*The role of soil biota during invasion of Impatiens glandulifera Royle and restoration at invaded sites.*

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# The role of soil biota during invasion of *Impatiens glandulifera* Royle and restoration at invaded sites.

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## Abstract

This thesis focuses on the exotic plant *Impatiens glandulifera*, which has invaded many habitats in the UK, including north-east England. Soil biota are frequently implicated in influencing plant invasions and here this is investigated by examining the soil-mediated impacts of *I. glandulifera* on native plant species. Select native plants, along with *I. glandulifera*, were grown in field-collected soil that had been invaded and not invaded by *I. glandulifera*. Sterilised *versus* unsterilised soil was used to test if any differences detected were mediated by soil microbes. Results showed that the growth of the native plant species was not necessarily negatively affected by growing in soils invaded by *I. glandulifera*. Evidence was also found that *I. glandulifera* may alter mycorrhizal colonisation of a native plant species in invaded soils. A consistent effect of soil origin was also found, which demonstrates the complexity and context-dependency of plant invasions. Findings from plant-soil interaction studies were then applied to the context of native plant restoration at invaded sites; a management approach often side-lined in invasive plant species control. The utility of two soil treatments were tested for *I. glandulifera* control, firstly addition of arbuscular mycorrhizal fungi (AMF), which is a significant contributor to plant biodiversity in natural systems; secondly, additions of activated carbon (AC), which is often used to negate the negative soil-mediated impacts of invasive plant species, through adsorption of allelochemicals. No effect of AMF on plant cover was detected and results suggested that AC may actually increase cover of *I. glandulifera* and thus may not be a suitable restoration tool.

# Table of contents

# Abbreviations and acronyms

| | |
|---|---|
| AARS | Allelopathic advantage against resident species |
| AC | Activated carbon |
| AMF | Arbuscular mycorrhizal fungi |
| df | Degrees of freedom |
| DMH | Degraded mutualisms hypothesis |
| DNA | Deoxyribonucleic acid |
| EICA | Evolution of increased competitive ability |
| EMH | Enhanced mutualisms hypothesis |
| ERCA | Evolutionary reduced competitive ability |
| ERH | Enemy release hypothesis |
| KOH | Potassium hydroxide |
| MG | Mesotrophic grassland |
| N | Nitrogen |
| NVC | National vegetation classification |
| NWH | Novel weapons hypothesis |
| PCR | Polymerase chain reaction |
| PLFA | Phospholipid fatty acid |
| PSF | Plant-soil feedback |
| RGR | Relative growth rate |
| RNA | Ribonucleic acid |

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# Chapter 1:

# Literature Review – The role of soil biota in plant invasions and their management.

## 1.1 INTRODUCTION

Only a small proportion of introduced plant species become naturalised, where their persistence no longer depends on recurring immigration, and then invasive in their non-native range (Richardson *et al.* 2000a). Yet, invasive plants represent a significant threat to native biodiversity in the ecosystems that they invade and thus represent a challenge to conservation (Vitousek *et al*. 1997; Mack *et al*. 2000). Through their impacts on native biodiversity, introduced plant species that become invasive are significant drivers of global environmental change and are an important signature of the recently proposed Anthropocene Epoch (Lewis & Maslin 2015).

Due to anthropogenic erosion of biogeographical barriers, plant invasions are a global phenomenon (Vitousek *et al*. 1997; Manchester & Bullock 2000) and it is estimated that 3.9% of global vascular flora have become naturalised as a consequence of human activity (van Kleunen *et al*. 2015). Plant invaders can impact native communities by becoming locally dominant and reducing resident species richness and diversity, which can alter both ecosystem structure and function (Vila *et al*. 2006; Hejda *et al*. 2009; Powell *et al*. 2011). Additionally, if the introduced species are able to form dense monocultures then competitive interactions among plants can be altered and impacts on native diversity become most severe (Hejda *et al*. 2009). However, the impacts of invasive plant species are difficult to generalise as they are highly context-specific and principally driven by invader identity and the type of ecosystem invaded (Hejda *et al*. 2009), which can complicate biological control efforts that operate on a community-level scale.

In addition to the context-dependency of plant invasions, controlling the consequences of introduced plant species has become particularly challenging because the mechanistic basis underlying invasiveness is often not well understood (van der Putten *et al*. 2007a) and attempts to characterise invasive species have had limited success (Manchester & Bullock 2000; Catford *et al*. 2016). Despite this it is important to identify any ecological factors that may regulate or contribute to plant invasiveness, because this knowledge can then be applied in the context of invasive species management and control.

Despite the lack of consistent mechanistic explanations that underlie the success of invasive plants, many mechanisms at the local plant and community scale fall into two categories, of plant traits and biotic interactions. For example, it has long been suggested that invasive plant species exhibiting higher phenotypic plasticity, the ability to express different phenotypes in different environments (Bradshaw 1965), than their native congeners may contribute to their naturalisation success (Baker 1965; Richards *et al*. 2006; Davidson *et al*. 2011). Plasticity in leaf area and biomass has been demonstrated (Richards *et al*. 2006; Dawson *et al*. 2012), which enable invaders to better capitalise on increases in resource availability in their introduced range. Another important characteristic acknowledged is the high

competitive ability achieved by invaders through having higher shade tolerance, high growth rates and fecundity (Cano *et al*. 2008; Ebeling *et al*. 2008; Dawson *et al*. 2009) in the introduced range. More recently, Oduor *et al*. (2016) have suggested that invaders are able to evolve local adaptations in order to occupy a broader range of habitats and thereby successfully establish in their introduced ranges.

Soil microbial communities can have considerable effects on individual plants and are considered principal regulators of plant community structure (Bever 1994; van der Putten 1997; Heijden *et al*. 1998; Klironomos 2002). These findings, together with the knowledge that invasive plant species in turn can modify the soil microbial community in introduced ranges to better suit their own performance (Belnap & Phillips 2001; Ehrenfeld 2003; Levine *et al*. 2006; Batten *et al*. 2008), has led ecologists to consider the role of plant-soil interactions in plant invasions. In this way the mechanistic basis of plant invasions is being explored by identifying ways in which soil biota may drive and regulate invasive success.

Presently, the most widely considered mechanism by which plant-soil interactions may facilitate invasions stems from the hypothesis that in their introduced ranges, invasive plant species experience decreased regulation by their natural enemies. This enemy release hypothesis (ERH) states that escape from natural enemies in the introduced range may increase the competitive ability of invasive plant species (Keane & Crawley 2002). These enemies may include belowground pathogens and bacteria, as well as aboveground herbivores. Subsequently, it has been shown that invasive plant species do not suffer less negative effects from soil biota than native plant species do (Kulmatiski *et al*. 2008). However, as each invasion event is context-dependent, other mechanisms of how soil biota may contribute to plant invasions have been proposed. These include direct mechanisms that involve an interaction between the introduced plants' roots and soil microbes (Dawson & Schrama 2016). This includes the evolution of increased competitive ability (EICA) hypothesis, which posits that invasive plant species gain a competitive advantage through a reallocation of resources from defence of enemies, to growth (Blossey & Nötzold 1995). Invasive plant species may also gain an advantage from novel mutualisms they may encounter in their non-native range, or by exploiting mutualisms in order to enhance their positive effect (Reinhart & Callaway 2006; Sun & He 2010; Callaway *et al*. 2011). In contrast, indirect mechanisms can involve interactions between the introduced plant and the plant community, through release of chemicals that act as novel weapons to competitors (Callaway & Ridenour 2004).

Currently, few invasive plant control methods utilise findings from plant-soil interactions (Kulmatiski & Beard 2006), yet soil biota consistently exhibit significant impacts on plant communities and so there is great potential to apply findings from plant-soil interactions to biological control (van der Putten *et al*. 2013). It is important, during invasive species control, to have a holistic approach, in which all

components of invasion are considered in a broad ecological perspective. Integrated control of invasive species may become increasingly important in the context of global change, as larger suitable areas of climatic space become available and are colonised. This review will evaluate the most prominent mechanisms by which soil biota can and is influencing plant invasions and highlight the potential ways in which soil biota can be used as a restoration tool for plant-invaded ecosystems.

## 1.2 THE ROLE OF SOIL BIOTA IN PLANT COMMUNITIES

### 1.2.1 Plant-soil feedbacks

The majority of plant invasion studies have focused on aboveground mechanisms (Levine *et al*. 2003), yet much of the biodiversity of terrestrial ecosystems is belowground (Torsvik *et al*. 1990). It is therefore not surprising that soil organisms are considered one of the main drivers of a number of ecological processes. Soil biota, including bacteria, fungi and microarthropods constitute soil food webs and contribute to nutrient cycling (Balestrini *et al.* 2015). Thus soil biota play an important role in the regulation of plant community composition and function, plant diversity and abundance, as well as in plant competitive interactions (van der Putten *et al*. 1993; Bever 1994; Klironomos 2002). Individual plants may utilise resources in different ways, creating unique plant-soil interactions and differentially accumulating structurally and functionally unique soil microbial communities in their rhizosphere (Zak *et al*. 2003; Johnson *et al*. 2004).

Soil microbes may drive diversity among plant communities through feedbacks created during plant-soil interactions. Dynamic plant-soil feedbacks (PSF) result from plant influences on the surrounding soil community, such as organic carbon provisioning, that subsequently affect plant performance through supply of available soil nutrients (Bever *et al*. 1997; Wardle *et al*. 2004). Each plant species differs in the strength of its PSF, where the net effect of these interactions can be positive, neutral or negative. It is the balance between the negative impacts of herbivory and soil-borne pathogens, and the positive impacts of mutualists such as nitrogen-fixing bacteria and mycorrhizal fungi, which determines feedback strength and whether the feedback will have a positive impact on plant performance (Bever *et al*. 1997; Bever 2002). Negative PSFs have been revealed as major facilitators of plant community diversity, through density-dependent control (van der Putten *et al*. 1993; Packer & Clay 2000; Klironomos 2002; Reinhart *et al*. 2003). Negative PSFs predominate in natural systems and constrain individual plant performance in soil occupied previously by the same species (Kulmatiski *et al*. 2008). This aids species turnover and consequently plant biodiversity, species coexistence and maintenance of high species diversity. Positive PSFs in comparison reduce diversity through increasing plant abundance of the benefitting species and facilitating dominance by a single or a few plant species (Klironomos 2002; Bever 2003).

### 1.2.2 Plant-soil feedbacks in plant invasions

PSFs have been widely tested and a wealth of evidence has reported the dynamic feedbacks demonstrated between plants and their associated soil biota (van der Putten *et al*. 1993; Bever 2003). For example, the complex competitive dynamics between *Brassica nigra* genotypes and their competitors was shown to be governed by local soil biota, principally by arbuscular mycorrhizal fungi (Lankau *et al.* 2011). The soil feedback approach has shifted the focus of invasion ecologists to

incorporate whole community-level processes in plant-soil interactions, rather than focusing on individual components (Bever *et al*. 1997). Additionally, literature is increasingly highlighting the relative importance of invasive plant species' responses to soil biota in predicting the invasive potential of some plant species (Reinhart & Callaway 2006; van der Putten *et al*. 2007a).

Species-specific plant-soil interactions are most often measured using a standard pot-based approach, which typically have two phases. In phase I, field-collected soil samples are cultivated by known plant species in order to condition the soil uniquely for that species (Kulmatiski & Kardol 2008; Dawson & Schrama 2016). Once a species-specific microbial community is assumed to have built up, known plant species are used as phytometers during phase II and grown in soil conditioned by themselves or by another species. Alternatively, plants are grown in sterilised or unsterilised 'home' soil. During a set period, plant growth is then measured, usually by harvesting aboveground biomass, producing a measure of the net effects of the soil biota (Dawson & Schrama 2016). A positive PSF is realised if plant productivity is higher in self-conditioned soil than soil conditioned by another species or when plant growth is higher in sterilised, compared to unsterilised soil. In contrast, a negative PSF is realised if the plant grows better in soil conditioned by another plant species. Cultivation of field soils during phase I is often time-limiting, so soils with known history and origin can also be used as the phase I soil (Kulmatiski & Kardol 2008). Using this approach may add more realism to the PSF study but the collected soils will also reflect the site conditions, which could cause variation in PSF that is not attributed to previous plant conditioning of the soil. To account for this, field soils can also be collected and used as inocula in a whole soil background (Kulmatiski & Kardol 2008).

Invasive plant species can preferentially alter the composition of the soil biota and reduce the abundance and diversity of particular soil microbial groups, such as ammonia-oxidising bacteria (Hawkes *et al*. 2005). This perturbation of stable soil microbial communities has been shown to negatively affect fundamental ecosystem processes, such as organic matter decomposition and nutrient cycling (Ehrenfeld 2003; Liao *et al*. 2008). Changes to the soil microbial community that are of large magnitude are expected to create greater feedbacks that may facilitate invasion of an exotic species and prevent native plant establishment (Kourtev *et al*. 2002; Batten *et al*. 2008; Scharfy *et al*. 2010). For example, the exotic grass *Bromus tectorum*, a western US invader, significantly decreases the species richness of microarthopod, fungi and nematode communities in the soil (Belnap *et al*. 2005) facilitating its own dominance. These belowground alterations induced by invasive plant species are highly variable creating species-specific measurable soil legacies (Elgersma *et al*. 2011).

The advent of advanced molecular techniques has allowed changes to soil microbial communities by plant invaders and their effects on PSFs to be quantified (Vandenkoornhuyse *et al*. 2002; Corbin & D'Antonio 2011). Phospholipid fatty acid (PLFA) analysis is a commonly used method to assess the

structural diversity of soil microbial communities as it gives an approximate measure of the abundances of different soil microbial groups (Bossio & Scow 1998; Kourtev *et al*. 2002). Specific groups of soil microbes produce unique fatty acids that can be extracted from soil samples and analysis of these groups can provide a fingerprint of the structure of soil communities (Wolfe & Klironomos 2005). The whole soil microbial community can be considered before and after an invasion event and PLFA can be used to provide a broad but sensitive indication of any shifts in community composition, particularly in relation to fungi and bacteria (Zelles 1999; Batten *et al*. 2008). Studies utilising PLFA analysis have correlated plant invasions with changes in soil microbial community structure (Kourtev *et al.* 2002; Stefanowicz *et al.* 2016). PLFA analyses can also be combined with substrate utilisation profiles, which provide a measure of soil microbial community function (Kourtev *et al*. 2002, 2003). PLFA analysis revealed a rapid shift towards a bacterial dominated community by the invasion of Japanese barberry (*Berberis thunbergii*) and Japanese silt grass (*Microstegum vimineum*) in North-eastern USA (Kourtev *et al.* 2003). Importantly when changes in the ratio of fatty acids in the soils was complemented with enzyme and substrate-induced respiration profiles, a decreased phosphorus availability was detected, along with a higher nitrification rate and pH. Thus, extending anal yses of invading exotics to belowground has revealed complex legacy effects and rapid long-term changes that may promote re-invasion. Other studies have also used PLFA to demonstrate the importance of changes to microbial enzymatic activity in plant invasions, such as facilitating invasion of *Eupatorium adenophorum* (Sun *et al*. 2013).

The use of molecular analyses in plant invasion studies has highlighted that invasive plant management should also consider alterations to belowground processes and functioning (Elgersma *et al*. 2011). Invader-induced changes in soil microbial structure and function have been shown to be largely determined by previous vegetation type (Elgersma *et al.* 2011). This indicates that the legacy effects, revealed by PLFA analysis, may contribute to re-invasion in the introduced range. In addition, analysis of changes in PLFAs, as a result of Japanese barberry invasion have revealed that these changes are not always proportional to invasion density, as previously thought. This has implications for management of invasive species, as keeping exotic plants at low densities may still have extensive ecosystem-level effects because through PSFs, processes such as carbon and soil organic matter storage can be altered by even a low density of invaders (Elgersma & Ehrenfeld 2011). Recently, PLFA analysis has been used to demonstrate for the first time that the effects of *Impatiens glandulifera* invasion, along with a greater mass of bacterial fatty acids, are not limited to the soil microbial community, but also foliar endophytes (Pattison *et al.* 2016). This demonstrates the utility of molecular analysis in creating a whole community approach to plant invasions.

PLFA analysis is generally regarded as the most powerful method used to detect changes in the structure of soil microbial communities (Ramsey *et al*. 2006) and attempts have been made to increase throughput

(Buyer & Sasser 2012). However, PLFA can only reveal broad shifts in microbial community composition and cannot resolve species-level changes (Ramsey *et al*. 2006). There is also difficulty proving a single biomarker is truly universal to a specific type of microbe, for example some common PLFAs can be found in both bacteria and arbuscular mycorrhizal fungi (Frostegard *et al*. 2011). PCR-based methods, such as terminal restriction fragment length polymorphism are of finer resolution and can provide population-level resolution (Ramsey *et al*. 2006). The advent of next-generation sequencing methods such as 454-pyrosequencing will allow assessment of more specific soil microbial legacy effects of invasions (Dawson & Schrama 2016).

Alterations to native soil microbial communities can create positive feedback effects for invasive plant species, creating less favourable microbial communities for native plant species (Levine *et al*. 2006). As a consequence of these soil microbial changes, invasive plant species have been shown to benefit from positive PSFs in their invaded range, in contrast to the negative feedbacks experienced by co-occurring native species (Belnap & Phillips 2001; Klironomos 2002; MacDougall *et al*. 2011; van der Putten *et al*. 2013). For example, the invaders *Centaurea maculosa* (Reinhart & Callaway 2006), *Chromolaena odorata* (Te Beest *et al*. 2009) and *I. glandulifera* (Pattison *et al*. 2016) have been shown to exhibit beneficial positive PSFs on themselves in comparison to native plant species.

The dissimilarities in the strength and direction of PSFs between exotic and native plant species has been employed to explain the facilitation of plant invasions (Klironomos 2002; Callaway *et al*. 2004a; Reinhart & Callaway 2004; Kulmatiski *et al*. 2008). Moreover, native plant species exhibit a greater variation in responsiveness to soil biota in comparison to invaders (Bennett & Strauss 2013). Invaders that respond less to soil biota in their introduced range may thus experience reduced regulation by soil pathogens, less negative feedback effects and increased performance. Native species may also produce types of PSF that benefit other species more than themselves, whereas invaders have been shown to produce feedback effects that do not affect other species in the community (Perkins & Nowak 2013). The asymmetry in the PSF effects experienced by non-natives provides them with a competitive advantage over native species, thereby in theory promoting their dominance in the invaded community. For example, the invasive grass *Aegilops triuncialis* showed no significant change in performance when grown in soils invaded by conspecifics and in soils invaded by the native plant community. This suggests that the species' alteration of the soil microbial community may contribute to a positive PSF (Batten *et al*. 2008). In contrast, native plant performance in *A. triuncialis*–invaded soil was significantly reduced, through decreased aboveground biomass and increased root mass ratio, which is suggestive of greater competition for soil nutrients (Batten *et al*. 2008). By altering the soil microbial community, non-native species can facilitate their invasion by translating their positive PSF effects into competitive effects that negatively impact native plant species.

Pot-based studies used to examine PSFs have become a useful tool to analyse the plant-soil interactions of invasive plant species at a community level. However, there is a bias towards simplified greenhouse studies, with the majority of studies focusing on grassland species (Kulmatiski & Kardol 2008). There is also considerable variation in the conclusions drawn from PSF studies, mainly due to the lack of consistency in the experimental methods that are used (Brinkman *et al*. 2010; Müller *et al*. 2016). A meta-analysis by (Brinkman *et al*. 2010) showed that measures used to calculate PSF values were more variable in positive than negative feedback values, suggesting some positive feedback values recorded could be inflated.

The use of small pots when assessing plant performance during PSF studies may affect the experimental outcome by magnifying the effects of competition relative to PSF effects, so called 'pot limitation' (Poorter *et al*. 2012). In some cases, the effect of soil biota may be overridden by competition for root space, thus any PSF effects cannot be partitioned from those of competition. For studies that compare plant performance in sterilised *versus* unsterilised soils, it is difficult to determine to what extent an increase in performance is enhanced by the absence of soil pathogens, or by the enhanced nutrient uptake in sterilised soils, which is caused by a flush of nutrients from dead soil microbes (Troelstra *et al*. 2001; McNamara *et al*. 2003). Experiments using sterilised soils thus often yield larger feedback values than those using conditioned soils (Brinkman *et al*. 2010). The choice of soil sterilisation method, such as gamma irradiation or autoclaving, has also been shown to differentially affect soil structure (Berns *et al*. 2008). The alteration of soil aggregation state produced by soil sterilisation should be taken into consideration, as this can affect soil microbial function. A combined approach that uses home and away soils alongside sterilised and unsterilised soils may be more appropriate in PSF studies (Abhilasha *et al*. 2008; Brinkman *et al*. 2010; Yuan *et al*. 2014; Del Fabbro & Prati 2015b).

More recent PSF studies have attempted to create more realistic soil-microbiome controls, for example, Müller *et al*. (2016) used common grassland species in phase I to condition the soil, instead of using a sterilised soil control. Methodological limitations also arise when using experimentally cultivated soils in PSF studies, which produce more negative effect sizes than studies using field-collected soils (Kulmatiski *et al*. 2008). The majority of PSF studies contain two phases, (Kulmatiski & Kardol 2008), but PSF experiments that include a third phase can be more informative, identifying the potential microbial mechanisms behind an observed PSF. In Phase III, the conditions that are associated with changes in plant growth are identified and then recreated (Bever 1994; Klironomos 2002; Kardol *et al*. 2007). A recent study demonstrated that PSFs found under glasshouse conditions are unlikely to be reflected in field conditions (Schittko *et al*. 2016). However, PSF effects are highly context-dependent and can change over short time periods (Hawkes *et al*. 2013). Moreover, the direction and strength of the feedback effects measured have been shown to depend on and vary greatly with the experimental approach used (Brinkman *et al*. 2010), highlighting the need for more continuity in PSF studies. Despite

the limitations of PSF studies and the varied conclusions that can be drawn from them, it is recognised that each study differs in its individual aims. Thus, some experimental approaches will be more suitable for use than others (Brinkman *et al*. 2010).

Pot-based PSF studies have been strengthened by more comprehensive biogeographical studies, which attempt to examine differences between PSFs of invasive plant species in their introduced and native ranges (Reinhart *et al*. 2003; Reinhart & Callaway 2004; Callaway *et al*. 2011). In addition to showing more positive PSFs in conspecific-conditioned soils, exotic plant species have been shown to experience more negative PSFs in their native ranges, compared to their invaded ranges, which may be attributed to release from belowground enemies (Callaway *et al*. 2004; Reinhart & Callaway 2006; Kulmatiski *et al*. 2008; Zuppinger-Dingley *et al*. 2011; Yang *et al*. 2013). It is suggested that the beneficial effects of soil biota in the invaded range accumulate as different soil biota are encountered in the novel range (Bever 2002; Reinhart & Callaway 2006). For example, Reinhart *et al*. (2003) showed that black cherry (*Prunus serotina*) experiences negative feedbacks in its native North American range, which has detrimental effects on plant performance but encourages interspecific competition. In contrast, in its invasive European range, black cherry exhibits more positive PSF effects facilitating its performance and thus dominance.

Few PSF studies currently utilise a biogeographical approach and compare the strength of feedbacks in native and introduced ranges (Hierro *et al*. 2005). Maron *et al*. (2014) showed that four of six exotic plant species studied exhibited more negative feedbacks when grown in native range soils, compared to when they were grown in soils from their introduced ranges. The difference in PSFs realised may explain why exotic plant species are able to achieve higher densities in their introduced ranges (Parker *et al*. 2013). Encountering a new suite of soil biota may change the selection environment of the introduced species. For example, change of biomass allocation patterns and resultant increases in competitive ability in *C. odorata* (te Beest *et al*. 2009). Presently, it is difficult to predict variation in the success of invasive plant species (Colautti *et al*. 2004; Hierro *et al*. 2005). Utilising comparative biogeographical approaches to PSF studies may increase understanding of the invasiveness of some exotic plant species and aid in their control (Kulmatiski *et al*. 2008).

## 1.3 THE PROPOSED MECHANISMS OF PLANT INVASIONS

It is generally considered that exotic plant species exhibit increased fitness, superior competitive ability and more positive PSFs in their introduced ranges, but the exact role of soil microbes in driving these processes is still contested. The plethora of research and evidence demonstrating the extensive impact exotic plant species can have on soil microbial communities has generated an array of mechanisms proposed to link the role of soil biota to exotic plant success. Plant-soil interactions have also been suggested to play a stronger role in invasive plant success that is currently acknowledged by most invasion theories (Catford *et al*. 2009). Mechanisms that can explain the role of soil microbes in invasions have recently been organised into direct and indirect routes (Bardgett & Wardle 2003; Dawson & Schrama 2016). Direct mechanisms include invasive plant interactions with specific microbes, such as arbuscular mycorrhizal fungi (AMF), and indirect mechanisms include invasive plant interactions with the plant community, via soil microbes and chemicals (Dawson & Schrama 2016).

