Honours Project Report

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School of Biological Sciences

The use of sheep grazing as a management tool for the control of the invasive species, giant hogweed (Heracleum mantegazzianum).

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Abstract

Invasive plant species are considered one of the biggest environmental threats seen across the world. One such species prevalent across much of Europe is giant hogweed, *Heracleum mantegazzianum*. There are several management strategies used to control outbreaks of *H. mantegazzianum*, with varying degrees of success. One strategy that has received very little attention within the literature is the use of livestock grazing. This method was tested using sheep on a site in Aberdeenshire, Scotland that had previously contained an extensive *H. mantegazzianum* invasion. The density of seedlings across the site was considerably reduced within three years of grazing. Investigation into the viability and longevity of the soil seedbank was conducted and found no seeds viable for germination from either soil from the site or seeds buried two years previously. However, as it is notoriously difficult to grow *H. mantegazzianum* under laboratory conditions, an experiment testing various growing conditions was conducted. This resulted in the germination of some seedlings, providing evidence for future attempts. In order to fully assess the impact of using grazing as a management tool, this study looked into the effects on the surrounding vegetation. Maps compiled over three years showed the changes in density and distribution of the different vegetation types across the site. These showed much more generalised areas of vegetation, showing one type of vegetation progressively dominating larger areas. Overall, sheep grazing has proven an effective solution for control of *H. mantegazzianum* and should be utilised in future management plans.

Introduction

Invasive species

The introduction of non-native species is one of the many anthropogenic actions that have severely influenced the natural environment (McNeely, 2001). The scale of invasive expansions as well as the negative effects on biodiversity, public health and ecosystem services makes invasive species one of the biggest global threats to the environment seen today (Mack, 2000; Walker & Steffen, 1997).

The introduction of species into new areas, either intentionally or unintentionally, is usually caused by contamination, carriers or the release of species kept for either private collections or food sources (Manchester & Bullock, 2000). Although not all introduced species become invasive, certain characteristics of some species allow the efficient utilisation of resources, resulting in extensive expansion. Invasive species have the ability to easily colonise and rapidly reproduce over a short period of time, enabling them to outcompete surrounding species and reduce the local biodiversity. A key factor in the success of many invasive species is their high level of fecundity, effective ways of dispersal and the ability to exploit the surrounding resources for their own benefit (Moravcová 2006). For invasive plant species, the ability to produce a large amount of seeds as well as an extensive seed bank reserve heavily contribute to their invasive success (Krinke, 2005).

Due to their resilient and persistent nature, controlling the spread of invasive species is one of the biggest challenges facing environmental conservation today (Allendorf, 2003). There are various methods used in the control of invasive plant species. These include manual cutting or mowing of plants, the application of chemical treatments, burning, removal of infected soil or the introduction of grazers to the area (Rejmánek, 2002). Regardless of the method, to ensure a successful control of an invasive species a strategic, long-term programme is required (Ditomaso, 2000).

Giant hogweed (*Heracleum mantegazzianum*)

A typical example of one such invasive species is giant hogweed (*Heracleum mantegazzianum*). Native to the Caucasus region of south west Asia, *H.*

mantegazzianum is now widespread through much of Europe (Pyšek, 2006). The species was introduced into Britain in the 19th Century and has become well established throughout most of the country (Pyšek, 2007a).

Tiley (1996) describes *H. mantegazzianum* as a tall, annual monocarpic herb with fast growing, extensive foliage. It depends solely on seed dispersal for reproduction (Pyšek, 2007b), multiple large flowers in compound umbels produce an enormous amount of seeds from late August to mid-October (Tiley, 96). *H. mantegazzianum* is most commonly found on banks of rivers and streams as the fast-flowing water system aids the distribution and movement of seeds (Pyšek, 1994). It is also often found in areas of rough ground and wastelands such as the verges of railway lines and roads. *H. mantegazzianum* contains phototoxic substances within its sap (Krinke, 2005) that can cause substantial burns to the skin and eyes when exposed to UV light (Boršić, 2015). This creates limitations to manually cutting back large, established infestations (Page, 2005).

Invasive traits of *H. mantegazzianum*

The ability of *H. mantegazzianum* to establish itself widely and in such high densities is due to a number of factors. As previously mentioned, *H. mantegazzianum* can grow to heights of up to five metres (Tiley, 96) with a number of large leaves covering a substantial area surrounding the plant. This results in *H. mantegazzianum* having the ability to shade out many other plant species that grow in close proximity. The competitive advantage this provides can attribute to its ability to spread rapidly within an area. *H. mantegazzianum* possesses hermaphrodite flowers (Page, 2005) and has the ability to fully self-fertilise without reduction in fruit production compared to natural fertilisation and without suffering from the effects of inbreeding (Perglová, 2007). This improves the efficiency of potential fertilisation as well as providing an advantage in areas with low insect densities. The unspecialised flowers allow pollination by a wide variety of species (Perglová, 2006), owing to the success of *H. mantegazzianum* in a variety of different habitats home to a variety of insect types.

Its ability to easily colonise disturbed and bare ground may provide a competitive advantage over other species in the surrounding area (Pergl, 2007). Germination of *H. mantegazzianum* occurs early between March and April (Moravcová, 2007), often before native species in the same area. This allows *H. mantegazzianum* to dominate early in the growing season and establish a foot-hold leading to further expansion.

With the negative impacts associated with the spread of *H. mantegazzianum*, extensive outbreaks require management and control measures. One of the main reason behind the need for control is the reduction *H. mantegazzianum* has on local species richness and biodiversity (Pyšek and Pyšek, 2005). Its ability to outcompete native plant species can have an influence on trophic levels and as a result, alter the entire community structure including herbivores, decomposers and pollinators (Levine, 2002). Widespread invasions can also cause alteration of the nutrient cycling in the soil (Levine, 2002). This further reduces the re-establishment of other species as well as limiting the ability for any future growth. Large infestations can result in the erosion of the soil. In areas near river banks, this erosion can contribute to the sediment being released downstream impacting the aquatic environment (Caffery, 1999). These factors all contribute to the need for control of *H. mantegazzianum* infestations.

<u>Seed ecology</u>

High fecundity and effective dispersal methods are key determinants in the success of an invasive species (Moravcova, 2006). Typical of many invasive plants, *H. mantegazzianum* has an extensive seed set which substantially contributes to its rapid spread and colonisation of new areas (Rejmanek & Richardson, 1996). Each single plant produces an enormous number of flowers both on the main umbel and the numerous secondary umbels (Tiley, 1996). The estimated number of seeds produced by each plant varies considerably (Moravcova, 2006; Krinke, 2005; Tiley, 1996) but it is thought to be in the region of 50,000 per plant. This enormous capacity for potential reproduction is a main factor in its ability to spread at such extensive rates.

<u>Seedbank</u>

The soil seedbank plays a key role in the success and persistence of *H. mantegazzianum*. Understanding this key stage of the life cycle effectively predicts how long the species is present within a site without further introduction of new seeds (Gioriaa and Pysek, 2016). This information is vital in understanding and controlling its spread (Matus, 2001). However, despite its importance, very little is known about the viability and length of *H. mantegazzianum's* seedbank.

Seeds require a period of cold stratification within the soil. During this time, morphophysiological development takes place to ensure embryo growth is completed and dormancy broken when the temperature increases (Moravcova, 2007). However, little research has been conducted on the length of cold period or depth of temperature required for maximum germination either in the field or under laboratory conditions.

There is very conflicting information in the literature about the length of time *H. mantegazzianum* seeds remain viable in the soil seedbank. Lundström (1989) claims seeds can persist for up to fifteen years in the soil seedbank, contradicted by Dodd (1994) suggesting the seedbank has a much shorter timescale of seven years. This is backed up by Manchester and Bullock (2000) that suggests a seven year management programme to ensure the species is completely eradicated from an area. Despite this, Andersen & Calov's 1996 study in Denmark showed no viable seeds in the soil seven years after the adult plants were removed suggesting a much shorter timescale for viable seeds in the seedbank. Moravcova's 2006 study examined buried seeds in the field to test their viability over several growing seasons. The results showed 8.8% of seeds remained viable after the first year, decreasing to just 1.2% by the third year. With these low numbers of viable seeds after just three years, a seedbank lasting up to seven years seems unlikely. There is a substantial requirement for more in depth research to be undertaken to establish the length of time that *H. mantegazzianum* seeds remain alive in the soil. In situations where seedling densities are low, there is a risk of the site being assumed eradicated of the invasion and the control methods stopped, only for dormant seeds to germinate and re-infest the area. Knowledge into the dynamics of the seedbank will help guide the timescale of control methods to produce the most effective results.

