Wissenschaftszentrum Weihenstephan
für Ernährung, Landnutzung und Umwelt

Lehrstuhl für Renaturierungsökologie

IT'S A MATTER OF SOURCE: THE COMPETITIVE EFFECTS OF DOMINANT SPECIES ON CALCAREOUS GRASSLAND FORBS

Emer Ann Walker

Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften

genehmigten Dissertation.

Vorsitzender: Univ.-Prof. Dr. H. Schäfer

Prüfer der Dissertation:

1. Univ.-Prof. Dr. J. Kollmann
2. apl. Prof. Dr. K. F. Auerswald

Die Dissertation wurde am 31.07.2014 bei der Technischen Universität München eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt am 13.10.2014 angenommen.

Gedruckt mit Unterstützung des Deutschen Akademischen Austauschdienst (DAAD)

Contents

Summary

Grassland restoration is a complex endeavour, which requires a thorough understanding of the ecosystem and how it functions to achieve success. It is therefore necessary to have a reliable knowledge of the interactions that occur between plants, particularly of the competition between dominant and subordinate species, to enhance the likelihood of reaching the restoration goals. This dissertation deals with some significant questions that are often overlooked by general guidelines regarding seed sources for grassland restoration. While the effects of mixing seed sources in restoration have received some research attention, there is inadequate knowledge concerning the effects of using cultivar and regional (locally sourced and propagated) seed in calcareous grassland restoration. This is especially lacking in relation to their interaction with growth form, seeding density and nutrient level.

Cultivars are selected for beneficial traits, some of which may include vigorous growth. This is likely to hinder the surrounding vegetation by suppressing establishment and growth. A first greenhouse experiment was established to investigate the competitive interactions between one dominant and three subordinate species from cultivar and regional sources. The main findings show that when grown without competition, all three subordinate species differed in their performance according to source. However, when grown with competition, cultivar subordinates produced more biomass than regional subordinates. Dominant species from the regional source was found to have a greater suppressive effect on the subordinate species than the dominant species cultivar. This experiment found mixed evidence in support of cultivar vigour and highlighted the suppressive effects of dominant species from regional sources.

As restoration often occurs on land previously used for agriculture, the soils may still have high residual levels of nutrients. Knowledge of the competitive ability of dominant species from cultivar and regional sources at different nutrient levels is limited. Thus, a second experiment was designed to examine the impact of nutrient level on the competitive effects of rhizomatous and tussock growth forms of *Festuca rubra* from different seed sources on a phytometer plant, i.e. *Dianthus carthusianorum*. Furthermore, seedling emergence differences between cultivar and regional dominant species were also examined. In general, cultivar *F. rubra* seed emerged faster than regional *F. rubra*, although there were no differences in their overall emergence. At medium and high nutrient levels, regional *F. rubra* seed was more competitive than

cultivar *F. rubra*. Generally, cultivars exhibited uniform patterns in emergence and competitive abilities at different nutrient levels, while regional seed showed greater variation. When conducting grassland restoration, the nutrient level of the soil should be considered in combination with the source of the seed used for grassland restoration.

A third experiment was established under limiting field conditions to determine the effects of seed source, growth form and seeding density of the dominant species on subordinates and the invasive non-native *Erigeron annuus*. The establishment and flowering of the subordinate species from cultivar and regional sources was also studied. Regional seed was generally found to have higher establishment than cultivars for both the dominant and subordinate species. Despite having greater establishment, regional subordinate species did not consistently produce more flowers than cultivars. The risk of invasion by *E. annuus* was reduced in the sub-plots containing regional *F. rubra* and those with the tussock growth form. Furthermore, there was very little increase in the establishment of *F. rubra* between the low density (800 seeds m^{-2}) and high density (1600 seeds m^{-2}) seeding treatments. This suggests that the inclusion of higher than recommended proportions of *F. rubra* in seed mixtures may be counterproductive in relation to the cost-effective establishment and the potential for increased competitiveness at higher densities. The source of the seed, as well as the growth form of the dominant species used in seed mixtures, can be used to reduce the risk of undesirable species establishing. However, this advantage may be offset by suppression of desired species. Additional research is required to determine which species composition and seeding densities of regional seed mixtures have the greatest potential to increase biotic resistance against unwanted and invasive species under a range of stressful and more benign environmental conditions.

The integrated approach of the three experiments from both the controlled greenhouse and field conditions has enabled the research to focus on some of the most important topics relating to the composition of seed mixtures used in grassland restoration. By combining the results, it is possible to gain a better understanding of the multifaceted processes at work during the establishment of a community with seed from cultivar and regional sources. This understanding will contribute to the long-standing debate relating to seed sources for restoration. As each restoration site is unique, the seed source used should be selected on the basis of the nutrient availability,

soil type and target vegetation community. The findings from this dissertation are highly relevant for restoration practitioners and can have immediate implementation in on-going and future restoration projects.

Zusammenfassung

Graslandrenaturierung ist ein komplexes Unterfangen, dessen Erfolg ein umfangreiches Verständnis des Ökosystems und dessen Funktionsweise voraussetzt. Um zu gewährleisten, dass die Ziele einer Renaturierung erreicht werden, ist außerdem ein umfassendes Wissen über die Interaktionen zwischen den einzelnen Pflanzen, insbesondere zwischen häufigen und seltenen Arten, notwendig.

Die vorliegende Dissertation befasst sich mit der Frage der Herkunft von Saatgut bei Renaturierungsvorhaben, die in Richtlinien und Praxisempfehlungen oft nicht berücksichtigt wird. Während es bereits Untersuchungen zu Auswirkungen von verschiedenen Saatmischungen gibt, fehlen Erkenntnisse über Effekte von Kultivaren im Vergleich zu regionalem Saatgut (sowohl lokal gesammelt als auch erzeugt) bei der Renaturierung von Kalkmagerrasen. Hier ist insbesondere die Frage von Bedeutung, welche Auswirkungen Wuchsform, Aussaatstärke und Nährstoffverfügbarkeit auf den Etablierungserfolg haben.

Kultivare werden nach nützlichen Eigenschaften selektiert, von denen einige ein starkes Wachstum bedingen, was wiederum zu einer Unterdrückung der umgebenden Vegetation führen kann. In einem ersten Gewächshausexperiment wurde die Konkurrenz von Kultivaren und regional erzeugtem Saatgut zwischen einer häufigen und drei seltenen Arten untersucht. Die Ergebnisse zeigen, dass alle drei seltenen Arten sich in ihrer Wuchsleistung unterscheiden, wenn sie ohne Konkurrenz wachsen konnten: *Buphthalmum salicifolium* zeigte keine Unterschiede zwischen Kultivar und regionaler Herkunft, wohingegen regional erzeugte *Dianthus carthusianorum* und ein Kultivar von *Linum perenne* deutlich mehr Biomasse produzierten als ihr jeweiliger Herkunftsgegenpart. Wenn diese drei Arten jedoch Konkurrenz ausgesetzt wurden, produzierten alle Kultivare mehr Biomasse als das regionale Saatgut. Bei der häufigen Art konnte beobachtet werden, dass die regionale Herkunft einen größeren Unterdrückungseffekt auf die seltenen Arten besaß, als der Kultivar. Mit diesem Experiment wurden zum einen Hinweise für die Konkurrenzstärke von Kultivaren gefunden und zum anderen gezeigt, dass häufige Arten aus regionaler Herkunft ebenfalls einen unterdrückenden Effekt besitzen können.

Da bei der Renaturierung oft Flächen im Fokus stehen, die vormals ackerbaulich genutzt wurden, zeichnen sich die Böden durch erhöhte Nährstoffgehalte aus. Es ist

nicht ausreichend bekannt, wie sich unterschiedliche Nährstoffgehalte auf Kultivare und regionales Saatgut von häufigen Arten auswirken.

Deshalb wurde ein zweites Experiment durchgeführt um den Einfluss von Nährstoffgehalt auf das Konkurrenzverhalten von ausläuferbildenden und horstig-wachsenden Formen von *Festuca rubra* aus unterschiedlicher Herkunft auf eine Phytometerart, *D. carthusianorum*, zu untersuchen. Zusätzlich wurden die Keimraten zwischen der unterschiedlichen Herkunft verglichen. Grundsätzlich keimte der Kultivar *F. rubra* schneller als die regional erzeugte Variante, obwohl die Gesamtkeimraten vergleichbar waren. Unter mittleren und hohen Nährstoffniveaus war die regional erzeugte Variante von *F. rubra* konkurrenzstärker als der Kultivar. Während die Kultivare ein einheitliches Verhalten bei Keimung und Konkurrenzfähigkeit unter verschiedenen Nährstoffniveaus zeigten, besaßen regional erzeugte Individuen eine größere Variation. Bei der Renaturierung von Kalkmagerrasen sollte daher neben der Herkunft des Saatguts auch der Nährstoffgehalt der Böden berücksichtigt werden.

Ein drittes Experiment wurde unter Freilandbedingungen durchgeführt, um die Auswirkung von Saatgutherkunft, Wuchsform und Saatstärke der häufigen Art auf seltene Arten und die invasive Fremdart *Erigeron annuus* zu untersuchen. Die Etablierung und Blüte von Kultivar und regionalem Saatgut wurde für die seltenen Arten ebenfalls untersucht. Sowohl für die häufigen als auch für die seltenen Arten zeigte das Saatgut aus regionaler Herkunft eine generell bessere Etablierung als das der Kultivare. Trotz der höheren Etablierung bildeten regional erzeugte seltene Arten aber nicht durchweg mehr Blüten aus als die Kultivare. Die Wahrscheinlichkeit einer Invasion von *E. annuus* wurde in den Untersuchungsplots mit regional erzeugtem *F. rubra* und Kultivaren mit der horstig-wachsenden Variante verringert. Des Weiteren zeigte *F. rubra* nur eine geringe Erhöhung der Etablierung zwischen der geringen (800 Samen m^{-2}) und der hohen (1600 Samen m^{-2}) Aussaatstärke. In Bezug auf eine kosten-günstige Etablierung und das Potential für noch höhere Konkurrenzstärke bei hohem Deckungsgrad deutet dieses Ergebnis darauf hin, dass die Verwendung von zu hohen Anteilen von *F. rubra* in Saatmischungen kontraproduktiv sein könnte. Die Herkunft der Samen, ebenso wie Wuchsform von häufigen Arten in Saatmischungen, kann genutzt werden, um unerwünschte Arten an der Etablierung zu hindern. Allerdings kann dieser Vorteil auch negativ sein, wenn erwünschte Arten ebenfalls unterdrückt werden. Weitere Untersuchungen sind notwendig, um zu bestimmen, welche

Artenzusammensetzung und Saatdichte bei regionalen Saatmischungen sowohl unter Stress als auch unter günstigen Umweltbedingungen das größte Potential zur Unterdrückung von unerwünschten und invasiven Arten besitzen.

Der integrative Ansatz der drei Experimente, die sowohl kontrollierte Gewächshausbedingungen als auch Freilandbedingungen umfassen, hat es ermöglicht, einige der wichtigsten Themenfelder in Bezug auf die Zusammensetzung von Saatmischungen bei der Renaturierung von Kalkmagerrasen zu untersuchen. Durch die Kombination der Erkenntnisse kann ein verbessertes Verständnis der vielschichtigen Prozesse während der Etablierung einer Artengemeinschaft mit Kultivaren und regional erzeugtem Saatgut erreicht werden. Diese Erkenntnisse tragen bei zu der derzeitigen Debatte über die Herkunft von Saatgut, das bei Renaturierung verwendet werden kann und darf. Da jede Renaturierungsfläche einzigartig ist, sollte die Saatgutherkunft, basierend auf Nährstoffverfügbarkeit, Bodentyp und Ziel-Artengemeinschaft, individuell ausgewählt werden. Die Ergebnisse dieser Dissertation sind für die Praxis von Renaturierungen wichtig und können direkt bei der Umsetzung von bereits laufenden und zukünftigen Projekten angewendet werden.

1. Introduction

1.1. General background and motivation

Grasslands are one of the most important biomes of the world (Gibson 2009). They have a widespread distribution and account for roughly 41% of the total terrestrial land cover (White et al. 2000). Ecologically, they support a high biodiversity at different scales (Spehn et al. 2005, Reitalu et al. 2014), while also providing many valuable ecosystem goods and services including food supply, maintenance of water regimes and carbon storage (White et al. 2000, Tilman and Polasky 2005, O'Mara 2012). The relationship between humans and grasslands is a complex one, which has evolved over many thousands of years. Semi-natural grasslands owe their existence and species richness to human interventions and continued maintenance (Pfadenhauer 2001, Poschlod and WallisDeVries 2002). However, in recent times the impact of anthropogenic activities on grasslands has become negative in many regions and consequently grasslands have suffered large declines, not only in relation to their area but also to the quality of these habitats (Lande 1998, Butaye et al. 2005).

Grasslands are vulnerable to many threats, some of the greatest include agricultural intensification (Walker et al. 2004), afforestation (Overbeck et al. 2013), habitat fragmentation (Butaye et al. 2005) and invasive non-native species (D'Antonio and Vitousek 1992, Gibson 2009). The growing demand for food has led to an increase in both the amount of land required for production purposes, as well as the intensity with which this land is used (O'Mara 2012). Agricultural intensification transforms historical grassland ecosystems into tracts of monoculture, with considerable losses to the plant and animal diversity (Tscharntke et al. 2005, Wesche et al. 2012). Furthermore, frequent fertilisation by farmers and atmospheric deposition from anthropogenic activities have considerable long-term effects on grassland communities adapted to low nutrient conditions (Willems and van Nieuwstadt 1996). Changes in land use due to agricultural intensification and urbanisation have also resulted in the fragmentation of grasslands (Pfadenhauer 2001). The natural dispersal of species and genotypes, which is necessary for ecosystem functioning, has become restricted due to fragmentation and changes in farming practices (Fischer et al. 1996), leading to isolated populations and species declines (Helm et al. 2006). Grassland communities, which have undergone declines in species diversity, are vulnerable to invasion by native and non-native weeds and have limited abilities to recover after an invasion (Dukes 2002). In certain circumstances, invasive non-native species can greatly alter grassland ecosystem functioning (Gibson 2009). Increases in primary productivity resulting from the growth of invasive non-native species can hinder native species establishment, modify soil nutrient cycles (Ehrenfeld 2003) and the frequency of disturbances, such as fire (D'Antonio and Vitousek 1992).

Grassland ecosystems have been well studied in most areas of the world; however, the restoration of threatened and degraded grasslands creates new challenges and requires innovative approaches. Over the past two decades, there have been considerable advances in the science of grassland restoration (Choi et al. 2008, Hobbs et al. 2011). Some of the main priorities of restoration are to quickly establish vegetation cover, to create communities that have long-term persistence and to restore ecosystem functioning (Kettenring et al. 2014). Investigations into methods of site preparation, seed harvesting, sowing techniques, species composition and the management of grasslands have led to greater understanding of the complex processes involved in grassland restoration and improvements towards achieving the restoration goals (Scotton et al. 2011). However, despite many advances, there is still much to be learned, especially in the area of seed material used for restoration.

As the desired vegetation community is usually limited or absent from the restoration site (Bakker and Berendse 1999), the reintroduction of plant species is one of the most important and fundamental tasks in the restoration of grasslands. The use of locally-sourced seed is widely recommended in grassland restoration (Bischoff et al. 2010). This recommendation is based upon the expectation that local seed material will have beneficial adaptations to the respective climate and soil conditions (Vander Mijnsbrugge et al. 2010). Furthermore, local seed is favoured genetically as the risks of maladapted genotypes (Vander Mijnsbrugge et al. 2010) as well as potential hybridisation with foreign genotypes are reduced (Hufford and Mazer 2003). Despite this recommendation, foreign genotypes, including cultivars, are still often included in seed mixtures (Conrad and Tischew 2011). Although this is a common occurrence, with estimates of up to 40% of commercial seed mixtures composed of cultivar grass seed (Conrad 2007), the effects of mixing seed sources in grassland restoration has received limited research. While the source of the seed is of fundamental importance, its influence on other factors, such as species growth form, seeding density and nutrient input and their interactions is largely unknown. Given the extent of cultivars in commercial seed mixtures, it is imperative to gain a greater knowledge of the effects that these foreign genotypes have in a restored grassland community.

1.2. Grassland restoration

As grasslands are diverse ecosystems with multiple interacting factors determining their species composition, the task of restoring them is also very complex. The entire restoration procedure is an elaborate process, which has been referred to as a 'jig-saw puzzle' (Clewell and Aronson 2013b). It requires expert planning and knowledge, not only of botanical and ecological aspects but also technical expertise of equipment and procedures (Scotton et al. 2011). There are many factors that can influence the success or failure of grassland restoration (Hutchings and Stewart 2002). In Figure 1, the most important factors are outlined. They can roughly be aligned into three categories: (i) site factors, (ii) species identity and (iii) seed quality. In general, these factors can be approached in a stepwise manner, beginning with the site factors.

Site factors, identified in the outer sections of the conceptual diagram, refer to the restoration goals, which should be considered in relation to the current site conditions, and the past and future land use of the site to be restored. These factors are of fundamental importance and should be carefully assessed prior to the start of the restoration work. Once the initial preparation work relating to the site factors has been organised, it is possible to decide upon the specific taxa to be included in the restoration.

The species identity will vary according to the habitat being restored. In many cases, seed of the target community will be diminished or absent completely (Bakker and Berendse 1999, Bossuyt and Honnay 2008). This is particularly true of calcareous grassland species, which have short-lived seed banks (Bossuyt et al. 2006). In areas which have limited source populations, the likelihood of natural colonisation is particularly low (Öster et al. 2009), and is also greatly impacted by the dispersal abilities of the seed (Coulson et al. 2001). Due to seed limitation, the restoration of grasslands usually requires the introduction of a diverse species assemblage of dominant, subordinate and transient species.

Seed quality considerations, identified in the centre of Figure 1, represent some of the finer details relating to the species that require consideration for restoration. The seed source is directly related to the goal of the restoration project and depends on whether there is greater emphasis on conservation of local species and genotypes or rapid restoration (Lesica and Allendorf 1999). Additional factors are growth form and seeding density, which are connected to cover and dominance of the vegetation community.

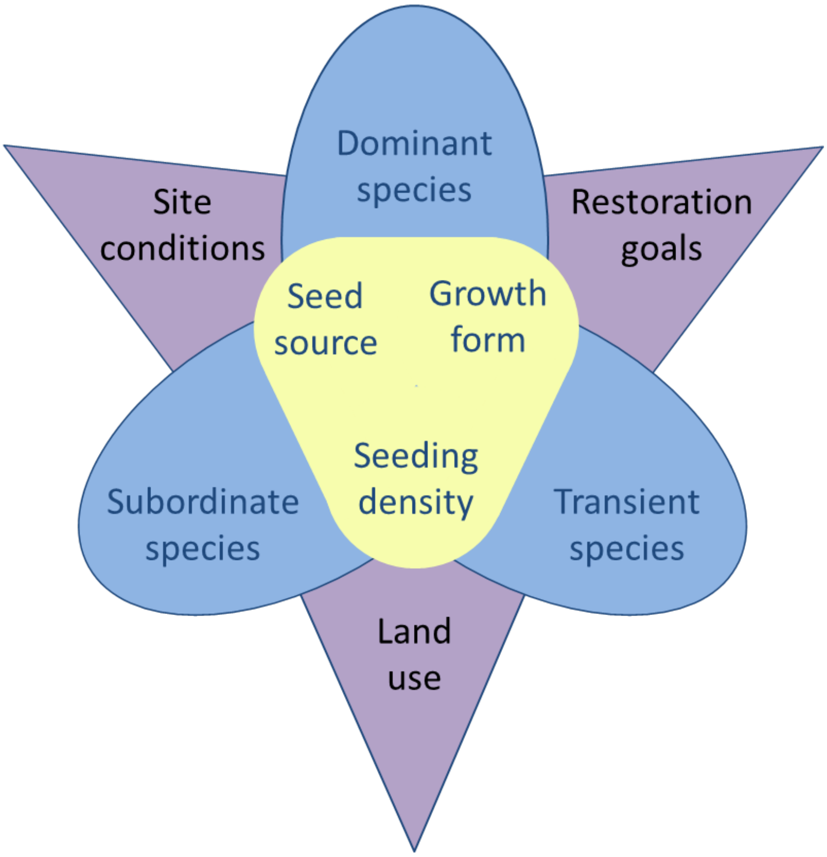

Figure 1 Clustering of the most important factors controlling grassland restoration. *Site factors* are outlined by the outer purple triangle sections, *species identity* is described by the dark blue ovals, and *seed quality* is found in the yellow section in the centre of the diagram.

Site factors

Site conditions

As the conditions vary considerably between restoration sites, each project will bring its own unique set of challenges. A thorough assessment of the site conditions is essential prior to the start of restoration work. Factors such as high altitudes, steep inclines or exposed slopes can increase the complexity of the restoration as additional consideration must be made in relation seeding techniques and soil stability (Matesanz et al. 2006, Pohl et al. 2009, Scotton et al. 2011). Many factors relating to soil impact on the success of community establishment and the likelihood of sustainable ecosystem functioning (Hutchings and Stewart 2002), including soil type, texture and structure (Scotton et al. 2011). Soils that have a limited water holding capacity, such as gravel soils, are particularly prone to drought, which may limit establishment potential and primary productivity (Kiehl and Pfadenhauer 2007). For many grasses, including *Festuca rubra*, moisture is a key germination trigger (Grime 2002). Extreme temperatures or droughts may result in considerable losses, especially for plants with little genetic variation (Knapp and Rice 1994). There is also much uncertainty as to the long-term effects of climate change, particularly in relation to restoration efforts and the goals (Hobbs 2012). Alterations to the weather patterns, temperature escalations, changes to precipitation patterns and increases in the frequency of extreme climate events will create additional challenges for restoration and threaten the success of restoring habitats to a target or reference historical ecosystem (Harris et al. 2006).

Land use

Land use change can have large impacts on the site conditions by altering vegetation and soil microbial communities (Steenwerth et al. 2002), water regimes (Nosetto et al. 2005), disturbance patterns (Gibson 2009) and soil conditions (Fu et al. 2000). Successful grassland restoration has been achieved in areas that experienced a wide range of land uses including mining (Kirmer et al. 2012), quarries (Novák and Prach 2010) and agricultural land (Török et al. 2011). The conversion of arable land into grassland is one of the most common forms of grassland restoration in Europe (Cramer and Hobbs 2007). In this form of restoration, one of the main challenges is the high residual nutrient levels in the soil. Artificial fertilisers used to increase agricultural productivity have long lasting effects on the soil, such as phosphorous, which may require up to 50 years to decline to acceptable levels (Fagan et al. 2008).

There is an inverse relationship between species diversity and nutrients (Janssens et al. 1998), so it may be necessary to reduce the fertility of the soil prior to restoration. There are a number of possible techniques which vary in their effectiveness and cost including biomass removal through mowing or grazing, manipulation of nutrient fluxes in the soil and topsoil removal (Marrs 1993, Pywell et al. 2002, Walker et al. 2004). In many cases, it is the high phosphorous concentrations in soils and not the overall nutrient content that pose problems for the community development (Fagan et al. 2008). High phosphorous concentrations favour the growth of grasses (Güsewell 2004, Fagan et al. 2008). Furthermore, arable soils may contain seed banks of undesirable species, which will compete with the restored grassland community (Pywell et al. 2003). The sowing of low density seed mixtures in combination with mowing may successfully supress arable weeds in restored grasslands but its success is likely to depend on the former land use (Török et al. 2012).

Restoration goals

Successfully restored ecosystems generally have 11 ecological attributes that contribute to their effective functioning, such as species composition, community structure and self-sustainability (Clewell and Aronson 2013a). These attributes should be combined with the specific targets of the site to establish clear restoration goals (Clewell and Aronson 2013b). The goals should be defined based on the size, conditions and the former land use of the site to be restored. Restoration goals are very diverse and are founded on a wide range of ecological perspectives that are often connected with socio-historical factors (Hobbs 2007, Miller and Hobbs 2007). Restoration is an interdisciplinary science and therefore the determination of the goals should be based upon the views of the involved experts and stakeholders (Hobbs 2012).

In many cases, the preservation of particular species or populations is one of the main priorities of restoration (Ruiz-Jaen and Aide 2005, Shackelford et al. 2013). This requires careful selection of species and also local genotypes to conserve genetic diversity in the new restoration site. However, it is important to consider that the introduction of any seed, even from other local sources, has the potential for genetic pollution (Aavik et al. 2012). Furthermore, by focusing only on local genotypes, other important factors such as the restoration of ecosystem functioning and the provision of ecosystem services may be neglected (Jones 2013a). Depending on the degree of habitat modification, the genotypes that are likely to best restore functioning and services may not always necessarily be of local origin (Jones 2013b). An additional priority may be to enhance the resilience of an ecosystem to stresses (Rapport et al. 1998), such as invasive non-native species. In addition to the ecological constraints, the goals of restoration are also limited by what is realistic financially and acceptable socially (Miller and Hobbs 2007).

Species identity

One of the priorities for grassland restoration is often to establish sustainable long-term vegetation cover, which is composed of a diverse mix of species and enhances biodiversity as well as the overall functioning of the ecosystem. A functional grassland community requires a variety of species that play different roles in the community. Each of these component species groups interacts with each other to influence the dynamics of the grassland community.

Dominant species

Dominant species are necessary for primary productivity and because of their large biomass, they also greatly contribute to the vegetation cover (Grime 1998). Grasses usually fulfil the role of the main dominant species in a grassland, and their growth and abundance can influence the performance of other species, such as the subordinates (McCain et al. 2010). Regeneration by clonal growth is common in the dominant species, some of them form dense tussocks, others extensive above- or belowground runners. In addition, dominant species help to maintain ecosystem functioning by stabilising ecosystems after non-random species loss (Smith and Knapp 2003), while their identity is also a regulating factor in invasion success (Emery and Gross 2006).

Subordinate species

The subordinate species are generally small in stature but contribute to the floristic diversity of the grasslands and tend to frequently co-occur with particular dominant species (Grime 1998, Gibson et al. 2012). When the growth of the dominant species is regulated by either environmental or biotic factors, subordinate species can achieve high levels of regeneration and considerable cover (Grime 2002). Additionally, they can fulfil the role of drivers in a community, as they can impact on ecosystem functioning and soil microbial communities (Mariotte et al. 2013).

Transient species

Transient species occur as seedlings or juveniles in a grassland community, although they usually exist as dominant or subordinate species in nearby communities (Grime 1998). They usually originate from seed rain, or remnants of the previous vegetation type that have remained in the seed bank, and depend very much on some gap dynamics (Grime 2002).

Seed quality

Seed source

Three seed sources are generally used in grassland restoration: wild, regional and cultivar seed. *Wild seed* is collected from areas located in close proximity to the restoration site. However, this can also be one of the main constraints, because the availability of near-by donor sites with suitable species composition can often be limited (Scotton et al. 2011). The seed resources within donor grasslands require careful management to avoid overharvesting, thereby threatening wild populations (Feucht 2010). Several techniques exist for the collection of wild seed from donor sites such as hay harvesting, seed striping, seed vacuuming and the transfer of topsoil (Kiehl et al. 2010). Both the seed collection method and the time of harvest can affect the species composition as seed from different species have different phonologies, which result in variation in ripening times (Riley et al. 2004). To increase the likelihood of collecting a suitably diverse range of seed from a variety of species, it may be necessary to collect wild seed throughout the course of the flowering season (Edwards et al. 2007, Scotton et al. 2011). Otherwise the seed from early- or late-flowering species may be excluded (Kiehl et al. 2006). Nevertheless, the species composition of wild seed can be difficult to manipulate, unlike that of regional and cultivar seed mixtures.

Although locally-sourced seed is recommended for restoration, the feasibility of using wild seed varies greatly depending on the locality. *Regional seed* is a suitable alternative to wild seed; it is locally-sourced from within a defined area and specifically propagated for use in seed mixtures. The defined areas in which a particular regional seed can be sown are known as seed transfer regions (Hufford and Mazer 2003). Clearly defined seed transfer zones are needed to ensure the preservation of adaptive traits within a population; while these exist for woody species in areas such as Flanders and the UK, there are only a limited number of seed zones for herbaceous species (Vander Mijnsbrugge et al. 2010).

In Germany, there are eight seed transfer zones referred to as 'regions of production'. These regions of production are further subdivided into 22 'provenance regions' throughout the entire country (Figure 2g) and are delineated according to the local climate and soil type as well as the genetic composition of the native species (DBU 2010). Ideally, seed that is produced in one provenance region is also sown in the same provenance region, but due to economic reasons, the seed transfer can occur between different provenance regions within a region of production (Scotton et al. 2011). Each regional seed species is sourced from at least 50 wild plants to preserve the genetic diversity of the species (VWW 2011). This seed is then sown directly or at the seedling stage in single species monocultures to produce regional seed. Generally, regional seed is propagated from the same parent material for up to four generations. After this, the process of wild seed collection and regional seed propagation is then repeated in order to avoid the loss of genetic diversity in the regional seed (Feucht 2010). Once the regional seed is ripe, it is harvested and the seed is separated from the remaining plant material by threshing and blowing techniques. The seed is then stored under cool temperatures (ca. 4 °C) until it is required for inclusion in a seed mixture.

Regulations are required to ensure transparency in the regional seed market (Scotton et al. 2011). Regional seed certification has been established to promote the market for regional seed as well as guaranteeing the traceability, minimum standards and quality of the product (VWW 2011). In Germany, the certification company ABcert is responsible for approving regional seed that complies with the rules and regulations regarding the seed collection methods and propagation procedures outlined by the Verband deutscher Wildsamen- und Wildpflanzenproduzenten e.V. (VWW 2011). This seed is then eligible to be certified as VWW-Regiosaaten®. The establishment of regulated regional seed production is a relatively recent occurrence and is currently only found in a few countries, such as Germany and Austria (REWISA-certificate) (Scotton et al. 2011). In relation to the production costs, time intensive procedures for gathering, propagating and cleaning regional seed combined with the relatively small scale of the provenance region market, result in higher seed prices. Therefore, depending on the scale of the restoration, this can result in considerable price differences.

