Biological invasions and restoration: trait-based design of seed mixtures, biotic resistance and competition with native species

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# Contents

Summary 1

Zusammenfassung 4

Resumen 8

General introduction 13

Chapter 1 33

Bioengineering effectiveness of seed mixtures for road verges: functional composition as a predictor of grassland diversity and invasion resistance

Chapter 2 63

Can plant invasions be prevented by limiting similarity? The role of seed size, seed density and resource availability during roadside revegetation

Chapter 3 91

Limiting similarity and Darwin’s naturalization hypothesis: understanding the drivers of biotic resistance against invasive plant species

General discussion 119

General conclusions 131

Publications and author contributions 133

Acknowledgements 135

References 137
Summary

Biological invasions are among the most important drivers of biodiversity loss, contributing to ecosystem degradation and complicating efforts to restore degraded ecosystems. A key question is whether or not we can prevent the establishment of invasive alien species (IAS) by designing resistant plant communities. In invasion ecology, most of the proposing hypotheses addressing biotic resistance of plant communities have focused on competitive interactions due to niche overlap between native and invasive species. In this dissertation, a theoretical approach is used for designing communities resistant to potential plant invaders. Thus, experiments were developed to test the diversity-invasibility, limiting similarity and Darwin’s naturalization hypotheses. The species selected for experimentation were native grassland plants used for restoration in central Europe and invasive plant species currently causing problems in that region. The overall aim of the dissertation was to investigate the invasion resistance of grassland communities created by engineered seed mixtures and intended for grassland restoration.

An alternative to restoration of historical grassland is the establishment of similar communities in new habitats like roadsides. However, these areas are notorious for IAS invasions due to irregular disturbance and high propagule pressure by traffic. Therefore, in Chapter 1 the bioengineering effectiveness of seed mixtures intended for the restoration of grasslands along roadsides was assessed. Here the communities have to cope with factors related to trafficability, while at the same time they should have a positive effect on both native biodiversity and IAS suppression. For doing so, a greenhouse experiment was done to study the performance of four regional and two non-regional seed mixtures on roadside substrate, with and without stony microhabitats, as a function of their seed source, functional group and trait composition. The alien Impatiens glandulifera Royle was used to test invasion resistance after establishment of these communities by adding seeds and later counting seedling emergence. In this experiment seed source and percentage legume seed significantly
affected vegetation cover, whereas stony microsites had negative effects on cover. Percentage grass biomass reduced species richness as well as invasion resistance. Functional diversity had a positive effect on suppressing the establishment of *I. glandulifera*, thus supporting the *diversity-invasibility hypothesis*. The chapter showed that using regional seeds is recommended for roadside restoration, yet only a careful balance of functional groups and plant traits within seed mixtures can ensure quick establishment, high species richness and invasion resistance.

Using again roadsides as model systems, **Chapter 2** investigates limiting similarity of seed size and seed-size-output strategies as a tool to design native communities resistant to IAS at an early stage of roadside revegetation. The *limiting similarity hypothesis* predicts that IAS are less likely to establish in communities comprising species with similar functional traits. Therefore, in a greenhouse experiment, communities with small- and large-seed-size-output strategies at two densities were confronted with one small- and one large-seeded IAS. Another experiment investigated the seed-size effect, by using small- and large-seed-size communities at constant density in competition with three small- and one large-seeded IAS at two nutrient levels. In terms of limiting similarity of seed-size-output strategy, small-seeded native communities at high density were highly effective in suppressing both seed-sized IAS, explained by a density effect. From the perspective of seed size alone, small-seeded IAS had lower performance when competing with by large-seeded communities and no differences were found for large-seeded IAS nor for nutrient addition. Hence, the limiting similarity hypothesis was only partially supported by the first experiment, while results were better explained by a density- and biomass-driven suppression effect. This indicates that testing limiting similarity in these terms is more complex than previously described.

Biotic resistance was then tested by confronting two hypotheses, that is the *limiting similarity* and *Darwin’s naturalization hypothesis*. The latter predicts that IAS closely related to the native community species would be less successful. **Chapter 3** combines both perspectives using the invasive alien plants *Ambrosia artemisiifolia* L. and *Solidago gigantea* Aiton and various grassland species. All plant species were classified into
groups based on eight functional traits, and the phylogenetic distance among the species was then calculated. In one greenhouse experiment, the two IAS at two propagule pressures were submitted to competition with communities comprising ten native species from the same functional group. While in a second experiment with pairwise competition the IAS were confronted to competing with native species selected from each functional group. Interestingly, responses of both IAS followed the same patterns in all experiments. The highest IAS suppression was observed at low propagule pressure in communities with a high proportion of grasses and fast-growing species, thus, not related to functional similarity according to the selected traits. Furthermore, suppression decreased with increasing phylogenetic distance to species of the native community until reaching an asymptote. In the pairwise competition experiment the suppression effect was better explained by phylogenetic distance than by trait similarity for both species. Overall, phylogenetic relatedness was a more suitable predictor of IAS suppression than functional group identity, thus, supporting Darwin’s naturalization hypothesis.

Overall, the findings of the dissertation demonstrate that a strong theoretical framework is a promising approach for designing communities aimed for restoration of areas prone to invasions. Furthermore, results indicate that is possible to address factors related to invasion resistance while at the same time deal with practical matters pertinent to for instance roadside revegetation. The dissertation strongly improves current knowledge on the effects of species composition of plant communities on biotic resistance. This allows developing new approaches for predicting, preventing and managing invasive species in areas subjected to restoration.
Zusammenfassung


Limiting Similarity der Strategie zur Größe der produzierten Samen unterdrückten die kleinsamigen heimischen Artengemeinschaften bei hoher Aussaatstärke die invasiven Fremdarten unabhängig von der Samengröße besonders effektiv, was durch einen Dichteeffekt erklärt werden kann. Wird nur die Samengröße betrachtet, zeigten die kleinsamigen invasiven Fremdarten in Konkurrenz mit großsamigen Artengemeinschaften geringere Biomasseentwicklung, während es keine Unterschiede für großsamige invasive Fremdarten oder Nährstoffzugabe gab. Somit haben die Ergebnisse des ersten Experiments die Limiting-Similarity-Hypothese nur zum Teil bestätigt. Sie wurden dagegen besser durch die dichte- und biomasseabhängige Verdrängung der Fremdarten erklärt. Dies deutet darauf hin, dass es deutlich komplexer ist, Limiting Similarity zu prüfen als zuvor beschrieben.

heimischen Arten bis zur Erreichung einer Asymptote ab. In dem paarweisen Konkurrenzversuch wurde der Verdrängungseffekt besser durch die phylogenetische Distanz erklärt als durch die Merkmalsähnlichkeit für beide Arten. Insgesamt war die phylogenetische Distanz besser geeignet, um die Verdrängung invasiver Fremdarten vorherzusagen als die Zugehörigkeit zu einer funktionellen Gruppe und bestätigt damit Darwin’s Naturalization-Hypothese.

Resumen

Las invasiones biológicas se encuentran entre los principales factores asociados a la pérdida de biodiversidad, contribuyendo a la degradación de ecosistemas y complicando esfuerzos para restaurar tales ecosistemas. Si existe la posibilidad de prevenir el establecimiento de especies invasoras (IAS por sus siglas en inglés) a través del diseño de comunidades resistentes, constituye una pregunta clave en este contexto. En la disciplina de la Ecología de invasiones, la mayoría de las hipótesis propuestas para abordar la temática de la potencial resistencia biótica de comunidades vegetales, se ha centrado en la superposición de nichos de especies nativas e invasoras a través de competencia por recursos. En esta disertación, el diseño de comunidades resistentes a potenciales invasores se basa en un enfoque teórico. De este modo, los experimentos presentados en esta tesis fueron desarrollados para poner a prueba las hipótesis de diversidad-invasibilidad (diversity-invasibility), de la similitud límite (limiting similarity) y de la Naturalización de Darwin. Se seleccionaron para experimentación especies nativas de pastizales normalmente usados para restauración ecológica en Europa central y especies invasoras conocidas por causar problemas en la misma área. El fin de la presente disertación es la investigación de los factores impulsando la resistencia biótica de comunidades de pastizales, especialmente diseñados por medio de mezclas de semillas y cuyo último objetivo es la restauración de pastizales.

El establecimiento de comunidades similares a pastizales históricos en nuevos hábitats como son los bordes de caminos, constituye una alternativa a la restauración de pastizales degradados. Sin embargo tales áreas se encuentran frecuentemente afectadas por IAS debido a la presencia de disturbios regulares y a la alta presión de propágulo relacionada con el tránsito automotriz. Por esto, la efectividad en términos de bioingeniería de ciertas mezclas de semillas utilizadas para la restauración ecológica en bordes de camino, fueron evaluadas en el Capítulo 1. En estas áreas, las comunidades deben cumplir con objetivos importantes para el tránsito automotriz, tener un efecto positivo en la diversidad y al mismo tiempo ser capaces de suprimir invasiones potenciales. Para lograr este objetivo, se llevó a cabo un experimento de invernadero
para estudiar el rendimiento de cuatro mezclas de semillas compuestas de material regional y dos de cultivares, utilizando sustrato del borde de un camino, con y sin microhábitats de substrato pedregoso, basado en el origen de las semilla, la composición de grupos y caracteres funcionales. La especie invasora *Impatiens glandulifera* Royle fue usada para probar la resistencia biótica de las comunidades post establecimiento. Esto se logró a través del sembrado de semillas posterior al establecimiento de la vegetación y luego al recuento de plántulas. En este experimento, el establecimiento de la vegetación fue significativamente afectado por factores tales como el área donde fueron producidos las semillas y el porcentaje de legumbres en la mezcla. Mientras los microhábitats de substrato pedregoso tuvieron un efecto negativo en la cobertura vegetal. Por otro lado, el porcentaje de biomasa de gramíneas redujo substancialmente la riqueza de especies y la resistencia biótica de las comunidades. La diversidad funcional tuvo un efecto positivo en la inhibición del establecimiento de *I. Glandulifera*, en consecuencia apoyando la hipótesis de diversidad-invasibilidad. Basado en los resultados de este capítulo, en el contexto de los bordes de camino se recomienda el uso de semillas producidas regionalmente para la restauración ecológica. Mientras se muestra su vez que solo un cuidadoso balance de factores tales como los grupos y caracteres funcionales incluidos en las mezclas de semillas, puede asegurar un rápido establecimiento de la vegetación, una alta riqueza de especies y una óptima resistencia biótica.

Utilizando nuevamente bordes de camino como sistema modelo, en el Capítulo 2 se investiga la similitud límite en términos del tamaño y la estrategia de producción relacionada al tamaño de semillas, como una herramienta para el diseño de comunidades resistentes a invasoras en una etapa temprana del establecimiento. La hipótesis de similitud límite predice que las especies invasoras serán menos propensas a establecerse en comunidades que posean caracteres funcionales similares a estas. En consecuencia, en un experimento de invernadero, comunidades compuestas por especies con estrategias de producción de semillas características de semillas pequeñas y grandes fueron sometidas a competencia con IAS de las mismas características a dos densidades de sembrado de especies nativas. En otro experimento, se investigó el
efecto de comunidades creadas solo con semillas pequeñas o grandes a una densidad constante en competencia con IAS de semillas pequeñas o grandes, utilizando dos niveles de agregado de nutrientes. En términos de la similitud límite en estrategia de producción de semillas, las comunidades con estrategia de semillas pequeñas a mayor densidad fueron altamente efectivas para suprimir los dos tamaños de semillas de IAS, como consecuencia de un efecto de la gran densidad de especies nativas. Desde la perspectiva solo del tamaño de semilla, las IAS con tamaño pequeño de semillas tuvieron un menor desempeño en competencia con las comunidades nativas compuestas por especies de semillas grandes, mientras que no se observó ninguna diferencia para las IAS de semillas grandes. Tampoco se encontró un efecto debido al agregado de nutrientes al substrato. Por lo tanto, la hipótesis de similitud límite fue solo parcialmente respaldada por los resultados del primer experimento, mientras que los resultados parecen ser mejor explicados por el efecto inhibitorio de la densidad y biomasa de la comunidad nativa. Esto demuestra la dificultad de probar esta hipótesis en estos términos.

La resistencia biótica fue entonces examinada por medio de la confrontación de dos hipótesis al mismo tiempo, esto es la hipótesis de similitud límite y de la Naturalización de Darwin. Esta segunda predice que las especies invasoras que estén estrechamente relacionadas con las especies nativas de la comunidad receptora, tendrán menos éxito en establecerse. El Capítulo 3 combina entonces las dos perspectivas usando dos especies invasoras (Ambrosia artemisiifolia L. y Solidago gigantea Aiton) junto con especies nativas de pastizal. Para esto, todas las especies fueron clasificadas en grupos basado en ocho caracteres funcionales y la distancia filogenética entre las especies fue también calculado. En un primer experimento de invernadero, las dos IAS a dos presiones de propágulo fueron sometidas a competencia con comunidades compuestas por diez especies nativas pertenecientes al mismo grupo funcional. En un segundo experimento con competencia de a pares de especies, las IAS fueron nuevamente confrontadas a competir con especies nativas seleccionadas de cada grupo funcional. Es interesante observar que en ambos experimentos las respuestas de las dos IAS siguieron patrones similares. La mayor inhibición de IAS fue observada a baja presión de
propágulo, en comunidades compuestas de una alta proporción de gramíneas y especies de rápido desarrollo. Consecuentemente, la inhibición no estuvo relacionada a similitud en términos de los caracteres que fueron seleccionados. Además, la supresión de IAS disminuyó cuando la distancia filogenética a las especies nativas de la comunidad aumentó hasta llegar a una asintota. En el experimento de competición de a pares el efecto inhibidor sobre las dos IAS fue mejor explicado por la distancia filogenética en vez de la similitud en los caracteres seleccionados. En conclusión, la relación filogenética demostró ser un mejor predictor de la inhibición de IAS comparado con la identidad en términos del grupo funcional, consecuentemente, afirmando la idea propuesta por la hipótesis de Naturalización de Darwin.

Las conclusiones de la presente tesis demuestran que basar el diseño de comunidades para la restauración ecológica de ecosistemas propensos a las invasiones, en un fuerte marco teórico es un enfoque prometedor. Además, los resultados indican que los factores conocidos por tener un efecto en IAS pueden ser probados en experimentos de invernadero, mientras que al mismo se puede hacer frente a cuestiones prácticas pertinentes para la revegetación en bordes de caminos. A su vez, esta tesis en gran parte aumenta el conocimiento actual sobre los efectos de la composición las comunidades vegetales en la resistencia biótica, lo cual permite el desarrollo de nuevos enfoques para la predicción, prevención y manejo de las especies invasoras en áreas sujetas a la restauración.
General introduction

“We must make no mistake: we are seeing one of the great historical convulsions in the world’s fauna and flora. We might say, with Professor Challenger, standing on Conan Doyle’s ‘Lost World’, with his black beard jutting out: ‘We have been privileged to be present at one of the typical decisive battles of history—the battles which have determined the fate of the world’. But how will it be decisive? Will it be a Lost World? These are questions that ecologists ought to try to answer.”

Elton (1958, pp 31-32)

1. Biological invasions – the motivation for the dissertation

Biological invasions along with habitat change, pollution, overexploitation of natural resources and climate change have been shown to be the most important drivers of biodiversity loss (Millennium Ecosystem Assessment 2005, CBD 2010). Invasive alien species (IAS) alter the biodiversity and ecosystem functions of many native communities (Levine et al. 2003, Gaertner et al. 2009, Hejda et al. 2009), including many abiotic characteristics (Eppinga et al. 2006, Gioria and Osborne 2010), thus, significantly changing the dynamics of the invaded ecosystem (Simberloff 2011, Vilà et al. 2011). The increased rates by which species are introduced both intentionally and unintentionally due to trade, travel and transport are expected to enhance these negative effects (Elton 1958, Lockwood et al. 2013, Simberloff et al. 2013).

Early references to invasions and their impacts date back to Darwin’s The Origin of Species (Darwin 1859). Nevertheless, it was Elton’s influential book Ecology of Invasions by Animals and Plants that increased the awareness of the effects which IAS may have
General Introduction

not only on ecosystems but as well on human health and economy (Elton 1958). This lead to an increased interest in understanding not only the impacts of IAS but also the underlying factors, explaining, for example, why certain plant communities are prone to invasions while others stay rather resistant to them (Bakker and Wilson 2004, Middleton et al. 2010). This last example has significant implications for restoration given that IAS can hinder efforts to restore degraded areas and is what motivates this dissertation. Despite the large body of research in invasion ecology, the study of IAS in the context of restoration is still an emerging scientific area of science, representing exciting opportunities for research.

2. Restoration ecology in the context of biological invasions

The increase of ecosystem degradation and land-use changes especially by transformation of natural ecosystems to arable land, forest plantations or urban development highlights the need for widespread restoration of historical or new ecosystems. Restoration ecology is defined by the Society for Ecological Restoration as the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed (SER 2004). The successful application of restoration measures requires the understanding of a range of ecological principles and thus, it involves several disciplines (Falk et al. 2006). For instance, given that a major aim of community ecology is to understand how species co-exist, ecological restoration and community ecology are integrally linked, and a synthesis of these fields promotes their mutual advancement (Temperton et al. 2004). The potential of restoration ecology has been clearly recognized to relate to fundamental topics in community ecology, such as succession (Baasch et al. 2012), disturbance (MacDougall and Turkington 2007), community assembly (Young et al. 2001), alternative states (Suding et al. 2004), and invasions (Funk et al. 2008, Hooper and Dukes 2010, Cleland et al. 2012).

The traditional approach of restoration has focused on the return of a degraded system to a state prior to the disturbance or damage (van Andel and Aronson 2012). Yet, in an
era of rapid change within the anthropocene, there are many uncertainties on how to restore dynamic communities best (Hobbs and Cramer 2008). Ultimately, the goal of restoration should be to achieve sustainable ecosystems with a certain species composition, desired state that is able to provide particular ecosystem services (Falk et al. 2006). The threat that IAS are known to pose to the long-term sustainability of ecosystems and associated services, makes necessary to take a preventive management approach against non-native species (Simberloff et al. 2013). If arrival cannot be prevented, inhibiting the establishment of IAS should be a priority of restoration, i.e. to reduce the ability of a certain invasive species population to survive and reproduce in a specific location (Lockwood et al. 2013). This is applicable in the context of restoration given that inhibiting the successful reproduction of IAS by vegetation management is essential to the success of a restoration project (D’Antonio and Meyerson 2002). IAS have been targets of control measures in agriculture and forestry for a long time, while in (near)natural systems, such as the ones targeted for restoration, they have been identified as a challenge only in the past decades (D’Antonio and Chambers 2006).

A common task for scientists and practitioners during ecological restoration is to reassemble communities on bare soil following the removal of degraded substrate or unwanted vegetation. Dispersal limitation and missing seed banks are frequently observed in such cases (Stampfli and Zeiter 1999, Hedberg and Kotowski 2010). This makes the introduction of propagules of target plant species necessary, e.g. by spreading hay or specific seed mixtures to accelerate and manipulate vegetation change (Young et al. 2005, Kiehl and Pfadenhauer 2007). This is why careful selection of adequate species is a vital step in restoration planning (Tormo et al. 2007, Bochet et al. 2010). Unfortunately, in many restoration projects commercial non-local seed material is used in seed mixtures due to their availability, higher establishment rate and lower seed costs (Rentch et al. 2005, Tinsley et al. 2006, García-Palacios et al. 2010). However, this may increase the frequency of future invasions, since at least some non-native species will probably spread from their initial location and invade surrounding areas. To avoid this, the use of native species in seed mixtures for restoration is highly recommended, also because of local adaptation advantages and often lower resource requirements.
(Petersen et al. 2004), support of associated native biodiversity (O'Dwyer and Attiwill 2000, Pfitsch and Williams 2009), landscape aesthetics (Albaladejo Montoro et al. 2000), and regional identity (Tinsley et al. 2006).