Management of *H. mantegazzianum*

There are multiple methods that have been used to control *H. mantegazzianum* with varied success. Treating adult plants with chemicals such as glyphosate is an effective and commonly used method (Wadsworth, 2000). However, there are limitations such as the need for repeated treatments to maintain a level of control as well the danger of chemicals to the surrounding vegetation and water courses. The mechanical cutting of plants is another commonly used method of control. The cutting back of umbels, stems or roots acts to either restrict growth, prevent seeding or kill the plant entirely (Nielsen, 2007). This is obviously very labourious work and poses the risk of being exposed to *H. mantegazzianum*'s toxic sap. Both of these methods can be successfully implemented if the area under treatment is small and easy to access. However, in larger infestations other means of control need to be utilised.

The grazing of livestock as a method of controlling *H. mantegazzianum* is one such option. This enables effective control without the need for strenuous work or risk of endangering human health. There are only a limited number of studies dedicated to this type of management. Andersen and Cavlov's 1996 study on a on the long-term effects of grazing of an infested meadow in Denmark showed a substantial decline in *H. mantegazzianum* density over just three years. A number of species including cows, goats and sheep will graze *H. mantegazzianum* (Tiley, 1996) and the selection of species should be based on the suitability of the site. Cows may be better suited to sites with larger infestations while sheep and goats would be more effective on smaller sites or on areas of steep, uneven ground conditions. The chosen animal's preference of additional species being grazed along with *H. mantegazzianum* must also be taken into consideration. Despite their ability to digest the plant, the animals are not immune to the risk of chemical burns. In light of this, livestock with dark pigmentation of the skin around the mouth are less likely to suffer from the burns caused by exposure to the toxins. Unlike manual methods, using grazing as a control for *H. mantegazzianum* will almost certainly have an influence on the other plant species within the study area. Hence, wider vegetation studies are required on top of monitoring of *H. mantegazzianum* to establish the true impact grazing is causing.

Any control methods used in the management of *H. mantegazzianum* must acknowledge the presence of the soil seedbank. It would be easy to assume the infestation is eradicated once no seedlings were present on site. This, however, would be overlooking the potential for dormant seeds within the soil to germinate and re-establish a population. Any control method must be conducted in conjunction with investigation into the seedbank.

Focus of this study

This study aims to investigate the continued effects of sheep grazing as a method of controlling *H. mantegazzianum* invasions. Limited research has been conducted on the use of grazing as a management strategy and the conclusions gathered can aid in further understanding of this method of control. This study is based on a site in Aberdeenshire and has previously been investigated in 2014 and 2015, allowing a comparison of data over the consecutive years.

The main focus of this study is the continued monitoring of the density and distribution of *H. mantegazzianum* seedlings under the influence of sheep grazing. Mapping the presence of seedlings allows a clear view of the current situation seen on the site. Comparison of maps from previous years clearly show any changes that have occurred under the influence of grazing. To determine whether the data gained from the maps is an accurate representation of *H. mantegazzianum* on the site, further tests both in the field and in the lab were carried out. To establish if seedlings are germinating but being grazed before identification can take place, small areas of restricted grazing were set up. This allows a more in depth insight into the true impact grazing is having on the distribution of *H. mantegazzianum* around the site.

There is the risk of assuming a *H. mantegazzianum* infestation has been eliminated once all flowering plants have been removed, only for seeds within the soil seedbank to germinate and reinstate a population. Hence, determining the viability and longevity of *H. mantegazzianum* seeds within the soil seedbank provides another key focus for this study. Taking soil cores from areas of previous high seedling densities and planting them under laboratory conditions helps determine whether *H. mantegazzianum* seeds are still present but dormant within the soil. The longevity of seeds can be tested further with the examination of bags containing *H. mantegazzianum* seeds buried in 2014. This will help determine whether any seeds remain and if they are still able to germinate after two years in the soil. Establishing the length of time seeds are viable is essential for formulating and implementing management strategies. This will provide further insight into *H. mantegazzianum's* seedbank and help aid future management plans.

A complicating factor in determining the size of *H. mantegazzianum's* soil seedbank is that seeds are notoriously difficult to germinate in a laboratory. A section of this study will focus on testing various growing conditions of fresh seeds to help establish the most suitable environment required for germination. Further understanding is required to determine the optimum period of time seeds spend in cold stratification as well the temperatures that best promote germination.

In addition to the investigation into *H. mantegazzianum*, this study will explore the secondary effects grazing is having on other plant species on the site. Mapping the general vegetation types allows the change in distribution of vegetation to be made with previous years as well as a comparison with an enclosed area restricted from sheep. This is vital in assessing the effectiveness of grazing as a method of control for invasive species and the impact on surrounding vegetation.

Research questions

1. Does continued sheep grazing reduce the density of *H. mantegazzianum* seedlings over time?
2. Are *H. mantegazzianum* seeds still viable for germination after two years buried in the soil?
3. Do variations in temperature and length of cold stratification increasing the chances of successfully germinating *H. mantegazzianum* seeds in the laboratory?
4. What effect does grazing have on the composition and distribution of the surrounding vegetation?

Methods

Study site

The site this study is focused on is located adjacent to farmland at Auldtown of Netherdale in rural Aberdeenshire, Scotland. The site is a fully fenced field of approximately 5 hectares situated near the banks of the River Deveron. The site consists of mixed vegetation dominated by multiple grass species, common nettle

(*Urtica dioica),* thistle *(Cirsium* ssp.) and *Juncus*. It also contains a plantation of rowan, oak, willow and birch trees. A small tributary of the Deveron runs through the entire site.

A heavy infestation of *H. mantegazzianum* caused noticeable damage to the site including soil erosion, reduction of native species and loss of soil nutrients (Fenn, 2012). The infestation was so severe that it led the Deveron, Bogie & Isla River Charitable Trust (DBIRCT) to add the site to their ongoing River Deveron Biosecurity Project in 2009. Previous control methods including chemical treatment and manual cutting of adult plants produced only minimal results. In early 2013, twenty black-faced sheep were introduced to the site in order to control the vegetation. The site has been closely studied in subsequent years and has undergone a substantial reduction in the density and distribution of *H. mantegazzianum* as well as the development of other vegetation. As a result of this, the grazing intensity was reduced to thirteen sheep in 2015 and kept at that level for the entirety of the 2016 study. Grazing commenced in March of each year and continued until the sheep were removed from the site in November.

Seedling density in the field

Seedling map

To investigate the density and distribution of *H. mantegazzianum* seedlings present at the site, two separate seedling density maps were created. These maps allow a comparison to be made between both the 2015 and 2014 maps on the distribution of seedlings. The same approach was followed as in the 2015 study to create a grid of the study site. The points were measured at approximately 15m apart and maintained in a straight-line grid format with the use of a digital compass direction. Each of the points were marked using a global positioning system (GPS) and the coordinates both digitally and manually recorded. At each point, a 1mx1m quadrat was placed and the number of *H. mantegazzianum* seedlings within the quadrat was counted. The quadrat was then flipped a further three times to create four individual quadrat records. This was conducted over two days in mid-October, 2016. This set up allows a comparison to the 2014 data that used a single 1mx1m quadrat per marked point as well as the 2015 data that, due to a lower number of seedlings, recorded a 1mx4m quadrat area to assess seedling density. Taking both sets of quadrat measurements allowed two separate maps to be created using the different sample sizes and a direct comparison to be made between both the 2014 and 2015 datasets. The GPS locations of each marked points were used to create a seedling density map of the site using geographic information system (GIS) software. These maps could then be compared to previous years to compare the density and distribution of *H. mantegazzianum* seedlings over time.

