

Aus dem Institut für Natur- und Ressourcenschutz
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**Basic and applied studies to evaluate management options for the regulation of Tansy Ragwort
(*Jacobaea vulgaris*)**

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Summary

Jacobaea vulgaris GAERTN. or common ragwort is a widespread noxious grassland weed that is native to Eurasia and invasive in many other continents. Since the pyrrolizidine alkaloids of the plant pose a health risk to cattle consuming this plant, its occurrence in high densities is a challenge for farmers all over the world. However, knowledge about reasons of ragworts invasiveness and environmentally sound management options of the plant in its home range is scarce. Therefore, we followed hypotheses-based approaches to explore the allelochemic and autotoxic potential of this plants litter to gain insights on why and how to manage the weed (Chapter 1 and 2) and tested several mechanical treatments to reduce population density and growth of ragwort, while fostering botanic diversity (Chapter 3).

Due to the posed research questions, climate chamber studies onto seed germination of several common and rare grassland species in extracts of ragwort litter have been studied (Chapter 1). Besides, common garden experiments of germination of ragwort under its litter and the influence of ragwort-conditioned soil have been put into practice (Chapter 2) as well as a long-term field experiment studying ragwort population density and growth under seven different mechanical and cultural treatments over a course of four years (Chapter 3).

The first study (Chapter 1) highlights the autotoxic potential of ragwort in delaying its germination. It also showed that rare species are not disproportionately more sensitive to ragwort allelochemicals than other plants. Finally, it demonstrated that allelopathy of ragwort in its home range does not seem to be a key factor for ragworts invasiveness as osmotic effects were similar to effects of ragwort extract. The second study (Chapter 2) reassures findings of the preceding study, underlining ragworts autotoxicity, but also showing that hampering of germination, facilitates seedling growth later on and is not reducing the weeds population growth. Thus, incorporating ragwort litter as management tool, seems not be effective. A third study (Chapter 3) underlines the importance of seedling recruitment for population growth. Hence, the data suggests cutting the plant during its bloom and to repeat this cut, when it starts to build flowers again within the same season, to effectively prevent seed production is a vital tool for population management. One cut treatments or seed addition treatments were not able to reduce seedling recruitment, population growth or population density.

Zusammenfassung

Jacobaea vulgaris GAERTN. (Jakobs-Kreuzkraut) ist ein weit verbreitetes giftiges Weideunkraut. Die Art ist in Eurasien heimisch und wird in verschiedenen Regionen weltweit als invasiver Neophyt angesehen. Da die Pflanze giftige Pyrrolizidin-Alkaloide enthält, stellt sie ein Gesundheitsrisiko für das Vieh dar, wenn die Pflanze auf der Weide oder in konserviertem Futter, wie Heu, aufgenommen wird. Daher stellt das Vorkommen von Jakobs-Kreuzkraut, v.a. in Massenbeständen, Landwirte in Bezug auf Grünlandmanagement weltweit vor große Herausforderungen. Es ist bisher nur wenig über die Ursachen der starken Zunahme der Art in ihrem natürlichen Verbreitungsgebiet bekannt und somit auch über Möglichkeiten, die Massenbestände naturschutzverträglich zu managen. In dieser Arbeit wurden hypothesenbasierte Ansätze gewählt, um sowohl das allelopathische und autotoxische Potential des Jakobs-Kreuzkrautes (Kapitel 1 und 2) als auch die Wirkung verschiedener Managementmethoden zur Regulierung des Jakobs-Kreuzkrautes in extensiven Weiden zu erfassen (Kapitel 3). Das so gewonnene Wissen soll in mögliche Managementoptionen zur Regulierung einfließen. Zudem wurden verschiedene Methoden auf ihr Potential hin untersucht die Populationsdichte und das Populationswachstum des Jakobs-Kreuzkrautes langfristig zu reduzieren, ohne gleichzeitig die botanische Diversität zu reduzieren (Kapitel 3).

Zur Erfassung des Keimungsverhaltens häufiger und seltener Grünlandarten, wenn diese Biomasse-Auszügen von Jakobs-Kreuzkraut ausgesetzt sind, wurden Experimente in Klimakammern durchgeführt (Kapitel 1). In einem Topfexperiment wurden unter Berücksichtigung von Bodeneffekten die intra- und interspezifischen Effekte von Jakobs-Kreuzkraut- und Grasstreu auf die Keimung und Keimlingsetablierung erfasst (Kapitel 2). In einem fünf Jahre andauernden Freilandexperiment (Kapitel 3) wurde der Einfluss von sieben verschiedenen Managementansätzen (verschiedene Mahdregime, verschieden Maßnahmen zur Erhöhung der Phytodiversität und Kombinationen von beiden) auf die Populationsdichte, -struktur und das Populationswachstum des Jakobs-Kreuzkrautes untersucht.

In der erstens Studie zeigte sich das autotoxische Potential der Art durch eine Verzögerung der Keimung (Kapitel 1). Eine höhere Sensitivität seltener Grünlandarten auf Biomasseauszüge des Jakobs-Kreuzkrautes konnte nicht festgestellt werden. Aus dieser Untersuchung wurde geschlossen, dass die Ausbreitung des Jakobs-Kreuzkrautes nicht in allelopathischen Eigenschaften des Jakobs-Kreuzkrautes liegt. Auch das Topfexperiment bestätigt zwar das Vorhandensein von autotoxischen Effekten während der Keimungsphase, allerdings führte diese Verringerung der Keimung letztendlich zu größeren und kräftigeren Keimlingen (Kapitel 2). Daher erscheint das Belassen der Streu des Jakobs-Kreuzkrautes auf einer Fläche nicht als sinnvolle Komponente in einer Strategie zur Regulierung von Massenbeständen des Jakobs-Kreuzkrautes.

Die Ergebnisse des Langzeitexperimentes, in dem über fünf Jahre die Wirkung von sieben Managementoptionen auf die Populationen des Jakobs-Kreuzkrautes untersucht wurden, unterstreichen die Bedeutung der Keimlingsrekrutierung für das Populationswachstum (Kapitel 3). Die Ergebnisse lassen einen Schnitt von Beständen des Jakobs-Kreuzkrautes zur ersten und zweiten Blüte als effektivste Methode zur Regulierung erscheinen. Die anderen Mahdregime und Methoden zur Erhöhung der Phytodiversität und Kombination von beiden führten nicht zu

einer geringeren Populationsdichte in den Massenbeständen des Jakobs-Kreuzkrautes. Aufgrund der negativen Effekte eines zweimaligen Schnittes auf die Fauna in extensiv genutzten Weidesystemen ist eine naturschutzfachliche Abwägung unabdingbar.

General Introduction

Semi-natural open habitats, such as grasslands, are among the most species-rich and threatened habitats in Europe (Wilson *et al.*, 2012). Since the 1950s, the cover of grasslands in Europe has declined by ca. 15% (Peeters, 2012). Therefore, the restoration of species-rich semi-natural grasslands has been a top priority of nature conservation activities in Europe since the 1970s (Walker *et al.*, 2004). Attempts to restore biodiversity are frequently hindered and occasionally prevented by the colonization of invasive native species (e.g. Pywell *et al.*, 2002; Pakeman *et al.*, 2005; Rasran and Jeromin, 2010). The pasture weed common ragwort (*Jacobaea vulgaris* GAERTN.) is often highly dominant in old-fields early after cessation of agricultural practices (van de Voorde *et al.*, 2012a) and could hinder the establishment of characteristic and threatened grassland species. Thus, ragwort poses a threat to plant species diversity (Maskell *et al.*, 2020). As ragwort is also toxic for horses, cattle, and to a lesser extent sheep (Wiedenfeld and Edgar, 2011) farmers risk the health of their animals grazing on ragwort-infested sites or feeding hay from meadows where ragwort occurs. Thus, they may decide to abandon or intensify grassland use, which would accelerate the loss of species-rich grassland. Consequently, control of common ragwort is prescribed in many countries (Leiss, 2011).

Ragwort is a monocarpic perennial herb native in Europe and Asia. After germination, the rosette is overwintering and usually flowers in the next year. Its Latin name refers to its heyday in end of June. However, flowering can be delayed if the first flowers were damaged by herbivory or cutting (van der Meijden and van der Waals-Kooi, 1979). As disturbance or injury promotes regrowth through roots and vegetative regeneration by crown buds the management of ragwort is challenging (Leiss, 2011). Ragwort is an early successional plant and mainly occurs in grasslands, where it is a character species of the Cynosurion Tx. 1947 (Oberdorfer, 1990) representing mesophilic pastures. It grows on ruderal sites, along roadsides, pastures, and grassland ecosystems in most temperate regions of the world, where it is an invader. It shows ecological characteristics of a pioneer species (Harper and Wood, 1957).

As ragwort is a very common weed in less intensively used grasslands, that are managed with the priority to maintain species diversity, herbicides or intensified fertilization are often banned, because of their negative side-effects on overall grassland diversity (Schmitz *et al.*, 2014). Chemicals can damage non-target plant species, including those the control measures are designed to protect (Matarczyk *et al.*, 2002), other trophic levels (Bünemann *et al.*, 2006) and, through run-off and drift, adjacent ecosystems (Relyea, 2005). This shows the demand for alternative ragwort management measures that are compatible with nature conservation goals in species-rich grasslands. Rather than simply targeting the weed species, approaches which

modify the whole ecosystem have great potential for sustainable and prolonged weed control (Pywell *et al.*, 2010).

The reason for ragwort's invasive success has not been understood, yet. While *J. vulgaris* is known as invader worldwide, it has recently also shown a strong increase and dominance in extensively managed grasslands in its native range across central Europe (Suter *et al.*, 2007). However, it is still unknown by which means the species can proliferate. Probably ragwort invades new places so fast, because of its numerous and light seeds and fast growth that enable it to spread rapidly in open areas (Wardle, 1987). Therefore, ragwort may outcompete other plants or, alternatively, profits from a non-resource mechanism such as allelopathy, where the release of chemical compounds hampers the establishment and growth of members of the recipient plant community (Hierro and Callaway, 2003). Allelopathy has been shown to play a relevant role in inhibiting or promoting plant species establishment and development in many natural and cultivated systems (da Silva *et al.*, 2017). Little is known about the extent to which this mechanism aids ragwort to suppress other plant species. Ahmed and Wardle (1994) showed that ragwort may weaken other plants by allelopathy in its invaded range in New Zealand. However, Pal *et al.* (2015) demonstrated that the allelopathic effect of a plant depends on its biogeographical context, and thus effects of ragwort on co-occurring plants in its home range might be different. As ragwort often occurs in grassland that is managed non-intensively to foster and maintain plant diversity (Huckauf *et al.*, 2017), ragworts allelopathic impact on rare plants could be an additional threat in grassland conservation.

On the other hand, allelopathic effects can also be autotoxic. A negative plant-soil-feedback has been found in *J. vulgaris* (Bezemer *et al.*, 2006a; van de Voorde *et al.*, 2011). Negative plant-soil-feedback is often ascribed to autotoxicity (Cesarano *et al.*, 2017). Indeed, autotoxicity has been found in ragwort (van de Voorde *et al.*, 2012a). Some authors suggest that autotoxicity acts as a density-dependent regulation mechanism to avoid population overcrowding (McNaughton, 1968; Singh *et al.*, 1999; Perry *et al.*, 2005). Thus, if autotoxic effects reduce ragwort density they might be used as a convenient management tool. If ragwort litter of mown ragwort individuals would reduce ragwort recruitment and growth, farmers could benefit from mowing ragwort-populated sites and leaving its litter on the grassland. Furthermore, germination under a dense litter layer might be reduced as light is required for germination (van der Meijden and van der Waals-Kooi, 1979). Thus, within this thesis, it is to evaluate whether there is a detectable negative influence of ragwort litter on ragwort germination and growth as germination is a very sensible process. The survival and persistence of plant populations

strongly depend on reproduction. As in other weeds, seed production is enormous in ragwort (Rasran and Jeromin, 2010). Each plant produces several thousand seeds per year (Wardle 1987). Seeds are dispersed by wind mainly within a short range of less than 15 m (McEvoy and Cox, 1987). Seed survival (up to 20 years) and germination rates (about 80 %) are high (Crawley and Nachapong, 1985). Like other grassland weeds as *Juncus effusus* or *Deschampsia cespitosa*, seedling recruitment of ragwort profits from trampling (Rasran and Jeromin, 2010; Eichberg and Donath, 2017). Seed germination and seedling establishment are the most critical stages in the life of plant individuals (Kitajima and Fenner, 2000).

Germination is affected by abiotic environmental conditions and the biotic environment, e.g. by competition from established vegetation (Bakker and Vries, 1992) or by litter (Jensen and Gutekunst, 2003; Loydi *et al.*, 2013). Litter influences micro-environmental conditions, is important for nutrient cycling, and can have prominent effects on seedling germination and growth via mechanical and chemical effects (Facelli and Pickett, 1991; Loydi *et al.*, 2013). It has been shown that litter can have both positive and negative effects on seedling germination and establishment (Loydi *et al.*, 2013, Xiong and Nilsson, 1999). Positive effects can occur due to higher soil humidity and a lower amplitude of diurnal temperature fluctuations beneath a low litter cover (Loydi *et al.*, 2014). If the litter layer is too high, it reduces the amount of light that reaches the seeds, which is needed for germination (van der Meijden and van der Waals-Kooi, 1979). In addition, thick litter layers can facilitate the spread of fungi that may cause decay of seeds (Baskin and Baskin, 2014). Litter may also exert chemical effects by nutrient immobilization, depletion of oxygen in the soil or toxicity of carbon dioxide produced by decomposers or by releasing phytotoxins (Bonanomi *et al.*, 2006; Loydi *et al.*, 2014). Through these mechanisms litter potentially influences community organization via delaying and reducing or facilitating seedling emergence or seedling growth (Loydi *et al.*, 2013, van der Putten *et al.*, 2013).

While rather artificial climate chamber and common garden experiments of litter effects are useful and can contribute to a mechanistic understanding of weed invasion and give first hints if e.g. management through litter effects is possible, field experiments are essential to test the relative effectiveness of different control measures at large temporal and spatial scales. Such long-term field studies are rare but are critical to increase the evidence base for conservation management (Pullin *et al.*, 2004).

In this context, management approaches which involve cutting or seeding can succeed both by damaging the invasive species directly and by decreasing the susceptibility of the plant community to invasion (Wilson and Clark, 2001; Sheley *et al.*, 2005; Jacobs *et al.*, 2006).

Studies of the effectiveness of cutting regimes on ragwort populations in grasslands are scarce (Roberts and Pullin, 2007) and so far ambiguous in their findings. While Siegrist-Maag *et al.* (2008) showed that frequent cutting decreases ragwort abundance and fertility, other studies find that the species has the ability to re-grow within a few weeks after being damaged or to switch to vegetative reproduction forming multiple rosettes (van der Meijden and van der Waals-Kooi, 1979; Wardle, 1987). Thus, slashing or mowing per se may not be sufficient to control tansy ragwort (McLaren and Faithfull, 2004).

In order to increase the effectiveness of cutting in suppressing weeds, the frequency and timing of cutting seems to be essential because the proportion of nutrients and energy invested in different plant parts varies among life-stages (Jung *et al.*, 2012; Carvalho *et al.*, 2014). In general, ragwort seems to channel the highest proportion of nutrients and energy into above-ground organs during bloom (Otzen, 1977). As ragwort grows back quickly after cutting (van der Meijden and van der Waals-Kooi, 1979), a second cut may be necessary before the second bloom. Since ragwort usually dies off after seed production (Cameron, 1935), cutting before seed dispersal may be another option that may not only prevent vegetative re-growth but also seed dispersal.

In addition to an adapted cutting regime, sowing other species may suppress unwanted weeds (DiTomaso, 2000), too. Such a suppression via increased plant diversity may result from increased competition at the establishment (Bullock, 2000; Pywell *et al.*, 2007), seedling (O'Dell *et al.*, 2007) or adult stage (Hess *et al.*, 2019). According to the biotic resistance hypotheses (Levine *et al.*, 2004), increased plant diversity may also increase plant community resistance against future invasion (Funk *et al.*, 2008).

Therefore, within this work, the effects of cutting regimes at different phenological stages of ragwort and restoration measures including mowing and seeding to increase plant species diversity as well as a combination of both on the development of ragwort populations have been tested.

Against this background, the research gaps and uncertainties that are addressed in each chapter of the thesis are to be described:

- Influence of allelopathy of ragwort on germination of grassland species and ragwort itself is insufficiently known (Chapter 1).
- Impact of ragwort litter on the emergence of its seedlings as possible management option has not been studied yet (Chapter 2).

- No assessment of the effectiveness of different ecologically based weed management systems that is mechanical and cultural weed control methods in ragwort in a study spanning several years exists (Chapter 3).

In **Chapter 1** of this thesis I describe the allelopathic and autotoxic potential of *J. vulgaris* on co-occurring species. To this end, I conducted germination experiments in climate chambers with seeds of a vast array of species growing next to *J. vulgaris* in natural grassland covering common and rare herb and grass species from four families and confronted these seeds with ragwort-extract in a germination chamber. This way, I wanted to evaluate if ragwort is inhibiting germination of other species generally or especially in already threatened plants, that are often poor competitors (Kempel *et al.*, 2020).

In my second study (**Chapter 2**), I explored the influence of ragwort litter on its germination under more natural conditions, to assess whether ragwort litter can be used as a management tool in population density regulation. Ragwort seeds were planted in a common garden with varying litter types and amounts, ranging from hay-litter, over a mixture of hay and ragwort litter to pure ragwort litter in high and low amounts and varying underground (ragwort conditioned vs. non-conditioned soil). After a few weeks biomass, plant height and leaf number of seedlings were measured and assessed.

Finally, the core of my work is presented in **Chapter 3** and consists of the study of the efficiency of different management tools that are based on the ecology and biology of ragwort to control its population growth and density without harming floral biodiversity. It is to compare the development of ragwort population density and growth under six different management regimes including various cutting and restoration techniques and the combination of both for four years. Number and stage classes of ragwort were assessed, and vegetation surveys conducted, to document the floral change caused by management regimes.

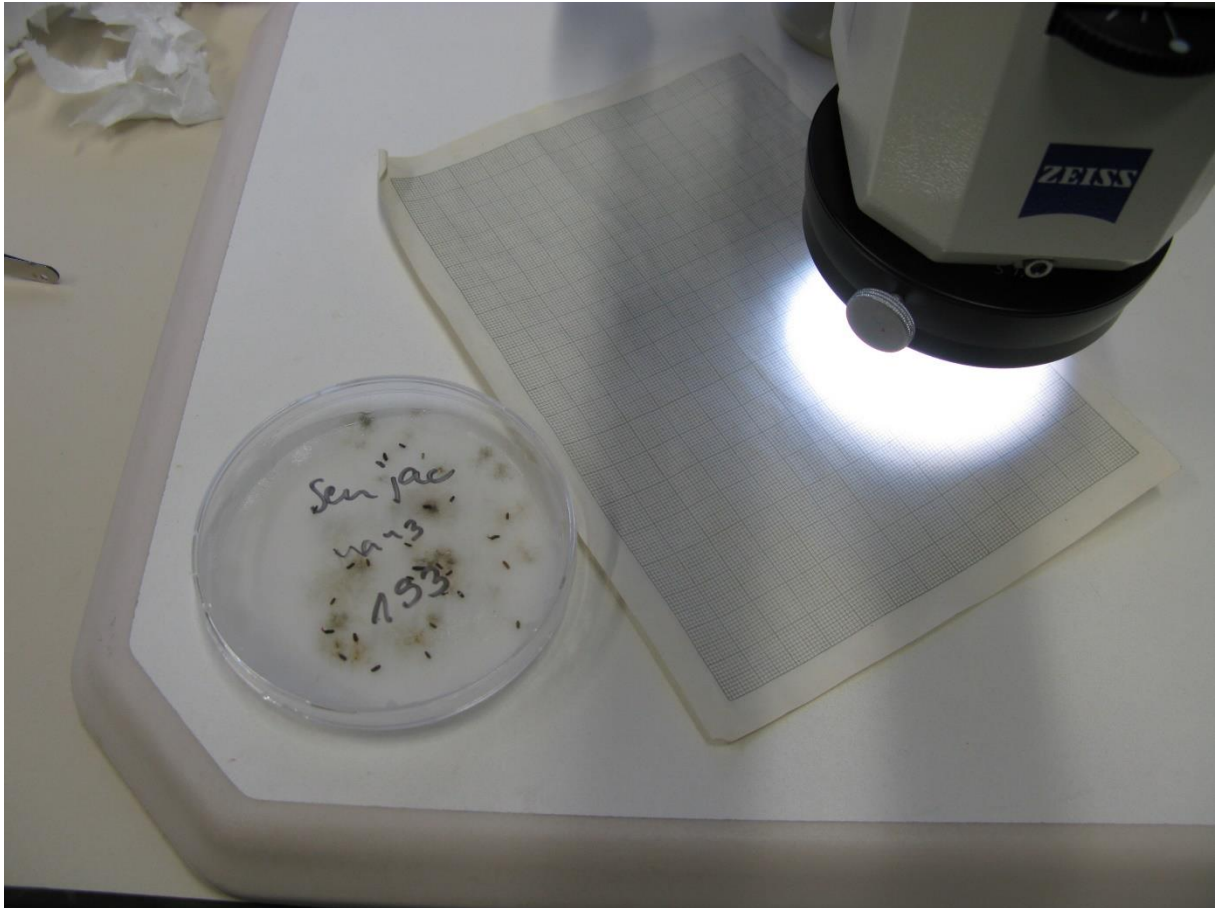
At the very end, I synthesize main findings of all studies in a comprehensive discussion and give management recommendations for farmers and further grassland managers.

Chapter 1 Allelopathic vs. autotoxic potential of a grassland weed - evidence from a seed germination experiment

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Abstract

Background: Mechanisms that affect invasiveness of non-indigenous species have received much attention, however, few studies have investigated invasions by native plants. Invasive native species such as common ragwort (*Jacobaea vulgaris*) may become noxious weeds in grasslands. Thus, they challenge farming and nature conservation by outcompeting fodder plants or rare herbs. One mechanism that can result in outcompeting co-occurring plants is to restrain their germination by allelopathy.

Aim: We evaluated the potential of *J. vulgaris* to suppress germination in common and rare grassland species by allelopathy.

Methods: In a germination chamber experiment, we exposed 22 rare and common species and *J. vulgaris* itself to ragwort leachate. We controlled for osmotic effects of the leachate by germination tests in mannitol solution and water. We assessed germination percentage, time and synchrony, and radicle length.

Results: Leachate reduced germination percentage, germination time, synchrony and radicle length. These effects were similar for *J. vulgaris*, indicating autotoxicity. Rare species germinated less than common species but were not more sensitive to the phytotoxic effects of *J. vulgaris*. Restraining effects of the leachate were similar to that of the mere osmotic effect.

Conclusion: Our results question allelopathy as the main driving mechanism behind *J. vulgaris* gaining dominance. However, the impact of *J. vulgaris* might depend on the composition of the invaded plant community due to species-specific effects of allelopathy.

Keywords: allelopathy, autotoxicity, grassland conservation, *Jacobaea vulgaris*, litter, pasture, phytotoxicity, ragwort,

Introduction

Semi-natural open habitats, such as grasslands, are among the most species-rich and threatened habitats in Europe (Wilson *et al.*, 2012). Since the 1950s, the cover of grasslands in Europe has declined by ca. 15% (Peeters, 2012). Land-use changes such as intensification, conversion to arable fields, or abandonment are well-known factors behind this decline (Carboni *et al.*, 2015).

The occurrence of pasture weeds may induce profound direct and indirect changes in grassland communities (Meiners *et al.*, 2001; Winter *et al.*, 2014). The common ragwort (*Jacobaea vulgaris* GAERTN.), which has a long history of spreading in grasslands around the world, is regarded as a noxious weed in its native and non-native ranges (Wardle, 1987; Suter *et al.*, 2007). While *J. vulgaris* has long been known to be invasive around the world, it has recently also shown a strong increase and dominance in extensively managed grasslands in its native range across central Europe (Suter *et al.*, 2007), where it might be regarded as an invasive species (see definition in Luken and Thieret, 1997; Valéry *et al.*, 2008). While the existence of 'native invaders' is hotly debated (Valéry *et al.*, 2009; Carey *et al.*, 2012; Blondel *et al.*, 2014), *J. vulgaris* has undoubtedly shown recent rapid spread and dominance in its native range (Suter *et al.*, 2007), two important prerequisites to coin it as a native invader (Luken and Thieret, 1997; Valéry *et al.*, 2008). However, it is still not known by which means it is able to proliferate.

Understanding the direct and indirect mechanisms that govern the success of native or non-native invasive plants is a scientific challenge (Catford *et al.*, 2009). Accordingly, little is known about the extent to which *J. vulgaris* directly affects plant diversity in grasslands be it via competition or allelopathy - the negative effect of one plant on the growth of another plant through the release of chemicals (Inderjit and Duke, 2003). We explored this by studying whether the increase of *J. vulgaris* in non-intensively managed grasslands can be linked to its allelopathic effects on co-occurring pasture species.

The survival and persistence of plant populations strongly depend on reproduction, thus seed germination and seedling establishment are the most critical stages in the life of plant individuals (Kitajima and Fenner, 2000). Germination is affected by abiotic environmental conditions and the biotic environment, e.g. by competition from established vegetation (Bakker and Vries, 1992) or by a litter layer (Jensen and Gutekunst, 2003; Loydi *et al.*, 2013). The presence of suitable microsites is crucial for successful germination and seedling establishment (Grubb, 1977). Litter can affect plant community structure through mechanical, physical and chemical mechanisms, and potentially influences community organisation via delayed and

reduced germination and/or reduced initial radicle growth (Loydi *et al.*, 2013). Thus, germination is the earliest phase in which allelopathic effects might control plant establishment. Accordingly, *J. vulgaris* may maintain and increase its dominance after its successful establishment through allelopathic suppression via secondary chemical compounds leached from dead plant remains (Ahmed and Wardle 1994).

While the allelopathic potential of *J. vulgaris* has already been confirmed in its non-native range in New Zealand (Ahmed and Wardle, 1994), evidence from within its native range is still missing. In fact, very little is known about the effect of secondary chemical compounds of plant species on themselves (Alías *et al.*, 2006), and we are only aware of one study that has assessed the autotoxic effects of *J. vulgaris* on germination (van de Voorde *et al.* 2012a). Evolutionary theory predicts that *J. vulgaris* would develop resistance to allelochemicals under frequent exposure to its own releases (Rabotnov, 1982). We hypothesised that allelopathy was a driving force of invasion success, and thus toxic effects of *J. vulgaris* on itself should be lower than on co-occurring resident species.

In its native range, *J. vulgaris* not only reduces the economic value of permanent grazing land but might also hamper the conservation and restoration of species-rich extensively managed grasslands (Gruber and Whytemare, 1997; Lawson *et al.*, 2004; Huckauf *et al.*, 2017). This is especially relevant since these grasslands are habitats for many rare and threatened grassland species (Kleijn *et al.*, 2009). Among other factors, rare species are often limited by biotic factors as they do not compete well with other plants (Candeias and Warren, 2016). They are naturally more prone to negative effects by environmental, genetic and demographic stochasticity (Gitzendanner and Soltis, 2000; Keller and Waller, 2002; Lapiedra *et al.*, 2015), and may be more susceptible to environmental changes induced by invading plant species (Dale, 1997; Vicente *et al.*, 2011). In this context, Rühl *et al.* (2016b) have found that rare arable weed species have more specific germination requirements than common species. Therefore, we expect that rare grassland species might also be more prone to the negative allelopathic effects of *J. vulgaris* during germination. Specific effects of *J. vulgaris* leachate on rare species are also relevant, since the establishment of a species-rich plant community is seen as one measure to reduce *J. vulgaris* populations (Bezemer *et al.* 2006a). Regularly, the seed mixtures applied for nature conservation purposes include rare species (Kiehl *et al.* 2010). Knowledge about the susceptibility of rare species towards allelopathy from *J. vulgaris* is of importance for planning grassland restoration measures, as these measures are often conducted on sites colonized by *J. vulgaris*.

With this in mind, we exposed seeds of 22 common and rare grassland species belonging to four plant families and *J. vulgaris* itself to: (1) *J. vulgaris*-leachate, (2) mannitol solution with the same osmotic potential as the leachate, or (3) distilled water. Subsequently, we assessed germination, mean germination time, germination synchrony and seedling radicle length (Ranal and Santana, 2006; Ranal *et al.*, 2009).

We hypothesised that: (1) negative effects on germination were caused by allelopathy rather than solely osmotic processes, (2) the germination of other species would be more negatively affected by exposure to *J. vulgaris* leachate than that of *J. vulgaris* itself, and (3) the negative effect of the leachate is more pronounced on rare compared to common pasture plants.

Material and methods

Study species

Jacobaea vulgaris is an herbaceous monocarpic plant species from the Asteraceae family native to Europe and Asia (Harper and Wood, 1957). It has been accidentally introduced to Australia, New Zealand and the USA (Bain, 1991). *J. vulgaris* prefers open patches and disturbed sites. It shows ecological characteristics of a pioneer species. In particular, its numerous and light seeds and fast growth enable it to spread rapidly in open areas (Wardle, 1987). Each plant produces several thousand seeds per year (Wardle, 1987). Seeds are mainly dispersed by wind over a distance of less than 15 m (McEvoy and Cox, 1987). Seed survival (up to 20 years) and germination rates (about 80%) are high (Crawley and Nachapong, 1985). *J. vulgaris* occurs on ruderal sites, along roadsides, pastures and grassland ecosystems in most temperate regions of the world. It is often regarded as a noxious weed due to its content of toxic pyrrolizidine alkaloids (Leiss, 2011).