Some controversy exists on whether it is enough to use native material indistinctively of its specific source area, or if seeds should be collected and produced in the area where they will be used. Regional seed material has been shown to create more diverse communities with higher cover (Gyssels et al. 2005), which could lead to successful suppression of IAS (Walker et al. 2015). Such effects of the specific seed source on the restored plant community make the careful identification of suitable seed material including the consideration of its specific origin another key issue for restoration planning. Similar classifications are needed for alien species; given that not all alien species become invasives and thus have different degrees of impact that need to be addressed in specific ways during restoration planning (Box 1 shows the definitions for native and non-native species used in this dissertation).
Box 1 – The origin of species in the context of restoration

The concept of ‘alienness’ denotes the idea of an organism not belonging to a certain place. In ecology this is mostly used as counterpart to ‘nativeness’ and thus, denotes species that did not evolve in a certain area (Head and Muir 2004). Moreover, species can be genetically alien to a certain area but still be closely related and may thus pose a threat to the genetic integrity of native populations which is also an important issue to be considered during restoration (Keller et al. 2000). In the context of invasion and restoration ecology, there is a need to classify alien species according to their potential to become a problem for restoration due to negative impacts. This assessment and definition of IAS is highly influenced by the context of the study and thus, can be related to different perspectives (Heger et al. 2013). In this dissertation I have used an ‘ecological perspective’ sensu Heger et al. (2013).

**Native regional species:** Native plant species from local provenances commercially produced and used in the area of origin (Vander Mijnsbrugge et al. 2010).

**Native cultivar species:** Cultivated native species used in its area of origin which has been selected for advantageous traits that confer greater competitive ability (Conrad and Tischew 2011).

**Alien plant species:** Plant species that occurs in a given area outside its region of origin because of intentional or accidental introduction as a result of human activities (Richardson et al. 2000b).

**Invasive plant species:** Alien plant species that sustains naturalized populations over many generations without human intervention, and that can produce reproductive offspring in very large numbers providing the species with the potential to spread over large areas (Richardson et al. 2000b).
Species selection for the design of plant communities intended for ecological restoration is influenced by the goals of the project. If the main focus is to design communities resistant to invasions, a promising approach is the assembly of a native community that would prevent the establishment of IAS through competitive interactions. This provides the opportunity to test a theory-based approach to community design, instead of trial-and-error attempts with results that are usually only of limited general applicability. With help of a strong theoretical framework, it may be possible to manipulate the composition of the community to be restored in such a way that it is resistant to invasions, while maintaining vital ecological functions (Laughlin 2014). Investigating the mechanisms driving resistance in native communities by means of a theoretical approach could reveal new insights relevant to the fields of invasion ecology, restoration ecology and community ecology (Funk et al. 2008). Although controversy still exists about whether scientific theory can effectively address the problems posed by IAS during ecological restoration (Cabin 2007, Giardina et al. 2007), in the past decade several studies have proposed frameworks for linking theoretical and applied approaches in this context (Funk et al. 2008, Laughlin 2014, Cadotte et al. 2015, Ostertag et al. 2015). Hence, combining insights from the theoretical field with empirical evidence could provide scientists and practitioners with effective tools for sustainably controlling IAS during ecological restoration.

**Applying competition theory to enhance biotic resistance**

A large proportion of studies on the ecology of communities prone to invasions has focused on competitive interactions among species, with great attention to the overlap of species niches (Lockwood et al. 2013). Specifically, studies have intended to understand the biotic and abiotic factors of an ecosystem that can inhibit the establishment of an invading species, which is referred to as ‘biotic’ or ‘ecological resistance’ (Elton 1958, Levine et al. 2004). Studies dealing with biotic resistance have strongly focused on competition (reviewed in Levine et al. 2004), but other factors such as herbivory (Maron and Vilà 2001) and the presence or absence of mutualisms...
Richardson et al. 2000a) may also affect community resistance. The biotic resistance hypothesis predicts that an increase in the number of resident species enhances the use of resources and therefore reduces the establishment probability of invaders (D’Antonio and Chambers 2006). The assumptions for this hypothesis are based on Lotka-Volterra equations indicating that an invader can be excluded by a competitor that is already present in the resident community, i.e. the exclusion principle (see Box 2; Lockwood et al. 2013). Some explanatory hypotheses have been developed to explain biotic resistance from different perspectives based on competition theory. Among the most widely known are the ‘diversity-invasibility’, ‘limiting similarity’ and ‘Darwin’s naturalization’ hypotheses (Fig. I. summarizes the main factors driving biotic resistance according to these three hypotheses).

The notion that diverse plant assemblages are more resistant to invasions due to a more exhaustive use of available resources compared to less diverse ones has been attributed to Charles Elton (1958). In the above-mentioned book Ecology of Invasions by Animals and Plants, diversity was described as richness of either species numbers or functional groups (see Levine and D’Antonio 1999, Pokorny et al. 2005). A large number of studies support this diversity-driven explanation for resistance (e.g. Tilman et al. 1997, Naeem et al. 2000, Hector and Bagchi 2007, Frankow-Lindberg 2012, Harrison et al. 2015), while others disagree (e.g. Robinson et al. 1995, Wiser et al. 1998). Overall, results from most studies recommend the increase of diversity in order to reduce the risk of establishment of invasive species, with clear implications for restoration and nature conservation (e.g. D’Antonio and Chambers 2006).
Fig. 1. The three main hypotheses of the dissertation and some factors of the biotic environment that can be used to test them. The hypotheses are proposed for explaining competitive biotic resistance of plant communities against IAS. All biotic factors have been shown to be good proxies of species niches and are, thus, related to resource acquisition.

Another perspective on biotic resistance comes from the limiting similarity hypothesis, which predicts that invaders that are very different from residents in their use of limiting resources would be able to establish given the availability of empty niches (MacArthur and Levins 1967, Abrams 1983). Funk et al. (2008) proposed this hypothesis as a method for assembling communities for ecological restoration based on functional similarity. They suggested that resistant communities could be accomplished by increasing native
species in the recipient community that have functional traits similar to those of potential invaders (see Box 2). The rationale behind using functional traits to test limiting similarity is based on their significance for resource acquisition, plant fitness and ecosystem processes (Funk et al. 2008). However, results of experimental studies using this trait-based approach to investigate biotic resistance have been partly contradictory (reviewed in Price and Pärtel 2013). A potential explanation for this could be related to the use of rather broad functional groups for testing biotic resistance, such as whether species are C3 or C4 grasses, legume and non-legume forbs (Emery 2007, Maron and Marler 2007, Young et al. 2009), or regarding species seasonality (Dukes 2002, Hooper and Dukes 2010). Therefore, a single or multiple traits could be used for testing limiting similarity depending on the focus of the study. Even if this approach provides the opportunity to choose species that might affect not only biotic resistance, but other ecosystem properties such as for instance carbon storage (Ostertag et al. 2015), it is still challenging to select the most relevant traits.

One of the earliest descriptions of the potential mechanisms related to the biotic resistance of a community is found in The Origin of Species (Darwin 1859). Here, Darwin hypothesized that invasive species closely related to the recipient community would be less likely to establish than IAS that are less closely related. This is known as ‘Darwin’s naturalization hypothesis’ and has been explain as the result of closely related species having similar niches, i.e. phylogenetic niche conservatism (see Box2; Prinzing 2001), and thus competing for the same resources (Daehler 2001). In other words, this hypothesis is based on the assumption that phylogenetic relatedness is associated with similarity in traits that play a role in determining species’ response to co-existence, also called the ‘phylogenetic signal’ sensu Blomberg and Garland (2002). Since then some inconsistencies have been found when testing this hypothesis, showing that spatio-temporal scales (Thuiller et al. 2010, Li et al. 2015b) and differences of some key traits in closely related species might affect the outcome of studies (Burns and Winn 2006, Funk and Vitousek 2007). Whether or not phylogenetic relatedness of the native community can influence the establishment of IAS is an interesting issue to be tested in the context of ecological restoration, given the great amount of genetic information available.
nowadays. Furthermore, up to now research analyzing this hypothesis has not been based on experimental studies but rather on post-invasion sites.

Overall, the various hypotheses for explaining biotic resistance of plant communities provide ecologists and practitioners with a basis for designing communities for areas that need to be restored and are prone to invasions (Funk et al. 2008). Nevertheless, in light of the controversial results found so far for all hypotheses, there is need for a deeper understanding of the mechanisms that drive resistance in a given ecosystem during restoration. One of the most promising ways to accomplish this is by manipulating the native species assemblage of plant communities and experimentally submitting them to competition with IAS. Testing biotic resistance of these communities with more than one IAS would allow furthering the generalization of the results to other IAS and systems. Furthermore, other factors known to modulate biotic resistance and affecting the success of IAS, such as propagule pressure (see Box 2; Miller et al. 2013, Byun et al. 2015) and disturbances related to increase resource availability (Davis et al. 2000) can also be experimentally tested in this context.

**Box 2. Setting the context of biotic resistance**

**Exclusion principle:** In an ecological community with limited resources, two species competing for the same resources cannot stably coexist (Hardin 1960, Levin 1970).

**Functional traits:** Morpho-physio-phenological characteristics of a species that have an effect on its resource capture and fitness (Lavorel and Garnier 2002).

**Phylogenetic niche conservatism:** The retention of ecological traits related to the species niche over time (Wiens et al. 2010).

**Propagule pressure:** Composite measure of the number of individuals and the number of release events of a species introduced to a new region (Lockwood et al. 2005).
3. Aims of this dissertation

The overall aim of this dissertation is to investigate whether we can predict the invasion resistance of grassland communities created by engineered seed mixtures and intended for grassland restoration. More specifically, the aim is to pinpoint which characteristics of the native community and species composition would describe the biotic environment and thus be crucial to ascertain the drivers of competition related to niche overlap among native and invasive species.

For doing so I investigate: (1) whether a theoretical approach can be used for explaining biotic resistance during restoration based on three hypotheses, i.e. the diversity-invasibility, limiting similarity and Darwin’s naturalization hypotheses; (2) whether the theoretical framework can be investigated from a trait-based or a phylogenetic approach. In terms of the trait-based perspective, I also attempt to investigate whether a single trait or multiple traits related to plant species' dispersal, establishment success, growth, persistence and competitive strength can be used as a proxy for biotic resistance. To this end, Chapter 1 focuses on the diversity-invasibility approach modulating the native community’s functional diversity during an early phase of restoration and studying the effect on the prevention of the establishment of one IAS. Chapter 2 takes a single-trait approach for testing the limiting similarity hypothesis in the context of early establishment of a native community. Chapter 3 combines two approaches, a multi-trait with a phylogenetic one, in order to test the limiting similarity and Darwin’s naturalization hypotheses. Specifically, the dissertation focuses on limiting similarity in functional group identity for designing resistant communities and then testing community’s ability for suppressing IAS depending on phylogenetic distance. An overview of the basic approach of the dissertation is given in Fig. II.

Overall, with the results of these chapters the dissertation aims to contribute to the design of suitable management strategies for different IAS during ecological restoration, providing a greater understanding not only on how to manage them, but also of the main characteristics and fundamental processes that make them successful invaders.
General Introduction

4. Methodological overview

4.1 Model systems and species

The use of particular species or systems has been encouraged for unraveling the interacting factors that affect invasions defined as ‘model system research’ (Kueffer et al. 2013). In the context of invasions, the study of a model species under different environmental conditions will allow a comprehensive evaluation of the factors and their interactions affecting the outcome of an invasion (Kueffer et al. 2013). Hence, I focus on native species used for restoration and on invasive species commonly problematic in
Central Europe. Specifically, I use plant species applied for restoring grassland(-like) systems, and IAS commonly found in disturbed ruderal areas such as river banks and roadsides in central Europe (Fig. III). Yet in Chapter 1 and 2, I use roadsides as a model system given that they are targeted for revegetation and prone to invasions.

**Grasslands**

Semi-natural grasslands are hotspots of biodiversity in Central Europe (Krauss et al. 2010). As ecosystem services they sequester carbon, provide food for grazers and prevent erosion (O’Mara 2012). Most semi-natural grasslands are located in places known to be formerly forested, their origin thus being linked to long-term human activities dating back to the presence of Neolithic settlers (Poschlod and WallisDeVries 2002). However, in recent years they have been submitted to destruction and fragmentation resulting from changed land use including afforestation, conversion to arable fields or abandonment (Buisson et al. 2006, Coiffait-Gombault et al. 2012). This has raised the interest to restore large areas of grasslands for example as compensation measures for construction work and urban developments (Tischew et al. 2010), for example on ex-arable fields (Vesk and Mac Nally 2006, Munro et al. 2009), in abandoned opencast mines (Bradshaw 1997, Tordoff et al. 2000), and along roadsides (Tormo et al. 2008, Bochet et al. 2010).

Grassland systems are well studied worldwide (Jones and Hayes 1999, Poschlod and WallisDeVries 2002, Dengler et al. 2014), including their restoration in the face of invasions (Dukes 2002, Emery and Gross 2007, Walker et al. 2014). Therefore, grasslands are perfect study systems, also because comparisons with other studies are possible and may lead to generalizations in regard to the mechanisms that affect their resistance to invasion.

**Invasive plants used as model species**

*Impatiens glandulifera* Royle

*Impatiens glandulifera* (Himalayan balsam) is an annual herb that belongs to the family Balsaminaceae and is native to the Western Himalayas. This species can reach heights of
2.5 m and has stems that are reddish-translucent, hollow, 5–50 mm in diameter, and simple or sometimes branched. Roots have depth of 10–15 cm, frequently produced adventitiously at the lower nodes and buttressing. It reproduces sexually with fruit capsules that shoot the ripe 3-mm seeds up to 7 m by the slightest touch (Beerling and Perrins 1993). *I. glandulifera* was introduced to Europe as an ornamental plant and is currently widely distributed throughout Europe. The fast growth of its seedlings and young plants allows the species to survive and often to replace native species even in competitive environments (Hulme and Bremner 2006). *I. glandulifera* is frequently found in moist ruderal sites such as river banks, waste sites and open woodlands, but has also been reported to occur in road verges being transported along them (Kollmann and Bañuelos 2004, Pacanoski and Saliji 2014). Therefore, this plant was used as test species in Chapter 1.

*Ambrosia artemisiifolia* Linné

*Ambrosia artemisiifolia* (Common ragweed) is an annual herb belonging to the family Asteraceae, native to North America. The characteristics of this species were recently summarized in the *Biological Flora of the British Isles* by Essl et al. (2015), where it is described as monoecious and wind-pollinated, with a variable height from 10 cm to 2.5 m. Moreover, it reproduces sexually by seeds, is known to form a persistent soil seed bank, and seeds require prolonged chilling to break dormancy. Following seedling emergence in spring, the rate of vegetative growth depends on temperature, but development occurs over a wide range of temperatures (Essl et al. 2015). *A. artemisiifolia* is present in almost all European countries at variable densities, having been introduced mainly as seed contaminant. It behaves as an annual pioneer species occupying disturbed habitats such as roadsides, waste places, construction sites, agricultural fields, disturbed or abandoned fields, waterways and urban areas. In heavily invaded areas it causes crop-yield losses and its pollen is highly allergenic, having thus negative impacts on public health (Gerber et al. 2011). Given that there is a special interest in developing more effective control measures of this species throughout Europe, it was used as a model in two chapters of this dissertation (Chapter 2 and 3).
Solidago gigantea Aiton

Solidago gigantea (Giant goldenrod) is a rhizomatous perennial herb native to northern America, also belonging to the Asteraceae. S. gigantea is a tall erect herb that has annual aboveground shoots and persistent belowground rhizomes and can rise to 2 m in height. It can reproduce sexually by seeds but mainly reproduces by clonal growth. It invades disturbed mesic sites such as riverbanks and roadsides. S. gigantea was introduced to Europe as an ornamental in the 18th century and became invasive about 100 years later (Weber and Jakobs 2005). It often forms dense monospecific stands, inhibiting native species establishment and drastically reducing diversity in invaded areas (Weber and Jakobs 2005). Since it causes problems in protected and reforested areas, it is a suitable candidate for testing biotic resistance in restored areas subjected to disturbance, and was used as such in Chapter 2 and 3.

Solidago canadensis Linné

Solidago canadensis (Canada goldenrod) is a rhizomatous perennial herb from the same family, native to North America. The plant produces annually aboveground shoots from its rhizome system and can reach up to 2 m height. Its main reproduction strategy is asexually by clonal growth, although it can also reproduce sexually by seeds (Werner et al. 1980). It was introduced to Europe in the 17th century. It invades disturbed areas such as abandoned fields and along roads and railroads where it has an effect similar to S. gigantea (Zwölfer 1976). Given that it is considered an aggressive invasive species, S. canadensis was selected as a model species in Chapter 2.

Erigeron annuus Linné

Erigeron annuus (Daisy fleabane) is a winter annual herb native to North America and another member of the Asteraceae. The plant reproduces mainly via agamospermy, but rare sexual events are presumed to occur. Seeds normally germinate in late summer, although the species overwinters as a rosette later producing stems in spring (Trtikova et al. 2010). It was introduced to Europe in the 17th century and since then it has become
abundant in roadsides and ruderal sites (Edwards et al. 2006). Given its high abundance in such areas it was used as a model species in Chapter 2.

**Fig. III.** Alien invasive species used as model species in this dissertation (Photos of *Solidago canadensis* and *S. gigantea* by Johannes Kollmann).

**Roadsides as model systems**

Roadsides (also referred to as ‘road verges’ in this dissertation) are widespread structures associated with human traffic constructions. Even if they are artificial, they play a central role as wildlife corridors connecting (near)natural areas and in some cases serving as habitats for native species. For instance, previous studies have found proper managed roadsides to be of great potential for the conservation of insects (Raemakers et al. 2001, Skórka et al. 2013) and for grassland plants (Hovd and Skogen 2005, Auestad et al. 2011). Therefore, they represent an attractive opportunity for the restoration of
more natural systems such as grasslands and also for improved design of seed mixtures to accomplish this (Kiehl and Pfadenhauer 2007). In order assure the success of these seed mixtures, several biotic and abiotic factors that might modulate species establishment have to be taken into account for most appropriate ecological design. Moreover, aspects related to engineering and traffic should be considered as well.

Among the biotic factors, the most significant are related to the dispersal and invasion of weedy species, since roadsides are prone to invasions and facilitate the dispersal of IAS (Hulme 2006). This process is enhanced by traffic, disturbance, nutrient-rich substrates and a lack of competing species (Haan et al. 2012). Furthermore, a still common practice is the use of non-regional (and sometimes non-native) plants for revegetation of roadsides. The use of regional native material is encouraged, since it has better adaption to local conditions as reflected by higher plant establishment and survival (Jones and Hayes 1999). From the abiotic perspective, some roadsides are constructed using stony substrate in order to assure stability and trafficability, which might negatively affect vegetation establishment by reducing the surface available, thus increasing competition for space. Other abiotic conditions in roadsides representing a challenge for species establishment are the high irradiation, extreme temperatures, low soil humidity, and irregular fertilization and disturbance (Forman et al. 2003). Consequently, species selection for seed mixtures intended for roadside revegetation becomes a decisive step in the planning of roadside revegetation (Bochet et al. 2010), since they should cope with high abiotic stress and suppress unwanted species, as for example IAS. Nevertheless, the careful planning of seed mixtures for roadsides in terms of these factors has received little attention so far.

4.2 Summary on the experimental approach and data analysis

All chapters in this dissertation make use of an experimental approach by means of manipulative greenhouse experiments (Fig. IV). Most experiments have fully randomized designs, except the second one in Chapter 3. Different statistical analyses were used according to the hypotheses targeted by each chapter: analysis of variance
(Chapter 1–3), Kruskal-Wallis test (Chapter 1–2), nonlinear regression models (Chapter 1 and 3) and cluster analysis (Chapter 3). Furthermore, in order to assess the suppression effect of the native species on the IAS, I used the relative competition index (RCI; Chapter 2 and 3). Most of the statistical analyses were performed using the open source software R Version 3.1.0 (R Development Core Team 2014), but for some analyses in Chapter 3 the Infostat software was used (Di-Rienzo et al. 2013).

