Reaktionskurven und Nischenquantifizierung von Gefäßpflanzen für Wiedereinbürgerung

Species response curves and niche quantification of vascular plants in the context of plant reintroduction

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- Acknowledgement: Additional thanks to the committee members and my husband.
- Chapter 8: Figure numbering was corrected and placement of figure 7.2 was changed.
- Chapter 9: Changed “this might lack of studies be due to” to “this lack of studies might be due to”

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Summary

The current loss of biodiversity is one of the most disastrous threats to the earth’s biosphere. The world is facing the biggest extinction event since the decline of the dinosaurs, with one third of all plant species being rare, endangered or at the risk of extinction. At local and regional scales the situation is even worse with habitat specialists being especially vulnerable to environmental changes that are thought to be the main reason for species declines. To save these species from extinction, it is important to understand the relationship between plants and their environment, identify the drivers of species distributions and rarity, and develop conservation techniques based on sound ecological knowledge.

Soil factors were found to be very important in determining species distributions at local and regional scales. However, they have rarely been considered in studies of species distributions so far. The present thesis examines the plant-environmental relationship with a special focus on soil parameters. Therefore, two approaches were followed – a modelling approach and an experimental approach. For the modelling approach, Huisman-Olff-Fresco (HOF) models were used to analyze the responses of herbaceous forest and grassland species along an indirect and a resource soil gradient. Species ecological niche parameters – optima and limits – were calculated and related to the species’ rarity, range size and population trend. The newly introduced niche limits determined the measures of rarity better than commonly used niche parameters and Ellenberg indicator values. Moreover, the limits revealed distinct differences in environmental tolerance between species with similar ecological optima. Thus, they provide important additional information to common niche measures in identifying species vulnerable to environmental change. One drawback of the HOF models is their sensitivity to unbalanced or small data sets. To build reliable models in response to soil factors also for rare species, it is of utmost importance to continuously collect small-scale, plot-based soil data and share it in online databases.

In the experimental approach, scientific knowledge on plant-soil interactions was used to facilitate the cultivation and acclimatization success in plants grown for reintroduction. Five regionally rare plant species were grown on three soil types and were partly inoculated with plant-growth-promoting-rhizobacteria. Plant growth and rhizobacteria communities were analyzed during cultivation and after transplanting the plant species to their new habitats. Germination and plant growth were strongly influenced by the soil, with slightly varying results regarding the preferred soil type among plant species. Overall, plants performed best on natural soil which was collected near the seed donor populations from their natural habitats. However, no strong long-term effects could be found in plant
growth after transplanting. Bacteria communities in the rhizosphere differed significantly between soil types and host plants. In contrast to the findings for plant growth, the bacteria communities still showed remarkable differences one year after transplanting, caused by the soil type used for cultivation. The results suggest that using suitable soil might boost cultivation success in reintroductions of rare plant species. The role of natural rhizobacteria communities in cultivation and acclimatization success during reintroduction needs further evaluation, however, promising results have already been found in plant community restoration when using microbial applications. The inoculation with plant-growth-promoting-rhizobacteria had no clear effect on neither plant growth nor the rhizobacterial community.

To conclude, the present thesis shows that soil factors play a major role in plant species distribution and rarity, as well as in practical conservation techniques, such as plant reintroduction. Both, the limits of ecological niches and soil microbes are important factors in conservation, which deserve more attention in future studies of rare plant species. Reliable predictions of species responses to environmental changes and widely applicable conservation techniques are needed to save endangered species from extinction.


Im Experiment wurden Kenntnisse über die Interaktionen zwischen Pflanzen und Boden genutzt, um den Erfolg bei der Anzucht und der Eingewöhnung von Pflanzen bei Wiedereinbürgerungen zu erhöhen. Fünf Pflanzenarten, die im Untersuchungsgebiet selten sind, wurden auf drei verschiedenen Bodentypen ausgesät und aufgezogen. Jeweils die Hälfte der Pflanzen wurde mit

Chapter 1

Introduction
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General introduction

The loss of biodiversity and the extinction of species have exceeded the planetary boundary by far and are considered to be among the biggest threats to Earth (Rockström et al. 2009; Hooper et al. 2012; Steffen et al. 2015). For every million species on the planet, we are losing approximately 100 species per year, which is about 1000 times more than at preindustrial times (De Vos et al. 2015). While these numbers are shocking, another bigger and more immediate threat is disregarded – the loss of local habitat specialists and the homogenization of habitats (McKinney & Lockwood 1999; Hewitt et al. 2010; Clavel et al. 2011). This may even have a more profound and noticeable effect on ecosystems and their services than global extinction rates (Newbold et al. 2015; Gonzalez et al. 2016). The most important causes of biodiversity loss are biotic invasions, overexploitation, pollution (e.g. nitrogen depositions), land use change (e.g. habitat loss or degradation) and climate change (Sala et al. 2000; Matesanz et al. 2010). Among the environmental threats to plants, climate change, determining global distribution, may also gain more importance on smaller scales in the future, but habitat availability and edaphic changes are much more important at the local and regional levels (Coudun et al. 2006; Bertrand et al. 2012). Within the scope of these alarming events, it is necessary to immediately take action to slow down extinction rates and conserve biodiversity and ecosystem functioning at all spatial scales. Therefore, there is an urgent need for an in-depth understanding of the interactions between species and the environment, as e.g. represented by the ecological niche concept, to make sound conservation decisions and improve the techniques used to save declining species from extinction.

The ecological niche

The ecological niche is one of the fundamental concepts of ecology. Although there have been different niche concepts (Grinnell 1917; Elton 1927), the commonly used definition is from Hutchinson (1957), who distinguishes between the fundamental and the realized niche. The fundamental niche can be seen as an n-dimensional space of environmental variables in which a species is able to survive and maintain a viable population. The species’ fundamental niche response towards a single environmental gradient is often assumed to follow a symmetric bell-shaped curve (Austin 2013b). In the presence of competitors, and of other biotic interactive factors, the species is restricted to its realized niche. A species’ realized (ecological) niche response might be displaced from the fundamental (physiological) response, resulting in skewed or bimodal response curves. The type, number and the relative importance of environmental variables varies between different species, according to their biotic and geographical context (Begon et al. 2006).
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Over the last decades, the niche concept has gained increasing attention again in the ecological, conservational and global change literature (Sax et al. 2013). The ecological niche describes and characterizes the ecological features of a species, and thus provides a basis for species distribution modelling and the prediction of future distribution shifts due to climate or other environmental changes. However, estimating the niche of a species is difficult, especially in plants, because it consists of a high number of environmental variables and is additionally influenced by a high number of biotic factors. Since our understanding of the nature of niches is still obscure, Turnbull (2014) called them “the dark matter” of ecology.

Nevertheless, the ecological niche is the basis for another ecological concept – specialization, the evolution of generalist and specialist species (Futuyma & Moreno 1988; Kassen 2002). Generalists are thought to have a wide tolerance towards heterogeneous and disturbed environments (wide niches), whereas specialists are likely to occur in homogeneous and relatively stable environments (small niches). Paleontological studies have shown that in past extinction events, the risk of extinction was smaller for generalists than for specialists (McKinney 1997). Thus, specialization is thought to be a major trait promoting extinction.

Niche theory is central to our understanding of species distributions. The impact of environmental variables on these distribution pattern varies on different spatial scales. On the geographic scale, plant distributions are correlated with physiological tolerances to climate, geology, and hydrologic and biogeochemical cycles (γ-niche) (Silvertown et al. 2006). On the landscape scale (β-niche), plant communities are shaped by dynamic processes including abiotic filters (e.g. climate, substrate and structure), biotic filters (e.g. competition, predation, dispersal and disturbance) and socioeconomic filters (Hobbs & Norton 2004). At the finest scale (α-niche), population persistence and site occupancy is mainly affected by fine-scale environmental factors like soil chemistry and nutrient availability. Additionally, physical variables such as soil water retention or solar radiation are determined by local topography, defining special microsites (Maschinski et al. 2012). These microsites have been found to be especially important during early life stages, because seedlings may require different conditions than adult plants (Wendelberger & Maschinski 2009).

Modelling species response curves

For the analysis of species responses towards the environment, species response models are used. They fit a model to species occurrence data along a measured environmental gradient. Two main components can be extracted from a response curve: the shape of the curve itself and a set of curve parameters. The shape of species responses needs to be determined for several reasons. First, findings test and advance ecological theory, e.g. by providing new insights into the fundamental question
whether species occur in defined communities or as an individualistic continuum (Austin & Gaywood 1994; Peper et al. 2011). Second, species responses help to develop more robust methods for vegetation analysis. This is especially important as some widely used ordination techniques (e.g. correspondence analysis) assume a symmetrical bell-shaped response (ter Braak 1985), which is not necessarily found under natural conditions. The third reason is to improve estimates of “indicator values”, e.g. Ellenberg indicator values, for environmental assessment (Lawesson 2003). Forth, a deeper understanding of species responses might increase prediction results of the present geographical and environmental distribution of species from sample surveys and, lastly, might improve simulations of distribution shifts due to climate change (Austin et al. 1994). Apart from the shape, several parameters, describing the ecological behavior of the species, can be derived from response curves. They are numerical descriptions of the species’ realized niche and can be easily used as variables in models and further analyses. Widely used response curve attributes are the optimum, describing the niche position, and the niche width, describing the range of acceptable environmental conditions for the species. Another important, yet often neglected, parameter is the niche limit, which indicates the threshold of feasible growth of the species (Figure 1.1). The limit offers critical information on the minimum and maximum requirements of a species, which is essential for understanding and predicting species reactions to climate and other environmental changes.

Figure 1.1: Species response curve along an environmental gradient. Niche parameters are the curve shape (dark blue), the optimum (green) and two measures of species limits. The 0.05 fixed limits (red) used in chapters 3-5 and the relative Central Borders (light blue) with the niche width used in chapters 3 + 5.
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In recent years computational power has increased tremendously, facilitating the development of new statistical methods. Several new approaches to model species responses have been implemented so far, most of which are based on regression techniques (Box 1). Symmetric bell-shaped (Gaussian) curves have often been and still are used to analyze species responses (ter Braak 1985; ter Braak & Looman 1986a; Roy et al. 2000). They have the big advantage that the whole range of curve parameters (optimum, limits, and width) can be easily calculated and compared between species. However, it has been shown that real data depict Gaussian forms only rarely (Økland 1986; Austin 1987; Austin & Gaywood 1994). In many cases, species show asymmetric and skewed responses, and even bimodal ones have been found. Thus, bell-shaped curves represent an oversimplified and incomplete picture of the species response. Beta-functions are a more flexible alternative to Gaussian curves, as they allow for skewed unimodal responses, but the practical relevance of the difference between these types has been questioned (Rydgren et al. 2003). Most flexible model shapes are offered by generalized additive models (GAM), and at first they also seem to be most unbiased, because the model shape is not selected by the scientist a priori but determined by the model (Heikkinen & Makipaa 2010; Benavides

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**Box 1: Statistical methods used to fit species response curves in vegetation science.**

**Generalized linear models (GLM):** GLMs are a generalization of ordinary linear regression using maximum likelihood. They allow for response variables that have different error distributions (Gaussian, Poisson, binomial). The response variable is connected to the linear model via a link function.

**Gaussian logistic regression:** A symmetric bell-shaped curve is fitted. The dependent variable is categorical in this regression model, e.g. it is binary (presence, absence). It is a special form of the generalized linear model.

**Beta-functions:** These curves are based on the beta distribution, which is a family of continuous probability distributions on the interval [0, 1]. They are parametrized by two positive parameters that control the shape of the response curve and are the exponents of the random variable. They belong to the generalized linear models.

**Generalized additive models (GAM):** This is a non-parametric expansion of GLM, in which the linear predictor depends linearly on unknown smoother functions of some predictors. Here, the shape of the response curve has not to be specified prior to the analysis.

**(extended) Huisman-Olff-Fresco models (HOF):** They are a hierarchical set of 5 (7) models with increasing complexity. They are fitted by means of logistic and non-linear regression techniques and increase in complexity.
& Vitt 2014). Unfortunately, they are sensitive to over-fitting and it is difficult to choose the right smoothing parameters. Both, smoother selection and the interpretation of the given shape, e.g. whether it is skewed or not, are found to be highly subjective choices (Oksanen & Minchin 2002; Heikkinen & Makipaa 2010). Another flexible set of response models are Huisman-Olff-Fresco (HOF) models. Originally, they consisted of a set of five hierarchical models with pre-determined shapes that increase in complexity (Huisman et al. 1993). It has been shown that they perform better than GLM or beta-functions (Oksanen & Minchin 2002; Lawesson et al. 2003). Recently, they were extended by Jansen and Oksanen (2013) and now comprise seven different model types: no response, increasing/decreasing response, plateau responses, as well as symmetric and skewed unimodal and bimodal curves. The extended model set was found to be a flexible and efficient tool for univariate response modelling (Jansen & Oksanen 2013), being more appropriate than GAM in many cases, as HOF models provide clear tests of skewness, kurtosis and niche attributes.

Application of niche theory in conservation

Niche theory has been invoked as a conservation tool in the past two decades (Maschinski et al. 2012). In regard to the reintroduction of rare plant species, three key applications can be identified. First, a niche-based model including stratified random sampling to improve rare species surveys was developed by Guisan et al. (2006). It searches for potential habitats based on the species niche and selects sampling sites only from these, which leads to a higher detection rate in shorter times. Second, the random placement of propagules within a reintroduction sites has been found to be inefficient. Thus, the selection of microsites based on niche characteristics, probably using the approach mentioned above, is recommended (Maschinski et al. 2012). Third, the niche concept, being the fundament of species distribution models, can be used for a broad categorization of potential habitats for reintroduction and to make sound conservation decisions. An example for this is given in Krause and Pennington (2012).

Reintroduction of rare plant species

For millennia, humans have moved organisms around the world for their own purpose. While this has in general yielded enormous benefit for human kind and development, it sometimes had disastrous impacts (IUCN/SSC 2013). Although we are nowadays aware of these risks, unintended, accidental translocations of plant and animal species still occur due to worldwide trade and travel. In contrast, intended translocations of rare and endangered species have been found to be an effective conservation tool, which is urgently needed in the face of increasing numbers of species being at the risk of extinction (Gilbert 2010) due to increasing habitat destruction, spreading invasive species and
climate change (Tilman & Lehman 2001; Walther et al. 2002; Karl & Trenberth 2003). Nevertheless, conservation translocations also bear multiple risks and should thus follow strategic and ethical guidelines, for example the “Guidelines for Reintroductions and Other Conservation Translocations”, published by the International Union for Conservation of Nature (IUCN/SSC 2013). In these guidelines, the authors define different modes of conservation translocations, differentiating between releases within or outside the indigenous range of the species and several purposes for the release (Box 2). In other publications the term reintroduction is commonly used as an umbrella term for these modes and will be used likewise in the present manuscript, if not stated otherwise.

First documented attempts of bringing rare plants to new suitable habitats were made in 1783, but the earliest plans of intended reintroductions are from 1955 (unpublished records from the Botanical Society of the British Isles, as cited in Dalrymple et al. 2012). Since then, reintroductions have developed into a recommended and well-established technique for mitigating plant species declines, and are nowadays promoted by responsible agencies, e.g. by the IUCN and the US Fish and Wildlife Service (US Fish and Wildlife Service 1999). Recent reviews of plant reintroduction success present varying results. Godefroid et al. (2011) found an average survival of 52% in reintroduced plant species, but much lower numbers when flowering or fruiting were considered. Interestingly, success rates were much higher based on a literature survey (78% survival), than in a questionnaire survey done with practitioners (33%). Guerrant (2012) analyzed the CPC International Reintroduction Registry (Center for Plant Conservation 2009) and found an overwhelming survival rate of more than 90% in the projects with known fate. However, it is too soon to declare victory, because these findings are suspected to be strongly biased due to short monitoring periods and the general bias of published literature towards successful projects as compared to failed ones (Pavlik et al. 1993; Guerrant 2012). The need for long monitoring periods to assess success in reintroductions has also been stretched by Drayton and Primack (2012), who found that the plants from their reintroduction project, which had previously been declared successful, had disappeared after 15 years. Apart from monitoring periods being too short, reintroductions have been criticized for lacking genetic considerations, insufficient knowledge about the demography of the donor populations and inadequate information on the biology and habitat needs of the species (Pearman & Walker 2004). According to practitioner experience, reintroduction failure is often caused by unfavorable habitat conditions despite efforts to use recipient conditions similar to those of wild populations (Dalrymple et al. 2012). This was especially often the case in unpredictable events, like drought (Maschinski et al. 2004; Jusaitis 2005; Batty et al. 2006) or disturbance (Leonard 2006; Maschinski & Duquesnel 2007; Drayton & Primack 2012).
Usually, recipient sites are selected based on habitat surveys using expert knowledge or coarse indicators like plant community type. It has been hypothesized that this procedure is not sufficient to identify critical parameters of the target species niche (Dalrymple et al. 2012). Other reasons for failure were linked to reproductive and developmental biology of the species, e.g. the usage of too few or too

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**Box 2: Definitions and classifications of translocations for conservation purposes, taken from the “Guidelines for Reintroductions and Other Conservation Translocations” (IUCN/SSC 2013). Text in quotation marks is directly quoted from the guidelines.**

“Translocation is the human-mediated movement of living organisms from one area, with release in another.”

“Conservation translocation is the intentional movement and release of a living organism where the primary objective is a conservation benefit: this will usually comprise improving the conservation status of the focal species locally or globally, and/or restoring natural ecosystem functions or processes.”

“Population restoration is any conservation translocation to within indigenous range, and comprises two activities:”

a) **Reinforcement** is the release into an existing population of conspecifics. The aim is to increase population size or diversity and thereby improve viability. Also called enhancement, augmentation, supplementation or restocking.

b) **Reintroduction** is the release inside its indigenous range from which it has disappeared. This aims to re-establish a viable population of focal species.

“Conservation introduction is the intentional movement and release of an organism outside its indigenous range.” Two types are recognized:

a) **Assisted colonization** is the release outside its range to avoid extinction of populations of the focal species. This is done when the protection from current and future threats is less feasible in the current range, e.g. as a protection against a changing climate. Synonyms are benign introduction, assisted migration and managed relocation.

b) **Ecological replacement** is the release outside its range to perform a specific ecological function. Alternative terms are ecological substitutes/proxies/surrogates, subspecific substitutions and analogue species.
small transplants (Dalrymple & Broome 2010; Drayton & Primack 2012). Nevertheless, in recent years some progress was made in understanding processes influencing the success of reintroductions and in developing more effective methods, mainly by analyzing past reintroduction efforts and by experimental testing of distinct hypotheses. Important findings being generally applicable are for example the improvement of survival by using adult plants instead of seeds or seedlings (Dalrymple et al. 2012; Guerrant 2012), better survival when introduction takes place in fall (Albrecht & McCue 2010), advantages of using only a single donor population (Stockwell et al. 2003) and the high potential of ex situ-sourced propagules (Dalrymple et al. 2012). Another promising approach, which has yet been overlooked, is the incorporation of knowledge on plant-soil interactions in restoration and reintroduction planning. Soil microbes are known to be key ecosystem components and might also play an important role in plant reintroduction success. Reintroduction practitioners face one major challenge: they work in an extremely complex system (nature) which differs greatly between species and places. This implies that many solutions found for specific reintroduction issues cannot easily be transferred to other projects. Therefore, much more time and effort is needed if we want to protect endangered species and win the race against ongoing species extinction.

Plant–soil interaction

Humans have been aware of and managed plant-soil interactions in agriculture and horticulture since ancient times, which led e.g. to the development of rotational cropping systems. Already Theophrastus (372-287 BC) suggested to mix different soils to “remedy defects and add heart to the soil”, which provides insight into the first utilization of bacteria to enhance plant growth (Tisdale & Nelson 1975). In 29 BC, Virgil identified that legume crops (lupine) were well established in rotation systems and were thus known to increase fertility.

Soil characteristics are among the most important environmental factors affecting plant growth and distribution on local (Gogol-Prokurat 2011) and landscape scale (Titeux et al. 2009; Bertrand et al. 2012). Soil offers physical support to plants, processes organic waste products and recycles nutrients, and influences water supply. It consists of living and non-living components, including air, water, minerals, organic matter, soil animals and microorganisms (Thomas & Packham 2007).