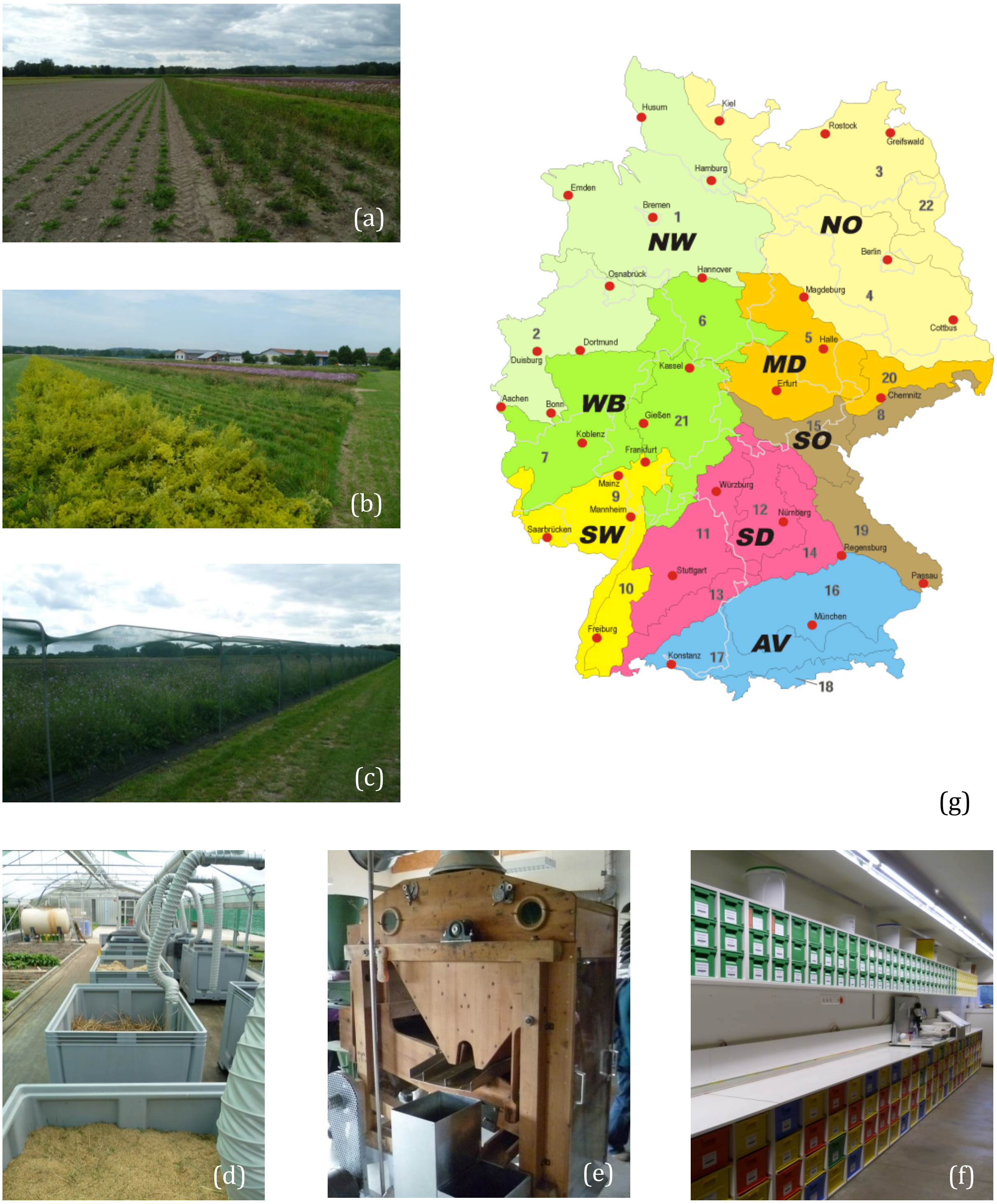

Figure 2 Regional seed propagation at the facilities of the regional seed producer, J. Krimmer, in provenance region 16 (Pulling, Bavaria, Germany). (a) and (b) Regional seed propagation; (c) protective cover over plants to prevent bird predation of seed; (d) drying containers for plant material; (e) machine for separating seed and plant material; (f) cold storage of harvested seed; (g) map of the eight production regions of regional seed in Germany outlined in different colours. Each production region is further divided into provenance regions, of which there are 22 in total throughout Germany (Source: VWW).

Cultivar seed is bred to be distinctive from other varieties of plants, yet still show uniformity within the cultivar population and have predictable performance (Aubry et al. 2005). Unlike regional and wild plants, which display considerable variation within population, the traits of cultivars remain relatively stable over time. Cultivar producers deliberately select for favourable heritable traits during the breeding process, which include reduced dormancy (Schröder and Prasse 2013a), enhanced root networks (Klopf and Baer 2011), improved physiological performance (Lambert et al. 2011), as well as higher flower production (McGrath et al. 2010). The origin of a cultivars can stem from a single plant, a single accession (plants collected from one locality), a mixed accession, an existing cultivar or mixtures of all of these combinations (Aubry et al. 2005). Once the desirable traits are present in the plants, these individuals are bred for seed increase. The entire process of developing a cultivar requires a number of plant generations to ensure that the traits remain stable (Aubry et al. 2005).

As cultivars can be mass-produced and are not restricted for use in a particular region, their market is greater than regional seed. Additionally, cultivar seed is considerably cheaper than regional seed (Conrad 2007). In order to reduce cost associated with restoration, seed mixtures sometimes contain mixes of cultivar grasses and regional forb species (Scotton et al. 2011). This can lead to problems in relation to the community composition, as cultivar grasses which exhibit enhanced vegetative growth, more effective nutrient capture or greater resistance to extreme climate events, pests and diseases potentially have better competitive abilities than regional seed (Wilsey 2010, Kettenring et al. 2014). Schröder and Prasse (2013b) found that cultivars and their hybrids have enhanced biomass production in comparison with wild counterparts, but only under favourable climate conditions. Despite their initial vigour due to selected traits, cultivars may be vulnerable to extreme conditions, which may reduce their long-term persistence in a community (Lesica and Allendorf 1999). Recent studies by Gibson et al. (2013) and Baer et al. (2013) found limited or no differences in the effects that cultivar and locally-sourced seed have on plant community assembly and net primary production, respectively. The use of cultivars could also lead to outcrossing with local populations. This may result in hybrid breakdown and disruption to co-adapted gene complexes (Hufford and Mazer 2003, Baer et al. 2013). Furthermore, reliance on cultivar genotypes to provide rapid vegetation establishment may have long-term detrimental consequences in relation to genetic diversity (Kettenring et al. 2014).

Growth form

The vegetation community is greatly influenced by the growth form of species, especially that of the dominant species, as they contributes greatly to the primary production. In grasslands, rhizomatous and tussock are the most prevalent growth forms of perennial grasses (Derner and Briske 2001) and they manipulate the above- and below-ground environments differently. Rhizomatous grasses benefit from their ability for rapid vegetative spread, particularly under high nutrient conditions (Grime 2002). However, the extensive surface growth may be compensated by reduced below-ground growth, for example, the rhizomatous grass *Festuca rubra* ssp*. rubra* was found to have reduced ability to stabilise deeper levels of soil (Feucht 2010). While rhizomatous grasses can produce homogenous swards, tussock grasses tend to create a more heterogeneous spatial distribution. This allows more canopy gaps (Bullock 2000), which may facilitate the establishment of other species through the availability of microsites. In general, tussock grasses predominate on severely nutrient-poor soils (Grime 2002), and accumulate greater pools of carbon and nitrogen in soils beneath them than rhizomatous grasses (Derner and Briske 2001).

The growth form of the subordinate species also plays an important role in their establishment and long-term persistence in grassland restoration. Plant height was found to be the major factor in the success of target species in urban grassland restoration (Fischer et al. 2013). As subordinate species are often small in stature, the risk of shading by taller grasses is high. Taller subordinate species may be less likely to experience shading by dominant species (Williams et al. 2005), which may result in better performance and seed dispersal potential than their smaller subordinate counterparts (Thomson et al. 2011). Low growth forms have also been found to have a higher extinction risk, particularly under changing land use patterns (Blomqvist et al. 2003, Williams et al. 2005). However, height may also be a disadvantage in grazed or mown grasslands, as prostrate rather than erect plants are favoured (Díaz et al. 2007). Growth forms with buds on or near the soil surface, such as hemicryptophytes and chamaephytes, typically increase after grazing pressure (Dupré and Ehrlén 2002, Kahmen and Poschlod 2008). Grazing, particularly by different species, creates gaps in the canopy structure (Loucougaray et al. 2004). As colonisation by seed can be slow, the ability for rapid lateral spread by plants on the edges of small gaps is particularly advantageous for grassland species (Marriott et al. 1997). Additionally, below-ground storage organs are particularly important for species in water-limited or drought prone environments, as they enable rapid recovery after periods of drought (Huber and van der Maarel 2007).

Seeding density

One of the distinctive advantages of using seed mixtures is the degree of flexibility available for species selection. Depending on the desired vegetation community, it is possible to include only the target species, unlike the unselective wild seed harvest (Scotton et al. 2011). Furthermore, the composition of species seed proportions with the seed mixture can also be altered to suit the requirements of the grassland. As a diverse grassland requires a mix of dominant, subordinate and transient species, the composition of the seed mixture must also be reflected in the initial seeding density of the species. The ratio of grasses to forbs varies according to the grassland type. Grasses can contribute to up to 80% weight of a seed mixture with the remaining proportion consisting of forbs (Hutchings and Stewart 2002). Generally, grasses can be expected to have good establishment rates in grassland restoration (Pywell et al. 2003). Therefore, the inclusion of competitive grasses in the seed mixture may disturb the dynamic balance of species, particularly when they are sown at high densities. Conrad and Tischew (2011) found that high densities of cultivar *F. rubra* contributed to excess biomass accumulation and suppression of target species.

Microsite limitation at the site is an important consideration when deciding the seeding density for sowing. The availability of microsites for plant establishment can be limited by both biotic and abiotic conditions (Zeiter et al. 2006). While the use of herbicide can alter biotic factors, such as plant competition, disturbance of the site by harrowing or rotovation can manipulate the abiotic conditions and increase the establishment of the target species (Kiehl et al. 2010). Species sown at high densities are more likely to reach a microsite and germinate (Turnbull et al. 2000). However, saturation of the microsites by using excess seed is likely to result in seed wastage.

As seed procurement can be an expensive factor in grassland restoration (Török et al. 2011), optimum seeding densities, which limit amounts of seed wastage, are desirable. As not every seed sown will germinate, it is important to calculate the seeding density of the seed mixture to compensate for failed germination, as factors such as seed storage and transportation may increase seed wastage (Merritt and Dixon 2011). Seed sown on raw soil can have up to a 60% transfer rate of successful establishment after one year and can potentially increase up to 80% after 5 years (Scotton et al. 2011).

1.3. Aims and objectives

The overall aims of this dissertation are to understand the effects and implications of using cultivar and regional seed sources in calcareous grassland restoration, particularly in relation to growth form, seeding density and nutrient level. Dominant and subordinate species have a dependent relationship. One of the aims is to explore the effects of cultivar and regional dominant species on cultivar and regional subordinate species. The effects of cultivar subordinate species in these competitive interactions warrant investigation. Growth form and seeding density are both important factors concerning seed mixtures, but there has been limited research into their interaction with seed source. An additional goal of this dissertation is to ascertain the implications of using certain seed source growth forms as well as seeding densities in grassland seed mixtures. The final goal of this research is to understand the effects of nutrient gradients on seed source and growth form, particularly in relation to the competitive effect of the dominant species. This knowledge will identify some of the most important factors that affect plant establishment and growth during the restoration process.

The interrelated nature of the three experiments in this dissertation, which is outlined in Figure 3, requires the use of complimentary studies to explore the abovementioned topics. This integrated approach will provide robust results and will contribute to the theoretical understanding of the complex processes at work during grassland restoration. Furthermore, the findings will be directly applied to seed selection in future grassland restoration and will assist restoration practitioners by highlighting key factors that need to be considered when restoring grasslands.

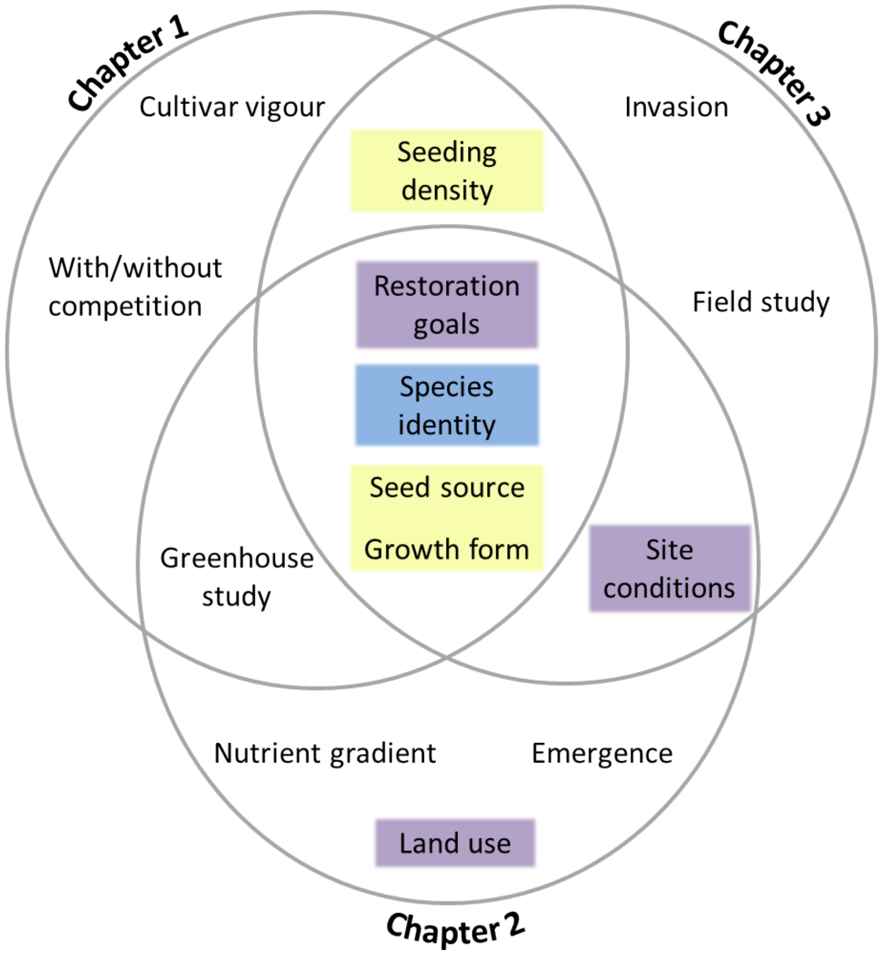

Figure 3 Conceptual diagram of the three dissertation chapters. Purple, blue and yellow text boxes relate to the *site factors*, *species identity* and *seed quality* referred to in Figure 1, respectively. Plain text identifies the specifics relating to each individual chapter.

Chapter 1

Competitive interactions between dominant and subordinate species from cultivar and regional sources: A greenhouse study involving three calcareous grasslands forbs

The main objective of the first experiment is to investigate the performance of cultivar and regional subordinate species without and in the presence of competition. An additional objective is to determine whether dominant species cultivars have greater suppression effects on the subordinate species than dominant species from regional sources. Performance is assessed using above-ground biomass measurements from a controlled greenhouse experiment. The implications of the findings are important in relation to the use of cultivar and regional seed in grassland seed mixtures.

Chapter 2

The influence of nutrient availability on the competitive effects of Festuca rubra *with different growth forms and seed sources*

Knowledge of the competitive ability of cultivar and regional dominant species at different nutrient levels is limited. Therefore, this experiment was established to determine the competitive effect of *Festuca rubra* from cultivar and regional sources and with rhizomatous and tussock growth forms across three nutrient levels on *Dianthus carthusianorum.* Additionally, differences in seedling emergence between cultivar and regional dominant species were also examined. Results from this experiment will assist in the selection of seed for use at nutrient enriched sites.

Chapter 3

Important factors relating to dominant and subordinate species: manipulations of seed source, growth form and seeding density in a calcareous grassland

The third chapter seeks to identify the most important factors relating to seed mixture composition. The effects of seed source, growth form and seeding density of the dominant species on subordinate species and the invasive species *Erigeron annuus* are investigated. Moreover, the establishment of the subordinate species from cultivar and regional sources is studied. The outcomes of this field experiment will have practical implications for the composition of seed mixtures for restoration of calcareous grasslands.

1.4. Study system

Calcareous grasslands

Calcareous grasslands are one of the most species-rich communities in Europe (Hutchings and Stewart 2002). They support a wide range of rare and threatened plant species (WallisDeVries et al. 2002), and are a particularly important habitat for a diverse range of butterflies and other invertebrates (van Swaay 2002, Krauss et al. 2003, Diacon-Bolli et al. 2013). Soils in calcareous grasslands are generally rich in calcium carbonate but poor in nutrients, with low concentrations of nitrogen and phosphorous (Hutchings and Stewart 2002). These limiting nutrient conditions support many specialist species (Maskell et al. 2010) and help increase the overall biodiversity.

Productivity in these grasslands is water-limited because of the high drainage capacity of the soil (Niklaus et al. 1998). Although calcareous grasslands harbour a rich biodiversity and have high aesthetic value (Fagan et al. 2008), they have suffered major declines over the past decades resulting largely from anthropogenic activities.

Changes in land use, which can principally be attributed to agricultural intensification, have caused extensive declines (Poschlod et al. 2005, Tscharntke et al. 2005). Soil eutrophication through fertilisation and atmospheric deposition hinders calcareous grassland vegetation, because they are adapted to low nutrient conditions (Römermann et al. 2008, Diacon-Bolli et al. 2013). Other factors, such as land abandonment and the limited use of traditional farming techniques, have also contributed to losses (Poschlod et al. 1998, Poschlod and WallisDeVries 2002, Römermann et al. 2008). The calcareous grasslands of Germany have declined by up to 60% over the past century (Krauss et al. 2004). In response to these declines and the importance of this unique and valuable habitat, calcareous grasslands are designated as a priority habitat in the European Commission Habitats and Species Directive (92/43 EEC) and are included in the NATURA 2000 network.

Efforts to restore calcareous grasslands over the past two decades have frequently centred on decreasing soil nutrient levels (Hutchings and Stewart 2002, Kiehl et al. 2006), while increasing species richness (Hedberg and Kotowski 2010, Kiehl et al. 2010), and improving management techniques (Fagan et al. 2008, Scotton et al. 2011). More recently, research into the effects of climate change on calcareous grasslands has also been published (Maalouf et al. 2012, Moser and Thompson 2014). The advances in the development of successful techniques for the restoration of calcareous habitats will ensure the preservation of this unique habitat under current and future climate conditions.

Study locations

Two of the experiments were conducted in a greenhouse facility of the Centre of Greenhouses and Laboratories Dürnast, Life Science Centre Weihenstephan, Technische Universität München, Germany. The remaining field experiment was carried out in the Munich Gravel Plain, at 'Gut Marienhof', which is located north of Munich (Figure 4). Meltwaters from the past ice ages deposited gravel in the Munich Gravel Plain, which resulted in the development of shallow pararendzinas soils with high drainage capacity

(Pfadenhauer 2001). This area was extensively covered in calcareous grassland until the middle of the nineteenth century, but agricultural intensification and artificial nutrient addition to the soils greatly reduced the calcareous grassland in size and connectivity (Pfadenhauer 2001).

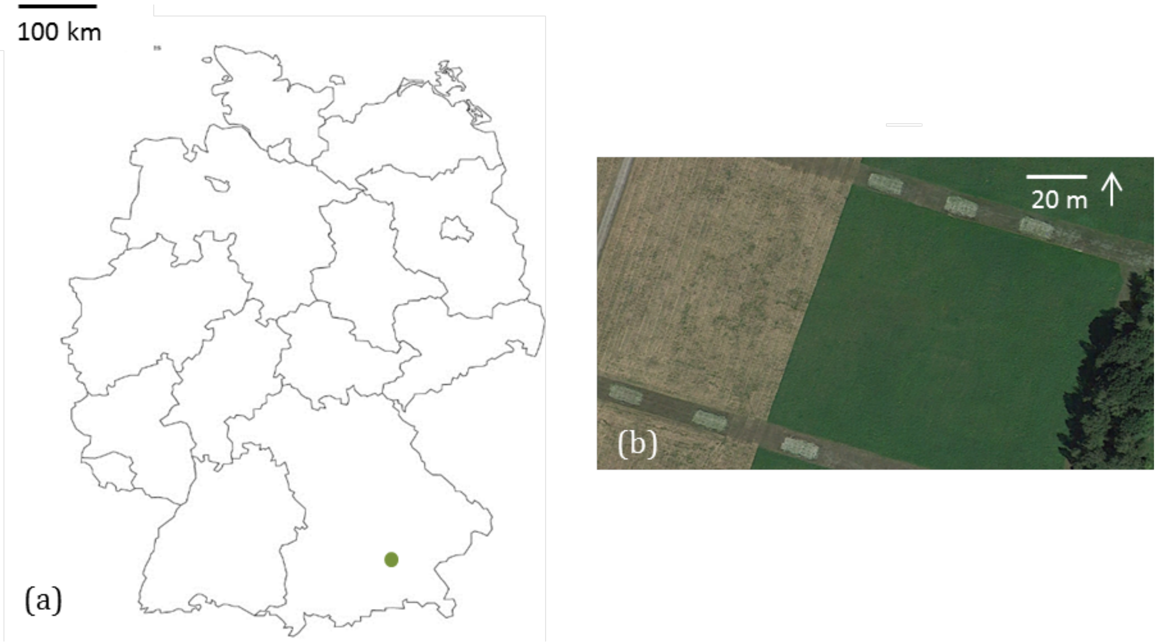

Figure 4 (a) Map of Germany with location of 'Gut Marienhof' marked in green. (Source: Bruce James Design Inc.) (b) Satellite image of field site. The six experimental blocks are seen in the middle of the two narrow roads (Source: Google Earth June 2013).

Study species

Dominant species

Festuca rubra (Poaceae) is commonly included in grassland mixtures and known to have competitive cultivars (Conrad and Tischew 2011). Therefore, it was selected as the dominant species for the experiments. The tussock perennial grass *Festuca rubra* ssp. *commutata* GAUDIN (synonym *Festuca nigrescens* Lam.) was used in all three experiments and is widely employed in grassland restoration due to its large ecological valance (Peratoner and Spatz 2004). It forms dense tussocks, which are generally dark green in colour with culms reaching up to 55 cm in height (Krautzer et al. 2004). The species, which is known for its competitive abilities, is commonly distributed in Alpine grasslands and pastures and the surrounding areas (Krautzer et al. 2004). *Festuca rubra* ssp. *rubra* L. was included in two of the experiments. It is a rhizomatous grass and former subspecies that produces widely spaced stolons and can produce culms up to 1 m in height (Hegi 1998) and roots of 50 cm (Oberdorfer 2001). The species can become problematic in seed mixtures due to its abundant growth (B. Feucht, pers. comm). It is found in pre-Alpine and Alpine grasslands and pastures (Oberdorfer 2001).

Subordinate species

Three perennial forbs from different families that are found in calcareous grasslands were selected as the subordinate species. An additional factor in the selection of subordinate species was the availability of both cultivar and regional seed. The species, *Buphthalmum salicifolium* L., *Dianthus carthusianorum* L. and *Linum perenne* L. are considered moderately rare in the lowlands of southern Germany but are commonly included in seed mixtures for their aesthetic properties. The hemicryptophyte, *B. salicifolium* (Asteraceae) ranges between 30–70 cm in height (Hegi 1979b). It produces leaves that are between 5–10 cm in length and yellow flowers roughly 3–5 cm in diameter (Sebald et al. 1996). *D. carthusianorum* (Caryophyllaceae) is a chamaephyte and produces a rosette with stems between 30–45 cm in height (Bloch et al. 2006). The species forms 5–10 pink flowers in clusters (Hegi 1979a). *L. perenne* (Linaceae) is also a hemicryptophyte. It has multiple upright stems that grow up to 60 cm in height and have light blue flowers 1.5–2 cm in diameter at the end of the stems (Hegi 1965). The dominant and subordinate species are identified in Figure 5.

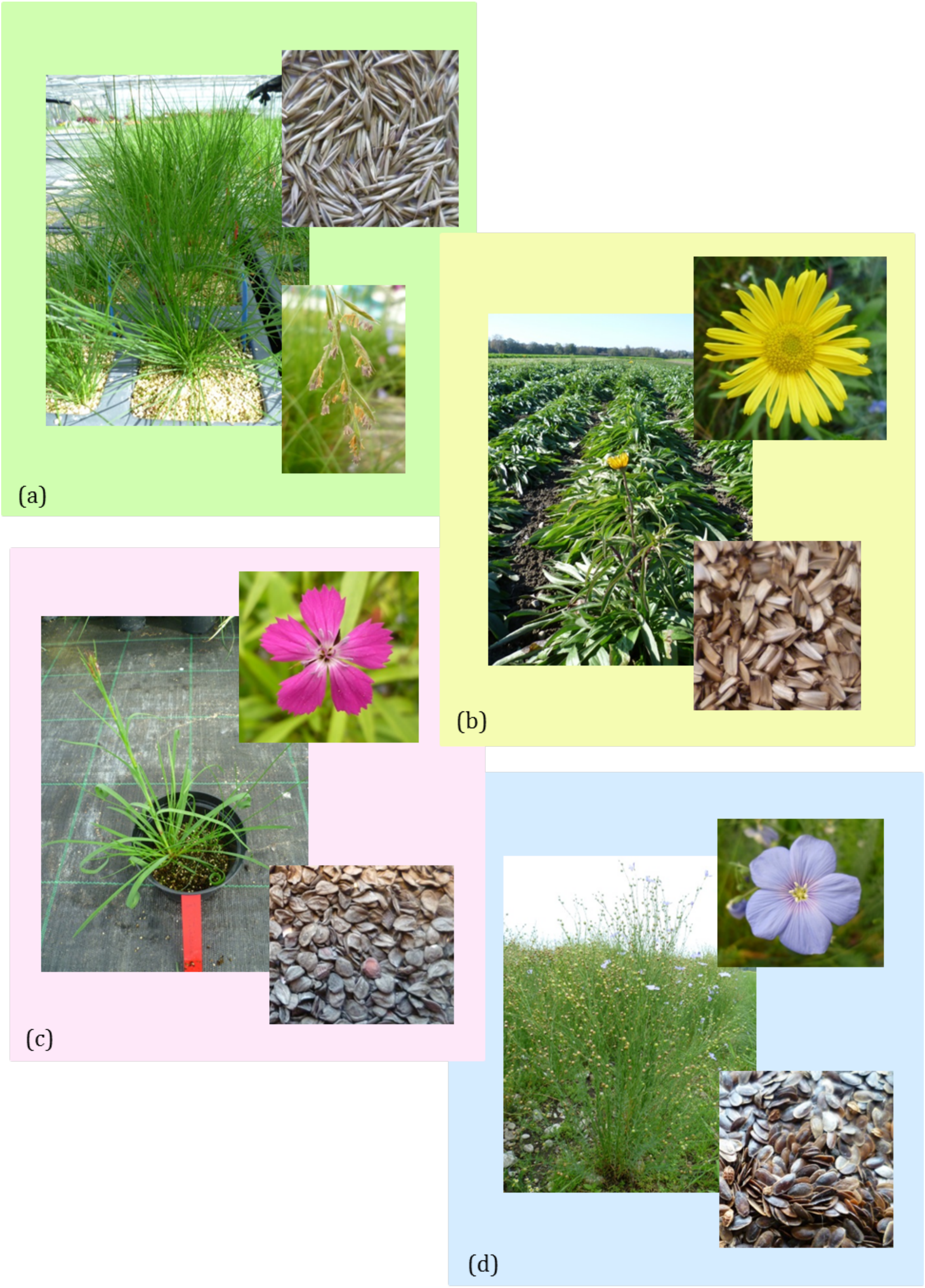

Figure 5 Growth form, flower and seed of the four study species of the PhD dissertation. Dominant species: (a) *Festuca rubra*; Subordinate species: (b) *Buphthalmum salicifolium*, (c) *Dianthus carthusianorum* and (d) *Linum perenne.*

2. Chapter 1

Mixed evidence for the cultivar vigour hypothesis: the case of calcareous grassland forbs in a matrix of *Festuca rubra*

2.1. Abstract

According to the cultivar vigour hypothesis, cultivars are frequently selected for traits such as increased biomass and resource capture, which may hinder the establishment of non-cultivars. Despite this concern, cultivar seed material is still used in grassland restoration. An alternative is wild plant material, which is commercially produced and used in the respective region ('regional seed'). We established a greenhouse experiment to examine the performance of cultivar and regional forb species with and without competition from cultivar and regional dominant grass species. This was conducted in order to understand the potential effects of sowing cultivar seed in grassland restoration projects. Three calcareous grassland forb species (*Buphthalmum salicifolium, Dianthus carthusianorum* and *Linum perenne*) and one dominant grass species (*Festuca rubra* ssp. *commutata*) were selected. One forb species was sown in the centre of each pot and either zero, two or four *F. rubra* ssp. *commutata* individuals were planted around it. Above-ground biomass, plant height, leaf length, leaf width, number of leaves and stems were measured. When grown without competitors, each forb species performed differently with only *L. perenne* exhibiting cultivar vigour. Cultivar forbs were less negatively affected by competition with *F. rubra* ssp. *commutata* than their regional counterparts, thus supporting the cultivar vigour hypothesis. Overall, regional *F. rubra* ssp. *commutata* suppressed the above-ground biomass of the forbs species more than the cultivar *F. rubra* ssp. *commutata,* thus highlighting the potential competitiveness of regional seed. Ultimately, the goals of the restoration project will determine the source of seed material to be used in the project. Due to the complex nature of the issues involved in restoration, it should be considered that the reliance on simple guidelines in relation to the source of seed material used in grassland restoration has its limitations.

2.2. Introduction

Calcareous grasslands are among the most species-rich habitats in Europe, particularly at small scales (Willems et al. 1993), and contain many rare and threatened species (WallisDeVries et al. 2002). Due to their semi-natural origin, calcareous grasslands require constant maintenance in the form of mowing or grazing to prevent successional changes (Kollmann and Poschlod 1997, WallisDeVries et al. 2002). In the past century, habitat loss has occurred in up to 60% of German calcareous grasslands (Krauss et al. 2004). These losses have been largely due to changes in land use (Poschlod et al. 2005), mostly agricultural intensification (Tscharntke et al. 2005), land abandonment (Poschlod and WallisDeVries 2002), and the decline of traditional farming techniques, such as grazing (Fischer et al. 1996). Due to these declines, it is necessary not only to conserve, but also to restore calcareous grasslands. Over the past decade, grassland restoration has become a rapidly developing field of research with many advances in the techniques used to restore grassland biodiversity and ecosystem function (Scotton et al. 2011).

Habitat fragmentation disrupts the mechanisms of gene flow (Diacon-Bolli et al. 2013). Furthermore, as seeds of many grassland species have little persistence and poor dispersal, seed banks may be impoverished (Bakker and Berendse 1999) or non-existent (Prach et al. 2013). This necessitates the re-introduction of seed to establish the desired vegetation. The source of the seed used for re-introduction largely depends on the goals of the restoration (Knapp and Rice 1994). Locally-sourced seed is widely recommended for use when the restoration goals are to restore biodiversity and ecosystem function (Bischoff et al. 2010). One of the main drawbacks of using locally-sourced seed is the limited availability of suitable donor sites. Material collected directly from donor sites within a provenance region, and then propagated and applied within the same provenance region is referred to as ‘regional seed’. The use of regional seed mixtures allows for greater control of the species composition and relative abundance within seed mixtures in comparison to the hay transfer method for plant re-introduction (Scotton et al. 2011). However, the availability and diversity of regional seed may be limited or completely absent in certain areas. Additionally, regional seed is more expensive than the widely available cultivars (Conrad 2007).

In cases where the aim of the restoration is to achieve an early and dense vegetation cover, the use of cultivars, especially for small areas which have undergone a high degree of disturbance, may be suitable (Lesica and Allendorf 1999). Cultivars are widely available and are often selected for advantageous traits, which may boost their competitive ability over non-cultivars (Fehr 1987). These traits include faster germination (Schröder and Prasse 2013a), greater basal area (lateral direction measurements; Wilsey 2010), and enhanced above-ground physiological performance (Lambert et al. 2011). The 'cultivar vigour hypothesis' (Wilsey 2010) proposes that traits which were intentionally selected for, such as enhanced resource capture, enable cultivars to produce greater above- and below-ground biomass than regional genotypes. Vigorous above-ground biomass production is a competitive advantage for cultivars, which is likely to hinder the establishment of late-successional grassland species (Gustafson et al. 2004). Despite these potential problems, cultivars continue to make up a large proportion of the seed in commercial seed mixtures (Conrad and Tischew 2011).

During grassland restoration, dominant grass species play an important role in directing the recovery of ecosystem processes (Baer et al. 2002). Dominant species are generally few in number, but often large in structure and contribute greatly to the biomass of the community (Grime 1998). Subordinate species, in contrast, are smaller in stature and contribute less to the primary productivity, but they are numerous and often co-occur with specific dominant species (Grime 1998). Mixed evidence has emerged concerning the effects of dominant species cultivars on vegetation development. Dominant species cultivars were found to suppress subordinate species in the studies of Baer et al. (2005) and Conrad and Tischew (2011). Baer et al. (2005) found that the dominance of one cultivar grass species, *Panicum virgatum*, was most likely facilitated by greater viability and higher seed production of the cultivar. Conrad and Tischew (2011) showed that *Festuca rubra* cultivars hinder the recruitment and development of desirable subordinate grassland species due to their high competitive ability, and the accumulation of above-ground biomass reduces the availability of safe sites. In contrast, Gibson et al. (2013) found limited evidence to suggest that the use of dominant cultivar and non-cultivar species impacts community composition during grassland development. This result was observed despite differences in the root networks and the physiological performance of the cultivar and non-cultivar plants used (Klopf and Baer 2011, Lambert et al. 2011). Above- and below-ground ecosystem function was unaffected by the seed source material of the dominant species (Baer et al. 2013). However, the success of the subordinate species, which amounted to half of the above-ground biomass for each of the plots, may have limited the effects of the dominant grasses (Baer et al. 2013). While most of the research on dominant species cultivars has been conducted in field experiments, there is a lack of studies on the cultivar vigour hypothesis under controlled conditions. Such studies allow a more mechanistic understanding of the effects of dominant species cultivars on subordinate species.