**Fig. IV.** Overview of the greenhouse experiments performed in the main chapters of this dissertation. In each experiment a specific seed mixture was sown into trays or pots to test the respective communities for their specific suppression of IAS.
5. Thesis outline

**Chapter 1**

The chapter *Bioengineering effectiveness of seed mixtures for road verges: Functional composition as a predictor of grassland diversity and invasion resistance* assesses seed mixtures intended for the restoration of grassland species in road verges in terms of their effect on species establishment, species diversity and invasion resistance of the resulting plant community. Moreover, it investigates how the resulting communities are affected by stony microhabitats, seed provenance, functional group and trait composition of the seed mixtures. In other words, this chapter aims to evaluate the effectiveness of seed mixtures for addressing engineering functions in roadsides while benefiting diversity. This is accomplished with a greenhouse experiment using native grassland species and testing them against the invasive alien *Impatiens glandulifera*.

**Chapter 2**

The chapter *Can plant invasions be prevented by limiting similarity? The role of seed size, seed density and resource availability during roadside re-vegetation* deals with a trait-based design of seed mixtures for roadsides revegetation in order to prevent the establishment of IAS. Here, I hypothesize that communities resistant to IAS should have similar traits, following the limiting similarity hypothesis, and selecting seed size and seed-size strategy as the predictors. This was studied by means of two greenhouse experiments. Native communities were designed using grassland species based on commercial seed mixtures tailored for roadside revegetation in Germany, and tested using the small-seeded invasive species *Erigeron annuus, Solidago canadensis, Solidago gigantea* and large-seeded *Ambrosia artemisiifolia*. 
Chapter 3

The chapter *Limiting similarity and Darwin’s naturalization hypothesis: understanding the drivers of biotic resistance against invasive plant species* studies whether the functional or the phylogenetic composition of a community govern its ability to control the success of invasive alien species (IAS). This was done by comparing two hypotheses, i.e. the limiting similarity and Darwin’s naturalization hypotheses. Both try to explain biotic resistance of a recipient community with limited niche opportunities for IAS, but one uses multiple species traits, while the other focuses on phylogenetic relationships as a proxy for niche similarity. This chapter is based on two greenhouse experiments, with communities consisting of grassland species and testing them with the invasive plants *Ambrosia artemisiifolia* and *Solidago gigantea*.

Overall, throughout these chapters the dissertation seeks to improve knowledge on the effects of species composition on biotic resistance of a community. By means of a new methodological approach combining a theoretical framework for the trait-based design of seed mixtures in order to restore resistant communities, this work strives to answer fundamental ecological questions in the field of invasion ecology. As a result, the dissertation will improve the ability for predicting, preventing and managing invasive species in areas subjected to restoration.
Bioengineering effectiveness of seed mixtures for road verges: functional composition as a predictor of grassland diversity and invasion resistance
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*These authors contributed equally to this work
Abstract

Many attempts have been made to restore semi-natural temperate grasslands that suffered substantial declines due to changes in land use in the past decades. An alternative to restoration of historical grassland is the establishment of analogous communities in new habitats like road verges. This study aims at assessing seed mixtures for road verges that cope with the need for bioengineering effectiveness while benefitting local biodiversity. We established a greenhouse experiment with microsite variation to study establishment, diversity and invasion resistance of four regional and two non-regional seed mixtures on road verge substrate, with and without stony microhabitats as a function of their seed source, functional group and trait composition. Vegetation cover was measured six and twelve weeks after sowing, as well as dry biomass of target and non-target species. Invasion resistance after establishment of native communities was tested by adding seeds of the alien Impatiens glandulifera Royle and later counting seedling emergence. Vegetation cover after six weeks was significantly affected by seed source and percentage legume seed, while stony microsites had negative effects on vegetation cover at both times. Percentage grass biomass reduced species richness, but had no influence on invasion resistance against non-target species. Functional dispersion (FDis) of rooting traits and aboveground biomass were not correlated with invasion resistance at the early stage of grassland development. Otherwise, the invasion of an alien species at a later stage was explained by seed mixture identity and thus, associated with lower diversity of rooting traits and lower species richness. Using regional seeds is recommended for road verge restoration, but only an appropriate balance of functional groups and traits within the seed mixtures can ensure quick establishment and high species richness.

Keywords: functional groups; greenhouse experiment; Impatiens glandulifera; local plant diversity; microsite variation; roadside revegetation; seed provenance
1. Introduction

Many semi-natural grasslands of the temperate zone are characterized by a high biodiversity (Kahmen et al. 2002). In the past decades changes in land use, like abandonment or agricultural intensification, followed by fragmentation, eutrophication and acidification have caused a marked reduction of these ecosystems (Bakker and Berendse 1999, Poschlod and WallisDeVries 2002). These habitat changes have resulted in declining populations of grassland plants and local species extinctions (Adriaens et al. 2006, Stehlik et al. 2007), although many grassland types and several species are protected by national and European laws. Thus, grassland conservation is no longer sufficient to stop these negative trends in biodiversity, while restoration might be a suitable alternative.

Several methods to restore historical grasslands have been successfully introduced (Willems 2001, Kahmen et al. 2002, Török et al. 2012), while establishment of grasslands on new sites is increasingly recognized as a suitable alternative (Kiehl and Pfadenhauer 2007), for example along road verges where open vegetation with low productivity is wanted (Rydgren et al. 2010). Road verges are common in most temperate regions and offer a chance to restore historical grassland types or to create new communities suitable for specific site conditions. They have great potential for insects (Raemakers et al. 2001), especially butterflies (Saarinen et al. 2005, Skórka et al. 2013), and for grassland plants (Hovd and Skogen 2005, Auestad et al. 2011), provided proper management (Tikka et al. 2000). Moreover, road verges do not only offer suitable habitats, but also serve as dispersal corridors within a potentially large habitat network (Tikka et al. 2001).

When planning road verge vegetation, the priority lies in engineering and traffic-related aspects, whereas ecological functions are less considered (Akbar et al. 2003). Before selecting appropriate plants it is important to determine the abiotic conditions the community will face, as for instance some road verges are constructed using stony substrate to increase stability and trafficability. This substrate leads to reduced effective surface for vegetation growth, and may result in stronger competition for space and
associated biotic interactions. However, due to local shading and reduced evaporation stones create safe sites for germination (Harper et al. 1965). Petersen et al. (2004) found that stones and associated microsites did indeed increase plant establishment when compared to open locations in re-vegetated road verges. However, more research is needed on the interaction between small-scale heterogeneity of microsites and specific seed mixtures for road verges.

In Germany, most road verges are still planted with standard commercial seed mixtures (‘Regel-Saatgutmischungen’, RSM), in this study called ‘non-regional’ mixtures, containing non-native species and agricultural cultivars (Hefter et al. 2010). The seed source is frequently not regional and most have a high proportion of grasses. The use of ‘regional’ seed material, i.e. native species from local provenances commercially produced and used in the area of origin, has several advantages, among them lower demands on resources (Petersen et al. 2004), greater plasticity under heterogeneous conditions (Grossman and Rice 2012), and suppression of invasive alien plants (Walker et al. 2015). These qualities should lead to higher grassland diversity, adequate cover of the target species and reduced soil erosion (Gyssels et al. 2005). This can be summarized as ‘bioengineering effectiveness’, whereby bioengineering is the use of living plants in order to fulfill engineering functions (Clark and Howell 1992).

A closer look at the composition of regional seed mixtures has received little attention so far. The proportion of functional groups can affect the bioengineering effectiveness of seed mixtures, as functional group richness increases biotic resistance of communities to invaders (Dukes 2001, Byun et al. 2013) and affects aboveground biomass (Hector et al. 1999). Legumes, for instance, facilitate establishment of other grassland species and thus increase vegetation cover at the initial stages of succession (Guo et al. 2012). Grasses, especially native ones, are known for negative effects on species diversity because of their potential dominance (Howe 1999). On the other hand, native perennial grasses have also the potential of creating an effective invasion resistance (Young et al. 2009, Sheley and James 2010), while low diversity can be linked to higher invasibility (Dukes 2002, Maron and Marler 2007). In terms of invasibility, a classification into functional groups based on morphological traits like aboveground biomass and rooting
architecture that reflect competition for space (Von Holle and Simberloff 2004) complements the classical grass-forbs-legume (GFL) scheme that so far cannot predict biotic resistance (Drenovsky and James 2010).

In the present study we examine compositional effects of different functional groups and traits on the bioengineering effectiveness of grassland mixtures for road verges, while the number of species is kept constant. Regional and non-regional commercial seed mixtures with different proportions of functional groups were compared in a greenhouse experiment with and without heterogeneous microsites produced by stones. Moreover, we tested community resistance against non-target species, i.e. native species present in the soil seed bank or dispersed by wind, and a common alien invasive species added as seeds after establishment of the native target community.

We test the following hypotheses: (1) Regional seed mixtures for grasslands show higher early vegetation cover than non-regional commercial mixtures, especially with heterogeneous microsites. (2) A higher proportion of legumes in the seed mixture leads to higher early cover. (3) A smaller proportion of grasses in the resulting community causes a higher species diversity and enhanced biotic resistance against invaders. And (4) diverse rooting architectures and high aboveground biomass reduce the invasion success during and after the establishment of the grassland community.

2. Material and methods

2.1. Seed mixtures

Four regional and two non-regional commercial seed mixtures were tested, each reduced to 23 grassland species to exclude species number effects (Table 1.1). The final 23 species were selected based upon the following criteria (in order of priority), i.e. same species, genus, family, life form, life span, vegetative propagation and storage organ as species of RSM7. The following mixtures were tested: (1) RSM7: non-regional commercial seed mixture widely used for planting road verges on moderately dry sites
in Germany (FLL, 2013). (2) RSM7R: same species and proportions as RSM7 but using regional seed material. (3) RSM16: Regional seed mixture tailored for southern Bavaria as a further development of the RSM concept, which aims for a nationally admitted framework for regional wildflower seed (Regelwerksausschuss Gebietseigenes Saatgut 2013; www.natur-im-vww.de). (4) RSM16C: as RSM16 but non-regional, with the grasses *Agrostis capillaris* L. and *Poa pratensis* L. replaced by cultivars; for all other wild provenances no cultivars were available. (5) ST: regional seed mixture developed by the University of Applied Sciences Weihenstephan-Triesdorf for road verges affected by de-icing salts and therefore containing salt-tolerant and low-growing plants. (6) RSMO: regional seed mixture developed by the seed producer Johann Krimmer (Pulling, Bavaria) with the objective to optimize the development of the RSM concept in relation to the special demands of road verge vegetation and regional grassland vegetation from the alliances Mesobromion erecti, Xerobromion or Cirsio-Brachypodion (Röder et al. 2006). Non-regional commercial seed were obtained from WeiSa KG (Bodenkirchen, Germany), regional seeds from Johann Krimmer.
Table 1.1. Composition of the six seed mixtures designed for grassland restoration in road verges. \(^a\)Non-regional seed mixtures, \(^b\)Cultivar species: *Agrostis capillaris* and *Poa pratensis*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Percentage seed content (wt%)</th>
<th>RSM7(^a)</th>
<th>RSM7R</th>
<th>RSM16</th>
<th>RSM16C(^a,b)</th>
<th>ST</th>
<th>RSM0</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Grasses</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Agrostis capillaris</em></td>
<td></td>
<td>–</td>
<td>–</td>
<td>5.0</td>
<td>5.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td><em>Anthoxanthum odoratum</em></td>
<td></td>
<td>–</td>
<td>–</td>
<td>10.0</td>
<td>10.0</td>
<td>8.0</td>
<td>–</td>
</tr>
<tr>
<td><em>Briza media</em></td>
<td></td>
<td>–</td>
<td>–</td>
<td>4.0</td>
<td>4.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Bromus erectus</em></td>
<td></td>
<td>5.0</td>
<td>5.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4.0</td>
</tr>
<tr>
<td><em>Festuca nigrescens</em></td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>16.0</td>
<td>15.0</td>
</tr>
<tr>
<td><em>Festuca ovina</em></td>
<td></td>
<td>51.9</td>
<td>51.9</td>
<td>–</td>
<td>–</td>
<td>25.0</td>
<td>15.0</td>
</tr>
<tr>
<td><em>Festuca rubra</em></td>
<td></td>
<td>10.0</td>
<td>10.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Festuca rubra</em> ssp. commutata</td>
<td></td>
<td>10.0</td>
<td>10.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Festuca rubra</em> ssp. trichophylla</td>
<td></td>
<td>10.0</td>
<td>10.0</td>
<td>–</td>
<td>–</td>
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<td>–</td>
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<tr>
<td><em>Helictotrichon pubescens</em></td>
<td></td>
<td>–</td>
<td>–</td>
<td>6.0</td>
<td>6.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Lolium perenne</em></td>
<td></td>
<td>10.0</td>
<td>10.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Poa angustifolia</em></td>
<td></td>
<td>–</td>
<td>–</td>
<td>30.0</td>
<td>30.0</td>
<td>20.0</td>
<td>30.0</td>
</tr>
<tr>
<td><em>Poa compressa</em></td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>5.0</td>
<td>10.0</td>
</tr>
<tr>
<td><em>Poa pratensis</em></td>
<td></td>
<td>–</td>
<td>–</td>
<td>15.0</td>
<td>15.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Percentage of grasses</strong></td>
<td></td>
<td>96.9</td>
<td>96.9</td>
<td>70.0</td>
<td>70.0</td>
<td>80.0</td>
<td>80.0</td>
</tr>
<tr>
<td><strong>Number of grasses</strong></td>
<td></td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td><strong>Legumes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><em>Anthyllis vulneraria</em></td>
<td></td>
<td>0.2</td>
<td>0.2</td>
<td>–</td>
<td>–</td>
<td>0.8</td>
<td>–</td>
</tr>
<tr>
<td><em>Lotus corniculatus</em></td>
<td></td>
<td>0.2</td>
<td>0.2</td>
<td>0.5</td>
<td>0.5</td>
<td>1.0</td>
<td>1.4</td>
</tr>
<tr>
<td><em>Medicago lupulina</em></td>
<td></td>
<td>0.2</td>
<td>0.2</td>
<td>0.5</td>
<td>0.5</td>
<td>1.0</td>
<td>–</td>
</tr>
<tr>
<td><em>Onobrychis viciifolia</em></td>
<td></td>
<td>0.8</td>
<td>0.8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Trifolium dubium</em></td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.2</td>
<td>–</td>
</tr>
<tr>
<td><em>Trifolium pratense</em></td>
<td></td>
<td>–</td>
<td>–</td>
<td>1.0</td>
<td>1.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Vicia cracca</em></td>
<td></td>
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**Number of forbs**: 13 13 13 13 13 16

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**Chapter 1**

Bioengineering Effectiveness of Seed Mixtures
2.2. **Non-target species**

Non-target species were introduced with the substrate by using soil collected from an unvegetated road verge mixed with peat-based standard garden substrate. Moreover, the half-open greenhouse allowed for input of seeds dispersed by wind. And finally, after establishment of the grassland communities, seed rain was imitated by adding propagules of the invasive alien *Impatiens glandulifera* Royle as a test species (see Appendix, Table S1.1 for further information on the seed material). Although this alien invasive species is mostly found in moist ruderal sites such as river banks, waste sites and open woodlands, it has also been reported from road verges, and proven to be transported along roads (Kollmann and Bañuelos 2004, Pacanoski and Saliji 2014).

2.3. **Experimental design**

The experiment was carried out from April 2014 until March 2015 at the Greenhouse Laboratory Center Dürnast, School of Life Sciences Weihenstephan, Technische Universität München (48°24’ N, 11°41’ E). For the first part of the experiment we used an a semi-open greenhouse with a glass roof but wire mesh walls, so that climate conditions were similar to outside conditions and some seed dispersal from the surroundings could occur. The mean temperature of this house was $16 \pm 4 \, ^\circ C$. After 24 weeks, the experiment was transferred to an unheated closed greenhouse that had a mean temperature of $8 \pm 3 \, ^\circ C$.

In the greenhouse each seed mixture and a control (no seed added) were established in plastic trays ($0.6 \, \text{m} \times 0.4 \, \text{m} \times 0.1 \, \text{m}$) at a density of $5 \, \text{g} \, \text{m}^{-2}$ mixed with $15 \, \text{g} \, \text{m}^{-2}$ of soy meal for easier scattering of the seeds. The substrate mixture consisted of 25vol% fine sieve fraction ($<5 \, \text{mm}$) of nutrient-poor calcareous rubble from a new road verge nearby completed in spring 2014, and 75vol% peat-based substrate (Einheits Erde®, Einheitserdewerke Werkverband e.V., Altengronau, Germany; N, 180 g m$^{-3}$; P, 240 g m$^{-3}$; K, 240 g m$^{-3}$; pH 5.8). Each seed mixture and the control were replicated six times, thus a total of 42 plastic trays were filled. To assess the effect of stones potentially acting as
safe sites on establishment of plants, trays were designed as split plots divided by a plastic spacer (0.05 m). One half of the trays received randomly distributed concrete rubble >5 cm from the road verge, covering ca. 50% of the area. The position of the trays was placed randomly, but to avoid cumulative effects, vicinity of the same seed mixtures was excluded. Irrigation was conducted when necessary by flooding all trays from below at the same watering frequency and duration.

2.4. Measurements

In late May, six weeks after sowing, early vegetation cover was measured by randomly placing a frame of 15 cm × 15 cm on both sides of the split plot (cf. de Bello et al. 2013). The early vegetation cover (%) was recorded using visual estimates. In early July, twelve weeks after sowing, vegetation cover was estimated for the whole area of each split plot side. At the same time all plants were harvested at ground level. To avoid edge effects, a separation into the interior vegetation within a 4 cm wide edge was done. For the interior part of each plot vegetation was sorted by species. On fresh material species number and identity were taken, while for unsown species only the number of individuals was recorded. The edge was divided into sown and unsown species, and noted if an unsown species of the interior part was also found in the edge. All samples were dried 3 days at 70 °C to determine dry biomass of each species.

Total biomass was collected again 24 weeks after sowing. This time, all plants were cut approximately 1 cm above ground level, and then dried following the same procedure. Two weeks later, 50 seeds of the invasive species I. glandulifera were added to each treatment combination including the control trays; seeds were not stratified and randomly scattered from above to simulate seed rain along roads. Four months after sowing (February 2015), the total number of emerged seedlings of I. glandulifera was counted from each treatment combination.
2.5. Data analysis

Statistical analyses were performed using R Version 3.1.0 (R Development Core Team, 2014). If data did not fulfill the conditions of normal distribution and homogeneity of variances, log transformation or for proportional data arcsine transformation were done. If homogeneity of variances and residual normal distribution could not be achieved, non-parametric Kruskal-Wallis test was used. The effect of seed source (non-regional vs. regional) of the seed mixtures on early vegetation cover was tested by two-way ANOVAs considering the microsite treatment and its potential effect on seed source. We used the tests also to show the differences in early cover between the six mixtures independently; pairwise comparisons were done with Tukey tests.

One-way ANOVA was performed to test the effect of legume seed content in the seed mixtures before sowing (wt%) on early vegetation cover. For legume seed content there were the categories 1.4% and 3.0%. An additional correlation with percentage legume biomass at harvest was done.

Percentage grass biomass at harvest was analysed by nonlinear regression models using the function `nls()` (Bates 1992) to show its influence on target species number (Bates and Watts 1988). We initially compared different models and subsequently selected the Michaelis-Menten function \( y = ax[b + x]^{-1} \) as the final model based on fitting and AIC values (Bates and Watts 1988). The effect of percentage grass biomass on invasive biomass, measured as the percentage of dry biomass of unsown species, was studied with a linear model.

A correlation between percentage grass biomass and grass seed content was conducted. Additionally, one-way ANOVA and Tukey contrasts were used to investigate the relationship between the six seed mixtures and percentage grass biomass or target species number. Differences in percentage of invasive biomass according to the seed mixtures were identified using Kruskal-Wallis tests.