Among the abiotic soil properties, soil pH has been found to be of great importance for the local distribution of plants, mainly for three reasons: first, high concentrations of H⁺ have been found to limit growth or being lethal to plants (Andersson 1992); second, a low pH increases the solubility of toxic aluminum ions in the soil, which is an additional negative factor (Andersson 1993); and third, important nutrients are not available for the uptake by plants if pH values are low. Plant species diversity has been found to be relatively low under acidic conditions in Central Europe (Dupré & Ehrlén
2002; Ewald 2003; Pärtel et al. 2004). Ongoing acidification caused by atmospheric depositions of nitrogen and sulfur might thus pose a high risk on many plant species (de Vries et al. 2007; Bowman et al. 2008). Other important edaphic parameters are soil texture and organic matter content. Mineral particles are categorized into three size ranges: clay (< 0.002 mm), silt (0.002 – 0.02 mm) and sand (0.02 – 2 mm). Their combination and their interaction with the organic matter mainly determine soil drainage, aeration and affect plant nutrition (Thomas & Packham 2007). Of course, also plant nutrition plays an important role in determining plant community composition and individual fitness. The most important macronutrients derived from the soil are nitrogen, phosphorus and potassium. Enhanced nitrogen deposition, caused by industrialization and agricultural fertilization, was found to reduce species richness in grassland due to the loss of low-fertility specialists (Stevens et al. 2004).

However, plant – soil interaction is not a one-way street, but rather a roundabout. Plants are affected by soil properties, but in turn able to influence soil properties. They do so via the secretion of root exudates, which alter chemical compounds in the soil, by impacting upon hydrological processes and surface temperature, and by providing resources for soil organisms (van der Putten et al. 2013). Plant derived changes of soil properties can also impact the ability of soil to support the same or other plant species or individuals (plant-soil feedback). They are believed to have a variety of effects on plant community dynamics, e.g. succession, invasion and plant abundance (van der Putten et al. 2013).

The third key player in plant–soil interactions are soil microbes. They are still poorly understood, despite their high abundance (van der Heijden et al. 2008). Soil microbes have a large influence on essential ecosystem processes, such as nutrient acquisition and cycling (Kowalchuk & Stephen 2001), carbon cycling (Högberg et al. 2001) and soil formation (Rillig & Mummey 2006). Moreover, they have been found to have a big impact on plant productivity, e.g. by pathogenic or growth promoting relationships. Several recent reviews on aboveground-belowground linkages recognized that plant–soil interactions play an essential role in ecosystem restoration (Suding et al. 2004; Eviner & Hawkes 2008; Kardol & Wardle 2010). Moreover, soil microbes have been suggested to significantly contribute to plant rarity (Klironomos 2002), due to a species specific accumulation rate of pathogens in the rhizosphere.

Plant growth promoting rhizobacteria

Plant growth promoting rhizobacteria (PGPR) represent a variety of soil bacteria, which are able to stimulate plant growth when grown in association with a suitable host plant (Vessey 2003). They usually colonize the rhizosphere, the root surface (both called rhizospheric) or the root interior (endophytic). About 2-5% of rhizobacteria exert beneficial effects on plant growth and thus belong to the PGPR (Kloepper & Schroth 1978). Most PGPR are Gram-negative rods, followed by Gram-positive
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rods, cocci and pleomorphics. In addition, several actinomycetes display plant growth promoting traits, especially as biocontrol agents against fungal pathogens (Bhattacharyya & Jha 2012). PGPR may promote plant growth directly or indirectly via the production and secretion of regulatory chemicals (Ahemad & Kibret 2014).

One way by which PGPR influence plant growth directly is by assisting in nutrient uptake. Some bacteria, e.g. *Azospirillum*, *Azotobacter* or *Rhizobium*, are able to fix atmospheric nitrogen (N\textsubscript{2}), making it available to their host plant (Kim & Rees 1994). This biological N\textsubscript{2} fixation (BNF) accounts for approximately two-thirds of the globally fixed nitrogen (Rubio & Ludden 2008). Second to nitrogen, phosphorus (P) is usually a limiting factor in plant growth. Although soil often contains a high amount of phosphorus, it is mostly in insoluble forms (Khan et al. 2007; Glick 2012). The solubilization of P is one of the most common mechanism of PGPR that increase nutrient availability (Richardson 2001). Well studied examples of P solubilizing bacteria are *Bacillus* (Pal 1998; Singh & Kapoor 1999) and *Pseudomonas* (Cattelan et al. 1999).

Apart from increasing the nutrient status of the plant, PGPR may also promote plant growth directly by modulating plant hormone levels (Ahemad & Kibret 2014). An increase in root weight (Bertrand et al. 2001; Vessey & Buss 2002), root length and surface area (Galleguillos et al. 2000; German et al. 2000; Holguin & Glick 2001) and the number of root hairs (Fallik et al. 1994) has frequently been reported as positive effects of inoculation with PGPR. Indole-3-acetic acid (IAA, auxin) has been found to be involved in virtually every aspect of plant growth, e.g. cell division, cell enlargement, root initiation and germination (Salisbury 1994; Spaepen & Vanderleyden 2011), and is regularly produced in PGPR, e.g. in *Azospirillum* and *Pseudomonas* (Barazani & Friedman 1999). Cytokinins, known to promote cell division, enlargement and tissue expansion, as well as gibberellins (gibberellic acid, GA), able to modify plant morphology, might also be common mechanisms of hormone induced growth promotion by PGPR (Salisbury 1994). Additionally, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, produced e.g. by *Pseudomonas* (Glick et al. 1998), disrupts the biosynthetic pathway of ethylene production. As ethylene can cause a reduction of root growth, its suppression results in root lengthening and enhances stress tolerance against flooding (Grichko & Glick 2001), heavy metal contamination (Burd et al. 1998), salt (Nadeem et al. 2007) and drought (Zahir et al. 2008). Aside from the phytohormones mentioned above, it is likely that more plant growth promoting or regulating substances play a role, which yet need to be discovered (Vessey 2003).

There are two mechanisms by which PGPR promote plant growth indirectly. The major indirect effect is through acting as biocontrol agents (Glick 2012). Causing a decrease in the inhibition of plant growth by pathogens via competition for nutrients, niche exclusion, induced systematic resistance (ISR) and antifungal metabolite production (Lugtenberg & Kamilova 2009). The second indirect type of growth
promotion is by stimulating a synergism between the host plant and a third-party rhizosphere microbe. PGPR aiding in other plant-symbiont relationships are called “helper” bacteria and have mainly been studied for the legume-rhizobia or the plant-fungi symbioses (Vessey 2003).

So far, the growth promoting modes of action of PGPR were often studied separately, but it is most likely that a single PGPR acts simultaneously in several ways. Antoun et al. (1998) found that strains of *Rhizobium* usually produced several growth promoting substances at the same time, but the composition differed between the stains. Moreover, it was shown that not all bacteria that have PGPR traits automatically stimulate plant growth in general (Antoun et al. 1998; Cattelan et al. 1999), but that they are only effective in certain host plant species. Nevertheless, PGPR have been found to be promising biofertilizers and biocontrol agents (Podile & Kishore 2007).

Aims and outline of the thesis

In summary, ongoing environmental changes lead to the extinction of many species and to a significant reduction of global biodiversity, notably with respect to local habitat specialists. To save plant species from extinction, the reintroduction of plants into new or former habitats has been recommended, but low success rates show that our knowledge about the species’ requirements – their niches - is still insufficient. Thus, there is an urgent need to study species–environment relationships to learn about the ecology of species, and to improve reintroduction methods based on sound ecological knowledge. The present thesis tackles this challenge by two approaches – the modelling approach and the experimental approach. The modelling approach aims to expand our understanding of the species’ realized niche in regard to its role in the determination of rarity, regional range size and extinction risk. In doing so, the focus is on the soil niche, because soil plays a major role in plant growth, and regional and local distribution. In addition, the experimental approach aims to develop new practices to propagate and grow plants for reintroductions including scientific knowledge about plant-soil interactions.

A brief summary of chapters 3 – 8, targeting the thesis’ objectives, is presented below (see also Table 1.1). In chapter 2, an introduction to the study area is given, including information on the studied forest and grassland communities and the rare species used for the reintroduction experiment. Appendices are presented in chapter 9.

**Chapter 3**

Soil parameters, such as pH, are known to influence plant distributions on regional and local scales. However, species responses along edaphic gradients are still not fully understood, especially regarding the importance of different niche characteristics for accurately describing a species’ niche. Under the
Introduction

The impact of strong environmental changes, species might no longer live in optimal habitats, thus niche limits have the potential to be better predictors of species occurrence than the commonly used optima. We calculate several niche limits and compare them to niche optima and Ellenberg indicator values in their ability to determine species range size and rarity. Moreover, we test their consistency between two regions and evaluated clustering patterns of the niche parameters.

Chapter 4

In the past, most species distribution models (SDM) were based on climate data only, although soil data might contribute significantly to increase prediction accuracy and find suitable habitats. Unfortunately, data on soil variables is still scarce, especially in combination with plant occurrences and at low resolutions. In this opinion article, we discuss the need for better data on soil variables to improve modelling results and show that calculated niche parameters, derived from HOF models, outperform Ellenberg indicator values in explaining regional range sizes and threat levels of forest and grassland species.

Chapter 5

To place modelling results into conservation action, these results need to be reliable and unbiased not only for common but also for rare species, which are the target organisms in conservation management. However, rare species have, by definition, also a low presence in vegetation surveys. Thus, smaller data sets are usually available for rare than for common plants. Data paucity is of concern, because it has been shown that models are influenced by the size and composition of the data set. We examine the effect of differing numbers of presences and frequencies of plant occurrences in a plot data set on species response curves and their niche attributes calculated with HOF models. Based on the results, we give advice on data requirements and handling for the unbiased calculation of the response curves.

Chapter 6

Ecological knowledge about the interaction between plants and soils, regarding biotic and abiotic factors, has not received much attention in reintroductory efforts so far, although the edaphic dimension has been shown to determine regional and local plant distribution. In this experiment, we implement some known principles of plant-soil interactions into common practice in growing plants for reintroductory, to test their potential to boost reintroductory success. The focus in this article is on plant growth during propagation, their capability to adapt to outside conditions and their fitness after two years in the field.
Chapter 7

Besides abiotic factors, biotic relationships play a major role in plant-soil interactions. Rhizosphere bacterial communities have been shown to influence many fundamental processes, including nutrient cycling, plant growth and plant community composition. By their engagement in plant nutrient acquisition or by being pathogenic, they also influence a species realized soil niche. To better understand the soil processes playing a role for success and failure in plant reintroductions, this chapter investigates the rhizosphere microbial community in a reintroduction experiment with special focus on the effect of PGPR.

Chapter 8

The results of the previous chapters are summarized and related to findings from other studies. I discuss the implications of the modelling results on ecological theory, statistical techniques and conservation. Moreover, I highlight the role of soil and soil bacteria in models and practical reintroduction trials, and give an introduction to the challenges in developing practice oriented conservation methods from ecological theory.
Table 1.1: Overview of the different chapters of this thesis, including a description of the main objectives.

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Main Objectives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter 1</td>
<td>... to provide an adequate background to the studies presented in this thesis and state the objectives of the thesis</td>
</tr>
<tr>
<td>Chapter 2</td>
<td>... to give an introduction to the study area and the studied species</td>
</tr>
<tr>
<td>Chapter 3</td>
<td>... to calculate species niche parameters (optima and different limits) from HOF models in regard to soil pH, compare them to each other and among regions and correlate them to measures of rarity and range size.</td>
</tr>
<tr>
<td>Chapter 4</td>
<td>... to discuss the need for better data on soil variables in relation to plant occurrences and to relate soil pH and P niche parameters, derived from HOF models and Ellenberg indicator values, to species threat levels and rarity.</td>
</tr>
<tr>
<td>Chapter 5</td>
<td>... to estimate the effects of data paucity (e.g. due to species rareness or insufficient environmental measurements) on HOF models and the niche attributes derived from them.</td>
</tr>
<tr>
<td>Chapter 6</td>
<td>... to evaluate the effect of different soil treatments, regarding biotic and abiotic factors, on growth and reintroduction success of rare plant species.</td>
</tr>
<tr>
<td>Chapter 7</td>
<td>... to better understand the processes influencing the plant-soil effects by investigating the microbial rhizosphere community in regard to their host plant, soil variables and reintroduction success.</td>
</tr>
<tr>
<td>Chapter 8</td>
<td>... to combine the main findings of the different studies and discuss them in a broader context.</td>
</tr>
<tr>
<td>Chapter 9</td>
<td>... to present additional Appendices.</td>
</tr>
</tbody>
</table>
References


Introduction


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Galleguillos, C., Aguirre, C., Miguel Barea, J. & Azcón, R. 2000. Growth promoting effect of two *Sinorhizobium meliloti* strains (a wild type and its genetically modified derivative) on a


Introduction


Chapter 1


Chapter 2

Study habitats and species
Characterization of the study area

Vegetation surveys and experiments were conducted within the German federal states of Lower Saxony and Bremen (including the Harz Mountain complex, Figure 2.1). Vegetation survey data for forest and grassland species used in chapters 3-5 were collected from all over the study area, whereas the experimental sites from chapter 6 + 7 were located in a smaller radius in and around Bremen.

Broadly speaking, the study area falls into the northern temperate zone of Central Europe and lies in a transitional zone between (sub-) oceanic climate in the north-west and a warm humid continental climate in the south-east. Mean annual temperature is 8 °C and mean annual rainfall ranges from 600 to 800 mm.

The region can be divided into two main areas, the north-western lowlands and the southern central German upland range, which differ in regard to climate, soil and historical management. In the lowlands, the landscape is flat to undulating, with elevations varying between 10 – 40 m above sea level (a.s.l.). The soils are mostly acidic podsol and secondary podsol or cambisol on more base-rich sites. The uplands provide a higher geodiversity in regard to relief, bedrock and soil. The elevation ranges here from 50-500 m a.s.l., but peaks at 1141 m a.s.l. in the Harz Mountains. Soils are as diverse as bedrocks ranging from base-poor sandstones to lime-rich soils.

Figure 2.1: Forest cover in Europe (left, Päivinen et al. 2001), Germany (mid) and in the study area (right, Bundesamt für Naturschutz et al. 2015). Coniferous forest is presented in dark green, broadleaved forest in light green and no forest in yellow (Europe) or white (Germany, study area).
Herbaceous forest communities used for response modelling

Forests cover 33% of the European land surface (FOREST EUROPE 2015), overall 30% of Germany (Wulf 2003) and 24.3% of Lower Saxony (Niedersächsisches Ministerium für den ländlichen Raum 2004b). However, the distribution pattern is patchy and especially in the north-western part of Lower Saxony the cover is very low and fragmented (Figure 2.1), being e.g. only 9.8% in the Weser-Elbe region near Bremen (Wulf & Kelm 1994). The potential natural forest vegetation in Germany is deciduous forests (except for high elevations). However, nowadays 60% of German woodlands are coniferous forests, mainly consisting of spruce plantations for timber production.

The forests investigated for chapters 3-5 belong to the phytosociological class of Querco-Fagetea, dominated by the orders Fagetalia (sylvaticae) and Quercetalia robori-petraeae. Detailed information on sampling sites and environmental conditions in the different datasets can be found in Gönnert (1989), Heinken (1995), Huntke (2002), Mast (1999); Pannek et al. (2013), Pflume (1999), Pollmann (2000); Rüther and Peppler-Lisbach (2007) and Wulf (1992) as well as in chapter 3. Overall, the data represents a set of diverse semi-natural forest communities and cover the whole spectrum of available soil acidity and moisture conditions.

In the lowlands, forest can broadly be divided into acidic and base-rich types. Naturally, base-rich woodland communities are rare in the lowlands and especially so in the Weser-Elbe region. They are usually constrained to ground depressions and sites connected to ground water (Wulf 1992), and harbor a species rich understory community, with common species being *Adoxa moschatelina*, *Anemone*

Figure 2.2: Lowland forest types on dry acidic soil, dry soil with intermediate pH and on wet and base-rich soils (top down).
nemorosa, Circe lutea, Gali um odoratum, Sanicula europaea and Stachys sylvatica (Figure 2.2). On wet soils, also species like Chrysoplenium alternifolium, Geum rivale, Paris quadrifolia and Stellaria nemorum can be found. On acidic, dry sites, the herbaceous community is sparse and species poor, e.g. characterized by Hedera helix, Oxalis acetosella or Pteridium aquilinum.

Grassland communities used for response modelling

The grassland dataset used in chapter 4 comprises 125 plots belonging to the phytosociological alliance Bromion erecti (Diekmann et al. 2014). These are semi-dry calcareous grasslands on infertile, high-pH soils that often occur on south-facing slopes. Typical species found in managed communities are e.g. Bromus pin natum, Festuca ovina agg., Lotus corniculatus and Cirsium acaule. In addition, these grasslands harbor a relatively high proportion of threatened plant species, including many orchids. In general, grassland cover is declining, which is attributed to agricultural intensification and land abandonment on the regional level (Bundesamt für Naturschutz 2014), and nitrogen depositions, drainage and altered management at the European level (European Commission 2008).

Plant species used for the reintroduction experiment

The common feature of the five species used in the reintroduction experiment (Chapters 6 and 7) is that they are rare in the study area and their numbers are declining. They represent three different types of ecosystems: dwarf-shrub heathland (Genista anglica), wet and dry sites of deciduous forests (Geum rivale and Phyteuma nigrum, respectively), and riversides (Euphorbia palustris and Senecio paludosus). The most important facts of each species can be found in Table 2.1. Distribution maps for local and regional scale, as well as pictures of the plants, are given in Figure 2.3.

Euphorbia palustris is an herbaceous perennial plant growing up to 1.5 m tall. It flowers from May to June and although within inflorescences male and female flowers are present, it is self-incompatible. The species is generally rare, protected by law in Germany and mainly found along large rivers (river corridor plants) (Wärner et al. 2011). The decrease in numbers is mainly caused by drainage and overgrowth by reeds and willows (Cordes et al. 2006).

Genista anglica is a shrubby, perennial, evergreen legume, which flowers from April to July. It is self-incompatible, non-clonal and its main pollinators are honeybees and bumblebees (Tsaliki & Diekmann 2010; Tsaliki & Diekmann 2011). Its fruits develop in long pods and seeds are expelled when being ripe. It is mainly found on dwarf shrub heathland and its distribution in Germany is restricted to the north-west.
Study habitats and species

It is a weak competitor which profits from extensive grazing. Almost no population with > 100 individuals are left in the study area (Cordes et al. 2006).

*Geum rivale* is a perennial plant flowering from April to July. It is mainly pollinated by bees, less often by beetle and flies, and is able to self-fertilize. It is widely distributed in Germany but in the study region it displays a patchy distribution and is mainly found in forests. When it is present, it usually occurs in big numbers, due to clonal reproduction (Cordes et al. 2006). In 2007, *G. rivale* was elected to be the “Blume des Jahres” in Germany, as a representative of decreasing wet meadow species.

*Phyteuma nigrum* is a self-incompatible, herbaceous perennial plant, which grows up to 70 cm. It flowers from May to July and is pollinated by bees and hoverflies. The center of its German distribution is in the south-west where it can be found in fresh meadows as well as in forests. In the study area, it is an indicator species for historically ancient forest on loamy soil (Cordes et al. 2006). Acidification, soil impoverishment and increasing wetness have been suggested to cause the decline of the species (Boerrigter 1995).

*Senecio paludosus* is a rare, perennial forb and grows up to 2m tall. It flowers yellow from June to August and is pollinated by insects. In Germany, it is mainly distributed along large rivers, on unused or only extensively used sites. The main reasons for its decline are lowered water levels and reduced flooding caused e.g. by diking (Diekmann & Bartels 2012).