In order to explore the validity of the cultivar vigour hypothesis in relation to dominant and subordinate species of calcareous grasslands, we set up a greenhouse experiment using cultivar and regional genotypes of both the dominant species, *Festuca rubra* ssp. *commutata* GAUDIN, and the three subordinate forb species, *Buphthalmum salicifolium* L., *Dianthus carthusianorum* L. and *Linum perenne* L. We examined the above-ground biomass of the subordinate plants with and without competition of the dominant species to test the following hypotheses: (1) cultivars of subordinate species will have more enhanced growth-related traits than regional subordinate species without and in the presence of competitors; (2) cultivars of the dominant species will suppress the subordinate species cultivar less than the regional subordinate species and (3) cultivars of the dominant species will develop more biomass than regional plants without competition. Confirmation of these hypotheses would support the cultivar vigour hypothesis.

2.3. Materials and methods

Species selection

The perennial grass *Festuca rubra* ssp*. commutata* (Poaceae) (synonym *Festuca nigrescens* Lam.) is known for its competitive abilities (Krautzer et al. 2004), and was therefore selected as the dominant species in this experiment. This species is widely used in regional seed mixtures and grassland restoration projects, and has a large ecological valence (Peratoner and Spatz 2004). *F. rubra* is a hemicryptophyte and forms dense tussocks with 20–55 cm culm height, and it can be found in grasslands and pastures in and around the Alps (Krautzer et al. 2004).

Three representative perennial calcareous grassland forbs from different families were selected as subordinate species. The species, *Buphthalmum salicifolium*, *Dianthus carthusianorum* and *Linum perenne*, were also chosen according to the requirement of having readily available cultivar and regional genotypes. They are considered to be moderately rare in the lowlands of southern Germany. *B. salicifolium* (Asteraceae) is a hemicryptophyte that grows to 30–70 cm in height (Hegi 1979b). *D. carthusianorum* (Caryophyllaceae) is a chamaephyte and produces 30–45 cm tall stems (Bloch et al. 2006). *L. perenne* (Linaceae) is another hemicryptophyte with multiple upright stems, which generally reach heights of 20–60 cm and are densely covered with leaves (ca. 2 cm in length; Hegi (1965)).

Cultivar *Festuca rubra* ssp*. commutata* 'Smaragd'(unspecified origin), was supplied by Saatzucht Steinach GmbH & Co. KG (Steinach, Germany). The cultivar subordinate species, *Buphthalmum salicifolium '*Sunwheel' (south-eastern Europe origin), *Dianthus carthusianorum* catalogue number DA162 (European Alps origin), and *Linum perenne* 'Himmelszelt' (unspecified origin) were obtained from Jelitto Staudensamen GmbH (Schwarmstedt, Germany). *D. carthusianorum* DA162 is a cultivated plant but not a registered cultivar. It was collected from wild material and propagated over ten generations, but it was most likely not crossed with other cultivars (Jelitto, pers. comm.). Regional dominant and subordinate seed were supplied by the regional seed producer Johann Krimmer (Pulling, Germany). The regional genotypes were produced from seed collected in the greater Munich area and were propagated for no more than four generations. Prior to the experiment, the seed material was stored in a refrigerator at 4 °C.

Experimental design

In February 2012, the competition experiment was established at the Centre of Greenhouses and Laboratories Dürnast, Life Science Centre Weihenstephan, Technische Universität München, Germany; it was completed in November 2012. No additional light was used in the greenhouse and the average temperature in the greenhouse during this period was 19 ± 6 °C.

The experiment followed a full factorial, partial additive design consisting of four blocks. Regional and cultivar genotypes of the three subordinate species were subjected to two treatments, i.e. 'dominant species genotype' (*F. rubra* ssp. *commutata* cultivar or regional, hereafter '*F. rubra*') and 'competition density' (either zero, two or four individuals of the dominant species per pot). An individual subordinate species was planted in the centre of a pot, and either zero, two or four *F. rubra* seeds of cultivar or regional genotype were sown equidistantly at the edges of the pot. Each treatment was replicated five times per block (20 replicates in total), but due to failed germination and seedling mortality the replicate numbers were reduced. As no significant effect was observed between two and four *F. rubra* individuals per pot for any of the subordinate species (Mann-Whitney *U*-test, $p > 0.05$), the data from the dominant species density treatments were combined for each subordinate species. This augmented the number of replicates for the analysis. In addition, cultivar and regional *F. rubra* was grown without the presence of the subordinate species. In the control pots, only one *F. rubra* individual was planted per pot.

Rectangular trays containing 15 pots were used in the experiment (Figure 6a); the pots measured 8 x 8 x 10 cm^3 and were filled with vermiculite substrate (Bayerische Gärtnerei-Genossenschaft eG, Aschheim, Germany). Each pot was randomly assigned to a treatment. In order to minimise edge effects, trays were aligned side by side on the greenhouse tables. However, due to the weight of the substrate material, particularly when wet, it was not feasible to rearrange the trays during the experiment. The pots were watered as often as necessary by flooding the tables from beneath, with each block receiving the same frequency and duration of watering. Six weeks after the initial sowing, each pot received 100 ml of fertilizer Ferty® 3 Grün (15% N, 10% P_2O_5, 15% K_2O) at 6‰ concentration.

Harvest

In July 2012, trait measurements from the subordinate individuals were taken according to Cornelissen et al. (2003). Stretched height was measured for *D. carthusianorum* and *L. perenne*, while the longest leaf length was measured for *B. salicifolium* and *D. carthusianorum* (Figure 6b). Leaf width and number of leaves per individual were only measured on *B. salicifolium* and the number of stems was counted on *L. perenne*. Limited flowering was observed during the summer months and thus not included in the analysis.

Above-ground biomass of subordinate and dominant species was randomly cut across the five blocks in November 2012 over a period of 2 weeks, when plant growth was strongly reduced. All plant material was dried for 48 hours at 70 °C and then stored for a number of weeks. Directly prior to weighing the above-ground biomass, the material was dried again for 48 hours at 70 °C. Subordinate species were weighed individually, while the total above-ground biomass of the dominant species individuals per pot was merged.

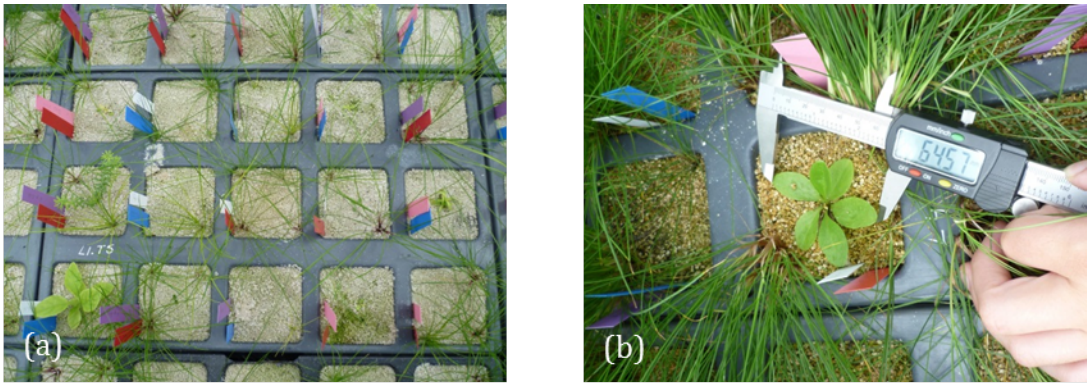

Figure 6 (a) Seedlings growth at ca. 8 weeks after sowing in rectangular tray with 15 pots, and (b) Vernier callipers used to measure leaf dimensions.

Data analysis

The differences in biomass among cultivar and regional genotypes of the subordinate species were analysed using the non-parametric Mann-Whitney U tests. Alternative analysis possibilities, such as factorial ANOVA were also explored. Due to the non-normal data distributions, even after transformation, and high mortality resulting in uneven replicate numbers, the Mann-Whitney U test was found to be the most suitable statistical test. Each subordinate species was analysed separately. The dominant species control biomass from cultivar and regional sources was analysed using a *t*-test. All of the statistical tests and graphics were conducted using the statistical software package SPSS Statistics 21.0.

2.4. Results

Subordinate species without and in the presence of competitors

When the subordinate species were grown without competition, only one of the three subordinate species, i.e. *L. perenne*, produced more above-ground biomass in the cultivar than the regional plants ($Z = -3.1$, $p < 0.001$, $n = 16$; Figure 7c). Cultivar *L. perenne* had a significantly greater height ($Z = -3.0$, $p < 0.001$, $n = 16$) and produced significantly more stems ($Z = -3.0$, $p < 0.001$, $n = 16$) than the regional genotype. In the case of *B. salicifolium*, there was no significant difference in the above-ground biomass of cultivar and regional genotypes ($Z = -0.3$, $p > 0.05$, $n = 10$; Figure 7a), or in any of the other traits examined. Contrastingly, regional *D. carthusianorum* produced significantly more above-ground biomass than the cultivar ($Z = -2.5$, $p < 0.05$, $n = 23$; Figure 7b). Regional *D. carthusianorum* individuals also had significantly greater height ($Z = -2.6$, $p < 0.01$, $n = 23$) and leaf length ($Z = -2.0$, $p < 0.05$, $n = 23$) than the respective cultivar.

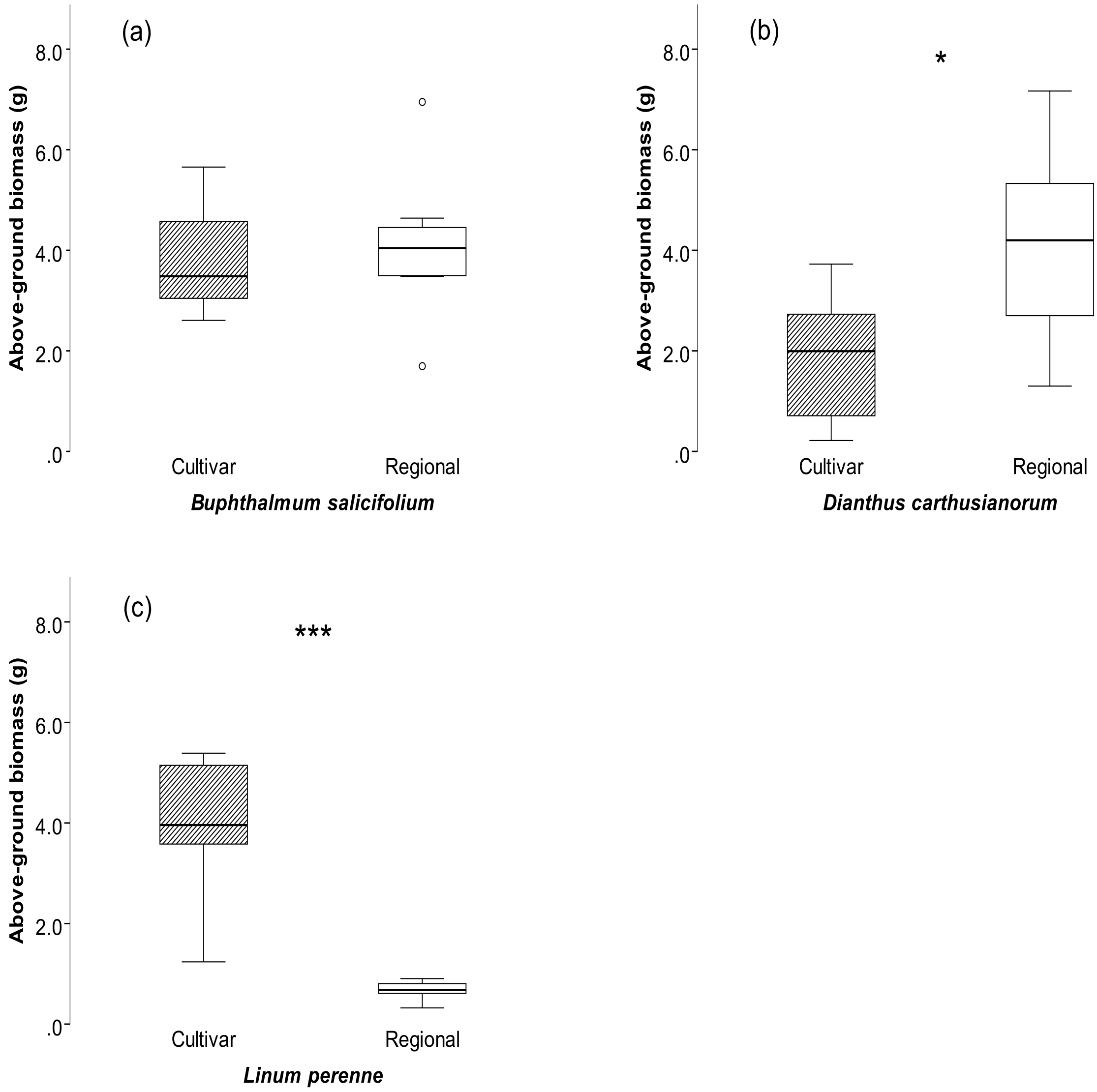

Figure 7 (a) Non-significant differences in cultivar ($n = 3$) and regional *Buphthalmum salicifolium* ($n = 7$) when grown without competitors (Mann-Whitney U-test, $Z = -0.3$, $p > 0.05$); (b) higher above-ground biomass of regional ($n = 9$) compared with cultivar *Dianthus carthusianorum* ($n = 14$), when grown without competitors (Mann-Whitney U-test, $Z = -2.5$, $p < 0.05$); and (c) higher above-ground biomass of cultivar *Linum perenne* ($n = 11$) compared with regional *L. perenne* ($n = 5$), when grown without competitors (Mann-Whitney U-test, $Z = -3.1$, $p < 0.001$). The striped box indicates the subordinate species cultivar; the white box indicates the regional subordinate species (* $p < 0.05$; *** $p > 0.001$).

No significantly different traits were observed for the cultivar and regional genotypes of any of the subordinate species grown with cultivar *F. rubra*. However, some differences were found in the traits of cultivar and regional genotypes of the subordinate species when grown in competition with regional *F. rubra*. Cultivar subordinate species exhibited greater above-ground biomass than regional subordinate species when grown in competition with either cultivar or regional *F. rubra*. Differences in the above-ground biomass were found to be significant only in the case of cultivar and regional *B. salicifolium* grown with regional *F. rubra*. Cultivar *B. salicifolium* produced significantly more above-ground biomass ($Z = -2.6$, $p < 0.01$, $n = 18$; Figure 8b), longer leaves ($Z = -2.9$, $p < 0.001$, $n = 18$), as well as a greater number of leaves ($Z = -3.3$, $p < 0.001$, $n = 18$), than regional *B. salicifolium* when grown with regional *F. rubra*. A significant difference in the height ($Z = -2.8$, $p < 0.01$, $n = 18$) of cultivar and regional *L. perenne* genotypes were also observed when grown with regional *F. rubra*. The height and number of stems of cultivar *L. perenne* was greater than its regional counterpart. Cultivar and regional genotypes of *D. carthusianorum* did not exhibit any observable differences when grown with either cultivar or regional *F. rubra* ($p > 0.05$).

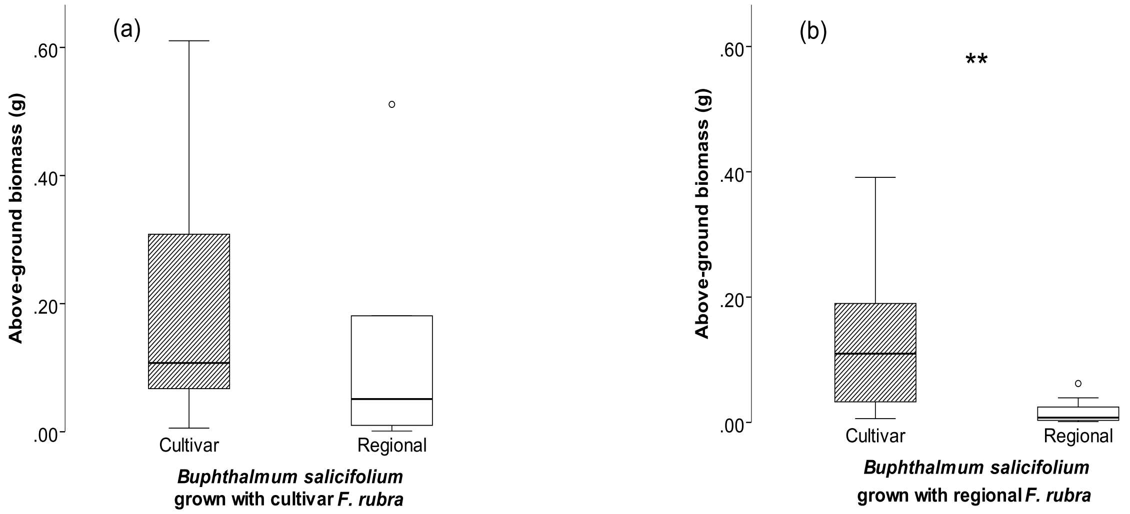

Figure 8 (a) Non-significant differences in biomass of cultivar ($n = 12$) compared with regional *B. salicifolium* ($n = 9$), when grown with cultivar *F. rubra* ssp. *commutata* (Mann-Whitney U-test, $Z = -1.1$, $p > 0.05$) and (b) Higher biomass of cultivar ($n = 11$) compared with regional *Buphthalmum salicifolium* ($n = 7$,) when grown with regional *F. rubra* ssp. *commutata* (Mann-Whitney U-test, $Z = -2.6$, $p < 0.01$). The striped box indicates the subordinate species cultivar; the white box indicates the regional subordinate species (** $p > 0.01$).

Dominant species suppression of subordinate species

Contrary to the second hypothesis, regional *F. rubra* was found to suppress the above-ground biomass of subordinate species more than cultivar *F. rubra*. The median values of the cultivar and regional subordinate species were greater under cultivar than regional *F. rubra*. However, only in the case of cultivar *D. carthusianorum* was there a significant difference in the above-ground biomass between dominant cultivar and regional species ($Z = -2.6$, $p < 0.05$, $n = 36$; Figure 9a). There was also a significant difference in the height ($Z = -2.5$, $p < 0.05$, $n = 36$) and leaf length ($Z = -2.3$, $p < 0.05$, $n = 36$) of cultivar *D. carthusianorum* when grown with cultivar and regional *F. rubra*. Greater height and leaf length was recorded with the cultivar *D. carthusianorum* genotypes exposed to cultivar rather than regional *F. rubra* competition. The traits measured from *B. salicifolium* and *L. perenne* genotypes did not exhibit any significant differences when exposed to cultivar or regional *F. rubra* ($p > 0.05$).

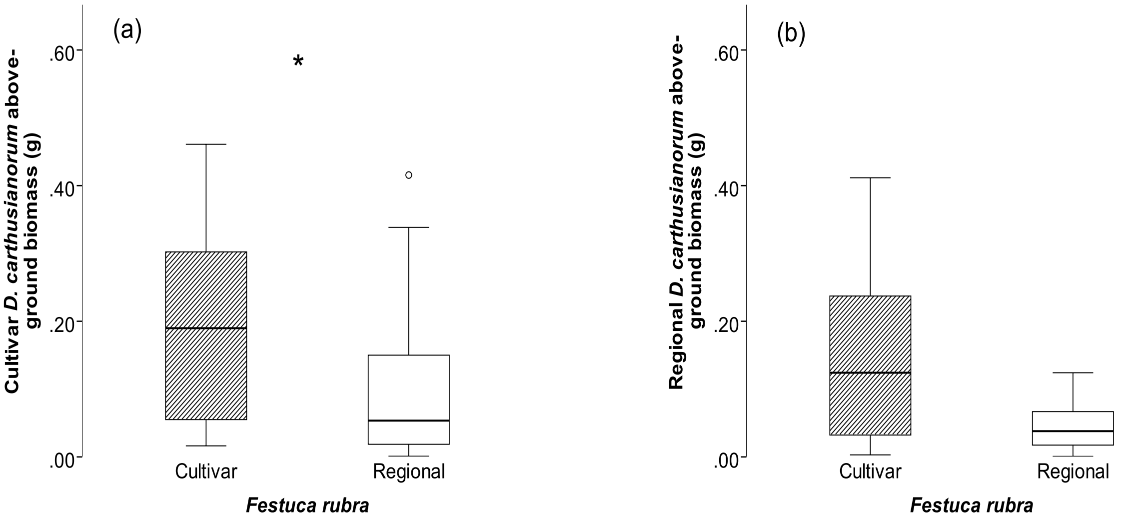

Figure 9 (a) Higher biomass of cultivar *Dianthus carthusianorum* when grown with cultivar *Festuca rubra* ssp. *commutata* (n = 18), compared with regional *F. rubra* ssp. *commutata* (n = 18) (Mann-Whitney U-test, $Z = -2.6$, $p < 0.05$); and (b) non-significant differences in biomass of regional *D. carthusianorum* when grown with cultivar (n = 15) and regional *F. rubra* ssp. *commutata* (n = 17) (Mann-Whitney U-test, $Z = -1.9$, $p > 0.05$). The striped box indicates the subordinate species cultivar growth with dominant species cultivar; the white box indicates the regional subordinate species growth with regional dominant species. (* $p < 0.05$).

Biomass production of dominant species

The results from the *F. rubra* control experiment showed that in the absence of competition, regional *F. rubra* produced more above-ground biomass than cultivar *F. rubra* (F = 1.66, $p < 0.02$, n = 34; Figure 10). This contrasts with the predicted outcome outlined by the third hypothesis.

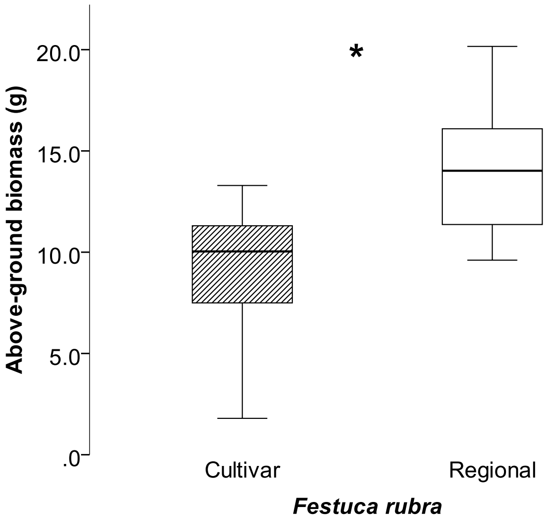

Figure 10 Higher biomass of regional *Festuca rubra* (n = 17) in comparison to cultivar *Festuca rubra* (n = 17) (t-test, F = 1.66, $p < 0.02$). The striped box indicates the dominant species cultivar; the white box indicates the regional dominant species. (* $p < 0.05$).

2.5. Discussion

Performance of subordinate species without competitors

In the absence of competition, cultivar subordinates were only found to produce more above-ground biomass in the case of *L. perenne*, while mixed results were observed for *B. salicifolium* and *D. carthusianorum*. Cultivar *L. perenne* produced significantly more stems than its regional counterpart and also had considerably greater root networks (E. A. Walker, unpubl. data), which strengthens the case for the cultivar vigour hypothesis in relation to this species. It cannot be excluded that at least some of these differences in vigour may be related to the maternal environmental conditions of the seed material. Regional seed material used in this experiment was propagated on nutrient-poor soils similar to those found in calcareous grasslands, while cultivars are generally cultivated under less stressful conditions. However, it should be noted that maternal effects are often most pronounced during the seedling stages of growth (Roach and Wulff 1987).

While significant differences were observed with the cultivar and regional genotypes of the other two subordinate species, the above-ground biomass production for cultivar and regional *B. salicifolium* was similar, although median biomass production was slightly greater for regional *B. salicifolium*. Leaf length, leaf width and number of stems also failed to show significant differences between the cultivar and regional genotypes. In the particular case of these *B. salicifolium* genotypes, the advantages of cultivar traits possibly cancelled out the advantages of regional traits, as predicted by the null hypothesis (Wilsey 2010). In some cases, cultivars may initially exhibit greater vigour. However, this is likely to come at a cost; their ability to survive harsher conditions and persist in the vegetation may be limited by their lack of local adaptation (Wilsey 2010, Schröder and Prasse 2013c).

In direct contrast to the findings of *L. perenne*, the above-ground biomass results from regional *D. carthusianorum* were significantly greater than of cultivar *D. carthusianorum*. One possible reason for this outcome is that the regional seed material used in this experiment was better adapted to the nutrient-poor conditions of the experiment than cultivar *D. carthusianorum*. This allowed the regional *D. carthusianorum* to grow taller and have longer leaves resulting in the production of more above-ground biomass. The leaf tips of many cultivar *D. carthusianorum* suffered from nutrient deficiency prior to the addition of fertiliser. However, only minor deficiencies were observed with regional *D. carthusianorum* individuals during this study (E. A. Walker, unpubl. data). It is therefore likely that in this experiment the nutrient demand of cultivar *D. carthusianorum* was greater than the nutrient demand of regional *D. carthusianorum*. As only one set of conditions was included in our study (low nutrient and ample water), it must be considered that the above-ground biomass response of cultivar and regional *D. carthusianorum* (as well as the other subordinate and dominant species) is likely to differ according to the amount of available nutrients. Under higher nutrient conditions, cultivars are expected to have the advantage, while adaptations to stressful nutrient conditions enabled regional *D. carthusianorum* to outperform cultivar *D. carthusianorum* in our study.

Performance of subordinate species in the presence of competitors

In the absence of competition, the three subordinate species responded very differently. However, in competition with cultivar and regional *F. rubra*, the cultivar genotype consistently produced more above-ground biomass than the regional genotype for each subordinate species. The findings were only significant in one case (regional *F. rubra* in competition with *B. salicifolium* genotypes), but the general trend of enhanced cultivar performance further supports the cultivar vigour hypothesis. More nutrient acquisition under competition is possibly responsible for the greater above-ground biomass of cultivar subordinate species. Cultivars have been found to have more extensive root networks, which would facilitate resource capture (Klopf and Baer 2011). Even though regional species are likely better adapted to the low nutrient conditions in the experiment, their efficiency at capturing resources when in competition with dominant species may not be as well developed as the subordinate cultivars. Interestingly, differences in the trait responses of the cultivar and regional subordinate species were only observed when grown with regional and not cultivar *F. rubra*. In this study, regional *F. rubra* had the greatest suppression effects on the subordinate species. Differences between the cultivar and regional genotypes of the subordinate species may only become apparent when they are exposed to a certain threshold of suppression. This may not have been reached, in this particular case, with cultivar *F. rubra*. Furthermore, as *Plantago lanceolata* root production was found to be stimulated in the presence of *F. rubra* competition (Padilla et al. 2013), it may also be possible that the root exudates of *F. rubra* trigger different root production responses in cultivar and regional subordinates. In our experiment, cultivar subordinates may have been less affected by *F. rubra* than the regional subordinates, which allowed them to produce more above-ground biomass.

As the source of subordinate species has not received much attention in grassland restoration literature, it was decided that the focus of this study would be on the effect of the dominant species on the subordinate species. However, it should be mentioned that the inverse effects of the subordinate species on the dominant species also exist but have not been included in this study.

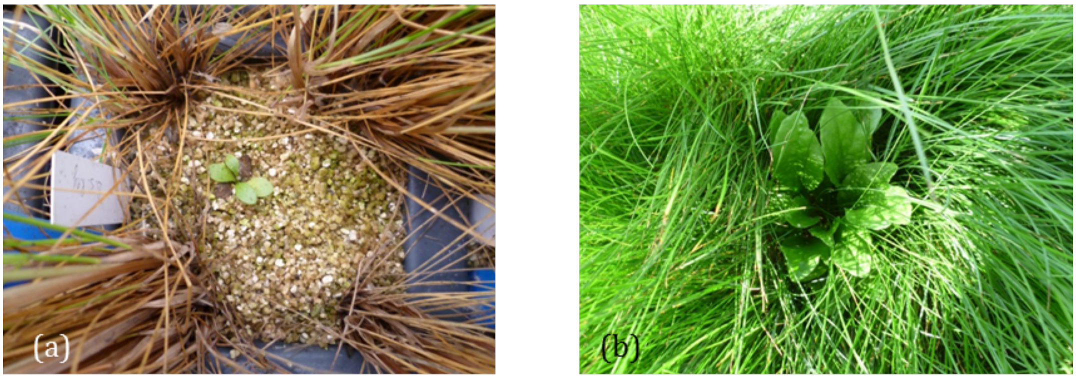

Figure 11 (a) *Buphthalmum salicifolium* in pot with four regional *Festuca rubra* individuals (ca. 10 months after sowing); (b) *Buphthalmum salicifolium* with grass competitors.

Effects of Festuca rubra

F. rubra cultivars are known for their negative competitive effects (Conrad and Tischew 2011). However, in our study regional *F. rubra* which produced more above-ground biomass and have the greater negative effects on the subordinate species. In comparison with the subordinate species grown with cultivar *F. rubra*, less above-ground biomass was observed for subordinate species grown with regional *F. rubra*, although this difference was only significant with cultivar *D. carthusianorum*. The superior performance of regional *F. rubra* in our experiment may have resulted from its better suitability to the low nutrient greenhouse conditions compared to that of cultivar *F. rubra*. In addition, allelopathic effects of *F. rubra* on root and shoot growth have been recorded (Bertin et al. 2003). It is plausible that the allelopathic capabilities of cultivar and regional *F. rubra* differ, which may have contributed to the greater suppression of subordinates by regional *F. rubra*. While the effects of dominant species cultivars in grassland restoration mixtures has been addressed (Baer et al. 2013, Gibson et al. 2013), the use of dominant regional or wild seed species, which may also be highly competitive, has received little attention. Our findings show that dominant regional species should also be considered for their competitive effects on the surrounding vegetation communities.

In this experiment, *L. perenne* frequently grew taller than the grasses and was therefore less affected by shading than *B. salicifolium* or *D. carthusianorum*. In contrast, rosette species, such as *B. salicifolium* in particular, are more vulnerable to the shading effects of dominant species (Figure 11a). It was observed that a number of the *B.*

salicifolium individuals failed to develop beyond the seedling stage when shaded by *F. rubra*. The effects of vigorous above-ground growth will impact the subordinate species differently depending on their growth forms.

2.6. Conclusions

Our results indicate that in a low nutrient greenhouse experiment, cultivar vigour is species-specific and related to the traits that the cultivar was selected for during the breeding process. The biotic and abiotic conditions present in this study reflect the nature of a controlled greenhouse experiment and may not accurately represent the conditions found in restoration sites. Under high nutrient conditions, the growth of cultivars may be favoured, which is a topic that warrants further investigation. The dominant species cultivar was not found to have the greatest suppressive effect on the subordinate species. It is therefore important not to overlook the effects and usage of competitive dominant species of regional source in seed mixtures. Subordinate species cultivars performed better than regional subordinate species when grown with the dominant species, which supports the cultivar vigour hypothesis. The inclusion of subordinate species cultivars in seed mixtures may negatively impact the growth of non-cultivar seed material. Certain growth forms, such as rosettes, are at a greater disadvantage than others under dense competition. This has consequences for the diversity of grassland forb species, as the vigorous growth of dominant species will impact certain growth forms more than others resulting in reduced ecosystem function. Thus, the selection of seed for restoration is a complex issue, and the source of the material should depend on the restoration goal. This study aims to highlight the complexity and limitations of simple guidelines in relation to the seed source used in grassland restoration.