<u>Cages restricting grazing</u>

To assess whether seedlings were germinating on the site but being grazed and removed before identification could take place, small cages were set up to prevent the sheep accessing the vegetation underneath. This would help to determine whether the low density of *H. mantegazzianum* seedlings on the site was due to the eradication of seeds within the seedbank or if viable seeds were still germinating but being controlled by grazing.

The cages were located in areas of previous high *H. mantegazzianum* seedling density according to the seedling map created by Downie in 2014. A total of ten cages were placed on the site and pegged to prevent them being moved by sheep. They were first added to the site in late September and remained there until in mid-November. The cages were checked and recorded during frequent visits to the site.

Seeds in the soil seedbank

<u>Soil cores</u>

To further test whether viable *H. mantegazzianum* seeds were still present in the seedbank, soil cores were taken from the site in mid-October. The location of these soil cores were determined using previous data taken from a density study in 2012. Four areas that displayed the highest densities from around the site were selected and a point randomly picked approximately in the centre of each of the areas. Ten numbers (from zero to ten) were generated using a random number generator and each number assigned a randomly generated compass directions (north, north east, east, south east, south, south west, west and north west). From this, the distance in metres and direction from the centre point was determined and the ten soil cores were extracted. The soil cores were sieved to reduce excess soil using a 4mm sieve.

They were put into cold storage at 5°C for three weeks to mimic the cold stratification conditions that would be experienced in the field.

Of the ten soil cores from each of the four areas, seven were planted in the greenhouse. The remaining soil cores were kept in cold storage for a further two weeks to determine whether the length of cold stratification effected the germination rates of *H. mantegazzianum.* In the greenhouse, 28 trays of 23x37cm were lightly filled with a layer of compost approximately 0.5cm thick and each soil core spread on top. The trays were kept in conditions of 20°C during the day and 10°C during the night on a daylight cycle of 16/8 hours. The trays were checked daily and watered when necessary.

<u>Germination experiment</u>

H. mantegazzianum is notoriously difficult to grow under laboratory conditions. In order to assess the most suitable growing conditions that encourages germination the following experiment was set up.

Adult *H. mantegazzianum* plants were located from the banks of the Urie River, Aberdeenshire at the beginning of October. Fresh and assumed viable seeds were collected from a multitude of these adult plants in three separate populations. These seeds were then stored at 5°C to mimic the cold stratification the seeds would have experienced in real life conditions in order to break dormancy and complete any morphophysiological development. In order to test whether the length of cold stratification altered the success of germination, the seeds were removed from cold storage and planted in the greenhouse after 2 weeks, 3 weeks and 4 weeks respectively. Trays of 17x23 cm were filled with 1.5 cm of Bulrush multi-purpose compost, the seeds then placed on top and lightly covered with the same soil. Each tray contained five replicates of ten seeds with each tray containing one population, resulting in three trays per treatment. Two temperatures were tested to determine the conditions that would produce the best germination results. Treatment one was set at a temperature of 20°C during the day and 10°C during the night while treatment two was set at a temperature of 20°C during the day and 18°C during the night. These temperatures were adapted from a similar germination study by Moravcova (2006). Both treatments were carried out under a 16/8 hour day cycle.

Trays were randomly allocated positions on the bench and frequently alternated. Trays were checked every day and watered when required. Any germination of seedlings was recorded and removed after identification.

An initial attempt of this experiment was undertaken using four phytotron machines at four different temperature settings. The seed trays were set up according to the procedure mentioned above and placed in the machines at day/night temperature of 10°C/10°C, 15°C/7°C, 20°C/7°C and 25°C/25°C respectively, based on a similar experiment by Moravcova (2006). The machines had a daylight cycle of 12 hours and were set at 75% humidity. Unfortunately, shortly after the experiment had begun, the machines broke down resulting in varied and fluctuating light and temperature settings. The trays were removed and placed in the greenhouse under the conditions mentioned above. This attempt was considered unreliable and, although left in the greenhouse for observation, was repeated under the experimental greenhouse conditions.

Seed bags

To investigate the long term viability of *H. mantegazzianum* seeds within the seedbank, seed bags were buried at the site in 2014. Downie (2014) used eight paired quadrats to select burial locations – one within nettles and one within grass. This design was used to help determine whether the type of vegetation surrounding the seedlings influenced the viability of the seed. For each site, five bags containing 25 seeds were buried under mesh and secured within the ground by pins. One bag from each of the sixteen sites was collected. The depth of the soil above the seed bags was recorded along with any other additional information that was thought to be of interest. The bags were stored in the fridge at a temperature of 5°C for three weeks to mimic the natural cold stratification period.

To test whether the seeds were still viable for germination after two years in the soil, a tetrazolium staining experiment was conducted based on the method used by Andersen (1996). When the seed bags were cut open, it was revealed that there were only a low number of intact seeds left inside. Despite this, five seeds from each of the sixteen seed bags were removed and tested. Ten fresh seeds collected from an adult population used in the germination experiment (see below), were used as a positive control to determine the effects of staining on fully viable seeds. Ten seeds from the same population were autoclaved to render them denatured were used as a negative control to test the assumption no staining would occur. The seeds were cut longitudinally from the tip using a scalpel without removal of the outside seed coat. The remaining half was discarded. The seeds were soaked in purified water for one hour. The tetrazolium solution was created by adding 1g of 2, 3, 5 triphyenyltetrazolium chloride to 100ml of autoclaved water and stored in a concealed container due to its sensitivity to light. After soaking, the seeds were sieved from the reduced osmosis water and soaked in containers of 20ml of tetrazolium solution. The container was covered to reduce light sensitivity and incubated at 22°C for four hours.

The seeds were removed and drained from the tetrazolium solution after the allotted time period. The seeds were large enough to be visually assessed without the use of a microscope and their colour easily determined (Appendix 1). Bright red colouration throughout was considered a sign of a viable seed. Pink colouration or areas of white were determined as weak seeds that were still partially viable but had a reduced chance of successful germination. Seeds that failed to be stained and remained white throughout the tissues were classed as dead and unable to germinate. The frequency that the colour appeared on each seed was recorded for all of the seed bags as well as both of the controls. The remaining contents within the bags were examined to determine whether there were any more seeds were present but went undetected.

Impact of grazing on surrounding vegetation

Vegetation distribution

To enable a comparison of the change in vegetation distribution over time caused by the effects of grazing, a vegetation map was created. This produced a record of the typical vegetation types around the site. Similar maps created in both 2014 and 2015 were compared to show the change in presence and distribution of vegetation types over the years. Using the 2015 map as an underlay, the change in borders and areas of vegetation types within the site were visually assessed and altered to create a hand-drawn paper map of the current year. GIS software was then used to construct a digital map from the paper map drawn in the field.

To gain a greater understanding of the composition of each vegetation type, Downie (2014) established two permanent 1mx1m plots within seven areas deemed to be typical of the vegetation types. These plots were revisited in 2015 and again during the current study. This allowed a detailed overview of any changes that may occur in vegetation composition over time. Once each quadrat was located using its GPS coordinates, all species of plant within it were recorded and their percentage cover estimated.

Field borders

To gain an understanding of the effect grazing has on vegetation composition and distribution in the field; a small area bordering the east end of the study site was utilised. This area of 52mx9.5m was fully fenced preventing sheep grazing as well as restricting any other factors that would influence vegetation distribution such as agricultural practices or machinery. *H. mantegazzianum* had previously been present but controlled chemically in 2012. This was the only area of its type adjacent to the site and because of this, the conducted experiment could not be replicated. Despite this, comparing the grazed and ungrazed areas would provide a key insight into the influence grazing was having on the surrounding vegetation.