### 1.3.1 Enemy release

Presently, one of the most popular and frequently tested hypotheses postulating how plant-soil interactions may facilitate invasions is the enemy release hypothesis (ERH), the idea that invasive plant species are not in a state of equilibrium in their introduced range (Hierro *et al*. 2005). The ERH states that exotic plant species 'escape' their specialist enemies, benefiting from reduced enemy regulation (Maron & Vila 2001; Keane & Crawley 2002; Mitchell & Power 2003), thus gaining a competitive advantage, allowing for an increased population growth. This hypothesis builds on the extensive PSF evidence demonstrating that native plant species suffer more negative effects of soil biota than invasive species do (Klironomos 2002; Kulmatiski *et al*. 2008) and that natural enemies are key regulators for plant populations, these enemies may include above-ground herbivores, pathogens and bacteria. Once introduced into their new range, exotic plant species lose co-evolved relationships with their specialist enemies, such that they experience a reduction in enemy attack and enemy diversity in comparison to their native ranges (Mitchell & Power 2003; Agrawal *et al*. 2005). Thus, the ERH assumes that specialist enemies will be absent in the novel range and that generalist enemies will have a greater impact on native congeners than exotic species because they have coevolved (Keane & Crawley 2002). This reduced enemy damage may translate into increased performance and fitness (Maron & Vila 2001). Invading species may also benefit from the presence of beneficial symbionts, such as AMF, in their introduced ranges, which provides a double competitive advantage against native plant species, if the mutualists preferentially benefit invasive species, alongside decreased enemy regulation (Knevel *et al*. 2004).

Results from tests of the ERH are inconsistent. Studies that have provided support for the ERH have traditionally measured insect herbivore richness, or have measured herbivory, showing reduced rates of

herbivory on introduced plant species, compared with native congeners, which can also be correlated with invasiveness (Agrawal *et al*. 2005; van der Putten *et al*. 2005). This can explain the increased abundance and biomass invasive species achieve in their introduced range, as they are not as regulated as native species (Liu & Stiling 2006). However, this reduction is skewed mainly towards specialist herbivores (Liu & Stiling 2006). More robust worldwide biogeographical comparisons have considered the link between increases in plant performance with natural enemy damage (Chun *et al*. 2010). For example, the natural arthropod enemy damage suffered by the invasive plant *I. glandulifera* in its native Indian Himalayan range, has been shown to significantly affect reproductive units, measured as the sum of the number of seeds, seed capsules and flowers of each plant, but not in the introduced range (Tanner *et al*. 2014).

Other studies lending support to the ERH have focused on soil-borne enemies, particularly soil pathogens (Reinhart *et al*. 2005). Typically, these studies have taken a PSF approach, demonstrating reduced invader performance in live *versus* sterilised native range soils (Reinhart & Callaway 2004; Reinhart *et al*. 2005; Maron *et al*. 2014). These studies often demonstrate that native soils have a stronger suppressive potential, compared to soils from the introduced range. For example, invasive *Prunus serotina* (black cherry) tree densities are regulated in their native range (eastern USA) range by oomycete pathogens of the genus *Pythium*. Both soil sterilisation and fungicide addition increased black cherry seedling survival (Reinhart *et al*. 2005), suggestive of enemy release. Pathogenicity analysis has shown that whilst black cherry may still encounter *Pythium* oomycetes in its introduced European range, the taxa that it encounters are much less virulent (Reinhart *et al*. 2010), indicating escape from the potentially most harmful enemies.

There appears to be as much evidence against the ERH as there is supporting it (Heger & Jeschke 2014). Further ERH community studies have demonstrated partial release, or release with weak beneficial effects to the invader (Beckstead & Parker 2003), and even no release at all. The performance of the invasive shrub *Centaurea odorata* did not differ when grown in soil from its native and non-native range (te Beest *et al*. 2009). Even multi-species studies have shown that enemy damage incurred by 12 exotic species and their native congeners in the Czech republic was of similar diversity and intensity, indicative of no escape from natural enemies (Dostál *et al.* 2013). Meta-analyses have reported inconsistent evidence for enemy release among invasive plant species, suggesting that even when enemy release occurs, it may not result in enhanced plant performance (Chun *et al*. 2010). Colautti *et al*. (2004) argue that in some studies, the ERH may have been uncritically accepted, without due consideration of other effects. Comparative ERH studies at the biogeographical scale generally do depict invader release from natural enemies and are often based on richness or diversity of enemy species on two different continents. However, this method for measuring release may be confounded by bias in research and sampling effort (Mitchell & Power 2003). Additionally, it is argued that without

knowledge of the net effect of enemies on their host, enemy richness measurements can only be considered evidence for an enemy reduction and not a release (Colautti *et al*. 2004).

Recently the complexity and variation of enemy release has been recognised, in order to account for the vast variation in the results from ERH studies. These extensions primarily suggest that enemy release is a dynamic process. As an invasive plant species becomes more established in its introduced range, it is expected to encounter new soil communities and thus accumulate new enemies, such as fungal pathogens, which have negative effects (Stricker *et al*. 2016). Thus the initial beneficial effects of enemy release, which allowed for initial colonisation and establishment in the introduced range, are expected to attenuate over time (Hawkes 2007; Mitchell *et al*. 2010). Negative PSF effects have been correlated with increased residence time of the invader and also with larger invader range sizes (Mitchell & Power 2003; Diez *et al*. 2010; Schultheis *et al*. 2015). Additionally, older invasive populations of *I. glandulifera* have been recently shown to suffer from higher herbivore attack rates than newly established invasive populations (Gruntman *et al*. 2017), confirming that enemy release is indeed dynamic and can deteriorate over time but may be evolutionarily recovered.

There is no doubt that some invasive plant species may experience a reduction in the enemies that they encounter in their introduced range, but this may not always be a complete release from enemies. However, there is no coherent evidence that enemy release is a general mechanism that contributes to plant invasiveness (Schultheis *et al*. 2015). A dynamic enemy release process, on the other hand, could contribute to explaining the divergent and context-dependent results of ERH studies, as residence time and range size may not always be taken into account. It has therefore become apparent that the broad interpretation of the ERH may provide little utility in explaining plant invasiveness. Instead, subdividing and differentiating the ERH into more precise concepts may allow for more rigorous empirical tests that can be more easily applied to understanding and predicting the effects of plant invasions (Colautti *et al*. 2004; Heger & Jeschke 2014).

### 1.3.2 Evolution of increased competitive ability

Many invasive plants exhibit increased growth, densities, larger size and thus in theory greater competitive ability in their introduced ranges (Jakobs *et al*. 2004; Ridenour *et al*. 2008; Vilà *et al*. 2011). Based on these observations, the ERH has been extended to the evolution of increased competitive ability (EICA) hypothesis. Developed in the context of allocation trade-offs, this hypothesis posits that exotic species that experience a release from their enemies, consequently evolving reduced allocation to defence traits, such as defence chemicals. Thus, exotic species in their introduced range experience a shift in selection for a greater allocation of resources to growth and reproduction (Blossey & Nötzold

1995). These genotypes have a competitive advantage over native plant species and it is this increase in competitive ability that is suggested to contribute to the success of exotic plant species.

Studies providing support for EICA demonstrate changes in plant allocation patterns in response to soil biota from the introduced range (Barney *et al*. 2009; te Beest *et al*. 2009) and post-invasion genetic differences, where invasive genotypes are largely more poorly-defended from herbivory (Siemann & Rogers 2001, 2003). Feng *et al*. (2011) have subsequently associated the observed shift in nitrogen allocation from cell walls (defence) to photosynthesis (growth), with a distinct energy use strategy for *Ageratina adenophora* in its introduced range. This has shed light on a possible mechanism for EICA and the resulting observations of increased vigour of exotic plant species in their introduced ranges.

Conflicting results from the tests of the EICA hypothesis and its predictions have found that in some instances, invasive-range populations of exotic species are not better competitors than native-range populations because they do not grow tallest (van Kleunen & Schmid 2003; Bossdorf *et al*. 2004; Vilà *et al.* 2010). Similarly, invasive-range populations have also exhibited similar or greater herbivore defence, as well as greater tolerance to herbivory (Ridenour *et al*. 2008; Huang *et al*. 2010). These refutations of the predictions of the EICA hypothesis have encouraged hypotheses that suggest selection may act against competitive ability if there is less competition in the invaded range (ERCA-Evolutionary Reduced Competitive Ability) (van Kleunen & Schmid 2003; Bossdorf *et al*. 2004). Inconsistent results for EICA highlights the fact that tests of the hypothesis are limited in their assessment of both aspects of the hypothesis; growth and defence (Bossdorf *et al*. 2005). Studies often find support for one aspect of the hypothesis, but not for the other, such as evidence for increased competitive ability of invasive plant species in their introduced range, but not decreased herbivore defence or tolerance (Ridenour *et al*. 2008). Many tests of the EICA are also biased towards extremely competitive invaders and use native plants that are weak competitors (Vila & Weiner 2004). It has subsequently been suggested that studies use more vigorous native species, in order to provide a more comprehensive indication of competitive ability (Joshi *et al*. 2014; Zheng *et al*. 2015a).

Overall current evidence suggests that there is little support for the predictions of the EICA hypothesis, which is complex to test (Felker-Quinn *et al*. 2013). The fact that tests of EICA are supported in some cases only under intraspecific competition suggest that the interactions considered in the EICA hypothesis may be species-specific (Joshi *et al*. 2014), making it difficult to utilise EICA in predicting the invasiveness of plant species. Consequently attempts to refine the EICA hypothesis have been made, which consider the evidence that invaders may not be completely released from their enemies (Colautti *et al.* 2004; Chun *et al*. 2010) and instead show a shift in herbivore communities, from specialists to generalists (Müller-Schärer *et al*. 2004).

### 1.3.3 Allelopathy

A well-studied mechanism frequently employed to explain naturalisation of non-native plant species, is allelopathy (Callaway & Ridenour 2004; Zheng *et al.* 2015b). Historically, allelopathy has been defined broadly as the effect of chemical compounds released into the environment on neighbouring plants (Rice 1984). A more recent definition refers to more specific plant-plant interactions where there is a negative effect of one plant on another, through the release of chemical compounds (Hierro & Callaway 2003). Allelopathy has both defensive and competitive characteristics, which are expressed directly or indirectly. Direct allelopathy occurs when allelochemicals are taken up by the target plant and have been shown to inhibit seed germination and root elongation (Hierro & Callaway 2003; Grove *et al.* 2013). Direct allelopathy can also involve interference with plant physiological processes, including inhibition of photosynthesis, DNA and RNA synthesis, as well as chlorophyll accumulation (Inderjit & Duke 2003). For example, (-)-catechin released by the invasive plant *C. maculosa* triggers a wave of reactive oxygen species at the root meristem of the target plant, ultimately causing root death (Bais *et al.* 2003). Allelopathic plants can also indirectly shift competitive balances between plants, such as by inhibiting soil-borne pathogens (Zhang *et al.* 2009) and by disrupting microbial mutualisms in the soil, such as those with mycorrhizal fungi (Schreiner & Koide 1993; Roberts & Anderson 2001; Stinson *et al.* 2006; Koch *et al.* 2011). The effects of allelopathic plants can vary by species (Hagan *et al.* 2013) but both direct and indirect allelopathy may shift the competitive balance of two interacting plant species, providing the allelopathic plant with an advantage (Callaway & Ridenour 2004). Thus, if an invasive plant species possesses allelopathic ability, it is plausible that this characteristic could facilitate an invasion.

Allelochemicals are released by plants into the soil through various routes, including in root exudates and leaf litter (Inderjit *et al.* 2011a). There is great diversity in the biochemical synthesised and released by allelopathic plants, many of which are species-specific (Hierro and Callaway 2003). As a result, allelochemicals are often complex molecules and their production and resultant avoidance of autotoxicity can be costly for allelopathic plants (Lankau 2008). This may explain why allelopathic compounds frequently have other ecological roles besides plant neighbour suppression, such as facilitation of nutrient uptake (Tharayil *et al.* 2009; Inderjit *et al.* 2011a).

Once allelochemicals are released into the soil, they may retain their bioactivity and persist in soils during the long term, creating a legacy specific to the allelopathic species (Blum 1998; Blum *et al.* 2000; Bossdorf *et al.* 2004). In contrast, different allelochemicals that are released can immediately affect neighbouring plant species, called immediate allelopathy (Del Fabbro & Prati 2015a). In the context of plant invasions, allelopathy legacies may be more important than immediate allelopathy, as legacies may hinder further native plant establishment. However evidence for allelopathic legacies is

limited, with the majority of invasive plant species reported to exhibit immediate allelopathy and lack persistent allelochemicals (Del Fabbro & Prati 2015a).

The varying concentrations measured of certain allelochemicals measured in the field has raised the suggestion that the ultimate effects of allelochemicals on target plants in natural environments may be influenced by the presence of soil microbes (Inderjit 2005; Cipollini *et al.* 2012). Soil microbes may influence allelopathic interactions by determining their magnitude and duration. Soil microbes may limit allelopathic effects in natural environments by degrading allelochemicals, increasing target plant tolerance to allelochemicals or by altering the phytochemical profiles of the allelopathic plants themselves (Cipollini *et al.* 2012).

Much of the reduction in allelopathic effects in the environment is due to microbial degradation of allelochemicals. Evidence for this has been demonstrated in studies that utilise sterilised and non-sterilised soil in growth bioassays (Kaur *et al.* 2009; Lankau 2010; Ehlers 2011). Soil microbes may utilise allelochemicals as carbon sources and thus alter their chemical character and resultant effects on target plants (Inderjit 1996; Blum 1998). Alternatively, soil microbes may degrade innocuous compounds released by plants, transforming them into toxic products that can interfere in plant-plant interactions (Gagliardo & Chilton 1992).

More common though, is the alleviation of allelopathic effects by soil microbes such as that shown for many invasive species (Li *et al.* 2015). Additionally, the allelochemicals of *Ageratina adenophora* were degraded more rapidly in soils with a longer invasion history (Li *et al.* 2015). This suggests that soil microbes may have the capacity to adapt to allelochemicals, enabling the microbes to degrade them (Inderjit & Cahill 2015). The degradation of allelochemicals by *A. adenophora* was also faster in invaded soils, suggesting that soils in invaded sites may accumulate microbes that degrade allelochemicals (Li *et al.* 2017). Evidence for allelopathy is typically demonstrated using bioassays in which leachate from an allelopathic plant is extracted and experimentally added to seedlings of a target plant species (Hierro & Callaway 2003; Dorning & Cipollini 2006; Zhang *et al.* 2009; Inderjit *et al.* 2011b; Vrchotová *et al.* 2011). Alternatively, plant leachate or litter can be added into the soil of a target plant species (Singh *et al.* 2005; Hagan *et al.* 2013).

Assessing allelopathic potential by experimentally extracting a single compound from living tissues may be an inaccurate representation of allelopathic potential. Allelochemicals can also be released from dead leaf litter and several compounds may work together to create an allelopathic effect (Hierro & Callaway 2003), which would not be accounted for in simple bioassays. Allelopathic bioassays therefore often overestimate the effects and residence times of allelochemicals, in comparison with realistic field conditions (Hierro & Callaway 2003).

Selecting the concentration of a particular chemical to use in an allelopathy bioassay is particularly troublesome, warranting criticism of allelopathy studies that use unrealistically high concentrations of a selected allelochemical. Experimental concentrations applied in bioassays may even overestimate phytotoxic concentrations by over an order of magnitude (Inderjit *et al*. 2008). The allelopathic potential of the invader *C. maculosa* has been well-documented but whether its primary allelochemical, (±)-catechin, is phytotoxic in natural soil conditions is controversial. *C. maculosa* exudes a racemic mixture of (±)-catechin, in which (-)-catechin has been shown to be more potent than the predominately antimicrobial (+)-catechin (Bais *et al.* 2003).

Soil concentrations of (±)-catechin have been shown to vary greatly, with field concentrations averaging 1.55 mg/g soil, but some sites have up to 7 mg/g soil (Perry *et al.* 2005). Determining reasonable concentrations of (±)-catechin to be used in allelopathic bioassays is thus challenging and makes it difficult to assess the realistic allelopathic potential of *C. maculosa*. Additionally, discrepancies in allelopathic bioassays, particularly those focusing on (±)-catechin may even be due to slight methodological differences that can produce highly different results (Bais & Kaushik 2010).

In order to overcome these uncertainties in phytotoxic concentrations of certain allelochemicals, studies have identified environmental sources of variation in the allelopathic effect of (±)-catechin. Concentrations of (±)-catechin in natural soils can vary according to root proximity, difference in soil sampling zones, age of *C. maculosa* invasion and also the time of sampling (Bais & Kaushik 2010; Tharayil & Triebwasser 2010). Additionally, the target plant species affected by the allelochemical vary in their sensitivity and resistance to (±)-catechin (Bais *et al.* 2003; Callaway *et al.* 2005; Perry *et al.* 2005), with some species suffering little inhibition from this allelochemical (Blair *et al.* 2005).

In addition to the uncertainty of the allelochemical concentrations, bioassays most often demonstrate only root-mediated allelopathy, with studies experimentally adding allelochemicals to plant roots under controlled conditions. However, this is not realistic as root-mediated allelopathy may not occur in a complex natural community, where biotic and abiotic factors, such as root density, allelochemical mobility and microclimate, may influence the ultimate allelopathic effect (Hierro & Callaway 2003). Thus evidence for allelopathy may be strengthened by demonstrating the movement of allelochemicals from the donor plant to the rhizosphere of the target plant (Staman *et al.* 2001).

In order to attempt to add environmental realism, studies have assessed allelopathic effect by growing study plant species on a more suitable substrate, such as soil. Activated carbon (AC) can be added to the growth substrate in order to manipulate the effect of allelochemicals (Inderjit & Callaway 2003; Prati & Bossdorf 2004; Murrell *et al.* 2011). AC has a large surface area and a high affinity to adsorb large organic compounds so is often used to reduce allelopathic interference (Callaway & Aschehoug

2000; Ridenour & Callaway 2001). The biomass of two native North American plant species when grown in competition with the invader *Centaurea diffusa* was increased with the addition of AC to the soil (Callaway & Aschehoug 2000), demonstrating an allelopathic advantage of invasive plant root exudates.

There are however limitations of the use of AC in studies of allelopathic interference. Lau *et al.* (2008) have shown that the addition of AC can affect both nutrient availability and plant growth, potentially confounding any results obtained. Although AC is considered a broad-spectrum adsorbent, it is not certain that all allelopathic chemicals will be adsorbed, which means that experiments resulting in no effect should be interpreted with caution (Inderjit & Callaway 2003). AC may also alter other substrate characteristics, such as pH or water retention, which must be considered during interpretation of results. Despite the experimental artefacts associated with AC use, AC currently remains one of the most effective methods for demonstrating the allelopathic advantages of invasive plant species.

Few studies have taken allelopathic studies out of the controlled environments of the greenhouse and laboratory (Inderjit & Callaway 2003; Hierro *et al.* 2005). Natural field soils and abiotic conditions that cannot easily be recreated in a greenhouse can limit the expression of allelopathic effect and the levels of allelochemicals in the environment (Inderjit & Weiner 2001; Inderjit 2005). Therefore, laboratory and greenhouse studies of allelopathy often lack the demonstration of the significance of allelopathy in natural communities. Field studies of allelopathy provide an opportunity to take into account environmental influences on allelopathy, such as residence times, and the chemical composition of compounds found in living plant tissues, as opposed to those extracted from soils or dead plant material (Hagan *et al.* 2013). Biotic stresses, such as herbivory have also been shown to influence release of allelochemicals, which is not considered in laboratory bioassays and greenhouse studies. *C. maculosa* releases greater amounts of (±)-catechin when attacked by herbivores, thus exhibiting a greater negative allelopathic interference effect on native plant species (Thelen *et al.* 2005). Allelopathy is not an autoecological process and thus, the significance of allelopathy in an ecological context should be considered in concert with its target community (Gómez-Aparicio & Canham 2008).

Field experiments of allelopathy offer the possibility to overcome the drawback of laboratory and glasshouse studies, demonstrating more than just the presence of a chemical, which is not evidence to demonstrate causative allelopathy (Seal *et al.* 2004). Bioassays are unable to demonstrate the conditionality of allelopathy in a variable environment (Inderjit & Callaway 2003), thus field studies are required to put allelopathic laboratory findings into an ecological context by demonstrating the release, arrival and effect of an allelochemical on its target under natural conditions.

Gómez-Aparicio & Canham (2008) conducted a realistic field study of the allelopathic effect of the invasive tree *Ailanthus altissima* by adding AC to invaded plots containing transplanted seedlings and experimentally sown seeds of three native species. The study showed that *A. altissima* significantly negatively affected seedling growth through allelopathic effects, but most importantly that these effects were species-specific. The discovery of species-specific allelopathic effects in the field may also be important for predicting the change in habitat composition caused by some invading non-native species.

Field studies can also demonstrate the relative significance of allelopathy in various ecological contexts. For example, Del Fabbro *et al.* (2014) showed that although the addition of AC to invaded field plots enhanced native seed germination, suppression of germination by invasive species was similar to that of the native community. This is important, as a bioassay would conclude that a particular allelochemical has a negative effect on native plant species and may incorrectly infer that this provides a competitive advantage to an invasive plant, when in fact in the field it may not. From the few field studies of allelopathy that have been performed, it is clear that allelopathic effects are highly context dependent and vary with the environment in which they are measured. This variation in allelochemical-environment interactions makes allelopathy difficult to demonstrate in field situations but is vital to establishing the ecological significance of allelopathy.

Although allelopathic interference is acknowledged as a mechanism underlying invasive plant success (Callaway & Ridenour 2004; Inderjit *et al.* 2008; Inderjit *et al.* 2011a), field studies have called into question the role allelopathy has in plant invasions. There is evidence that allelochemicals are not unique to invaders, but contribute to creating plant diversity in natural systems (Greer *et al.* 2014). Invasive plants however may possess different allelochemicals to those produced by native non-invasive species, but the significance of these differences is still debated, and therefore so is the role of allelopathy in plant invasions (Mallik & Pellissier 2000; Barto *et al.* 2010; Lind & Parker 2010; Kim & Lee 2011; Del Fabbro *et al.* 2014).

In order to disentangle complicated allelopathic effects in natural environments, a multidisciplinary approach to allelopathy must be taken. Field studies have suggested that the significance of allelopathy in the study of invasive species cannot effectively be considered in isolation of its target community and thus have limited ecological relevance (Gómez-Aparicio & Canham 2008). Conclusions drawn solely from laboratory or greenhouse studies must therefore be interpreted with care, and should be supplemented with field studies to apply any mechanistic findings to the relevant ecological context. Additionally, the under-reporting of negative results should be addressed (Hierro & Callaway 2003), because in some cases, an allelochemical isolated from an invasive plant in the laboratory and experimentally applied to a target species under controlled conditions and concentrations may not be ecologically relevant under natural conditions.

Allelopathy may play a significant role in invasive plant success, but the action of these allelochemicals in concert with the target community must be considered. Although Scharfy *et al.* (2011) found that the invasive forbs studied displayed greater allelopathic effects than native forbs, this difference was much smaller than expected. Thus, in order to assess the relevance of allelopathy to plant invasions, suitable comparisons should be made to determine if laboratory-demonstrated allelopathic effects manifests in the recipient ecosystem. Allelopathy research has been perceived as lacking the rigour and respectability of other fields (Romeo 2000), most probably due to the neglect of the soil component and the mixed direct evidence for allelopathic interference. Inderjit & Weiner (2001) propose placing allelopathy in the context of soil chemical ecology. In this way, future studies can add environmental relevance, considering all the abiotic and biotic components of the soil that may influence allelopathic interference.

### 1.3.4 Novel weapons

The high competitive ability of invasive plants in their introduced range has also been attributed to the effects of allelochemicals that some invasive plant species produce and release. The novel weapons hypothesis (NWH) suggests that allelochemicals produced by invasive plant species function as novel biochemical weapons in their introduced range, mediating new plant-soil interactions (Callaway & Ridenour 2004). Biochemicals novel to the introduced range of the invader provide a disproportionate allelopathic advantage against naïve plant neighbours (Callaway & Aschehoug 2000; Callaway & Ridenour 2004). Native species in the introduced range are assumed not to have adapted to or evolved counter-defences against novel allelochemicals, which consequently negatively affect plant performance.

A key prediction of the NWH is that native plant species in the introduced range should be more vulnerable to the allelochemicals produced by invasive plant species, than are plants from the native range of the invasive plant species. Additionally, invasive populations of the exotic species are assumed to have stronger allelopathic effects on naïve species than populations from the native range, due to a lack of co-evolutionary history (Callaway & Ridenour 2004; Thorpe *et al.* 2009; Inderjit *et al.* 2011a). *Centaurea diffusa* has been shown to exhibit stronger suppressive effects on North American (invasive range) plant communities, which are more susceptible to invasion by *C. diffusa* than on Eurasian (native range) communities (Callaway & Aschehoug 2000; Vivanco *et al.* 2004). The novelty of allelochemicals has also been investigated in other invasive plant species. In the exotic plant *Solidago canadensis*, a lower investment of allelochemicals in the native range has been shown (Abhilasha *et al.* 2008) and garlic mustard (*Alliaria petiolata*) has been shown to possess a phytochemical profile distinct from other closely related Brassicaceaeous species, which supports the NWH (Barto *et al.* 2010).

The novelty that provides invasive plant species with an initial post-introduction advantage can wane with time (Zhang *et al*. 2010a; Lankau 2011). For example, allelochemical investment by *A. petiolata* over time can decline with invasion history and microbial taxa at site of more recent invasions were found to be more sensitive to allelopathic glucosinolates, than were taxa at historically invaded sites (Lankau 2011). This is evidence that the novelty of allelochemicals and thus their role as weapons in competitive interactions with interspecific competitors can be unstable across evolutionary time. In addition to evidence of an evolutionary decline in allelochemical production by invaders, native plant species may also evolve in response to allelochemicals. Surviving native individuals from the invasive range of *Centaurea maculosa* in North America had higher tolerances to the invader's allelopathic effects than individuals from communities that did not experience *C. maculosa* invasion (Callaway *et al.* 2005).

