Observing the tripartite interaction between three invasive plant species, alongside the ecological restoration of biodiversity in habitats invaded by Japanese knotweed.

Calista Collins

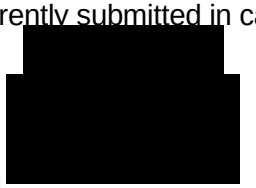
[redacted]

2022

Submitted to Swansea University in fulfilment of the requirements for the Degree of MRes Bioscience

DECLARATION

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.



Signed ..... (candidate)

Date 16/06/2022...

STATEMENT 1

This thesis is the result of my own investigations, except where otherwise stated. Where correction services have been used, the extent and nature of the correction is clearly marked in a footnote(s).

Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

Signed ..... (candidate)

Date ... 16/06/2022...

STATEMENT 2

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available to outside organisations.

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I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loans after expiry of a bar on access approved by the Swansea University, and for the title and summary to be made available to outside organisations.

Signed .....  ..... (candidate)

Date ..... 16/06/2022.....

The University's ethical procedures have been followed and, where appropriate, that ethical approval has been granted.

Signed.....  .....

Date..... 16/06/2022.....

Statement of Expenditure

Incidental costs/materials

- Seedling trays £15.42
- Capillary matting £44.85

Statement of contributions

Contributor Role	Contributors
Conceptualization	CC, DCE, SCH, MSF, DJ
Data Curation	CC, SH, DJ
Formal Analysis	CC, DCE, MSF
Investigation	CC, DCE, SCH, MSF, DJ
Methodology	CC, DCE, SCH, MSF, DJ
Project Administration	CC, DCE, SCH, MSF, DJ
Resources	SH, DJ
Software	R Studio

Ethics approval

College Ethics Committee/AWERB Group DECISION on Ethical Review

Application Details

Project Title: Ecological restoration of biodiversity in habitats invaded by Japanese knotweed

Applicant Name: Calista Collins

Submitted by: Daniel Eastwood

Full application details can be found in College Environmental Risks Application .

Having examined the information included in the above application with Reference No. STU_BIOL_161783_290121154048_1, this Committee has decided to:

☑ **Approve this application**

with the following reputation risk to the University

☑ Low Risk ☐ Moderate Risk ☐ High Risk

Any amendments to approved proposals should be emailed to College Ethics Committee for review: cosethics@swan.ac.uk

☐ **Reject this application and allow for resubmission provided the ethical issues raised by the College Ethics Committee/AWERB Group below are addressed**

☐ **Return for minor amendment/clarification (please resubmit using the 'Resubmit minor amendment' option for a quick turnaround for approval)**

Comments:

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The COS ethics committee approve this proposal (05/02/2021):

********** REVIEWER 1 - 02/02/2021 Recommendation: APPROVED (Low Risk)**********
There aren't any details on the experiments that will be performed, but it is clear that the biosecurity risks have been
evaluated and minimised, and the work will be carried out under licence.