An area to the east end of the site was selected for comparison due its close proximity to the border of the study site. Five transects were set up along the area at approximately 10m apart. Along each of these transects, three quadrats were placed at approximately 4.5m apart. Within each quadrat, all plant species were identified and their percentage cover recorded. To allow a comparison with the grazed study site, the same sized plot was established on the immediate other side of the fence, within the study site. As above, five transects were created within the study site measured at 10m intervals. Each transect had three quadrats spaced at 4.5m apart and the plant species and their percentage cover recorded. A comparison can be made between the vegetation composition and density between the two areas to better assess the effects grazing is having on the study site.

Statistical analysis

The data collected was tested using non-parametric tests. Fisher's exact test was chosen to compare the number of seedlings present on the site in both 2014 and 2015 with the data collected in this study. The statistical programme Minitab 17 was used for this analysis. All graphs were created in excel using data collected over all three years. The programme ArcGIS version 10.3.1 was used to create the seedling density maps as well as the vegetation distribution map.

Results

Seedling density in the field

The density of *H. mantegazzianum* seedlings in 2016 is low across the entire site. In comparison with the data collected in 2014 that used a 1mx1m quadrat area at each point, there is a substantial reduction in seedling densities (Fig. 1). The 2016 data shows only a very small number of seedlings present, slightly grouped in three separate areas across the site with the remaining points having no seedlings present at all (Fig. 1b). This is quite a contrast in comparison to the 2014 map that has very high densities recorded in the south west corner of the site (Fig. 1a). There is no evidence of this hotspot in the 2016 data.

To allow comparison with the data collected in 2015 that used a 1mx4m area at each point a second density map was created (Fig. 2). In 2015, the highest densities are distributed in four separate groups in relatively close proximity to the burn. The maximum number of seedlings for any of the points found within the 1mx4m area did not exceed 9 (Fig. 2a). In comparison, the 2016 data shows very low densities of seedlings across most of the site (Fig. 2b). A small area situated at the centre of the site shows the highest density of seedlings but in a much lower frequency than is seen in the 2015 data. A visual comparison of the 2016 maps compared to both the 2014 and the 2015 maps shows a substantial decline in the distribution of *H. mantegazzianum* seedlings across the entire site.

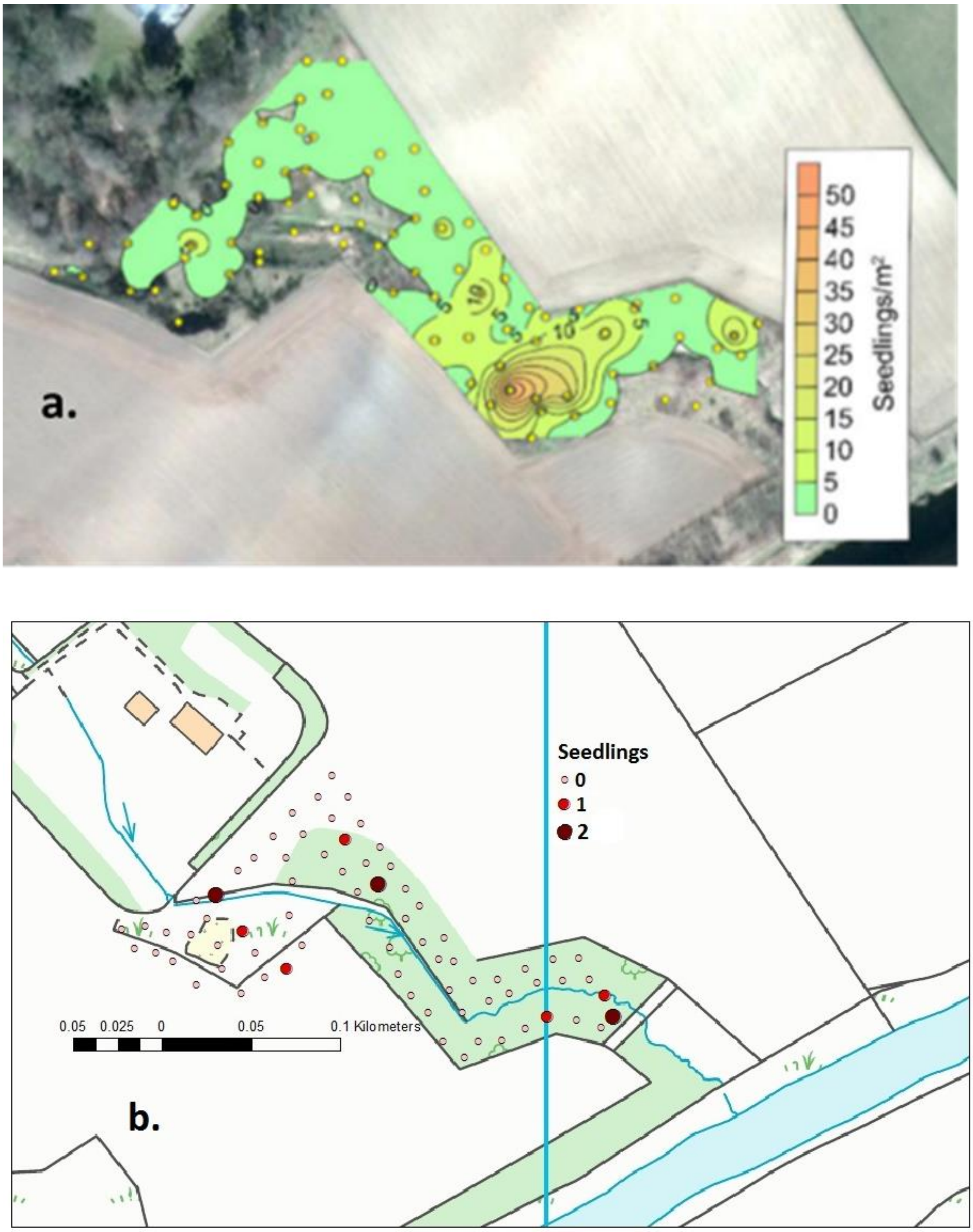

Fig. 1: *H. mantegazzianum* seedling densities on the Auldtown of Netherdale site in 2014 (**a.**) and 2016 (**b.**) per 1mx1m square. *Fig. 1a* is taken from Downie (2014). Scale bar refers to *Fig. 1a* only. The dots in Fig. 1b represent number of seedlings recorded.