Table 2.1: Important information on the plant species used in the reintroduction experiments. Given are habitat preferences, plant community affiliation (Bundesamt für Naturschutz 2017), red list status (Garve 2004), expected population development in the study area (Cordes et al. 2006), Ellenberg indicator values (Ellenberg et al. 2001) and biological characteristics (Bundesamt für Naturschutz 2017). Abbreviations are Tx = Tüxen, Br-Bl = Braun-Blanquet, ‘ = 19XX, Koch = W. Koch.
<table>
<thead>
<tr>
<th>Family</th>
<th><em>Euphorbia palustris</em></th>
<th><em>Genista anglica</em></th>
<th><em>Geum rivale</em></th>
<th><em>Phyteuma nigrum</em></th>
<th><em>Senecio paludosus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>German name</td>
<td>Sumpf-Wolfsmilch</td>
<td>Englischer Ginster</td>
<td>Bach-Neikenwurz</td>
<td>Schwarze Teufelskralle</td>
<td>Sumpf-Greiskraut</td>
</tr>
<tr>
<td>Main Habitat</td>
<td>Marsh area</td>
<td>Dwarf shrub heath, mat-grass meadow</td>
<td>Marsh area</td>
<td>Fresh meadows, perennial forb vegetation, mountainous shrubbery, fir and deciduous forests</td>
<td>Nutrient-rich waters</td>
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<tr>
<td>Secondary Habitat</td>
<td>Nutrient-rich perennial forb and weed vegetation, nutrient-rich waters</td>
<td>Perennial forb vegetation, mountainous shrubbery, swamp forest</td>
<td></td>
<td></td>
<td>Marsh area</td>
</tr>
<tr>
<td>Study area</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Associations</td>
<td>Veronico longifolii-Euphorbietaion palustris (Korneck '63)</td>
<td>Genisto anglicae-Callunetum (Schwickerath '33 em. Tx '75)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alliances</td>
<td>Senecion fluviatilis (Tx '50 em. Tx '67), Magnocaricion (Koch '26)</td>
<td>Violion caninae (Schwickerath '44)</td>
<td>Calthion (Tx '37), Alno-Ulmion (Br-B1 et Tx '43), Adenostylon alliariae (Br-B1 '25)</td>
<td>Carpinion (Issler '31 em. Oberdorfer '53)</td>
<td>Magnocaricion (Koch '26)</td>
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<td>Molinietaion caeruleae (Koch '26)</td>
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<td>Germany 3 Study area 3</td>
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<td>Germany Decreasing Study area Decreasing</td>
<td>Decreasing</td>
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<td>L 8 T 6 K 6 F 8, fluctuating R 8 N 2 S 1</td>
<td>L 6 T 5 K 5 F 8, fluctuating R 4 N 4 S 0</td>
<td>-</td>
<td>7</td>
<td>7</td>
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<td>Biology</td>
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<td>Chamaephyta</td>
<td>Hemicryptophyta</td>
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<td></td>
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<td>Insects</td>
<td>Insects, self</td>
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<td>Epichory</td>
<td>Anemochory</td>
<td>Anemochory, Epichory, Myrmecochory</td>
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<td>Competition-Stress</td>
<td>Competition-Stress</td>
<td>Competition</td>
<td>Intermediate</td>
<td>Competition-Stress</td>
</tr>
</tbody>
</table>
Soil pH determines range size

Figure 2.3: Spatial distribution of the study species at regional (Weser-Elbe area) and national (Germany) scale, and pictures of seedlings and adult plants. Regional maps are taken from Cordes et al. (2006), national maps are available from Bundesamt für Naturschutz (2017).
References


Bundesamt für Naturschutz 2017. FloraWeb: Website accessed on 23.08.2017


Because environmental change may force species to live closer to their ecological limits than to their optima, we examined the importance of soil pH response limits for determining the regional range sizes and threat levels of German forest plants. The results suggest that limits are better predictors of current and future species distributions than optima.
Abstract

Questions

Knowledge about the response curves of species along soil variable gradients is scarce. Because environmental change may force species to live closer to their ecological limits than optima, we examined and compared the importance of response limits, response optima and Ellenberg indicator values regarding soil pH for determining the regional range sizes and threat levels of species.

Location

Deciduous forests in the lowlands and uplands of northern Germany

Methods

We used Huisman-Olff-Fresco models to examine the species responses of forest vascular plants along a soil pH gradient with a particular focus on rare species. Separately for the two regions, optima and limits across all species were related to range size, change in regional range size over the past decades and threat level.

Results

Lower pH limits showed an aggregation around pH 4 and were consistent across regions, whereas upper pH limits were not clumped and not significantly correlated between regions. In the lowlands, species with relatively high lower pH limits were less widespread, had decreased more over time and were more threatened than species able to grow on very acid soils. Lower limits were more closely related to regional range size and rarity than optima and Ellenberg R values. These patterns were not found for upland species, probably due to a more complex interaction between pH and other soil factors.

Conclusion

The results reinforce the importance of soil variables and their interactions for the occurrence of plant species and suggest that limits may be better predictors of current and future species distributions than optima and Ellenberg indicator values. Still, crucial information is missing even for common species. Therefore, there is an urgent need for more and better species occurrence data relative to edaphic factors in order to identify the species' needs and their potential responses to environmental change.
Introduction

The study of plant species’ responses along environmental gradients and research exploring the consequences of global change for the occurrence of species are essential for informed conservation decisions. Therefore, the quantification of species niches is considered to be of fundamental importance for basic and applied ecology (Rushton et al. 2004).

While climatic variables are known to influence plant species occurrence on broad scales, soil properties are important for their small-scale distributions, as shown by Coudun et al. (2006) and Bertrand et al. (2012). In their model, the inclusion of soil variables not only contributed significantly to the definition of niche space and species distribution, but also enabled them to find corridors and refugia for species in the face of climate change. One problem of the use of soil variables as predictive tools for local plant species occurrence is their scarcity, due to the fact that site-specific sampling involves time-consuming field work and laboratory measurements. Furthermore, often different methods are applied for sampling and measuring. The use of soil variables, e.g. in large-scale species distribution models, is also complicated by the small-scale heterogeneity of soils. Another problem is that the parameters measured today may no longer reflect the species’ real preferences owing to a time lag between environmental change and species response, which may lead to a so-called extinction debt. Therefore, field measurements of important environmental drivers are often replaced by an indirect assessment of habitat quality by means of indicator values (especially those developed by Ellenberg et al. (2001)), which quantify the ecological behaviour of species integrated over time. Despite their common application (see Diekmann 2003), indicator values have some drawbacks, like the difficulty to transform the scores to physical numbers (Wamelink et al. 2005). In many situations, however, measured values are preferable and in some cases inevitable, for example when calculating critical loads of atmospheric deposition or when predicting the suitability of habitats for reintroductions.

Species’ response curves along measured environmental gradients are usually characterized by their optima and amplitudes (e.g. ter Braak & Looman 1986b; Peppler-Lisbach 2008b), allowing the comparison between different species. Although response curves have earlier been regarded as being either sigmoid or Gaussian (Whittaker 1956; Gauch 1982), they can adopt different forms (Økland 1990), and recent studies have used statistical techniques able to cope with non-Gaussian responses, like Generalized Additive or Huisman-Off-Fresco (HOF) models. These modelling approaches produce three kinds of useful outputs. First, they allow a calculation of niche characteristics; second, they enable us to predict the probability of occurrence of a species at a specific point along the measured gradient; and third, they allow the prediction of suitability of a (not yet occupied) site for a species.
With ongoing habitat change, the reintroduction of rare and dispersal-limited species has become an important practice in nature conservation. The re-establishment of species, however, often fails, probably because of poor site selection due to insufficient knowledge about the edaphic requirements of the target species (Maschinski & Haskins 2012). From a conservation point of view, the quantification of response curves to soil variables such as moisture, pH and nutrients, especially of rare species, is urgently needed to assess the match between species presence and site conditions of remaining populations, and to improve the success rate of future reintroductions.

Another consequence of the ongoing environmental changes is that many species already at present – and increasingly so in the future – may be forced to live in environments that are closer to their physiological or ecological limits than to their optima, which would lead to skewed species-habitat relationships. If this is true, the optima of species’ response curves, as reflected e.g. in Ellenberg indicator values, may be less relevant for practical conservation measures than the species’ minima and maxima or threshold values with defined probabilities of occurrence. These limits define marginal habitats in which species are just able to survive, whereas, beyond these limits, the species no longer meet their basic physiological needs or become competitively excluded. Knowledge of such limits is thus crucial for being able to prevent a further loss of habitats for threatened species. An aggregation of limits of many species in a plant community might indicate that these are determined by physiological constraints rather than by biotic interactions, and in this case the limits are likely to be more stable across larger spatial scales than optima known to vary between different regions. The conservational relevance of species limits vs. species optima can be tested by correlating both with measures of range size, local abundance or threat level. A suitable variable for analyzing these relationships is soil pH that usually shows a close correlation to several other soil factors (Peppler-Lisbach 2008a).

In this study, we examined the responses of forest vascular plants along a soil pH gradient in Germany, being particularly interested in the ecological significance of relative and fixed pH limits. The main research questions were:

1) Where is the position of species limits along the gradient, and are there any patterns of aggregation of limits within a certain pH range (boundary clumping)?

2) Are the pH limits of species and their underlying patterns consistent across different regions?

3) Is the rarity of species correlated with the position of their limits?

4) Are the above relationships for limits stronger than those for the species’ optima or Ellenberg indicator values?
Soil pH determines range size

Methods

Study area & species

The study was carried out in two distinct geographical regions in Germany; the North German lowlands and the Central German Upland Range, hereinafter referred to as “lowlands” and “uplands” (Figure 3.1). Vegetation and soil data were compiled from several published sources and, for the lowlands, additionally from a survey carried out in summer 2013 that served to enlarge the data set, especially for rare plant species. In total, 1470 plots were available from the lowlands (897 plots from the literature) covering a pH range between 2.37 and 7.22, while the data set for the uplands comprised 1173 plots with pH values varying between 2.47 and 8.05. Plots were evenly distributed along the gradient in the uplands, but showed a peak around pH 3 in the lowlands (for further details, see Appendix 3.1 and Appendix 3.2).

The soils in the lowlands are mostly acidic podsols or secondary podsols and cambisols on more base-rich sites. The landscape is flat to slightly undulating, with elevations varying mostly between 10 and 40 m a.s.l. Today, the region is predominantly used for agriculture, with forests covering on average 14% of the area. The woodland consists mainly of coniferous plantations, while deciduous forests are largely confined to the less common base-rich sites.

Compared to the lowlands, the uplands have a higher geodiversity in terms of relief, bedrock and soils. Elevation mainly ranges between 50 and 500 m a.s.l., but reaches up to 1141 m a.s.l. in the Harz Mountains. The soil conditions are highly diverse, including both very base-poor sandstones and lime-rich soils. Agriculture in the uplands was less intensive in the past than in the lowlands, and therefore a larger area (32%; Niedersächsisches Ministerium für den ländlichen Raum 2004a) is still covered by forests.

Figure 3.1: Map of Germany with contour lines of the federal state boundaries. The study area is mainly situated in Lower Saxony. The black part marks the sampling area for lowland data, the region for the upland sample data is marked in white.
In order to confine the species sample to a reasonably cohesive ecological unit, we considered only herbaceous taxa with a preference of growing in closed forests or forest edges in the lowlands (group K1.1 and K1.2 according to Schmidt et al. 2011), overall 61 species. In the uplands, 17 of these species are also found in more open habitats and are assigned to group K2.1.

**Chemical analysis**

In each plot of the 2013 survey, mixed soil samples were collected from the upper soil layer (0-10 cm, without litter layer) and pooled. Each sample was air-dried and passed through a 2-mm sieve. For pH measurements, 10 g of soil and 25 ml of CaCl₂ buffer solution were mixed for 90 minutes, after which pH was analyzed with a standard glass electrode. A sub-sample was also measured in KCl buffer solution and in H₂O to enable us to convert the pH data from the literature to standardized values of pH\textsubscript{CaCl₂}. We followed the procedure proposed by Conyers and Davey (1988) of using regression equations for the conversion of pH values: $pH\textsubscript{CaCl₂} = \frac{pH\textsubscript{H₂O} - 0.694}{0.933}$ ($R^2 = 0.92, P < 0.001, N = 72$) and $pH\textsubscript{CaCl₂} = \frac{pH\textsubscript{KCl} + 0.092}{1.05}$ ($R^2 = 0.99, P < 0.001, N = 72$), see Appendix 3.3.

**Determination of conservation status and range size**

The threat level status of the species (distinguishing endangered, vulnerable, near-threatened and least concern) was obtained from the red list of vascular plants in Lower Saxony and Bremen (Garve 2004). For data analysis, the threat level categories were coded contrasting to the red list as “0” for least concern, “1” for near-threatened, “2” for vulnerable and “3” for endangered species.

Regional range size and temporal change in regional range size were determined using a 1:25000 Ordnance Survey map (Garve 2007). This floristic map covers the area of Lower Saxony and Bremen, is divided into 473 grid cells (11 km x 11 km), and gives information on species occurrence for two time periods: I) before 1981 and II) between 1982 and 2003. Regional range size, defined as area of occupancy (cf. Gaston et al. 1997), was determined by counting the number of occupied grid cells over the recent inventory period. We did so separately for the lowlands (360 grid cells) and the uplands (113 grid cells). To determine the temporal change in range size, we calculated the percentage change in grid cell occupancy by comparing the regional range size of both time periods and standardizing the outcome by the regional range size of the former time period, resulting in positive values for increasing and negative values for decreasing occupancy.

A second approach for determining the population trend was used for parts of the lowlands, the Weser-Elbe region. Here, the flora is among the best known in Germany (Cordes et al. 2006) and gives
Soil pH determines range size

an estimation of population trends for the region based on expert opinion for the reference period from 1935 to 2004, which we used as a proxy for the trends of species in the whole lowlands. No comparable information was available for the uplands.

**Modeling species response & niche boundaries**

Species' response curves relative to soil pH were modeled with hierarchical logistic regression with the program R (v. 3.1.3, http://www.r-project.org; R Foundation for Statistical Computing, Vienna, AT), using the package eHOF, version 3.1.3 (Jansen & Oksanen 2013). The Huisman-Olff-Fresco (HOF) models were first introduced by Huisman et al. (1993) as a set of five hierarchical models with increasing complexity. Recently, Jansen and Oksanen (2013) expanded them to encompass seven types, from simple linear to bimodal skewed responses (Appendix 3.4). The HOF modelling approach selects the best fit out of the pre-determined model types for each species, using statistical information criteria (here: Akaike information criterion) and bootstrapping to stabilize the model choice. A minimum of 10 occurrences for each species is required (Jansen & Oksanen 2013).

The following set of model parameters were extracted from the model curves:

**Species' optimum**

The species' optimum was defined as that value along the gradient where the species had its highest probability of occurrence or, in case of model III (plateau response), as the midpoint of the plateau. Optima, derived from re-modelling the plateau response species with bell-shaped curves, were highly correlated with the midpoint optima ($r_p = 0.86$, $P < 0.001$, $N = 26$).

**Relative tolerance limits**

Relative species tolerance limits, the Central Borders, were calculated as implemented in the eHOF package. These are specified fractions of the curve maxima ($\text{max} \times e^{-0.5}$) (Heegaard 2002) and are calculated separately for the left (LowCB) and right (UppCB) hand side of the optimum (see Appendix 3.4 for visualization).

**Fixed tolerance limits**

These describe the points of the response curve where the probability of occurrence values reach 0.05, quantifying the limits of feasible growth of the species, irrespective of the overall frequency in the datasets (prevalence). The threshold of 0.1 as suggested by Austin et al. (1990) would have excluded a large number of less common species. The fixed tolerance limits are in the following called 0.05 limits or LowLim and UppLim for the left- and right-hand side of the response curve, respectively.
**Prevalence based threshold**

As fixed tolerance limits might be biased by the different frequencies of species in the dataset, with frequent species tendencies to have broader pH niches, prevalence based species thresholds (LowPrevT, UppPrevT) were calculated. These are defined as those points where the response curve intersects the line representing the overall percent frequency in the data set (prevalence). For example, a species present in half of all plots has its LowPrevT where the response curve falls below 0.5 left of the optimum. The prevalence based thresholds thus identify those points along the gradient where the probability of occurrence becomes lower than by chance given the total frequency of the species.

For several species the full set of parameters could not be estimated, because parameter calculations strongly depend on the shape of the response curve, e.g. no clear optimum could be estimated for bimodal response curves. This results in different sample sizes (n) between analyses.

**Statistical analysis**

Altogether 54 species in the lowlands and 53 species in the uplands reached the required minimum number of occurrences for the HOF modelling approach. First, we quantified the boundary clumping of the species for the two types of limits (central borders and 0.05 limits) using Morisita’s Index (MI). In a null model where range boundaries are randomly scattered across a given set of sites, the index value is expected to be 1.00. A value greater than 1.00 indicates that range boundaries are more clumped than expected and vice versa (Morisita 1962). In addition, we used chi-square analysis to test whether there were significant deviations of range boundaries from the null model (Hoagland & Collins 1997). To examine possible shifts of species behavior between the lowlands and uplands of Germany, upper and lower limits from both regions were correlated with each other. Furthermore, the measures of species’ rarity in northern Germany (threat level, regional range size, temporal change in regional range size) were correlated with the pH optima and limits. Unless otherwise stated, Spearman’s rank correlation was applied in all analyses, using the statistical program R (v. 3.1.3, http://www.r-project.org; R Foundation for Statistical Computing, Vienna, AT).

**Results**

**Shape of response curves**

Overall only one species (Milium effusum in the uplands) followed a HOF-type I response, meaning that almost all species were affected by soil pH. In the lowlands, the predominant model shapes were plateau (type III, 48%) and unimodal curves (type IV & V, 36%). Monotonic responses played a minor role (9%) in this region. In the uplands, 30% of the species showed unimodal, 19% plateau-like and 17%
Soil pH determines range size monotonic responses. Among the monotonic and plateau species, the probability of occurrence increased in most cases with increasing pH. Bimodal curves were obtained for 7% in the lowlands and 32% in the uplands. In both regions, many of the unimodal and bimodal responses were skewed.

**Evaluation of limits**

LowLims were significantly correlated with the number of occurrences in both regions (lowlands: $r_s = -0.44$, $P = 0.012$, $N = 31$; uplands: $r_s = -0.50$, $P = 0.005$, $N = 30$), with more common species having their lower limits on more acidic soils. This was not the case for UppLims (lowlands: $r_s = 0.47$, $P = 0.078$, $N = 15$; uplands: $r_s = 0.468$, $P = 0.051$, $N = 18$). Central boarders (all CBs: $r_s < 0.12$, $P \geq 0.377$, $N = 53-54$) and prevalence based thresholds were uncorrelated with the species' frequencies (lowlands: LowPrevT $r_s = -0.17$, $P = 0.264$, $N = 47$, UppPrevT $r_s = 0.21$, $P = 0.339$, $N = 22$; uplands: LowPrevTs $r_s = 0.03$, $P = 0.861$, $N = 42$), except for the UppPrevTs in the uplands ($r_s = 0.38$, $P = 0.045$, $N = 28$).

![Figure 3.2](image) Figure 3.2: Correlations between lower and upper 0.05 limits and the corresponding prevalence thresholds, given separately for (a, b) lowlands and (c, d) uplands. Spearman rank correlation coefficients ($r_s$) and $P$ values are given in each panel. Grey shades reflect the number of plots in which the species was found.
To further examine whether the 0.05 limits were biased by species' frequencies they were related to the unbiased CBs and PrevTs. LowLim and UppLim were significantly positively correlated with the LowCB and UppCB, respectively, both in the lowlands (lower: $r_s = 0.73$, $N = 31$; upper: $r_s = 0.86$, $N = 15$) and in the uplands (lower: $r_s = 0.61$, $N = 30$; upper: $r_s = 0.68$, $N = 18$, all $P < 0.002$). Highly significant relationships were also found between fixed 0.05 limits and prevalence based thresholds (all $P < 0.001$), with very high correlation coefficients for the upper limits (lowlands: $r_s = 0.87$, $N = 12$; uplands: $r_s = 0.89$, $N = 15$) and somewhat lower values for the lower limits (lowlands: $r_s = 0.69$, $N = 31$; uplands: $r_s = 0.62$, $N = 30$).

**Clustering and regional differences**

Across all species, we found a significant aggregation of the limits in the lowlands. Combined lower and upper limits were strongly clumped at pH values between 4 and 4.5 (MI = 1.67; $\chi^2 = 39.21$, $P < 0.001$), mainly caused by a large number of species having their lower limits in this pH range (Figure 3.3). In the uplands, LowLim and UppLim were somewhat aggregated between pH 2.5 and 4.5, again mainly explained by a clumping of LowLim values, but this pattern was not significant (MI = 1.15; $\chi^2 = 17.08$, $P = 0.073$).

![Figure 3.3](image)

**Figure 3.3:** Stacked barplots showing the positions of the species’ pH limits along the soil gradient. Light grey bars indicate lower limits, whereas dark grey bars show upper limits, separately for lowlands (left) and uplands (right).