To identify the effect of functional diversity on invasion resistance, the functional traits rooting depth, rooting density and aboveground density were used. For all species
rooting depth (deep, middle, shallow) and rooting density (dense, middle, sparse) were taken from Kutschera and Lichtenegger (1982) and Kutschera et al. (1992). With the functional traits and the presence-absence species data, functional dispersion (FDIs) was assessed for each plot. Presence-absence data FDIs is a good measure of the differences in functional diversity and can be computed using the FD package in R (Laliberté and Legendre 2010). The effect of FDIs on invasion resistance was tested by a linear model. For the six seed mixtures we analysed differences in FDIs with Kruskal-Wallis test and associated post-hoc tests.

Two-way ANOVA was used to test the effects of seed mixture, stone addition and the interaction on total biomass of the seed mixtures before sowing I. glandulifera. Effects of seed mixture, stone addition, FDIs and total biomass of the seed mixture were also tested on I. glandulifera seedling emergence, using a generalized linear model (GLM) with Poisson error distribution (Cameron and Trivedi 1990). Several models using the three suggested explanatory variables were assessed. Subsequently, the final model was selected based on AIC values; this model complied with the assumption of equidispersion. Finally, to test differences in seedling emergence and total biomass among the seed mixtures, multiple comparisons were performed under Tukey’s procedure using the R package Agricolae (De Mendiburu 2014).

3. Results

3.1. Effects of seed source and microsites on early vegetation cover

Vegetation cover after six weeks was influenced significantly by seed source and microsite treatment with higher cover for regional communities ($F_1 = 14.03, p < 0.0001$) without stony microsites (ANOVA, $F_1 = 5.08, p < 0.05$), albeit with no interaction ($F_1 = 0.89, p > 0.05$). Although there were no significant differences in early vegetation cover for stone-free ($F_1 = 4.07, p > 0.05$) nor for stony plots ($F_1 = 1.16, p > 0.05$; Fig. 1.1a), the treatments using regional plants had slightly higher early vegetation cover values. After twelve weeks no significant difference in late vegetation cover was observed over all
plots depending on seed source ($F_1 = 0.05, p > 0.05$), within stone-free ($F_1 = 0.17, p > 0.05$) or stony plots ($F_1 = 0.38, p > 0.05$), while stone-free plots had still significant higher vegetation cover ($F_1 = 43.0, p < 0.0001$; Fig. 1.1b).

The seed mixtures RSM16/RSM7 were the only pair differing significantly in terms of early vegetation cover, with RSM16 having a higher cover than RSM7 ($F_5 = 2.81, p < 0.05$; Appendix, Fig. S1.1).

**Fig. 1.1.** Vegetation cover of non-regional and regional seed mixtures grown in stone-free or in stony trays six weeks and twelve weeks after sowing. Different letters indicate significant differences ($p < 0.05$).
3.2. Compositional effects on early vegetation cover, diversity and invasion resistance

Legume seed content of the seed mixture had a significant positive effect on vegetation cover after six weeks ($F_1 = 4.07, p < 0.05$; Fig. 1.2), while the positive correlation with percentage legume biomass after twelve weeks was weak ($r_s = 0.23, p < 0.05$).

![Early vegetation cover vs Legume seed content](image)

**Fig. 1.2.** Vegetation cover in seed mixtures comprising to different legume seed proportions after six weeks. Different letters indicate significant differences ($p < 0.05$).

Percentage grass biomass had a significant effect on the number of target species. Higher percentage grass biomass led to a decrease in species number, particularly when grass biomass exceeded 95% (Michaelis-Menten model, $a = 18.43, b = 4.92, p < 0.0001$, where $a$ is the asymptote and $b$ the half-maximum scale parameter; Fig. 1.3a). There were two points were the richness of species was low even under 95%, thus, the model was evaluated with and without these outliers. Since the outcome did not change significantly and both were replicates of the same treatment RSM7, we chose the model including the two points. No effect of percentage grass biomass on percentage invasive biomass occurred (adjusted $R^2 = −0.007, p > 0.05$, Fig. 1.3b). Grass seed content and percentage grass biomass were positively correlated ($r = 0.73, p < 0.001$).
The six seed mixtures showed significant differences in percentage grass biomass ($F_5 = 4.16, p < 0.01$), with RSM7 and RSM7R having a higher grass biomass than the other five mixtures (Appendix, Fig. S1.2). Species number also differed significantly ($F_5 = 178.3, p < 0.001$), with RSM7 having the lowest diversity followed by RSM7R, which differed from the other seed mixtures and between each other (Appendix, Fig. S1.2).

At the early phase of grassland establishment, biomass of non-target species differed among the six seed mixtures (Kruskal-Wallis test, $p < 0.0001$; Appendix, Fig. S1.2). RSM7R showed highest invasion resistance measured as percentage biomass of unsown species (median = 1.1%), which differed from the two non-regional seed mixtures RSM7 (3.9%) and RSM16C (4.3%) having the highest biomass of non-target species. The seed mixtures varied significantly in FDis (Kruskal-Wallis test, $p < 0.0001$; Appendix, Fig. S1.3), with ST and RSMO having highest FDis, both differing significantly from RSM7 and RSM16, and RSMO differing from RSM16C. However, FDis showed no significant effect on biomass of non-target species at this early phase ($R^2 = -0.014, p > 0.1$; Fig. 1.4).

**Fig. 1.3.** (a) Relationship between grass biomass and number of target species using a Michaelis-Menten model ($y = 18.43x (4.92 + x^{-1})$), and (b) between percentage grass biomass and invasive biomass (lm: adjusted $R^2 = -0.007$). White dots indicate samples with grass biomass causing a species reduction of at least 50% (11.5 species).
3.3. Compositional effects on late vegetation cover and invasion resistance

After 24 weeks, total biomass showed significant differences among seed mixtures (ANOVA, $F = 6.38$, $p < 0.0001$) and microsite treatment (ANOVA, $F = 44.8$, $p < 0.0001$), albeit with no interaction (ANOVA, $F = 0.62$, $p > 0.05$). The seed mixtures RSM7 differed significantly from the control with no seed addition, showing the highest total biomass compared to the other seed mixtures (Appendix, Fig. S1.4). Stone-free treatments had always higher biomass compared to treatments covered with stones.

Invasion resistance at this stage measured as seedling emergence of *I. glandulifera* was determined by seed mixtures identity and FDis, whereas the effects of microsite and total biomass were not significant (GLM (*Poisson*): seed mixture, $LR = 19.1$, $p < 0.001$; FDis, $LR = 3.90$, $p < 0.04$; microsite, $LR = 1.46$, $p > 0.05$; total biomass, $LR = 1.37$, $p > 0.05$). The least resistant seed mixtures were RSM7 and RSM7R with almost as much seedlings as the unsown controls (Fig. 1.5). Furthermore, these mixtures showed the highest variability and the lowest values of FDis (RSM7: mean = 0.41 ± 0.06; RSM7R: mean =

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**Fig. 1.4.** Relationship between functional dispersion (FDIs) and percentage invasive biomass (lm: adjusted $R^2 = -0.014$).
0.45 ± 0.04). There were no significant differences in invasion resistance among the other seed mixes.

Fig. 1.5. Seedling emergence of the invasive alien *Impatiens glandulifera* in the six grassland mixtures and the control with no sown native seed. Grey boxes identify non-regional seed mixtures and white boxes regional seed mixtures; abbreviation of the mixtures explained in the methods section. Different letters indicate significant differences (*p* < 0.05).

4. **Discussion**

4.1 *Seed source and microsite effects on vegetation development*

Our results suggest that the origin of the seeds plays a significant role only during the first stages of grassland establishment. Positive seed source effects were found on early vegetation cover, and confirmed the considerations about regional seeds having higher bioengineering effectiveness than non-regional commercial seeds. Although this effect had no statistical significance within treatments, the results showed a clear tendency for
better establishment of regional seeds in all treatments. These findings contrast current practice favouring non-regional seed mixtures for road verges in order to accomplish fast plant cover development (Kirmer and Tischew 2006). While grassland cultivars are often bred for optimal performance under favourable site conditions, regional species might be more suitable for stressful conditions (Vander Mijnsbrugge et al. 2010, Walker et al. 2014), as for example road verges. Consequently, the better adaptation of regional seeds to the local conditions overcomes cultivar vigour. Furthermore, our results indicate that sowing densities of 5 g m\(^{-2}\) are sufficient to achieve adequate early vegetation cover. Although this value exceeded the mean sowing density used for grassland restoration in former croplands (see Török et al. 2011), it was still within standards used in practical studies with site-specific seed mixtures (Kiehl et al. 2010). This is an important aspect, because high seeding densities of more than 20 g m\(^{-2}\), as they are used for common seed mixtures like RSM7 to ensure enough plant cover, impair the performance of target species (Stolle 2006).

Some weeks later non-regional and regional seed mixtures had similar vegetation cover; this fact illustrated that seed source as a driving factor for vegetation cover had no relevance in later stages and that plant establishment was highly dynamic in the first weeks after sowing. Surveys at small-scaled time intervals comparable to our experiment therefore deliver important findings when restoring grassland in areas that rely on fast vegetation development, for example areas highly vulnerable to erosion (Gyssels et al. 2005). Moreover, no positive microsite effect of the stone treatment was observed, and regional seeds did not respond to stones with higher establishment. In all cases plots with stone treatment showed reduced vegetation cover compared to stone-free plots. An explanation of these results may be the stronger effect of reduced surface for plant growth caused by the presence of stones, compared to positive microsite effects for plant emergence. Moreover, our experiment was carried out in a greenhouse with not drought periods, which are characteristic in grasslands and road verges (Hofmann and Isselstein 2005).

When we examine vegetation cover in terms of the six seed mixtures, the RSM7 mixture exhibited lowest vegetation cover followed by its regional counterpart, RSM7R, while
the other non-regional seed mixture, RSM16C, had third smallest cover. These findings are relevant since RSM7 is a seed mixture extensively used in Germany, whereas RSM16 was specially designed for southern Bavaria. Therefore, our results strengthen the significance of choosing seed mixes that not only use regional seed material but also that are tailored for the respective region in order to obtain high early vegetation cover.

4.2. **Percentage legume seed and increasing early vegetation cover**

Vegetation cover in the early stage after sowing was affected positively by the proportions of legumes in the seed mixture. Since correlation between the proportion of legume seed and the eventual legume biomass was very low and legumes were sparsely represented in established biomass, legume seeds rather than legume biomass might have contributed to early vegetation cover of other species in the seed mixtures. Our findings support those of Li et al. (2007), who showed that on abandoned fields the increase of soil organic carbon and total nitrogen through legumes facilitated aboveground biomass and vegetation cover of target species rather than legume nitrogen fixing. Moreover, they demonstrated that legumes were able to suppress dominant species, especially from the seed bank, and therefore reduce diversity. In consequence, when establishing seed mixtures for road verges the percentage of legume species has to be chosen carefully to get a balance between facilitating effects and maintenance of local diversity.

4.3. **Grass biomass and its effect on community composition and stability**

Grass abundance of the experimental grassland communities had a strong negative effect on the number of target species, and there was a reduction of >50% in the number of target species when grasses made up >95% of total biomass. With the exception of two plots the number of species was similar for all grass contents below this critical value. As the correlation between grass abundance in established biomass and percentage grass seed was high, the proportion of grass seed in a particular seed
mixture can be used as an indicator of target community diversity for site conditions and time scales similar to our experiment. Moreover, detailed splitting into seed mixtures gave similar results regarding the negative relationship between percentage grass biomass and species diversity. Our results are therefore consistent with those who found that species richness is negatively correlated with the productivity of dominant grass species (McCain et al. 2010, Pfeifer-Meister et al. 2012).

Regarding percentage non-target species biomass from the seed bank and wind-dispersed plants from the surroundings at the early phase of the community establishment, grass biomass could not be used as a predictor. There was no relationship between grass abundance and invasion resistance, although species diversity was negatively affected by grass biomass. However, the lack of a negative effect of the grasses on the non-target species at this early stage is not surprising. For instance, Török et al. (2010) found that only after the second year the perennial grasses were able to dominate the fields and successfully suppress short-lived weeds. In terms of seed mixtures, both RSM7 and RSM7R contained almost 100% grasses, but the invasion resistance of RSM7R at this stage was significantly higher. This can be explained by the fact that native perennial grasses such as *Bromus erectus* Huds., *Festuca ovina* L., *Festuca rubra* L. and *Lolium perenne* L. in RSM7R are able to compete with invasive seeds and to shift biotic interactions for the benefit of native target species (Corbin and D’Antonio 2004). Surprisingly, this effect was seen in early vegetation stages, although Corbin and D’Antonio (2004) postulated this for later stages. Like RSM7R, its non-regional counterpart, RSM7, consisted of perennial grasses, but the lack of adaptation to specific site conditions, might have reduced perennial grass vigour and therefore thwarted invasion resistance. The same applies to RSM16C with the consequence that the two non-regional seed mixtures had highest percentage invasive biomass. Therefore, more detailed aspects of seed mixture compositions affected invasion resistance.

Another aspect to highlight is the relatively low early richness (23 spp.) of our seed mixtures compared to what can be found in natural and near-natural grasslands (40–50 spp.; cf. Dengler et al. 2014). This, however, is a rather common practice in grassland
restoration, where what is considered high-diversity seed mixtures comprise in average about the same amount of species we used. Moreover, mixtures containing lower species richness can result in semi-natural grasslands provided that the initial conditions and management techniques are suitable (Török et al. 2010).

### 4.4. The effect of functional dispersion on invasion resistance

Seed source or functional groups were no good predictors of invasion resistance to non-target species during the early stage of vegetation establishment. However, we did find an effect of functional dispersion of rooting traits and aboveground biomass measured at earlier stages when seeds of *I. glandulifera* were added. This trend was not explained only by biomass, and thus could be due to higher diversity of rooting. Our results support the idea that richness of functional traits representing competitive ability and niche filling, have a stronger positive influence on invasion resistance than richness per se (Funk et al. 2008).

Furthermore, identity of seed mixtures was a good predictor of resistance to invasion by *I. glandulifera*. Here, contrary to what was found for non-target species at an earlier phase, the least successful mixtures were RSM7 and RSM7R. This can be described as consequence of the lower species richness detected during vegetation establishment, since these mixtures presented strong differences compared to the others. The outcome supports what was found in terms of diversity of rooting traits. Nevertheless, the differences with early results on non-target species can be explained due to the lack of full development of the vegetation, and native species not yet filling the available niches. Interestingly, these less resistant mixtures are most widely used for moderately dry road verges in Germany, thus we suggest that experiments should be carried out to test if these trends persist in the long term.
5. Conclusions

Many aspects must be taken into account when choosing seed mixtures that comply with the engineering constraints of road verges, with the associated site conditions while also benefitting biodiversity. Our results confirm that regional seeds perform best regarding establishment and produce highest vegetation cover in the early stages after sowing. Microsite effects of stone treatments and interactions with seed sources are less relevant, but as the experiment was done in a greenhouse, field conditions of road verges for restoration were not reproduced exactly. Functional groups like legumes or grasses are important drivers of community composition. The prediction of invasion resistance depends on the stage of grassland development. At an early stage neither seed source nor specific seed mixture composition or functional dispersion could explain percentage of non-target species biomass. Nevertheless, the invasion of an alien species was associated with lower diversity of rooting architecture and lower species richness later during establishment. Future restoration of road verges needs to be more critical about seed source and composition which contribute strongly to the bioengineering effectiveness of grassland mixtures.

Acknowledgements

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Supporting information to Chapter 1: Bioengineering effectiveness of seed mixtures for road verges: functional composition as a predictor of grassland diversity and invasion resistance.

Appendix, Fig. S1.1. Differences in initial vegetation cover between the seed mixtures used for road verge grasslands (ANOVA, $F_5 = 2.81, p < 0.05$). Grey boxes show non-regional commercial mixtures, white boxes regional seeds; abbreviations of seed mixtures are explained in the methods sections. Different letters indicate significant differences ($p < 0.05$).
Appendix, Fig. S1.2. (a) Differences in percentage of grass biomass among the seed mixtures (ANOVA, $F_5 = 4.164, p > 0.01$); (b) Number of target species number by seed mixtures (ANOVA, $F_5 = 178.3, p < 0.001$); and (c) Percentage of non-target species biomass per seed mixture (Kruskal-Wallis test, $p < 0.0001$). Grey boxes reveal non-regional seed mixtures; white boxes reveal regional seed mixtures.
Appendix, Fig. S1.3. Functional dispersion (FDis) values per seed mixture (Kruskal-Wallis test, $p < 0.0001$). Grey boxes reveal non-regional seed mixtures; white boxes reveal regional seed mixtures.
Appendix, Fig. S1.4. Total biomass production of seed mixtures and control with no seed addition (ANOVA, $F = 6.38, p < 0.0001$). Grey boxes identify non-regional seed mixtures and white boxes regional seed mixtures; different letters indicate significant differences ($\alpha = 0.05$).
Appendix, Table S1.1. Locations where seed material of *Impatiens glandulifera* was collected from sides of roads and trails in southern Germany (October 2013). The procedure for the seed harvest was performed by selecting around five plants per patch and collecting all seeds from 3–4 fruits per plant, then repeating the technique 50 m away.

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Chapter 2

Can plant invasions be prevented by limiting similarity? The role of seed size, seed density and resource availability during roadside revegetation
The content of this chapter was submitted for publication as follows:

Yannelli F A, Hughes P, Kollmann J. Can plant invasions be prevented by limiting similarity? The role of seed size, seed density and resource availability during roadside revegetation.
Abstract

Roadsides constitute an opportunity for biodiversity restoration, but are prone to the invasion of alien plant species (IAS). We investigated limiting similarity in seed-size-output strategy and seed size as a tool to design native communities resistant to IAS at an early stage of roadside revegetation. In a first greenhouse experiment, communities with small- and large-seed-size-output strategies at two densities were confronted with one small- and one large-seeded IAS. The height of IAS, aboveground biomass, and plant regrowth recovery were measured after 4, 8 and 8–16, respectively. Another experiment investigated the seed size effect, by using small- and large-seed-size communities at constant density confronted to competition with three small- and one large-seeded IAS at two nutrient levels. Height, specific leaf area and aboveground biomass of invasive species were measured after eight weeks. IAS with large-seeded-output strategy responded to initial competition by growing taller than the native canopy, avoiding light competition. Small-seeded native communities at high density were highly effective in suppressing both seed-sized IAS, explained by a density effect. The recovery rate showed no significant differences across treatments. Large-seeded IAS reacted to competition by allocating biomass to the stem, while small-seeded allocated more to leaves, supporting findings of our first experiment. Small-seeded IAS were better suppressed by large-seeded communities and no differences were found for large-seeded IAS nor for nutrient addition. Limiting similarity in seed-size-output strategy or seed size did not completely explain resistance to IAS, but was better explained by a density- and resource-driven suppression effect.

Keywords: IAS; biotic resistance; density-driven suppression; resource-driven suppression; restoration
1. Introduction

Human activities result in land-use modification and ecosystem degradation with negative effects on biodiversity and ecosystem services worldwide (Millennium-Ecosystem-Assessment 2005). The intensification of land use and the transformation of near-natural areas to croplands or urban areas call for restoration of historical or new ecosystems in many regions (Mitsch 2012). Examples are restored ecosystems on ex-arable fields (Vesk and Mac Nally 2006, Munro et al. 2009), on abandoned opencast mines (Bradshaw 1997, Tordoff et al. 2000) and along roadsides (Tormo et al. 2008, Bochet et al. 2010).

Roadsides are ubiquitous landscape elements in most countries, with a significant role as wildlife corridors that connect natural areas and serving as habitats for native species. Networks of roadside corridors have become more important with increasing landscape fragmentation (Auffret and Cousins 2013), e.g. due to construction work and land-use change. However, roadsides also allow dispersal and establishment of invasive alien plant species (IAS; Hulme 2006), facilitated by traffic, irregular disturbance, nutrient-rich soil and a lack of competing species (Haan et al. 2012). The situation is worsened when the revegetation of roadsides is carried out with non-native or contaminated seed material (Tinsley et al. 2006). Consequently, species selection becomes a decisive step in the planning of roadside revegetation (Bochet et al. 2010), and the current challenge is to design plant communities that can prevent the establishment of IAS.

