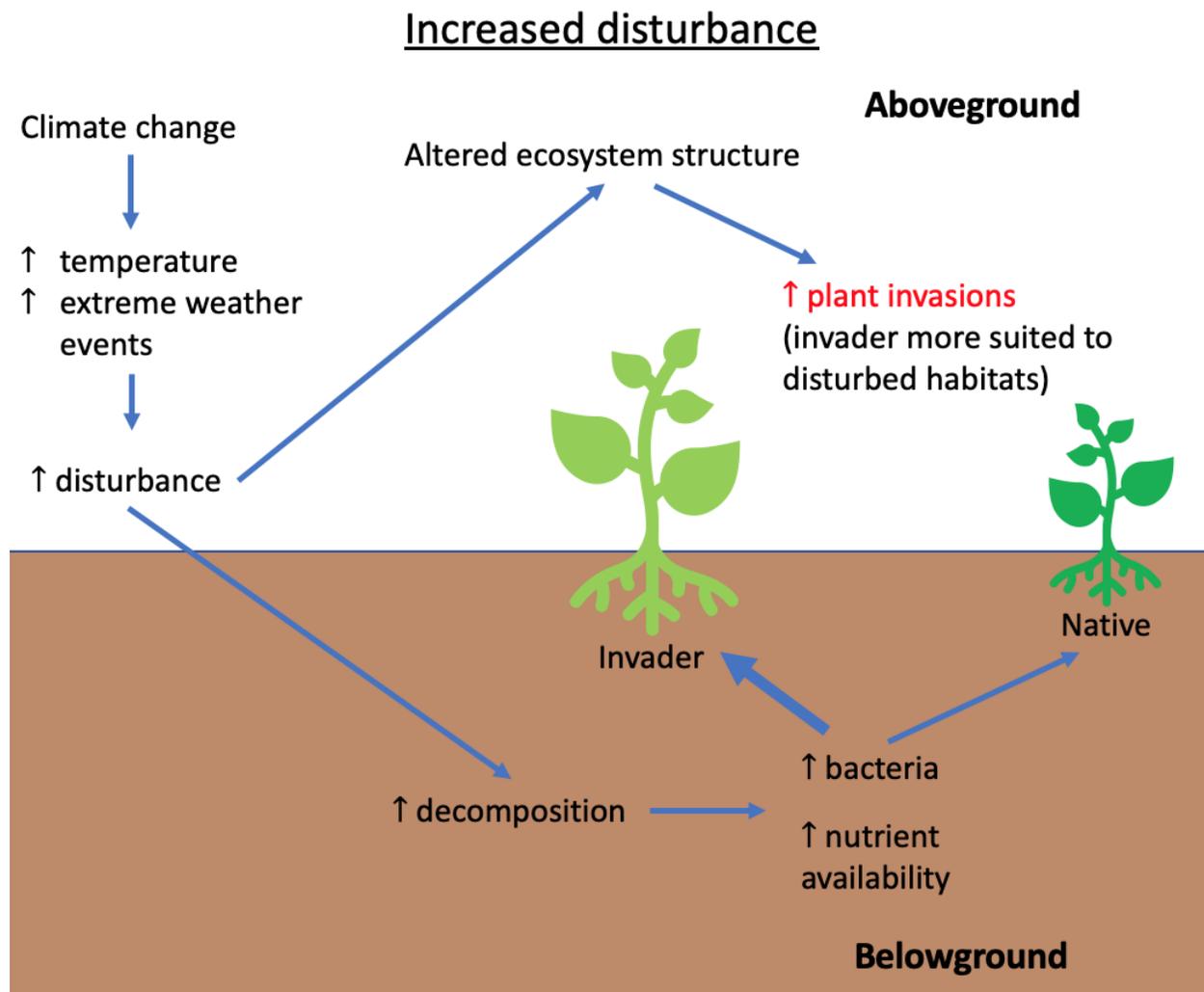


**Fig 2.** A potential scenario demonstrating the effects of climate change-induced changes in the soil microbial community composition on plant invasions.

Figure 2 illustrates a possible plant invasion scenario under the effects of climate-change induced alterations in the soil microbial community. The increase in temperature and extreme weather events under climate change may result in a greater frequency of drought events. Drought has been shown to amplify the temperature sensitivity of bacteria and fungi, and may mediate the effects of PSF (Briones *et al.*, 2014). AMF, alongside a more diverse soil microbiome, have been shown to improve resistance to drought stress in associated plant communities (Bowled *et al.*, 2017; Bogati & Walczak, 2022; Malacrino *et al.*, 2020). The increased frequency of plant invasions under climate change may result in decreased colonisation by AMF, alongside shifts in the bacterial community (Pattison *et al.*, 2016; Gaggini *et al.*, 2018). This alteration in the

microbial community, combined with lower soil moisture as a result of climate change, may decrease the growth and competitive ability of the native species community whilst increasing the proportional growth of invasive species (Bowled *et al.*, 2017; Bogati & Walczak, 2022; Malacrino *et al.*, 2020).

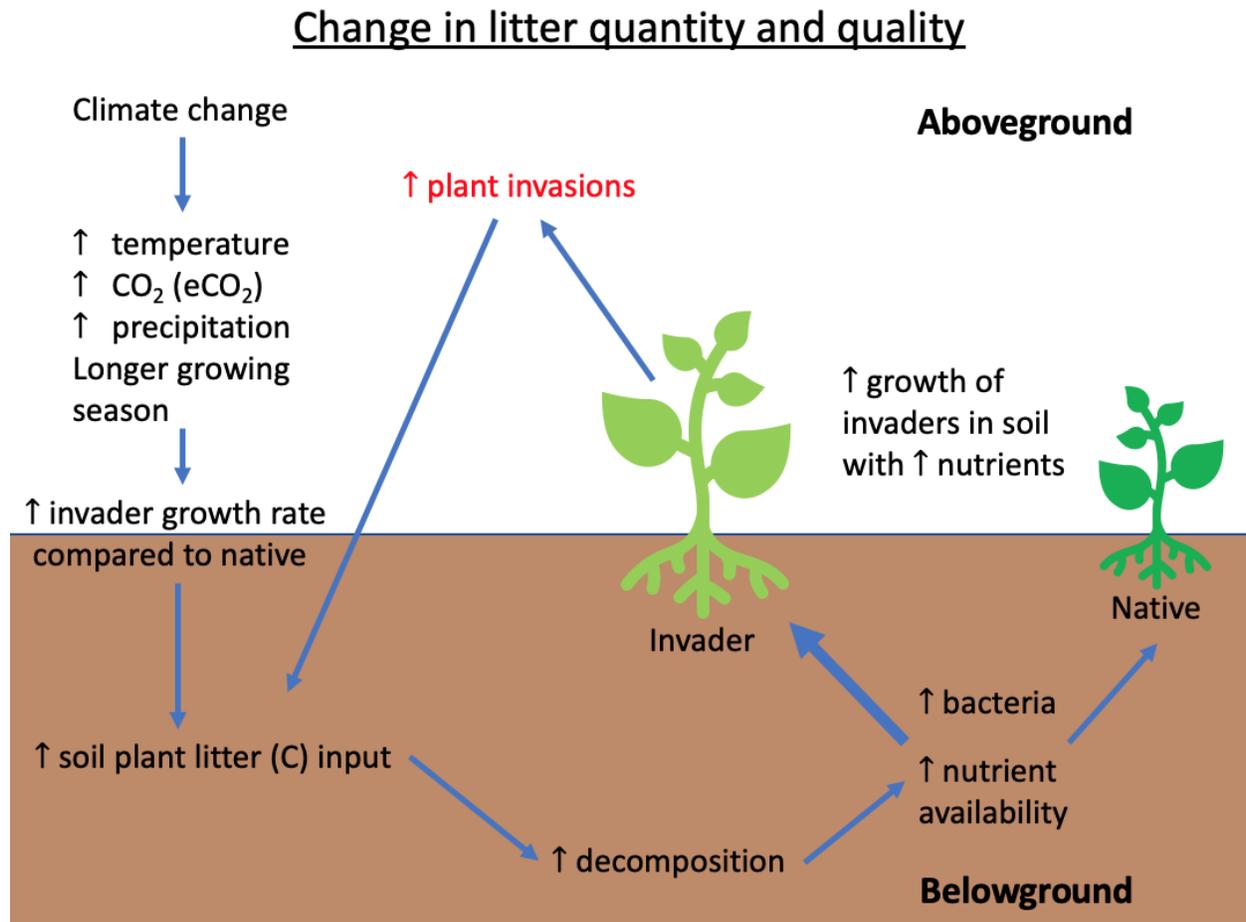
### 1.4.2 DISTURBANCE



**Fig 3.** A potential scenario demonstrating the effects on plant invasions of climate change-induced increases in environmental disturbance. Size of arrows indicate effect size.

Figure 3 describes a potential invasion scenario under increased disturbance as a result of climate change. Climate change is expected to increase temperature, alter precipitation patterns, and result in more extreme weather events, leading to drastic habitat disturbance (Harley *et al.*, 2011; Parasiewicz *et al.*, 2019). These disturbance events in particular are expected to facilitate the spread and establishment of invasive species, principally through altering resource availability and ecosystem structure (Davis *et al.*, 2000; Orbán *et al.*, 2021). This may occur as a result of the higher resource-use efficiency, phenotypic plasticity, and competitive ability observed in many invasive plants, with these species being better-able to utilise resources in a disturbed environment, as well as tolerate substandard conditions, compared to native species (Dawson *et al.*, 2010; Cuda *et al.*, 2015; Cavaleri & Sack, 2020). Alternatively, disturbance may alter the soil microbial community, with increased decomposition leading to greater nutrient availability; invasive species may be better-equipped to utilise these nutrients than native species (Cuda *et al.*, 2015).

### 1.4.3 SOIL LITTER CHANGES



**Fig 4.** A potential scenario demonstrating the effects of climate change-induced alterations in litter quantity and quality on plant invasions. Size of arrows indicate effect size.

Figure 4 describes a potential invasion scenario under changes in litter quantity and quality as a result of climate change. Invasive species have high resource-use efficiency, phenotypic plasticity, and broad environmental tolerances, with these being important traits for facilitating entry into novel habitats (Davidson *et al.*, 2011; Higgins & Richardson, 2014). Climate change is predicted to increase temperature, resource availability, and result in a longer growing season; it is not a stretch to expect that these characteristics will impart advantages over native species under environmental changes effected by climate change (Harley *et al.*, 2011; Parasiewicz *et al.*, 2019). As a result of the increased growth of invaders, more organic matter may enter the soil, with the potential to affect nitrogen cycling and decomposition (Wang *et al.*, 2015; Liao *et al.*,

2008). In turn, this may affect the soil microbial community, increasing bacterial biomass, increased soil nutrient availability, and greater invader growth (Zhang *et al.*, 2019). This may result in a positive feedback loop, whereby invasive plants are more successful, further alter soil chemistry, and facilitate greater plant invasion.

#### **1.4.4 SUBSTITUTING SPACE FOR TIME TO PREDICT PLANT INVASIONS**

Predicting the effects of rising temperature on invasive and native PSFs can be attempted through examining how plant-soil feedback effects and microbial communities vary along a latitudinal gradient. Lu *et al.* (2018) examined variation in the soil community of the invasive *Alternanthera philoxeroides* and its native congener *A. sessilis* across a latitudinal gradient from 22 °N to 36.6 °N in China. They found that soil biota community structure differed across latitude as a result of both climate and soil properties. Root-knot nematode abundance as well as soil fungal pathogen diversity decreased with latitude, possibly as a result of higher pH and lower temperatures. Interestingly, native plant growth increased with increasing latitude of soil collection (Lu *et al.*, 2018). This may have been due to a decreased negative effect of soil-borne enemies at higher latitudes, with enemy abundance and diversity decreasing as latitude increases. The invasive plant showed no latitudinal pattern in plant-soil interactions, likely as a result of the invasive species possessing a more effective defence against the native plant's soil enemies (Lu *et al.*, 2018).

If we substitute space for time, these results suggest that invasive species may possess an intrinsic advantage over natives under warmer temperatures as a result of climate change; however, this is conditional on enemies of natives increasing in abundance, and that these enemies negatively affect native species more than invaders. Native species are more negatively affected by soil pathogens in warmer temperatures due to increases in abundance and diversity, whereas invasive species show identical responses in both 'colder' and 'warmer' treatments. This approach of substituting space for time is not fool-proof, however, as many factors that change along with latitude may not be present with the environmental alterations posed by climate change, and thus the use of latitudinal experiments as a proxy for climate change can

only hint at possible results. Further manipulative experimental studies including treatments that simulate facets and effects of climate change are required to better-understand how PSFs may change and affect invasions under future climate change.

## 1.5 CONCLUSION

This literature review showcases the ability of plants to alter their surrounding soil, which can in turn result in feedback and subsequently affect plant performance in the surrounding community. These plant-soil feedbacks manifest in myriad ways, including mutualisms, allelopathy, and alterations in soil abiotic conditions. In the context of plant invasions, PSFs are usually considered to be positive for conspecifics, and negative for native plants. Invaders directly and indirectly foster these positive PSF loops to favour their own growth and survival whilst negatively affecting those of their neighbouring natives (Kulmatiski *et al.*, 2006; van der Putten *et al.*, 2013). However, how climate change will affect these dynamics is a complex subject requiring further study, as illustrated in Fig. 1.

Climate change is predicted to impact soil microbial communities alongside invasive plant species. The simple models explored in section 1.4.1, 1.4.2, and 1.4.3 offer an introduction to the variable interconnections of PSF and climate change. The effects of climate change may affect PSFs directly, as in the scenarios showcased in Fig. 2 and Fig. 3, where the temperature sensitivity of bacteria and fungi is amplified; this may mediate the effects of PSFs and cause a shift in the microbial community, such as an increase or decrease in bacterial abundance or richness (Briones *et al.*, 2014; Gaggini *et al.*, 2018). A more diverse soil microbiome has been shown to buffer the effects of drought; losses in microbial diversity and shifts in the structure of microbial populations, combined with climate change stresses, could negatively affect the performance of native plant species (Bogati & Walczak, 2022; Malacrino *et al.*, 2020).

Alternatively, climate change may impact PSFs indirectly through its effects on invasive plants, such as increasing competitiveness or facilitating faster growth. More frequent plant invasions may negatively affect AMF colonisation, which, as discussed earlier, has the potential to result in lower drought-stress tolerance in native plant species (Pattison *et al.*, 2016; Bowled *et al.*, 2017;

Bogati & Walczak, 2022; Malacrino *et al.*, 2020). The PSF effects of invaders appear to differ greatly by species, however, and thus it can be expected that invasive plant PSF responses to climate change may also be species-specific as well (Qin *et al.*, 2014; Cuda *et al.*, 2017).

*I. glandulifera* has been mentioned many times in this literature review, and is an invader that exhibits many of the PSF effects discussed earlier, such as enemy release, mutualisms, allelopathy, and PSF inconsistencies. This broad array of PSF possibilities makes it an excellent choice to use when investigating the effects of climate change on the role PSF plays in plant invasions. As a result, *I. glandulifera* was chosen to be the subject of this current study, and its presence will hopefully result in at least some of the plant invasion PSF factors detailed in this review. The effect of *I. glandulifera* on AMF is of particular interest. As *I. glandulifera* is only weakly dependent on AMF, it can be expected that plant communities dominated by *I. glandulifera* may show decreasing root AMF colonisation and total soil AMF density (Zybeck *et al.*, 2016; Pattison *et al.*, 2016; Grove *et al.*, 2017; Coakley & Petti, 2021). Whether this is the case under the effects of climate change is yet to be discovered.

The specific role of microorganisms in PSF has been the subject of much study in recent years (Aires *et al.*, 2021). Zilber-Rosenberg and Rosenberg (2008) argue that as the microbiomes associated with plants affect such far-reaching components as plant development, interactions with the surrounding environment, adaptation, and ultimate survival, individual plant phenotypes should be taken as the sum of the host and its associated microbial gene expressions. In this ‘hologenome’ theory of evolution, the roles of bacteria, fungi, and other microorganisms in plant invasions are potentially as important as host physiology alone (Rosenberg and Zilber-Rosenberg, 2018). More studies of this type are required to fully understand PSFs dynamics during invasions how these are affected by climate change.

The experimental chapters in this thesis attempt to explore the complex dynamics of PSFs, plant invasion, and climate change. Teasing out the many complicated interactions between these factors has only recently become possible with modern techniques such as next-generation sequencing, and thus represent an exciting new area of study. The second chapter of this thesis investigates the effects of climate change-induced alterations in water availability, with *I. glandulifera* and a native community grown in a range of watering treatments simulating different water availability scenarios under climate change. The third chapter explores the

temperature effects of climate change in tandem with its potential impact on invasive plant soil conditioning. Finally, the fourth chapter is a DNA metabarcoding study of the soil bacterial and fungal communities under the effects of climate change and *I. glandulifera* soil conditioning, to further investigating the drivers of invasive species plant-soil feedbacks. The findings of these three studies will have important implications for future efforts to manage invasive species, and provide a roadmap for further study in this area.

## Chapter 2:

### The effects of water surplus and deficit on the competitive ability of *Impatiens glandulifera* relative to a native plant community

#### ABSTRACT

Modern ecosystems are undergoing unprecedented shifts as a result of climate change and increased biological invasions. However, there is a dire lack of research to aid in predicting their combined outcomes. In addition to increased temperature, climate change is expected to alter precipitation patterns and result in more extreme weather events, leading to habitat disturbance and affecting resource availability. This may favour invasive species, which often have higher growth rates and resource-use efficiency than native species. A growth experiment was performed to assess the impact of water availability on a community of *I. glandulifera* and four native species. A range of watering treatments were used to simulate different water availability scenarios under climate change. Plant physical parameters were recorded, demonstrating that *I. glandulifera* consistently showed a greater competitive ability than native species in the community, even under watering treatments that negatively affected the invader. These findings suggest that in future ecosystems subjected to the precipitation and extreme weather effects of climate change, riparian invaders such as *I. glandulifera* are poised to show increased competitive abilities. This has important implications for future efforts to manage invasive species.

## 2.1 INTRODUCTION

Modern communities are undergoing unprecedented shifts as a result of climate change and increased biological invasions (Sala *et al.*, 2000; van Kleunen *et al.*, 2015). However, although the individual effects of these phenomena have been subject to a large amount of ecological study, there is a lack of work investigating invasions and climate change effects combined (Gong *et al.*, 2020).

Climate change has the potential to alter the distribution of invasive species, allowing them to survive and establish viable populations in areas that were previously inaccessible (Gong *et al.*, 2020). For example, Osland and Feher (2019) found that warming winter temperatures in the southeastern United States has the potential to facilitate the spread of non-native Brazilian pepper northward, transforming ecosystems. In North East England, the location of this experiment, climate change is expected to increase temperature, alter precipitation patterns, and result in more extreme weather events, leading to drastic habitat disturbance (Harley *et al.*, 2011; Parasiewicz *et al.*, 2019). These disturbance events in particular are expected to facilitate the spread and establishment of invasive plants, principally through altering resource availability and providing a ‘window of opportunity’ where competitors are absent or impaired (Davis *et al.*, 2000; Orbán *et al.*, 2021).

Invasive plants have often been shown to owe their success to an ability to capitalise on increased resources, often being characterised by higher growth rates, resource-use efficiency, and fecundity than native species, even under suboptimal conditions (Kleunen *et al.*, 2010; Dawson *et al.*, 2011; Funk, 2013; Ens *et al.*, 2015; Jelbert *et al.*, 2015). They are commonly well-suited to changes in the environment, with high phenotypic plasticity and broad environmental tolerances being important traits for facilitating entry into novel habitats (Davidson *et al.*, 2011; Higgins & Richardson, 2014). We might then expect that these characteristics will impart advantages over native species under environmental changes effected by climate change, such as altered precipitation patterns and higher temperatures.

Although the competitive abilities of invasive plants have been shown in many studies, the timing of competition (that is, the differences between invasions at the seedling stage of *I. glandulifera* as opposed to those by a fully-established plant) has been demonstrated to affect the

success or failure of invasions (Beckmann *et al.*, 2011; Cuda *et al.*, 2015; Gioria & Osborne, 2014). Gioria *et al.* (2016) found that many invasive species in grassland communities have a short-term germination advantage, entering the seedling stage earlier than natives and subsequently taking advantage of this period of low competition with fast growth. However, the high vulnerability of the seedling stage and associated risks with early germination can negatively affect the establishment of invasive species (Cuda *et al.*, 2015; Gioria *et al.*, 2016). Climate change has the potential to affect germination timing and subsequent seedling survival of both native and invasive species through increased temperatures and altered precipitation, among other effects (Walther *et al.*, 2002; Gioria *et al.*, 2016).

Many studies with experimental manipulation of different factors related to invasion success have been performed in the past. White *et al.* (2009) found that the potentially invasive annual subtropical grass *Digitaria sanguinalis* achieved maximum biomass in disturbed environments with high water availability. Other experimental manipulation experiments have shown that invasive plants show greater nutrient use efficiency across a gradient of soil nutrient availability (Knauf *et al.*, 2021). Invasive plants are not always shown to outcompete natives in experiments of this type, however; studies such as McGlone *et al.* (2012) have found that the competitive ability of an invasive plant may differ between species, such as the invasive grass *B. tectorum* being outcompeted by the natives *E. elymoides* and *P. smithii* across a range of soil water and nutrient levels. Although some natives have been shown to compete successfully against alien invaders, a large body of evidence shows invasive plants grow and germinate faster, consume disproportionately more resources, and show higher phenotypic plasticity than native plants suggests that invasions will increase under the greater disturbance wrought by climate change (Dawson *et al.*, 2010; Cuda *et al.*, 2015; Cavaleri & Sack, 2020). As a result, more work is needed to fully explain the association between disturbance, resource availability, and invasive success, especially when the timing of the invasion is involved (Leishman & Thomson, 2004; Gioria *et al.*, 2016).

This study focused on the effects of varying water ability on the competitive ability of the invasive annual *Impatiens glandulifera* versus a community of four commonly co-occurring native plants in the Northeast England. *I. glandulifera* is a very successful invader in the region, with a wide tolerance for a range of environmental conditions including low soil moisture (Cuda

*et al.*, 2014; Pattison *et al.*, 2016; Cuda *et al.*, 2017). This study addresses the following questions: (1), does variation in water, reminiscent to the extreme weather predicted to occur under climate change, affect the competitiveness of *I. glandulifera*, and (2), will the timing of a simulated flood or drought event during the beginning or middle the growing period affect this competitiveness?

## **2.2 METHODOLOGY**

### **2.2.1 SITE DESCRIPTION**

The experiment took place inside a greenhouse (54°45'41.8"N 1°34'28.5"W) owned by the Durham University Department of Biosciences in Durham, UK. Interior temperature was regulated automatically via the opening and closing of the greenhouse roof.

### **2.2.2 NATIVE SPECIES SELECTION, SEED AND SOIL COLLECTION**

In October 2020, seed pods from 23 individual *Impatiens glandulifera* plants were collected from three invaded sites around Durham, UK (Fig. 5). Seeds were collected from locally dominant populations of *I. glandulifera*. The seed capsules were opened inside a lab, and ten seeds from each of the 23 plants (N=230) were put into cold-wet stratification buried in wet sand at 4°C for two months to encourage germination. As some *I. glandulifera* seeds failed to germinate, a number of seedlings of the same developmental stage as those germinated from seed were harvested directly from the surrounding area. These additional seedlings had their roots sterilised with 1% hypochlorite solution for 2 minutes prior to inclusion in the experiment to minimise the risk of contamination from the natural soil microbiome. In addition to the invader, seeds of four native species often occurring near *I. glandulifera* were obtained from seed banks originally sourced from wild populations. These four natives were *Rumex obtusifolius*, *Epilobium hirsutum*, *Centaurea nigra*, and *Chamaenerion angustifolium*. The natives were chosen both due to their co-occurrence with *I. glandulifera* as per our personal observations around the study area, but also as representatives of a simulated riparian community; riparian ecosystems are especially threatened by *I. glandulifera*. These native seeds (N=460) were also put into cold-wet

stratification buried in wet sand at 4°C for two months. Following the stratification, all seeds were sown on sterilised sand within a greenhouse under constant light and 21°C ambient temperature and left to germinate for two weeks. Successfully germinated seeds were transplanted into 3L pots, as described in “2.2.3: Experimental Design”.

The experimental substrate used was J Arthur Bowers plant-grade topsoil. A total of 3L of soil was used in each pot, the maximum capacity.



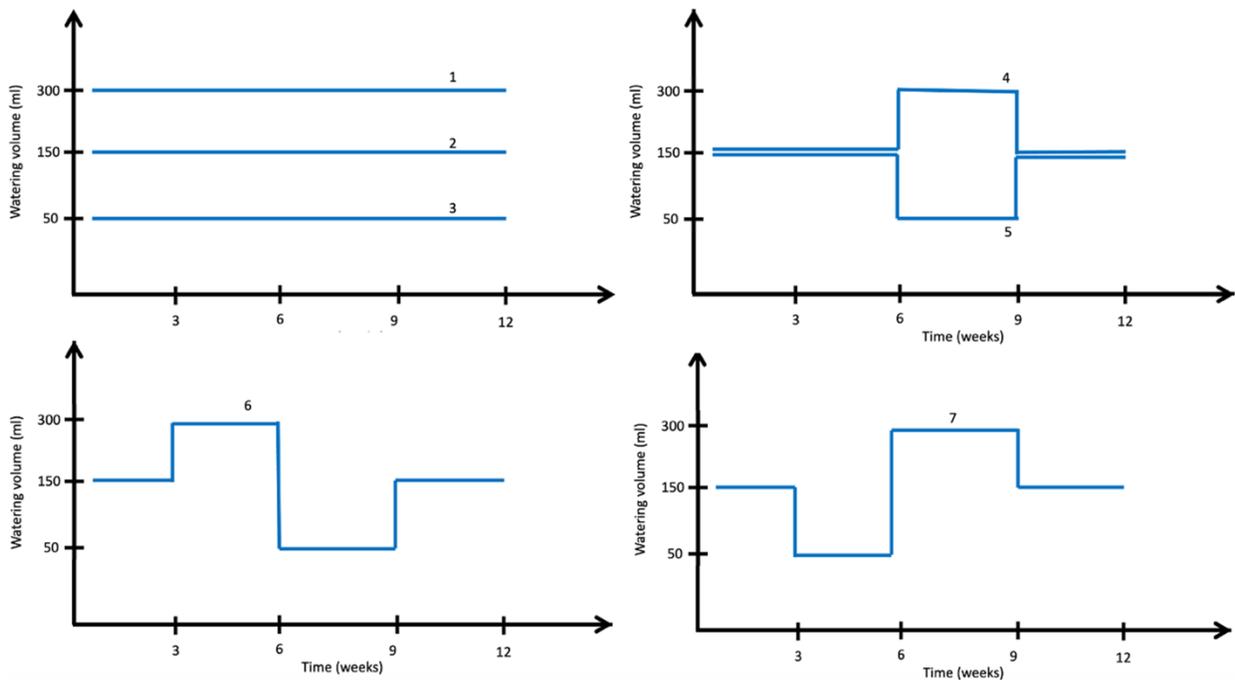
*Fig. 5. Location of the sites where seeds of *Impatiens glandulifera* were collected. (Image via Google Maps).*

### **2.2.3 EXPERIMENTAL DESIGN**

In total 70 pots, each 20cm in diameter, were filled with 3L of soil. Seedlings from all species were randomly selected from those that successfully germinated and were transplanted into the pots, with one invasive species planted in the centre and the four natives forming a square

approximately halfway between the centre and pot edge. This resulted in an experimental block design of 70 pots arranged in a grid.

Pots were assigned to the following seven watering treatments (Fig. 6) replicated 10 times: constant low (drought, 50mL x3 per week), constant medium (150mL x 3 per week), constant high (flood, 300mL x3 per week), high pulse (150mL x 3 per week for three weeks, followed by 300mL x3 per week for six weeks, and then back to 150mL per week for the final three weeks), low pulse (150mL x 3 per week for three weeks, followed by 50mL x3 per week for six weeks, and then back to 150mL per week for the final three weeks), high-to-low (300mL x 3 per week for first half of experiment, followed by 50mL x3 per week for the latter half), and low-to-high (50mL x 3 per week for first half of experiment, followed by 300mL x3 per week for the latter half).



**Fig. 6.** The seven watering treatments applied in this study.

Plants were grown for a total period of 12 weeks, with pots watered three times a week according to the volume of water required for their treatment (Table 1). The short growing period helped to limit ‘pot-size’ effects, as plants were unable to grow to maturity. Pots were randomised again halfway through the experiment to combat unevenness in light availability in the greenhouse. During the growing period, the height of every individual plant was measured at weeks 6, 9, and 12. Height was measured from the base of the plant to the tallest element of the plant (leaves included).

After the 12-week growing period, plants were cut off at soil level and washed free of soil, then divided into stems, leaves, and cotyledons/undeveloped leaves. All biomass was then dried at 60°C for 48 hours and weighed.

*C. angustifolium* failed to grow larger than seedling size and did not survive in a number of pots.

#### **2.2.4 STATISTICAL ANALYSES**

All data sets were checked for normality and homogeneity of variances prior to analysis. Data for height and biomass were analysed by species using a 1-way ANOVA in R. Height, aboveground biomass, and volume water content were natural log-transformed. Proportional aboveground biomass (percentage of total aboveground biomass made up by *I. glandulifera*) was logit-transformed. Post-hoc comparisons were performed using Tukey’s HSD test for multiple comparisons, via the ‘emmeans’ R package. Specified contrasts were between treatments 1-2, 3-2, 4-2, 5-2, 6-2, 7-2, 1-4, 6-4, 3-5, 7-5, and 7-6, as these were the contrasts best suited to our experimental aims. All analysis was conducted in R, version 3.3.1 (R Core Team, 2017). A statistics table of significant effects can be found in Appendix 1.

#### **2.2.5 MEASURING SOIL WATER CONTENT**

Soil moisture was measured every three weeks using a soil moisture meter (HH2 WET Sensor). These measurements took place 24 hours following watering. Prior to measurement, soil calibration coefficients  $b_0$  and  $b_1$  were obtained according to the WET User Manual (v1.6) and

entered into the HH2 ‘custom calibrations’ section. Finding the appropriate calibration coefficients is imperative to proper use of the WET sensor, as water content measurements can vary depending on the soil type.

To measure the calibration coefficients, three 250 mL pots (volume,  $L$ ) of the soil used in the experiment were watered to saturation and had their permittivity,  $E'_w$ , measured with the WET sensor. These samples were then weighed again to give  $W_w$ . Samples were then oven-dried until completely dry, and then had their dry weight measured to give  $W_0$ . Permittivity of the dry samples was then measured using the WET sensor.

The first calibration coefficient,  $b_0$ , was found using the formula  $b_0 = \text{sqrt}(E'_0)$ .  $b_0 = 1.2$ .

Volumetric water content ( $\theta_w$ ) was then calculated as  $\theta_w = (W_w - W_0) / L$ . Finally,  $b_1$  was calculated as  $b_1 = (\text{sqrt}(E'_w) - \text{sqrt}(E'_0)) / \theta_w$ .  $b_1 = 10.9$ . Calculated values were the mean of the three pots.

## **2.3 RESULTS**

### **2.3.1 BIOMASS**

#### **2.3.1.1 ABOVEGROUND BIOMASS**

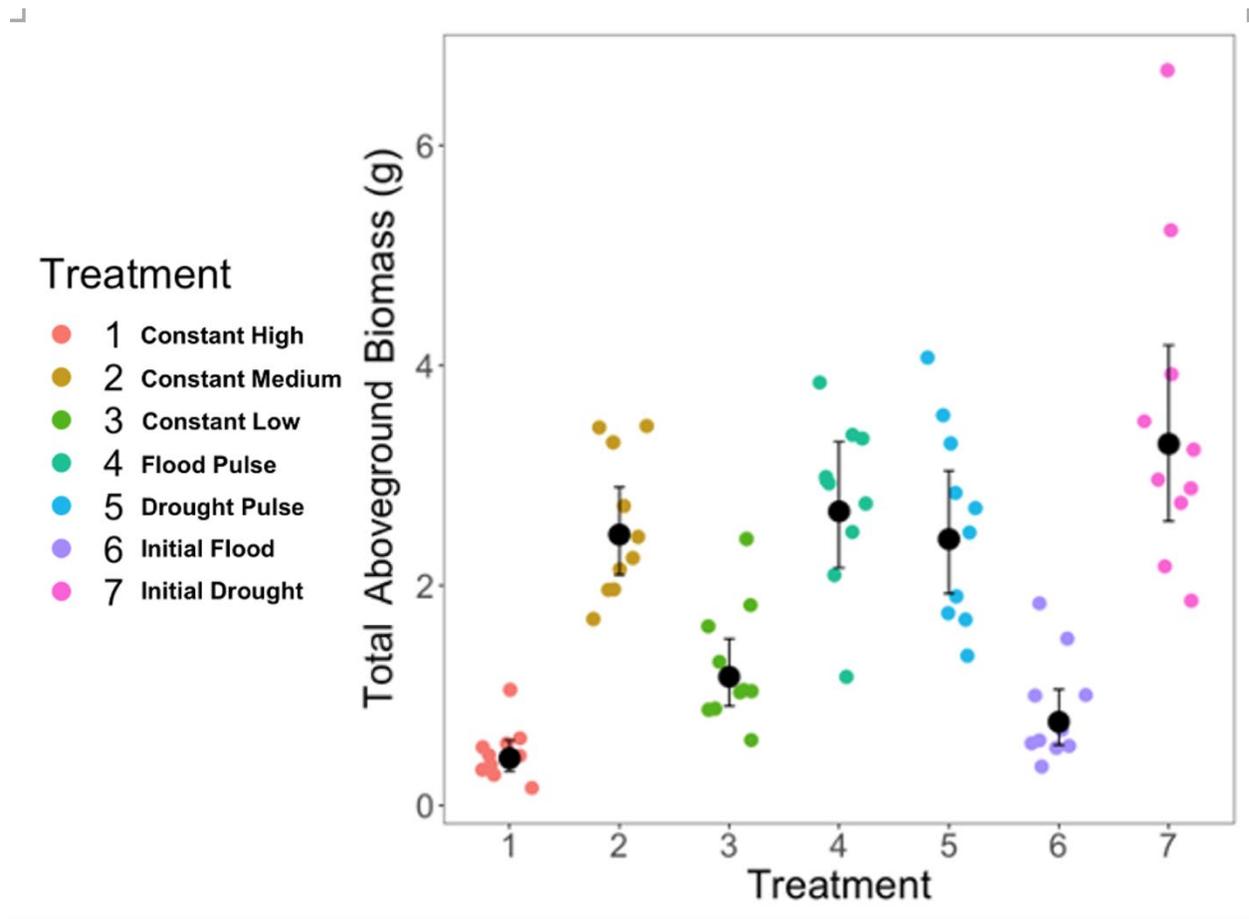
##### Total Aboveground Biomass

A one-way ANOVA revealed there was a significant effect of watering treatment on total aboveground biomass, which includes both invasive and non-invasive species ( $F_{6, 63} = 35.66$ ,  $p < 0.001$ ).