To calculate the clustering of the CBs, we omitted all the borders that were truncated at the gradient ends to avoid artificial clumping. The results for the lowlands were similar to those for the LowLim, with the main clustering of species at pH values between 3.5 and 4 (MI = 1.38; $\chi^2 = 39.14$, $P < 0.001$).
Soil pH determines range size

In the uplands, no significant aggregation of the CBs was found (MI = 1.07; χ² = 17.5, P = 0.132). As the 0.05 limits showed the most distinct patterns, all further analyses were done with LowLims and UppLims only.

A comparison of LowLim and UppLim between lowlands and uplands showed that the LowLims of most species remained constant across regions (Figure 3.4). However, some species shifted their LowLim towards more base-rich conditions in the uplands compared to the lowlands (e.g., *Paris quadrifolia* with pH 4.21 vs. 5.46). In contrast, *Stachys sylvatica* showed a LowLim of pH 3.51 in the lowlands but a value of 2.86 in the uplands. No significant correlation between lowlands and uplands was found for the UppLims, which might be an effect of the low sample size (N = 5). In general, more species shifted their limits to more base-rich sites in the uplands, which is not surprising, considering the much higher abundance of calcareous, high-pH soils in this region.

![Figure 3.4](attachment:image.png)

**Figure 3.4**: Correlation between the (a) lower limit and (b) upper limit of the studied species between lowlands and uplands. Spearman rank correlation coefficients (rs), P values and sample size (N) are given in each panel. The dashed line corresponds to the y=x diagonal.
**Determination of rarity**

In the lowlands, the LowLims of species were significantly positively correlated with their threat level, i.e., species confined to more base-rich soils were more threatened than species able to tolerate more acidic soils (Tab. 1). There was no such trend in the uplands. In both regions, species with low LowLim values had larger range sizes than more base-demanding species. Interestingly, none of the measures of rarity, range size or change in range size was significantly related to the UppLim of species (Tab. 1).

It has to be noted that range size was positively correlated to frequency in the datasets (lowlands: $r_s = 0.44$, $P < 0.001$, $N = 61$; uplands: $r_s = 0.34$, $P = 0.013$, $N = 31$). In both regions, no significant relationship was found between the survey based change in range size and the LowLim. The experts’ estimation of the population trends in the lowlands, however, indicated a decline of species not able to tolerate low pH values.

Table 3.1: Correlations of lower limits, upper limits and optima of species for soil pH and Ellenberg’s R values with measures of species rarity in northern Germany (threat level, regional range size, temporal change in range size based on counts of formerly and currently occupied grid cells (survey based change in RRS) and experts’ estimation of the population trend (for lowlands only)). Spearman rank correlation coefficients ($r_s$) as well as $P$ values and sample sizes ($N$) are given, with significant values shaded. Analyses were carried out separately for lowlands and uplands.

<table>
<thead>
<tr>
<th></th>
<th>Lower Limit</th>
<th>Upper Limit</th>
<th>Optimum</th>
<th>Ellenberg R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_s$ $P$</td>
<td>$r_s$ $P$</td>
<td>$r_s$ $P$</td>
<td>$r_s$ $P$</td>
</tr>
<tr>
<td><strong>Lowlands</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threat level</td>
<td><strong>0.54</strong> 0.002</td>
<td><strong>0.31</strong> 0.262</td>
<td><strong>0.34</strong> 0.016</td>
<td><strong>0.37</strong> 0.005</td>
</tr>
<tr>
<td>Regional Range Size (RRS)</td>
<td>-0.55 0.001</td>
<td>-0.47 0.081</td>
<td><strong>-0.46</strong> &lt;0.001</td>
<td><strong>-0.39</strong> 0.003</td>
</tr>
<tr>
<td>Survey based change in RRS</td>
<td>0.03 0.885</td>
<td>0.17 0.55</td>
<td>-0.12 0.404</td>
<td>-0.23 0.085</td>
</tr>
<tr>
<td>Expert estimation of population trend</td>
<td>-0.51 0.004</td>
<td>-0.3 0.275</td>
<td><strong>-0.32</strong> 0.023</td>
<td><strong>-0.27</strong> 0.045</td>
</tr>
<tr>
<td></td>
<td>(N = 31)</td>
<td>(N = 15)</td>
<td>(N = 50)</td>
<td>(N = 55)</td>
</tr>
<tr>
<td><strong>Uplands</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threat level</td>
<td>0.29 0.121</td>
<td>0.3 0.22</td>
<td>0.17 0.328</td>
<td>0.16 0.249</td>
</tr>
<tr>
<td>Regional Range Size</td>
<td><strong>-0.37</strong> 0.043</td>
<td>0.07 0.785</td>
<td>0.05 0.774</td>
<td>-0.11 0.415</td>
</tr>
<tr>
<td>Survey based change in freq.</td>
<td>-0.07 0.73</td>
<td>0.39 0.11</td>
<td>-0.06 0.725</td>
<td>0.01 0.939</td>
</tr>
<tr>
<td></td>
<td>(N = 30)</td>
<td>(N = 18)</td>
<td>(N = 35)</td>
<td>(N = 55)</td>
</tr>
</tbody>
</table>

The optima of species in the uplands were on average 0.68 pH units higher than those in the lowlands, mainly caused by the different pH maxima (7.22 in lowlands, 8.04 in uplands). Species optima in the lowlands were positively correlated with their threat level (Tab. 1): Species having their optima on more base-rich soils were rarer than species having their optima on more acidic soils. Range size and expert-based population trends were negatively correlated with the optima of species in the lowlands. The correlation coefficients for the above optima were throughout lower than those for the 0.05 limits.
Soil pH determines range size

Ellenberg’s R values showed the same trends as LowLims and optima with similar or even lower $r_s$ values than the optima. Thus, measures of species regional range size in the lowlands were best described by the LowLims, followed by Optima and Ellenberg’s R values. For the uplands, no significant correlations were found. In both regions, the optima for soil pH were positively correlated with the reaction values of Ellenberg (lowlands: $r_s = 0.60, P < 0.001, N = 45$; uplands: $r_s = 0.42, P = 0.015, N = 33$). For calculated parameters of single species, see Appendix 3.5.

Discussion

In the past, high groundwater levels secured soil buffering and thereby the availability of nutrients in lowland forests. The conversion of the majority of forest sites to agricultural land, associated with the general loss of habitats, fragmentation and particularly a pronounced drainage of the area, led to a strong decrease in moist, base-rich and fertile forest habitats and hence to a decline of basophilous forest species (Döring-Mederake 1991). In contrast, the soils of upland forests are often better buffered by the carbonate bedrock, and here the close relationship between pH, moisture content and nutrient availability is decoupled, with soil pH being independent from the water regime and low nutrient sites being present on both extremes of the pH gradient. Upland forests are also less fragmented and all in all offer better conditions for basophilous forest species. These differences in abiotic conditions determine the strength of the impact of the pH gradient on the distribution of plant species in the two regions.

The decoupling of soil pH and nutrients might cause the high number of bimodal responses in the uplands (32%), due to competitive exclusion at intermediate pH values and nutrient-poor conditions on low and high-pH soils. In the lowlands, we found a remarkably high number of plateau responses, corresponding to the findings of Peppler-Lisbach (2008b), who concluded that this is due to the fact that few forest soils in the North German lowlands exceed pH values of 6.0 so that many species do not reach their potential upper pH limits. Care must be taken especially when optima, amplitudes and Central Borders are calculated, as they can be heavily biased when the full gradient of possible values is not realized.

Limits are likewise biased if the gradient is incomplete, such as the upper limit in the lowlands. However, the acidic extremes were comparable in both regions (pH minimum: 2.37 in lowlands and 2.40 in uplands) representing the lowest pH values generally realized in forests in Germany.

Another source of uncertainty in fixed response limits is the varying frequency of species in the dataset (prevalence), because it is assumed that rare species (with a low number of occurrences) necessarily
have narrower niches than common species (Peppler-Lisbach 2008b). Indeed, although we adjusted the species’ frequencies in the dataset by additionally sampling rare species, LowLims were correlated with the percentage frequency, with low prevalence being associated with higher limits in both regions. No such relationships were found for most of the other curve parameters (UppLims, CBs, PrevTs). To be able to judge how far our results were biased by this correlation, we tested whether the fixed limits were positively related to the presumably unbiased CBs, and especially the prevalence based thresholds (Fig. 2). In fact there was a strong correlation, suggesting that the bias introduced by the different frequencies of species is of minor importance. However, it needs to be kept in mind when evaluating the results.

Statistical models based on observational field data include competition (Guisan & Zimmermann 2000; Austin 2002) and may only yield insight into the realized niche sensu Hutchinson (1957). The boundary clumping at pH values between 3.5 and 4.5 (lowlands) and between 3.0 and 4.0 (uplands) was mainly caused by the strong aggregation of lower limits. A previous study by Falkengren-Grerup and Tyler (1993a) experimentally identified this pH range (3.2 - 4.3) as a physiological threshold for several forest vascular plants, including 13 species also analyzed in the present study, caused by high H⁺ concentrations and Al toxicity. For the lowlands in northern Germany it was shown that the highest species turnover rates in forest plants were related to a steep increase in the contents of exchangeable Al and a high C/N ratio at this pH range (Peppler-Lisbach & Kleyer 2009). It is unlikely that the lower limits are induced by negative biotic interactions, as highly competitive species usually have a high nutrient demand and are incapable of growing on low-pH soils with low nutrient availability (Ellenberg & Leuschner 1996). We believe that the lower limits in our study reflect the physiological thresholds of a major part of the species in the community.

In contrast, the upper limits do not show a strong aggregation pattern along the gradient, nor do they contribute much to the clumping at values between pH 3.0 and 4.5, nor do they form a maximum at higher pH levels. This indicates that the upper limits of most species are determined by biotic interactions rather than by physiological limitation. The lower soil pH limits were strongly positively correlated between the two regions, supporting that these limits represent physiological thresholds for most species. Even though also the physiological limits might differ slightly due to interacting abiotic factors, e.g. pH and soil moisture (Pakeman et al. 2008), they are probably more consistent than parameters of the response curves that are also influenced by biotic interactions (Diekmann & Lawesson 1999).

The lower limits of species were good predictors of threat level and regional range size in the lowlands where acidic soils dominate. Here, accordingly, species are less common the more base-demanding they are. Species optima and Ellenberg R values show similar patterns, but with weaker correlations.
Soil pH determines range size

The significant relationship between the expert-based estimates of population trends and lower limits (and optima) indicates a decline of species intolerant of low pH values, as already observed by Falkengren-Grerup (1986). No relationship was found for the survey-based change in range size. This seems to be contradictory, but population decline usually starts with a thinning of populations, a process that might be discovered by an expert, but not yet manifests in a reduction in the number of occupied grid cells. In the uplands, the only significant correlation was found between the lower pH limit and regional range size, suggesting that acid-tolerant species are more widespread. In general, habitat availability for basophilous species is much higher in the uplands, and hence many of these species considered as threatened in the lowlands are quite common there. Moreover, the explanatory power of the pH gradient for the occurrence of species may be less strong compared to the lowlands, due to the uncoupling between pH, moisture and nutrients. In contrast, the upper limits of species were unrelated to all measures of range size. This may partly be attributed to the low number of replicates, but also indicates that the observed upper limits are ecologically less meaningful.

Range size was not independent of species frequency in the datasets, which was also true for the LowLims. This means that the strength of the correlation between LowLims and range size found in our study is probably overestimated, because it may be reinforced by an underlying correlation between LowLims and species frequency, which also questions the correlation between LowLims and range size in the uplands. In the lowlands, however, all curve parameters (apart from UppLims) showed coincident patterns.

Prevalence-based thresholds as relative limits taking into account the total frequency of species have the advantage of being statistically unbiased. They have the disadvantage that they do not give realistic estimates of limits at least for common species. *Oxalis acetosella*, for example, was present in 41.4% of all plots in the lowlands and therefore had a relatively high prevalence based threshold of 3.10, although it is commonly found in sites with much lower pH values (and other, more base-demanding species such as *Galium odoratum* had a lower PrevT). When using 0.05 limits it is impossible to judge whether the low LowLim of a common species is partly an artifact of its overall high frequency, or whether it is frequent because of low LowLim and thus high amplitude. As they are absolute and comparable between species, they are ecologically more meaningful compared to prevalence based thresholds, especially for predictive purposes.

Conclusions

Our results suggest a large ecological importance of lower soil pH limits of species: these appeared to be relatively stable across regions and were more closely related to measures of regional range size, threat level and population decline than optima and indicator values in the lowlands. In the uplands,
soil pH was less effective in predicting species rareness, due to a more complex interaction with other soil factors. However, even though soil pH is the edaphic factor most frequently measured in vegetation studies, data are scarce especially for many rare species, and for the large majority of other soil factors information is lacking even for common taxa. Hence, there is an urgent need for more data and for compiling a comprehensive database of species responses relative to soil variables. This would enable us to understand the complex and often small-scale abiotic patterns determining species occurrence. For plants, successful reintroductions are possible only if suitable habitats can be identified and suitability can be quantified.
Soil pH determines range size

References


Habitat destruction and change in terms of an altered edaphic environment are the main factors behind the decline of many plant species in Central Europe. Thus, more attention should be paid to local processes and the responses of species along soil gradients. For successful conservation it is necessary to identify optimal and marginal habitats of species.
Abstract

Species distribution modelling has largely focused on larger spatial scales and the significance of climatic variables for future species ranges. In this study, we argue that more attention should be paid to local processes and the responses of species along soil gradients, as habitat destruction and change in terms of an altered edaphic environment are the main factors behind the decline of many plant species in Central Europe. Examples from deciduous forests and calcareous dry grasslands show that response optima and especially response limits relative to soil pH and phosphorus availability are more closely related to the range sizes and threat levels of species than the traditionally applied Ellenberg indicator scores, and that species assumed to have similar preferences show considerable, ecologically relevant differences in their thresholds. There is an urgent need for collecting more and better soil data and for analyzing the relationships between the spatial distribution of plant species and edaphic variables on regional and local scales, in order to identify optimal and marginal habitats of species as a pre-requisite for their successful conservation.

Zusammenfassung

Species response limits

A major goal of ecology is the scientific study of the distribution and abundance of organisms and of those factors that affect distribution and abundance. One important applied output of ecology is thus to create models relating the occurrence of organisms to a suite of environmental factors and to define ecological niches of species. These can in turn be used to make predictions about the responses of species to changing environments and about their potential distributions. Such models allow us, for example, to identify areas not yet occupied that might be suitable for the species in the future.

Over the last years, species distribution models (SDM) have become increasingly popular in ecology, especially in the framework of climate change research. SDM relate the distribution of organisms to a suite of environmental predictors, mostly on large spatial scales and by primarily using climatic variables (Guisan & Thuiller 2005). Even though it has since long been recognized that many range limits of species are closely related to specific values of climatic variables, notably temperature (e.g., Iversen 1944), more quantitative approaches have emerged only about 10-15 years ago when new modelling techniques and large climate data bases became available (Elith et al. 2006). The popularity of SDM in ecology is reflected in the recent exponential increase in the number of publications on the topic. A search in the Web of Science on December 16, 2014, resulted in 1513 findings of papers using the term “species distribution models”, about 2/3 of which published over the past three years. Few of the above publications integrate variables other than climatic, among the few exceptions being recent papers by Coudun et al. (2006), Bertrand et al. (2012), Chambers et al. (2013), Dubuis et al. (2013) and Beauregard and de Blois (2014).

On more regional or local scales with an often more or less uniform climate, models focusing on climate alone appear not to be meaningful (see also Beck et al. 2012). From the viewpoint of a plant ecologist and conservation biologist, more attention should here be paid to factors linked to habitat loss and habitat change, being the main causes of species loss in most regions of the world. In Germany, these two processes, often caused or accompanied by the addition of nutrients due to e. g. atmospheric deposition and fertilization, are the most important factors behind the decline of vascular plant species (Korneck et al. 1998). At the global scale, the addition of nitrogen and phosphorus to ecosystems has been identified as a process where the planetary boundary of what the Earth can tolerate - without facing unacceptable environmental changes - has been exceeded (Rockström et al. 2009; Steffen et al. 2015). The distribution of many plant species especially in regions with a low climatic and topographic heterogeneity is mainly a function of bedrock and soil conditions. In the lowlands of North-western Germany, for example, the absence of many calciphilous species (such as the orchids Orchis militaris and O. morio; Garve (2007)) is caused by the scarcity of lime-rich, high-pH soils and not by any climatic factor, as these species show an extent of occurrence far beyond the region. Furthermore, the
dramatic decline of many stress-tolerant species with a low competitive ability is mainly caused by eutrophication and competitive exclusion, and not primarily by climate change (Korneck et al. 1998; Ellenberg & Leuschner 2010). This means that, while the future large-scale movement of species due to climate change will be prompted and in parts be controlled by climatic variables, local establishment in new areas (apart from being dependent on the species' dispersal capacities) will also be strongly affected by the availability of suitable sites subject to global change (e.g., Riofrío-Dillon et al. 2012).

A main problem for modelling species responses along soil gradients is data shortage. Many climatic variables can easily be extracted from climate data portals such as WorldClim (e.g., Hijmans et al. 2005; www.worldclim.org). Although soil data are increasingly made available in spatial grids and databases (for example, soilgrids.org or eusoils.jrc.ec.europa.eu/ESDB_Archive/ESDB/Index.htm), soil variables vary on much smaller spatial scales so that grid-based information is not always representative of the site in which a plant grows. In addition, soil sampling involves time-consuming field work and often expensive laboratory measurements. The few available soil data have often been obtained using different methods with regard to, for example, sampling depth or chemical analysis, which further complicates comparability and interpretation. Therefore, measurements of important environmental drivers in terms of soil variables have in most cases been replaced by an indirect assessment of habitat quality by means of indicator values. These quantify the ecological behaviour of species integrated over time, instead of reflecting conditions at a specific moment. In Europe, one widely used system of indicator values is that of Ellenberg et al. (2001), who developed indicator scores for three climatic factors (light, temperature and continentality) and four edaphic factors (soil moisture, reaction [pH], nitrogen and salt). These indicator values have been widely and successfully used in ecological research (Diekmann 2003) and are indispensable in historical studies when environmental measurements for the past are not available (for a recent study in the context of global change, see Riofrío-Dillon et al. 2012). However, a drawback for the application of indicator values in SDM is that they are not easily transformed to real values (Wamelink et al. 2005). Another general problem consists in the risk to obtain biased results when using mean indicator values for the interpretation of ordinations of vegetation data (Zelený & Schaffers 2012). More importantly, indicator values describe the response optima of species relative to environmental variables in the field, but do not include any information on response limits. In the same way as the large-scale geographic distribution of species is limited by climatic variables, their small-scale distribution is limited by soil variables: it is a valid assumption that there are many edaphic physiological thresholds beyond which species are not able to survive (for soil pH, see Falkengren-Grerup & Tyler 1993a). In the field, species are further affected by competition. We know very little about these species limits, which is alarming as many species already at present are forced to live in environments that do not offer optimal conditions, but rather represent marginal habitats. If climate change prompts a movement of species
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towards the north, most areas in the new potential range will not offer edaphically optimal conditions, but rather marginal habitats just sufficient to enable the species to survive.

In this paper, we argue that knowledge about species limits is crucial for the modelling of species distributions on regional and local spatial scales, and highly important also in the context of practical nature conservation. Plant re-introductions may fail if the requirements and responses of the target species are not taken into account and unsuitable sites are selected. We also argue that parameters derived from species' response curves are an important tool for the interpretation of vegetation data, and that measurements obtained from large regional data sets can be superior to Ellenberg values that in many cases do not properly reflect the species' ecological responses across the whole of Central Europe. More specifically, this study aims (1) to show that the range size and the threat status of species are better explained by response limits than by optima, especially when the latter are estimated based on indicator values, and (2) to demonstrate that response limits may differ considerably also between species considered to have very similar ecological optima.