If the production of allelochemicals by invasive plant species enhances the ability of the invader to compete with other species, one might expect increased allelochemical production to rapidly evolve at the invasive range (Callaway & Ridenour, 2004; Inderjit *et al.* 2006; Bossdorf 2013). The allelopathic advantage against resident species (AARS) hypothesis, derived from the NWH (Callaway & Ridenour 2004) predicts direct selection of competitive traits, including quantities of allelochemicals, because of a greater selection pressure for advantageous traits in the introduced range (Inderjit *et al.* 2006). Evidence supporting the AARS demonstrates greater concentrations of allelochemicals produced by invasive plant species in their introduced, compared to their native ranges, such as that for *C. maculosa* and *C. diffusa* (Bais *et al.* 2003; Vivanco *et al.* 2004).

Difficulty arises in demonstrating empirically that enhanced allelochemical production among invasive plant species has evolved (Yuan *et al.* 2012). No study has yet examined the novelty and evolution of allelopathic effects in concert, but recently attempts have been made to do so (Zheng *et al.* 2015b; Gruntman *et al.* 2016). Gruntman *et al.* (2016) showed that invasive populations of *I. glandulifera* (Himalayan balsam), more strongly inhibited germination of a vigorous native competitor, *Urtica dioica*. This finding supports the notion that allelopathy can evolve in the introduced range. However, the study found little support for the NWH; allelochemicals produced by invasive Himalayan balsam did not have a differential effect upon *U. dioica* from the native and introduced range (Gruntman *et al.* 2016).

A new hypothesis, the evolution of enhanced weaponry, has recently been proposed that incorporates the EICA hypothesis and the NWH (Uesugi & Kessler 2013; Zheng *et al.* 2015b; Gruntman *et al.* 2016). Allelopathic ability may increase the competitive ability of an invasive plant species and consequently the invader may experience direction selection for the production of greater amounts of allelochemicals (Yuan *et al.* 2012). Thus, the evolution of enhanced allelochemical production provides a mechanism

for EICA in invasive plant species. This new hypothesis, however, assumes a physiological trade off exists between allelopathy and herbivore defence (Bossdorf 2013; Uesugi & Kessler 2013), which would provide an inherent advantage to non-natives in their invasive range.

Although based only on populations from the native range, Uesugi & Kessler (2013) provided initial evidence for a link between the EICA hypothesis and the NWH. They showed that *Solidago altissima* individuals from New York, USA, were selected for enhanced allelopathic ability in the absence of herbivores, thus demonstrating allelopathy as an alternative mechanism for EICA. A link between EICA and NWH has been strengthened by evidence of a trade-off between allelopathy and defence, but in the context of a different invasive plant species, *Chromoloaena odorata* (Zheng *et al.* 2015b).

The majority of evidence that provides support for the NWH was obtained under controlled conditions and bioassays, whereas field evidence is limited (Del Fabbro *et al.* 2014). Evidence is also only based on a subset of exotic species and few studies have compared responses in invasive and native ranges (Qin *et al.* 2013). Field evidence both supports and refutes the NWH. The first field biogeographical evidence concluded that (±)-catechin exuded by *C. maculosa* is a novel weapon, but the study was only carried out at one site in each the native and invasive range of *C. maculosa* (Thorpe *et al.* 2009). In contrast, a more robust study examining three plant invaders in concert, found that even though allelochemicals were released by *I. glandulifera*, *Solidago gigantea* and *Erigeron annuus*, they did not suppress native germination more than the native plant community (Del Fabbro *et al.* 2014). This suggests that in some cases, invasive plant species may not create negative soil conditions for native communities in their invasive range (Del Fabbro & Prati 2015b). The NWH has also been contradicted with regards to the novelty of allelochemicals that it predicts should promote invasive plant success. Evolutionary novelty can also theoretically suppress invasions because novel weapons may be ineffective against enemies in the new introduced range to which they have not co-evolved with (Lind & Parker 2010).

A meta-analysis of the role of NWH in tree invasion studies has shown that stronger effect sizes were present in support for the hypothesis than alternative hypotheses (Lamarque *et al.* 2011). However, this study was only based on tree invasions and thus is not comparable to other commonly studied high impact exotic invaders, such as *Centaurea maculosa*, *Solidago canadensis* and *Impatiens glandulifera*. Comparisons of secondary compounds found in invasive and native North American plant species found that compounds present in exotic species were more unique than those found in the native flora (Cappuccino & Arnason 2006).

Allelochemicals, such as glucosinolates and (±)-catechin produced by *A. petiolata* and *C.* maculosa, respectively, are clearly detrimental to native plant communities. These chemicals may provide the

invader with an initial advantage when introduced to the invasive range, but the role of novel weapons in sustaining invasion success and the mechanism of allelopathy remain unclear. At present, the lack of field studies raises questions about the ecological relevance of the NWH, which is not as well studied as other plant invasion hypotheses. However, the potential role of novel weapons in driving increased competitive ability could provide another mechanism to explain some plant invasions.

## 1.4 THE ROLE OF MUTUALISMS IN PLANT INVASIONS

### 1.4.1 Arbuscular mycorrhizal fungi

The potential role of mutualisms in driving plant invasions is less clear than the role of antagonistic pathogens but is receiving increasing research attention (Richardson *et al*. 2000b). In particular, arbuscular mycorrhizal fungi (AMF), which are rarely considered in invasion theories (Catford *et al.* 2009), are becoming increasingly associated with plant invasions because of their fundamental importance in species' ecology (Herre *et al*. 1999). AMF form biotrophic relations with a host plant, developing inside plant roots and in the soil forming an extensive extraradical network (Jeffries *et al*. 2003). The mycorrhizal fungi receive fixed carbon in exchange for increased nutrient availability for the host plant, such as for phosphorous (Smith & Read 1997). Nutrient transfer occurs through arbuscules in the plant root cells (Parniske 2008). AMF also provide resistance to pathogens, protect against toxic stresses and aid in building soil structure (Newsham *et al.* 1995; Andrade *et al.* 1998; van der Putten *et al*. 2001). Plant-AMF symbioses are the most abundant and evolutionary ancient mycorrhizal relations, with AMF associating with approximately 75% of vascular plants (Smith & Read 2008).

As AMF play a significant role in transport of resources within an ecosystem, they are keystone mutualists and their own diversity is an important determinant of plant biodiversity and ecosystem functioning (O'Neill *et al*. 1991; van der Heijden *et al.* 1998; Smith & Read 2008; Johnson *et al*. 2012). AMF are important mediators of competitive interactions between plant species, through their influence on nutrient uptake (Hartnett *et al.* 1993; Shah *et al*. 2009). Mycorrhizal fungi substitute the role of root nutrient uptake of host plants, thus the plant is able to reallocate resources from growth to defence and enhance its competitive ability (Berta *et al.* 1993; Vance *et al.* 2003). AMF also differentially increase resource acquisition of select host plant species, enabling coexistence of multiple plant species (Caldwell *et al*. 1985; Allen & Allen 1990).

The interaction between plant species and AMF contributes to PSFs, with each plant varying in its response to and resource exchange with a species of AMF (Bever 2002). As a result of this differential response, the plant-AMF interaction is not always mutualistic (Francis & Read 1995; Klironomos 2003; Reinhart & Callaway 2006), with AMF often generating negative feedbacks on plant growth. Consequently, plant species vary in their dependency on mycorrhizal fungi (Klironomos 2003). The differential effects, relations and dependencies of different plant species with AMF has encouraged plant invasion theories to encompass the role of mycorrhizal fungi in explaining the mechanisms behind some plant invasions.

### 1.4.2 Degraded mutualisms

AMF have been implicated in plant invasion success, particularly through the discovery that several invasive plant species have evolved a low dependency on mycorrhizal fungi, compared to native plants (Richardson *et al.* 2000b; van der Putten *et al*. 2007b; Seifert *et al*. 2009; Bunn *et al*. 2015). Several invaders, have even shown a dampened growth response when colonised by mycorrhizal fungi (Bunn *et al.* 2015) such as *I. glandulifera* (Tanner *et al*. 2014), suggesting it may be inherently beneficial to avoid the costs of a mycorrhizal association. Additionally, the lack of association with AMF has also been shown to be beneficial for invaders in a competitive environment (Waller *et al*. 2016).

As a result of this low dependency on AMF in comparison with native plant species, invaders can take advantage of this differential AMF dependence and selectively alter the abundance and composition of the AMF community, creating more negative PSFs for native mycotrophic plant species (Hawkes *et al.* 2006; Shah *et al.* 2009; Vogelsang & Bever 2009). The degraded mutualisms hypothesis (DMH) formally integrates this idea that non-mycorrhizal invaders can facilitate their own invasion by reducing the fungal abundance and density in occupied soils, thereby disrupting native plant-AMF associations (Vogelsang & Bever 2009). Alteration of the AMF community has been primarily shown to occur through indirect allelopathy, with invaders producing novel allelochemicals that inhibit mycelial growth (Stinson *et al.* 2006; Cipollini *et al.* 2012). Evidence comparing soil biota at invaded and uninvaded sites, often using genetic sequencing technologies, has shown that this depletion of fungal abundance and diversity in invaded ecosystems may leave long-lasting legacy effects in the invaded soil microbial community, further increasing the invasibility of the ecosystem (Mummey & Rillig 2006; Day *et al.* 2015).

The DMH has been well-studied in invasive populations of *A. petiolata*, which indirectly interfere with the formation of mycorrhizal associations with native host plants, and which alter AMF community composition through production of allelopathic glucosinolates that act as novel weapons (Barto *et al*. 2010). It has been shown that in *A. petiolata*-invaded patches, molecular diversity of local AMF in host roots is significantly reduced (Koch *et al*. 2011), with *A. petiolata* preventing germination of spores of AMF, and reducing native plant growth (Roberts & Anderson 2001). Stinson *et al*. (2006) provide sound support for the DMH as they show a positive relationship between reduction in native plant growth and mycorrhizal dependency. This inhibition of mycorrhizal fungi in the invaded range by *A. petiolata* is far stronger than that in the native range, providing evidence for a novel competitive advantage gained during invasion (Callaway *et al*. 2008)

More recent evidence has shown that having a low dependency on AMF may not be advantageous to invasive plant species in the long-term. Lankau (2011) showed that the anti-fungal impact of *A. petiolata* may lose evolutionary novelty as the invasive population ages. Microbial communities

invaded by *A. petiolata* may even begin to develop some resistance to the effects of invasion, showing some signs of recovery (Lankau 2011). Moreover, *A. petiolata* may not generally suppress AMF communities in host roots. Instead, the invader may selectively suppress fungi association with certain plant species, with many communities showing some tolerance to the fungal effects of *A. petiolata* invasions (Burke 2008; Barto *et al*. 2011). A mechanistic model by which *A. petiolata* indirectly suppresses select competing plants has been explored, with depletion of the AMF network shown to create symptoms of carbon stress in host plants (Brouwer *et al*. 2015).

### 1.4.3. Enhanced mutualisms

Contrary to the DMH, some non-native plant species form highly mutualistic associations with AMF, which may facilitate invasions through positive plant-soil feedbacks (Reinhart & Callaway 2006; Shah *et al*. 2009). The enhanced mutualisms hypothesis (EMH) suggests that some invaders encounter mutualists that facilitate their establishment more strongly than mutualists in their native range (Reinhart & Callaway 2006). When colonising a new area, the invader may associate with a new mutualistic partner not present in its native range, which may promote naturalisation by creating highly positive feedbacks (Richardson *et al*. 2000b; Callaway *et al*. 2011). Additionally, it is posited that invasive plants may gain a mutualist advantage by associating with a wider range of AMF (van der Putten *et al.* 2007a; Pringle *et al.* 2009; Moora *et al.* 2011).

Evidence for the EMH typically demonstrates a selective change in AMF community composition that has a positive influence on the invaders growth, compared to native plant species (Fumanal *et al.* 2006; Hawkes *et al*. 2006; Lekberg *et al.* 2013). The prolific invasion of *Solidago canadensis* in southeast China has been shown to be facilitated by altering AMF species composition, through allelopathy (Sun & He 2010; Yuan *et al.* 2014). The invader selectively increases the AMF species *Glomus geosporum*, which promotes its own growth (Zhang *et al.* 2010b). Simultaneously, *S. canadensis* also decreases another AMF species, in order to reduce native species mycorrhizal associations.

In contrast to *S. canadensis,* invasion by *C. maculosa* has also be shown to be facilitated by mutualists, but indirectly through interspecific competition (Marler *et al.* 1999; Mummey *et al.* 2005). AMF was found to exert no direct effect on *C. maculosa* but did increase the competitive effect of the invader on a competing native, *Festuca idahoensis* (Marler *et al.* 1999). It was subsequently shown through carbon isotope analysis that AMF facilitate the transfer of carbon from *F. idahoensis* to *C. maculosa*. The biomass of the invader was greatest in the presence of both AMF and *F. idahoensis*, suggesting *C. maculosa* indirectly benefits from the presence of a competitor, through carbon parasitism, mediated by AMF (Carey *et al.* 2004).

Although both the DMH and EMH are sound mechanistic explanations for the invasiveness for *A. petiolata, S. canadensis* and *C. maculosa*, a recent meta-analysis showed little species-wide support for either hypothesis (Bunn *et al.* 2015). The analysis showed that mycorrhizal colonisation could be better explained with plant functional group, rather than invasive status. Evidence shows that plant invasions may not influence evolutionary trajectories by selecting for or against AMF associations. However, AMF are clearly pivotal in influencing direct and indirect plant competitive interactions, which some invasive plant species are able to take advantage of to facilitate their invasion. It has recently been shown that being a facultative mycorrhizal species can be advantageous for invasive spread (Menzel *et al*. 2017), so in some cases AMF associations with invasive plant species may not directly lead to invasion, but aid the process.

## 1.5 INVASIVE PLANT CONTROL AND RESTORATION OF INVADED ECOSYSTEMS

### 1.5.1 Current control methods for invasive plant species

Invasive plant species are a significant global threat and are themselves agents of global change. In the UK, 10-12 new non-native species become established each year and 10-15% of these non-native species that become established cause adverse impacts (Defra 2015). These impacts range from local-scale ecological alterations, to large-scale and long-term reductions in overall biodiversity, as well as economic and social impacts (Mack *et al*. 2000; Simberloff *et al*. 2013). The cost of invasive non-native species in the UK is at least £1.7 billion each year (Defra 2015). The Invasive Non-Native Species Framework strategy for Great Britain sets out strategic aims to minimise the risks and impacts of non-native invasive species, including controlling already established invasive species (Defra 2015). As a result of the considerable environmental and socioeconomic costs, substantial effort is concentrated at the start of the invasion pathway, with the aim of preventing non-native species becoming established and naturalised in the UK. The need to predict and anticipate invasions has become the focus of much research in invasion biology, as this is the most cost effective and desired approach to control invasive non-native species. Patterns of invasions are largely directed by global trade networks (Chapman *et al*. 2017), and in the UK the majority of established non-native plant species have been introduced for ornamental purposes, such as garden plants. As a result, the UK seeks to develop Pathway Action Plans to reduce the risk of new invasive species introductions (Defra 2015). Horizon scanning for potential threats of new invasive exotic species and the creation of comprehensive global (GloNAF; https://glonaf.org) and regional databases (e.g. GB Non-native Species Information Portal (Roy *et al*. 2014) of exotic plant species has helped to unify policy implementation and invasive plant management.

Despite the plethora of research focused on unifying invasive species databases and improving prediction power for invasive species, by far a more challenging problem is the control of non-native plant species once they have become naturalised in the UK. The greater part of invasive plant species control aims to deplete the seed bank, gradually reducing invasive populations (Davis 2006). One of the most common methods of invasive plant species control is mechanical removal of invasive plants, which can include overgrazing, prescribed burning and pulling of individual plants. Chemical control, using foliar sprays of herbicides is often used in conjunction with physical plant removal. For example, attempted control of the invader garlic mustard (*A. petiolata*) in North America often requires cutting, fire and glyphosate herbicide application (Carlson & Gorchov 2004).

Notwithstanding the ease of implementation of these strategies, mechanical control is often labour-intensive and chemical control using herbicide is costly, and both methods require repeated annual treatments. In some cases, mechanical control through pulling may actually promote invasive plant

spread, through disturbance (Murphy *et al*. 2007) and strategic implementation of control may be required, to target particular focal populations, for which adequate knowledge may not be available. Consideration of the potential non-target effects of herbicide use may be warranted and methods often require continued ongoing long-term monitoring after treatments are applied to detect any adverse effects. Different treatments may need to be applied at select times and particular growth stages in order to produce the best effectiveness, and to take into account complex population dynamics of the target species (Pardini *et al*. 2009; Davis *et al*. 2014). The inconsistency of results and success of many control methods (Carlson & Gorchov 2004; Hochstedler *et al.* 2007; Pardini *et al.* 2009) suggests that implementation of control methods is highly context-dependent and each individual plant species will require its own strategic action plan, which undoubtedly will be costly to managers. In some cases, where aggressive management may not be feasible or even exacerbate the problem, the best option may be passive management of a particular plant species and to focus on bringing about behavioural changes to reduce invasive plant spread (Rinella *et al.* 2009).

To many, biological control (biocontrol) provides a sustainable and more cost-effective method of invasive plant species control as often only an initial cost of research and exploration is required. Biocontrol theory is based on re-establishing the relationship of invaders with their co-evolved natural enemies (Callaway & Aschehoug 2000; Keane & Crawley 2002). Biocontrol programmes aim to reduce the fitness and competitiveness of the invader and are often considered the 'green' alternative to other control methods, including herbicide use. Introduction of a natural enemy is usually considered where there are no other options for invasive plant control, given the scale of the infestation, and the environmental sensitivity of chemical and mechanical methods (Seastedt 2015). For example, Japanese knotweed (*Fallopia japonica*) is a widespread and persistent invasive plant in the UK, resisting chemical control, causing building damage and posing a risk to flood management through its damage to riverbanks. In 2011, a highly specialist psyllid (*Aphalara itadori*) was released in order to control the weed (Shaw *et al.* 2011). So far the agent looks promising at controlling abundances of Japanese knotweed, especially since it has been shown not to breed on any other native plant species. However, as with other biocontrol agents, maintaining a viable population in harsh conditions, such as over-winter survival, may be challenging (Shaw *et al.* 2011).

Even though some biological control programmes have been successful, there are many cases where biocontrol agents introduced to suppress invasive species have failed (Williamson 1996; Denoth *et al*. 2002). For example, 13 insect species have been introduced to control *C. maculosa*, all failing (Müller-Schärer & Schroeder 1993; Pearson & Callaway 2003), which may be attributed to the incredibly context specific nature of biocontrol (Seastedt 2015). Both abiotic and biotic conditions in the introduced range need to be taken into account when screening for a biological control agent, and monitoring success after agent introduction. Disturbance regimes, temperature and nutrient fluxes can

affect not only the fitness of the target invasive plant, but the performance of the introduced biocontrol agent, which could hinder a biocontrol programme if not considered. Additionally, biotic factors, such as plant-soil feedbacks may also influence the success of biocontrol efforts (Bever 2003; Maron *et al.* 2014), as well as rapid evolution documented for many invasive plant species and their introduced agents (Sax *et al.* 2007; Mcevoy *et al.* 2012; Turner *et al.* 2014), which may produce unexpected host shifts and thus limited biocontrol success (Seastedt 2015).

Utilising biological control in invasive plant species management can be controversial, as biocontrol agents themselves are often non-native species. Success of biocontrol is also uncertain and there is a substantial risk that that the introduced agent can become invasive and have damaging unforeseen impacts on non-target species and the environment (Simberloff & Stiling 1996; Simberloff 2012). A classic example of this is that of the weevil *Rhinocyllus conicus*, introduced to North America in 1969 from Europe to control musk thistle (*Carduus nutans*), which subsequently attacked many native species (Louda & O'Brien 2002).

Despite these caveats, a meta-analysis of the effectiveness of biocontrol targeted at invasive plant species showed that overall biocontrol significantly reduces various growth parameters of target plants (Clewley *et al.* 2012), indicating that biocontrol can return positive impacts on the invaded ecosystem. Additionally, safety procedures and risk assessments are improving (Simberloff 2012), which is greatly improving confidence in biocontrol programmes. In the case of highly vigorous invasive plants, there is also the significant opportunity cost of ongoing adverse impacts caused by invasive plants (Suckling & Sforza 2014). For other invasive plants, the possible environmental benefits of biocontrol may not be worth the inherent risks so an alternative management strategy will be required.

### 1.5.2. Utilising knowledge of soil ecology in invasive plant management

Approaches to managing plant invasions often lack a broader ecological perspective (Krueger-Mangold *et al.* 2006) such as restoring multiple aspects of the invaded ecosystem. This may be a significant reason why control efforts for long-established invasive plants have had limited success (Norton 2009; Kettenring & Adams 2011; Simberloff *et al.* 2013). Even if an invasive plant is successfully been removed, restoration attempts are often hampered by a failure to establish a diverse native species community in its place (Foster & Gross 1998; Averett *et al.* 2004), which is essential to restore ecosystem functioning and services that a plant invasion may have compromised, as well as helping to prevent re-invasion. The recognition that invasive plant species significantly influence linkages between aboveground and belowground components of ecosystems (Inderjit & van der Putten 2010) has prompted restoration efforts to consider how knowledge of plant-soil interactions can contribute to restoration efforts (Heneghan *et al.* 2008; Kardol & Wardle 2010; Ohsowski *et al.* 2012).

The soil community greatly influences individual plant performance and community composition through PSFs and plant invaders typically alter biotic and abiotic components of the soil communities that they invade, such as nutrient cycling (Ehrenfeld 2003; Hawkes *et al.* 2005; Vinton & Goergen 2006; Weidenhamer & Callaway 2010) and mutualisms (Richardson *et al.* 2000b; Stinson *et al.* 2006; Vogelsang & Bever 2009). Therefore not considering the need to re-establish soil communities alongside aboveground communities may hinder ecological restoration after plant invasions (Thrall *et al.* 2005; Eviner & Hawkes 2008; Kardol & Wardle 2010). The soil legacies that invasive plant species create through alteration of the soil community can be persistent and thus interfere with restoration attempts, meaning invader removal alone is not sufficient (Kardol *et al.* 2007; Marchante *et al*. 2009; Corbin & D'Antonio 2011; Jordan *et al*. 2012).

Despite the extensive research surrounding PSFs and soil legacies created by invasive plant species, few control methods consider plant-soil relationships, so there is still potential to include knowledge of soil ecology in invasive plant management (Wolfe & Klironomos 2005; Kulmatiski & Beard 2006; Perkins & Hatfield 2016). Consideration of the role of soil communities in driving and responding to plant invasions has generated empirical studies of restoration efforts that attempt to remediate the negative PSFs experienced by native plant species in soils invaded by exotic plant species. One approach is to amend the nutrient content of soils, in order to alter competition between plants (Tilman *et al.* 1999) to restore native plant diversity. For example, the abundance of invasive *Bromus tectorum* in the western USA was reduced with soil amendments that reduced potassium and phosphorus availability to the invader (Belnap *et al.* 2003; Newingham & Belnap 2006). The success of restoration of sites persistently invaded by *Bromus inermis* in the Great Plains of the US using phosphorus amendments also emphasises the potential role of soil amendments in augmenting restoration success (Grygiel *et al.* 2012). However the effects of soil amendments can be influenced by abiotic variability, such as annual climatic changes and the time of application, which may affect the success of such soil amendments (Newingham & Belnap 2006). Additionally, soil nutrient additions may interact with other abiotic factors, such as water availability to reduce invasive plant success, which should be considered when selecting and applying amendments to invaded sites (Blumenthal 2009).

Another, less frequently used approach to recover the negative PSFs of native plant species at invaded sites is the use of activated carbon (AC). For invasive plant species that have been shown to possess allelopathic abilities, AC can be used to reduce the negative effects of root exudates and alter plant-soil interactions by sequestering allelopathic compounds (Callaway & Aschehoug 2000; Bais *et al.* 2005; Mangla *et al.* 2008). AC may also reduce microbial substrate concentrations and subsequently reduce microbial activity, which contributes to the strong positive PSFs experienced by invasive plant species (Bever 2003; Bais *et al.* 2004; Duffy *et al.* 2004; Gage 2004; Kulmatiski 2011; Nolan *et al.* 2014). AC may further reduce the positive feedbacks experienced by invasive plant species in their introduced

range by adsorbing allelochemicals that inhibit plant pathogens (Bais *et al.* 2004), thus decreasing plant defensive ability of invaders. Lastly, AC can also reduce nutrient mineralisation rates through its sequestering of nitrogen and phosphorous (Kulmatiski & Beard 2006), thereby altering plant competitive interactions and removing the competitive advantage incurred by exotics (Davis *et al.* 2000; Lake & Leishman 2004).

Greenhouse studies have shown that native plant species growth in invaded soils, or in competition with invasive plants is often increased with the addition of AC (Callaway & Aschehoug 2000; Ridenour & Callaway 2001; Inderjit & Callaway 2003). Thus this has prompted studies to use AC in field restorations (Kulmatiski & Beard 2006; Kulmatiski 2011; Nolan *et al.* 2014). Although the addition of AC to experimental field plots decreased the growth of some invasive plant species, other invaders responded positively and natives negatively, to AC addition (Kulmatiski & Beard 2006). The ability of AC to manipulate both abiotic and biotic components of the soil makes it an attractive restoration tool. Although its effects may be species-specific, manipulating plant-soil interactions using AC may be a novel and promising approach to restoring communities invaded by a particular introduced plant (Kulmatiski & Beard 2006; Kulmatiski 2011; Nolan *et al.* 2014).