Tukey’s HSD test for multiple comparisons showed that the mean aboveground biomass in the pots was significantly lower than the constant medium treatment (treatment 2) in the flooding ( $p < 0.001$ ), drought ( $p < 0.001$ ), and initial flood ( $p < 0.001$ ) treatments. Additionally, the flood ( $p = 0.6515$ ) and drought ( $p = 0.9255$ ) pulses, as well as the initial drought ( $p < 0.617$ ) treatment,

showed no overall difference in aboveground biomass when compared to the constant medium watering treatment.

The timing of a watering treatment significantly affected aboveground biomass. The constant flood treatment had significantly less biomass than the flood pulse ( $p < 0.001$ ), an effect shared by the constant drought treatment, which also had significantly less biomass than the drought pulse treatment ( $p < 0.001$ ). A flood at the start of the experiment, treatment 6, resulted in significantly lower aboveground biomass than treatment 7, the initial drought ( $p < 0.001$ ).

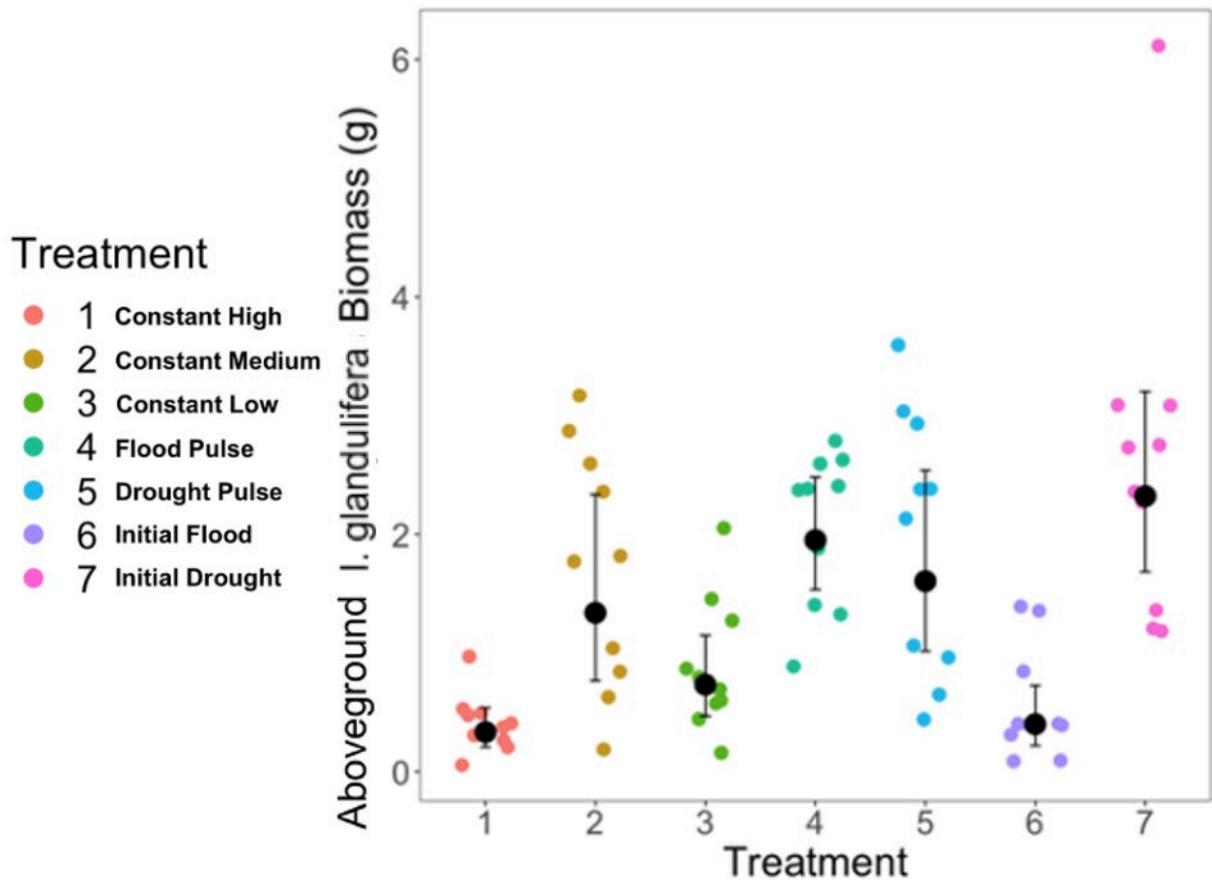


**Fig 7.** Total aboveground biomass, measured using dried weight (g) of all species grown in soil under 12 weeks of varying watering treatments. Black circles represent the mean, and thin lines represent  $\pm 1$  standard error. Coloured dots represent actual data points recorded.

## Aboveground Biomass of *I. glandulifera*

A one-way ANOVA revealed there was a significant effect of watering treatment on the aboveground biomass of *I. glandulifera* ( $F_{6, 63} = 11.46, p < 0.001$ ).

Tukey's HSD test for multiple comparisons showed that the mean aboveground biomass (g) of *I. glandulifera* was significantly lower than the constant medium treatment (treatment 2) in the constant high ( $p < 0.001$ ) and initial flood ( $p < 0.001$ ) treatments. Interestingly, the flood pulse treatment showed no significant difference in *I. glandulifera* biomass compared to the constant medium treatment ( $p = 0.249$ ), and was significantly higher than the constant high ( $p < 0.001$ ) and initial flood ( $p < 0.001$ ) treatments, showing that there is a temporal element associated with a decrease in biomass due to excess water. The drought ( $p = 0.067$ ), drought pulse ( $p = 0.579$ ), and initial drought ( $p = 0.094$ ) treatments showed no significant difference in *I. glandulifera* biomass compared to the constant medium treatment.



**Fig 8.** Aboveground biomass of *I. glandulifera*, measured using dried weight (g) of *I. glandulifera* plants grown in soil under 12 weeks of varying watering treatments. Black circles represent the mean, and thin black lines represent  $\pm 1$  standard error. Coloured dots represent actual data recorded.

## Aboveground Biomass of Natives

### A) *C. angustifolium*

A one-way ANOVA revealed there was a significant effect of watering treatment on aboveground biomass of *C. angustifolium* ( $F_{6, 56} = 5.454$ ,  $p < 0.001$ ).

Tukey's HSD test for multiple comparisons showed that the mean aboveground biomass (g) of *C. angustifolium* was significantly lower than the constant medium treatment (treatment 2) only in the constant high ( $p = 0.024$ ) treatment. In the initial drought treatment, however, *C. angustifolium* had a significantly higher aboveground biomass than in the constant medium treatment ( $p = 0.016$ ). All other treatments showed no significant difference in biomass compared to the constant medium.

Although the initial flood treatment did not differ significantly from the constant medium treatment ( $p = 0.156$ ), it showed significantly lower aboveground biomass than in the initial drought treatment ( $p < 0.001$ ).

### B) *R. obtusifolius*

A one-way ANOVA revealed there was a significant effect of watering treatment on aboveground biomass of *R. obtusifolius* ( $F_{6, 63} = 20.17$ ,  $p < 0.001$ ).

Tukey's HSD test for multiple comparisons showed that the mean aboveground biomass (g) of *R. obtusifolius* was significantly lower than the constant medium treatment (treatment 2) in the constant high ( $p < 0.001$ ) and initial flood ( $p = 0.002$ ) treatments. The flood pulse treatment, however, showed no significant difference in biomass to the constant medium treatment ( $p =$

0.955). Both the constant high ( $p < 0.001$ ) and initial flood ( $p = 0.001$ ) treatments had significantly lower *R. obtusifolius* biomass than the flood pulse treatment. All other treatments showed no significant difference in biomass compared to the constant medium.

The initial drought treatment, whilst not having significantly different *R. obtusifolius* biomass to the constant medium watering ( $p = 0.887$ ), had significantly higher biomass than treatment 6, the initial flood ( $p = 0.002$ ).

### C) *E. hirsutum*

A one-way ANOVA revealed there was a significant effect of watering treatment on aboveground biomass of *E. hirsutum* ( $F_{6,63} = 6.095$ ,  $p < 0.001$ ).

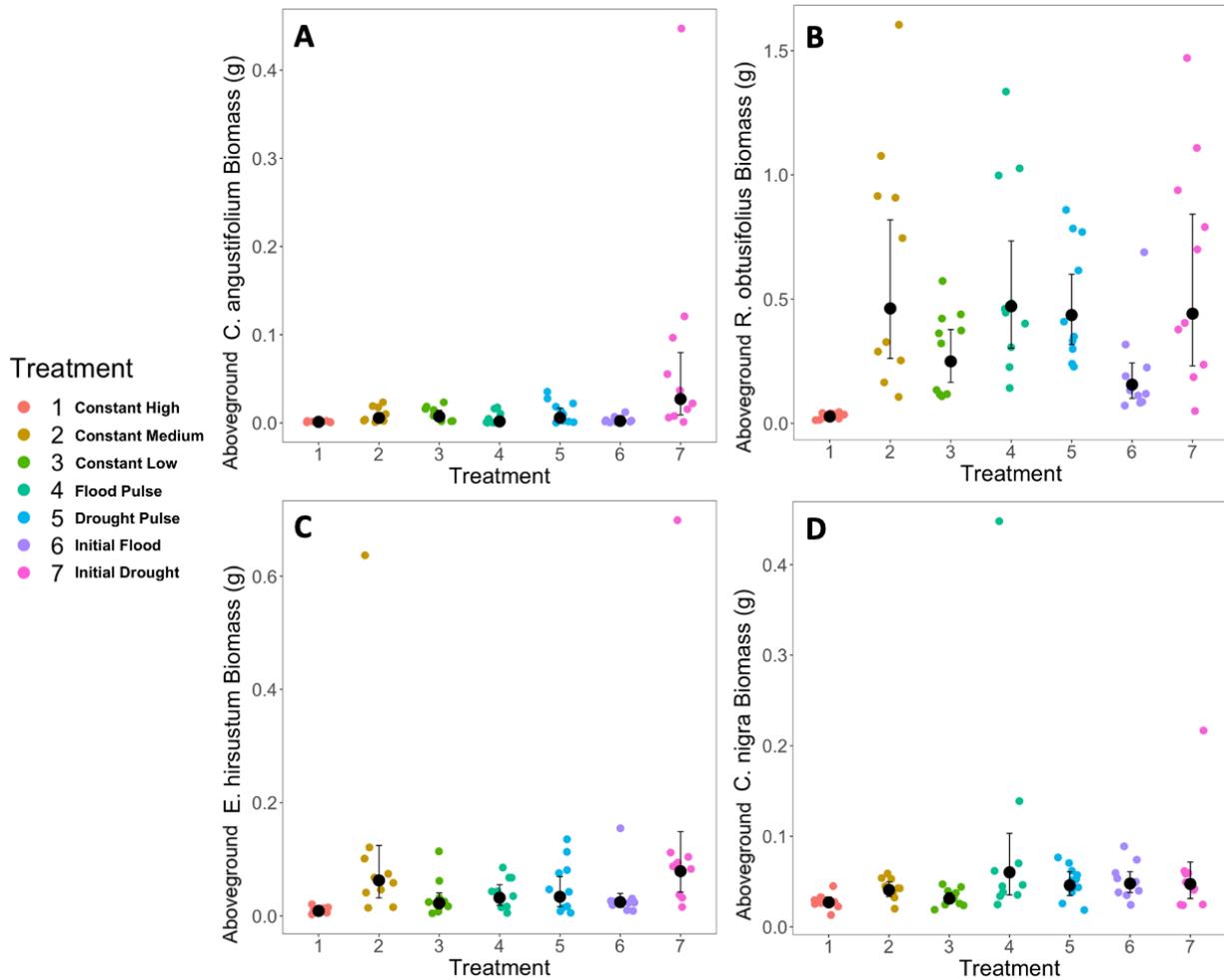
Tukey's HSD test for multiple comparisons showed that the mean aboveground biomass (g) of *E. hirsutum* was significantly lower than the constant medium treatment (treatment 2) in the constant high ( $p < 0.001$ ), constant low ( $p = 0.017$ ), and initial flood ( $p = 0.026$ ) treatments. The flood pulse treatment, however, showed no significant difference in biomass to the constant medium treatment ( $p = 0.110$ ). The constant high treatment had significantly lower biomass than the flood pulse treatment ( $p = 0.003$ ), however the initial flood, whilst significantly lower in biomass than the constant medium, did not result in significantly different biomass to the flood pulse treatment ( $p = 0.5089$ ).

The initial drought treatment had slightly higher *E. hirsutum* biomass than the drought pulse ( $p = 0.044$ ), and significantly more biomass than the initial flood treatment ( $p = 0.006$ ).

### D) *C. nigra*

A one-way ANOVA revealed there was a significant effect of watering treatment on aboveground biomass of *C. angustifolium* ( $F_{6,63} = 2.964$ ,  $p = 0.013$ ).

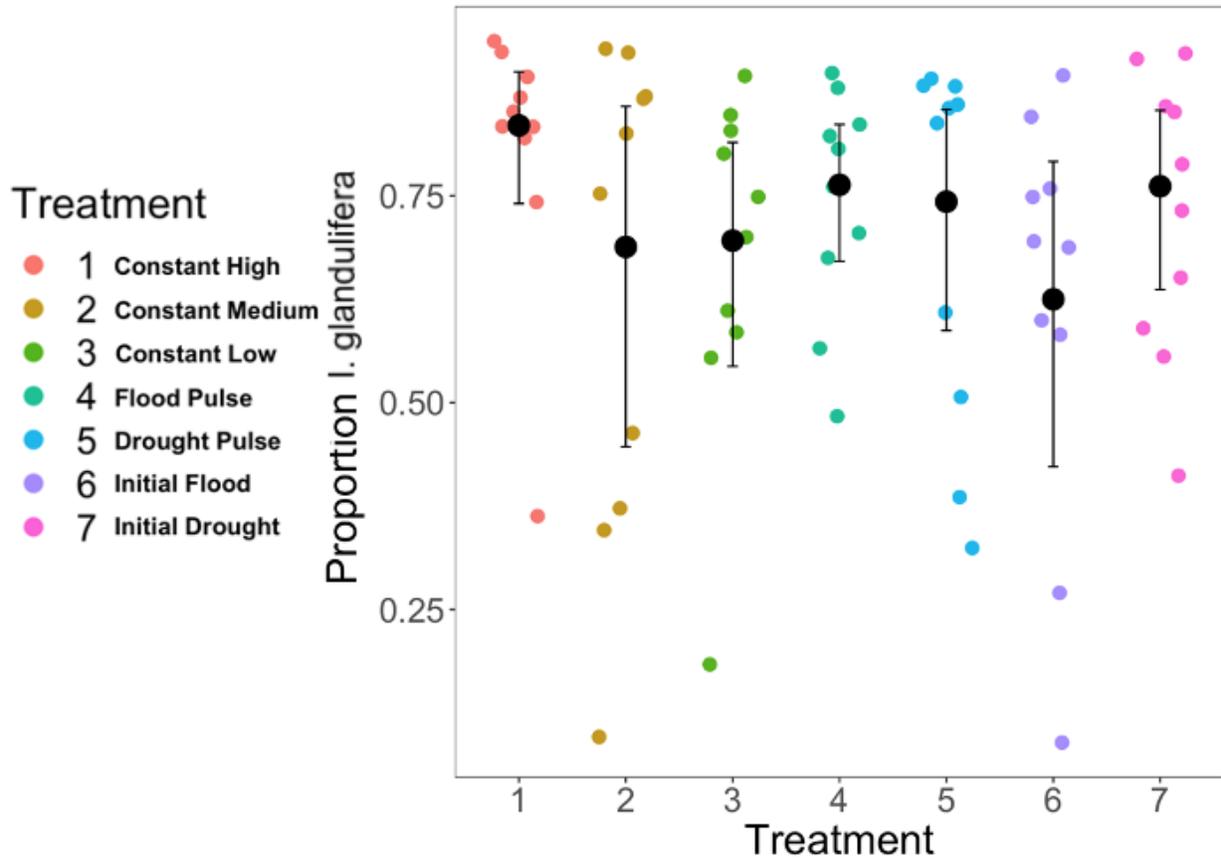
Tukey's HSD test for multiple comparisons showed that the mean aboveground biomass (g) of *C. nigra* was significantly lower in the constant high treatment when compared to the flood pulse ( $p < 0.001$ ). There were no other significant differences in biomass between the watering treatments.



**Fig 9.** biomass of native species A) *C. angustifolium*, B) *R. obtusifolius*, C) *E. hirsutum*, and D) *C. nigra*, measured using dried weight (g) of plants grown in soil under 12 weeks of varying watering treatments. Black circles represent the mean, and thin black lines represent  $\pm 1$  standard error. Coloured dots represent actual data recorded.

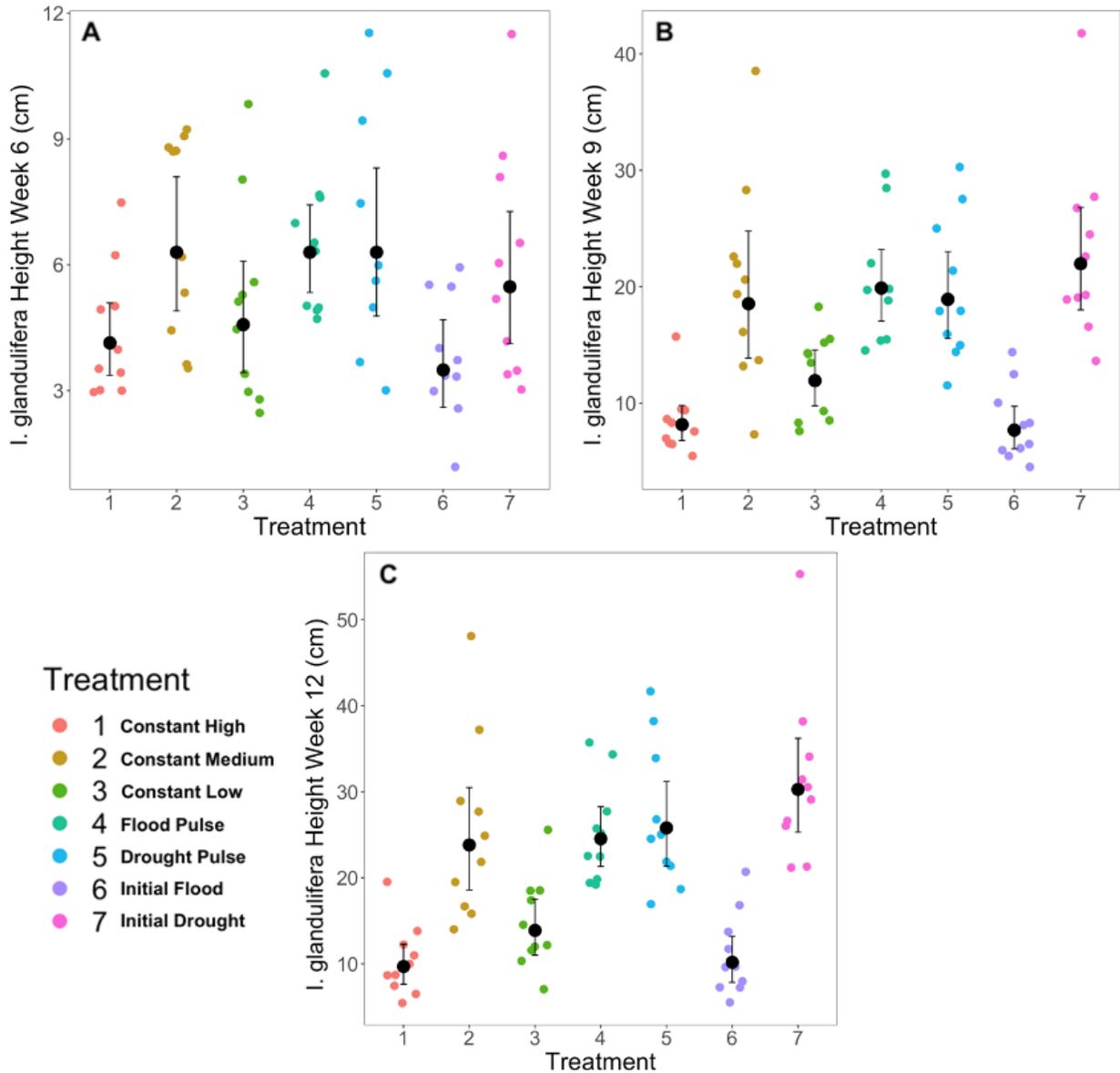
### 2.3.1.2 PROPORTIONAL *I. glandulifera* ABOVEGROUND BIOMASS

A one-way ANOVA revealed there was no significant effect of watering treatment on proportional aboveground biomass of *I. glandulifera* ( $F_{6, 63} = 0.992$ ,  $p = 0.439$ ). *I. glandulifera*, regardless of treatment, made up the majority of aboveground biomass in the pot.



**Fig 10.** Proportional *I. glandulifera* aboveground biomass, measured using dried weight (g) of *I. glandulifera* plants grown in soil under 12 weeks of varying watering treatments. Black circles represent the mean, and thin black lines represent  $\pm 1$  standard error. Coloured dots behind represent actual data recorded.

### 2.3.2 HEIGHT



**Fig 11.** *I. glandulifera* height at A) week 6, B) week 9, and C) week 12, measured using the height from the base to the tallest point (cm) of *I. glandulifera* plants grown in soil under varying watering treatments. Black circles represent the mean, and thin black lines represent  $\pm 1$  standard error. Coloured dots behind represent actual data recorded.

### *I. glandulifera* Height at Week 6

A one-way ANOVA revealed there was an effect of watering treatment on the height of *I. glandulifera* six weeks into the experiment ( $F_{6, 63} = 3.469, p = 0.005$ ).

Tukey's HSD test for multiple comparisons showed that the mean height (cm) of *I. glandulifera* plants was significantly lower than the constant medium (treatment 2) treatment in the constant high ( $p = 0.022$ ) and initial flood ( $p = 0.002$ ) treatments. Interestingly, the constant low ( $p = 0.074$ ) and initial drought ( $p = 0.433$ ) treatments showed no significant difference in height compared to the constant medium treatment.

The initial drought treatment also showed significantly greater mean height than the initial flood treatment (0.016).

### *I. glandulifera* Height at Week 9

A one-way ANOVA revealed there was a significant effect of watering treatment on the height of *I. glandulifera* nine weeks into the experiment ( $F_{6, 63} = 17.39, p = 0.005$ ).

Tukey's HSD test for multiple comparisons showed that the mean height (cm) of *I. glandulifera* plants was significantly lower than the constant medium (treatment 2) treatment in the constant high ( $p < 0.001$ ), constant low ( $p = 0.005$ ), and initial flood ( $p < 0.001$ ) treatments.

Contrary to the pattern shown in the constant flood and initial flood treatments, whilst plants in the constant low treatment were significantly smaller than in the constant medium, the initial drought treatment had no difference in mean height ( $p = 0.242$ ) compared to the constant medium. This was also the case when comparing the constant drought to the drought pulse treatment, where the constant drought showed significantly lower mean height ( $p = 0.003$ ). The initial drought treatment also had significantly greater height than the initial flood treatment ( $p < 0.001$ ).

The mean height of *I. glandulifera* plants in the flood pulse treatment was significantly higher than both the constant high ( $p < 0.001$ ) and initial flood ( $p < 0.001$ ) treatments.

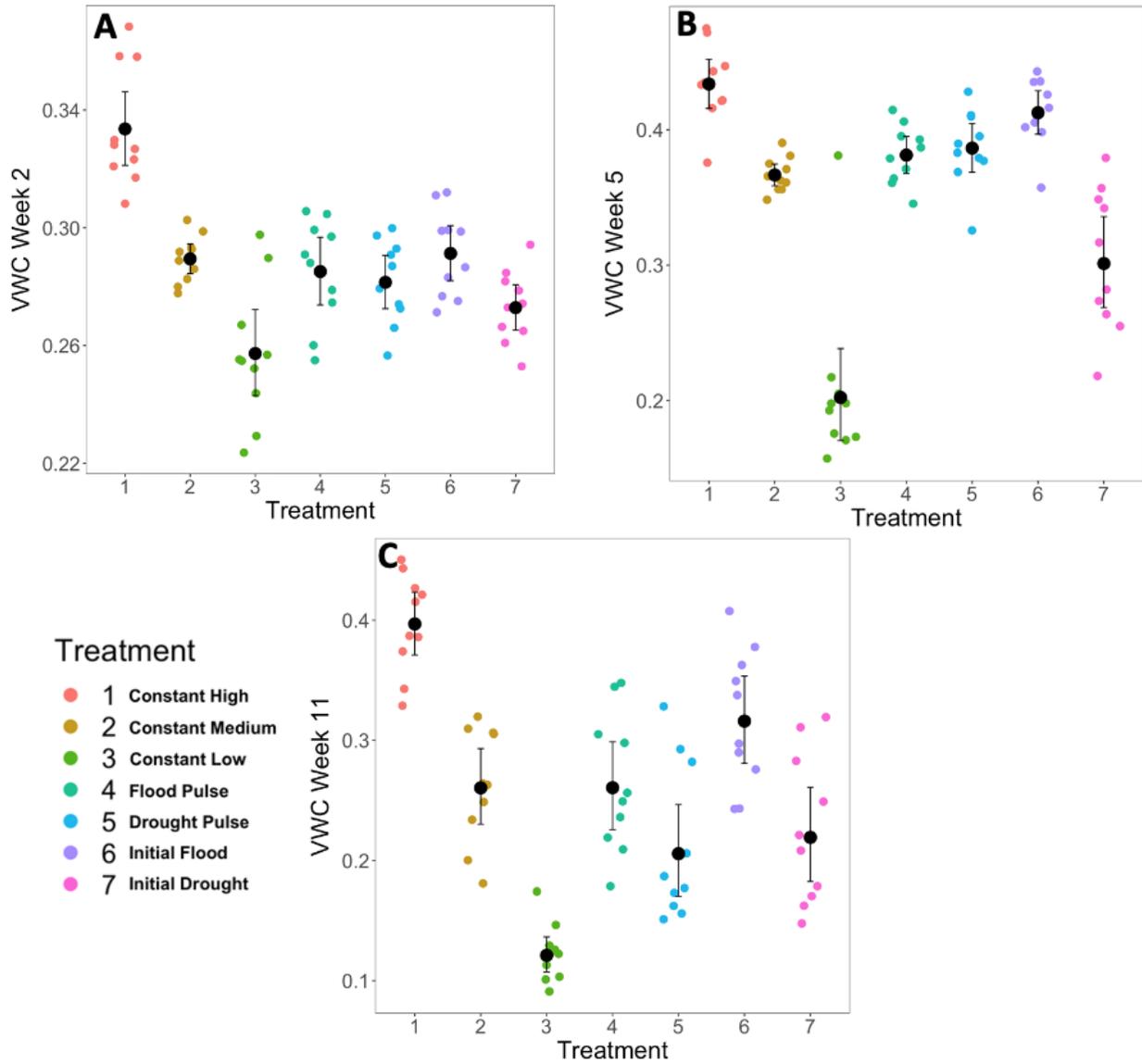
### *I. glandulifera* Height at Week 12

A one-way ANOVA revealed there was a significant effect of watering treatment on the height of *I. glandulifera* nine weeks into the experiment ( $F_{6, 63} = 19.15, p < 0.001$ ).

Tukey's HSD test for multiple comparisons showed that the mean height (cm) of *I. glandulifera* plants was significantly lower than the constant medium (treatment 2) treatment in the constant high ( $p < 0.001$ ), constant low ( $p < 0.001$ ), and initial flood ( $p < 0.001$ ) treatments. Similar to previous weeks, the constant high ( $p < 0.001$ ) and initial flood ( $p < 0.001$ ) treatments were also significantly smaller than the flood pulse treatment.

The constant low treatment was significantly smaller than the drought pulse treatment ( $p < 0.001$ ), however there was no difference in mean height between the drought pulse and initial drought treatment ( $p = 0.314$ ). As before, the initial drought treatment was significantly taller on average than the initial flood treatment ( $p < 0.001$ ).

### 2.3.3 SOIL VOLUME WATER CONTENT



**Fig 12.** Soil volume water content (%) of pots for each watering treatment at A) week 2, B) week 5, and C) week 11, measured using a soil moisture meter. Black circles represent the mean, and thin black lines represent  $\pm 1$  standard error. Coloured dots behind represent actual data recorded.

#### Pot Soil Volume Water Content at Week 2

A one-way ANOVA revealed there was a significant effect of watering treatment on the pot WWC two weeks into the experiment ( $F_{6, 63} = 19.65, p < 0.001$ ).

Tukey's HSD test for multiple comparisons showed that the mean VWC (%) of the pots in the constant high treatment was significantly higher than the constant medium treatment ( $p < 0.001$ ). The constant drought ( $p < 0.001$ ) and initial drought ( $p = 0.027$ ) treatments had a significantly lower mean VWC than the constant medium.

As expected, the constant high treatment VWC was significantly higher than the flood pulse treatment ( $p < 0.001$ ), and the constant low treatment had significantly lower VWC than the drought pulse treatment ( $p < 0.001$ ).

The initial drought treatment had a significantly lower mean VWC than the initial flood treatment ( $p = 0.015$ ).

### Pot Soil Volume Water Content at Week 5

A one-way ANOVA revealed there was a significant effect of watering treatment on the pot VWC nine weeks into the experiment ( $F_{6, 63} = 44.54, p < 0.001$ ).

Tukey's HSD test for multiple comparisons showed that the mean VWC (%) of the pots in the constant high treatment was again significantly higher than the constant medium (treatment 2) treatment ( $p < 0.001$ ), however the initial flood treatment was also significantly higher after at week five ( $p = 0.019$ ). The constant drought ( $p < 0.001$ ) and initial drought ( $p < 0.001$ ) treatments also had a significantly lower mean VWC than the constant medium.

The flood pulse ( $p = 0.438$ ) and drought pulse ( $p = 0.297$ ) treatments showed no significant difference in mean VWC compared to the constant medium.

The constant high treatment mean VWC was significantly higher than the flood pulse treatment ( $p < 0.001$ ), however the initial flood treatment showed no significant difference in mean VWC when compared to the flood pulse ( $p = 0.107$ ).

The drought pulse treatment had significantly higher mean VWC than the constant low ( $p < 0.001$ ) and initial drought ( $p < 0.001$ ) treatments.

The initial drought treatment again had a significantly lower mean VWC than the initial flood treatment ( $p < 0.001$ ).

### Pot Soil Volume Water Content at Week 11

A one-way ANOVA revealed there was a significant effect of watering treatment on the pot VWC nine weeks into the experiment ( $F_{6, 63} = 29.43, p < 0.001$ ).

Tukey's HSD test for multiple comparisons showed that the mean VWC (%) of the pots in the constant high ( $p < 0.001$ ) and initial flood ( $p = 0.038$ ) treatments were again significantly higher than the constant medium (treatment 2) treatment. The constant drought ( $p < 0.001$ ) treatment had a significantly lower mean VWC than the constant medium, however the initial drought ( $p = 0.081$ ) treatment showed no significant difference in soil water content compared to the constant medium.

In week 11, the effect of the drought pulse was finally reflected in the VWC data, with the drought pulse soil being significantly dryer than the constant medium ( $p = 0.019$ ). The flood pulse, however, remained non-significant compared to the constant medium ( $p = 0.995$ ).

The constant high treatment mean VWC was significantly higher than the flood pulse treatment ( $p < 0.001$ ). The initial flood treatment also had significantly higher VWC than the flood pulse treatment ( $p = 0.038$ ).

The constant high treatment mean VWC was significantly higher than the flood pulse treatment ( $p < 0.001$ ), and the initial flood treatment had significantly higher mean VWC than the flood pulse ( $p = 0.038$ ).

The drought pulse treatment had significantly higher mean VWC than the constant low ( $p < 0.001$ ) treatment, however the initial drought and drought pulse treatments showed no significant difference in mean VWC ( $p = 0.529$ ).

The initial drought treatment again had a significantly lower mean VWC than the initial flood treatment ( $p < 0.001$ ).