A potential method for designing plant communities that suppress IAS is the trait-based approach proposed by Funk et al. (2008). These authors suggested assembling communities for ecological restoration based on the limiting similarity hypothesis, which states that IAS will be unlikely to establish if native species with similar traits are present in the resident plant community (Funk et al. 2008). In other words, niche overlap could allow native species to prevent the establishment of invaders with similar resource acquisition strategies (Hooper and Dukes 2010). Most studies define similarity based on very broad functional categories such as phenology (Dukes 2002, Hooper and Dukes 2010), life forms (Maron and Marler 2007, Young et al. 2009), or a combination of life
form and photosynthetic pathways (Symstad 2000, Emery 2007). Support for limiting similarity in this context has been found for invasive forbs, albeit not for grasses (Price and Pärtel 2013). However, to put the usefulness of this approach to a more rigorous test it is necessary to focus on more specific functional traits that influence the competitive response of species to environmental factors.

One reason that this has not yet been done sufficiently is that it is challenging to identify the most important traits that determine community resistance to IAS in the context of roadside revegetation. Often little information is available on the soil seed bank and dispersal of native or invasive alien plant species. Moreover, the abiotic conditions of roadsides are harsh including high irradiation, extreme temperatures, low soil humidity, irregular fertilization and disturbance (Forman et al. 2003). The plant traits to consider should, therefore, be those that have an effect on species performance when faced with high abiotic stress, and on resource uptake and establishment. Seed size is a good proxy for plant performance, since it is related to seedling emergence and establishment (Westoby et al. 2002), while in terms of a ‘seed-size-output’ strategy there is a trade-off between seed mass and number of seeds produced per plant (Turnbull et al. 1999, Jakobsson and Eriksson 2000). This means that because of the resource cost, plants with a ‘large-seed strategy’ usually produce fewer seeds compared to small-seeded species. The amount of resources in the seed is positively correlated with seedling size, survival and establishment success (Westoby et al. 2002), while small-seeded species often have higher dispersal, larger soil seed banks and compensate the lower initial resources by higher growth rates (Maranon and Grubb 1993, Leishman et al. 2000, Moles et al. 2004).

According to these considerations, it seems possible to create native communities that are resistant to small- or large-seeded IAS based on the limiting similarity choosing among different seed sizes of (potential) IAS and different species of the native community. Applied at the establishment phase of a community (e.g. at the beginning of roadside revegetation measures), this seed-size approach could be translated into the native community exerting two kinds of suppression on the IAS depending on whether we focus on seed-size-output strategy or just seed size. For instance, in a community based on seed-size-output strategy, density-driven suppression (i.e. suppression due to...
high numbers of individuals) of IAS by small-seeded native species could occur due to the reduction of the amount of resources available for invading species (Moles and Westoby 2004). One should look at the relative seed density of IAS and native species, since this may also affect resistance of the invaded community and thus, could be another significant factor to be considered in terms of management. Looking at seed size alone, high resource-driven suppression can be exerted by large-seeded species due to the initial advantage of having a higher amount of resources compared to the small-seeded species. Considering this while choosing species for roadside revegetation could thus significantly enhance the competitive ability and establishment success of native species compared to IAS (Leishman 2001). Another aspect to consider when addressing resource-based suppression of IAS is the patchy resource availability commonly found on roadsides, which often favours invasions because of unused resources (Davis et al. 2000). Therefore, an increase in soil nutrient levels could lead to increased biomass of the plants growing in it, thus increasing the competitive strength of the community (Poorter and Nagel 2000).

In this study we investigated the use of limiting similarity in seed-size-output strategy and seed size at an early stage of revegetation as a tool to design roadside seed mixtures of native species that would result in communities more resistant to IAS. Furthermore, we assessed if this resistance would be explained by density-driven or resource-driven mechanisms. In a greenhouse experiment we targeted limiting similarity in terms of seed size and seed output by designing small- and large-seeded communities at two densities, and confronting them with small- and large-seeded IAS. Here we tested the following hypotheses: (1) small-seeded native communities exert higher suppression than large-seeded native communities on small-seeded IAS due to the limiting effect of similarity between seed output strategies, i.e. similarity in high density of individuals results in higher competition for resources. (2) Large-seeded native communities are more successful than small-seeded native communities at suppressing large-seeded IAS, because of higher resource availability during the initial establishment phase. (3) The addition of higher number of seeds of the native communities would enhance suppression in both cases by increasing density-driven
suppression, but also makes small-seeded communities more successful at suppressing both large- and small-seeded IAS due to less resource availability for invaders.

In a second greenhouse experiment we investigated the seed size effect. We designed small- and large-seeded native communities using a constant density and tested them against three small- and one large-seeded IAS. Here we hypothesized that: (4) depending on the seed size of the IAS and the competition pressure resulting from this, native communities differ in their resource allocation to leaves or stems. Because of these differences and in line with the limiting similarity hypothesis, (5) small-seeded IAS are stronger suppressed by small-seeded native communities and large-seeded IAS by large-seeded communities. Finally, we expected that (6) the suppression effect on the IAS decreases with nutrient availability.

2.  Material and methods

2.1.  Plant material

For the experiments we selected common IAS from roadsides in central Europe (Kowarik 2010; Essl et al. 2015). In the first experiment we used small-seeded *Solidago gigantea* Aiton and large-seeded *Ambrosia artemisiifolia* Linné, while the second one included small-seeded *Erigeron annuus* Linné, *Solidago canadensis* Linné and *S. gigantea*, and large-seeded *A. artemisiifolia*. Initially the second experiment was planned for equal numbers of large- and small-seeded IAS, but this was not possible due to the lack of germination of some large-seeded species. Seed material was collected in southern Germany and eastern Austria; further information on the origin of seed material can be found in Appendix, Table S2.1. When needed, seeds were cold-wet stratified on filter paper in Petri dishes at 4 °C for four weeks.

Native species were selected based on a common roadside mixture for Germany (categories ‘Böschungen’ and ‘Straßenbegleitgrün’; Rieger-Hofmann GmbH, Blaufelden-Raboldshausen). This species-rich mixture is tailored for roadside revegetation.
consisting mainly of grassland species, which aims to obtain fast cover and different rooting depth. Trait information on all species, such as seed mass, life span and life form, was obtained from the BiolFlor (Klotz et al. 2002) and the LEDA trait database (Kleyer et al. 2008). The two seed size levels (small vs. large) were defined according to the 0.25–0.75 percentile from the average seed mass of all species of the roadside seed mixture (<0.3 mg and >1.3 mg). In order to avoid results to be related to a single replicated community type, in both experiments the community composition of each replicates differed. In the case of the first experiment the community composition of the seed mixtures was randomly selected from the species pool, consisting of five combinations of ten native grassland species. These were chosen according to the mean seed mass, including two species of grasses and the same number of different life span in all target community mixtures (Appendix, Table S2.2). The same procedure was followed for the second experiment; though this time the mixtures consisted of five combinations of five native grassland species. Again the species were selected according to the mean seed mass, including two species of grasses in all target community mixtures (Appendix, Table S2.3). All native seed material was obtained from the local seed producer Johann Krimmer (Pulling, Germany).

2.2. Experimental design

Both experiments were carried out in unheated greenhouses at the Centre of Greenhouses and Laboratories Dürnast, School of Life Sciences Weihenstephan, Technische Universität München, Freising (48°24′N, 11°41′E). The first experiment ran over four months (early May until early September 2013) in a greenhouse with an average temperature of 22 ± 7 °C, while the second experiment lasted eight weeks (mid-May until mid-July 2014), and had a temperature of 21 ± 6 °C.

Experiments followed a completely randomized design with two factors replicated five times. In the first experiment, IAS were separately subjected to two treatments, i.e. ‘native community seed size’ (small- or large-seeded) and ‘native community seeding density’ (low at 1 g m⁻², or high at 10 g m⁻²) in order to test limiting similarity of the
seed-size-output strategy. Invasive species seeds were added at 1 g m\(^{-2}\) by mixing them with the native seeds and scattering them on top of the soil (Appendix, Fig. S2.1). The amount of IAS seeds used was chosen following average values of viable seeds found in soil seed banks for *A. artemisiifolia* and extrapolated to *S. gigantea* (Rothrock et al. 1993; Fumanal et al. 2008). Five replicates of each IAS species were grown as a control in the absence of competition with native communities. All communities were grown in 40 x 30 x 6 cm\(^3\) plastic trays.

In the second experiment we submitted four IAS to two treatments, i.e. ‘native community seed size’ (small-seeded or large-seeded) and ‘nutrient addition’ (nutrient addition or none). Native and invasive species were pre-grown separately in order to avoid the loss of replicates due to mortality of IAS individuals. Three weeks after sowing, one IAS individual was placed in the middle of the pot surrounded by a native species at a density of 15 g m\(^{-2}\) (Appendix, Fig. S2.1). The density was calculated according to the 3 g m\(^{-2}\) recommended by the local seed producer (J. Krimmer, pers. comm.), but corrected for the five times higher volume of soil used in this experiment to make it comparable to the first experiment. The ratio of seedlings in each pot was kept constant by calculating the number of seeds needed based on dividing the total sowing density by the average mass of all species used. Also in order to avoid density effects, the seed number was adjusted according to percentage of plant emergence which was obtained through prior germination trials of the native species in climatic chambers. As controls, monocultures of the eight invasive alien species were also subjected to the nutrient treatments. The nutrient treatment consisted of 10 ml of 1.5‰ Ferty\(^{®}\) 3 (15% N, 10% P\(_2\)O\(_5\), 15% K\(_2\)O), four and six weeks after sowing. For this experiment, 3 L round plastic pots were used. In both experiments peat-based substrate was used (Einheits Erde\(^{®}\), Einheitserdewerke Werkverband e.V., Altengronau, Germany; N, 180 g m\(^{-3}\); P, 240 g m\(^{-3}\); K, 240 g m\(^{-3}\); pH 5.8), and pots were initially watered every day from above until germination and plant establishment, then changed to watering when needed from beneath allowing the pots to soak for one hour.
2.3. Measurements

In the first experiment, the height of ten individuals was measured four weeks after sowing following Cornelissen et al. (2003). In early June 2013, eight weeks after sowing, plants were harvested at 1 cm above ground level. A separation into the interior vegetation within a 5 cm-wide edge was done to avoid edge effects, and the material from the edges was discarded. When harvesting, the plants were separated into IAS or native species. All samples were dried for three days at 65 °C to determine dry biomass of native and invasive species. Total biomass was collected again 12 and 16 weeks after sowing, following the same procedure. To assess the treatment effects after the first harvest, a relative recovery growth rate (RGRr) was calculated as the difference of the natural log of the IAS aboveground biomass values measured at week 12 and 16, divided by the period of time in between measurements in months (cf. Pérez-Harguindeguy et al. 2013).

In mid-July 2014, eight weeks after sowing, we did all measurements of the second experiment. Height, specific leaf area (SLA) of IAS, and aboveground biomass of both native and invasive species were measured. SLA was determined for three healthy leaves (including petiole) scanned with the computer software ImageJ (Rasband, W.S., U.S. National Institutes of Health, Bethesda, MD, USA, http://imagej.nih.gov/ij/, 1997–2014); leaves were then oven-dried to determine biomass. On fresh plant material, IAS and native species were distinguished, and the aboveground biomass of IAS was separated into leaves and stems in order to identify differences in biomass allocation. Dry biomass was done following the same procedure as above.

2.4. Data analysis

All statistical analyses were performed using R Version 3.1.0 (R Development Core Team 2014). The effect of seed size and density of native communities on small- and large-seeded IAS was evaluated in three stages, i.e. effects of the treatments after four weeks were identified by the height of IAS, after eight weeks by using aboveground biomass,
and based on plant regrowth recovery after 8–16 weeks. In order to assess the suppression effect of the native communities on the IAS in terms of height and aboveground biomass, we calculated the relative competition index (RCI) using these variables by the following formula (Weigelt and Jolliffe 2003):

\[
\text{RCI}_\gamma = \frac{Y_{\text{Control}} - Y_{\text{Treatment}}}{Y_{\text{Control}}}
\]

here \(\gamma\) corresponds to the values for height or aboveground biomass in control and under treatment. In this context, a high suppression effect of the treatment will result in RCI values closer to one, whereas negative ones would mean that the measured variable had higher values under treatments compared to the control. Since the experiment was completely randomized, an average \(\gamma\) of all replicates could be used for the statistical analysis. Non-parametric Kruskal-Wallis test was used for identifying differences in this index, because the data did not fit a normal distribution, homogeneity of variances and residual normal distribution. Subsequently, a multiple pairwise comparison among treatments was done with R package *pgirmess*.

The effect of seed size and density of small- and large-seeded IAS on RGRr of the IAS was assessed using two-way ANOVAs, and multiple comparisons were calculated under Tukey’s procedure using the R package *agricolae*.

In the second experiment, IAS were grouped together depending on whether they were small- or large-seeded to search for general patterns related to the effect of limiting similarity in terms of the seed size. To identify differences in the IAS response to competition according to resource availability associated to size seed and nutrient addition, differences in biomass allocation to leaves, stem and SLA were assessed by using ANOVA. Furthermore, we analysed differences in suppression on the two seed-sized IAS, according to seed size of the native community and nutrient addition, using the RCI and Kruskal-Wallis test. We also looked for differences in aboveground biomass production according to seed size of the native communities using a log transformation of the data and subsequently performing ANOVA.
3. Results

3.1. Limiting similarity on seed-size-output strategy and the effect of density

Community competitive effect measured as RCI for the height of the small-seeded *S. gigantea* four weeks after sowing was not influenced by the seed-size-output strategy of the native community or density, including no interaction effect ($p > 0.05$; Fig. 2.1a). In contrast, the RCI calculated from the aboveground biomass of the small-seeded IAS was significantly different for the native community with different seed sizes (Kruskal-Wallis test, $H = 11.0, p < 0.001$); with no differences between the density treatments ($H = 0.47, p > 0.05$), but with significant interactions ($H = 16.2, p < 0.01$; Fig. 2.1b). Thus, eight weeks after sowing, the RCI for aboveground biomass was closer to 1 when competing with small-seeded communities and significantly higher when small-seeded native species were sown at high density. No differences were found for RGRr of the small-seeded species in terms of native seed size (ANOVA, $F = 0.24, p > 0.05$) or density ($F = 0.14, p > 0.05$), meaning that the IAS plants produced the same rate of biomass across all treatment combinations after the first harvest.

When RCI for height was evaluated for the large-seeded *A. artemisiifolia*, we found significant differences according to native community strategy, density and the interaction. The overall negative values pointed to taller plants when facing competition than in the controls, across the experiment. Plants grew significantly taller when competing with small-seeded native communities (Kruskal-Wallis test, $H = 5.86, p < 0.05$), and also under high density ($H = 4.65, p < 0.05$). When considering the interaction, plants growing in small-seeded communities were considerably taller than in all other treatments ($H = 11.7, p < 0.01$; Fig. 2.1a). RCI for invasive biomass of the large-seeded strategist indicated higher suppression under competition with small-seeded native communities (Kruskal-Wallis test, $H = 11.6, p < 0.001$), albeit with no density effect ($H = 2.23, p > 0.05$). The interaction effect was shown by higher suppression when *A. artemisiifolia* was submitted to competition with small-seeded communities at high density, followed by the same native community at low density and finally lower suppression was found in the two densities of the large-seeded
communities ($H = 14.4, p < 0.01$; Fig. 2.1b). The large-seeded species showed no
differences in RGRr with regards to seed size of the native community (ANOVA, $F = 0.06,$
$p > 0.05$) or density ($F = 2.64, p > 0.05$).

![Graphs showing relative competition index (RCI) for height and biomass](image)

**Fig. 2.1.** Relative competition index (RCI) measured in the first experiment for a) height under competition with communities of native species with small- and large-seeded strategy (‘Ssm’ vs ‘Lsm’), for small-seeded *Solidago gigantea* and large-seeded *Ambrosia artemisiifolia* (four weeks after sowing); and b) aboveground biomass under competition with small- and large-seeded strategy native communities for *S. gigantea* and *A. artemisiifolia* (eight weeks after sowing). Different letters indicate significant differences ($p < 0.05$; mean ± SE).
3.2.  *Plant allocation and resource availability*

We found clear differences in resource allocation according to seed size of the IAS, albeit with no differences regarding nutrient addition (ANOVA, $p > 0.05$). Under competition, small-seeded IAS allocated more biomass to leaves, whereas large-seeded IAS responded with higher allocation to stem structures (ANOVA, $F = 45.3, p < 0.0001$). Moreover, small-seeded IAS displayed significantly higher mean SLA values compared to large-seeded IAS (ANOVA, $F = 55.2, p < 0.0001$).

When we assessed biomass suppression of IAS due to competition with small- and large-seeded native communities, we found significant differences only for small-seeded IAS (Fig. 2.2), although native communities differed in the amount of total aboveground biomass (ANOVA, $F = 21.6, p < 0.0001$; Fig. 2.3). These showed higher suppression when submitted to competition with large-seeded native communities (Kruskal-Wallis test, $H = 4.85, p < 0.05$). Nutrient addition did not result in differences of suppression for any of the treatment combinations for the two seed type of IAS (Kruskal-Wallis test, $p > 0.05$).

**Fig. 2.2.** Relative competition index (RCI) measured for aboveground biomass in the second experiment, under competition with small- and large-seeded native communities (‘Ssm’ vs ‘Lsm’), for small- and large-seeded IAS (eight weeks after sowing). Means ± SE; different letters indicate significant differences ($p < 0.05$).
Fig. 2.3. Total native species biomass according to their seed size. Significant differences were found according to if the communities had a large- or small-seed strategy native (‘Lsm’ vs ‘Ssm’). Different letters indicate significant differences ($p < 0.05$).

4. Discussion

4.1. Seed-size strategy and density-driven suppression

Our results indicate clear differences on how IAS responded to initial competition given by the seed-size strategy and thus, related to initial resource availability and resource capture success of the native community. The small-seeded *S. gigantea* showed no differences in terms of height at this early stage and had overall smaller seedlings compared to *A. artemisiifolia*. On the other hand, the large-seeded *A. artemisiifolia* responded to initial competition by growing taller than the canopy, allowing the species to avoid light competition, and this effect was enhanced with higher density of the native community. These results agree with studies proving that a correlation exist between seed size and seedling height (Gross 1984, Moles and Westoby 2004). In other words, large seeds result in larger seedling which allows the plants to overcome obstacles to reach the light through the competing vegetation canopy (Westoby et al. 1996). Small-seeded species, however, rely on the higher number of seed produced and
on disturbance for establishment, since they are less successful underneath closed canopies (Leishman et al. 2000). Overall, these are important aspects because the survival and establishment of some few tall individuals could lead to seed production and thus, to future spread of the IAS.

When we examined the suppression effect of the seed mixtures on both small- and large-seeded IAS, we found that a density-driven effect prevailed. Irrespective of the seed size of the IAS, small-seeded native communities at high density significantly suppressed the establishment of invasives. The success of high native seeding density as a limit for IAS colonization has also been reported before from other systems. For instance, a study performed during grassland restoration, found that the use of high-density seeding specially of high-richness mixtures generally reduced the cover of exotic species (Carter and Blair 2012). Furthermore, when planted at high densities, wet meadow native species have shown to suppress the invasive *Phalaris arundinacea* (Reinhardt Adams and Galatowitsch 2008). Here the suppression was increased when IAS were added at high densities, supporting the idea of limiting similarity in terms of density. These results can be explained as an effect of the positive correlation between sowing densities and establishment success of the sown species. Specifically, when the planting density of native species is high, the number of potentially established individuals increases and so does the resource acquisition, resulting in higher competition for them (Lockwood et al. 2005). This is consistent with the considerably lower success of large-seeded native communities, since the overall sowing density was lower due the larger mass of the seeds at the same sowing weight compared to the small-seeded mixtures. Moreover, along with the lower density, poor germination of some species included in the seed mixtures could enhance the modest performance.

There were no effects of seed size or density of the seed mixtures on relative growth recovery rate eight weeks after the first harvest. In other words, both IAS produced the same rate of biomass after the first harvest independently of the treatments and thus, there were no further differences in suppression effect. This could be explained as a consequence of the decreased aboveground competition after all plants were harvested 1 cm aboveground and would highlight the importance of light acquisition as a limiting
factor over belowground competition. The absence of treatment effects in later harvests indicates that IAS recovery was only related to their ability to resprout from the basal buds.