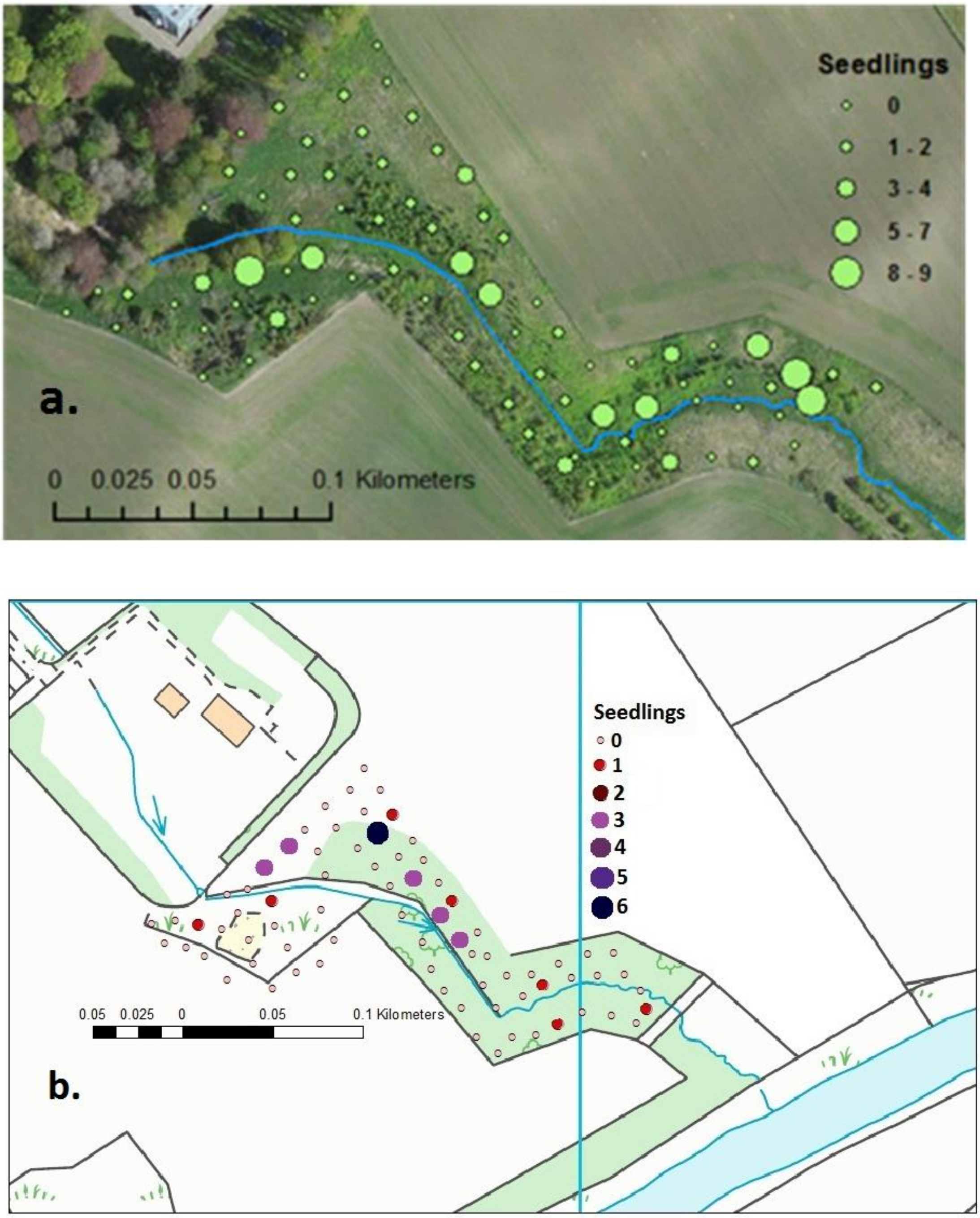

Fig. 2: *H. mantegazzianum* seedling densities on the site in 2015 (**a.**) and 2016 (**b.**) per 1mx4m square. *Fig. 2a* is taken from O'Sullivan (2015). Dots in both figures represent number of seedlings recorded.

To further assess the seedling densities between 2014 and 2016 the number of quadrats that *H. mantegazzianum* seedlings were present in out of the total numbers of quadrats recorded were compared. Out of the 82 quadrats recorded in 2014, seedlings were present in 34 of them. In turn, out of the 75 quadrats recorded in 2016, only 8 occupied seedlings. This produces a highly significant difference ($p<0.001$) showing a substantial reduction in the seedling density between the two years. This test was repeated for the following year by comparing the 2015 data with 2016. This again showed a highly significant difference ($p<0.001$) in the density of seedlings across the site.

To show the seedling densities over all three years, the percentage of plots with seedlings present over 2014, 2015 and 2016 was compared (Fig. 3). There is a wide distribution of seedling frequencies in 2014 with the number of seedlings present in a single plot ranging from low frequencies of 1 and 2 seedlings per plot right up to 50. The 2015 and 2016 data show a much reduced frequency of seedlings with a maximum of 9 seedlings recorded in 2015 and only 6 in 2016. There is a much higher percentage of plots that contain only one seedling in the 2016 data compared to the previous two years as well as a much more reduced distribution of numbers. This shows the substantial decline in plots containing seedlings at the site over the three years.

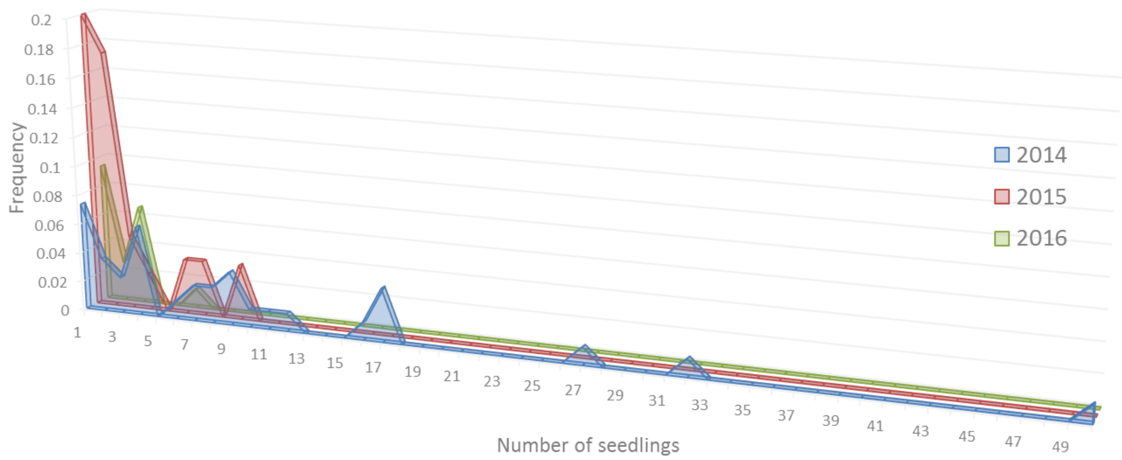

Fig. 3: The frequency in which the number of seedlings occur in each plot represented as a percentage of the total number of plots. Data shown for 2014, 2015 and 2016.

The cages placed on the site to further assess the reliability of the above data showed no results of any growth. Of the 10 cages that were placed in areas of previous high density, no *H. mantegazzianum* seedlings emerged underneath them for the duration of the study.

Seeds in the soil seedbank

The various experiments investigating the soil seedbank provided some key findings. From the 28 soil cores taken from the site and planted in the greenhouse, there was no germination of any *H. mantegazzianum* seedlings. However, this is not conclusive evidence that no seeds remain on site as germinating *H. mantegazzianum* in the lab is difficult. In order to determine the viability of seeds that are definitely known to be present, the 16 seed bags were opened and inspected.

There was no germination of any of the seeds planted from any of the three populations, in either of the treatment temperatures, during the three tested cold stratification periods. However, two seedlings germinated from the initial treatment that underwent varied conditions during the breakdown of the phytotron machines. The two seedlings were both from the same population and germinated at the treatment temperature of 20°C/10°C. Unfortunately, it is almost impossible to reproduce the conditions experienced by these seeds for further replication.

On examination, it was found only 8 intact seeds were left out of all the bags. What remained were a limited number of outer seed coats without any seeds inside. It was impossible to determine whether these missing seeds had previously germinated or had decayed away before germination. Half of the bags were fully examined and it was concluded that no more seeds remained other than the ones already counted, these bags were classed as accurate representation of all 16 bags in total. From this it can be concluded that out of a potential 400 seeds (25 seeds in each of the 16 bags) only 8 remained intact enough to be tested. Out of these 8, 3 seeds were lightly stained pink and determined as viable but weak and with a small chance of germination. The remaining 5 seeds failed to stain any colour and were classed as dead.

Impact of grazing on surrounding vegetation

Grazing not only impacts *H. mantegazzianum* but also the other vegetation present on the site. In a comparison between 2014, 2015 and 2016, the site has undergone some change in vegetation type and distribution (Fig. 4). There will be some variability in the mapping due to the fact all three years were recorded by different people.

Nettles and thistles remained a dominant presence throughout the site. Dense patches of nettles coincided with similar densities found in 2015 with an average cover of >20% covering a third of the plots surveyed. Although the average percentage cover of both nettles and thistles within the plots remained at similar levels over the three years studied, the vegetation maps show the increase in distribution across the site (Fig. 4). Comparing all three years, it can be seen that both nettles and thistles extend substantially further into areas where they previously did not occur such as the east and west ends of the site.

Juncus has also substantially increased its distribution throughout much of the site over the three-year period. Covering only an average of 9% of the plots in 2014, its abundance doubled to 18% in 2015 and then to approximately 19.5% the following year. In relation to the site as a whole, *Juncus* featured in only a small area located to the west of the site, on the banks of the burn in 2014 (Fig. 4a). The area increased further the following year (Fig. 4b) but still remaining close to the waterside. However, the distribution in 2016 shows an extensive development of *Juncus* along most of the banks of the burn as well as extending further into other areas of the site (Fig. 4c). This relatively quick expansion can have a substantial impact on the future vegetation cover and will require close monitoring to fully assess its impacts on the surrounding vegetation and the site as a whole.