Other research has focused on remediating PSFs created by invasive plant species by increasing positive plant-soil interactions, such as mutualisms. Increasing AMF diversity can significantly improve plant community diversity and productivity (van der Heijden *et al*. 1998; Vogelsang *et al*. 2006). In invaded communities that rely heavily on the presence of mutualists or sites where plant mutualisms have been degraded, plant inoculation with mycorrhizal fungi may aid restoration and establishment of native plant species (Richter & Stutz 2002; Jeffries *et al.* 2003; Eschen *et al.* 2009; Shah *et al.* 2009; Kardol & Wardle 2010; Middleton *et al.* 2015; Koziol & Bever 2017). Moreover there is evidence that plant communities with a high diversity of AMF may be more resistant to plant invasions (Shah *et al.* 2009), which would increase the sustainability of restoration efforts. AMF additions may also increase the growth of later successional plant species (Middleton & Bever 2012), which may override competitive advantages invasive plant species possess, such as early germination and increased growth with disturbance.

Soil remediations using fungal inoculants have however had only partial success so far. Perkins & Hatfield (2016) found that addition of fungal inoculant could reduce the performance of invaders *Bromus inermis* and *Poa pratensis*, but these effects were highly context-dependent and no single treatment increased native plant performance. Additionally, this study was conducted in a greenhouse setting, so the potential to apply these results to field restorations is currently limited. Other studies have found inconsistent support for the use of AMF inoculants in improving restoration success (Rowe *et al.* 2007; Middleton & Bever 2012; Paluch *et al.* 2013; Middleton *et al.* 2015). Commercial fungal

inoculum has been recommended as a soil enhancement agent to increase native plant performance (Schwartz *et al.* 2006; Ohsowski *et al.* 2012), despite the poorly understood ecological ramifications and context-dependency. Moreover, the success of fungal inoculants has been shown to be highly dependent on the host plant identity (Klironomos 2003; Ehinger *et al.* 2009; Mummey *et al.* 2009). In some cases, where some highly mycorrhizal invasive plants may increase fungal abundance (Lekberg *et al.* 2013), the use of fungicide may be more beneficial at increasing native species performance (Perkins & Hatfield 2016). Overall, soil remediations that use fungal additions may provide some improvement to restoration efforts where the invasive plant is not mycorrhizal and has severely degraded the fungal community. Land-use history must also be considered when assessing the potential for fungal-based soil restoration (Paluch *et al.* 2013), and it has been recommended that locally sourced fungal inocula be most effective (Schwartz *et al.* 2006; Middleton *et al.* 2015). More empirical field studies on the long-term effects of the use of fungal inocula in restoration efforts is required in order to evaluate the sustainability of this as a restoration method.

The UK currently has a strategy dedicated to long-term management and control of invasive species, however only 20 species are currently actively controlled as active management is often not feasible (Defra 2015). Thus there is a need to develop more advanced control methods that are easily implemented, with more integrated control so that efforts provided are resourceful and effective. Many strategic priorities are still under development so invasive plant species research could greatly inform these priorities. The incorporation of soil ecological knowledge into restoration efforts is still in its infancy (Aronson *et al.* 1993; Harris *et al.* 2006; Kardol & Wardle 2010). Restoration practitioners should aim to utilise findings from soil ecological research to inform management, as in some cases of persistent plant invaders, it may provide a novel but effective approach to restoration of invaded plant communities.

## 1.6 CONCLUSION

It is now well-acknowledged that soil biota and the PSFs they create can play a significant role in structuring native plant communities and their functioning (Bever *et al*. 1997; van der Heijden *et al*. 1998; Klironomos 2002). The mechanistic basis of some plant invasions is not completely understood, thus inclusion of the role of soil biota in plant invasions may offer unique and valuable insight, including in predicting the invasive potential of some non-native plant species (Reinhart & Callaway 2006; van der Putten *et al.* 2007a). Invasive species, compared to their native congeners often exhibit superior performance, which has led to identification of 'invasion syndromes' for particular species that include attributes such as phenotypic plasticity (Richards *et al.* 2006; Skálová *et al.* 2012), early germination (Perglová *et al.* 2009) and high fecundity (Cano *et al.* 2008; Ebeling *et al.* 2008). However, the soil-mediated drivers of plant invasions are receiving more research attention (Kourtev *et al.* 2002; Levine *et al*. 2006; Batten *et al.* 2008).

Some non-native plant species create more positive PSFs in their introduced range, compared to native plant species, which may significantly contribute to their success (Klironomos 2002; van der Putten *et al.* 2013, 2016). The mechanisms by which these feedbacks are created, including enemy release, EICA and degrading of fungal mutualisms (Blossey & Nötzold 1995; Keane & Crawley 2002; Vogelsang & Bever 2009) are highly variable and dynamic, both in their direct and indirect contribution to plant invasions, and in the methods used to test their role in invasions. All of the mechanisms reviewed here are highly context-dependent, and each mechanism is often described based on observations of a single species, making any general inferences problematic. So far, these mechanisms and hypotheses have been useful in contributing to a greater understanding of plant invasiveness. However, as the wealth of evidence accumulates, these mechanisms that have such broad interpretations may have less utility in the future. Instead, an integrated framework in which aspects of all mechanisms can be considered in concert for a particular species, may be more useful (Jeschke 2014). It is clear that the generality of a mechanism is complex and it is also important to consider that some mechanisms may not be restricted to invaders. It is the combination of non-mutually exclusive mechanisms, alongside particular plant traits, such as phenotypic plasticity, that may drive a non-native plant to become invasive in its introduced range.

Although the mechanisms discussed here are well-studied in some particularly damaging invasive plant species, less is known if and how these mechanisms, and indeed PSFs, will be altered in the future, due to anthropogenic global change (van der Putten *et al.* 2016). Increased temperature, for example, may stimulate an increase in microbial activity in the short term (Dorrepaal *et al.* 2009), potentially increasing positive PSFs, whereas drought may reduce abundances of soil biota (Kardol *et al*. 2010). The response of soil biota to global changes should be incorporated into predictive models of invasive species spread and distributions.

The increased understanding of the role of soil biota in driving and responding to plant invasions is and will continue to be crucial to predicting future plant invasions and adequately managing current invasions. The economic, social and ecological costs of invasive plant species are profound, with invaders re-defining biogeographical barriers and altering the composition of the world's biotas (Hejda *et al.* 2009; Vilà *et al.* 2011; Pyšek *et al.* 2012; Simberloff 2014). Current and future land-use changes are likely to create new and empty niches for invasive plants to occupy (Masters & Norgrove 2010). Therefore, it is crucial for biodiversity conservation to attempt to mitigate against the most acute impacts. Although biocontrol presents the most promising and sustainable management options, it is initially expensive and is controversial. Many invasive plant species management tools do not consider restoration and re-establishment of native species in the post-invaded ecosystem, which opens up the potential to attempt to utilise knowledge of soil ecology to improve restoration efforts. By considering how plant-soil interactions might drive and respond to plant invasions, the soil system should also be restored whilst trying to physically control an invasive plant species. More large-scale field studies are required to determine if low-cost options, such as AC additions and fungal inoculants, can have some utility in restoring highly invaded ecosystems following invader removal.

The following experimental chapters will attempt to link the PSFs of an invasive plant with an applied context; habitat restoration, with a consideration of how plant-soil interactions may mediate plant invasion. Few studies link findings from PSF experiments to field studies, which is important to verify that the PSFs and their resultant effects observed under controlled conditions, occur in variable field conditions. The second chapter will examine the impact of an invasive plant, *I. glandulifera*, on subsequent growth of commonly co-occurring natives to test the impact of PSFs created by *I. glandulifera* on successful native species, often not considered in traditional PSF studies. The second chapter is then complemented with a field study in Chapter 3 that attempts to apply findings from the first experiment to test if soil-based management approaches may help habitat restoration efforts following invasion of *I. glandulifera*. Restoration efforts often do not consider management approaches that attempt to reverse the positive PSFs of invaders demonstrated in pot-based studies, in order to reduce the negative impacts of an invader, such as reduced plant diversity.

# Chapter 2:

# The soil-mediated impacts of invading *Impatiens glandulifera* on the growth of native plant species.


## ABSTRACT

Invasive plant species may modify the outcome of plant-soil interactions by selectively altering the soil microbial community. This phenomenon is well-studied in some invasive plant species, but little is known about the association of *I. glandulifera*, a widespread invader in the UK, with soil microbial communities during its prolific invasions. Furthermore, it is unknown whether or how these soil microbial alterations affect native plant species. A pot experiment was conducted, in which *I. glandulifera* and selected co-occurring native plant species were grown in invaded *versus* uninvaded, and sterilised *versus* unsterilised field-collected soil from sites of *I. glandulifera* invasion. Subsequent growth rates and aboveground biomass were recorded, which showed a significant site-dependent effect. Overall, *I. glandulifera* grew better in invaded soils, which is indicative of a positive plant-soil feedback. The growth and biomass production of the native plant species was dependent on the soil they were grown in, suggestive of a strong effect of the local soil biota at each field site. Arbuscular mycorrhizal fungal (AMF) colonisation of two native plant species, was also dependent on soil origin, but some evidence was found that *I. glandulifera* may alter AMF communities in invaded patches. These results show that, although *I. glandulifera* may alter the soil microbial communities, these effects on native plant species are highly context-dependent and influenced by the local soil biota.

## 2.1 INTRODUCTION

It is now well-recognised that the aboveground and belowground components of an ecosystem are inextricably linked (Wardle *et al.* 2004; Batten *et al.* 2008). Individual plants can selectively alter their rhizosphere community through root exudates and leaf litter, as well as alter soil nutrient availability (Westover *et al.* 1997; Grayston *et al.* 1998). In turn, soil microorganisms subsequently affect plant performance and plant community composition, through pathogenic and mutualistic effects, and nutrient cycling (Burdon 1993; Newsham *et al.* 1995; Holah & Alexander 1999; Packer & Clay 2000; van der Putten *et al.* 2001; Mitchell & Power 2003). These linked processes create feedbacks that can extend to whole ecosystem processes and functioning (van der Putten *et al.* 2001). Plants may experience negative soil feedbacks, where reduced growth is caused by accumulation of specific pathogens, which benefits growth of co-occurring plants thus maintaining species diversity (Bever *et al.* 1997; Klironomos 2002; Bever 2003). Positive feedbacks occur when beneficial microbes, such as mycorrhizal fungi and nitrogen fixers, are accumulated. In contrast with negative feedbacks, positive feedbacks may drive species dominance and thus may diminish plant species diversity.

The strong influence of plant-soil feedbacks in driving the outcome of plant-plant interactions (van der Heijden *et al.* 1998; Packer & Clay 2000) has stimulated interest of the role of the soil community in plant invasion ecology (Callaway & Aschehoug 2000; Wolfe & Klironomos 2005; Inderjit & van der Putten 2010). Invasive species are a significant component of global change (Vitousek *et al.* 1997), with far-reaching impacts that can be ecologically, socially and economically damaging (Strayer *et al.* 2006; Hejda *et al.* 2009; Ehrenfeld 2010). Thus it is important to determine the factors contributing to the success of the most damaging invasive plants. Evidence suggesting that some invasive plant species may experience more positive plant-soil feedbacks compared to native plant species in their introduced range (Klironomos 2002; van der Putten *et al.* 2007b; Inderjit & van der Putten 2010) has stimulated research to consider the role of soil biota in both driving and limiting plant invasions. Non-native plant species may strongly alter the composition of microbial communities of the soils that they invade with potentially major consequences for ecosystem functioning (Hawkes *et al.* 2005; Pimentel *et al.* 2005; van der Putten *et al.* 2007a; Vilà *et al.* 2010). These novel feedbacks that are created by invaders altering microbial communities have been suggested to contribute to their invasiveness (Richardson *et al.* 2000b; Callaway *et al.* 2001, 2004a; van der Putten *et al.* 2005; Wolfe & Klironomos 2005; Inderjit & van der Putten 2010). As a consequence of these soil microbial changes that preferentially favour their own growth, invasive plant species have been shown to benefit from positive PSFs in their invaded range, in contrast to the negative feedbacks experienced by co-occurring native species (Belnap & Phillips 2001; Klironomos 2002; Levine *et al.* 2006; van der Putten *et al.* 2013).

There are a multitude of belowground explanations by which invasive plant species are suggested to enhance their invasiveness, which are not mutually exclusive. Non-native plant species may displace

native species, or reduce their growth through gaining a competitive advantage from escaping soil-borne pathogens (Keane & Crawley 2002). The loss of co-evolved relationships with pathogens may subsequently lead to the evolution of enhanced competitive ability of the invader, as more resources are freed up from plant defence and allocated to plant growth (Blossey & Nötzold 1995; Uesugi & Kessler 2013). Alternatively, invasive plant species may utilise their allelopathic ability to direct inhibit plant species growth (Callaway & Ridenour 2004; Prati & Bossdorf 2004; Cappuccino & Arnason 2006; Inderjit *et al.* 2008, 2011a; Del Fabbro *et al.* 2014; Zheng *et al.* 2015) or degrade beneficial mutualisms in the soil (Cappuccino & Arnason 2006; Stinson *et al*. 2006; Vogelsang & Bever 2009).

The impacts of invasive plant species on native plant species are typically investigated in greenhouse pot studies that grow plant species of interest in soils from different origins, and sterilised and unsterilised soils (Callaway *et al.* 2004a; van der Putten *et al.* 2007b; Batten *et al.* 2008; Kulmatiski & Kardol 2008; Dawson & Schrama 2016). These studies have shown that invasive plant species most often grow best in 'home' soils and that native plant species show reduced growth in soils conditioned by invasive plant species (Kulmatiski & Kardol 2008; Dawson & Schrama 2016). Differences in the performance of invasive compared to native plant species grown on different soils is frequently used as evidence that many plant invasions can be driven by soil biota (Reinhart & Callaway 2004).

*Impatiens glandulifera* is a gregarious invader that rapidly outcompetes native plant species (Beerling & Perrins 1993). *I. glandulifera* has been shown to maintain its high fitness by exhibiting considerable phenotypic plasticity enabling it to exploit a wide range of environmental conditions (Pahl *et al.* 2013). As a result, in invaded areas the invader can cause significant changes in plant community composition (Hejda & Pyšek 2006). Comparisons of invasive and native populations of *I. glandulifera* have shown that introduced populations exhibit greater performance than populations in the native range, which are highly regulated by natural enemies (Tanner *et al.* 2014). *I. glandulifera* is also weakly dependent on AMF and may actively deplete the mycelial network in invaded soils (Tanner *et al.* 2013; Ruckli *et al.* 2014a). Thus it has been posited that *I. glandulifera* may create a more positive plant-soil feedback (Pattison *et al.* 2016) for itself by degrading mutualisms experienced by native plant species, reducing their fitness (Vogelsang & Bever 2009; Tanner & Gange 2013). In addition to its effects on soil biota through reduction in mutualistic effects, there is evidence that *I. glandulifera* releases allelopathic naphthoquinones that inhibit plant germination and prevent fungal mycelial growth (Ruckli *et al.* 2014b). These multiple lines of evidence demonstrate the pervasive impacts of invading *I. glandulifera* on native plants and their associated soil microbial communities.

There is evidence that some native plant species may tolerate invasive plant species and adapt to the allelochemicals of long-established and abundant invasive plant species and thus co-exist with an invader (Callaway *et al.* 2005; Thorpe *et al.* 2011). Soil biota at invaded sites may respond to invasion

by adapting to allelochemicals of invasive plants and degrading them over time, reducing any invasive legacies created (Lau 2006; Inderjit & Cahill 2015; Li *et al.* 2015, 2017). Few studies have explored the soil-mediated impacts of *I. glandulifera* and the resulting effects on the growth of native plant that coexist at sites invaded by *I. glandulifera*. Growth of coexisting species in invaded sites has not yet been compared with non-invaded sites. This may shed light on to the impacts of *I. glandulifera* on invaded soils and the resultant impact on native plant species in terms of plant growth. Native plants grown in invaded soil predicted to exhibit decreased performance and here this, and the presence of a positive PSF created by *I. glandulifera* was tested for by growing *I. glandulifera* and common co-occurring native species in invaded and uninvaded soils. In order to test if the impacts of *I. glandulifera* on native plant species performance is mediated by soil biota, sterilised soil was also used, which is expected to increase performance of the native plant species tested. Additionally, the mycorrhizal colonisation of native plant species was quantified in order to determine if *I. glandulifera* affects performance of natives by depleting the mycelial network in invaded soils, and if this has an impact on the performance of common native plants.

## 2.2. METHODOLOGY

### 2.2.1 Site descriptions

Three experimental field sites of *I. glandulifera* invasion were selected in Durham, Northern England, UK (Figure 1). The first was located in a managed mesotrophic grassland. The site is dominated by a monoculture of invasive *I. glandulifera* with the commonest native herbaceous species being *Urtica dioica* and *Festuca rubra*, among others. The second and third sites were located in an ancient deciduous woodland with a mixture of tree species, including ash (*Fraxinus excelsior*), sycamore (*Acer pseudoplatanus*), oak (*Quercus robur*) and beech (*Fagus sylvatica*). Commonly occurring plant species in the woodland include *U. dioca*, *Rumex obtusifolius* and *Heracleum sphondylium* and the grass species *Dactylis glomerata* and *Milium effusum*. The woodland is frequently managed by local volunteers. Management and restoration activities include strimming of vegetation, *I. glandulifera* pulling, as well as planting and sowing of woodland plants.


***Figure 1.*** *Location of the three experimental field sites, Durham, UK, where seeds and soil were collected. Ordnance Survey Grid references are included for each site. [Image © Google Earth].*

### 2.2.2 Native species selection and seed collection

A single phase pot experiment was conducted in order to measure the growth of native plant species that co-occur with invasive *I. glandulifera*, in invaded soils from different origins. In October 2016, seeds from three invasive populations of *I. glandulifera* were collected at the three field sites, from patches where *I. glandulifera* is locally dominant. Seeds of *I. glandulifera* were collected from at least 30 individuals at each site and dry stored for approximately four months. Based on observations of the native vegetation at each site, two common forbs, *Rumex obtusifolius* and *Urtica dioica*, and a common grass *Dactylis glomerata* were selected. *U. dioica* in particular was chosen for the study because of its high competitive ability and co-occurrence with *I. glandulifera* in both its native and invaded ranges (Beerling & Perrins 1993). Seeds of *R. obtusifolius* were collected in the same manner and locations as those of *I. glandulifera*. *D. glomerata* and *U. dioica* seeds were obtained from Emorsgate Seeds (https://wildseed.co.uk/), which were harvested from stock plants that were originally sourced from wild plant populations. All seeds were cold-wet stratified at 5°C for eight weeks before being transferred onto trays of sterilised sand on the 27th April 2017 and subsequently left to germinate for two weeks. The seeds were housed in a greenhouse with an average temperature of 21°C and under natural light conditions. Seeds that successfully germinated were transplanted into 200 ml pots according to the experimental design (see below).

### 2.2.3 Soil collection

Soil was collected directly from the field sites where *I. glandulifera* seeds were collected, to improve the ecological realism of the study and create a more spatially explicit design. One site was selected at the grassland site and two at the woodland site. At each site, a *I. glandulifera*- invaded patch and an immediately adjacent non-invaded patch were selected. Soil was sampled from multiple randomly selected points within each patch and bulked together. The soil was collected using hand trowels, at approximately 15 cm in depth. Approximately 14 L of soil was collected per patch, per site and transported back to the greenhouse and stored for one day, after which it was sieved to remove coarse material. Soil collected from the first woodland site was particularly wet, so samples were placed in a drying oven for 50°C for two hours. 1.4 L of soil from each patch (invaded *versus* non-invaded), from each site was not sterilised and kept to be used as a live inoculum. The remaining 12.6 L of soil per patch, per site was sterilised by autoclaving at 121°C for 35 minutes and left to cool overnight. This was to create a background substrate to be used in the experiment. Sterilisation is a method commonly used in plant-soil feedback experiments to assess the influence of soil biota on plant growth (Brinkman *et al.* 2010; Perkins *et al.* 2013; Dawson & Schrama 2016).

### 2.2.4 Experimental design

Single germinated seedlings were transplanted into 210 ml pots containing soils with two treatments; invaded or uninvaded, and sterilised or unsterilised soil. Each treatment was applied to soil from the three separate field sites, and for each of the four plant species, resulting in 48 pots per block (Figure 2).

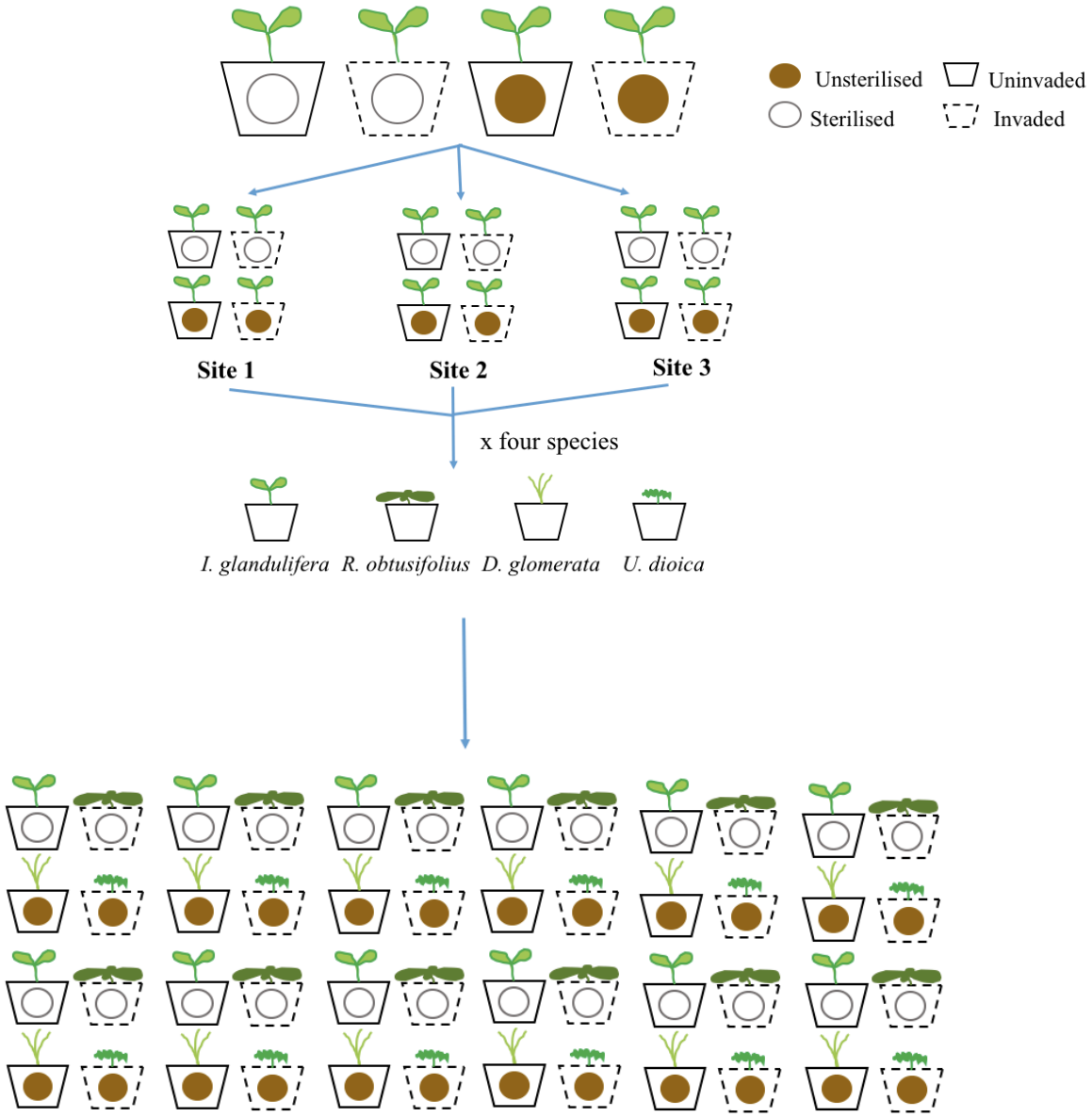


***Figure 2.*** *Experimental set up of a single block in the pot experiment. The fully factorial design includes two soil treatments; sterilised versus unsterilised soil and invaded versus uninvaded soils. Soils were then sourced from three field sites and three native plant species (*R. obtusifolius, D. glomerata and U. dioica*) and* I. glandulifera *were tested. This resulted in 48 pots per block, with a total of seven blocks for the experiment.*

Pots in the unsterilised treatment contained unsterilised field-collected soil in a 1:3 mix with a sterilised soil background from the same field site (and either invaded or uninvaded). The sterilised treatment contained only soil that had been sterilised from a particular site, and that was invaded or uninvaded. The whole design was replicated seven times, resulting in a total of 336 pots. All pots were placed on the same bench in the greenhouse, with individual pots within each block arranged randomly and each block's position on the bench rotated weekly. The seedlings were left to establish for one week and watered daily with approximately 50 ml of water. After this week, individual plant height, length of the longest leaf and number of leaves was recorded weekly for each individual plant. Data were collected for five weeks in total, which minimised pot limitation (Poorter *et al.* 2012), as individual plants were not allowed to grow to a large size. Plant height was used to calculate a relative growth rate for each individual plant over the experimental period using Equation 1, where *T1* is the first time point and *T2* is the final time point.

$$\text{Plant height} = \left( \frac{\text{height}_{T2} - \text{height}_{T1}}{\text{no. weeks}} \right) \quad [1]$$

At week five, aboveground biomass of all surviving plants was harvested, dried at 70°C for three days before the biomass was weighed. Individuals of *U. dioica* had low survival rates, resulting in small sample sizes for this species.