In this study, we tested the effect of *J. vulgaris*-leachate on the germination of 24 grassland species typical for non-intensively managed grasslands, e. g. pastures (Natura 2000 code 6510) or meadows, dry grasslands (Natura 2000 code 6210) and *J. vulgaris* itself (Table 1). Six species for each of the most species-rich plant families in mesic grasslands (Ellenberg and Leuschner, 2010) Asteraceae, Fabaceae, Caryophyllaceae, and Poaceae -, were selected for the study as each of them frequently co-occur with ragwort. Moreover, Poaceae and Fabaceae are very important for farming on grasslands (Klapp, 1965; Tallwin and Jefferson, 1999). Each family was represented by species that were either rare or common in northern Germany (Mierwald, 2006). Common species often co-occur with ragwort, whereas rare species are

confronted with the invasive plant on sites where they are sown for restoration measures. The mix of families and species tested recognizes phylogenetic effects and therefore increases the explanatory reliability of the results as effects occurring in all families and species are not likely to be limited to special phyla (Gitzendanner and Soltis, 2000; Leishman *et al.*, 2000). Seed viability was tested for each species (25 seeds, $n = 2$) with 1% tetrazolium chloride solution (ISTA, 2015) (Table 1). The germination of *Trifolium campestre* and *T. pratense* was extremely low (Table 1) as most of the seeds already germinated during stratification. Consequently, both species were omitted from the statistical analyses.

Table 1 Study species, with information on plant family, Red List status (Mierwald, 2006), mean \pm SE of seed viability according to a tetrazolium chloride test with 25 seeds per replicate ($n = 2$) and mean \pm SE of germination percentage in distilled water (control treatment). Nomenclature according to Buttler *et al.*, 2017).

Family and species	Red List status	Germination (%)	Viability (%)
Poaceae			
<i>Agrostis capillaris</i>	LC	82 \pm 7	80 \pm 3
<i>Briza media</i>	EN	61 \pm 9	90 \pm 14
<i>Dactylis glomerata</i>	LC	79 \pm 7	98 \pm 11
<i>Festuca ovina</i>	NT	64 \pm 4	70 \pm 3
<i>Holcus lanatus</i>	LC	64 \pm 8	76 \pm 6
<i>Lolium perenne</i>	LC	90 \pm 7	94 \pm 8
Fabaceae			
<i>Lotus corniculatus</i>	NT	68 \pm 20	100 \pm 0
<i>Medicago lupulina</i>	LC	42 \pm 17	91 \pm 7
<i>Trifolium campestre</i>	NT	4 \pm 2	84 \pm 0
<i>Trifolium dubium*</i>	LC	22 \pm 16	88 \pm 6
<i>Trifolium pratense</i>	LC	2 \pm 2	10 \pm 3
<i>Trifolium repens*</i>	LC	35 \pm 9	64 \pm 23
Caryophyllaceae			
<i>Cerastium holosteoides</i>	LC	75 \pm 5	70 \pm 14
<i>Dianthus deltoides</i>	EN	99 \pm 1	98 \pm 3
<i>Lychnis flos-cuculi</i>	VU	18 \pm 8	20 \pm 6
<i>Silene dioica</i>	LC	29 \pm 4	82 \pm 3

<i>Silene vulgaris</i>	NT	70±11	92±0
<i>Stellaria graminea</i>	LC	57±7	88±11
Asteraceae			
<i>Achillea millefolium</i>	LC	100±0	92±0
<i>Centaurea jacea</i>	NT	39±8	72±17
<i>Crepis capillaris</i>	LC	97±3	94±3
<i>Hypochaeris radicata</i>	LC	96±6	96±6
<i>Jacobaea vulgaris</i>	LC	68±22	65±7
<i>Leontodon hispidus</i>	EN	50±19	56±6
<i>Leucanthemum ircutianum</i>	NT	84±4	70±3

* These species were excluded from the analyses due to their low germination percentage.

LC = Least Concern; NT = Near Threatened; VU = Vulnerable; EN = Endangered

Experimental design

We carried out a germination experiment to test the potential allelopathic effects of *J. vulgaris* on selected co-occurring species, rare species and itself in laboratory conditions. In our experiment, we quantified the effects of *J. vulgaris* above-ground biomass leachate, mannitol solution (D-mannitol; Roth, Karlsruhe, Germany), and distilled deionised water (factor levels [k] = 3) on germination of the selected co-occurring common and rare pasture species and itself (k = 25). We are aware of the recent suggestions by da Silva *et al.* (2017) about the assessment of allelopathic effects. They call for experiments to be carried out under conditions as close to natural as possible, preferably in field conditions, including species co-occurring with the potentially allelopathic species. Even though our experiment does not meet all preconditions postulated by da Silva *et al.* (2017), our approach is widely accepted for an initial assessment of the allelopathic potential of plants (Gomes *et al.*, 2017).

The mannitol solution, having the same osmotic potential as the leachate (- 0.4 MPa, measured with Osmomat 030, gonotec), was considered an osmotic effect control for the leachate treatment. Distilled deionised water served as a control for osmotic and/or allelopathic effects. Each factor combination was replicated five times resulting in 15 Petri dishes (9 cm diameter) per species and 375 dishes in total. Sterile Petri dishes were laid out with a double layer of filter paper before 50 seeds of one study species were equally spread across each dish. *J. vulgaris* seeds were hand-collected from at least 20 individuals on each of eight pastures across

Schleswig-Holstein (Northern Germany) from August to September 2015 and mixed for the experiment. Seeds of co-occurring and rare species were obtained from commercial regional seed suppliers (Rieger-Hofmann GmbH, Blaufelden-Raboldshausen, Germany and Cruydt-hoeck, Groningen, the Netherlands).

Five Petri dishes per species were treated with 4 mL of either of *J. vulgaris* leachate, mannitol solution or distilled deionised water. The leachate treatment was prepared by soaking 400 g of dry leaves and stalks in 4 L of deionized water for 72 h (Hovstad and Ohlson, 2008). This amount of biomass corresponds to the average litter quantity in the field for grasslands with medium production of biomass (400 g m⁻²; Donath *et al.*, 2004) and the volume of water corresponds to the average amount of rain on a rainy day during spring (between 4.0 and 5.4 L m⁻²). Leaves and stalks were collected from rosettes and mature (flowering stage) *J. vulgaris* plants on fallow land near Kiel in Schleswig-Holstein (53°43'N 9°12'E–54°25'N 10°40'E), at the end of the growing season in October 2015. After collection, the biomass was dried at 60°C for 72h, and afterwards stored at room temperature until the start of the experiment.

Petri dishes were randomly distributed in a germination chamber (Versatile Environmental Test Chamber MLR-352; Panasonic) with diurnally fluctuating temperatures (20/10 °C) and 12 h of light and 12 h of dark period. Petri dishes in the germination chamber were sealed in transparent plastic bags in groups of six to avoid excessive water evaporation (Loydi *et al.*, 2015a). For four weeks prior to the incubation, seeds received a cold-wet stratification treatment in the climate chamber at 4 °C (Hölzel and Otte, 2004). The experiment lasted five weeks. Twice every week, germinated seeds were counted, their radicle length measured and then removed. The radicle length was measured for five randomly selected germinated seeds per Petri dish at most. Germination was defined as the emergence of the radicle.

Data analysis

Germination data were used to calculate the dependent variables germination percentage (G) as the percentage of all germinated seeds from the initial number of seeds and mean germination time (MT) as the weighted mean of the time to germination, using days since the start of the experiment as time measurement. The number of seeds germinated in the intervals of time established for data collection was used as weight (Ranal and Santana 2006). Furthermore, we calculated germination synchrony (Z) indicating the germination variability over time, ranging from 0 to 1 (high values indicate highly synchronous germination) (Ranal and Santana, 2006).

These parameters and radicle length (RL) were included in the statistical analyses. We conducted two separate sets of analyses, one for the test of the allelopathic effects of *J. vulgaris* leachate on the co-occurring species and one to assess the autotoxic effect of *J. vulgaris*.

The data evaluation started with the definition of an appropriate statistical model based on generalised least squares (Carroll and Ruppert, 1988) and accordingly on a linear model for the analysis of the autotoxicity of *J. vulgaris*. Graphical residual analysis revealed the data to be approximately normally distributed and to be heteroscedastic. Dependent variables were tested against the factorial explanatory variables species identity (22 species) and germination environment (*J. vulgaris* litter leachate, mannitol solution, distilled water) as well as their interaction. Based on this model an analysis of variance (ANOVA) was conducted, followed by multiple contrast tests for heteroscedastic data (Hasler and Hothorn, 2008, 2008) to compare rare vs. common species. Additionally, the germination percentage was also evaluated at the species level by conducting one-way analyses of variance (ANOVA) followed by a Tukey HSD test after significant results in the ANOVA. Analyses were made in R (R Core Team, 2016) using the packages nlme, MASS, car, multcomp, SimComp and ggplot2.

Results

Across species, the germination parameters were more negatively affected by *J. vulgaris* leachate than by distilled water, while mannitol showed mostly an intermediate effect. All germination parameters (G, MT, Z, RL) were interactively affected by treatment and species identity (Table S1). The interaction effect was quantitative in nature (Figure S1), i.e. only the size but not the sign of the effect of the germination environment changed with species identity. The response pattern that germination was best in distilled water and worst in leachate occurred in all species, albeit it was not significant in all single-species analyses. For 10 out of 22 tested species, germination was significantly reduced by the *J. vulgaris* leachate as compared to distilled water or the mannitol treatment (Figure S1). Mean germination percentage was significantly lower by about 25% in *J. vulgaris* leachate and mannitol compared to distilled water (Figure 1A). Across species, significant differences among all three treatments were found in mean germination time (Figure 1B). Seeds needed twice the time for germination when exposed to the leachate as compared to distilled water (Figure 1B). Synchrony of germination was significantly lower by about 33% in *J. vulgaris* leachate than in distilled water (Figure 1C). Radicles of seedlings in the leachate were one-third of the length of seedlings in distilled water (Figure 1D), and half of those in mannitol.

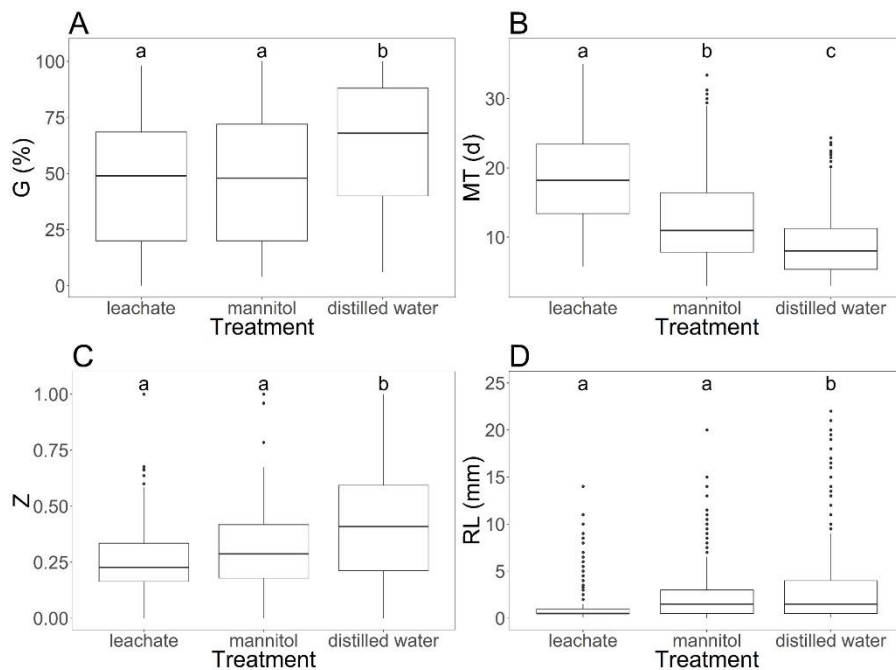


Figure 1 Germination percentage (G), mean germination time (MT), germination synchrony (Z) and radicle length (RL) in leachate, mannitol or distilled deionised water across 22 species tested, except *J. vulgaris* after five weeks. In the box plots middle lines represent median, boxes represent the first and third quartiles, lower and upper bars represent the minimum and the maximum and points represent outliers (i.e. points above 1.5 SD). Different letters indicate significant differences ($P \leq 0.05$; Tukey HSD) between treatments.

The response of germination characteristics of *J. vulgaris* showed a pattern concurrent with the response pattern across species. Germination percentage was about 10% lower in the leachate and mannitol solution compared to distilled water. As for several other species (nine out of 22 (Figure S1), there were no significant differences in germination percentages among treatments (see Figure 2A). Yet, germination took longest when seeds were exposed to leachate and was about three times faster in distilled water; germination time in mannitol was intermediate (Figure 2B). The difference in germination time for *J. vulgaris* in distilled water and in its own extract was 25 days, ca. 7 days longer than the average delay over all species. Seeds in the extract and mannitol treatment germinated least synchronously (Figure 2C), concomitant to the general pattern. In accordance with the general pattern, roots in the leachate were less than half as long as in distilled water and intermediate in mannitol (Figure 2D).

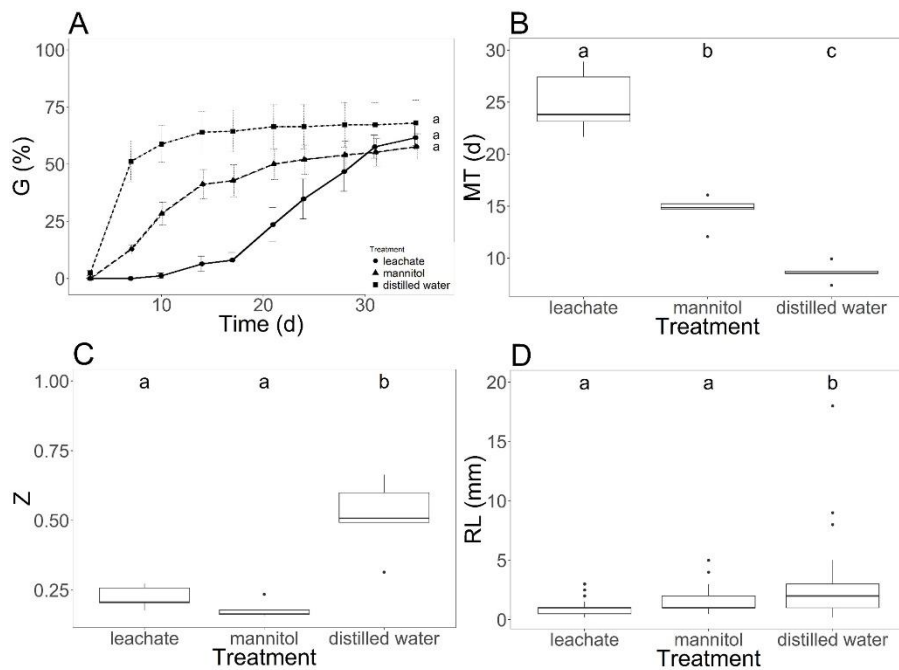


Figure 2 Cumulative germination percentage (G, mean \pm SE), mean germination time (MT), germination synchrony (Z) and radicle length in leachate, mannitol or distilled water obtained for *J. vulgaris* after five weeks. For explanation of the boxplot refer to Figure 1. Different letters indicate significant differences ($P \leq 0.05$; Tukey HSD) between treatments.

Rare and common species differed significantly in their germination pattern (Figure 3). Regardless of the treatment, germination of rare species ($42.5 \pm 4.6\%$, mean \pm SE) was on average lower than that of common species ($56.2 \pm 1.7\%$). Moreover, the medians of the germination percentage between rare and common species differed much more in the mannitol than in the leachate treatment (Figure 3A). The germination percentage was lowest in the leachate and the mannitol treatment. This response pattern was similar in case of mean germination time (Figure 3B).

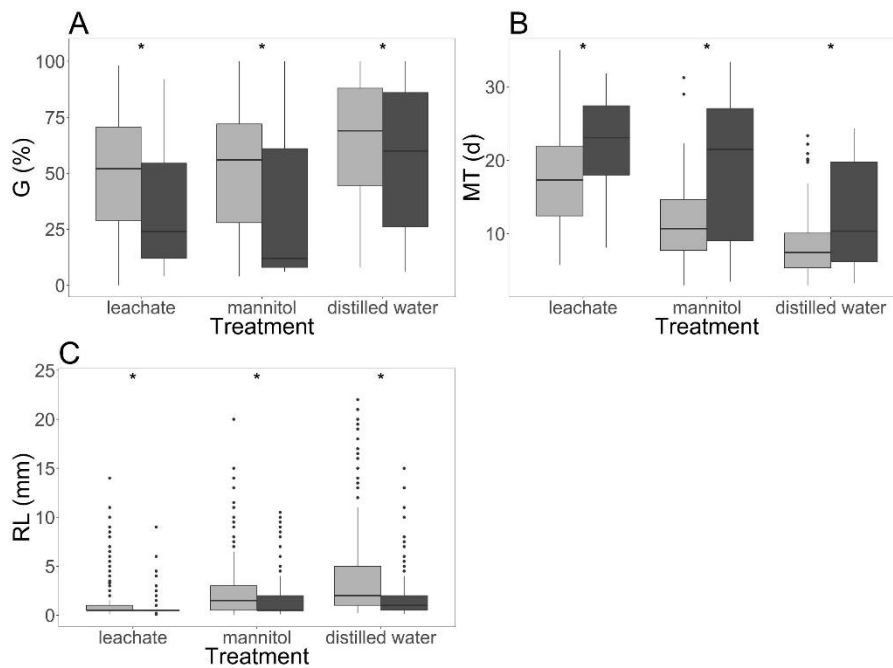


Figure 3 Response of germination percentage (G [%]), mean germination time (MT [d]) and radicle length (RL [mm]) of common (light grey) and rare species (dark grey) when exposed to *J. vulgaris* leachate, mannitol and distilled water after five weeks. For explanation of the boxplot refer to Figure 1. Asterisks indicate significant differences ($P \leq 0.05$) between rare and common species.

The response of radicle length was concurrent with the general significance pattern, i.e. radicles were shortest in the leachate and longest in distilled water (Figure 3C). The differences between rare and common plants were smallest in the leachate and highest in distilled water. Germination synchrony showed no significant differences between the two species groups.

Discussion

We found that *J. vulgaris* leachate negatively affected seed germination of co-occurring grassland species and its own, however, the effects were equal to those of the osmotic treatment. When exposed to the leachate, co-occurring plant species showed reduced percentage and synchrony of germination, increased germination time and a decrease in radicle length. Compared to Ruprecht *et al.* (2008), who found the leachate of a dominant grass to reduce the germination of co-occurring herbs by between 33 and 94%, the measured effects of *J. vulgaris* leachate were much lower, ranging from a reduction of 4 to 47% in germination. However, the delay of germination by about 9 days in our study was comparable to the delay caused by the dominant grass in the study by Ruprecht *et al.* (2008) and even stronger than the effect found for non-native invasive forbs on native plants (Loydi *et al.*, 2015a).

To find toxic effects of the above-ground plant parts of *J. vulgaris* is not unexpected. Bonanomi *et al.* (2006) have shown that allelopathy, especially of forbs, is a widespread phenomenon. In general, the delayed mean germination time and reduced radicle length that we observed as a response suggest chemical competition. Delayed germination of neighbouring species can be advantageous, since this creates a temporal advantage against co-occurring species during emergence (Rühl *et al.*, 2016a). Interestingly germination delay of *J. vulgaris* through its own leachate was stronger than the average germination delay of the other species. Even differences in the timing of seedling emergence of only a few days can have profound effects on community assembly due to priority effects (Körner *et al.*, 2008; Urban and de Meester, 2009; Viana *et al.*, 2016). The reduction of radicle growth of co-occurring species by *J. vulgaris* leachate might give it an advantage during its establishment. Since *J. vulgaris* leachate also inhibited its own radicle growth and germination, however, such a potential competitive advantage does not occur.

At the species level, the current experiment revealed significant negative effects for less than half of the tested species. This indicates relatively weak allelopathic effects for *J. vulgaris*, which is in line with (Wille *et al.*, 2013), who have found only limited evidence for allelopathic effects of the invasive *Heracleum mantegazzianum* on co-occurring plant species. Furthermore, we found relatively strong negative osmotic effects on germination parameters. The osmotic potential of ragwort leachate in our study, - 0.40 MPa, was close to that of Ludewig *et al.* (2014), who have found that a potential of - 0.5 MPa significantly reduced the germination of 80 % of their study species. Differences between the osmotic and the allelopathic effect on germination parameters were often only marginal in our study. Accordingly, germination percentage and germination synchrony of seeds exposed to leachate were significantly lower compared to those in distilled water but not to those in mannitol. Therefore, we conclude that *J. vulgaris* exerted only a weak allelopathic effect on seed germination compared to the pure osmotic leachate effects. Thus, we reject our hypothesis that reduced germination performance is caused by allelopathy of the *J. vulgaris* leachate.

While the observed species-specific responses in multiple-species experiments are not unexpected (Baskin and Baskin, 2014; van Kleunen *et al.*, 2014), our results contrast with the findings of Ahmed and Wardle (1994). They found an allelopathic effect, i.e. reduced germination, on all five study species exposed to *J. vulgaris* leachate from above-ground tissue. Since Ahmed and Wardle (1994) also controlled for osmotic effects in their experiment, effects of the secondary chemical compounds seem to have been stronger compared to our study. This

pattern might have been caused by methodological differences in acquiring the net allelopathic effect of the leachate (calibration curve in their study vs. treatment of osmotic potential similar to that of leachate in our study) or differences in timing of plant material collection.

Ahmed and Wardle (1994) have mimicked a grassland system, which was cut within the growing season and therefore used freshly grown material. In contrast, we assessed the allelopathic potential of naturally deposited litter, typically found late in the growing season (Loydi *et al.* 2013). It is well documented that concentrations of secondary metabolites, such as pyrrolizidine alkaloids, change greatly during the growing season, with a peak early during the vegetation period and a continued decrease until the end of the vegetation period (Johnson *et al.*, 1985; de Boer, 1999). Later in the vegetation period, plants either produce less toxins or transfer them from senescing organs to roots or seeds where protection is still ecologically worthwhile (Jung *et al.*, 2012). Thus, it is likely that seeds in Ahmed and Wardle (1994) were exposed to higher concentrations of allelopathic compounds than in our study.

Wink (1993) has noted that more than 50 alkaloids have been reported to have allelopathic properties within nearly all structural types of alkaloids. Pyrrolizidine alkaloids are very frequent in *J. vulgaris*, but there are few reports of their allelopathic activity (Reigosa *et al.*, 2006). Other allelochemical compounds found in *J. vulgaris* such as sesquiterpene lactones (Ambika, 2013) or the almost omnipresent coumarin (Ruprecht *et al.*, 2008; Razavi, 2011) might also be causative for the negative effect of *J. vulgaris* leachate on the germination of seeds (Chon and Kim, 2004).

It is also important to note, that the study by Ahmed and Wardle (1994) was carried out in New Zealand, where both *J. vulgaris* and the potentially affected species (e.g. *Lolium perenne*, *Trifolium repens*) had been introduced from Europe. Joshi and Vrieling (2005), and Doorduyn and Vrieling (2011) have found that *J. vulgaris* had increased levels of toxic compounds in its non-native range. This pattern and the results of our study are therefore in line with Pal *et al.*, (2015), who have demonstrated that the allelopathic effect of a plant depends on its biogeographical context and that plants often have a stronger impact on neighbouring plants in their non-native range.

Equally to affecting germination of co-occurring species, we found *J. vulgaris* to negatively affect its own seed germination. This effect was especially strong for mean germination time being prolonged in *J. vulgaris* leachate. Thus, our hypothesis of ragwort hindering other species more than itself must be rejected. The strong autotoxic effects we observed suggest that their contribution to structuring plant communities with *J. vulgaris* could be higher than allelopathic

effects and may also contribute to *J. vulgaris* being a typical plant species of early successional stages, vanishing after a first population burst and thus known to be a “notoriously cyclic plant” (Holly *et al.*, 1952). In accordance with that notion, van de Voorde *et al.* (2012a) have shown the autotoxicity of *J. vulgaris* in a germination experiment on glass beads and in soil. In addition, Bezemer *et al.* (Bezemer *et al.*, 2006a) and van de Voorde *et al.* (2012b) have demonstrated that *J. vulgaris* underperformed in soils conditioned by itself. The latter authors reported autotoxic effects using pieces of roots and shoot tissue (medium- to high concentration of extracts) from 12-week-old plants, which may not contain the same amount of toxins as litter at the end of the growing season. Again, seasonality might play a role in explaining the difference between their and our results. As litter usually comes from senescent individuals that flowered and shed seeds, it seems more realistic that seeds will be affected with litter from older shoots (Ahmed and Wardle, 1994).

Although, the presence of strong autotoxic effects seems counterintuitive at first glance, autotoxicity may just be a simple evolutionary consequence of the allelopathic ability of a plant (Rabotnov, 1982), since complete resistance to its own toxins is energetically expensive (Bais *et al.*, 2006). Given that intraspecific interactions frequently occur in many species (Cheplick, 1992; Dyer, 2004), strong resistance to within-species allelochemicals may be expected to be commonplace. Alternatively, autotoxicity might serve as population control by the species itself, to avoid intraspecific competition (Renne *et al.*, 2014). Thus, in densely populated areas non-germinated seeds may benefit from autotoxicity by inducing seed dormancy, without the loss of viability (Picman and Picman, 1984; Bais *et al.*, 2006). *J. vulgaris* is capable of producing several hundred thousand seeds per plant, which usually exhibit very poor dispersal (Wardle, 1987). In fact, most of the seeds are deposited within a 5 m radius from the mother plant (McEvoy and Cox, 1987) and build up a persistent soil seed bank (Harper and Wood, 1957). Consequently, *J. vulgaris* might indeed experience seedling competition during regeneration from the seed bank following soil disturbance (Wardle 1987). Thus, spreading germination in time through autotoxicity can be advantageous for the establishment and long-term persistence of *J. vulgaris* populations (Edwards *et al.*, 1988).

When looking at the effects of invasive weeds on nature conservation efforts, an assessment of the allelopathic effect on rare herbs is of special interest. Differences in mean germination between rare and common species were more pronounced in the mannitol than in the leachate treatment (Figure 3). Thus, although our results confirm that rare species seem more sensitive to reduced water availability (mannitol treatment) (Rühl *et al.*, 2016b), germination of rare

species seemed not to be particularly sensitive to *J. vulgaris* leachate per se and instead to any litter leachate that reduces water potential. Therefore, the presence of *J. vulgaris* does not appear to preclude their (re-)establishment. In fact, increasing biodiversity by sowing rare species could even prevent invasions by *J. vulgaris* according to the biotic resistance hypotheses (Levine *et al.*, 2004), which is in accordance with the general recommendation to keep a closed sward to prevent establishment and spread of *J. vulgaris* (Suter *et al.*, 2007).

Conclusion

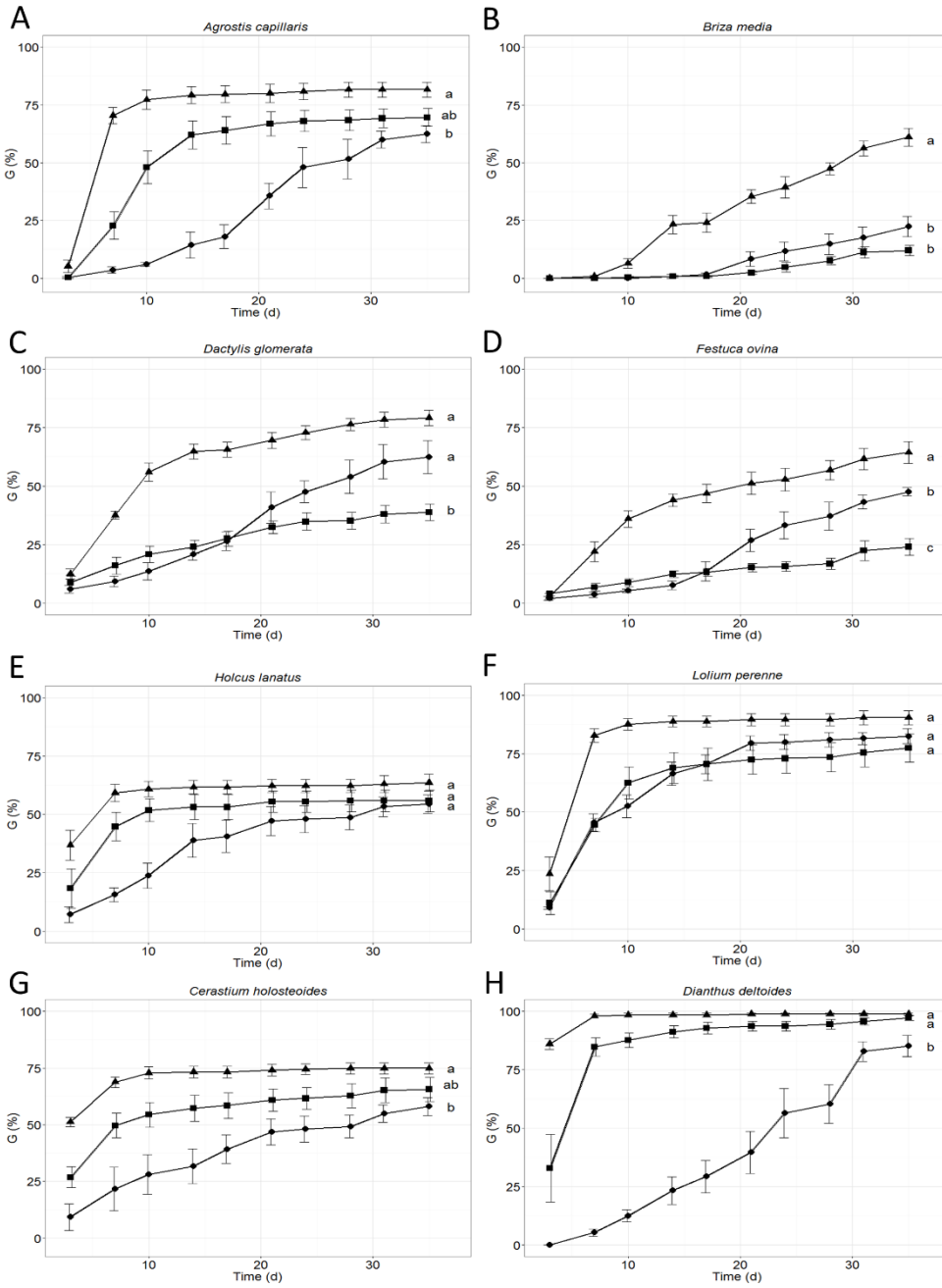
Our results provide indications for weak allelopathic effects of *J. vulgaris* in its native range. It appears doubtful that allelopathy is the driver of its invasion success in its home range. Nevertheless, as measured effects were very species-specific, the cumulative impacts of allelopathy might have profound effects on the composition of a plant community during succession. Regarding the impacts of *J. vulgaris* on nature conservation and restoration measures, our results suggest that the presence of *J. vulgaris* does not preclude re-establishment of rare and endangered species. Yet, since plant community assemblage is a complex interplay of a wider array of abiotic and biotic interactions, it is also crucial to identify the relative importance of direct and indirect plant-plant interactions, e.g. allelopathy and competition, under field conditions.