3. Chapter 2

Seed source interacts with nutrient availability to mediate the competitive effect of a dominant grass on a subordinate grassland species

3.1. Abstract

Cultivars are selected for advantageous traits, which may enable them to have a greater competitive effect, particularly under high nutrient conditions. Sites with high nutrient availability may favour the development of dominant grasses rather than subordinate species, especially in systems that are usually nutrient limited, such as calcareous grasslands. The aim of this experiment was to determine whether the seed source influences the competitive effect of a dominant grass species on a subordinate species, and whether nutrient availability interacts with competitive effect. A greenhouse experiment with three nutrient treatments was established; *Dianthus carthusianorum* was chosen as the subordinate 'phytometer' to detect variation in the competitive effect of genotypes of *Festuca rubra* seed sources. The focal species was sourced from 13 cultivar and 11 commercially propagated, but not selected regional sources. No significant difference in the total number of emerged *F. rubra* seedlings from either source was observed, but cultivar *F. rubra* showed greater seedling emergence potential (this was calculated for each *F. rubra* source as the total number of emerged seedlings times the day after which ≥50% of the totally emerged seedlings were present). Wild sources of *F. rubra* had a greater competitive effect on the subordinate species than cultivars, but only in the medium and high nutrient treatments. Grasses from wild sources showed greater variability in emergence and competitive effect on *D. carthusianorum* than cultivar grasses. Contrary to the general notion that cultivars are more competitive than wild populations, the strong competitive abilities of wild *F. rubra* may hinder the establishment of forb-rich vegetation, contingent on nutrient availability.

3.2. Introduction

One of the most fundamental questions in ecological restoration is where to source the plant material. Many species found in calcareous grasslands have limited dispersal and no persistent seed bank (Stöcklin and Fischer 1999). Therefore, re-introducing desirable species as part of the restoration effort is usually required. Seed of regional origin is widely recommended for use in restoration, to protect the genetic integrity of regional populations (Hufford and Mazer 2003), and is expected to have some adaptation to the regional environmental conditions (Vander Mijnsbrugge et al. 2010). Cultivar seed of grass species is still included in seed mixtures, such as along road verges (Conrad and Tischew 2011), despite concerns relating to maladaptation and hybridisation of non-local genotypes (Hufford and Mazer 2003).

Cultivars have been selected for traits that are deemed favourable in relation to their morphology, physiology or reproduction (Fehr 1987). Traits such as faster and more abundant germination (Chrobock et al. 2011, Schröder and Prasse 2013a), rapid growth (Fehr 1987), enhanced nutrient uptake (Klopf and Baer 2011) and stress tolerance (Lesica and Allendorf 1999) can be beneficial to the establishment of grasses when the goal is to provide rapid vegetation cover, stabilise the soil and reduce unwanted species. Cultivar seed is widely available and generally costs less than regional seed sources, resulting in it being frequently included in seed mixtures (Conrad 2007). Commercially propagated, regionally produced seed, which has not been selected for particular traits, is an alternative for larger-scale projects. Regional seed is defined as seed which is collected from donor sites within a designated provenance region and then propagated for use within that region (Scotton et al. 2011). Although cultivars have many enhanced traits compared to non-cultivars, their robustness over time, particularly in relation to stochastic effects may be less than local or regional seed material (Schröder and Prasse 2013c). Moreover, the environmental conditions at a site are likely to affect the ability of the source of the seed used for restoration.

Naturally occurring or human-made nutrient gradients are found within and among grassland habitat patches. In recent times, nutrient loads in calcareous grasslands have generally increased due to more intensive agricultural practices and to atmospheric nitrogen deposition (Bobbink et al. 1998). Intensive agriculture requires soil enrichment in the form of artificial fertiliser addition to improve soil fertility (Bakker and Berendse 1999). The high nutrient loads that remain in the soil after fertilisation threaten species diversity of typically nutrient-poor calcareous grasslands (Critchley et al. 2002, Jacquemyn et al. 2003). Although nitrogen levels in the soil will decline relatively quickly over the course of a decade through leaching and biomass removal, phosphorous may require up to 50 years to return to pre-fertilisation levels (Fagan et al. 2008). Cultivars are often bred for efficient nutrient uptake, and their use in high nutrient soils may be problematic if they have a competitive advantage (Klopf and Baer 2011). In addition, dominant grasses benefit disproportionally from elevated soil nutrient conditions, which may further reduce community composition, especially during the early stages of grassland restoration (Walker et al. 2004). The community-level effects of competition from dominant species, however, may be mediated by site conditions (Freckleton et al. 2009, Hart and Marshall 2013). Therefore, the source (i.e. cultivar or regional) of the seed material and the nutrient conditions of a site are likely to influence competitive interactions between co-existing species.

Dominant species are few in number, but often relatively large and thus constitute a major component of the community biomass, whereas subordinate species, are generally smaller and contribute less biomass (Grime 1998). The ratio of dominant to subordinate species within a seed mixture is important in order to establish species-rich communities (Packard and Mutel 1997, Klopf et al. 2014). As cultivars are selected for their advantageous traits, such as early emergence and rapid growth, a temporal asymmetric competitive advantage may have consequences for future community dynamics, as outlined in the priority effect (Young et al. 2001, Körner et al. 2008). On the other hand, a recent study by Gibson et al. (2013) showed that the seed source of dominant species had only a limited influence on community composition over 4 years. However, further research is warranted to test the overall hypothesis that cultivars are stronger competitors.

The aim of this experiment was to determine whether seed source (cultivar versus regional) interacts with nutrient availability to influence the competitive effect of a dominant species. A greenhouse study was established to test the following hypotheses: 1) *Festuca rubra* cultivars emerge faster and in higher numbers than regional *F. rubra*; 2) *F. rubra* cultivars have a greater competitive effect on a regional subordinate species than regional *F. rubra*; 3) seed mass and speed of emergence are correlated with the competitive effect of the *F. rubra* sources; and 4) competitive effect increases with increasing soil nutrient content.

3.3. Materials and Methods

Species selection

Festuca rubra L. (Poaceae) is a dominant grass frequently used in grassland mixtures and is known to have highly competitive cultivars (Conrad and Tischew 2011). In this experiment, the competitive effect of both *Festuca rubra* ssp. *commutata* GAUDIN and *Festuca rubra* ssp. *rubra* L. from cultivar and regional sources was investigated. *F. rubra* ssp. *commutata* forms dense, dark green tussocks and generally produces culms 20–55 cm in height (Krautzer et al. 2004). *F. rubra* ssp. *rubra* has a rhizomatous growth form and can grow to heights of 1 m (Hegi 1998). Both species are found in grasslands and pastures in the pre-alpine and alpine region in central Europe (Hegi 1998). For analysis purposes, *Festuca rubra* ssp. *commutata* and *Festuca rubra* ssp. *rubra* were combined (‘*F. rubra*’), resulting in 13 cultivar *F. rubra* and 11 regional *F. rubra* (Table 1).

The subordinate species *Dianthus carthusianorum* L. (Caryophyllaceae) was selected for use as a ‘phytometer’ to detect variation in the competitive effect of a dominant grass species. *D. carthusianorum* is a rosette forb with approx. 30–45 cm tall flowering stems (Bloch et al. 2006). The species is insect pollinated and produces 5–10 pink flowers in clusters (Hegi 1979). It is characteristic of calcareous grasslands in central Europe (Oberdorfer 2001), but rare in calcareous grasslands of the German pre-alpine lowland (T. Conradi, unpubl. data). The species is commonly used in re-vegetation due to its aesthetically appealing flowers. *D. carthusianorum* seed was obtained from Johann Krimmer, a regional producer in Pulling, Upper Bavaria. Regional seed was originally sourced from wild populations within the provenance region and propagated for up to four generations without intentional selection for specific traits.

Table 1 Cultivar and regional *Festuca rubra* genotypes used as the dominant species under different nutrient treatments. Plant biomass, height and number of tillers were determined after 12 weeks. Abbreviations: Cult., cultivar; Reg. , regional; Rhiz., rhizomatous; and Tus., tussock.

Grass source	Growth form	“Cult.” or provenance	Commercial supplier	*n*	Mean seed mass (g)	Mean biomass (g)	Mean height (cm)	Mean no. tillers
Cult.	Rhiz.	"Maxima"	DLF-Trifolium	5	0.853	0.568	30.5	17
Cult.	Rhiz.	"Roland"	DLF-Trifolium	5	0.121	0.970	36.1	22
Cult.	Rhiz.	"Rossinante"	DLF-Trifolium	4	0.768	1.003	35.2	21
Cult.	Rhiz.	"Sergei"	DLF-Trifolium	5	0.269	1.066	35.0	22
Cult.	Rhiz.	"Shadeway"	DLF-Trifolium	4	0.115	0.597	38.1	14
Reg.	Rhiz.	Dummersdorf	Landschaftspflegeverein Dummersdorfer Ufer e.V.	5	0.327	0.154	24.3	8
Reg.	Rhiz.	Halle	Matthias Stolle	5	0.559	1.134	50.5	15
Reg.	Rhiz.	Pulling	Johann Krimmer	4	0.713	0.682	33.7	18
Cult.	Tus.	"Azurit"	Saatzucht Steinach GmbH &Co KG	5	0.106	0.238	26.3	9
Cult.	Tus.	"Blenheim"	DLF-Trifolium	5	0.773	0.400	24.9	13
Cult.	Tus.	"Capriccio"	DLF-Trifolium	5	0.780	0.689	28.4	23
Cult.	Tus.	"Carina"	DLF-Trifolium	5	0.770	0.340	26.0	16
Cult.	Tus.	"Casanova"	DLF-Trifolium	4	0.842	0.643	26.7	14
Cult.	Tus.	"Legende"	DLF-Trifolium	5	0.622	0.439	22.5	14
Cult.	Tus.	"Salut"	Saatzucht Steinach GmbH &Co KG	5	0.870	0.446	27.2	12
Cult.	Tus.	"Smaragd"	Saatzucht Steinach GmbH &Co KG	5	0.106	0.602	24.3	23
Reg.	Tus.	Blaufelden	Rieger-Hofmann GmbH	5	0.118	0.941	40.5	14
Reg.	Tus.	Glandorf	Rieger-Hofmann GmbH	5	0.861	0.600	30.5	14
Reg.	Tus.	Jessishoe	Rieger-Hofmann GmbH	4	0.447	1.028	39.5	21
Reg.	Tus.	Lommatzsch	Rieger-Hofmann GmbH	5	0.727	0.810	46.1	16
Reg.	Tus.	Lübeck	Rieger-Hofmann GmbH	4	0.446	0.107	22.3	11
Reg.	Tus.	Neumarkt	Rieger-Hofmann GmbH	5	0.950	0.936	35.9	19
Reg.	Tus.	Pulling	Johann Krimmer	5	0.844	0.680	30.3	12
Reg.	Tus.	Wetzlar	Rieger-Hofmann GmbH	5	0.697	0.797	29.0	15

Experimental design

A greenhouse experiment was established in the Centre of Greenhouses and Laboratories Dürnast, Life Science Centre Weihenstephan, Technische Universität München, Germany, to investigate the competitive effect of the two seed sources of the dominant grass on our phytometer in contrasting levels of nutrient availability. The experiment ran for 12 weeks (early June until late August 2013) in an unheated greenhouse which had a mean temperature of 22 ± 7 °C.

The phytometer, *D. carthusianorum*, was subjected to two treatments: *F. rubra* genotype source (cultivar or regional) and a nutrient treatment (control, medium, high). Each *F. rubra* –*D. carthusianorum* combination was replicated five times per nutrient treatment. Round pots (9 cm diameter) were lined at the base with 100 cm^3 of potting soil (Floradur B Seed) and filled with 250 cm^3 of vermiculite (Bayerische Gärtnerei-Genossenschaft e.G., Aschheim, Germany). Two seeds of both *D. carthusianorum* and *F. rubra* (from either a cultivar or regional source) were sown per pot to account for failed germination. Once seedlings emerged, excess seedlings were removed to ensure that each pot contained one *D. carthusianorum* and one *F. rubra* seedling. Some of the *F. rubra* seeds failed to emerge, resulting in unequal replicates for some nutrient treatments.

The pots were watered twice a week from beneath and allowed to soak for 1 hour. Throughout the experiment the pots were randomised weekly to prevent edge effects. All pots were initially placed on one table and later transferred to three separate tables shortly before the first nutrients were added to prevent cross contamination of the nutrient treatments by watering. Five weeks after sowing, nutrients were added to each pot in the medium and high treatments by pouring 100 ml of the fertilizer Ferty Grün 3 (15% N, 10% P_2O_5, 15% K_2O) at a concentration of 6‰ into each pot (9.4 g m^{-2}). Two weeks after the initial nutrient treatment, pots in the high nutrient treatment received a second dose of Ferty Grün 3 at the same concentration. The control treatment received no additional nutrients.

Trait measurements

For each cultivar and regional *F. rubra* source, the seed mass was measured as the mean weight of five replicates of 100 seeds. Seedling emergence was examined at day 9, 13, 17 and 22 after sowing. Twelve weeks after sowing, the stretched height of *D.*

carthusianorum plants was measured according to the protocol outlined in Cornelissen et al. (2003). The aboveground biomass of phytometer plants was harvested directly following the height measurements by cutting the biomass 1 cm above the surface of the vermiculite. All biomass was then oven dried at 70°C for 48 hours prior to weighing.

Data analysis

The median number of emerged seedlings per day of observation was calculated (from a maximum of ten emerged seeds per treatment). These values were also used to calculate the seedling emergence potential (SEP). SEP for each genotype was calculated as the total number of emerged seedlings times the day after which ≥50% of the totally emerged seedlings were present. Thus, SEP accounts for speed of emergence and the total number of emerged seedlings. After checking for normality, differences in variability were tested for the total number of emerged seedlings (until day 22 after sowing) and seedling emergence potential were assessed with F-tests. Differences in the total number of emerged seedlings and SEP between cultivar and regional grasses were tested with two-sample t-tests.

Spearman's rho was used to determine whether a correlation existed between SEP or seed mass and competitive effect based on biomass and height. This was measured separately in the three nutrient treatment levels.

The competitive effect of each cultivar and regional *F. rubra* source (CE_{gen}) on the phytometer *D. carthusianorum* was calculated based on biomass and height values separately as

$$CE_{gen} = P/P_{gen}$$

with P being the phytometer biomass or height in the respective nutrient treatment level without intra- and interspecific competition (one individual), and P_{gen} being the phytometer biomass or height with competition from the respective *F. rubra* source. Thus, a high value for competitive effect of a *F. rubra* source indicates a high net effect of competition on the phytometer that is independent of the direct response of the phytometer to the environment (Chesson 2000, Adler et al. 2013). As each grass genotype was grown with the phytometer species in multiple replicate pots, the mean competitive effect value for each grass genotype was calculated and used in all subsequent analyses. An analysis of variance was used to test for the effect of grass source, nutrient treatment, and their interaction on competitive effect based on phytometer biomass and height, respectively. As competitive effect values could not be transformed to normality and because of the unbalanced design, permutation tests were used to obtain estimated *p*-values based on sums of squares, drawing random samples from all possible permutations until the estimated standard deviation of the *p*-value was below 10% of the estimated *p*-value (Anscombe 1953). Permutation tests were performed with package 'lmPerm' (Wheeler 2010) in R.

3.4. Results

Differences in seedling emergence between cultivar and regional genotypes

F. rubra cultivars had a significantly higher seedling emergence potential (Figure 12a, $t = -2.14$, $p = 0.04$), whereas the total number of emerged seedlings did not differ between the cultivar and regional source groups ($t = -1.54$, $p = 0.14$). *F. rubra* sources differed significantly in variability of the total number of emerged seedlings ($F = 3.99$, $p = 0.03$), with a higher variability in regional genotypes (Figure 12b), whereas variability in seedling emergence potential was only marginally different between both sources ($F = 2.82$, $p = 0.09$).

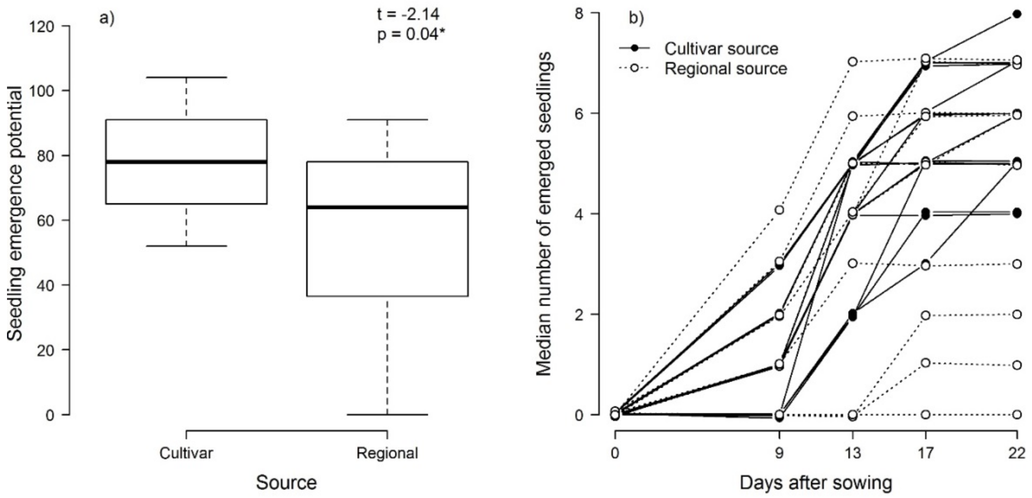

Figure 12 (a) Differences in seedling emergence potential, which is calculated as the total number of emerged seedlings times the day after which ≥50% of the totally emerged seedlings were present, between regional and cultivar genotypes of *Festuca rubra*. (b) Rate of emergence in regional and cultivar grass genotypes; random noise was added to avoid overlapping points.

The influence of seed mass and species emergence potential on competitive effect

We found no correlation between seed mass and competitive effect based on biomass (rho for low nutrient treatment = –0.07, medium = –0.1, high = –0.25) or on height (rho for low = –0.32, medium = –0.19, high = –0.17). In addition, there was no correlation between SEP and competitive effect based on biomass (rho for control nutrient treatment = 0.06, medium = 0.41, high = –0.17) or height (rho for control nutrient treatment = 0.25, medium = 0.22, high = –0.06).

Competitive effect of dominant grass genotypes along the soil nutrient gradient

When competitive effect was calculated from phytometer biomass, source (cultivar versus regional), nutrient treatment, and their interaction on competitive effect were significant (Table 2). It should be noted however, that the borderline-significant *p*-values for source and nutrient treatment sometimes changed to non-significant depending on the randomly drawn subset of permutations. Genotypes from regional sources had a smaller competitive effect than cultivars in the control nutrient treatment, but the opposite relationship was observed in the medium and high nutrient treatments (Figure 13a). Competitive effect values for regional sources were highest at the medium nutrient treatment and lowest at control (low) nutrient treatment. Competitive effect values for cultivars were highest at intermediate nutrient treatment, too, but lowest at high nutrient treatment. Therefore, the difference in competitive effect based on biomass between cultivar and regional sources was largest at higher nutrient conditions.

When the competitive effect was calculated using phytometer height, nutrient treatment only had a strongly significant effect, whereas grass source and its interaction with nutrient treatment had no effect (Table 2). This result was unaffected by the subsets of permutations used to obtain *p*-values. Although this interaction was not significant, regional genotypes were again more competitive than cultivars when nutrient treatment was low, and lower values when nutrient treatment was medium and high (Figure 13b). Both regional and cultivar genotypes had highest competitive effect values at intermediate and lowest values at high nutrient supply (Figure 14).

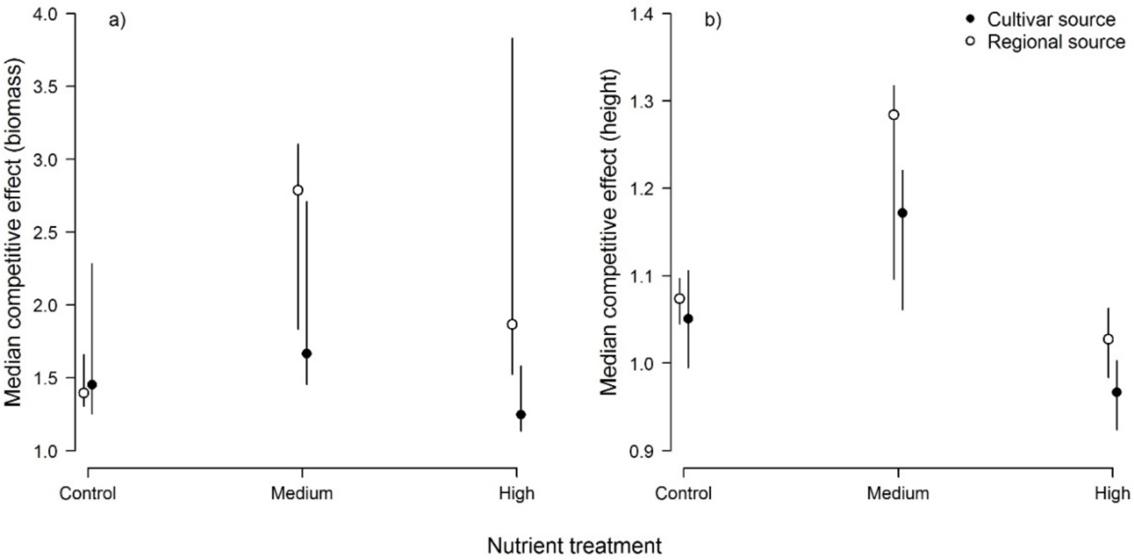

Figure 13 The competitive effect of cultivar and regional *Festuca rubra* genotypes based on (a) phytometer biomass and (b) height. *Dianthus carthusianorum*, a subordinate species of calcareous grasslands, was used as phytometer. Points indicate median values; bars show upper and lower quartiles, respectively.

Table 2 The effects of grass source (cultivar versus regional seed), nutrient treatment and their interaction on the competitive effect (based on phytometer biomass and height) of dominant grass genotypes used for grassland restoration. A subordinate species of calcareous grasslands, *Dianthus carthusianorum*, was used as phytometer. ANOVA table with estimated *p*-values obtained from permutation tests; *ss* are unique sums of squares of the effects.

	df	*ss*	*ms*	Permutations	*p*
Biomass					
Grass source	1	3.03	3.03	2000	0.048
Nutrient treatment	2	5.06	2.53	2048	0.046
Interaction	2	8.63	4.31	5000	0.004
Residuals	65	55.77	0.86		
Height					
Grass source	1	0.03	0.03	628	0.139
Nutrient treatment	2	0.43	0.21	5000	<0.001
Interaction	2	0.04	0.02	713	0.224
Residuals	65	0.78	0.01		

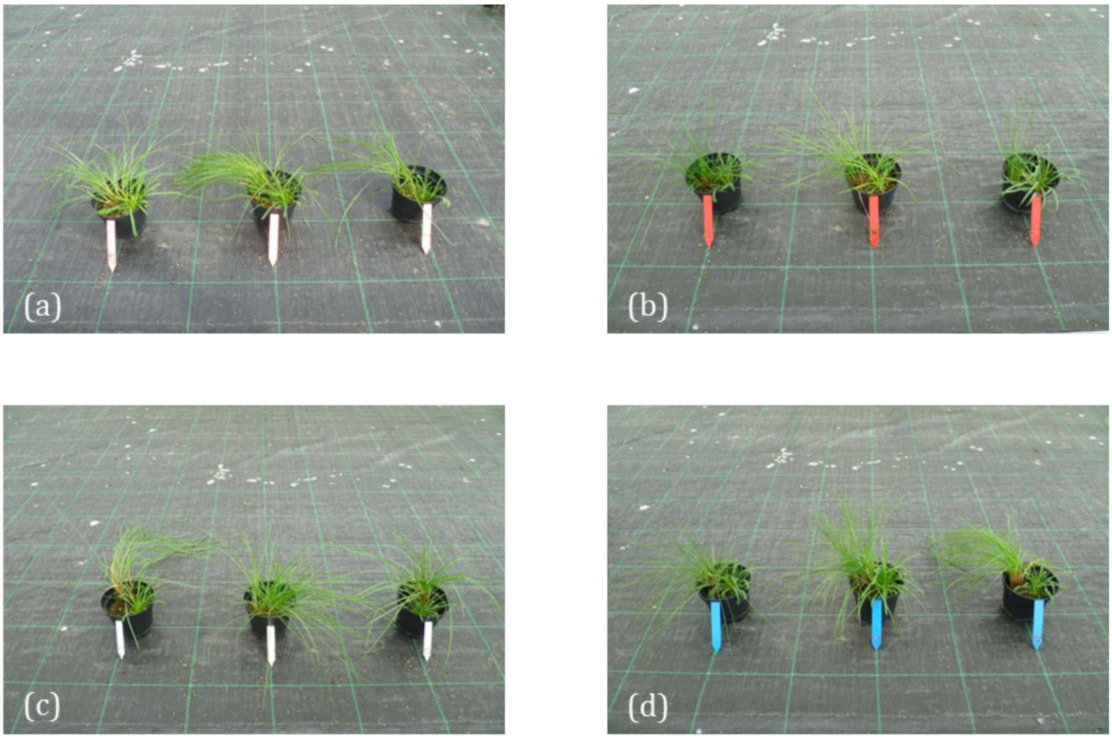

Figure 14 Examples of the four *Festuca rubra* genotypes in competition with *Dianthus carthusianorum*. In each photograph the pot on the left is the control, the central pot is from the medium treatment and the pot on the right is from the high nutrient treatment. (a) Cultivar rhizomatous 'Sergei'; (b) Cultivar tussock 'Salut'; (c) Regional rhizomatous, Pulling; and (d) Regional tussock, Neumarkt.

3.5. Discussion

It was found that *F. rubra* cultivars had significantly higher seedling emergence potential (SEP) in comparison with regional *F. rubra* genotypes. As cultivars are often bred for efficiency, this can result in reduced seed dormancy (Schröder and Prasse 2013a) and thus, higher SEP. Despite having a higher SEP, *F. rubra* cultivars did not emerge significantly better than regional *F. rubra* seed. These results contrast with the findings of Schröder and Prasse (2013a) as the *Plantago lanceolata* and *Lotus corniculatus* cultivars in their study germinated significantly faster and in higher abundance than wild seed material. Our study included more seed sources (13 cultivar and 11 regional) than Schröder and Prasse (2013a), which may make our findings in relation to the performance of cultivars more robust. Unlike regional *F. rubra* sources, *F. rubra* cultivars displayed little variation in their emergence patterns. Variation in emergence may have useful implications for restoration, as it may enhance the ability to survive stochastic environmental events, such as drought or frost, and thus boost the likelihood of the long-term survival of a genotype (Evans and Dennehy 2005). Seed that has a high dormancy is particularly advantageous in environments that frequently experience unfavourable conditions (Rice and Dyer 2001), as there is a greater likelihood of some seed will survive and establish after the period of harsh conditions.

Our results show that the competitive effect of *F. rubra* cultivars on *D. carthusianorum* biomass was relatively stable across the nutrient treatments tested in this experiment. Cultivars are bred for their uniformity and applicability under a wide range of conditions (Aubry et al. 2005), which may also result in relative standardisation of their performance and, potentially, competitive effect in different environments. Baer et al. (2003) found that cultivars of the dominant grass, *Panicum virgatum*, had good establishment across a range of soil depths and nutrient conditions due to a range of trait variation. In contrast, regional *F. rubra* sources are propagated for use within a particular region and therefore may be more likely to have a narrower optimal range of nutrient conditions than *F. rubra* cultivars.

We did not find a relationship between the seed mass and the competitive effect of the *F. rubra* seed sources. Additionally, the SEP of the *F. rubra* sources was not found to be correlated to the competitive effect based on either biomass or height. Although SEP is a useful indicator of seedling emergence, the emergence potential of seed material can vary greatly depending on the test conditions and levels of inbreeding in source populations. High cultivar germination in the laboratory or greenhouse does not ensure the same performance under field conditions (Oliveira et al. 2012).

While an increase in the competitive effect for both cultivar and regional *F. rubra* sources occurred between the control and medium nutrient treatments for phytometer biomass and height, the competitive effect declined in the high nutrient treatment. The competitive effect of *F. rubra* cultivars in the high nutrient treatment was even lower than in the control nutrient treatment. One reason for this may have been that *D. carthusianorum* profited more from the second nutrient supply than *F. rubra*, which may have helped to counteract the competitive effects of the *F. rubra* sources. The growth response, and hence competitive effect, of *F. rubra* is likely to depend on neighbouring species as well as on nutrient levels. Abraham et al. (2009) found that *F. rubra* did not exhibit a positive growth response from nitrogen addition in the presence of *Bromus diandrus*, although it was nitrogen limited in the monoculture treatment. As *F. rubra* is often found on soils with intermediate nutrient levels (Vázquez De Aldana and Berendse 1997), it may be possible that the high nutrient level treatment was not as suitable for *F. rubra* growth. Interestingly, *F. rubra* was found to have the highest total biomass in the low fertility treatment in a study by Vázquez De Aldana and Berendse (1997), which may highlight its difficulty performing in high nutrient conditions.

The extent of variation in competitive effect of regional *F. rubra* sources on phytometer biomass increased along the nutrient gradient. It also demonstrated that regional *F. rubra* appears to have greater capacity to respond to the different treatments than cultivars. This may highlights the adaptive capacity of regional seed to respond to a variety of environments, which is of great relevance for restoration. However, the competitive effect of regional *F. rubra* sources may be more difficult to predict at higher nutrient treatments than *F. rubra* cultivars, which performed relatively uniformly throughout the nutrient gradient. In a greenhouse study using some of the same genotypes, regional *F. rubra* was found also found to have a greater negative effect on subordinate species than *F. rubra* cultivars (Walker et al. in press). The selection of seed material used for grassland restoration should be made in combination with an assessment of site productivity. The effect of irrigation was not investigated as part of this study. It should be noted that in a restoration setting, the combination of high nutrient contents and high precipitation is likely to increase growth and competitiveness of the grasses, regardless of their source (Bobbink et al. 1998). However, the competitive effect of *F. rubra* on the surrounding vegetation would be moderated by the effect of species with similar niches.

Implications for practice

- Cultivar seed of *F. rubra* is not always more competitive than regional seed material, particularly at higher soil nutrient levels.
- Although cultivar *F. rubra* had a greater seedling emergence potential (i.e., more seedlings emerged faster), the cultivar and regional *F. rubra* seed material did not differ significantly in their overall emergence. A less uniformly rapid emergence, as observed in the regional *F. rubra* genotypes, might be a beneficial trait, as such populations are less prone to stochastic environmental fluctuations.
- Cultivar *F. rubra* genotypes have a more stable performance over a wide range of environmental conditions in comparison to the regional *F. rubra* genotypes, which display greater variation in emergence and competitive effect.

- The level of site productivity should be considered in combination with the source of the seed material used for grassland restoration, as they can both play a role in determining competitive plant interactions, particularly for dominant species.

4. Chapter 3

Pick and mix: the effect of seed source, growth form and seeding density for grassland restoration

4.1. Abstract

Question. What is the relative significance of seed source, growth form and seeding density contributing to the success of restoration projects?

Location. Experimental field site with bare gravel in the lowlands north of Munich (Southern Germany).

Methods. The research question was investigated for calcareous grasslands using a dominant grass (*Festuca rubra*) and three subordinate forbs (*Buphthalmum salicifolium*, *Dianthus carthusianorum, Linum perenne*). A randomized split-block design consisting of six blocks was established with two growth forms of *F. rubra* (rhizomatous, tussock) from two provenances (cultivar, regional) were seeded at two densities (800, 1600 seeds m^{-2}), and inter-seeded with forbs from regional and cultivar sources (100 seeds m^{-2}). Each combination of plant source, growth form and seeding density of the dominant species was replicated six times. The number of individuals of *F. rubra* (Year 1), subordinate species (Year 1 and Year 2) and a spontaneously invading alien plant (*Erigeron annuus*, Year 2) were counted. Total cover of vascular plants in each sub-plot and average number of flowering individuals were recorded (Year 2).

Results. Regional seed generally had higher establishment than its cultivar counterparts in both the dominant and subordinate species. Growth form and seeding density of *F. rubra* differed little in their effects on the establishment and individual numbers of seeded forbs. Fewer *E. annuus* invaded sub-plots with regional *F. rubra,* and sub-plots with the tussock growth form. Only in *D. carthusianorum* regional rather than cultivar subordinates produced more flowers.