### 2.2.5 Measuring root colonisation by arbuscular mycorrhizal fungi

In order to assess if there was a relationship between plant growth and mycorrhizal association of each species, a subsample of belowground biomass from the experimental pots was harvested. *I. glandulifera* is known to be weakly dependent on mycorrhizal fungi (Beerling & Perrins 1993), whereas *R. obtusifolius* is largely non-mycorrhizal. The mycorrhizal dependency of *D. glomerata* and *U. dioica* is unclear so root analyses were undertaken to assess how strongly these species associate with AMF and if this association is affected by the soil conditions created by *I. glandulifera*. Roots were harvested from surviving *D. glomerata* and *U. dioica* plants that were grown in unsterilised soils from all three sites and from both invasion treatments. A total of 23 *U. dioica* plants and 18 *D. glomerata* plants were sampled and roots washed with water and then stored in water for four days at 5°C.

To assess fungal colonisation, roots in each sample were bleached by placing them in 10% potassium hydroxide solution in a water bath at 80°C for approximately five minutes. Samples were then washed and stained with a 5% Parker® Quink Black Ink and vinegar (acetic acid) solution and placed into a water bath again at 80°C for approximately five minutes. After washing again thoroughly until clear, the stained roots were stored in test tubes in 50% glycerol at room temperature in a dark cupboard for one week to allow excess ink to be removed. The stained roots in each sample were then mounted on

slides for microscopic analysis. Using light microscopy at 100x magnification, the presence of mycorrhizal structures was recorded systematically per 100 views per slide. The presence of vesicles (Figure 3a), arbuscules (Figure 3b) and hyphae (Figure 3b) was recorded. From this, percentage colonisation of the plant roots by arbuscular mycorrhizae was calculated, expressed as a percentage of the total number of views per slide.

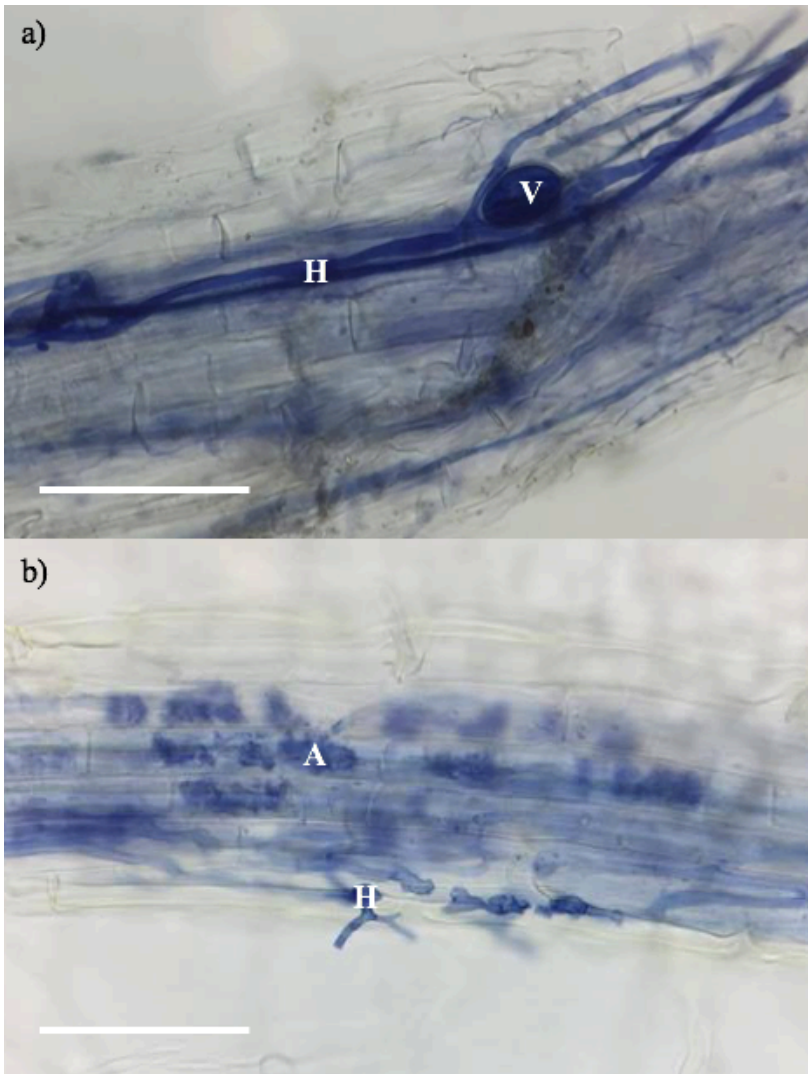


***Figure 3.*** *Light microscope images of arbuscular mycorrhizal structures identified in roots of* D. glomerata*, bleached with KOH and stained with ink. a) AMF vesicle (V) associated with hyphae (H). b) Clusters of AMF arbuscules (A) and hyphae (H). Scale bars: 75µm.*

### 2.2.6 Statistical analyses

Data for relative growth rate and final aboveground biomass were analysed separately per species in a linear mixed effects model using the 'lmerTest' package in R. Invasion status, sterilisation and soil origin were set as fixed factors, with block number set as a random factor. Interactions between factors

were considered and subsequent post-hoc comparisons were performed to determine significant differences between groups of treatments. Post-hoc comparisons were performed using the 'glht' function from the R package 'multcomp'. Group comparisons were specified according to the significant main effects and interactions between factors that were detected. Root data were also analysed with a separate linear mixed effects model per species, with invasion status and site as fixed effects, and block as a random factor. Post-hoc comparisons for these data were also performed using the 'glht' function. All analyses were conducted in R, version 3.3.1 (R Core Team, 2017).

## 2.3 RESULTS

### 2.3.1. Growth rate

#### 2.3.1.1. *I. glandulifera*

There was a significant three-way interaction between invasion, sterilisation and soil origin for relative growth rate, using height, for *I. glandulifera* ($F_{2,72}$ = 4.15, $P$ = 0.01). Overall, there was a significant main effect of site ($F_{2,72}$ = 12.45, $P$ < 0.001), with individuals exhibiting greater growth in soils from site one than two ($t_{72}$ = 4.98, $P$ < 0.001; Figure 4) and better growth in soils from site three, compared to site two ($t_{72}$ = -2.83, $P$ = 0.01; Figure 4). There was no overall difference in growth in soils between sites one and three ($t_{72}$ = 2.15, $P$ = 0.09).

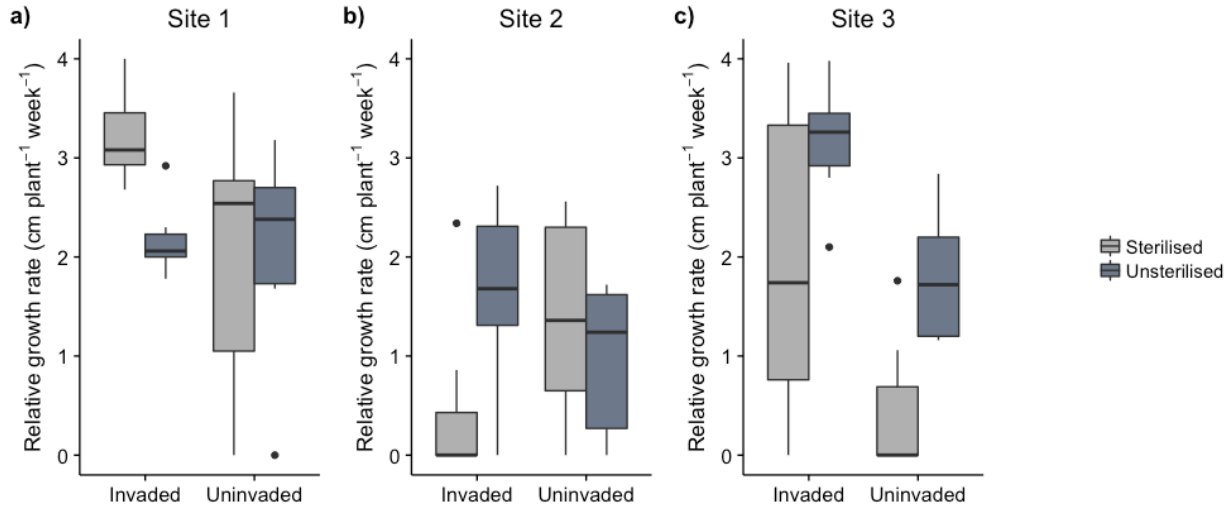


***Figure 4**. Relative growth rate, using height measured (cm plant$^{-1}$ week$^{-1}$) of* I. glandulifera *plants grown in invaded versus uninvaded soils, sterilised versus unsterilised soils, in field-collected soils from three sites; a) site one, b) site two, c) site three. Bold lines of the boxplots represent the median, thin lines on boxplots represent upper and lower quartiles, with boxes representing the interquartile range. Lines above and below boxplots represent maximum and minimum observations excluding outliers. Black dots are outliers representing any data points more or less than 1.5x interquartile range.*

For plants grown in soils from site one, *I. glandulifera* growth rate was significantly higher in sterilised invaded soils than uninvaded sterilised soils ($z$ = 2.71, $P$ = 0.05; Figure 4a), this effect was also seen in plants grown in soils from site three ($z$ = 2.99, $P$ = 0.01; Figure 4c) and in plants that were grown in unsterilised soils from site three ($z$ = 2.67, $P$ = 0.05; Figure 4c). In uninvaded soils from site three, sterilisation negatively affected *I. glandulifera* growth rate ($z$ = 2.62, $P$ = 0.05; Figure 4c). There were no significant differences between groups in site two soils (Figure 4b).

### 2.3.1.2 *R. obtusifolius*

For *R. obtusifolius* plants, there was a significant three-way interaction between invasion and sterilisation status of the soil, and soil origin ($F_{2,66} = 4.04$, $P = 0.01$). There was also a significant main effect of site ($F_{2,66} = 4.52$, $P = 0.01$) and *R. obtusifolius* plants that grew in soils from site one had a greater growth rate than those grown in site two ($t_{66} = 2.82$, $P = 0.01$; Figure 5) and three soils ($t_{66} = 2.31$, $P = 0.05$; Figure 5).

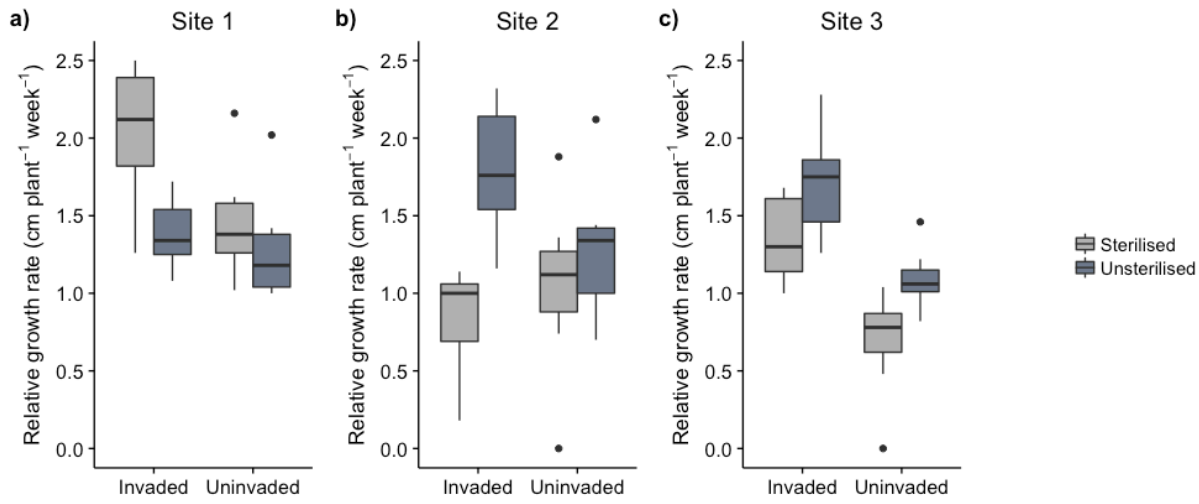


***Figure 5***. *Relative growth rate, using height measured (cm plant$^{-1}$ week$^{-1}$) of* R. obtusifolius *plants grown in invaded versus uninvaded soils, sterilised versus unsterilised soils, in field-collected soils from three sites; a) site one, b) site two, c) site three. Bold lines of the boxplots represent the median, thin lines on boxplots represent upper and lower quartiles, with boxes representing the interquartile range. Lines above and below boxplots represent maximum and minimum observations excluding outliers. Black dots are outliers representing any data points more or less than 1.5x interquartile range.*

Sterilisation positively influenced growth rate of *R. obtusifolius* plants that were grown in invaded soils from site one ($z = 3.01$, $P = 0.01$; Figure 5a), and growth rate was also higher in plants that grew in sterilised invaded soils, compared to sterilised uninvaded soils ($z = 2.67$, $P = 0.05$; Figure 5a). In plants that grew in site two soils, the only significant comparisons were between sterilised and unsterilised invaded soils, where sterilisation decreased growth rate of *R. obtusifolius* plants ($z = 4.45$, $P < 0.001$; Figure 5b). In soils from site three, plants exhibited a greater growth rate in both sterilised ($z = 3.95$, $P < 0.001$; Figure 5c) and unsterilised ($z = 3.48$, $P = 0.001$; Figure 5c) soils from invaded patches, compared to uninvaded patches.

### 2.3.1.3 *D. glomerata*

A three-way interaction between soil origin, sterilisation and invasion was also detected for growth rate of *D. glomerata* plants ($F_{2,66}$ = 10.09, $P$ < 0.001). Soil origin was also highly significant ($F_{2,66}$ = 29.62, $P$ < 0.001) and *D. glomerata* plants again showed the highest growth rates in soils from site one compared to sites two ($t_{66}$ = 7.50, $P$ < 0.001; Figure 6) and three ($t_{66}$ = 5.23, $P$ < 0.001; Figure 6).

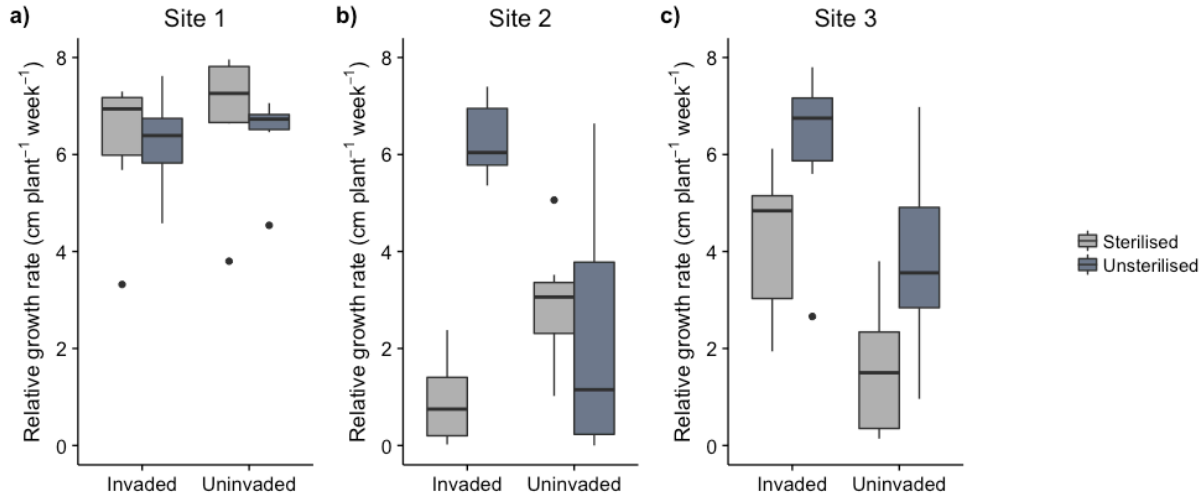


***Figure 6**. Relative growth rate, using height measured (cm plant$^{-1}$ week$^{-1}$) of* D. glomerata *plants grown in invaded versus uninvaded soils, sterilised versus unsterilised soils, in field-collected soils from three sites; a) site one, b) site two, c) site three. Bold lines of the boxplots represent the median, thin lines on boxplots represent upper and lower quartiles, with boxes representing the interquartile range. Lines above and below boxplots represent maximum and minimum observations excluding outliers. Black dots are outliers representing any data points more or less than 1.5x interquartile range.*

The growth rate of *D. glomerata* individuals that were grown in soils from site one was not significantly influenced by invasion or sterilisation (Figure 6a). However in site two soils, sterilisation negatively affected plant growth in invaded soils ($z$ = 6.13 $P$ < 0.001; Figure 6b) and individuals performed better in unsterilised soil from invaded patches, compared to uninvaded patches ($z$ = 4.91, $P$ < 0.001; Figure 6b). *D. glomerata* individuals also exhibited greater growth rates in invaded sterilised ($z$ = 2.92, $P$ = 0.01; Figure 6c) and unsterilised ($z$ = 2.83, $P$ = 0.05; Figure 6c) soil, compared to soil from uninvaded patches.

### 2.3.1.4 *U. dioica*

Soil origin significantly affected growth rate of *U. dioica* ($F_{2,72}$ = 36.59, $P$ < 0.001) and plants grew better in soils from site one compared to site two ($t_{72}$ = 7.73, $P$ < 0.001; Figure 7) and three ($t_{72}$ = 7.04, $P$ < 0.001; Figure 7). A significant interaction was detected between sterilisation and soil origin ($F_{2,72}$ = 9.58, $df$ = 2, $P$ < 0.001) and between invasion and soil sterilisation ($F_{1,72}$ = 4.03, $df$ = 1, $P$ = 0.01).

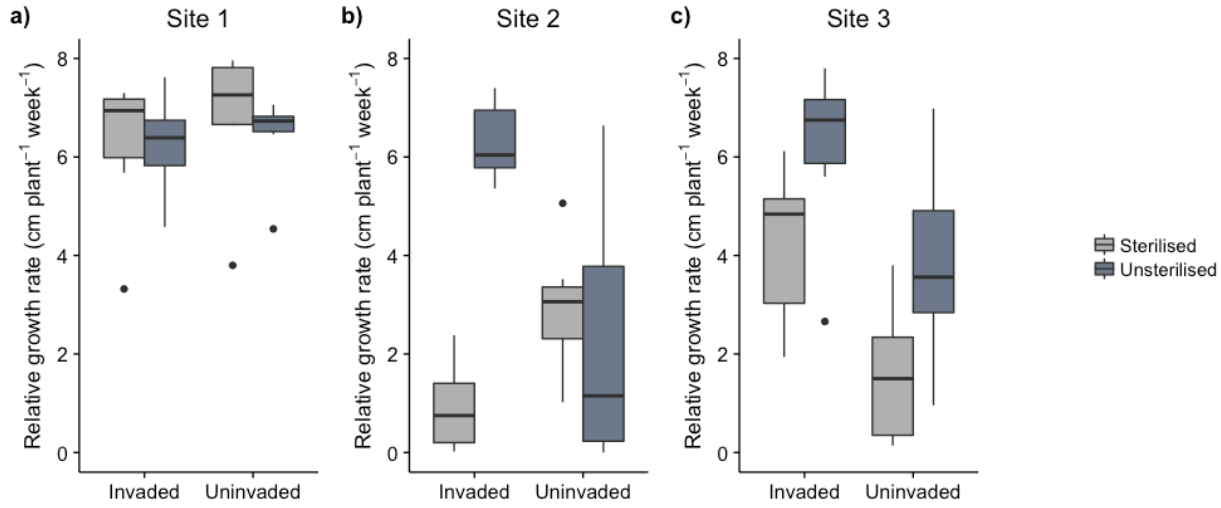


***Figure** 7. Relative growth rate, using height measured (cm plant$^{-1}$ week$^{-1}$) of* U. dioica *plants grown in invaded versus uninvaded soils, sterilised versus unsterilised soils, in field-collected soils from three sites; a) site one, b) site two, c) site three. Bold lines of the boxplots represent the median, thin lines on boxplots represent upper and lower quartiles, with boxes representing the interquartile range. Lines above and below boxplots represent maximum and minimum observations excluding outliers. Black dots are outliers representing any data points more or less than 1.5x interquartile range.*

The growth rate of *U. dioica* plants in site one soils was significantly greater in sterilised soils ($z = 3.37$, $P = 0.001$; Figure 7a), but sterilisation negatively affected growth in site three soils ($z = 2.41$, $P = 0.01$; Figure 7c). There were no significant comparisons between *U. dioica* growth rate in invaded and uninvaded soils, in any of the soils (Figure 7; Appendix 1).

### 2.3.2 Biomass

#### 2.3.2.1 *I. glandulifera*

For biomass of *I. glandulifera* plants there was a significant interaction between invasion and soil origin ($F_{2,66} = 4.15$, $P = 0.01$) and also between soil sterilisation and soil origin ($F_{2,66} = 7.84$, $P < 0.001$). There was also a main effect of site ($F_{2,66} = 14.73$, $P < 0.001$) where *I. glandulifera* biomass was significantly greater when plants were grown in soils from site one compared to soils from site two ($t_{66} = 5.30$, $P < 0.001$; Figure 8) and site three ($t_{66} = 3.68$, $P = 0.001$; Figure 8).

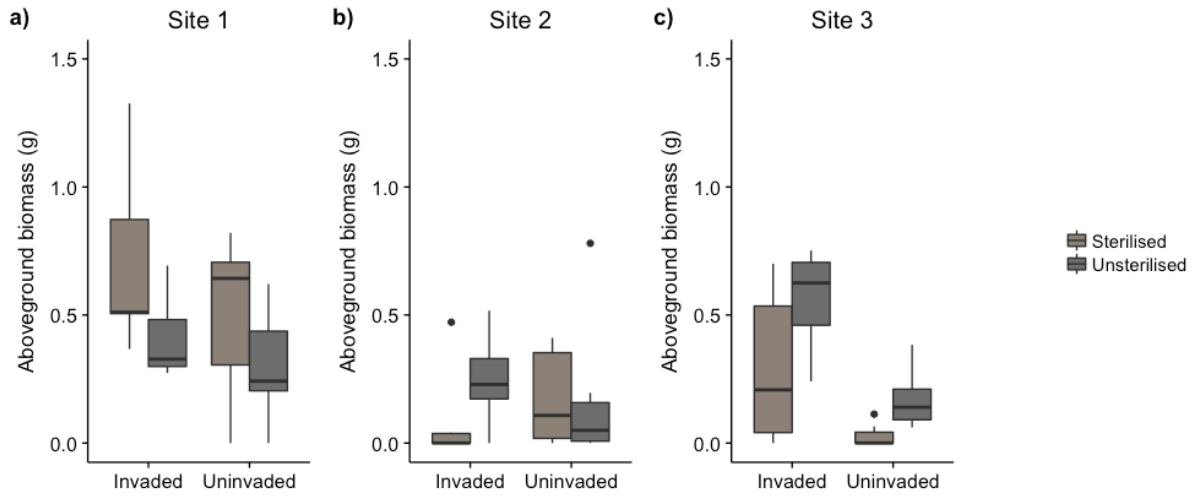


***Figure 8.*** *Dry aboveground biomass of* I. glandulifera *plants grown in invaded versus uninvaded soils, and sterilised versus unsterilised soils, in field-collected soils from three sites; a) site one, b) site two, c) site three. Bold lines of the boxplots represent the median, thin lines on boxplots represent upper and lower quartiles, with boxes representing the interquartile range. Lines above and below boxplots represent maximum and minimum observations excluding outliers. Black dots are outliers representing any data points more or less than 1.5x interquartile range.*

Biomass of *I. glandulifera* plants was significantly greater in site three soils that were invaded, compared to uninvaded ($z$ = 3.67, $P$ < 0.001; Figure 8c), but there were no differences in biomass produced in invaded *versus* uninvaded soils from sites one ($z$ = 1.74, $P$ = 0.227; Figure 8a) or two ($z$ = 0.10, $P$ = 0.999; Figure 8b). Sterilisation had a positive effect on *I. glandulifera* biomass production in site one soils ($z$ = 2.68, $P$ = 0.01; Figure 8a), a negative effect in site three soils ($z$ = 2.24, $P$ = 0.05; Figure 8c) and no significant effect in site two soils ($z$ = 0.86, $P$ = 0.77; Figure 8b).

#### 2.3.2.2 *R. obtusifolius*

There was a significant three-way interaction between invasion, sterilisation and soil origin for biomass of *R. obtusifolius* plants ($F_{2,72}$ = 3.37, $P$ = 0.01). There was a significant main effect of site ($F_{2,72}$ = 30.08, $P$ < 0.001) and *R. obtusifolius* biomass was significantly higher when plants were grown in soils from site one compared to site two ($t_{72}$ = 7.61, $P$ < 0.001; Figure 9) and site three ($t_{72}$ = 5.13, $P$ < 0.001; Figure 9).