********** REVIEWER 2 - 01/02/2021 Recommendation: APPROVED (Low Risk)**********
No concerns from me. Sufficient biosecurity protocols are in place
```

Risk assessments

Lab Risk Assessment			
*Grey boxes must be completed by field leader			
College/ PSU	CoS	Assessment date	January 2021
Location	Swansea University	Assessor	Calista Collins
Activity	Lab work/greenhouse – Seed bank assessment	Approved by	
		Review date (if applicable)	
Associated documents			

Part One: Risk Assessment

What are the hazards?	Who might be harmed ?	How could they be harmed?	What are you already doing?	Do you need to do anything else to manage this risk?
Inhalation of dust/debris from soil	Staff Student	May cause irritation to the chest	Safety PPE: including dusk mask when sampling if necessary.	
Contaminated material leaving the lab e.g. accidental spread of invasive species (due to potential seeds and seedlings in the soil).	Staff Student	Carrying potentially harmful material the persons	The autoclave will be used to rid of all plant material and excess soil to excludethe risk of speading invasive plant matter. Ensure autoclave training has been completed before use.	

What are the hazards?	Who might be harmed ?	How could they be harmed?	What are you already doing?	Do you need to do anything else to manage this risk?
Hot greenhouse conditions.	Staff Student	Low ventilation	In hot weather conditions, take regular nreaks, carry water and a mobile ohone at all times. Make sure to avoid greenhouse work on particularly hot days.	
Contact with rat droppings/flees	Staff Student	May cause illness	Where the risk of contact with rats is likely (e.g. in greenhouse), wear latex/nitryl gloves and wash hands regularly. Avoid contact with eyes/mouth.	
Contact with microbes in the soil	Staff Student	May cause illness	When in contact with soil/compost, wear appropriate gloves and wash hands when finished.	
Tripping hazards	Staff Student	Physical injury	Keep all walkways clear by ensuring bags and coats are stored safely.	
Digestion of potentially dangerous substances	Staff Student	Illness following digestion	No food or drink allowed to be in or eaten within the lab/greenhouse. Avoid touching your face, particularly the mouth.	
Faulty equipment	Staff Student	Risk of injury or environmental hazard	Check all equipment including glassware is in good working order before conducting experiments and reporting to supervisors if all is not in order.	
Contaminated worksurfaces	Staff Student	Spread of potentially harmful substances	Wipe and clean all workbenches after use. Clean all spillages. Clean all equipment used. Remove contaminated gloves before using phones, keyboards, and handles. Wash your hands regularly	
Autoclave burns	Staff Student	Burns to skin	Ensure appropriate autoclave training has been received before it is used.	
COVID-19 transmission from infected	Staff Student	Transmission of disease. Illness	Follow all COVID-19 safety measures including: • Wear appropriate PPE such as a face covering/mask • Always abide by social distancing • No more than the maximum amount of people in one lab at a time • Regular hand washing • Avoid touching face • Disinfect surfaces before and after use. • Do not go onto campus if feeling unwell: self-isolate	

Field Risk Assessment			
*Grey boxes must be completed by field leader			
College/ PSU	CoS	Assessment date	January 2021
Location	The Invasives Research Centre (Taff's Well, privately owned land near Cardiff)	Assessor	
Activity	Fieldwork for MRes – vegetation sampling (throughout spring and summer), setting up and monitoring plot trials (on-site).	Approved by	
		Review date (if applicable)	
Associated documents	• Participant list		

Part One: Risk Assessment

What are the hazards?	Who might be harmed?	How could they be harmed?	What are you already doing?	Do you need to do anything else to manage this risk?
Remote areas	Project members	Difficulty in getting aid if injured or in need of help.	No lone working permitted - Buddy system in place under the university guidelines. Always alert supervisor(s) when entering and leaving the field site. Exchange contact details, such as phone numbers, with fellow researchers/staff.	

What are the hazards?	Who might be harmed?	How could they be harmed?	What are you already doing?	Do you need to do anything else to manage this risk?
Uneven ground	Project members	Falling over Physical injury	Wear appropriate footwear and PPE for the terrain. Proceed with caution. Site induction will be conducted with supervisors. Obvious hazards are flagged and will be recorded on site map to minimize risk.	
Sun exposure	Project members	Dehydration Sun burn	Wear sunscreen on sunny days. Ensure adequate food and water brought to site for personal consumption.	
Lifting heavy objects	Project members	Injury caused by dropping heavy object on oneself or strain.	Ensure a buddy/co-worker is on-site to oversee or help at a distance. Do not attempt to lift objects if you think it is too heavy to safely handle.	
COVID-19 transmission	Project members And potentially members of public/family members	Transmission of disease	Travel in separate private vehicles. Working in an outdoor location. Abiding by local Governmental COVID-19 safety measures: • Maintaining 2m+ distance • Working in small groups • Suitable PPE • Use of hand-sanitiser • Sanitise shared equipment • Field-site is on private land, therefore there is no access to the public • Do not travel to the field site if feeling unwell or exhibiting any symptoms • Ensure 'Buddy' is a member of the same household • Wear a face mask • Avoid touching face • Completed Covid training course on Canvas; appropriate permissions will be obtained before travelling.	
Tripping	Project	Physical injury	Make sure the site is well-known to all before data collection is	

What are the hazards?	Who might be harmed?	How could they be harmed?	What are you already doing?	Do you need to do anything else to manage this risk?
hazards	members		conducted to avoid tripping over objects such as debris, pots, or vegetation. Wear appropriate footwear. Be aware of surroundings and take care on site.	
Dehydration	Project members	Exhaustion. Exposure.	Ensure you bring plenty of water to the site and keep drinking throughout the day.	
Extreme weather	Project members	Stuck in remote areas during extreme weather conditions	Carefully check weather forecast. Ensure you are wearing appropriate clothing. Do not enter the site alone. Leave site if weather conditions become unsafe.	
Brambles or other sharp objects; biting/stinging insects; hazardous plants	Project members	May pierce and cut skin which could lead to infection. Potential adverse reaction to bites and stings/contact with noxious plants (e.g. bees, wasps, stinging nettle). Contact with hogweed can induce UV-triggered reaction on skin.	Carry first aid kit. Wear appropriate clothing: long sleeves and trousers where possible. If experiencing adverse reactions, seek medical attention and notify supervisor(s). Be aware of surroundings. If potentially disturbed bee/wasp nest, leave immediate area to reduce risk. Learn to identify hogweed to ensure distance can be kept from plants – avoid contact with this plant. Site induction will also identify risk associated with presence of this species.	
Potential spread of invasive species	The surrounding environment	May adversely impact local biodiversity	Biosecurity measures are in place Equipment and clothing/footwear will be cleaned before leaving site	
Travel to and from site	Project members, and potentially family members and members of the public	Spread of covid-19; car accident (motorway travel)	Travel in private transport separately. Only travel with members of the same household and support bubbles.	
Unstable ground near riverbank – proximity to river	Project members	Falling; drowning	Work does not require entry into river – ensure safe distance is maintained from riverbank.	

Contents page

Acknowledgments

I would like to thank my supervisors, Prof Dan Eastwood, Prof Mike Fowler, Dr Sophie Hocking, and Dr Dan Jones for all of the help they have given me over throughout my Master's. My field assistants: Paddy Colledge, Ellie Evans, Alexie Jenkins, and my Mum, Nick, and sister, Isa, for all keeping me company during data collection, and keeping my seedlings watered!

List of Tables and Figures

Abbreviations

R. japonica – Reynoutria japonica

S. canadensis – Solidago canadensis

I. glandulifera – Impatiens glandulifera

F_v/F_m *– Chlorophyll fluorescence*

For pot trial treatment groups:

RJ - *Reynoutria japonica*

SC – *Solidago canadensis*

IG – *Impatiens glandulifera*

(17 L) – Treatment with additional 17 L Hadopot™

For community analysis

HD – High functional diversity species mix

LD – Low functional diversity species mix

A – Amenity grass species mix

HDM – High functional diversity species mix with matting

LDM – Low functional diversity species mix with matting

AM – Amenity grass species mix with matting

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Submitted to Swansea University in fulfilment of the requirements for the Degree of MRes Biosciences.

i. Lay summary

Japanese knotweed is an invasive plant species that causes global problems for local biodiversity and ecosystems. Control methods are ineffective and incur large costs, and often leads to the wasteful use of herbicides and increased knotweed spread. Long-term restoration data are lacking to understand how well control methods have worked over-time. One of the characteristics that determines the invasive potential of invasive plant species, such as Canadian goldenrod, is the release of organic compounds known as allelochemicals which can negatively affect the health of native species. Therefore, such compounds may also have negative effects on other problematic invasive species, such as Japanese knotweed. To test this hypothesis, a pot trial was set up, which aimed to determine whether Canadian goldenrod negatively affected the health of Japanese knotweed aboveground growth. To do this, the two species were grown together in the same treatment pot, and the weekly growth rates and chlorophyll fluorescence (a measure of photosynthesis efficiency) were compared to treatments where the two species were isolated from each other, so belowground interactions could not occur. Secondly, the pot trial aimed to assess the interactions between Japanese knotweed and Canadian goldenrod alongside another invasive species, Himalayan balsam. In this three-part experiment, the growth rate and chlorophyll fluorescence of interacting plants were measured weekly and compared against single species-only controls. Canadian goldenrod had the highest mean chlorophyll fluorescence in all treatments it was involved in. Himalayan balsam had the highest mean growth rate in the three-part treatment. The chlorophyll fluorescence of Canadian goldenrod was always higher than that of Japanese knotweed in both interaction and single species treatments. The functional traits of native plants in supressing recurrence and secondary invasion in land herbicide treated for Japanese knotweed control was assessed as part of an ongoing restoration study (started in 2018). This included observing the effectiveness of different seed mixes to aid land restoration and prevent secondary invasions. Community and seedbank analysis confirmed that high and low functional diversity seed mixes provided the highest species diversity and species richness within treatment plots. This long-term restoration data collection should continue to increase information surrounding post-knotweed treatment restoration management.

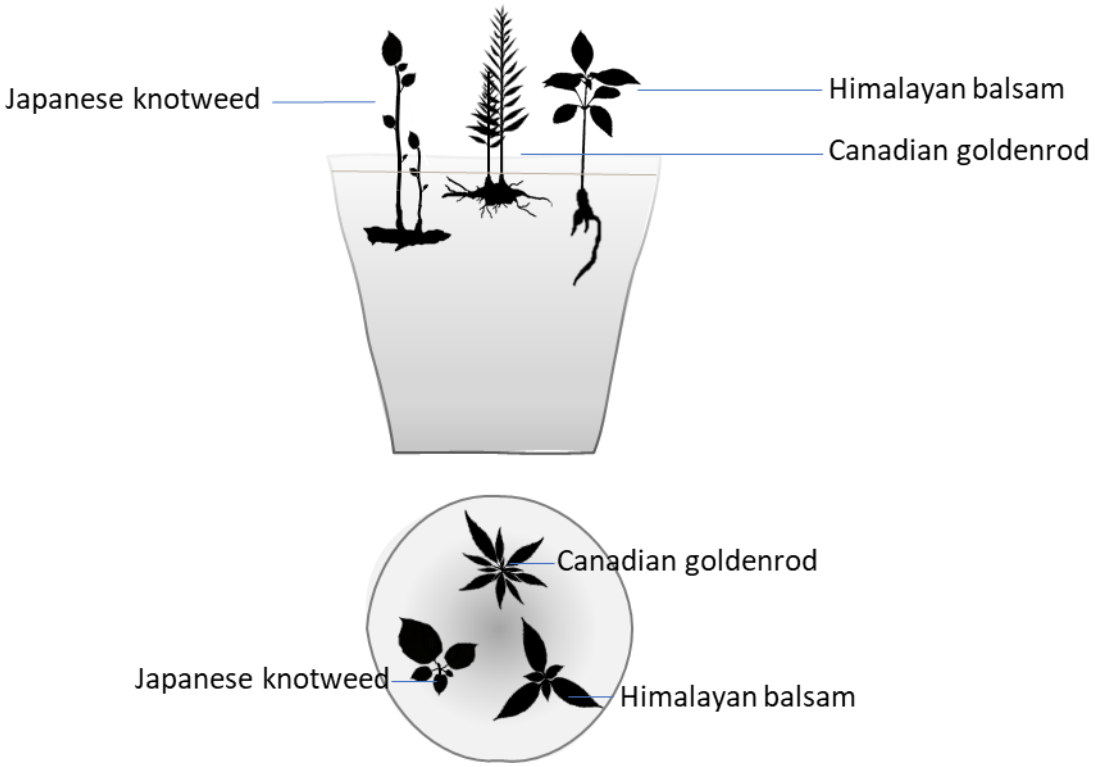

Figure X. Schematic of the study design for the tripartite aspect of the pot trial experiment. The three invasive species: Japanese knotweed (Reynoutria japonica), Himalayan balsam (Impatients glandulifera), and Canadian goldenrod (Solidago canadensis) were grown in the same pot to observe whether there may have been above- or belowground interactions. Single species control pots were also prepared for the study.

ii. Abstract

Reynoutria japonica (Japanese knotweed) is an invasive species that negatively impacts local biodiversity and ecosystems globally. Control methods are inefficient and laborious, and long-term restoration data is sparse.

One of the characteristics that contributes to the invasiveness of a plant is allelopathy, which has been recorded in *Solidago canadensis* (Canadian goldenrod). Allelochemicals can reduce the fitness of native species, outlined by the native weapons hypothesis, and therefore may negatively impact other invasive species, such as *R. japonica*.

To test this, a pot trial was set up, which firstly aimed to determine whether *S. canadensis* negatively affected the health of *R. japonica* aboveground growth. The two species were grown together with and without isolation treatments. The weekly growth rates and chlorophyll fluorescence were measured and compared to single species controls. Secondly, the pot trial aimed to assess the interactions between *R. japonica*, *S. canadensis*, and *Impatiens glandulifera* and growth rate and chlorophyll fluorescence were measured weekly. *S. canadensis* had higher mean chlorophyll fluorescence in its pairwise and tripartite treatments than the *R. japonica* and *I. glandulifera* ($p = 0.0016$ and $p < 0.0111$, respectively). *I. glandulifera* had the highest mean growth rate in the tripartite treatment ($p = 0.0001$). *S. canadensis* had higher chlorophyll fluorescence than *R. japonica* when grown together or separately.

To understand the long-term effects of using native plant functional traits in habitat restoration, I continued the data collection of Hocking, 2021, to measure the restorative success of various specific seed mixes sown on land previously dominated by *R. japonica* and treated with glyphosate. Plants was selected due to functional traits which aided land restoration and prevented secondary invasions. Community and seedbank analyses of plots with the tailored restorative seed mixes were carried out to assess the subsequent species diversity and richness. High and low functional diversity seed mixes provided the highest species diversity and richness within treatments.

1. Introduction

An invasive species is defined as one that arrives in a habitat it has not previously occupied, then establishes a population autonomously (Simberloff, 2010). Invasive species populations threaten biodiversity, ecosystem integrity, agriculture, aquaculture, and public health (Lee, 2002). Invasive plants may also have an economic impact; for example, the annual cost of managing invasive non-native species to the UK economy was estimated at £1.7 billion in 2010 (Williams *et al*., 2010). The transfer of alien species across geographical barriers has been facilitated by the increased transport for global trade (Mooney & Cleland, 2001). This not only causes a wider distribution of the alien species, but also an increase in their invasive potential through increasing the size of established populations. These alien species may experience a 'lag phase,' which can occur due to Allee effects, spatial dynamics, and logistic population growth, as newly introduced species may maintain low population levels before they enter the expansion phase and become 'invasive' (Marsico *et* al., 2010; Mooney & Cleland, 2001).

Invasive species have the potential to modify ecosystems (Simberloff, 2010) through the introduction of new functional traits in invaded habitats (Charles and Dukes, 2008). These species shift native species richness and abundance, and alter fire regimes, water quality, and biogeochemical cycles (Crowl *et al*., 2008). Invasive species can also induce evolutionary changes of native species via competitive exclusion, niche displacement, hybridisation, and predation (Lee, 2002; Mooney & Cleland, 2001). These changes have led to the extinction of species in invaded ecosystems (Simberloff, 2010; Mooney & Cleland, 2001). As a result, there are many differing policies globally that aim to reduce the transport and release of non-native species; for example, at an international level, the Convention on Biological Diversity (CBD) requires nations to work to prevent the introduction, spread, and export of all types of invasive species (Keller *et al*., 2011).

I. Japanese knotweed