Data sets and methods
Response models of species were constructed with data sets from two habitat types focusing on two different edaphic gradients. Data set 1 included 1460 sample plots of deciduous forests from the lowlands of North-western Germany (situated between the regions Westmünsterland and Westmecklenburgisches Seenhügelland), complemented by values of soil pH representing a complex-gradient in nutrient status (Michaelis et al., unpubl. data). The pH was measured in a CaCl$_2$-solution, or measured in H$_2$O or KCl and later transformed to pH (CaCl$_2$). The transformations were based on 72 soil samples for which measurements were carried out in all three solutions (H$_2$O, CaCl$_2$ and KCl), which enabled us to construct regression equations for the conversion of pH values:

\[
pH_{CaCl_2} = \frac{pH_{H_2O} - 0.694}{0.933} \quad (R^2 = 0.92, P < 0.001) \text{ and } \\
pH_{CaCl_2} = \frac{pH_{KCl} + 0.092}{1.05} \quad (R^2 = 0.99, P < 0.001).\]

The data set comprises the full range of forest types found in the study region. Analyses were carried out exclusively with herbaceous species that show a preference of growing in closed forests or forest edges (Schmidt et al. 2011) and that had a minimum frequency of 10 occurrences, altogether 61 species.

Data set 2 included vegetation samples of dry calcareous grasslands in the sub-atlantic, hilly regions of North-western Germany (Niedersachsen). To avoid confounding management effects, plots were only
retained if the sites were still grazed or mown and not abandoned, and if there was no evidence of recent fertilization. In total 125 sample plots and 60 species were used for the analysis. In each of these plots, soil samples were collected and analyzed in the laboratory. Whereas soil pH was consistently high in these grasslands and only plays a minor role for the differentiation of the vegetation, one of the critical factors affecting the occurrence of many dry grasslands species is the availability of nutrients, especially phosphorus (P). P contents were determined with flow-injection analysis after extraction with ammonium lactate (for more details, see Diekmann et al. 2014).

The regional range size of species was determined as area of occupancy by counting the number of occupied grid squares in the lowlands (forests) and uplands (dry grasslands) of the federal states of Niedersachsen and Bremen based on topographical maps (Garve 2007). The threat level of the species was obtained from the red list of vascular plants for the same region (Garve 2004).

Species response curves relative to either soil pH or P were calculated with Huisman-Olff-Fresco (HOF) modelling based on hierarchical logistic regression using the R package eHOF, version 1.3 (Jansen & Oksanen 2013; R Developmental Core Team 2013). These models distinguish seven types of curves, from simple monotonously increasing or decreasing curves to a bimodal skewed response. For each species, the HOF approach selects the best fit out of the pre-determined model types. The species optimum of the selected response curve is defined as the value along the gradient where the species has its highest probability of occurrence or, in case of a plateau response, at the midpoint of the plateau. For the modelling, all species abundance values were transformed to presence-absence values. To determine the species limits with regard to soil pH or P, we calculated those points of the HOF model response curves where the probability of occurrence reaches 0.05. Two examples of response curves are shown in Figure 4.1.

The ecological relevance of the species’ response optima and limits vs. Ellenberg scores was assessed by relating all variables to range size by means of simple linear regression. For data set 1, only the lower pH limits were considered, because the large majority of herbaceous forest specialists prefer base-rich sites and reach a (likely physiological) threshold at the lower end of the pH gradient, i.e. on moderately acid or highly acid soils, but not at the upper end. In contrast, the dry grassland species in data set 2 mostly showed upper P limits, probably predominantly caused by competition. Differences for optima, limits and Ellenberg scores between red list categories were examined with Kruskal-Wallis tests. All statistical analyses were carried out with the programme package R (R Developmental Core Team 2013). The compositional variation of the vegetation of both deciduous forests and dry grasslands was analyzed with Detrended Correspondence Analysis (DCA) using the ‘decorana’ function of the VEGAN package in R (Oksanen et al. 2015).
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Figure 4.1: Two examples of HOF models showing the responses of *Paris quadrifolia* (*n* = 54) along the soil pH gradient in deciduous forests (left) and *Hippocrepis comosa* (*n* = 14) along the soil P gradient in dry calcareous grasslands (right). The dotted grey line shows the probability of occurrence = 0.05, and its intersection with the response curve corresponds to the lower limit (pH) or upper limit (P).

Results

Among the forest species, the predominant model shapes were plateau (type III) and (optimum) unimodal curves (types IV & V), while monotonic (type II) and bimodal responses (types VI & VII) played a minor role (and none of the species was independent of soil pH). The responses of dry grassland species to soil P were much different: 18 species were found to be unrelated to phosphorus availability, and the most frequent model type was the monotonic response. Bimodal responses were not observed.

For the deciduous forest species, range size (no. of occupied grid cells) decreased with an increasing Ellenberg R score, i. e., species with an indicated higher pH optimum were less widespread (Figure 4.2). The range size of dry grassland species was positively correlated with the Ellenberg N score, meaning that species with higher nutrient demands were more common than species tolerant of low nutrient availability. When using measured optima instead of indicator values, identical relationships were obtained, but with slightly higher (forests) and lower (grasslands) correlation coefficients (*r*).
Figure 4.2: Relationship between range size and ecological responses of species along edaphic gradients in deciduous forests (soil pH; left) and dry calcareous grasslands (log-transformed soil P; right) in North-western Germany. The upper panels show Ellenberg scores, the panels in the middle measured optima, and the lower panels measured lower and upper limits, respectively. In the lower right panel, 15 grassland species are marked with open circles, all having Ellenberg N scores of 2 and thus being very similar in their preference of low nutrient availability.
The same effects on range size were also found for the measured lower limits for soil pH (negative) and upper limits for soil P (positive), but here both $r_s$ values exceeded those for the indicator scores. The relationship between range size and the upper limit for soil P was also examined separately for those 15 species having Ellenberg N scores of 2 and thus being very similar in their preference of low nutrient availability (open circles in the lower right panel of Figure 4.2). There was also a significantly positive correlation ($r_s = 0.686$, $p = 0.044$), meaning that the species most tolerant of low P availability and/or those being least competitive had the smallest range size. The seven species with the lowest upper P limits are all considered as threatened or near-threatened. Optima and upper limits for P were positively correlated ($r = 0.460$, $p = 0.036$, $n = 41$).

Figure 4.3: Differences between species’ threat categories (red list status; near threatened and threatened species were merged into the category ‘threatened’) in Ellenberg scores (upper panels), measured optima (middle panels) and measured limits (lower panels) for soil pH in deciduous forests (left) and for soil P in dry calcareous grasslands (right) in North-western Germany. The statistical results refer to Kruskal-Wallis tests.
Deciduous forest species with higher Ellenberg R scores were more threatened than those with lower scores (Figure 4.3). This pattern became more pronounced for the measured pH optima and was especially striking for the measured lower limits for pH. For the dry grasslands, threatened species showed significantly lower Ellenberg N scores than species of least concern, whereas there was only a marginally significant difference in P optima between red list categories. In contrast, the differences of upper P limits between threat categories were clearly more pronounced than those for the P optima.

Figure 4.4: Relationship between the species scores along DCA axis 1 and ecological responses of species along edaphic gradients in deciduous forests (soil pH; left) and dry calcareous grasslands (log-transformed soil P; right) in North-western Germany. The upper panels show Ellenberg scores, the panels in the middle measured optima, and the lower panels measured lower and upper limits, respectively.
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The main gradients in species composition for both habitat types were captured by the first axis of the Detrended Correspondence Analysis (in both data sets the gradient length for axis 1 was > 3). The scores of deciduous forest species along DCA axis 1 were significantly positively related to the Ellenberg R scores and pH optima (Figure 4.4). The relationship between DCA scores and lower pH limits was even closer. For the dry grasslands, the measured P optima performed best among the three species response variables for explaining the variation in DCA scores for axis 1, followed by the upper P limits and the Ellenberg N scores.

Discussion

The results of the exemplary analyses can be summarized as follows: first, measured response limits performed consistently better than Ellenberg scores in explaining the regional range size and threat status of species. Second, the limits were also related to the range sizes of those species that were considered having the same optima with respect to a specific environmental factor. And third, measured optima were superior to Ellenberg scores in explaining the variation of vegetation data. We will first try to shortly interpret the results and then discuss what these mean with respect to our initial hypotheses.

Even though the species’ optima and limits were significantly related to each other in both habitat types, they differed in their ability to explain the regional range size of species. Overall, response limits were more closely related to the area of occupancy and the threat status of species than both indicated and measured optima. Many species (or their populations) do not or no longer occur in preferred environments, but are confined to habitats with less favourable or even marginally favourable conditions. Several forest specialists, such as *Hepatica nobilis*, prefer high-pH and moderately moist soils and face the problem that base-rich forest sites in the North-west German lowlands are generally also relatively wet and therefore not particularly suitable. Such species do not have – and perhaps never had – sites in the region that would represent an optimal environment. Similarly, many dry grassland species lack sites with optimal conditions, especially where nitrogen deposition and / or the addition of phosphorus, often accompanied by reduced management, lead to an increase in taller-growing, more competitive species (Diekmann et al. 2014). The importance of phosphorus-deficient soils is reflected in Figure 4.2 and Figure 4.3, showing that the species with the lowest upper P limits (such as *Euphrasia officinalis*, *Helictrotrichon pratense* and *Hippocrepis comosa*) were also the least widespread and most threatened ones. At least on a regional scale, the distribution of plant species appears to be more closely related to the extreme ends of the species' response curves than to their optimum positions.
The differentiation of the vegetation as reflected by the position of species scores in the DCA ordination was well explained by the measured optima. This is not surprising, because the ordination scores represent the locations of the realized-niche positions of species and not their niche boundaries (Wasof et al. 2013). Unexpected was that the measured optima outperformed the Ellenberg scores, which indicates that, on a regional scale, measurements and response curves based on measurements may describe the ecological behaviour of species better than expert-based indicator values and their averages. Similar results were found by e.g. Diekmann and Falkengren-Grerup (1998) and Gégout et al. (2003).

Being aware that the extent of this study is limited and that the results must be considered as preliminary, we nonetheless conclude the following:

(1) At least in regional vegetation studies, species optima derived from measurements of soil variables show a higher explanatory power than indicator values. They also have the advantage to represent true values that can be compared between regions, ecosystems and species without the need for transformation. Studies on niche characteristics (such as niche breadth and position) of plants have often been based on indirect assessments of species’ behaviour and turnover along gradients (e.g., Fridley et al. 2007; Wasof et al. 2013), which often involves analytical problems and a lack of transferability to field conditions. A measurement-based approach might contribute to make studies on ecological niches more realistic.

(2) When aiming to predict the potential or future distribution of plant species on a regional scale, measured response optima and especially limits need to be considered. The importance of edaphic variables for predicting plant distributions has already been emphasized by Thuiller (2013) and put in practice by, for example, Dubuis et al. (2013) and Beauregard and de Blois (2014). Rare species were shown to have narrower habitat preferences in terms of soil parameters than common species (Wamelink et al. 2014). As already noted, a practical problem is the shortage of available environmental data. Another drawback is the high spatial heterogeneity of most soil variables that makes it difficult to integrate these variables in SDM on a coarse spatial resolution (Thuiller 2013). The problem can partly be rectified by using units on a much smaller spatial scale such as classical sample plots. Another possible solution was offered by Bertrand et al. (2012) who used an indirect estimation of soil pH for 1 km² grid cells based on the species composition and the modelled response of species to pH. Response optima and limits assessed on a regional scale are invaluable for a refinement of large-scale SDM. For example, the general prediction of a climate change-induced shift of highly base-demanding species towards the north in Scandinavia can be modified by taking into account the relative scarcity of high-pH soils in northern Europe and the relatively high lower pH limits of many species in Central Europe (Ewald 2003).
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Figure 4.5: Model of species response (colored optimum curves) under scenarios of increasing fertility (black exponential lines). A species is able to persist in an environment if its response curve exceeds a probability of occurrence (limit) at a point where enough sites with a suitable fertility are available. In the example, species 1 and 2 have the same optima, but differ in their abundance and limits. At point ‘A’ representing current conditions, both species can persist because the number of sites with an optimal fertility is sufficiently large. Point ‘B’ describing a future scenario of increased fertility is beyond the threshold of species 2, but still allows species 1 to persist.

For predictions of plant distributions in climatically relatively homogeneous regions, information on species responses to finer-grained (edaphic) variables is crucial (see Beck et al. 2012), as shown by Kelly et al. (2014) in a study on invasive plants. Our results suggest that response optima and Ellenberg scores both perform reasonably well, but do not succeed to differentiate between species with highly similar preferences but diverging limits (Figure 4.2). On a course spatial scale such as a topographic grid cell, there is often a close agreement between occurrence and the general ecological behaviour of species, which is reflected in many flora maps documenting the importance of bedrock types for the distribution of acidophilous vs. calciphilous species (Netzwerk Phytodiversität Deutschland (NetPhyD) & Bundesamt für Naturschutz 2014). Within these broadly defined groups of plants, however, limits appear to work better than optima. If the landscape in Central Europe becomes increasingly homogenized due to the omnipresent processes of land use intensification and eutrophication, limits
will likely gain even a higher importance. This is depicted in Figure 4.5 showing the responses of two species along a fertility gradient under a scenario of increasing nutrient availability. Species with identical optima but different overall abundances and limits are expected to respond differently to future eutrophication. Thus, the optima of species may just give a rough indication of their ecological behaviour in changing ecosystems.

(3) Knowing that edaphic species thresholds matter and that at the same time edaphic limits are not yet quantified for most species and variables is alarming, because the conservation of species will depend on a thorough understanding of the ecological niches of species and where these are met, now and in future. Given the long tradition of vegetation science in Central Europe and in other parts of the world, with hundreds of thousands of plots being available, we still know little about the species’ niches and especially their limits. In our opinion, we need: (a) To carry out more measurements of edaphic variables, especially of pH and nutrient contents or availabilities, both in vegetation plots and in a systematic manner across regions. More standardized procedures for these measurements would be desirable. (b) To use these data to model the species’ response curves and their variation across different biogeographical regions in order to determine optima and limits, both for single variables and for combinations of variables. The latter will help us to quantify the niches of species, being a difficult but central task of ecology (Turnbull 2014). (c) To examine the relationship between the spatial distribution and responses of species to identify the most critical factors for the persistence of plant populations, and to incorporate this knowledge into predictions of future range sizes. Ultimately, detailed knowledge about the edaphic pre-conditions for the survival of plants will be crucial for the conservation of species as well as for a successful re-establishment of populations at restored sites. We especially need to know the response limits beyond which the species are no longer able to survive.
References


We show that the ecological response of a species modelled with Huisman-Olff-Fresco models might change completely with differing data attributes (presence and prevalence), while taken from the same data set. This fact is rarely taken into account in recent studies using this method, although “just applying” the models bears the risk of biased results and wrong ecological interpretations.
Abstract

The study aimed to examine the effects of different numbers of presences and frequencies (proportions) of occurrences of species in a plot data set of forest vegetation on the species response curves and their niche attributes, based on Huisman-Olff-Fresco models (HOF). We modeled responses of 72 to 105 herbaceous forest species along a pH gradient under 14 different random sampling scenarios by varying the number of presences and absences used for model fitting. Mean niche attributes were calculated from 100 repetitive runs for each scenario and species. Re-prediction success of HOF models among the repetitive runs was highest when the total number of plots was high and the frequency of occurrences was low. With low plot numbers and high frequencies, less complicated model types (no response or monotonically increasing/decreasing responses) predominate. Measures of species niche boundaries (limits & borders) and niche width were strongly influenced by changes in sampling characteristics. With an increasing number of presences and an increasing frequency, limits and borders shifted to more extreme values, leading to wider niches. In contrast, species optima showed almost no change between the scenarios. Thus, the detected ecological response of a species often depends on the size of the data set and the relation between presences and absences of a species. In general, high data quantities are required for reliable response curve modeling with HOF models, which prevents the assessment of the responses of many rare species. To avoid undesired bias by differing sampling characteristics when comparing niches between different species or between data sets, the data basis used for model fitting should be adjusted according to the niche attribute in question, for example by keeping the frequency of the species constant.
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Introduction

Understanding species responses along environmental gradients is of great ecological importance and has received much attention by scientists for many years. Analyzing the shape of the response curves is necessary to advance ecological theory (Austin 1999) and also of practical interest, because some widely used ordination techniques (such as correspondence analysis, CA) are based on the assumption of symmetrical responses (ter Braak 1985). Hence, information about the species responses along gradients is the basis for numerical community analysis and has important implications for continuum theory (Oksanen & Minchin 2002). Moreover, the shape also determines model attributes, such as optima or niche limits, which can further be used to describe the ecological behavior of species in a simplified manner.

Various methods are available to examine species responses. For example, Gaussian logistic regression, as a special case of generalized linear models (GLMs), allows the modeling of symmetric bell-shaped response curves, which can be used to calculate numerical summaries of the species niche, e.g. the ecological optimum (ter Braak & Looman 1986a). Gaussian logistic regression has been shown to be a robust technique (Roy et al. 2000; Coudun & Gégout 2005), but the assumption of an unimodal symmetric shape of the niche has sometimes been heavily criticized (Austin et al. 1994). Generalized additive models (GAMs) are more flexible in their model shape, but also more difficult to apply and interpret, due to problems with smoother selection and over-fitting (Oksanen & Minchin 2002; Heikkinen & Makipaa 2010).

Huisman-Off-Fresco (HOF) models provide a reasonable compromise between statistical correctness, flexibility and ecological interpretability for modeling species responses. They have first been introduced by Huisman et al. (1993) as a set of five hierarchical models with an increasing complexity, which were in accordance with ecological theory about species response patterns along gradients. The original paper distinguished the types: no response, increasing or decreasing response with or without plateau as well as skewed and non-skewed unimodal responses (Figure 5.1 a-d). The original model set-up was implemented as package “gravy” in the R statistical environment by Oksanen and Minchin (2002). This model framework has recently been expanded by Jansen and Oksanen (2013) to encompass seven ecological niche patterns, being implemented as the new R package “eHOF”. Apart from the five model types previously mentioned, two bimodal (skewed and symmetric) response shapes were included to cope with species that are restricted to gradient extremes due to competition (Figure 5.1 e-f and Appendix 5.1). Although HOF models can take several different shapes, the niche parameters can easily be calculated and used for further analyses. In previous studies, HOF models have been shown to be superior to more restrictive methods (such as GLM or beta functions) and to
perform well compared with GAMs that are more flexible (Oksanen & Minchin 2002; Jansen & Oksanen 2013).

Figure 5.1: Examples of HOF models (types II-VII) showing the responses of species along a pH gradient. A species with model type I shows no response along the gradient (b shows *Chrysosplenium alternifolium*). Thick vertical solid lines describe the position of the optima, thin vertical solid lines denote the upper and lower central borders. The dotted grey line corresponds to a probability of occurrence of $y = 0.05$, and its intersection(s) with the response curve marks the lower and / or upper limit.

Over the past years, HOF models were widely used in vegetation science and paleoecology, but only rarely in animal ecology. While the main application was to model the responses of single species (Holmes et al. 2011; Toledo et al. 2012; Michaelis et al. 2016), HOF models were also used to analyze changes in species richness (Suchrow et al. 2015), species cover (Sheppard et al. 2014) or species
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turnover (Peper et al. 2011) along different types of gradients. The attributes of the response curve analyzed most often were the shape (in 97% of studies), followed by different kinds of niche boundary measures (limits, borders, thresholds, tolerances; 35%) and the species optimum (32%; Appendix 5.3).

Despite the frequent use of HOF models, a basic problem of their application is that plot data on species occurrences in combination with environmental measurements in these plots is limited, mainly because the measurement of most environmental factors is time- and cost-intensive. In addition, many species have a low frequency (proportion of presences out of the total number of plots) in data sets even if the overall available number of plots is high, simply because species are often rare in nature for various reasons. Data paucity is of concern, because model fit, results and comparability are influenced by the size and composition of the data set used for modeling. It has been shown that the results of Gaussian logistic regression and of other modeling approaches (incl. GAMs) are heavily affected by the species frequency in the data set and the overall number of data points (Coudun & Gégout 2006; Wisz et al. 2008). However, only 53% of all studies that have used HOF models over the last six years (Appendix 5.3) report the frequencies or the exact number of data points available for each modelled species. Among those, the variability in size and features of the data sets from which models were built is very high: the number of plots varied between 26 and 2691 (median = 207), the minimum number of required species presences ranged from three to 40 (median = 10) and the minimum frequency was within the range of 0.004 to 0.432 (median = 0.04). Moreover, model procedures and evaluations are usually not described in much detail. Thus, it is likely that HOF models are often applied to the data with default package settings and without any prior data adjustment, model stability evaluation or model repetitions. Jansen and Oksanen (2013) stated that re-prediction of a unimodal shape works excellent with the “eHOF” package and the stability checks implemented therein, but only if sufficient plot data with an even distribution along the gradient in question is available. Their tests are in line with a suggestion made by Coudun and Gégout (2006) for Gaussian logistic regression to use at least 50 occurrences to obtain reliable results. Unfortunately, this precondition leads to the exclusion of many less common species from studies, which is why it is rarely followed (Appendix 5.3).