Overall, the areas of vegetation have become much more generalised over the three years with one type of vegetation dominating larger areas. This could be a secondary effect of using grazing as a control method. Potential changes in vegetation such as these must be taken into consideration when formulating management plans for the control of a targeted species.

Fig. 4a: Spatial representaion of the types of vegetation present on the study site in 2014. The map is taken from Downie (2014). A legend for this map is included in Appendix 2.

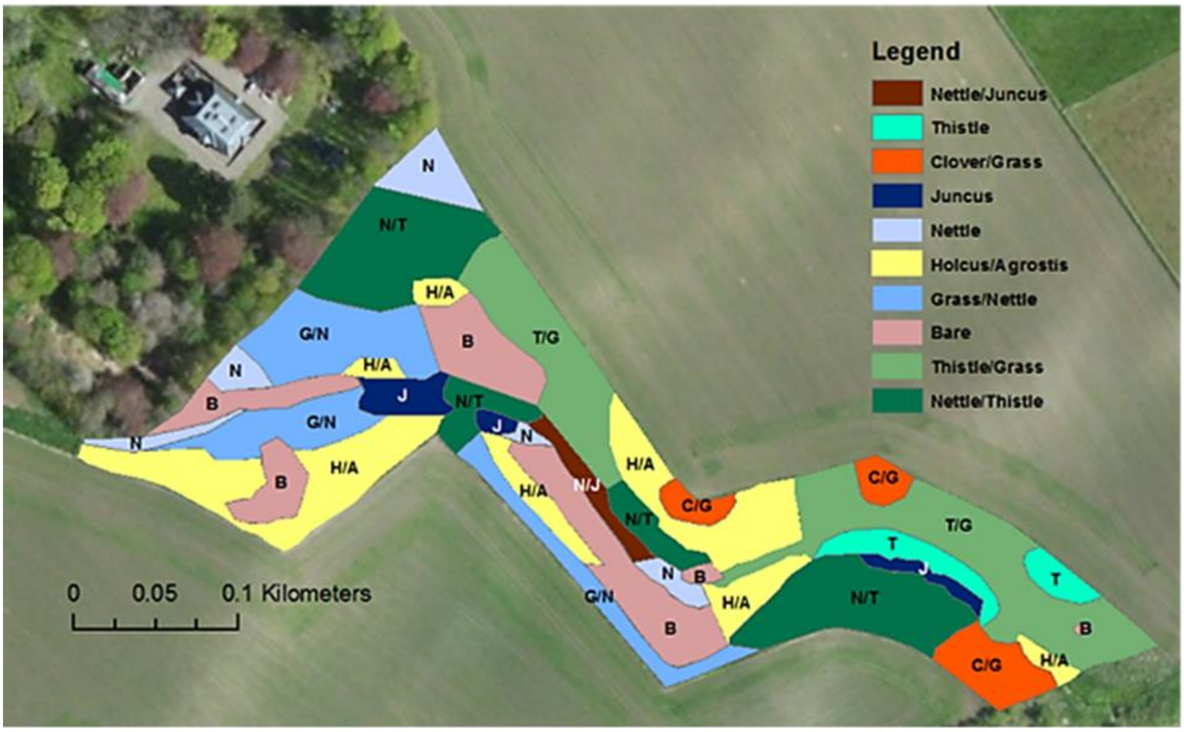

Fig. 4b: Spatial representation of the types of vegetation present on the study site in 2015. The map is taken from O'Sullivan (2015).

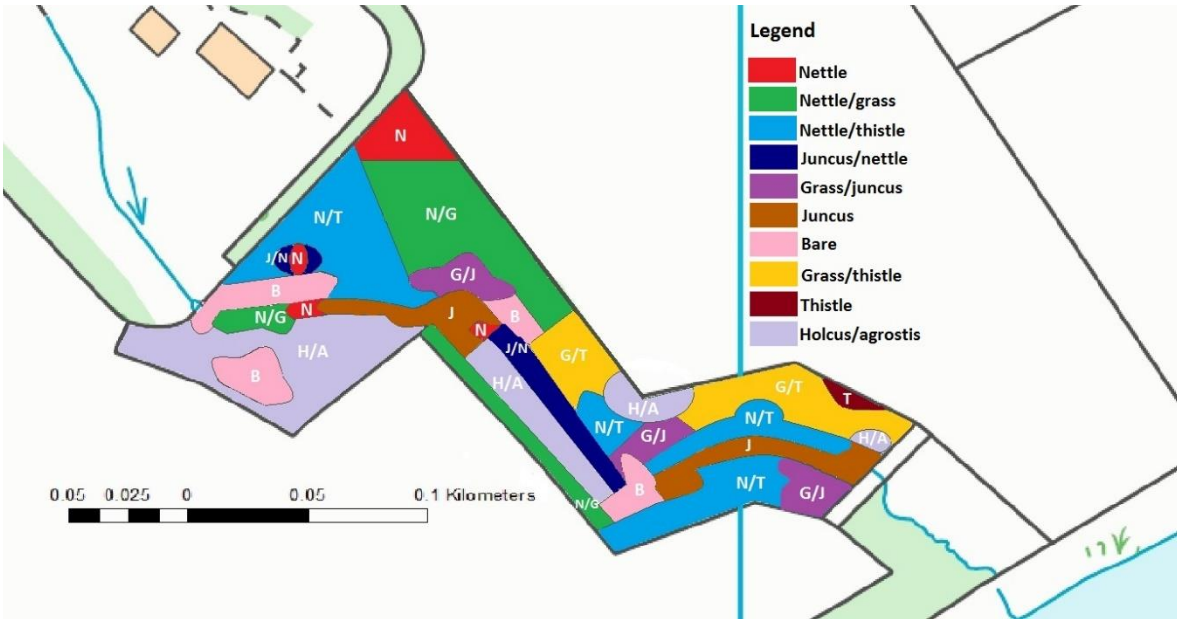

Fig. 4c: Spatial representation of the types of vegetation present on the study site in 2016.

In order to assess the impact of grazing on the study site, an analysis of vegetation types was conducted. In comparison to the grazed study site, a key aspect of the ungrazed area was the diversity of different plant species present. The grazed area contained only 11 species and was heavily dominated by grasses and moss. The ungrazed area however, contained 23 different species of plant with much more even distribution of species between the transects. There were multiple species such as *Heracleum sphondylium, Lathyrus pratensis* and *Festuca ovina* present within the plots of the ungrazed area that were not seen in the grazed area.

Of all the species recorded on both the grazed and ungrazed study areas, Fig. 5 depicts the percentage cover of the most abundant. As can be seen, *Agrostis* spp. is common throughout but is much more abundant in the grazed area as opposed to the ungrazed. The ungrazed area also contains a high proportion of *Epilobium* spp. that is only present in the grazed area in very low abundance. Similarly, the grass *Dactylis glomerata* appears regularly in the grazed area but is not recorded at all in the quadrats of the grazed section.