Supplementary Information

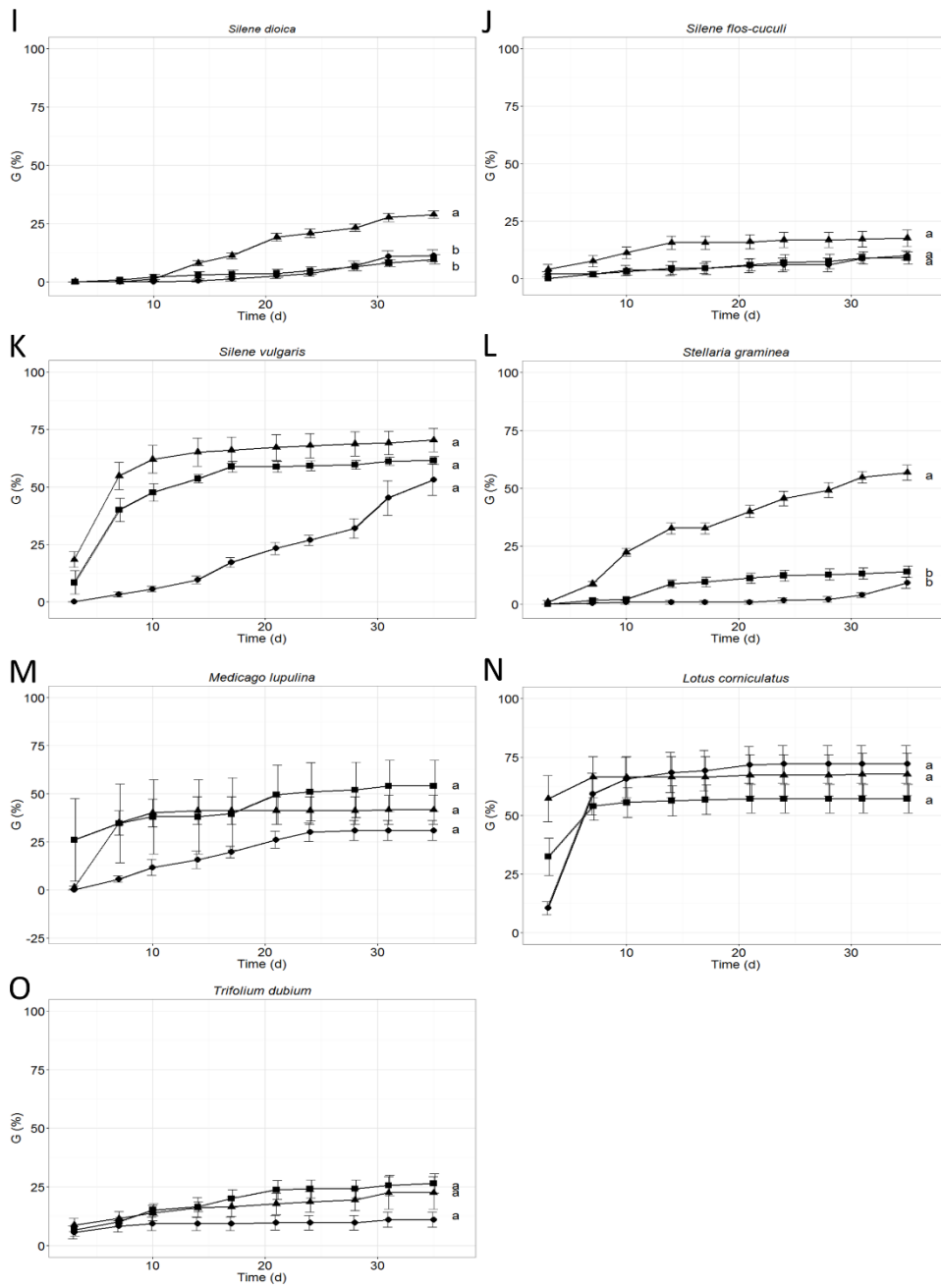
S1 Table ANOVA results for the effects of treatment (i.e. *J. vulgaris* leachate, mannitol solution, distilled deionised water), species identity and their interaction on germination percentage (G [%]), mean germination time (MT [d]), synchrony of germination (Z [unit less]) and radicle length (RL [mm]); df = degrees of freedom, P = error probability. Data of *Jacobaea vulgaris* were excluded from this analysis.

Source of variation	df	Germination		Mean Germination Time		Germination Synchrony		Root Length	
		X ²	P	X ²	P	X ²	P	X ²	P
Species	21	5708.62	< 0.0001	3409.07	< 0.0001	1473.88	< 0.0001	1308.45	< 0.0001
Treatment	2	316.96	< 0.0001	1167.11	< 0.0001	171.76	< 0.0001	201.56	< 0.0001
Species*Treatment	42	315.8	< 0.0001	464.16	< 0.0001	432.95	< 0.0001	324.65	< 0.0001

CHAPTER 1 ALLELOPATHIC VS. AUTOTOXIC POTENTIAL OF A GRASSLAND WEED - EVIDENCE FROM A SEED GERMINATION EXPERIMENT



CHAPTER 1 ALLELOPATHIC VS. AUTOTOXIC POTENTIAL OF A GRASSLAND WEED - EVIDENCE FROM A SEED GERMINATION EXPERIMENT



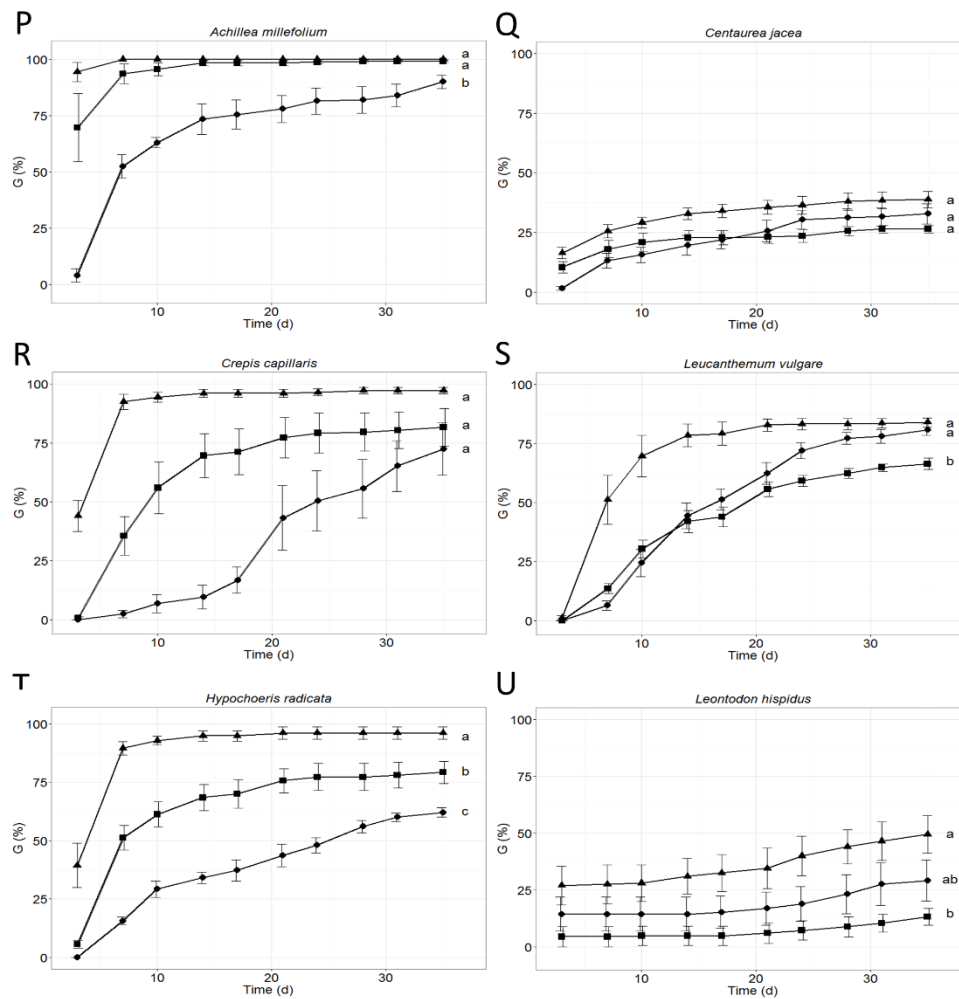


Figure S1 Cumulative germination percentage (G [%]) of the study species over time (days), averaging the data of the five replicates (mean ± SE). Different letters indicate significant differences ($P \leq 0.05$; Tukey-HSD) in the final germination percentage between treatments (circle = leachate, triangle = distilled water, square = mannitol). Please note: *Trifolium campestre* and *T. pratense* were omitted from the analysis. Please refer to Figure 2 for the data of *Jacobaea vulgaris*.

Chapter 2 Conspecific and heterospecific litter effects on seedling emergence and establishment in ragwort (*Jacobaea vulgaris*)

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Abstract

Jacobaea vulgaris GAERTN. or common ragwort is a widespread noxious grassland weed that is subject to different regulation measures worldwide. Seedling emergence and growth are the most crucial stages for most plants during their life cycle. Therefore, heterospecific or conspecific litter as well as soil-mediated effects may be of relevance for ragwort control. Our study examines the effects of conspecific and heterospecific litter as well as ragwort conditioned soil on seedling emergence and growth.

We conducted pot experiments to estimate the influence of soil conditioning (with, without ragwort), litter type (grass, ragwort, grass-ragwort-mix) and amount (200 g/m², 400 g/m²) on *J. vulgaris* recruitment. As response parameters, we assessed seedling number, biomass, height, and number of seedling leaves.

We found that 200 g/m² grass litter led to higher seedling numbers, while litter composed of *J. vulgaris* reduced seedling emergence. Litter amounts of 400 g/m² had negative effects on the number of seedlings regardless of the litter type. Significant effects of the soil conditioned by ragwort on seedling emergence were negligible. Results for biomass, plant height and leaf number showed opposing patterns to seedling numbers. Seedlings in pots treated with high litter amounts and seedlings in ragwort litter became heavier, grew higher and had more leaves.

The study confirms that the amount and composition of litter strongly affect seedling emergence and growth of *J. vulgaris*. Moreover, while conspecific litter and high litter amounts negatively affected early seedling development in ragwort, those seedlings that survived accumulated more biomass and got taller than seedlings grown in heterospecific or less dense litter. Therefore, ragwort litter has negative effects in ragwort germination, but positive effects in ragwort growth.

Keywords: *Jacobaea vulgaris*, mulching, litter, autotoxicity, ragwort, phytotoxicity

Introduction

Jacobaea vulgaris GAERTN. or common ragwort is a widespread noxious grassland weed that is native to Europe, Asia, and Siberia and invasive in North America, New Zealand, and Australia. Since the pyrrolizidine alkaloids of the plant pose a health risk to cattle that consume this plant (Wardle, 1987), its occurrence in high densities is a challenge for grassland management. Consequently, control of common ragwort is prescribed in the UK: “Weeds Act 1959” and “Ragwort Control Act 2003”, Ireland: “Noxious Weed Act 1936”, New Zealand:

“Noxious Plant Act 1978”, Australia: “Weed Management Act 1999”, and Friesland, province of the Netherlands “Verordening Jakobskruid 2007”(Leiss, 2011). Therefore, several studies have aimed to find effective and efficient ways to manage this grassland weed (McEvoy *et al.*, 1991; Jacobs and Sing, 2009; Suter and Lüscher, 2011; Leiss, 2011). Some of these studies came to the conclusion that specific cutting regimes are a promising approach (Siegrist-Maag *et al.*, 2008). However, the toxicity of ragwort biomass precludes its use as hay or silage and the high number of long-living seeds rules out composting ragwort biomass (Huckauf *et al.*, 2017; Kaltner *et al.*, 2018). Consequently, the biomass is often disposed of as special waste, which is costly (Huckauf *et al.*, 2017). Yet, the occurrence of conspecific allelopathic effects (Wang *et al.*, 2011b; Möhler *et al.*, 2018) suggests that leaving ragwort litter on mown grassland could be an additional component in managing ragwort abundance. As a first step to evaluate the validity of this approach, we experimentally tested this management option.

Litter influences micro-environmental conditions, is important for nutrient cycling and can have prominent effects on seedling germination and growth via mechanical and chemical effects (Facelli and Pickett, 1991; Loydi *et al.*, 2013). Litter has been shown to have both positive and negative effects on seedling germination and establishment (Loydi *et al.*, 2013, Xiong and Nilsson, 1999). Positive effects were due to higher soil humidity and a lower amplitude of diurnal temperature fluctuations beneath a low litter cover (Loydi *et al.*, 2014). Still the size of litter effects seedling germination and establishment do also depend on the amount of litter present. If litter layer is too high, it reduces the amount of light that reaches the seeds, which reaching seeds, where seeds of ragwort depend on a certain light amount to germinate (van der Meijden and van der Waals-Kooi, 1979). In addition, thick litter layers that preserve a relative high humidity can facilitate the spread of mould that may cause decay of seeds. Van der Meijden and van der Waals-Koi showed that ragwort seedlings need light for germination. If not enough light is reaching the seeds, dormancy will be induced. On the other side they also demonstrated that germination was facilitated under light cover with sand in compared to no cover. They found that seeds need a certain moisture to germinate. Short periods of frost or drought can induce dormancy even if favourable conditions are restored again. After germination seedlings can be hampered in their growth or even die if litter amounts are too high. Thus Van der Meijden and van der Waals-Koi (1979) found significantly less seedlings in pots with high vegetation cover. Litter may also exert chemical effects by nutrient immobilization, depletion of oxygen in the soil or toxicity of carbon dioxide produced by decomposers or by releasing phytotoxins (Bonanomi *et al.*, 2006; Loydi *et al.*, 2014; Möhler *et al.*, 2018). Through these mechanisms

litter potentially influences community organization via delaying and reducing or facilitating seedling emergence or seedling growth (Loydi *et al.*, 2013, van der Putten *et al.*, 2013).

Litter type is another important factor that can influence whether seed germination and seedling establishment is facilitated or hampered (Donath and Eckstein, 2008). It changes conditions for germination and growth as chemical as well as physical structure depend on the characteristics of species (Donath and Eckstein, 2008). Grass litter, for example, might be less hampering than large-leaved forb litter due to a lower amount of phytotoxins and an easier to penetrate structure (Bonanomi *et al.*, 2006; Donath and Eckstein, 2008). Under natural conditions, however, litter is not usually monospecific, e.g. in grasslands it consists of a mixture of forb and grass litter. The effects of litter from plant communities are driven by species-specific litter properties (Questaed and Eriksson, 2006) and may either be purely additive as hypothesized in the mass-ratio-hypothesis (Grime, 1998) or non-additive, as litter types may interact. In this context, Gartner and Cardon (2004) found that more than half of the litter mixtures they reviewed behaved in a non-additive way concerning factors such as mass loss, nutrient transfer or decomposer composition.

It is intuitive to hypothesize that conspecific litter facilitates the establishment of conspecific seeds. Yet, as intraspecific competition was found to be more intense than interspecific competition (Antonovics and Levin, 1980; Adler *et al.*, 2018), inhibiting effects may also be expected. While many studies have shown facilitating effects of adult plants on conspecific seedlings (Pleasants and Jurik, 1992; Roll *et al.*, 1997; Montesinos *et al.*, 2006), particularly in weed species (McEvoy, 1984; Wardle *et al.*, 1991; Reinhart *et al.*, 2006), there are also studies demonstrating the opposite (Singh *et al.*, 1999; Suzuki *et al.*, 2003). Some authors did not find evidence of unidirectional conspecific facilitation (Hovstad and Ohlson, 2009). In line with the latter, studies on ragwort showed the negative autotoxic potential of ragwort litter and soil close from ragwort plants (van de Voorde *et al.*, 2012a; Möhler *et al.*, 2018). Also, Bezemer *et al.* and Van de Voorde *et al.* found a negative intraspecific plant-soil-feedback for ragwort, which they assumed is probably driven by pathogens that accumulate in the soil community (Bezemer *et al.*, 2006a; van de Voorde *et al.*, 2011). Under these circumstances, negative effects of ragwort litter and ragwort conditioned soil on ragwort performance (e.g. lower biomass) may either be additive, i.e. result in stronger inhibitory effects than they would do individually, or the combined effect is not additive, i.e. combined effects are lower or higher than the sum of both effect sizes. However, the influence of litter during seedling emergence might be stronger than during seedling growth, since at the latter stage impacts of competition are of higher

importance than direct litter effects (Loydi *et al.*, 2015a). Thus, if litter reduces seedling density through self-thinning, surviving seedlings might have an advantage later on, as intraspecific competition would be reduced (Begon *et al.*, 2011).

The present study is to our knowledge the first to test the influence of the combined effects of litter type, amount and soil conditioning on seedling emergence and growth in ragwort. To this end, we set up a common garden experiment, where we assessed the conspecific and heterospecific effects of litter on the number of emerged ragwort seedlings, their biomass, height, leaf number and specific leaf area. We posed the following questions:

I. Does litter type influence the emergence and growth of ragwort seedlings?

Assuming conspecific negative effects, we expect lower numbers of ragwort seedlings to emerge from beneath conspecific litter compared to pure grass litter or a mixture of grass and ragwort litter. We expect similar patterns to occur in seedling growth after emergence.

II. Does litter amount influence seedling emergence and growth of ragwort and does this depend on litter type?

We expect negative litter effects to increase with a higher litter cover and to be stronger with increasing litter amount. We also expect the size of litter effects to increase with the portion of ragwort litter in the litter cover. Thus, we expect the interaction of litter type and amount on seedling emergence and growth to be lowest in high amounts of ragwort litter. Seedling performance is hypothesized to be higher under medium ragwort amounts and highest under low amounts of grass litter.

III. Does ragwort-conditioned soil influence conspecific seedling emergence and growth? If so, do these effects interact with the litter effects?

We hypothesized an inhibitory effect of ragwort-conditioned soil on its own emergence and growth as negative plant-soil feedback for this species has been observed. We expect these effects to be additive for ragwort litter.

Material and methods

Study species

Jacobaea vulgaris L. syn. *Senecio jacobaea* GAERTN. or common ragwort is a herbaceous monocarpic plant from the Asteraceae family (Harper and Wood, 1957). It is a

hemicryptophyte, forming a rosette in the first year and flowering in the second year (Harper and Wood, 1957). It prefers open patches and disturbed sites. Thus, it occurs on ruderal sites, along roadsides, pastures, and grassland ecosystems in most temperate regions of the world. It is often regarded as a noxious weed due to its content of toxic pyrrolizidine alkaloids (Leiss, 2011). Ragwort shows ecological characteristics of a pioneer species, in particular its numerous light seeds and fast growth enable ragwort to spread rapidly in open areas (Wardle, 1987). Each plant produces several thousand seeds per year (Wardle, 1987). Seeds are mainly dispersed by wind over a distance of usually less than 15 m (McEvoy and Cox, 1987). Seed survival (up to 20 years) and germination rates (about 80%) are high (Crawley and Nachapong, 1985).

Experimental design

We studied the effects of *litter amount* ($k = 2$; 2 (low) vs. 4 (high) g/dm²), *litter type* ($k = 3$; ragwort litter, grass litter, 1:1 mix of ragwort and grass litter) and *conditioned soil* ($k = 2$; soil conditioned with ragwort vs. unconditioned soil) on seedling emergence and growth. The litter amounts applied correspond to the range of average productivity of extensively managed grasslands in Northern Germany (Smit *et al.*, 2008). Loydi *et al.* (2013) found positive litter effects to occur at these litter amounts. Thus, the litter amounts applied represent litter quantities that ragwort seeds are exposed to naturally and that seem to affect germination.

Seeds of *J. vulgaris* were hand-collected from at least 20 plant individuals on each of eight extensively managed pastures across Schleswig-Holstein from August to September 2015 and mixed for the experiment. These seeds were dry stored in darkness at room temperature until sowing on 21st April 2016. An initial viability test with a 1% tetrazolium chloride solution (ISTA, 2015) on additional seed batches showed that most seeds (> 80%) were viable. *J. vulgaris* litter and conditioned and unconditioned soil was collected from rosettes on a ragwort-infested extensive cattle pasture near Preetz, Germany (54.227175, 10.242809) in April 2016. We assumed soil to be affected by ragwort, i.e. conditioned, if at least four ragwort-plants were growing within 0.5 m². We refer to unconditioned soil when soil was taken from the center of an area (diameter 10 m) where no ragwort was growing. Litter was collected, cleaned, and dried for two days at room temperature and for 24 hours at 40 °C to constant weight. This way we ensured that chemicals potentially affecting germination were not changed. For grass litter, we used dried plant material from a grassland mainly consisting of sweet grass (Poaceae) species.

We set up a completely randomized experimental design. We prepared pots of 1 dm³ volume (c. 10 × 10 × 10 cm) for each litter amount × litter type × soil conditioned (12 combinations) replicated eight times. To assess for seedlings emerging from the soil taken from ragwort stands we additionally included 20 control pots, ten with ragwort conditioned soil and ten filled with unconditioned soil. In none of these pots, ragwort seedlings emerged. Half of the pots were filled with a 6:1 mixture of commercial potting soil (Fruhstorfer Erde®, Type P, Industrie-Erdenwerke Archut GmbH, Germany) and ragwort-conditioned soil or unconditioned soil, respectively. We followed the protocol of van de Voorde *et al.* (2012b) concerning inoculation and mixing of soil, as it led to significant differences between conditioned and unconditioned soil in their experiment.

In late April 2016, we sowed 50 seeds in each experimental pot and added litter on top of it. Pots were placed randomly in a common garden in Kiel, Germany (54.346883, 10.106829), surrounded by another row of pots (to reduce desiccation) and protected by a light metal grid to avoid litter being blown away. Pots were kept constantly moist by regular watering.

Germination started in mid May 2016. Seedlings that emerged above the litter cover were counted weekly until the tenth week after the experiment started. After the tenth week, we counted the total number of leaves per plant and measured the height of the highest plant in the pot. We determined specific leaf area for five pots per combination by cutting the youngest fully developed leaves of three plants. These were scanned and leaf area in mm² measured by the programme IMAGEJ (Schindelin *et al.*, 2015). Afterwards leaves were dried at 60 °C and weighed to the closest milligram. The same was done with total aboveground biomass.

Data analysis

We tested for differences in number of emerged seedlings and measurements of seedling growth represented by: total biomass per pot and per plant, plant height, leaf number and specific leaf area. We used a three-factorial ANOVA to analyze the effect of litter type, litter amount and soil type, and their interactions. Residuals were examined visually to assess validity of assumptions for analysis of variance, i.e. normal distribution, and variance homogeneity. Data for biomass per pot and biomass per plant had to be log transformed to meet assumptions of analysis of variance (natural logarithm). Subsequent to significant ANOVA results post-hoc Tukey-HSD test was employed to test for individual differences. In addition, we used contrasts to specifically test whether the effect of mixed litter on response variables was mid-way between that of grass and ragwort alone (additive effect of pure type litters in the mixture).

Since not general litter effects but specific effects of the different litter types on seedling emergence and growth of ragwort were the focus in our study, we did not include the controls in our model but included them for visual comparison in the figures. In addition, we analyzed the controls for soil effects as well as for differences to the litter types across litter amount using contrasts. As a measure for the relative contribution of each factor and their interactions to the total variability of the response parameters of interest, we used the ratio of the sum of squares of the factor or interaction of interest to the total sum of squares (i.e. for all factors, their interactions and error). All analyses were done using R version 3.6.1 (R Core Team, 2019).

Results

In general, our results showed significant main effects of litter type and litter amount on all response variables: seedling number, biomass per plant and pot, plant height and mean leaf number. In contrast, the soil factor (conditioned vs. unconditioned) was not significant for any response variables under the litter treatments. Only the case of the separate analysis of controls soil revealed a significant effect on plant height. Our analyses revealed two significant interactions between main factors. In the case of biomass per plant and pot and seedling height we found a significant two-way *litter type* \times *litter amount* interaction. Effects of the treatments on specific leaf area were not significant (results not shown).

Seedling emergence

We found that while both, litter type and litter amount, affected seedling emergence significantly, the amount of explained variance was more than twice as high for litter type than litter amount (38.22 % vs. 16.58 %; Table 1). Number of emerged seedlings differed significantly between all three litter types (Fig. 1). In addition, contrast analyses of the control against litter types revealed that the litter mixture had no significant effect on seedling number compared to the control ($p = 0.30$), while in the control seedling number differed significantly from grass and ragwort litter ($p = 0.016$ and $p \leq 0.0001$, respectively). From beneath grass litter, seedling emergence was significantly raised by about 50% compared to ragwort litter (18.1 ± 1.0 vs. 27.6 ± 0.8), while emergence from beneath mixed litter was intermediate (22.8 ± 0.8) (Fig. 1). Litter effects were additive, i.e. the effect size of mixed grass-ragwort litter did not deviate significantly from the expected mean effect of pure grass and ragwort litter ($p = 0.99$). Across litter types higher litter amounts of 400 g/m^2 , significantly reduced seedling numbers by about one fifth, compared to low litter cover of 200 g/m^2 (24.2 ± 1.0 vs. 20 ± 1) (Fig 1).

Table 1. Results of a three-way ANOVA on the effects of litter type, litter amount and soil on seedling number and measures of seedling growth (biomass per plant, plant height and mean leaf number).

Source of variation	df	Seedling number				Ln Biomass per pot				Height				Mean leaf number			
		MSQ	F	<i>P</i>	vc	MSQ	F	<i>P</i>	vc	MSQ	F	<i>P</i>	vc	MSQ	F	<i>P</i>	vc
Litter type	2	726.8	38.107	≤ 0.0001	38.22	10.824	95.940	≤ 0.0001	55.23	234.63	98.618	≤ 0.0001	56.51	1.602	7.868	≤ 0.0001	11.64
Litter amount	1	630.4	33.053	≤ 0.0001	16.58	12.355	5.415	≤ 0.0001	13.82	105.84	44.486	≤ 0.0001	12.74	5.505	27.037	≤ 0.0001	20.02
Soil	1	42.7	2.237	0.138	1.12	0.008	0.072	0.427	0.18	0.07	0.030	0.864	0.01	0.108	0.532	0.468	0.40
Litter type x litter amount	2	52.3	1.371	0.259	1.38	1.252	1.127	≤ 0.0001	5.75	21.84	9.181	≤ 0.0001	5.26	0.542	2.663	0.076	3.94
Litter type x soil	2	21.3	0.558	0.575	0.56	0.043	0.125	0.333	0.64	0.14	0.059	0.943	0.04	0.104	0.512	0.601	0.75
Litter amount x soil	1	1.0	0.055	0.816	0.03	0.004	0.001	0.924	0.00	3.15	1.326	0.253	0.40	0.012	0.059	0.809	0.04
Litter type x litter amount x soil	2	0.1	0.1	0.004	0.00	0.049	0.038	0.336	0.19	3.99	1.679	0.192	0.96	0.139	0.684	0.507	1.01
Error	84	1602.0			42.12	0.104			24.18	2.38			24.07	0.204			62.18

d.f. = degrees of freedom; MS = mean sum of squares; vc (%) = relative contribution of individual factors and their interactions to total variation.

Significant effects ($P < 0.05$) are given in bold.