## 2.4 DISCUSSION

Invasive species often have higher growth rates and resource-use efficiency than natives, even when conditions are sub-optimal. The altered resource availability caused by climate change-induced extreme weather, such as floods and droughts, has the potential to facilitate the spread and establishment of invasive species (Davidson *et al.*, 2011; Higgins & Richardson, 2014). This may occur as a result of the higher resource-use efficiency, phenotypic plasticity, and competitive ability observed in many invasive plants, with these species being better-able to utilise resources in a disturbed environment, as well as tolerate substandard conditions, compared to native species (Dawson *et al.*, 2010; Cuda *et al.*, 2015; Cavaleri & Sack, 2020). However, previous studies have shown that the increased competitive ability of invasive species may not hold true across the entire temporal period of plant invasions, with increased vulnerability at the seedling stage (Gioria & Osborne, 2014); the effects of climate change on this occurrence, if any, lacked research. The results presented in this study further confirm the increased competitive ability of invasive plants, and suggest that their spread and establishment may increase under the extreme weather wrought by climate change.

This study showed a significant effect of watering treatment on the growth and competitive ability of *I. glandulifera* compared to a simulated native community from the UK's Northeast. From the aboveground biomass in the pots from each of the seven treatments, it is clear that all plants, native and invasive, perform significantly worse under constant high and constant low watering, as well as when a 'flood' occurs at the beginning of the growing period, during the seedling stage. Flooding, or simply an overabundance of water, negatively affects plant growth in a number of ways, mainly through a reduction in soil oxygen, as well as altered water, carbohydrate, and nutrient uptake (Kozlowski, 1984; Visser *et al.*, 2003). Stomata usually close soon after a flooding event, and remain so for an extended period of time; this has obvious consequences for plant growth. These effects are especially detrimental to plants in the growing stage, when they are highly vulnerable to any changes in the environment (Kozlowski, 1984).

The effect of watering treatment on the aboveground biomass of *I. glandulifera* was similar to that of the pot biomass. The constant high and initial flood treatments resulted in much lower biomass than the other treatments, none of which encouraged significantly greater growth. The same effect was observed in the native species *R. obtusifolius* and *E. hirsutum*, both of which

grew worse in the flooding and initial flood treatments. *C. angustifolium* and *C. nigra*, however, were only negatively affected by the constant high treatment; no other watering regime had a significant effect on biomass. These results suggest that floods have the potential to negatively affect the growth of both invasive and native species. This effect also has a temporal element; the flood pulse treatment, treatment four, had no significant effect on pot biomass, nor any of the individual plant species, be they invasive or native. Therefore, a constant high water level and a flood at the beginning of a plant's development may have a much greater effect than a single flooding event mid-way through the growing cycle.

Drought stress affects growth through a lack of resource availability, and associated pressures such as lower photosynthesis and root mineral uptake (da Silva *et al.*, 2013). The constant low treatment, however, did not result in a significantly lower quantity of biomass in *I. glandulifera* or any of the natives. This suggests that the significantly lower pot biomass in the constant low treatment was a result of combined effects across all of the species growing slightly worse under a drought, however not on an individually significant level. This result supports the finding that, at least in this simulated community, climate change-induced floods will have a much greater impact on plants than drought, however both extreme weather events will negatively impact plant biomass. The drought pulse also had no significant effect on biomass for any of the species, suggesting that a short period of drought during the year will not have as major an impact, if any, as a long flood, especially a flood at the initial stage of a plant's life cycle. Unlike the flooding treatment, an initial drought did not significantly affect pot biomass; drought stress appears to facilitate better recovery than flooding stress. A recent meta-analysis found that drought stress affects plant biomass allocation, with a significant increase in root mass fraction and a decrease in stem, leaf, and reproductive mass (Eziz *et al.*, 2017). It can be hypothesised that during the initial drought treatment, the invasive and native species allocated greater biomass to the roots, allowing them to recover quickly when a high quantity of water was supplied following the six-week initial drought. However, this study did not address biomass allocation; it would be interesting to observe if this effect holds true for *I. glandulifera* and the simulated native community across the temporal gradient in this study (constant low, drought pulse, and initial drought), as well as to investigate biomass allocation under the flooding treatments.

The height of *I. glandulifera* in each watering treatment followed similar patterns to that of biomass, as expected. After six weeks of growth, *I. glandulifera* in the constant high and initial flood treatments were significantly smaller than those in the pots from the other five watering regimes. As the experiment continued, the height differential between the constant high and initial flood treatments continued to increase, with average height appearing to halt at around 10cm at week nine, and continuing to the end of the experiment after 12 weeks. Additionally, although the constant low treatment showed no significant difference in height to the constant medium after six weeks of growth, after nine weeks *I. glandulifera* plants were significantly smaller than the medium; this effect continued until the end of the experiment. The detrimental effects of the constant high, initial flood, and constant low treatments seemed to compound over the course of the experiment. This was especially the case for the diminutive growth shown by the invader in the ‘initial flood’ treatment; the negative effects of a flood at the seedling stage may have been significant enough to severely affect growth for the rest of the plant growing period, even when the watering regime returned to normality. The findings of this study, especially the protracted loss of height/biomass caused by a brief flooding event at the beginning of a plant’s growing period, add support to similar findings in the literature, such as those of Gioria & Osborne (2014).

Interestingly, the tallest individual plant was found in the initial drought treatment, being above 50cm tall at the end of the experiment, and whilst non-significant, the mean height of plants in this treatment was slightly higher than even the constant medium. It is clear that a short period of drought at the beginning of an invasive plant’s growing period is not necessarily negative, at least in this simulated community; actually, it may foster increased competitive ability. Invasive species are known for their increased resource-use efficiency, and it is possible that *I. glandulifera* was less-affected by the initial drought than the native species. This would allow it to quickly grow to a more developed stage than the natives, and be better poised to utilise the increased water availability once the initial drought treatment ceased in week six.

Although the effect of each watering treatment on pot biomass appears to reflect the condition of both the native species and *I. glandulifera*, the proportion of pot biomass taken up by *I. glandulifera* makes clear that these biomass effects are overwhelmingly driven by the invader. *I. glandulifera* made up the majority of plant biomass in every treatment, even those that

negatively impacted on its growth. There was no significant difference in mean proportion of aboveground biomass taken up by *I. glandulifera*; this supports the literature suggesting invasive species have an inherent greater competitive ability than native species in its invaded ecosystem, even when its growth is negatively impacted by an extreme weather event, such as those simulated by the constant high and initial flood treatments.

Using a soil moisture meter confirmed the impact of an invader on water availability; treatments where *I. glandulifera* did well, such as treatment 7, the initial drought, continued to show a low volume water content percentage even when the ‘drought’ finished and the pots were given five-times the water. These were also the pots where *I. glandulifera* grew the tallest; it may have been that the increased evapotranspiration of these tall plants, in addition to extensive root systems and resource-use efficiency, kept pot volume water content low, stifling the growth of natives.

Using a moisture meter to measure pot volume water content allowed for confirmation that the various watering treatments had a significant effect on soil moisture, and thus that any subsequent changes in biomass or height were a result of these treatments, and not other, unexpected variables. The treatments did result in the expected changes in volume water content throughout the 12-week growing period, especially in the three ‘constant’ treatments: constant high was always significantly higher than constant medium, with constant low always significantly lower. The initial drought treatment was also effective in significantly lowering volume water content compared to the other treatments. The flood pulse and drought pulse treatments, however, never resulted in a significantly higher or lower soil moisture level when compared to each other or the constant medium. The volume of water applied for these treatments may not have been extreme enough to significantly affect pot water content. Alternatively, the length of the pulse, three weeks, may have required a longer period to take effect. However, a short extreme weather event in the field may also fail to significantly alter soil volume water content under a similar time-frame, suggesting that these treatments may be effective simulations of a real-life short drought or flood event. Regardless of any significant changes in soil volume water content, the inclusion of a drought or flood pulse was important to investigate the impact of the timing of an extreme weather event on the competitive ability of *I. glandulifera*.

When evaluating the results of this study, it must be kept in mind that *I. glandulifera* is an annual plant, and the four natives are all biennial. These differences in growing period partially explain the incredibly large differences of biomass between the *I. glandulifera* and the four natives.

Whilst *I. glandulifera* must focus its resources on growing as quickly as possible during its sole growing period to grow as tall as possible and out-shade other plants, the natives have a longer period of time to accumulate biomass; this growth may have been allocated to the roots, which were not measured in the study. Additionally, as an annual *I. glandulifera* has a greater need and ability to plastically respond to environmental stresses than biennial species, which may explain its proportional biomass dominance even in those treatments that negatively affected its growth. Although these native species may follow different life cycles, their co-occurrence with the invasive *I. glandulifera* is well documented in the UK, and *I. glandulifera* continues to spread and outcompete native species throughout the country. In addition, *I. glandulifera* is an incredibly successful invader even among other invasive species of the same genus; Cuda *et al.*, (2015) found that compared to two *Impatiens* species, the invasive *I. parviflora* and native *I. noli-tangere*, *I. glandulifera* overtopped both congeners early into the experiment, increasing in superiority throughout the growing season. This was down to the ability of *I. glandulifera* to grow continuously throughout the whole vegetation period, an advantage seemingly demonstrated in the present study as well (Cuda *et al.*, 2015). The competitive ability of *I. glandulifera* will be further explored in the context of climate change in the following chapter.

The evidence presented in this study, especially the proportional dominance of pot biomass regardless of watering treatment demonstrated by *I. glandulifera*, suggests that riparian invaders such as *I. glandulifera* are poised to benefit from the effects of climate change on native plant communities. Extreme weather events caused by climate change, such as floods and droughts, as well as general climate-induced changes in water availability, are set to increase disturbance in these ecosystems; this has great potential to increase the invasibility of these areas. The sheer speed at which invasive species, or at least annuals such as *I. glandulifera*, accumulate biomass, as well as their increased competitive ability and plasticity, grant these species inherent advantages over natives, which will only be exacerbated under the effects of climate change. Even when the growth of *I. glandulifera* is negatively affected, such as in the constant high and initial flood treatments, the proportional biomass dominance exhibited by the invasive species is unaffected, exhibiting its advantages over native species. The timing of an extreme weather

event appears to affect the dominance of riparian invasive species little; *I. glandulifera* remained more competitive than the natives even in the initial flood treatment, during its seedling stage. Native species undoubtedly suffer when an invasive plant succeeds, with low aboveground biomass potentially as a result of lower resource availability and increased shading by the invader, accentuated by inherent advantages possessed by invasive species over natives. Greater efforts must be put into limiting the spread and impact of invasive riparian species such as *I. glandulifera*, as their negative impacts are only set to increase under the effects of climate change.

## Chapter 3:

The effects of temperature and soil conditioning on the competitive ability of *Impatiens glandulifera* relative to a native plant community

### ABSTRACT

Invasive plant species such as *I. glandulifera* have the ability to alter the biotic and abiotic components of soil, resulting in plant-soil feedback chains that affect the performance of plants within the surrounding community. This is often positive for the invader, and negative for native plant species. However, little is known about the impact, if any, of increased temperature due to climate change on these PSFs. A two-phase pot experiment was conducted to assess the impact of initial soil conditioning by *I. glandulifera* and a community of natives on the subsequent competitive ability of *I. glandulifera* in a community with native species, as well as to observe the effects of increased temperature on this dynamic. Plant physical parameters were recorded, confirming both a positive PSF and greater competitive ability of *I. glandulifera*, but also that these effects can complement the impacts of climate change to negatively affect the native community. These findings suggest that in future ecosystems subjected to the effects of climate change, invasive species have the potential to become even more competitive than the native community. This has important implications for future efforts to manage invasive species.

### 3.1 INTRODUCTION

There is a large repository of evidence demonstrating that plant species are able to alter the biotic and abiotic components of soil, resulting in feedback chains that subsequently affect the performance of plants within the surrounding community (Kulmatiski *et al.*, 2008; Beals *et al.*, 2020). These plant-soil feedbacks (PSFs) can result in increased growth and survival when positive; however, when negative can have the opposite effect (Bennett & Klironomos, 2018; Beals *et al.*, 2020). PSFs are often positive for invasive plant species in their invaded range yet negative between native and exotic plants in these communities; this can result in increased growth and survival of invasive species, facilitating further invasions and often resulting in monotypic stands at the expense of natives (Kulmatiski *et al.*, 2006; van der Putten *et al.*, 2013). The expansion and soil-conditioning effects of alien plants can cause losses not only to the structure and function of invaded ecosystems, but also on a social and economic level (Linders *et al.*, 2019; Diagne *et al.*, 2021).

One possible pathway for invasive plant species to negatively impact natives is through the alteration of arbuscular mycorrhizal fungi (AMF) communities. Nearly 90% of plant species can develop mutualistic connections with AMF via their roots to significantly increase belowground surface area, resulting in greater nutrient uptake and an improvement in plant growth (Bowles *et al.*, 2016; Rouphael *et al.*, 2015; Begum *et al.*, 2019). However, invasive plant species are often less-dependent on AMF than natives, and there is a growing body of research demonstrating that invaded soils have lower AMF densities and colonisation, likely as a direct result of the invasive species (Zybeck *et al.*, 2016; Pattison *et al.*, 2016; Grove *et al.*, 2017). Alternatively, invasive species may continue to associate with AMF and other fungi, instead affecting bacterial community composition, such as increasing bacterial biomass (Pattison *et al.*, 2016; Zhang *et al.*, 2019). These alterations of the soil microbial community composition preferentially favour the growth and competitive ability of invasive species as a positive PSF, whilst co-occurring native species are left to suffer the effects of negative feedbacks (van der Putten *et al.*, 2013; Ruckli *et al.*, 2014).

The role of plant-soil feedbacks in plant invasions has become an area of great interest in recent years (van der Putten *et al.*, 2013). However, the impact of climate change on these dynamics requires further study. Climate modelling for North East England, the location of this

experiment, predicts that the area will become warmer and drier under the effects of climate change (Harley *et al.*, 2011; Parasiewicz *et al.*, 2019). Temperature and drought stress, alongside pressures from invasive plant species, are likely to lead to decreased native plant performance and diversity (Duell *et al.*, 2019). The effects of climate change may also impact PSFs, often to the benefit of invaders. Drier soil conditions as a result of increased temperature and drought events decrease the availability of plant nutrients, especially nitrogen and phosphorus (Suriyagoda *et al.*, 2014; Bowles *et al.*, 2017). Bowles *et al.* (2017) found that the increased root surface area and subsequent nutrient uptake offered by AMF to their host plants increased resistance to drought stress. As some invasive plant species have shown the ability to decrease AMF colonisation and density in soil, their presence may affect AMF-regulated drought stress tolerance in natives, lending a competitive advantage to the invaders (Zybeck *et al.*, 2016). Many plants also show greater drought stress tolerance when their associated microbial community is more diverse, as well as when certain bacteria and fungi groups are present, such as plant growth-promoting microbial groups (Bogati & Walczak, 2022). Plant invasions have been shown to cause losses in microbial diversity and shifts in the structure of microbial populations, and combined with climate change stresses, could negatively affect the performance of native plant species (Malacrino *et al.*, 2020).

In addition to plant-microbe interactions, many invasive species have higher growth rates, resource-use efficiency, and fecundity than native species, even under suboptimal conditions (Kleunen *et al.*, 2010; Dawson *et al.*, 2011; Funk, 2013; Ens *et al.*, 2015; Jelbert *et al.*, 2015). These characteristics facilitate entry into the novel habitats invaded by these species, and are associated with broad environmental tolerances; it is likely that they will offer invasive species better tolerance to the environmental stresses caused by climate change as well (Davidson *et al.*, 2011; Higgins & Richardson, 2014). The inherent physiological advantages and plant-soil feedbacks of invasive plants have the potential to combine with the effects of climate change and create a ‘perfect storm’ for increased plant invasions in the future.

*Impatiens glandulifera* is one of the most widespread invasive plant species in the UK, and has been shown to alter soil chemistry and microbial communities (Pattison *et al.*, 2016). In the past 20 years, *I. glandulifera* has started to expand from its usual riparian habitat into nearby forest areas, demonstrating its impressive range of environmental tolerances (Cuda *et al.*, 2020). *I.*

*glandulifera* is only weakly dependent on AMF for growth, with corresponding invaded soil only showing sparse colonisation by AMF (Tanner & Gange, 2013). Pattison *et al.* (2016) showed in an experiment similar to the present study that when grown in *I. glandulifera*-conditioned soil, *I. glandulifera* was taller, produced more leaves, grew faster, and had higher biomass than in non-conditioned soil; this suggests a positive PSF. AMF root colonisation in conditioned soil was half that of control soil, whilst bacterial biomass increased almost two-fold in conditioned versus control soil (Pattison *et al.*, 2016). This may be an effect of AMF being starved of carbon usually acquired from its plant host as it fails to form a mutualism with *I. glandulifera* in communities dominated by this invader (Coakley & Petti, 2021). Alternatively, this could be an effect of allelopathy; *I. glandulifera* has been shown to produce 1,4-naphthoquinone (2-MNQ), a potential allochemical capable of inhibiting the growth of nearby native plants or disrupting their soil microbial community (Perglová *et al.*, 2009; Coakley & Petti, 2021). As previously discussed, these *I. glandulifera* soil-conditioning effects have great potential to negatively impact the growth of native species; how these effects combine with increased temperatures and drought events under future climate change is a topic requiring further study.

Competition between invasive and native plant species is typically explored through greenhouse pot studies utilising soil from various origins, such as previously-invaded, native, or sterile soil. Many of these have found that invasive plants grow best in soils most alike their native ranges, and that native species are negatively affected when grown in soil ‘conditioned’ by invaders (Dawson & Schrama, 2016; Pattison *et al.*, 2016). This study utilised a similar approach to determine the PSF effects of *I. glandulifera*; however, the soil conditioning stages were performed in growth chambers set to emulate present-day and future (under the warming effects of climate changing) temperature regimes. Our objectives were to address the impacts of increased temperature due to climate change on 1) soil conditioning by *I. glandulifera*, and 2) the competitive ability of *I. glandulifera* relative to a native plant community. As an extension to this study, soil extracts were taken prior to and following conditioning of pots by *I. glandulifera* and a native community, in order to extract bacterial and fungal community DNA for downstream sequencing and metabarcoding. This allowed for quantitative and qualitative analysis of the microbial community associated with these species, and offers a possible explanation of the results of this study.

## 3.2 METHODOLOGY

### 3.2.1 SITE DESCRIPTIONS

The experiment took place inside two Weiss Fitotron growth chambers with an internal area of 1.2m by 0.75m owned by the Durham University Department of Biosciences in Durham, UK. One growth chamber was set to follow present-day daily temperatures, and the other simulated temperatures under the effects of climate change. Specifically, the present-day chamber followed a temperature regime modelled off of the average hourly temperature in May and June in Durham from 2010 to 2019 (Appendix 2), whereas the ‘future’ chamber had hourly temperature set points 3°C higher than the present chamber, reflecting the ‘intermediate warming’ scenario for North East England described in the UKCP18 report. This resulted in temperatures in the present-day chamber ranging from 10°C to 18.5°C, and temperatures of 13°C to 21.5°C in the future chamber.

The light levels in the chamber also followed those of Durham during May and June (Table 1), with a low level of 50  $\mu\text{moles}/\text{m}^2/\text{s}$  at ‘dawn’ from 04:30 to 5:00, followed by full brightness of 500  $\mu\text{moles}/\text{m}^2/\text{s}$  during the ‘day’ from 05:00 to 21:00, then a subsequent period of low light of 50  $\mu\text{moles}/\text{m}^2/\text{s}$  at ‘twilight’ from 21:00 to 21:30, and full darkness at ‘night’ from 21:30 to 04:30. This pattern was repeated daily throughout the experiment.

**Table 1:** *Light intensity per hour in the growth chambers. The full hourly temperature regime from each chamber can be found in Appendix 3.*

<b>Time period</b>	<b>Hours</b>	<b>Light intensity</b>
Dawn	04:30 to 5:00	50 $\mu\text{moles}/\text{m}^2/\text{s}$
Day	05:00 to 21:00	500 $\mu\text{moles}/\text{m}^2/\text{s}$
Twilight	21:00 to 21:30	50 $\mu\text{moles}/\text{m}^2/\text{s}$
Night	21:30 to 04:30	0 $\mu\text{moles}/\text{m}^2/\text{s}$

### 3.2.2 NATIVE SPECIES SELECTION AND SEED COLLECTION

In October 2020, seed pods from 23 individual *Impatiens glandulifera* plants were collected from three invaded sites around Durham, UK (Fig. 2). Seeds were collected from locally dominant populations of *I. glandulifera*. In addition to the invader, seed families from three native species often occurring near *I. glandulifera* were obtained from seed banks originally sourced from wild populations. These three natives were *Epilobium hirsutum*, *Jacobaea vulgaris*, and *Silene dioica*. The natives were chosen for their co-occurrence with *I. glandulifera*, noted from personal observations around the study area. Additionally, some species, most notably *C. angustifolium* and *C. nigra*, did not show much growth in the previous experiment; the new native species *J. vulgaris* and *S. dioica* may perform better in this experiment. Seed capsules from all species were opened inside a lab, and ten seeds from each of the *I. glandulifera* seed families as well as all of the native seeds were put into cold stratification at 4°C for two months to encourage germination. Following the stratification, seeds from all species were sown on sterilised sand within a greenhouse under constant light and 21°C ambient temperature and left to germinate for two weeks. Successfully germinated seeds were transplanted into 750mL pots, as described in “3.2.4: Experimental Design”.



**Fig. 13.** Location of the sites where seeds of *Impatiens glandulifera* were collected. (Image via Google Maps).

### 3.2.3 SOIL COLLECTION

Soil was collected in-situ from 10 different riparian areas around Durham that had not been previously invaded by *I. glandulifera*. Collected soil was mixed in a 1:1 ratio with sterilised sand, and 750mL of the resulting substrate was used for each pot in the experiment, the maximum pot capacity. The ‘soil origin’ (i.e. which of the 10 soil samples were mixed with sand) was controlled throughout the experiment, being noted and kept exclusive, with no mixing of soils from different origins. Prior to the experiment or any mixing with sand, samples were collected from each of the 10 soil groups and kept refrigerated for use in the soil nutrient content (3.2.5.1 “soil nutrient content”) and soil microbial community analyses (chapter four).

### 3.2.4 EXPERIMENTAL DESIGN

In addition to the role of increased temperature on the growth and competitiveness of invasive species, this experiment also explored how plant-soil feedbacks may be altered by the effects of climate change. To evaluate these changes, this experiment utilised a two-stage approach, first ‘conditioning’ soil with *I. glandulifera* and the three natives in phase 1, and then observing the effects of this soil conditioning through the growth and success of *I. glandulifera* and the natives in the second phase. This ‘two-phase’ approach follows Kulmatiski and Kardol, 2008, as well as Pattison et al., 2016; however, these experiments did not evaluate the effect of temperature on soil conditioning by *I. glandulifera*.

In the first phase, 60 pots of 10.5cm diameter and 13cm height were filled with 750mL of the soil-sand substrate and divided between the two growth chambers, 30 in each, with one chamber set to follow a ‘present-day’ temperature regime, and the other with ‘future’ temperatures simulating the effects of climate change. Seedlings were planted 1cm below the soil surface according to group treatment. In each chamber, the 30 pots were split into groups of 10, with each group containing a different community type, as well as 10 ‘control’ pots with no plants. The three communities were ‘invader’ (I), with three *I. glandulifera* seedlings, ‘community’ (C), with the three native species *Epilobium hirsutum*, *Jacobaea vulgaris*, and *Silene dioica*, and ‘community + invader’ (CI), which had the three natives arranged around a single *I. glandulifera*

seedling in the centre of the pot (Appendix 3). The 30 pots in each growth chamber were arranged randomly, approximately 5cm apart in a five-by-eight grid design. This distance was deemed sufficient to prevent cross-pot colonisation; additionally, pots were observed during watering to ensure no additional seedlings were growing. Chamber effects were accounted for by swapping plants and programs (present and future temperatures) between the two chambers halfway through the experiment.

The experiment ran for nine weeks, allowing the plants to grow to maturity. The plants were watered twice weekly, initially with 100mL/week, however this increased to 150mL/week during the final two weeks of the experiment as the growing plants were beginning to show signs of dehydration. All pots were given 50mL of 1g L<sup>-1</sup> Universol Green low-Phosphate fertiliser solution during weeks 4 and 7 in addition to the 100mL of water.

**Table 2.** *Number of pots allocated to each type of community under each temperature treatment in phase 1*

Community composition	Growth chamber	
	Current climate	Future climate
<i>I. glandulifera</i> (I)	10	10
Native species (C)	10	10
None (control) (X)	10	10

Following the nine-week growing period, aboveground biomass from all pots was harvested. Roots were left intact in the ‘invader’ and ‘natives’ pots, and were harvested separately in the ‘natives + invader’ treatment.

Once aboveground biomass had been removed, 5g samples of soil from the invader, natives, and control pots in each growth chamber were collected and refrigerated for use in the soil microbial community analyses in chapter 4. Three samples were taken from each pot and combined to

provide a representation of the general microbial community found in the soil. Soil samples were also used to analyse nutrient contents in each pot, described in 3.2.5.1 “soil nutrient content”. All soil was then left to air-dry for 1 week.

Following the air-drying, the second phase of the experiment commenced. The 60 pots with ‘conditioned’ soil from phase 1 (20 from the ‘invader’ treatment, 20 from the ‘natives’ treatment, and 20 control pots, with 10 pots of each temperature treatment within those groups) were planted with seedlings 1cm below the soil surface. All plants had the same arrangement of plants as the ‘invader + natives’ treatment from the first phase: a single *I. glandulifera* seedling in the centre of the pot, with the three native species *Epilobium hirsutum*, *Jacobaea vulgaris*, and *Silene dioica* surrounding it (Appendix 4). The 60 pots were then placed into the same growth chamber treatment they had received in phase 1, 30 in the ‘present day’ temperature regime, and 30 in the ‘future’ chamber, a constant 3°C warmer than the ‘present’ chamber. The chambers followed the same temperature and light treatment as in the first phase. The pots were arranged randomly, approximately 5cm apart in a six-by-five grid design. Chamber effects were accounted for by swapping plants and programs (present and future temperatures) between the two chambers halfway through the experiment.

**Table 3.** *Number of pots from each soil origin allocated to the two temperature treatments in phase 2*

Soil Origin	Growth chamber	
	Current climate	Future climate
Invaded (I)	10	10
Community (C)	10	10
Control (X)	10	10

The experiment again ran for nine weeks, allowing the plants to grow to maturity. The plants were watered twice weekly, with 150mL/week. All pots were given 50mL of 1g L<sup>-1</sup> Universol

Green low-Phosphate fertiliser solution fortnightly in addition to the 150mL of water to decrease the chance of nutrient availability limiting growth.

Following the 9-week growing period, the height of each individual plant was measured. This was done from the base of the plant to the tallest element of the plant (leaves included). Aboveground biomass was then harvested and washed free of soil, then divided into stems, leaves, and cotyledons/undeveloped leaves. Belowground biomass was also harvested from five pots each from the ‘invader’ and ‘native’-conditioned soil in the two growth chambers (n=20). Roots were washed free of soil. All harvested biomass was then dried at 60°C for 48 hours and weighed.

### **3.2.5 SOIL NUTRIENT CONTENT**

Soil nutrient content was measured following phase one of the experiment to investigate the effect of temperature and pot community on soil nutrients, as well as to ensure that pot nutrient content was similar between all treatments prior to the initiation of the experiment’s second phase. Additionally, if the nutrient analysis shows that nutrient levels are consistent between treatments yet *I. glandulifera* was more competitive than the native species, then it can be inferred that there may be another explanation for this dominance, for example plant-soil feedbacks in the soil microbiome. Forty soil extracts were used, 10 from the original soil samples prior to any mixing with sand, and five from each treatment in phase 1, not including ‘invader + community’.

Each soil sample first underwent water extractions to obtain a nutrient solution. This was done by running 25mL of deionised water through each sample on Whatman #1 filter paper and collecting the filtrate. This resulted in approximately 15mL of extract per sample. Some samples required two filtration runs due to the presence of solid soil matter within the filtrate.

Following the water extraction, each sample was then further filtered down to 0.2µm to remove fine particulate matter before analysis. The resultant filtrate then underwent ion chromatography performed by the Durham University Geography department to obtain the concentration (mg/L) of fluoride, chloride, nitrite, nitrate, sulphate, and phosphate in each sample.

### 3.2.6 STATISTICAL ANALYSES

All data sets were checked for normality and homogeneity of variances prior to analysis. Data for height and biomass were analysed by species using a two-way ANOVA with interaction in R, with soil sample site as a random controlling factor. Height; total, belowground, and aboveground biomass; volume water content; and soil nutrient content were natural log-transformed. Proportional biomass of each species was logit-transformed. Root mass fraction was calculated as root dry mass divided by total plant dry mass. Post-hoc comparisons were performed using Tukey's HSD test for multiple comparisons via the 'emmeans' R package. All analysis was conducted in R, version 3.3.1 (R Core Team, 2017). A statistics table of significant effects can be found in Appendix 5.

### 3.2.7 SOIL VOLUME WATER CONTENT

Soil moisture was measured in weeks 5, 7, and 9 using a soil moisture meter (HH2 WET Sensor) in order to quantify the direct effects of the temperature treatments on the soil. Prior to measurement, soil calibration coefficients  $b_0$  and  $b_1$  were obtained according to the WET User Manual (v1.6) and entered into the HH2 'custom calibrations' section. Finding the appropriate calibration coefficients is imperative to proper use of the WET sensor, as water content measurements can vary depending on the soil type.

To measure the calibration coefficients, three 250mL pots (volume,  $L$ ) of the soil used in the experiment were dampened and had their permittivity,  $E'_w$ , measured with the WET sensor. These samples were then weighed to give  $W_w$ . Samples were then oven-dried for 72 hours, and then had their dry weight measured to give  $W_0$ . Permittivity of the dry samples was then measured using the WET sensor.

The first calibration coefficient,  $b_0$ , was found using the formula  $b_0 = \sqrt{E'_0}$ .  $b_0 = 1.96$ .

Volumetric water content ( $\theta_w$ ) was then calculated as  $\theta_w = (W_w - W_0) / L$ . Finally,  $b_1$  was calculated as  $b_1 = (\sqrt{E'_w} - \sqrt{E'_0}) / \theta_w$ .  $b_1 = 11.48$ . Calculated values were the mean of the three pots.

## 3.3 RESULTS

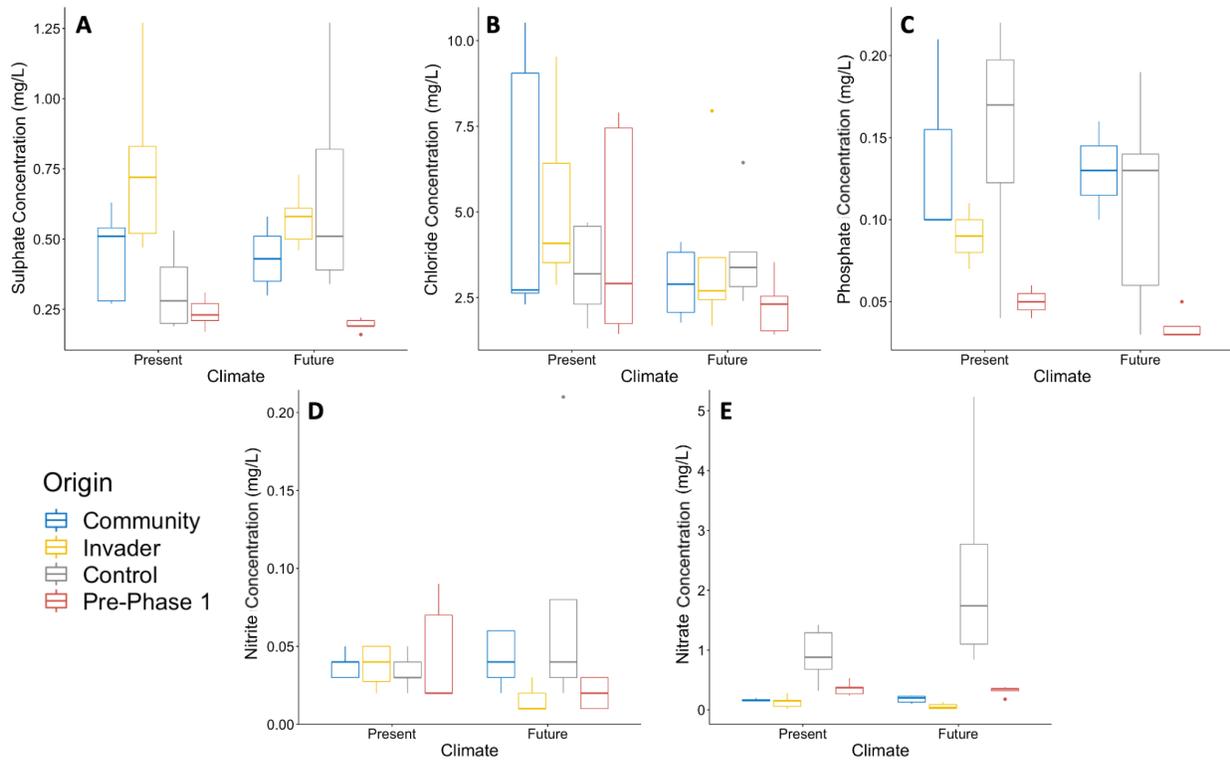
### 3.3.1 SOIL NUTRIENT CONTENT

A sequence of two-way ANOVAs were performed to investigate the effects of climate ('present' and 'future' temperatures) and pot origin (that is, the 'community', 'invader', and control soils) on the concentration of soil nutrients following the nine-week growing period of phase one. Pre-phase one soil was excluded from the statistical analyses as it was not one of the experimental treatments, but is included in Fig. 14 as a general indication of original soil nutrient levels prior to phase one.

There was a statistically significant interaction between the effects of climate and pot origin on the concentration of sulphate in the 30 'treatment' pots examined for nutrient content ( $F_{2, 24} = 3.731$ ,  $p = 0.039$ ). Overall, there was a significant main effect of origin ( $F_{2, 24} = 3.852$ ,  $p = 0.035$ ), with higher concentrations of sulphate in pots from the invader treatment than the control treatment ( $p = 0.048$ ).