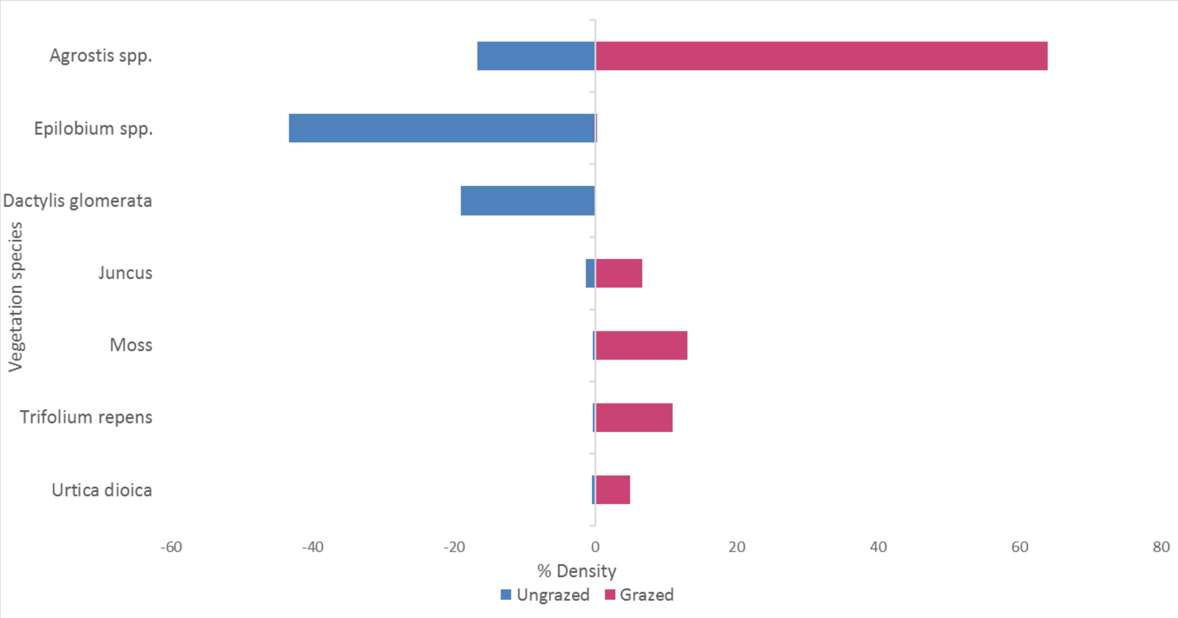

Fig. 5: The percentage density of seven of the most abundant species present in the quadrats of both the grazed and ungrazed areas.

Dominated predominately by *Agrostis* spp., the remaining species that appear in the grazed area are at much lower densities. The influence of grazing shows a substantial difference in the diversity of plant species between the two study areas.

Discussion

Seedling density in the field

Based the evidence collected over the three years, the use of sheep grazing as a management tool appears to successfully reduce the density and distribution of *H. mantegazzianum* seedlings on the site. The seedling map created in 2016 shows very few seedlings present. In comparison with both the 2014 and 2015 data, the number of seedlings has undergone a substantial reduction across the entire site. In support of this, there is a substantially significant difference in the number of seedlings present in 2014 compared with the number of seedlings recorded in 2016, as well as between 2015 and 2016. There is no evidence of the 'hotspot' areas that are shown on the 2014 data being represented in the data collected in either 2015 or 2016. Similarly, the areas of highest density in 2015 are not necessarily the same areas that the highest seedling densities were found the following year. These results, along with a high number of plots over all three years that contain no seedlings at all suggests that sheep grazing reduces the density of *H. mantegazzianum,* across the site over the three years. The few seedlings that were recorded in 2016 were found in areas of dense vegetation, often in nettles, that may have deterred the sheep from grazing.

Due to the fact the sheep have effectively grazed the site since early 2013 and no adult plants were recorded going to seed in either 2013 or 2014 (Downie, 2014), the seedlings present would have most likely germinated from the soil seedbank. It is possible that fresh seeds have entered the site either by water or wind from plants out with the study area, however with no knowledge of a *H. mantegazzianum* population in close proximity, this seems unlikely. The length of time the seeds remain viable within the soil could be another contributing factor in the decline of seedlings recorded in 2016. It is easy to assume from the evidence that grazing alone will entirely eradicate *H. mantegazzianum* from the study site. It's possible however, that seedlings are germinating on the site but are being grazed and removed before identification can take place, creating the appearance that the infestation has been completely controlled.

In order to test this, the cages were installed on the site to gain a better understanding of the impact grazing was having. These small restricted areas would allow an insight into the theoretical conditions of the site in the absence of grazing. This explores whether seedlings do germinate on the site but are being grazed and removed before identification and recording can take place or if there are simply no seedlings present on the site at all. This would also provide evidence to support the viability of seeds within the seedbank if germination was occurring without the presence of fresh seeds.

From the results gathered, no *H. mantegazzianum* seedlings germinated under the cages. This supports the evidence that no seedlings are germinating on the site, rather than seedlings germinating but being removed later by sheep. Furthermore, the cages were placed in areas that would be expected to have a high density of seeds in the soil seedbank. The lack of germinating seedlings would suggest either a proportion of seeds remain dormant or only a low number of seeds remain viable within the seedbank. It is possible the cages were placed on the site too late in the growing season meaning non-dormant seeds would have germinated earlier in the year and already been grazed. However, the presence of seedlings found on the seedling map suggests *H. mantegazzianum* germination continues into late summer and as a result can be influenced by the effects of grazing.

<u>Seeds in the soil seedbank</u>

Due to the lack of seedlings present on the site, according to both the seedling density map and the cages restricting grazing, it would be easy to assume *H. mantegazzianum* has been eradicated from the site. To make this assumption however, would be ignoring the possibility of viable but dormant seeds present within the soil seedbank. There is a risk of prematurely removing the sheep from the site assuming the infestation has been controlled, only for seeds within the seedbank to germinate and re-establish on the site. Investigation of the soil cores allows an examination of the composition of seeds within the soil.

The fact that no germination of *H. mantegazzianum* seedlings occurred within any of the soil cores can possibly provide further evidence that grazing has substantially reduced its presence on the site and that the soil seedbank has been considerably depleted. However as previously stated, *H. mantegazzianum* is exceedingly difficult to grow under laboratory conditions. The lack of germination within the cores could suggest a reduction of *H. mantegazzianum* seeds but could equally be due to the unsuitable growing conditions experienced in the lab, preventing any seedlings to emerge. Because of this, further investigation is required into the optimum growing conditions to promote germination in the lab. Only then will a lack of germination be conclusive evidence that *H. mantegazzianum* seeds have been eradicated from the soil.

The germination experiment using fresh seeds enabled these complex growing conditions to be tested. The use of fresh seeds rules out the possibility that no seeds are present in the first place, such as from within the soil cores.

Despite only two seedlings germinating within the study period, both from the abandoned initial treatment, three further seedlings germinated in late December/early January. Two of these seedlings germinated under the temperature condition of 20°C/18°C while the remaining seedling germinated at 20°C/10°C. All three seedlings had undergone different lengths of time in cold stratification; 2 weeks, 3 weeks and 4 weeks respectively. The variation of conditions that promoted the growth of these seedlings shows that further investigation is required to narrow down the optimum conditions that will provide the most effective outcome for germination. However, it is possible to conclude from this result, that a longer time period is required for *H. mantegazzianum* seeds to germinate than was allowed within this study.

The limited germination success could also be because the length of time the seeds spent in cold stratification was insufficient to break dormancy. In the field, seeds experience colder temperatures and for longer periods of time that exceeded the maximum of 4 weeks given in this experiment. If a similar experiment was repeated, it would be advisable to keep the seeds in cold storage for a much longer period of time as well as testing more temperature variations to best determine the ideal growing conditions for *H. mantegazzianum*.

Investigation of the seed bags allows a more definitive outcome of the conditions of seeds within the soil seedbank. The fact that so few seeds remained intact within the seed bags suggests that the seedbank is substantially reduced. From the few seeds that were recovered, none were considered viable with a high change of germination. These findings provide key evidence on the length of time that *H. mantegazzianum* seeds remain viable within the soil. The literature is unclear in this area and suggests seeds remain viable from anywhere between 2 and 15 years. The evidence collected in this study suggests the length of time seeds remain viable is substantially shorter than these studies suggest. As previously stated, some seeds must still be able to germinate considering seedlings were found on the site. However, the abundance of these viable seeds must be substantially reduced.

Impact of grazing on surrounding vegetation

In order to assess the true impact grazing is having on the study site as a whole; the focus cannot be restricted solely to *H. mantegazzianum.* The side effects grazing is having on the distribution and density of other species present on the site must also be taken into consideration. Grazing encourages the dominance of graze tolerant species while suppressing other species that are less so. This leads to a much more generalised vegetation distribution and an overall reduction in species diversity. The vegetation maps over the three consecutive years allows a clear comparison of the change in vegetation composition of the site. This enables the patterns of different vegetation types to be monitored over time.