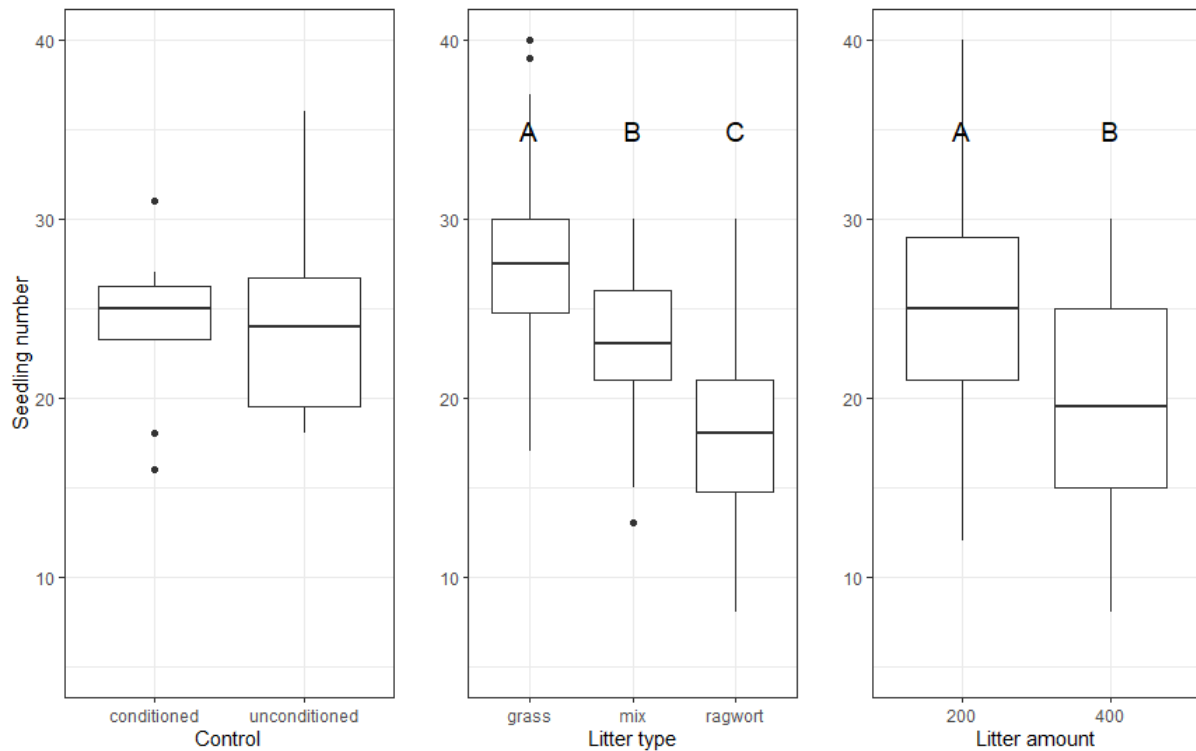


Fig 1. Seedling number: effects of soil conditioning on the control, litter type (grass, mix, ragwort (left)) and different litter amounts (200 g/m² litter and 400 g/m² (right)). In the box plots middle lines represent median, boxes represent the first and third quartiles, lower and upper bars represent the minimum and the maximum and points represent outliers (i.e. points above 1.5 SD). Upper case letters indicate significant main effects between treatments (TukeyHSD, $P \leq 0.05$).

Seedling growth

More than half of the variance in biomass per pot was explained by litter type, whereas litter amount only explained around one tenth of the variance (Table 1). The significant interaction, which accounted only for 6% of the variance, was caused by increased biomass with increasing litter amount when litter contained ragwort compared to no effect of amount in grass litter (Table 1; Fig. 2). Across litter amounts biomass per pot was more than doubled for seedlings that grew in ragwort litter compared to those in grass litter (1.1 ± 0.1 g vs. 0.4 ± 0.1 g) (Fig. 2). Interestingly, seedling biomass per pot in the litter mixture (0.9 ± 0.1 g) lay between that of seedlings growing in pure grass or ragwort litter. In contrast to seedling emergence, the litter type effects were not additive, i.e. seedling biomass per pot in the mixed litter treatment lay significantly above the mean of seedling biomass in grass and ragwort litter ($p = 0.001$, Fig. 2). Across litter types, biomass per plant and per pot increased with increasing litter amount applied

(0.56 ± 0.03 g in low litter vs. 1.00 ± 0.08 g in high litter). Contrast analyses of the control against the three litter types revealed no significant difference of biomass per pot between the control and grass litter ($p = 0.40$) but significant differences in biomass between control and litter mixture as well as ragwort litter (both $p \leq 0.0001$). Seedling biomass per plant showed almost the same response patterns but the litter effect was additive ($p = 0.88$, Supporting information Fig S1).

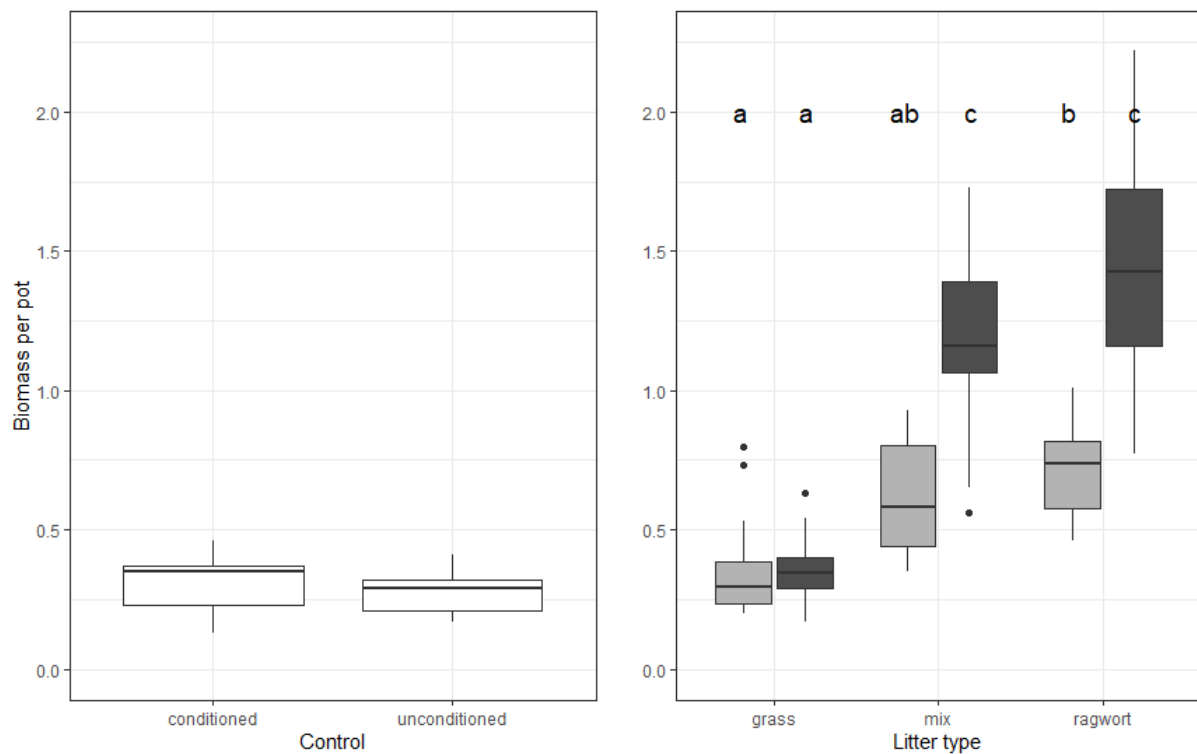


Fig 2. Biomass per pot - effects of soil conditioning on the control and the two-way interaction of litter type and litter amount. In the box plots middle lines represent median, boxes represent the first and third quartiles, lower and upper bars represent the minimum and the maximum and points represent outliers (i.e. points above 1.5 SD). Grey: 200 g/m² litter, black: 400 g/m² litter. Lower case letters indicate significant interactions between treatments (TukeyHSD, $P \leq 0.05$).

Seedling height was largest for seedlings growing in high amounts of ragwort litter (Fig. 3). Across amounts seedlings in ragwort litter grew almost three times as tall as in grass litter (8.1 ± 2.8 cm vs. 2.8 ± 0.9 cm) and about one third taller compared to the litter mixture (6.5 ± 0.3 cm). Plant height was significantly influenced by the main factors litter type and litter amount as well by the interaction of these factors (Fig 3). More than half of the variance was explained

by litter type (Table 1). Again, litter type effects were not additive, i.e. seedling height per pot in the mixed litter treatment lay significantly above the mean of seedling height in grass and ragwort litter ($p = 0.001$, Fig. 3). Contrast analyses of the control against the three litter types revealed no significant difference of height between the control and grass litter ($p = 0.39$) but significant differences between control and litter mixture as well as ragwort litter (both $p \leq 0.0001$). In contrast, litter amount had a much smaller, still significant effect, i.e. higher litter amounts led to taller seedlings (Fig. 3). Seedling height is the only response variable where the control was significantly influenced by soil type, i.e. ragwort conditioned soil led to significantly taller seedlings (3.9 ± 0.5 cm vs. 2.6 ± 0.3 cm).

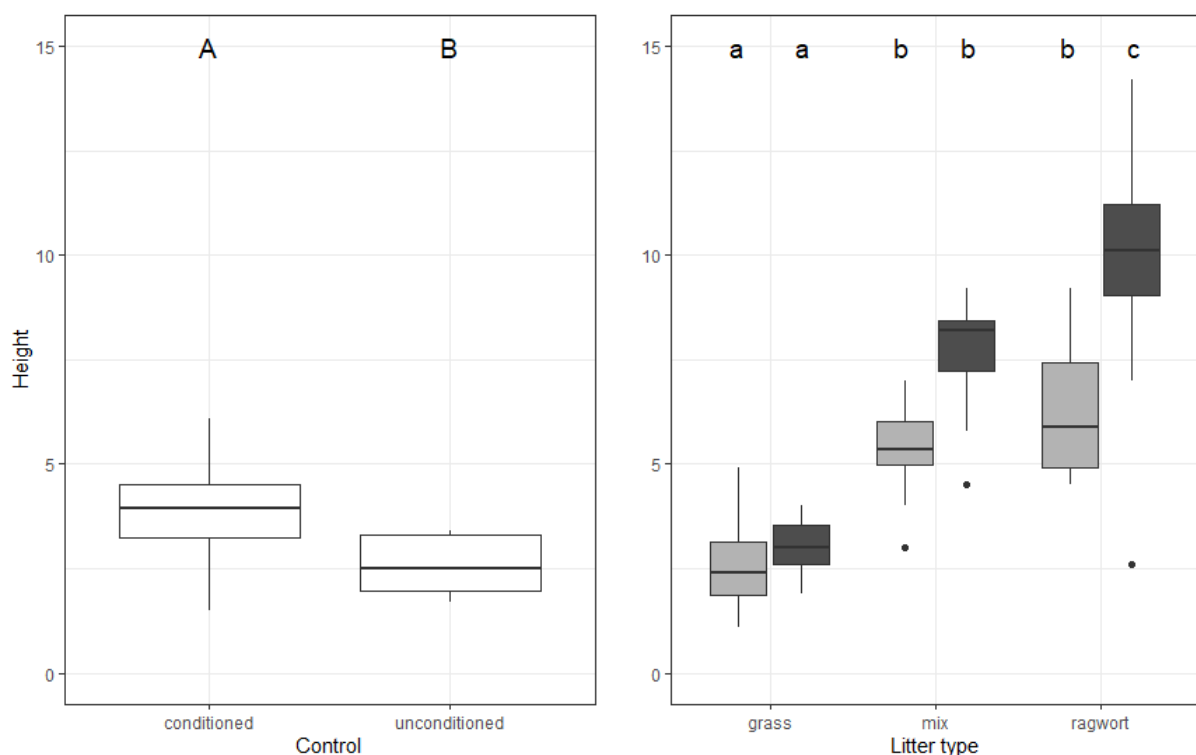


Fig 3. Seedling height - effects of soil conditioning on the control and the two-way interaction of litter type and litter amount. In the box plots middle lines represent median, boxes represent the first and third quartiles, lower and upper bars represent the minimum and the maximum and points represent outliers (i.e. points above 1.5 SD). Grey: 200 g/m² litter, black: 400 g/m² litter. Lower case letters indicate significant interactions between treatments (TukeyHSD, $P \leq 0.05$).

The mean leaf number of plants growing in ragwort litter was highest compared to in plants growing in grass litter and the combination of both litter types (ragwort 3.59 ± 0.12 , grass 3.15 ± 0.05 , mixture 3.31 ± 0.08). Analyses of variance revealed that mean leaf number was

significantly affected by litter type and amount but not their interaction (Table 1; Fig 4). The variance explained by litter amount was twice as high as that explained by litter type (Table 1). Thus, mean leaf number is the only measured variable that seems to be stronger influenced from litter amount than litter type. Effects of litter type were additive, i.e. the leaf number in the litter mixture did not differ significantly from the expected mean effect of pure grass and ragwort litter ($p = 0.49$, Fig. 4). Plants growing in high litter amounts (400 g/m^2) had significantly more leaves per plant than plants growing in the low litter amounts (200 g/m^2). Contrast analyses revealed no significant difference in leaf number between the plants growing in the control and in any of the three litter types ($p > 0.05$).

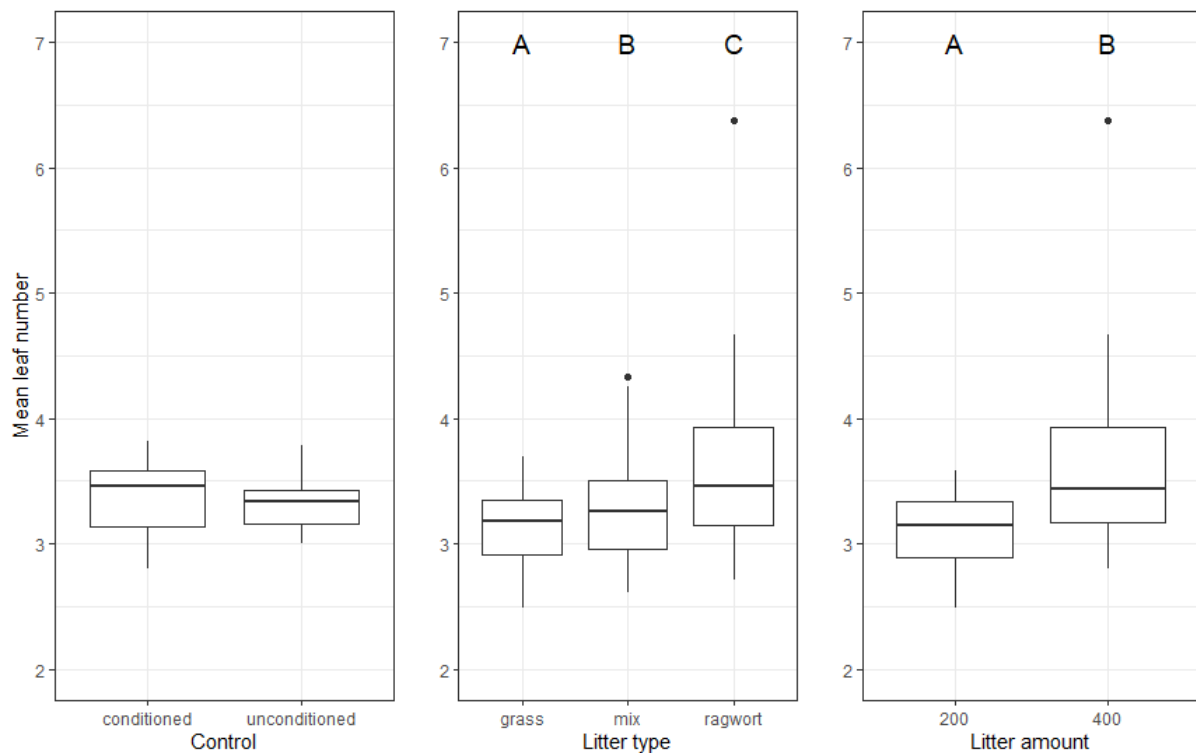


Fig 4. Mean leaf number under soil conditioning in the control, different litter types (grass, mix, ragwort (left)) and different litter amounts (200 g/m^2 litter, 400 g/m^2 litter (right)). In the box plots middle lines represent median, boxes represent the first and third quartiles, lower and upper bars represent the minimum and the maximum and points represent outliers (i.e. points above 1.5 SD). Upper case letters indicate significant main effects between treatments (TukeyHSD, $P \leq 0.05$).

Discussion

Our experimental results suggest that litter type as well as litter amount do influence seedling emergence and growth, whereas conditioned soil did not. It is interesting to note that although

ragwort litter reduced the number of conspecific emerging seeds, seedlings actually performed better in ragwort litter showing increased biomass, height and leaf numbers.

Litter type accounted for a much higher portion of the variance than litter amount. In contrast, other studies reported that litter amount was more relevant than litter type (Myster, 2006; Loydi *et al.*, 2015b). This highlights the species specific nature of litter effects (Bonanomi *et al.*, 2006). One explanation for this pattern could be that ragwort litter is indeed autotoxic and negatively influences the emergence of conspecific seedlings (Möhler *et al.*, 2018).

Seedling emergence was highest in grass litter, lowest in ragwort litter and intermediate in the litter mixture. Germination was highest in medium compared to no or high litter amounts. In contrast, all parameters measuring seedling growth (biomass, height, mean leaf number) were highest in high and/or ragwort litter. The positive effect of grass litter and medium litter amounts on seedling emergence might be due to physical litter effects like preserving a relatively constant temperature and soil moisture (Eckstein and Donath, 2005). In our experiment, the same amount of grass litter covered a larger proportion of the soil surface than ragwort or mixed litter (high amount: grass $90 \pm 3\%$, mixed $85 \pm 4\%$ and ragwort $67 \pm 8\%$, low amount: grass: $85 \pm 3\%$, mixed $70 \pm 9\%$, ragwort $37 \pm 5\%$). In accordance with a study by Eckstein and Donath (2005), which revealed a rise in soil moisture when litter cover increased, we assume that seedlings experienced more favourable water availability in grass litter and medium litter amounts. In high litter amounts however, negative effects of litter prevailed over positive effects. Laboratory experiments of van der Meijden *et al.* (1979) demonstrated that seed germination of ragwort increased with higher soil humidity. We also observed that ragwort litter decayed faster than grass litter (personal observation). This is in line with general findings that litter of forb species such as ragwort tends to decay faster than grass litter (Shaw and Harte, 2001). Schuster *et al.* (2017) found that the mass of ragwort litter remaining after 30 days was only half of the amount of grass (*Alopecurus pratensis*) litter. Since the protective properties of a litter cover against desiccation and temperature extremes increases with litter amount present, seedlings emerging from beneath faster decaying ragwort litter might have experienced less protection than those seedlings emerging beneath grass litter (Eckstein and Donath, 2005; Loydi *et al.*, 2014). Thus, the negative effect of ragwort litter on ragwort seedlings emergence seems to be a result of fewer physical benefits seedlings experienced from conspecific litter compared to grass litter. In addition, leachate effects may also depend on the specific decomposition rate of different litter types. As leachates released from the litter are declining very rapidly after senescence (Sydes and Grime, 1981), intensity of seedlings' exposure to leachate depends on

the decomposition rate of the litter present. Thus, ragwort seeds and seedlings emerging from ragwort litter, which decays fast (Schuster *et al.*, 2017), were only exposed to the ragwort leachate for a short time. Consequently, we assume that leachate effects on seedling emergence were more pronounced than on seedling growth.

In general, the amelioration of the physical environment by litter, rather than its chemical composition, seems to be the main factor boosting seedling growth in short time scales (Quested and Eriksson, 2006). However, the size of chemical autotoxic effects and therefore their relative importance in relation to physical and mechanical or other biotic effects are species-specific (2017). Several studies show the occurrence of autotoxicity of ragwort (van de Voorde *et al.*, 2012a; Möhler *et al.*, 2018), which suggests that chemical effects are an additional mechanism for lower seedling emergence from beneath ragwort litter. Van de Voorde (2012a) reported decreasing germination with increasing concentrations of ragwort extract. As ragwort produces many seeds with high germination potential and low dispersal ability (Crawley and Nachapong, 1985, Wardle, 1987, McEvoy and Cox, 1987) autotoxic effects may avoid intraspecific competition in ragwort and thus may lead to lower seedling numbers but increased seedling vitality.

In contrast of Van de Voorde's (2012a) notion of a negative soil-feedback in ragwort, we found only weak indications of effects of ragwort conditioned soil on seedling emergence and growth (Table 1). In fact, the only significant effect of conditioned soil occurred on seedling height in controls, i.e. seedlings in conditioned soil grew bigger than in unconditioned soil. This finding is in contrast to findings of van de Voorde (2012a), who reported autotoxic effects of ragwort on its seedling development. However, on average the observed difference in plant height between ragwort seedlings growing in conditioned versus those growing in unconditioned soil including control and litter addition treatments was only 2 mm (less than 4 % of the average height of about 54.5 mm). Therefore, we expect that this difference is too small to be of ecological relevance. In other studies, looking at several seedling cohorts, those cohorts usually differed several centimeters in height (Seiwa, 2000; Pausas *et al.*, 2003; Quintana *et al.*, 2004).

Mixed litter resulted in fewer emerged seedlings than in grass litter, but more emerged seedlings compared to ragwort litter, which suggests that the effects of grass and ragwort litter simply added up to a neutral effect. This supports the mass-ratio hypothesis by Grime (Grime, 1998) for the factors seedling number, biomass per plant and mean leaf number, i.e. mixed litter effects did not deviate positively nor negatively from the expected combined effects. However, for biomass per pot and plant height, effects in mixed litter were higher than could be assumed

from the simple addition of grass and ragwort. Thus, ragwort growth profited in single aspects from mixed litter layers. Thus, from a management point of view, leaving litter on ragwort infested sites does slightly facilitate ragwort growth when biomass of ragwort and grass is balanced. However, if the site is rather productive and ragwort is dominating the standing biomass, germination of its seedlings might indeed be limited. When litter cover is too high, negative effects prevail over positive effects. Accordingly, compared to the control treatment, seedling emergence was lowest from beneath 400 g of litter and not reduced from beneath 200 g of litter cover. The reduced seedling emergence of ragwort from beneath 400 g of litter is very likely linked to the relatively small seed mass of ragwort (0.22 mg; Fenner, 1983). The smaller the seeds are, the less resources a seed can spend to successfully penetrate through a litter cover (Donath and Eckstein, 2010). In addition, small seeded species, like ragwort, tend to depend on higher light levels for germination than large seeded species (Milberg *et al.*, 2000, Baskin and Baskin, 2014). Since photosynthetic active radiation (PAR) decreases with increasing litter cover (Eckstein and Donath, 2005) not only will germination be lower, but germinated seedlings will also lack light resources for successful penetration of the litter cover. This is also in accordance with van der Meijden and van der Waals-Kooi (1979), who found that emergence in ragwort significantly decreased when seeds were placed beneath a sand cover of more than 4 mm. Furthermore, Facelli *et al.* (1988) showed that ruderal species, such as ragwort, tend to be more sensitive and thus more likely to be negatively affected by high amounts of litter. Therefore, ragwort seedlings might be reduced by a mulching regime on productive sites where litter amounts are medium to high.

In contrast to the findings for seedling emergence, seeds in ragwort litter built up more biomass, grew higher and tended to have more leaves than seedlings in grass litter. Biomass and height are the two variables where we found a significant interaction between litter type and litter amount. This could be explained by a reduction in competition for the few seedlings that emerged in ragwort litter. The interaction was quantitative and weak compared with the effects of litter type and amount alone (Table 1). Seedlings emerging from beneath high amounts of litter and/or ragwort litter built up the highest biomass and got tallest. This is also diametrical to the findings of van de Voorde *et al.* (2012a), who reported lower biomass for seedlings in ragwort influenced environments. These conflicting results could be due to different methodology. While van de Voorde *et al.* (2012a) harvested seedling biomass after 19 days, seedlings in our study were harvested about 50 days later. While van de Voorde's results were supported by our early observations (seedlings looked smaller and thinner early during early development in ragwort litter), seedlings started to grow bigger after ragwort litter had

decomposed. Thus, the longer duration of our experiment compared to the experiment by van de Voorde *et al.* (2012a) allowed us to observe a change in the importance of litter effects in relation to intraspecific density dependent competitive effects (Smith-Ramesh and Reynolds, 2017). This is further supported by the fact that lower seedling numbers from beneath grass litter resulted in seedlings of higher biomass and height. Loydi *et al.* (2015b) already identified, that while litter plays a major role for seedling emergence, for instance, via phytotoxins, competition is more important for seedling development later during seedling growth. Significantly increased mean leaf numbers in seedlings in high litter amounts and in ragwort litter compared to plants in grass or mixed litter might be linked to lower intraspecific competition as there were fewer seedling numbers in the ragwort pots or by less physical hampering compared to grass litter (Donath and Eckstein, 2010). However, indirect positive effects of reduced seedling emergence in boosting seedling biomass could be especially pronounced in situations where, as in the present experiment, intraspecific competition prevails and may overlay different environmental constraints under field conditions (Cavieres and Peñaloza, 2012; Loydi *et al.*, 2013).

Another explanation for superior performance of emerged seedlings in high litter amounts and ragwort litter might be an improved availability of nutrients (Facelli and Pickett, 1991), as more biomass contains more nutrients and ragwort litter decomposed quite fast. This is supported by our observation that even though the number of seedlings in the control pot and in the ragwort litter pot with 200 g/m² was about the same and thus competition among seedlings was comparable, biomass of seedlings growing in ragwort litter pots was still higher. However, it is questionable if nutrients from decaying litter are converted to plant available nutrients within 70 days. Effects of different litter amounts on nutrient availability are generally relatively low (Loydi *et al.*, 2015b). Another reason for smaller seedlings in lower amounts of litter could be due to the missing investment of seedlings in higher growth when they have already reached light (Olson and Wallander, 2002). Siebenkäs *et al.* (2015) compared seedling growth in the same developmental stage as seedlings in our experiment and found that plants tend to grow higher when there is more shade. Thus, seedlings may grow higher in higher litter amounts to avoid its shade and to reach light. Renne *et al.* (2006) found that small differences in height of seedlings early on can produce large differences in their final biomass indicating that early shading can translate in higher heights, even if shade is gone.

Conclusion

We found both negative and positive effects of ragwort litter on conspecific seedling emergence and growth. While negative effects can be most likely linked to autotoxic chemical and physical ragwort litter effects in the stage of seedling emergence, those seedlings that emerged successfully may have profited from a lower intraspecific competition and may had more resources to invest than seedlings that emerged beneath grass or mixed litter. Due to the ambivalent nature of our results, these findings cannot easily be translated into management recommendations for ragwort-infested sites. However, the effects of mixed litter on ragwort recruitment were either neutral or even promoted ragwort growth. Therefore, when we s and ragwort, we expect that leaving litter containing ragwort biomass on the grassland will have no negative effect on ragwort population density or growth. As already our results for a simple controlled experiment do not lead to a clear hint of the effectiveness of ragwort litter in reducing ragwort population density, we do not expect pronounced effects in an experiment under more natural conditions.

Acknowledgements

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Supplementary Information

S2 Table. Results of a three-way ANOVA on the effects of litter type, litter amount and soil on ln biomass per plant.

Source of variation	df	Ln Biomass per plant			
		MSQ	F	<i>P</i>	vc
Litter type	2	0.024	72.685	≤ 0.0001	39.99
Litter amount	1	0.027	83.155	≤ 0.0001	22.87
Soil	1	0.000	0.851	0.359	0.23
Litter type x litter amount	2	0.008	24.878	≤ 0.0001	13.68
Litter type x soil	2	0.000	0.013	0.987	0.01
Litter amount x soil	1	0.000	0.056	0.814	0.02
Litter type x litter amount x soil	2	0.000	0.169	0.845	0.09
Error	84	0.000			23.11

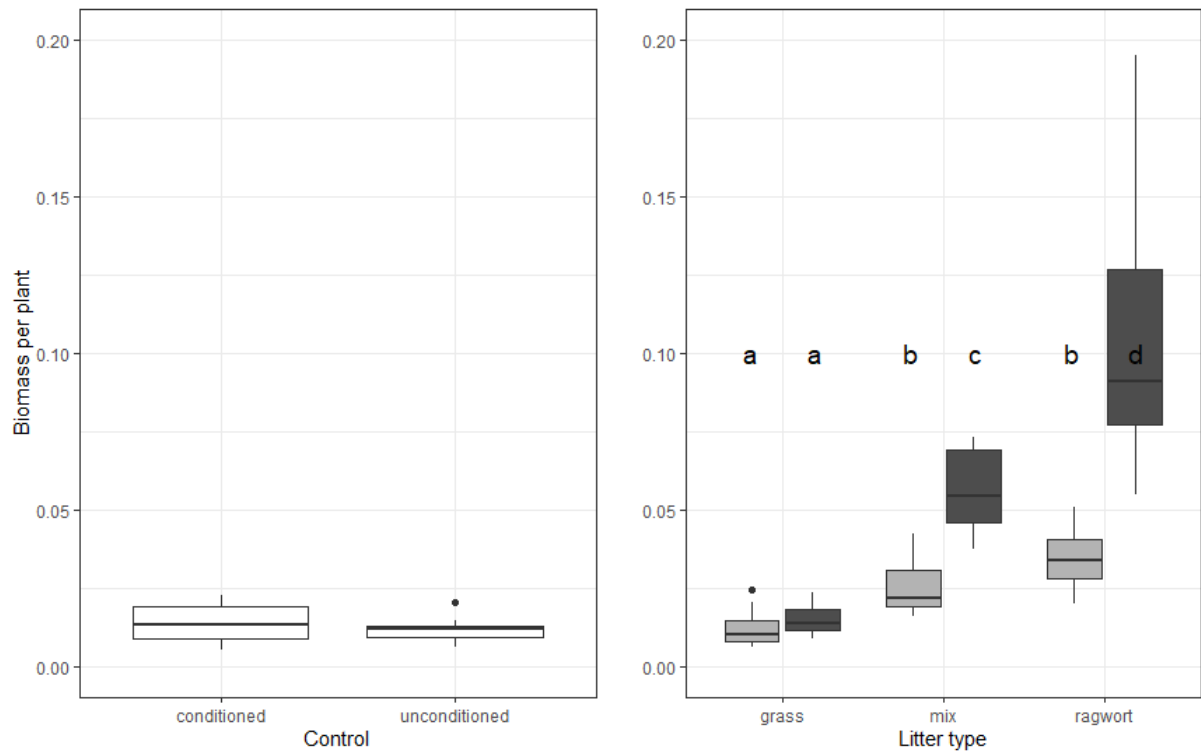


Fig S1. Biomass per plant - effects of the two-way interaction of litter type and litter amount. In the box plots middle lines represent median, boxes represent the first and third quartiles, lower and upper bars represent the minimum and the maximum and points represent outliers (i.e. points above 1.5 SD). Grey: 200 g/m² litter, black: 400 g/m² litter. Lower case letters indicate significant interactions between treatments (TukeyHSD, $P \leq 0.05$).