To our knowledge, the only recent study taking into account the potential bias in HOF modeling was done by Reinecke et al. (2016) who used an extensive procedure to overcome the problems caused by varying numbers of plots, varying frequencies of species in different regions and uneven sampling along the gradient. In addition, they fitted 50 models, based on random subsets of their data, created an average curve and derived their niche parameters from this average curve, instead of using the parameters provided by the “eHOF” package directly. But their approach still does not take into account between-species differences in data availability, which might lead to differences in model parameters irrespective of the underlying niche features, and thus to biased ecological interpretations.
Given the influence of data paucity and variability on the results of other modeling approaches, we suspect HOF models to be affected by these factors too. This in turn is likely to bias the results, especially if different studies are to be compared or if data sets contain common and rare species at the same time, which is usually the case. To evaluate the impact of the number of presences and species frequency within and between data sets on HOF models and to find a way to correctly apply this method in practical work, we asked the following questions:

Are the species response curves resulting from HOF models influenced by the different numbers of presences and frequencies?

If so, does this lead to directional or unpredictable changes in the attributes of the response curves (shape, optimum, limits, niche width), and how extensive are these changes?

Can we simply apply HOF models to a data set and then compare the niche attributes of rare and common species, or do we have to process our data set differently to get unbiased results?

Methods

Data set

Vegetation and soil pH data were compiled from three published vegetation surveys from the geographical region of the Central Upland Range in Germany (Mast 1999; Pflume 1999; Pollmann 2000). The data set comprised 1219 plots from all understory community types found in semi-natural deciduous forests in the study region. Soil samples were collected from the upper 10 cm of soil (without litter) and analyzed in different buffer solutions. To be able to compare the different pH values, we converted them all to pH$_{\text{CaCl}_2}$, as described in Michaelis et al. (2016). The soil pH ranges from 2.44 to 8.05 and covers the whole gradient found in the forests of this area. Most plots were obtained from acidic soils whereas the number of sites decreased towards more base-rich conditions (Appendix 5.2). Plot size varied between 50 and 200 m$^2$, depending on the area of homogeneous understory vegetation. For the statistical analysis, we only used herbaceous plants, as they are less directly influenced by forest management compared to trees and because they are supposed to respond to different pH regimes within the upper 10 cm of soil. The data was analyzed as presence/absence.

Modeling approach

Huisman-Olff-Fresco models were fitted in the R statistical program (v. 3.3.1; R Developmental Core Team (2016)) using the package “eHOF” (Jansen and Oksanen (2013), version 3.2.2). To improve
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modeling results even for small data sets, the stability of model choice was double-checked by (1) bootstrapping (100 samplings, default package setting) to ensure model robustness, and (2) the Akaike information criterion corrected for small data sets (AICc, Burnham and Anderson (2002), default setting). In case the two procedures differed in their choice for the best model type, the bootstrapping model was preferred. A minimum number of 10 presences and absences in the data set was set as a pre-condition for modeling in the package.

HOF models were applied to 14 different training combinations of presence and frequency constructed by random sampling from the original data set (Table 5.1). With a constant number of presences, the frequency was changed by varying the number of absences. These are hereinafter referred to as Pre10, Pre25, Pre50, Pre100 and Fre0.068, Fre0.116, Fre0.5, Fre0.714, respectively, and cover a wide range of situations that can be found in ecological studies, from very rare to very common species in small and big data sets. In total 105 species from the original data set met the pre-condition of a minimum of 50 occurrences within the data set to be used in the analysis of scenarios Pre10, Pre25 and Pre50, whereas only 72 species could be used for scenario Pre100 requiring a minimum of 100 occurrences. The scenario Pre100:Fre0.068 could not be modeled due to data shortage of the original data set (1470 plots would have been needed), whereas the scenario Pre10:Fre0.714 did not meet the pre-condition of the “eHOF” package. For the number of presences and frequencies of the species in the original data set, see Appendix 5.1.

Table 5.1: Modeling set-up with 14 different combinations of presence and frequency based on random sampling. Four different presence scenarios (number of randomly selected presences being 10, 25, 50 or 100) combined with four different frequency scenarios (by varying the number of absences) were modeled. Two combinations could not be applied due to model restrictions or data paucity.

<table>
<thead>
<tr>
<th>Presence</th>
<th>0.068</th>
<th>0.116</th>
<th>0.5</th>
<th>0.714</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10 / 138</td>
<td>10 / 76</td>
<td>10 / 10</td>
<td>-</td>
</tr>
<tr>
<td>25</td>
<td>25 / 345</td>
<td>25 / 190</td>
<td>25 / 25</td>
<td>25 / 10</td>
</tr>
<tr>
<td>50</td>
<td>50 / 689</td>
<td>50 / 380</td>
<td>50 / 50</td>
<td>50 / 20</td>
</tr>
<tr>
<td>100</td>
<td>-</td>
<td>100 / 762</td>
<td>100 / 100</td>
<td>100 / 40</td>
</tr>
</tbody>
</table>

Presence / Absence
For each data combination (14) and each species (105/72), model fitting was repeated 100 times, resulting in a total number of 137,100 HOF models. From the 100 repetitions, mean niche parameters, a model stability index and the probability of getting a certain niche parameter were calculated for every species - data combination. Moreover, differences in model choice (which model types were chosen) were evaluated visually.

Curve/niche parameters

The following model parameters, numerically describing different features of a species niche, were calculated from the HOF models:

General species response along the gradient (curve shape)

The shape of the curve is given by the seven different model types of the HOF approach (Figure 5.1 and Appendix 5.1). As an estimate of model shape stability, we calculated the Index of Qualitative Variation (IQV). This index is zero when all repeated runs arrive at the same model shape, whereas it is one when all model types are chosen equally often (Mueller & Schuessler 1961). With \( n \) being the number of categories (model types) and \( p \) being the proportion for each category, the index is calculated as:

\[
IQV = \frac{1 - \sum_{i=1}^{n} p_i^2}{\frac{1}{n} * (n - 1)}
\]

Species optimum

The species optimum describes the highest probability of occurrence of the species along the pH gradient. It can easily be extracted from models of type II, IV and V. In case of model type III (plateau), the optimum is defined as being the midpoint of the plateau. No single optimum can be calculated for the bimodal curve types. Two different optima were distinguished: optimum\(_{\text{any}}\) and optimum\(_{S1}\). In case of the optimum\(_{\text{any}}\), an optimum was assigned to a species whenever an optimum was found in the 100 repetitions, even if this happened only once. For optimum\(_{S1}\), a value was assigned only if a minimum of 51 repetitions resulted in an optimum.

Fixed tolerance limits

The fixed limits are the points of the curve where the probability of occurrence reaches 0.05. They quantify the statistical limits of occurrence of the species, irrespective of species’ fitness or general commonness/rareness in the data set or survey area. The fixed tolerance limits are in the following called 0.05 limits or LowLim and UppLim for the left- and right-hand side of the response curve,
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respectively. In bimodal models, the outermost 0.05 limits are calculated. We assumed a species to have a LowLim$$_{51}$$ or UppLim$$_{51}$$ if at least 51% of model repetitions allowed calculating values. LowLim$$_{any}$$ and UppLim$$_{any}$$ were assigned if at least one run gave a corresponding limit.

Relative tolerance limits and niche width

A procedure to calculate relative tolerance limits for each species is already implemented in the “eHOF” package: the central (CB) borders following Heegaard (2002). These are defined as the response values being equal to a specified fraction of the curve maximum: max * e$$^{-0.5}$$. Similar to the fixed limits, central borders are calculated separately for the left (LowCB) and right (UppCB) hand side of the optimum. Again, the outermost borders are used in bimodal responses. The niche width is defined as the distance between the lower and upper central border.

Statistical analysis

The statistical analysis was done in R (R Developmental Core Team 2016). To evaluate the effects of the varying numbers of presences (Pre) and frequencies (Fre) on the niche parameters (IQV, optima, limits, width), (generalized) linear mixed models ((G)LMM) were used (“lme4” package). Explanatory variables (Pre, Fre) were scaled and centered (for easier interpretation of the results and model fitting except for the IQV analysis). Species was set as random intercept to allow between-species variation in pH preference. Pre and Fre were also included as random slopes, accounting for different responses of single species towards Pre and Fre changes. Building the model like that allows treating each species as an independent replicate within each of the 14 scenarios (and for each niche parameter), resulting in 105/72 replicates (code example in S3 Table). For niche parameters (e.g. position of the optimum along the gradient), a normal distribution was assumed. Changes in the number of niche parameters (e.g. optima) derived from the repetitive runs were analyzed as count data. In general, these are proportions, because the maximal number of repetitive runs is known (100). In the present case, the binominal models failed to converge and overdispersion was an issue. The analysis as count data with a Poisson distributed model was a compromise and the best approximation, because the results follow the overall trend. Model selection and evaluation followed Zuur et al. (2009) and Bolker et al. (2009). P-values for the (G)LMMs were calculated using the Anova function from the “car” package.

To see in how far niche parameters for the species were related between the different scenarios and whether there was a directional or unpredictable change, Spearman correlation matrices were used (package “psych”).
Results

Optima\textsubscript{51}, LowLims\textsubscript{51} and UppLims\textsubscript{51} showed similar trends as their corresponding any-parameters, but their usage reduced the size of the data set by up to 90%. Thus, we decided to only present the results for optima\textsubscript{any}, LowLims\textsubscript{any} and UppLims\textsubscript{any}. Results of 51-parameters, figures of all parameters and a detailed overview of model outputs can be found in Appendix 5.4 and Appendix 5.5.

Choice of model types and their variability

The less complicated model curves of type I and II strongly predominated when the number of presences was low (Pre10) or the frequency was high (Fre0.714). Model choices varied more between the different types in scenarios that include an overall high number of data points and a low frequency (Figure 5.2).

Figure 5.2: Frequency distribution of model types chosen in the different Pre:Fre scenarios. Dps gives the number of overall data points used for model fitting.

The pattern of variation in model choice differed between species and between scenarios. Figure 5.3 a) shows that there was no correlation for the chosen model type between scenarios when the number of data points differed strongly. This means that aside from the underlying ecological response of
species, the chosen model type strongly depended on the size of the data set. This effect can partly be mitigated by equalizing the number of presences or frequencies between data sets.

Overall, model variability was very high with IQV being 0.7 on average (Figure 5.4 a). The index significantly decreased with an increasing number of presences (est. = -0.006, p < 0.001). In the Pre10 scenario, the variability decreased with increasing frequency, i.e., the chances to re-predict a certain model type became higher with an increasing ratio of presences to absences. In the Pre25, there was no change in the IQV with changing frequency, whereas in Pre50 and Pre100 the variability showed a strong increase with increasing frequency. The interaction between Pre and Fre was highly significant (est. = 0.005, p < 0.001). In the high presence scenarios (Pre50, Pre100) the overall variability was lower than in the low presence scenarios. The best results in terms of the lowest variability (IQV 0.4) were obtained in the Pre100:Fre0.068 combination, which is also representing the scenario with the highest number of data points used for model building (862; see Table 5.1). However, when comparing Pre100:Fre0.714 (very common species present in most plots) with Pre10:Fre0.068 (rare species present in a low proportion of plots) it becomes clear that variability was reduced in the Pre100 even if the number of data points (140 vs. 148) was comparable.

Optimum

The probability of obtaining an optimum curve (types II, III, IV & V) in 100 repetitive runs across all species was 54%. When the frequency increased from 0.068 to 0.714, the probability increased by 1%, a weak change despite the fact that the trend of getting more optima curves with increasing frequency was significant (est. = 0.022, p < 0.001). This frequency-induced increase was more pronounced with high presence numbers (est. = 0.008, p = 0.024).

The positions of species optima were not influenced by the number of presences. However, we found a significant influence of frequency (est. = 0.041, p < 0.001) and of the interaction between presence and frequency (est. = -0.033, p = 0.002). Overall, the optima increased with rising frequency, but this tendency was reversed in the Pre100 scenario. For Pre10 the maximum shift was 0.1 pH units, whereas it was -0.01 pH units for Pre100 (Figure 5.4 b). The variation between species increased with an increasing number of presences and extended towards the extremes of the pH gradient. In all scenarios, the optima positions were closely positively correlated between the different scenarios, with correlation coefficients ranging from 0.79 to 0.97, with a trend towards weaker relationships between the Pre100 scenarios and all others (Figure 5.3 b).
Figure 5.3: Spearman-correlation matrices for all Pre:Fre scenarios. Given are: a) model type (chosen most often from 100 repetitive model fittings for each species), b) optimum, c) lower limit and d) upper limits. Central numbers show the correlation coefficients. In a) axes are sorted based on the number of data points used for fitting. For b) – d) axes are sorted by frequency and presence numbers.
Lower 0.05 limits and central borders (acidic side of the gradient)

The chances of estimating a lower limit were heavily influenced by frequency (est. = -0.618, p < 0.001) and to a smaller extent by the number of presences (est. = 0.077, p < 0.001). While an increase in presences from 10 to 100 led to a 5% higher probability to detect a limit at low frequency levels, an increase in frequency otherwise led to a reduction in the chance of observing a lower limit at the 0.05 level of up to 37%.

The LowLim\textsubscript{any} of species were strongly influenced by the number of presences (est. = -0.152, p < 0.001) and the frequency (est. = -0.205, p < 0.001) in the data used for model fitting. Across species, the LowLim\textsubscript{any} shifted to more extreme (low) pH values by 0.4 units with an increasing number of presences.
and increasing frequency, respectively. This resulted in the highest pH LowLims\textsubscript{any} in the Pre10:Fre0.068 scenario and the lowest pH LowLims\textsubscript{any} in the Pre100:Fre0.717 scenario, with a difference of 0.8 pH units (Figure 5.4 c). The higher the frequency, the lower was the variation among the LowLim\textsubscript{any} values. LowLims\textsubscript{any} from different scenarios were closely correlated with an exception being the Pre50:Fre0.5 scenario, which was not or only weakly correlated to all other scenarios (Figure 5.3 c).

Lower central borders also varied depending on the scenario, which is in accordance with the results for LowLims\textsubscript{any}. There was a shift towards more acidic conditions with an increasing number of presences (est. = -0.048, p < 0.001) and an increasing frequency (est. = -0.178, p < 0.001), with a significant interaction effect (est. = -0.032, p < 0.001). Across species, lowest pH borders (pH 3.36) were found for the scenario where species were very common and highly frequent (Pre100:Fre0.714). On the other hand, borders under more base-rich conditions (pH 3.86) were detected for the rarest species scenario (Pre10:Fre0.068). Overall, the maximum shift between these scenarios was less pronounced than for the lower limits, indicating that the lower borders were less influenced by differences in the data sets than the lower limits. This was confirmed by the strong correlations between scenarios (coefficients 0.75 – 0.99).

Upper 0.05 limits and central borders (base-rich side of the gradient)

The number of species with detectable ecological limits towards high pH values was significantly reduced with high presence numbers (est. = -0.354, p < 0.001) and high frequencies (est. = -0.879, p < 0.001), with frequency being more influential. For example, the most contrasting scenarios Pre10:Fre0.068 and Pre100:Fre0.714 differed by 33 species that had a limit in the first, but not in the second scenario.

The position of the UppLim\textsubscript{any} along the pH gradient was shifted to more base-rich values with increasing presence (est. = 0.292, p < 0.001) and increasing frequency (est. = 0.173, p < 0.001). For many species, this resulted in UppLims\textsubscript{any} predicted to be outside of the pH gradient measured in this study, and thus the number of species having an UppLim\textsubscript{any} decreased. A mean increase of 0.4 pH units was found along the frequency gradient for Pre25. Changing the number of presences from 25 to 100 in Fre0.714 led to an increase in UppLim\textsubscript{any} by 0.5 pH (Figure 5.4 d). The correlations between the UppLims\textsubscript{any} of different scenarios were very high among the low frequency settings. In the high frequency scenarios, the correlation coefficients were much lower, caused by stronger and less systematic changes in UppLim\textsubscript{any} values and a smaller data set. This was especially true for the Pre100:Fre0.5 case (Figure 5.3 d).
Again, the borders showed the same trend as the limits, but less distinct. They shifted to higher values with species getting more common, both regarding the number of presences (est. = 0.158, p < 0.001) and frequency (est. = 0.044, p < 0.001), with Pre being the main driver. Maximal pH shift across all species was 0.5 pH units. The correlation matrix between the Pre100 scenarios and other scenarios was relatively weak, but in general all coefficients were high (0.6 – 0.97).

Niche width

Given that both lower and upper central borders shifted towards the extremes with an increasing number of presences and an increasing frequency in the data set, it is not surprising that species niches got wider when both parameters increased (Pre: est. = 0.238, p < 0.001; Fre: est. = 0.193, p < 0.001). Thus, irrespective of their ecological preferences or their occurrence in the original (natural) data set, species had wider niches when they were better presented in the data set used for model fitting. Mean niches spanned a range of 3.2 pH units and could shift, depending on the scenario, up to 0.5 units. The main variation between scenarios was found between low and high frequency scenarios in the correlation matrix, but, as found for the central borders, relationships were in general quite strong (coef. 0.63 – 0.98).

Discussion

The Huisman-Olff-Fresco models are clearly influenced by the number of presences and the frequency of species in a data set. Response curves and their attributes differed significantly between the tested scenarios, irrespective of the underlying (“true”) ecological behavior of the species, despite the fact that several model stability mechanisms were used (bootstrapping, cAIC, repetitive random subsampling and averaging of results). However, the impact and effect size of changes in species frequency and presence differed between the niche attributes derived from the models. Thus, it is obvious that HOF model results cannot simply be compared between rare and common species, differing in presence and frequency, or between data sets of different size. To assess the extent of this possible bias and propose a solution, it is necessary to examine each model parameter separately.

Most previous studies using HOF models analyzed the shape of the species response curve along the gradient, i.e. the model type. The re-prediction success in model types was very low. Even for the - in terms of the total number of plots - largest data scenario (Pre100:Fre0.116) the mean Index of Variation across species was still 0.4. This result, however, overestimates the actual differences between the curves, because the IQV takes the different model types as being completely different categories. Visual inspection of the curves revealed that these in many cases, differed only slightly, although different model types were chosen: bimodal models with an extremely flat second hump, for
example, are very similar to unimodal curves. These results confirm the findings by Jansen and Oksanen (2013) that re-prediction rates rapidly decrease in small data sets and with uneven sampling distribution. Mohler (1983) suggested to sample more extensively at the gradient extremes to increase prediction success in Gaussian regression, and thus to intentionally use an unbalanced data set. In the present data set, the acidic extreme of the gradient was sampled intensively, while the more base-rich part of the gradient was sampled less well. Despite the high sampling intensity at low pH, curve shape and also lower limits and lower central borders varied strongly. When models are fitted with few data points or high frequencies, usually curves of type I to III are selected. Models that are more complicated can only be found with a high total number of plots in combination with low frequencies. This result is expectable, because the AICc penalizes, apart from the addition of parameters to the model, small sample size. Using the Bayesian Information Criterion (BIC) instead, adds an even stronger penalty for small sample size, which results in a stronger restriction in model choice and in the selection of simple models in all data scenarios (data can be found in Appendix 5.6). This further adds to the observation that results obtained with small data sets are not reliable. In general, accuracy of response or distribution models usually improves with additional of information, but plateaus exist (Stockwell & Peterson 2002). The most rapid improvement of model accuracy in logistic regression was found to take place in scenarios with less than 20 presence points in the study by Stockwell and Peterson (2002). We also found an increased variety in the frequency distribution of model types between ten and 25 presences in the present data (Figure 5.2), but the within repetition variation, i.e. the IQV, did not improve much. Hence, our data support the findings that a minimum of 50 occurrences is necessary to obtain reliable re-prediction results and maximal model accuracy (Stockwell & Peterson 2002; Coudun & Gégout 2006). In addition, our results suggest that a low frequency of occurrence in the data set is important as well. In general, a high re-prediction success of curve shapes (or model types) can be achieved only with a high total number of plots, which are evenly distributed along the whole gradient, and free choice of the model types by a low frequency of the single species in this data set, regardless of which IC is used. In case between-species differences are to be compared, the frequency should be kept constant for all species, regardless of their natural occurrence rates.