Invasion success varies between species and has often been linked to growth rate and resource use, alongside the competitive advantage of invasive plants following release from their natural enemies (Murrell *et al*., 2011). From a competitive standpoint, Japanese knotweed, *R. japonica* (previously *Fallopia japonica* var. *japonica*), is a very successful invasive species (Murrell *et al*., 2011). *R. japonica* belongs to the Polygonaceae family, but due to the instability of taxonomy and differing terminology within Polygonaceae, it has been referred to three different genera: *Fallopia japonica*, *Reynoutria japonica*, and *Polygonum cuspidatum* (Bashtanova *et al*., 2009).

Native to Japan, Korea, China, and Taiwan, *R. japonica* is a seral species found on volcanic slopes, making it an effective primary coloniser of disturbed land (Gillies *et al*., 2016; Colleran *et al.,* 2014; Bashtanova *et al*., 2009; Del Tredici, 2017). It is a tall, herbaceous perennial plant with underground woody rhizomes (storage systems) at maturity (Fennel *et al*., 2018). Riparian and disturbed areas are highly vulnerable to the growth of monospecific Japanese knotweed stands (Bailey *et al*., 2009), due to their influx of nutrients, plentiful light, and frequent deposition of new propagules (Dauer & Jongejans, 2013). Riparian habitats are unique and dynamic ecosystems with

complex disturbance regimes: communities found along riverbanks are prone to invasion and are amongst the most invaded ecosystems world-wide (Hejda & Pyšek, 2006; Čuda *et al*., 2017).

Tolerance of poor soil conditions, allows *R. japonica* to grow under a wide range of soil pH. This wide pH tolerance, coupled with the ability of *R. japonica* to develop monospecific stands rapidly, ultimately results in reduced native plant diversity in the invaded area (Gillies *et al*., 2016; Dauer & Jongejans, 2013). Rapid monospecific stand development is attributed to the vigorous rhizome system of *R. japonica*, and the capability to grow from rhizome and stem fragments (Gillies *et al*., 2016; Mandak *et al*., 2003). *R. japonica* can also reproduce sexually, via seeds that can produce several inter-and intraspecific hybrids, which then expand through vegetative growth (Grimsby *et al*., 2007). The seeds are often not viable in climates that are colder than its native range; therefore, in countries such as the UK, root and rhizome growth is responsible for the spread (McLean, 2010).

The dense *R. japonica* canopy restricts the growth of established vegetation in various ecosystems, such as riparian areas (Gerber *et al*., 2008). This occurs via several mechanisms, including via reducing species richness and abundance of native understory herbs, shrubs, and juvenile trees (Bashtanova *et al*., 2009; Fennel *et al*., 2018)**.** Rhizomes also release secondary products with allelochemical properties, thus affecting the availability of mineral nutrients for native plants, potentially causing native plant mortality, and consequentially disrupted faunal diversity (Bashtanova *et al*., 2009)**.** The resorption of nutrients by *R. japonica* alters nutrient cycling and productivity of both riparian forest soils and aquatic food webs by sequestering available nitrogen and reducing the quantity of nutrients input through litterfall (Urgenson, 2006). Furthermore, ecosystem services in riparian zones are reduced (Fennel *et al*., 2018), such as via obstruction of important corridors for invertebrates and vertebrates (Gerber *et al*., 2008). Structural damage can also occur through impeded water flow and facilitated riverbank erosion affecting bank stability and flood defences, building foundations, drainage works, and pavements (Bashtanova *et al*., 2009), which has created economic impacts, particularly in the UK (Fennel *et al*., 2018).

The introduction of *R. japonica* into Europe and North Americas occurred in the nineteenth and became strongly established by the twentieth century (Walls, 2010; Hollingsworth & Bailey, 2000). In the UK, a female (male sterile) *R. japonica* sent to Kew Gardens, London, soon became distributed throughout Victorian parks and gardens (Fennell *et al*., 2018). As early as 1898, Victorian gardeners voiced concerns regarding the plant's invasiveness (Fennell *et al*., 2018). The first recording of *R. japonica* outside of cultivation was in South Wales in 1886 and by 1996 it had been recorded in 1584 of the 2862 10 km^2 of the Biological Records Centre mapping system of the British Isles (Hollingsworth & Bailey, 2000; Fennell *et al*., 2018). The spread of *R. japonica* has been encouraged by anthropogenic disturbance, such as the disposal of rhizome-contaminated soil, and natural disturbance, such as flooding (Jones *et al*., 2018).

Invasive species such as *R. japonica* colonise new areas despite low genetic variation (Richards *et al*., 2012; Walls, 2010). This was shown through Randomly Amplified Polymorphic DNA (RAPD) analysis demonstrating that there was a single widespread clone of *R. japonica* in the UK (Hollingsworth & Bailey, 2000). The ability to outcompete locally adapted species despite such genetic bottlenecks is interesting evolutionarily and ecologically.

a. **Control measures**

Due to ability of *R. japonica* to propagate reproductively by intra- and interspecific hybridisation, and vegetatively via rhizome fragments, eradication is difficult (Bashtanova *et al*., 2009). Mechanical, chemical, and biological techniques have been used in attempts to eradicate the plant (Delbart *et al*., 2012). Limited understanding of the effect of such techniques on the long-term ecology and management of rhizome-forming invaders has led to ineffective and labour-intensive treatments, alongside unnecessary herbicide use (Jones *et al.,* 2020). Complete excavation of small stands, and large-scale excavations combined with chemical control (the preferred method for removal in building sites) incur significant costs, with potential low efficiency (Bashtanova *et al*., 2009; Delbart *et al*., 2012). This method of removing entire rhizomes may create more environmental issues, such as those related to disposal of plant material (Delbart *et al*., 2012). Belowground rhizomes biomass can extend to several metres in diameter and depth, meaning without full eradication, stands can recover from long-term physical, herbicide-based, and integrated control treatments (Jones *et al*., 2020). *R. japonica* can grow from rhizome fragments as small as 0.7 g (Dauer & Jongejans, 2013); consequently, management strategies such as cutting and mowing can promote spread, due to the resultant dispersal of rhizome fragments (Bashtanova *et al*., 2009). These strategies can also cause habitat damage due to heavy equipment on riparian/roadside vegetation (Jones *et al*., 2020).

Herbicide treatments such as glyphosate have proven to be successful in controlling, but not eradicating, *R. japonica* (Jones *et al*., 2018; Delbart *et al*., 2012; Skinner *et al*., 2012). Methods of glyphosate delivery have also been optimised to reduce herbicide use whilst maximising plant basal cover and stem density; for example, studies have shown that spraying foliage rather than stem injections leads to more effective glyphosate coverage (Jones *et al*., 2018). Other herbicides, such as imazapyr, have proven effective control of *R. japonica*; however, imazapyr is no longer authorised for European use (Delbart *et al*., 2012). The use of native species mixes is another *R. japonica*-control strategy aimed at promoting the growth of natural vegetation and excluding *R. japonica* (Skinner *et al*., 2012; Delbart *et al*., 2012). The competitive benefits of these mixes may not become apparent for several years; in the USA, natural species mixes, with up to two years of glyphosate application, successfully started to compete with *R. japonica* only after two years, when native plants reached >80% coverage of invaded plots (Skinner *et al*., 2012). Integrated control strategies such as this, alongside increased prevention efforts and public awareness campaigns would be beneficial to successfully control the invasive species (Delbart *et al*., 2012).

a. **Allelopathy**

The production of various bioactive secondary compounds by *R. japonica* result in allelopathic effects, with positive or inhibitory interactions with other species (Gross, 2003). Allelopathy is the mechanism behind the novel weapon hypothesis, which proposes that invasive plants produce advantageous allelochemicals that significantly affect the fitness of native species due to their lack of previous exposure to the allelochemicals in the surrounding environment, making them particularly sensitive to these compounds (Dommanget *et al*., 2012; Abhilasha *et al*., 2008; Rice, 1974). Therefore, this characteristic further improves the competitive ability of *R. japonica* as allelopathic

compounds can inhibit neighbouring native plants directly, or indirectly via disruptions of beneficial belowground microbial mutualisms, altered soil resources, and soil fauna at different trophic levels via the addition of both nutrients and secondary metabolites to soil (Kalisz *et al*., 2021; Abgrall *et al*., 2018). Studies have indicated that certain native species, can exert allelopathic inhibition on invasive species and contribute to community resistance to invasion. Though there are few studies on the allelopathic potential of native species, it was reported that soil from later successional stages of a forest community had stronger inhibitory effects on an invasive weed, *Picea mariana* (Chen et al., 2017). Allelochemicals are diverse in chemical structure, and are released from plant parts by leaching, root exudation, volatilisation, or residue decomposition to susceptible plants (Abhilasha *et al*., 2008).

R. japonica emits multiple chemicals that combine to give the plant its competitive advantage. These chemicals include resveratrol, emodin and (–)-epicatechin (Tucker Serniak, 2016). A hybrid between *R. japonica* and *Fallopia sachalinesis, F.* x *bohemica*, has demonstrated allelopathic effects on native species through its leaf litter and/or drained soil, which affects the growth rather than the germination of natives, and subsequently causes significant life-history shifts in the dominant native species (Parepa *et al*., 2012). Furthermore, perennial species, like *R. japonica*, have a stronger allelopathic effect than annual species capable of allelopathy, and have shown exclusively negative effects on test species such as oat (*Avena sativa L*.), oilseed rape (*Brassica napus subsp. oleifera*) and sunflower (*Helianthus annuus L*.) (Novak *et al*., 2018).

Leachates from *R. japonica* have inhibited the growth of Salicaceae species cuttings, which was linked to the emission of polyphenol compounds (Dommanget *et al*., 2012). These leachates induced changes in soil nitrogen composition; however, not all species of Salicaceae were affected equally, suggesting that species more resistant to allelopathic compounds should be chosen for greater restoration success (Dommanget *et al*., 2012).

II. <u>The risk of secondary invasions</u>

In the management of invasive species, the control of one species may lead to a niche opening for another to exploit (secondary invasion); furthermore, little is known about how invasive species with differing phenotypic traits interact and form an invasion hierarchy in nature. Two other problematic invasive plants that may encroach into natural systems via secondary invasions are Himalayan balsam (*Impatients glandulifera*) and Canadian goldenrod (*Solidago canadensis*). Both species are invaders of disturbed land (Čuda *et al*., 2017; Bielecka & Królak, 2019**;** Gusev 2015) and have demonstrated potential belowground interactions via allelopathy (Smith, 2013; Vrchotová, 2011; Abhilasha *et al*., 2008; Zandi *et al*., 2020; Anžlovar & Anžlovar, 2019). Like *R. japonica, S. canadensis* is a perennial rhizome-forming plant, that tolerates a wide range of environmental conditions and modifies natural ecosystems due to its dense thickets that limit water and light access for other species and its allelopathic effects on other plant species (Gusev, 2015; Dudek *et al*., 2016; Anžlovar & Anžlovar, 2019). Alternatively, *I. glandulifera* is an annual plant, that reproduces successfully due to the attractiveness of its zygomorphic flowers to pollinators, and its ability to disperse up to 2,500 seeds each up to 5 m from the parent plant (Bartomeus *et al*., 2010; Clements *et al*., 2007). This dispersal

method results in monotypic stands, which prevent the establishment of native plants and increase the erosion risk of venerable stream banks when the shallow-rooted balsams die back (Clements *et al*, 2007).

Controlled biogeographical comparisons have provided evidence that the invasive success of *S. canadensis* is not due to the competitive advantage of natural enemy release, nor the introduction of vigorous novel genotypes (Abhilasha *et al*., 2008). Instead, a potential explanation for the invasive success of *S. canadensis* is the novel weapons hypothesis (Abhilasha *et al*., 2008; Zandi *et al*., 2020; Anžlovar & Anžlovar, 2019).

Studies have shown *that R. japonica* and *S. canadensis* can be found in the same invaded areas (Smerdu *et al*., 2020). The Advanced Invasives Field Site is a privately owned field site located in Taff's Well (Wales), which has been historically dominated by *R. japonica* invasions, and *I. glandulifera* and *S. canadensis* invasions. The site has supported projects investigating the effectiveness of different glyphosate application methods on *R. japonica,* alongside long-term post-treatment land recovery, following the additions of native seed mixes to plots treated with glyphosate. On-site field observations made in 2018 showed that on a patch of ground where *S. canadensis* was growing, no *R. japonica* was growing amongst or surrounding the *S. canadensis* stand. This firstly elicited the question of why *R. japonica* was not growing in this area, and secondly of how *S. canadensis* may be inhibiting the growth of *R. japonica* on the patch land. As some studies have explored the potential use of allelopathy as a tool to control exotic plant invasion, and allelopathy of native species is recognized as an important trait in selection of native species to control the invasive plants (Chen *et al*., 2017), the hypothesis was formed that the inhibition of *R. japonica* may be derived from allelopathic active compounds emitted from *S. canadensis.* This further led to queries surrounding the hierarchy of the three names invasive species found at the Advanced Invasives Field Site, and how the species would interact.

II. Long term restoration collection

The lack of long-term restoration data following *R. japonica* treatment can lead to ineffectual management strategies (Jones *et al*., 2020). The integration of initial Japanese knotweed treatment and subsequent restoration methods can control *R. japonica* spread whilst preventing secondary invasions (Jones *et al*., 2018; Delbart *et al*., 2012). As mentioned previously, seed mixes of native species can be an effective restoration method, following glyphosate spraying (Skinner *et al*., 2012; Jones *et al*., 2018). Long term restoration data has occured at the Advanced Invasives field site since 2018, when tailored seed mixes were sown within plots previously treated with glyphosate (Hocking, 2021; Jones *et al*., 2018). Seed mixes have reportedly provided the most successful native plant recover when compared to other restoration methods, such as cutting (Hall *et al*., 2021). Although seed mixes can be expensive, studies have shown that the cost of treating secondary invasions in treatments where seeds had not been sown as part of the restoration treatment, were considerably higher (Hall *et al*., 2021). Testing different species mixes through initial modelling before application can provide cost-effective and successful restoration mixes that provide high plant cover (Kimball *et al*., 2015).

Hocking (2021) implemented seed mixes which included species that were from the NVC restoration reference community, and were already found on the field site, to promote native plants whilst selecting species with appropriate functional traits to increase invasion resistance (Sheley *et al*., 2006). These seed mixes tailored to the site included a high functional density, low functional density mix, and amenity grass mix, to assess which seed mix had the best restoration success.