Conclusions. Seed source and growth form seem to be the most significant factors for designing seed mixtures for restoration of calcareous grasslands.

4.2. Introduction

The soil seed banks of calcareous grassland are composed of short-lived, transient species (Bossuyt et al. 2006). Instead of investing in seed longevity, many grassland species focus their resources on the competitive ability of seedlings or clonal spread, particularly in environments with safe site limitations (Maron and Gardner 2000, Bossuyt and Honnay 2008). Species transfer using fresh hay or seed mixtures can be used to restore diverse grassland communities (Hedberg and Kotowski 2010, Kiehl et al. 2010). However, a balanced species composition, containing appropriate amounts of dominant, subordinate and transient species is required for the creation of a grassland community that provides sufficient vegetation cover as well as floristic diversity (Kiehl et al. 2010).

Dominant species, such as many grasses, are few in number, but are a major component of the primary production in grasslands, mainly due to their large size or clonal spread (Grime 1998). Smaller species, known as subordinate species, greatly contribute to the floristic diversity, but little to the primary productivity and they often co-occur with dominant species (Grime 1998, Gibson et al. 2012). Usually, dominant species control the abundance of the subordinate species, but in the case of disturbance, such as ploughing or fire, subordinate species can quickly take advantage of newly available nutrients before the dominant species re-establish (Grime 2002). Transient or ruderal species also occur in communities, often only as seedlings or juvenile individuals, though they are present in neighbouring communities as either dominant or subordinate species (Grime 2002). Seed mixtures used in grassland restoration require an appropriate assortment of species, in order to reach the goals of the restoration project.

The goals of restoration are diverse, including the re-introduction of a single species, improvement of heavily degraded ecosystems and the conversion of urban wasteland (Ehrenfeld 2000, Klaus 2013). Regional genotypes are widely recommended for restoration, as natural selection affects species traits allowing adaptation to the regional environmental conditions (Kawecki and Ebert 2004). In cases where the environmental conditions have not been greatly altered, regional seed is expected to be most suitable for restoration (Jones 2013b). Furthermore, the introduction of potentially disadvantageous foreign genotypes may be avoided by using regional seed (Hufford and Mazer 2003). Scientific evidence for these assumptions is equivocal: (Kiehl and Pfadenhauer 2007, Prach et al. 2013) found that regional seed mixtures yielded successful results for both small- and large-scale restoration, however, other studies have shown that regional genotypes do not always perform best (Bischoff et al. 2006, Leimu and Fischer 2008). Nevertheless, regional seed has been increasingly commercially propagated, branded as 'source-identified'. The respective producers propagate native plants from a defined provenance for up to four generations in monocultures, avoiding intentional selection, for later use within the same region (Scotton et al. 2011).

However, cultivar seed is still a widely used alternative to regional seed, constituting a large proportion of the plants constituting commercial seed mixtures (Kiehl et al. 2010), especially those used in urban grasslands (Klaus 2013). Beneficial traits are intentionally selected for in cultivars, and traits such as greater above-ground biomass and nutrient acquisition may prove to be competitively advantageous in relation to regional genotypes as predicted by the 'cultivar vigour hypothesis' (Wilsey 2010). Novel environments may challenge regional plants, as their adaptations may no longer be well suited to the modified conditions. In such cases, cultivars may be a suitable alternative (Jones 2013b), although their long-term persistence under extreme events is uncertain (Lesica and Allendorf 1999).

The growth form of the dominant species can enhance or decrease cultivar vigour. Rhizomatous grasses spread laterally either above- or belowground and produce dense swards of vegetation cover, which hinders the recruitment of seeded individuals and spontaneous immigration of target species. Baer et al. (2005) and Conrad & Tischew (2011) reported such suppressive effects in cultivars of rhizomatous, dominant grass species, i.e. *Panicum virgatum* and *Festuca rubra*, respectively. Conversely, the patchworks of light and soil gaps created by tussock grasses may facilitate establishment of other species (Bullock 2000), especially in short grasses (Wilsey 2010).

Optimal sowing densities are required to achieve the desired community composition and avoid seed wastage. High seeding densities can result in faster establishment of the desired species, but also increase the demand for resources (Lindborg 2006, Török et al. 2011). Generally, seed mixtures sown at densities of 3–5 g m^{-2} produce adequate results, provided they contain a suitable selection of species (Scotton et al. 2011). In regional seed mixtures, the proportions of grasses and forbs are generally assigned according to the relative abundance of species in grasslands within the provenance regions (B. Feucht, pers. comm.). Commercial cultivar mixtures do not account for differences in species composition across provenance regions, and often bulk up seed mixtures with grass seed, which sometimes make up 25–40% of the seed mixture (Conrad 2007). Conrad & Tischew (2011) found that the high proportion of cultivars of the dominant species *Festuca rubra* in a seed mixture reduces establishment and growth of other vegetation.

Suppression of arable weeds, ruderal and invasive alien species is usually desired during grassland restoration (SER 2004). Regional seed is expected to have competitive advantages over these species (Aldrich 2002). However, the effect of the seed source used in seed mixtures in relation to biotic resistance of the restored grassland have not been fully addressed, particularly concerning invasive alien species. Additionally, it is essential that the target species start reproducing to the benefit of population dynamics and pollinators. Moreover, restoration in urban settings requires a rich flower display for public benefit and acceptance. It may also be expected that regional seed enhances individual flowering.

Seed source, growth form of the dominant grass and seeding density are thus major factors that contribute to the success, or potential failure, of the restoration project. As these factors are often confounded in previously published studies, there is a lack of knowledge regarding how they interact during community establishment in grassland restoration. The aim of the present study is to determine the establishment of the dominant species *Festuca rubra* L. with respect to its genotype (seed source x growth form) and sowing density, and how these factors affect the establishment of three subordinate species of calcareous grassland (*Buphthalmum salicifolium* L., *Dianthus carthusianorum* L., *Linum perenne* L.) from cultivar and regional sources. Additionally, the effect of *F. rubra* genotype and sowing density on the risk of colonisation was investigated after invasion by the alien *Erigeron annuus* (L.) PER.

A field experiment on bare gravel was established to prove the following hypotheses: (1) regional dominant and subordinate species have greater establishment than the respective cultivars under limiting field conditions; (2) tussock *F. rubra* forms less cover than rhizomatous *F. rubra*, and therefore increases the establishment of subordinate species and of risk of invasion by the alien species; (3) low-density seeding of *F. rubra* results in less cover, thereby increasing the establishment of subordinate species and alien invasion and (4) regional subordinate species produce more flowers than cultivars under limiting field conditions.

4.3. Materials and methods

Experimental site

This field experiment was carried out within the tree nursery at ‘Gut Marienhof’, north of the city of Munich (N48°17´, E20°55´, 468 m a.s.l.). Unmanaged gravel beds were selected as a suitable target site for the experiment as they were similar in soil type and structure to those used for restoration of calcareous grasslands around Munich after topsoil removal (cf. Temperton et al. 2012). Soil at the site had a pH of 7.5 and very low concentrations of phosphorous (3 mg P_2O_5 100 g^{-1}) and potassium (3 mg K_2O 100 g^{-1}) (T. Conradi, unpubl. data). Total precipitation for the area during the study period June 2012 through July 2013 was 1193 mm, mean monthly temperature 9.5 °C, and number of sunshine hours was 2144. Three-month periods of above-average temperature and precipitation occurred during the first summer (June to August 2012: 1.8 °C, 42 mm a.a.) and during winter (November 2012 to January 2013: 1.6 °C, 23 mm a.a.), and spring to early summer 2012 (April through June) was marked by both above-average precipitation and below-average number of sunshine hours (28 mm a.a., 85 hs b.a.; data 2012–2013 and 30-yr means from the nearby stations ‘Freising’ and ‘Weihenstephan’, respectively; Agrarmeteorologie Bayern 2014).

Before sowing the experiment, all vegetation was removed from the site to enhance seed establishment. This was achieved by spraying the site twice with the systemic herbicide ‘Roundup’, in mid-October 2011 and in mid-March 2012 (Figure 15). The site was harrowed in April 2012 and raked by hand directly before sowing in June 2012.

Figure 15 (a) Site being sprayed with herbicide for the second time (March 2012); (b) harrowing of the blocks; (c) marking block and plot outlines; and (d) sowing seed in the sub-plots. In the centre of the photograph, two of the sub-plots have been sown with seed and soy and appear slightly lighter brown in colour. The plots were marked by fixed stakes and a mobile timber frame, which was divided into sections was used to indicate the position of the sub-plots.

Study species

Seed mixtures used in restoration frequently include the dominant species *Festuca rubra* L. (Poaceae). Two growth forms of *F. rubra* were used in this experiment, i.e. the rhizomatous *F. rubra* ssp. *rubra* L. and the tussock form *F. rubra* ssp. *commutata* GAUDIN (synonym *Festuca nigrescens*). The latter is versatile and often used in grassland restoration due to its high ecological amplitude and resistance to mowing and grazing (Peratoner and Spatz 2004). The species has culms of 20–55 cm in height and grows in dense perennial tussocks (Krautzer et al. 2004). The former subspecies, *F. rubra* ssp. *rubra,* develops widely spaced stolons and has culms of up to 1 m (Hegi 1998). The two subspecies are common in meadows and pastures of the (pre)alpine regions of central Europe to altitudes of 3000 m for *F. rubra* ssp. *commutata,* and 2500 m for *F. rubra* ssp. *rubra* (Hegi 1998). Cultivar *F. rubra* ssp. *commutata* 'Smaragd' and cultivar *F. rubra* ssp. *rubra* 'Roland 21' were supplied by Saatzucht Steinach GmbH & Co. KG (Steinach, Germany). Regional grass seed was propagated close to the study site (ca. 5 km) and was supplied by the seed producer Johann Krimmer (Pulling, Germany).

Buphthalmum salicifolium (Asteraceae), *Dianthus carthusianorum* (Caryophyllaceae) and *Linum perenn*e (Linaceae) are forbs representative of calcareous grassland. These subordinate species were also selected due to their availability as both cultivar and regional seed. *B. salicifolium* grows to 30–70 cm in height (Hegi 1979). The species frequently occurs in (pre)alpine southern Germany (Oberdorfer 2001). *D. carthusianorum* is a rosette forming species with stems 30–45 cm in height (Bloch et al. 2006). The species is considered moderately common and characteristic in calcareous grasslands of Germany (Oberdorfer 2001). *L. perenne* has multiple upright stems with numerous leaves and 20–60 cm in height (Hegi 1965). The species is rare in calcareous grasslands of southern Germany (Oberdorfer 2001). Jelitto Staudensamen GmbH (Schwarmstedt, Germany) supplied the cultivar seed for the three subordinate species; *Buphthalmum salicifolium* 'Sunwheel', *Dianthus carthusianorum* 'DA162', and *Linum perenne* 'Himmelszelt'. *Dianthus carthusianorum* DA162 was not a registered cultivar but a cultivate plant, which was originally sourced from wild plant materials and propagated for at least ten generations. During this time, it may have hybridized with *Dianthus* cultivars. The regional seed producer J. Krimmer supplied also the regional subordinates; the seed was stored at 4 °C before sowing.

Native species, including *Calamintha acinos* (L.) Clairv., *Echium vulgare* L. and *Verbascum lychnitis* L., also established to some degree in the sub-plots during the first and second year of the experiment. In Year 2, the alien species *Erigeron annuus* (L.) PER. invaded the experiment. The species was introduced from North America (Oberdorfer 2001) and is widespread in Germany due to effective wind-dispersal that allows rapid colonisation of ruderal habitats (Edwards et al. 2006). It is a winter annual, which overwinters as a rosette flowering in the following summer (Stratton 1992). The species was present in a random distribution on all six experimental blocks that had been disturbed in 2012 during the setup of the experiment. No *E. annuus* plants were observed in the undisturbed area.

Experimental design

The experiment had a randomized split block design consisting of six blocks (each 5 m x 10 m; Figure 16). The blocks were spread randomly along the two gravel strips, which ran parallel to each other ca. 100 m apart; three blocks were established per strip. Each block was divided into five 3.5 m x 1.0 m plots, which were further subdivided into 0.5 m x 0.5 m sub-plots (420 total). The sub-plots were either left (i) unsown ('control'), (ii) sown as a *F. rubra* control at low density (LD, ca. 800 seeds m^{-2}) or high density (HD, ca. 1600 seeds m^{-2}), (iii) sown as a subordinate species control (100 seeds 0.25 m^{-2}), or (iv) sown as a treatment, i.e. one *F. rubra* genotype at low or high density and 100 seeds 0.25 m^{-2} of one subordinate species. Each *F. rubra* and subordinate species control and treatment combination was repeated once per block (total six replicates). The experiment was manually sown in early-June 2011. Soy meal (80 g m^{-2}) was mixed with the seed to assist the even sowing of the sub-plots.

The *F. rubra* low-density seeding was based on the amount recommended for calcareous grassland in the provenance region (13% per 30 kg ha^{-1} or roughly 0.4 g m^{-2}; J. Krimmer, pers. comm.). The high-density seeding was twice the recommended amount. Average weight of 100 *F. rubra* seeds was measured five times for each genotype. Seed numbers for the LD and HD treatments were calculated from this average weight.

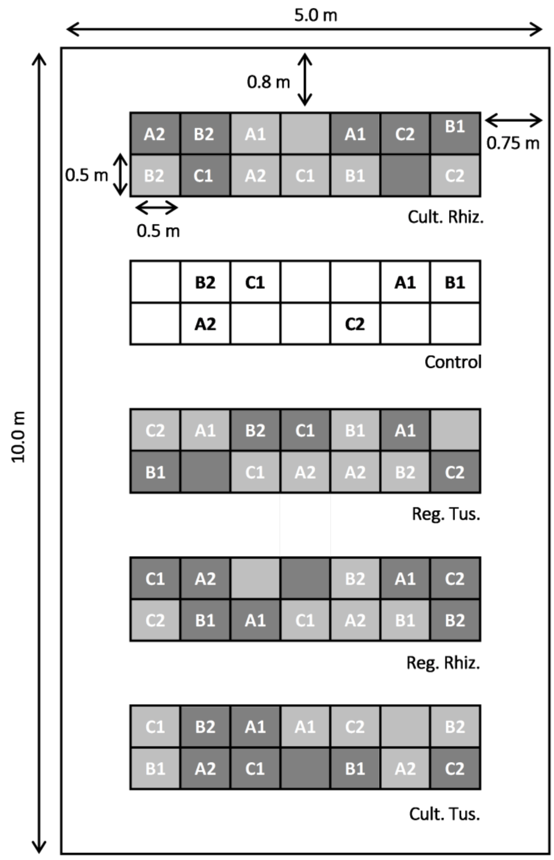

Figure 16 One of the six blocks (10.0 m x 5.0 m) used in the field experiment; each block consisted of five plots (3.5 m x 1.0 m). One of the four *Festuca rubra* genotype treatments was sown per plot, one plot left unseeded (control). Plots were subdivided into 14 randomly assigned sub-plots (0.5 m x 0.5 m). White sub-plots (without text) are unsown ('control') treatments; white sub-plots with text are subordinate species control treatments (without *F. rubra*). Letters in the sub-plots indicate the subordinate forbs *Buphthalmum salicifolium* (A), *Dianthus carthusianorum* (B) and *Linum perenne* (C). Numbers indicate cultivar (1) and regional subordinates (2). Light grey and dark grey sub-plots (without text) are *F. rubra* low and high seeding density controls, respectively. Light grey and dark grey sub-plots with text are *F. rubra* low and high seeding density treatments with the subordinate species.

Measurements

At the end of July 2012 ('Year 1'), 8 weeks after sowing, seedlings of the *F. rubra* genotypes and of the three subordinate species were counted in all sub-plots. In May 2013 ('Year 2'), the established plants of subordinate species and of the invasive alien *E. annuus* were counted. The number of flowers per plant, which only appeared in Year 2, was recorded for the three subordinate forbs during two visits (7 and 31 July 2013). Each sub-plot was photographed at that time for determining total cover of vascular plants; moss cover was <1%.

Data analysis

The data was tested for normality and, when necessary, they were transformed using square-root transformations. A three-way mixed analysis of variance was used to determine whether the fixed factors of *F. rubra* genotype and seeding density, or the random factor 'block' had a significant effect on the establishment of *F. rubra* genotypes in Year 1, and on total vascular plant cover of the plots in Year 2. The effects of the fixed factors '*F. rubra* genotype', 'seeding density' and 'subordinate species source', and the random factor 'block', on the risk of colonisation of the sub-plots were tested in a four-way mixed analysis of variance.

The effects of *F. rubra* genotype, seeding density, subordinate species source and block on the establishment of the subordinate species in Year 1 and 2 was also determined by a four-way mixed analysis of variance; each subordinate species was tested separately. Pair-wise post-hoc Tukey tests were conducted on the significant variables. Moreover, *t*-tests were used to determine differences in establishment between cultivar and regional subordinate species on control sub-plots in Year 1 and 2. Differences between cultivar and regional sources in the mean number of flowers per plant were analysed using a Mann-Whitney *U* test. All of the data analysis and figures was conducted in statistical software package SPSS Statistics 21. The software program ImageJ was used to determine the percentage vegetation cover of the sub-plots. The total number of green pixels in an area of known size was calculated from the sub-plot photographs.

4.4. Results

Festuca rubra establishment and cover

The establishment of the dominant grass in Year 1 differed between the *F. rubra* genotypes ($F_{3,240} = 15.2$, $p < 0.000$) and the two seeding densities ($F_{1,240} = 140.1$, $p = 0.000$), with a significant block effect ($F_{5,240} = 31.9$, $p < 0.000$; Figure 17). There was also an interaction between *F. rubra* genotype and block ($F_{15,240} = 0.77$, $p < 0.001$), resulting in slightly higher regional *F. rubra* establishment in one gravel strip. The high seeding density resulted in lower relative emergence than the low density, and despite doubling the number of seeds, only about only ten additional seedlings emerged per sub-plot in the high-density treatment.

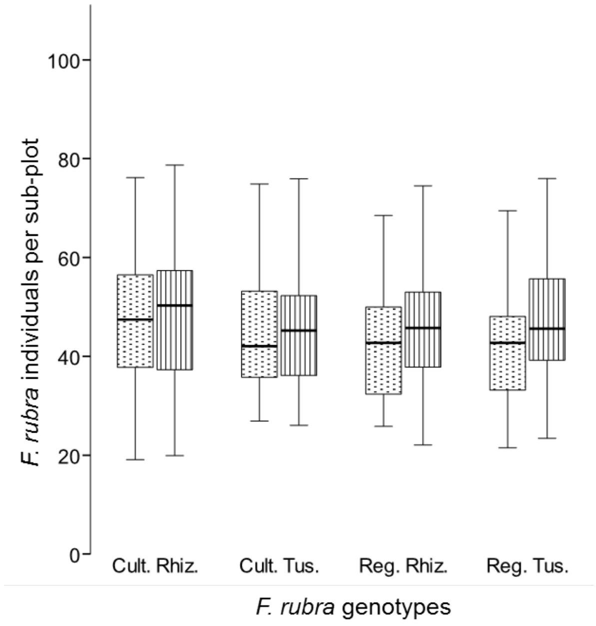

Figure 17 Significant differences in establishment between the *Festuca rubra* genotypes ($F_{3,240}$ = 15.2, $p < 0.000$), the two seeding densities ($F_{1,240}$ = 140.1, $p = 0.000$) and the six blocks ($F_{5,240}$ = 31.9, $p < 0.000$) in Year 1. There was also a significant interaction between *F. rubra* genotypes and block ($F_{15,240}$ = 0.77, $p < 0.001$). Dotted boxes, low-density seeding treatment; striped boxes, high-density seeding treatment.

While the regional tussock *F. rubra* had the greatest mean establishment per sub-plot (HD = 44%, LD = 35%), the regional rhizomatous *F. rubra* had the lowest mean establishment per sub-plot (HD = 36%, LD = 25%) of all *F. rubra* genotypes. The cultivar rhizomatous *F. rubra* had on average slightly better establishment (HD, 41%; LD, 31%) per sub-plot than the cultivar tussock *F. rubra* (HD, 37%; LD, 26%). In a separate germination trial, cultivar rhizomatous *F. rubra* had the greatest germination at 20 °C for 20 days (cultivar rhizomatous, 97%; regional tussock, 92%; cultivar tussock, 82%; regional rhizomatous, 74%) (E.A. Walker, unpubl. data). Lower germinability, especially in relation to the cultivar, may therefore have contributed only to the relatively low establishment of regional rhizomatous *F. rubra*.

In Year 2, the *F. rubra* genotypes and densities had no significant effect on the total cover of the sub-plots ($F_{3,366}$ = 1.6, $p > 0.05$; $F_{1,366}$ = 3.0, $p > 0.05$, respectively) but there were significant differences between the blocks ($F_{5,366}$ = 17.6, $p < 0.000$; Figure 18). Over the course of the experiment, several unsown species, including *E. annuus*, established in the sub-plots. These species contributed to between 20–40% of the total percentage cover of the sub-plots.

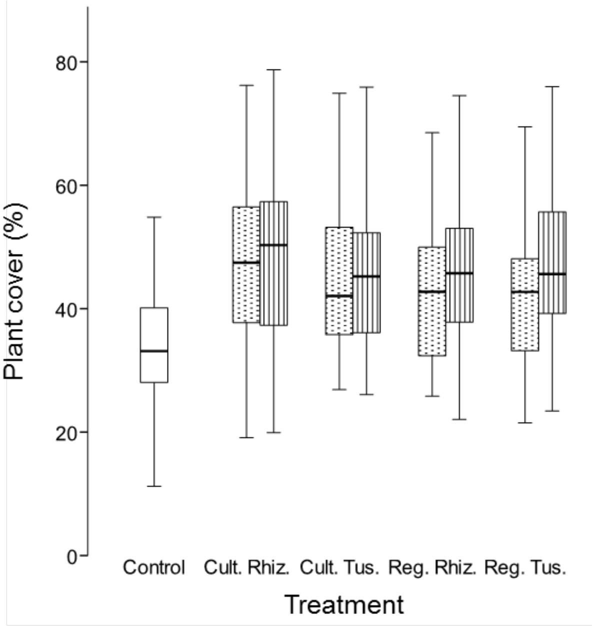

Figure 18 No significant differences in the total plant cover between *Festuca rubra* genotypes and seeding density ($F_{3,366}$ = 1.6, p > 0.05; $F_{1,366}$ = 3.0, p > 0.05, respectively) in Year 2, although there was a significant block effect ($F_{5,240}$ = 15.1, p < 0.000). White box, unsown treatment; dotted box, low-density treatment; striped box, high-density treatment.

Subordinate species establishment and performance

In Year 1, there were no significant effects of *F. rubra* genotype, density, block or subordinate species source on the establishment of *B. salicifolium,* while in Year 2 subordinate species source ($F_{1,15}$ = 9.08, p = 0.030; Figure 19) and block ($F_{5,15}$ = 4.10, p = 0.033) had significant effects on the establishment of this species. Cultivar *B. salicifolium* had significantly higher establishment and two plots in particular showed lower emergence than the other four. In both Year 1 and Year 2, cultivar *B. salicifolium* had greater establishment in the control sub-plots than regional *B. salicifolium,* although the differences were not significant (Year 1: t = –1.41, p = 0.19; Year 2: t = –1.58, p = 0.15).

In *D. carthusianorum,* seed source had a significant effect on establishment in Year 1 ($F_{1,15}$ = 63.6, p = 0.001; Figure 19) with some variation among blocks ($F_{5,15}$ = 3.93, p = 0.041). In Year 2, *D. carthusianorum* source ($F_{1,15}$ = 164.3, p < 0.000) and *F. rubra* genotype ($F_{3,15}$ = 4.92, p = 0.014) had significant effects on the number of individuals present per sub-plots. In both years, regional *D. carthusianorum* had greater establishment, although fewer individuals occurred in the regional *F. rubra* treatments. Regional *D. carthusianorum* had significantly greater establishment than cultivar *D. carthusianorum* in Year 1 (t = 3.05, p = 0.012) and Year 2 (t = 4.13, p = 0.002).

The establishment of the third subordinate species, *L. perenne,* was significantly affected by the interaction of *L. perenne* source and block in Year 1 ($F_{5,15} = 5.36$, $p = 0.008$; Figure 19). Cultivar *L. perenne* established better in the blocks on one gravel strip and establishment of both sources was low in one block. No significant effects were found in Year 2. No significant differences were observed in the establishment of cultivar and regional *L. perenne* in the sub-plots in either Year 1 ($t = -0.11$, $p = 0.91$) or Year 2 ($t = 0.24$, $p = 0.82$), although regional *L. perenne* performed better than cultivar *L. perenne* in Year 2.

The number of flowers produced by cultivar and regional *B. salicifolium* differed not significantly in July of Year 2 (Mann Whitney $U = 165$, $n_1 = 11$, $n_2 = 33$, $p > 0.05$). Regional *D. carthusianorum* had a significantly greater average number of flowers than cultivar *D. carthusianorum* ($U = 162.5$, $n_1 = 47$, $n_2 = 16$, $p = 0.001$), while cultivar *L. perenne* produced significantly more flowers on average than regional plants ($U = 350.5$, $n_1 = 41$, $n_2 = 51$, $p < 0.000$; Table 3).

Table 3 The mean number of individuals of the *Festuca rubra* genotypes (Year 1), *Buphthalmum salicifolium*, *Dianthus carthusianorum*, *Linum perenne* (Year 1 and Year 2) and *Erigeron annuus* (Year 2). Percentage cover values of the sub-plots (Year 2) and the mean number of flowers for July (Year 2) are included. Abbreviations: Cult., cultivar; Reg., regional; Rhiz., rhizomatous; Tus., tussock; LD, low density treatment; HD, high density treatment; and Inds., Individuals.

Species	x̄	**Year**	**Treatment**								
			Control	Cult. Rhiz.		Cult. Tus.		Reg. Rhiz.		Reg. Tus.	
				LD	HD	LD	HD	LD	HD	LD	HD
F. rubra	Inds.	1		31	41	26	37	25	36	35	44
	% cover	2	35	48	49	46	46	42	47	43	48
E. annuus	Inds.	2	40	40	36	32	32	37	33	28	28
B. salicifolium	Cult. inds.	1	9	6	8	8	8	9	6	6	6
		2	13	8	10	9	9	11	9	8	9
	Cult. flowers	2	1	0	1	1	1	1	1	1	1
	Reg. inds.	1	6	7	7	6	7	6	6	6	5
		2	8	10	7	7	8	7	9	8	9
	Reg. flowers	2	1	1	0	0	0	0	0	0	1
D. carthusianorum	Cult. inds.	1	11	11	13	14	14	11	10	13	9
		2	12	13	13	11	13	10	10	12	9
	Cult. flowers	2	1	0	1	1	0	0	0	0	1
	Reg. inds.	1	22	20	24	24	20	16	14	18	16
		2	23	23	25	21	18	16	14	21	19
	Reg. flowers	2	2	3	2	3	3	3	3	4	3
L. perenne	Cult. inds.	1	26	20	27	22	24	24	24	24	20
		2	27	23	24	21	22	22	18	18	19
	Cult. flowers	2	8	3	4	5	7	5	3	4	3
	Reg. inds.	1	26	26	22	26	23	22	24	21	21
		2	28	29	25	24	28	25	26	24	23
	Reg. flowers	2	1	2	3	2	2	1	1	1	1

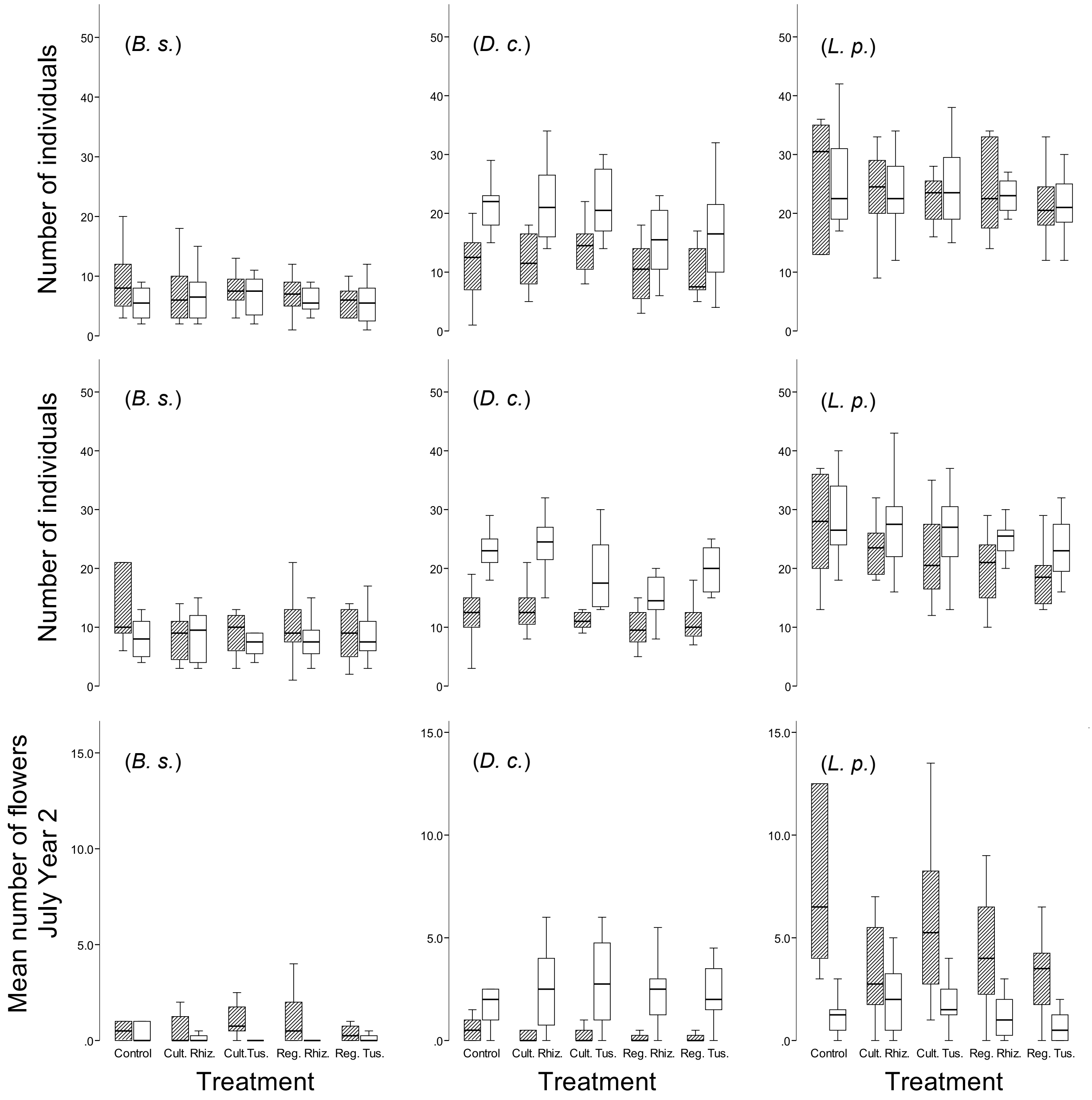

Figure 19 No significant differences of subordinate species source, *Festuca rubra* genotype, seeding density or block on the establishment of *Buphthalmum salicifolium* (*B. s.*) in Year 1; while a significant effect of *B. salicifolium* source ($F_{1,15}$ = 9.08*) and block ($F_{5,15}$ = 4.10*) occurred in Year 2 (U = 15.5, $n_1 = n_2$ = 6 ^{ns}); and differences in the number of flowers produced by cultivar and regional *B. salicifolium* in Year 2 (Mann Whitney U = 165, n_1 = 11, n_2 = 33 ^{ns}). A significant effect of subordinate species source ($F_{1,15}$ = 63.6**) and block ($F_{5,15}$ = 3.93*) was found on the establishment of *Dianthus carthusianorum* (*D. c.*) in Year 1; significant differences between the subordinate species source ($F_{1,15}$ = 164.3***) and *F. rubra* genotype ($F_{3,15}$ = 4.92*) in Year 2; and regional *D. carthusianorum* had more flowers than cultivar plants in Year 2 (U = 162.5, n_1 = 47, n_2 = 16 ****). A significant effect of subordinate species source and block interaction ($F_{5,15}$ = 5.36**) on the establishment of *Linum perenne* (*L. p.*) in Year 1; no significant difference between the subordinate species source, *Festuca rubra* genotype, seeding density or block with respect to establishment of *L. perenne* in Year 2; while cultivar *L. perenne* produced more flowers than regional plants (U = 350.5, n_1 = 41, n_2 = 51 *****). Striped boxes: cultivars; white boxes: regional seed (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ^{ns}, $p \geq 0.05$).