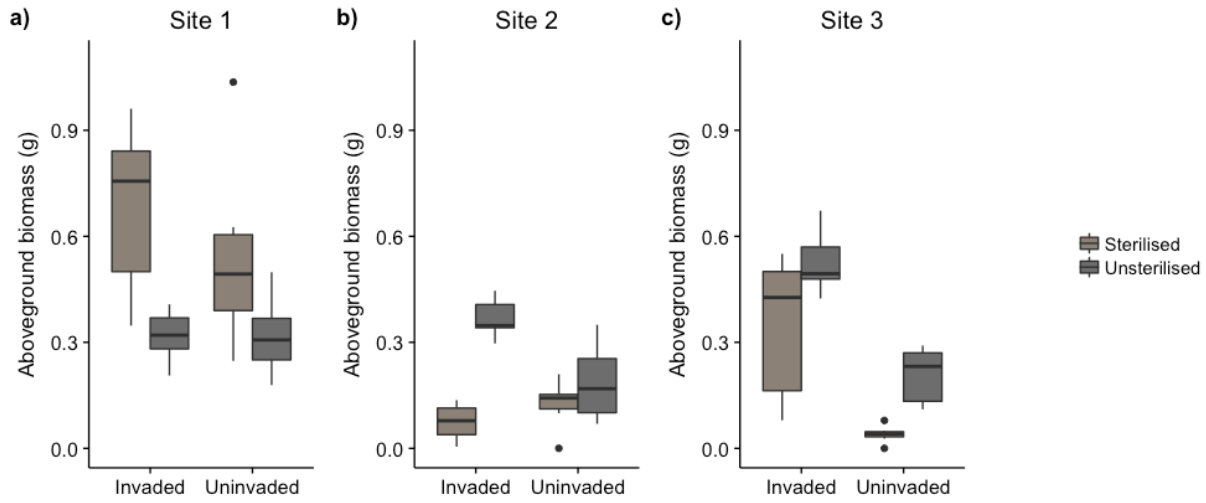


***Figure 9.*** *Dry aboveground biomass of* R. obtusifolius *plants grown in invaded versus uninvaded soils, and sterilised versus unsterilised soils, in field-collected soils from three sites; a) site one, b) site two, c) site three. Bold lines of the boxplots represent the median, thin lines on boxplots represent upper and lower quartiles, with boxes representing the interquartile range. Lines above and below boxplots represent maximum and minimum observations excluding outliers. Black dots are outliers representing any data points more or less than 1.5x interquartile range.*

Aboveground biomass of *R. obtusifolius* plants was significantly greater in sterilised invaded ($z = 4.97$, $P < 0.001$) and sterilised uninvaded soils ($z = 3.06$, $P = 0.01$) from site one, compared to unsterilised soils. (Figure 9a). In site two soils, biomass production in invaded soils was negatively affected by soil sterilisation ($z = 4.09$, $P < 0.001$; Figure 9b), but not in uninvaded soils ($z = 0.83$, $P = 0.99$; Figure 9b). Biomass production of *R. obtusifolius* was greater in both sterilised ($z = 4.17$, $P < 0.001$) and unsterilised ($z = 4.45$, $P < 0.001$) soils from invaded patches in site three (Figure 9c).

### 2.3.2.3 *D. glomerata*

For *D. glomerata* plants, soil origin was significant in influencing plant biomass ($F_{2,66} = 62.97$, $P < 0.001$) and *D. glomerata* biomass was greatest in soils from site one compared to sites two ($t_{66} = 10.64$, $P < 0.001$; Figure 10) and three ($t_{66} = 8.42$, $P < 0.001$; Figure 10). There was a significant two-way interaction between invasion and sterilisation ($F_{1,66} = 7.78$, $P = 0.001$), between invasion and soil origin ($F_{2,66} = 6.20$, $P = 0.001$) and between sterilisation and site ($F_{2,66} = 17.54$, $P < 0.001$).

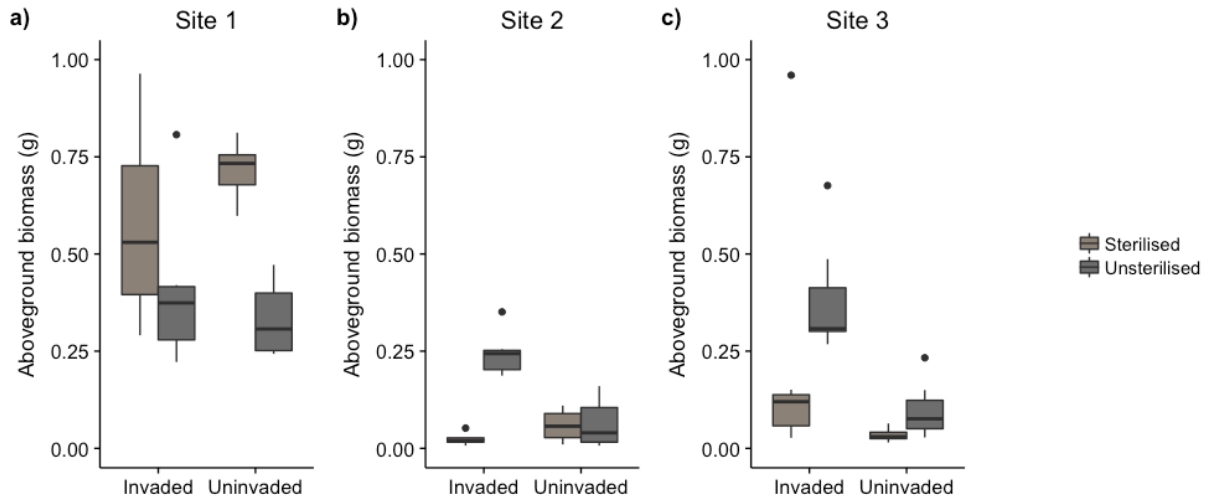


***Figure 10.*** *Dry aboveground biomass of* D. glomerata *plants grown in invaded versus uninvaded soils, and sterilised versus unsterilised soils, in field-collected soils from three sites; a) site one, b) site two, c) site three. Bold lines of the boxplots represent the median, thin lines on boxplots represent upper and lower quartiles, with boxes representing the interquartile range. Lines above and below boxplots represent maximum and minimum observations excluding outliers. Black dots are outliers representing any data points more or less than 1.5x interquartile range.*

Invasion status of the soil significantly influenced plant biomass in soils from site three (Figure 10), where biomass production of *D. glomerata* plants was greater in invaded, compared to uninvaded soils ($z = 3.46$, $P = 0.001$; Figure 10c). Plants that grew in unsterilised soils from invaded patches produced more biomass than plants grown in unsterilised soils from uninvaded patches ($z = 2.25$, $P = 0.05$; Figure 10). Sterilisation only affected biomass of plants grown in soils from site one (Figure 10), where plants produced significantly more biomass in soils that were sterilised ($z = 4.49$, $P < 0.001$; Figure 10a).

### 2.3.2.4 *U. dioica*

As with the other species, biomass production of *U. dioica* plants was significantly affected by soil origin ($F_{2,66} = 15.38$, $P < 0.001$; Figure 11). Biomass was greater when plants were grown in soils from site one compared to site two soils ($t_{66} = 5.19$, $P < 0.001$; Figure 11) and site three soils ($t_{66} = 4.27$, $P < 0.001$; Figure 11). There was also a significant interaction between sterilisation and soil origin ($F_{2,66} = 6.08$, $P = 0.001$), where plant biomass was greater in site one soils that had been sterilised ($z = 2.73$, $P = 0.01$; Figure 11a). There were no significant differences between *U. dioica* biomass grown in sterilised *versus* unsterilised soils from site two ($z = 0.71$, $P = 0.86$; Figure 11b) or three ($z = 2.10$, $P = 0.10$; Figure 11c).

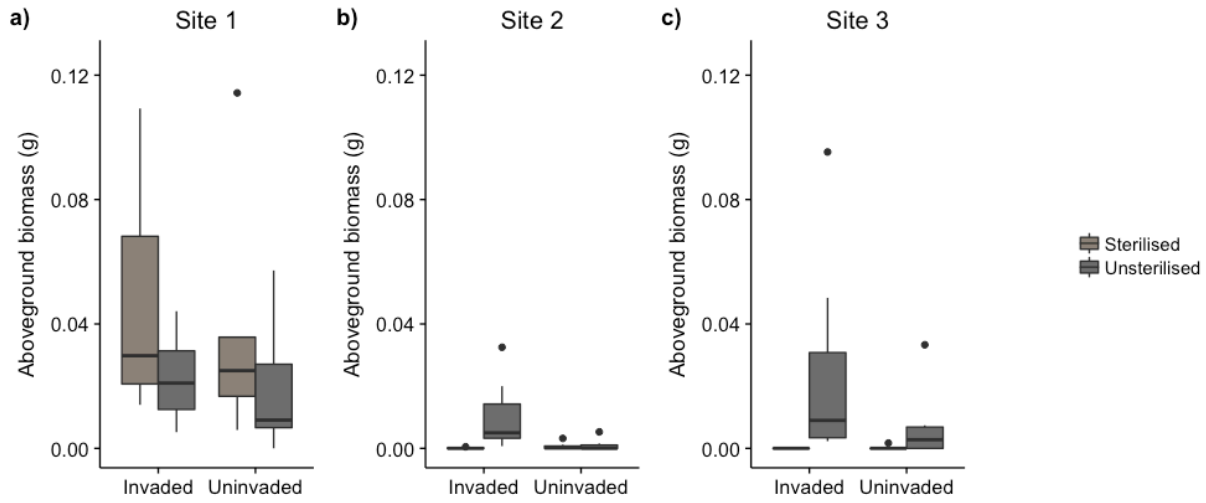


***Figure 11.*** *Dry aboveground biomass of* U. dioica *plants grown in invaded versus uninvaded soils, and sterilised versus unsterilised soils, in field-collected soils from three sites; a) site one, b) site two, c) site three. Bold lines of the boxplots represent the median, thin lines on boxplots represent upper and lower quartiles, with boxes representing the interquartile range. Lines above and below boxplots represent maximum and minimum observations excluding outliers. Black dots are outliers representing any data points more or less than 1.5x interquartile range.*

### 2.3.3 Root arbuscular mycorrhizal colonisation

Soil origin significantly affected mycorrhizal colonisation of *D. glomerata* roots ($F_{2,10}$ = 8.20, $P$ = 0.001), with plant roots grown in site three soils being colonised significantly more by arbuscular mycorrhizal fungi than roots from plants grown in site one ($t_9$ = -3.93, $P$ = 0.001; Figure 12a). There was a significant interaction between invasion and site ($F_{2,3}$ = 3.15, $P$ = 0.05), with mycorrhizal colonisation higher in uninvaded soils from site one ($z$ = 2.16, $P$ = 0.05; Figure 12a). There was no significant difference between mycorrhizal colonisation of *D. glomerata* roots grown in invaded *versus* uninvaded soils from site two ($z$ = 1.24, $P$ = 0.51; Figure 12a) or site three ($z$ = 1.68, $P$ = 0.25; Figure 12a).

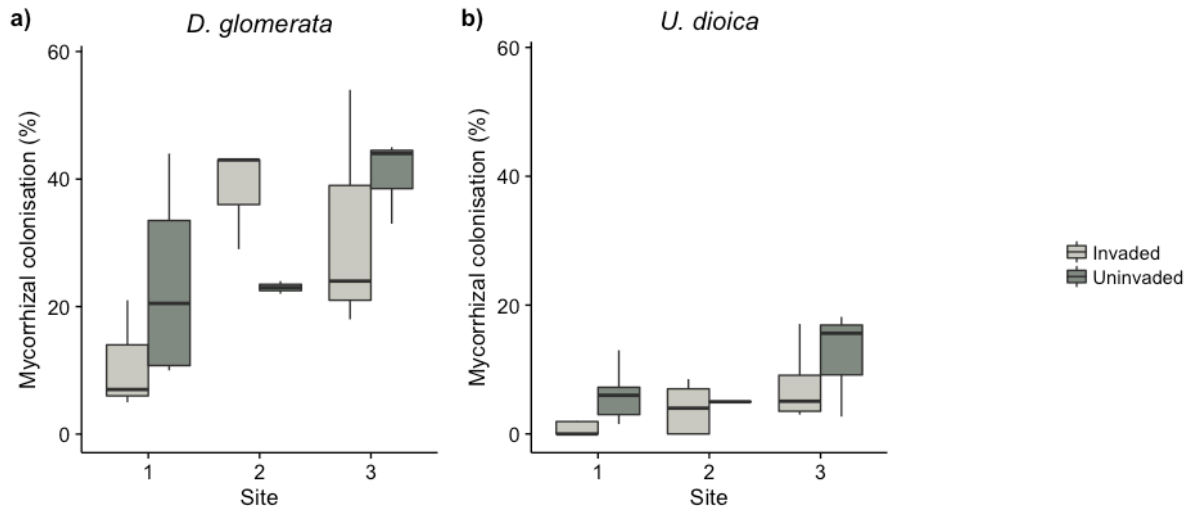


***Figure 12.*** *Mycorrhizal colonisation, measured by the number of mycorrhizal structures present in a subsample of roots, from a)* D. glomerata *and b)* U. dioica *plants grown in unsterilised field-collected soils from three sites. Bold lines of the boxplots represent the median, thin lines on boxplots represent upper and lower quartiles, with boxes representing the interquartile range. Lines above and below boxplots represent maximum and minimum observations excluding outliers.*

Soil origin significantly affected mycorrhizal colonisation of *U. dioica* plant roots ($F_{2,17}$ = 3.59, $P$ = 0.01), with arbuscular mycorrhizal colonisation being significantly higher in plant roots grown in soils from site three than roots grown in site one soils ($t_{17}$ = -2.62, $P$ = 0.01; Figure 12b). There were no significant differences in arbuscular mycorrhizal colonisation of *U. dioica* roots grown in site two soils compared to site one ($t_{17}$ = -0.32, $P$ = 0.95; Figure 12b) and site three soils ($t_{17}$ = -1.65, $P$ = 0.25; Figure 12b). Invasion status of the soil in which *U. dioica* plants were grown in did not significantly affect arbuscular mycorrhizal colonisation of roots ($F_{1,17}$ = 2.33, $P$ = 0.15; Figure 12b).

## 2.4 DISCUSSION

The concept of individual plant species interacting with their soil environment, creating a feedback has become well-recognised in invasion ecology (van Grunsven *et al*. 2007; Callaway *et al.* 2004a; Klironomos 2002). Multiple lines of evidence suggest that invasive plant species can introduce novel mechanisms of plant-soil interactions and alter soil microbial communities, which subsequently affect native plant productivity and growth (Batten *et al.* 2008; Mangla *et al.* 2008; Beckstead *et al.* 2010; Li *et al.* 2014; Day *et al.* 2015). However, the results presented here have shown that the effects of invasive plant species on native plant growth, mediated through the soil community, are not always straightforward.

In this study I showed a significant effect of soil origin in plant-soil interactions. The growth of invasive *I. glandulifera* and selected native plant species was site-dependent, which makes discerning the soil-mediated effects of *I. glandulifera* difficult. All species demonstrated the greatest relative growth rates (RGRs) in soils from site one and, as expected, *I. glandulifera* exhibited evidence of a positive PSF, with both RGR and biomass production being greatest in invaded, compared to uninvaded soils. This is in agreement with more definitive evidence of *I. glandulifera* creating a strong positive feedback (Pattison *et al.* 2016) and more general evidence of non-native invasive plants growing better in their 'home' soils (van der Putten *et al*. 2007b; Kulmatiski *et al*. 2008; Pendergast *et al*. 2013). It is commonly thought that mutualisms, such as associations with AMF, are most responsible for driving this positive feedback for non-native plants (Klironomos 2002; Zhang *et al*. 2010b; Bever *et al.* 2012).

Previous evidence has shown that invasive *I. glandulifera* has a low mycorrhizal dependency and exhibits reduced mycorrhizal colonisation when grown in home soil, compared to when grown in soil conditioned by other species (Pattison *et al*. 2016). These findings are in line with the predictions of the degraded mutualisms hypothesis, where invasive plants may reduce beneficial mutualists in invaded soils, to the detriment of native plant species performance (Vogelsang & Bever 2009). On the contrary, this study found that at site two and three, *I. glandulifera* produced more biomass and had a higher growth rate in invaded soils that had not been sterilised, indicative of a mutualistic effect. However, sterilisation of site one soils positively affected *I. glandulifera* biomass and RGR, which suggests the mutualistic associations of *I. glandulifera* may be dependent on the environmental context and local soil conditions. The soil biota present at site one may be more inhibitory towards plant growth than the soil biota at site three, which showed evidence of a mutualistic effect on plant growth. Indeed, there is evidence that the benefits of mycorrhizal associations may be context-specific; for example plant response to mycorrhizal symbiosis may vary with soil fertility and the complexity of the soil community, among other factors (Hoeksema *et al.* 2010). *I. glandulifera* plants grown in site two soils were not affected by invasion and sterilisation, as measured by aboveground biomass or RGR, which suggests that *I. glandulifera* may not make any significant changes to the soil biota in these soils.

Alternatively, there may be some differences in other factors that were not measured in this study due to time constraints, for example soil nutrients, which may have contributed to the observed results.

The native plant species *U. dioica* and *R. obtusifolius* also showed a similar increase in growth with sterilisation of site one soils on growth and biomass production, as *I. glandulifera,* suggesting this may be the action of generalist pathogenic biota that may have a stronger effect than the difference in soil biota between invaded and uninvaded patches. *D. glomerata* biomass also responded positively to sterilisation of site one soils, but with no effects on RGR, suggesting that it may be a good competitor at *I. glandulifera*- invaded sites. Roots of *U. dioica* growing in site one soils showed significantly lower rates of mycorrhizal colonisation than those from site three, suggesting the mycorrhizal community at site one may be depleted or the site conditions are less favourable for mycorrhizal symbiosis. However, no difference in mycorrhizal colonisation of *U. dioica* roots in invaded and uninvaded soils was detected. Contrary to this, roots of *D. glomerata* that grew in site one invaded soils exhibited reduced mycorrhizal colonisation compared to uninvaded soils. This supports the notion that *I. glandulifera* may deplete mycorrhizae in the areas it invades (Ruckli *et al*. 2014a; Pattison *et al*. 2016) but this effect is context- and species-specific as this effect was not seen in soils from sites two and three.

The majority of the invasive plant literature finds that native plant species perform poorly when grown in soil conditioned by invasive plant species (Batten *et al.* 2008; Suding *et al.* 2013). The results of the present investigation contrast with this consensus, as both *R. obtusifolius* and *D. glomerata* performed well in soils from invaded patches, despite mycorrhizal colonisation of *D. glomerata* being higher in uninvaded soils. Additionally, removal of soil biota by sterilisation in site two invaded soils, significantly decreased growth of *D. glomerata* and *R. obtusifolius*. This suggests that the soil conditions created in *I. glandulifera*- invaded patches may generally benefit the native plant species studied, as well as *I. glandulifera* itself, possibly through the action of soil mutualists. There is evidence that high abundances of mutualists can be supported during some plant invasions, and not always with a concurrent high plant diversity (Lekberg *et al*. 2013). Although these generalist mutualists may also associate with native species, they may disproportionately affect invasive *I. glandulifera*, allowing it to reach high local abundances. Cui & He (2009) also found evidence that soil biota may have positive mutualistic effects on both invasive and native plant species, but that the invader may benefit more from mutualistic soil biota. Alternatively, *I. glandulifera* may increase levels of, for example, N-fixing microbes, increasing local nutrient availability (Ehrenfeld 2003; Batten *et al*. 2008). Due to the high plasticity of this invader (Skálová *et al.* 2012), *I. glandulifera* may be able to better capitalise on local nutrient availability, compared to native plant species.

The precise mechanistic explanations for the observed patterns during *I. glandulifera* invasions is not entirely clear from these results. It is expected that if *I. glandulifera* reduces mycorrhizal colonisation

of natives growing in invaded soils (Ruckli *et al*. 2014a), then mycorrhizal associations would be significantly lower in plant roots that had grown in invaded soils. However, this effect was highly dependent on the soil origin and only seen in *D. glomerata* roots grown in site one soils. The effect of soil mutualists on plant growth may thus be context-dependent, and possibly affected by the local soil biota, which may affect the overall outcome of plant-soil interactions. For example, the soil biota at site two and three, which had a positive effect on plant growth may act to degrade any allelochemicals by *I. glandulifera* that act on fungal mutualists (Inderjit *et al.* 2011a; Cipollini *et al.* 2012; Li *et al.* 2015). The presence and distribution of AMF is not only contingent on the presence of an invader, but abiotic soil conditions, such as pH (Dumbrell *et al*. 2010). Therefore, no firm conclusion can be made, without further supporting evidence that *I. glandulifera* is influencing the presence of AMF in this study. Additionally, there have been suggestions that plant functional group, rather than invasive ability, may be a more significant factor influencing plant-AMF interactions (Lekberg *et al*. 2013).

Similarly, all native species' RGR and biomass showed a negative response to soil sterilisation and increased growth in invaded soils from the third site, suggestive of a mutualistic effect of the local soil biota. Roots from both *D. glomerata* and *U. dioica* grown in soils from site three had the highest mycorrhizal colonisation, suggesting that the soil biota present in this soil was most mutualistic and contained high amounts of mycorrhizal fungi, which may have increased the nutrient acquisition of these species, thus increasing their growth rate. Less is known about the allelopathic influence of invasive *I. glandulifera* on the growth of co-occurring native plant species in the field, as opposed to germination inhibition demonstrated in bioassays (Ruckli *et al*. 2014b; Gruntman *et al*. 2017). This study found no evidence that any allelochemicals produced by *I. glandulifera* and released into the soil have any negative effects on native plant performance. However, soil microbes have been shown to significantly affect allelopathy (Inderjit *et al*. 2011a; Cipollini *et al.* 2012), so any allelochemicals released in invaded soils may actually have been degraded by adapted soil microbes (Li *et al.* 2015).

Plant-soil interactions are often evaluated in isolation of the whole ecological community, but the use of soil samples from three field sites in this study has primarily shown the environmental context-dependency of plant-soil interactions during plant invasions. The consideration of the role of other ecological processes in plant-soil interactions is crucial to applying findings meaningfully in different contexts. PSFs can vary greatly in a heterogeneous environment, such as with time, so applying individual plant-soil interactions to a community context may not be representative of complete community conditions (Casper *et al*. 2008; Hawkes *et al*. 2013). Environmental factors, such as plant-plant interactions and resource availability can significantly impact soil communities and the outcome of PSFs (Johnson *et al*. 2003; Larios & Suding 2015). This response is also specific to the plant species in question (Bever 1994; Klironomos 2002; Bezemer *et al*. 2006; Manning *et al*. 2008), so it is important to add ecological realism and address this context-dependency. Relating the effects of soil

biota to non-native plant invasions has revealed discrepancies, which suggests that soil communities may not always have consistent effects on invasion trajectories (Levine *et al*. 2004). Nonetheless, this investigation has shown that both *I. glandulifera* and the native plant species studied perform better on invaded, compared to uninvaded soils and this effect is consistent.

Although the present study has shown that the soil microbial community can affect plant species growth and biomass production, this study cannot determine if these effects were indeed mediated by invasive *I. glandulifera*. Additionally, other soil conditions, such as nutrient content, bacterial, nematode and microarthropod biomass were not investigated. This makes the exact mechanisms driving these plant responses unclear, as these factors can also drive feedback effects (Casper *et al.* 2008; Harrison & Bardgett 2010; Pattison *et al.* 2016). The outcome of feedback effects can also be time dependent, with some feedback effects taking a significant time to develop (Bonanomi *et al*. 2005; Kulmatiski & Beard 2011). Therefore, the plant species growth observed here may primarily reflect the study time period, as feedback effects can become apparent over an array of timescales (van der Stoel *et al*. 2002; Grman & Suding 2010). These results are most relevant to early plant growth stages, with the possibility that the feedback effects here could be dynamic and be different later in the growing season.

This investigation has provided preliminary evidence that AMF may be involved in influencing native plant growth during *I. glandulifera* invasions. An important finding demonstrated here is that invasion of *I. glandulifera* may not necessarily limit the growth of the native plant species studied in invaded soils. The greater growth of *I. glandulifera* and the native plant species on invaded soils was shown to be partially dependent on the effects of the invader on soil microbes. However, further work is required to investigate the variation in these effects and identify the specific soil microbes involved. This study has shown that in field contexts, the local soil biota and site environmental conditions may significantly influence the outcome of plant-soil interactions, which many greenhouse studies do not reflect. In order to elucidate to the ecologically relevant soil-mediated impacts of plant invasions, the site dependency of such effects should be considered. The present findings, that *I. glandulifera* exhibits a positive PSF compared to common native plant species, potentially through its effects on local AMF, is next applied in the following chapter to the context of restoring native plant diversity at invaded sites. Another aspect of *I. glandulifera* invasion, allelopathy, is also addressed through the use of soil remediations to decrease the negative effects of allelopathy in the field.

# Chapter 3:

# Belowground restoration methods for control of invasive *Impatiens glandulifera*.


## ABSTRACT

Invasive plant species have considerable and far-reaching impacts on plant biodiversity, and thus it is important to mitigate against these impacts. The majority of management approaches to control invasive plant species often do not consider native plant restoration once an invasive plant has been successfully removed, yet there is evidence that this could help prevent re-invasion. The importance of plant-soil feedbacks (PSFs) in determining plant community structure and function is well-recognised and consequently ecologists are utilising knowledge of PSFs to remediate the negative soil conditions created by invasive plant species. *I. glandulifera* is one such invader that alters soil microbial communities through allelopathy and disruption of mutualisms, but no assessment of the utility of soil treatments in its control have yet been made. Here I test a combination of two treatments, activated carbon (AC) and arbuscular mycorrhizal fungal (AMF) spores, in a field experiment at two sites of *I. glandulifera* invasion. Percentage cover of invading *I. glandulifera* and co-occurring native plant species was recorded. Contrary to expectations, *I. glandulifera* responded positively to additions of AC and no effect of AMF addition was detected. Consequently, treatments including AC and AMF may not be useful in attempts to ecologically restore native plant communities invaded by *I. glandulifera*.