There was also a significant effect of origin on nitrate concentration ( $F_{2, 24} = 47.05$ ,  $p < 0.001$ ) with both the invader ( $p < 0.001$ ) and community ( $p < 0.001$ ) treatments having lower pot nitrate concentration than the control pots. The invader treatment also had significantly lower nitrate ( $p = 0.022$ ) than the community treatment.

There were no significant differences in chloride and nitrite concentration between the three soil origin treatments and present and future temperatures. Extraction of phosphate failed in a large number of samples, so phosphate concentration was not analysed.



**Fig 14.** Concentration (mg/L) of A) sulphate, B) chloride, C) phosphate, D) nitrite, and E) nitrate in soil extracts prior to ('Pre-Phase 1', red boxplot) and following ('Community', blue boxplot; 'Invader', yellow boxplot; 'Control', grey boxplot) the temperature treatments applied in phase one, measured using ion chromatography. Bold lines on the boxplots represent the median, with boxes representing the interquartile range, and horizontal lines representing maximum and minimum observations excluding outliers. Thin vertical lines above and below the boxplots represent upper and lower quartiles. Dots are outliers representing any data points more or less than 1.5x interquartile range.

### 3.3.2 BIOMASS

#### 3.3.2.1 TOTAL BIOMASS (ABOVEGROUND AND BELOWGROUND)

##### A) *I. glandulifera*

*I. glandulifera* plants grown in the future temperature treatment had significantly higher total biomass than those in present temperatures ( $F_{1, 16} = 4.96$ ,  $p = 0.0407$ ). There was no effect of soil origin on total *I. glandulifera* biomass.

##### B) *S. dioica*

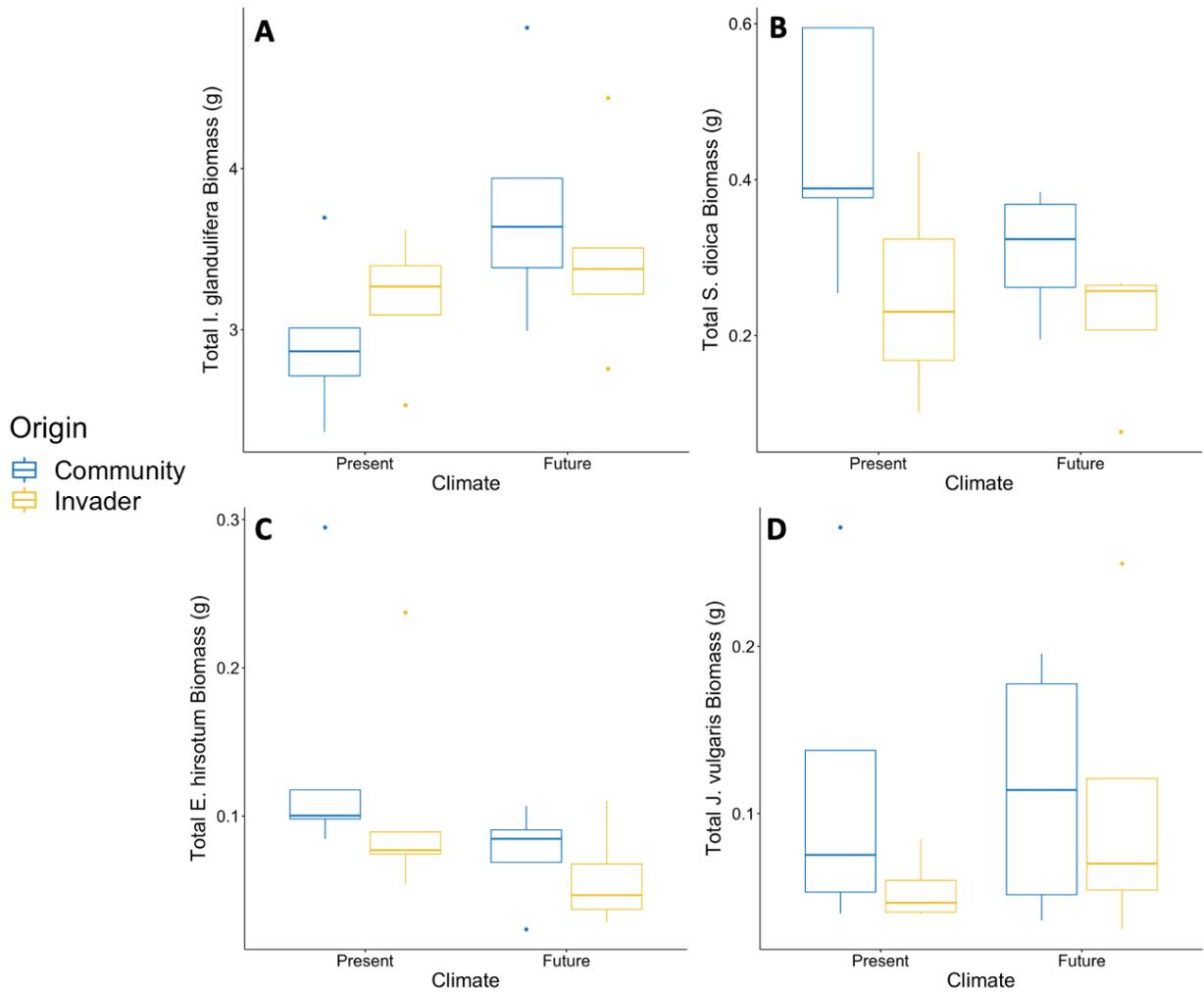
*S. dioica* plants grown in previously-invaded soil had significantly lower total biomass than those in uninvaded soil ( $F_{1, 16} = 5.850$ ),  $p = 0.030$ ). There was no effect of climate on total *S. dioica* biomass.

##### C) *E. hirsutum*

*E. hirsutum* plants grown in simulated ‘future’ temperatures under the effects of climate change had significantly lower total biomass than those in ‘present-day’ temperatures ( $F_{1, 16} = 5.054$ ),  $p = 0.0412$ ). There was no effect of soil origin on total *E. hirsutum* biomass.

##### D) *J. vulgaris*

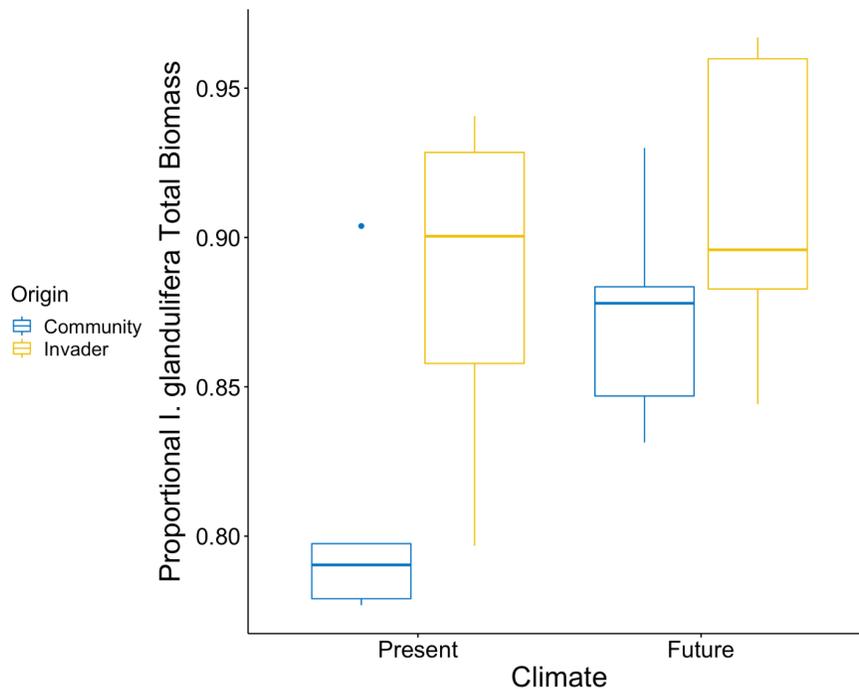
There was no statistically significant effect of climate or soil origin on total *J. vulgaris* biomass.



**Fig 15.** Total biomass of A) *I. glandulifera*, B) *S. dioica*, C) *E. hirsutum*, and D) *J. vulgaris* from each soil origin ('Community', blue boxplot; 'Invader', yellow boxplot) following the temperature treatments applied in phase two, measured using dried weight (g) of each species. Bold lines on the boxplots represent the median, with boxes representing the interquartile range, and horizontal lines representing maximum and minimum observations excluding outliers. Thin vertical lines above and below the boxplots represent upper and lower quartiles. Dots are outliers representing any data points more or less than 1.5x interquartile range.

### 3.3.2.2 PROPORTIONAL *I. glandulifera* TOTAL BIOMASS

*I. glandulifera* made up the majority of total pot biomass regardless of soil origin or climate, always making up on average >75% of total biomass in each treatment. However, *I. glandulifera* made up a significantly greater proportion of total pot biomass when grown in soil previously conditioned by *I. glandulifera* (the ‘Invader’ origin) ( $F_{1, 16} = 5.512$ ,  $p = 0.032$ ) compared to soil which had only had native species growing in phase one. There was no effect of climate on proportional *I. glandulifera* total biomass.



**Fig 16.** Proportion of total biomass made up by *I. glandulifera* from each soil origin (‘Community’, blue boxplot; ‘Invader’, yellow boxplot) following the temperature treatments applied in phase two, measured using dried weight (g) of each species. Bold lines on the boxplots represent the median, with boxes representing the interquartile range, and horizontal lines representing maximum and minimum observations excluding outliers. Thin vertical lines above and below the boxplots represent upper and lower quartiles. Dots are outliers representing any data points more or less than 1.5x interquartile range.

### 3.3.2.3 ABOVEGROUND BIOMASS

#### A) *I. glandulifera*

There was a significant effect of soil origin on the aboveground biomass of *I. glandulifera* ( $F_{1, 50} = 4.143$ ,  $p = 0.022$ ). *I. glandulifera* plants grown in pots left unconditioned in phase one had significantly higher aboveground biomass than those grown in pots from the ‘community’ treatment in phase one ( $p = 0.016$ ). There was no effect of climate or any of the other soil origins on aboveground *I. glandulifera* biomass.

#### B) *S. dioica*

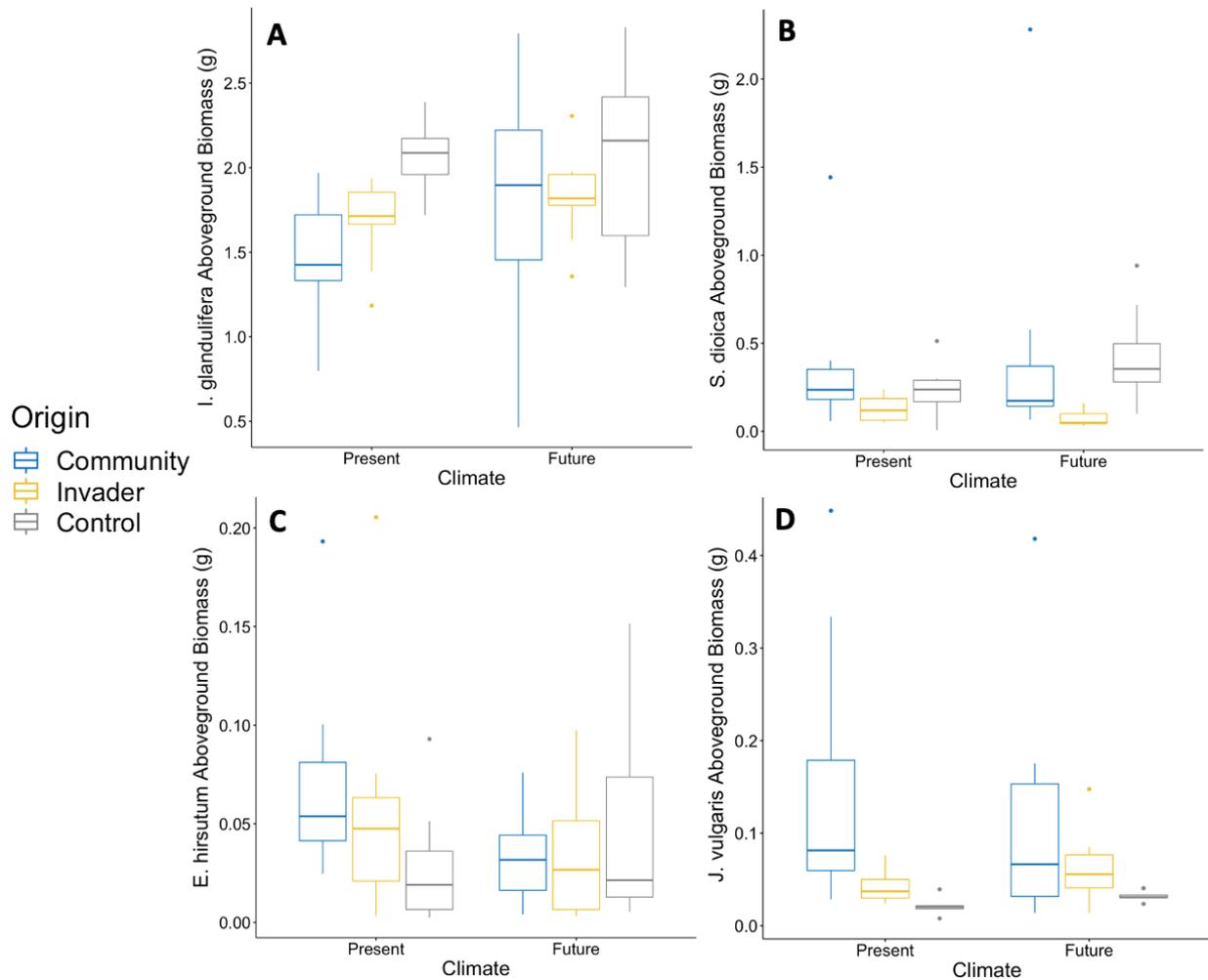
There was a significant effect of soil origin on the aboveground biomass of *S. dioica* ( $F_{1, 50} = 9.823$ ,  $p < 0.001$ ). *S. dioica* plants grown in pots conditioned by *I. glandulifera* in phase one of the experiment had significantly lower aboveground biomass than in pots from the ‘community’ treatment ( $p < 0.001$ ). Additionally, *S. dioica* plants in the control treatment had significantly greater aboveground biomass than those in the invader-conditioned pots ( $p = 0.002$ ). There was no significant difference in aboveground biomass between the control and community pots, as well as between pots in the ‘present’ or ‘future’ temperatures.

#### C) *E. hirsutum*

There was no statistically significant effect of climate or soil origin on aboveground *E. hirsutum* biomass.

#### D) *J. vulgaris*

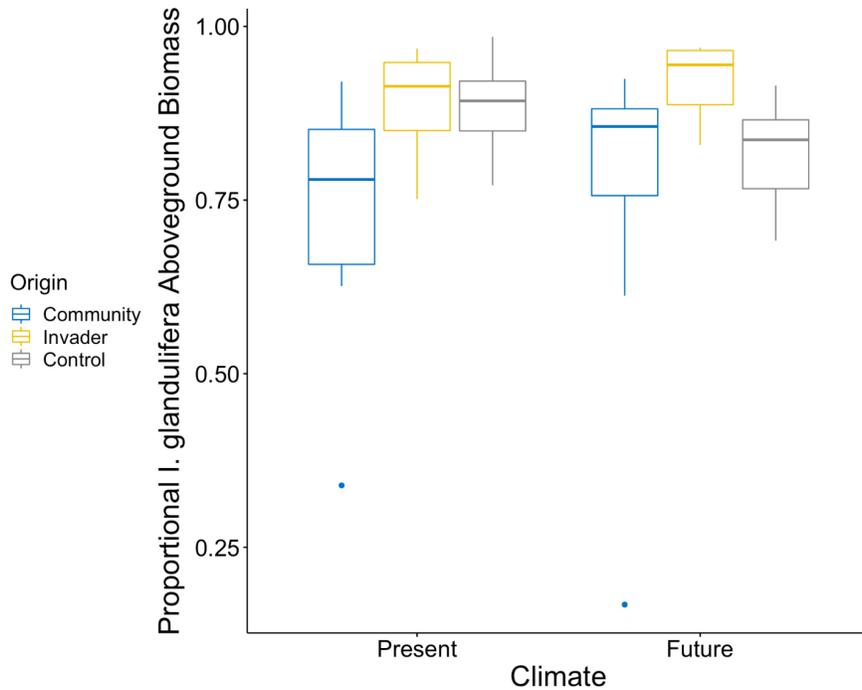
There was a significant effect of soil origin on the aboveground biomass of *J. vulgaris* ( $F_{1, 38} = 8.657$ ,  $p < 0.001$ ). *J. vulgaris* plants grown in pots from the control treatment in phase one of the experiment had significantly lower aboveground biomass than in pots from the ‘community’ treatment ( $p < 0.001$ ). There was no significant difference in aboveground biomass between any of the other soil origin treatments, as well as between pots in the ‘present’ or ‘future’ temperatures.



**Fig 17.** Aboveground biomass of A) *I. glandulifera*, B) *S. dioica*, C) *E. hirsutum*, and D) *J. vulgaris* from each soil origin ('Community', blue boxplot; 'Invader', yellow boxplot, 'Control', grey boxplot) following the temperature treatments applied in phase two, measured using dried weight (g) of each species. Bold lines on the boxplots represent the median, with boxes representing the interquartile range, and horizontal lines representing maximum and minimum observations excluding outliers. Thin vertical lines above and below the boxplots represent upper and lower quartiles. Dots are outliers representing any data points more or less than 1.5x interquartile range.

### 3.3.2.4 PROPORTIONAL *I. glandulifera* ABOVEGROUND BIOMASS

*I. glandulifera* made up the majority of aboveground pot biomass regardless of soil origin or climate, always making up on average >75% of aboveground biomass in each treatment. However, like for total biomass, there was a significant effect of origin on proportional *I. glandulifera* aboveground biomass ( $F_{2, 50} = 10.46, p < 0.001$ ), with the invader making up a significantly greater proportion of aboveground biomass when grown in soil previously conditioned by *I. glandulifera* (the ‘Invader’ origin) compared to soil which had only had native species growing in phase one ( $p < 0.001$ ). There was no effect of climate on proportional *I. glandulifera* total biomass.

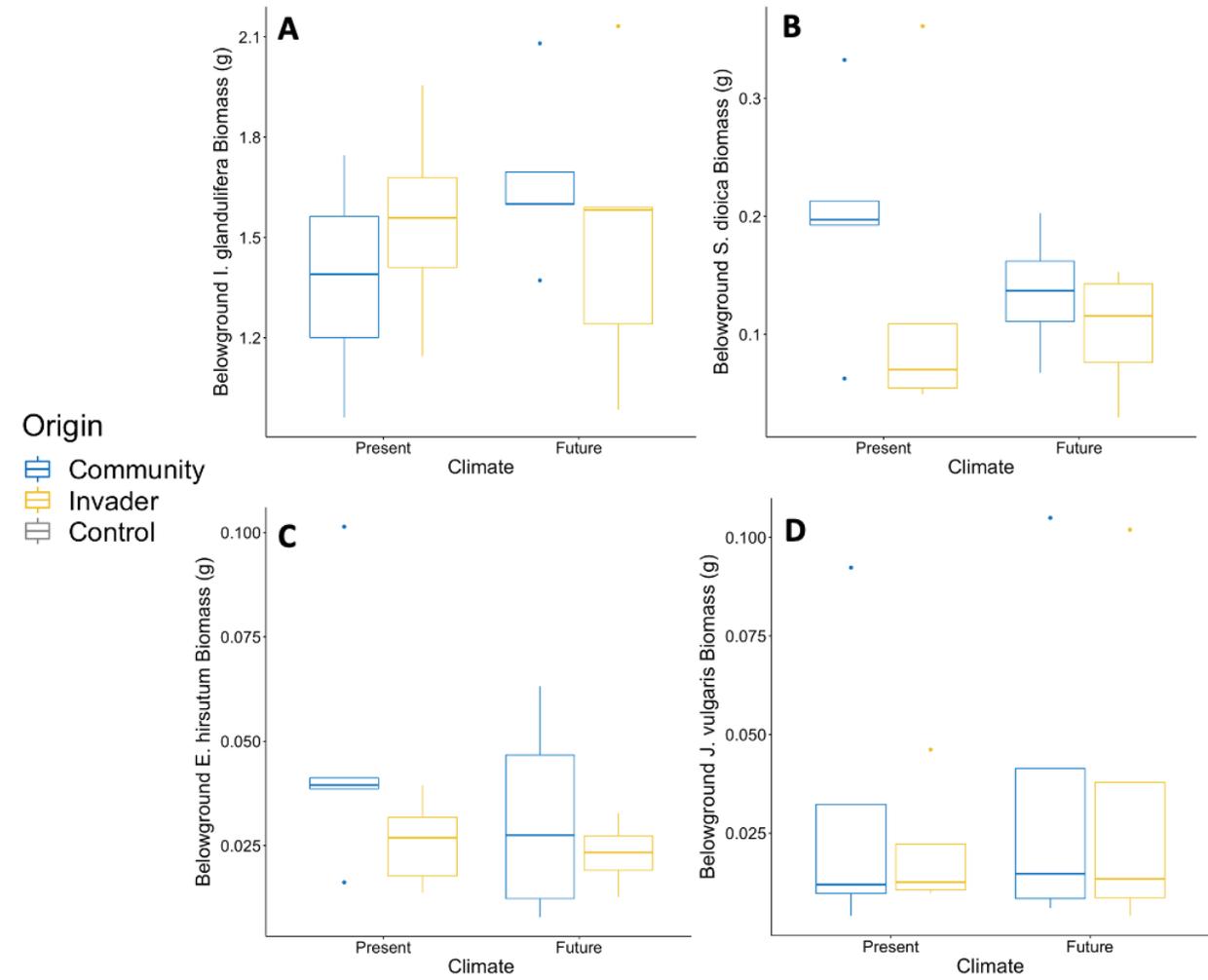


**Fig 18.** Proportion of aboveground biomass made up by *I. glandulifera* from each soil origin (‘Community’, blue boxplot; ‘Invader’, yellow boxplot; ‘Control’, grey boxplot) following the temperature treatments applied in phase two, measured using dried weight (g) of each species. Bold lines on the boxplots represent the median, with boxes representing the interquartile range, and horizontal lines representing maximum and minimum observations excluding outliers. Thin vertical lines above and below the boxplots represent upper and lower quartiles. Dots are outliers representing any data points more or less than 1.5x interquartile range.

### 3.3.2.5 BELOWGROUND BIOMASS

#### Belowground Biomass of Individual Species

There was no statistically significant effect of climate or soil origin on belowground biomass for any of the four species.

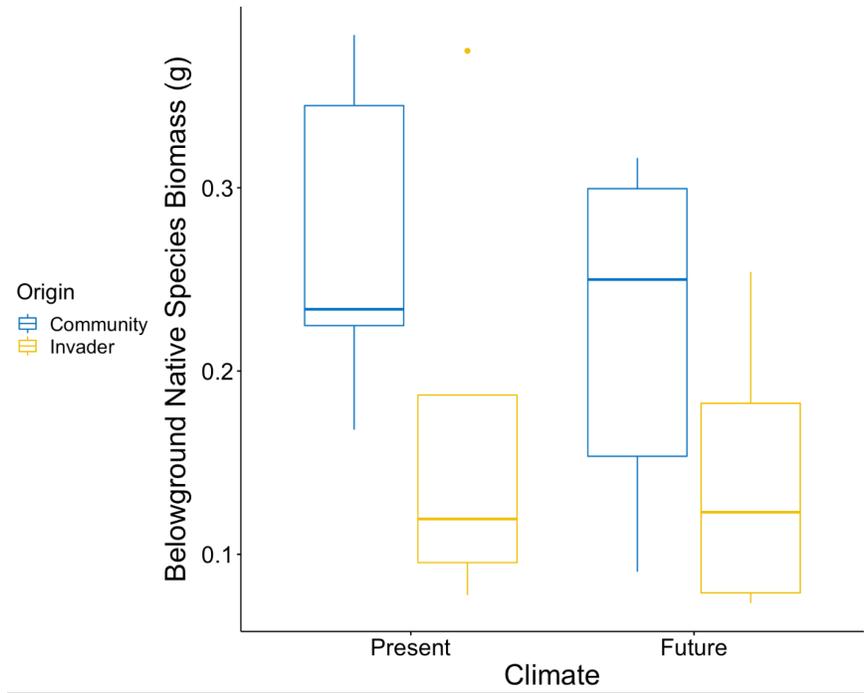


**Fig 19.** Belowground biomass of A) *I. glandulifera*, B) *S. dioica*, C) *E. hirsutum*, and D) *J. vulgaris* from each soil origin ('Community', blue boxplot; 'Invader', yellow boxplot) following the temperature treatments applied in phase two, measured using dried weight (g) of each species. Bold lines on the boxplots represent the median, with boxes representing the interquartile range, and horizontal lines representing maximum and minimum observations excluding outliers. Thin vertical lines above and below the boxplots represent upper and lower

quartiles. Dots are outliers representing any data points more or less than 1.5x interquartile range.

### Belowground Biomass of Native Species

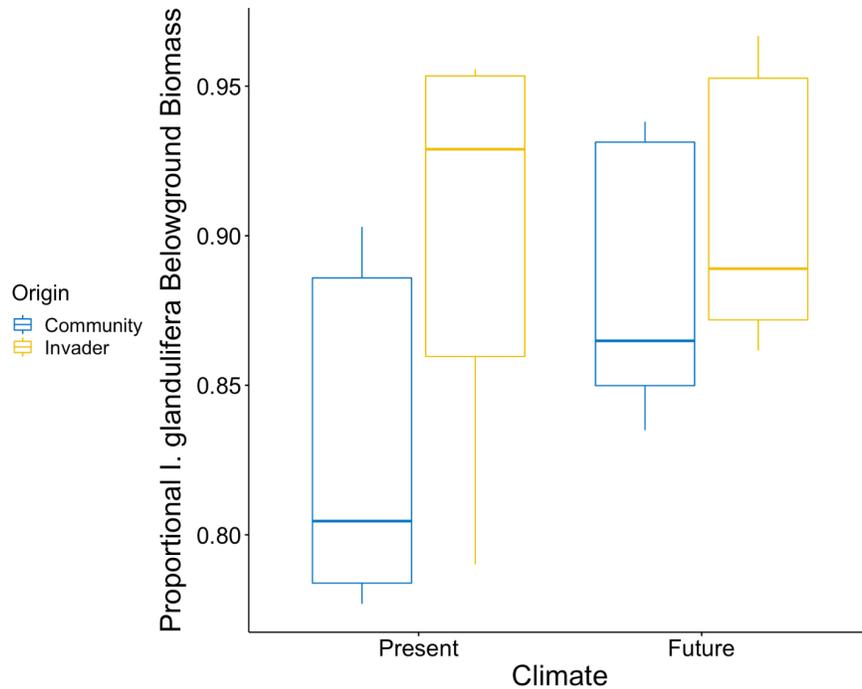
Although there was no statistically significant effect of climate or soil origin on belowground biomass for the three native species individually, when their belowground biomass is taken as a whole, there is a significant effect of soil origin ( $F_{1, 16} = 5.12, p = 0.038$ ). The combined belowground biomass of natives is significantly lower in soil previously conditioned by *I. glandulifera* than in soil originating from the ‘community’ treatment in phase one.



**Fig 20.** Combined belowground biomass of the three native species *S. dioica*, *E. hirsutum*, and *J. vulgaris* from each soil origin (‘Community’, blue boxplot; ‘Invader’, yellow boxplot, ‘Control’, grey boxplot) following the temperature treatments applied in phase two, measured using dried weight (g) of each species. Bold lines on the boxplots represent the median, with boxes representing the interquartile range, and horizontal lines representing maximum and minimum observations excluding outliers. Thin vertical lines above and below the boxplots represent upper and lower quartiles. Dots are outliers representing any data points more or less than 1.5x interquartile range.

### 3.3.2.6 PROPORTIONAL *I. glandulifera* BELOWGROUND BIOMASS

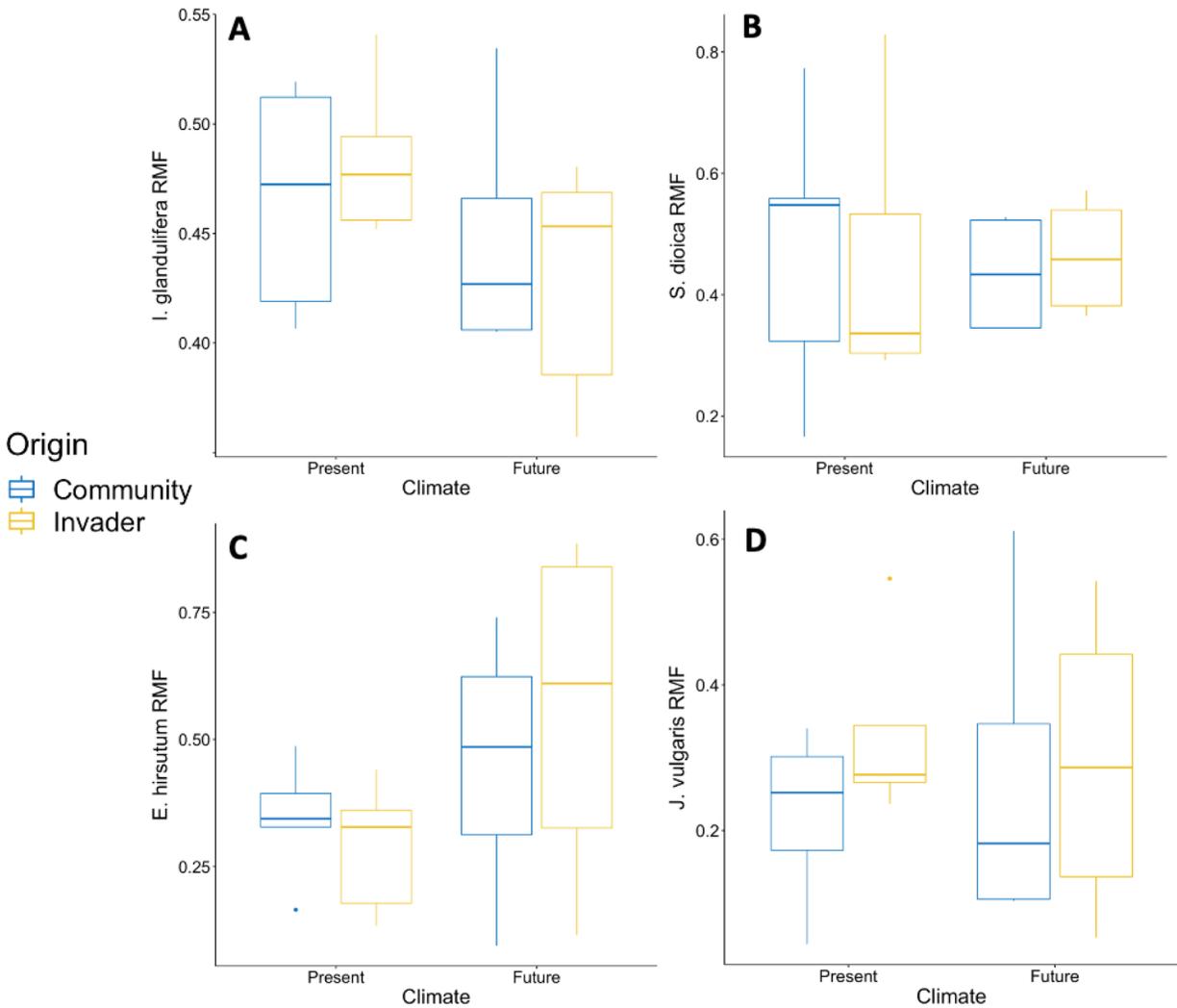
*I. glandulifera* again made up the majority of belowground pot biomass regardless of soil origin or climate, always making up on average >80% of belowground biomass in each treatment. However, unlike for total and aboveground biomass, there was no statistically significant effect of climate or soil origin on proportional *I. glandulifera* belowground biomass.



**Fig 21.** Proportion of belowground biomass made up by *I. glandulifera* from each soil origin ('Community', blue boxplot; 'Invader', yellow boxplot; 'Control', grey boxplot) following the temperature treatments applied in phase two, measured using dried weight (g) of each species. Bold lines on the boxplots represent the median, with boxes representing the interquartile range, and horizontal lines representing maximum and minimum observations excluding outliers. Thin vertical lines above and below the boxplots represent upper and lower quartiles. Dots are outliers representing any data points more or less than 1.5x interquartile range.

### 3.3.2.7 ROOT MASS FRACTION (RMF)

There was no statistically significant effect of climate or soil origin on RMF for any of the four species.



**Fig 22.** Root mass fraction of A) *I. glandulifera*, B) *S. dioica*, C) *E. hirsutum*, and D) *J. vulgaris* from each soil origin ('Community', blue boxplot; 'Invader', yellow boxplot) following the temperature treatments applied in phase two, measured using dried weight (g) of each species. Bold lines on the boxplots represent the median, with boxes representing the interquartile range, and horizontal lines representing maximum and minimum observations excluding outliers. Thin vertical lines above and below the boxplots represent upper and lower quartiles. Dots are outliers representing any data points more or less than 1.5x interquartile range.

### 3.3.2 HEIGHT

#### A) *I. glandulifera*

*I. glandulifera* plants grown in the future temperature treatment were significantly taller than those grown in present temperatures ( $F_{1, 50} = 13.4, p < 0.001$ ). There was no effect of soil origin on the height of *I. glandulifera*.