Invasion by Erigeron annuus

The invasion by *E. annuus* differed significantly between the *F. rubra* genotypes ($F_{3,283}$ = 6.96, $p < 0.000$; Figure 20), and among blocks ($F_{5,283}$ = 44.9, $p < 0.000$), and there was an interaction between *F. rubra* genotype and block ($F_{15,283}$ = 2.34, $p = 0.004$). *E. annuus* establishment on one block was particularly low, while the blocks on one gravel strip had higher establishment. A post-hoc Tukey test showed that regional tussock *F. rubra* had a significantly lower risk of colonisation than regional rhizomatous *F. rubra* (p = 0.029), cultivar rhizomatous *F. rubra* ($p < 0.000$) and the control sub-plots ($p < 0.000$). No significant effect was observed between regional tussock *F. rubra* and cultivar tussock *F. rubra* (p = 0.34). Sub-plots with regional *F. rubra* showed the least risk of colonisation, whereas the sub-plots with the highest risk of colonisation were the control sub-plots.

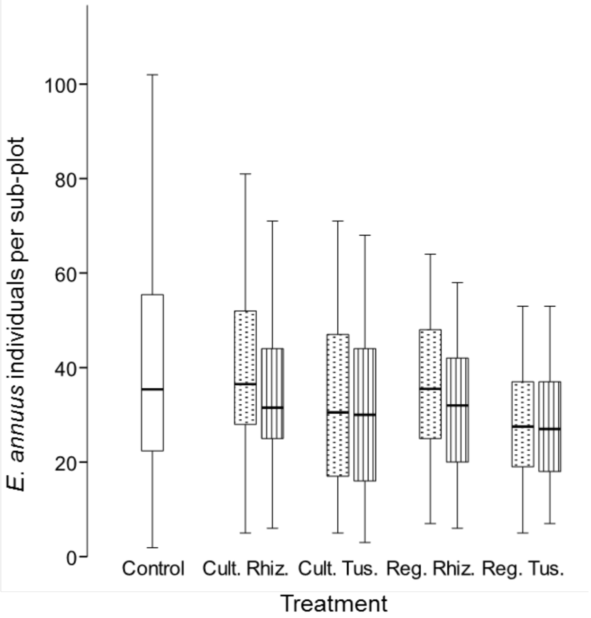

Figure 20 The invasion of *Erigeron annuus* differed significantly between the *Festuca rubra* genotypes ($F_{3,283}$ = 6.96, $p < 0.000$), among blocks ($F_{5,283}$ = 44.9, $p < 0.000$) and there was an interaction between *F. rubra* genotype and block ($F_{15,283}$ = 2.34, $p = 0.004$). White box: unsown treatment; dotted box: low-density treatment; striped box: high-density treatment.

Figure 21 (a) Block with seedlings in early July 2012 (ca. 6 weeks after sowing); (b) Emergence on gravel soils at the site (early July 2012); (c) Vegetation cover in late May 2013 and (d) Flowering of *Buphthalmum salicifolium*, *Dianthus carthusianorum*, *Linum perenne* and *Erigeron annuus* in the sub-plots (early July 2013).

4.5. Discussion

Establishment of dominant and subordinate species

Under the limiting conditions, the regional tussock *F. rubra* had the greatest mean and total establishment of the four grass genotypes and performed better than its cultivar counterpart. Calcareous grassland seed mixtures used in the Munich area recommend the regional tussock *F. rubra* due to its suitability to the soil and climate conditions (J. Krimmer, pers. comm.); our study supports this recommendation. However, rhizomatous cultivar *F. rubra* had the second best establishment and may have greater establishment under less limiting conditions. Interestingly, the cultivar tussock *F. rubra* and the regional rhizomatous *F. rubra* genotype had similar establishment, although comparatively low establishment of regional rhizomatous *F. rubra* may be due to lower germinability of older seed.

Although regional *F. rubra* had the best initial establishment, the establishment of grasses was found not only to depend on source, but also on the growth form of the species. The timing of sowing also contributes to the community composition; grasses have an establishment advantage when sown in autumn, while forbs are favoured by spring sowing (Scotton et al. 2011). As this experiment was sown in early June, the full potential of *F. rubra* emergence may have been limited, because the species is generally germinates in autumn (Grime 2002).

While the performance of the cultivar or regional sources varied among the subordinate species, after 2 years the regional seed had better establishment in two of the three subordinate species in the control sub-plots. Significant differences were only observed with regional *D. carthusianorum*, which had significantly better establishment in the control during Year 1, and significantly more individuals than cultivar *D. carthusianorum* in Year 2. Regional *L. perenne* had a greater number of individuals than cultivar *L. perenne* only in Year 2. Throughout the experiment, cultivar *B. salicifolium* performed better than regional *B. salicifolium*. One reason for the poor establishment of regional *B. salicifolium* could have been the quality of the seed used, which was 3 years old at the time of sowing. The regional seed used for *D. carthusianorum* and *L. perenne* was harvested less than a year before the experiment, while the information relating to the age of cultivar seed was not obtainable from the suppliers.

The source of the subordinate species had a limited effect on the establishment of *B. salicifolium* and *L. perenne* in the *F. rubra* treatments in Year 1, although cultivar *B.*

salicifolium and regional *L. perenne* generally had better establishment than their respective counterparts in Year 2. During both years, regional *D. carthusianorum* had significantly better establishment than cultivar *D. carthusianorum* in the *F. rubra* treatments. A greenhouse study using the same seed found that regional *D. carthusianorum* and cultivar *L. perenne* produced significantly more above-ground biomass than their source counterparts, while no significant differences were detected between the two sources of *B. salicifolium* (Walker et al. in press).

Due to the lack of adaptations to the regional climate and local soil conditions, cultivar *F. rubra* treatments were expected to favour the subordinate species establishment rather than regional *F. rubra*. It was observed that the establishment of the subordinate species was generally higher in the cultivar *F. rubra* treatments across both years for *D. carthusianorum* and only in Year 2 for *L. perenne*. However, differences in the performance of seed sources may only become evident over longer periods of time, particularly during stressful conditions. The failure of cultivar *Lotus corniculatus* during harsh winter conditions in a study by Schröder & Prasse (2013c) and the failure of forb species, many of them cultivars in a three year study (Hitchmough 2000) supports the idea that regional seed may prove more suitable for establishment and also long-term vegetation cover.

Growth form of Festuca rubra

Contrary to the second hypothesis, the percentage cover of sub-plots with rhizomatous grasses was not greater than that of the tussock grasses. Therefore, there was not reduced establishment of the subordinate species or decrease the risk of invasion by the invasive alien *E. annuus*. The ability of the rhizomatous growth form to produce stolons was possibly hampered by the adverse site conditions of the bare gravel (Figure 21 b), thus allowing the subordinate species to have better establishment, and facilitating immigration of other species, such as *E. annuus* and the ruderals. Establishment for the subordinate species was highest in the cultivar rhizomatous *F. rubra* and not in the cultivar tussock *F. rubra* (with the exception of cultivar *D. carthusianorum*) as had been expected. The poorer performance of the cultivars could have been advantageous for the establishment of the subordinates. However, the establishment of grasses was found not only to be dependent on source, but also on the growth form of the species. This may account for the fact that the two growth forms of *F. rubra*, contrary to observations from earlier studies (Baer et al. 2005, Conrad & Tischew 2011), varied little in their effects on individual number of the subordinate species. Furthermore, the subordinate species may have undergone biotic filtering influenced by the *F. rubra* genotypes: Gustafson et al. (2014) showed that the intraspecific variation in the dominant species influences the genetic composition of the subordinate species.

There was a significant effect of *F. rubra* genotype on the risk of invasion. Sub-plots with the tussock growth form had slightly lower median establishment of *E. annuus* in comparison to the rhizomatous and control sub-plots. Although there was no major difference in the total percentage cover between the *F. rubra* treatments, there was a decline in the numbers of *E. annuus* found on the sub-plots sown with regional *F. rubra* in comparison with the control and cultivar *F. rubra* sub-plots. While other studies have examined the effects of seed mixtures on weed suppression (Lepŝ et al. 2007, Török et al. 2012), this study examines the effect of source and growth form. The results show that regional seed may help reduce the risk of invasion of alien species on restoration sites.

The number of *D. carthusianorum* and *L. perenne* individuals in the regional *F. rubra* treatments declined slightly; however, a greater decline was observed with the number of *E. annuus* in the regional *F. rubra* treatments. The successful restoration of a grassland requires a suitable mix of species with a variety of functional traits, some of which should be similar traits to those of potential invaders (Funk et al. 2008). The competitive ability of the dominant species should allow for a balance between the establishment of target species and the reduction of colonisation by unwanted species.

Seeding density of Festuca rubra

There were no significant seeding density effects on the establishment of the subordinate species or on the risk of invasion of the sub-plots, which was likely due to the limited differences in the density treatments. Given the fact that the quantity of seed was doubled, the difference in established seedlings between the high-density (1600 seeds m^{-2}) and low-density treatment (800 seeds m^{-2}) was very small in Year 1. The establishment of *F. rubra* was possibly limited by the number of safe sites (Maron and Gardner 2000), as the soil consisted mostly of nutrient-poor gravel that had a low field capacity (T. Conradi, unpubl. data). Conrad & Tischew (2011) found that cultivar *F. rubra* sown at high densities (3200 seeds m^{-2}) were particularly competitive in the chernozem soils. In our experiment, the low-density treatment was carried out according to the recommended sowing density of 13% *F. rubra* (0.4 g m^{-2}) in calcareous grassland mixtures in the study region (J. Krimmer, pers. comm.). Under these conditions, seed mixtures with higher proportions of *F. rubra* may have limited increases in establishment, which may result in seed wastage. In less limiting environments, the use of additional *F. rubra* seed may have detrimental effects on the community resulting from increased competitiveness (Conrad and Tischew 2011).

As this experiment examined the specific effects of source, growth form and seeding density, the use of few individual species was favoured over a seed mixture. The sub-plots were sown at a lower seed density than would be used in a restoration setting, thus allowing for the establishment of ruderal species. Though some of the ruderal species may have assisted the establishment of *F. rubra* genotypes and subordinate species by providing safe sites and microclimates for establishment in the gravel substrate, they also competed with these species for space and nutrients.

Flowering of subordinate species

The average number of flowers produced by the subordinate forbs varied between seed sources. The hypothesis that regional subordinate species produce more flowers than cultivar subordinates under limiting field conditions was only proven in the case of *D. carthusianorum*. Cultivar *L. perenne* produced significantly more flowers on average than its regional counterpart, and although cultivar *B. salicifolium* also produced more flowers than regional *B. salicifolium*, there was no significant difference between the two sources. Under limiting conditions, it was expected that regional subordinate species would be better adapted to the regional conditions; therefore, enabling in greater flower production. However, as cultivars are bred for traits, such as flowering (McGrath et al. 2010), the selection for these traits may outweigh the adaptations of regional subordinate species. In areas that are at risk from unwanted species, it is essential that the desired target species flower and produce viable seed quickly to ensure their long-term survival in the vegetation community. The risk of a cryptic invasion (Hufford and Mazer 2003, Vander Mijnsbrugge et al. 2010) exists if cultivar species are more successful at producing flowers and seed. Furthermore, the greater displays of flowers that are frequently selected for in cultivars may distract pollinators from visiting regional species resulting in a decline in their seed output, as was seen by the invasive species *Impatiens glandulifera* (Chittka and Schurkens 2001). In an urban setting, however, the enhanced flower aspect is desirable, and mixing sources might be acceptable.

4.6. Conclusions

Under limiting conditions of the experimental site, regional seed generally had higher establishment than cultivars in both the dominant and subordinate species. However, regardless of its greater establishment, regional subordinate species did not always produce more flowers than cultivars. Greater numbers of *D. carthusianorum* and *L. perenne* were present in the cultivar rather than in the regional *F. rubra* treatments. There was also evidence to suggest that the risk of invasion by *E. annuus* was reduced by the superior growth of regional *F. rubra*. While the two growth forms of *F. rubra* varied little in their effects on the subordinate species, the tussock growth form also played a role in the suppression of *E. annuus* establishment. The inclusion of higher than recommended proportions of *F. rubra* seed in mixtures for restoring calcareous grassland may be counterproductive in relation to the cost-effective establishment as well as the potential for increased competitiveness at higher densities. Despite its short duration, this experiment demonstrates that the source of the seed, as well as the growth form of the dominant species used in seed mixtures decreases the risk of undesirable species establishing. However, this advantage may be offset by suppression of desired species. Further investigations are needed to determine which species composition and seeding densities of regional seed mixtures has the best potential to increase biotic resistance against unwanted species under stressful and more benign environmental conditions.

5. Discussion

The selection of seed for grassland restoration is a complex undertaking that should be conducted based upon the best available knowledge. Results from the three chapters show that the interplay of *site factors*, *species identity* and *seed quality* outlined in Figure 1 play an important role in determining the establishment of dominant and subordinate species in calcareous grasslands. The general outcomes and conclusions of each chapter are shown in Figure 22. By combining the findings from the experiments, it is possible to get greater understanding of the impacts that seed source, growth form, seeding density and nutrient level have in grassland restoration, and therefore improve seed mixtures.

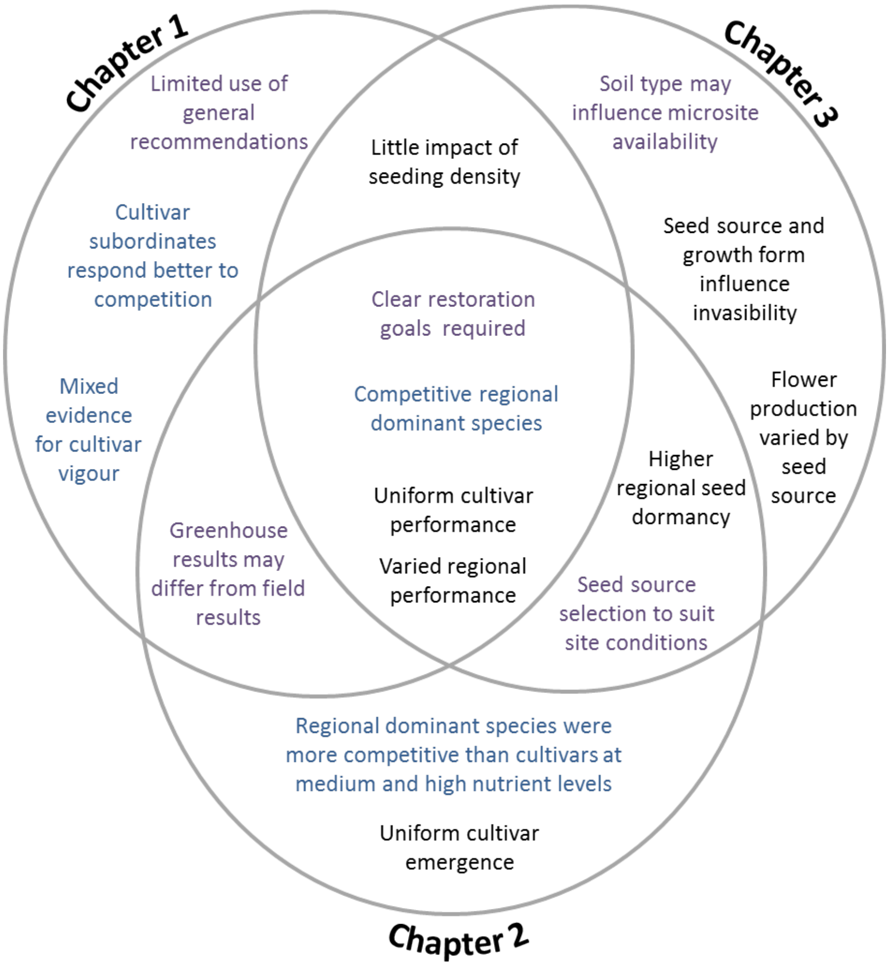

Figure 22 Conceptual diagram outlining the main conclusions and recommendations from each of the three chapters. Purple, blue and black text boxes relate to the *site factors*, *species identity* and *seed quality* referred to in Figure 1 and Figure 3.

5.1. Site factors

Soil nutrient conditions

The nutrient level at a restoration site is likely to contribute to the performance of seed in grassland restoration. Soil that has received high amounts of fertiliser during intensive agricultural production may have limited potential for the development of long-term highly diverse vegetation communities (Pywell et al. 2002). The slow decline of residual nutrients in the soil (Fagan et al. 2008) pose additional challenges for plant communities adapted to low nutrients. In Chapter 2, it was found that the source of the seed as well as the nutrient level of the soil affects the competitive ability of *F. rubra*. Unexpectedly, regional *F. rubra* had a greater competitive effect on the above-ground biomass of *D. carthusianorum* in the medium and high nutrient treatment. While there have been a number of studies that have focused on the effects of cultivars on non-cultivar species in relation to their vigour (Wilsey 2010), effects on ecosystem functioning (Baer et al. 2013) or influence community assembly (Gibson et al. 2012), the risks and effects of competitive dominant regional species have so far not been highlighted, particularly at higher nutrient levels. This is an important topic, which deserves greater attention, as regional seed material is widely recommended, often without due considerations for the soil conditions at the site to be restored. Highly competitive grasses pose a greater risk of becoming abundant and suppressing the other vegetation (Walker et al. 2004), thus limiting biodiversity.

The conditions established in Chapter 2 as part of the nutrient availability treatment do not completely reflect the conditions that seed material would experience under field conditions with elevated nutrient levels. The germination and emergence of seed may differ in a site that had higher nutrient levels at the time of sowing, which did not occur in this experiment. Additionally, the time of nutrient addition may also influence the growth of a species depending on the life cycle stage. This was possibly seen in the response of the grasses under the high nutrient treatment. It is likely that *D. carthusianorum* benefited more from the second dose of nutrients, thus lowering the competitive effect of *F. rubra*. Further research on the performance of cultivar and regional seed could be conducted in the future under elevated soil nutrient conditions in field experiments.

5.2. Species identity

Plant growth

The cultivar vigour hypothesis predicts that cultivars have enhanced growth traits due to selective breeding (Wilsey 2010). It was therefore surprising that regional *F. rubra* performed so well in the experiments. Regional *F. rubra* had the strongest competitive effect in Chapter 1 and also in the medium and high nutrient levels in Chapter 2. Additionally, the tussock form had greater initial establishment under field conditions in Chapter 3. From these results it was possible to conclude that cultivar vigour is not universally found in all cultivars and is likely to be influenced by environmental conditions and the neighbouring competitors.

In Chapter 1, the performance of the cultivar subordinates varied according to whether competitors were present or absent. In the absence of competitors, all three subordinates responded differently. Cultivar *L. perenne* and regional *D. carthusianorum* produced significantly more above-ground biomass than their source counterparts, while there were no significant differences in the above-ground biomass of the two sources of *B. salicifolium*. Interestingly, when grown in the presence of competition, it was the cultivar subordinates that produced more biomass than the regional subordinates for all three of the subordinate species. This would imply that cultivar subordinates may respond better to competition than regional subordinates. In the field experiment, the subordinate species were in competition with cultivar or regional *F. rubra*, as well as *E. annuus* and the ruderals species. Under these circumstances, regional *D. carthusianorum* and *L. perenne* were found to have significantly greater establishment than their cultivar subordinates in the second year. Regional subordinate species may have had an advantage in the field because they have adaptations to the local soil and climate conditions (Vander Mijnsbrugge et al. 2010), which may have surpassed the competitive advantages of the cultivars grown under greenhouse conditions. Wilsey (2010) suggested that cultivars may be disadvantaged under field conditions due to local adaptations of locally-sourced seed. The artificial conditions of the greenhouse may have suited the cultivars better than field conditions, thus improving their competitive ability under these conditions. Furthermore, Oliveira et al. (2012) found a decline in germination from laboratory to greenhouse and field experiments. This highlights the limitations of controlled environments, such as laboratories and greenhouses, at predicting the outcome of the complex interactions that occur under field conditions. In order to get a realistic impression of how a seed mixture will behave at a particular site, it is advisable to establish small scale plots of seed mixtures prior to the restoration work to predict how the seed will perform.

Flowering is a key life cycle trait (Pywell et al. 2003), which is required for the success of a viable long-term vegetation community in a restored grassland. The production of flowers soon after establishment is particularly favourable, because the likelihood of new seed establishing is higher when the vegetation cover is low and microsites are still available (Pywell et al. 2003). This allows the species to get a better foothold in the community prior to the immigration of unsown species. Due to the adaptations that regional seed have to their environmental conditions (Rice and Emery 2003), regional subordinate species were expected to produce a greater number of flowers under the limiting conditions of the field site. However, this was found to be true only in the case of *D. carthusianorum*. As regional *D. carthusianorum* also had significantly better establishment in both years of the experiment, this genotype appeared to be well suited to the field site conditions. Although the field conditions may have been limiting, cultivar *L. perenne* produced a significantly greater amount of flowers than its regional counterpart. Cultivar seed, which has been selected for increased inflorescences, may become problematic if the abundance of flowers also results in increased viable seed production (Lesica and Allendorf 1999). In addition to the risks of genetic pollution of the local genotypes, the use of cultivars may also result in a cryptic invasion of the local community (Saltonstall 2002).

Invasive species suppression

Dominant species identity plays a role in determining the suppression of non-native species (Emery and Gross 2006). In Chapter 3, the seed source and growth form of the dominant species were factors in the suppression of unwanted species, *E. annuus*. As there was no significant difference in the above-ground vascular plant cover between the four *F. rubra* genotypes, the suppression effect of regional *F. rubra* may have resulted from below-ground competition. Regional *F. rubra* may have produced a more extensive root architecture, which may have hindered the establishment of *E. annuus*. The subordinate species also had slightly reduced establishment in the regional *F. rubra* treatments; however, this effect was not as great as the reduction in *E. annuus* individuals. Invasive forbs may have large effects in grass-dominated environments, as they may alter the functional trait composition and soil processes (Scharfy et al. 2011). As the subordinate species were sown at the same time as *F. rubra*, they are likely to have established before the arrival of invasive alien *E. annuus* to the site. *E. annuus* may have faced greater competition for establishment, particularly in the regional *F. rubra* sub-plots. Although it may be possible to reduce the risk of colonisation by unwanted species by selecting dominant regional seed material, competitive regional seed is also likely to impact on the establishment of the desired species.

It is noteworthy to mention that *F. rubra* is allelopathic (Bertin et al. 2003). This presents an alternative explanation, which may have occurred singly or in combination with the greater root network. The allelopathic effects of *F. rubra* may possibly differ between the cultivar and regional plants. Interestingly, cultivar fescues, including *F. rubra*, are being developed for their allelopathic weed suppression abilities (Bertin et al. 2003). However, the results from Chapter 3 indicate that under field conditions, regional *F. rubra* has better suppression abilities. Furthermore, *E. annuus* also has allelopathic tendencies (Scharfy et al. 2011). Although it was not studied as part of the field experiment, the vulnerability of the *F. rubra* sources to the allelopathic effects of *E. annuus* may have also differed.

An array of management techniques, including grazing and mowing at specific times (DiTomaso 2000), can also be used to control invasive species in grasslands. However, it is often most advantageous to tackle the problem of invasive species before they become widespread in a habitat. As the findings from Chapter 3 show, the risk of invasion should be considered from the onset of the restoration work. Therefore, the selection and use of suitable seed sources and growth forms, which reduce the risk of invasive species establishment, is advised in grassland restoration.

5.3. Seed quality

Emergence and dormancy of seed sources

The establishment of vegetation cover is a one of the most fundamental priorities in grassland restoration (SER 2004). Sites that are at risk from soil erosion (Gros et al. 2004) or invasion from unwanted species (Young et al. 2009) are particularly vulnerable and require high establishment and vegetation cover during the early stages of restoration. Cultivars are expected to have high germination due to their selection for reduced dormancy (Schröder and Prasse 2013a). Fast, abundant germination is a desirable trait in cultivars. A study by Schröder and Prasse (2013a) found that cultivars germinated faster and more abundantly than wild seed. While the results from Chapter 2 support the findings of Schröder and Prasse (2013a) in relation to faster cultivar emergence, the results deviate concerning cultivar abundance. In comparison to regional seed, cultivars were not necessarily more abundant. Then again, in Chapter 2 cultivar seed also had more reliable emergence than the regional plant material, which varied more across the different sources. This was to be expected as cultivars are bred for uniformity (Aubry et al. 2005). Cultivar seed displayed fast emergence, good levels of abundance and performed consistently in comparison to the greater variation displayed by regional seed. While some of the regional seed sources had faster emergence and abundance than the cultivars, other regional seed sources were considerably lower. For restoration practitioners, the predictability and reliability of seed performance may be an important consideration when determining the seed used for restoration. As these results were from a greenhouse experiment, they cannot be directly compared to the performance of the seed source under field conditions in its provenance region. Nevertheless, lower emergence in the greenhouse can be indicative of lower emergence in the field, as was found with regional *B. salicifolium*. Differences in emergence may relate to the age of the seed and storage conditions, such as temperature and moisture level (Probert et al. 2009), which was not available from all seed suppliers.

In Germany, the market for seed production on a provenance region scale is smaller than the widespread production of cultivars. Regional seed producers therefore vary their assortment of species each year due to supply and demand and space restrictions on the production facilities. In any given year, the diversity of species available within a particular provenance region differs (Scotton et al. 2011). Seed produced in previous years may have lower viability, as was seen with three year old regional *B. salicifolium* seed. The storage requirements of each species may also vary (van der Valk et al. 1999), thus making it challenging for seed producers to store all of the seed under optimal conditions. Although regional seed is expected to fail less under field conditions due to maladapted genotypes, seed viability is imperative. Thus, it is advisable to use the youngest seed available for restoration, as the seed viability is likely decline in storage (Baskin and Baskin 2001). Low seed viability reflects the risks of ‘hit and miss' establishment, which could be particularly detrimental to the success of a restoration project if seeding only occurs once (Jones 2013b).

While reliability and reduced dormancy of cultivars can provide fast results (Schröder and Prasse 2013a), there are also problems with such a uniform germination pattern. Reduced seed dormancy, also increases the vulnerability of the cultivar seedlings to stochastic events (Lesica and Allendorf 1999). During the early seedling stage, seedlings are particularly vulnerable to drought, frost, flooding or excessive herbivory. Therefore seed that has higher dormancy can avoid these potential dangers if not all of the viable seed germinate at once (Knapp and Rice 1994). In environments that frequently experience unfavourable conditions, it is advantageous to have increased dormancy (Rice and Dyer 2001). This is due to the greater chance of seed establishing after the first year, when populations have suffered a decline caused by adverse conditions. The natural variation in seed germination that is still present in regional seed may be useful in such circumstances. Interestingly, while the number of cultivar *D. carthusianorum* and *L. perenne* individuals remained similar throughout the two years of the field experiment, the number of regional individuals of these species increased, which supports the idea of higher seed dormancy in regional seed. Although increased seed dormancy is an advantage, the age of the seed is also important as the likelihood of the seed remaining viable in the soil declines over time (Baskin and Baskin 2001).

Sowing time also effects the establishment of species, and the likelihood for successful establishment will vary according to the harvest method, site conditions, soil preparation and climatic conditions (Scotton et al. 2011). While it is advisable to sow the seed during the optimal season for establishment, it may not always be feasible as restoration projects are often under time restrictions. According to the results of the field study, the chance of establishment for cultivars appears to be highest during the first year, as very little increase in the number of individuals was observed for the cultivar subordinate species during the second year. The use of regional seed mixtures may allow greater flexibility in relation to the sowing time, due to the higher likelihood of individuals also establishing in the following year. This may be related to the conditions in which the maternal plants were grown, as plants grown in more variable conditions were found to have more vigorous older seed (Rice and Dyer 2001).

Growth form

Dominant species help to structure the grassland communities, partly through their large contribution to biomass production (Grime 1998). In Chapter 2 and Chapter 3, the form of this biomass production, either rhizomatous or tussock, was investigated to determine whether the growth form of *F. rubra* impacts on its suppressive effects and whether this was also combined with the source effects. The pot size (Poorter et al. 2012) and the duration of the experiment in Chapter 2 may have limited the differences in the growth form effects of *F. rubra*. In the field experiment, the four *F. rubra* genotypes significantly differed in their establishment in the first year. Regional *F. rubra* tussock had the greatest establishment in Year one, followed by the cultivar *F. rubra* rhizomatous. While the conditions for establishment were equal, the likelihood of above-ground biomass expansion may have differed between the two growth forms. The expansion of tussocks in small pockets of soil may have been easier than the production of rhizomes in the gravel environment of the field site.

Plant height is the most important trait in relation to the measure of competitive ability in urban grasslands (Fischer et al. 2013). The importance of height in relation to the performance of the different growth forms of the subordinate species was seen in Chapter 1. Both *B. salicifolium* and *D. carthusianorum* produce rosettes in their first year and generally bolt with flowers in the following year (Oberdorfer 2001, Bloch et al. 2006). In the greenhouse experiment, *B. salicifolium* produced a low, flat rosette, while the rosettes of *D. carthusianorum* were taller and had a tussock appearance. In contrast, *L. perenne* produced tall upright stems. *B. salicifolium* was particularly disadvantaged by its low growth form as the grasses quickly restricted the amount of available light in the greenhouse experiment in Chapter 1. The close arrangement of the pots in the greenhouse experiment was likely to have intensified the shading effect of *F. rubra*. Nonetheless, high density seed mixtures in the field may also create intense shading, particularly for low lying species. Regular mowing or grazing would limit the biomass accumulation of the dominant species and may favour low growing species (Kahmen and Poschlod 2008), such as *B. salicifolium*. In order to ensure that a restored grassland is functionally diverse, the competitive ability of the dominant species, regardless of whether it is from a cultivar or regional source, must be carefully considered in the seed mixture. This may reduce the risks associated with vigorous above- and below-ground growth and biomass accumulation, which are likely to suppress vulnerable species.

Seeding density

The seeding density of *F. rubra* in Chapter 1 and in Chapter 3 had very limited effects on the subordinate species. In the greenhouse experiment, the pots were likely to have been the limiting factor in the above- and below-ground growth of *F. rubra*, which resulted in no significant differences in the above-ground biomass between the two and four *F. rubra* individuals per pot (Poorter et al. 2012). Based on this result, it was decided that only one *F. rubra* and one *D. carthusianorum* individual would be sown per pot in Chapter 2. In the field experiment, the doubling of *F. rubra* seed in the high density treatment resulted in only a very slight increase in the number of *F. rubra* individuals established in the first year and the percentage cover of the sub-plots in the second year. The relatively low establishment was likely to have been limited by the availability of microsites in the limiting soil conditions, or the limited recruitment opportunities at the time of sowing (Turnbull et al. 2000).

F. rubra often constitutes large portions of commercial seed mixtures (Conrad 2007). This may result in a large amount of seed wastage if the seed is sown in environmental conditions similar to those of the field experiment or an abundance of the species under more fertile conditions (Conrad and Tischew 2011). Seeding density is particularly important in relation to the diversity of the vegetation community. Seed mixtures should therefore be created with suitable densities of each species rather than bulking up on one or a few species, such as grasses. As the niche requirements of species in high diversity seed mixtures are likely to be more varied than low diversity seed mixtures, there may be a reduced risk of establishment failure (Lepŝ et al. 2007). This may assist in the creation of a long-term vegetation community at restoration sites.