Without long-term restoration data, the extent of treatment success is impacted due to the lack of long-term monitoring. Hence, the importance of long-term restoration provides economic and environmental benefits, as costly ineffective invasive plant treatments are reduced, whilst allowing the greatest restorative biodiversity of previously invaded areas (Kimball *et al*., 2015; Jones *et al*., 2018; Hocking, 2021).

III. Aims and objectives

Firstly, this project aimed to investigate the interaction of *S. canadensis* on *R. japonica*. This was to provide an insight into the use of allelochemicals from *S. canadensis* as a *R. japonica* management method. To investigate this aim, a pot trial was conducted to observe the growth and interactions of the two species within the same environmental conditions and using treatments that potentially exposed *R. japonica* to the allelochemicals of *S. canadensis*. Single species control groups were used to determine whether any treatment exerted detrimental effects on *R. japonica*.

To understand the interactions between these two species, the initial measurement was plant emergence (Yes/No (Y/N)), which was used as an early indicator of plant health, as plants overcome abiotic stress during emergence and growth (Nouman *et al*., 2012). Plant growth rate was chosen as a parameter of competition as growth depends on the ability of a plant to compete for resources, such as light, to grow and maintain access to these resources (Freckleton *et al*., 2009; Moles *et al*., 2009).) Chlorophyll fluorescence (F_v / F_m) of each species was measured. The chlorophyll fluorescence provides information about Photosystem II, such as the extent to which Photosystem II is using the energy absorbed by chlorophyll and the extent to which it has been damaged by excess light, thus enabling estimates of photosynthetic performance (Maxwell & Johnson, 2000). A higher maximal possible value of fluoresce indicates a healthy non-stressed plant, therefore the F_v / F_m -values provide indication into whether the plant is stressed and may not be as effective at competing for resources (Murchie & Lawson, 2013).

The hypothesis was that *S. canadensis* would outcompete *R. japonica*; therefore, all height, chlorophyll fluorescence and emergence data would be higher for *S. canadensis* and that allelochemicals may have affected *R. japonica*.

Secondly, as a continuation of the pot trial, this project aimed to observe a tripartite species interaction between *R. japonica*, *S. canadensis*, and *I. glandulifera*, to observe whether there was a dominant competitor between the invasive species, and ultimately whether one invasive species can be utilised to control another. Again, plant emergence (Y/N), plant height (cm), and chlorophyll fluorescence (F_v / F_m) were measured to assess the competitive ability of each species. *I. glandulifera* was hypothesised to have the fastest growth rate, due to its characteristic

rapid growth rate. *S. canadensis* was hypothesised to have the highest chlorophyll fluorescence due to its emissions of allelochemicals that may lower the fitness of the other two species.

This project thirdly aimed to contribute to the production of long-term restoration data of previously glyphosate-treated *R. japonica* plots. Therefore, long-term data collection was continued from an ongoing project at the Advanced Invasives Field Site, which focussed on land-restoration post- *R. japonica* treatment. Seedbank analysis and community analysis that had been conducted for the previous three years, of treatment plots (treated with *R. japonica* control strategies since 2013) that had additionally been treated with varying seed mixes, were repeated. The seedbank analysis aimed to assess the habitat restoration success of the different seed mixes, using the species richness and abundance of the seedbank as indicators of this. The community analysis was carried out to assess the species richness and species diversity of the previously treated plots during the summer growing season.

2. Methods

I. Pot trial parameters, design, and layout

The pot trial location (the Advanced Invasives field site) was on fully contained private land with no public access. The growth of the invasive plants was conducted with full biocontrol measures and following our licence agreement from the Environment Agency. The field site had previously been invaded by *Reynoutria japonica* and had been affected by a secondary *Impatiens glandulifera* invasion, alongside historic *Solidago canadensis* growth. The pot trial will be held on the site for multi-year study, to enable long-term data collection.

The experimental design (Table 1) consisted of 11 treatment groups, each with ten replicates (n = 110) set up between 13/04/21 and 26/04/21 using 50 L Hadopots™ for all treatment groups. This ensured there would be enough growing room for tripartite and allelopathy experiments (Figure 1). The 50 L Hadopots™ were half-filled at first to easily position and reposition the pots. Once the Hadopots™ were in position, they were filled up to ¾ full. Soil pH and conductivity were tested using a Multimeter and Multimeter calibration fluid (Hanna Instruments Ltd).

Three controls contained approximately (+/- 0.3 g) 10 g of *R. japonica* rhizome only (RJ Control), 10 g of *S. canadensis* rhizome only (SC control), and 10 g of *I. glandulifera* seedlings only (IG Control). To eliminate potential bias via the growth of plants in smaller 17 L pots (that were placed in filled 50 L pots), controls of each rhizomatous species were grown in 17 L pots (RJ Control (17 L) and SC Control (17 L)). These smaller bags were used to compare competitivity parameters of both rhizomatous species grown together (5 g each) when no allelopathy could occur due to additional 17 L pots that isolated one species, and therefore compare these results to the treatment with no barrier between the two species (RJ + SC), so potential allelopathy could occur.

Treatments RJ + IG and SC +IG included 5 g of rhizome/seedlings to test *R. japonica* and *S. canadensis* interactions with *I. glandulifera*, to compare findings with the tripartite interaction experiment, which included 3.3 g of *R. japonica* and *S. canadensis* rhizome and 3.3 g of *I. glandulifera* seedlings.

The substitutive experimental design was chosen to ensure comparability of response variables across treatments (Li & Hara, 1999), as changes of proportion and rhizome/seedling density may make the interpretation of results difficult (Rivaie, 2016). Substitutive designs can also be valuable for studying the effects of single factors on the outcome of interference between species (Rivaie, 2016), which in this experiment is the potential allelopathic compounds. The study design also ensured there was standardisation between treatments.

Table 1. Treatment groups (10 replicates per treatment, n = 110), for the pot trial experimental design.

Treatment	Species rhizome	Overall planting weight per pot (g)	Additional features
RJ control	*R. japonica*	10	No 17L Hadopot™
SC control	*S. canadensis*	10	No 17L Hadopot™
IG control	*I. glandulifera*	10	No 17L Hadopot™
RJ control (17 L)	*R. japonica*	10	17L Hadopot™
SC control (17 L)	*S. canadensis*	10	17L Hadopot™
RJ + SC	*R. japonica* and *S. canadensis*	5	No 17L Hadopot™
RJ(17 L) + SC	*R. japonica* and *S. canadensis*	5	*R. japonica* in 17L Hadopot™
RJ + SC(17 L)	*R. japonica* and *S. canadensis*	5	*S. canadensis* in 17L Hadopot™
RJ + IG	*R. japonica* and *I. glandulifera*	5	No 17L Hadopot™
SC + IG	*S. canadensis and I. glandulifera*	5	No 17L Hadopot™
Tripartite	*R. japonica, S. canadensis and I. glandulifera*	3.3	No 17L Hadopot™

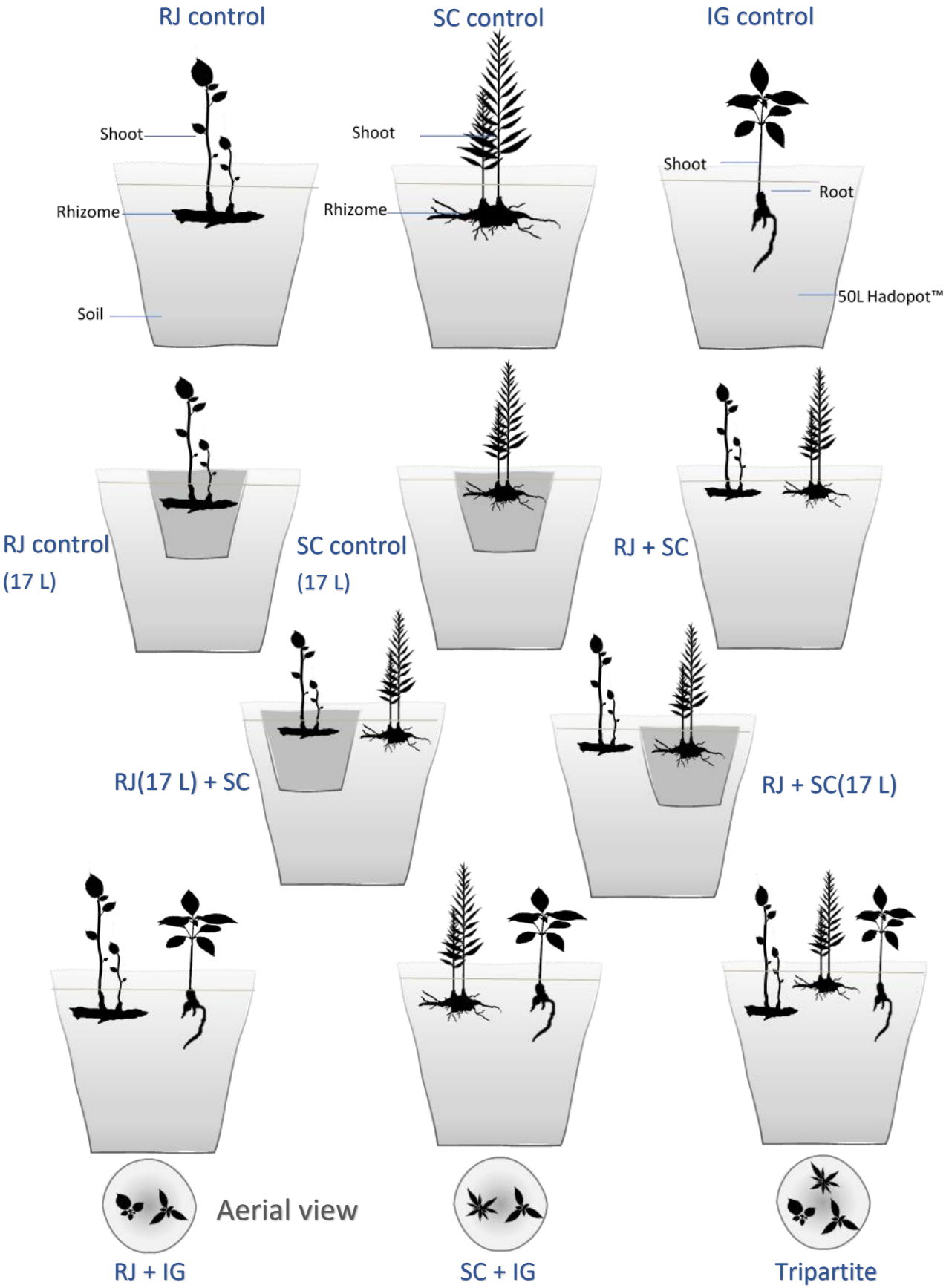

Figure 1. Pot trial experimental design. RJ (Reynoutria japonica) control; SC (Solidago canadensis) control; IG (Impatiens glandulifera) control, RJ control (17 L) – R. japonica control within 17 L Hadopots™; SC control (17 L) – S. canadensis control within 17 L Hadopots™; RJ + SC – R. japonica and S. canadensis within the same pot, no 17 L Hadopots™; RJ(17 L) + SC – R. japonica and S. canadensis within same pot, R. japonica in 17 L Hadopots™; RJ + SC (17 L) - R. japonica and S. canadensis within same pot, S. canadensis in 17 L Hadopots™; RJ + IG – R. japonica and I. glandulifera within same pot, no 17 L Hadopots™; SC + IG – S. canadensis and I. glandulifera within same pot, no 17 L Hadopots™; and Tripartite – R. japonica, S. canadensis, and I. glandulifera, no 17 L Hadopots™

Pots were arranged in groups of 20, with four columns of five pots. Columns of pots in the same treatment groups were distanced by 10 cm, and 8 cm between rows. Treatments were distanced by 12 cm within the groups of 20, and each group was distanced by 30 cm (Figure 2). As the pot trial was set up on a flat open area of land with no shading, the treatment pots were not stratified.

The use of 50 L pots ensured there would be enough growing room for tripartite and allelopathy experiments. *I. glandulifera* seedlings were planted later (one week) than *R. japonica* and *S. canadensis* rhizomes due to a delay in the permit approval to plant *I. glandulifera*. However, this did represent the ecological interaction of *I. glandulifera* with an established stand of the two rhizome-forming species.

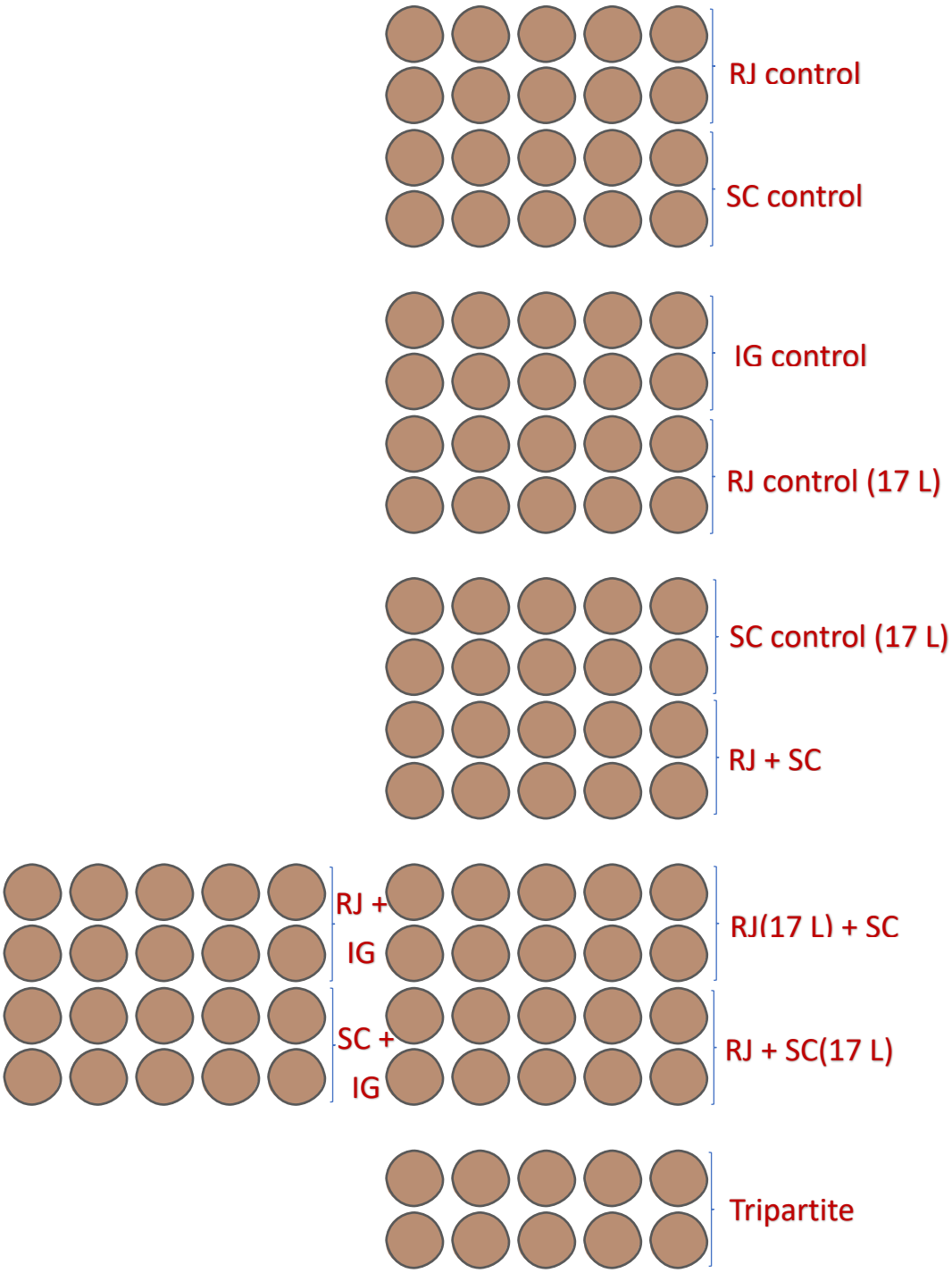

Figure 2. schematic representation of the pot trial experimental design (n=110) at the Advanced field site, Taff's Well. All 11 treatment groups had ten replicates. RJ (Reynoutria japonica) control; SC (Solidago canadensis) control; IG (Impatiens glandulifera) control, RJ control (17 L) – R. japonica control within 17 L Hadopots™; SC control (17 L) – S. canadensis control within 17 L Hadopots™; RJ + SC – R. japonica and S. canadensis within the same pot, no 17 L Hadopots™; RJ(17 L) + SC – R. japonica and S. canadensis within same pot, R. japonica in 17 L Hadopots™; RJ + SC (17 L) - R. japonica and S. canadensis within same pot, S. canadensis in 17 L Hadopots™; RJ + IG – R. japonica and I. glandulifera within same pot, no 17 L Hadopots™; SC + IG – S. canadensis and I. glandulifera within same pot, no 17 L Hadopots™; and Tripartite – R. japonica, S. canadensis, and I. glandulifera, no 17 L Hadopots™

a. Rhizome re-planting following planting of *Convolvulus arvensis,* not *R. japonica*

Initial planting of *R. japonica* and *S. canadensis* rhizomes took place on 21/04/21 and 22/04/21. Rhizomes were dug up for each species on 21/04/21 and cut to the correct mass (with trimmed ends to encourage growth) for each treatment (10 g, 5 g, or 3.3 g), using a hack knife and weighing scales. Standardisation by rhizome weight combined with high treatment replication was chosen as the most amenable method available, each species demonstrates similar ecology and rhizome physiology; approximately two active buds per rhizome piece. Once weighed, the number of buds were recorded for each rhizome. *R. japonica* rhizomes were planted (in 7 cm wells) on 21/04/21, and *S. canadensis* on 22/04/21, with the buds facing up, and covered with soil from the site. *I. glandulifera* seedlings were weighed and planted on 28/05/21 following the permit acceptance. This was done by transplanting seedlings from untreated areas, removing the soil from the roots, and weighing seedlings. As *I. glandulifera*-containing treatments could contain 1-3 seedlings to reach the required mass, the number of seedlings was recorded for each pot. Seedlings were initially between 5 cm to 16 cm in height. During weeding on 08/06/21, it became apparent that field bindweed (*Convolvulus arvensis*) rhizome had mistakenly been planted instead of *R. japonica* in some treatment groups. In other pots, deformed *R. japonica* grew due to rhizome collection from areas of the site historically sprayed with herbicide. Some *S. canadensis* rhizomes also failed to shoot in pots, which may have been due to the 24-hour gap between weighing and planting. Due to discrepancies in >70% of the pot trial, all rhizomes were dug up on 09/06/21, and freshly dug and weighed rhizomes were re-planted in their correct treatment groups the same day, which completed the set up (Figure 3). Although this approach did this chance legacy effects within the soil, time management regarding the use of new soil would have been too time consuming, and the number of replicated meant we were confident any specific effects would be apparent.

A.

B.

C.

Figure 3. Treatment plots. A – Pot trial pre-S. canadensis planting (27/05/21), B - Data collection week 1 (09/07/21), C - data collection (week 6).

II. Pot trial data collection

Shoot emergence for rhizomatous plants was recorded for 26 days after which no further emergence was detected. Treatments where no emergence occurred were still monitored throughout the experiment to confirm zero emergent growth. Data collection initiated 09/07/2021 and continued for eight weeks. Non-intrusive data collection

was chosen, due to the timeframe of the study, to maximise the use of the growing season, as project set-up began in Spring, and data collection ended in late Summer. Therefore, following this thesis, potential intrusive data could be collected such measuring the mass of rhizome growth post-experiment.

a. Chlorophyll fluorescence (F_v/F_m)

Chlorophyll fluorescence was measured using a HandyPEA+ (Hansatech instruments Ltd). The HandyPEA+ provided an F_v/F_m output (ratio of variable fluorescence (F_v) over the maximum fluorescence value (F_m)), which indicated the maximum quantum efficiency of photosystem II and indicates plant photosynthetic performance. Healthy plants generally achieve an F_v/F_m result of 0.85, values lower than this have been exposed to stress, biotic or abiotic, which has reduced the capacity for photochemical quenching within PSII (Hansatech Instruments Ltd, 2021).

Prior to measurements, the HandyPEA+ was set to autogain: a level of gain automatically selected to ensure the maximisation of signal levels but also ensuring that the F_m signal level remain within the upper range of the instrument. Hansatech Instruments Ltd leaf clips were clipped onto leaves of each plant that were large enough to be clipped. The leaf clips were slid to the shut mode to shield the fluorescence detector, and mainly to dark-adapt a section of the leaf prior to measurement. Leaf clips remained closed on leaves for at least fifteen minutes to ensure leaf sections were fully dark-adapted for accurate chlorophyll fluorescence yield. Post-15 minutes, the HandyPEA+ sensor unit was attached to the leaf clip, and the slider slid open to provide uniform illumination over the area of leaf exposed by the leaf clip (4 mm diameter) for accurate F_v/F_m outputs. The F_v/F_m unit was recorded, and the process repeated for every plant in every treatment pot. This enabled means to be calculated if there was more than one shoot/stem for the same species in a pot.

b. **Growth rate (cm/week)**

Plant height was measured to the nearest 0.1 cm. Plants were measured from the base of emergence to the highest point of the plant. All plants of each species were measured, to provide means if there was more than one shoot/stem of the same species of plant planted/emerged. Growth rates were calculated between week to week.

c. **Data analysis**

All data analysis was carried out in R studio (R Core Team, 2020). ANCOVA analysis was carried to determine whether there were significant relationships ($p < 0.05$) between chlorophyll fluorescence and growth between species in pairwise or tripartite treatments. Tukey post-hoc comparisons were conducted using the lsmeans package (Length, 2016), to determine whether species in pairwise and tripartite treatments had significantly different growth rates, and significantly different chlorophyll fluorescence ($p < 0.05$).

A generalised linear model (GLM) was used to determine whether there was a significant relationship between the likelihood of emergence of rhizomatous species depending on their treatment groups ($p < 0.05$). A post-hoc Shapiro-Wilk test was conducted to test the normality of the samples.

III. Long-term restoration community analysis