The optima of species were less influenced by differing numbers of presences or frequencies than all other niche attributes. Although significant differences between the scenarios were found, the actual effect sizes were very small. For example, differences of up to 0.1 pH units are within the range of variation that can be expected when combining data from different sources, with samples being measured in different labs or solvents. Thus, we do not see the need for any data manipulation when considering optima, neither regarding number of presences nor regarding frequency.

The fixed 0.05 limits were very sensitive to changes in the number of presences and in frequency. An increase in the number of presences led to a shift to more extreme values on the left- and right-
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hand side of the gradient, respectively, and to fewer species having their limits within the gradient range. An increase in frequency, in general, resulted in more extreme limits and a high chance for the response curve to no longer cross the 0.05 line. However, it also caused a strong clustering of limits at a certain pH level, which induces a shift to less extreme limits for some species, causing weaker correlations between the scenarios and making the effects of data differences on single species less predictable. The clustering was probably caused by the fact that, in high frequency scenarios, most species showed responses of type I – III with a very high probability of occurrence along the gradient and very steep slopes towards the gradient ends. To be able to compare fixed limits between species or data sets, we therefore suggest to use low frequencies or to enlarge the gradient by additional sampling to allow for smooth slopes, variation of limit positions and to avoid clustering. Moreover, it is necessary to keep both presence numbers and frequency constant.

The central borders were somewhat less influenced by manipulations of the data set than the limits. They also shifted to more extreme values with increasing number of presences and frequency, and suffered from the same clustering effect. The niche width suffered from the shift of the lower central borders to more acidic conditions and from the shift of upper borders to more base-rich conditions. This additive effect led to a high sensitivity of the niche width towards changes of presences and frequencies. Thus, we suggest applying occurrence data with a low frequency and keeping frequency and number of presences constant when comparing different species with each other or across data sets.

In the present study, curve parameters were derived by calculating mean parameter values from 100 repetitive model runs with random sampling. Optima and limits were heavily influenced by unexpected outlier curves, resulting in misleading optima or limit values. For example, it is likely that a species is not facing its limit within the gradient range when 99 curves give no limit and a limit is assigned in only one repetition. It thus seems to be more reasonable to use the optima and limits, but this reduced the present data set by up to 90% and led to similar results. An alternative approach is applied by Reinecke et al. (2016) who created an average curve from repetitive runs and derived their parameters from it. In how far and to what extent these parameters are influenced by the number of presences and frequency remains to be tested.

Based on our results, it appears to be difficult to establish the "true" niche characteristics of species, because all model attributes, apart from the optimum, are heavily influenced by the properties of the data set. In practice, we believe that it is necessary to adjust a data set in terms of species presences and frequencies to compare niches between species or between different data sets, according to the above-mentioned criteria. Of course, this data manipulation has a strong impact on the results, i.e. the absolute values, but at least the effects then are consistent within the study. In general, a high number
of presences combined with an overall low frequency seems to be the best option to obtain good results from HOF models. In the present data set, only 30% of the plant species met the conditions of having 50 occurrences or more, hence 70% of the species could not be modeled. These restrictions caused by data paucity are not unique to vegetation science, but can also be found in animals. For example, in Mexican bird populations, the median of available data points was 83 for all species with a heavy right skewed frequency distribution (Peterson et al. 1998). The high requirements with respect to the properties of the data set for successfully modeling species response curves and the fact that few species meet these requirements, even in big data collections, stress the need for continued plot-based sampling of species and associated environmental data, and for further effort to make existing data accessible to the scientific community. Moreover, detailed information on the data used for response modeling should be given in future studies in order to enable comparisons across data sets and for meta-analyses.
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References


Chapter 5


Different soil types have a strong influence on germination, cultivation and partly even long time performance of rare plant species grown for reintroduction, whereas this is not the case for the inoculation with plant growth promoting rhizobacteria. The value of artificial cultivation soils might probably be enhanced by a microbial inoculum extracted from natural soils.
Abstract

The interaction between plants, soils and microbes has not received much attention in reintroduction efforts so far, although it is widely known that edaphic conditions play an essential role in local plant distribution. To analyze in how far adaption to natural soil conditions and the use of plant growth promoting rhizobacteria (PGPR) influence reintroduction success, five rare plant species from three habitat types in north-west Germany were exposed to three different soil types, with or without the application of PGPR inoculum. The plants were bred in the greenhouse and transplanted to the field after which growth was monitored for two years. Natural soil from the field yielded the best results for four out of five species, indicating that the “home soil advantage” might have great potential value in the cultivation and reintroduction of rare plants. Commercial potting soil resulted in good to intermediate growth in forest and riverside species, but was the least successful option for the heathland species. Overall, growth was least good on an artificially mixed substrate, mimicking physicochemical soil properties from the natural soil, probably due to incomplete soil forming processes. The application of PGPR had no effect on germination rates and on plant growth in the field, and no consistent effect during cultivation. Based on these results, the use of a generalist PGPR inoculum does not provide a significant benefit to reintroduction efforts. In contrast, the use of home soil or home soil inoculum might have a great potential to boost reintroduction success.
Soil and bacteria in reintroduction

Introduction

The reintroduction of rare and endangered plant species has become a widespread technique in conservation and restoration ecology. The creation of new populations is used to counteract the problems associated with habitat loss, landscape fragmentation, reproductive isolation and climate change (Quinn et al. 1994; Drayton & Primack 2012). Reintroduction success rates differ between studies and range from 33% to 90% (Godefroid et al. 2011; Guerrant 2012), but Drayton and Primack (2012) found that after 15 years almost all reintroduced populations, which had been declared successful before, had disappeared. Thus, high success rates might be overestimated due to short monitoring periods or a bias towards successful projects in the literature (Pavlik et al. 1993). Reintroductions have been criticized for not considering genetics, a lack of demographic knowledge of the donor populations and inadequate information on the species’ habitat needs (Pearman & Walker 2004). However, a considerable amount of reintroductions fail in the first years, when reproduction and genetics only play a minor role for the survival of the reintroduced individuals. Likely, reintroductions often fail due to unfavorable habitat conditions, probably because most reintroduction sites are selected based on fairly coarse indicators, such as the general habitat type. For many species, the critical factors determining the species’ niche and its survival have not yet been identified (Dalrymple et al. 2012).

The process of acclimatization has been identified as a major challenge when reintroducing plants to new environments, because propagules raised ex situ are, by definition, not adapted to their new natural habitat (Haskins & Pence 2012). Plant leaves can be adapted to natural conditions (in terms of, for example, solar radiation and climate) during cultivation by transferring the pots from the greenhouse to outside beds. In contrast, to adapt plants and their root system to natural soil conditions with respect to abiotic and biotic factors is more challenging. It is well documented that physicochemical soil properties strongly influence root growth and morphology (Neumann et al. 2014). Nutrient availability (Ericsson 1995), water content (Hu et al. 2009), pH (Andersson 1992), organic matter (Quan & Liang 2017) and compaction (Sinnett et al. 2008) have all been identified to play important roles in root development. Apart from the plants, also the soil biota are strongly influenced by soil properties. Both soil biota and plants are in turn able to alter the soil, e.g. by root exudates or the production of soil enzymes (Neumann et al. 2014; Quan & Liang 2017). Therefore, soil properties, plants and soil biota form a complex system with direct and indirect interactions, causing positive or negative feedbacks for plant growth (Bever et al. 2010).

Little attention is payed to the care and treatment of plant roots during reintroductions, especially regarding the role of soil microbes (Haskins & Pence 2012). This might partly be attributed to the fact that microbial communities, particularly natural ones, are still poorly understood (Haselwandter 1997).
However, the application of ‘beneficial microbes’ has been studied for several decades in crop species (Okon & Labandera-Gonzalez 1994). They have been successfully used in agriculture and horticulture industries (Schwartz et al. 2006) and shown to promote root growth (Smith & Read 1997). Among the few studies done on the impact of soil and microbial factors on the reintroduction of rare plant species, contradictory results were found. Arbuscular mycorrhiza fungi were shown to have beneficial effects on *Uniola* species in dune restoration (Sylvia 1989), on the acclimatization of in vitro propagated plantlets of *Quercus euboica* in soil and their survival after transplantation to the field (Kartsonas & Papafotiou 2007), and on the growth of several endangered Hawaiian plants (Koske & Gemma 1995). In contrast, Klironomos (2002) found strong negative effects on plant performance in some rare species when the plants were grown on soil that had been inoculated with substrate on which other plants of the same species had been grown before (home soil). Similar results were found for temperate tree species (Packer & Clay 2000) and for three endangered violet species (Eckstein & Otte 2005). All authors argue that this effect is caused by an accumulation of pathogens and harmful fungi.

There is an ongoing debate whether a home soil advantage can generally be assumed or not (Kindell et al. 1996; Montalvo & Ellstrand 2000; Packer & Clay 2000; Nijjer et al. 2007; Brenes-Arguedas et al. 2008; Grøndahl & Ehlers 2008).

An option to avoid potential negative effects of home soil, but still profit from soil bacteria, is the application of selected bacterial groups known to have special plant growth promoting traits. These so-called plant growth promoting rhizobacteria (PGPR) inhabit the rhizosphere of plants and produce various regulatory chemicals that promote plant growth and development. These bacteria are free-living and can assist plants in resource acquisition, as biocontrol agents or by altering plant hormone levels (Ahemad & Kibret 2014). Although some PGPR genera have been shown to be beneficial to a variety of plants and are considered as promising biofertilizers (see Ahemad & Kibret 2014 and literature cited therein), they have rarely been used for optimizing the propagation success in plant reintroductions.

In the present study, we examined the impact of different soil types and PGPR on the propagation and acclimatization success in five rare plant species from three different habitat types. Plants were propagated in potting soil, in natural soil collected near the seed donor populations (home soil), and on an artificially mixed soil that mimicked some important physical parameters of the natural soil. Potting soil is easy to use and was expected to bear good results during propagation, but was at the same time assumed to produce plants having problems to acclimatize to the field. Natural soil is more difficult to obtain in huge amounts, especially without endangering the wild donor population at the collection sites. Due to pathogens, propagation results on natural soil were expected to be lower than on the other soil types. In contrast, acclimatization in the field should be less stressful to the transplants, as they are already adapted to natural soil factors. The mixed soil was hypothesized to
bear overall good results, because seedlings are not exposed to potential pathogens and plant root systems could already adapt to physical soil conditions similar to those in natural soils. Half of the individuals in each soil treatment was inoculated with plant growth promoting rhizobacteria, which were expected to increase plant fitness in all soil treatments.

Methods

Study region and plant species
The study was conducted in the regions of Bremen and Lower Saxony in the northern lowlands of Germany. Today, the area is predominantly used for agriculture and the remains of semi-natural habitats are highly fragmented. Five persistent herbaceous plants were selected as model species in this study: Euphorbia palustris, Genista anglica, Geum rivale, Phyteuma nigrum and Senecio paludosus (Appendix 6.1 and Appendix 6.2). They represent three different habitat types – tall herbaceous vegetation of river corridors (E. palustris, S. paludosus), heathland (G. anglica) and deciduous forest (G. rivale, P. nigrum) – and are all relatively rare in the study region. Still, for all five species there existed seed donor populations (one for each species) being sufficiently big to minimize the risk for the population due to seed sampling. Mature seeds of all species were collected in summer 2013 and kept at 4 °C in the dark until the start of the experiments. The seeds of G. rivale and E. palustris were stratified in Petri dishes on moist paper for 6 and 8 weeks before sowing. The seeds of G. anglica were carefully sandpapered and kept in water for one week to initialize germination before sowing.

Experimental setup
In March 2014, the seeds were sown to big, shallow bowls under six different treatments, combining three different soil types (natural, mixed, potting) with the presence or absence of the inoculation with plant growth promoting rhizobacteria (PGPR). During seedling stage the plants were pricked out and planted into individual pots (0.5 l volume) containing the same treatment conditions. During germination, seedling stage and after pricking the plants were kept in the greenhouse. For acclimatization to outside conditions, they were transferred to a cold frame in June and kept there until transplantation to the field in September 2014. The overall number of available seeds was determined by the size of the donor populations (for each treatment, E. palustris: 300, G. anglica: 500, G. rivale: 500, P. nigrum: 2000, S. paludosus: 500). Due to low germination rates in some species the intended number of 45 transplantation replicates for each species and treatment could not be reached. This was especially true in P. nigrum and on mixed soil in E. palustris (Appendix 6.3). After transplantation to the field, the plants were monitored for two years to evaluate acclimatization and reintroduction success of individuals. Mature plants were transplanted to three field locations, with
two plots in each location. Treatments were evenly distributed between sites, and the replicates were assigned randomly. The suitability of transplantation sites was evaluated based on the following criteria: 1) the species was already present at the location, 2) the species had successfully been transplanted to the area in prior reintroductions, and 3) expert knowledge on the habitat requirements of the species.

Soil treatment

Three different soil types were used (Appendix 6.4): The natural soil was collected from the field near the seed donor populations of the plants shortly before sowing. It was sieved to exclude stones, large roots and large soil animals. To test whether the target species were present in the seed bank and might bias the counts of germinated seedlings, a control germination test was conducted parallel to the experiment. The mixed soil treatment was created to model the natural soil in regard to soil pH, organic matter content and soil texture. These three soil features are indicative of important soil properties, e.g. water holding capacity and nutrient availability, and can easily be measured in the field. Natural soil parameters were measured from soil samples taken during seed sampling (Appendix 6.5) and used as template to build the artificial soil mixture from commercially available substrates, i.e. quartz sand, potting soil (KNO Bremen), peat, silt (Florisol® rubra) and clay (Florisol® TM profi, 10030.006, Stephan Schmidt Gruppe). The substrates were mixed based on their dry weight and moistened shortly before sowing. For the potting soil treatment the same potting soil was used as in the mixed treatment. For germination the potting soil was mixed with quartz sand (approx. 50:50) to increase water percolation and reduce nutrient availability. During prickling, the seedlings were transferred to pure potting soil. Because the nutrient content in the potting soil treatment was much higher than in the other treatments, and reduced growth rates during propagation due to nutrient shortage in the other treatments should be prevented, all plants were provided with a small amount of slow-release fertilizer after prickling.

Microorganism treatment

A commercially available mixture of plant growth promoting rhizobacteria (PGPR) was used for the microorganisms (MO) treatment (Vitabac®, Bactivia GmbH). This mixture contained five rhizobacteria (Azospirillum brasilense, Azotobacter chroococcum, Bacillus subtilis, B. megaterium and Pseudomonas fluorescence), which can also be found naturally in low numbers in regional soils and show several plant growth promoting traits, e.g. stimulation of germination and root growth or increase the availability of nutrients (for details, see Appendix 6.6). The mixture was delivered as white powder, which was diluted in water and applied to the pots by watering repeatedly after sowing. Following the producer’s instruction (personal communication), for each liter of soil 0.4g Vitabac® was applied (corresponding to 2x10⁵ colony-forming units) in G. anglica, G. rivale and P. nigrum. Due to the high
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contents of clay and silt, the soils for *E. palustris* and *S. paludosus* were very compact and part of the PGPR inoculum passed the pots along the seam without percolating the soil. To ensure an appropriate inoculation of the soil, the watering procedure was repeated, leading to a final application rate of 1g Vitabac® per liter of soil.

**Plant fitness measurements**

As the five model species differ from each other in their general appearance and growth, various mainly non-destructive plant characteristics were used to evaluate the fitness of individuals (Appendix 6.7). Overall germination rates were measured by counting the number of seedlings for all species and treatments during the first eight weeks after sowing. In the germination tests for the natural soil treatments, one seedling of both *E. palustris* and *G. rivale* germinated from the natural seedbank, hence their germination rates were slightly corrected based on the amount of soil used.

To estimate the fitness of individual plants, plant height (i.e., length of the main branch) was measured for *E. palustris*, *G. anglica* and *S. paludosus* and branching for the first two species. In the field, *G. anglica* developed a bushy growth habitat and side shoots developed into the dominant branches whereas the original main branch was often grazed by animals. Thus, overall plant fitness was assessed on a 4-step ordinal scale in the field, measured independently by 2-4 persons and averaged. Mean leaf length was assessed for *G. rivale*, *S. paludosus* and *P. nigrum* by measuring the length of the three biggest leaves of each plant and calculating the mean. Moreover, the number of leaves was counted for *P. nigrum*. As a proxy for long-time fitness and reproduction potential several flowering characteristics were assessed, e.g. the number of flowering individuals, flowering intensity (4-step ordinal scale) or the number of inflorescences per individual. The variables measured varied depending on the growth form and reproduction of the species. Survival in the field was estimated for all species.

**Statistical analysis**

All statistical analyses and graphics (“ggplot2”) were done with the statistical software R (R Developmental Core Team 2016). Count data were analyzed with generalized linear models (GLM) for Poisson distributed data or with negative binomial errors with the R packages “stats”, “MASS”, “car” and “AER”. Ordinal variables were analyzed with ordinal regression (“ordinal”). The analyses of continuous measurements were done with linear models (LM) or linear mixed models (LMM), using the packages mentioned before and the “lme4” package. Post-hoc contrasts for treatments were done with Tukey-Tests and thereby automatically corrected for multi-testing (packages “multcomp”, “lsmeans” and “multcompView”). Except for *E. palustris*, no significant effect of spatial autocorrelation was found for field locations and plots, thus these were not included in the models as random factors for the other species. For some species - treatment interactions the number of
replicates decreased strongly over the years, therefore it was not possible to statistically analyze some of the fitness parameters over longer time periods. These parameters were therefore analyzed separately for the different sampling times. For a detailed description of the specific methods used to analyze the different fitness parameters, see Appendix 6.8.

Results

Germination

Germination rates varied strongly between species. Overall, *P. nigrum* and *E. palustris* had very low rates with 2% and 4%, respectively, but germination continued after the measurements were stopped. A higher proportion of seeds germinated in *G. rivale* (40%), *S. paludosus* (41%) and *G. anglica* (61%). For all species, germination rates and seedling numbers differed strongly between soils, but not between PGPR treatments. In *E. palustris*, *G. rivale* and *S. paludosus*, most seedlings were found on natural soil, an intermediate number on potting soil and fewest on mixed soil. For *E. palustris*, these differences were significant only between natural and mixed soils (p = 0.002), whereas seedlings of *G. rivale* germinated significantly better on natural and potting soil than on mixed soil (p < 0.001). Germination rates differed significantly between all soil types in *S. paludosus* (all p < 0.001). For *P. nigrum*, the highest germination was found on natural soils (p < 0.001), while seeds on potting and mixed soil performed poorly. In contrast to the other species, *G. anglica* showed the best germination rates on the mixed soil, followed by natural soil (p = 0.024) and potting soil (p = 0.014; natural vs. potting p < 0.001).

Seedlings and juvenile plants (from pricking to transplantation)

*Euphorbia palustris* seedlings grew significantly taller on natural soil, compared to potting soil and mixed soil (p < 0.001). On natural and potting soil the PGPR inoculum slightly decreased the height of seedlings, whereas it had an opposite effect in mixed soil (p = 0.038; Figure 6.1). At transplantation, plants grown on natural and potting soil performed significantly better than grown on mixed soil (Chisq = 54.6, p < 0.001). Plants with bacterial inoculum grew taller in natural and mixed soil, but not on potting soil (interaction: Chisq = 7.28, p = 0.026). In addition, *E. palustris* individuals produced on average 6 to 7 shoots on natural and potting soil, but only 2 shoots on mixed soil (Chisq = 42.02, p < 0.001); there was no influence of the bacterial inoculum (Chisq = 3.51, p = 0.061).
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Figure 6.1: Growth parameters of all five plant species during the experimental time for all combinations of soil and bacterial treatments. Given are plant height (in cm) for *E. palustris*, *G. anglica* and *S. paludosus*, leaf length (in cm) for *G. rivale* and number of leaves for *P. nigrum*. Numbers below the boxplots indicate the number of replicates.
On natural soil the plant height of *G. anglica* seedlings was highest, followed by mixed soil and potting soil (all \( p < 0.001 \)). Moreover, plants grown on natural soil produced significantly more branches (mean 6) than plants on mixed (3.3) or potting (1.5) soil \( (p < 0.001) \). The PGPR did not play a significant role at the seedling stage. At transplantation, the length of the main branch was also affected by the soil treatment \( (p < 0.001) \), but not by the bacterial inoculum \( (p = 0.071) \). Plants grew biggest and produced most branches on natural soil (10.4), followed by mixed (6.2) and potting soil (3; branching: \( p < 0.001 \)). The PGPR increased branching on potting soil only \( (by \ 2.7, \ p < 0.001) \) (Figure 6.1).