It is a common concern that the reduction of one invasive species may encourage another to take its place. This can be seen in the increased abundance of vegetation dominated by both common nettle (*Urtica dioica)* and thistles (*Cirsium spp*.) over a growing proportion of the site. It can be assumed a reduction in *H. mantegazzianum* densities has allowed both thistles and nettles to increase in abundance. According to the data over the three years, a large proportion of the grassland areas are being increasingly integrated and often completely taken over by nettles or thistles (sometimes both). The defence mechanisms on these plants make them less palatable for the sheep, possibly aiding in their extensive spread.

Although thistles and nettles do not pose the risks associated with *H. mantegazzianum*, the underlying ecological threat and the reduction of biodiversity is still a concern. The monoculture that results from an extensive spread of one particular species often prevents the establishment of other plant life, leading to a much more generalised vegetation type. This can lead to the overall reduction in biodiversity within the area. In addition to this, an expansion of unpalatable or well defended plants substantially reduces the area suitable for livestock grazing. This can have potential implications for the economic income of farmers and livestock keepers.

Nettles and thistles have undergone a major expansion throughout the site but not nearly as extensively as *Juncus*. As mentioned in the previously, *Juncus* was at relatively low densities in 2014 and increased only slightly in 2015. However, the 2016 vegetation map showed *Juncus* had spread considerably, originally occupying areas close to the burn and now expanding further into the site. Large areas previously populated with nettles and thistles as well as grasses have either been integrated by *Juncus* or occupied completely. This relatively fast development of vegetation can be due to the effect grazing has on reducing the densities of other plant species, allowing *Juncus* to expand and dominate out with its original range. Further monitoring of the spread of Juncus is required to fully evaluate its growing impact on the site as well as the potential of becoming over-dominant and invasive.

It can clearly be seen that grazing has a positive impact on reducing the density *of H. mantegazzianum* on the study site. However, the reduction in one species allows other species present to take advantage of the now available space and resources. Continued monitoring of the vegetation development is required to fully understand the further impact grazing has on the site as a whole.

In order to examine the effects of grazing on vegetation cover in more detail, a comparison was made between a grazed and an ungrazed area of the site. The results show almost double the number of plant species present in the ungrazed quadrats as opposed to the grazed. This suggests that species more tolerable to grazing can dominate and outcompete other species that are less able to cope with the pressure of being grazed. This results in a homogeneous spread of vegetation on the grazed site compared with the area restricted from grazing. This is supported by Andersen's 1996 study on *H. mantegazzianum* that found grazing of an invaded site encouraged a less species rich community dominated by graze tolerant plants. The more diverse selection of species within the ungrazed area shows the extent of the impact grazing is having on the site.

The potential change in vegetation composition caused by grazing must be taken into consideration when formulating a management plan for *H. mantegazzianum*. Grazing can effectively control an invasion but at the same time cause a substantial reduction in the richness of other species and overall diversity of the study site. As previously stated, grazing can increase the density of other invasive plants which further reduces the number of species present. Grazing as a management tool cannot simply be assessed by its effectiveness in controlling the target species. The secondary impacts it has on other species and the site as a whole must also be taken into close consideration.

Concluding remarks

The findings from this study show the use of sheep grazing is successful in controlling an extensive invasion of *H. mantegazzianum*. It is possible this investigation was conducted too late in the growing season meaning non-dormant seeds would have germinated earlier in the year and already been grazed. However, the fact that seedlings were found on the site suggests *H. mantegazzianum* germination continues into late summer and as a result, can be influenced by the effects of grazing.

Investigation into the seedbank is essential in determining whether the invasion is completely eradicated from the site. The limited growth from the soil cores may provide some evidence supporting the depletion of the seedbank. However, only when seeds can be germinated within a laboratory on a larger scale, will the lack of growth be conclusive evidence that *H. mantegazzianum* has been eradicated from the soil. The experiment to test germination provides some evidence on these complex conditions and can be used to better inform future attempts.

The seed bags provided evidence that the seedbank remains viable for a substantially shorter period of time than previously thought. Understanding the length of time seeds remain viable in determining the length of time a control measures need to be implemented after the densities of seedlings are substantially reduced. If the evidence points to the complete eradication of viable seeds from within the soil with little chance of fresh seeds being introduced on to the site, a time limit can be set on the need for continued management.

The influence grazing has on the surrounding vegetation also must be taken into close consideration. The areas of vegetation have become much more generalised with one type of vegetation dominating larger areas. There is evidence that a reduction in *H. mantegazzianum* has allowed the expansion of other species such as nettles, thistles and *Juncus* on the site. Replacing one invasive with another is a danger that requires close monitoring to fully assess its impact.

Future studies should continue to focus on the densities of *H. mantegazzianum* on the site under the influence of grazing. Gaining a better understanding of the optimum conditions to promote germination will also allow the viability of the seedbank to be more effectively determined. Overall, the use of sheep grazing has proved very successful and should be considered an effective solution for future control of *H. mantegazzianum* invasions.

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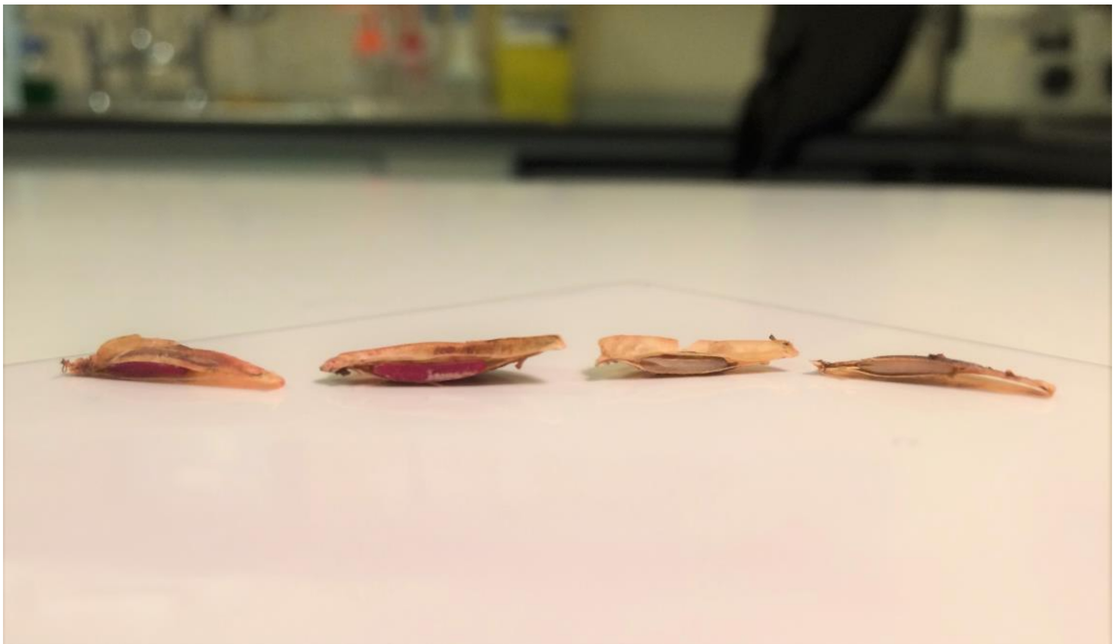
Appendix

Appendix 1: *H. mantegazzianum* seeds cut longitudinally and stained with tetrazolium. These samples are from the control experiment. The two seeds on the left are fresh seeds and are stained red throughout showing they are viable for germination. The two seeds on the right are autoclaved fresh seeds from the same population and remain unstained showing they are unviable for germination.



Appendix 2: Legend for *Fig. 4a* the spatial representaion of the types of vegetation present on the study site in 2014 adapted from Downie (2014).

Abbreviation	**Vegetation type**
N	Nettle
CV	Cirsium vulgare
HA	Holcus/Agrostis
GN	Grass/Nettle
GC	Grass/Clover
CAG	Cirsium arvense/Grass
J	Juncus
B	Bare