Chapter 3 Regulation of tansy ragwort (*Jacobaea vulgaris* GAERTN.) by varied cutting and restoration measures

Manuscript in preparation

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Abstract

The growth of the noxious grassland weed *Jacobaea vulgaris* GAERTN. (ragwort) in pastures is a threat to grazing animals. This is especially true when it dominates vegetation cover, which often occurs on non-intensively used pastures that are managed with the aim to maintain and promote biodiversity. Thus, management of the plant without harming the surrounding vegetation is warranted. We tested several mechanical and cultural methods to reduce the presence and spread of ragwort. Seven study sites in Northern Germany (Schleswig-Holstein) were treated with various measurements, including mowing and seeding, hay transfer, mowing regimes at different times and frequencies, and combinations of mowing and seeding. Our results show that cutting within the bloom of the plant at the end of June and again four weeks later, when the plant is in its second bloom, was the most effective treatment in reducing ragwort abundance. This was the only treatment leading to a significant reduction in population growth rate and density without reducing surrounding plant species richness.

Keywords: Grassland weed; grassland restoration; population dynamics; biodiversity; pasture, *Senecio jacobaea*

Introduction

Jacobaea vulgaris GAERTN. or tansy ragwort is a widespread noxious grassland weed that is native to Europe, Asia, and invasive in North America, New Zealand, and Australia (Harper and Wood, 1957). As the plant's pyrrolizidine alkaloids pose a health risk to cattle when consumed (Wardle, 1987), the control of tansy ragwort is a primary management goal of many farmers and in some countries even prescribed in their legislation (Leiss, 2011). In intensive grasslands, high fertilizer input, high cutting frequency, and chemical weed management precludes the occurrence of ragwort. The weed's control is challenging, though, in low input grasslands that are managed for high plant and animal diversity, yet prone to massive ragwort spread (Huckauf *et al.*, 2017). Taking this into account, we tested different management strategies that aim at controlling *J. vulgaris* without jeopardizing wild plant diversity in low input grasslands.

Several studies have aimed to find effective and efficient ways to manage ragwort (McEvoy *et al.*, 1991; Jacobs and Sing, 2009; Suter and Lüscher, 2011; Leiss, 2011). Some of these studies focused on chemical control measures (Leiss, 2011), intensified fertilization (Suter *et al.*, 2007) or the introduction of antagonists to reduce ragwort density (James *et al.*, 1992; Hawkes and

Johnson, 1978). While these measures can be very effective, their negative side-effects on overall grassland diversity precludes their widespread application, especially in semi-natural non-intensively managed species-rich grasslands (Schmitz *et al.*, 2014) or are more promising in the weed's non-native range (Cameron 1935; Schmidl, 1972; McEvoy *et al.* 1991). Studies on alternative ragwort management measures that are compatible with nature conservation goals in species-rich grasslands, in contrast, are scarce (Roberts and Pullin, 2007).

Therefore, we tested the effects of cutting regimes at different phenological stages of ragwort and restoration measures to increase plant species diversity as well as a combination of both on the development of ragwort populations.

Studies of the effectiveness of cutting regimes on ragwort populations in grasslands are scarce (Roberts and Pullin, 2007) and so far ambiguous in their findings. While Siegrist-Maag *et al.* (2008) showed that frequent cutting decreases ragwort abundance and fertility at least within one year, other studies find that the species has the ability to re-grow within a few weeks after being damaged or to switch to vegetative reproduction forming multiple rosettes (van der Meijden and van der Waals-Kooi, 1979; Wardle, 1987). Thus, slashing or mowing per se may not be sufficient to control tansy ragwort (McLaren and Faithfull, 2004).

In order to increase the effectiveness of cutting in suppressing ragwort, the frequency and timing of cutting seems to be essential because the proportion of nutrients and energy invested in different plant parts varies among life-stages (Jung *et al.*, 2012; Carvalho *et al.*, 2014). In general, ragwort seems to channel the highest proportion of nutrients and energy into above-ground organs during bloom (Otzen, 1977). During this time, ragwort nectar and pollen is also intensively utilized by bees (Neumann and Huckauf, 2016) that transfer the alkaloids into their honey (Gottschalk *et al.*, 2018). Thus, a cut just before flowering may not only affect the plant's vitality but also reduce undesired alkaloid content in honey. As ragwort grows back quickly after cutting (van der Meijden and van der Waals-Kooi, 1979), a second cut may be necessary before the second bloom. While this cutting regime may prevent generative reproduction, it may well induce vegetative reproduction and clonal growth and a switch to a perennial life cycle (Harper and Wood, 1957). Since ragwort usually dies off after seed production (Cameron, 1935), cutting before seed dispersal may be another option that may not only prevent vegetative re-growth but also seed dispersal.

In addition to an adapted cutting regime, sowing other species may suppress unwanted weeds (DiTomaso, 2000), too. Such a suppression via increased plant diversity may result from increased competition at the establishment (Bullock, 2000; Pywell *et al.*, 2007), seedling

(O'Dell *et al.*, 2007) or adult stage (Hess *et al.*, 2019). According to the biotic resistance hypotheses (Levine *et al.*, 2004), increased plant diversity may also increase plant community resistance against future invasion (Funk *et al.*, 2008). Several studies showed that the invasion of potential problematic plant species can be prevented through increasing plant species diversity (e.g. Baasch *et al.*, 2012, Oakley *et al.*, 2013). Introduced species might be functionally similar in resource use to target weeds and may increase the competitive pressure on the weed species. Sowing desired plant species also reduces the occurrences of open soil patches, which would otherwise facilitate the establishment of unwanted plant species (Bullock, 2000; Pywell *et al.*, 2007). Lawson *et al.* (2004) showed that ploughing and sowing effectively controlled ragwort in the first vegetation period after grassland restoration. However, long-term effects have not yet been studied.

If species enrichment proves an effective measure in ragwort control, it could easily be combined with the overall goals of ecological restoration to re-establish species-rich grassland communities, which have declined dramatically during the last decades (Peeters, 2012). Since both loss of species-rich grasslands (Wesche *et al.*, 2012) and the spread of *J. vulgaris* (Huckauf *et al.*, 2017) were especially pronounced in Northern Germany, species-enrichment might serve the interests of both land managers as well as nature conservationists. Various techniques of species enrichment are commonly applied in restoration projects (Kiehl *et al.*, 2014). While seeding seed mixtures is frequently applied for the restoration or re-creation of grasslands across Europe (Kiehl *et al.*, 2014), transferring freshly cut, seed-containing biomass from species-rich grasslands (green hay) is a more laborious but successful method to restore grasslands with rare and locally adapted plants (Kiehl *et al.*, 2010). When green hay is transferred, disturbance to an existing sward has been shown to enhance seedling establishment (Donath *et al.*, 2006). Under high seed pressure from *J. vulgaris*, however, sward disturbance will also enhance the establishment of ragwort (Suter *et al.*, 2007); thus, slot drilling and broadcast sowing without sward disturbance may be promising techniques (Foster *et al.*, 2007) to combine with the adapted cutting regimes tested to control the establishment and growth of ragwort. We also expected species-enrichment measures and cutting regimes to have a higher impact on *J. vulgaris* suppression when applied simultaneously rather than separately, as McEvoy *et al.*, 1988) found that the combined effect of herbivory and competition reduced ragwort density. Therefore, we also applied a combination of cutting regimes and species-enrichment measures, where the cutting regime is thought to weaken the ragwort individuals already present and the increased plant diversity reduces new establishment of *J. vulgaris* in the aftermath.

Contrary to deliberate action against tansy ragwort, there is indication that simply waiting for ragwort to disappear naturally might be another promising management approach (van der Meijden and van der Waals-Kooi, 1979; Bezemer *et al.*, 2006b; van de Voorde *et al.*, 2012a). Harper and Wood (1957), for example, have observed a hump-shaped population development of ragwort, with a population boom followed by sudden decline. In addition, Bezemer *et al.* (2006a) reported a natural decrease in ragwort after four years without any further management and linked the decrease to a negative plant-soil-feedback (van de Voorde *et al.*, 2011). Nonetheless, deliberate action to reduce ragwort populations is essential during the plant's population booms to reduce threat to domestic animals.

The objective of our study was to find the optimal treatment, timing, and frequency for grassland management on non-intensive pastures that leads to a maximal reduction in ragwort and a minimal loss of co-occurring vegetation. We studied six different management options for five years.

We addressed the following research questions:

1. Which treatment is most effective in reducing the abundance and population growth rate of ragwort?
2. What are the effects of the different treatments on the co-occurring grassland vegetation?

Material and Methods

Study species

Tansy ragwort, *Jacobaea vulgaris* (Asteraceae; syn. *Senecio jacobaea* L.), is native to Europe and Asia, but has been introduced in Northern America, Australia, and New Zealand (Harper and Wood, 1957). Despite being regarded as a character species of mesophilic pastures of the alliance Cynosurion Tx. 1947 (Oberdorfer, 1990) in its native range, its potential poisonousness leads to its rating as a noxious pasture weed worldwide (Leiss, 2011). Its occurrence is common in open disturbed sites, such as ruderal sites, road verges, fallow land, and temperate non-intensively managed grasslands (Harper and Wood, 1957; Wardle, 1987).

J. vulgaris is a mostly monocarpic perennial herb (Fig. 2) (van der Meijden and van der Waals-Kooi, 1979). After germination of seeds in late summer or autumn, the rosette overwinters and usually flowers the next year, with a flowering peak at the end of July. However, flowering can

be delayed if site conditions are not optimal or if first flowers are damaged by herbivory or cutting (van der Meijden and van der Waals-Kooi, 1979).

Ragwort has several biological traits that support its spread and hamper its regulation. It produces thousands of light, wind-dispersed seeds and grows quickly at suitable sites (Wardle, 1987), making it a typical pioneer species of ruderal or disturbed sites (Harper and Wood, 1957). Seeds show a high germination rate (about 80 %) and are long-term persistent (Crawley and Nachapong, 1985). The plant tolerates disturbance during different life stages, e.g. an early cut of the inflorescence does not preclude post-ripening of vital seeds (Eisele *et al.*, 2011) and disturbance or injury to the vegetation bud promotes regrowth through roots and crown buds (Leiss, 2011).

Study sites

In 2015, we began management experiments on seven pastures with presence of ragwort classified as Cynosurion grasslands (Leuschner and Ellenberg, 2017). Sites were situated in two geographical regions (Moraine, Hill land) in Northern Germany (see Fig. 1, Table S1, Supporting Information) on sandy soils (Heydemann, 1997).



Fig. 1 – Study area in Schleswig-Holstein, Northern Germany.

At least 5 out of 7 sites were formerly used as arable land. The current non-intensive nature-conservation grazing scheme applied by the managing nature conservation organisation aims at maintaining and promoting biodiversity (Ssymank *et al.*, 2015). At all sites, ragwort abundance was very high with on average 60 ± 5 ragwort plants per square meter (excluding seedlings) at the start of the experiment.

Study design

Six different grassland management schemes, two measures of milling and species enrichment, two cutting treatments, and two combinations of seeding (as a measure of species-enrichment) and cutting, against a control with no measures applied (Table 1), were assessed in a randomized block design for their effects on tansy ragwort. On each of the seven study sites, each of the six treatments was applied within an area of 90 m² (block). All study sites were under non-intensive grazing during the study. To minimize edge effects, ragwort and the co-occurring vegetation were assessed within three permanently marked 9 m² plots in the central part of each block.

Table 1 Treatments

Name	Description
Biodiversity 1	Milling and broadcast-seeding in 2015, then left untouched in following years
Biodiversity 2	Milling and green hay (Kiehl <i>et al.</i> , 2010) spread over the ground in 2015, then left untouched in following years
Flower cut	Flowers cut before each bloom (end of June and beginning of August) in every year (2015-2019)
Seed cut	Flowers cut before seeds disperse (mid/end of July) in every year (2015-2019)
Combination 1	Mowing and drill-seeding in 2015, then left untouched in following years
Combination 2	Mowing and broadcast-seeding in first two years (2015-2016), mowing for whole study period (2015-2019 mid/end of July)
Control	No treatment, still grazed as all other treatments

In both *biodiversity* treatments, the plots were milled to a depth of 30 cm prior to seed addition in autumn 2015. For the *biodiversity 1* treatment, 5 g/m² of the “fertile meadow mixture” (Rieger-Hoffmann seed supplier) containing 20 plants (30 % forbs 70 % grasses) commonly found and collected in northeast Germany (see Table S2, Supporting Information) were sown and rolled. For the *biodiversity 2* treatment, freshly cut plant material was transferred from species-rich donor-sites nearby. Thereafter, cattle were fenced out of the seed-enriched area some weeks to allow seedling establishment. For the *combination* treatments, 1.5 g/m² of the seed mixture mentioned above (see Table S2, Supporting Information) was sown by slit drill (*combination 1*) or by hand in two subsequent years (2015, 2016; *combination 2*) after mowing the standing vegetation. Sowing took place in September and October 2015 and additionally in October 2016 for *combination 2*. Treatments with removal of the aboveground biomass were either mown with a bar mower or mulched. The cutting bar was set at 30 cm above ground, while mulching took place at ground level. Cuttings remained on the plots. Mowing took place between June and August every year from 2015 to 2019. The *flower cut* treatment was mulched in late June and before the second blooming peaked. The *seed cut* treatment was mulched before seed dispersal in mid to late July. Cutting of the *combination 2* treatment in late July was continued annually until the end of the experiment in July 2019.

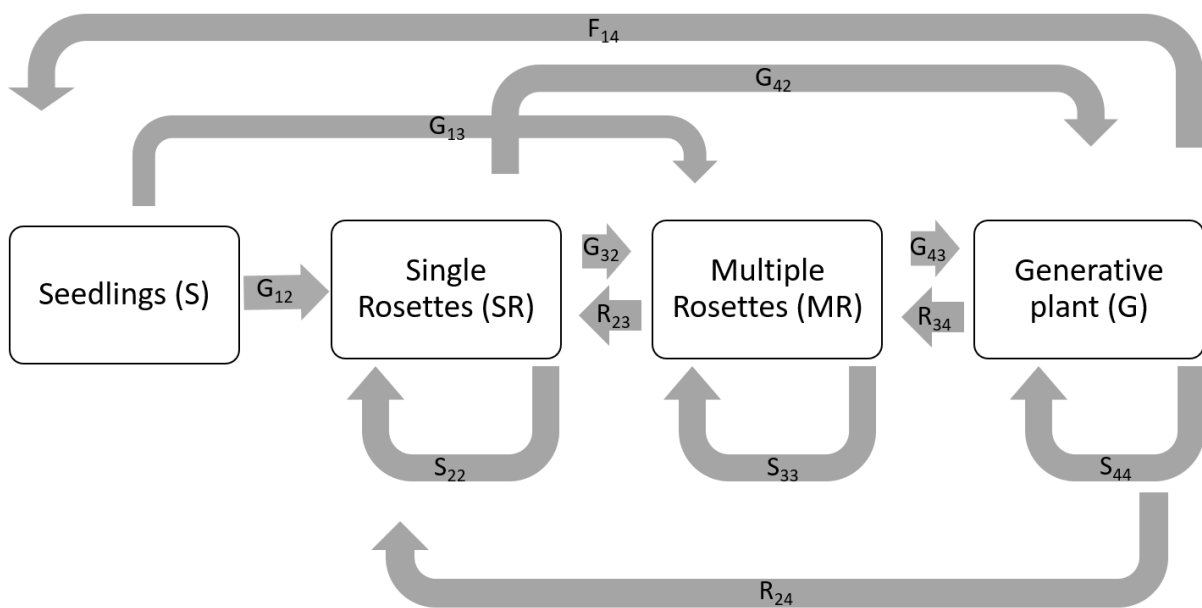
Data sampling

Within the central 1 m² of the permanently marked 9 m² plots, the position, and numbers of stalks of each ragwort individual were recorded every year between 2015 and 2019. Recorded individuals of ragwort were assigned to one of three life stages: (i) seedling (S) with two leaves ≤ 4 mm in width [which could include one- or two-year-old plants as they are indistinguishable from seedlings], (ii) rosettes (with one single rosette (SR) or multiple rosettes (MR)), and (iii) generative (G) with flower stalks. Altogether, 40 466 individuals for one or more transitions were monitored, summing up to 100 199 observations. Three plots were misplaced by farm management in 2017.

The initial species composition of the vegetation was recorded from June to August 2015. In subsequent years, vegetation assessment according to the Braun-Blanquet scale (Braun-Blanquet, 1932) took place between May and August before plots were cut. Red List status of recorded species was taken from the regional Red List for Schleswig-Holstein (Mierwald, 2006). Target species were defined as character species for species-rich grasslands in Northern Germany according to Schmidt *et al.*, 2019.

Transition matrix model and population dynamics

Based on the frequency distribution of recorded life stages, a 4x4-transition matrix (based on S, SR, MR, and G) was constructed for each study site, treatment, and year (Fig 2). Each matrix element (a_{ij}) was calculated from the number of individuals in stage j in year t that passed to stage i in year $t + 1$, divided by the column total of stage j (Caswell, 2001). Fecundity was determined by dividing the number of generative plants (G) in year t by the number of seedlings (S) in the following year $t + 1$ (Jung *et al.*, 2010).



		Stage at time t			
		S	SR	MR	G
Stage at time $t + 1$	S	0	0	0	F_{14}
	SR	G_{21}	S_{22}	R_{23}	R_{24}
	MR	G_{31}	G_{32}	S_{33}	R_{34}
	G	0	G_{42}	G_{43}	S_{44}

Fig. 2 – Life cycle graph for *J. vulgaris* and the corresponding transition matrix. Boxes indicate life stages, arrows represent the possible transitions between them, and letters show the connection between each transition and its matrix entry (F, fecundity; G, growth; S, stasis).

The population growth rate was calculated by pooling all plants of the single life stages for all study sites for each treatment and year. A 95 % confidence interval was established by bootstrapping the data (5 000 iterations) for each treatment and year (Caswell, 2001) in R (R Core Team, 2019). For fertility values, we used mean fertilities per treatment and year.

Life-table response experiments (LTREs) were conducted using matrices based on vital rates to analyze the contribution of different vital rates to the difference in the population growth rate ($\Delta\lambda$) between each treatment and the control (Caswell, 2001). Each matrix element is a product of the lower-level vital rates: survival (σ_j), stasis ($\gamma_{i=j}$), growth ($\gamma_{i>j}$), retrogression ($\gamma_{i<j}$), and reproduction (Φ_{ij}) (Franco and Silvertown, 2004). All analyses were performed with the program POPTOOLS version 3.2 (Hood, 2011).

Statistical Analysis

Population growth was analyzed using mixed effect models (Laird and Ware, 1982; Verbeke and Molenberghs, 2000). The first model included a pseudo factor as a fixed factor. This pseudo factor consisted of the actual factors *treatment* and *year*. This was necessary because the actual factors *treatment* and *year* are not orthogonal for population growth. The second model included the fixed factors *treatment* and *year* without an interaction effect. The two models were compared to test for significance of the interactive effect. As the interactive effect was not significant, the second model with *study site* as an additional random factor was used for further analyses. Residuals were visually checked for normality and heteroscedasticity. Based on this model, a Pseudo R^2 was calculated (Nakagawa and Schielzeth, 2013) and an analysis of variances (ANOVA) conducted, followed by multiple contrast tests (e.g., see Bretz *et al.*, 2011) in order to compare the influence of factors levels of *treatment* and *year*, respectively. To test if treatments differed from the control, we used a Dunnett test.

Numbers of plants and their life stages in response to *year* were studied using analysis of variances (ANOVA) for each treatment. For each study site, average numbers of plants per square-meter from the three plots per block were used. To identify differences between the treatments, years, and life stages, Tukey's HSD (honestly significant difference) post hoc test was applied.

To examine species diversity, we calculated species richness and evenness (McCune *et al.*, 2002). To investigate whether species composition fluctuated more strongly in the treatments compared to the control plots, the temporal species turnover rate for each population and treatment was calculated as $(NR + D)/(n_t + n_{t+1})$ (Mühlenberg, 1989). NR denotes the number of species per plot that were newly recorded in year $t + 1$ but did not occur on the plot in year t . D

is the number of species that disappeared during the transition from year t to year $t + 1$. n_t and n_{t+1} denote the species numbers in year t and $t + 1$, respectively.

All statistical analyses were calculated using the statistical software R (R Core Team, 2019).

Results

Population dynamics of J. vulgaris

Abundance and life stages

The abundance of ragwort individuals did not differ significantly between treatments; however, a trend was identified (ANOVA $F_{6, 204} = 1.955$ $P = 0.073$). Year had a significant effect on ragwort abundance ($F_{4, 204} = 6.6478$, $P < 0.0001$).

The seven treatments led to three distinct response patterns (Fig. 3). Both *biodiversity* treatments showed an initial decline in ragwort abundance but a rebound after the fourth year. The *combination* treatments, the *seed cut* treatment, and the control showed an oscillating pattern with falling and rising ragwort abundance every other year. Under all treatments, the abundance of *J. vulgaris* individuals was on average lower in the fourth year of the study compared to the first year. Similarly, the abundance of ragwort rosettes and flowering plants was slightly lower at the end of the study in 2019 compared to its start in 2015 under all treatments (52 ± 8 in 2019 vs. 60 ± 5 in 2015). Only the *flower cut* treatment led to a continuous decrease in ragwort abundance, while statistically this was only a trend ($F_{4,30} = 2.558$, $P = 0.059$).

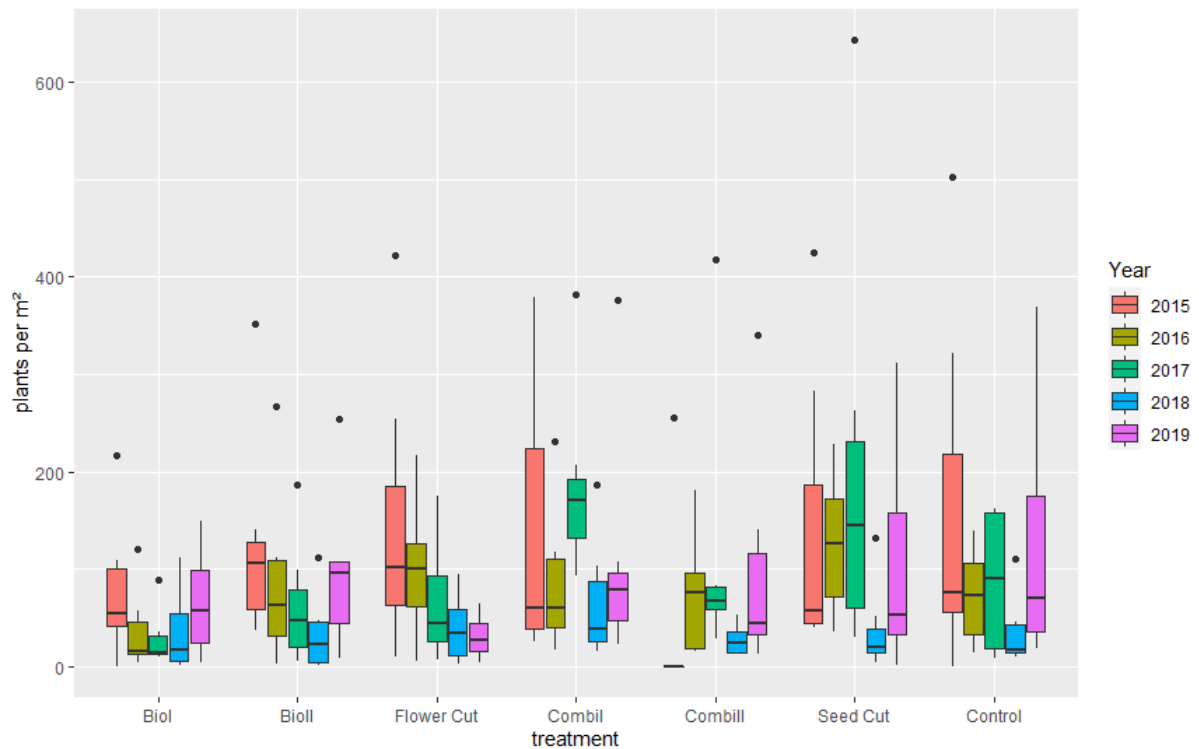


Fig. 3 Abundance of ragwort plants per 1 m² according to treatment and year. In the box plots, middle lines represent the median, boxes represent the first and third quartiles, lower and upper bars represent the minimum and the maximum, and points represent outliers (i.e. points above 1.5 SD). There were no significant differences between treatments (TukeyHSD, $P \leq 0.05$).

Fluctuations in ragwort abundance in non-milled sites between years were mainly driven by differences in the numbers of vegetative plants, while the number of flowering plants showed an almost continuous decrease during the study period (see Fig. 4; ANOVA $F_{4,65} = 10.46$, $P < 0.0001$). This decrease was significant within *biodiversity* treatments, *seed cut*, and *combination I* treatments.

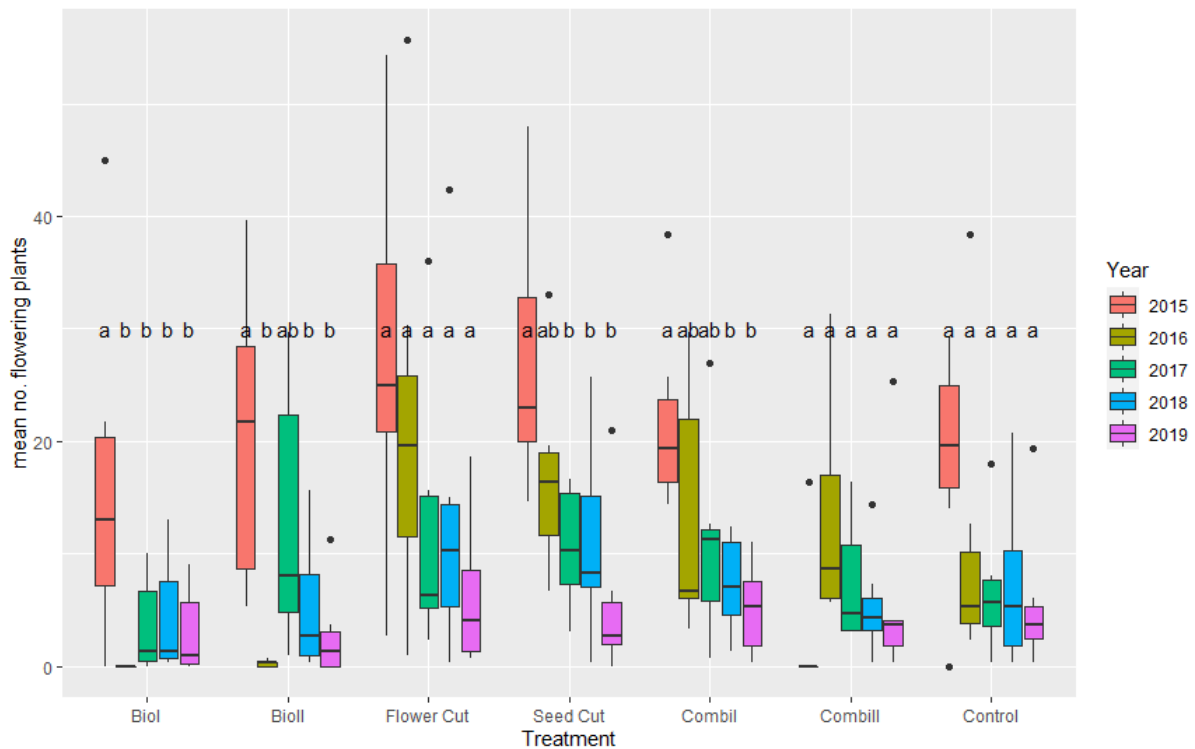


Fig. 4 Abundance of flowering ragwort plants per 1 m² according to treatment and year.

In the box plots, middle lines represent the median, boxes represent the first and third quartiles, lower and upper bars represent the minimum and the maximum, and points represent outliers (i.e. points above 1.5 SD). Different letters indicate significant differences between the years within the treatments (TukeyHSD, $P \leq 0.05$).

Population growth and Life-Table-Response-Experiments

Population growth rates did not differ significantly between treatments ($F_{6,153} = 1.541$, $P = 0.168$). For the *flower cut* treatment, the population growth rate was significantly below one throughout the study period, thus indicating a constant decline in population size (Fig. 5). All other treatments did not lead to a constant decline in population sizes but showed considerable variation between years. Interestingly, years with relatively high population growth rates were followed by years with lower population growth rates, which led to a significant year effect on λ (ANOVA $F_{3, 158} = 15.35064$, $P < 0.0001$). The substantial population growth setback in 2018 (year 17-18) was prominent.

ANOVA of our mixed model including *treatment* and *year* as main factors and *study site* as a random factor revealed that *year* rather than *treatment*, mainly influenced the survival and population growth rates of ragwort. In the dry year of 2018, ragwort density and abundance

decreased but increased even more in the subsequent moist year 2019. Consequently, there was a positive linear relationship between the amount of rainfall and the abundance of seedlings found in the study plots (regression $y = -55.5360 + 1.0612 * N$, $F_1 = 17.57$, $P < 0.0001$).