The tripartite experiment aimed to observe the associations between invasive species and help to understand the potential of invasive species interactions due to the potential of secondary invasions of *Impatiens glandulifera* and *Solidago canadensis* at the Advanced Invasives Field Site. The second part of this study was to continue long-term data collection regarding land restoration post-*Reynoutria japonica* invasion/treatment which is also influenced by secondary invasions. This was done by assessing the effectiveness of specific seed mixes (with selectively chosen species to prevent secondary invasions from other invasive species, and to promote native plant cover) through community analysis and seedbank analysis,

a. Community analysis

Community analysis of three 225 m^2 plots occurred in three blocks: Block 2, Block 3, and Block 4 at the Advanced Invasives field site (Figure 4). Each block consisted of three 225 m^2 replicated plots with the seed mix restoration experimental design described by Hocking 2020. The rationale for the seed mixes was due to the functional species traits and community NVC category, which produced two varying functional diversity mixes, alongside an amenity grass seed mix (Table 2). The plots used from each block were 2I, 2H, and 2D for Block 2, 3I, 3E, and 3G for Block 3, and 4F, 4J, and 4H for Block 4. Each plot was further subdivided into nine 3 m^2 restoration treatment subplots of three seed mixes with/without combination of matting and three independent passive (no seed mix) control subplots (Table 3).

Figure 4. Aerial site map of the Advanced Invasives field site. Soil cores were taken from and community analysis occurred in Blocks 2 (blue), 3 (Green), and 4 (orange). Figure taken from Hocking (2021).

Table 2. Table taken from Hocking (2021). Displaying species composition in the high functional diversity mix, and the low diversity mix (Competition mix)

Table 2.13. Species composition of seed mixes and description of associated traits.

Seed mix	Species	Life form	C-S-R	Clonality	SLA	Plant height (cm)
High functional diversity	*Arrhenatherum elatius*	Protohemicryptophyte	C	Perennial tussock-forming grass	24.3	150
	Poa annua	Hemicryptophyte	R	Not clonal	37	20
	Vicia sativa	Therophyte	R	Not clonal	21.2	90
	Lotus corniculatus	Hemicryptophyte	S (C/R)	Not clonal	22.8	40
	Achillea millefolium	Hemicryptophyte	C/S/R	Rhizome forming	21.4	45
	Papaver rhoeas	Therophyte	R	Not clonal	27.5	60
Competition mix	*Festuca rubra*	Hemicryptophyte	C/S/R	Rhizome forming	17.7	75
	Agrostis capillaris	Hemicryptophyte	C/S/R	Rhizome forming	30.8	62
	Vicia sativa	Therophyte	R	Not clonal	21.2	90
	Trifolium repens	Chamaephyte	C/R	Creeping; rooting at nodes	38.8	20
	Achillea millefolium	Hemicryptophyte	C/S/R	Rhizome forming	21.4	45
	Gallium verum	Hemicryptophyte	C/S	Rhizome forming	30	75

Table 3. Treatment groups repeated in each subplot (n = 81).

Name	Treatment (Seed mix / matting)
A	General amenity grass mix
AM	General amenity grass mix + matting
LD	Low functional diversity seed mix (competition mix)
LDM	Low functional diversity seed mix + matting
HD	High functional diversity seed mix
HDM	High functional diversity seed mix+ matting
ST	Control
SM	Control
C	Control

Community analysis occurred on 24/08/2021 and 26/08/2021. Species in each treatment of each plot were surveyed, the plants identified to species level and the percentage coverage of each species in the 3 m^2 plots recorded.

The percentage coverage and species richness (count of different species) was used to calculate the Shannon Diversity index (H') of each treatment group. The species richness and Shannon Diversity index were compared across

treatments using ANOVA statistical testing, and post-hoc Kruskal-Wallis chi squared analysis to further test associations.

b. Seedbank assessment

Soil samples were collected the same blocks and subplots surveyed in the community analysis. Only one of the three control treatments (C) was surveyed for each 225 m^2 subplot (n = 63, Table 4)

We used a hand-held soil corer to take soil cores from each 3 m^2, which penetrated the soil up to 19 cm. Enough soil was sampled to fill a small polyethene bag, which needed three to five soil cores from each treatment to fill, depending on the soil texture. This made sure there would be enough soil in seedling trays. Soil cores were taken from the centre, and two to five corners of the 3 m^2 treatment plots, which were amalgamated into pooled samples for each plot. Sampling depth varied depending on the belowground *R. japonica* rhizome depth, and the density of aboveground litter. The pooled samples labelled with the treatment group, date of collection and plot ID. Soil cores were collected between 02/03/21 and 12/04/2021. The gap in collection was attributed to Swansea University's reprocessing of risk assessments related to fieldwork activities.

Soil samples were taken to Swansea University Singleton campus, for glasshouse germination between 15/04/2021 and 19/04/2021. All samples were weighed to the nearest gram using battery powered scales and based on the lowest core weight obtained (range 427 to 1584 g), 420 g of each sample was used. A thin layer of sterile compost was spread at the bottom of each seedling tray with drainage holes to prevent leakage. Each 420 g soil sample was then spread thinly over the thin layer of compost in each treatment seedling tray, and watered. Trays were 37.5 x 24 cm, and 23 x 17 cm. and the weight recorded. A layer of topsoil 3 cm deep was added to seedling trays with drainage holes, the soil samples spread evenly on top and labelled with the treatment group and site ID, and watered. Soil samples from the same plot were stratified randomly on the greenhouse bench to prevent bias and standardise the growing conditions (Figure 5).

Figure 5. Stratified arrangement of soil samples in Swansea University Greenhouse 2, collected from the Advanced Invasives Field site, Taff's Well.

Soil samples were initially watered twice weekly to stop seedlings drying out and aid germination. However, as no seedling had emerged by June 2021, watering was increased to daily and capillary matting with reservoirs (trays with no holes) were added under the trays.

The seedlings were given more time to grow to facilitate species identification, which started one month after germination. Seedlings were identified to species level using ID books, namely *The Vegetative Key to the British Flora* (Poland & Clement, 2009), *Seedlings of the Northern-Western European Lowland* (Muller, 1978), and *The Wild Flower Key* (Rose, 2006) and the abundance recorded. Species that were hard to identify were transplanted out of their trays and into pots to allow them to grow further. Once seedlings identification was complete, all seedling and soil samples were autoclaved prior to disposal.

3. Results

I. Morphological developments of the invasive species during pot trials

a. General observations

Following planting on 09/06/2021, *Reynoutria japonica* and *Solidago canadensis* rhizomes began to shoot aboveground three weeks later. Data collection of height (cm) and chlorophyll fluorescence (F_v/F_m) began four weeks after rhizome-planting (Figure 5) and continued for eight weeks. All *Impatiens glandulifera* seedlings (71) established without mortality. Shoot mortality of a single shoot of one replicate of the *S. canadensis* single species control in the 17 L Hadopot™ (T4) occurred after four weeks of data collection, but all other emerged plants survived throughout the experiment. As *I. glandulifera* seedlings were planted with initial heights between 5 cm to 6 cm, the plants which developed appeared to overgrow other species that they were in competition with during the assessment period (Figure 6).

Figure 6. A seven-week growth comparison between RJ + SC(17 L) - R. japonica not in Hadopot™, S. canadensis in 17 L Hadopot™ (T8) - and the Tripartite treatment (R. japonica, S. canadensis, and I. glandulifera, no 17 L Hadopots™) from a growth period, one week prior to the first data collection (left), and week 6 of data collection (right). B. shows the effects of I. glandulifera seedling planting, resulting in shorter R. japonica and S. canadensis plants in the Tripartite interaction (T11) compared to RJ + SC(17 L).

b. Plant growth rates

All growth rates of plants in treatments involved in the paired and tripartite experiments significantly changed over time when species is an influencing factor (RJ + SC ($F_{3,\ 192}$ = 5.623, p = 0.001027), RJ(17 L) + SC ($F_{3,\ 241}$ = 5.988, p = 0.0.0005955), RJ + SC(17 L) ($F_{3,\ 141}$ = 5.788, p = 0.0.0009241), RJ + IG ($F_{3,\ 192}$ = 4.291-15, p = 0.001027), SC + IG ($F_{3,\ 205}$ = 37.86, p < $2.2e^{16}$), and Tripartite ($F_{5,\ 246}$ = 5.623, p = $5.641e^{-11}$). *Impatiens glandulifera* always had the average highest weekly growth rate in its multispecies treatments (RJ + IG; SC + IG; and Tripartite), however its growth rate slowed down over the eight weeks (Figure 7). *Reynoutria japonica* always had the lowest average growth rate within multispecies treatments (T6-T9 RJ + SC; RJ(17 L) + SC; RJ + SC(17 L); and Tripartite (Appendix A)). *S. canadensis* had higher average growth rates than *R. japonica* in treatments with both species (T6-T9 RJ + SC; RJ(17 L) + SC; RJ + SC(17 L); and Tripartite) but did not have higher mean growth rates than *I. glandulifera. R. japonica* and *S. canadensis* control groups within 17 L Hadopot™ had smaller mean growth rates (4.44 cm/week and 6.58 cm/week respectively) than controls not in single species 17 L Hadopots™ (4.56 cm/week and 7.54 cm/week).

The growth rates of *R. japonica* and *S. canadensis* in treatment RJ + SC were not significantly different from each other across the entire time course; however, growth rates of both species increased over time, unlike the control for *R. japonica* only. Although the growth rate of *R. japonica* was initially higher than that of *S. canadensis* (mean growth rate of 5.21 cm/week and 3.13 cm/week respectively between Week 1 and Week 2 (Time 1)), by Time 2 (growth rate between Week 2 and 3), *S. canadensis* had a higher mean growth rate of 0.19 cm than *R. japonica.*

The growth rates of *R*. japonica and *I. glandulifera* in RJ + IG were significantly different (p < 0.05) under a post hoc Tukey test. While the growth rates of both species decreased over time, the growth rate of *I. glandulifera* was always higher than *R. japonica,* but decreased from 18.94 cm/week between Week 1 and 2 (Time 1), to 3.37 cm/week between Week 7 to 8 (Time 7), and *R. japonica* from 5.12 cm/week to 0.72 cm/week for the same time intervals. In treatment SC + IG, the growth rates of *S. canadensis* and *I. glandulifera* were significantly different (p < 0.05). This treatment was the only treatment where the growth rate of *S. canadensis* decreased over time, from 7.34 cm/week between Weeks 1 and 2, to 1.15 cm/week between Weeks 7 and 8. The growth rate of *I. glandulifera* was always higher than that of *S. canadensis* and was higher than its control until time interval 6 (Weeks 6 to 7), where the growth rate dropped below the control growth rate. The growth rates observed in *S. canadensis* and *I. glandulifera,* and *R. japonica* and *I. glandulifera* interactions were significantly different (p = 0.0001 for both interactions) from the tripartite treatment measurements.

In the tripartite experiment, the growth rate of *I. glandulifera* was again the highest of all species, and higher that its single species control measurement. *S. canadensis* was the only species in this treatment to have an increasing growth rate over time; however, this was below the control growth rate for *S. canadensis*. The growth rate of *R. japonica* was also lower than its control.

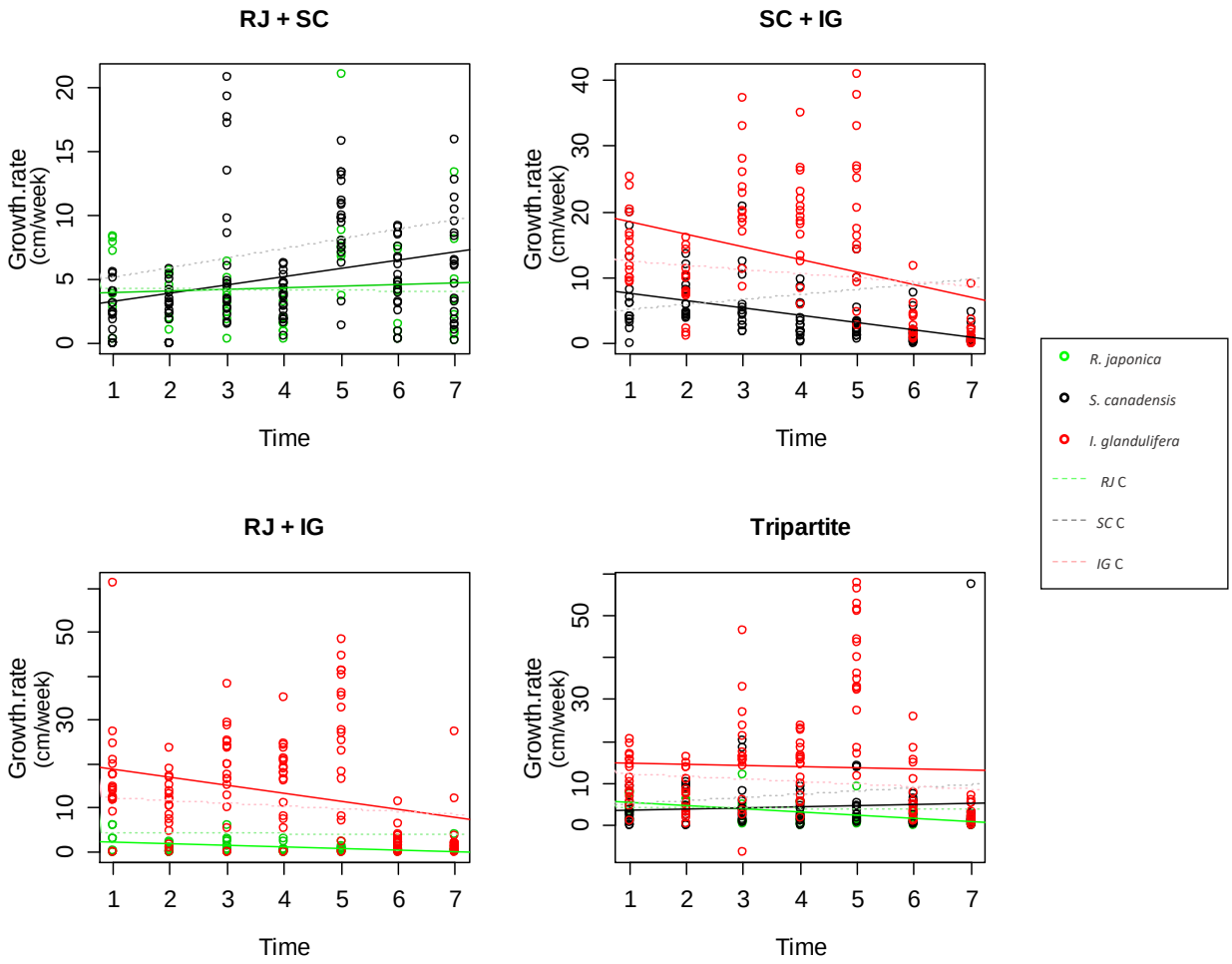

Figure 7. The growth rate (cm / week) of species between weeks of treatments; RJ + SC (top left) – R. japonica *and* S. canadensis *within the same pot, no 17 L Hadopots™;* RJ + IG (bottom left) – R. japonica *and* I. glandulifera *within same pot, no 17 L Hadopots™;* SC + IG (top right) – S. canadensis *and* I. glandulifera *within same pot, no 17 L Hadopots™ and* Tripartite (bottom right) – R. japonica, S. canadensis, *and* I. glandulifera, *no 17 L Hadopots™. Regression lines added for each species, with baseline regression lines of* the weekly growth rates of *controls to their corresponding treatments. Species colours: green - R. japonica, black- S. canadensis, and red - I. glanulifera. Control basline regressions for controls without 17 L Hadopots™;* R. japonica = *light green*, S. canadensis = *grey, and* I. glandulifera = *pink. Plot created in R Studio. Time is not denoted by weeks, as change in growth rate was calculated from the preceding week, rather '1' stands for the change of growth rate between week 1 and week 2 of data collection, '2' the change from week 2 to week 3 of data collection, '3' the change from week 3 to week 4, '4' from week 4 to week 5, '5' from week 5 to 6, '6' from week 6 to 7, and '7' from week 7 to 8.*

c. Plant chlorophyll fluorescence ratios

For the single species tretaments, *Solidago canadensis* had the highest average F_v/F_m values that continued to increase over the eight week period. The average F_v/F_m values for *Reynoutria japonica* also increased over the growing period, however the values were constantly lower than that of *S. canadensis*. *Impatiens glandulifera* had the lowest average F_v/F_m values over the growing period, which descreased over time. High F_v/F_m values of *S. canadensis* were consistent across all treatments it was included in compared to other plants, including RJ + SC. Although, in RJ + SC, the F_v/F_m values of *S. canadensis* were lower that its control values. The highest mean F_v/F_m ratio for *S. canadensis* was in SC + IG, with 0.812 compared to 0.77 of *I. glandulifera*. Isolation into smaller 17 L Hadopots did not seem to affect F_v/F_m values (Appendix B).

Chlorophyll fluorescence significantly differed over time between species in treatment groups (Figure 8). This indicated that there were statistically different relationships between F_v/F_m ratio and time, and F_v/F_m ratio and species across the multispecies treatments ($p < 0.05$). (RJ + SC; RJ(17 L) + SC; RJ + SC(17 L); RJ + IG; SC + IG; and Tripartite - $F_{3, 204} = 21.34$, $p = 4.595e^{-12}$; $F_{3, 269} = 30.42$, $p = 2.2e^{-16}$; $F_{3, 153} = 13.82$, $p = 5.054e^{-18}$; $F_{3, 195} = 30.42$, $p = 0.01611$; $F_{3, 230} = 17.76$, $p = 2.109e^{-10}$; and $F_{5, 274} = 4.555$, $p = 0.00052$ respectively).

Of the 10 g controls, *S. canadensis* within 17 L Hadopots™ had the highest mean F_v/F_m ratio across the eight weeks (initial reading of 0.795 and final reading of 0.812). *S. canadensis* controls not in 17 L Hadopots™ had the largest increase of F_v/F_m over the eight weeks (initial reading of 0.779 and final reading of 0.809). Both *R. japonica* controls with and without 17 L Hadopots™ had increasing F_v/F_m values over the eight weeks (initial readings of 0.760 and 0.780, final reading of 0.776 and 0.784). The mean F_v/F_m ratios for the *I. glandulifera* control decreased over the eight-week period (from 0.794 to 0.790).

All multispecies treatments, excluding RJ + IG, had significant pairwise chlorophyll fluorescence differences over time between species. Weekly chlorophyll fluorescence values for *R. japonica* and *S. canadensis* significantly differed in treatment RJ + SC ($p < 0.05$). The F_v/F_m ratio for *S. canadensis* increased (initial ratio of 0.798 and final of 0.819) and closely followed its control mean F_v/F_m, whereas the mean F_v/F_m ratio for *R. japonica* decreased (initial ratio of 0.779, final ratio of 0.763). The mean *S. canadensis* F_v/F_m ratio over eight weeks was also significantly higher than *R. japonica* in treatments RJ(17L) + SC and RJ + SC(17L) ($p < 0.05$).