## 3.1 INTRODUCTION

Invasive non-native plant species are a significant threat to global biodiversity, with wide-ranging impacts that present a problem to management efforts (Mack *et al*. 2000; Richardson *et al*. 2000a). Where invasive plants become established, they often become dominant in a community, altering plant competitive interactions, reducing native plant species richness and diversity and changing habitat structure (Vitousek *et al*. 1997; Asner *et al*. 2008). Invasive plants have far-reaching ecosystem effects, disrupting pollination (Traveset & Richardson 2006), altering disturbance regimes (Mack & D'Antonio 1998) and altering ecosystem processes and functioning, including carbon cycling and water use (Richardson *et al*. 2000a; Ehrenfeld 2003; Liao *et al*. 2008). Owing to the extensive impacts of invasive plant species, there great effort is afforded to control invasive species, at multiple stages of their invasion.

Management of invasive plant species and their impacts involves targeting efforts and resources at particular stages of the invasion pathway. Considerable effort is afforded to preventing non-native plants from becoming established and naturalised in the UK (Roy *et al*. 2014), but if this does occur managing the resultant impacts of an invasive plant is more difficult. Efforts to manage naturalised exotic plant species includes physical and chemical removal of the invader, and biological control. Current methods for invasive plant control in the UK primarily focus on physical removal whereas restoration of the invaded ecosystem with native plant species, once the invader has been successfully removed, is often side-lined. Restoring native plant biodiversity to the invaded community can aid in recovery of fundamental ecosystem processes, such as nutrient cycling, and help to prevent further invasions (Averett *et al*. 2004; Simberloff *et al*. 2013).

The significant role of plant-soil interactions in structuring plant communities (van der Putten 1997; Klironomos 2002) has led to some restoration ecologists to consider using belowground processes, such as PSFs, in order to control invasive plant species and increase native establishment (Kardol & Wardle 2010). PSFs describe the alterations of the soil environment by plants that feedback to influence subsequent plant growth. PSFs of invasive plant species may selectively alter the physical, chemical and biotic components of the soil in their introduced ranges (Ehrenfeld 2003; Kourtev *et al*. 2003; Hawkes *et al*. 2005; Mummey & Rillig 2006). Relative to native plant species, invasive plant species have been shown to create more positive PSFs for themselves, which may facilitate their invasion (Klironomos 2002; Levine *et al*. 2006; Reinhart & Callaway 2006; Kulmatiski *et al*. 2008; Corbin & D'Antonio 2011; van der Putten *et al*. 2013). For example, invasive plant species may exude allelochemicals that directly inhibit seedling germination (Hierro & Callaway 2003), or invaders may disrupt mutualisms, such as those involving AMF (Stinson *et al*. 2006; Callaway *et al*. 2008; Vogelsang & Bever 2009). These various mechanisms that create differential PSFs between invaders and natives may hinder restoration efforts. Even after an invasive plant has been physically removed, a remaining

soil legacy in the form of allelopathic chemicals or suppressed AMF abundance may decrease the subsequent establishment success of native species (Kulmatiski & Beard 2006; Grman & Suding 2010; Jordan *et al*. 2012). Consequently, this has led to the idea of remediating PSFs in invaded soil communities to increase native establishment (Bach *et al*. 2012; Ohsowski *et al*. 2012; Perkins & Hatfield 2016).

So far, restoration efforts aimed at remediating PSFs have had mixed success and may be highly context-dependent (Eviner & Hawkes 2008). Microbial treatments have included the use of fungal inoculants (Ohsowski *et al*. 2012; Perkins & Hatfield 2016), designed to enhance positive mutualistic plant-fungal interactions. Activated carbon (AC) is a relatively low-cost adsorbent that is regularly used in studies to reduce the negative allelopathic effects exerted by invasive plants (Callaway & Aschehoug 2000; Ridenour & Callaway 2001; Inderjit & Callaway 2003). As a result, AC is increasingly being used in restoration efforts to remove allelopathic effects and reduce microbial populations that drive strong positive feedbacks with invaders (Kulmatiski & Beard 2006; Kulmatiski 2011; Nolan *et al*. 2014).

Himalayan balsam (*Impatiens glandulifera*) is a vigorous annual invasive plant which, since its intentional introduction to the UK in 1839, has experienced an increase in abundance and distribution (Beerling 1993). Owing to its fast growth and explosive dehiscence of its seed capsules allowing for seed dispersal up to 5 m (Beerling & Perrins 1993), *I. glandulifera* is able to form dense monocultures in woodlands and riparian areas around the UK. The superior competitive ability of *I. glandulifera* consequently negatively affects the ecosystems it invades, significantly reducing plant species diversity (Hulme & Bremner 2006) and altering ecosystem services, such as reducing bee pollination of native plants species (Chittka & Schurkens 2001). In addition to whole-ecosystem impacts, *I. glandulifera* has been shown to reduce other plant species growth through allelopathy (Scharfy *et al*. 2011; Vrchotová *et al*. 2011; Ruckli *et al*. 2014b; Gruntman *et al.* 2016) and depletion of the mycelial network of AMF (Tanner & Gange 2013; Ruckli *et al*. 2014a; Pattison *et al*. 2016). In this way, it is posited that *I. glandulifera* is able to create a positive PSF that may contribute to its invasive success (Pattison *et al*. 2016).

Current control of *I. glandulifera* in the UK is focused aboveground and includes 'balsam bashing' work parties to physically remove stands of *I. glandulifera* each year. This management is labour- and time-intensive, relies on generous volunteers and is often ineffective at reducing local abundances of the invader. Recently, work has identified a suitable biocontrol candidate for *I. glandulifera* control in the UK, a rust fungus native to the western Himalayas (Tanner *et al*. 2015). Although this rust has been released at authorised locations, UK populations of *I. glandulifera* vary greatly in their susceptibility to the rust fungus, suggesting another rust strain will be required to control the invader (Varia *et al*. 2016).

Biocontrol however, is a controversial management option, which if often associated with unforeseen consequences of introducing an addition exotic species, and is also expensive (Simberloff 2012; Simberloff & Stiling 2016).

Overall, control for *I. glandulifera* is difficult and strategies are still lacking for some habitat types (Dawson & Holland 1999; Wadsworth *et al*. 2000). The economic cost of invasive non-native species to the UK is estimated to be around £1.7 billion (Williams *et al*. 2010), thus there is a need to invest in control and restoration methods that are more cost-effective. Therefore, a soil microbial management approach that reduces the abundance and/or establishment of the invasive plant in question, whilst simultaneously increasing the restoration potential of native plant species, would be highly desirable. This investigation aimed to assess the potential for soil remediation at sites invaded by *I. glandulifera*, in order to inform subsequent management efforts. Soil treatments of AC and mycorrhizal fungi were added to experimental field plots in a fully factorial design and subsequent effects on *I. glandulifera* and native plant species were measured. Prior to this, the early germination of *I. glandulifera* (Beerling & Perrins 1993) compared to co-occurring native species at the experimental sites was confirmed and measured, so as to best inform when soil treatments would be best applied.

## 3.2 METHODOLOGY

### 3.2.1. Germination of *I. glandulifera* versus native species

Preliminary data were gathered to assess the differential germination of *I. glandulifera* and co-occurring native plant species. At the three field sites mentioned in Chapter 2, 20 randomly selected 1 $m^2$ plots were set up in invaded patches of *I. glandulifera*. In each plot, the number of *I. glandulifera* and native seedlings was counted. Additionally, the percentage cover of *I. glandulifera*, other natives and moss was recorded. Weekly repeated measurements were conducted for four weeks, starting on the 6th March 2017 and ending on the 31st March 2017.

### 3.2.2 Field soil treatments

A field experiment was performed to examine the effect of soil manipulations on the establishment of native species in *I. glandulifera*- invaded patches. Experimental field plots were set up at the same two field study sites, described in 2.2.1. Prior to experimental set-up, the grassland site was prepared by rotavating the soil twice, and hand raking to break up existing *I. glandulifera* seedlings and leave bare, evenly-tilled soil. After this, eight 5 $m^2$ blocks of four 1 $m^2$ plots were marked out at 2 m in two rows (Figure 13). A buffer zone of 0.5 $m^2$ was left between each plot in a block. This resulted in a total of 32 experimental plots.

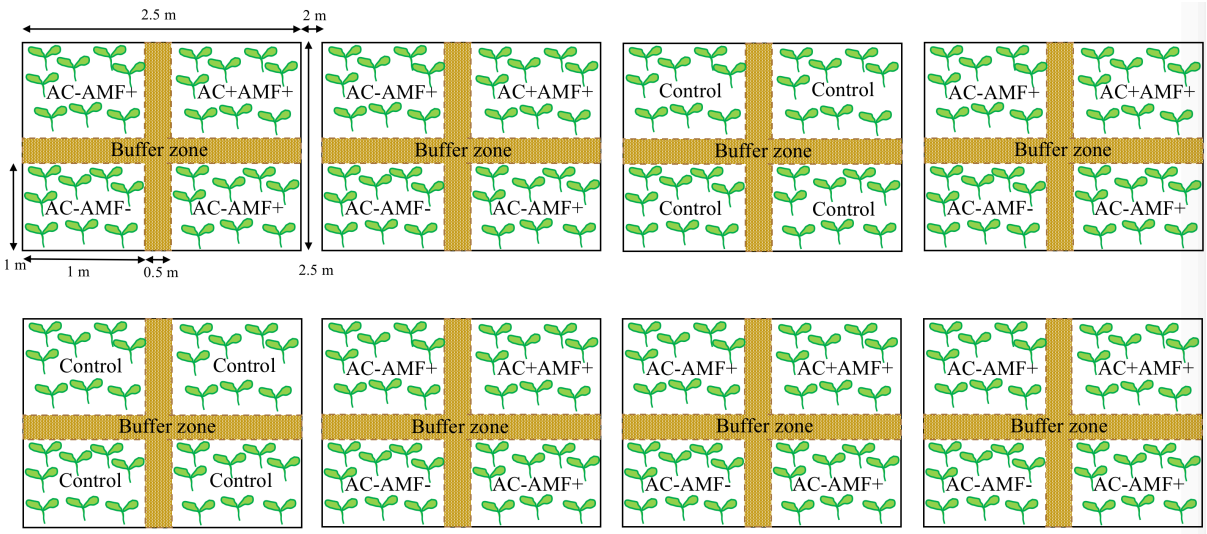


***Figure 13.*** *Experimental design of the field setup at the grassland site, with eight 5 $m^2$ experimental blocks each with four 1 $m^2$ plots of which contained a randomly-assigned combination of two treatments; arbuscular mycorrhizal fungi (AMF) and activated carbon (AC). The two plots marked control were raked only. A buffer zone of 0.5 $m^2$ was also set up between individual plots. AMF+/AC+ represent plots that received AMF/AC treatments and AMF-/AC- represent plots that received no AMF/AC treatments.*

In early April, the plots in six randomly chosen blocks were treated with 1.6 L of AC, 150 g of AMF spores and 150 g of an AMF-free carrier, which acted as a control, in a fully factorial design. The AMF and the AMF carrier were sourced from Symbiom (https://www.symbiom.cz/en), and the AC from Alcotec (http://www.the-home-brew-shop.co.uk/acatalog/Activated_Carbon.html). Each treatment was sprinkled evenly across each plot and hand-raked into the top 10 cm of soil. AC is a highly adsorptive compound, indiscriminately binding organic compounds, including allelopathic root exudates (Inderjit & Callaway 2003). Each plot in each of these six blocks received one of the four combinations of the two treatments. The two remaining blocks were kept as control blocks and were raked, but received no treatments.

All four plots in each block, apart from the control blocks, also received 4 g of a custom seed mix representative of a MG1 plant community, obtained from Emorsgate Seeds (https://wildseed.co.uk/). The MG1 seed mix was a composition of 80% grasses and 20% forbs. The mix included *Dactylis glomerata*, *Agrostis stolonifera*, *Poa pratensis*, *Lolium perenne*, *Festuca rubra*, *Avenula pratensis*, *Plantago lanceolata*, *Achillea millefolium*, *Anthriscus sylvestris* and *Centaurea nigra*.

The experimental set-up at the woodland site (Figure 14) was similar to the grassland site, with AMF and AC treatments, and four 1 $m^2$ plots in a block, but there were no control blocks or buffer zones between plots due to the limited number and size of suitable patches of *I. glandulifera* that could be identified at the site. The MG1 seed mix was not added to any of the plots in order to observe effects of the present seed bank. In addition, six 4 $m^2$ blocks were located in randomly chosen heterogeneous patches of *I. glandulifera* within the woodland. This resulted in a total of 24 experimental plots. The plots were checked regularly at the two sites and adjacent *I. glandulifera* plants encroaching on the blocks were cut back to prevent overcrowding and shading out of other plant species. In each plot at each site *I. glandulifera* percentage cover, grass percentage cover, forb percentage cover, and sward height was recorded. Four repeated measurements were carried out fortnightly for seven weeks at each site. At week seven, *I. glandulifera* individuals had been removed by unknown members of the public at site two, resulting in missing data for one block at site two during that week.

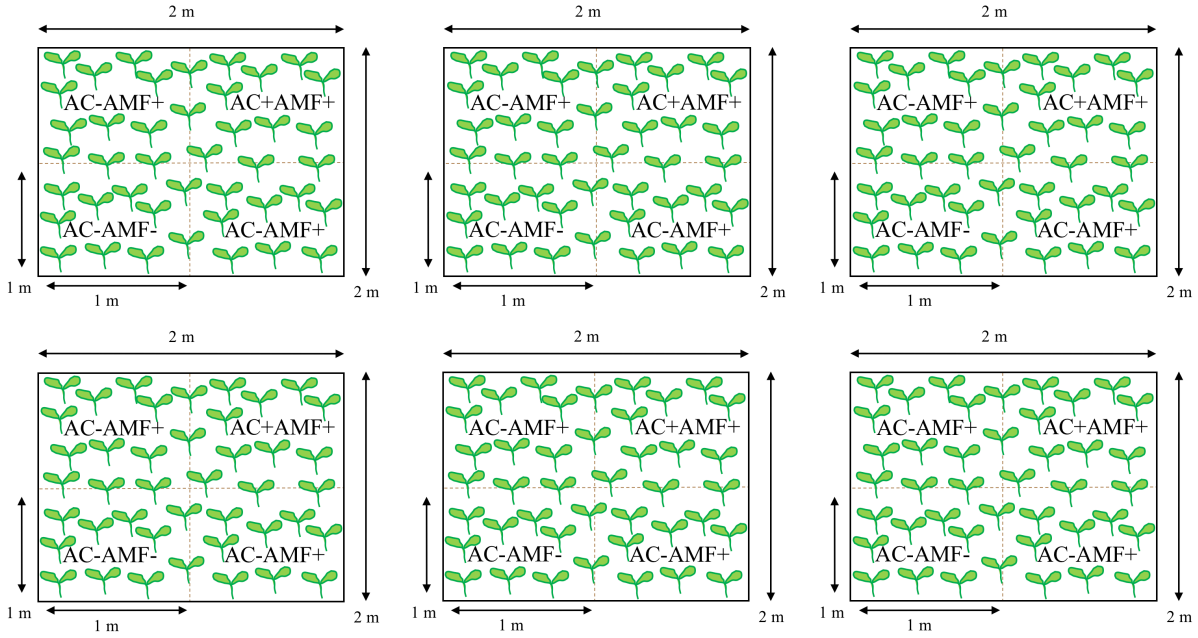


***Figure 14.*** *Experimental design of the field setup at the woodland site, with six 4 m² experimental blocks each with four 1 m² plots. Each individual block was randomly located in a patch of* I. glandulifera *at the woodland site. Each plot contained a randomly-assigned combination of two treatments; arbuscular mycorrhizal fungi (AMF) and activated carbon (AC). AMF+/AC+ represent plots that received AMF/AC treatments and AMF-/AC- represent plots that received no AMF/AC treatments.*

### 3.2.3 Statistical analyses

Count data for the germination investigation did not fit a classical Poisson distribution. So seedling counts were normalised using a log (+1)-transformation and placed as the response variable in a linear mixed effects model from the lmerTest package in R. Week of observation, species (*I. glandulifera* or native) and site were set as fixed factors and plot number, where observations were made, was set as a random factor to account for repeated measurements. Interactions between factors were considered and subsequent post-hoc comparisons were performed to determine significant differences between groups of treatments. Post-hoc comparisons were performed using the 'glht' function from the R package 'multcomp', Group comparisons were specified according to the significant main effects and interactions between factors that were detected.

Percentage cover data were normalised using a logit-transformation and data from each site were analysed in a separate linear mixed effects model. AMF and AC addition, along with species recorded (*I. glandulifera*, native forb or native grass) and week of observation were set as fixed factors. The replicate block was included as a random factor. Data from the control blocks at site one, which received only raking and no soil treatments, were not included in this model. Interactions between factors were considered and subsequent post-hoc comparisons were performed to determine significant differences

between groups of treatments. Post-hoc comparisons were performed using the 'glht' function from the R package 'multcomp'. Group comparisons were specified according to the significant main effects and interactions between factors that were detected. All analyses were conducted in R, version 3.3.1 (R Core Team, 2017).

## 3.3 RESULTS

### 3.3.1 Field germination

The number of *I. glandulifera* seedlings (log-transformed+1) that were counted were on average across sites and observations, an order of magnitude significantly greater than the number of seedlings of all other native plant species, ($F_{1,266}$ = 371.77, $P$ < 0.001; Figure 15), and more seedlings were counted at site one than at site two across all weeks of observation ($F_{1,38}$ = 122.51, $P$ < 0.001; Figure 15).

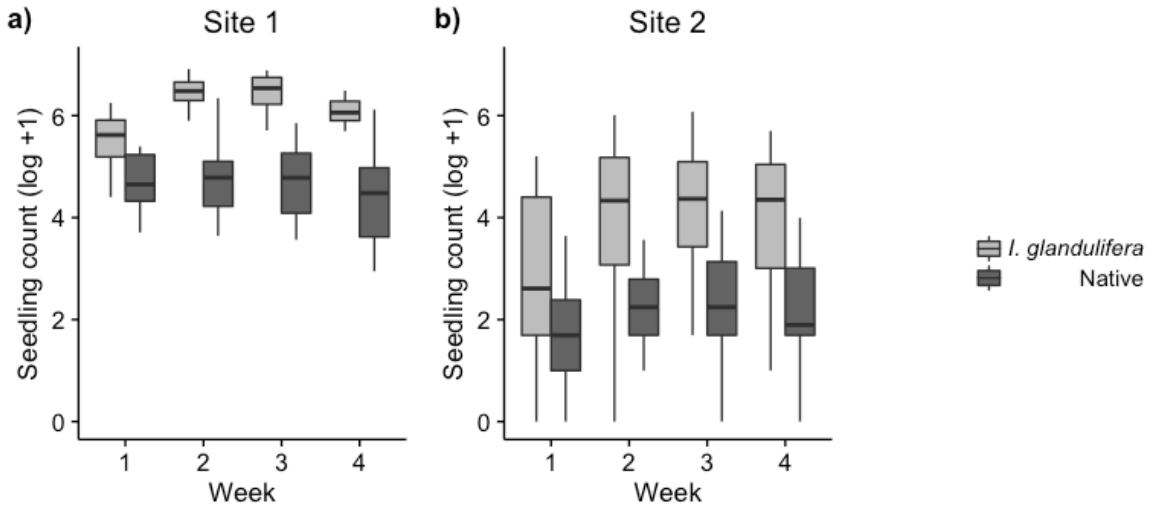


***Figure 15.*** *The number of seedlings counts (log +1) at each of the field study site;. a) Site one and b) Site two, across the four-week study period, for* I. glandulifera *and native plant species. Bold lines of the boxplots represent the median, thin lines on boxplots represent upper and lower quartiles, with boxes representing the interquartile range. Lines above and below boxplots represent maximum and minimum observations excluding outliers. Normalised transformed data were presented as original data did not follow the expected Poisson distribution.*

There was a significant two-way interaction between week of observation and species identity ($F_{3,266}$ = 4.87, $P$ = 0.01) and contrasts showed that at each week, seedling count of *I. glandulifera* was greater than that of native plant species (Figure 15; Appendix 2). The greatest significant difference between species seedling counts was at week three ($z$ = 11.03, $P$ < 0.001; Figure 15), where approximately 70 more seedlings of *I. glandulifera* were recorded, than native plant seedlings. For the duration of the experimental period, seedling count of native plant species remained at a similar level (Figure 15; Appendix 2), whereas seedling count of *I. glandulifera* significantly increased from week one to week four ($z$ = 4.89, $P$ < 0.001; Figure 15), but began to level off at week two (Figure 15; Appendix 2).

### 3.3.2 Percentage cover

#### 3.3.2.1 Site one

During the first week of measurements, percentage cover was significantly different between species ($F_{2,44}$ = 105.11, $P$ < 0.001; Figure 16). *I. glandulifera* cover was significantly higher than that of native forbs ($t_{44}$ = 10.29, $P$ < 0.001) and grasses ($t_{44}$ = 13.99, $P$ < 0.001) in the experimental plots and the cover of native forbs was significantly higher than that of native grasses ($t_{44}$ = 3.71, $P$ = 0.001). Plant percentage cover was generally greater in plots that did not contain added AC ($F_{1,44}$ = 3.03, $P$ = 0.05; Figure 16). The addition of AMF did not significantly affect percentage cover in the plots during week one ($F_{1,44}$ = 0.01, $P$ = 0.93; Figure 16).

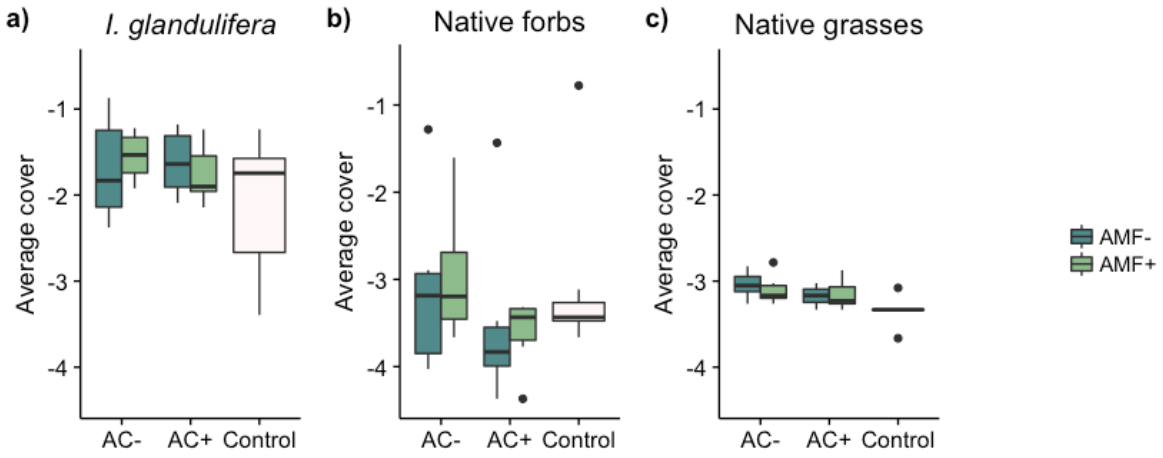


***Figure 16.*** *The effect of AMF and AC addition on percentage cover (logit transformed) recorded at site one, during the first week of observations for a)* I. glandulifera*, b) native forbs and c) native grasses. AC- denotes no addition of AC, and AC+ denotes addition of AC. The control treatments refers to blocks in the experiment that received only raking. Bold lines of the boxplots represent the median, thin lines on boxplots represent upper and lower quartiles, with boxes representing the interquartile range. Lines above and below boxplots represent maximum and minimum observations excluding outliers. Black dots are outliers representing any data points more or less than 1.5x interquartile range. Normalised transformed data were presented as original data did not follow the expected normal distribution.*

There was a significant interaction between AC addition and species, for percentage cover during week three ($F_{2,44}$ = 2.65, $P$ = 0.05), there were no significant differences between percentage cover of any species with AC addition, and without AC (Figure 17; Appendix 3a). At week three, cover of *I. glandulifera* was significantly greater than native forbs in the presence ($z$ = 6.08, $P$ < 0.001) and absence ($z$ = 3.70, $P$ = 0.001) of AC (Figure 17). Similarly, *I. glandulifera* was also greater than native grass cover in plots with ($z$ = 9.68, $P$ < 0.001) and without ($z$ = 6.41, $P$ < 0.001) AC addition (Figure 17).