4.2. **Seed size as a predictor of biomass allocation and resource driven suppression**

Clear differences in resource allocation under competition were observed in our second experiment. Interestingly, small-seeded IAS allocated more biomass to the leaves than to stems and this was also supported by results for SLA. Otherwise, large-seeded IAS allocated more biomass to stem structures, which is in agreement with the results for height in the first experiment. The contrast in resource allocation can, therefore, explain the IAS different responses to competition. Our results suggest that the higher initial resource availability given by the seed, provides large-seeded species with the opportunity to grow above the native canopy and thus, the investment in leaves biomass is not crucial. Whereas when confronted to high competition, small-seeded species attempt to increase light capture by investing in leaves.

When addressing suppression of IAS according to seed size, it is interesting to notice that communities of native species differed in the amount of total biomass produced according to their seed size, with large-seeded communities producing higher quantities. Nevertheless, this was only translated into higher suppression in the case of the small-seeded IAS, where large-seeded native communities were found to exert a higher influence. Therefore, when density is held constant across the experiment, suppression seems to be driven by seed resources and would point to size asymmetric competition. In other words, large-seeded native communities produce larger individuals that are able to obtain higher amount of the contested resources, which in this case is light (Schwinning and Weiner 1998). Similar results have been found in an experiment with multispecies mixtures using three seed size classes, where a seed-size driven competitive hierarchy among seedlings was found. Therefore, in terms of competition large seeds consistently won over smaller seeds (Leishman 2001). Another study carried out in Swedish grasslands found analogous results. Here larger-seeded
species negatively affected recruitment of smaller-seeded species when three species of Asteraceae were sown into gaps of the community, with no effect found of smaller-seeded species on larger ones (Eriksson 1997).

Nutrient addition did not generate significant differences in any of the response variables measured. Nutrient content could not explain higher success measured as higher biomass. This is contrary to previous work in this area suggesting that nutrient increases should lead to an increase in biomass; that is a decrease in the suppression effect (Poorter and Nagel 2000). A possible explanation for this is that the soil used for the experiment was already nutrient-rich relative to what the plants required. Even in the treatments with no nutrient addition a great amount of resources was still unused and thus, nutrient availability was not a limiting resource across the experiment. Since nutrients were not limiting, an increase did not produce the effect of increased invasibility proposed by Davis et al. (2000).

4.3. Predicting biotic resistance through limiting similarity during roadside revegetation

In our work, limiting similarity was addressed from two perspectives, that is, from the seed-size-output strategy and from plain seed size. Our results show that addressing similarity in these terms is a complex approach, since the effects of some considered factors exceeded the similarity. For instance, one could argue that limiting similarity in the case of seed-size-output strategy used as a tool to design communities resistant to small-seeded IAS was successful; nonetheless the density effect exerted a great suppression on large-seeded IAS as well. While there was no successful way to strictly address limiting similarity in terms of large-seeded IAS from both perspectives. Hence, a better approach would be to focus on density- and resource-driven suppression, although in essence the successful density-driven suppression is not far from what explains biotic resistance by means of limiting similarity.
This hypothesis is based on resource partitioning and the assumption that functionally similar species would use resources in the same way by occupying similar niches, therefore increasing interspecific competition (MacArthur and Levins 1967; Abrams 1983). In this context and according to our results, the underlying process behind density-driven suppression is similar, while in this case niches are occupied by increased density of individuals. Because our findings are consistent for all the selected IAS, and given that native communities had a changing community composition, we are confident that our results are general and not explained by species identity alone. Lastly, since the experiment was carried out in a greenhouse, further testing in field situations would include other environmental influences not considered in our study and facilitate generalization.

5. Conclusions and implications for management

To our initial question on whether or not plant invasions can be prevented during revegetation by limiting similarity in seed-size strategy or seed size, we conclude that a better approach would be to focus on density- and resource-driven suppression, respectively. When focusing on seed-size-output strategy at an initial vegetation establishment phase, the large-seeded IAS responded to initial competition by growing taller than the canopy, allowing the species to avoid light competition. A density effect explained suppression and was evidenced by the greater effect of small-seeded native communities at high density independently of the IAS seed-size strategy; although the recovery rate after the first harvest was related to the ability of the IAS to resprout from the basal buds and did not differ across the treatments. In terms on seed size, large-seeded IAS were found to respond to competition by allocating biomass to the stem, while small-seeded allocated more to leaves. Consequently, when density in the communities was held constant, small-seeded IAS were better suppressed by large-seeded communities and explained by this seed-resource-driven suppression. In our experiment, nutrient addition did not produce differences because of the high amount of resources already present in the soil thus, not making nutrients a limiting factor.
Based on our findings we recommend that when seed mixtures for revegetation are based on seed weight, practitioners should favour the use of small-seeded species due to their high density and success at suppressing all sized IAS. While at constant density, large-seeded native communities are to be preferred because of higher suppression effect driven by seed resource. Overall, our study shows that ecological principles such as the limiting similarity in particular and resource partitioning in general, can be used to design resistant native communities meant for roadside revegetation projects.

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Supporting information to Chapter 2: Can plant invasions be prevented by limiting similarity? The role of seed size, seed density and resource availability during roadside revegetation.

Appendix, Fig. S2.1. Experimental design to test limiting similarity in terms of seed-size-output strategy and density. Both invasive alien species (IAS) were added separately to each treatment combinations and replicated five times. Five replicates of the native communities and the IAS were grown as control without competition.
Appendix, Fig. S2.2. Experimental design to test limiting similarity in terms of seed size and nutrient availability. The eight invasive alien species were subjected to the nutrient addition treatment without the native community influence and the same with the native communities in absence of invasive species as controls. All treatments and controls were replicated five times.
**Appendix, Table S2.1.** Locations and dates where invasive alien species seed material was collected from roads sides and trails.

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<th>Species</th>
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<tbody>
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<td></td>
<td>Marienhof, Germany</td>
<td>48.291204N, 11.689709E</td>
<td></td>
</tr>
<tr>
<td><em>Solidago canadensis</em></td>
<td>Freising, Germany</td>
<td>48.412119N, 11.739362E</td>
<td>Oct. 2013</td>
</tr>
<tr>
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<td></td>
<td>48.291204N, 11.689709E</td>
<td></td>
</tr>
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</table>
Appendix, Table S2.2. Seed mixtures’ species composition for the first experiment. All replicates differed in community composition by random selection of species according to seed size and life spam. Nomenclature follows Wisskirchen & Haeupler (1998).

<table>
<thead>
<tr>
<th>Species</th>
<th>Small-seeded communities (% seed content in total weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Replica 1 2 3 4 5</td>
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<tr>
<td><strong>Grasses</strong></td>
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<tr>
<td><em>Agrostis capillaris</em></td>
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</tr>
<tr>
<td><em>Briza media</em></td>
<td>- - - - 10.0</td>
</tr>
<tr>
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</tr>
<tr>
<td><em>Phleum phleoides</em></td>
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</tr>
<tr>
<td><em>Poa angustifolia</em></td>
<td>- 10.0 - - -</td>
</tr>
<tr>
<td><em>Poa pratensis</em></td>
<td>- - 10.0 - -</td>
</tr>
<tr>
<td><strong>Forbs</strong></td>
<td></td>
</tr>
<tr>
<td><em>Achillea millefolium</em></td>
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</tr>
<tr>
<td><em>Campanula rotundifolia</em></td>
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<td><em>Linaria vulgaris</em></td>
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<tr>
<td><em>Origanum vulgare</em></td>
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<tr>
<td><em>Papaver rhoes</em></td>
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</tr>
<tr>
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<td><em>Thymus praecox</em></td>
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<tr>
<td><em>Thymus pulegioides</em></td>
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</tr>
<tr>
<td><em>Verbascum thapsus</em></td>
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</tr>
</tbody>
</table>

* – continued on next page –
### Large-seeded communities

(% seed content in total weight)

<table>
<thead>
<tr>
<th></th>
<th>Bromus erectus</th>
<th>Helictotrichon pratense</th>
<th>Helictotrichon pubescens</th>
<th>Koeleria pyramidata</th>
</tr>
</thead>
<tbody>
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<td><strong>Grasses</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>Bromus erectus</em></td>
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</tr>
<tr>
<td><em>Helictotrichon pratense</em></td>
<td>10.0</td>
<td>-</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td><em>Helictotrichon pubescens</em></td>
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<td>10.0</td>
<td>10.0</td>
<td>-</td>
</tr>
<tr>
<td><em>Koeleria pyramidata</em></td>
<td>-</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td><strong>Forbs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Agrimonia eupatoria</em></td>
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<td>10.0</td>
<td></td>
<td>-</td>
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<tr>
<td><em>Anthericum ramosum</em></td>
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<td>10.0</td>
<td>-</td>
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<tr>
<td><em>Anthyllis vulneraria</em></td>
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<td>-</td>
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<td>-</td>
</tr>
<tr>
<td><em>Centaurea cyanus</em></td>
<td>-</td>
<td>-</td>
<td>10.0</td>
<td>-</td>
</tr>
<tr>
<td><em>Centaurea scabiosa</em></td>
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<td>-</td>
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</tr>
<tr>
<td><em>Dorycnium germanicum</em></td>
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<td>10.0</td>
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<tr>
<td><em>Euphorbia cyparissias</em></td>
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<td>10.0</td>
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<tr>
<td><em>Genista tinctoria</em></td>
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<td><em>Hippocrepis comosa</em></td>
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<td>-</td>
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<tr>
<td><em>Knautia arvensis</em></td>
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<td>10.0</td>
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</tr>
<tr>
<td><em>Pastinaca sativa</em></td>
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<tr>
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<td><em>Ranunculus bulbosus</em></td>
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<td><em>Rhinanthus glacialis</em></td>
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<td>-</td>
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<td><em>Rhinanthus minor</em></td>
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<td>-</td>
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<tr>
<td><em>Salvia pratensis</em></td>
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<td>-</td>
<td>10.0</td>
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</tr>
<tr>
<td><em>Sanguisorba minor</em></td>
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</tr>
<tr>
<td><em>Scabiosa canescens</em></td>
<td>10.0</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>
**Appendix, Table S2.3.** Species composition of the seed mixtures for the second experiment. All replicates differed in their species composition by random selection of the species according to seed size. The number of seeds was calculated based on dividing the total sowing density by the average mass of all species used. In order to avoid density effects, the seed number was adjusted according to percentage of plant emergence, previously obtained through prior germination trials of the native species in climatic chambers. Nomenclature follows Wisskirchen & Haeupler (1998).

<table>
<thead>
<tr>
<th>Species</th>
<th>Small-seeded communities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Replica 1</td>
</tr>
<tr>
<td>Grasses</td>
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</tr>
<tr>
<td><em>Bromus erectus</em></td>
<td>X</td>
</tr>
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<td><em>Helictotrichon pubescens</em></td>
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<tr>
<td>Forbs</td>
<td></td>
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<tr>
<td><em>Centaurea cyanus</em></td>
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<tr>
<td><em>Centaurea scabiosa</em></td>
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<tr>
<td><em>Knautia arvensis</em></td>
<td>X</td>
</tr>
<tr>
<td><em>Salvia pratensis</em></td>
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</tr>
<tr>
<td><em>Sanguisorba minor</em></td>
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<tr>
<td>Large-seeded communities</td>
<td></td>
</tr>
<tr>
<td>Grasses</td>
<td></td>
</tr>
<tr>
<td><em>Poa compressa</em></td>
<td>X</td>
</tr>
<tr>
<td><em>Agrostis capillaris</em></td>
<td>X</td>
</tr>
<tr>
<td>Forbs</td>
<td></td>
</tr>
<tr>
<td><em>Achillea millefolium</em></td>
<td>X</td>
</tr>
<tr>
<td><em>Origanum vulgare</em></td>
<td>X</td>
</tr>
<tr>
<td><em>Papaver rhoeas</em></td>
<td>X</td>
</tr>
<tr>
<td><em>Plantago media</em></td>
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</tbody>
</table>
Chapter 3

Limiting similarity and Darwin’s naturalization hypothesis: understanding the drivers of biotic resistance against invasive plant species
The content of this chapter was submitted for publication as follows:

Abstract

Several hypotheses have been proposed to explain biotic resistance of a recipient community by reducing niche opportunities for invasive alien species (IAS). The *limiting similarity hypothesis* predicts that IAS are less likely to establish in communities comprising species with similar functional traits. Similarly, *Darwin’s naturalization hypothesis* states that IAS closely related to the native community would be less successful. This study combines both perspectives to improve our understanding of the factors that control biotic resistance. For testing both hypotheses we used the invasive alien plants *Ambrosia artemisiifolia* L. and *Solidago gigantea* Aiton and grassland species commonly sown for ecological restoration in central Europe. We classified all plant species into groups based on functional traits, and calculated the phylogenetic distance among the species. In a first greenhouse experiment, we submitted the two IAS at two propagule pressures to competition with communities comprising 10 native species from the same functional group. In a second experiment, we submitted the IAS to pairwise competition with native species selected from each functional group. Responses of both IAS followed the same patterns in all experiments. From a community perspective, the highest IAS suppression was observed at low propagule pressure in communities with a high proportion of grasses and fast growing species, thus, not related to functional similarity according to our selected traits. Suppression decreased with increasing phylogenetic distance to species of the native community until reaching an asymptote. In the pairwise competition experiment, the suppression effect was better explained by phylogenetic distance than by trait similarity for both species. Phylogenetic relatedness was a good predictor of IAS suppression, providing support to Darwin’s naturalization hypothesis. Functional group identity was less suitable to predict biotic resistance in the experimental communities, weakening the limiting similarity hypothesis.

**Keywords:** *Ambrosia artemisiifolia*; biological invasions; functional traits; IAS; priority effect; propagule pressure; restoration ecology; *Solidago gigantea*
1. Introduction

The invasion of alien plant species has become a major concern worldwide due to their numerous ecological, economic and social impacts (Pimentel et al. 2000). Understanding what makes certain communities resistant to invasions and which factors control the success of invasive alien species (IAS) is essential for designing appropriate control measures (Bakker and Wilson 2004, Middleton et al. 2010). Biotic resistance can be defined as the ability of the native community to thwart the invasion success of IAS (Levine et al. 2004). Although it has been widely investigated in plant communities, specifically in order to understand how competition with resident plants can control plant invasions (Elton 1958, Levine et al. 2004), identifying which processes drive biotic resistance in communities is still poorly understood (Lockwood et al. 2013).

Possibly the best known explanation for biotic resistance dates back to Charles Elton’s Ecology of Invasions by Animals and Plants, where he postulated that a higher number of species in a community makes greater use of resources (Elton 1958). Thus, a high diversity of native species will pre-empt limiting resources and limit establishment of non-native species due to niche overlap (May and MacArthur 1972). Most of the supporting evidence for this idea stems from small-scale competition experiments (Levine et al. 2004, Hector and Bagchi 2007, Harrison et al. 2015), whereas at large spatial scales, the diversity-invasibility hypothesis has not received much empirical support (Stohlgren et al. 1999, Fridley et al. 2007). Furthermore, the hypothesis assumes a linear relationship between species number and niche filling, but fails to elucidate the role of redundancy and complementarity in for instance resource acquisition (Diaz and Cabido 2001).

Another perspective on biotic resistance stems from Charles Darwin. In The Origin of Species (Darwin 1859), he postulated that IAS that are phylogenetically closely related to the native species of a community would be less successful colonizers than IAS that are less closely related to the native species. This hypothesis is called Darwin’s naturalization hypothesis and was explained with the higher likelihood of species being ecologically similar and thus competing for the same resources (Daehler 2001). Even
though some studies have supported this prediction (Rejmánek 1996, Strauss et al. 2006), others have highlighted how spatio-temporal scales affect the outcome when addressing similarity in these terms (Thuiller et al. 2010, Li et al. 2015b). Darwin’s perspective relies on the assumption that species’ phylogenetic relatedness is associated with similarity of traits determining species co-existence, i.e. the ‘phylogenetic signal’ sensu Blomberg and Garland (2002). Therefore, relatedness can serve as a proxy for trait similarity, potentially providing information on traits that have not been quantified so far. Nevertheless, it has also been found that closely related species can present divergences in key traits affecting the invasibility of a community (Burns and Winn 2006, Funk and Vitousek 2007).

Understanding biotic resistance from a functional trait perspective could be a more promising approach (Funk et al. 2008). The limiting similarity hypothesis proposes that the successful establishment of IAS would be unlikely if the native species of the recipient community hold similar functional traits and thereby similar resource acquisition strategies as the invader (MacArthur and Levins 1967, Abrams 1983). In other words, trait similarity between the native community and the IAS would lead to increased competition for resources (Funk et al. 2008). Most studies have tested similarity by focusing on broad functional categories, i.e. phenology (Hooper and Dukes 2010), life forms (Maron and Marler 2007, Young et al. 2009), or a combination of life forms and photosynthetic pathways (Symstad 2000, Emery 2007). Though this grouping might overlook traits relevant for seedling establishment in an existing community, explaining why studies using invasive forbs found support for the limiting similarity hypothesis while others using invasive grasses did not (Price and Pärtel 2013). Plant species can be grouped into ‘functional groups’ based on similarity in several traits related to competitive ability or resource use. Such functional groups might better reflect species’ niche overlap and therefore biotic resistance through limiting similarity (Byun et al. 2013). Moreover, given that species within a functional group can have distinctive responses to environmental factors or biotic interactions, biotic resistance can be enhanced by functional redundancy of the experimental communities (Díaz and Cabido 2001).
An application of an improved understanding of the drivers of biotic resistance can be found in the field of restoration ecology (Funk et al. 2008). The various theories mentioned above can be tested when designing invasion-resistant communities, yet the functional trait-based approach provides practitioners also with the opportunity to choose species whose traits may affect other ecosystem properties (Suding et al. 2008). By designing potentially resistant communities based on functional similarity, it is possible to understand how the native functional structure can influence invasion success. Assessing the same communities by focusing on Darwin’s naturalization hypothesis would indicate whether or not trait-based similarity resistance is in fact closely related to the phylogenetic structure of the native community. Apart from the different perspectives to look at biotic resistance, several other factors related to invasion success need also be accounted for. Some studies have found that resistance can be modified by species identity and by the number of individuals arriving in the community, i.e. propagule pressure (Miller et al. 2013, Byun et al. 2015). Yet interactions between invasion resistance and the role of propagule pressure have received little attention so far. Experimentally testing invasion resistance from more than one perspective, using more than one IAS and contrasting propagule pressures, would help disentangle the factors that control biotic resistance.

In this study, we investigate whether limiting similarity in terms of functional traits can be used as a proxy for predicting biotic resistance of plant communities to IAS. Moreover, we assess if this resistance can also be associated to phylogenetic relatedness, as explained by Darwin’s naturalization hypothesis. We used two IAS confronted with native grassland species commonly sown in restoration projects in central Europe. With functional trait information obtained from two databases, we classified all species into functional groups and calculated the phylogenetic relatedness distance of each native species to both IAS. In a first greenhouse experiment we designed redundant communities, each one composed by a constant number of native species from the same functional group, and introduced two IAS at two propagule pressure levels. In a second greenhouse experiment, we cultivated the IAS pairwise with native species selected from each functional group.
Overall, we hypothesized that in our experimental system the establishment success of IAS will, at least partly, be explained by: (1) functional similarity, where invaders are less successful in communities with species from the same functional group; (2) propagule pressure, with greater invader success associated with a higher number of propagules; and (3) phylogenetic relatedness, where invaders are more successful in communities dominated by phylogenetically distant species.