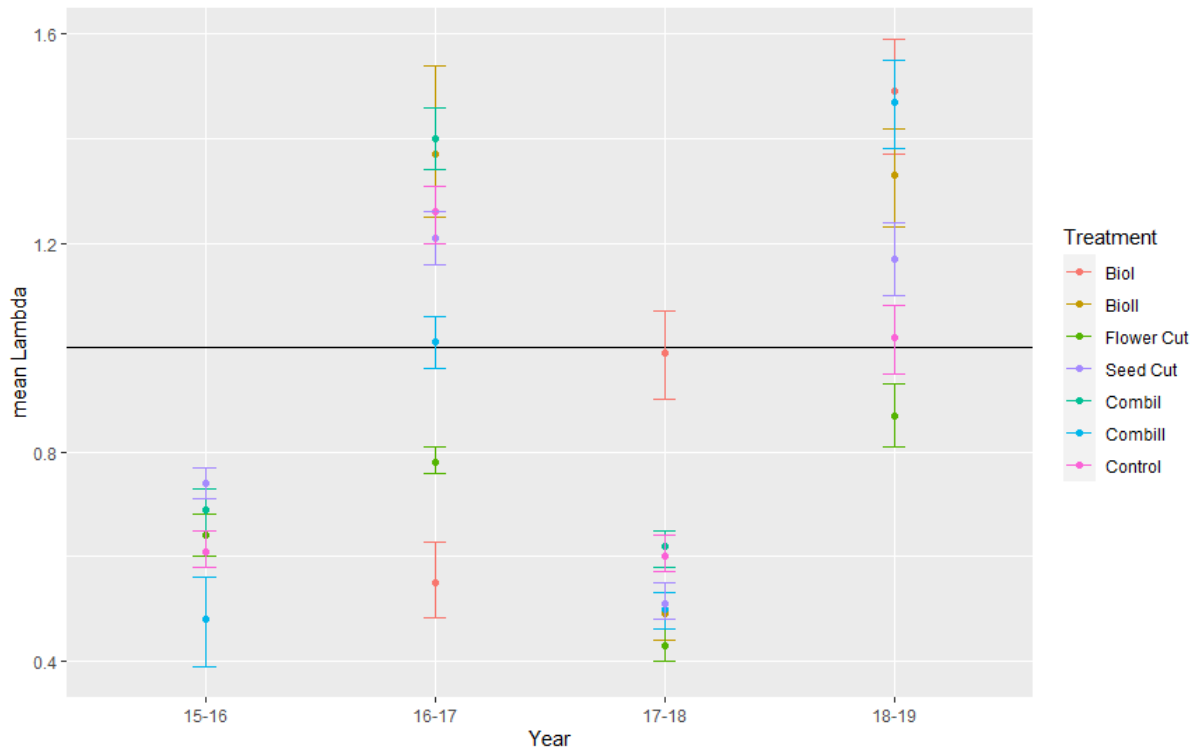


Fig. 5 Population growth rates for treatments according to years. Shown are the means \pm 95 % CI. Treatments did not differ significantly. Constant population is marked with the line $\lambda = 1$.

The LTRE analysis showed that differences in the population growth rate ($\Delta\lambda$) between each treatment and the control were mainly the result of differences in generative reproduction and survival (Fig. 6). Another reason for higher population growth rates in treatments compared to the control was a higher survival of plants in the treatments than in the control. The analysis also demonstrates that the influence of vital rates on population growth in the *flower cut* treatment is very different from all other treatments. Here stasis and retrogression have a considerable impact on positive population growth, as these vital rates are much higher than in the control.

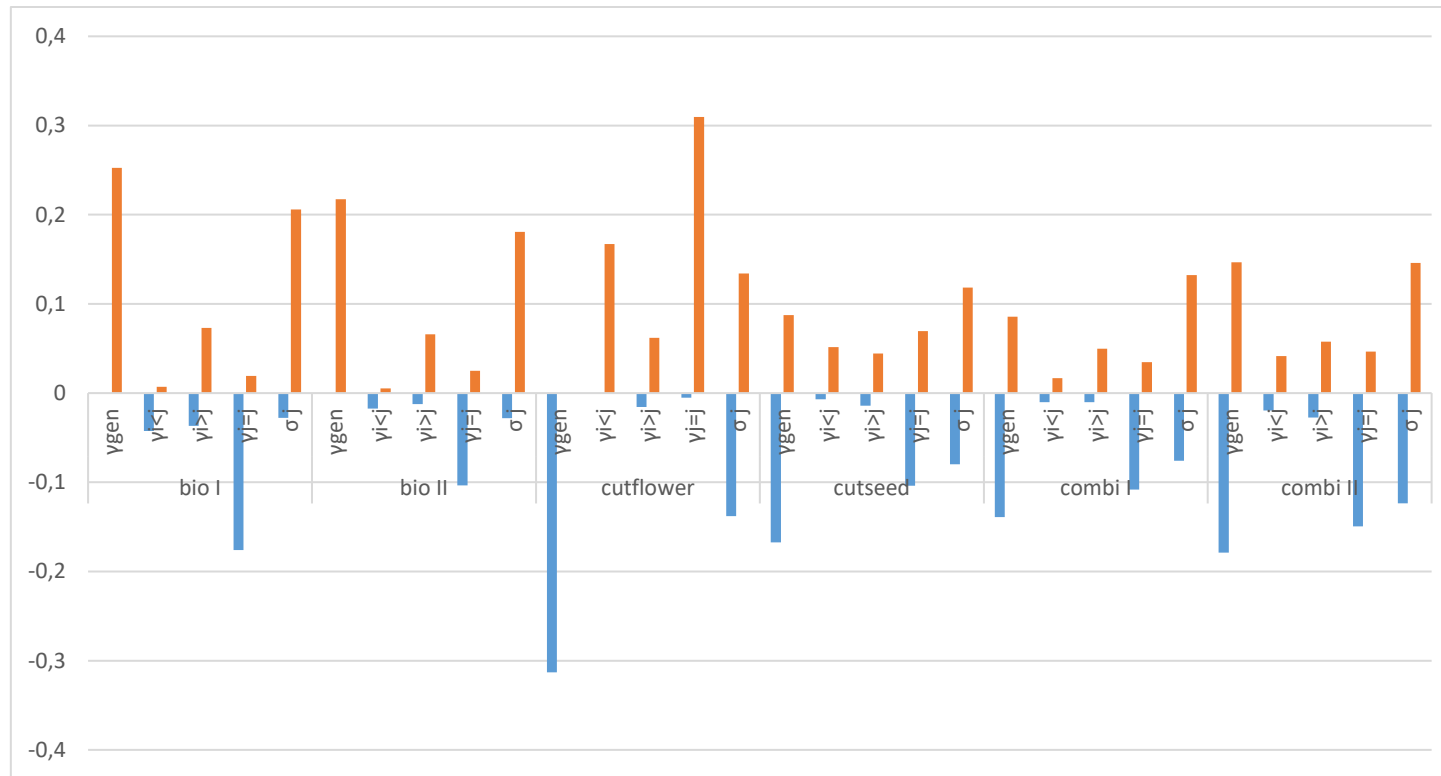


Fig. 6. Contribution of vital rates to the difference in population growth rate ($\Delta\lambda$) between the control and other treatments (bio I, *biodiversity 1*; bio II, *biodiversity 2*; cutflower, *flower cut* treatment; combi I, *combination* treatment 1; combi II, *combination 2*; cutseed, *seed cut* treatment) in the pooled *J. vulgaris* population, as determined by LTRE (life-table response experiment) analysis. Bar sections above zero display the summed positive contributions and bar sections below zero the summed negative contributions.

The elasticity analysis revealed that three main transitions are crucial for population growth: i) the transition from seedling to single rosette (G_{21}), ii) from single rosette to flowering plant (G_{24}), and iii) from flowering plant to seedling (F_{14}). The importance of these transitions did not differ between treatment or year and all transitions contributed equally to population growth.

Changes in vegetation composition

The overall mean number of vascular plants per 1 m² was 23 ± 1 in 2015 and 29 ± 1 in 2019. Species richness differed between treatments (ANOVA $F_{6,210} = 3.204$, $P < 0.005$). The seed addition in *biodiversity* and *combination* treatments led to species enrichment. About five more plant species were found on plots with seed addition (*biodiversity* and *combination*) compared to control plots. In the last year of the experiment only species richness in the *combination* treatments was significantly higher than in the control treatment. All management measures but the control enhanced species richness (Fig. 7).

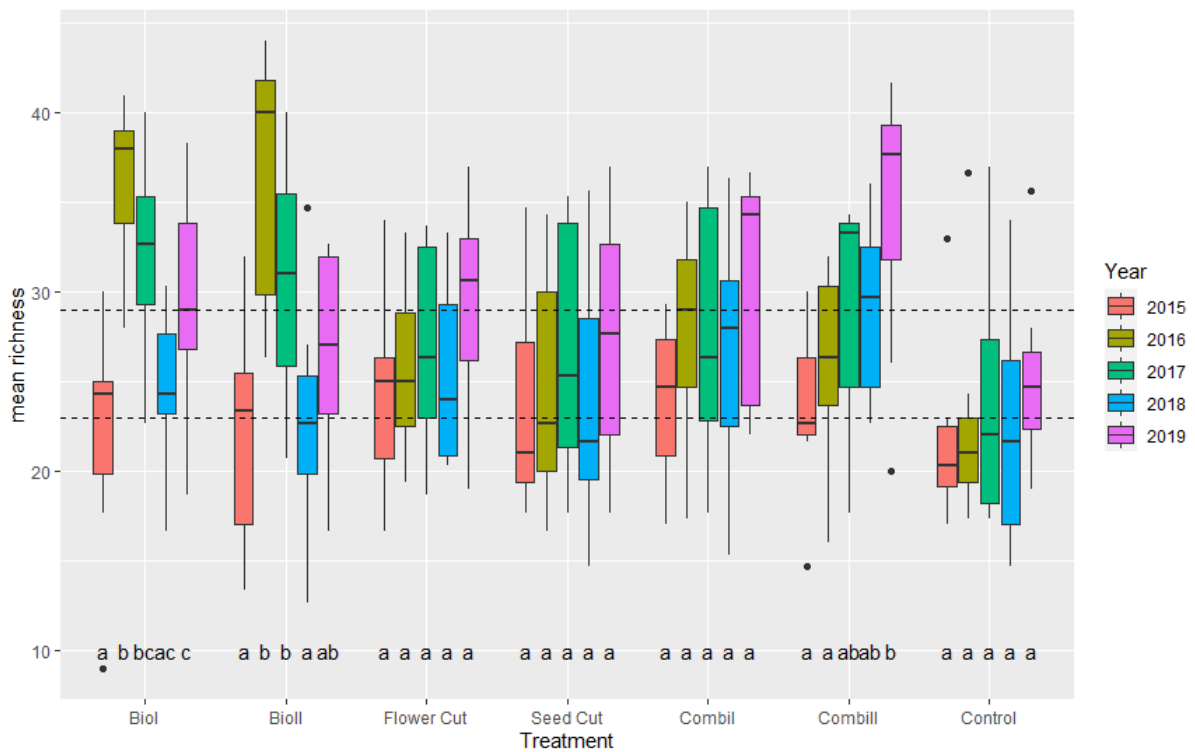


Fig. 7 Species richness according to year and treatment. For explanation of the boxplot, refer to Figure 3. Different letters indicate significant differences ($P \leq 0.05$; Tukey HSD) between years within one treatment. Dashed lines indicate mean species richness in 2015 (lower line) and 2019 (upper line).

Richness patterns were varying over the study period (Fig. 7) and were significantly different between treatments and years (ANOVA treatment $F_{6, 168} = 3.893$, $P < 0.005$; year $F_{3, 168} = 3.813$, $P < 0.05$). While in the first and second years after seed addition, the *biodiversity* treatments resulted in the highest species richness, in 2018 and 2019 the *combination* treatments were the most species rich. During all study years, the control treatment resulted in the lowest species richness, also only significantly different from the *biodiversity* treatments. Both cutting regimes had slightly but not significantly higher species richness compared to the control. Throughout the study period, species evenness was highest under treatments that included mowing (*flower* and *seed cut, combination 2*).

The number of red-listed plant species differed significantly between treatments (ANOVA $F_{6, 189} = 31.08$, $P < 0.0001$). Most endangered plants occurred in the *biodiversity 1* treatment (mean: 3 endangered species per 1 m² vs. 0 or 1 species in the control). Plants categorized as characteristic valaubale grassland species (Schmidt *et al.*, 2019) were highest in numbers in the *biodiversity 1* treatment, followed by hay transfer (*biodiversity 2*) and *combination* treatments with mowing and sowing (Fig. 8). *Biodiversity* treatments and the *combination 2* treatment led to significantly higher numbers of endangered plants than in the control.

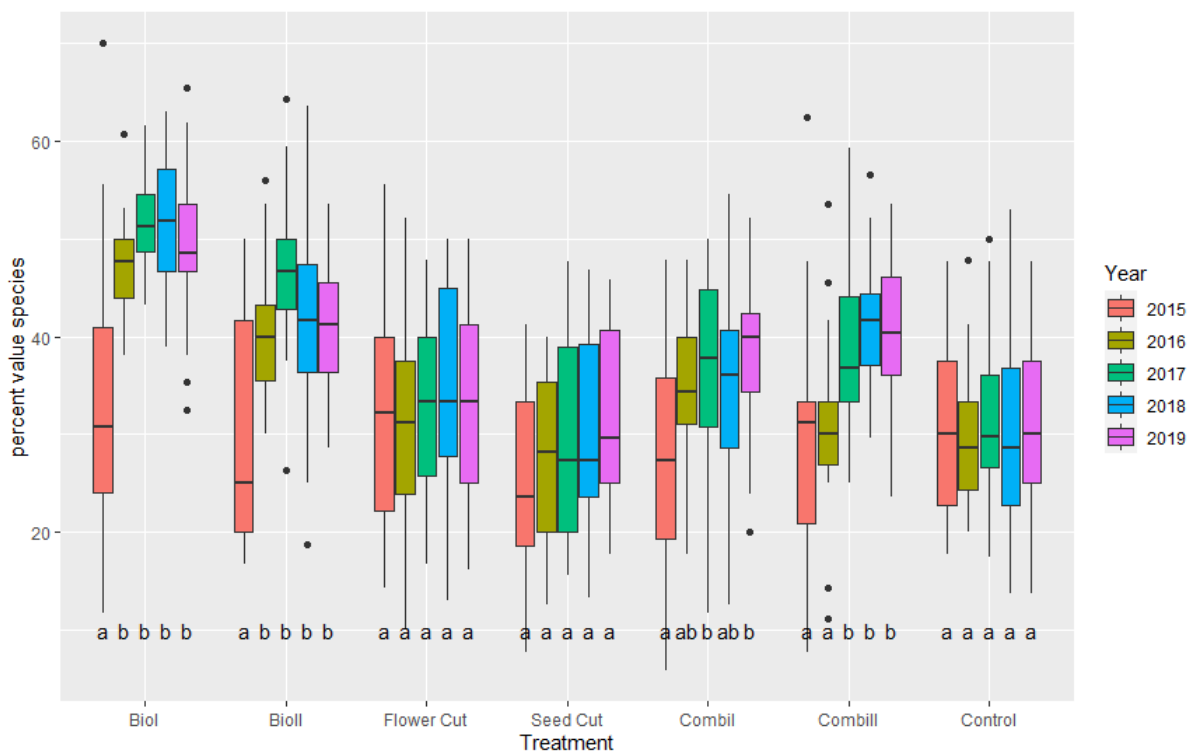


Fig. 8 Percentage of value species per year and treatment. For explanation of the boxplot, refer to Figure 3. Different letters indicate significant differences ($P \leq 0.05$; Tukey HSD) between years within one treatment.

Turnover rates of the vegetation differed significantly between treatments (ANOVA $F_{6, 140} = 16.74$, $P < 0.0001$) and were significantly highest under treatments with milling in combination with seeding or plant material transfer, i.e. *biodiversity 1* and 2, followed by *combination 2*, which combined sowing and mowing (Fig. S3, Supporting Information). Turnover rates of pure cutting treatments, *combination 1*, and the control were significantly lower and did not differ from each other. The percentage of target species contributing to the turnover rate was highest for *biodiversity 1* (37 ± 0.02 %) and *combination 2* (35 ± 0.03 %) and lower for *combination 1* (29 ± 0.02 %) even though these differences were not significant.

Discussion

Effects of regulation measures on J. vulgaris

Of the six management regimes tested, the *biodiversity* treatments led to a drastic decrease in ragwort abundance in the first two to three years (Fig. 3). The decrease was especially pronounced, significant, and lasting in the *biodiversity 1* treatment for the flowering stage (Fig. 4). Although the general abundance decline in the *biodiversity* treatments after the first years was not significant, the results concur with previous findings that ragwort densities decline as a result of sowing grassland species into the sward or after soil disturbance (Lawson *et al.*, 2004; Bezemer *et al.*, 2006a).

Declines in ragwort abundance following soil disturbance, such as ploughing, seem counterintuitive at first since establishment of ragwort is generally favored by soil disturbance (Suter *et al.*, 2007). Consequently, soil disturbance was combined with sowing grassland species (Lawson *et al.* 2004; Bezemer *et al.* 2006a). Lawson *et al.* (2004) revealed a reduction in ragwort densities in the first year after ploughing and sowing grassland species. Similarly, Bezemer *et al.* (2006a) reported reduced ragwort abundance on a former arable field in those plots that were sown with grassland species compared to species-poor control plots without seed addition over a period of eight years.

According to the biotic resistance theory (Levine *et al.* 2004), invasibility in species-rich systems should be lower than in species poor ones. While this might be true in the long run, as Bezemer *et al.* (2006a) showed, at shorter temporal scales, species-rich plant communities may also be invaded if open soil patches remain that favor the establishment of light-demanding pioneers such as ragwort (van der Meijden and van der Waals-Kooi, 1979). Thus, we cannot confirm the results of Bezemer *et al.* (2006a) and Lawson *et al.* (2004) that ragwort density declines as a result of sowing grassland species into the sward or after soil disturbance as we

did not find lower ragwort numbers in sown plots compared to untreated control plots in the four years after milling and sowing took place. We assume that this is due to differences in the initial setting of the study by Bezemer *et al.* (2006a) and our study. While in our study, grazed grassland was the control baseline, i.e. cattle caused continuous small-scale disturbances throughout the study, Bezemer *et al.* (2006a) began with an un-vegetated control, which was mown once a year. Repopulation of the early successional ragwort might not have been prevented by a maximized diversity but rather by a very dense sward, as ragwort demands high light levels for germination, which prevail after soil disturbance (van der Meijden and van der Waals-Kooi, 1979). In our case, these conditions prevailed on milled and sown plots, which had around 33% higher bare ground cover than control plots (Fig. S4, Supporting Information).

The LTRE analysis showed that the population growth on milled sites was due to an increased generative reproduction (Fig. 5), which profited from lower levels of competition. This increase in ragwort might also have occurred due to changes in the soil biota after soil milling. While soil biota is thought to suppress ragwort growth over time (Bezemer *et al.*, 2006a; van de Voorde *et al.*, 2011), soil disturbance might set soil biota back to an earlier developmental state, where negative effects on ragwort are less pronounced. Kulmatiski *et al.* (2006) reported increased growth of invaders in tilled soil due to changes in the soil community, which supports this assumption.

Besides milling, mowing is a commonly used tool to control noxious weeds (DiTomaso, 2000). It can prevent seed production, reduce carbohydrate reserves, and give advantages to desirable perennial grasses. Our results show that the success of mowing depends on timing and frequency. While mowing once before seed dispersal failed to reduce the population growth rate or abundance of ragwort plants, mowing twice shortly before the bloom resulted in a growth rate significantly and constantly below one and constantly decreased ragwort abundance. This concurs with the finding that the optimal time for mowing noxious weeds is usually the flowering stage before seed development (DiTomaso, 2000). This too is true for ragwort, as the highest proportion of nutrients and energy is channeled into aboveground organs during bloom (Otzen 1977). Yet, as the plant quickly grows back, a second cut within the second bloom is essential to effectively prevent seed development and population growth.

According to the pattern of population growth in the *flower cut* treatment, the LTRE analysis showed that the lower population growth rate in *flower cut* compared to the *control* treatment was mainly caused by the suppression of generative reproduction, the most important means of invasion and mass colonization of disturbed sites for pioneer plants (Harper and Wood, 1957).

Additionally, repeated mowing leads to a denser sward and thus less open soil patches, which prevents emergence of ragwort seedlings. Furthermore, we found that continuous mowing twice a year for more than two years is needed to drive down ragwort abundance. Otherwise, high numbers of plants either remaining in their life stage or becoming rosettes again will grow back the next year and prolific blooming will only be postponed.

In accordance with our results, Siegrist-Maag *et al.* (2008) also found that cutting ragwort early and at least two times significantly weakens the plant. However, the percentage of plants that died directly after cutting was relatively low in their study (between 6%-10%), while mortality rates in all of our treatments were much higher (50%). The LTRE analysis showed that impeding the fertility transition, i.e. the production of germinable seeds, was the decisive property of the *flower cut* treatment causing ragwort to decline, particularly in 2017, two years after the treatment began. The need to mow repeatedly over several years to successfully control ragwort is also supported based on the estimated survival rates in the *flower cut* treatment.

Our LTRE-analysis showed that survival of ragwort plants in the *flower cut* treatment in 2016/17 was higher than in the control. This is in accordance with findings of Crawley and Gillman (1989), who found that plants defoliated by the cinnabar moth (*Tyria jacobaeae*) die less than those setting seeds probably due to higher resource investments involved in seed production than in regrowth in the subsequent year. Cumulative effects of constant mowing regimes were also found by Winter *et al.* (2014), who showed that cutting twice per year only once, supported the development of generative plants based on ragwort's adaptation to drastic grazing by herbivorous insects (McEvoy and Rudd, 1993). Increased retrogression and stasis of plants that are ready to bloom in the next year reflects this mechanism in our LTRE analysis.

Finding the optimal cutting date after which ragwort will not bloom again and produce vital seeds again is difficult. Contrary to the effects of repeated cutting (*flower cut*), cutting once before seeds disperse did not influence population dynamics or ragwort abundance. Even though more flowering plants completed their lifecycle and died, new seedlings emerging from post-ripened seeds compensated this mortality. In ragwort, the first germinable seeds occur with the first withered capitula (Eisele *et al.*, 2011). Eisele *et al.* suggest cutting ragwort, when 10 % of the capitula begin to flower within half of the generative plants. However, under field conditions where the phenology of ragwort is not synchronous, this stage is rather difficult and laborious to assess and occurs only a few days before the emergence of the first germinable seeds. If cut too early however, enough resources still remain for vegetative reproduction (Otzen, 1977).

Rosettes and seedlings constituted the largest proportion of ragwort plants, which is in accordance with Siegrist-Maag *et al.* (2008), who also found that 50% of ragwort populations are made up of rosettes. Yet, ragwort population structure and growth rates were highly variable across years, such that year had a stronger effect on population growth than management. We observed an oscillating pattern with falling and rising ragwort abundance every other year in *seed cut*, *combination*, and *control* treatments (Fig. 3). Extreme weather conditions, such as exceptionally hot or dry summers, affect population growth through immediate effects on vital rates (Lennartsson and Oostermeijer, 2001; Hüls *et al.*, 2007). The low rainfall in 2018 caused a decline in the population growth rate from 2017 to 2018 and a density decrease at all sites under all treatments. In accordance with our general observation of increased seedling numbers when rainfall was higher, we found that the population growth rate from 2018 to 2019 increased again after increased precipitation in 2019 (Fig. 5). Crawley and Gillman (1989) found that autumn and spring rains encourage ragwort biomass growth. Ragwort populations probably profited from more light on the ground from 2018 to 2019 because the sward was less dense after the drought of 2018. Crawley and Gillman (1989) also found that plant recruitment was microsite limited and depended very much on weather and activity of rabbits, boars, and moles, i.e. enough light and water for germination (van der Meijden and van der Waals-Kooi, 1979). In our experiment, trampling and grazing from cattle can create windows of opportunity for ragwort establishment (Eichberg and Donath, 2017).

Our analysis showed that all transitions from seedling to rosette, from rosette to flowering plant, and from flowering plant to seedling contribute equally to population growth rate. This is in accordance with Dauer *et al.* (2012), who found that transitions from rosette to flowering plant and from flowering plant to rosette were especially important for population growth.

Development of species richness and vegetation composition

Botanical richness in our study either remained constant or increased under the tested treatments. Thus, none of the measures applied to regulate ragwort counteracted the goal of preserving or increasing species richness in grasslands. Highest species richness occurred in the combination treatment of seeding and cutting (*combination 2*), where resident species and newly sown species coexisted, whereas species diversity in *control* treatments stayed lowest. *Combination 2* also reached high evenness-indices, as did the *flower cut* treatment. In general, evenness was significantly higher under mowing regimes (*flower cut*, *seed cut*, and *combination 2*), in which more species were probably able to establish due to reduced competition by dominant grasses (Smith *et al.*, 2018). Diversity patterns of the treatments changed over time.

Biodiversity treatments were most species rich in the first two years after milling and seeding or transferring green hay. However, species numbers and cover of target species in *biodiversity* treatments declined after the third year (Fig. 7 - 8). Other studies also showed that initially high species numbers in sown treatments decreased after some years (Bezemer *et al.*, 2006a; Jongepierová *et al.*, 2007; Fritch *et al.*, 2011). One reason for this decrease is that ruderal species and former arable weeds occur in the first years after milling and vanish later on (Donath *et al.*, 2006; Schmiede *et al.*, 2012). Therefore, species turnover was highest in mill-sow treatments (*biodiversity* treatments 1 and 2) and seed-addition treatments (*combination* treatments 1 and 2), whereas turnover in the pure cutting treatments (*flower* and *seed cut*) did not differ from the control.

Species turnover in *biodiversity* and *combination* treatments led to a higher share of characteristic grassland species. Percentage of characteristic grassland species was highest in *biodiversity* and *combination* treatments (Fig. 8). As numerous other studies showed, without bridging dispersal limitation by actively introducing seeds, it is rather unlikely that species-rich grasslands will develop on former arable land (Pywell *et al.*, 2002; Hölzel and Otte, 2003; Lawson *et al.*, 2004). This is due to the disappearance of characteristic grassland species in intensively used and fertilized agricultural landscapes (Leuschner *et al.*, 2013). This is especially problematic within modern agricultural landscapes in Northern Germany, which are characterized by severe habitat fragmentation and biodiversity losses in grasslands (Kollmann *et al.*, 2019).

The percentage of characteristic grassland species in *biodiversity* treatments was approximately 40%, which was 10 % higher than in treatments without seed addition. While the number of species in the *biodiversity* treatments decreased again, the number of species in the *combination* 2 treatment increased constantly over the study period and exceeded the number of species in the *biodiversity* treatments. This increase in species richness may have been supported by sowing grassland species twice, which lowers the risk that establishment fails due to unfavorable germination conditions when plants are sown in one year only. Besides the positive effect of two sowing applications, the establishment of higher species numbers in the *combination* 2 treatment might have been supported by the yearly cut, which is known to favor the occurrence of forbs over grasses (Briemle, 2007). In our *control* plots, dominance of grasses and occurrence of ruderal species was higher than in all other plots. This suggests that residual soil fertility on ex-arable land may reduce the survival of characteristic grassland species due to competition from grasses (Marrs, 1993).

Most promising in promoting threatened plants is mowing and sowing (*biodiversity I*), probably because germination conditions for seeds in mowed plots are better than in non-mowed plots. In the latter, the accumulation of living and dead biomass and the encroachment of competitor species can hinder germination and the establishment of grassland species (Schmiede *et al.*, 2012; Valkó *et al.*, 2016). However, there is also a noticeable effect of over-seeding in our study (seed addition by hand) – probably because cattle not only produce open areas for the establishment of ruderal species like ragwort but also for other species that happen to be near to disturbed areas (Faust *et al.*, 2011). As cattle are quite heavy, their trampling can result in relatively large bare patches, potentially increasing establishment opportunities for sown seeds. Various studies (Janicka and Pawluśkiewicz, 2020, Eckstein *et al.*, 2012) showed that gap size in the sward is an important element of successive seed introduction. This is due to improved early development of the sown species when gaps are present.

Conclusion

Slight ragwort decline occurred under all treatments and consequently we found no significant differences in ragwort abundance between the applied treatments and the control. Even the most effective treatment, the *flower cut* treatment, which was significant by trend and the only treatment resulting in a population growth rate below one during the complete study period, did not lead to a complete disappearance of ragwort but allowed on average five ragwort plants per square meter to occur. Therefore, we do not have an easy answer for practitioners on how to manage ragwort populations in nature conservation grasslands.

We probably did not find clear differences between the treatments as ragwort is a successional plant adapted to disturbance and quickly reacts to windows of opportunity being continuously created by grazing animals or as a result of drought. Moreover, as our treatments were designed to minimize unwanted harmful impacts on grassland ecosystems, we refrained from drastic measures. Nonetheless, our results reveal some promising approaches for ragwort regulation that do not jeopardize nature conservation efforts.

The *flower cut* treatment, which led to a constant decline in ragwort resembles a traditional non-intensive meadow management technique, which supports high plant species and animal species richness. Still, the two cuts that were employed for this treatment are not without potential harm for invertebrates. These drawbacks must be weighed against the treatment's regulating effect on ragwort densities. This might restrict the application of this measure to sites with high conflict potential.

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Discussion and synthesis

The main aim of this thesis was to find environmentally sound management opportunities for the grassland weed tansy ragwort on non-intensively used pastures that are not contradictory to maintenance and promotion of species-rich grasslands. It turns out, that allelopathy in ragwort seems not to be a main driver of invasion success. Furthermore, autotoxicity occurs in ragwort but is not effective in reducing ragwort growth. Finally, the only efficient treatment in reducing ragwort population density and growth rate tested is a mowing regime within the bloom of ragwort. As this mowing regime did not reduce plant diversity, it is possible to reduce ragwort populations without jeopardizing wild plant diversity in low input grasslands.