Maternal effects

The performance of the seed used in these experiments may be related to maternal effects. Plants that have been grown under favourable environmental conditions may transfer these advantages or disadvantages relating to seed dormancy or germination traits into the seed of the next generation (Roach and Wulff 1987). Seed mixtures used in grassland restoration often contain mixes of seed and species from different areas. Regional species are occasionally transferred between province regions and the larger regions of production, when there are insufficient quantities of species (Scotton et al. 2011). As different growing conditions occur even within the same provenance region, it is likely that different maternal effects will be transferred to the seed by the mother plants. This is also true for the conditions the propagation conditions of cultivars. Due to the practicalities involved in grassland restoration, it is necessary to sow seed material directly from the seed suppliers. Therefore, the experiments conducted as part of this dissertation aimed to deal with realistic problems, such as maternal effects in the field, faced by restoration practitioners relating to the source of seed used in grassland restoration. It may also be possible for seed producers to use maternal effects beneficially in restoration, as seed could be bred for their suitability in a wide variety of habitats or under specific conditions (Espeland and Hammond 2013). The three experiments in this dissertation focused largely on the establishment and growth of species, as the impacts of material effects are most strongly expressed during the early life cycle in plants (Roach and Wulff 1987), although they can also influence later growth stages as well (Helenurm and Schaal 1996).

5.4. Application of findings

While the results of these three chapters relate to calcareous grasslands, the findings have broader implications for restoration in other grassland habitats as well. If the introduction of seed is required for restoration, then seed source is a relevant factor for restoration of any habitat. The findings from Chapter 2 showed that cultivars have relatively uniform patterns of predictability, which can be advantageous under certain circumstances. Then again, the performance of cultivars in the field experiment was generally not as good as the regional seed over the two year study period. The long-term effects of cultivar and regional performance under limiting field conditions could not be deduced from the duration of the study. However, in the first 2 years of the field experiment, regional seed had generally better establishment and the dominant species had greater suppression of *E. annuus*, which suggests that they are suited to the site conditions. The long-term persistence of cultivars may be hampered by their sensitivity to stochastic events (Lesica and Allendorf 1999, Schröder and Prasse 2013c). Furthermore, they may be more vulnerable than regional seed in the long-term, as they have uniform emergence and reduced dormancy (Knapp and Rice 1994, Schröder and Prasse 2013a), which may limit their recovery after disturbances.

Rhizomatous grasses often have rapid regeneration potential (Lepŝ et al. 2007, Török et al. 2010). The findings from Chapter 3 show that in nutrient limited environments with heavily gravelled soils, the expansion of rhizomatous grasses may be limited. This may imply that the performance of the dominant species growth form is influenced greatly by the site conditions. While rhizomatous *F. rubra* grasses were not problematic at the densities sown in Chapter 3, at higher densities they became very abundant and suppressing in the ‘chernozem over loess’ soils in the study by Conrad and Tischew (2011). This highlights the interaction between growth form, seeding density and site conditions which should be considered for every grassland restoration. Although cultivar rhizomatous grasses were not problematic in this calcareous grassland experiment, they may be more problematic in other grasslands with more favourable soil conditions. Furthermore, the performance of the subordinate species may be influenced by the management regime of the grassland. If regular mowing or grazing does not occur, then low-lying species may be particularly disadvantaged by the accumulation of dominant species biomass over long periods.

Seeding density is an important practical factor in restoration, both ecologically and financially. The limited increase in *F. rubra* seed between the low and high densities in the field experiment highlights the need for careful seed density selection in seed mixtures. Suitable niche microsites are required for germination (Turnbull et al. 2000). The use of high diversity seed mixtures may be more desirable in grassland restoration, as they have a lower risk of saturating the microsites with one particular species and also avoid the likelihood of one species becoming dominant.

The restoration of grasslands is often constrained by high residual nutrient levels (Walker et al. 2004). Although regional seed is generally recommended for widespread use in restoration, the implications of seed source and soil nutrient level have not been previously addressed in detail. In Chapter 2, it was found that regional species have a greater competitive effect at medium and high nutrient levels, which had not been expected. This finding has important implications for other grassland restoration projects as the use of regional dominant species may greatly suppress the growth of subordinate species. As regional seed also had greater variation, it may be more difficult to predict the outcome of a restoration project sown with regional seed. In the majority of cases, regional seed is likely to perform well, or even better than cultivars in its local provenance region. However, the low emergence of some of the regional *F. rubra* seed in Chapter 2 suggests that this may not always be the case.

6. Conclusions

Grassland restoration is a complex undertaking, which must be approached in a logical, systematic way to enhance the likelihood of success. By categorising the main considerations into *site factors*, *species identity* and *seed quality* factors, it is easier to approach the challenges of restoration in a step-wise manner.

In relation to the *site factors*, there are a number of considerations that should be evaluated prior to the initiation of restoration. While there is a lot of merit in the recommendation to use regional seed in grassland restoration, the source of the seed used for landscaping should always be considered in relation to the site conditions. The competitive abilities of regional seed, particularly in relation to the dominant species, may be enhanced by high soil nutrient levels present in the restoration site. This will result in a greater suppression of the subordinate species, which play an important part for biodiversity and ecosystem functioning in grasslands. Furthermore, site conditions have considerable influence over the relative performance of the grass growth forms and the availability of microsites. The expansion of rhizomatous grasses, for example, may be hindered in gravel soils with little humus. These soil conditions may also be harsh environments for germination, particularly in times of drought or extreme temperatures. This raises the importance of microsites for seed germination. Increasing the seeding density beyond the carrying capacity of the soil is unlikely to result in large increases in plant establishment.

Dominant species regulate the growth of subordinate species. In contrast to expectations, the regional dominant species was found to have a greater competitive effect than cultivar dominant species. While the cultivar dominant species remained relatively stable in their competitive effect on the subordinate species across a nutrient gradient, the dominant species of regional source showed greater variation and exhibited the greatest competitive effect at medium nutrient levels. It was also found that the source of the dominant species can affect the invasibility of site. The invasive non-native *E. annuus* had lower establishment in sub-plots with regional *F. rubra*, particularly those with the tussock growth form. Subordinate species from cultivar and regional sources perform differently in the greenhouse and field experiments. Regional subordinate species had greater establishment in the field when grown with the dominant species and other ruderals. However, cultivar subordinate species performed better in the presence of competition with dominant species in the greenhouse.

The source of the seed used in restoration had wide-reaching implications in relation to the performance of dominant and subordinate species in calcareous grassland restoration. These implications are intricately connected with other factors including growth form, seeding density and nutrient level. Therefore, the source of the seed applied in restoration work should be considered in connection with the other influencing factors. Cultivars generally provide consistent results regarding their emergence and competitive effect across a nutrient gradient, which contrasts with the greater variation shown by the regional seed in these traits. The occurrence of cultivar vigour was not universally found in all of the cultivars and is likely to depend the environmental conditions, such as nutrient availability, and the degree of competition that the plant experiences. Despite having consistent traits, cultivars did not generally perform as well in the field experiment as the regional plants. Although the long-term performance of cultivars was not assessed as part of this dissertation, it can be concluded that regional seed performed better in the first 2 years after establishment. Furthermore, regional seed had higher dormancy, which resulted in greater establishment in the second year of the study. This may be advantageous for restoration, particularly if sowing does not occur at the optimum time of the year.

The findings of this dissertation will help to further the theoretical understanding behind the debate on the source of seed to be used in grassland restoration. Rather than basing the recommendations on general guidelines for regional seed, the selection of seed should be founded on the site conditions and goals of the restoration work. Each restoration site is unique and should have its own prescribed remedy based on the nutrient availability, soil type and target vegetation community. Thus, the findings from the three experiments are highly relevant for restoration practitioners and can be immediately implemented in on-going and future restoration projects.

7. Recommendations

The recommendations developed based on the results of this dissertation have to be differentiated depending on the scientific and practical progress in restoration ecology of the different countries. While, for example, in Europe and North America research and application in regional seed for grassland restoration is quite advanced, the situation is less favourable, for example, in Brazil and China where the regional genetic differentiation of most target species is unknown, and no companies have been established that propagate and sell wild seed of native species.

7.1. Countries with developed ecological restoration

The source of the seed used for grassland restoration is of fundamental importance. Therefore, if a range of seed sources is available, the selection of the most suitable seed source should be based on the site conditions and the restoration goals (Figure 23). Generally, regional seed is the most appropriate for grassland restoration as it has high establishment under field conditions, strong competitive abilities and maintains the genetic integrity of the regional species pool. This is likely to assist long-term population survival and resilience of the target vegetation. However, regional seed also display greater variability in their competitive effect at different levels of nutrient availability compared with cultivar seed. Thus, the choice of the most appropriate seed material depends on site conditions and the intended vegetation cover. Cultivars have relatively uniform emergence and competitive abilities, but their performance under field conditions was lower than that of regional seed.

Based on the results of this dissertation, there are no incentives to select cultivar rather than regional seed for grassland restoration, apart from the obvious difference in price. However, it is advisable to test different seeding densities of the seed mixture under field conditions prior to restoration to determine the performance of the seed under field conditions.

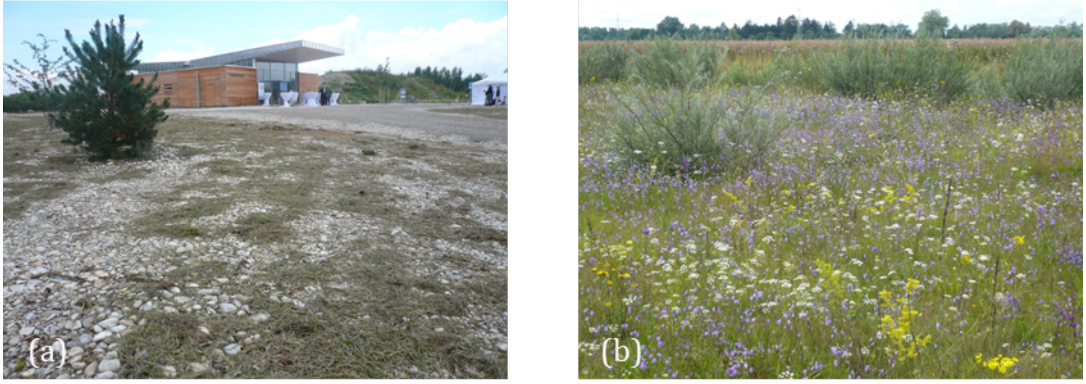

Figure 23 Examples of restoration in the Munich Gravel Plain. (a) Restoration using hay transfer on gravel soils at the Heidehaus, München-Freimann; and (b) successful restoration at Kissinger Bahngruben using hay transfer. Source of photographs: J. Kollmann.

7.2. Countries with developing ecological restoration

Great progress has been made in the advancement of grassland restoration in recent years. However, advancements are only being slowly implemented in countries like Brazil (Figure 24), which have limited or developing support networks for restoration. These networks rely on the knowledge of experts, the commitment and understanding of the involved communities and the necessary infrastructure for successful restoration. When any part of this network fails to function properly, the likelihood of successful restoration can be severely challenged.

While using regional seed is generally advisable for grassland restoration, in many cases this is not an available option in countries with developing ecological restoration. Often, there may only be a limited supply of commercial cultivar seed of native seed available, or in other cases not even cultivars of native species are obtainable. In such cases, it is important to approach the problem of species and seed selection in a step-wise manner from *site factors* to *species identity*, and finally the *seed quality*.

Once the *site factors* of the grasslands to be restored have been determined, a suitable selection of dominant, subordinate and transient species should be established based upon the restoration goals. In cases where the species composition is the main priority, only native species should be selected, preferably from local sources but if not, then cultivars can be used. When the main priority is to restore ecosystem function, then native species with similar functions should be given precedence, followed by the use of cultivars. While the use of cultivars may be unavoidable in circumstances where there are very limited seed availability options, they should always be used cautiously in grassland restoration as their long-term performance and impacts are not fully understood. Ideally, the seed mixture should be tested under field conditions prior to restoration, preferably at different seeding densities to determine the most suitable species composition ratios.

Future goals should include efforts to develop cultivars of the native species that can be used in widespread restoration projects. Once native species are available as cultivars, priority should be given to the development of regional seed production as a sustainable economic activity in rural regions of countries with developing ecological restoration. The establishment of regional seed production would help solving problems relating to native seed availability, while also creating locally based, ecological employment opportunities. Although this is a lengthy process, which entails the delineation of seed transfer zones and the development of propagation techniques, knowledge gained from regional seed production in other areas could also be transferred and assist the developmental process. Furthermore, greater recognition and appreciation of grasslands as providers of important ecosystem services and as areas of unique biodiversity are needed to promote the sustainable usage and management of grasslands. This requires an integrated restoration approach that focuses not only on policy changes, but also the education of local farmers and land owners who directly manage the grasslands.

Figure 24 Examples of grassland management and land use in Rio Grande do Sul, Brazil. (a) Recently burned grassland (background area with trees) and unburned grassland (foreground) in Parque Estadual do Tainhas, Campos da Cima da Serra; impoverished grasslands in (b) logged pine plantation in Parque Aparados da Serra, Cambará do Sul (source: J.-M. Hermann); and (c) logged eucalyptus plantations (Pampas region) and (d) rice fields on former grasslands (Pampas region).

Co-author contributions

Many people contributed to the individual chapters in this thesis. This is an outline of the co-author contributions to each of the three chapters.

Chapter 1

Harald Meimberg, Johannes Kollmann, Julia-Maria Hermann and I developed the conceptual idea and experimental design of this experiment. I established the experiment in the greenhouse and was responsible for all of the observations and measurements made during the experimental period. Gabriele Pilger assisted me with seed sowing. Christiane Koch, Florencia Yannelli and Ingrid Kapps participated in the cutting plant biomass. I conducted all of the statistical analyses. I wrote the text of the chapter, which was improved and edited by Johannes Kollmann. Overall, I estimate that my contributions amounted to about 80% of total co-author contributions.

Chapter 2

Harald Meimberg and I created the initial idea and design of this experiment. The greenhouse experiment was established with the assistance of Olivia Fröhlich. Three interns (Yede Zhu, Pauline Böttcher-Graf and Emilie Vajda) helped with the measurements and biomass collection. Timo Conradi undertook all of the statistical analyses and wrote the data analysis and result section of the chapter. I wrote the remaining text of the chapter, which was then edited by Johannes Kollmann and Timo Conradi. My contributions to this chapter amounted to roughly 70% of total co-author contributions.

Chapter 3

Johannes Kollmann, Julia-Maria Hermann and I developed the concept and initial experimental design. Harald Meimberg also gave suggestions and practical advice throughout the experiment. Nils Beese, Timo Conradi and Julia Prestele assisted me in the establishment the experiment. I conducted the vast majority of the field measurements by myself, but I did receive some help from Charlotte Mason and Olivia

Fröhlich. I was responsible for the statistical analyses. Julia-Maria Hermann reviewed literature for inclusion and I wrote the text of the chapter. This text was improved and edited by Johannes Kollmann and Julia-Maria Hermann. About 75% of the total co-author contributions for this chapter resulted from my contributions.

Acknowledgements

This PhD would not have been possible without the guidance and assistance of many people. Although not every contribution is acknowledged in this text, I am extremely grateful for all contributions, no matter how big or small.

Firstly, I would like to thank Johannes Kollmann for his excellent supervision. Over the past number of years, I have benefited enormously under his direction and from his ideas. I am extremely grateful to him for being my supervisor. His efficiency and reliability are the attributes of a superb professor, from which I have learned greatly and am very thankful for.

Harald Meimberg contributed many great ideas and suggestions for my PhD and I greatly appreciated his contributions over the past 3 years. Especially, his creativity and approach in stressful situations were of benefit to me. His input has enriched my research and broadened my knowledge of genetics.

Julia-Maria Hermann provided advice and expertise throughout my PhD and am very thankful to her for her significant input. Her expert overview of the literature relating to grassland restoration and seed sources, combined with her knowledge of the local area and useful contacts have greatly enhanced my experiments. Furthermore, Julia assisted me with my many linguistic challenges over the last few years.

I would also like to give a special thanks to my fellow PhD student and co-author, Timo Conradi. Throughout my PhD, Timo kindly shared his detailed knowledge of grassland systems, for which I am very grateful. It was a pleasure to work with him as a co-author. He is super.

My PhD was financed by the Deutscher Akademischer Austauschdienst (DAAD). Their support over the past three years has allowed me to pursue my goals and I am very thankful for this opportunity. I also received a consumables grant from the Dr.-Ing. Leonhard-Lorenz-Foundation, which was of great assistance. During my PhD, I also received a TUMBRA Scholarship for a research stay at the Botany Department of the

Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil. Through this research trip, I expanded my views on restoration and learned about the challenges facing restoration practitioners in areas where restoration is only developing. I am indebted to Gerhard Overbeck and his colleagues for making my research stay such an eye-opening and memorable experience.

There are many high points during a PhD, but also some challenging times. For providing encouragement and extremely useful practical advice, I am much indebted to my mentor, Klaus Wiesinger. His contributions and personal support are very much appreciated.

The knowledge and assistance of Johann Krimmer have been invaluable to this PhD. I am deeply grateful to him for his interest and commitment to this project. Furthermore, I would like to thank him for assistance with the field site preparation.

The staff of Greenhouse staff of the Centre of Greenhouses and Laboratories Dürnast, Life Science Centre Weihenstephan of the Technische Universität München provided expert knowledge and assistance for my greenhouse experiment. I greatly appreciate their contributions, especially those of Ivonne Jüttner, Sabine Zuber, Bärbel Breulmann and Margot Reith.

I am deeply grateful for the assistance of a number of individuals from the Bayerische Landesanstalt für Landwirtschaft. Helmut Schneider and Robert Bloier provided helped with the establishment of my field experiment. Susanne Mosch assisted me with the germination trials. Furthermore, I would like to thank the staff of Klärwerk Gut Marienhof for access to the field site.

I am grateful to Brigit Feucht and Ernst Rieger for their insight into regional seed production. Jelitto Staudensamen GmbH and Saatzucht Steinach GmbH &Co KG also provided useful information relating to cultivar seed. Additionally I would like to thank the individuals and seed companies that supplied seed for my experiments. These include Johann Krimmer, Matthias Stolle, DLF-Trifolium, Jelitto Staudensamen GmbH,

Landschaftspflegeverein Dummersdorfer Ufer e.V., Rieger-Hofmann GmbH, Saatzucht Steinach GmbH &Co KG and Wildsaaten.

There were many people who helped me with the practical elements of my experiments. Their time and assistance have helped me hugely and I am extremely grateful for their contributions. A big thanks goes to Ingrid Kapps, Pauline Böttcher-Graf, Olivia Fröhlich, Tim Heger, Niklas Kroner-Weigel, Charlotte Mason, Gabriele Pilger, Emilie Vajda and Yede Zhu.

I am very grateful to the staff of the Chair of Restoration Ecology, first of all for their friendly welcome, and later the support and constructive comments throughout my PhD. It has been a pleasure to work with them and I value their contributions and lively discussions greatly. A special mention must be given to Michaela Bücherl, who is a multi-talented secretary and a wonderful cook. Thank you all very much.

Sara Altenfelder, Timo Conradi, Naret Guerrero Moreno, Christiane Koch, Anna Pahl, Julia Prestele, Wolf Saul, Maximiliane Schümann and Florencia Yannelli deserve a very big thank you. These fellow PhD students provided all-round support through my PhD. Their willingness to help in any way they could, to listen, problem solve, translate and entertain have been invaluable over the past three years. They helped me to enjoy the highs and lows of the PhD with a smile.

A few people require species acknowledgement in recognition of their contributions. Julia Prestele helped to establish the field experiment and provided useful tips and advice through the PhD. I am very grateful to Gearóid Ó Murchú and Conor Ryan for their comments and improvements to the text and Christiane Koch for translating the summary into German.

My family have always encouraged me to pursue my goals and I am very grateful to them for their constant encouragement and support. Their frequent calls helped me through the ups and downs and kept me up-to-date with the goings-on in Ireland. I was always well informed about the state of the weather and the local bingo wins thanks to my Granny ☺

It would not have been possible for me to get this far and pursue this PhD without the absolute support of two close friends. Thank you for being there when I needed a helping hand.

Last but not least, I would like to thank Nils Beese. Throughout the PhD, he has demonstrated his versatile field work skills, as well as excellent proof reading and translation abilities. He even improved his own gardening skills along the way! His humour, patience and love through the good and the not so good times, greatly helps me to persevere. Most of all, thank you for being you and for believing in my potential to become a qualified Dr.E(a)mer.

Thank you to those who contributed to this dissertation. They helped to make it an extremely fun and memorable experience.

References

Aavik, T., P. J. Edwards, R. Holderegger, R. Graf, and R. Billeter. 2012. Genetic consequences of using seed mixtures in restoration: a case study of a wetland plant *Lychnis flos-cuculi*. Biological Conservation **145**:195–204.

Abraham, J., J. Corbin, and C. D'Antonio. 2009. California native and exotic perennial grasses differ in their response to soil nitrogen, exotic annual grass density, and order of emergence. Plant Ecology **201**:445–456.

Adler, P. B., A. Fajardo, A. R. Kleinhesselink, and N. J. B. Kraft. 2013. Trait-based tests of coexistence mechanisms. Ecology Letters **16**:1294–1306.

Aldrich, J. H. 2002. Factors and benefits in the establishment of modest-sized wildflower plantings: a review. Native Plants Journal **3**:67–86.

Anscombe, F. J. 1953. Sequential estimation. Journal of the Royal Statistical Society. Series B (Methodological) **15**:1–29.

Aubry, C., R. Shoal, and V. Erickson. 2005. Grass cultivars: their origins, development, and use on national forests and grasslands in the Pacific Northwest. USDA Forest Service, Portland, OR, USA. Available from: http://www.fs.fed.us/wildflowers/Native_Plant_Materials/documents/cultivars_maindoc_040405_appendices.pdf (accessed 20 July 2013)

Baer, S. G., J. M. Blair, S. L. Collins, and A. K. Knapp. 2003. Soil resources regulate productivity and diversity in newly established tallgrass prairie. Ecology **84**:724–735.

Baer, S. G., S. L. Collins, J. M. Blair, A. K. Knapp, and A. K. Fiedler. 2005. Soil heterogeneity effects on tallgrass prairie community heterogeneity: an application of ecological theory to restoration ecology. Restoration Ecology **13**:413–424.

Baer, S. G., D. J. Gibson, D. J. Gustafson, A. M. Benscoter, L. K. Reed, R. E. Campbell, R. P. Klopf, J. E. Willand, and B. R. Wodika. 2013. No effect of seed source on multiple aspects of ecosystem functioning during ecological restoration: cultivars compared to local ecotypes of dominant grasses. Evolutionary Applications **7**:323–335.

Baer, S. G., D. J. Kitchen, J. M. Blair, and C. W. Rice. 2002. Changes in ecosystem structure and function along a chronosequence of restored grasslands. Ecological Applications **12**:1688–1701.

Bakker, J. P., and F. Berendse. 1999. Constraints in the restoration of ecological diversity in grassland and heathland communities. Trends in Ecology & Evolution **14**:63–68.

Baskin, C. C., and J. M. Baskin. 2001. Seeds: ecology, biogeography, and evolution of dormancy and germination. Academic Press, San Diego, CA, USA.

Bertin, C., R. N. Paul, S. O. Duke, and L. A. Weston. 2003. Laboratory assessment of the allelopathic effects of fine leaf fescues. Journal of Chemical Ecology **29**:1919–1937.

Bischoff, A., L. Crémieux, M. Smilauerova, C. S. Lawson, S. R. Mortimer, J. Dolezal, V. Lanta, A. R. Edwards, A. J. Brook, M. Macel, J. A. N. Leps, T. Steinger, and H. Müller-Schärer. 2006. Detecting local adaptation in widespread grassland species — the importance of scale and local plant community. Journal of Ecology **94**:1130–1142.

Bischoff, A., T. Steinger, and H. Müller-Schärer. 2010. The importance of plant provenance and genotypic diversity of seed material used for ecological restoration. Restoration Ecology **18**:338–348.

Bloch, D., N. Werdenberg, and A. Erhardt. 2006. Pollination crisis in the butterfly-pollinated wild carnation *Dianthus carthusianorum*? New Phytologist **169**:699–706.

Blomqvist, M. M., P. Vos, P. G. L. Klinkhamer, and W. J. ter Keurs. 2003. Declining plant species richness of grassland ditch banks – a problem of colonisation or extinction? Biological Conservation **109**:391–406.

Bobbink, R., M. Hornung, and J. G. M. Roelofs. 1998. The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. Journal of Ecology **86**:717–738.

Bossuyt, B., J. Butaye, and O. Honnay. 2006. Seed bank composition of open and overgrown calcareous grassland soils – a case study from Southern Belgium. J Environ Manage **79**:364–371.

Bossuyt, B., and O. Honnay. 2008. Can the seed bank be used for ecological restoration? An overview of seed bank characteristics in European communities. Journal of Vegetation Science **19**:875–884.

Bullock, J. M. 2000. Gaps and seedling colonization. CABI, Wallingford, UK.

Butaye, J., D. Adriaens, and O. Honnay. 2005. Conservation and restoration of calcareous grasslands: a concise review of the effects of fragmentation and management on plant species. Biotechnology, Agronomy, Society and Environment **9**:111–118.

Chesson, P. 2000. Mechanisms of maintenance of species diversity. Annual Review of Ecology and Systematics **31**:343–366.

Chittka, L., and S. Schurkens. 2001. Successful invasion of a floral market. Nature **411**.

Choi, Y. D., V. M. Temperton, E. B. Allen, A. P. Grootjans, M. Halassy, R. J. Hobbs, M. A. Naeth, and K. Torok. 2008. Ecological restoration for future sustainability in a changing environment. Ecoscience **15**:53–64.

Chrobock, T., A. Kempel, M. Fischer, and M. van Kleunen. 2011. Introduction bias: cultivated alien plant species germinate faster and more abundantly than native species in Switzerland. Basic and Applied Ecology **12**:244–250.

Clewell, A., and J. Aronson. 2013a. Ecological attributes of restored ecosystems. Pages 89–112 Ecological Restoration. Island Press/Center for Resource Economics.

Clewell, A., and J. Aronson. 2013b. Project Planning and Evaluation. Pages 169–196 Ecological Restoration. Island Press/Center for Resource Economics.

Conrad, M. K. 2007. Effectiveness and cost measures for the establishment of species-rich grasslands: development and application of a procedure for efficiency controls. Technical University Berlin.

Conrad, M. K., and S. Tischew. 2011. Grassland restoration in practice: do we achieve the targets? A case study from Saxony-Anhalt/Germany. Ecological Engineering **37**:1149–1157.

Cornelissen, J. H. C., S. Lavorel, E. Garnier, S. Díaz, N. Buchmann, D. E. Gurvich, P. B. Reich, H. ter Steege, H. D. Morgan, M. G. A. van der Heijden, J. G. Pausas, and H. Poorter. 2003. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. Australian Journal of Botany **51**:335–380.

Coulson, S. J., J. M. Bullock, M. J. Stevenson, and R. F. Pywell. 2001. Colonization of grassland by sown species: dispersal versus microsite limitation in responses to management. Journal of Applied Ecology **38**:204–216.

Cramer, V. A., and R. J. Hobbs. 2007. Old fields: dynamics and restoration of abandoned farmland. Island Press, Washington, DC, USA.

Critchley, C. N. R., B. J. Chambers, J. A. Fowbert, R. A. Sanderson, A. Bhogal, and S. C. Rose. 2002. Association between lowland grassland plant communities and soil properties. Biological Conservation **105**:199–215.

D'Antonio, C. M., and P. M. Vitousek. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annual Review of Ecology and Systematics **23**:63–87.

DBU. 2010. Entwicklung und praktische Umsetzung naturschutzfachlicher Mindestanforderungen an einen Herkunftsnachweis für gebietseigenes Wildpflanzensaatgut krautiger Pflanzen. Project Report, Universität Hannover.

Derner, J. D., and D. D. Briske. 2001. Below-ground carbon and nitrogen accumulation in perennial grasses: a comparison of caespitose and rhizomatous growth forms. Plant and Soil **237**:117–127.

Diacon-Bolli, J. C., P. J. Edwards, H. Bugmann, C. Scheidegger, and H. H. Wagner. 2013. Quantification of plant dispersal ability within and beyond a calcareous grassland. Journal of Vegetation Science **24**:1010–1019.

Díaz, S., S. Lavorel, S. U. E. McIntyre, V. Falczuk, F. Casanoves, D. G. Milchunas, C. Skarpe, G. Rusch, M. Sternberg, I. Noy-Meir, J. Landsberg, W. E. I. Zhang, H. Clark, and B. D. Campbell. 2007. Plant trait responses to grazing – a global synthesis. Global Change Biology **13**:313–341.

DiTomaso, J. M. 2000. Invasive weeds in rangelands: Species, impacts, and management. Weed Science **48**:255–265.

Dukes, J. S. 2002. Species composition and diversity affect grassland susceptibility and response to invasion. Ecological Applications **12**:602–617.

Dupré, C., and J. Ehrlén. 2002. Habitat configuration, species traits and plant distributions. Journal of Ecology **90**:796–805.

Edwards, A. R., S. R. Mortimer, C. S. Lawson, D. B. Westbury, S. J. Harris, B. A. Woodcock, and V. K. Brown. 2007. Hay strewing, brush harvesting of seed and soil disturbance as tools for the enhancement of botanical diversity in grasslands. Biological Conservation **134**:372–382.

Edwards, P. J., D. Frey, H. Bailer, and M. Baltisberger. 2006. Genetic variation in native and invasive populations of *Erigeron annuus* as assessed by RAPD markers. International Journal of Plant Sciences **167**:93–101.

Ehrenfeld, J. G. 2000. Defining the limits of restoration: the need for realistic goals. Restoration Ecology **8**:2–9.

Ehrenfeld, J. G. 2003. Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems **6**:503–523.

Emery, S. M., and K. L. Gross. 2006. Dominant species identity regulates invasibility of old-field plant communities. Oikos **115**:549–558.

Espeland, E. K., and D. Hammond. 2013. Maternal effects on growth and competitive ability in a commonly used restoration species. Native Plants Journal **14**:231–242.

Evans, M. E. K., and J. J. Dennehy. 2005. Germ banking: bet-hedging and variable release from egg and seed dormancy. The Quarterly review of biology **80**:431–451.

Fagan, K. C., R. F. Pywell, J. M. Bullock, and R. H. Marrs. 2008. Do restored calcareous grasslands on former arable fields resemble ancient targets? The effect of time, methods and environment on outcomes. Journal of Applied Ecology **45**:1293–1303.

Fehr, W. R. 1987. Principles of cultivar development. Macmillan Publishing Company, New York, USA.

Feucht, B. 2010. Production and use of native plant seeds in Germany.*in* SALVERE: semi-natural grassland as a soure of biodiversity improvement. Regional workshop in the Czech Republic. Available at: http://www.salvereproject.eu/downloads/workshop_cz/PP4Output224ProceedingsCzechRegionalWorkshopMay2010b.pdf#page=47 (access 13 March 2014)

Fischer, L. K., M. von der Lippe, and I. Kowarik. 2013. Urban grassland restoration: which plant traits make desired species successful colonizers? Applied Vegetation Science **16**:272–285.

Fischer, S. F., P. Poschlod, and B. Beinlich. 1996. Experimental studies on the dispersal of plants and animals on sheep in calcareous grasslands. Journal of Applied Ecology **33**:1206–1222.

Freckleton, R. P., A. R. Watkinson, and M. Rees. 2009. Measuring the importance of competition in plant communities. Journal of Ecology **97**:379–384.

Fu, B., L. Chen, K. Ma, H. Zhou, and J. Wang. 2000. The relationships between land use and soil conditions in the hilly area of the loess plateau in Northern Shaanxi, China. Catena **39**:69–78.

Funk, J. L., E. E. Cleland, K. N. Suding, and E. S. Zavaleta. 2008. Restoration through reassembly: plant traits and invasion resistance. Trends in Ecology & Evolution **23**:695–703.

Gibson, D. J. 2009. Grasses and grassland ecology. Oxford University Press, Oxford, UK.