2. Material and methods

2.1. Plant species, trait selection and functional grouping

We used two highly problematic IAS commonly found in disturbed areas in central Europe (Kowarik 2003), i.e. Ambrosia artemisiifolia Lineé and Solidago gigantea Aiton. Seeds of A. artemisiifolia were collected in ‘Neue Donau’, eastern Austria (48°16’N, 16°22’E), while S. gigantea was collected along the river Isar in Freising, southern Germany (48°24’N, 11°41’E). The experimental pool of native grassland species contained 54 species occurring with a frequency ≥10% in more than 100 surveys of calcareous grasslands in the agricultural landscapes north of Munich, Germany (T. Conradi, unpubl. data). The native seed material was obtained from the local seed producer Johann Krimmer (Pulling, Germany). Species nomenclature follows (Wißkirchen and Haeupler 1998).

Functional traits of the native and invasive species were used for classifying them into functional groups. Eight traits were selected, i.e. canopy height at maturity, life form, shoot morphology, root morphology, leaf dry matter, specific leaf area (SLA), seed mass and longevity. Traits such as canopy height at maturity, SLA and seed mass have been found to be correlated with invasiveness (Hamilton et al. 2005). The selected traits are proxies for species dispersal, establishment success, growth, persistence and competitive ability (Westoby et al. 2002, Funk et al. 2008), thus related to community assembly processes (Kraft et al. 2008). While longevity, i.e. if the species is perennial or annual, accounts for a temporal niche overlap (Wilsey et al. 2011). Information on the
traits was obtained from the BiolFlor (Klotz et al. 2002) and LEDA databases (Kleyer et al. 2008).

For functional grouping, the non-numerical functional traits were transformed into dummy variables with binary values, and later converted to continuous values using a principal coordinate analysis. This was accomplished using Jaccard’s distance measure saving the first five principal coordinates. Based on all functional traits, a cluster analysis was conducted using Gower’s similarity coefficient among species and Ward as the linkage method. Three functional groups were identified after clustering all species according to similarities in functional traits, i.e. FG 1–3 (Fig. 3.1); both IAS were classified in FG 2. The functional group named FG1 contained a high number of grass species, whereas FG3 had none, and FG2 was intermediate with four grass species (for trait characteristics of the functional groups see Appendix, Table S3.1). To check if clusters significantly differed from each other, a multivariate analysis of variances was performed with the same variables, showing significant differences among the clusters (F = 13.6, p < 0.0001). All functional group analyses were performed using Infostat software (Di-Rienzo et al. 2013).
Fig. 3.1. Classification of 54 grassland species and two invasive plant species (IAS) by trait similarity into three functional groups. Clustering based on the selected functional traits (canopy height at maturity, life form, shoot morphology, root morphology, leaf dry matter, specific leaf area, seed mass and longevity), using Gower’s similarity coefficient among species and the Ward linkage method.
2.2. **Phylogenetic analyses**

Phylogenetic analyses were performed using R version 3.1.0 (R Development Core Team 2014). A dated phylogenetic tree of all angiosperms was used as a backbone (Zanne et al. 2014) for constructing a phylogenetic tree of all species used in our experiments, generated by phyloGenerator (Pearse and Purvis 2013) within the R package `pez`. With the same package, we computed the distance between species pairs within the phylogenetic tree using branch lengths and calculated distance-based metrics (Appendix, Fig. S3.1). Furthermore, we checked for a phylogenetic signal in the quantitative functional traits selected using Abouheif’s test (Abouheif 1999) in the R package `adephylo`. This test corroborates the assumption that closely related species share similar ecological characteristics, i.e. functional traits. Our results partially supported this assumption by showing phylogenetic signals for seed mass and leaf mass ($p < 0.05$; Table S3.2).

2.3. **Experimental design**

Two greenhouse experiments were carried out at the Centre of Greenhouses and Laboratories Dürnast, School of Life Sciences Weihenstephan, Technical University of Munich (48°24′N, 11°41′E). The first experiment started in early July 2013 and ran for eight weeks in a semi-open greenhouse with a glass roof but wire mesh walls, so that climatic conditions were similar to the conditions outside with an average temperature of $18 \pm 7$ °C. The second experiment started in June 2014 and was carried out during eight weeks in an unheated closed greenhouse with an average temperature of $21 \pm 6$ °C.

The first experiment had a fully randomized design with five replicates and two treatments. The treatment functional group identity of the community consisted of four levels, i.e. FG1, FG2a, FG2b and FG3. There were two sets of communities designed from FG2 due to the higher amount of species included in this group and given the significance of both IAS being included in that group. The composition of each community was set by randomly selecting ten native species from each FG identified.
(community composition in Appendix, Table S3.3). If two species from the same genus were selected by chance, one was replaced by another species. The target community had a seed density of 3 g m$^{-2}$, which corresponds to common practice in grassland restoration in Central Europe (Kiehl et al. 2010). The amount of seeds added for each native species was calculated by dividing this value by the ten native species. The second treatment of the experiment was propagule pressure of the IAS that consisted of two levels, i.e. high pressure (HP) with 1 g m$^{-2}$, and low pressure (LP) with 0.03 g m$^{-2}$. The low propagule pressure was equivalent to the amount of seeds of one native species in the community, while the high level was selected according to average values of viable seeds found in soil seed banks published for *A. artemisiifolia* and extrapolated to *S. gigantea* for better comparison (Rothrock et al. 1993, Fumanal et al. 2008). Seeds of IAS were mixed with the native seeds and scattered on top of the soil. Monocultures of each IAS species at the two propagule pressures were grown as a control. All communities were grown in 40 x 30 x 6 cm$^3$ plastic trays in peat-based substrate (Einheits Erde®, Einheitserdewerke Werkverband e.V., Altengronau, Germany; N, 180 g m$^{-3}$; P, 240 g m$^{-3}$; K, 240 g m$^{-3}$; pH 5.8).

The second experiment was a competition experiment with an additive design (Snaydon 1991), replicated four times. For this experiment we randomly selected four out of ten species of each functional group and combined each of them pairwise with one of the IAS. The IAS was planted in the middle of a pot and surrounded by four equidistantly sown individuals of a native species from the pool. Additionally, each IAS was cultivated separately without competition of native species as controls. All treatments were grown in rectangular trays containing 16 pots measuring 8 x 8 x 10 cm$^3$ and using the same substrate as the first experiment. In both experiments, pots were initially watered every day from above until germination and plant establishment, then changed to watering on demand by flooding the tables and allowing the pots to saturate for one hour.
2.4. **Measurements**

In both experiments the biomass was harvested at 1 cm above ground eight weeks after sowing. In the first one, the harvesting area was placed in the centre of each tray, with 4 cm distance to the edges. This was not necessary in the second experiment. The aboveground biomass was sorted by species, and all samples were dried for three days at 65 °C and weighted immediately after this period.

2.5. **Data analysis**

All statistical analyses were performed using R version 3.1.0 (R Development Core Team 2014). From the first experiment, the biomass data of both invasive and native species were log transformed, because of lacking normal distribution and homogeneity of variances. The effect of communities’ functional group identity on the IAS at the two propagule pressures was identified by using two-way ANOVAs and multiple pairwise comparisons with Tukey tests using the R package *agricolae*. Differences in native biomass production among the communities were also identified by means of two-way ANOVAs and pairwise subsequent comparisons.

In order to test Darwin’s naturalization hypothesis, the weighted mean distance of the native species to each IAS (WMDNS) and the distance to the most abundant native species (DMANS) of each community were calculated (Thuiller et al. 2010). The WMDNS was computed by weighting the mean distance to the invader according to the amount of biomass produced per native species in the community. The effect of WMDNS and DMANS on the invasive species biomass were studied by means of nonlinear regression models using the function *nls()*. We compared different models (including a linear and logarithmic model) and selected a 2-parameter asymptotic exponential model based on AIC values (Bates and Watts 1988): \( y = a(1 - e^{-bx}) \). The relationship between diversity and IAS biomass was investigated with linear models, and differences in total native species biomass among the FG communities were analysed using ANOVA.
For the second experiment, we calculated the relative competition index (RCI) using aboveground biomass, to assess the competitive effect of the native species on the IAS using the following formula (Weigelt and Jolliffe 2003):

$$\text{RCI}_\gamma = \frac{\gamma_{\text{Control}} - \gamma_{\text{Treatment}}}{\gamma_{\text{Control}}}$$

where $\gamma$ corresponds to the values for total aboveground biomass in control and under treatment. In this context, the absence of an effect results in an RCI value of zero, a high suppression effect of the treatment results in RCI values closer to one, and facilitative effects result in negative values. Subsequently, we used the RCI values to identify whether suppression is explained by trait or phylogenetic distance to the IAS, using linear models.

3. Results

3.1. Effects of limiting similarity by functional group identity and propagule pressure

Results from the first experiment showed that IAS biomass was significantly affected by the functional identity of the community and by propagule pressure. Biomass of *A. artemisiifolia* was highly suppressed by community FG1, while it did not differ among the other FGs (ANOVA, $F = 9.07, p < 0.001$; Fig.3.2). Moreover, lower values of IAS biomass were found at low propagule pressure of the IAS ($F = 7.03, p < 0.05$). The same pattern was found for *S. gigantea*, with higher suppression exerted by community FG1 followed by FG3 (ANOVA, $F = 79.8, p < 0.001$; Fig. 3.2), and higher suppression at low propagule pressure ($F = 31.8, p < 0.001$). FG1 showed the highest amount of native biomass compared to the other FGs irrespective of the competing IAS, followed by FG3 and with no differences between the two FG2 communities (*A. artemisiifolia*: $F = 28.2, p < 0.001$; *S. gigantea*: $F = 26.5, p < 0.001$).
Fig. 3.2. Aboveground biomass of *Ambrosia artemisiifolia* and *Solidago gigantea* when sown with different native communities. Note the reduced aboveground biomass of both IAS when sown with the FG1 community. Monocultures of the IAS served as control. Different letters indicate significant differences ($p < 0.05$; pairwise comparisons with Tukey tests).
3.2. **Effect of phylogenetic distance on biotic resistance**

The phylogenetic analysis showed that the two IAS are closely related. Also, the interaction between the IAS and the communities in terms of phylogenetic distance led to similar patterns for both invasive species. When looking at WMDNS, phylogenetic distance influenced IAS establishment by a decrease in biomass at close distances in the case of both IAS (\(A. \text{artemisiifolia}\): 2-parameter asymptotic exponential model, \(a = 3.35, p < 0.001, b = 0.04, p < 0.05; \) \(S. \text{gigantea}\): 2-parameter asymptotic exponential model, \(a = 2.98, p < 0.001, b = 0.04, p = 0.07\), Fig. 3.3). Specifically, suppression decreased with increasing phylogenetic distance of the community until reaching an asymptote. In terms of DMANS, results followed the same trends. For both invasive species, lower values of IAS biomass were found in communities where the most abundant species were closely related (\(A. \text{artemisiifolia}\): 2-parameter asymptotic exponential model, \(a = 3.12, p < 0.001, b = 0.01, p < 0.05; \) \(S. \text{gigantea}\): 2-parameter asymptotic exponential model, \(a = 3.14, p < 0.001, b = 0.01, p < 0.05\); Fig. 3.3).

![Fig. 3.3. Non-linear regression between phylogenetic distance of the IAS with species of the native community and IAS biomass, for Ambrosia artemisiifolia and Solidago gigantea. Note the decrease in suppression for both IAS with increasing phylogenetic distance to the native communities. Regressions were performed using a 2-parameter asymptotic exponential model \((y = a(1 - e^{-bx}))\). Each graph shows the values and](image-url)
fitted model resulting from the weighted mean distance of the native species to each IAS (WMDNS, in grey), and those resulting from the distance to the most abundant native species (DMANS, in black).

3.3. Species-specific effects of phylogenetic vs trait similarity under competition

When each IAS was exposed to competition with one native species at a time, the suppression effect was better explained by phylogenetic distance than by trait distance for both species. There was no significant effect of trait distance on IAS suppression described by the RCI in the case of both IAS (linear model: *A. artemisiifolia*: adjusted $R^2 = -0.01, p > 0.05$; *S. gigantea*: adjusted $R^2 = 0.01, p > 0.05$; Fig. 3.4). Phylogenetic distance had a negative effect on IAS, suppressing the species as defined by RCI, yet results were only statistically significant for *S. gigantea* (linear model, adjusted $R^2 = 0.13, p < 0.05$; Fig. 3.4), even if a similar trend was found for *A. artemisiifolia* (adjusted $R^2 = 0.05, p = 0.059$; Fig. 3.4). In other words, a higher competitive effect of the native species was observed when species were closely related with the IAS, and this pattern was consistent for both IAS.
Fig. 3.4. Relationship between the RCI measured for the biomass of each IAS and the distance in trait similarity (graphs on the left, linear model: *Ambrosia artemisiifolia*: adjusted $R^2 = -0.01$, $p > 0.05$; *Solidago gigantea*: adjusted $R^2 = 0.01$, $p > 0.05$), and with the phylogenetic distance (graphs on the right, *A. artemisiifolia*: adjusted $R^2 = 0.05$, $p = 0.059$; *S. gigantea*: adjusted $R^2 = 0.13$, $p < 0.05$). Dashed grey lines separate positive and negative RCI values, related to suppression and facilitation, respectively.

4. Discussion

4.1. The role of functional group identity and propagule pressure in community resistance

Our results indicate a clear effect of the community functional group identity on the establishment of the IAS. Functional Group 1 was the most successful community in suppressing both invaders with similar trends, while no significant differences were found among the other FGs. However, this is contrary to our hypothesis of higher
suppression of the IAS in communities from the same functional group (FG2) due to limiting similarity in functional traits. Some of the most important characteristics of FG1 that may explain resistance is the higher number of grass species (six out of the ten species of the mixture) and the faster establishment observed compared to the other native communities, indicating a priority effect. The abundance of grass species in a community has been found to correlate positively with invasion resistance in field experiments during grassland restoration (Török et al. 2010), especially when the seed mixtures consisted of at least 70% perennial grasses (Lepš et al. 2007). Grasses are efficient in making use of the soil upper layers’ resources due to their extensive root systems, thus decreasing available resources for invaders (Fargione et al. 2003). Additionally, grasses have been found to negatively influence exotic species recruitment due to phenological overlap (Longo et al. 2013).

The faster development of some of the native species, particularly of the grass *Agrostis capilaris* and the forb *Achillea millefolium* might also explained IAS suppression. Generally, invasive species have been found to take the advantage of a temporal window of opportunity by early growth and development compared to native species in invaded areas (Gioria and Osborne 2014). This agrees with our findings in the less resistant communities, yet in FG1 the early development of native species most likely pre-empted resources and subsequently constrained the establishment of the IAS (Cleland et al. 2014). Thus, results from our experiment point to a temporal advantage of native species in terms of resource uptake over both IAS, demonstrating a priority effect not accounted for in our trait selection. A study conducted using Californian grassland plants supports this assumption by finding natives to display strong priority effects when germinating before invasive species (Cleland et al. 2014). The generally higher values of aboveground biomass found in community FG1 support the suppositions of a priority effect. In other words, higher aboveground biomass reflects a size-asymmetric competition by the native species increased resource acquisition (Lulow 2006). Overall, our results are in agreement with Byun et al. (2013) who found functional group identity to be a good proxy for biotic resistance, based on a functional
group containing early- and fast-growing species and suggesting its success to be related to priority effect and higher biomass yield.

Our results also show that suppression was higher at lower propagule pressure values of the IAS, nevertheless the higher success of FG1 compared to the other communities was still maintained even at the high values. Propagule pressure has been found to be a good predictor of the number of surviving invaders over time (Von Holle and Simberloff 2005), associated to the increased chances of niches being occupied by IAS. Yet the invasion success at increased propagule pressure might have a saturation point beyond which additional seeds will not increase invasion proportionally (Byun et al. 2015), which was not captured by our treatment levels. Moreover, we stress that biotic resistance was not able to completely exclude IAS during the time our experiment was running, even at low propagule pressure. Instead, *A. artemisiifolia* produced seeds, which could lead to successful recruitment if the experiment had not been stopped after eight weeks. Finally, in our experiment we only considered the number of IAS individuals arriving at the community (‘propagule size’), whereas we did not consider the temporal component given by the number of release events (‘propagule number’; Lockwood et al. 2005). Still, the number of events can play a significant role when the repeated release of propagules into a location can help sustain a population even when the initial release had an inadequate size or timing (Lockwood et al. 2005).

### 4.2. Limiting similarity vs Darwin’s naturalization hypothesis

The underlying assumption of both the limiting similarity and Darwin’s naturalization hypothesis is that we can predict invasion success by comparing the IAS with the functional or phylogenetic composition of the native community (Ordonez 2013). With both experiments, we found no supporting evidence for limiting similarity in terms of functional distance based on the selected traits; nonetheless, we found evidence that phylogenetic distance can be used as a predictor for invasion success.
Several experimental studies performed by assembling communities have found supporting results for limiting similarity based on traits such as phenology, life forms and photosynthetic pathways (Dukes 2002, Emery 2007, Young et al. 2009, Hooper and Dukes 2010), while others based on family, life forms and functional traits did not (Lulow 2006, Emery 2007, Byun et al. 2013). We can explain the lack of positive results in this context as a result of several factors. A first explanation is related to the use of trait databases as sources for information. Trait information is measured for fully matured adult plants and may not reflect the effect of limiting similarity during the establishment phase, with the exception of seed mass (Hooper and Dukes 2010). Another limitation of our classification is the assumption that all species within a functional group are competitively equivalent (Hubbell 2005), while competitive hierarchies can result from abiotic conditions or priority effects (Firn et al. 2010). Additionally, the lack of a pattern explaining suppression in terms of trait similarity could be due to high variability in trait information. In other words, when the trait information is analysed through cluster analysis, even if some of the traits show a pattern explaining resistance, such a pattern could be masked by other traits included in the analysis. Assessing limiting similarity in these terms proves to be challenging when trying to obtain trait information for all niche dimensions. As pointed out before, we might have failed to include traits related to temporality that could have been helpful to explain our results in terms of limiting similarity.

In terms of Darwin’s naturalization hypothesis, our results confirm that phylogenetic relatedness explained suppression of both IAS under competition with native species. Thus supporting the idea that phylogenetic similarity of communities can be used as predictor of biotic resistance as found by previous studies (Rejmánek 1996, Strauss et al. 2006). Furthermore, it agrees with a previous study that found the average size of A. artemisiifolia to increase with phylogenetic distance in an experimental setting (Li et al. 2015b). At a community level, however, we did not find a linear relationship between phylogenetic distance and IAS biomass; instead, suppression increased at low values of WMDNS and DMANS, while at higher values a saturation point existed representing a limit to how much better IAS could establish. This is not surprising, given that there is a
limited amount of resources such as nutrients or light that species can make use of. The similarity in suppression patterns found for both selected metrics can be explained as a result of the high abundance of native closely related species in FG1 such as *Achillea millefolium*. These findings support the idea that the most important role in biotic resistance is played by a limited number of dominant species (Thuiller et al. 2010).

Biotic resistance in our experiments was better described in terms of phylogenetic distance, showing that relatedness is a better indicator of biotic resistance than trait similarity. These results support Darwin’s naturalization hypothesis but not the limiting similarity hypothesis. The most likely explanation lies in the failure to include extra or more accurate niche-wise traits. Overall, our experiments provide evidence of the changes in community composition given by earlier species replacement by IAS that is not accounted for in most studies dealing with Darwin’s naturalization hypothesis, where only post-establishment alien populations and established native communities are considered (Li et al. 2015a). Finally, our results support the idea of designing resistant communities for instance in the case of restoration, based on Darwin’s naturalization hypothesis rather than on limiting similarity of functional aspects.

5. Conclusions

Limiting similarity given by functional group identity was not a good predictor for biotic resistance in our study according to the traits we selected. When both IAS were subject to competition with native communities, suppression seems to be related to grass species abundance and earlier establishment of native compared to invasive species, indicating a priority effect. Higher suppression was observed at low propagule pressure but this did not prevent *A. artemisiifolia* to produce seeds, which can lead to potential recruitment and thus to weak biotic resistance in the long term. Additionally, comparing suppression according to distance in trait similarity and phylogenetic distance, biotic resistance was better predicted by phylogenetic relatedness as explained by Darwin’s naturalization hypothesis. This indicates that we might have failed to include important
niche-related functional traits, for instance related to priority effects. Overall, our results have significant implications for managing plant invasions especially when communities have to be planned for restoration projects. In order to reduce the success of IAS, early growing native species that are closely related to IAS present in the area should be preferred.