There was not a significant relationship between the mean F_v/F_m ratios over time for treatment RJ + IG. The mean F_v/F_m ratios for *R. japonica* and *I. glandulifera* both decreased over the growing period. *I. glandulifera* followed a close pattern to its control. In contrast to the positive regression for the *R. japonica* control, *R. japonica* within the treatment shows a negative regression for its mean F_v/F_m. In treatment SC + IG, there was a significant difference between the F_v/F_m ratios of the two species over the eight weeks ($p < 0.001$). While *S. canadensis* shows a positive regression, and *I. glandulifera* shows a negative regression, the mean F_v/F_m values for *S. canadensis* are constantly higher than *I. glandulifera* and that of its control over the eight weeks (initial ratio of 0.807, final ratio of 0.817), whereas *I. glandulifera* has mean F_v/F_m ratios below its control comparative over the eight weeks (initial ratio of 0.721, final ratio of 0.749).

Within the Tripartite treatment, significant differences are shown between *S. canadensis* and *I. glandulifera* ($p <0.05$) and *R. japonica* and *S. canadensis*. Both *S. canadensis* (initial ratio of 0.802, final ratio of 0.815) and *R. japonica* (initial ratio of 0.775, final ratio of 0.800) show positive regressions of mean F_v/F_m ratios over eight weeks that are higher than their control comparatives. The mean F_v/F_m of *I. glandulifera* decreases over eight weeks, following the regression of its control closely (initial ratio of 0.787, final ratio of 0.760).

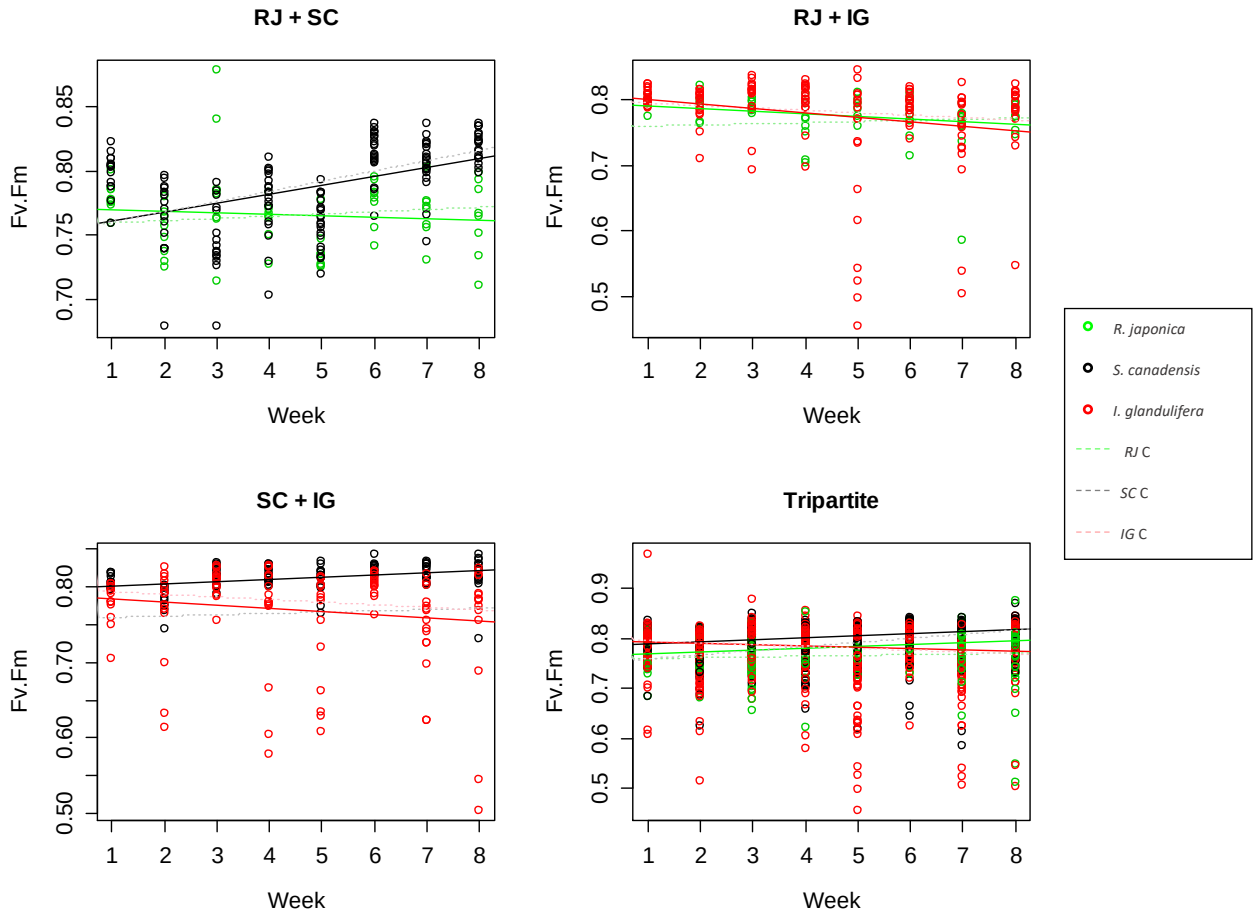

Figure 8. Changes of chlorophyll fluorescence (F_v/F_m) over the eight-week period for each species within treatments: RJ + SC (top left) – R. japonica and S. canadensis within the same pot, no 17 L Hadopots; RJ + IG (bottom left)– R. japonica and I. glandulifera within same pot, no 17 L Hadopots™; SC + IG (top right)– S. canadensis and I. glandulifera within same pot, no 17 L Hadopots™ and Tripartite (bottom right) – R. japonica, S. canadensis, and I. glandulifera, no 17 L Hadopots™. Regression lines added for each species, with baseline regression lines of F_v/F_m controls species ratios to their corresponding treatments. Species colours: green - R. japonica, black - S. canadensis, and red - I. glanulifera. Control basline regressions for controls without 17 L Hadopots™; R. japonica = light green, S. canadensis = grey, and I. glandulifera = pink. Plot created in R Studio.

a. Rhizomatous species emergence

The only case where *S. canadensis* did not emerge was in a single pot of the Tripartite treatment (Figure 9). No emergence of *R. japonica* was recorded in two pots of RJ + SC, one pot of RJ(17 L) + SC, three pots of RJ + SC(17 L), four pots of RJ + IG, and three pots of the Tripartite treatment. A binomial generalised linear model (glm) revealed that plant emergence was significantly influcenced by the treatment group ($Z = 4.169$, $p = 3.06e^{-05}$). A Shaprio-Wilk normality test confimed that the chance of emergence was significantly different between treatment groups ($W = 0.36662$, $p < 2.2e^{-16}$).

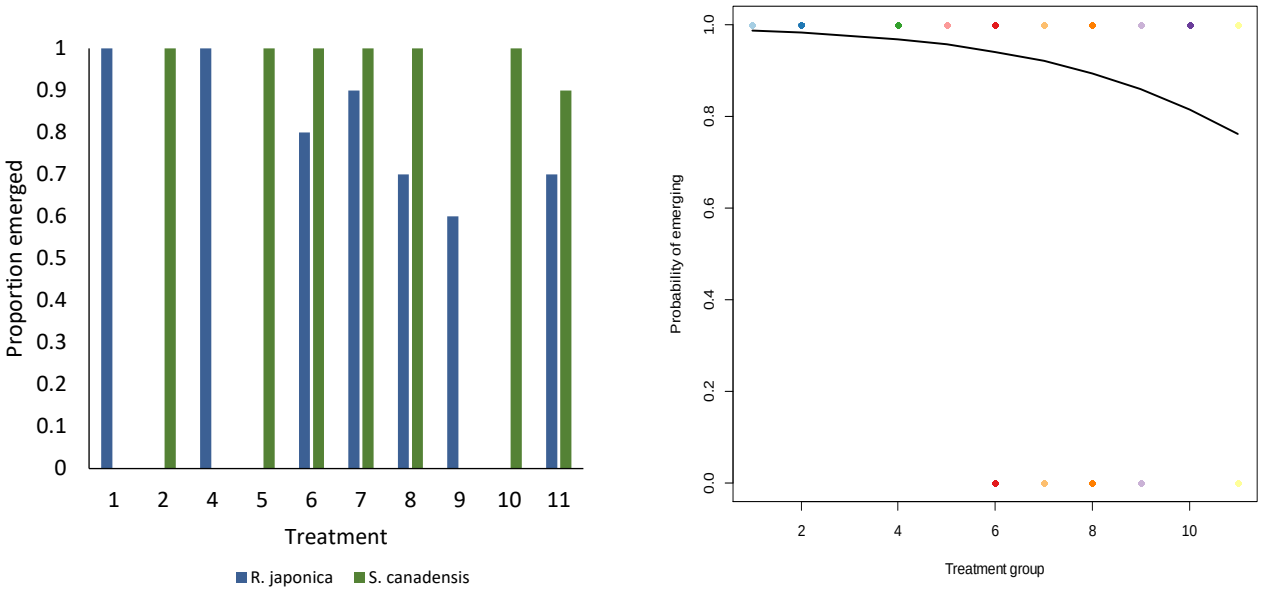

Figure 9. Left: proportion of rhizomatous plants emerged for each treatment. This data was collected at the beginning of the growth period (early July), and at the end of data collection (04/09/2021) to determine whether plants had emerged late. Dark blue; R. japonica, and dark green; S. canadensis. Right: binomial representation of the propability of plant emergence, depending on treatment. Treatment group is denoted by numbers, where 1 = RJ control, 2 = SC control; 3 = IG control (not included as no rhizome forming species present), 4 = RJ control(17 L), 5 = SC control (17 L), 6 = RJ + SC, 7 = RJ(17 L) + SC, 8 = RJ + SC(17 L), 9 = RJ + IG, 10 = SC + IG, 11 = Tripartite. All data points for each plant of each species R. japonica, S. canadensis, and I. glandulifera

II. Long-term restoration data – biodiversity analysis of land previously dominated by *Reynoutria japonica*

a. Community analysis

Community analysis of post- *Reynoutria japonica* treatment regeneration plots occurred at the Advanced Invasives Field Site. The community analysis took place in three main blocks (block 2, block 3, and block 4), which were each comprised of three subplots (Table 5). As there are three subplots to each main blocks, comparisons could be made due to replicates. The seedmixes/treatments included: A - amenity grass mix, AM - amenity grass mix + matting, LD - low density species mix, LDM - low density species mix + matting, HD - High density seed mix, HDM - High density seed mix + matting, and ST, SM, and control - three types of control treatment. Each of these nine treatments were set up in a 3 m x 3 m section of each subplot (n = 81).

Table 5. Description of the subplots that comprise each Block. Plot 2 contains subplots 2I, 2H, 2D. Plot 3 contains 3I, 3G, 3E. Plot 4 contains4F, 4J, 4H.

Block	Subplot
Block 2	2I, 2H, 2D
Block 3	3I, 3G, 3E
Block	4F, 4J, 4H

The community analysis resulted in 99 species (Appendix C) identified over all nine subplots and treatments. For control SM treatmnets in subplots 3G and 3E, no data was recorded due to inasscessability (n = 79).

Commonly occuring (in all nine subplots) species included: *Festuca rubra* (red fescue), which had a mean percentage coverage of 34.22%, and was found in three or more treatment groups of each subplot; *Holcus lanatus* (Yorkshire fog), which occurred in seven or more treatment groups of each subplot, with a mean percentage coverage of 20.178%; *I. glandulifera*, which was found in seven or more treatment groups of each subplot, with a mean percentage coverage of 14.97; *R. japonica*, which was found in five or more treatment groups of each subplot, with a mean percentage coverage of 10.32%; *Rubus fruticosus* (bramble), which was found in three or more treatment groups of each subplot, with a mean percentage coverage of 14.65%; *Artemisia vulgaris* (common mugwort), which occurred in one to seven treatments of each subplot, with a mean percentage coverage of 16.81%; *Lotus corniculatus* (Bird's-foot trefoil), which occurred in one to four treatment groups in each of the subplots, with a mean percentage coverage of 16.71%; and *Galium aparine* (cleavers), which occurred in one to seven treatment groups in each of the subplots, with a mean percentage coverage of 2.4%. Species, such as *Equisetum arvense* (field horsetails), did not occur in every subgroup, but occurred in high percentages of treatments it was found in (30.24%), *E. arvense* was found in four to nine treatment groups within the five subplots it was growing in.

Mean H' for each treatment group (n = 9 for each treatment) ranged from 1.899 to 2.168, with low density matting (LDM) treatments producing the lowest mean Shannon diversity index (H') and high density (HD) treatments producing the highest mean H'. Highest mean species richness was recorded in a control group, ST (15.78), and lowest in the high-density + matting (HDM) treatment group (11.56). Both HD and low density (LD) mix treatments had higher mean H' and species richness values than treatments with matting added to the HD/LD treatments (HD – 2.168 and 13.67 respectively, HDM – 2.00 and 11.56 respectively, LD – 2.06 and 12.56 respectively, and LDM – 1.90 and 11.78 respectively), demonstrating that both HD and LD species mixes may encourage higher biodiversity without matting. ANOVA testing between mean plot Shannon Weiner diversity index (H') and species richness showed that both variables did not have a significant relationship with the treatment group (Figure 10). Post hoc Kruskall-Wallis chi squared analysis confimed this.

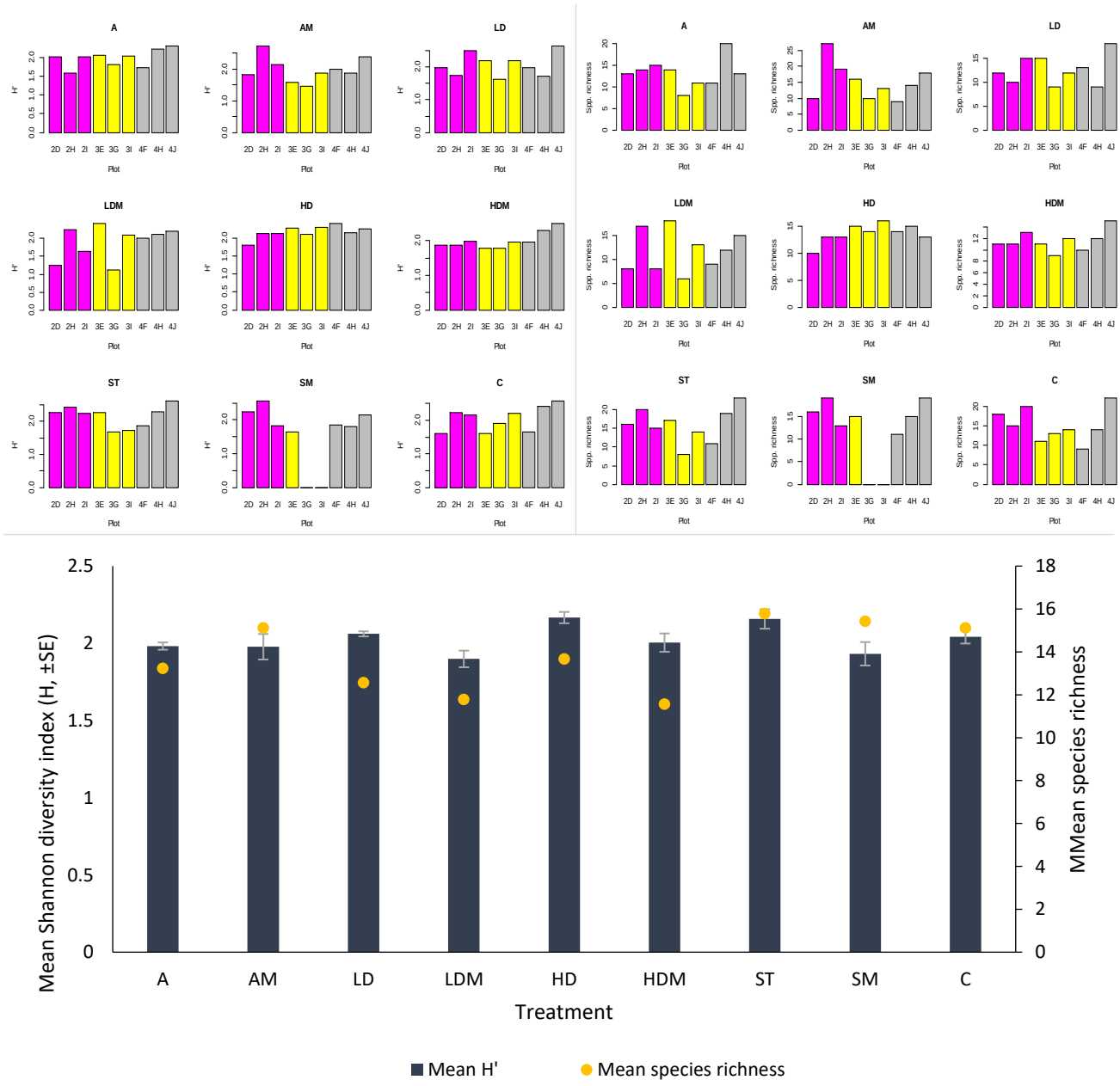

Figure 10. Shannon Diversity index (H' – left) and species richness (right) for each treatment group (A - amenity grass mix, AM - amenity grass mix + matting, LD - low density species mix, LDM, low density species mix + matting, HD - High density seed mix, HDM - High density seed mix + matting, ST, SM and control are three types of control treatment) for each plot (2- pink(D, H, I), 3- yellow(E, G, I), and 4- grey(F, H, J) surveyed at the Advanced Invasives field site. Bottom: mean H' and species richness for each treatment.

b. Seedbank analysis

Soil samples taken from the Advanced Invasives field site were used for the seedbank experiment to investigate whether functional species from the seed mixes were still found in the seedbank, and to compare the species diversity and species richness of the seedbank with the community analysis. These soil samples were taken from each subplot (see Table 5). Each of the nine subplots encompassed 3 m x 3 m plots, each treated with seedmixes/treatments: A - amenity grass mix, AM - amenity grass mix + matting, LD - low density species mix, LDM - low density species mix + matting, HD - High density seed mix, HDM - High density seed mix + matting, and C - control treatment. Each of these nine treatments were set up in each subplot (n = 63).

Seedlings began to germinate three weeks after the addition of capillary matting and reservoirs which aided watering (Figure 11). Data collection began one month later. All seedling trays produced viable seedlings.

Figure 11. Left: Seedbank analysis experiment with added capillary matting on 27/05/2021. Right: an example tray of seedlings at Swansea University, taken during data collection, 04/08/2021.

Identification of species during the seebank study yielded 42 species (Appendix D). All species were identified to species level, with the exception of a *Rosa spp*. and a species of Aguilegia. *Achillea millefoilium* (yarrow), *Festuca rubra* (red fescue), *Buddleia davidii* (Buddleia), and *Epilobium tetragonum* (square-stalked willowherb) were the three species with the highest mean seedling count (4.52, 4.62, 4.81, and 6.3 respectively). *Holcus lanatus* (Yorkshire fog), *A. millefolium*, *F. rubra*, and *E. tetragonum* were found in all subplots (2I, 2G, 2E, 3I, 3G, 3E, 4F, 4J, and 4H). *A. millefolium* was found in treatments with low density (LD or LDM) seed mixes in eight of the nine subplot groups, *H. lanatus* was also found in low density seed mix (LD or LDM) treatments (seven out of nine subplots), and amenity grass mix (A or AM) treatments (also seven out of nine subplots). Species that were initially present in one or two mixes occurred in treatments without these species in the original mixes: *A. millefolium* was initially part of HD and

LD seed mixes, however the species was also found in A and AM treatments. *F. rubra* was part of the LD and A species mixes, but within the seedbank experiment it was found in every treatment (A, AM, LD, LDM, HD, HDM and C).

ANOVA testing confirmed that there was not a signicant relationship between the Shannon diversity index and treatment nor the species richness and treatment group. LD treatments had the highest mean H' and species richness of 1.29 and 5.22 respectively, and HDM treatments the mean lowest H'and species richness of 0.91 and 3.22 respectively (Figure 12) of all treatment groups. The mean species richness and H' for HD treatments were 4 and 1.1 respectively. The mean species richness of A treatments was 4.4, and H' of A treatments was 1.056. AM treatments had a mean species diversity of 4, and H' of 1.03. LDM treatments groups had a mean species richness of 0.94, and H'of 3.56. The control (C) groups had a mean species richness of 3.36, and H'of 0.91.

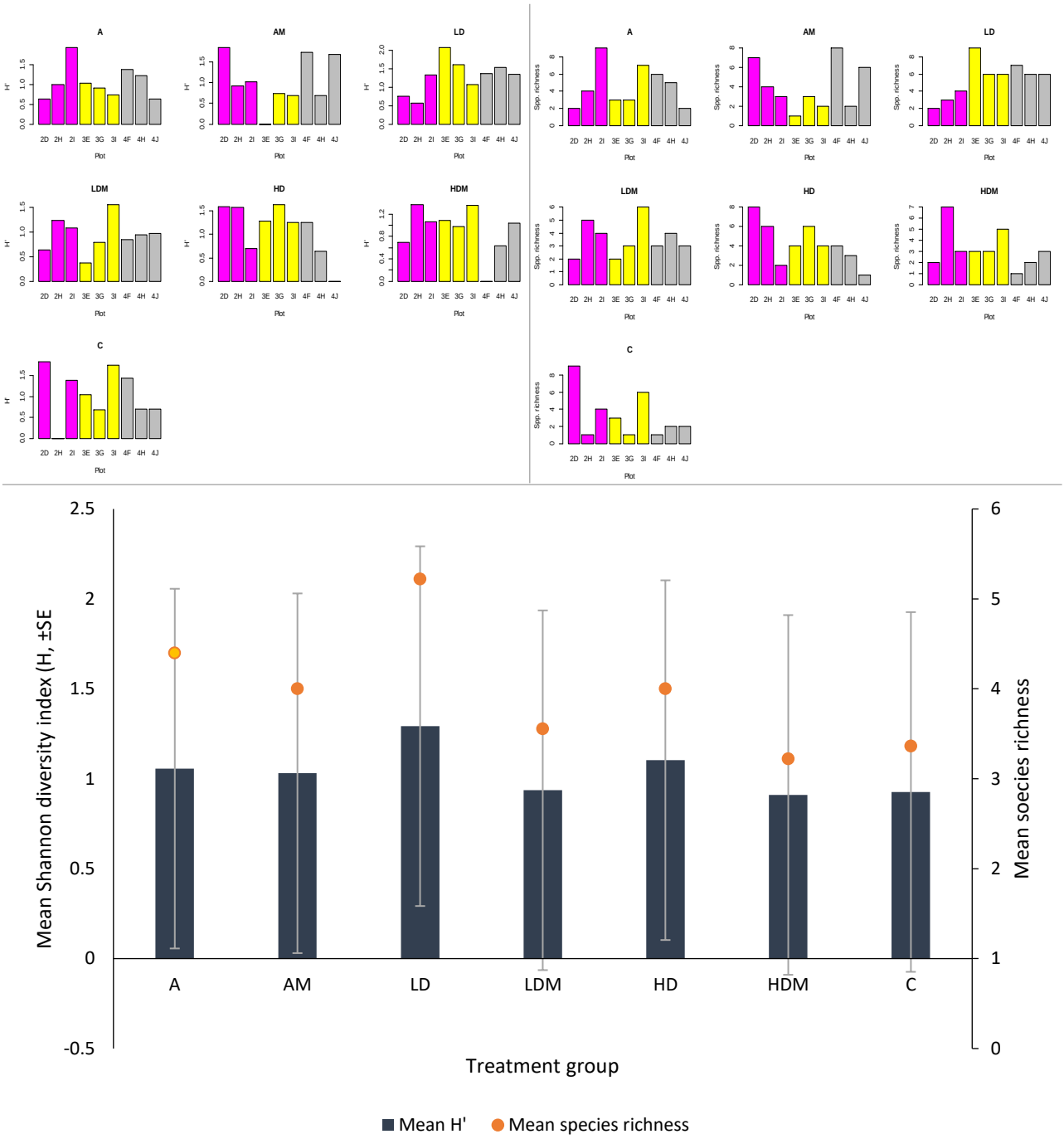

Figure 12. Shannon Diversity index (H' – left) and species richness (right) for each treatment group (A - amenity grass mix, AM - amenity grass mix + matting, LD - low density species mix, LDM, low density species mix + matting, HD - High density seed mix, HDM - High density seed mix + matting, ST, SM and control are three types of control treatment) for seedling tray (2- pink(D, H, I), 3- yellow(E, G, I), and 4- grey(F, H, J) surveyed at Swansea University. Bottom: mean H' and species richness for each treatment seedling tray.

4. Discussion

I. Measuring the invasive potential of *Solidago canadensis, Reynoutria japonica*, and *Impatiens glandulifera*

In the management of invasive species, the control of one species may lead to a niche opening for another to exploit; furthermore, little is known about how invasive species with differing phenotypic traits interact and form an invasion hierarchy in nature. These experiments tested the growth and interaction between three invasive species, *Reynoutria japonica, Solidago canadensis*, and *Impatiens glandulifera*, either paired or in tripartite interactions to investigate these questions. The interaction pot trial provided measurements over 11 different interactions and control experiments, for three key fitness traits over eight weeks. The post-herbicide treatment analysis of *R. japonica* management and restoration was further tested by community analysis and seedbank analysis to compare the effectiveness of different restoration strategies in the form of seed mixes and matting.

a. Rhizomatous species emergence

Although the lower *R. japonica* emergence within pairwise and tripartite treatments in accordance with the hypothesis, the results showed that this is probably not due to the allelopathy of *S. canadensis*, rather belowground competition stemmed from *I. glandulifera*. The least number of *R. japonica* plants emerged was in treatment RJ + IG, where no emergence occurred in four replicate pots. *I. glandulifera* can alter soil moisture, soil pH, and increase available phosphrous and micorbial activities (Ruckli *et al*., 2013). Therefore, *I. glandulifera* may have altered the belowground environment to an extent that did not favour the growth of *R. japonica* buds, as the only replicate of *S. canadensis* that did not emerge was in the tripartite interaction, this further suggests that *I. glandulifera* may be the dominant invasive species regarding the explanation for belowground competition. However, node mortality may have occurred as *R. japonica* did not emerge in a total of six replicates over three pairwise treatments with *S.* canadensis, due to stressful environmental conditions such as restricted space (Dauer & Jongejans, 2013). As the control treatments for *R. japonica* had 100% emergence, belowground competitive capacities of *I. glandulifera* and *S. canadensis* may have affected *R. japonica* emergence.

b. Effects of plant interactions on plant growth rate over time

Growth rate is a major component in the competitive interactions between plants (White *et al.,* 2016) which influences the ability of a plant to compete for light (Moles *et al*, 2009). Overall, over the eight-week period *I. glandulifera* had the highest growth rate compared to *R. japonica* and *S. canadensis* in all treatments associated with the tripartite interaction section of the pot trial. This was as expected, as *I. glandulifera* has a rapid growth rate and tall height, hence its reputation as Europe's tallest plant annual (Pyšek & Prach, 1995; Hejda & Pyšek, 2006; Bartomeus *et al*., 2010). The fast growth rate of *I. glandulifera* can be associated with its large leaf area, and less root mass compared to the other two species (Lambers & Poorter, 1992).

The data suggests that the growth rates of both *I. glandulifera* and *R. japonica* declined towards the end of the season, as data collection terminated in early September, which signifies the end of the growing season for *R. japonica* and *I. glandulifera* (Ellison *et al*., 2019; Gala-Czekaj *et al*., 2021; Nguyen, 2002). At the end of the growing season, resources

that drive aboveground biomass production are reallocated to belowground organs, for example rhizomes, (Soetaert *et al*., 2004), and the production of seeds for annual plants (Bennett *et* al., 2011). Nevertheless, for *S. canadensis* this is not the case, the mean growth rate still increased at the end of data collection for all but one treatment. This is because the growing season for *S. canadensis* is later, and growth still occurs during autumn. The flowering time is also later than *R. japonica and I. glandulifera* (Boise, 2020). As the control treatments for *R. japonica* and *I. glandulifera* had lower mean growth rates at the end of data collection compared to the beginning, it cannot be said that that the lower growth rates at the end of data collection are caused by allelopathic agents emitted by *S. canadensis* in this experiment, rather as the growing season slowed, so did the growth rates of *R. japonica* and *I. glandulifera* plants. These findings show that the slightly later growing season of *S. canadensis* means it has a different consequent peak competitive influence than *R. japonica* and *I. glandulifera* (Dudek *et al*., 2016). The effectiveness of glyphosate spraying can have varying effectiveness depending on the growing season of plants, for example, the germinability of non-rhizomatous invasive species seeds, such as *I. glandulifera*, is most effective early in the growing season (Rice *et al*., 2018), hence spraying all invasive species at the same time may not be efficient. Herbicide application would therefore differ between *R. japonica* and *S. canadensis*, due to the different peak growing seasons of *R. japonica* (early-mid-summer (Jones *et al*., 2018)) compared to *S. canadensis*, where herbicide application would be more effective in mid to late-autumn, as glyphosate spraying for *R. japonica* is recommended in late summer to mid-autumn, when resource is being moved from the aboveground biomass into the belowground rhizome, so the herbicide has greatest effect (Jones *et al*., 2018).