#### B) *S. dioica*

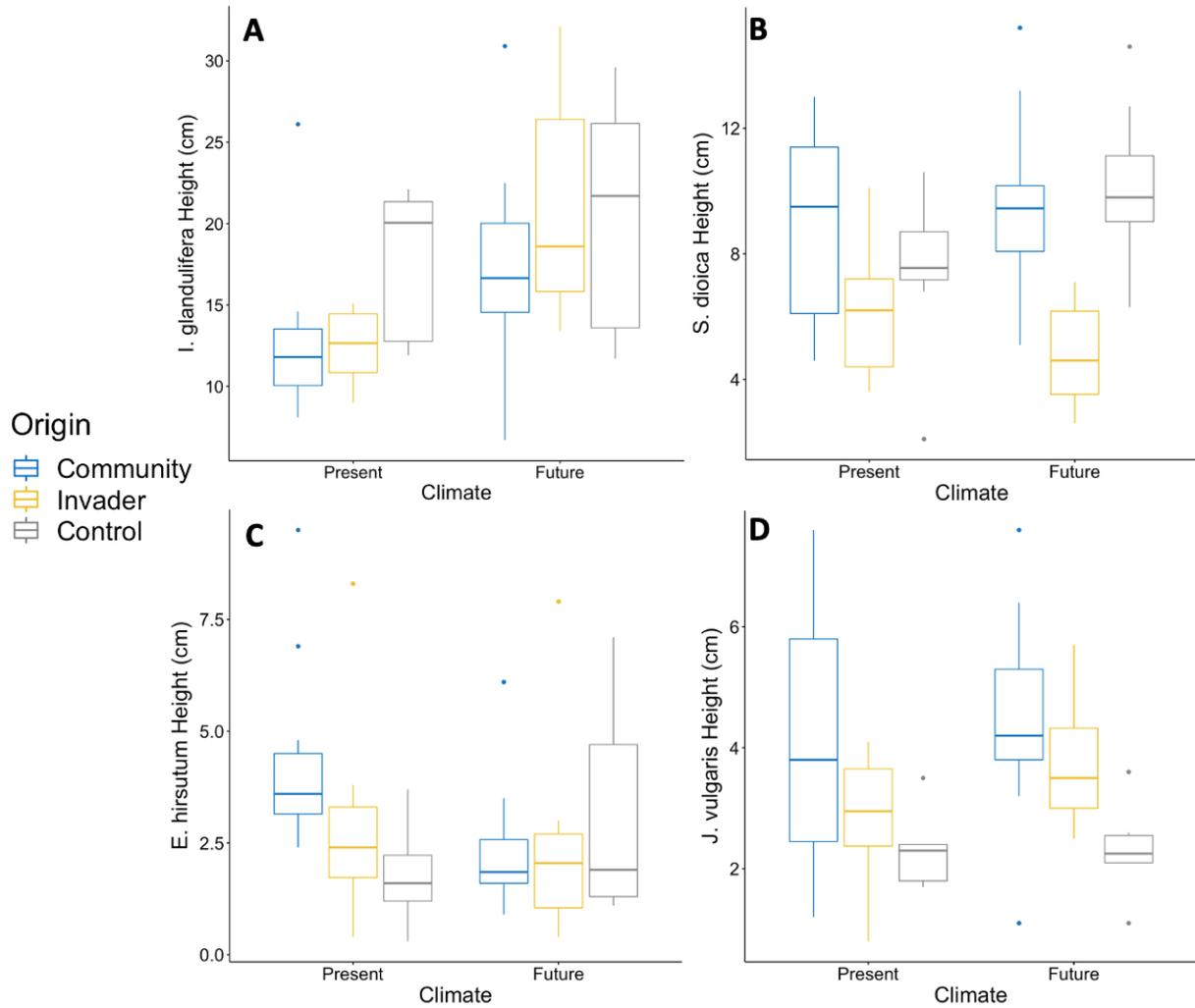
There was a statistically significant interaction between the effects of climate and pot origin on the height of *S. dioica* ( $F_{2, 50} = 3.548, p = 0.036$ ). Overall, there was a significant main effect of origin ( $F_{2, 50} = 12.527, p < 0.001$ ), with *S. dioica* plants significantly smaller in pots conditioned by *I. glandulifera* than the community ( $p < 0.001$ ) and ‘control’ pots ( $p = 0.001$ ).

#### C) *E. hirsutum*

There was no statistically significant effect of climate or soil origin on the height of *E. hirsutum*.

#### D) *J. vulgaris*

There was a significant effect of soil origin on the height of *J. vulgaris* ( $F_{2, 40} = 4.448, p = 0.018$ ). *J. vulgaris* plants grown in pots from the control treatment in phase one of the experiment were significantly smaller than in pots from the ‘community’ treatment ( $p = 0.013$ ).

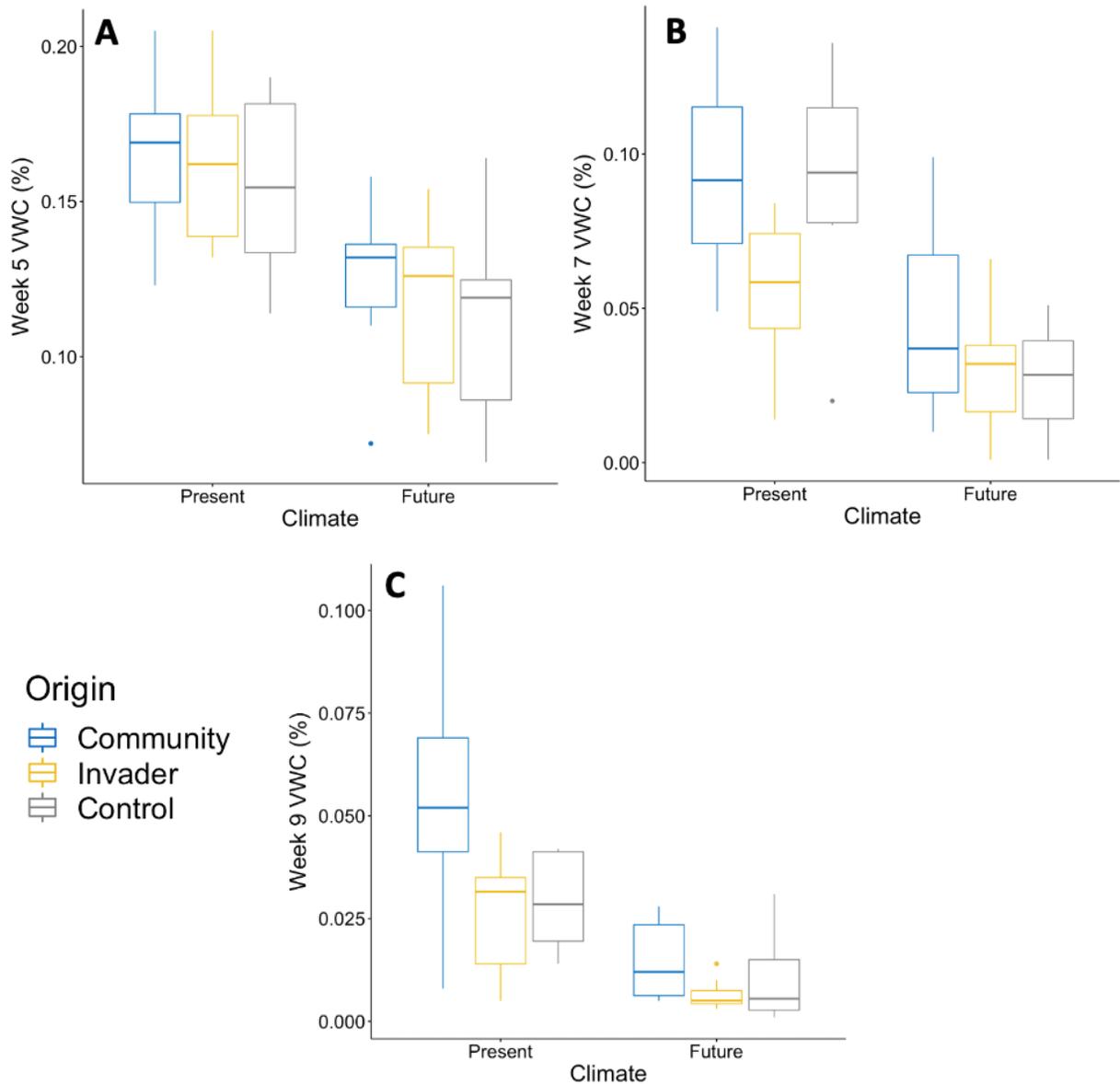


**Fig 23.** Height (cm) of A) *I. glandulifera*, B) *S. dioica*, C) *E. hirsutum*, and D) *J. vulgaris* from each soil origin ('Community', blue boxplot; 'Invader', yellow boxplot; 'Control', grey boxplot) following the temperature treatments applied in phase two, measured using dried weight (g) of each species. Bold lines on the boxplots represent the median, with boxes representing the interquartile range, and horizontal lines representing maximum and minimum observations excluding outliers. Thin vertical lines above and below the boxplots represent upper and lower quartiles. Dots are outliers representing any data points more or less than 1.5x interquartile range.

### 3.3.3 SOIL VOLUME WATER CONTENT (VWC)

Throughout the duration of phase two, the 40 pots in the ‘future’ climate chamber had significantly lower percentage VWC than those in the ‘present’ temperature (week 5,  $F_{1, 50} = 31.35, p < 0.001$ ; week 7,  $F_{1, 50} = 22.83, p < 0.001$ ; week 9,  $F_{1, 50} = 44.61, p < 0.001$ ).

Additionally, in week nine, the final week of the experiment, there was also a significant effect of soil origin on VWC; pots from the ‘invader’ origin had significantly lower percentage VWC than those from the ‘community’ origin ( $p = 0.016$ ).



**Fig 24.** Pot volume water content (%) in A) week five, B) week 7, and C) week 9, from each soil origin ('Community', blue boxplot; 'Invader', yellow boxplot; 'Control', grey boxplot) following the temperature treatments applied in phase two, measured using a moisture meter. Bold lines on the boxplots represent the median, with boxes representing the interquartile range, and horizontal lines representing maximum and minimum observations excluding outliers. Thin vertical lines above and below the boxplots represent upper and lower quartiles. Dots are outliers representing any data points more or less than 1.5x interquartile range.

### 3.4 DISCUSSION

Invasive plants have the capability to alter biotic and abiotic soil components in their invaded range, often resulting in positive PSFs for conspecifics (Kulmatiski *et al.*, 2008; Beals *et al.*, 2020). These PSFs can have direct and indirect negative impacts on native plant species in these communities (Kulmatiski *et al.*, 2006; van der Putten *et al.*, 2013). Climate change is expected to increase temperature and drought events, both of which have the potential to affect PSFs and soil dynamics; thus, climate change may impact soil conditioning by invasive plants, and plant invasions as a whole (Duell *et al.*, 2019). In this study, I have shown that in addition to *I. glandulifera* demonstrating a positive PSF that results in a greater competitive ability relative to a native plant community, the invader is also more competitive in warmer temperatures. These findings have important consequences for invasive plant management in the coming decades under the effects of climate change.

In soil previously conditioned by *I. glandulifera*, the invader showed a greater competitive ability than in soil conditioned by natives or in control soil, making up a larger proportion of community aboveground biomass. However, pot conditioning had no direct effect on the aboveground or belowground biomass of *I. glandulifera*; this suggests a positive, or at least less-negative, PSF effect on *I. glandulifera* compared to the native plant community. In these invader-conditioned pots, native plant species grew worse, especially *S. dioica*, which had lower biomass both aboveground, and in total as the sum of aboveground and belowground biomass. This is supported by the literature, where conditioned soil in invaded communities preferentially favours the growth and competitive ability of invasive species as a positive PSF, whilst co-occurring

native species suffer the effects of negative feedbacks (van der Putten *et al.*, 2013; Ruckli *et al.*, 2014).

The greater biomass proportion of *I. glandulifera* in invader-conditioned pots could simply be explained as a result of the greater resource-use efficiency exhibited by many invasive species (Kleunen *et al.*, 2010). The concentration of nitrate, a crucial nitrogen source for plants, was significantly lower in pots conditioned by *I. glandulifera* than the native-conditioned or control pots prior to phase two (Raven, 2003). This is most likely a result of increased nutrient uptake by the community of *I. glandulifera* previously growing in these pots during phase one. The higher growth rates and resource-use efficiency of the invader could be predicted to result in *I. glandulifera* making up a greater biomass proportion in the native-conditioned and control pots with their higher starting concentrations of nitrate; however, this was not the case. Although *I. glandulifera* had the greatest aboveground biomass in the control pots with their higher nitrate concentrations, this had no significant effect on proportional aboveground biomass, which was highest in invader-conditioned pots. Additionally, fertiliser was regularly applied to all pots throughout the experiment, counteracting any nutrient-limiting effects on the plant community. This suggests that, as in the similar study conducted by Pattison *et al.* (2016), there was a positive PSF effect in *I. glandulifera*-conditioned pots. This may have been the result of root exudates or alterations in the soil microbial community (Pattison *et al.*, 2016).

Phosphorous, available to plants in the form of phosphate, is also critical for plant growth (Raghothama, 1999). Unfortunately, nutrient analysis on pot phosphate levels failed in the majority of samples. This could have been a result of the method of nutrient analysis; phosphate may have been bound to clay in the soil, less labile and unable to be extracted through the water filtration method utilised in this study. Establishing whether phosphate drawdown by *I. glandulifera* was as great as that of nitrate would further support the conclusion that *I. glandulifera* exhibited positive PSFs in this study.

In addition to the effect of soil conditioning, *I. glandulifera* also showed positive responses to growing in simulated temperatures under future climate change. Total biomass of *I. glandulifera*, the sum of aboveground and belowground biomass, was significantly greater in the future climate than the present. Additionally, *I. glandulifera* plants were taller in the future growth

chamber. The increased temperature in the future chamber had the opposite effect on the native species: *E. hirsutum* had lower total biomass, and there was a significant interaction between climate and soil origin on the height of *S. dioica*; the combination of a warmer climate and the soil conditioning effects of *I. glandulifera* resulted in *S. dioica* not growing as tall as in the present-day growth chamber or in the native-conditioned and control soils. There was no effect of climate or soil origin on the height of *E. hirsutum*; this species tended to grow horizontally rather than vertically. *J. vulgaris* consistently had very low biomass, and grew significantly worse than this only in the control pots. This may be a result of *J. vulgaris* having a weak competitive ability even against other natives; a study by McEvoy *et al.* (1993) found *J. vulgaris* to be a poor competitor, with increased competition in diverse plant communities leading to reduced growth (McEvoy *et al.*, 1993).

There are a number of possible explanations for the positive effects of temperature on *I. glandulifera*, and negative effects on the native community. First, *I. glandulifera*, as an annual, prioritises fast growth, quickly becoming taller than the natives in both climate chambers. The higher intrinsic growth rates and resource-use efficiency of the invasive *I. glandulifera* compared to the perennial natives may have allowed it to better-perform in warmer temperatures, resulting in its increased height in the future chamber (Kleunen *et al.*, 2010). Additionally, the warmer temperatures may have incited *I. glandulifera* to attempt to flower faster, resulting in greater vertical growth. This resulted in a feedback loop whereby as *I. glandulifera* grew taller, it shaded the native plants beneath it, inhibiting their growth. This shading effect has been observed in-situ in sites invaded by *I. glandulifera*; light-dependent species in particular are more negatively affected by *I. glandulifera* than those that are able to survive with less light, as the fast growth of the invader can shade smaller native plants in the surrounding community (Coakely & Petti, 2021). However, although shading by *I. glandulifera* most likely did have a detrimental effect on the growth of the native community, the natives, especially *S. dioica*, grew worse in *I. glandulifera*-conditioned soil regardless of temperature (and the taller invaders in the future climate), suggesting there were other factors contributing to the increased competitive ability of *I. glandulifera*.

The differences in temperature did have a corresponding effect on soil volume water content, with pots in the future chamber consistently having lower water levels than in the present

chamber when measured with a soil moisture meter. This effect became more pronounced as the experiment progressed. The prolonged lower levels of moisture may have resulted in drought-like effects in the future chamber. This may be a combined effect of both the increased temperature and greater height of *I. glandulifera* in the future chamber, with increased evapotranspiration by the taller invader; this could be a topic of study for future research with a lysimeter. Additionally, in the final week of the experiment there was an effect of soil origin on volume water content, with pots conditioned by *I. glandulifera* having significantly less water than native-conditioned or control pots. This may be a result of increased evapotranspiration by *I. glandulifera* in the invader-conditioned pots, in which they make up a significantly greater proportion of pot biomass. Taken together, these findings suggest that in soil invaded by *I. glandulifera*, both increased temperature under climate change and the soil legacy of invasion will affect evapotranspiration, potentially decreasing soil water content.

Drought amplifies the temperature sensitivity of bacteria and fungi, perhaps mediating the effects of PSF (Briones *et al.*, 2014). This effect may be exacerbated when occurring alongside the PSF effects of plant invasions, which involve alterations in the activity and structure of the soil microbial community, alongside abiotic soil conditions (Qin *et al.*, 2014; Cuda *et al.*, 2017). AMF, alongside a more diverse soil microbiome, have been shown to improve resistance to drought stress in associated plant communities (Bowled *et al.*, 2017; Bogati & Walczak, 2022; Malacrino *et al.*, 2020). The increased competitive ability demonstrated by *I. glandulifera* in the future climate and in invader-conditioned soil may be a result of losses in microbial diversity and shifts in the structure of microbial populations due to the presence of the invader. This alteration in the microbial community, combined with lower soil moisture as a result of climate change and increased evapotranspiration by successful invasive plant species, may have decreased the growth and competitive ability of the native species community (Bowled *et al.*, 2017; Bogati & Walczak, 2022; Malacrino *et al.*, 2020).

Below the ground, there was no effect of either climate or origin on the root biomass of any of the study species. However, when taken as the sum of all belowground native biomass, native root biomass was significantly lower in soil conditioned by *I. glandulifera*. This may be a result of the allelopathy or other PSF effects by the invader, as there was no increase in *I. glandulifera* biomass above or below ground in the invader-conditioned soil, only a significant decrease in

native belowground biomass (Perglová *et al.*, 2009; Coakley & Petti, 2021). *I. glandulifera* again made up the majority of the belowground biomass proportion, however there was no effect of climate or soil origin on this dominance. Interestingly, neither climate nor soil origin had an effect on the root-mass fraction of any species. Nishar *et al.* (2017) found lower root biomass of plants in warmer soils, as plants invested more into aboveground growth in these conditions. However, that study had a much greater range of soil temperatures, and the 3°C of warming in the future chamber may not have been enough to facilitate a change in root biomass allocation.

This study illustrated the ability of *I. glandulifera* to effect a positive PSF in its invaded soil, to the benefit of its conspecifics and the detriment of the native community. Additionally, this soil conditioning has the potential to complement the effects of climate change, decreasing soil moisture and possibly decreasing drought stress resistance mechanisms in native species through alterations of the soil microbial community. Whilst *I. glandulifera* remains the greatest proportion of biomass in both present-day and future temperatures, natives in its soil community respond negatively to the effects of climate and invader soil conditioning, and even combinations of the two. The extent to which the soil microbial community mediates this effect, if any, is addressed in chapter 4. These findings suggest that in future ecosystems subjected to the effects of climate change, invasive species have the potential to become even more competitive than the native community. This has important implications for future efforts to manage invasive species.

## Chapter 4:

The effects of temperature and climate on the soil microbial community associated with *Impatiens glandulifera* and a native plant community

### ABSTRACT

Invasive plant species such as *I. glandulifera* have the ability to alter the microbial community in invaded soil. These plant-soil feedbacks are often positive for invasive plant species, yet negative for native plants in these communities; this can result in increased growth and survival of the invader, and facilitate further invasions. However, little is known about the precise dynamics of these changes in the soil microbial community, as well as the impact, if any, of increased temperature due to climate change on these PSFs. A two-phase pot experiment was conducted to assess the impact of initial soil conditioning by *I. glandulifera* and a community of natives on the subsequent competitive ability of *I. glandulifera* in a community with native species, as well as to observe the effects of increased temperature on this dynamic. Soil extracts were taken prior to the second phase of this experiment, with soil DNA being extracted for sequencing and metabarcoding of the soil bacterial and fungal communities. Overall, bacterial diversity was lower in invaded pots than those grown with a native community. Additionally, growth in a simulated future climate resulted in greater bacterial diversity, which constituted a shift in the bacterial community compared to soil from present-day temperatures. Fungal diversity and community composition remained the same regardless of soil origin and climate. These results show a potential effect of *I. glandulifera* soil conditioning on microbial communities, as well as belowground bacterial and fungal responses to increased temperature under climate change. Additionally, this study illustrates that the field of invasive plants, as well as ecology as a whole, can benefit from metabarcoding studies similar to the one presented here.

## 4.1 INTRODUCTION

As discussed in chapter 3, there have been many studies confirming that plant species have the ability to alter their surrounding soil, which in turn results in feedback and subsequently affects plant performance in the surrounding community. Additionally, a number of invasive plants are able to effectively ‘hijack’ their soil, directly and indirectly fostering positive PSF, or at least less negative, loops that favour their own growth and survival whilst negatively affecting those of their neighbouring natives (Kulmatiski *et al.*, 2006; van der Putten *et al.*, 2013). The role of microorganisms in this process of invasion has been the subject of much study in recent years (Aires *et al.*, 2021). Zilber-Rosenberg and Rosenberg (2008) argue that as the microbiomes associated with plants affect such far-reaching components as plant development, interactions with the surrounding environment, adaptation, and ultimate survival, individual plant phenotypes should be taken as the sum of the host and its associated microbial gene expressions. In this ‘hologenome’ theory of evolution, the roles of bacteria, fungi, and other microorganisms in plant invasions are potentially as important as host physiology alone (Rosenberg and Zilber-Rosenberg, 2018).

*I. glandulifera* has the potential to impact both soil bacterial and fungal communities. In particular, as *I. glandulifera* is only weakly dependent on AMF, in plant communities dominated by *I. glandulifera* the fungi may fail to form a mutualism and acquire carbon from a plant host, decreasing root AMF colonisation and total soil AMF density (Zybeck *et al.*, 2016; Pattison *et al.*, 2016; Grove *et al.*, 2017; Coakley & Petti, 2021). Pattison *et al.* (2016) showed that when grown in *I. glandulifera*-conditioned soil, *I. glandulifera* was taller, produced more leaves, grew faster, and had higher biomass than in non-conditioned soil; this suggests a positive PSF. AMF root colonisation in conditioned soil was half that of control soil, whilst bacterial biomass increased almost two-fold in conditioned versus control soil (Pattison *et al.*, 2016). *I.*

*glandulifera* has the potential to impact both soil bacterial and fungal communities via allelopathy: *I. glandulifera* has been shown to produce 1,4-naphthoquinone (2-MNQ), a potential allochemical capable of inhibiting the growth of nearby native plants or disrupting their soil microbial community (Perglová *et al.*, 2009; Coakley & Petti, 2021). In-vitro studies support this hypothesis, with extracts of 2-MNQ from *I. glandulifera* negatively impacting a range of native

species from the invaded range of *I. glandulifera*, such as the AMF-associating *Dactylis glomerata* and *Urtica dioica* (Bieberich *et al.*, 2018; Coakley & Petti, 2021).

Invasive plant species have been shown to alter the activity and structure of soil bacterial communities, however the effects appear to differ greatly by species (Qin *et al.*, 2014; Cuda *et al.*, 2017). Zhang *et al.* (2019) found that invasive plants “increased bacterial biomass by 16%, detritivore abundance by 119% and herbivore abundance by 89%” via their litter, whilst in the rhizosphere, “invasive plants reduced bacterial biomass by 12%, herbivore abundance by 55% and predator abundance by 52%, but increased AM fungal biomass by 36%”. Interestingly, this is the opposite effect to that found in *I. glandulifera* by Pattison *et al.* (2016). There are a number of studies that have found *I. glandulifera* to have negligible impacts on native plant species and soil communities (Diekmann *et al.*, 2016; Cuda *et al.*, 2017).

Climate change is also predicted to impact soil microbial communities alongside invasive plant species. As discussed previously, North East England, the location of this experiment, is predicted to become warmer and drier under the effects of climate change in the coming decades (Harley *et al.*, 2011; Parasiewicz *et al.*, 2019). Increased temperature and drought can affect soil microbial responses to climate change in a number of ways (Classen *et al.*, 2015). For example, drought amplifies the temperature sensitivity of bacteria and fungi, perhaps mediating the effects of PSF (Briones *et al.*, 2014). AMF have been shown to improve resistance to drought stress in their host plants (Bowles *et al.*, 2017). A more diverse soil microbiome has been shown to buffer the effects of drought; losses in microbial diversity and shifts in the structure of microbial populations, combined with climate change stresses, could negatively affect the performance of native plant species (Bogati & Walczak, 2022; Malacrino *et al.*, 2020).

The results of chapter 3 justified an exploration of the soil microbiome of pots prior to phase two of the temperature experiment. This experimental design allowed the results of phase two to be interpreted in the context of changes in the microbial community as a result of temperature or soil conditioning. Following phase two, *I. glandulifera* consistently performed better in its own soil, growing more biomass and taking up a greater proportion of pot biomass, whilst the native species, especially *S. dioica*, grew significantly worse in *I. glandulifera*-conditioned soil. These results compliment the literature suggesting *I. glandulifera* fosters positive PSFs to increase the

growth of conspecifics, to the detriment of the native plant community. This effect occurred regardless of soil nutrient levels prior to phase two; nitrate availability was significantly lower in pots conditioned by *I. glandulifera*, yet the invader consistently grew better in these pots, implying the presence of another factor positively impacting *I. glandulifera*. In addition to biomass, *I. glandulifera* also grew significantly taller in temperatures simulating a future climate under climate change, and pots within the future chamber consistently had lower water content than in the present temperature chamber. Whether the lower water content and higher temperatures had an impact on the soil microbial community, and potentially the height of *I. glandulifera*, remains to be explored.

This metabarcoding analysis of the soil microbial community following conditioning of soil in phase one of the temperature experiment aims to address multiple issues. These include the frequent contradictions in papers investigating the effects of plant invasions on PSFs and the soil microbial community, the intriguing results of chapter 3, and the lack of studies investigating the interplay of soil conditioning by invasive plants and the effects of climate change. Filling the gaps in our understanding of this topic will contribute not only to understanding how plant invasions occur, but also to aid in prediction and prevention of invasions in the future under climate change (Kowalski *et al.*, 2015; Coakley & Petti, 2021).

## **4.2 METHODOLOGY**

### **4.2.1 SAMPLING**

Analysis of bacterial and fungal communities was performed on soil samples obtained prior to ‘phase two’ of the temperature experiment, as well as a small number of samples from the original soil prior to mixing with sand. Three subsamples from each pot were combined to create a representative soil sample as described in 3.2.3, “Soil Collection”. In total, 129 samples underwent DNA sequencing, with a single sample from the pre-phase 1 soils being omitted due to PCR failure: ten from the ‘original’ soil samples, taken in-situ around Durham (“original”, samples 1-10), 59 from the 60 pots filled with a soil-sand substrate mix prior to phase 1 of the temperature experiment (“pre”, samples 11-69), and 60 from the same 60 pots after 9-weeks of plant growth in phase 1 (“after”, samples 70-129) (Table 4). The “after” samples were further

segmented according to the climate and origin their pot was allocated to in phase 1: the ‘present’ temperature chamber (“present”, samples 70-99), ‘future’ temperature chamber (“future”, samples 100-129), pots conditioned by *Impatiens glandulifera* (“invader”, samples 80 - 89 and 110 - 119), pots conditioned by the three native species (“community”, samples 90 - 99 and 120 - 129), and the control pots with no plant species (“control”, samples 70 - 79 and 100 - 109) (Table 5).

**Table 4.** *Soil type associated with each sample number*

Sample number	Soil Type
1-10	“Original”
11-69	“Pre”
70-129	“After

**Table 5.** *Climate and origin treatments associated with each sample number*

Sample number	Climate	Origin
70 - 79	“Present”	“Control”
80 - 89		“Invader”
90 - 99		“Community”
100 - 109	“Future”	“Control”
110 - 119		“Invader”
120 - 129		“Community”

Soil samples had a volume of 5mL, and when collected from pots (“pre” and “after”) were taken using a soil corer at the root depth of the plant species. All tools were treated with a bleach solution between sampling to prevent cross-contamination. All soil samples were immediately transferred to a freezer to await DNA extraction and sequencing. Samples from the original soil and pre-phase 1 pots were used in order to compare the in-situ microbiome with that of the pots post-phase 1.

#### **4.2.2 DNA EXTRACTION AND AMPLICON SEQUENCING**

Total genomic DNA was extracted from all soil samples using the DNeasy Powersoil Pro Kit (QIAGEN) according to the manufacturer’s “Quick-Start Protocol”. After extraction, the concentration of DNA was tested using a nanodrop. Prior to PCR amplification, DNA was standardised to 1ng/μl.

To characterise the bacterial community, the V3-V4 region of the bacterial 16s rRNA was amplified using the primer set with Illumina adaptors recommended in the Illumina “16s Metagenomic Sequencing Library Preparation” protocol (Illumina, 2013). Primers are bold and underlined to distinguish them from the Illumina adaptors. These were the forward primer 5’TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CCTACGGGNGGCWGCAG**3’ and the reverse primer 5’GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**GACTACHVGGGTATCTAATC****C**3’. The PCR reaction mix consisted of 7.5μl 2X QIAGEN Multiplex PCR Master Mix, 0.2μl of each primer at 10μM concentration, 6.1μl nuclease-free water, and 1μl of the standardised template DNA. The PCR conditions were: initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 53 °C for 30 seconds, and extension at 72 °C for 30 seconds, with a final elongation of 72 °C for 10 minutes.

The fungal locus-specific PCR was initially undertaken using the general eukaryote internal transcribed spacer (ITS) reverse primer ITS4 in combination with the ‘newly-designed’ forward fungal-specific ITS2 region primer ITS7o, as described in Kohout *et al.*, 2014 and modified from the ITS7 primer published in Ihrmark *et al.*, 2012. This combination was chosen due to its high resolution power for glomeromycota, the phylum of arbuscular mycorrhizal fungi (AMF), which

have been shown to be negatively affected following invasion by *I. glandulifera*. Unfortunately, amplicon sequencing using this primer pair continually failed under a range of PCR conditions, thus it was decided that another fungal ITS primer pair would be used. We settled on another ITS2 region amplifier, the ITS86F forward primer (5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGAATCATCGAATCTTTGAA 3') and general eukaryote reverse primer ITS4 (5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCTCCGCTTATTGATATGC 3'). This pair was chosen based on a study which found the ITS86F/ITS4 primer pair outperformed other commonly used fungal primer pairs in PCR efficiency, coverage, number of reads, and species-level OTUs obtained (Op De Beeck *et al.*, 2014). A PCR trial was performed comparing ITS7o/ITS4, ITS86F/ITS4, and a commonly-used fungal primer pair, ITS1/ITS4, to identify which forward primer was best-suited to our soil extracts. The ITS86F/ITS4 primer pair consistently resulted in the brightest bands on a gel electrophoresis (Appendix 6). A gradient PCR with annealing temperatures 50°C, 53°C, and 55°C was performed to identify the optimum PCR conditions for amplification with this new ITS86F/ITS4 primer pair. Annealing at 55°C produced the brightest bands on gel electrophoresis following PCR (Appendix 7). The PCR reaction mix consisted of 7.5µl 2X QIAGEN Multiplex PCR Master Mix, 0.2µl of each primer at 10µM concentration, 6.1µl nuclease-free water, and 1µl of the standardised template DNA. The PCR conditions were: initial denaturation at 95 °C for 5 minutes, followed by 40 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 45 seconds, with a final elongation of 72 °C for 10 minutes.

In all cases, negative controls were included, and the locus-specific PCRs were done in triplicate, resulting in 780 samples. Following PCR, the products were run on 2% agarose gel electrophoresis (1.6g agarose, 80mL 0.5X TAE, 0.8uL ethidium bromide) to ensure bands of the expected size and brightness were present. Once this was confirmed, 5µl of the PCR product from the three replicates of the samples from each locus were pooled, resulting in 260 samples (130 each for bacteria and fungi) with 15µl per sample. After pooling, all samples were purified to eliminate primers, impurities, and primer-dimer sequences using Ampure XP beads per the instructions for use (Beckman Coulter, 2016).

Once purified, DNA was tested for quantity following the standard Qubit protocol (Thermo Fisher Scientific). Each sample was standardised to 50ng/μl, and then each sample was combined by locus in equal concentrations, resulting in the final 130 samples for sequencing.

The 130 samples were then indexed according to the Illumina protocol. The PCR reaction mix consisted of 25μl Kapa HiFi Master Mix, 2.5μl of each primer at 10μM concentration, 10μl nuclease-free water, and 10μl of the pooled PCR product containing both loci. The PCR conditions were: initial denaturation at 95 °C for 3 minutes, followed by denaturation at 98 °C for 30 seconds, then 15 cycles of denaturation at 98°C for 10 seconds, annealing at 63 °C for 30 seconds, and extension at 72 °C for 3 minutes. Following the indexing PCR, the products were run on 2% agarose gel electrophoresis to ensure bands of the expected size and brightness were present. Once this was confirmed, a second purification bead cleaning was performed using Ampure XP beads to remove excess index primers.

Once purified, the indexed products were again tested for quantity following the standard Qubit protocol. The samples were then pooled into a single tube in equal concentrations. The final sample was analysed for quality with a TapeStation to check size and concentration of the DNA fragments, and the concentration of indexed samples in the final pool was identified through qPCR. The pooled library was then sequenced using the Illumina MiSeq platform. TapeStation analysis, qPCR, and Illumina MiSeq was performed by the Durham University genomics facility.