Ragwort is no threat to biodiversity

Within this study, no strong allelopathic effects on germination and growth of common or rare co-occurring plants occurred, which levels out allelopathy as a main driver of invasion success (Chapter 1). Indeed, authors that did find allelopathic effects of ragwort in bioassays failed to do so under field conditions (Wardle *et al.*, 1995). Based on the results, it is very unlikely that ragwort will prevent the growth of endangered plants through allelochemicals. Probably ragwort has other ways to outcompete co-occurring plants by shading or a more efficient resource use (Harper and Wood, 1957; McEvoy, 1985). However, ragwort seedlings are as seedlings of other problematic grassland weeds such as *Juncus effusus* commonly assessed as weak competitors that will just germinate in competition free bare soil (Cameron, 1935; Lazenby, 1955; Suter *et al.*, 2007). Indeed, fieldwork (Chapter 3) has shown that ragwort frequently co-occurs with many different plant species including red listed species, that are as light demanding as ragwort (L¹ 8) such as *Potentilla argentea* (L 9) or *Silene vulgaris* (L 8). Thus, even though ragwort may dominate pastures optically in its bloom, there was no evidence that it excludes other plant species through its presence. In addition, in milled sites sown with endangered species, ragwort and sown species co-occurred and did so for several years (Chapter 3). Therefore, it is unlikely that ragwort itself is a threat to botanical diversity or hampers establishment of species-rich grasslands. Other studies investigating the effect on weeds on biodiversity found that invasion of weeds started after floral diversity had already declined (Rasran and Jeromin, 2010) and that the main problem of weed species was the hampering of immigration of rare species (Agnew, 1961). High ragwort densities are probably not the cause for species decline, much more they reflect manmade changes such as disturbance and changed

¹ Ellenberg's Indicator Value for light derived from Jäger (2017)

livestock management, which foster ragwort establishment and decline of other species (Maskell *et al.*, 2020). Rather, establishment of species-rich grassland on old-fields that are often highly dominated by ragwort, is due to seed-limitation as our fieldwork shows (Chapter 3) that plant diversity was promoted by all seeding treatments but not by cutting or grazing alone, even if cutting reduced ragwort densities considerably (Hutchings and Booth, 1996; Hölzel and Otte, 2003).

Thus, in terms of nature conservation, ragwort is no problematic invasive species. It harbors a huge variety of insect herbivores (Kassebeer, 2015) and according to our findings, it does not reduce botanical richness either. In addition, it is a native species and character species for pastures (Oberdorfer 1990) and no new invader. Therefore, it is unlikely that ragwort changes ecosystem functions, or irreversibly alternates the abiotic and biotic environment in a way, that would prevent the semi-natural grassland plant community to flourish.

Ragwort litter reduces germination but is an unsuitable tool for ragwort regulation

Nevertheless, ragwort is a problematic weed because of its toxicity when consumed by grazers (Wiedenfeld and Edgar, 2011) and can cause severe economic losses for farmers (Coombs *et al.*, 1996; Fowler *et al.*, 2016). Therefore, management is still warranted. Autotoxicity, which occurred in the germination experiments and in our common garden experiment is finally overlain by other processes in seedling development. Therefore, it cannot be used in ragwort management. Rather autotoxicity fulfills its ecological sense in population regulation as it leads to enough strong individuals that survive and form a healthy population and prevents overcrowding (Renne *et al.*, 2014). Data of the field experiment (Chapter 3) supports this assumption, as there was no significant or constant decline in ragwort densities on plots where ragwort litter was left. Besides, although many more seedlings grew in the seed cut treatment (Chapter 3), this did not translate in higher numbers of rosettes or flowering plants. Thus, many seedlings must have died either of fierce intraspecific competition or by density-regulating autotoxic allelochemicals in the litter.

The litter (Chapter 2) and field experiments (Chapter 3) did not find evidence for a negative plant-soil feedback. Even though, slight decreases especially in flowering plants occurred and a hint, that ragwort establishment might be hampered soil effects as in some milled sites many more seedlings emerged than on non-milled sites, there was no significant constant general decline in plant numbers on control plots. This findings do not mirror a strong negative plant-soil-feedback, that has been reported in other studies (Bezemer *et al.*, 2006a; van de Voorde *et al.*, 2011). Probably soil disturbance by grazers was too intense for such a feedback to be

detectable in plant numbers. Therefore, relying on self-regulation of ragwort, in non-intensively used pastures ragwort density will probably not reduce (Chapter 3).

Generally, the experiments showed that a slight litter layer improves ragwort germination (Chapter 2). This is in accordance with other studies on the effect of litter on germination (Loydi *et al.*, 2013). Thus, the high number of seedlings found in the seed containing plant material-transfer treatment (*biodiversity 2*) seems to be caused by favorable germination conditions on milled soil – free of competition by resident species - under a slight litter cover namely less fluctuating temperatures and protection from desiccation (Loydi *et al.*, 2013). Therefore, milling and transfer of seed containing plant material are less effective in regulating ragwort especially in ragwort-populated surroundings.

Reducing ragwort without harming plant biodiversity is possible

All cutting and restoration measures applied in this study did either not change or even promoted plant species richness (Chapter 3). Results of the third experiment demonstrated that milling and seeding was very effective in promoting species richness especially for characteristic grassland species. It reduced flowering ragwort fast and prolonged. Milling and transfer of seed containing plant material is also possible and reduces flowering ragwort plants, but more ragwort seedlings occurred in this treatment. Transfer of characteristic grassland-species in species impoverished landscapes is also possible with these methods (Kiehl *et al.*, 2010). However, repopulation of ragwort on ploughed ground, and thus population growth, is not stopped in biodiversity treatments. This is in accordance with findings for other weeds such as *Juncus effusus*. For this plant soil perturbation and seeding harbors more risk as potential as *J. effusus* produces similar to ragwort exceedingly many seeds (8 million seeds per square yard), which also stay vital in the seed bank for many years. Thus, as both, neither *J. vulgaris* nor *J. effusus*, are seed limited (Lazenby, 1955), soil disturbance can lead to population growth of those weeds.

The only treatment where ragwort abundance and density decreased constantly and significantly was the *flower cut* treatment, with mowing twice a year, when ragwort was in its bloom. This treatment has also been recommended for many other grassland weeds such as *J. effusus*, *Deschampsia cespitosa*, *Rumex obtusifolius* and *Equisetum palustre* (Rasran and Jeromin, 2010). Mowing must be repeated for several years and as hay from ragwort infested grassland cannot be sold, this option might be unattractive for farmers. However, regulation by herbicides will also not be possible without (high) costs (Fowler *et al.*, 2016). Furthermore, simultaneous grazing in low densities on these pastures is still possible as long as grazers find

enough alternative grasses and herbs and are excluded from the freshly mown meadow to prevent consumption of cut ragwort that loses its bitterness (Neitzke and Berendonk, 2011). Concerning nature conservation aspects, *flower cut* lead to higher evenness and number of plant species stayed stable under this treatment. However, cutting of plants may effect numerous animals (Humbert *et al.*, 2009). These drawbacks must be weighed against the regulating effect on ragwort densities.

If grassland nature-reserves shall be upgraded with more characteristic grassland species without risking a burst of ragwort numbers, mowing combined with overseeding has proven to be a valuable treatment (Chapter 3). Probably a combination of mowing twice and seeding after the second cut can aid both, reducing ragwort density and increasing species numbers. Several studies showed that the invasion of potential problematic plant species can be prevented through increasing plant species diversity (e.g. Bezemer *et al.*, 2006a; Baasch *et al.*, 2012, Oakley *et al.*, 2013). However, this needs to be investigated in more detail.

Control of ragwort by doing nothing as Bezemer *et al.*(2006b) mentioned it, will not lead to predictable and fast success in pastures. However, in pure meadows that are not grazed or frequently dug up by moles, boars, or rabbits, control without any disturbance might work as germination of ragwort in a dense sward is very unlikely (van der Meijden and van der Waals-Kooi, 1979).

Conclusion and management recommendations

As the enhancement of biodiversity is a primary objective in many non-intensively used grassland systems, for which farmers often receive remuneration, the thesis allows some recommendations to be made. In addition to pastures and meadows, these recommendations may also be applied to the maintenance of field margins, and the management of grassland nature reserves.

First, it needs to be defined if action against ragwort is needed. In grasslands managed with the aim to promote biodiversity and when health of grazing animals is assured by the presence of enough alternative plant material to consume, farming can be continued, and explicit management is not needed. If management is needed and plant diversity shall be sustained, mowing twice a year will reduce ragwort population growth and density, when applied for several years.

One-cut per year as well as no cut at all on the other hand will not reduce ragwort density. However, the number of flowering plants might decrease. Milling and seeding will reduce ragwort initially but will not prevent remigration. The latter is very likely to occur due to bare

soil patches. Thus, reducing sward damage by cattle is also an important element as seed recruitment depends essentially on open soils.

Based on the results of this thesis a differentiated management of ragwort should be aimed at, considering the various objectives of nature conservation, agriculture and the health risk to animals and humans. Coordinated instructions for action are important, based on which it is possible to check for every single site whether management is necessary and, if so, how this can be achieved in an environmentally sound, means sustainable way, and therewith also in an economical manner.

References

- Adler PB, Smull D, Beard KH, et al. 2018.** Competition and coexistence in plant communities: Intraspecific competition is stronger than interspecific competition. *Ecology Letters* **21**: 1319–1329. doi:10.1111/ele.13098.
- Agnew ADQ. 1961.** The ecology of *Juncus effusus* L. in North Wales. *Journal of Ecology* **49**: 83–102. doi:10.2307/2257425.
- Ahmed M, Wardle DA. 1994.** Allelopathic potential of vegetative and flowering ragwort (*Senecio jacobaea* L.) plants against associated pasture species. *Plant Soil* **164**: 61–68. doi:10.1007/BF00010111.
- Alías JC, Sosa T, Escudero JC, Chaves N. 2006.** Autotoxicity against germination and seedling emergence in *Cistus ladanifer* L. *Plant Soil* **282**: 327–332. doi:10.1007/s11104-005-6066-y.
- Ambika SR. 2013.** Multifaceted attributes of allelochemicals and mechanisms of allelopathy. In: Cheema ZA, Farooq M, Wahid A, eds. *Allelopathy*. Berlin, Heidelberg: Springer Berlin Heidelberg, 389–405.
- Antonovics J, Levin DA. 1980.** The ecological and genetic consequences of density-dependent regulation in plants. *Annual Reviews of Ecological Systems*. **11**: 411–452.
- Basch A, Kirmer A, Tischew S. 2012.** Nine years of vegetation development in a postmining site: Effects of spontaneous and assisted site recovery. *Journal of Applied Ecology* **49**: 251–260. doi:10.1111/j.1365-2664.2011.02086.x.
- Bain JF. 1991.** The biology of Canadian weeds: 96 *Senecio jacobaea* L. *Canadian Journal of Plant Science* **71**: 127–140.
- Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. 2006.** The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual review of plant biology* **57**: 233–266. doi:10.1146/annurev.arplant.57.032905.105159.
- Bakker JP, Vries Y. 1992.** Germination and early establishment of lower salt-marsh species in grazed and mown salt marsh. *Journal of Vegetation Science* **3**: 247–252. doi:10.2307/3235686.
- Baskin CC, Baskin JM. 2014.** *Seeds: ecology, biogeography, and evolution of dormancy and germination*, 2nd edn. Amsterdam: Elsevier.
- Begon M, Townsend CR, Harper JL. 2011.** *Ecology: From Individuals to Ecosystems*, 4th edn.: Blackwell Publishing.
- Bezemer TM, Harvey JA, Kowalchuk GA, Kopershoek H, van der Putten WH. 2006a.** Interplay between *Senecio jacobaea* and plant, soil, and aboveground insect community composition. *Ecology* **87**: 2002–2013.
- Bezemer TM, van der Putten WH, Rienks F. 2006b.** Niets doen loont bij Jakobskruiskruidplaag. *De Levende Natuur* **107**: 214–216.
- Blondel J, Hoffmann B, Courchamp F. 2014.** The end of Invasion Biology: Intellectual debate does not equate to nonsensical science. *Biological Invasions* **16**: 977–979. doi:10.1007/s10530-013-0560-6.
- Bonanomi G, Sicurezza MG, Caporaso S, Esposito A, Mazzoleni S. 2006.** Phytotoxicity dynamics of decaying plant materials. *New Phytologist* **169**: 571–578. doi:10.1111/j.1469-8137.2005.01611.x.
- Braun-Blanquet J. 1932.** *Plant Sociology: The Study of Plant Communities*. New York: McGraw-Hill.
- Bretz F, Hothorn T, Westfall P. 2011.** *Multiple comparisons using R*. London: Chapman and Hall/CRC.
- Briemle G. 2007.** Empfehlungen zu Erhalt und Management von Extensiv- und Biotopgrünland. *landinfo* **2**: 16–22.
- Bullock JM. 2000.** Gaps and seedling colonization. In: Fenner M, ed. *Seeds: The Ecology of Regeneration in Plant Communities*. Wallingford, UK: CAB International.
- Bünemann EK, Schwenke GD, van Zwieten L. 2006.** Impact of agricultural inputs on soil organisms—a review. *Soil Research*. **44**: 379. doi:10.1071/SR05125.

- Buttler KP, Thieme M, Mitarbeiter. 2017.** *Florenliste von Deutschland – Gefäßpflanzen: Version 9.* <http://www.kp-buttler.de>.
- Cameron E. 1935.** A study of the natural control of ragwort (*Senecio jacobaea* L.). *Journal of Ecology* **23**: 265–322.
- Candeias M, Warren RJ. 2016.** Rareness starts early for disturbance-dependent grassland plant species. *Biodiversity and Conservation* **25**: 2771–2785. doi:10.1007/s10531-016-1202-y.
- Carboni M, Dengler J, Mantilla-Contreras J, Venn S, Török P. 2015.** Conservation value, management and restoration of Europe’s semi-natural open landscapes. *Hacquetia* **14**: 5–17. doi:10.1515/hacq-2015-0017.
- Carey MP, Sanderson BL, Barnas KA, Olden JD. 2012.** Native invaders – challenges for science, management, policy, and society. *Frontiers in Ecology and the Environment* **10**: 373–381. doi:10.1890/110060.
- Carrol RJ, Ruppert D. 1988.** *Transformation and Weighting in Regression.* New York: Chapman and Hall.
- Carvalho S, Macel M, Mulder PPJ, Skidmore A, van der Putten WH. 2014.** Chemical variation in *Jacobaea vulgaris* is influenced by the interaction of season and vegetation successional stage. *Phytochemistry* **99**: 86–94. doi:10.1016/j.phytochem.2013.12.004.
- Caswell H. 2001.** *Matrix Population Models: Construction, Analysis, and Interpretation*, Second Edition. Massachusetts: Inc. Publishers.
- Catford JA, Jansson R, Nilsson C. 2009.** Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. *Diversity and Distributions* **15**: 22–40. doi:10.1111/j.1472-4642.2008.00521.x.
- Cavieres LA, Peñaloza A. 2012.** Facilitation and interference at the intraspecific level: Recruitment of *Kageneckia angustifolia* D. Don (Rosaceae) in the montane sclerophyllous woodland of central Chile. *Perspectives in Plant Ecology, Evolution and Systematics* **14**: 13–19. doi:10.1016/j.ppees.2011.09.003.
- Cesarano G, Zotti M, Antignani V, Marra R, Scala F, Bonanomi G. 2017.** Soil sickness and negative plant-soil-feedback: A reappraisal of hypotheses. *Journal of Plant Pathology* **99**: 545–570. doi:10.4454/jpp.v99i3.3960.
- Cheplick GP. 1992.** Sibling Competition in Plants. *Journal of Ecology* **80**: 567–575.
- Chon S-U, Kim Y-M. 2004.** Herbicidal potential and quantification of suspected allelochemicals from four grass crop extracts. *Journal of Agronomic Crop Science* **190**: 145–150. doi:10.1111/j.1439-037X.2004.00088.x.
- Coombs EM, Radtke H, Isaacson D, Snyder SP. 1996.** Economic and regional benefits from the biological control of tansy ragwort, *Senecio jacobaea*, in Oregon. *Proceedings of the IX international symposium on biological control of weeds*: 489–494.
- Crawley M, Nachapong M. 1985.** The establishment of seedlings from primary and regrowth seeds of ragwort (*Senecio jacobaea*). *Journal of Ecology* **73**: 255–261.
- Crawley MJ, Gillman MP. 1989.** Population dynamics of cinnabar moth and ragwort in grassland. *Journal of Animal Ecology* **58**: 1035–1050.
- da Silva ER, Overbeck GE, Soares, Geraldo Luiz Gonçalves. 2017.** Something old, something new in allelopathy review: What grassland ecosystems tell us. *Chemoecology* **27**: 217–231. doi:10.1007/s00049-017-0249-x.
- Dale VH. 1997.** The relationship between land-use change and climate change. *Ecological Applications* **7**: 753–769.
- Dauer JT, McEvoy PB, van Sickle J. 2012.** Controlling a plant invader by targeted disruption of its life cycle. *Journal of Applied Ecology* **49**: 322–330. doi:10.1111/j.1365-2664.2012.02117.x.

- de Boer NJ. 1999.** Pyrrolizidine alkaloid distribution in *Senecio jacobaea* rosettes minimises losses to generalist feeding. *Entomologia Experimentalis et Applicata* **91**: 169–173. doi:10.1046/j.1570-7458.1999.00480.x.
- DiTomaso JM. 2000.** Invasive weeds in rangelands: Species, impacts, and management. *Weed Science* **48**: 255–265. doi:10.1614/0043-1745(2000)048[0255:IWIRSI]2.0.CO;2.
- Donath TW, Eckstein RL. 2008.** Grass and oak litter exert different effects on seedling emergence of herbaceous perennials from grasslands and woodlands. *Journal of Ecology* **96**: 272–280. doi:10.1111/j.1365-2745.2007.01338.x.
- Donath TW, Eckstein RL. 2010.** Effects of bryophytes and grass litter on seedling emergence vary by vertical seed position and seed size. *Plant Ecology* **207**: 257–268.
- Donath TW, Hölzel N, Bissels S, Otte A. 2004.** Perspectives for incorporating biomass from non-intensively managed temperate flood-meadows into farming systems. *Agriculture, Ecosystems & Environment* **104**: 439–451. doi:10.1016/j.agee.2004.01.039.
- Donath TW, Hölzel N, Otte A. 2006.** Influence of competition by sown grass, disturbance and litter on recruitment of rare flood-meadow species. *Biological Conservation* **130**: 315–323. doi:10.1016/j.biocon.2005.12.022.
- Doorduyn LJ, Vrieling K. 2011.** A review of the phytochemical support for the shifting defence hypothesis. *Phytochemistry reviews proceedings of the Phytochemical Society of Europe* **10**: 99–106. doi:10.1007/s11101-010-9195-8.
- Dyer AR. 2004.** Maternal and sibling factors induce dormancy in dimorphic seed pairs of *Aegilops triuncialis*. *Plant Ecology* **172**: 211–218. doi:10.1023/B:VEGE.0000026339.61069.33.
- Eckstein RL, Donath TW. 2005.** Interactions between litter and water availability affect seedling emergence in four familial pairs of floodplain species. *Journal of Ecology* **93**: 807–816. doi:10.1111/j.1365-2745.2005.01015.x.
- Eckstein RL, Ruch D, Otte A, Donath TW. 2012.** Invasibility of a nutrient-poor pasture through resident and non-resident herbs is controlled by litter, gap size and propagule pressure. *PLoS one* **7**: e41887. doi:10.1371/journal.pone.0041887.
- Edwards ME, Harris EM, Wagner FH, Cross MC, Miller GS. 1988.** Seed germination of American Pokeweed (*Phytolacca americana*): Laboratory techniques and autotoxicity. *American Journal of Botany* **75**: 1794–1802.
- Eichberg C, Donath TW. 2017.** Sheep trampling on surface-lying seeds improves seedling recruitment in open sand ecosystems. *Restoration Ecology* **26**: S211–S219. doi:10.1111/rec.12650.
- Eisele N, Tonn B., Pekrun C., Elsaesser M. 2011.** Influence of different cutting dates on regrowth and achene germination capacity of *Senecio jacobaea*. In: Pötsch, E. M., Krautzer, B. & Hopkins, A. (Eds.): *Grassland Science in Europe*, 196–198.
- Ellenberg H, Leuschner C. 2010.** *Vegetation Mitteleuropas mit den Alpen: In ökologischer, dynamischer und historischer Sicht*. Stuttgart: Ulmer.
- Facelli JM, Montero CM, Leon RJC. 1988.** Effect of different disturbance regimen on seminatural grasslands from the subhumid pampa. *Flora - Morphology, Distribution, Functional Ecology of Plants* **180**: 241–249.
- Facelli JM, Pickett STA. 1991.** Plant litter: Its dynamics and effects on plant community structure. *The Botanical Review* **57**: 1–32.
- Faust C, Eichberg C, Storm C, Schwabe A. 2011.** Post-dispersal impact on seed fate by livestock trampling – A gap of knowledge. *Basic and Applied Ecology* **12**: 215–226. doi:10.1016/j.baae.2011.02.009.
- Fenner M. 1983.** Relationships between seed weight, ash content and seedling growth in twenty-four species of Compositae. *New Phytologist* **95**: 697–706.

- Foster BL, Murphy CA, Keller KR, Aschenbach TA, Questad EJ, Kindscher K. 2007.** Restoration of prairie community structure and ecosystem function in an abandoned hayfield: A sowing experiment. *Restoration Ecology* **15**: 652–661. doi:10.1111/j.1526-100X.2007.00277.x.
- Fowler SV, Gourlay AH, Hill R. 2016.** Biological control of ragwort in the New Zealand dairy sector: An ex-post economic analysis. *New Zealand Journal of Agricultural Research* **59**: 205–215. doi:10.1080/00288233.2016.1170050.
- Franco M, Silvertown J. 2004.** A comparative demography of plants based upon elasticities of vital rates. *Ecology* **85**: 531–538. doi:10.1890/02-0651.
- Fritch RA, Sheridan H, Finn JA, Kirwan L, hUallacháin DÓ. 2011.** Methods of enhancing botanical diversity within field margins of intensively managed grassland: A 7-year field experiment. *Journal of Applied Ecology* **48**: 551–560. doi:10.1111/j.1365-2664.2010.01951.x.
- Funk JL, Cleland EE, Suding KN, Zavaleta ES. 2008.** Restoration through reassembly: Plant traits and invasion resistance. *Trends in Ecology & Evolution* **23**: 695–703. doi:10.1016/j.tree.2008.07.013.
- Gartner TB, Cardon ZG. 2004.** Decomposition dynamics in mixed-species leaf litter. *Oikos* **104**: 230–246.
- Gitzendanner MA, Soltis PS. 2000.** Patterns of genetic variation in rare and widespread plant congeners. *American Journal of Botany* **87**: 783–792. doi:10.2307/2656886.
- Gomes MP, Garcia QS, Barreto LC, Pimenta LPS, Matheus MT, Figueredo CC. 2017.** Allelopathy: An overview from micro- to macroscopic organisms, from cells to environments, and the perspectives in a climate-changing world. *Biologia* **72**: 113–129. doi:10.1515/biolog-2017-0019.
- Gottschalk C, Huckauf A, Dübecke A, et al. 2018.** Uncertainties in the determination of pyrrolizidine alkaloid levels in naturally contaminated honeys and comparison of results obtained by different analytical approaches. *Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment* **35**: 1366–1383. doi:10.1080/19440049.2018.1468929.
- Grime JP. 1998.** Benefits of plant diversity to ecosystems: immediate, filter and founder effects. *Journal of Ecology* **86**: 902–910.
- Grubb PJ. 1977.** The maintenance of species-richness in plant communities: The importance of the regeneration niche. *Biological Reviews* **52**: 107–145. doi:10.1111/j.1469-185x.1977.tb01347.x.
- Gruber E, Whytemare A. 1997.** The return of the native?: *Sidalecea hirtipes* in coastal Oregon. In: Kaye TN, Liston A, Love RM, Luoma DL, Meinke RI, Wilson MV, eds. *Conservation and management of native plants and fungi: Proceedings of an Oregon Conference on the Conservation and Management of Native Vascular Plants, Bryophytes, and Fungi*. Corvallis, Oregon, 121–124.
- Harper JL, Wood WA. 1957.** Biological Flora of the British Isles: *Senecio jacobaea*. *Journal of Ecology* **45**: 617–637.
- Hasler M, Hothorn LA. 2008.** Multiple contrast tests in the presence of heteroscedasticity. *Biometrical Journal* **50**: 793–800. doi:10.1002/bimj.200710466.
- Hawkes RB, Johnson GR. 1978.** *Longitarsus jacobaeae* aids moth in the biological control of tansy ragwort. In: Freeman TE, ed. *Proceedings of the IV international symposion on biological control of weeds*.
- Hess MCM, Mesléard F, Buisson E. 2019.** Priority effects: Emerging principles for invasive plant species management. *Ecological Engineering* **127**: 48–57. doi:10.1016/j.ecoleng.2018.11.011.
- Heydemann B. 1997.** *Neuer biologischer Atlas: Ökologie für Schleswig-Holstein und Hamburg*. Neumünster: Wachholtz Verlag.
- Hierro JL, Callaway RM. 2003.** Allelopathy and exotic plant invasion. *Plant and Soil* **256**: 29–39. doi:10.1023/A:1026208327014.
- Holly K, Woodford EK, Blackman GE. 1952.** The control of some perennial weeds in permanent grassland by selective herbicides. *Agriculture* **59**: 19–32.

- Hölzel N, Otte A. 2003.** Restoration of a species-rich flood meadow by topsoil removal and diaspora transfer with plant material. *Applied Vegetation Science* **6**: 131–140. doi:10.1111/j.1654-109X.2003.tb00573.x.
- Hölzel N, Otte A. 2004.** Ecological significance of seed germination characteristics in flood-meadow species. *Flora - Morphology, Distribution, Functional Ecology of Plants* **199**: 12–24. doi:10.1078/0367-2530-00132.
- Hood GM. 2011.** *POPTOOLS*. Canberra.
- Hovstad KA, Ohlson M. 2008.** Physical and chemical effects of litter on plant establishment in semi-natural grasslands. *Plant Ecology* **196**: 251–260. doi:10.1007/s11258-007-9349-y.
- Hovstad KA, Ohlson M. 2009.** Conspecific versus heterospecific litter effects on seedling establishment. *Plant Ecology* **204**: 33–42.
- Huckauf A, Rabe I, Aboling S, et al. 2017.** *Umgang mit dem Jakobs-Kreuzkraut: Meiden - dulden - bekämpfen*, 4., überarbeitete Auflage, August 2017. Flintbek, Molfsee: Landesamt für Landwirtschaft Umwelt und ländliche Räume des Landes Schleswig-Holstein (LLUR); Stiftung Naturschutz Schleswig-Holstein.
- Hüls J, Otte A, Eckstein RL. 2007.** Population life-cycle and stand structure in dense and open stands of the introduced tall herb *Heracleum mantegazzianum*. *Biological Invasions* **9**: 799–811. doi:10.1007/s10530-006-9082-9.
- Humbert J-Y, Ghazoul J, Walter T. 2009.** Meadow harvesting techniques and their impacts on field fauna. *Agriculture, Ecosystems & Environment* **130**: 1–8.
- Hutchings MJ, Booth KD. 1996.** Studies on the feasibility of re-creating chalk grassland vegetation on ex-arable land: I. The potential roles of the seed bank and the seed rain. *Journal of Applied Ecology* **33**: 1171–1181.
- Inderjit, Duke SO. 2003.** Ecophysiological aspects of allelopathy. *Planta* **217**: 529–539. doi:10.1007/s00425-003-1054-z.
- ISTA. 2015.** Chapter 6: Biochemical test for viability: the topographical tetrazolium test. *International rules of seed testing* **2015**: 6. doi:10.15258/istarules.2015.06.
- Jacobs J, Sing S. 2009.** *Ecology and management of tansy ragwort (Senecio jacobaea L.)*. Montana.
- Jacobs JS, Sing SE, Martin JM. 2006.** Influence of herbivory and competition on invasive weed fitness: Observed effects of *Cyphocleonus achates* (Coleoptera: Curculionidae) and grass-seeding treatments on spotted knapweed performance. *Environmental Entomology* **35**: 1590–1596.
- Jäger EJ(H). 2017.** *Rothmaler - Exkursionsflora von Deutschland: Gefäßpflanzen: Grundband*, 21st edn. Berlin: Springer Spektrum.
- James RR, McEvoy PB, Cox CS. 1992.** Combining the cinnabar moth (*Tyria jacobaeae*) and the ragwort flea beetle (*Longitarsus jacobaeae*) for control of ragwort (*Senecio jacobaea*): An experimental analysis. *Journal of Applied Ecology* **29**: 589–596.
- Janicka M, Pawluśkiewicz B. 2020.** The increasing in the floristic diversity of the abandoned Arrhenatherion elatioris meadows by dicotyledonous species oversowing. *Journal of Ecological Engineering* **21**: 168–179. doi:10.12911/22998993/112679.
- Jensen K, Gutekunst K. 2003.** Effects of litter on establishment of grassland plant species: The role of seed size and successional status. *Basic and Applied Ecology* **4**: 579–587. doi:10.1078/1439-1791-00179.
- Johnson AE, Molyneux RJ, Merrill GB. 1985.** Chemistry of toxic range plants.: Variation in pyrrolizidine alkaloid content of *Senecio*, *Amsinckia*, and *Crotalaria* species. *Journal of agricultural and food chemistry* **33**: 50–55. doi:10.1021/jf00061a015.
- Jongepierová I, Mitchley J, Tzanopoulos J. 2007.** A field experiment to recreate species rich hay meadows using regional seed mixtures. *Biological Conservation* **139**: 297–305. doi:10.1016/j.biocon.2007.07.026.