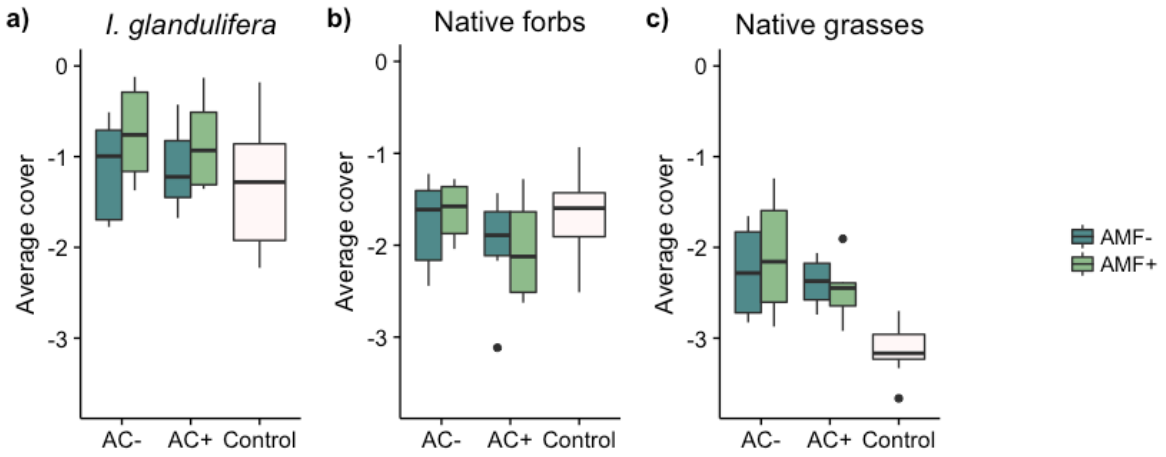


***Figure 17.*** *The effect of AMF and AC addition on percentage cover (logit transformed) recorded at site one, during the third week of observations for a)* I. glandulifera*, b) native forbs and c) native grasses. AC- denotes no addition of AC, and AC+ denotes addition of AC. The control treatments refers to blocks in the experiment that received only raking. Bold lines of the boxplots represent the median, thin lines on boxplots represent upper and lower quartiles, with boxes representing the interquartile range. Lines above and below boxplots represent maximum and minimum observations excluding outliers. Black dots are outliers representing any data points more or less than 1.5x interquartile range. Normalised transformed data were presented as original data did not follow the expected normal distribution.*

There was no significant effect of either AMF ($F_{1,44} = 0.59$, $P = 0.45$) or AC addition ($F_{1,44} = 0.23$, $P = 0.64$) on percentage cover at week five and *I. glandulifera* percentage cover remained higher than both native forbs ($t_{44} = 5.82$, $P < 0.001$) and grasses ($t_{44} = 10.68$, $P < 0.001$). At week seven, there was a significant interaction between AC addition and species recorded ($F_{2,48} = 2.81$, $P = 0.05$). Percentage cover of *I. glandulifera* was not affected by the addition of AC (Appendix 3b). *I. glandulifera* percentage cover was greater than grass cover, regardless of AC addition (Figure 18; Appendix 3b). However, *I. glandulifera* cover was only greater than native forb cover in the presence of AC ($z = 2.57$, $P = 0.05$; Figure 18). Addition of AMF did not affect percentage cover of any species in the experimental plots ($F_{1,48} = 1.05$, $P = 0.31$; Figure 18).

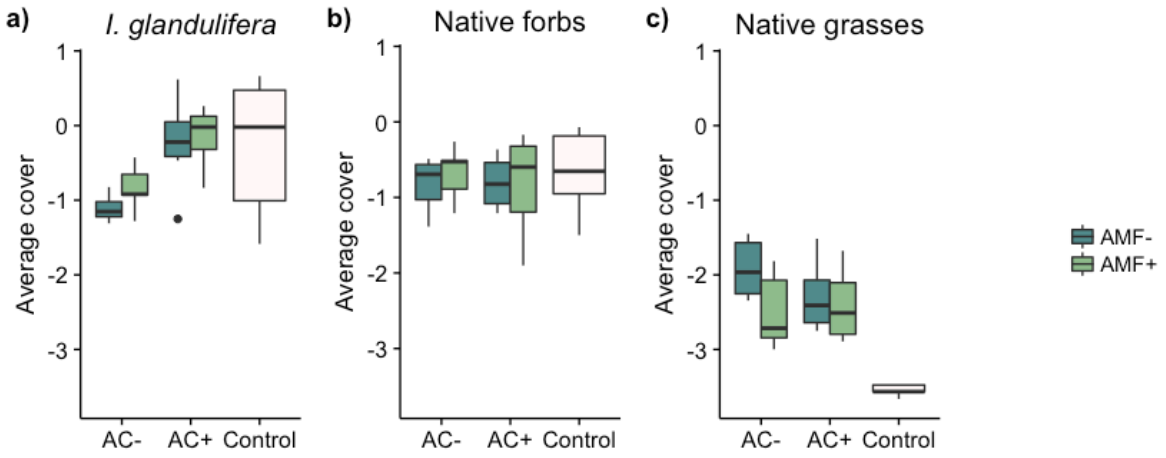


***Figure 18.*** *The effect of AMF and AC addition on percentage cover (logit transformed) recorded at site one, during week seven of observations for a)* I. glandulifera*, b) native forbs and c) native grasses. AC- denotes no addition of AC, and AC+ denotes addition of AC. The control treatments refers to blocks in the experiment that received only raking. Bold lines of the boxplots represent the median, thin lines on boxplots represent upper and lower quartiles, with boxes representing the interquartile range. Lines above and below boxplots represent maximum and minimum observations excluding outliers. Black dots are outliers representing any data points more or less than 1.5x interquartile range. Normalised transformed data were presented as original data did not follow the expected normal distribution.*

### 3.3.2.2 Site two

At site two, the addition of AMF or AC did not have a significant effect on species percentage cover at any time period (Table 1). Percentage cover was significantly different among the species recorded at each week of observation (Figure 19; Appendix 4a) and *I. glandulifera* percentage cover was significantly greater than native grass cover at each week of observation (Figure 19; Appendix 4b). *I. glandulifera* cover was only greater than native forb cover during week one (Figure 19; Appendix 4b).

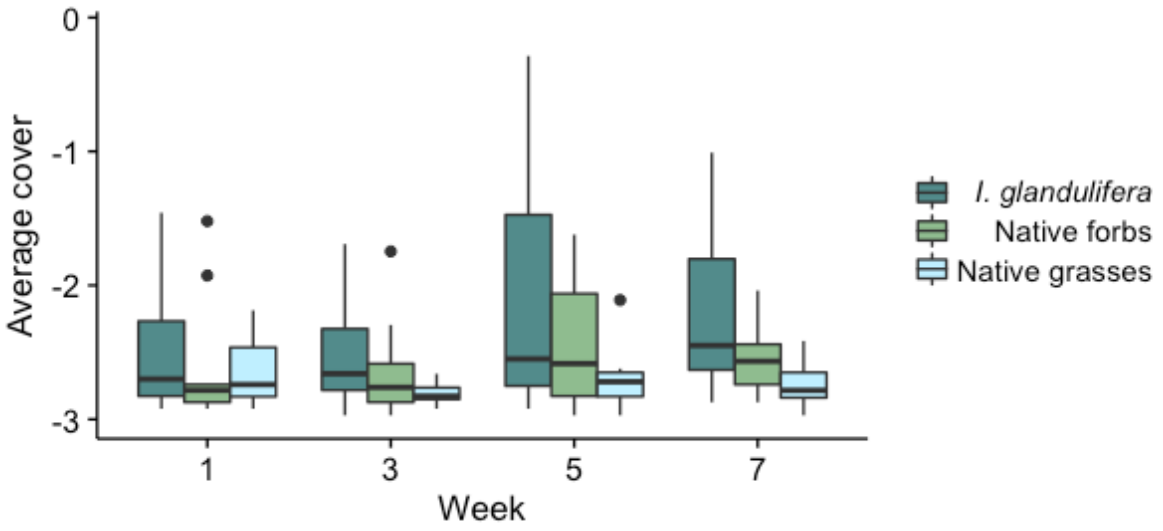


***Figure 19.*** *The change in percentage cover (logit transformed) of species recorded at site two, over the experimental period. Bold lines of the boxplots represent the median, thin lines on boxplots represent upper and lower quartiles, with boxes representing the interquartile range. Lines above and below boxplots represent maximum and minimum observations excluding outliers. Black dots are outliers representing any data points more or less than 1.5x interquartile range. Normalised transformed data were presented as original data did not follow the expected normal distribution.*

***Table 1.*** *Results of a linear mixed effects model testing for the effects of AMF and AC addition on the percentage cover of species recorded at site two, across the experimental period (weeks 1 – 7). * denotes an interaction between factors.*

| Week | Fixed factor | *F* | *df* | Residual *df* | *P* |
|---|---|---|---|---|---|
| 1 | AMF | 0.12 | 1 | 55 | 0.73 |
| | AC | 0.04 | 1 | 55 | 0.84 |
| | AMF*AC | 1.25 | 1 | 55 | 0.27 |
| | AMF*Species | 1.10 | 2 | 55 | 0.34 |
| | AC*Species | 0.06 | 2 | 55 | 0.94 |
| | AMF*AC*Species | 0.13 | 2 | 55 | 0.88 |
| 3 | AMF | 0.92 | 1 | 55 | 0.34 |
| | AC | 0.33 | 1 | 55 | 0.57 |
| | AMF*AC | 0.58 | 1 | 55 | 0.45 |
| | AMF*Species | 1.04 | 2 | 55 | 0.36 |
| | AC*Species | 0.57 | 2 | 55 | 0.57 |
| | AMF*AC*Species | 0.84 | 2 | 55 | 0.44 |
| 5 | AMF | 0.59 | 1 | 55 | 0.45 |
| | AC | 0.12 | 1 | 55 | 0.73 |
| | AMF*AC | 0.25 | 1 | 55 | 0.62 |
| | AMF*Species | 1.07 | 2 | 55 | 0.35 |
| | AC*Species | 0.15 | 2 | 55 | 0.86 |
| | AMF*AC*Species | 0.36 | 2 | 55 | 0.70 |
| 7 | AMF | 0.11 | 1 | 51 | 0.74 |
| | AC | 0.27 | 1 | 51 | 0.60 |
| | AMF*AC | 0.42 | 1 | 51 | 0.52 |
| | AMF*Species | 0.05 | 2 | 51 | 0.95 |
| | AC*Species | 0.27 | 2 | 51 | 0.76 |
| | AMF*AC*Species | 0.14 | 2 | 51 | 0.87 |

## 3.4. DISCUSSION

The potential role of plant-soil feedbacks in the plant invasion process is now well recognised, yet this soil ecological knowledge is rarely acknowledged in invasive plant species control efforts. This may explain why many attempts at restoring native plants to invaded areas has had limited success (Eviner & Hawkes 2008). The role of soil microbes has only recently become integrated into restoration ecology, which has stimulated research investigating the use of soil manipulations to augment invasive species control (Kulmatiski & Beard 2006; Harris 2009; Kardol & Wardle 2010; Ohsowski *et al*. 2012; Nolan *et al*. 2014).

In this investigation, in the field germination of *I. glandulifera* seedlings was greater than that of native plant seedlings, suggestive of a synchronous germination of the seedbank coupled with fast growth (Beerling & Perrins 1993). In the soil manipulation experiment, *I. glandulifera* cover was greater than the cover of native forbs and grasses, at each site, at each time period of the experiment, which was expected due to the high growth rate of *I. glandulifera* and plasticity which allows it to persist in varying environmental conditions (Perglová *et al*. 2009; Skálová *et al*. 2012).

The role of activated carbon in influencing the percentage cover of all species in this experiment is less clear. During the first week at site one, percentage cover of native species was greater in plots that contained added AC. These findings are in agreement with studies that show that the allelopathic effects of *I. glandulifera* can be alleviated with AC addition (Gruntman *et al*. 2014). The allelopathic napthoquinones that are exuded by *I. glandulifera* can inhibit native species growth, providing a competitive advantage to the invader (Vrchotová *et al*. 2011; Pattison *et al*. 2016), thus if this advantage is removed competing native species growth may be increased. Indeed, AC addition has also been shown to increase native plant species growth in soils invaded by other exotic plant species (Kulmatiski & Beard 2006; Kulmatiski 2011; Nolan *et al*. 2014).

It was assumed in this investigation that AC addition may increase native plant species growth through the removal of the competitive advantage afforded to *I. glandulifera* through allelopathy, by adsorbing organic compounds (Callaway & Aschehoug 2000; Inderjit & Callaway 2003). However, the effects of AC on subsequent plant growth may also be microbially-mediated. AC indiscriminately binds organic molecules, so may interfere with plant-microbe communication, reducing microbial activity disrupting potential positive plant-soil feedbacks (Kulmatiski & Beard 2006; Abhilasha *et al*. 2008; Kulmatiski 2011; Nolan *et al*. 2014). However, in this study it is unlikely that the effects of AC were caused by decreasing microbial activity, as percentage cover of *I. glandulifera* at week seven was greater in plots that contained AC, compared to plots without AC, suggesting AC addition may provide an advantage to *I. glandulifera* growth. Other studies have also found that some exotic plant species respond positively to AC addition (Kulmatiski & Beard 2006; Weißhuhn & Prati 2009). Additionally AC may

result in an increase in nutrient availability, such as phosphorus, which could stimulate microbial activity, promoting plant growth (Weißhuhn & Prati 2009). However, this investigation lacks the available data on the microbial community and its activity to be able to discern if this was the case.

At week seven, *I. glandulifera* cover was however only greater than native forb cover in the presence of AC, suggesting that seeding of the treatment plots may have been beneficial in increasing forb percentage cover during the course of the experiment. At site two, there was no effect of AC on plant species percentage suggesting that the effects of AC, in addition to being species specific, may also be context-dependent.

At both experimental field sites there was no significant effect of AMF addition on plant species percentage cover. *I. glandulifera* has been previously shown to alter AMF communities in invaded soils, depleting the mycelial network of native plant species and incurring a competitive advantage in the process (Tanner *et al*. 2013; Ruckli *et al*. 2014a; Pattison *et al*. 2016). In line with this, it was assumed in this investigation that additions of AMF to experimental field plots may consequently increase native forb and grass cover however the results of this investigation contradicted this assumption. Our results also contrast other studies which depict and increase in native plant species growth in invaded soils of other exotics that are treated with AMF inoculants (Ohsowski *et al*. 2012; Paluch *et al*. 2013; Middleton *et al.* 2015; Koziol & Bever 2017). Plant species vary in their responses to mycorrhizal colonisation (Klironomos 2003) and *I. glandulifera* performance has been shown to be negatively affected by AMF colonisation (Tanner *et al*. 2014).

Subsequent sampling of plant species for assessment of root mycorrhizal colonisation was not conducted in this study due to time constraints, although it was assumed that mycorrhizal colonisation of plant roots would be higher for plant species growing in the individual field plots that were treated with added AMF due to a greater abundance of AMF. However, these results suggest that AMF addition had no subsequent positive or negative effect on plant growth, which is unexpected. The addition of AC has been shown to modify plant-AMF relationships, through increasing nutrient availability such that the mutualism becomes less profitable for the host plant and AMF colonisation is decreased (Weißhuhn & Prati 2009). However, it is unlikely that this may have occurred in the current experiment, as no interaction was detected between AC addition and AMF addition in the experimental plots. Without root sampling, analyses of the soil biota and soil nutrient levels in the treated plots, a mechanistic explanation for the observed results cannot be elucidated.

The present results have shown that additions of activated carbon and AMF to *I. glandulifera*-invaded soils may provide little utility in attempts to ecologically restore native plant communities invaded by *I. glandulifera*. These results highlight the context-dependent nature of the use of AC as a restoration

tool, which is likely driven by invader identity and the target ecosystem. Instead, ensuring that native plant species germinate in equal abundances as *I. glandulifera*, by using custom seed mixes at sites, may prove useful for increasing the native seedbank and subsequent growth and competitive ability. Annual hand pulling of *I. glandulifera* at severely invaded sites before flowering and recent biocontrol efforts (Tanner *et al*. 2015) may also prove fruitful management tools. No single approach will likely be effective in controlling *I. glandulifera*, as this study has shown a highly complex nature to plant invasions, which should be considered when approaching management options.

# Chapter 4:
# Final conclusions

Invasive plant species are a significant component of anthropogenic global change and consequently are a threat to native plant biodiversity in their introduced ranges (Mack *et al*. 2000; Lewis & Maslin 2015). Evidence has shown that the number of the world's flora that will become naturalised in their introduced range will increase, and consequently the negative impacts they bring with them will also increase (van Kleunen *et al*. 2015; Seebens *et al.* 2017). The new biotic and abiotic conditions caused by climate change will also create new plant distributions for exotic species (Bellard *et al*. 2013). Thus it is important to study the impacts of invasive plant species on native flora in their introduced ranges, particularly for long-established and gregarious invaders, in order to better understand the mechanistic basis behind plant invasions and inform restoration efforts.

The literature reviewed in Chapter 1 has demonstrated the need to explore the mechanistic basis behind plant invasions. In particular, the role of plant-soil feedbacks and soil microbes in driving and influencing plant invasions is convincing. The wealth of evidence for the perturbation of PSFs and alteration of soil microbial communities during plant invasions is conclusive, and is therefore useful in predicting the invasiveness of exotic plant species and their potential impacts. Studies however often lack the ability to apply findings to an ecologically meaningful context, which can limit their utility. Plant-soil interactions are now an integral part of invasion ecology, which should be more widely recognised and integrated into management efforts. Soil remediations and restoration of native soil microbial communities at invaded sites have the potential to offer a contemporary focus and whole community approach to invasive plant management. More research, however, is required to discern if this knowledge has utility in management of invasive plants now naturalised in the UK.

The aim of this study was to assess the role of soil microbes in invasion of a prolific plant species, *Impatiens glandulifera*, non-native to the UK, via its impacts on native plant species. This mechanistic study was then supplemented by applying soil ecological knowledge to the restoration of sites invaded by *I. glandulifera*, through the use of soil treatments. *I. glandulifera* was selected as a study species because of its rigorous invasion of riparian areas in the north-east of England. Additionally, in comparison to other invasive plant species, it's plant-soil interactions and the role of these in its invasion are less well studied. Recently *I. glandulifera* was shown to exhibit a positive PSF (Pattison *et al*. 2016), which implicates plant-soil interactions in its invasion and thus warrants further study.

In Chapter 2, through a glasshouse study pot experiment I showed that there was significant difference in the growth of the native plant species studied, when they were grown in soil from sites invaded and not invaded by *I. glandulifera*. The significant effect of sterilisation on plant species growth that was demonstrated also implicates soil microbes in mediating invader-induced effects on native plant growth. I found evidence to corroborate the positive PSF created by *I. glandulifera* and depletion of the AMF community, which implicates plant-soil interactions in its invasion. However contrary to expectations, I found that the native plant species studied grew better on soils collected from patches of *I. glandulifera* invasion. These results are particularly interesting, especially given the wealth of literature reviewed in Chapter 1, which finds that in general native plant species exhibit greater performance in soils conditioned by conspecifics, rather than invasive plant species. These results suggest that in the case of some native plant species, the soil-mediated impacts of *I. glandulifera* may not necessarily limit plant growth.

The use of soil collected from field sites was intended to add ecological realism to the glasshouse study, but also provided key findings with respect to the context-dependency of plant-soil interactions. This suggests that caution should be taken when trying to apply findings from laboratory and greenhouse studies to the field, as other factors, such as local site conditions and soil biota, can significantly affect the final outcome of a plant-soil interaction. The consistent effect of soil origin on plant growth that was found, may be a more important factor in influencing plant-soil interactions than is currently acknowledged. However, the PSF effects measured were created during the early growth stages of the plant species studied, which may not be representative of the PSFs during later growth stages. Further work is therefore required to investigate the site conditions that result in the observed effects.

The results of Chapter 2, that the invasion of *I. glandulifera* may involve alterations to soil microbial communities, can be applied to the context of ecological restoration. This was the aim for Chapter 3, which tested the utility of two soil treatments in aiding restoration of invaded sites. Similarly, this study also showed a highly site dependent effect of the two treatments on subsequent plant cover, at sites with different characteristics. This study showed, in accordance with other studies, that the addition of activated carbon to invaded soils may actually increase the growth of the target invader, but only at one site. The mechanism by which AC affected plant cover could not be determined in this study and warrants further research. I also showed that the addition of AMF spores to invaded soils did not produce any resultant effect on plant species cover, when applied early in the growing season. This was a surprising result, since AMF abundance and diversity is frequently correlated with plant biodiversity (van der Heijden *et al*. 1998) and fungal inocula is becoming increasingly recommended to improve soil conditions and resultant native plant biodiversity (Ohsowski *et al*. 2012; Middleton *et al*. 2015). Whether AMF addition could be useful in increasing native plant biodiversity and growth at *I.*

*glandulifera*-invaded sites later in the growing season remains to be studied, then again seeding sites with a mix of native plant species may aid establishment of competing native species.

The results demonstrated here are important more broadly in the context of plant invasions and also in ecological studies. I have shown that the invasion of *I. glandulifera* is multifaceted and can manifest itself in different ecological contexts, with resultant effects that do not necessarily conform to a consensus. Integrating approaches, using results from both glasshouse and field studies however, means that results can have value to real-world applications, such as restoration. This study is a reminder that plant invasions are heterogeneous, complex and that the results of ecological studies do not always correspond to predictions. Invasive plant species remain a significant problem for ecologists and restoration practitioners, so it is important to continue research efforts into emerging fields, such as plant-soil interactions, in order to have the best chance at mitigating against plant invasions.

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# Appendices

**List of Appendices**

**Appendix 1.** *Results of post-hoc tests testing for significant comparisons of relative growth rates of* U. dioica *plants grown invaded versus uninvaded soils, from three field sites.*

| Site | Difference (cm plant$^{-1}$ week $^{-1}$) | *P* |
|---|---|---|
| 1 | 0.13 | 0.688 |
| 2 | 0.05 | 0.967 |
| 3 | 0.28 | 0.103 |

**Appendix 2.** *Results of post-hoc tests testing for significant comparisons in seedling counts of* I. glandulifera *and natives at different time points throughout the experiment. P values in bold represent significant differences.*

| Comparison | | Difference (log +1 counts) | *P* |
|---|---|---|---|
| *I. glandulifera* week 1 | Native week 1 | 1.07 | **<0.001** |
| *I. glandulifera* week 2 | Native week 2 | 1.70 | **<0.001** |
| *I. glandulifera* week 3 | Native week 3 | 1.85 | **<0.001** |
| *I. glandulifera* week 4 | Native week 4 | 1.83 | **<0.001** |
| *I. glandulifera* week 1 | *I. glandulifera* week 4 | 0.82 | **<0.001** |
| *I. glandulifera* week 1 | *I. glandulifera* week 2 | 0.94 | **<0.001** |
| *I. glandulifera* week 2 | *I. glandulifera* week 3 | 0.14 | 0.980 |
| *I. glandulifera* week 3 | *I. glandulifera* week 4 | 0.26 | 0.637 |
| Native week 1 | Native week 4 | 0.06 | 1.000 |
| Native week 1 | Native week 2 | 0.31 | 0.432 |
| Native week 2 | Native week 3 | 0.01 | 1.000 |
| Native week 3 | Native week 4 | 0.24 | 0.726 |

**Appendix 3.** *Results of post-hoc tests testing for significant comparisons in percentage cover of* I. glandulifera*, native forbs and native grasses, with and without addition of AC to experimental field plots at site one during a) week three and b) week seven. AC- denotes no addition of AC, and AC+ denotes addition of AC. P values in bold represent significant differences.*

a) Week three.

| Comparison | | Difference (logit % cover) | *P* |
|---|---|---|---|
| *I. glandulifera* AC+ | *I. glandulifera* AC- | 0.43 | 0.452 |
| Forb AC+ | Forb AC- | 0.19 | 0.950 |
| Grass AC+ | Grass AC- | 0.43 | 0.434 |
| *I. glandulifera* AC+ | Forb AC+ | 1.59 | **<0.001** |
| *I. glandulifera* AC+ | Grass AC+ | 2.54 | **<0.001** |
| *I. glandulifera* AC- | Forb AC- | 0.97 | **0.001** |
| *I. glandulifera* AC- | Grass AC- | 1.68 | **<0.001** |

b) Week seven

| Comparison | | Difference (logit % cover) | *P* |
|---|---|---|---|
| *I. glandulifera* AC+ | *I. glandulifera* AC- | 0.57 | 0.107 |
| Forb AC+ | Forb AC- | 0.01 | 1.000 |
| Grass AC+ | Grass AC- | 0.24 | 0.869 |
| *I. glandulifera* AC+ | Forb AC+ | 0.62 | **0.050** |
| *I. glandulifera* AC+ | Grass AC+ | 2.18 | **<0.001** |
| *I. glandulifera* AC- | Forb AC- | 0.04 | 1.000 |
| *I. glandulifera* AC- | Grass AC- | 1.37 | **<0.001** |

**Appendix 4.** *Results of a) linear mixed effects models for site two, showing the significant effect of species recorded on percentage cover over the experimental period, weeks one to seven and b) post-hoc tests testing for the significant differences between percentage cover of the three species recorded;* I. glandulifera*, native forbs and native grasses across the experimental period. P values in bold represent significant differences.*

a)

| Week | *F* | *df* | Residual *df* | *P* |
|---|---|---|---|---|
| 1 | 23.13 | 2 | 55 | **<0.001** |
| 3 | 31.30 | 2 | 55 | **<0.001** |
| 5 | 25.15 | 2 | 55 | **<0.001** |
| 7 | 25.37 | 2 | 51 | **<0.001** |

b)

| Week | Comparison | | Difference (logit % cover) | *P* |
|---|---|---|---|---|
| 1 | *I. glandulifera* | Forb | 0.31 | **0.020** |
| | *I. glandulifera* | Grass | 0.76 | **<0.001** |
| | Forb | Grass | 0.44 | **0.001** |
| 3 | *I. glandulifera* | Forb | 0.20 | 0.060 |
| | *I. glandulifera* | Grass | 0.65 | **<0.001** |
| | Forb | Grass | 0.46 | **<0.001** |
| 5 | *I. glandulifera* | Forb | 0.36 | 0.060 |
| | *I. glandulifera* | Grass | 1.05 | **<0.0001** |
| | Forb | Grass | 0.69 | **0.0001** |
| 7 | *I. glandulifera* | Forb | 0.23 | 0.13 |
| | *I. glandulifera* | Grass | 0.79 | **<0.001** |
| | Forb | Grass | 0.56 | **<0.001** |