The initial study predictions hypothesised that when *R. japonica* was exposed to *S. canadensis* (RJ + SC), the growth rate would be significantly lower than when the two species were grown in isolation and to the controls, due to the ability of *S. canadensis* to inhibit growth (Abhilasha *et al*., 2008), however this is not the case. When the two species were exposed to each other in the pairwise treatment, both growth rates increased over time, even though the general growth rates of other treatments indicated the end of the *R*. *japonica* growth season. Thiébaut *et al.* (2019), found that alongside the inhibitory aspect of allelopathy, allelopathic invasive species can stimulate the biomass growth of neighbouring plants. Unsurprisingly, it is usually native species that received the inhibitory aspect, and other invasive plants that can benefit from allelopathy (Thiébaut *et al*., 2019). Further analysis would confirm this hypothesis, as this contradicts the initial on-site observation that initiated this experiment, but if this was the case for *S. canadensis* and *R. japonica*, public awareness for the prevention of the spread of both species is more vital than before, to avoid "invasional meltdown"' and even denser thickets of both species (Thiébaut *et al*., 2019; Delbart *et al*., 2012; Gusev, 2015).

c. Effects of plant interactions on chlorophyll fluorescence

Chlorophyll fluorescence is a reliable indicator of plant stress in response to environmental stresses such as heat, drought, nutrition, and growth stress (Feng *et al*., 2015). The F_v/F_m ratio provides us with information that can be missed if plant health estimated were solely based on the growth rate of a plant. The mean F_v/F_m ratios for each species showed that all plants were generally healthy (Zhori *et al*., 2015) and smaller pots did not cause stress due to lack of space. Unlike the mean species growth rate, which showed *I. glandulifera* as the best competitor of the three invasive

species due to the taller height, the chlorophyll fluorescence results demonstrated that *S. canadensis* was consistently healthier than the other species in all treatments it was included in (Figure 8), if only chlorophyll fluorescence was being considered. This correlates with observations made above, in relation to the growth rate reaching peaks in late-summer to mid-autumn, as chlorophyll fluorescence decreases after peak growth season and full bloom (Greer, 2005), which reflects the different invasive potential peaks of plants with slightly different growing seasons (Dudek *et al*., 2016).

The lowest F_v/F_m ratio for *R. japonica* was in treatment RJ + SC. The significant difference between the F_v/F_m ratio of *R. japonica* and *S. canadensis* in this treatment may be an indication of allelopathy, as the F_v/F_m ratio is lower than treatments where *R. japonica* and *S. canadensis* are isolated from each other with 17 L Hadopots™. The significant relationship between both *S. canadensis* and *R. japonica*, and *S. canadensis* and *I. glandulifera,* but not *R. japonica* and *I. glandulifera* may indicate an influence from *S. canadensis* on the other two invasive species. Although the release of allelochemicals can reduce the chlorophyll fluorescence of a plant (Xie *et* al., 2019), this may show that *R. japonica* and *I. glandulifera* experienced more stress to abiotic conditions than *S. canadensis*, as *S. canadensis* consistently had the highest chlorophyll fluorescence values compared to the other two species. Therefore, it cannot be confirmed that allelochemicals emitted by *S. canadensis* caused the lower chlorophyll fluorescence of *R. japonica*, rather *S. canadensis* may have had a greater ability to tolerate abiotic environmental stressors, such as lack of space and resources (Zhang *et al*., 2008). Potential allelopathic activity can only be tested with chemical analysis of the soil, and further isolation of the chemical to test it in a pure form, and the use polydimethylsiloxane (PDMS) microtubing to construct sampling devices to monitor the release of lipophilic allelochemicals from plant roots (Weidenhamer *et al*., 2014). If *S. canadensis* allelochemicals were present, then their adverse effects may be a method for controlling *R. japonica*. The soil was slightly acidic (pH 5.45) and can be ruled out as the cause of stress. Due to the lack of evidence, the hypothesis that *S. canadensis* secretes allelochemicals that hinder the growth and health of *R. japonica* cannot be supported by these experiments.

The lack of *R. japonica* emergence in replicates of five pot trial treatments indicates the potential of belowground interactions. However, other findings, such as the potential of *I. glandulifera* to alter belowground conditions and therefore affect *R. japonica* emergence; and the generally higher chlorophyll fluorescence of *S. canadensis* (in its single-species control), do not support the hypothesis that allelopathy is responsible for adverse effects on *R. japonica*, which was hypothesised following an on-site observation. healthier *S. canadensis* plants, as the single species control treatments also show that *S. canadensis* has higher F_v/F_m ratios than the other two species. Extending the pot trial would provide a deeper insight into the plant interactions, as allelopathy can become stronger over time (Weidenhamer *et al*., 2014).

I. Long-term restoration data – biodiversity analysis of land previously dominated by *Reynoutria japonica*

a. Community analysis

Seed mixes did show differences in the mean species richness and diversity for this year's restoration data collection. The highest species diversity and richness was found in high functional diversity (HD) seed mixes (H'= 2.168, which is a moderate diversity index (Ifo *et al*., 2016)), and the lowest in low functional diversity + matting treatments (Figure 10). Species diversity was higher in seed mix treatments that did not have matting (HD, LD, and A) than those that did. Additional matting has been shown to enhance the success of revegetation (Roberts & Seastedt*,* 2019); however, this was not the result in this community analysis. Therefore, tailored seed mixes may not require the use of matting to provide regeneration following the treatment of *Reynoutria japonica*.

The integration of *Reynoutria japonica* control measures (glyphosate spraying) alongside restoration measures (application of tailored seed mixes (Hocking, 2021)) has shifted the dominance away from *R. japonica* within treatments plots, and towards plants such as *Festuca rubra* (included in the high density and amenity grass seed mixes) and *Holcus lanatus* (a grass species not included in any mixes). As species not found within the seed mixes were present across each subplot (in the case of *H. lanatus,* and *Galium aparine,* in every subplot) it can be said that the use of seed mixes of plants with functional traits suited for restoration of *R. japonica*-invaded habitats, can facilitate the return of native plants back to the invaded area, which is often the goal of community restoration (Lockwood & Pimm, 1999). Although *R. japonica* and *I. glandulifera* were found across all treatments (A, AM, LD, LDM, HD, HDM, ST, SM, C), they were no longer found in monotypic stands due to the integrated restoration methods, considering that plots were once dominated by *R. japonica* stands, and now *R. japonica* has average percentage cover of 10.32% for treatment groups it was found in (and *I. glandulifera* with a mean percentage coverage of 14.97%) the seed mixes were successful. The restoration treatments show that biodiversity can be developed alongside the managed invasive species, which provides a hopeful outlook that even if *R. japonica* eradication may not occur, the possible use of tailored seed mixes with plants to fill environmental niches (Cordell *et al*., 2016) can displace *R. japonica* and encourage native plant species back to the invaded area for biodiversity and ecological services benefits (Gascon *et al*., 2015).

a. Seedbank analysis

The seedbank analysis indicates that the low functional diversity (LD) seed mix provides the highest mean species diversity , followed by the HD seed mix (Figure 11). These two mixes were also the top two (HD the highest) mixes with the highest mean H' for the community analysis. This demonstrates that these are the best mixes to continue habitat restoration of land previously dominated by *R. japonica*. These findings can be confirmed with the continuation of long-term restoration data.

Long-term data collection has shown that researched seed mixes (Hocking et al., 2021) have the potential to encourage diverse native species communities back into land previously dominated by *R. japonica*, and therefore can contribute to post-invasion land recovery. The selection of plants from the NVC restoration reference community which could already be found in the local habitat, provided the creation of beneficial seed for regeneration and encouragement of

native species back into treatment plots, which was displayed with the moderate species diversities of HD and LD seed mixes. Functional species involved in the seed mixes have also remained in the seedbank. Long-term restoration data can also provide insight into the succession of native species reestablishment, as late-successional species dominate restoration later as they allocate resources to belowground growth, allowing them to be more tolerant of stressful environmental conditions compared to early successional species (Sheley *et al*., 2006). The continuation of data collection will help provide insights to the succession of native plants at the Advanced Invasives field site.

Regeneration by seeding is an important tool in restoration, more native species at higher rates can lead to greater restoration successes (Barr *et al*, 2017). Modelling to determine the combination of plants for a species mix to identify the species most suited to restoration i.e. species that can survive stressful environmental conditions and contain functional traits that increase invasion resistance (Sheley *et al*., 2006), lead to cost-effective efforts that can restore biodiversity and ecosystem services (Kimball *et al*., 2015). Among restoration methods, seed mixes have resulted in the most successful native plant recovery when compared to other restoration methods, such as cutting. (Hall *et al*., 2021). With efficient control and restoration strategies, such as ecosystem modelling, actions can be implemented to reduce the negative impacts of invasive species, whilst enhancing the benefits to native species and ecosystems (Kopf *et al.,* 2017).

The results of the seedbank analysis show similarities to results of the community analysis, as species such as *Achillea millefolium* and *Festuca rubra* were found in all subplots across both analyses, and two of the most abundant species for the seedbank analysis. *A. millefolium* was initially part of HD and LD seed mixes, however within the seedbank experiment and community analysis the species was found in A and AM treatments in the former, and all treatments excluding SM in the latter. *F. rubra* was part of the LD and A species mixes, but within the seedbank experiment it was found in every treatment. These results show that viable restoration species are still present in the seedbank. Species dispersal is evident across treatments, as species such included in sole species mixes, such as *Arrhenatherum elatius* (HD) was found across multiple treatments (A, AM, LD, HD, HDM).

The continuation of restoration data since 2018 has demonstrated the successful results that can be achieved when integrating restoration strategies (Delbart *et al*., 2012). Although *R. japonica* was still present in at least five treatment plots in all subplots, it did not dominate, and high species richness could still be achieved in its presence due to the seed mixes, particularly LD and HD mixes, whose species are still found in the seed bank after three years of data collection. These findings can be compared to data collection from previous years, to understand whether *R. japonica* coverage has increased or decreased with time. These methods of *R. japonica* control can help to minimise the use of excessive herbicide applications (Jones *et al*., 2018), accidental spread of *R. japonica* rhizome via mowing and cuttings (Bashtanova *et al*., 2009), and attempts at complete excavation which often has questionable efficiency (Bashtanova *et al*., 2009; Delbart *et al*., 2012).

II. Improvements

To develop this project, an additive approach to the pot trial should be conducted in parallel to the substitutive design that was chosen in the pot trial of this project, this design is possible in nature (Li & Hara, 1999) and would eliminate the density dependent results that may occur in substitutive designs (Rivaie, 2016). I would plant *I. glandulifera* seedlings closer to the time of emergence of the rhizomatous species, this way, the level of shading by *I. glandulifera* on *R. japonica* and *S. canadensis* may be tested under more field-relevant conditions, as the initial heights would be more even. The data collection should start at an earlier period in the year, such as late-May or at the beginning of June, this would ensure data collection is carried out throughout the growing season, and the end of growing season would not influence the results. These timeframes would have been possible if I had not planted the rhizome of the incorrect species (*Convolvulus arvensis* instead of *R.japonica*). Instead of using a substitutive approach, calculating one unit of biomass for each species may limit the competitive disparities and facilitate comparability, as 5 g of *R. Japonica* versus 5 g of *S. canadensis* contain very different bud numbers for example, and vices versa. All data collection was aboveground due to the timeframe of this project, which was during the summer growing season, and therefore non-intrusive data collection was chosen. In the future, belowground response variables may be extracted, as patterns may show different trends belowground, for example as *R. japonica* and *S. canadensis* are rhizomatous species, more energy may be invested into lengthening the rhizome, whereas *I. glandulifera* is not rhizomatous and therefore can invest more energy in aboveground growth. Extra analysis to test the soil for the presence of any allelochemicals will give a more quantitative analysis of the belowground environment, and by continuing pot trial for a few more years to determine longer term interaction dynamics if any interactions are developed; followed by the weighing of belowground rhizomes to see whether rhizomes have been affected by belowground interactions.

5. Conclusion

The dominating growth rate of *Impatiens glandulifera* was expected due to the fast growth rate that categorises the invasive species. The growth of *I. glandulifera* outcompeted *Reynoutria japonica* and *Solidago canadensis* for light, hence the growth rates were lower than their baseline control for *R. japonica* and *S. canadensis* in the tripartite experiment. *Reynoutria japonica* had a higher growth rate than its control in the treatment with no isolation from *S. canadensis*, and therefore there may have potentially been signs of positive allelopathy between the two invasive species.