Raw sequence data were demultiplexed by the Durham genomics facility and supplied in Casava 1.8 paired-end demultiplexed fastq format. Microbial bioinformatics were performed with QIIME 2 2021.8 (Bolyen *et al.*, 2019). Sequence data were denoised and trimmed by 5bp at the left end and truncated at 273bp at the right end to remove forward primers and low-quality sequences, and trimmed by 5bp at the left end and truncated at 207bp to remove reverse primers and low-quality sequences with DADA2 (Callahan *et al.*, 2016) (via q2-dada2). All amplicon sequence variants were aligned with mafft (Katoh *et al.*, 2002) (via q2-alignment) and used to construct a phylogeny with fasttree2 (Price *et al.*, 2010) (via q2-phylogeny). Taxonomy was assigned to bacterial ASVs using the q2-feature-classifier (Bokulich *et al.*, 2018a) classify-sklearn naive Bayes taxonomy classifier against the Greengenes 13\_8 99% OTU reference sequences (DeSantis, T. Z. *et al.*, 2006). Fungal ASVs were assigned taxonomy using the same commands and open taxonomy unit (OTU) reference sequences from the Unite database

(Abarenkiv, K. *et al.*, 2010). OTUs were clustered at >97% similarity. From the resulting OTU table for both bacteria and fungi, unassigned sequences and OTUs observed only one or two times were removed.

For bacteria, a total of 1,469,832 reads were produced with an average of 11,394 reads per sample, which were clustered into 28,089 OTUs. For fungi, a total of 3,163,988 reads were produced with an average of 24,527 reads per sample, which were clustered into 5,283 OTUs.

### **4.2.3 MICROBIAL COMMUNITY ANALYSES**

Further microbial community analysis was conducted in R (R Core Team, 2021) using the feature tables, taxonomy tables, phylogenetic trees, and metadata from QIIME2. The R analysis was performed using the phyloseq (McMurdie & Holmes, 2013), vegan (Oksanen *et al.*, 2018), ggpubr (Kassambara, 2018), ggplot (Wickham, 2009), and tidyr (Wickham & Henry, 2018) packages.

For statistical purposes, samples were normalised by rarefaction according to a rarefaction curve for use in downstream alpha and beta diversity analyses, with a set seed of 1. The bacterial samples were rarefied to 4,700 sequences per sample, whilst the fungal samples were rarefied to 3,500 sequences per sample.

Analysis was performed separately for bacteria and fungi, with the dataset being separated according to a metadata file. To compare the microbial community ‘before’ and ‘after’ phase 1 of the temperature experiment, the full dataset of 129 samples (‘original’, ‘pre’, and ‘after’) was used. Following this, the dataset was segmented into only the 60 ‘after’ samples (samples 70 - 129), which were then analysed to observe differences in the microbial community due to the effects of growth chamber (‘present’, ‘future’) and soil origin (‘invader’, ‘community’, ‘control’). These 60 samples were also labelled according to a combination of growth chamber and soil origin for visualisation purposes: future - invader (FI), present - invader (PI), future - community (FC), present - community (PC), future - control (FX), and present - control (PX).

## $\alpha$ -Diversity: Microbial Community Richness and Diversity

Microbial community richness was estimated using the number of observed OTUs, with evenness calculated according to the Shannon index. Boxplots were generated to visualise the two  $\alpha$ -diversity estimates in the different groups of samples according to their metadata labels. Data were tested for a normal distribution using the Shapiro-Wilk test. As data were not normally distributed, the non-parametric Kruskal-Wallis test was performed to determine significant differences, followed by a post-hoc Dunn test with Bonferroni corrections for multiple testing using the R package `dunn.test` (Dinno, 2017). A statistics table with all results can be found in Appendix 8.

## $\beta$ -Diversity: Microbial Community Composition and Structure

The microbial community composition and structure were assessed using the Bray-Curtis distance matrix, calculated using the OTU matrix. Dissimilarities between communities were visualised on non-metric multidimensional scaling (NMDS) ordination plots using the `metaMDS` function (R package 'vegan').

Following the NMDS visualisations, statistical analysis was done using the `adonis` function (R package 'vegan') to perform two-factor Permutational Multivariate Analysis of Variance (PERMANOVA), comparing the overall community composition between groups for the following factors: 'climate' ('present' or 'future') and soil origin ('origin': 'invader', 'community', and 'control'). To control for variation between the 10 original soil types, the `adonis` 'strata' command to restrict permutations within soil type as a random effect was included in the PERMANOVA. For significant interactions or when soil origin was a significant factor, a separate pairwise PERMANOVA was performed with a Bonferroni correction to adjust for multiple hypothesis testing. When appropriate, the adjusted  $p$ -values ( $p_{adj}$ ) were reported. A statistics table with all results can be found in Appendix 8.

## Microbial Community Visualisations

The relative abundances of bacteria and fungi phyla and classes were observed through the creation of a stacked taxonomy barplot. For these taxonomic summary figures, non-rarefied data

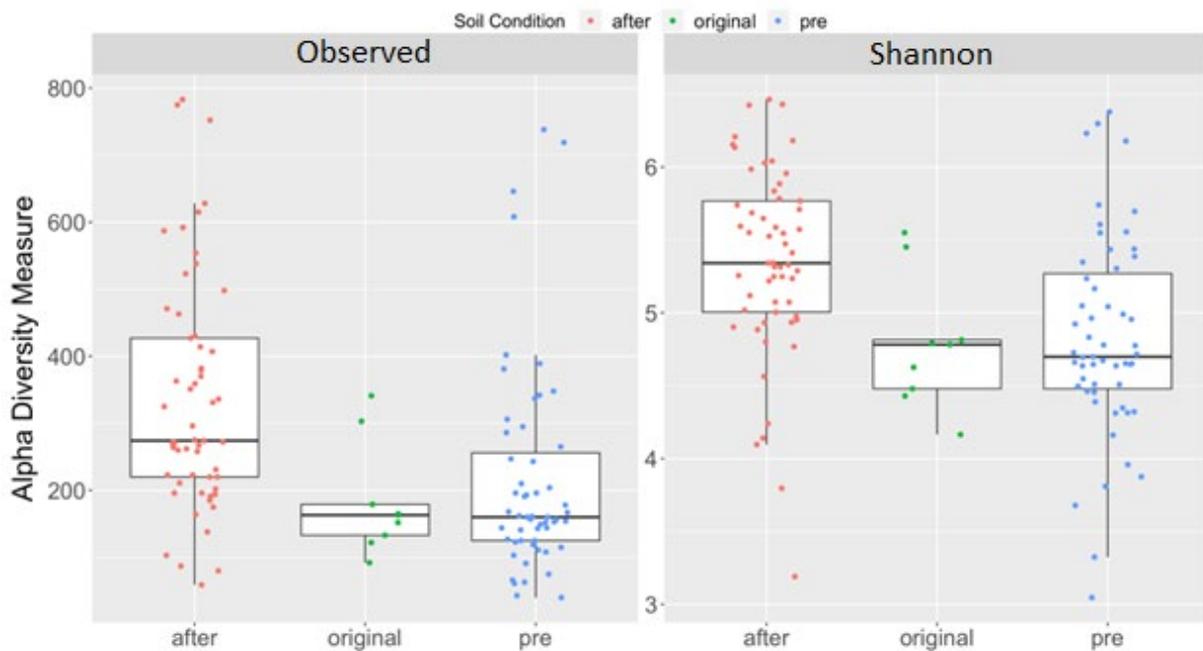
were used to preserve the ‘actual’ proportion of OTUs analysed. For both bacteria and fungi, taxa were agglomerated at the phylum and class level, transformed to relative abundance, and then filtered for low-abundance taxa that made up less than 1%. For comparison of the microbial community before and after phase 1, barplots were arranged by ‘original’, ‘pre’, and ‘after’. Taxonomic figures made from the microbial community post-phase 1 were arranged according to the combinations of ‘climate’ and ‘origin’: FI, PI, FC, PC, FX, and PX.

## 4.3 RESULTS

### 4.3.1 BACTERIA

#### 4.3.1.1 ALPHA DIVERSITY

##### All Samples

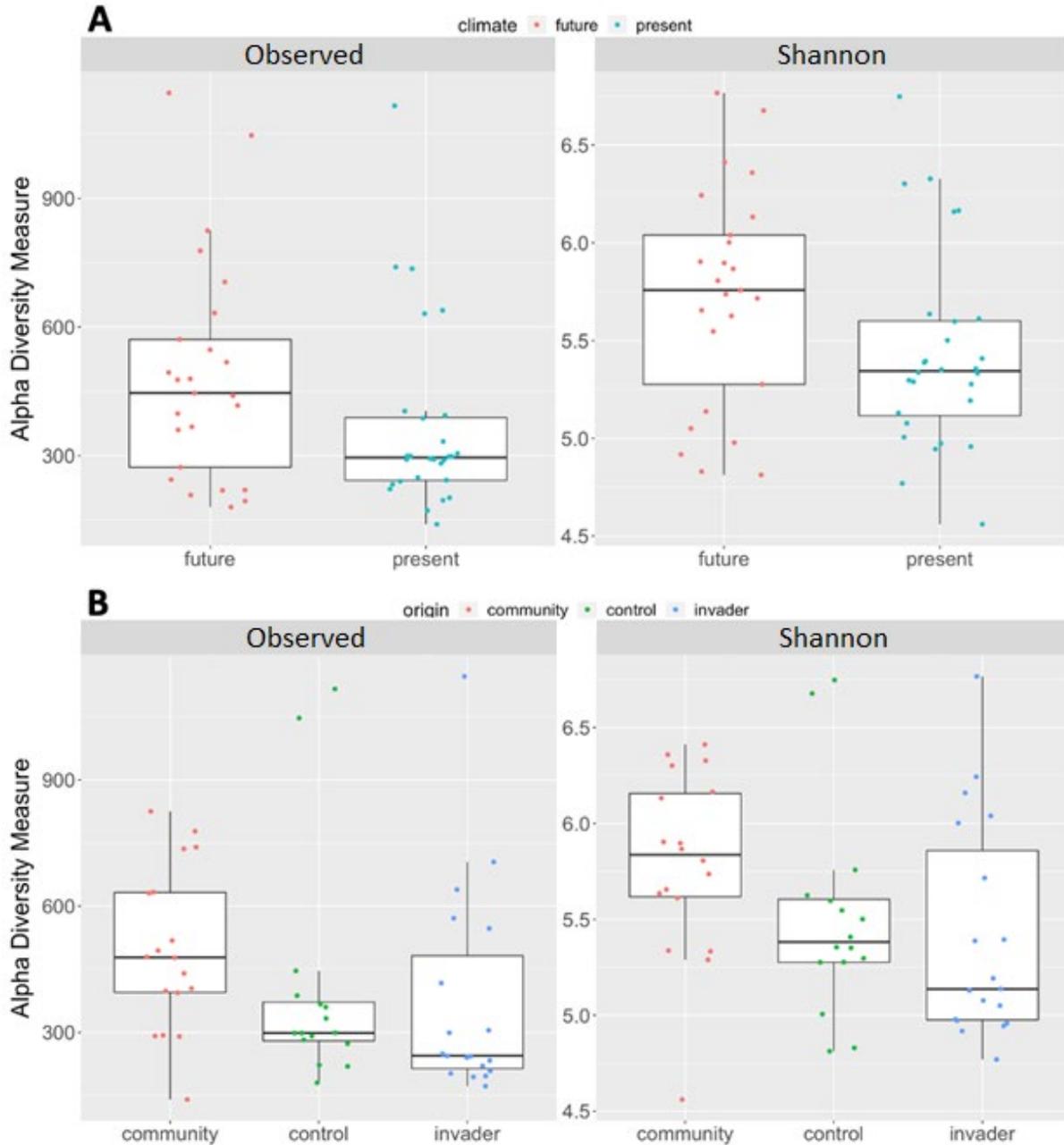


**Fig 25.** Boxplot for bacterial alpha-diversity using observed OTUs and Shannon diversity indices of bacterial communities prior to and after phase 1 of the temperature experiment. Bold lines on the boxplots represent the median, with boxes representing the interquartile range, and horizontal lines representing maximum and minimum observations excluding outliers. Thin

*vertical lines above and below the boxplots represent upper and lower quartiles. Coloured dots represent actual data points recorded.*

The calculated diversity indices (Observed OTUs and Shannon diversity,  $p$ -values presented in this order throughout) showed significant differences between the soil bacterial communities prior to ('original' and 'pre') and post-phase 1 ('after') (Kruskal-Wallis test,  $\chi^2 = 23.99$ ,  $p < 0.001$  /  $\chi^2 = 21.18$ ,  $p < 0.001$ ). Bacterial community observed OTUs and Shannon diversity were significantly higher in post-phase 1 soil compared to samples pre-phase 1 (Dunn test,  $p_{adj} < 0.001$  /  $p_{adj} < 0.001$ ), as well as the 'original' soil (Dunn test,  $p_{adj} = 0.008$  /  $p_{adj} = 0.013$ ). There was no significant difference in the alpha diversity indices between samples from the 'original' and 'pre' soils.

Post-Phase 1



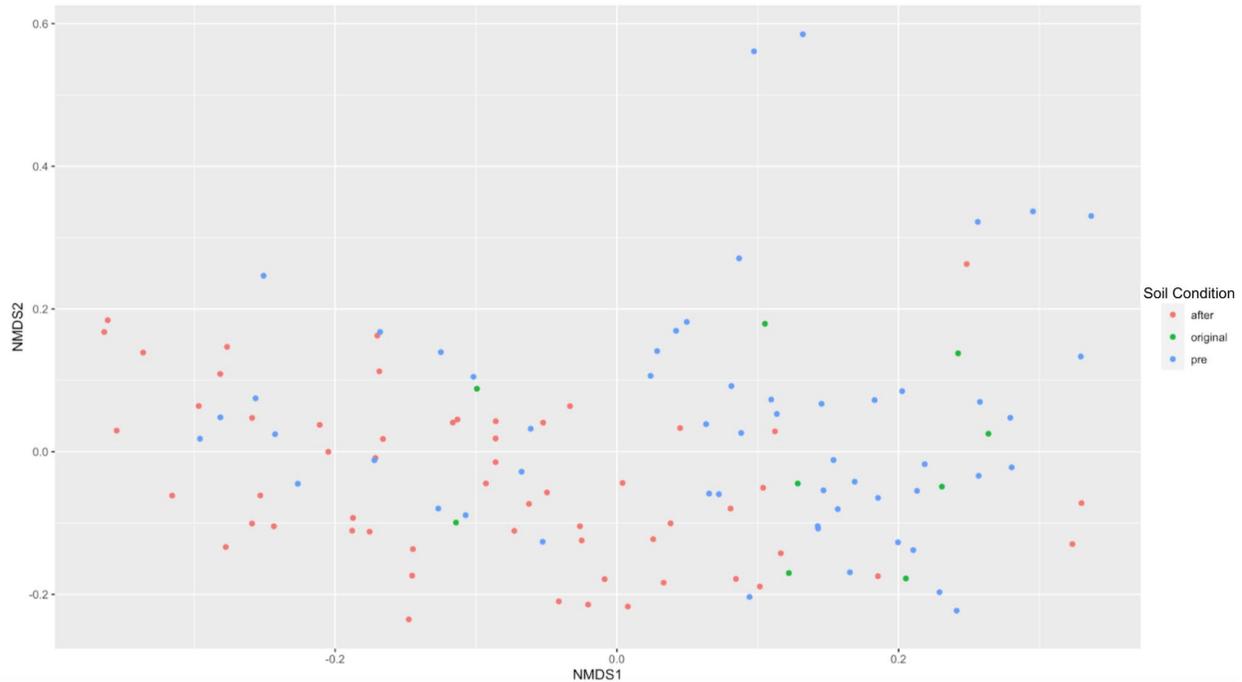
**Fig 26.** Boxplot for bacterial alpha-diversity using observed OTUs and Shannon diversity indices of bacterial communities after phase 1 of the temperature experiment, with samples separated by A) climate and B) soil origin. Bold lines on the boxplots represent the median, with boxes representing the interquartile range, and horizontal lines representing maximum and

*minimum observations excluding outliers. Thin vertical lines above and below the boxplots represent upper and lower quartiles. Coloured dots represent actual data points recorded.*

The calculated diversity indices showed significant differences between the soil bacterial communities post-phase 1 both for climate ('future' and 'present') (Kruskal-Wallis test,  $\chi^2 = 3.842, p = 0.03$  /  $\chi^2 = 4.054, p = 0.022$ ) and soil origin ('invader', 'community', and 'control') (Kruskal-Wallis test,  $\chi^2 = 7.238, p = 0.03$  /  $\chi^2 = 7.240, p = 0.03$ ). Both alpha diversity indices had significantly higher values in samples from the growth chamber set to a simulated 'future' temperature regime compared to those in the 'present-day' chamber. For soil origin, both observed OTUs and Shannon diversity were lower in soil from pots invaded by *Impatiens glandulifera* when compared to pots grown with a native community during phase 1 of the temperature experiment (Dunn test,  $p_{adj} = 0.014$  /  $p_{adj} = 0.015$ ).

### 4.3.1.2 BETA DIVERSITY

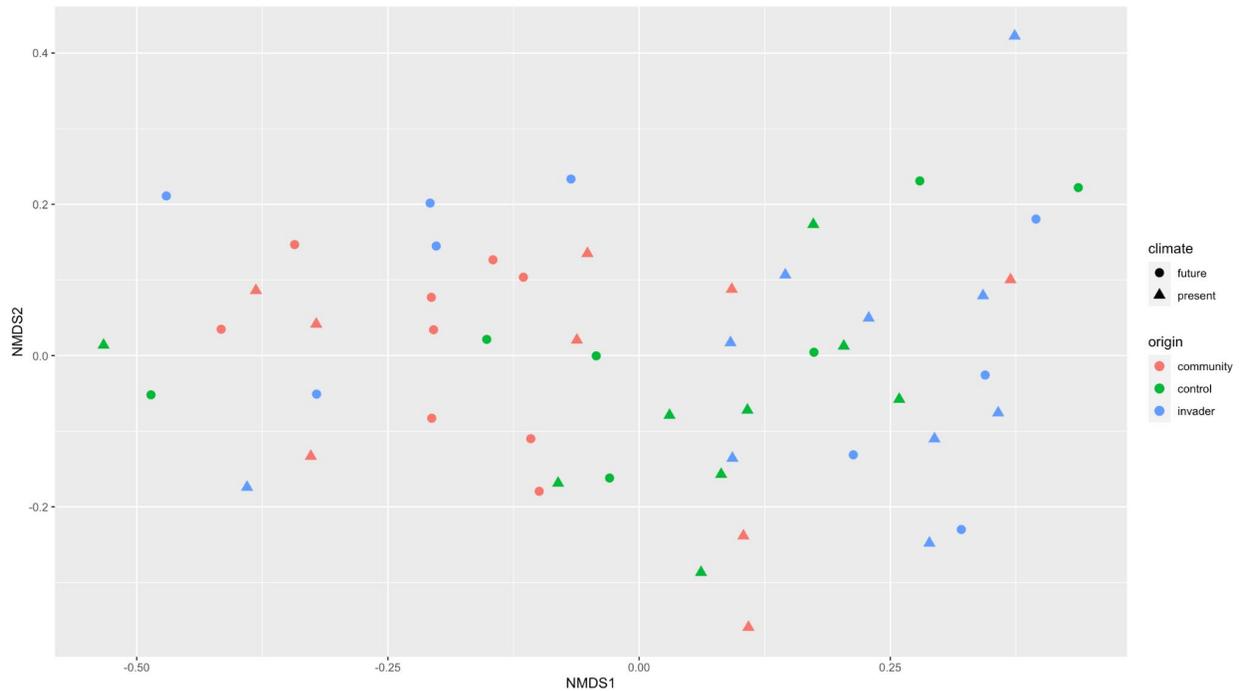
#### All Samples



**Fig 27.** Non-metric multidimensional scaling (NMDS) plot of bacterial community composition among the original soil samples (green), as well as prior to (blue) and after (red) phase 1 of the temperature experiment. The NMDS is based on OTU community similarity using a Bray-Curtis matrix, with three dimensions and stress = 0.188.

A PERMANOVA showed a separation of bacterial communities for the original, pre, and post-phase 1 samples ( $F_{2, 118} = 1.101, p = 0.001$ ). Pairwise tests showed that within the three soil types, there was a significant difference in composition between bacterial communities of the pots from before and after phase 1 ( $p_{adj} = 0.003$ ). There was no significant difference in bacterial community composition between the original soil samples and the pre-phase 1 samples.

## Post-Phase 1



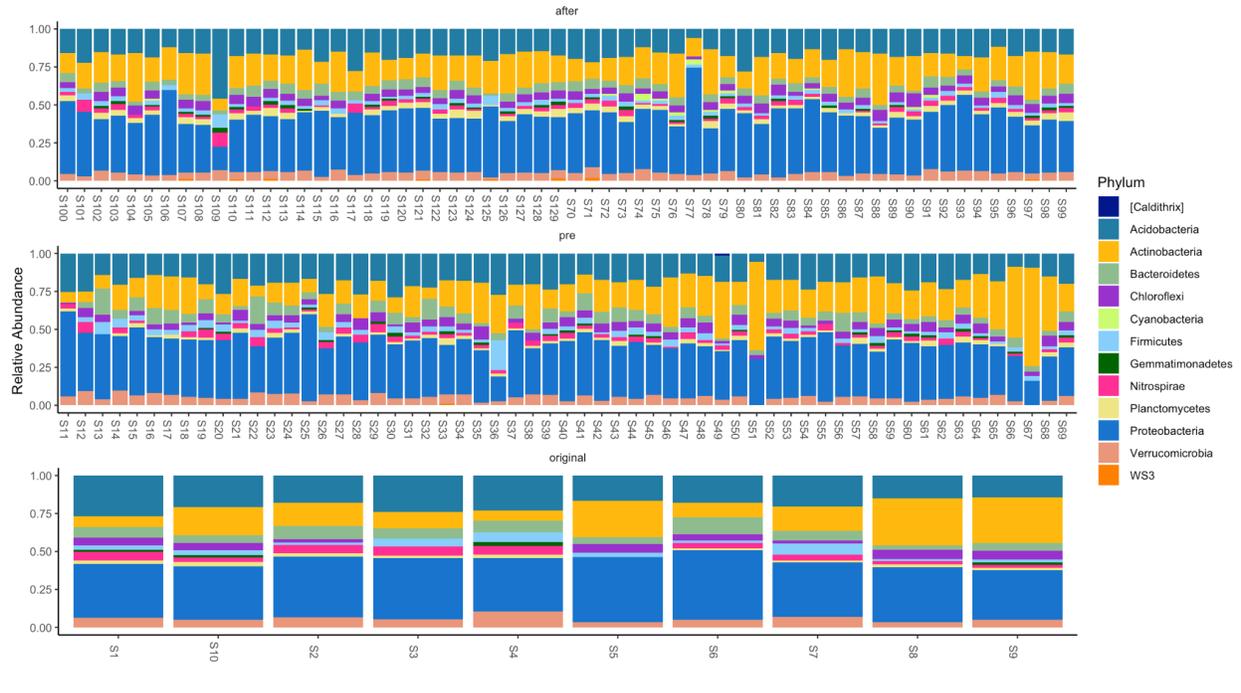
**Fig 28.** Non-metric multidimensional scaling (NMDS) plot of bacterial community composition among the soil samples after phase 1 of the temperature experiment. Samples from the future climate chamber are represented by a circle, whilst those from the present chamber are represented by a triangle. Samples from the community pots are red, those from the control pots are green, and those from the invader pots are blue. The NMDS is based on OTU community similarity using a Bray-Curtis matrix, with three dimensions and stress = 0.174.

A PERMANOVA showed a separation of bacterial communities for climate ( $F_{1, 47} = 1.050$ ,  $p = 0.031$ ) and origin ( $F_{2, 47} = 1.038$ ,  $p = 0.027$ ), with no interaction. Pairwise tests showed that within the three soil origins, there was a significant difference in composition between bacterial communities of the control and native community pots ( $p_{adj} = 0.045$ ).

### 4.3.1.3 TAXONOMIC SUMMARY

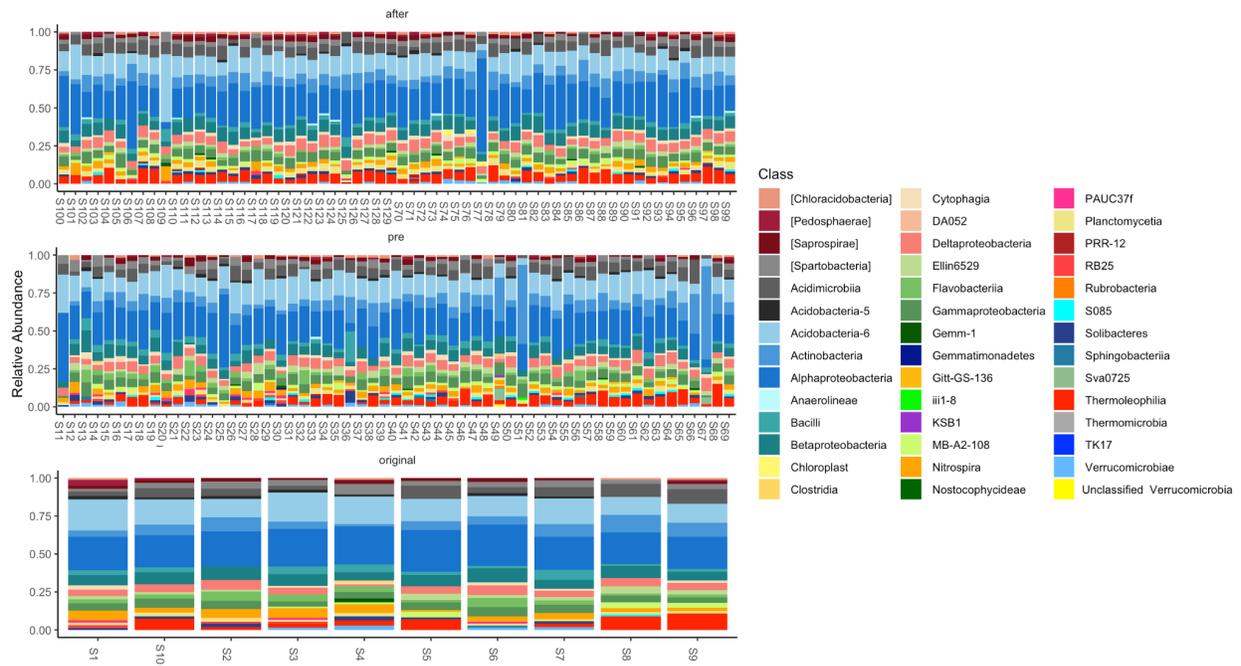
#### All Samples

#### Phylum



**Fig 29.** Stacked taxonomy barplot showing relative abundances of bacteria phyla making up each sample, filtered to remove less-abundant samples making up less than 1% of the community. Barplots are separated into samples from original, pre-phase 1, and post-phase 1 soil, with sample number shown on the x-axis. 13 phyla are represented in this plot.

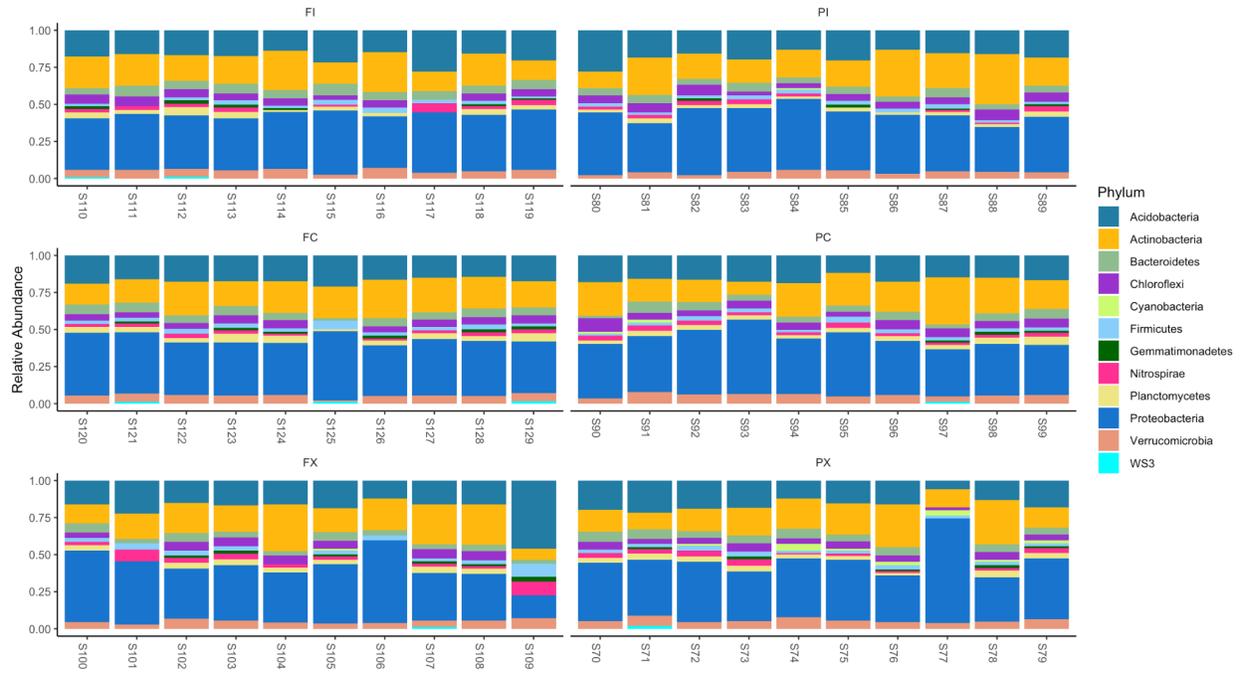
## Class



**Fig 30.** Stacked taxonomy barplot showing relative abundances of bacteria classes making up each sample, filtered to remove less-abundant samples making up less than 1% of the community. Barplots are separated into samples from original, pre-phase 1, and post-phase 1 soil, with sample number shown on the x-axis. 42 phyla are represented in this plot.

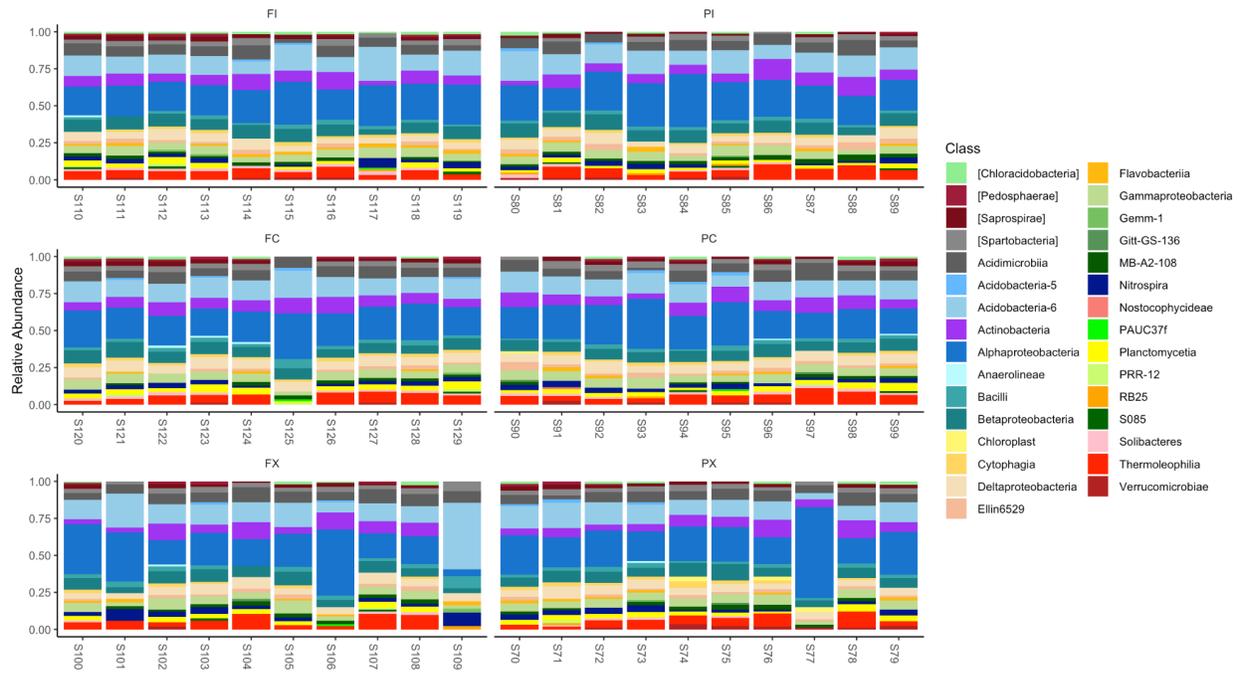
## Post-Phase 1

### Phylum



**Fig 31.** Stacked taxonomy barplot showing relative abundances of bacteria phyla making up each sample, filtered to remove less-abundant samples making up less than 1% of the community. Barplots are separated into groups from combinations of the two climates and three origins in phase 1 of the temperature experiment: future - invader (FI), present - invader (PI), future - community (FC), present - community (PC), future - control (FX), and present - control (PX), with sample number shown on the x-axis. 12 phyla are represented in this plot.