- Joshi J, Vrieling K. 2005.** The enemy release and EICA hypothesis revisited: Incorporating the fundamental difference between specialist and generalist herbivores. *Ecology Letters* **8**: 704–714. doi:10.1111/j.1461-0248.2005.00769.x.
- Jung LS, Eckstein RL, Otte A, Donath TW. 2012.** Above- and below-ground nutrient and alkaloid dynamics in *Colchicum autumnale*: Optimal mowing dates for population control or low hay toxicity. *Weed Research* **52**: 348–357. doi:10.1111/j.1365-3180.2012.00923.x.
- Jung LS, Winter S, Kriechbaum M, Eckstein RL, Donath TW, Otte A. 2010.** Regulation of meadow saffron (*Colchicum autumnale* L.) in extensively managed grasslands. *Grassland Science in Europe*: 660–662.
- Kaltner F, Rychlik M, Gareis M, Gottschalk C. 2018.** Influence of storage on the stability of toxic pyrrolizidine alkaloids and their N-Oxides in peppermint tea, hay, and honey. *Journal of agricultural and food chemistry* **66**: 5221–5228. doi:10.1021/acs.jafc.7b06036.
- Kassebeer C. 2015.** *Erfassung phytophager Insekten an Jakobs-Kreuzkraut in Schleswig-Holstein*. Damlos.
- Keller LF, Waller DM. 2002.** Inbreeding effects in wild populations. *Trends in Ecology & Evolution* **17**: 230–241. doi:10.1016/S0169-5347(02)02489-8.
- Kempel A, Vincent H, Prati D, Fischer M, Taylor A. 2020.** Context dependency of biotic interactions and its relation to plant rarity. *Diversity and Distributions* **26**: 758–768. doi:10.1111/ddi.13050.
- Kiehl K, Kirmer A, Donath TW, Rasran L, Hölzel N. 2010.** Species introduction in restoration projects: Evaluation of different techniques for the establishment of semi-natural grasslands in Central and Northwestern Europe. *Basic and Applied Ecology* **11**: 285–299. doi:10.1016/j.baae.2009.09.002.
- Kiehl K, Kirmer A, Shaw NL, Tischew S. 2014.** *Guidelines for native seed production and grassland restoration*. Newcastle upon Tyne: Cambridge Scholars Publishing.
- Kitajima K, Fenner M. 2000.** Ecology of seedling regeneration. In: Fenner M, ed. *Seeds – the ecology of regeneration in plant communities*. Wallingford: CABI Publishing, 331–359.
- Klapp E. 1965.** *Grünlandvegetation und Standort: nach Beispielen aus West-, Mittel- und Süddeutschland*. Berlin: Parey.
- Kleijn D, Kohler F, Báldi A, et al. 2009.** On the relationship between farmland biodiversity and land-use intensity in Europe. *Proceedings in Biological sciences* **276**: 903–909. doi:10.1098/rspb.2008.1509.
- Kollmann J, Kirmer A, Tischew S, Hölzel N, Kiehl K. 2019.** *Renaturierungsökologie*. Berlin: Springer Spektrum.
- Körner C, Stöcklin J, Reuther-Thiébaud L, Pelaez-Riedl S. 2008.** Small differences in arrival time influence composition and productivity of plant communities. *New phytologist* **177**: 698–705. doi:10.1111/j.1469-8137.2007.02287.x.
- Kulmatiski A, Beard KH, Stark JM. 2006.** Soil history as a primary control on plant invasion in abandoned agricultural fields. *Journal of Applied Ecology* **43**: 868–876. doi:10.1111/j.1365-2664.2006.01192.x.
- Laird NM, Ware JH. 1982.** Random-effects models for longitudinal data. *Biometrics* **38**: 963–974.
- Lapiedra O, Sol D, Traveset A, Vilà M. 2015.** Random processes and phylogenetic loss caused by plant invasions. *Global Ecology and Biogeography* **24**: 774–785. doi:10.1111/geb.12310.
- Lawson CS, Ford MA, Mitchley J. 2004.** The influence of seed addition and cutting regime on the success of grassland restoration on former arable land. *Applied Vegetation Science* **7**: 295–266.
- Lazenby A. 1955.** Germination and establishment of *Juncus effusus* L.: The effect of different companion species and of variation in soil and fertility conditions. *Journal of Ecology* **43**: 103–119.

- Leishman MR, Wright IJ, Moles AT, Westoby M. 2000.** The evolutionary ecology of seed size. In: Fenner M, ed. *Seeds: The ecology of regeneration in plant communities*. Wallingford: CAB International, 31–57.
- Leiss KA. 2011.** Management practices for control of ragwort species. *Phytochemistry Reviews* **10**: 153–163. doi:10.1007/s11101-010-9173-1.
- Lennartsson T, Oostermeijer JGB. 2001.** Demographic variation and population viability in *Gentianella campestris*: Effects of grassland management and environmental stochasticity. *Journal of Ecology* **89**: 451–463.
- Leuschner C, Ellenberg H. 2017.** *Ecology of Central European Non-Forest Vegetation: Coastal to Alpine, Natural to Man-Made Habitats*: Springer.
- Leuschner C, Wesche K, Meyer S, et al. 2013.** Veränderungen und Verarmung in der Offenlandvegetation Norddeutschlands seit den 1950er Jahren: Wiederholungsaufnahmen in Äckern, Grünland und Fließgewässern. *Berichte der Rheinhold-Tüxen-Gesellschaft* **25**: 166–182.
- Levine JM, Adler PB, Yelenik SG. 2004.** A meta-analysis of biotic resistance to exotic plant invasions. *Ecology Letters* **7**: 975–989. doi:10.1111/j.1461-0248.2004.00657.x.
- Loydi A, Donath TW, Eckstein RL, Otte A. 2015a.** Non-native species litter reduces germination and growth of resident forbs and grasses: allelopathic, osmotic or mechanical effects? *Biological Invasions* **17**: 581–595.
- Loydi A, Donath TW, Otte A, Eckstein RL. 2015b.** Negative and positive interactions among plants: Effects of competitors and litter on seedling emergence and growth of forest and grassland species. *Plant Biology* **17**: 667–675. doi:10.1111/plb.12287.
- Loydi A, Eckstein RL, Otte A, Donath TW, Gómez-Aparicio L. 2013.** Effects of litter on seedling establishment in natural and semi-natural grasslands: A meta-analysis. *Journal of Ecology* **101**: 454–464. doi:10.1111/1365-2745.12033.
- Loydi A, Lohse K, Otte A, Donath TW, Eckstein RL. 2014.** Distribution and effects of tree leaf litter on vegetation composition and biomass in a forest–grassland ecotone. *Journal of Plant Ecology* **7**: 264–275. doi:10.1093/jpe/rtt027.
- Ludewig K, Zelle B, Eckstein RL, Mosner E, Otte A, Donath TW. 2014.** Differential effects of reduced water potential on the germination of floodplain grassland species indicative of wet and dry habitats. *Seed Science Research* **24**: 49–61. doi:10.1017/S096025851300038X.
- Luken JO, Thieret JW. 1997.** *Assessment and management of plant invasions*. New York: Springer.
- Marrs RH. 1993.** Soil Fertility and Nature Conservation in Europe: Theoretical Considerations and Practical Management Solutions. In: Begon M, Fitter AH, eds. *Advances in Ecological Research*: Academic Press, 241–300.
- Maskell LC, Henrys P, Pescott OL, Smart SM, Acosta ATR. 2020.** Long-term trends in the distribution, abundance and impact of native “injurious” weeds. *Applied Vegetation Science* **39**: 1–13. doi:10.1111/avsc.12518.
- Matarczyk JA, Willis AJ, Vranjic JA, Ash JE. 2002.** Herbicides, weeds and endangered species: management of bitou bush (*Chrysanthemoides monilifera* ssp. *rotundata*) with glyphosate and impacts on the endangered shrub, *Pimelea spicata*. *Biological Conservation* **108**: 133–141.
- McCune B, Grace JB, Urban DL. 2002.** *Analysis of Ecological Communities*. Glendeden Beach: MjM Software Design.
- McEvoy PB. 1984.** Seedling dispersion and the persistence of ragwort *Senecio jacobaea* (Compositae) in a grassland dominated by perennial species. *Oikos* **42**: 138–143.
- McEvoy PB. 1985.** Depression in ragwort (*Senecio jacobaea*) abundance following introduction of *Tyria jacobaeae* and *Longitarsus jacobaeae* on the central coast of Oregon. *Proceedings of the VI international symposium on biological control of weeds*: 57–64.

- McEvoy PB, Cox C, Coombs E. 1991.** Successful biological control of ragwort, *Senecio jacobaea*, by introduced insects in Oregon. *Ecological Applications* **1**: 430–442.
- McEvoy PB, Cox CS. 1987.** Wind dispersal distances in dimorphic achenes of ragwort, *Senecio jacobaea*. *Ecology* **68**: 2006–2015.
- McEvoy PB, Cox CS, James RR, Rudd NT. 1988.** Ecological mechanisms underlying successful biological weed control: Field experiments with ragwort, *Senecio jacobaea*. In: Delfosse ES, ed. *Proceedings of the VII international symposium on the biological control of weeds*. Melbourne, Australia: Commonwealth Scientific and Industrial Research Organization, 55–66.
- McEvoy PB, Rudd NT. 1993.** Effects of vegetation disturbances on insect biological control of tansy ragwort, *Senecio jacobaea*. *Ecological Applications* **3**: 682–698.
- McLaren D, Faithfull I. 2004.** *Landcare notes: Ragwort - Management*.
- McNaughton SJ. 1968.** Autotoxic feedback in relation to germination and seedling growth in *Typha latifolia*. *Ecology* **49**: 367–369. doi:10.2307/1934475.
- Meiners SJ, Pickett STA, Cadenasso ML. 2001.** Effects of plant invasions on the species richness of abandoned agricultural land. *Ecography* **24**: 633–644. doi:10.1034/j.1600-0587.2001.240602.x.
- Mierwald U. 2006.** *Die Farn- und Blütenpflanzen Schleswig-Holsteins. Rote Liste., 4. Fassung, Datenstand Dez. 2005*. Flintbek.
- Milberg P, Andersson L, Thompson K. 2000.** Large-seeded species are less dependent on light for germination than small-seeded ones. *Seed Science Research* **10**: 99–104. doi:10.1017/S0960258500000118.
- Möhler H, Diekötter T, Herrmann JD, Donath TW. 2018.** Allelopathic vs. autotoxic potential of a grassland weed—evidence from a seed germination experiment. *Plant Ecology & Diversity* **11**: 539–549. doi:10.1080/17550874.2018.1541487.
- Montesinos D, García-Fayos P, Mateu I. 2006.** Conflicting selective forces underlying seed dispersal in the endangered plant *Silene diclinis*. *International Journal of Plant Science* **167**: 103–110.
- Mühlenberg M. 1989.** *Freilandökologie*. Heidelberg: Quelle & Meyer.
- Myster RW. 2006.** Species-specific effects of grass litter mass and type on emergence of three tall grass prairie species. *Ecoscience* **13**: 95–99.
- Nakagawa S, Schielzeth H. 2013.** A general and simple method for obtaining R² from generalized linear mixed-effects models. *Methods in Ecology and Evolution* **4**: 133–142. doi:10.1111/j.2041-210x.2012.00261.x@10.1111/(ISSN)2041-210X.TOPMETHODS.
- Neitzke A, Berendonk C. 2011.** *Jakobskreuzkraut (Senecio jacobaea): Eine Giftpflanze auf dem Vormarsch*, 3rd edn.
- Neumann H, Huckauf A. 2016.** Tansy ragwort (*Senecio jacobaea*): A source of pyrrolizidine alkaloids in summer honey? *Journal für Verbraucherschutz und Lebensmittelsicherheit* **11**: 105–115. doi:10.1007/s00003-015-0986-0.
- Oakley CA, Knox JS, Symstad A. 2013.** Plant species richness increases resistance to invasion by non-resident plant species during grassland restoration. *Applied Vegetation Science* **16**: 21–28. doi:10.1111/j.1654-109X.2012.01202.x.
- Oberdorfer E. 1990.** *Pflanzensoziologische Exkursionsflora*, 6th edn. Stuttgart: E. Ulmer.
- O'Dell RE, Young SL, Claassen VP. 2007.** Native roadside perennial grasses persist a decade after planting in the Sacramento Valley. *California Agriculture* **61**: 79–84.
- Olson BE, Wallander RT. 2002.** Effects of invasive forb litter on seed germination, seedling growth and survival. *Basic and Applied Ecology* **3**: 309–317. doi:10.1078/1439-1791-00127.
- Otzen D. 1977.** Life forms of three *Senecio* species in relation to accumulation and utilization of nonstructural carbohydrates. *Acta Botanica Neerlandica* **26**: 401–409.

- Pakeman RJ, Small JL, Le Duc MG, Marrs RH. 2005.** Recovery of moorland vegetation after aerial spraying of bracken (*Pteridium aquilinum* (L.) Kuhn) with Asulam. *Restoration Ecology* **13**: 718–724. doi:10.1111/j.1526-100X.2005.00091.x.
- Pal RW, Chen S, Nagy DU, Callaway RM. 2015.** Impacts of *Solidago gigantea* on other species at home and away. *Biological Invasions* **17**: 3317–3325. doi:10.1007/s10530-015-0955-7.
- Pausas JG, Ouadah N, Ferran A, Gimeno T, Vallejo R. 2003.** Fire severity and seedling establishment in *Pinus halepensis* woodlands, eastern Iberian Peninsula. *Plant Ecology* **169**: 205–213.
- Peeters A. 2012.** Past and future of European grasslands.: The challenge of the CAP towards 2020. In: Goliński P, Warda M, Stypiński P, eds. *Grassland - a European Resource?: Proceedings of the 24th General Meeting of the European Grassland Federation, Lublin, Poland, 03 - 07 June 2012*. Poznań: Garmond Oficyna Wydawnicza, 17–32.
- Perry LG, Thelen GC, Ridenour WM, et al. 2005.** Dual role for an allelochemical: (±)-catechin from *Centaurea maculosa* root exudates regulates conspecific seedling establishment. *Journal of Ecology* **93**: 1126–1135.
- Picman J, Picman AK. 1984.** Autotoxicity in *Parthenium hysterophorus* and its possible role in control of germination. *Biochemical Systematics and Ecology* **12**: 287–292.
- Pleasants JM, Jurik TW. 1992.** Dispersion of seedlings of the prairie compass plant, *Silphium laciniatum* (Asteraceae). *American Journal of Botany* **79**: 133–137.
- Pullin AS, Knight TM, Stone DA, Charman K. 2004.** Do conservation managers use scientific evidence to support their decision-making? *Biological Conservation* **119**: 245–252. doi:10.1016/j.biocon.2003.11.007.
- Pywell RF, Bullock JM, Hopkins A, et al. 2002.** Restoration of species-rich grassland on arable land: Assessing the limiting processes using a multi-site experiment. *Journal of Applied Ecology* **39**: 294–309. doi:10.1046/j.1365-2664.2002.00718.x.
- Pywell RF, Bullock JM, Tallowin JB, Walker KJ, Warman EA, Masters G. 2007.** Enhancing diversity of species-poor grasslands: An experimental assessment of multiple constraints. *Journal of Applied Ecology* **44**: 81–94. doi:10.1111/j.1365-2664.2006.01260.x.
- Pywell RF, Hayes MJ, Tallowin JB, et al. 2010.** Minimizing environmental impacts of grassland weed management: Can *Cirsium arvense* be controlled without herbicides? *Grass and Forage Science* **65**: 159–174. doi:10.1111/j.1365-2494.2010.00735.x.
- Quested H, Eriksson O. 2006.** Litter species composition influences the performance of seedlings of grassland herbs. *Functional Ecology* **20**: 522–532.
- Quintana J, Cruz A, Fernández-González F, Moreno JM. 2004.** Time of germination and establishment success after fire of three obligate seeders in a Mediterranean shrubland of central Spain. *Journal of Biogeography* **31**: 241–249.
- R Core Team. 2016.** *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. Vienna, Austria: R.
- R Core Team. 2019.** *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Rabotnov TA. 1982.** Importance of the evolutionary approach to the study of allelopathy. *Ekologia* **12**: 5–8.
- Ranal MA, Santana DGd. 2006.** How and why to measure the germination process? *Brazilian Journal of Botany* **29**: 1–11. doi:10.1590/S0100-84042006000100002.
- Ranal MA, Santana DGd, Ferreira WR, Mendes-Rodrigues C. 2009.** Calculating germination measurements and organizing spreadsheets. *Brazilian Journal of Botany* **32**: 849–855.
- Rasran L, Jeromin H. 2010.** Problempflanzen im Fokus des Naturschutzmanagements von Dauergrünlandflächen (Literaturstudie). *Berichte der Deutschen Gesellschaft für Moor- und Torfkunde* **40**: 119–136.

- Razavi SM. 2011.** Plant coumarins as allelopathic agents. *International Journal of Biological Chemistry* **5**: 86–90.
- Reigosa MJ, Pedrol N, González L. 2006.** *Allelopathy: A physiological process with ecological implications*. Dordrecht: Springer.
- Reinhart KO, Maestre FT, Callaway RM. 2006.** Facilitation and inhibition of seedlings of an invasive tree (*Acer platanoides*) by different tree species in a mountain ecosystem. *Biological Invasions* **8**: 231–240.
- Relyea RA. 2005.** The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecological Applications* **15**: 618–627.
- Renne IJ, Sinn BT, Shook GW, et al. 2014.** Eavesdropping in plants: Delayed germination via biochemical recognition. *Journal of Ecology* **102**: 86–94. doi:10.1111/1365-2745.12189.
- Renne IJ, Tracy BF, Colonna IA. 2006.** Shifts in grassland invasibility: Effects of soil resources, disturbance, composition, and invader size. *Ecology* **87**: 2264–2277. doi:10.1890/0012-9658(2006)87[2264:SIGIEO]2.0.CO;2.
- Roberts PD, Pullin AS. 2007.** The effectiveness of management interventions used to control ragwort species. *Environmental management* **39**: 691–706. doi:10.1007/s00267-006-0039-7.
- Roll J, Mitchell RJ, Cabin RJ, Marshall DL. 1997.** Reproductive success increases with local density of conspecifics in a desert mustard (*Lesquerella fendleri*). *Conservation Biology* **11**: 738–746.
- Rühl AT, Donath TW, Otte A, Eckstein RL. 2016a.** Impacts of short-term germination delay on fitness of the annual weed *Agrostemma githago* (L.). *Seed Science Research* **26**: 93–100. doi:10.1017/S0960258516000106.
- Rühl AT, Eckstein RL, Otte A, Donath TW, Bekker R. 2016b.** Distinct germination response of endangered and common arable weeds to reduced water potential. *Plant Biology* **18**: 83–90. doi:10.1111/plb.12331.
- Ruprecht E, Donath TW, Otte A, Lutz Eckstein R. 2008.** Chemical effects of a dominant grass on seed germination of four familial pairs of dry grassland species. *Seed Science Research* **18**: 239–248. doi:10.1017/S0960258508096190.
- Schindelin J, Rueden CT, Hiner MC, Eliceiri KW. 2015.** The ImageJ ecosystem: An open platform for biomedical image analysis. *Molecular Reproduction and Development* **82**: 518–529.
- Schmidl L. 1972.** Biology and control of ragwort, *Senecio jacobaea* L., in Victoria, Australia. *Weed Research* **12**: 37–45. doi:10.1111/j.1365-3180.1972.tb01185.x.
- Schmidt, Jürgen, Petersen W. 2019.** *Kartieranleitung und Biototypenschlüssel für die Biotopkartierung Schleswig-Holstein: mit Hinweisen zu den gesetzlich geschützten Biotopen sowie den Lebensraumtypen gemäß Anhang I der FFH-Richtlinie*, 5th edn. Flintbek.
- Schmiede R, Otte A, Donath TW. 2012.** Enhancing plant biodiversity in species-poor grassland through plant material transfer – the impact of sward disturbance. *Applied Vegetation Science* **15**: 290–298. doi:10.1111/j.1654-109X.2011.01168.x.
- Schmitz J, Hahn M, Brühl CA. 2014.** Agrochemicals in field margins – An experimental field study to assess the impacts of pesticides and fertilizers on a natural plant community. *Agriculture, Ecosystems & Environment* **193**: 60–69. doi:10.1016/j.agee.2014.04.025.
- Schuster MJ, Kreyling J, Berwaers S, et al. 2017.** Drought inhibits synergistic interactions of native and exotic litter mixtures during decomposition in temperate grasslands. *Plant Soil* **415**: 257–268.
- Seiwa K. 2000.** Effects of seed size and emergence time on tree seedling establishment: importance of developmental constraints. *Oecologia* **123**: 208–215.
- Shaw RM, Harte J. 2001.** Control of litter decomposition in a subalpine meadow–sagebrush steppe ecotone under climate change. *Ecological Applications* **11**: 1206–1223.
- Sheley RL, Jacobs JS, Svejcar TJ. 2005.** Integrating disturbance and colonization during rehabilitation of invasive weed-dominated grasslands. *Weed Science* **53**: 307–314. doi:10.1614/WS-04-152R2.

- Siebenkäs A, Schumacher J, Roscher C. 2015.** Phenotypic plasticity to light and nutrient availability alters functional trait ranking across eight perennial grassland species. *AoB PLANTS* **7**. doi:10.1093/aobpla/plv029.
- Siegrist-Maag S, Lüscher A, Suter M. 2008.** Reaktion von Jakobs-Kreuzkraut auf Schnitt. *Agrarforschung (Switzerland)* **15**: 338–343.
- Singh HP, Batish DR, Kohli RK. 1999.** Autotoxicity: Concept, organisms, and ecological significance. *Critical Reviews in Plant Science* **18**: 757–772.
- Smit HJ, Metzger MJ, Ewert F. 2008.** Spatial distribution of grassland productivity and land use in Europe. *Agricultural Systems* **98**: 208–219.
- Smith AL, Barrett RL, Milner RNC. 2018.** Annual mowing maintains plant diversity in threatened temperate grasslands. *Applied Vegetation Science* **21**: 207–218. doi:10.1111/avsc.12365.
- Smith-Ramesh LM, Reynolds HL. 2017.** The next frontier of plant–soil feedback research: unraveling context dependence across biotic and abiotic gradients. *Journal of Vegetation Science* **28**: 484–494.
- Ssymank A, Ellwanger G, Raths U. 2015.** Beweidung als Management in NATURA-2000 Gebieten. In: Heinz Sielmann Stiftung D, ed. *Naturnahe Beweidung und NATURA 2000: Ganzjahresbeweidung im Management von Lebensraumtypen und Arten im europäischen Schutzgebietssystem NATURA 2000*. Duderstadt, Germany.
- Suter M, Lüscher A. 2011.** Measures for the control of *Senecio aquaticus* in managed grassland. *Weed Research* **51**: 601–611. doi:10.1111/j.1365-3180.2011.00875.x.
- Suter M, Siegrist-Maag S, Connolly J, Lüscher A. 2007.** Can the occurrence of *Senecio jacobaea* be influenced by management practice? *Weed Research* **47**: 262–269.
- Suzuki RO, Kudoh H, Kachi N. 2003.** Spatial and temporal variations in mortality of the biennial plant, *Lysimachia rubida*: Effects of intraspecific competition and environmental heterogeneity. *Journal of Ecology* **91**: 114–125.
- Sydes C, Grime JP. 1981.** Effects of tree leaf litter on herbaceous vegetation in deciduous woodland: II. An experimental investigation. *Journal of Ecology* **69**: 249–262. doi:10.2307/2259829.
- Tallowin JRB, Jefferson RG. 1999.** Hay production from lowland semi-natural grasslands: A review of implications for ruminant livestock systems. *Grass and Forage Science* **54**: 99–115.
- Urban MC, de Meester L. 2009.** Community monopolization: local adaptation enhances priority effects in an evolving metacommunity. *Proceedings of the Royal Society. Biological sciences* **276**: 4129–4138. doi:10.1098/rspb.2009.1382.
- Valéry L, Fritz H, Lefeuvre J-C, Simberloff D. 2008.** In search of a real definition of the biological invasion phenomenon itself. *Biological Invasions* **10**: 1345–1351. doi:10.1007/s10530-007-9209-7.
- Valéry L, Fritz H, Lefeuvre J-C, Simberloff D. 2009.** Invasive species can also be native... *Trends in Ecology & Evolution* **24**: 585. doi:10.1016/j.tree.2009.06.017.
- Valkó O, Deák B, Török P, et al. 2016.** High-diversity sowing in establishment gaps: a promising new tool for enhancing grassland biodiversity. *Tuexenia* **36**: 359–378.
- van de Voorde TFJ, Ruijten M, van der Putten WH, Bezemer TM. 2012a.** Can the negative plant–soil feedback of *Jacobaea vulgaris* be explained by autotoxicity? *Basic and Applied Ecology* **13**: 533–541. doi:10.1016/j.baae.2012.08.012.
- van de Voorde TFJ, van der Putten WH, Bezemer TM. 2012b.** The importance of plant–soil interactions, soil nutrients, and plant life history traits for the temporal dynamics of *Jacobaea vulgaris* in a chronosequence of old-fields. *Oikos* **121**: 1251–1262. doi:10.1111/j.1600-0706.2011.19964.x.

- van de Voorde TFJ, van der Putten WH, Martijn Bezemer T. 2011.** Intra- and interspecific plant-soil interactions, soil legacies and priority effects during old-field succession. *Journal of Ecology* **99**: 945–953. doi:10.1111/j.1365-2745.2011.01815.x.
- van der Meijden E, van der Waals-Kooi RE. 1979.** The population ecology of *Senecio jacobaea* in a sand dune system: I. Reproductive strategy and the biennial habit. *Journal of Ecology* **67**: 131–153.
- van der Putten WH, Bardgett RD, Bever JD, et al. 2013.** Plant-soil feedbacks: The past, the present and future challenges. *Journal of Ecology* **101**: 265–276. doi:10.1111/1365-2745.12054.
- van Kleunen M, Dawson W, Bossdorf O, Fischer M. 2014.** The more the merrier. Multi-species experiments in ecology. *Basic and Applied Ecology* **15**: 1–9.
- Verbeke G, Molenberghs G. 2000.** *Linear mixed models for longitudinal data*: Springer.
- Viana DS, Cid B, Figuerola J, Santamaria L. 2016.** Disentangling the roles of diversity resistance and priority effects in community assembly. *Oecologia* **182**: 865–875. doi:10.1007/s00442-016-3715-1.
- Vicente J, Randin CF, Gonçalves J, et al. 2011.** Where will conflicts between alien and rare species occur after climate and land-use change? A test with a novel combined modelling approach. *Biological Invasions* **13**: 1209–1227. doi:10.1007/s10530-011-9952-7.
- Walker KJ, Stevens PA, Stevens DP, Mountford JO, Manchester SJ, Pywell RF. 2004.** The restoration and re-creation of species-rich lowland grassland on land formerly managed for intensive agriculture in the UK. *Biological Conservation* **119**: 1–18. doi:10.1016/j.biocon.2003.10.020.
- Wang R, Yang X, Song Y, et al. 2011.** Allelopathic potential of *Tephrosia vogelii* Hook. f.: Laboratory and field evaluation. *Allelopathy Journal* **28**: 53–62.
- Wardle DA. 1987.** The ecology of ragwort (*Senecio jacobaea* L.): A review. *New Zealand Journal of Ecology* **10**: 67–76.
- Wardle DA, Ahmed M, Nicholson KS. 1991.** Allelopathic influence of nodding thistle (*Carduus nutans* L.) seeds on germination and radicle growth of pasture plants. *New Zealand Journal of Agricultural Research* **34**: 185–191.
- Wardle DA, Nicholson KS, Rahman A. 1995.** Ecological effects of the invasive weed species *Senecio jacobaea* L. (ragwort) in a New Zealand pasture. *Agriculture, Ecosystems & Environment* **56**: 19–28. doi:10.1016/0167-8809(95)00636-2.
- Wesche K, Krause B, Culmsee H, Leuschner C. 2012.** Fifty years of change in Central European grassland vegetation: Large losses in species richness and animal-pollinated plants. *Biological Conservation* **150**: 76–85. doi:10.1016/j.biocon.2012.02.015.
- Wiedenfeld H, Edgar J. 2011.** Toxicity of pyrrolizidine alkaloids to humans and ruminants. *Phytochemical Reviews* **10**: 137–151. doi:10.1007/s11101-010-9174-0.
- Wille W, Thiele J, Walker EA, Kollmann J. 2013.** Limited evidence for allelopathic effects of giant hogweed on germination of native herbs. *Seed Science Research* **23**: 157–162. doi:10.1017/S096025851300007X.
- Wilson JB, Peet RK, Dengler J, Pärtel M. 2012.** Plant species richness: The world records. *Journal of Vegetation Science* **23**: 796–802. doi:10.1111/j.1654-1103.2012.01400.x.
- Wilson MV, Clark DL. 2001.** Controlling invasive *Arrhenatherum elatius* and promoting native prairie grasses through mowing. *Applied Vegetation Science* **4**: 129–138.
- Wink M. 1993.** Allelochemical Properties or the raison d'être of alkaloids. *The Alkaloids* **1993**.
- Winter S, Jung LS, Eckstein RL, et al. 2014.** Control of the toxic plant *Colchicum autumnale* in semi-natural grasslands: Effects of cutting treatments on demography and diversity. *Journal of Applied Ecology* **51**: 524–533. doi:10.1111/1365-2664.12217.
- Xiong S, Nilsson C. 1999.** The effects of plant litter on vegetation: A meta-analysis. *Journal of Ecology* **87**: 984–994. doi:10.1046/j.1365-2745.1999.00414.x.

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