As the F_v/F_m ratio of *S. canadensis* was the highest mean ratio over all treatments, we can assume that it is outcompeting the other two invasive species in some way, as chlorophyll fluoresce can be used as an indicator of plant health (Jia *et al*., 2019). Nonetheless, this cannot be attributed to allelopathy due to the low F_v/F_m ratios of the *R. japonica* and *I. glandulifera* controls. Further tests, such as soil sampling and the continuation of the pot trial may provide a deeper insight into whether there are any belowground interactions.

The continuation of long-term data has revealed that species from tailored seed mixes, designed to withstand secondary invasions, can remain in the seedbank of land undergoing restoration, and can therefore promote native species back into invaded areas, while reducing the risk of secondary invasions. The seed mixes that yielded the highest species diversity in the community analysis were high- and low-density mixes, which should therefore be considered when implementing tailored seed mixes as a method for land restoration post-invasive species control treatments.

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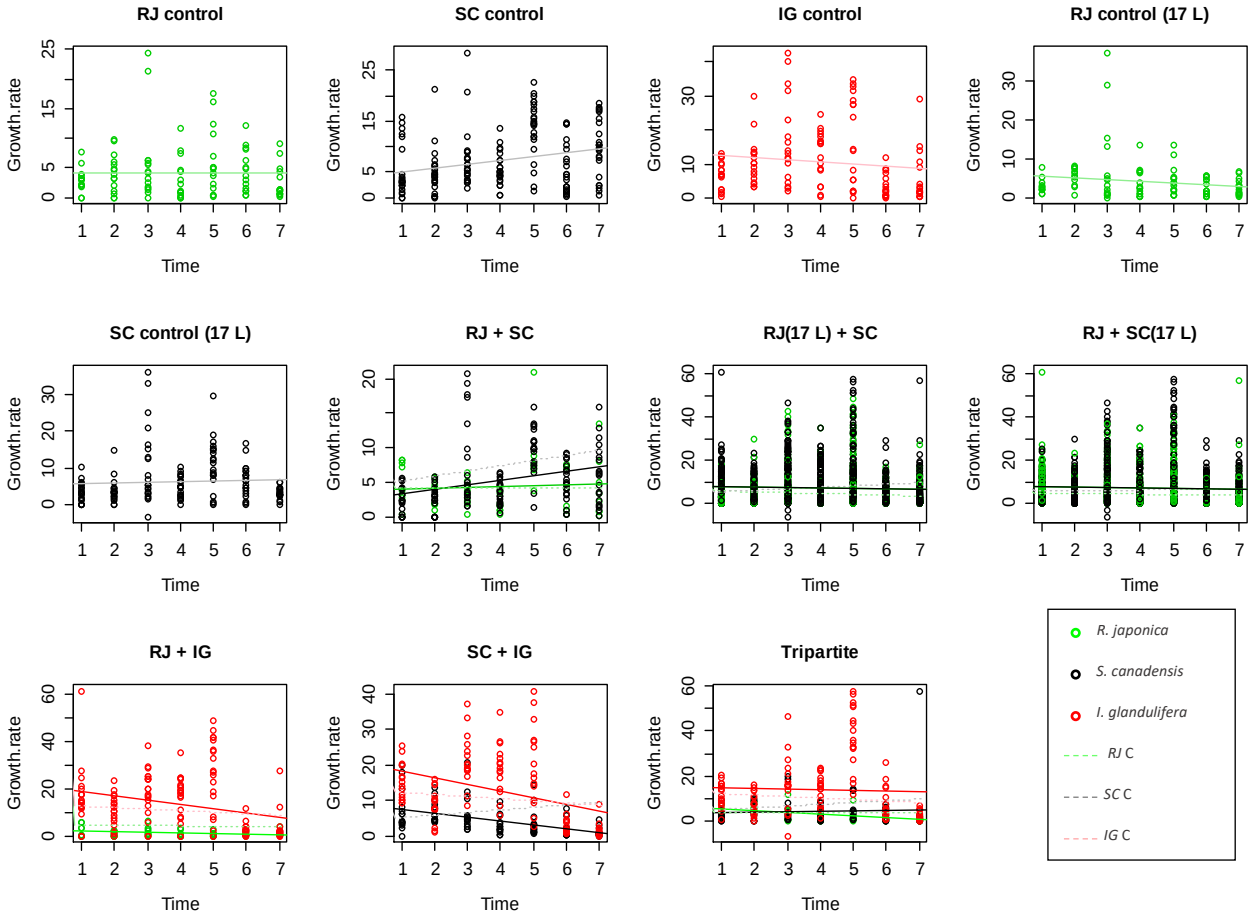
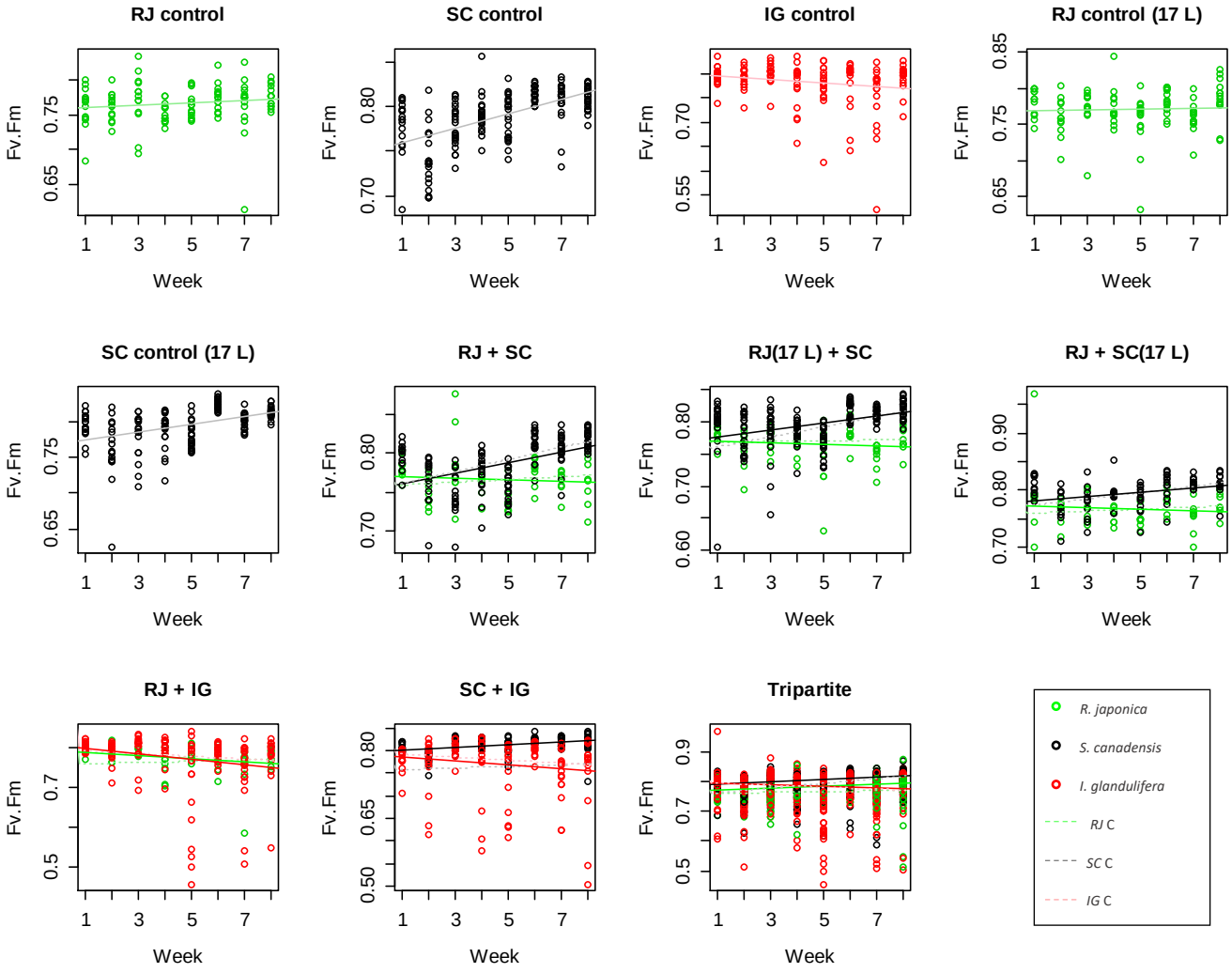
7. Appendices

Appendix A



Appendix A. The growth rate (cm / week) of species between weeks of each treatment group: RJ (Reynoutria japonica) *control*; SC (Solidago canadensis) *control;* IG (Impatiens glandulifera) *control*, RJ control (17 L) – R. japonica *control within 17 L Hadopots™;* SC control (17 L) – S. canadensis control within 17 L *Hadopots™;* RJ + SC – R. japonica *and* S. canadensis *within the same pot, no 17 L Hadopots™;* RJ(17 L) + SC – R. japonica *and* S. canadensis *within same pot,* R. japonica *in 17 L Hadopots™;* RJ + SC (17 L) - R. japonica *and* S. canadensis *within same pot,* S. canadensis *in 17 L Hadopots™;* RJ + IG – R. japonica *and* I. glandulifera *within same pot, no 17 L Hadopots™;* SC + IG – S. canadensis *and* I. glandulifera *within same pot, no 17 L Hadopots™; and* Tripartite – R. japonica, S. canadensis, *and* I. glandulifera, *no 17 L Hadopots™. Regression lines added for each species, with baseline regression lines of* the weekly growth rates of *controls to their corresponding treatments. Species colours: green - R. japonica, black - S. canadensis, and red- I. glanulifera. Control basline regressions for controls without 17 L Hadopots™;* R. japonica = *light green*, S. canadensis = *grey, and* I. glandulifera = *pink. Control basleine regressions for controls with 17 L Hadopots™:* R. japonica = *pink and* S. canadensis = *grey. Plot created in R Studio. Time is not denoted by weeks, as change in growth rate was calculated from the preceding week, rather '1' stands for the change of growth rate between week 1 and week 2 of data collection, '2' the change from week 2 to week 3 of data collection, '3' the change from week 3 to week 4, '4' from week 4 to week 5, '5' from week 5 to 6, '6' from week 6 to 7, and '7' from week 7 to 8.*

Appendix B



Appendix B. Changes of chlorophyll fluorescence (F_v/F_m) *over the eight-week period for each species within each treatments:* RJ (Reynoutria japonica) *control*; SC (Solidago canadensis) *control;* IG (Impatiens glandulifera) *control*, RJ control (17 L) – R. japonica *control within 17 L Hadopots™;* SC control (17 L) – S. canadensis control within 17 L *Hadopots™;* RJ + SC – R. japonica *and* S. canadensis *within the same pot, no 17 L Hadopots™;* RJ(17 L) + SC – R. japonica *and* S. canadensis *within same pot,* R. japonica *in 17 L Hadopots™;* RJ + SC (17 L) - R. japonica *and* S. canadensis *within same pot,* S. canadensis *in 17 L Hadopots™;* RJ + IG – R. japonica *and* I. glandulifera *within same pot, no 17 L Hadopots™; and* Tripartite – R. japonica, S. canadensis, *and* I. glandulifera, *no 17 L Hadopots™. Regression lines added for each species, with baseline regression lines of* F_v/F_m *controls species ratios to their corresponding treatments. Species colours: green - R. japonica, black - S. canadensis, and red- I. glanulifera. Control basline regressions for controls without 17 L Hadopots™;* R. japonica = *light green*, S. canadensis = *grey, and* I. glandulifera = *pink. Control basleine regressions for controls with 17 L Hadopots™:* R. japonica = *pink and* S. canadensis = *grey. Plot created in R Studio.*

Appendix C

Appendix C. Species list of all species identified during the community analysis (99 species), including the plot/s and treatment/s the species were found in alongside their average percentage cover in said plots (n = 79). The seedmixes/treatments include: A - amenity grass mix, AM - amenity grass mix + matting, LD - low density species mix, LDM - low density species mix + matting, HD - High density seed mix, HDM - High density seed mix + matting, and ST, SM and control - three types of control treatment.

		Plots and treatments the species was found in									
		2			**3**			**4**			
Scientific name	**Common name**	**I**	**H**	**D**	**I**	**G**	**E**	**F**	**J**	**H**	Mean %age cover
Acer pseudoplatanus	Sycamore	A, LD, HD, SM, C	A, AM, LDM, ST, SM, C	SM	AM, LDM, ST, C			AM	C	AM, LDM, ST, SM	3.04
Achillea millefolium	Yarrow	AM, LD, LDM, HD, HDM, C	AM, LD, LDM, HD, HDM, ST	LD, HD, HDM	AM, LD, LDM, HD, HDM, ST, C	LD, HD, HDM	LD, LDM	LD, LDM, HD, HDM	LD, HD, C	LD, LDM, HD, HDM, C	21.24
Aegopodium podagraria	Ground elder	HD, HDM, C			A		A, HD, SM, C		LD, C	LD, HD	3.73
Agrostis capillaris	Common bent grass	A, AM, LD, LDM, HD, HDM, ST, SM, C	SM, C	LSM, ST, SM	A, AM, HDM		A, LDM, ST		ST, SM, C		36.16
Agrostis stolonifera	Creeping bent grass		AM, ST				HD				17.33
Alliaria petiolata	Garlic mustard								LD, ST, SM		3
Angelica sylvestris	Wild angelica			C							1
Anthoxanthum odoratum	Sweet vernal grass		AM								1
Anthriscus sylvestris	Cow parsley	A		ST, SM		AM, LDM			AM, LDM, ST	ST	2.2
Aquilegia sp.	Aquilegia spp.				C						1

Arctium minus	Common burdock			C					AM, LDM, ST		8.67
Argentina anserina	Silverweed			A, LD, HDM					AM, LD, SM		1.8
Arrhenatherum elatius	False oat grass	AM, LD, HDM, ST, SM	AM, LD, LDM, HD, HDM, ST	AM, LD, ST, SM, C	LD, HDM, ST		A, LD, LDM, HD, ST	A, AM, LD, LDM, HDM, ST	A, AM, LD, HD, ST, SM, C	AM, HD, HDM, SM	10.56
Artemisia absinthium	Common wormwood		LD								2
Artemisia vulgaris	Common mugwort	LD, HD, SM, C	A, LD, LDM, HD, C	A, C	A, AM, LD, LDM, HDM, ST, C	AM,	LDM, HD, HDM, C	LD, SM	AM, HDM, ST	A, HD, SM, C	16.88
Betula pendula	Silver birch	AM, C									2.5
Buddleja davidii	Buddleia	A, SM, C	ST				LD, LDM, SM	SM			9.56
Cardamine hirsuta	Hairy bittercress		SM		ST				C		1
Centaurea nigra	Lesser knapweed			SM, ST					AM	HDM	2.5
Centaurea scabiosa	Greater knapweed									ST	4
Chamaenerion angustifolium	Rosebay willowherb		A		A, HDM					HDM, C	14.2
Cirsium dissectum	Meadow thistle			ST, C		HD, HDM, ST, C			HD, ST, SM	A, AM, LD, LDM, HD, HDM, SM, ST, C	21.57
Cirsium vulgare	Spear thistle		AM, LDM, HDM, SM, ST	HD, HDM, ST	AM		A, LD, ST	LD, HD, ST, C	AM, LDM, AT, SM	A, LD, LDM, ST	4.4
Convolvulus arvensis	Field bindweed	LD, HD, HDM, ST, SM, C	AM, LDM, HD, HDM, SM	A, AM, LD, LDM, HD, HDM, ST, C	A	LDM, HD, C	A, LD, HD, HDM, SM	A, AM, LD, LDM, HD, HDM, ST, SM, C	AM, LDM HD, HDM, C	A, LDM, HD	12.25

Cornus sanguinea	Common dogwood		C								1
Crataegus monogyna	Hawthorne				HD						3
Dactylis glomerata	Cock's-foot	LD, LDM, C	AM, LD, LDM, HD, C	C	AM, LD, HD, SM				LDM, ST, C	LDM, ST	5.78
Epilobium hirsutum	Greater willowherb					HD	A, LD, LDM, ST		LD		16.17
Epilobium montanum	Broad-leaved willowherb	SM			LD				LDM, ST, C		4
Epilobium tetragonum	Square-stalked willowherb	A, AM, ST	A, AM, ST, SM	HDM, ST, SM, C	ST		HDM	HDM	LD, ST, SM, C		2.35
Equisetum arvense	Field horsetail				AM, LDM, HD, ST, C		A, AM, LD, LDM, HD, HDM, SM, ST, C	A, HD, ST, SM	A, LD, HDM, ST, SM, C	AM, LD, LDM, HDM, C	30.24
Erigeron canadensis	Canadian fleabane	AM, HDM, ST, SM	A								13.2
Eupatorium cannabinum	Hemp-agrimony	A, AM, LD, HDM, SM, C	A, AM, LD, HD, ST, SM, C	C	C	HD	AM, LD, LDM, HD, ST, SM, C	A, LD, HD, SM, C			13.55
Festuca ovina	Sheep's fescue			LDM			A				10
Festuca rubra	Red fescue	A, AM, LD, LDM, HD, HDM, SM, C	A, AM, LD, ST	AM, LD, ST, SM, C	AM, LD, LDM, ST	A, AM, LD, LDM, HD, HDM, ST, C	LD, LDM, HD, ST	A, LD, LDM	AM, LD, SM	A, AM, LD, LDM, HD, C	34.22
Fraxinus excelsior	Ash	ST	SM						LD, SM		1
Galium aparine	Cleavers	A, AM, LD, HDM, ST	A, HD, HDM, ST, SM, C	LD, LDM, HD, HDM, SM	A, LDM, HD, HDM	HDM	AM, HDM, ST	A, AM, LDM, HD, HDM, D	A, AM, LD, LDM, HD, SM, C	AM, HDM, C	2.4
Galium verum	Lady's bedstraw	LD, LDM, C	LD, LDM	LD, LDM	LDM, ST	LD, LDM	LD, LDM	LD, LDM		LD, LDM	20.18
Geranium dissectum	Cutleaf cranesbill			A							2

Geranium robertianum	Herb Robert	ST	ST								1.5
Geranium rotundifolium	Round-leaved cranesbill			C							1
Geum urbanum	Wood avens	AM, C			HDM						1.98
Glechoma hederacea	Ground-ivy								HDM		1
Hedera helix	Common ivy								C		25
Helminthotheca echioides	Bristly oxtongue	AM									2
Heracleum sphondylium	Common hogweed		AM		HD		A, HD, ST, SM, C		A, AM, SM		6.4
Hesperis matronalis	Dame's rocket								LD		3
Hirschfeldia incana	Hoary mustard	ST			HD, LD		AM		A, AM, LD, LDM, HD, HDM, ST, SM, C	ST, SM, C	11.82
Holcus lanatus	Yorkshire fog	A, LD, LDM, HD, ST, HD	AM, LDM, HD, HDM, ST, SM, C	AM KDM, HD, HDM, ST, SM, C	A, AM, LD, LDM, HD, HDM, ST, C	A, AM, HD, HDM, ST, C	LDM, HD, HDM, ST, SM, C	A, AM, LD, LDM, HD, HDM, ST, SM, C	AM, LDM, HD, HDM, ST, SM, C	A, AM, LD, LDM, HD, HDM, ST, SM, C	20.178
Hypericum perforatum	Perforate St John's-wort		AM		C						4.5
Hypericum tetrapterum	Square-stalked St John's-wort							HD			5
Hypochaeris radicata	Common catsear	A, AM	HDM								1.33
Impatiens glandulifera	Himalayan balsam	A, AM, LD, LDM, HD, ST,SM, C	A, AM, LDM, HD, HDM, ST, SM, C	A, AM, LD, HD, HDM, ST, C	A, AM, LD, LDM, HD, HDM, ST, C	A, AM, LD, LDM, HD, HDM, ST, C	A, AM, LD, LDM, HD, HDM, ST	AM, LD, LDM, HD, HDM, ST, SM, C	A, AM, LD, LDM, HD, HDM, ST, SM, C	A, AM, LD, LDM, HD, HDM, ST, SM, C	14.97
Jacobaea vulgaris	Common ragwort		AM			C			HDM		8
Juncus effusus	Soft rush		ST, C								3

Lapsana communis	Common nipplewort	ST									2
Linaria vulgaris	Common toadflax			A		A, AM					3.33
Lolium perenne	Perennial ryegrass			C	HD	AM, HD		AM, HD	A, AM		13.5
Lotus corniculatus	Birds-foot trefoil	HD, HDM	AM, LDM, HD, HDM	HD	HD, HDM	HD, HDM	HDM, ST	HD, HDM, ST	HDM	HD, HDM, ST, C	16.71
Melilotus officinalis	Common melilot			ST, SM			AM, LD, SM			A, SM	5.57
Mentha arvensis	Wild mint		AM		HD, C		LDM				4
Oenothera biennis	Common evening-primrose		A, SM, C	C, SM		A, AM, ST, C	ST		A, AM, LD, HD, ST, SM	A, HD, ST	5.43
Petasites hybridus	Common butterbur						A				10
Phalaris arundinacea	Reed canary grass		SM				AM		C, ST	AM, ST, SM	6.86
Phleum pratense	Timothy	HDM		HDM, SM					HDM, ST	HDM	3.33
Plantago lanceolata	Ribwort plantain	A, HDM	AM	A, AM, LD, HD, SM	AM	C	AM, LDM		A, AM, ST, C	ST, SM	3.17
Plantago major	Greater plantain	C	SM	AM, C						ST, SM	3.67
Prunella vulgaris	Selfheal	AM, ST									3.5
Pteridium aquilinum	Bracken fern								A, LD, HD, C		12.75
Quercus robur	Oak	A	A, AM, HD, ST						LDM, C		3
Ranunculus acris	Meadow buttercup							1			1
Ranunculus repens	Creeping buttercup	C	A, AM, LDM, ST	A, LD, ST	ST	A, LD, HD, HDM, ST	AM		ST, SM, C	A	2.83

Reynoutria japonica	Japanese knotweed	A, AM, LD, LDM, HD, HDM, ST, C	A, AM, LD, LDM, HD, HDM, ST, SM, C	A, AM, LD, LDM, HDM, ST, C	A, AM, LD, LDM, HD, HDM, ST, C	A, AM, LD, LDM, HD, HDM, ST, C	AM, HD, HDM, SM, C	A, AM, LD, LDM, HD, HDM, ST, SM, C	A, AM, LD, LDM, HD, HDM, ST, C	A, AM, LD, LDM, HD, HDM, ST, SM, C	10.32
Rosa spp.	Rosa spp.	AM									1
Rubus fruticosus	Bramble	A, AM, LD, LDM, HD, HDM, ST, SM, C	A, AM, LDM, HDM, ST, SM	A, AM, LD, LDM, HD, HDM, ST, C	A, AM, LDM, HD, HDM, ST	AM, LD, HD, HDM, ST	A, AM, LD, LDM, ST, SM, C	AM, LD, HDM, ST, SM, C	A, LD, SM	A, HD, SM	14.65
Rumex acetosa	Common sorrel									C	1
Rumex obtusifolius	Broad-leaved dock			AM, C	LD				AM, ST	AM, ST	3
Salix aurita	Eared willow	AM	C						SM		26
Saponaria officinalis	Common soapwort			SM	HD				HDM, ST, SM	A, HD, ST, SM	4.67
Scrophularia nodosa	Common figwort	C	AM				ST	A, HD	A	AM	7.43
Silene dioica	Red campion			SM			AM, SM, C		C		2.4
Smyrnium olusatrum	Alexanders		SM								1
Solidago canadensis	Canadian goldenrod			A							8
Sonchus oleraceus	Smooth sow-thistle		AM, HDM, SM								1.67
Stachys sylvatica	Hedge woundwort						LDM, HD, SM				2
Symphyotrichum lanceolatum	White pinnacle aster									HDM	5
Tanacetum parthenium	Feverfew		C								4
Tanacetum vulgare	Common tansy						A				4
Taraxacum officinale	Dandelion		AM, HD	ST, SM	LD, HD	A, ST		A		SM	2.5

Torilis arvensis	Spreading hedgeparsley			SM							3
Trifolium campestre	Hop trefoil						ST		A	A, SM	1.5
Trifolium repens	White clover	LD, C	AM, LDM		HD, ST	HD				A, HD, ST	1.4
Tussilago farfara	Coltsfoot			C							1
Ulex europaeus	Common gorse		LDM			C					3
Umbilicus rupestris	Navelwort		AM								3
Urtica dioica	Common nettle	HD	LD, LLDM, HD, ST, C	A, LD, LDM, HD, HDM, ST	A, LD, LDM, C		A, AM, LD, LDM, HD, HDM, ST, SM, C	SM, C	AM, HD, C	A, AM	9.42
Veronica serpyllifolia	Thyme-leaved speedwell	SM									3
Vicia hirsuta	Hairy tare vetch			C							2
Vicia sativa	Common vetch				AM, A			A, ST			2.5

Appendix D

Appendix D. Species list of all species identified during the community analysis (99 species), including the plot/s and treatment/s the species were found in alongside their average percentage cover in said plots (n = 64). A - amenity grass mix, AM - amenity grass mix + matting, LD - low density species mix, LDM, low density species mix + matting, HD - High density seed mix, HDM - High density seed mix + matting, and C – control group).

		Plots and treatments the species was found in									
		2			**3**			**4**			
Scientific name	**Common name**	**I**	**H**	**D**	**I**	**G**	**E**	**F**	**J**	**H**	Mean abundance
Achillea millefolium	Yarrow	LDM, LD	HDM, LD, A, LDM	HD, HDM	A, HD, LDM, HDM	LD, LDM, HDM, HD	LDM	LDM	AM, LDM	LD, LDM	4.521739
Agrostis capillaris	Common bent grass	A		LDM							2.5
Arabidopsis thaliana	Mouse ear cress			AM							2
Arrhenatherum elatius	False oat grass			AM, HD			LD, HD	AM, A, LD	LD, AM, A	HDM, AM	3.17
Artemisia vulgaris	Common mugwort	AM		C, AM	C, A, HDM	LDM	HDM, C, LD, HD	AM, LD		C	2.94
Buddleja davidii	Buddleia	LDM, C, A		C, HD		LDM		AM, A, HD		LD	4.81
Cardamine hirsuta	Hairy bittercress						LD		LD, HDM		1
Centaurea nigra	Common knapweed						LD				2
Chamaenerion angustifolium	Rosebay willowherb				HDM					C, HDM	2.6
Cirsium dissectum	Meadow thistle			C		HD					4
Cirsium vulgare	Spear thistle						A			HD, LD, LDM	1.97
Convolvulus arvensis	Field bindweed			AM							1
Cymbalaria muralis	Ivy-leaved toadflax							AM			1
Dactylis glomerata	Cock's-foot	LDM	LDM	C		LD	LD			C	2.67

Digitalis purpurea	Fox glove		HDM								1
Epilobium hirsutum	Greater willowherb					HD	A				1.5
Epilobium tetragonum	Square-stalked willowherb	HD, A	HDM, AM, LD, A	C, HD	C, LDM	LD, C	HDM, LD	AM, A, LD	LD, AM	A	6.3
Erigeron canadensis	Canadian fleabane	HDM	A						LD		4
Eupatorium cannabinum	Hemp-agrimony	A		C			C, AM, HD				2.4
Festuca rubra	Red fescue	HDM, A	AM, LD, A	LDM, AM, LD, HD	C, AM	LD, A, AM	LD, HD, LDM	A, LDM, LD	LDM, HDM	C, HD, LD, LDM, AM	4.62
Gallium aparine	Cleavers				LDM						1
Galium verum	Lady's bedstraw	LDM	LD		A		LD			LD	1.5
Geum urbanum	Wood avens	C			A, HDM		A				2
Holcus lanatus	Yorkshire fog	AM, HD, A	LDM	C, AM, HD, HDM, A	A, HD, AM, LDM, HDM	LD, A, HDM, HD	HDM, LD	AM, A, LDM, HDM, HD, LD	AM, LDM, HDM	A, C, LD, LDM	4
Jacobaea vulgaris	Common ragwort		AM								1
Linaria vulgaris	Common toadflax					AM					1
Lolium perenne	Perennial ryegrass				HD	AM		HD			2.8
Lotus corniculatus	Bird's-foot trefoil			HD	HD						4
Oenothera biennis	Common evening-primrose					A		LD	C, LD, AM, A	A, HD	1.78
Plantago lanceolata	Ribwort plantain	HDM							C		2
Ranunculus repens	Creeping buttercup			LD		HDM					1

Rubus fruticosus	Bramble	A	HDM, AM, LDM	AM, A	A, LDM	LD, HD		AM, LD	LD		2.23
Scrophularia nodosa	Common figwort	A			C			AM, HD			2.25
Senecio vulgaris	Common groundsel	A	HDM								3
Solanum nigrum	Black nightshade			C						A	1
Sonchus oleraceus	Smooth sow-thistle		HDM						AM		1
Tanacetum parthenium	Feverfew		C								1
Trifolium repens	White clover					HD					1
Urtica dioica	Common nettle			LD, HD	C, A, LDM		C		HD	A, C	1.89
Veronica persica	Bird's-eye speedwell		HDM		C						1
Vicia hirsuta	Hairy tare vetch										1
Vicia sativa	Common vetch	AM		C				A			2