## Class

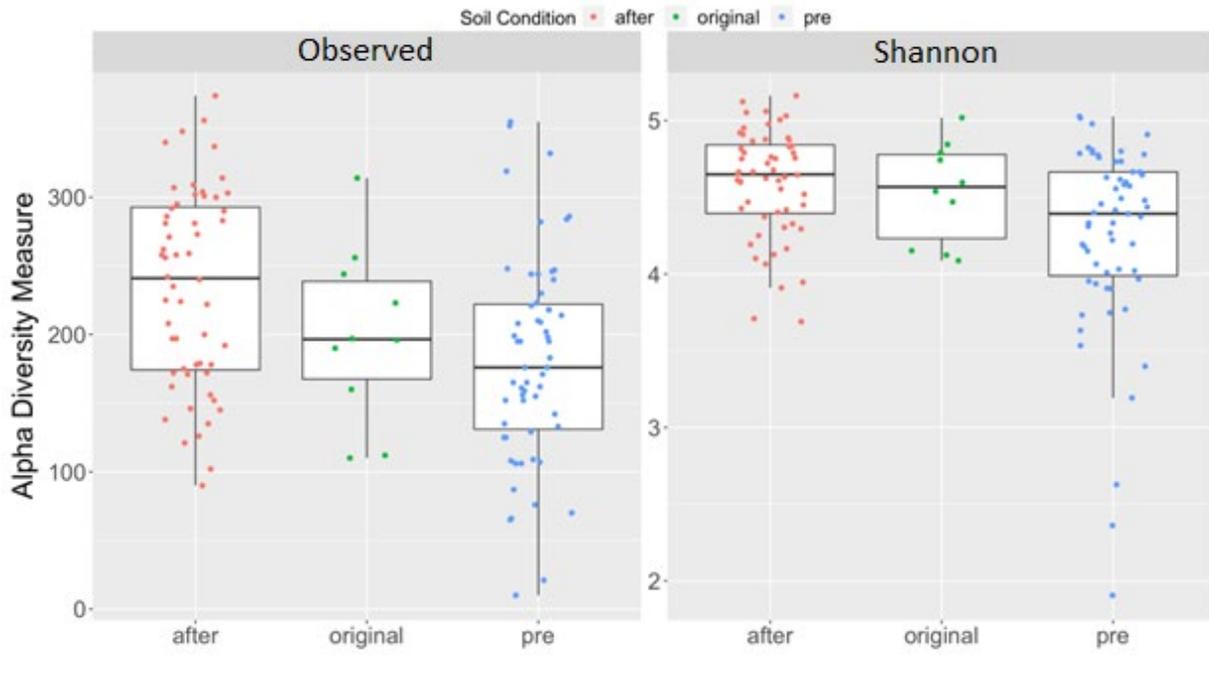


**Fig 32.** Stacked taxonomy barplot showing relative abundances of bacteria classes making up each sample, filtered to remove less-abundant samples making up less than 1% of the community. Barplots are separated into groups from combinations of the two climates and three origins in phase 1 of the temperature experiment: future - invader (FI), present - invader (PI), future - community (FC), present - community (PC), future - control (FX), and present - control (PX), with sample number shown on the x-axis. 31 phyla are represented in this plot.

## 4.3.2 FUNGI

### 4.3.2.1 ALPHA DIVERSITY

#### All Samples

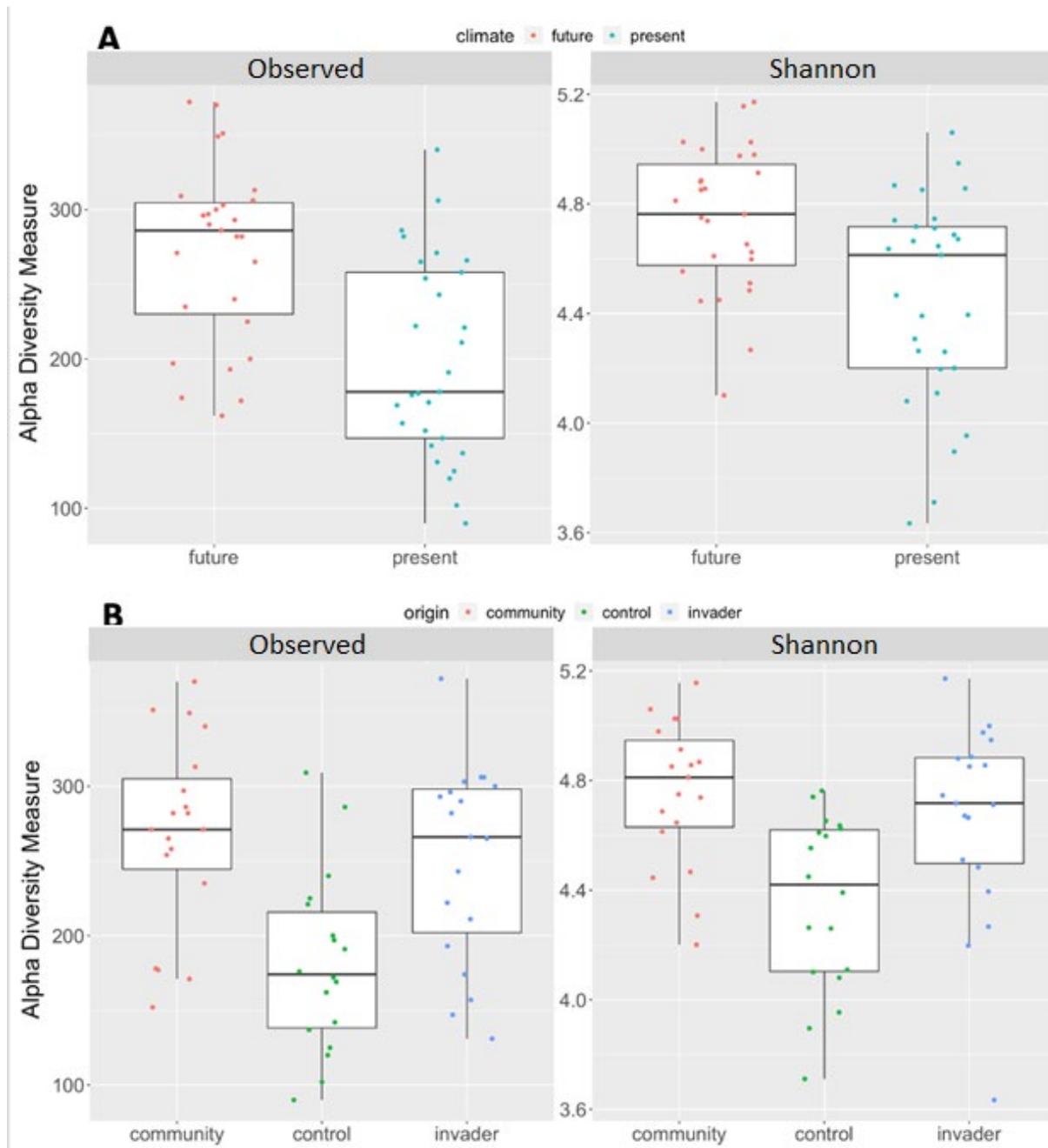


**Fig 33.** Boxplot for bacterial alpha-diversity using observed OTUs and Shannon diversity indices of fungal communities prior to and after phase 1 of the temperature experiment. Bold lines on the boxplots represent the median, with boxes representing the interquartile range, and horizontal lines representing maximum and minimum observations excluding outliers. Thin vertical lines above and below the boxplots represent upper and lower quartiles. Coloured dots represent actual data points recorded.

The calculated diversity indices (Observed OTUs and Shannon diversity,  $p$ -values presented in this order throughout) showed significant differences between the soil fungal communities prior to and post-phase 1 (Kruskal-Wallis test,  $\chi^2 = 13.16$ ,  $p = 0.001$  /  $\chi^2 = 11.892$ ,  $p = 0.003$ ). Fungal community observed OTUs and Shannon diversity were significantly higher in soil after phase 1 compared to samples taken from before the temperature experiment (Dunn test,  $p_{adj} < 0.001$  /

$p_{adj} < 0.001$ ). There were no significant differences in the alpha diversity indices between samples from the ‘original’ and ‘pre’ soils, as well as between the ‘original’ and ‘after’ soils.

### Post-Phase 1



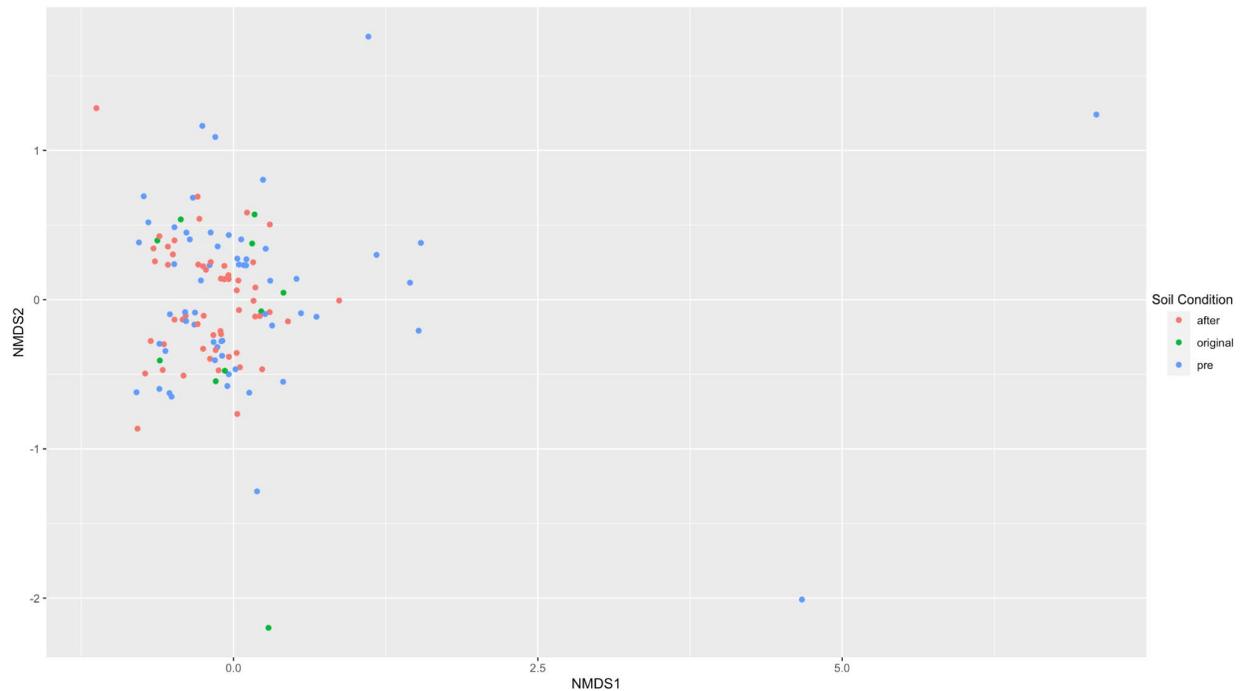
**Fig 34.** Boxplot for fungal alpha-diversity using observed OTUs and Shannon diversity indices of bacterial communities after phase 1 of the temperature experiment, with samples separated by

*A) climate and B) soil origin. Bold lines on the boxplots represent the median, with boxes representing the interquartile range, and horizontal lines representing maximum and minimum observations excluding outliers. Thin vertical lines above and below the boxplots represent upper and lower quartiles. Coloured dots represent actual data points recorded.*

The calculated diversity indices showed significant differences between the soil fungal communities post-phase 1 both for climate (Kruskal-Wallis test,  $\chi^2 = 14.66$ ,  $p < 0.001$  /  $\chi^2 = 8.094$ ,  $p = 0.002$ ) and soil origin (Kruskal-Wallis test,  $\chi^2 = 13.49$ ,  $p < 0.001$  /  $\chi^2 = 14.92$ ,  $p < 0.001$ ). Like bacteria, both alpha diversity indices had significantly higher values in samples from the growth chamber set to a simulated ‘future’ temperature regime compared to those in the ‘present-day’ chamber. For soil origin, both observed OTUs and Shannon diversity were lower in soil from the control pots when compared to both pots grown with a native community during phase 1 of the temperature experiment (Dunn test,  $p_{adj} < 0.001$  /  $p_{adj} < 0.001$ ), as well as those grown with only *Impatiens glandulifera* (Dunn test,  $p_{adj} = 0.007$  /  $p_{adj} = 0.005$ ).

### 4.3.2.2 BETA DIVERSITY

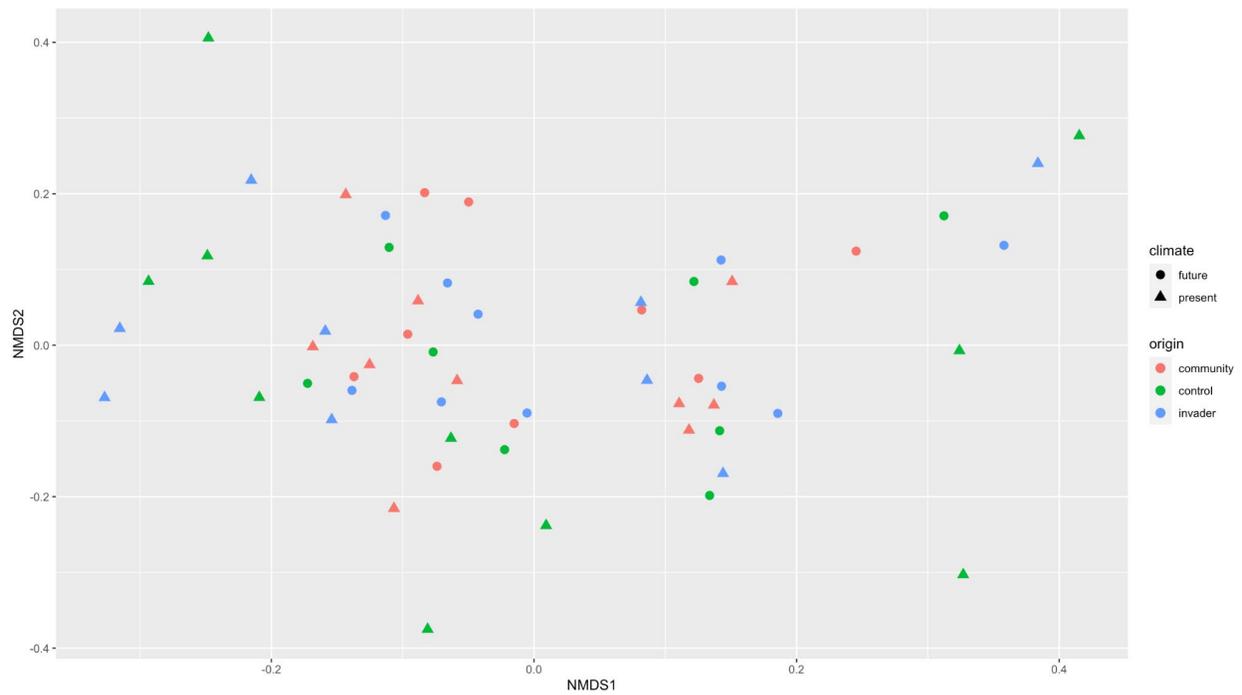
#### All Samples



**Fig 35.** Non-metric multidimensional scaling (NMDS) plot of fungal community composition among the original soil samples (green), as well as prior to (blue) and after (red) phase 1 of the temperature experiment. The NMDS is based on OTU community similarity using a Bray-Curtis matrix, with two dimensions and stress = 0.148.

A PERMANOVA showed no significant separation of fungal communities for the original, pre, and post-phase 1 samples ( $F_{2, 122} = 1.166, p = 0.183$ ).

## Post-Phase 1



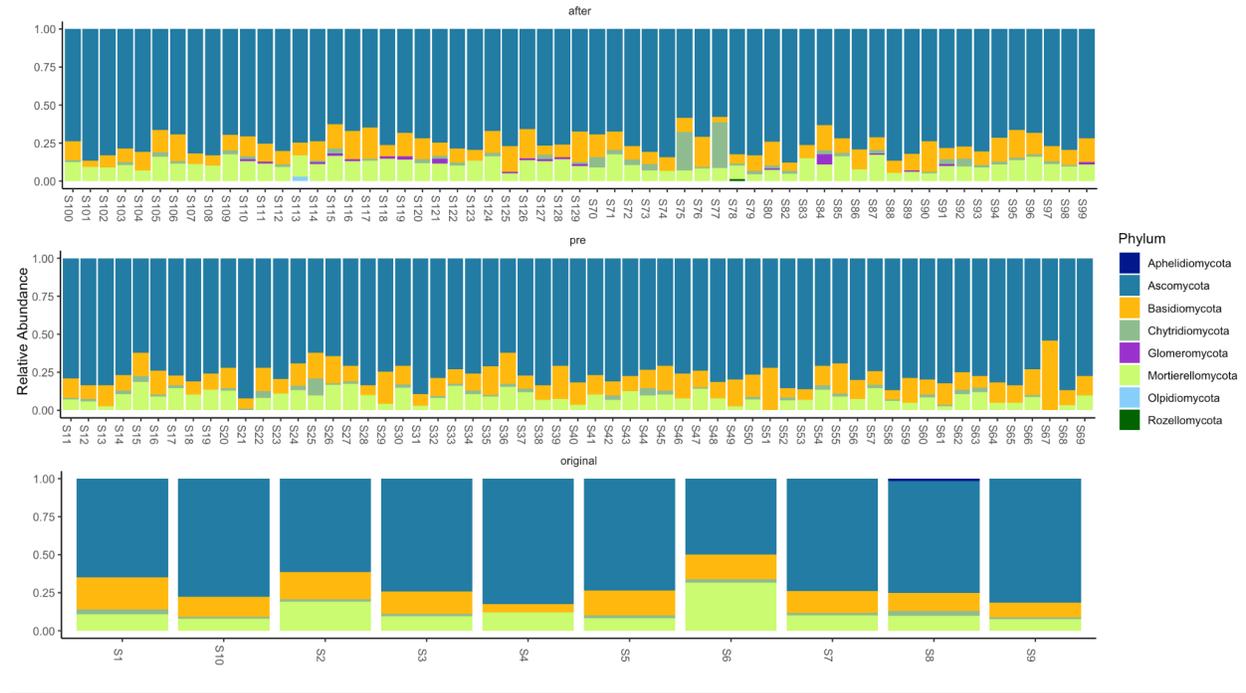
**Fig 36.** Non-metric multidimensional scaling (NMDS) plot of fungal community composition among the soil samples after phase 1 of the temperature experiment. Samples from the future climate chamber are represented by a circle, whilst those from the present chamber are represented by a triangle. Samples from the community pots are red, those from the control pots are green, and those from the invader pots are blue. The NMDS is based on OTU community similarity using a Bray-Curtis matrix, with two dimensions and stress = 0.165.

A PERMANOVA showed no separation of fungal communities for climate ( $F_{1, 50} = 0.995$ ,  $p = 0.416$ ) and origin ( $F_{2, 50} = 0.7014$ ,  $p = 0.977$ ), with no interaction.

### 4.3.2.3 TAXONOMIC SUMMARY

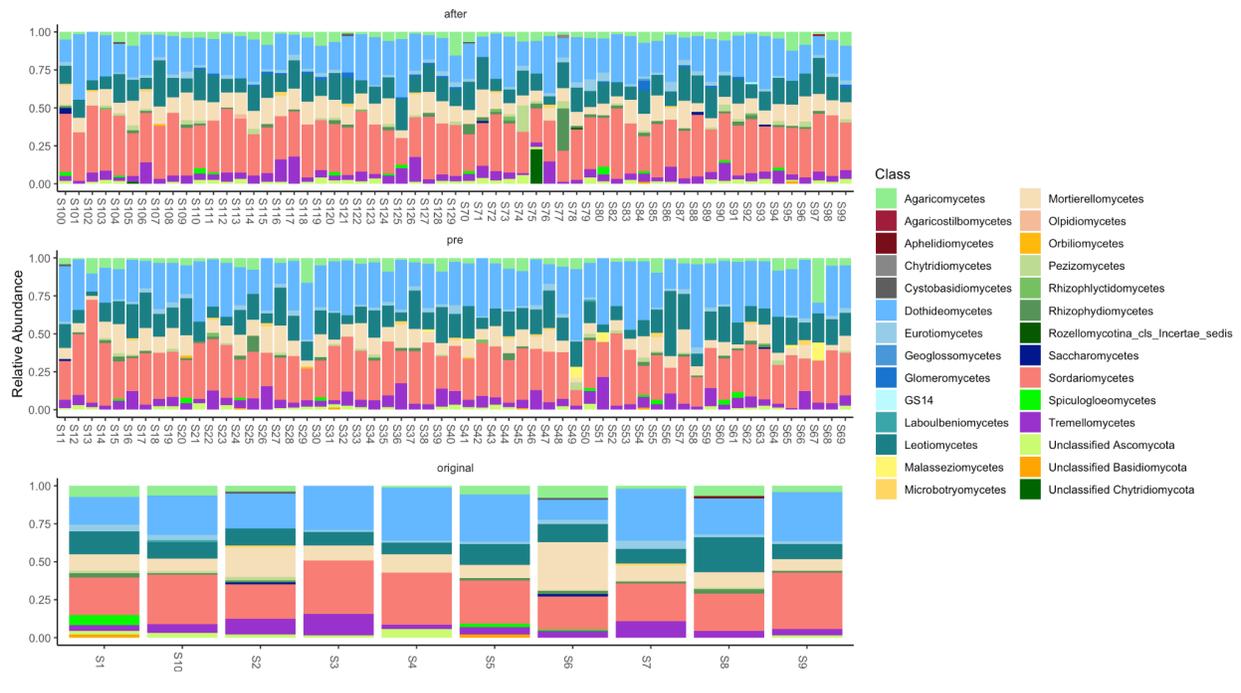
#### All Samples

#### Phylum



**Fig 37.** Stacked taxonomy barplot showing relative abundances of fungal phyla making up each sample, filtered to remove less-abundant samples making up less than 1% of the community. Barplots are separated into samples from original, pre-phase 1, and post-phase 1 soil, with sample number shown on the x-axis. 8 phyla are represented in this plot.

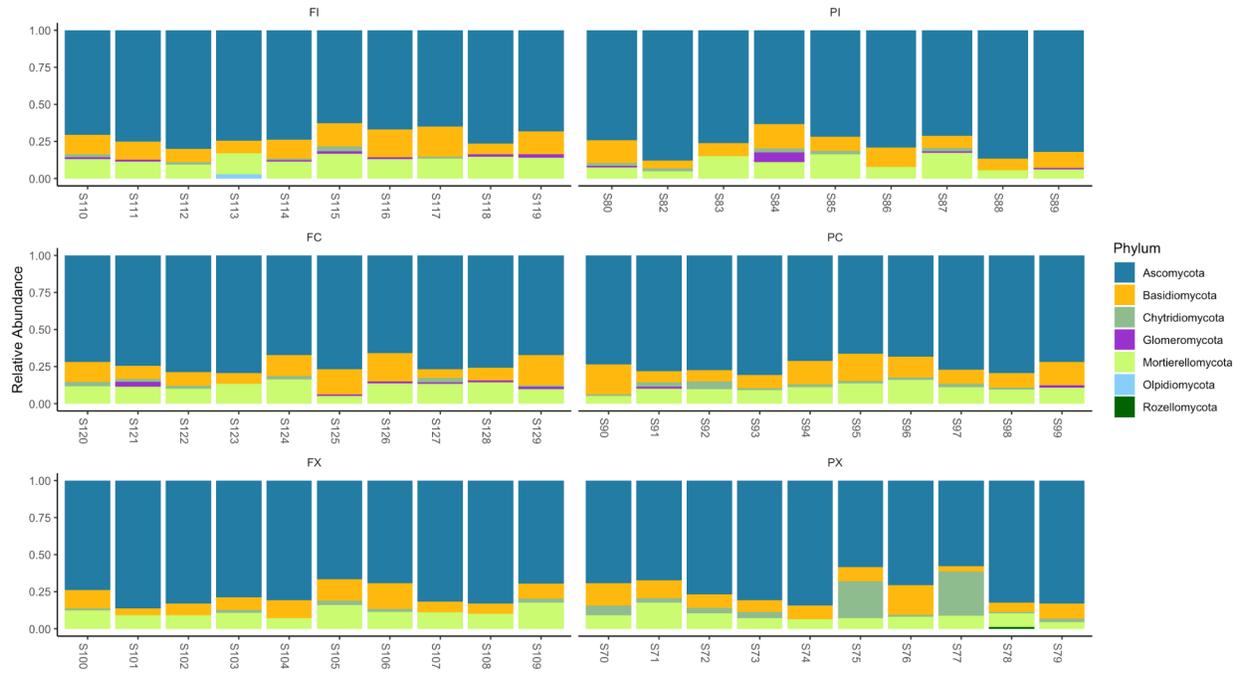
## Class



**Fig 38.** Stacked taxonomy barplot showing relative abundances of fungal classes making up each sample, filtered to remove less-abundant samples making up less than 1% of the community. Barplots are separated into samples from original, pre-phase 1, and post-phase 1 soil, with sample number shown on the x-axis. 28 phyla are represented in this plot.

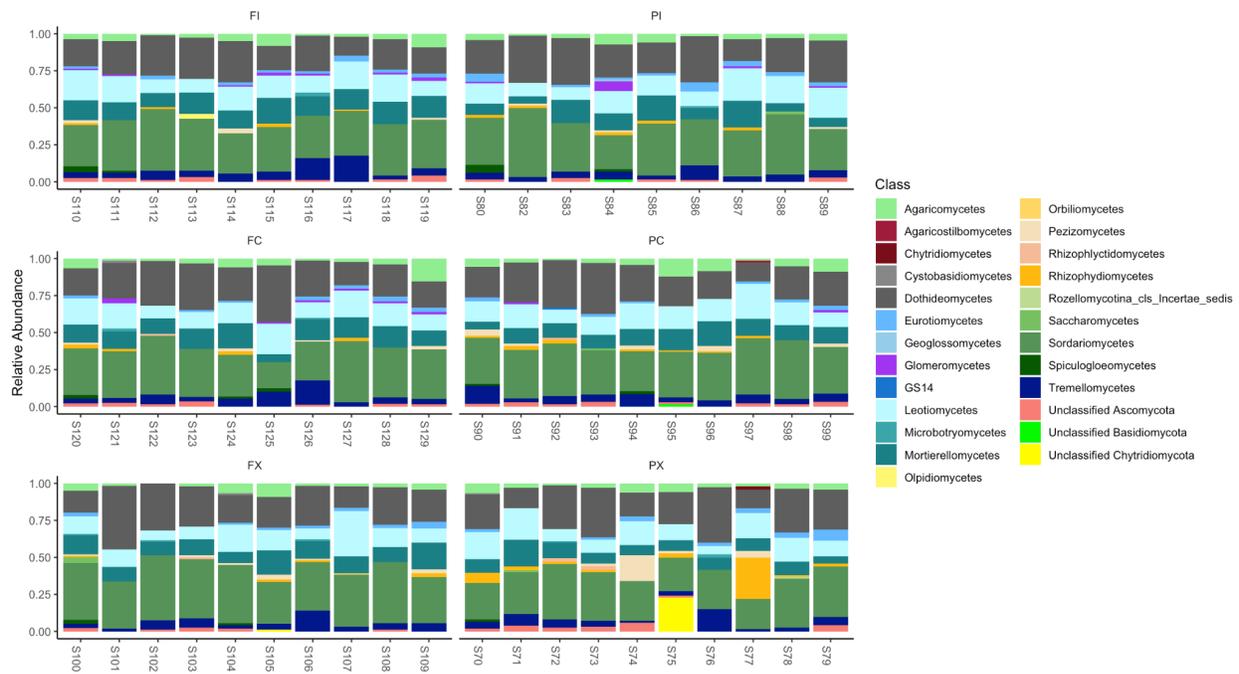
## Post-Phase 1

### Phylum



**Fig 39.** Stacked taxonomy barplot showing relative abundances of fungal phyla making up each sample, filtered to remove less-abundant samples making up less than 1% of the community. Barplots are separated into groups from combinations of the two climates and three origins in phase 1 of the temperature experiment: future - invader (FI), present - invader (PI), future - community (FC), present - community (PC), future - control (FX), and present - control (PX), with sample number shown on the x-axis. 7 phyla are represented in this plot.

## Class



**Fig 40.** Stacked taxonomy barplot showing relative abundances of fungal classes making up each sample, filtered to remove less-abundant samples making up less than 1% of the community. Barplots are separated into groups from combinations of the two climates and three origins in phase 1 of the temperature experiment: future - invader (FI), present - invader (PI), future - community (FC), present - community (PC), future - control (FX), and present - control (PX), with sample number shown on the x-axis. 25 phyla are represented in this plot.

## 4.4 DISCUSSION

The results of Chapter 3 showed that *I. glandulifera* produced a positive PSF, increasing its competitive ability in invader-conditioned soil and negatively affecting the native community. Climate change was also demonstrated to play a role in soil dynamics, with a possibility that alterations of the microbial community in invaded soil decreased drought stress resistance of natives in future temperatures, alongside other general shifts in the bacterial and fungal communities. At this point, many soil conditioning studies halt their analysis, or continue with

general qualitative analyses of the microbial community. The DNA sequencing and metabarcoding analysis in this chapter, however, goes one step further, allowing for quantitative comparisons of the bacterial and fungal communities of soil conditioning. This analysis also extends to the effect of climate change on the soil microbial community as well, and how this impacts soil conditioning by *I. glandulifera*.

The bacterial and fungal communities associated with the plants in this study were diverse, with millions of reads clustered into tens of thousands of OTUs. Soil bacterial and fungal alpha diversity was higher for both observed OTUs and Shannon diversity after the soil conditioning treatment of phase 1 when compared to both the original soil samples and the same pots prior to planting of either *I. glandulifera* or a native community. This is to be expected, as all plants, whether native or invasive, alter their surrounding soil microbial community (Kulmatiski *et al.*, 2008; Dawson & Schrama, 2016; van der Putten *et al.*, 2016; Beals *et al.*, 2020). As such, growing plants in soil for nine weeks should increase alpha diversity compared to the soil prior to growth. In this case, this was true for both observed OTUs, an indication of community richness, and Shannon diversity, which incorporates both species richness and their relative abundance. These indices show that not only was the soil microbial community richer in species following phase one, but also that those bacterial and fungal species were distributed more evenly.

Additionally, there was no significant difference in alpha diversity for either diversity index in both bacteria and fungi between the original soil samples and those mixed with sterile sand. This supports the temperature study as being an appropriate simulation of the microbial community of an actual ecosystem in Northeast England, as both bacteria and fungi richness and evenness in the experiment pots are very close to those from the original soil extracts. The taxa barplots showcasing relative abundances of bacterial and fungal phyla in this study are similar to those from natural soil. Janssen (2006) performed an analysis of 32 libraries of bacterial 16s rRNA genes to understand the general composition of soil bacterial communities. The most abundant bacterial phyla found in the bacterial communities of this study were proteobacteria, actinobacteria, and acidobacteria, which are also among the most abundant phyla found by Janssen (Janssen, 2006). The most abundant fungal phyla by far in this study was ascomycota, which also matches the literature (Schoch *et al.*, 2009; Egidi *et al.*, 2019). The closeness of the

microbial community in this pot conditioning experiment to that of actual ecosystems lends credence to the findings of chapters 3 and 4, whilst also demonstrating that metabarcoding studies such as this one are appropriate for more diverse applications in the field of plant invasions, and ecology as a whole.

Pattison *et al.* (2016) found that soil conditioning by *I. glandulifera* decreased AMF root colonisation to half that of control soil, whilst bacterial biomass increased almost two-fold in the conditioned soil. Interestingly, the results of this study contrast with those findings; there was lower bacterial diversity for both observed OTUs and Shannon diversity in invaded pots than native pots, and no difference in fungal diversity between the two conditions. This is more consistent with a recent meta-analysis by Zhang *et al.* (2019), which showed that in the rhizosphere invasive species “reduced bacterial biomass by 12%, but increased [AMF] fungal biomass by 36%” in invaded soils. Gaggini *et al.* (2018), found similar results in soil invaded by *I. glandulifera*: there was a reduction in bacterial community activity, whilst the fungal community had higher OTU richness in the presence of *I. glandulifera*. This reduction in bacterial diversity may be a result of allelopathy, perhaps as a result of naphthoquinones or 1,4-naphthoquinone (2-MNQ), which can affect soil microbial communities (Gaggini *et al.*, 2018; Cipollini *et al.*, 2012; Coakley & Petti, 2021). For the fungal community in this study, the control pots had lower fungal diversity than the invader and community pots, showing that there was an effect of plant presence on fungal richness. The lack of any contrasts in fungal diversity between invaded and native community pots may be evidence that *I. glandulifera* was forming mutualisms with AMF, similar to the mutualisms in the native community pots. Whilst *I. glandulifera* has been shown to only be weakly dependent on AMF, recent studies have shown that in its invaded range, *I. glandulifera* can form mutualisms with the fungus, with a colonisation rate of 10-90% (Tanner *et al.*, 2014; Gucwa-Przepióra *et al.*, 2016; Gaggini *et al.*, 2018). The large range of AMF colonisation in *I. glandulifera* suggests that, as has been shown for other plants, association with AMF may depend on soil conditions, rather than the invader strictly avoiding symbiosis with AMF (Carrenho *et al.*, 2007; Gaggini *et al.*, 2018). Alternatively, as noted by Gaggini *et al.* (2018), if AMF colonisation was lower in invaded pots in this study, non-mycorrhizal species may have increased in abundance to compensate. Unfortunately, time constraints prevented changes in AMF abundance from being directly measured in this study. However, the taxa resolution of this study was fine enough that these

differences could be elucidated, and it would be interesting in a future study to see if 1) mycorrhizal fungi abundance was impacted by *I. glandulifera*, and 2) if non-mycorrhizal fungi increased in abundance in response, as they may have benefited from increased carbon resources sourced from extra root biomass of *I. glandulifera*.

Beta-diversity analysis showed a clear separation of the bacterial communities in pots from the present and future climate chambers. This is in line with other studies which found that bacteria do respond to changes in soil temperature, with different phyla having contrasting responses to warming (Briones *et al.*, 2014; Fang *et al.*, 2021). Fang *et al.* (2021) found that proteobacteria, gemmatimonadetes, and chloroflexi, all taxa shown in the bacterial taxa barplots of this study, were insensitive to temperature changes; as a result, they may have further dominated the bacterial community in future temperatures compared to those of the present-day chamber. However, the alpha diversity analysis showed that bacterial richness was actually greater in the future climate. This may be a result of increased bacterial turnover and activity at increased temperatures (Dutta & Dutta, 2016). Bacteria that are better-adapted to warmer temperatures, such as those with a higher growth rate, may have fared better in the future climate chamber, and resulted in increased bacterial diversity. Future analysis of this dataset may focus on whether these potentially ‘thermophilic’ taxa did show a quantitative increase in future soils.