Gibson, D. J., A. J. Allstadt, S. G. Baer, and M. Geisler. 2012. Effects of foundation species genotypic diversity on subordinate species richness in an assembling community. Oikos **121**:496–507.

Gibson, D. J., S. G. Baer, R. P. Klopf, L. K. Reed, B. R. Wodika, and J. E. Willand. 2013. Limited effects of dominant species population source on community composition during community assembly. Journal of Vegetation Science **24**:429–440.

Grime, J. P. 1998. Benefits of plant diversity to ecosystems: immediate, filter and founder effects. Journal of Ecology **86**:902–910.

Grime, J. P. 2002. Plant strategies, vegetation processes, and ecosystem properties. 2nd edition. Wiley, Chichester, UK.

Gros, R., L. Jocteur Monrozier, F. Bartoli, J. L. Chotte, and P. Faivre. 2004. Relationships between soil physico-chemical properties and microbial activity along a restoration chronosequence of alpine grasslands following ski run construction. Applied Soil Ecology **27**:7–22.

Güsewell, S. 2004. N : P ratios in terrestrial plants: variation and functional significance. New Phytologist **164**:243–266.

Gustafson, D. J., D. J. Gibson, and D. L. Nickrent. 2004. Competitive relationships of *Andropogon gerardii* (Big Bluestem) from remnant and restored native populations and select cultivated varieties. Functional Ecology **18**:451–457.

Gustafson, D. J., C. Major, D. Jones, J. Synovec, S. G. Baer, and D. J. Gibson. 2014. Genetic sorting of subordinate species in grassland modulated by intraspecific variation in dominant species. PLoS ONE **9**:e91511.

Harris, J. A., R. J. Hobbs, E. Higgs, and J. Aronson. 2006. Ecological restoration and global climate change. Restoration Ecology **14**:170–176.

Hart, S. P., and D. J. Marshall. 2013. Environmental stress, facilitation, competition, and coexistence. Ecology **94**:2719–2731.

Hedberg, P., and W. Kotowski. 2010. New nature by sowing? The current state of species introduction in grassland restoration, and the road ahead. Journal for Nature Conservation **18**:304–308.

Hegi, G. 1965. Illustrierte Flora von Mittel-Europa. Band V, Teil 1. Carl Hanser Verlag, München, Germany.

Hegi, G. 1979a. Illustrierte Flora von Mittel-Europa. Band III, Teil 2 2nd edition. Verlag Paul Parey, Berlin, Hamburg, Germany.

Hegi, G. 1979b. Illustrierte Flora von Mittel-Europa. Band VI, Teil 3. 2nd edition. Paul Parey Verlag, Berlin, Hamburg, Germany.

Hegi, G. 1998. Illustrierte Flora von Mitteleuropa. Band I, Teil 3. 3rd edition. Verlag Paul Parey, Berlin, Hamburg, Germany.

Helenurm, K., and B. A. Schaal. 1996. Genetic and maternal effects on offspring fitness in *Lupinus texensis* (Fabaceae). American Journal of Botany **83**:1596–1608.

Helm, A., I. Hanski, and M. Pärtel. 2006. Slow response of plant species richness to habitat loss and fragmentation. Ecology Letters **9**:72–77.

Hitchmough, J. D. 2000. Establishment of cultivated herbaceous perennials in purpose-sown native wildflower meadows in South-west Scotland. Landscape and Urban Planning **51**:37–51.

Hobbs, R. J. 2007. Setting effective and realistic restoration goals: key directions for research. Restoration Ecology **15**:354–357.

Hobbs, R. J. 2012. Environmental management and restoration in a changing climate. Pages 23–29 Restoration Ecology. John Wiley & Sons, Ltd., Chichester, UK.

Hobbs, R. J., L. M. Hallett, P. R. Ehrlich, and H. A. Mooney. 2011. Intervention ecology: applying ecological science in the twenty-first century. BioScience **61**:442–450.

Huber, R., and E. van der Maarel. 2007. Plant species turnover in an alvar grassland under different environmental conditions and at different scales. Acta Phytogeographica Suecica **88**:43–54.

Hufford, K. M., and S. J. Mazer. 2003. Plant ecotypes: genetic differentiation in the age of ecological restoration. Trends in Ecology & Evolution **18**:147–155.

Hutchings, M. J., and A. J. A. Stewart. 2002. Calcareous grasslands. Pages 419–442 *in* M. R. Perrow and A. J. Davy, editors. Handbook of ecological restoration: restoration in practice. Cambridge University Press, Cambridge, UK.

Jacquemyn, H., R. Brys, and M. Hermy. 2003. Short-term effects of different management regimes on the response of calcareous grassland vegetation to increased nitrogen. Biological Conservation **111**:137–147.

Janssens, F., A. Peeters, J. R. B. Tallowin, J. P. Bakker, R. M. Bekker, F. Fillat, and M. J. M. Oomes. 1998. Relationship between soil chemical factors and grassland diversity. Plant and Soil **202**:69–78.

Jones, T. A. 2013a. Ecologically appropriate plant materials for restoration applications. BioScience **63**:211–219.

Jones, T. A. 2013b. When local isn't best. Evolutionary Applications **6**:1109–1118.

Kahmen, S., and P. Poschlod. 2008. Effects of grassland management on plant functional trait composition. Agriculture, Ecosystems & Environment **128**:137–145.

Kawecki, T. J., and D. Ebert. 2004. Conceptual issues in local adaptation. Ecology Letters **7**:1225–1241.

Kettenring, K. M., K. L. Mercer, C. Reinhardt Adams, and J. Hines. 2014. Editor's choice: Application of genetic diversity – ecosystem function research to ecological restoration. Journal of Applied Ecology **51**:339–348.

Kiehl, K., A. Kirmer, T. W. Donath, L. Rasran, and N. Hölzel. 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.

Kiehl, K., and J. Pfadenhauer. 2007. Establishment and persistence of target species in newly created calcareous grasslands on former arable fields. Plant Ecology **189**:31–48.

Kiehl, K., A. Thormann, and J. Pfadenhauer. 2006. Evaluation of initial restoration measures during the restoration of calcareous grasslands on former arable fields. Restoration Ecology **14**:148–156.

Kirmer, A., A. Baasch, and S. Tischew. 2012. Sowing of low and high diversity seed mixtures in ecological restoration of surface mined-land. Applied Vegetation Science **15**:198–207.

Klaus, V. H. 2013. Urban grassland restoration: a neglected opportunity for biodiversity conservation. Restoration Ecology **21**:665–669.

Klopf, R., S. Baer, and D. Gibson. 2014. Convergent and contingent community responses to grass source and dominance during prairie restoration across a longitudinal gradient. Environmental Management **53**:252–265.

Klopf, R. P., and S. G. Baer. 2011. Root dynamics of cultivar and non-cultivar population sources of two dominant grasses during initial establishment of tallgrass prairie. Restoration Ecology **19**:112–117.

Knapp, E. E., and K. J. Rice. 1994. Starting from seed: Genetic issues in using native grasses for restoration. Ecological Restoration **12**:40–45.

Kollmann, J., and P. Poschlod. 1997. Population processes at the grassland–scrub interface. Phytocoenologia **27**:235–256.

Körner, C., J. Stöcklin, L. Reuther-Thiébaud, and S. Pelaez-Riedl. 2008. Small differences in arrival time influence composition and productivity of plant communities. New Phytologist **177**:698–705.

Krauss, J., A.-M. Klein, I. Steffan-Dewenter, and T. Tscharntke. 2004. Effects of habitat area, isolation, and landscape diversity on plant species richness of calcareous grasslands. Biodiversity & Conservation **13**:1427–1439.

Krauss, J., I. Steffan-Dewenter, and T. Tscharntke. 2003. How does landscape context contribute to effects of habitat fragmentation on diversity and population density of butterflies? Journal of Biogeography **30**:889–900.

Krautzer, B., G. Peratoner, and F. Bozzo. 2004. Site-specific grasses and herbs: seed production and use for restoration of mountain environments. Food and Agriculture Organisation of the United Nations, Rome, Italy. Available at: http://www.fao.org/docrep/007/y5576e/y5576e0f.htm#bm15 (access 12 December 2014)

Lambert, A. M., S. G. Baer, and D. J. Gibson. 2011. Intraspecific variation in ecophysiology of three dominant prairie grasses used in restoration: cultivar versus non-cultivar population sources. Restoration Ecology **19**:43–52.

Lande, R. 1998. Anthropogenic, ecological and genetic factors in extinction and conservation. Researches on Population Ecology **40**:259–269.

Leimu, R., and M. Fischer. 2008. A meta-analysis of local adaptation in plants. PLoS ONE **3**:e4010.

Lepŝ, J., J. Doleẑal, T. M. Bezemer, V. K. Brown, K. Hedlund, M. Igual Arroyo, H. B. Jörgensen, C. S. Lawson, S. R. Mortimer, A. Peix Geldart, C. Rodríguez Barrueco, I. Santa Regina, P. Ŝmilauer, and W. H. van der Putten. 2007. Long-term effectiveness of sowing high and low diversity seed mixtures to enhance plant community development on ex-arable fields. Applied Vegetation Science **10**:97–110.

Lesica, P., and F. W. Allendorf. 1999. Ecological genetics and the restoration of plant communities: mix or match? Restoration Ecology **7**:42–50.

Lindborg, R. 2006. Recreating grasslands in Swedish rural landscapes – effects of seed sowing and management history. Biodiversity & Conservation **15**:957–969.

Loucougaray, G., A. Bonis, and J.-B. Bouzillé. 2004. Effects of grazing by horses and/or cattle on the diversity of coastal grasslands in western France. Biological Conservation **116**:59–71.

Maalouf, J.-P., Y. Le Bagousse-Pinguet, L. Marchand, E. Bâchelier, B. Touzard, and R. Michalet. 2012. Integrating climate change into calcareous grassland management. Journal of Applied Ecology **49**:795–802.

Mariotte, P., C. Vandenberghe, C. Meugnier, P. Rossi, R. D. Bardgett, and A. Buttler. 2013. Subordinate plant species impact on soil microbial communities and ecosystem functioning in grasslands: Findings from a removal experiment. Perspectives in Plant Ecology, Evolution and Systematics **15**:77–85.

Maron, J. L., and S. N. Gardner. 2000. Consumer pressure, seed versus safe-site limitation, and plant population dynamics. Oecologia **124**:260–269.

Marriott, C. A., J. M. Fisher, K. J. Hood, and M. A. Smith. 1997. Persistence and colonization of gaps in sown swards of grass and clover under different sward managements. Grass and Forage Science **52**:156–166.

Marrs, R. H. 1993. Soil fertility and nature conservation in Europe: theoretical considerations and practical management solutions. Advances in Ecological Research **24**:241–300

Maskell, L. C., S. M. Smart, J. M. Bullock, K. E. N. Thompson, and C. J. Stevens. 2010. Nitrogen deposition causes widespread loss of species richness in British habitats. Global Change Biology **16**:671–679.

Matesanz, S., F. Valladares, D. Tena, M. Costa-Tenorio, and D. Bote. 2006. Early dynamics of plant communities on revegetated motorway slopes from southern Spain: is hydroseeding always needed? Restoration Ecology **14**:297–307.

McCain, K. N. S., S. G. Baer, J. M. Blair, and G. W. T. Wilson. 2010. Dominant grasses suppress local diversity in restored tallgrass prairie. Restoration Ecology **18**:40–49.

McGrath, S., T. R. Hodkinson, T. M. Charles, D. G. Zen, and S. Barth. 2010. Variation in inflorescence characters and inflorescence development in ecotypes and cultivars of *Lolium perenne* L. Grass and Forage Science **65**:398–409.

Merritt, D. J., and K. W. Dixon. 2011. Restoration seed banks – a matter of scale. Science **332**:424–425.

Miller, J. R., and R. J. Hobbs. 2007. Habitat restoration – do we know what we're doing? Restoration Ecology **15**:382–390.

Moser, B., and K. Thompson. 2014. Self-incompatibility will slow climate driven northward shift of two dominants of calcareous grasslands. Biological Conservation **169**:297–302.

Niklaus, P. A., D. Spinnler, and C. Körner. 1998. Soil moisture dynamics of calcareous grassland under elevated CO_2. Oecologia **117**:201–208.

Nosetto, M. D., E. G. Jobbágy, and J. M. Paruelo. 2005. Land-use change and water losses: the case of grassland afforestation across a soil textural gradient in central Argentina. Global Change Biology **11**:1101–1117.

Novák, J., and K. Prach. 2010. Artificial sowing of endangered dry grassland species into disused basalt quarries. Flora **205**:179–183.

O'Mara, F. P. 2012. The role of grasslands in food security and climate change. Annals of Botany **110**:1263–1270.

Oberdorfer, E. 2001. Pflanzensoziologische Exkursionsflora für Deutschland und angrenzende Gebiete. Eugen Ulmer, Stuttgart.

Oliveira, G., A. Nunes, A. Clemente, and O. Correia. 2012. Testing germination of species for hydroseeding degraded Mediterranean areas. Restoration Ecology **20**:623–630.

Öster, M., K. Ask, C. Römermann, O. Tackenberg, and O. Eriksson. 2009. Plant colonization of ex-arable fields from adjacent species-rich grasslands: The importance of dispersal vs. recruitment ability. Agriculture, Ecosystems & Environment **130**:93–99.

Overbeck, G. E., J.-M. Hermann, B. O. Andrade, I. I. Boldrini, K. Kiehl, A. Kirmer, C. Koch, J. Kollmann, S. T. Meyer, S. C. Müller, C. Nabinger, G. E. Pilger, J. P. P. Trindade, E. Vélez-Martin, E. A. Walker, D. G. Zimmermann, and V. D. Pillar. 2013. Restoration ecology in Brazil – time to step out of the forest. Brazilian Jounral of Nature Conservation **11**:92–95.

Packard, S., and C. F. Mutel. 1997. The tallgrass restoration handbook: for prairies, savannas, and woodlands. Island Press, Washington, DC, USA.

Padilla, F. M., L. Mommer, H. de Caluwe, A. E. Smit-Tiekstra, C. A. M. Wagemaker, N. J. Ouborg, and H. de Kroon. 2013. Early root overproduction not triggered by nutrients decisive for competitive success belowground. PLoS ONE **8**:e55805.

Peratoner, G., and G. Spatz. 2004. Organic seed propagation of *Festuca nigrescens* Lam. (*Festuca rubra* ssp. *commutata* Gaud.). Pflanzenbauwissenschaften **8**:40–46.

Pfadenhauer, J. 2001. Some remarks on the socio-cultural background of restoration ecology. Restoration Ecology **9**:220–229.

Pohl, M., D. Alig, C. Körner, and C. Rixen. 2009. Higher plant diversity enhances soil stability in disturbed alpine ecosystems. Plant and Soil **324**:91–102.

Poorter, H., J. Bühler, D. van Dusschoten, J. Climent, and J. A. Postma. 2012. Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. Functional Plant Biology **39**:839–850.

Poschlod, P., J. P. Bakker, and S. Kahmen. 2005. Changing land use and its impact on biodiversity. Basic and Applied Ecology **6**:93–98.

Poschlod, P., S. Kiefer, U. Tränkle, S. Fischer, and S. Bonn. 1998. Plant species richness in calcareous grasslands as affected by dispersability in space and time. Applied Vegetation Science **1**:75–91.

Poschlod, P., and M. F. WallisDeVries. 2002. The historical and socioeconomic perspective of calcareous grasslands – lessons from the distant and recent past. Biological Conservation **104**:361–376.

Prach, K., I. Jongepierová, and K. Řehounková. 2013. Large-scale restoration of dry grasslands on ex-arable land using a regional seed mixture: establishment of target species. Restoration Ecology **21**:33–39.

Probert, R. J., M. I. Daws, and F. R. Hay. 2009. Ecological correlates of *ex situ* seed longevity: a comparative study on 195 species. Annals of Botany **104**:57–69.

Pywell, R. F., J. M. Bullock, A. Hopkins, K. J. Walker, T. H. Sparks, M. J. W. Burke, and S. Peel. 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.

Pywell, R. F., J. M. Bullock, D. B. Roy, L. I. Z. Warman, K. J. Walker, and P. Rothery. 2003. Plant traits as predictors of performance in ecological restoration. Journal of Applied Ecology **40**:65–77.

Rapport, D. J., R. Costanza, and A. J. McMichael. 1998. Assessing ecosystem health. Trends in Ecology & Evolution **13**:397–402.

Reitalu, T., A. Helm, M. Pärtel, K. Bengtsson, P. Gerhold, E. Rosén, K. Takkis, S. Znamenskiy, and H. C. Prentice. 2014. Determinants of fine-scale plant diversity in dry calcareous grasslands within the Baltic Sea region. Agriculture, Ecosystems & Environment **182**:59–68.

Rice, K., and A. Dyer. 2001. Seed aging, delayed germination and reduced competitive ability in *Bromus tectorum*. Plant Ecology **155**:237–243.

Rice, K. J., and N. C. Emery. 2003. Managing microevolution: restoration in the face of global change. Frontiers in Ecology and the Environment **1**:469–478.

Riley, J. D., I. W. Craft, D. L. Rimmer, and R. S. Smith. 2004. Restoration of magnesian limestone grassland: optimizing the time for seed collection by vacuum harvesting. Restoration Ecology **12**:311–317.

Roach, D. A., and R. D. Wulff. 1987. Maternal effects in plants. Annual Review of Ecology and Systematics **18**:209–235.

Römermann, C., O. Tackenberg, A.-K. Jackel, and P. Poschlod. 2008. Eutrophication and fragmentation are related to species' rate of decline but not to species rarity: results from a functional approach. Biodiversity & Conservation **17**:591–604.

Ruiz-Jaen, M. C., and T. M. Aide. 2005. Restoration success: how is it being measured? Restoration Ecology **13**:569–577.

Saltonstall, K. 2002. Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. Proceedings of the National Academy of Sciences **99**:2445–2449.

Scharfy, D., A. Funk, H. Olde Venterink, and S. Güsewell. 2011. Invasive forbs differ functionally from native graminoids, but are similar to native forbs. New Phytologist **189**:818–828.

Schröder, R., and R. Prasse. 2013a. Cultivation and hybridization alter the germination behavior of native plants used in revegetation and restoration. Restoration Ecology **21**:793–800.

Schröder, R., and R. Prasse. 2013b. Do cultivated varieties of native plants have the ability to outperform their wild relatives? PLoS ONE **8**:e71066.

Schröder, R., and R. Prasse. 2013c. From nursery into nature: a study on performance of cultivated varieties of native plants used in re-vegetation, their wild relatives and evolving wild x cultivar hybrids. Ecological Engineering **60**:428–437.

Scotton, M., A. Kirmer, and B. Krautzer. 2011. Practical handbook for seed harvest and ecological restoration of species-rich grasslands. CLEUP, Padova, Italy.

SER. 2004. SER International primer on ecological restoration. Society for Ecological Restoration. Available at: http://www.ser.org/resources/resources-detail-view/ser-international-primer-on-ecological-restoration (access 15 May 2014)

Shackelford, N., R. J. Hobbs, J. M. Burgar, T. E. Erickson, J. B. Fontaine, E. Laliberté, C. E. Ramalho, M. P. Perring, and R. J. Standish. 2013. Primed for change: developing ecological restoration for the 21st century. Restoration Ecology **21**:297–304.

Smith, M. D., and A. K. Knapp. 2003. Dominant species maintain ecosystem function with non-random species loss. Ecology Letters **6**:509–517.

Spehn, E. M., A. Hector, J. Joshi, M. Scherer-Lorenzen, B. Schmid, E. Bazeley-White, C. Beierkuhnlein, M. C. Caldeira, M. Diemer, P. G. Dimitrakopoulos, J. A. Finn, H. Freitas, P. S. Giller, J. Good, R. Harris, P. Högberg, K. Huss-Danell, A. Jumpponen, J. Koricheva, P. W. Leadley, M. Loreau, A. Minns, C. P. H. Mulder, G. O'Donovan, S. J. Otway, C. Palmborg, J. S. Pereira, A. B. Pfisterer, A. Prinz, D. J. Read, E. D. Schulze, A. S. D. Siamantziouras, A. C. Terry, A. Y. Troumbis, F. I. Woodward, S. Yachi, and J. H. Lawton. 2005. Ecosystem effects of biodiversity manipulations in European grasslands. Ecological Monographs **75**:37–63.

Steenwerth, K. L., L. E. Jackson, F. J. Calderón, M. R. Stromberg, and K. M. Scow. 2002. Soil microbial community composition and land use history in cultivated and grassland ecosystems of coastal California. Soil Biology and Biochemistry **34**:1599–1611.

Stöcklin, J., and M. Fischer. 1999. Plants with longer-lived seeds have lower local extinction rates in grassland remnants 1950–1985. Oecologia **120**:539–543.

Stratton, D. A. 1992. Life-cycle components of selection in *Erigeron annuus*: I. phenotypic selection. Evolution **46**:92–106.

Temperton, V. M., L. L. A. Märtin, D. Röder, A. Lücke, and K. Kiehl. 2012. Effects of four different restoration treatments on the natural abundance of ^{15}N stable isotopes in plants. Frontiers in Plant Science **3**:1–12.

Thomson, F. J., A. T. Moles, T. D. Auld, and R. T. Kingsford. 2011. Seed dispersal distance is more strongly correlated with plant height than with seed mass. Journal of Ecology **99**:1299–1307.

Tilman, D., and S. Polasky. 2005. Ecosystem goods and services and their limits. Page 78 *in* R. D. Simpson, M. A. Toman, and R. U. Ayres, editors. Scarcity and growth revisited: natural resources and the environment in the new millenium. Resources for the Future, Washington, DC, USA.

Török, P., B. Deák, E. Vida, O. Valkó, S. Lengyel, and B. Tóthmérész. 2010. Restoring grassland biodiversity: sowing low-diversity seed mixtures can lead to rapid favourable changes. Biological Conservation **143**:806–812.

Török, P., T. Miglécz, O. Valkó, A. Kelemen, B. Deák, S. Lengyel, and B. Tóthmérész. 2012. Recovery of native grass biodiversity by sowing on former croplands: is weed suppression a feasible goal for grassland restoration? Journal for Nature Conservation **20**:41–48.

Török, P., E. Vida, B. Deák, S. Lengyel, and B. Tóthmérész. 2011. Grassland restoration on former croplands in Europe: an assessment of applicability of techniques and costs. Biodiversity & Conservation **20**:2311–2332.

Tscharntke, T., A. M. Klein, A. Kruess, I. Steffan-Dewenter, and C. Thies. 2005. Landscape perspectives on agricultural intensification and biodiversity – ecosystem service management. Ecology Letters **8**:857–874.

Turnbull, L. A., M. J. Crawley, and M. Rees. 2000. Are plant populations seed-limited? A review of seed sowing experiments. Oikos **88**:225–238.

van der Valk, A., T. Bremholm, and E. Gordon. 1999. The restoration of sedge meadows: seed viability, seed germination requirements, and seedling growth of *Carex* species. Wetlands **19**:756–764.

van Swaay, C. A. M. 2002. The importance of calcareous grasslands for butterflies in Europe. Biological Conservation **104**:315–318.

Vander Mijnsbrugge, K., A. Bischoff, and B. Smith. 2010. A question of origin: where and how to collect seed for ecological restoration. Basic and Applied Ecology **11**:300–311.

Vázquez De Aldana, B. R., and F. Berendse. 1997. Nitrogen-use efficiency in six perennial grasses from contrasting habitats. Functional Ecology **11**:619–626.

VWW. 2011. Rules and regulation for the certification of 'VWW-Regionsaaten®'. Verband deutscher Wildsamen- und Wildpflanzenproduzenten e.V. Available at: http://www.natur-im-vww.de/en/download/vww-rules-and-regulations.pdf (access 20 June 2014)

Walker, K. J., P. A. Stevens, D. P. Stevens, J. O. Mountford, S. J. Manchester, and R. F. Pywell. 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.

Walker, E. A., H. Meimberg, and J. Kollmann. (in press). Mixed evidence for the cultivar vigour hypothesis: the case of calcareous grassland forbs in a matrix of *Festuca rubra*. Ecological Engineering

WallisDeVries, M. F., P. Poschlod, and J. H. Willems. 2002. Challenges for the conservation of calcareous grasslands in Northwestern Europe: integrating the requirements of flora and fauna. Biological Conservation **104**:265–273.

Wesche, K., B. Krause, H. Culmsee, and C. Leuschner. 2012. Fifty years of change in Central European grassland vegetation: large losses in species richness and animal-pollinated plants. Biological Conservation **150**:76–85.

Wheeler, B. 2010. lmPerm: Permutation tests for linear models. R package version 1.1–2

White, R. P., S. Murray, and M. Rohweder. 2000. Pilot analysis of global ecosystems: grassland ecosystems. World Resources Institute, Washington, DC, USA. Available at: http://pdf.usaid.gov/pdf_docs/Pnacs567.pdf (access 9 June 2014)

Willems, J. H., and M. G. L. van Nieuwstadt. 1996. Long-term after effects of fertilization on above-ground phytomass and species diversity in calcareous grassland. Journal of Vegetation Science **7**:177–184.

Williams, N. S. G., J. W. Morgan, M. J. McDonnell, and M. A. McCarthy. 2005. Plant traits and local extinctions in natural grasslands along an urban–rural gradient. Journal of Ecology **93**:1203–1213.

Wilsey, B. J. 2010. Productivity and subordinate species response to dominant grass species and seed source during restoration. Restoration Ecology **18**:628–637.

Young, S. L., J. N. Barney, G. B. Kyser, T. S. Jones, and J. M. DiTomaso. 2009. Functionally similar species confer greater resistance to invasion: implications for grassland restoration. Restoration Ecology **17**:884–892.

Young, T. P., J. M. Chase, and R. T. Huddleston. 2001. Community succession and assembly: comparing, contrasting and combining paradigms in the context of ecological restoration. Ecological Restoration **19**:5–18.

Zeiter, M., A. Stampfli, and D. M. Newbery. 2006. Recruitment limitation constrains local species richness and productivity in dry grassland. Ecology **87**:942–951.

Affidavit

I hereby declare that the dissertation titled:

It's a matter of source: the competitive effects of dominant species on calcareous grassland forbs

prepared under the guidance and supervision of

Prof. Dr. Johannes Kollmann and Prof. Dr. Harald Meimberg

at the Chair of Restoration Ecology and submitted to the degree-awarding institution of: Center of Life and Food Sciences Weihenstephan of TUM is my own, original work undertaken in partial fulfilment of the requirements for the doctoral degree. I have made no use of sources, material or assistance other than those specified in § 6 (6) and (7), clause 2.

(X) I have not employed the services of an organisation that provides dissertation supervisors in return for payment or that fulfils, in whole or in part, the obligations incumbent on me in connection with my dissertation.

(X) I have not submitted the dissertation, either in the present or a similar form, as part of another examination process

( ) The complete dissertation was published in ______________________________

The degree-awarding institution
Center of Life and Food Sciences Weihenstephan
has approved prior publication of the dissertation.

(X) I have not yet been awarded the desired doctoral degree nor have I failed the last possible attempt to obtain the desired degree in a previous doctoral programme.

(-) I have already applied for admission to a doctoral programme at the school or college of
At ____
By submitting a dissertation on the topic____________________________
With result:____

I am familiar with the publicly available Regulations for the Award of Doctoral Degrees of TUM, in particular §28 (Invalidation of doctoral degree) and § 29 (Revocation of doctoral degree). I am aware of the consequences of filing a false affidavit.

(X) I agree, (-) I do not agree

that my personal data is stored in the TUM alumni database.

Munich, ____

Curriculum Vitae

Name	**Emer Ann Walker**
Date of birth	12th August 1986
Address	Angerstr. 12a, 85354 Freising
E-mail	e.walker@tum.de

Education

Oct. 2011 – Nov. 2014	Doctoral studies (Dr. rer. nat.) at the Chair of Restoration Ecology, Technische Universität München (TUM)
Oct. 2009 – Sept. 2011	Research Master in Applied Ecology (M. Sc.), Environmental Research Institute, University College Cork, Ireland
2005 – 2009	Bachelor degree in Environmental Science (B. Sc.), University College Cork, Ireland
1999 – 2005	Christ King Girls Secondary School, Turner's Cross, Cork, Ireland

Research stays abroad

Nov. 2012 – Dec. 2012	Research stay at the Federal University of Rio Grande do Sul, Porto Alegre, Brazil
Jun. 2009 – Sept. 2009	ERASMUS Work Placement at the University of Copenhagen, Denmark

Scholarships and funding

Aug. – Dec. 2014	Deutscher Akademischer Austauschdienst (DAAD) Ph.D. completion support grant
Oct. 2011 – Jul. 2014	DAAD research grant for doctoral candidates
Nov. – Dec. 2012	TUMBRA scholarship from the DAAD for travel costs and stipend in Brazil
Apr. 2012	Leonhard-Lorenz-Stiftung from the Chair of Construction Chemistry, TUM, for research consumables
Jun. – Sept. 2009	ERASMUS Work Placement funding for research stay at University of Copenhagen

Publications

Internationally reviewed publications

Walker, E.A., Conradi, T., Meimberg, H. & Kollmann, J. (in press) Seed selection for grassland restoration: Competitive effect of a dominant grass is mediated by seed source and nutrient availability. *Restoration Ecology*

Walker, E.A., Hermann, J.-M. & Kollmann, J. (in press) Grassland restoration by seeding: Seed source and growth form matter more than density. *Applied Vegetation Science*

Walker, E.A., Meimberg, H. & Kollmann, J. (2014) Mixed evidence for the cultivar vigour hypothesis: the case of calcareous grassland forbs in a matrix of *Festuca rubra. Ecological Engineering* **71**:301–307

Overbeck G.E., Hermann J.-M., Andrade B.O., Boldrini I.I., Kiehl K., Kirmer A., Koch C., Kollmann J., Meyer S.T., Müller S.C., Nabinger C., Pilger G.E., Trindade J.P.P., Vélez-Martin E., **Walker, E.A.**, Zimmermann D.G. & Pillar V.D. (2013) Restoration ecology in Brazil - Time to step out of the forest. *Brazilian Journal of Nature Conservation* **11**:92–95

Wille, W., Thiele, J., **Walker, E.A.** & Kollmann, J. (2013) Limited evidence for allelopathic effects of giant hogweed on germination of native herbs. *Seed Science Research* **23**:157–16

Conference proceedings - oral and poster presentations

Hermann, J.-M., Koch, C., **Walker, E.A**. & Kollmann, J. (2013) Sowing seeds of doubt: Use of native and non-native cultivars and implications for grassland restoration. *5th World Conference on Ecological Restoration*, Madison, WI, USA, Oct. 6 – 11, 2013 (Oral presentation)

Walker, E.A., Meimberg, H., Hermann, J.-M. & Kollmann, J. (2012) Provenance effects on plant establishment and competition during grassland restoration. *European Conference on Ecological Restoration*, České Budějovice, Czech Republic, Sept. 9 – 14, 2012 (Oral presentation)

Walker, E.A., Conradi, T., Meimberg, H.& Kollmann, J. (2014) Nature and nurture: how site productivity and seed source of a dominant species affect a subordinate grassland species. *9th European Conference on Ecological Restoration,* Oulu, Finland, Aug. 3 – 8, 2014 (Poster presentation)

Walker, E.A., Meimberg, H., Hermann, J.-M. & Kollmann, J. (2013) Mixed evidence for the *Cultivar Vigour Hypothesis* – The case of grassland forbs in a matrix of *Festuca rubra*. *Ecological Society of Germany, Austria and Switzerland Annual Meeting*, Potsdam, Germany, Sept. 9 – 13, 2013 (Poster presentation)

Walker, E.A., Emmerson, M. & Whelan, P.M. (2010) The importance of ecoregions in the determination of susceptibility to plant invasions – an example from Ireland. *NEOBIOTA Conference*, Copenhagen, Denmark, Sept. 14 – 17, 2010 (Poster presentation)

Wille, W., **Walker, E.A.**, Kollmann, J. (2010) Furanocoumarins of *Heracleum mantegazzianum* – a 'novel weapon'? *NEOBIOTA Conference*, Copenhagen, Denmark, Sept. 14 – 17, 2010 (Poster presentation)