Acknowledgements

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Supporting information to Chapter 3: Limiting similarity and Darwin’s naturalization hypothesis: understanding the drivers of biotic resistance against invasive plant species.

Appendix, Fig. S3.1. Phylogeny of all native and invasive species used for our experiment generated by using a phylogenetic tree of all angiosperms as a backbone from Zanne et al. (2014). Colours indicate whether species were IAS or in the case of natives to which functional group they belonged according to the cluster analysis.
Appendix, Table S3.1. Characteristics of each functional group classified based on a cluster analysis using information on eight functional traits obtained from two databases, for all native and invasive species. In the case of qualitative traits, when more than one of these traits can be found in each functional plant group, the most representative trait included in each is shown in bold (highest mean > 0, when transformed to dummy values). For quantitative traits, characteristics of each group are presented by the mean and standard deviation (mean ± SD).

<table>
<thead>
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<th>Functional group</th>
<th>Functional group</th>
<th>Functional group</th>
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<td><strong>Rhizome,</strong> runner, pleiocorm, tuft</td>
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<td>SLA (mm²mg⁻¹)</td>
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<td>Dry leaf mass (mg)</td>
<td>276.9±88.8</td>
<td>236.8 ± 60.6</td>
<td>195.3 ± 54.7</td>
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Appendix, Table S3.2. Abouheif test results for the four quantitative traits used in the classification of functional groups. Traits holding significant phylogenetic signal are shown in bold.

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General discussion

In view of the threat that alien invasive species pose to ecosystem diversity and services, their management becomes an indisputable obligation (Hobbs and Harris 2001). This dissertation used a theoretical approach for the design of communities that would be resistant to potential invasions and tested these experimentally in order to advance our understanding of the processes behind biotic resistance. The numerous hypotheses posed for explaining biotic resistance of plant communities provide us with a starting point, but other factors related to the community or species composition might specifically affect how native species prevent IAS establishment by occupying available niches. In the context of ecological restoration this could be evidenced by manipulating the seed mixtures species composition and submitting the resulting communities to competition with IAS (Chapters 1–3). Overall, the aim of this dissertation was to identify which characteristics of the native community and species composition aimed for restoration, would describe the biotic environment and thus be significant in order to determine the drivers of competition related to niche overlap among native and invasive species. In the following section I discuss the results of the three chapters concerning the factors that have an effect on biotic resistance and thus should be taken into account for the design of seed mixtures for grassland restoration in areas prone to invasions. Moreover, I will summarize additional factors found in this dissertation to be important for the outcome of vegetation establishment during roadside revegetation and thus, have significant implications for restoration.

1. **Community diversity and composition as a predictor of biotic resistance**

The effect on biotic resistance that diversity in terms of species or functional group richness may have, is a highly discussed topic in the field of invasion ecology (Elton 1958, Lockwood et al. 2013). The rationale behind this idea is that higher number of species or functional groups would increase the chances of native species pre-empting
limiting resources, thus leaving fewer resources for IAS (MacArthur and Levins 1967). This is based on the expectation that the community traits will be well dispersed over the available niche space, limiting establishment of new species (Funk et al. 2008). This agrees with results from Chapter 1 where functional diversity measured from rooting traits was found to negatively affect the establishment of *I. glandulifera*. Nevertheless, at the early stage of establishment suppression was not explained by species richness, supporting the idea that high number of species does not always relate with trait dispersion and thus, niche filling.

A lack of correlation between species richness and functional diversity in terms of biotic resistance can be explained as a result of a non-linear relationship between species number and niche filling. In other words, co-occurring species in a community can share similar traits and thus increase the functional redundancy of the trait space, still living vacant niches (Díaz and Cabido 2001). Furthermore, positive relationship between diversity and invasibility can occur if facilitating effects emerge (e.g. given by the presence of nitrogen fixing species), allowing new species such as IAS to establish (Fridley et al. 2007). However, *I. glandulifera* was found to have better established in communities with lower species richness, indicating that the lack of full development of the vegetation might lead to available niches (Chapter 1).

Overall, results presented in this dissertation agree with the idea that there is a positive relationship between diversity and biotic resistance. Most likely as a consequence of resource use complementarity driven by diversity in rooting characteristics. As a result, in terms of the design of seed mixtures for restoration, by increasing the number of species with different in rooting traits one can assure resource capture, leaving fewer resource available for invaders. Additionally, it is important to plan other factors related to species composition that might have an effect in the resulting community's diversity during vegetation establishment. In this dissertation, the proportion of grass species was found to have an effect on the number of target species (Chapter 1) and on the overall resistance of the native community (Chapter 3).
1.1. Grass biomass and its effect on community diversity and resistance

Other factor pertinent to both community diversity and biotic resistance is the proportion of grasses, given that it is known to have an effect on both and thus should be taken in consideration when designing seed mixtures for restoration. Grass abundance in a community has been found to negatively correlate with species richness (McCain et al. 2010, Pfeifer-Meister et al. 2012), while often having a positive effect on invasion resistance (Törökö et al. 2010, Longo et al. 2013), especially when the seed mixtures consisted of at least 70% perennial grasses (Lepš et al. 2007). Nevertheless, in Chapter 1, grass biomass was found to have a strong negative effect on the number of target native species leading to a decrease in invasion resistance. That is, when more than 95% community biomass was composed by grass species, native species richness was reduced by 50%, increasing IAS establishment.

In contrast, results from Chapter 3 point to a positive effect of grass abundance on invasion resistance, given that the most successful communities at suppressing IAS had a high proportion of grass species in the seed mixture. Here, success in invasion resistance was potentially explained as a consequence of efficient in making use of the resources in the upper soil horizons due to the common extensive root systems found for grasses (Fargione et al. 2003) and phenological overlap (Longo et al. 2013). Furthermore, a study by Lepš et al. (2007) concluded that the abundance of grass species has a positive effect on biotic resistance during grassland restoration when the seed mixtures consist of at least 70% perennial grasses. Therefore, these inconsistencies might indicate that grass proportion would positively affect community resistance to invasions but that a threshold exists from where the increase in the proportion of grass species would switch to having a negative effect due to low diversity. Overall, when designing seed mixtures the proportions of grass species should be carefully selected and according to results shown, this should preferably not exceed 70%.
2. **Limiting similarity: the role of density, biomass and timing on resistance**

Several studies have tried to use the limiting similarity hypothesis either to describe patterns found in natural systems or in order to design communities that could resist invasions during restoration (Funk et al. 2008, Thompson et al. 2010, Larson et al. 2013, Price and Pärtel 2013). As in the case of the diversity-invasibility hypothesis, biotic resistance is linked to the ability of native species to fill available niches, but in this case by increasing the similarity in species having similar traits related to resource acquisition (MacArthur and Levins 1967, Abrams 1983). With the increasing availability of trait information on plant species worldwide that can be easily accessed through trait databases (Klotz et al. 2002, Kleyer et al. 2008, Kattge et al. 2011), there is a great interest in using limiting similarity by functional traits as a method for assembling resistant communities during restoration (Funk et al. 2008, Laughlin 2014, Ostertag et al. 2015). This dissertation addressed limiting similarity from two perspectives, i.e. using a single- and multi-trait approach. In Chapter 1, seed-size-output strategy and plain seed size was used in order to design native communities that by having a similar effect with resource uptake strategies with IAS during establishment would be resistant to them. Chapter 3 addressed similarity in terms of functional groups by classifying all species into functional groups using seven traits obtained from databases, i.e. canopy height at maturity, life form, shoot morphology, root morphology, leaf dry matter, specific leaf area (SLA), seed mass and longevity. These traits were selected because their effect on species dispersal, establishment, growth, persistency and competitive ability (Westoby 1998, Cornelissen et al. 2003, Hamilton et al. 2005).

From the seed size perspective, results were partially positive indicated that testing limiting similarity in these terms is a complex issue. Specifically, IAS with small-seed-output strategy were successfully suppressed by communities sharing the same strategy. Yet the high density of individuals exerted a great suppression on large-seeded IAS as well. In terms of seed-size, results from Chapter 1 did not support the limiting similarity hypothesis with large-seeded communities only being successful at suppressing small-seed IAS and no differences among treatments for large-seeded IAS. These conclusions point instead to other emerging factors explaining biotic resistance of
the communities, i.e. density and biomass-driven suppression. On the other hand, from the multi-trait approach, IAS suppression was explained as a consequence of priority effects rather than limiting similarity according to the selected traits, contrary to what hypothesized. Nevertheless, this experiment provides little evidence for limiting similarity, as even if priority effect was not accounted for in the selected traits, the success in suppression was a result of similarity in time of development.

2.1. Density, biomass and priority as predictors of size-asymmetric competition

The effect of density is explained as a consequence of increased chances of establishment when the number of native species is high (Turnbull et al. 2000). Consequently, reflecting size-asymmetric competition resulting by the increased native species’ resource acquisition (Lockwood et al. 2005). Therefore, as concluded in Chapter 2, native species seeding density could be used as a tool for developing communities resistant to invasions during grassland restoration. Nonetheless, given that seed material can be expensive (Török et al. 2011), seed quantities should be carefully planned in order not to saturate available space thus resulting in seed waste.

In all three main chapters biomass was found to be negatively correlated to IAS establishment. As in the case of density, biomass-driven suppression also reflects size-asymmetric competition for available resources. In other words, the ability of the native community to capture the contested resources would be translated into biomass yield and would lead to high competition for them (Lulow 2006).

Nevertheless, according to results from this dissertation, differences in the overall biomass of the native community can come from a high proportion of grass species (Chapter 1), native species having bigger seeds (Chapter 2) and early establishment of fast growing species in a community (Chapter 3). Some of these aspects can be included in order to increase invasion resistance of seed mixtures used for ecological restoration. Nonetheless, since biomass accumulation might hamper the development of vulnerable
and low growing species, additional mowing or grazing should be carried out in order to prevent the dominance of few species.

Priority effects related to early establishment and fast-growing native species were found to have an effect on biomass and thus, on the overall resistance of the community to IAS (Chapter 3). The fast development of species such as the grass *Agrostis capillaris* and the forb *Achillea millefolium*, improved the chances of native species to pre-empt resources and inhibit the establishment of co-occurring IAS. As discussed in Chapter 3, invasive species have been found to have early growth and development compared to native species in invaded areas (Gioria and Osborne 2014). This allows them to profit from a temporal window of opportunity in communities where species have slower development. Consequently, when designing communities based on the limiting similarity hypothesis, traits related to temporality in resource uptake as well as those correlated with competitive ability should be taken into consideration.

Overall, the effect of density, biomass and priority can be also explained by modifying the limiting similarity hypothesis. As discussed in Chapter 2, this hypothesis is based on the assumption that niche partitioning exist and that native species will increase interspecific competition with IAS by occupying similar niches (MacArthur & Levins 1967; Abrams 1983). Here, the underlying processes behind positive results in communities’ resistance to invasions were related to similar niches being occupied but explained in terms of space and temporality. In the context of target IAS known to produce large numbers of seeds, limiting similarity can be translated into density-driven suppression by increased numbers of native individuals occupying ‘space niches’. Whereas limiting similarity in terms of resource acquisition can be accomplished by selecting species with similar strategies space and temporal niches. The challenge remains in selecting the most appropriate traits that could reflect these mechanisms.
3. The role of phylogenetic distance in preventing IAS establishment

The evolutionary perspective of biotic resistance, as a way to determine the likelihood of an invasive species to successfully establish in a community according their phylogenetic position has become more common with the increase of detailed genetic information (Ordonez 2013, Li et al. 2015a, Li et al. 2015b, Marx et al. 2015). In the context of Darwin’s naturalization hypothesis, communities composed by phylogenetically closely related native species to the arriving IAS would be more resistant than those integrated by less related species. In other words, alien species might be able to colonize communities by filling phylogenetic space not used by native species (Ordonez 2013). Results presented in Chapter 3 support this assumption by showing a negative effect of relatedness of the native community on the invasive species biomass. Therefore, in agreement with studies postulating that phylogenetic similarity of communities can be used as predictor of biotic resistance (Rejmánek 1996, Strauss et al. 2006). Nonetheless, Darwin’s naturalization hypothesis is based on the underlying assumption that phylogenetic relatedness is associated with species similarity of traits determining species co-existence. While in Chapter 3 there was a lack of correlation of between the effects of phylogenetic relatedness and trait similarity according to the selected traits. A plausible explanation for this could be that closely related species can present variances in key traits affecting the invasibility of a community (Burns and Winn 2006, Funk and Vitousek 2007). Therefore, traits responsible for the resistance effect found in one of the communities did not correspond to traits selected in Chapter 3.

4. Integrating all aspects for explaining biotic resistance

Several factors have been investigated throughout this dissertation in order to elucidate the fundamental mechanisms driving biotic resistance in plant communities against IAS. In invasion ecology, most hypotheses addressing biotic resistance have focused on competitive interactions that would ultimately be translated into niches overlap
between native and invasive species (Elton 1958, Levine et al. 2004, Lockwood et al. 2013). This dissertation shows that niche overlap can be described as a result of different factors related to resource acquisition as summarized in Fig. V. In Chapter I, a clear positive effect of functional diversity on resistance was found, supporting the diversity-invasibility hypothesis. As a result, evidencing that an increase in functional dispersion in a community can lead to niche overlap; decreasing the chances of available niches to be occupied by IAS. From the limiting similarity hypothesis perspective, I found only partial support, if strictly focusing on the selected traits. Therefore, showing that trying to obtain trait information representing all niche dimensions for assessing limiting similarity can be challenging. While a better way to explain suppression was by breaking down similarity in terms of the effect of community density, biomass, priority effect and composition. As pointed out before, we might have failed to include traits reflecting species time of development that could have been helpful for explaining our results in terms of limiting similarity. On the other hand, biotic resistance was well described in terms of phylogenetic distance, providing evidence for Darwin’s naturalization hypothesis. This supports the idea of choosing closely related native species for designing communities resistant to co-occurring IAS, in the context of ecological restoration.
Although biotic resistance is considered to be one of the most persistent conceptual paradigms in invasion ecology, identifying which processes drive biotic resistance in communities is still challenging (Kennedy et al. 2002, Lockwood et al. 2013). Moreover, further questions remain unsolved, that is on how effective biotic resistance can be and on whether is possible to completely prevent invasions (Levine et al. 2004). Results from Chapter 3, showed that biotic resistance was not able to completely exclude IAS during the time our experiment was running, even at low IAS propagule pressure. Therefore, in agreement with what found by Levine et al. (2004) plant-plant interactions alone are apparently not able to completely suppress IAS.
5. **Other factors to be considered during restoration – Implications of roadside revegetation**

In two chapters of this dissertation roadsides were used as model systems for testing the different hypothesis in order to design communities resistant to invasions. Yet preventing the success of IAS is usually only one goal for roadside revegetation. While other aspects such as fast cover, diversity and the effect that the abiotic environment might have on the resulting community should also be considered. From the perspective of obtaining a fast cover of the surface to be revegetated, a positive effect of using regional material and including a high proportion of legumes in seed mixtures was found in Chapter 1. This reaffirms the need of using regional seed material in order to obtain fast vegetation development (Kirmer and Tischew 2006) and decreases the risk of altering the genetic integrity of native populations or introducing vigorous cultivars that might reduce species diversity (Gustafson et al. 2014). The percentage of legumes in the seed mixture was also found to have a positive effect on vegetation cover at an early stage of development (Chapter 1). Nevertheless, because of the effect that legumes might have on the abiotic environment by nutrient enrichment, the percentage of legume species has to be chosen carefully in order to get a balance between facilitating effects and maintenance of local diversity.

Abiotic characteristics known to be common in roadsides and that become challenging to vegetation establishment and invasion resistance, are the presence of stones (i.e. microsites) and nutrient enrichment. Even though microsites created by the stony substrate often used in roadsides can create safe sites for plant germination by protecting seedlings from desiccation, in Chapter 1 no positive effects were observed. Instead, plots with stone treatment showed reduced vegetation cover compared to stone-free plots. The lack of positive effects was explained due to the benign conditions found in the greenhouse.

In terms of nutrient availability, it did not have the expected effect facilitating the establishment of IAS compared to treatments with no nutrient addition (Chapter 2; Davis et al 2000). Again, the artificial conditions of the greenhouse experiment this time
given by nutrient-rich substrate might explain the absence of an effect. That is, the soil used for the greenhouse experiment already had plenty of resources necessary for the plant, leaving a great amount of unused nutrients. Hence, some of the abiotic conditions found in roadsides could not be accurately imitated by a greenhouse experiment thus, pointing to the need of further testing under field conditions. Though, the more controlled conditions of the greenhouse decreased the level of noise in the results by eliminating other effects. Given the consistent results using different grassland composition and different IAS, there is a good potential for some generalization of the results found in this dissertation.
General conclusions

The negative impact of invasive alien plant species on biodiversity and ecosystem functions have fostered an increased interest in understanding the factors explaining biotic resistance of plant communities used for restoration. The dissertation combines a theoretical approach with various greenhouse experiments for designing communities resistant to invasions. The species selected for experimentation are native grassland plants used for restoration and common European IAS. As model ecosystems roadsides were chosen to assess the success of such communities in a restoration context.

The dissertation assessed biotic resistance from the perspective of the diversity-invasibility, limiting similarity and Darwin’s naturalization hypotheses. These hypotheses also provide evidence for other emerging factors that should be taken into account when designing seed mixtures for restoration. They are based on the idea that by increasing the number of species in a community or the similarity between the invaders and the native community, fewer resources would be left for the IAS. The results of the dissertation are in agreement with the idea of a positive relationship between diversity and biotic resistance. Nevertheless, since the proportion of grasses might have a negative effect on diversity and thus on IAS, when designing seed mixtures the proportions of grass species should be carefully selected. If the amount of grass species is correctly chosen, the positive effects on biotic resistance might prevail. Only partial support was found for the limiting similarity hypothesis from a plant trait perspective. Instead, the effect of density, biomass and priority had a clearer effect on IAS establishment. On the other hand, the dissertation supports Darwin’s naturalization hypothesis, i.e. a positive effect of relatedness on biotic resistance. Nonetheless, biotic resistance of plant communities was not able to completely exclude IAS during the time the experiments were running, even at low propagule pressure of IAS.

Other results with implications for roadside revegetation related to fast cover, diversity and the effect that the abiotic environment might have on the resulting communities were discussed as well. A positive effect of using regional material and including a high
proportion of legumes in early cover was found, reaffirming the arguments in favour of regional seed material to obtain fast vegetation development. Microsites created by the stony substrate often used in roadsides had a negative effect on vegetation cover, explained as a result of benign conditions in the greenhouse. Nutrient availability had no effect on the establishment of IAS, since nutrients were not a limiting factor in the potting substrate. Hence, some of the abiotic conditions found in roadsides could not be accurately described by a greenhouse experiment even if these experiments allowed for the generalization of other mechanisms related to species competitive interactions.

Overall, the results obtained in this dissertation improve the current understanding of the effect of species composition on biotic resistance of a community. This supports the idea that a theoretical framework is a promising approach for designing communities aimed for restoration of areas prone to invasions. The results of the dissertation will help developing new methods for controlling invasive species during land management.
Publications and author contributions

The following publications derive from this thesis, for each of which the author contributions are also outlined:

Chapter 1

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Author contributions: JK contributed with the basic idea for the experiment. KS, ML and myself, prepared, conducted and collected data from the experiment. The statistical analysis and draft of the manuscript was done by KS and myself. All authors did literature research. JK significantly contributed to structuring and revising the manuscript for publication.

Chapter 2


Author contributions: The general idea for these experiments were conceived by FY and JK. PH and I, carried out the experiment and collected data for analysis. Statistical analysis were planned and implemented by myself. I drafted the manuscript, which JK and PH significantly improved with their comments.
Chapter 3


Author contributions: FY and CK conceived the initial idea and JK contributed with suggestions to improve the experiment. CK and myself jointly performed the experiment and collected the data. I planned the statistical analysis and drafted the first manuscript. CK, JMJ and JK improved the focus and content of the manuscript.
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