There was no clear separation in the bacterial and fungal communities between soils from the different conditioning treatments, and the fungal community appeared not to shift between the two climates. This is interesting, as soil conditioned by *I. glandulifera* had lower bacterial alpha-diversity. The lack of any community-wide shifts in bacterial composition suggest that while soil conditioning by *I. glandulifera* does affect the bacterial community on a certain scale, this effect is not pronounced enough to indicate a total shift in the belowground community. The lack of changes in fungi beta-diversity, on the other hand, may simply be a result of *I. glandulifera* forming AMF mutualisms similar to those of the native community, or providing greater carbon resources for non-AMF fungi (Tanner *et al.*, 2014; Gucwa-Przepióra *et al.*, 2016; Gaggini *et al.*, 2018). Also, the role of AMF in alleviating plant drought stress implies a certain level of adaptation to endure warmer climates (Bowles *et al.*, 2017). Whether this effect would persist if the experiment ran for longer than nine weeks is a question of great importance. Alternatively, this may have been the result of primer choice: whilst the ITS86F/ITS4 primer pair has been

shown to have good coverage, read numbers, and OTUs, it may not be as adept at sequencing AMF (Op De Beeck *et al.*, 2014). This relative paucity of AMF fungal groups in this study is dissimilar to the fungal communities found in other studies, supporting the notion that this primer pair may have less sensitivity to AMF (Tanner *et al.*, 2014; Gucwa-Przepióra *et al.*, 2016; Gaggini *et al.*, 2018). A future study utilising the forward fungal-specific ITS2 region primer ITS7o, which has been shown to have greater sensitivity for AMF, may aid in solving this issue (Ihrmark *et al.*, 2012; Kohout *et al.*, 2014). However, the ITS4/ITS7o primer pair failed to amplify in this study, so the PCR process may require some reworking if this primer pair is to be used successfully. The lack of any bacterial and fungal community-wide separation between soils from different origins and climates, however, is not unsupported in the literature; there are studies that have found *I. glandulifera* to have negligible impacts on native plant species and soil communities (Diekmann *et al.*, 2016; Cuda *et al.*, 2017).

The conflicting results of the effects of *I. glandulifera* on the soil microbiome from Pattison *et al.* and this study may be a result of different methodological approaches. Whereas this study was performed in pots placed inside a sealed growth chamber, the Pattison *et al.* study was performed outside, in a site cleared of vegetation. Whilst alpha diversity and taxonomic analysis showed that the diversity and relative phyla abundances of soil microbes in our pots closely matched those of ‘original’ soil samples obtained from the field, it is impossible to fully replicate natural conditions in a sealed environment (Passioura, 2006). However, although this may be the case, the clear positive PSFs exhibited by *I. glandulifera* in chapter 3 shows that there is still merit to this approach, especially when investigating the combined effects of soil conditioning and climate change. Additionally, the microbial community shifts under the effects of *I. glandulifera* soil conditioning observed in this study are similar to those of Gaggini *et al.* (2018), illustrating that PSFs often depend on the surrounding conditions. The findings of this study can be interpreted in the context of its method, and taken as one possible outcome of soil conditioning by invasive plant species.

When taken in the context of climate change, the alpha and beta diversity findings have contrasting results. On the one hand, a more diverse soil microbiome is able to buffer the effects of drought; soil conditioning by *I. glandulifera* led to losses in microbial alpha diversity, which, combined with climate change stresses, could negatively affect the performance of native plant

species (Bogati & Walczak, 2022; Malacrino *et al.*, 2020). However, growth in simulated future temperatures under the effects of climate change resulted in increased bacterial species richness, with the soil bacterial communities of pots from the two climates showing a clear separation in community composition. This increase in bacterial diversity may buffer the drought effects of climate change; however, this may not be the case in invaded ecosystems. Fungal diversity and community composition remained the same regardless of soil origin and climate, implying that fungal communities may be able to remain the same regardless of invasion status or warmer temperatures. This effect is heavily-reliant on the presence or absence of AMF, which aid plants in drought stress tolerance. However, the results of chapter 3 show a clear positive PSF of *I. glandulifera*, as well as a possible effect of climate on the competitive ability of the invader. It may be the case that AMF abundance was decreased by the presence of *I. glandulifera* and replaced by non-mycorrhizal species, as suggested in Gaggini *et al.* (2018); this effect could be missed by the diversity analyses. Alternatively, as the fungal taxa barplots show only a small relative abundance of glomeromycota, there may have simply been only a small initial inoculum of AMF at the start of the experiment, not enough to show an effect in this study. One of the first additional analyses that should be performed on this dataset is a differential abundance analysis to identify if *I. glandulifera*-conditioning or climate did have a significant effect on glomeromycota. It would also be of great benefit to analyse the functional profiles of some of the bacterial and fungal taxa found in the soils from each conditioning origin, as well as between the two climate treatments; this could be achieved through further use of phyloseq in R, or through bioinformatics software such as PICRUSt. This analysis may reveal further details of the changes in the soil microbiome due to the multifaceted interactions of plant invasions and climate change.

The findings of this chapter indicate that the field of plant invasions can benefit from more studies of this type, with which we can better understand the dynamics of plant invasions from a holobiont perspective. By identifying possible bacterial and fungal taxa, alongside the differences in these microbial communities, this study opens a path for greater understanding of the mechanisms underplaying soil conditioning and climate-related PSF responses to invasion by *I. glandulifera*, and plant invasions in general.

## Chapter 5:

### Final conclusions

Plant invasions are a major driver of global change in modern times, reducing the diversity of and displacing native species, as well as altering the activity and structure of soil microbial and fungal communities through plant-soil feedbacks (van Kleunen *et al.*, 2015; Morón *et al.*, 2009; Hejda *et al.*, 2009; Batten *et al.*, 2006; Pattison *et al.*, 2016). These alterations have the potential to affect larger ecosystem-wide processes such as nitrogen cycling and litter decomposition (Wang *et al.*, 2015; Liao *et al.*, 2008). Legacy effects of these changes can persist after the removal of the invader, increasing the challenge and cost of restoring invaded sites to their uninvaded state (Stefanowicz *et al.*, 2017). Climate change has the potential to impact the effects of plant invasions and PSFs in general, however more knowledge on this dynamic is required in order to counteract the effects of invasive plants in the coming decades.

The literature review of chapter 1 illustrated the many ways plant-soil feedbacks can manifest, and the way in which invasive species utilise PSFs to facilitate further invasions. There is a large repository of evidence showcasing the threat of invasive plants, many focusing on PSFs; however, there is a dearth of studies investigating the effects of climate change on this dynamic. Climate change, like plant invasion, is predicted to impact soil microbial communities, and therefore the two can be expected to at least interact with each other, if not show complementary effects (Briones *et al.*, 2014; Gaggini *et al.*, 2018). The specific roles of bacteria and fungi in these PSF dynamics, if any, is also an area requiring more research.

This study explored how invasive plants, specifically *I. glandulifera*, utilise PSFs to alter the biotic and abiotic components of soil in order to facilitate invasions, and how this phenomenon responds to climate change. *I. glandulifera* was an appropriate species for this study as it displays a broad array of well-documented PSF mechanisms, allowing for analysis of these dynamics under the warming and precipitation effects of climate change (Zybeck *et al.*, 2016; Pattison *et al.*, 2016; Grove *et al.*, 2017; Coakley & Petti, 2021).

The first experiment in this study, the water availability experiment in chapter 2, demonstrated that *I. glandulifera* consistently showed a greater competitive ability than native species in the community, even under watering treatments that negatively affected the invader. The annual invader accumulated biomass much faster than the native species under all watering treatments; it was also less negatively affected by the flood and drought treatments, exhibiting its competitive advantage over native plants. These findings suggest that riparian invaders such as *I. glandulifera* are poised to benefit from the extreme weather events and altered precipitation under the effects of climate change. As a result, greater efforts must be put into limiting the spread and impact of invasive riparian species such as *I. glandulifera*, as their negative impacts are only set to increase under the effects of climate change.

Building off of the findings of chapter 2, chapter 3 explored the role of warmer temperatures under climate change on the soil conditioning effects and competitive ability of *I. glandulifera*. Similar to the previous experiment, the effects of climate change were positive for the invasive plant, and negative for a native community. Additionally, soil conditioning and increased temperatures even appeared to complement each other, to the detriment of the competitive ability of native plants. One mechanism for this effect may be the combination of drought in higher temperatures alongside lower native drought stress resistance as a result of *I. glandulifera* soil conditioning altering the soil microbial community (Qin *et al.*, 2014; Cuda *et al.*, 2017; Bowled *et al.*, 2017; Bogati & Walczak, 2022). The extent to which the soil microbial community was altered by temperature or soil conditioning in this experiment is addressed in chapter 4.

Chapter 4 was a metabarcoding analysis of the soil microbial community following conditioning in phase one of the temperature experiment. The results of this analysis were thus interpreted in the context of the results from chapter 3. First, it must be remarked that the metabarcoding analysis in this chapter worked successfully. Bacterial and fungal diversity increased following the first phase of the experiment, as expected, and taxa barplots displaying the relative abundances of microbial phyla follow the same distribution as those of soil in the field. If a study of this scale and complexity can be achieved by a single researcher in a University laboratory, as was the case in this experiment, then this approach can and should be used in other plant invasion and ecology studies.

Overall, bacterial diversity was lower in invaded pots than those grown with a native community. Additionally, the future climate resulted in greater bacterial diversity, and an overall shift in the bacterial community compared to soil from present-day temperatures. However, fungal diversity and community composition remained the same regardless of soil origin and climate. These results are especially interesting in light of chapter 3; the hypothesised mechanism soil conditioning, that of a decrease in soil AMF colonisation, did not occur. This highlights a factor of plant invasions mentioned in the literature review of chapter 1: plant invasions mechanisms are not homogenous. This study was more in line with that of Gaggini *et al.* (2018), which suggested *I. glandulifera* may actually form mutualisms with mycorrhizal fungi, and that the decrease in bacterial diversity may be an effect of allelopathy. Further analysis of the dataset from this study is warranted to discover if this is the case.

This study exhibited the effects of *I. glandulifera* soil conditioning on microbial communities, as well as microbial responses to increased temperature under climate change. When taken together with the physical responses of *I. glandulifera* to climate change, such as increased biomass and greater tolerance of extreme instances of water availability, it is clear that climate change positively affects the competitive ability of *I. glandulifera* relative to a native plant community. The findings of these three chapters have important implications for future efforts to manage invasive species, as well as approaches to ecological studies as a whole. More research in this vein must be conducted on other invasive species and in other climate change contexts to elucidate the effects of climate change on plant invasions in the context of PSFs, allowing us to better mitigate the effects of plant invasions in the future.

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# APPENDICES

## List of Appendices

Appendix 1: Statistics table for results of chapter 2 .....	135
Appendix 2: Pot species arrangement in chapter 2 .....	138
Appendix 3: Average hourly temperature in May and June in Durham, 2010-2019 .....	139
Appendix 4: Pot species arrangement in chapter 3 .....	140
Appendix 5: Statistics table for results of chapter 3 .....	141
Appendix 6: Comparison of fungal primers .....	143
Appendix 7: Gradient PCR .....	144
Appendix 8: Statistics table for results of chapter 3 .....	145

**Appendix 1:** Statistics table for results of chapter 2

Variable	ANOVA Results				Tukey HSD Results			
	Species/Week	F	df	<i>p</i>	Pair	<i>p</i>	Lower CI	Upper CI
Total biomass	All	35.66	6	<0.001	1 - 2	<0.001	2.11	-1.39
					3 - 2	0.001	-1.11	-0.38
					6 - 2	<0.001	-1.54	-0.81
					1 - 4	<0.001	-2.19	-1.47
					3 - 5	0.002	-1.09	-0.37
					6 - 5	<0.001	-1.52	-0.80
					7 - 6	<0.001	1.10	1.83
Aboveground biomass	<i>I. glandulifera</i>	11.46	6	<0.001	1 - 2	<0.001	-2.03	-0.74
					6 - 2	<0.001	-1.85	-0.56
					1 - 4	<0.001	-2.41	-1.11
					3 - 5	0.018	-1.43	-0.14
					7 - 5	<0.001	-2.23	-0.94
					7 - 6	<0.001	1.11	2.40
	<i>C. angustifolium</i>	5.454	6	<0.001	1 - 2	<0.001	-2.03	-0.74
					6 - 2	<0.001	-1.85	-0.56
					1 - 4	<0.001	-2.41	-1.11
					6 - 4	<0.001	-2.23	-0.94
					3 - 5	0.018	-1.43	-0.14
					7 - 5	<0.001	-2.03	-0.74
	<i>R. obtusifolius</i>	20.17	6	<0.001	1 - 2	<0.001	-3.48	-2.17
					6 - 2	0.001	-1.74	-0.43
					1 - 4	<0.001	-3.49	-2.19
					6 - 4	0.001	-1.76	-0.45
					7 - 6	0.002	0.39	1.70
	<i>E. hirsutum</i>	6.095	6	<0.001	1 - 2	<0.001	-2.80	-1.14
					3 - 2	0.017	-1.85	-0.19
					6 - 2	0.026	-1.77	-0.12
					1 - 4	0.003	-2.13	-0.47
					7 - 5	0.044	0.02	1.68
					7 - 6	0.006	0.35	2.01
					1 - 4	0.001	-1.25	-0.35

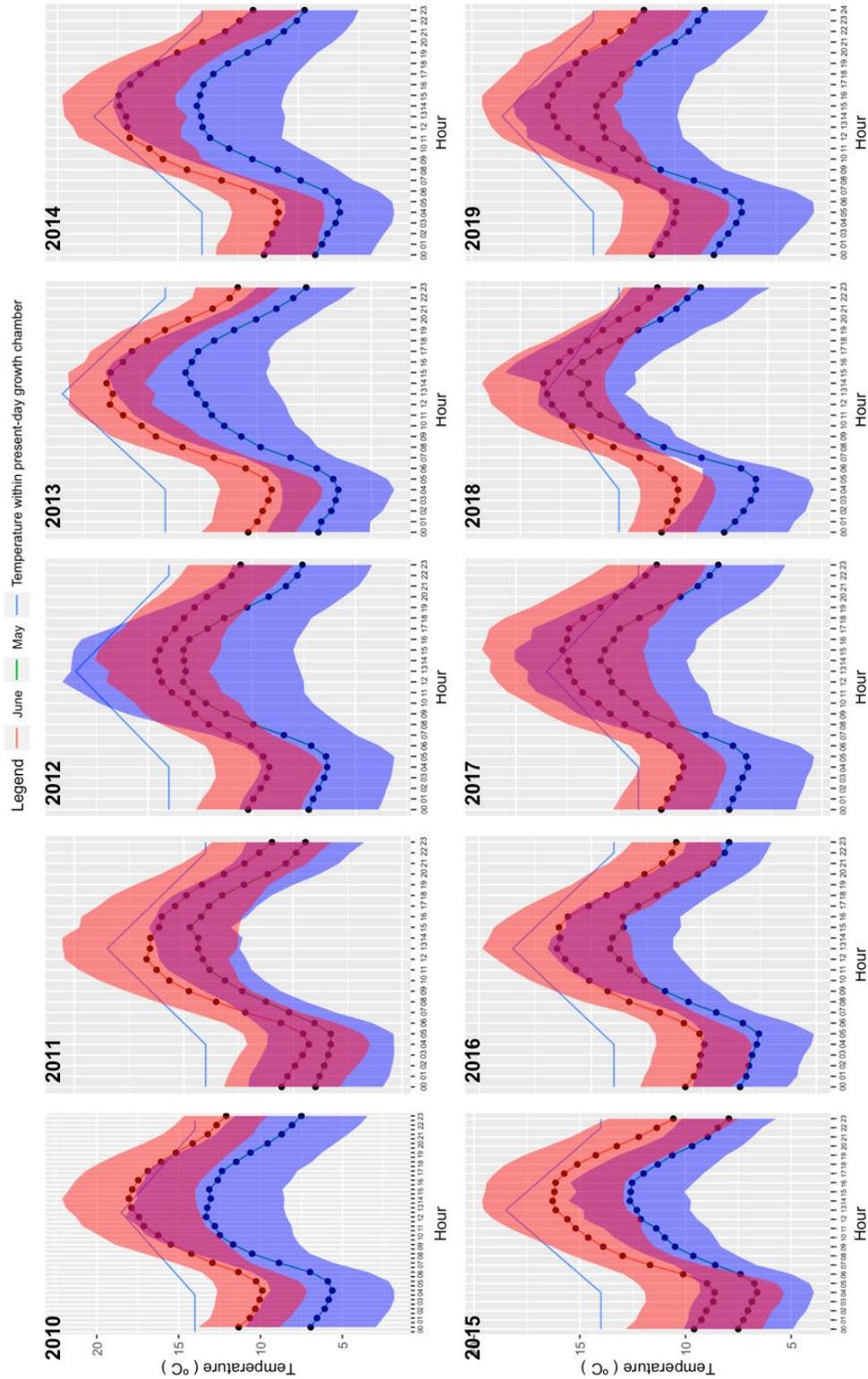
<b>Proportional aboveground biomass</b>	<i>I. glandulifera</i>	0.992	6	0.439	No significant effect				
<b>Height</b>	6	3.469	6	0.005	1 - 2	0.022	-0.79	-0.06	
					6 - 2	0.002	-0.95	-0.23	
					1 - 4	0.022	-0.79	-0.06	
					6 - 4	0.002	-0.95	-0.23	
					7 - 6	0.016	0.09	0.81	
	9	17.39	6	<0.001	1 - 2	<0.001	-1.12	-0.52	
					3 - 2	0.005	-0.74	-0.14	
					6 - 2	<0.001	-1.18	-0.58	
					1 - 4	<0.001	-1.19	-0.59	
					6 - 4	<0.001	-1.25	-0.65	
					3 - 5	0.003	-0.76	-0.16	
					7 - 6	<0.001	0.75	1.35	
	12	19.15	6	<0.001	1 - 2	<0.001	-1.20	-0.59	
					3 - 2	0.001	-0.84	-0.23	
					6 - 2	<0.001	-1.15	-0.54	
					1 - 4	<0.001	-1.24	-0.63	
					6 - 4	<0.001	-1.19	-0.58	
					3 - 5	<0.001	-0.92	-0.31	
					7 - 6	<0.001	0.78	1.39	
	<b>Soil volume water content</b>	2	19.65	6	<0.001	1 - 2	<0.001	0.13	0.28
						3 - 2	<0.001	-0.24	-0.09
7 - 2						0.027	-0.15	-0.01	
1 - 4						<0.001	0.15	0.30	
3 - 5						0.001	-0.20	-0.05	
7 - 5						0.249	-0.11	0.03	
7 - 6						0.015	-0.16	-0.02	
5		44.54	6	<0.001	1 - 2	0.001	0.12	0.44	
					3 - 2	<0.001	-0.99	-0.66	
					6 - 2	0.019	0.03	0.35	
					7 - 2	<0.001	-0.46	-0.13	
					1 - 4	0.009	0.06	0.38	
					6 - 4	0.107	-0.03	0.29	
					3 - 5	<0.001	-1.07	-0.75	
7 - 5	<0.001	-0.54	-0.22						

					7 - 6	<0.001	-0.65	-0.33
					1 - 2	<0.001	0.37	0.88
					3 - 2	<0.001	-1.19	-0.68
					5 - 2	0.019	-0.56	-0.05
					6 - 2	0.038	0.02	0.53
	11	29.43	6	<0.001	1 - 4	<0.001	0.37	0.88
					6 - 4	0.038	0.02	0.53
					3 - 5	<0.001	-0.89	-0.38
					7 - 6	<0.001	-0.75	-0.24

**Appendix 2:** *Pot species arrangement in chapter 2 at the start and end of the experiment. I. glandulifera in the centre.*



**Appendix 3:** Average hourly temperature in May and June in Durham, UK, modelled using the R package 'ggplot'. Growth chamber temperatures represented by the solid blue line.



**Appendix 4:** *Pot species arrangement in chapter 3. I. glandulifera in the centre.*

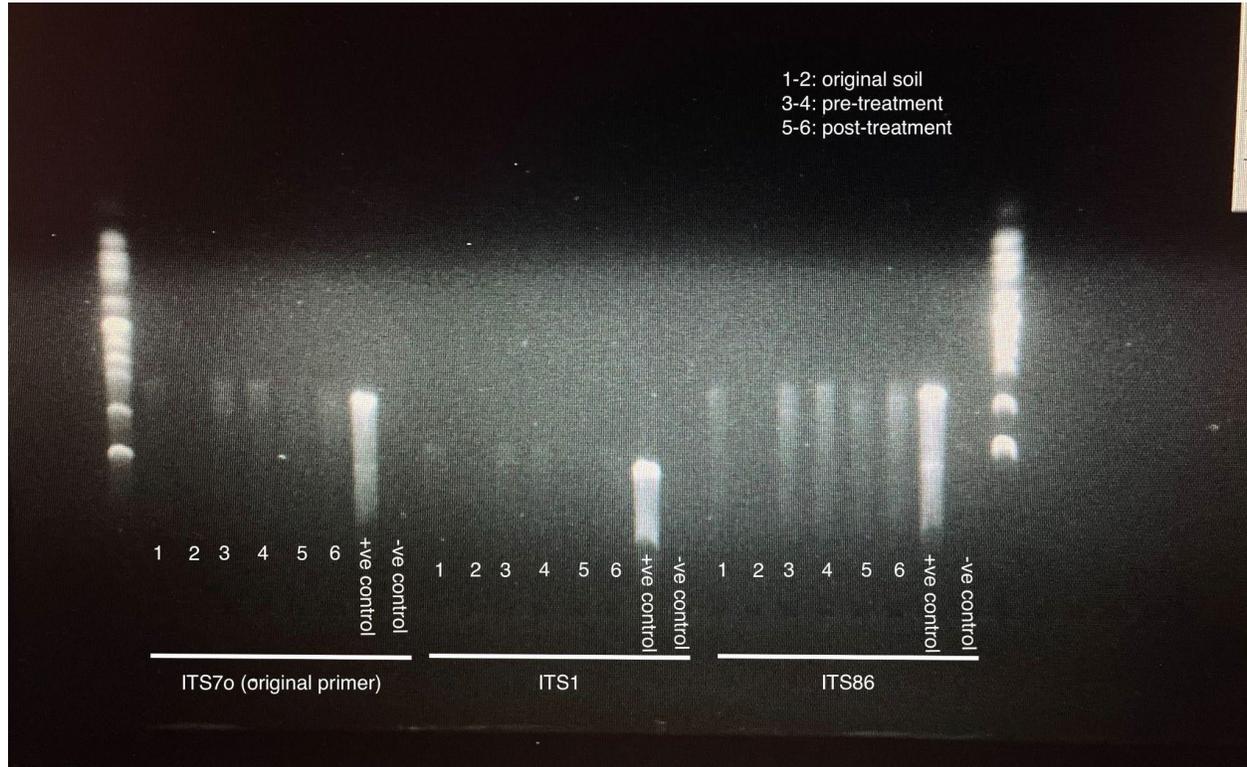


**Appendix 5:** Statistics table for results of chapter 3

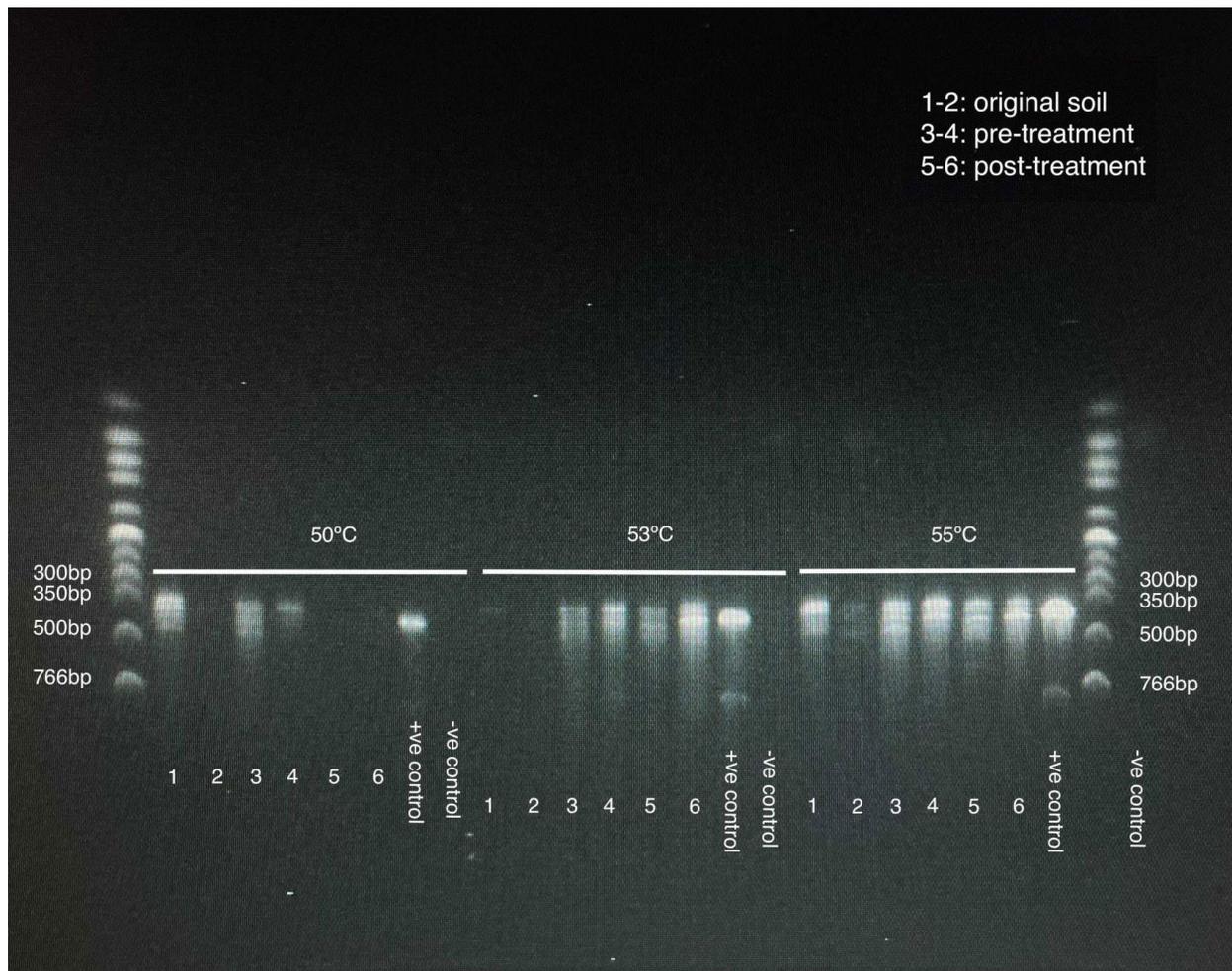
Variable	Nutrient/ Species/Week	ANOVA Results				Tukey HSD Results			
		Factor	F	df	p	Pair	p	Lower CI	Upper CI
Soil nutrient content	Sulphate concentration	Climate				F - P			
		Origin	3.852	2	0.354	I - C			
						X - C			
						X - I			
	Climate * Origin	3.731	2	0.388	PX - PI	0.017	-1.64	-0.12	
	Chloride concentration	Climate				F - P			
	Nitrite concentration	Climate				F - P			
	Nitrate concentration	Climate				F - P			
		Origin	47.05	2	<0.001	I - C	0.221	-1.75	-0.12
						X - C	<0.001	1.18	2.81
X - I	<0.001	2.12	3.75						
Total biomass	<i>I. glandulifera</i>	Climate	4.959	1	0.041	F - P	0.041	0.01	0.16
	<i>S. dioica</i>	Climate				F - P			
		Origin	5.85	1	0.03	I - C	0.03	-1.03	-0.06
	<i>E. hirsutum</i>	Climate	5.054	1	0.041	F - P	0.041	-1.20	-0.03
	<i>J. vulgaris</i>	Climate				F - P			
Proportional total biomass	<i>I. glandulifera</i>	Climate				F - P			
		Origin	5.512	1	0.032	I - C	0.035	0.05	1.11
Aboveground biomass	<i>I. glandulifera</i>	Climate				F - P			
		Origin	4.143	2	0.022	I - C			
						X - C	0.014	0.05	0.50
						X - I			
	<i>S. dioica</i>	Climate				F - P			
		Origin	9.823	2	<0.001	I - C	<0.001	-1.80	-0.42
						X - C			
	X - I	0.002	0.34	1.80					
<i>E. hirsutum</i>	Climate				F - P				
<i>J. vulgaris</i>	Climate				F - P				

		Origin	8.657	2	<0.001	I - C			
						X - C	<0.001	-1.95	-0.51
						X - I			
<b>Proportional aboveground biomass</b>	<i>I. glandulifera</i>	Climate				F - P			
		Origin	10.46	2	<0.001	I - C	<0.001	0.58	1.90
						X - C			
X - I									
<b>Belowground biomass</b>	All	Climate				F - P			
<b>Belowground biomass</b>	Natives as a whole	Climate				F - P			
		Origin	5.115	1	0.038	I - C	0.038	-1.01	-0.03
<b>Proportional belowground biomass</b>	<i>I. glandulifera</i>	Climate				F - P			
<b>Root mass fraction</b>	All	Climate				F - P			
<b>Height</b>	<i>I. glandulifera</i>	Climate	13.43	1	<0.001	F - P	<0.001	0.14	0.48
	<i>S. dioica</i>	Climate				F - P			
		Origin	12.53	2	<0.001	I - C			
						X - C			
						X - I			
	Climate *	3.548	2	0.036					
	<i>E. hirsutum</i>	Climate				F - P			
	<i>J. vulgaris</i>	Climate				F - P			
		Origin	4.448	2	0.018	I - C			
						X - C	0.013	-0.10	-0.01
X - I									
<b>Soil volume water content</b>	Week 5	Climate	31.35	1	<0.001	F - P	<0.001	-0.51	-0.24
	Week 7	Climate	22.83	1	<0.001	F - P	<0.001	-1.66	-0.68
	Week 9	Climate	44.61	1	<0.001	F - P	<0.001	-1.79	-0.96
		Origin	4.686	2	0.014	I - C	0.016	-1.29	-0.11
						X - C			
X - I									

**Appendix 6:** Comparison of fungal primers *ITS7o*, *ITS1*, and *ITS86F*, with reverse primer *ITS4*.



**Appendix 7:** Results of a gradient PCR using the ITS86F/ITS4 primer pair.



**Appendix 8:** Statistics tables for results of chapter 3

Variable	Taxa/Included samples	Kruskal-Wallis Rank Sum Test Results			Dunn Test Results			
		Factor	$\chi^2$	df	<i>p</i>	Pair	<i>p</i>	Difference
Alpha diversity: observed OTUs	16s all samples	Soil condition	24	2	<0.001	original - after	0.008	2.77
						pre - after	<0.001	4.62
						pre - original	1.000	-0.34
	ITS all samples	Soil condition	13.2	2	<0.001	original - after	0.327	1.23
						pre - after	<0.001	3.62
						pre - original	0.691	0.74
	16s post-phase 1	Origin	7.24	2	0.03	control - community	0.090	1.90
						invader - community	0.014	2.60
						invader - control	0.813	0.61
	ITS post-phase 1	Origin	13.5	2	<0.001	control - community	0.000	3.45
						invader - community	0.821	0.60
						invader - control	0.007	-2.86
16s post-phase 1	Climate	3.84	1	0.05	present - future	0.025	1.96	
ITS post-phase 1	Climate	14.7	1	<0.001	present - future	<0.001	3.83	
Alpha diversity: Shannon index	16s all samples	Soil condition	21.2	2	<0.001	original - after	0.013	2.62
						pre - after	<0.001	4.33
						pre - original	1.000	-0.33
	ITS all samples	Soil condition	11.9	2	0.003	original - after	0.862	0.56
						pre - after	<0.001	3.42
						pre - original	0.281	1.20
	16s post-phase 1	Origin	7.24	2	0.03	control - community	0.084	1.91
						invader - community	0.015	2.59
						invader - control	0.852	0.57
	ITS post-phase 1	Origin	14.9	2	<0.001	control - community	<0.001	3.66
						invader - community	0.684	0.75
						invader - control	0.005	-2.92
16s post-phase 1	Climate	3.84	1	0.05	present - future	0.025	1.96	
ITS post-phase 1	Climate	8.09	1	<0.001	present - future	0.002	2.84	

Variable	Taxa/ Origin	PERMANOVA Results				Pairwise PERMANOVA Results		
		Factor	F.model	df	<i>p</i> (>F)	Pair	<i>p</i>	<i>p</i> <sub>adj</sub> (Bonferroni)
Beta diversity	16s all samples	Soil condition	1.101	2	0.001	after - original	0.068	0.10
						after - pre	0.001	0.00
						original - pre	0.891	0.89
	ITS all samples	Soil condition	1.117	2	0.183	after - original	0.465	0.70
						after - pre	0.027	0.08
						original - pre	0.901	0.90
	16s post- phase 1	Climate	1.0502	1	0.031	P - F	0.034	0.034
		Origin	1.038	2	0.027	C - X	0.015	0.05
						C - I	0.092	0.14
						X - I	0.192	0.19
	Climate * Origin	1.0057	2	0.358				
	ITS post- phase 1	Climate	0.995	1	0.416	P - F	0.272	0.272
		Origin	0.701	2	0.977	C - X	0.615	0.99
						C - I	0.989	0.989
X - I						0.938	0.989	
Climate * Origin	0.257	2	0.982					