In *G. rivale* seedlings, both treatments, i.e. soil \( (p < 0.001) \) and bacterial inoculum \( (p = 0.037) \), played a significant role for the length of leaves. The biggest leaves were on average found on mixed soil, followed by potting and natural soil. Bacteria inoculum influenced leaf size of seedlings negatively. In transplants, the same relationship between leaf length and treatments was found as in seedlings (Figure 6.1).

Seedlings of *P. nigrum* produced significantly more leaves when grown on natural soil compared to the other soil types \( (p < 0.001) \), with no or a slightly positive effect of the bacterial inoculum. The leaves of plants grown on natural soil were significantly bigger than on other soils \( (\text{mean} \ 1.2 \ \text{cm}, \ \text{mixed} \ 0.9 \ \text{cm}, \ \text{potting} \ 0.8 \ \text{cm}; \ p < 0.001) \). Again, soil inoculum had no significant effect (Figure 6.1). At transplantation individuals grown on natural soil had produced twice as many leaves as in the other treatments \( (p < 0.001) \). PGPR had only a weak influence in form of a significant interaction with sampling time \( (p = 0.032) \). Mean leaf length was 2.3 cm in transplants, with no effect of the bacteria. In contrast, the soil treatment influenced leaf length \( (p < 0.001) \): the plants growing on natural soils produced leaves which were up to twice as long compared to the other treatments, being 3.3 cm for natural, 1.9 cm for mixed and 1.6 cm for potting soil.

In seedlings of *S. paludosus* significantly longer leaves were found on natural soil \( (3.16 \ \text{cm}) \), compared to potting soil \( (2.62 \ \text{cm}) \) and mixed soil \( (2.45 \ \text{cm}; \ p = 0.029) \). The bacterial treatment reduced mean leaf size on natural and potting soil by 0.7 cm and 0.5 cm, respectively. On contrary, on mixed soil the bacteria caused an increase in leaf length of 1.2 cm \( (\text{interaction} \ : \ p = 0.001) \). When *S. paludosus* was transplanted to the field, plant height was significantly higher on natural and potting than on mixed soil \( (p < 0.001; \ Figure \ 6.1) \). Bacterial inoculum had a negative effect on plant height on mixed and natural soil, but no effect on potting soil \( (\text{for inoculum} \ p = 0.006; \ \text{interaction} \ : \ p = 0.044) \). The leaves on natural and potting soil were significantly longer than on mixed soil \( (\text{natural} \ = 9.97 \ \text{cm}, \ \text{potting} \ = 9.51 \ \text{cm}, \ \text{mixed} \ = 8.27 \ \text{cm}; \ p < 0.001) \). Bacterial inoculation caused a reduction of leaf size in all treatments by 0.3 to 1 cm \( (p = 0.002) \).
**Adult stage (first and second year in the field)**

Adult plants of *E. palustris* grew taller on natural and potting soil than on mixed soil (Chisp = 54.6, p < 0.001). Bacterial inoculum did not affect plant height in the natural soil treatment, but the positive and negative trends seen before in mixed and potting soil, respectively, became more pronounced (interaction: Chisq = 7.28, p = 0.026). Even after two years in the field, plants grown on mixed soil were on average smaller than those grown on natural or potting soil (Figure 6.1).

In *G. anglica*, in both monitoring years, soil (both: p < 0.001) and bacterial (year one: p = 0.007; year two: p < 0.001) treatments had significant effects on plant fitness. After one year, three different groups were found: (1) the highest fitness was observed in plants propagated on natural soil without PGPR, (2) intermediate fitness levels were achieved by plants grown on mixed soil and on natural soil with PGPR and (3) the smallest plants grew on potting soil. No significantly different groups were identified in the second field year (although there was a trend towards bigger plants on mixed and potting soil compared to natural soil, Figure 6.2).

![Figure 6.2](image_url)

**Figure 6.2:** Fitness of *Genista anglica* individuals in the field, estimated in four categories. Shown are only those individuals that were still alive. The numbers above the x-axis are the numbers of replicates. As different numbers of individuals survived in the treatments and years, the y-axis refers to a different number of replicates for each bar.
The leaf size of *G. rivale* in the field was similar to leaf size in transplants. After one year in the field, plants still grew significantly better on mixed soil than on potting and natural soil (*p* < 0.001). The PGPR inoculum increased leaf length on all soil types (*p* = 0.037), but in general absolute differences between all treatments were small. For the second year after transplantation, only few individuals survived, which is why the big differences found between soils and bacteria are not reliable, although they were significant (Figure 6.1).

For *S. paludosus*, being in their first year in the field, mean plant height was only slightly higher than at transplanting in all treatments. The only factor significantly influencing plant height was grazing by animals (for soil: *p* = 0.046). Two year after transplanting, neither grazing nor soil nor bacterial treatment had an impact on plant height alone (Figure 6.1).

**Survival and reproduction potential**

In all species the number of individuals with an unknown fate was extremely high, because neither their aboveground shoots could be relocated nor their soil-bound labels. It is likely that most of the unknown individuals were dead, because great effort was put into the monitoring. Based on this assumption, the number of surviving individuals decreased strongly during the course of the experiment in all species (Table 6.1). Highest survival rates were observed in *E. palustris* with 61% of transplanted individuals still being alive (but not flowering) after two years. In *G. anglica*, only 42% of the plants survived until the second year, but in the first year, already 34 individuals (15.7%) of the surviving plants were flowering. Their flowering intensity was significantly higher being grown on natural soil compared to the other treatments (*p* < 0.001).

Both forest species showed very low survival rates. Only 9% of *G. rivale* transplants survived to the second year and no individual of *P. nigrum* could be relocated. However, both species flowered already in their first year after transplantation. A flowering rate of 46% of the surviving individuals was observed in *G. rivale*. As the flowers were often grazed by deer, the number of flowering shoots was analyzed, showing a significantly lower number in individuals being treated with PGPR inoculum than those without, especially in mixed and potting soil (overall *p* = 0.013; interaction *p* = 0.020). In addition, *G. rivale* individuals produced several ramets, but these could not clearly be related to distinct individuals. In *P. nigrum* all surviving 24 individuals flowered, producing 1-6 inflorescences per individual. Inflorescence shoot height was between 20 and 70 cm (mean = 43 cm) and the length of the inflorescence ranged from 2 to 9 cm (mean 4 cm). No statistical tests were done due to the small data basis. For *S. paludosus*, survival rates dropped to 22% after two years and only 10.5% of these individuals produced flowers.
Table 6.1: Overview of the survival of the plants during the experiment. Given are the number of living and dead (or with unknown fate) individuals. The category “unknown” was assigned when neither the plant nor the soil-bond label could be relocated during monitoring.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Transplanted</th>
<th>after 1 year</th>
<th>after 2 years</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Euphorbia palustris</em></td>
<td>alive</td>
<td>236</td>
<td>177</td>
</tr>
<tr>
<td></td>
<td>dead/unknown</td>
<td>45</td>
<td>79</td>
</tr>
<tr>
<td><em>Genista anglica</em></td>
<td>alive</td>
<td>269</td>
<td>217</td>
</tr>
<tr>
<td></td>
<td>dead/unknown</td>
<td>53</td>
<td>158</td>
</tr>
<tr>
<td><em>Geum rivale</em></td>
<td>alive</td>
<td>270</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>dead/unknown</td>
<td>157</td>
<td>246</td>
</tr>
<tr>
<td><em>Phyteuma nigrum</em></td>
<td>alive</td>
<td>143</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>dead/unknown</td>
<td>119</td>
<td>NA</td>
</tr>
<tr>
<td><em>Senecio paludosus</em></td>
<td>alive</td>
<td>265</td>
<td>148</td>
</tr>
<tr>
<td></td>
<td>dead/unknown</td>
<td>117</td>
<td>208</td>
</tr>
</tbody>
</table>

Discussion

The establishment of new populations of rare plant species has enormous potential conservation value. The use of ex situ material has already made the difference between extinction and continued survival and offers further valuable conservation options (Guerrant 2012). Although the acclimatization to field conditions has been identified as one of the critical hurdles in reintroduction success, pre-adaptation, especially to soil conditions, has rarely been taken into account so far (Haskins & Pence 2012). In practice, a reasonable compromise between reintroduction success and effort must be found. Thus, the present study evaluates two procedures that possibly increase adaptation to natural soil properties without asking for substantial extra effort.

The soil type influenced cultivation results strongly and lasting in our experiment. This finding is supported by Quan and Liang (2017) who found that soil types with different physicochemical properties had substantial effects on the growth and development of plants. They showed a significant influence of soil texture, pH and organic matter on the growth of a medical plant. The same conclusions were drawn by Neumann et al. (2014) who found that growth characteristics and root exudates differed strongly between soil types, even in the absence of soil stress factors, probably due to soil-specific microbiomes and their interaction with the plant roots. Thus, physicochemical as well as microbial soil properties may affect plant growth on different soil types.
In the present study, cultivation on natural soil resulted in the highest germination rates in all four forest and riverside species, and intermediate germination rates in *G. anglica*. Moreover, plants of *E. palustris*, *G. anglica*, *P. nigrum* and *S. paludosus* had the highest fitness and best growth on natural soil throughout the cultivation period. Even a long term benefit in the field was found for *E. palustris* and *G. anglica*. These results support the assumption that home soil offers a variety of benefits, e.g. optimal edaphic conditions for the ecotype or a suite of pre-adapted beneficial microbes. A number of studies support the home soil advantage for a variety of plant species (Kindell et al. 1996; Carrillo-Garcia et al. 2000; Montalvo & Ellstrand 2000; Grøndahl & Ehlers 2008). In contrast to the other species, growth in *G. rivale* was lower on natural soil compared to the other substrates, even in adult plants after having been in the field for one year. In line with this, several studies found negative effects of home soil on herbaceous and tree species due to an accumulation of soil pathogens in the root vicinity (Packer & Clay 2000; Klironomos 2002; Eckstein & Otte 2005; Nijjer et al. 2007). The natural soil for *G. rivale* was sampled from a huge and dense population, being the dominant species at this site, while all other studied species showed a rather sparse occurrence at their sites. Thus, a pathogen accumulation is likely to be higher in *G. rivale* compared to the other species.

The construction of an artificial soil, resembling natural physiochemical properties, was only successful in regard to texture, organic matter content and most relevant nutrients. Acidic soil pH, however, changed to neutral over the course of the experiment, probably because the buffer capacity of the soil components was underestimated. Moreover, the mixed soil structure deviated strongly from natural soil, especially when the silt and clay contents were high (in all species except *G. anglica*). Natural soils consisted of loose fragments, whereas mixed soils formed compact blocks in the germination bowls, with very low water percolation and aeration capacity. This was probably responsible for the low germination success in most species, because compact soils and increased mechanical impedance were found to inhibit root growth (Sinnett et al. 2008; Neumann et al. 2014). *G. anglica* was the only species with good germination on the mixed soil, but their mixture contained sand only. In the long term, growth of the two riverside species, having the highest clay content in their soils, was negatively influenced by the mixed soil. *G. anglica* and *P. nigrum* showed intermediate growth on mixed soil, whereas *G. rivale* had its best performance there. We hypothesized that plants respond positively to the semi-natural soil properties, but this was only partly true. The absence of beneficial microorganisms decreased growth compared to natural soil in *G. anglica* and *P. nigrum*, whereas *G. rivale*’s fitness was not limited by pathogens. The fact that the overall negative effect on germination was no longer found in seedlings and transplants can be explained by different stages of soil forming processes. While seeds were sown on the freshly mixed substrate, the substrate in the pricking pots already had a more advanced soil structure. Most aggregation and association processes in the soil happen within the first three months (Pronk et al. 2012), thus artificial soils develop quickly. In the
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present experiment, the preparation of mixed soils several weeks or months prior to sowing would probably have led to better results for growth and germination, especially in the clayey soils.

On potting soil, all species showed good to intermediate growth rates, except *G. anglica*, who’s natural soil properties differ markedly from potting soil. Low growth rates for seedlings and transplants can thus best be explained by the high water holding capacity in the pure potting soil used after prickling.

Germination rates and plant fitness were expected to be facilitated by the inoculation with plant growth promoting rhizobacteria. Their ability to promote plant growth has been shown in numerous studies in agricultural and natural systems. In contrast, our results do not show an overall positive effect, neither for certain species or habitat types, nor certain times during cultivation. No effect of the PGPR was found during germination and in the second field year. For the time in between, no consistent picture can be drawn, because positive, negative and neutral effects vary among species, growth parameters and soils. The absolute differences between growth parameters of treated and untreated plants, however, were rather small in most cases. One of the main constraints on the use of free-living, host-independent PGPR is their inconsistent behavior caused by the complex interactions between soil, plants and organisms, and the fact that they have to survive and multiply in the rhizosphere without the protection of a symbiotic niche (Kloepper & Beauchamp 1992). In the present study, bacteria might have not been able to establish stable populations in the soil above the critical size to cause a relevant growth promotion. There may be several reasons for insufficient bacterial growth, e.g. sub-optimal inoculum application (Kokalis-Burelle et al. 2006), competition with the native soil community (Weinbaum et al. 1996) or unsuitable soil conditions. Based on our findings, we draw the conclusion that the application of generalist, commercial PGPR inoculum does not provide substantial benefits to the cultivation of plants for reintroduction. A promising alternative is microbial inoculum from reference ecosystems, because it is easily accessible, cost-effective, provides the whole spectrum of the soil microbial community and has already shown to provide benefit to restoration projects (Middleton & Bever 2012; Maltz & Treseder 2015).

In summary, different soil types had a strong influence on germination, cultivation and partly even long time performance of rare plant species grown for reintroduction, whereas this was not the case for the inoculation with plant growth promoting rhizobacteria. Natural or home soil was a good cultivation medium for most species tested, but might introduce soil pathogens. A way to minimize this risk would be to collect soil from the vicinity of a donor population, but not from areas directly next to the species, especially when the species is dominating the area. Otherwise, cultivation soil mixed from several substrates can also yield good results regarding plant growth, as long as water percolation is good and soil texture is not completely different from the natural soil. The application of PGPR inoculum had no effect on plant fitness. Thus, the value of artificial cultivation soils might probably be enhanced better
by a microbial inoculum extracted from natural soils. To further disentangle the interaction between plants, soils and microbes, and to be able to develop practice-oriented solutions, more collaborative research between soil, plant and molecular scientists is needed.
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Chapter 7

Reintroduced plant species and their rhizobacterial communities are strongly affected by soil properties and less by plant growth promoting rhizobacteria

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submitted

The application of unspecific PGPR had no clear effect on plant growth or rhizosphere communities, probably because they are not able to compete with established microbial communities. In contrast, different soil types influenced plant growth as well as bacteria communities strongly and lasting even in the field.
Abstract

Interactions between plants, soil and microbes affect the fitness of individual plants and the dynamics of plant communities. This fact has yet rarely been considered to overcome challenges in plant reintroductions, such as the adaptation to natural conditions. To analyze the potential benefits, three rare plant species were propagated on different soil treatments (natural, artificially mixed and potting soil) with or without the application of plant growth promoting rhizobacteria (PGPR) and transplanted to the field. Plant fitness and the bacterial rhizosphere community was sampled from seedlings in the greenhouse and adults after they have been in the field for one year. Rhizosphere (microbial) communities were analyzed by molecular techniques of fingerprinting and sequencing. Seedlings of *Genista anglica* and *Senecio paludosus* grew best on natural soil, followed by potting and mixed soil, whereas *Geum rivale* showed the opposite trend. In the field, a longtime benefit of the natural soil was found for one of the species. Rhizobacteria communities differed strongly between plant species, soil types and growth stage. The natural soil treatment was rapidly taken over by the native microbes in the field, whereas this was not true for the other soil types. The PGPR had no clear effect, neither on plant growth, nor on the bacterial community. Thus, the substrate used for the propagation of transplants has a strong and longtime effect on plant fitness and the rhizosphere bacteria of the plants. The use of natural soil or natural microbial inoculum might help to overcome adaptation issues in species reintroductions.
Rhizosphere microbial communities contribute to fundamental ecosystem processes, including nutrient acquisition (van der Heijden et al. 2008), nitrogen cycling (Kowalchuk & Stephen 2001), and soil formation (Rillig & Mummey 2006). Moreover, they may facilitate plant growth and root health, or act as plant pathogens, and thereby strongly influence plant health and plant community structure (van der Heijden et al. 2008). On the other hand, rhizosphere microbes are affected by the abiotic environment and the host plant and its’ root exudates. Thus, plants, soil microbes and soil abiotic properties form a complex system of interactions that is still poorly understood (van der Heijden et al. 2008; Bever et al. 2010). The rhizosphere microbial community consists of bacteria, fungi (including mycorrhiza), oomycetes, viruses and archaea, which are feeding on rhizodeposits (Philippot et al. 2013). While the proportional contribution of the different microbes to the overall plant-soil interaction is still unclear, two main mechanisms can be distinguished: direct effects via the formation of mutualistic or pathogenic relationships between plants and microbes, and indirect effects caused by free-living microbes that alter nutrient supply and resource acquisition (van der Heijden et al. 2008). Rhizosphere bacteria that have positive effects on plant growth are called plant growth promoting rhizobacteria (PGPR). Their application in agriculture and horticulture has been studied for more than 55 years and they have been successfully applied as biofertilizers and biocontrol agents in industrial greenhouse plant propagations (Okon & Labandera-Gonzalez 1994; Ahemad & Kibret 2014). The mechanisms by which certain PGPR interact with the plants have been reviewed by Vessey (2003) and more recently by Ahemad and Kibret (2014), but mainly in the context of crop species.

There is an increasing awareness that soil microbes also play an important role in the restoration of natural ecosystems and in the conservation of biodiversity (Philippot et al. 2013). Recent reviews conclude that the consideration of plant-soil interactions is essential for the effective restoration of terrestrial ecosystems (Eviner & Hawkes 2008; Kardol & Wardle 2010). However, the interactions between above- and belowground can vary greatly, and therefore general, unifying theories do not meet the requirements to predict site-specific plant community development at restored sites. The consideration of plant-soil-microbe interactions regarding whole communities is a challenging task and more research combined with experience from practitioners is needed to understand the context dependence of successes and failures in community restorations (Eviner & Hawkes 2008). Nevertheless, soil microbes offer a great potential in facilitating restoration success, especially when used in simpler systems, for example for the propagation of rare plant species for reintroduction purposes.

The introduction of threatened plant species to new or former habitats is nowadays a commonly used method to meet problems associated with habitat loss, fragmentation and reproductive isolation.
The finding that many reintroductions fail during the first years suggests that this is caused by either unfavorable environmental conditions at the new site or unsuccessful acclimatization of individuals to outside conditions (Dalrymple et al. 2012; Haskins & Pence 2012). The highest survival rates were found for adult plants (instead of seeds or seedlings) used for reintroduction (Dalrymple et al. 2012), but their propagation is also the most expensive method, which is why propagation and transplantation processes should be optimized. In this context, only little attention has been payed to the treatment of plant roots and to above- belowground interaction in reintroduction trials (Haskins & Pence 2012). Although it is widely known that edaphic parameters and microbial communities have a great influence on plant growth, only few studies have investigated the effect of different soils and soil microbes on the propagation and transplantation of rare plant species.

Abiotic soil parameters, such as moisture capacity, have been found to be the limiting factor for the introduction of the endangered shrub *Purshia subintegra* in Arizona, USA, and strongly influenced the cultivation of the medical herb *Lycoris aurea* that has decreasing natural populations in China (Maschinski et al. 2004; Quan & Liang 2017). Soil from the natural environment (including natural microbe communities) increased survival of *Quercus euboica* seedlings compared to a general potting media mix (Kartsonas & Papafotiou 2007) and also mattered for the survival of *Pachycereus pringlei* seedlings in the Sonoran desert. In the latter case, a PGPR inoculum additionally increased growth, having a stronger effect on nutrient-poor soils compared to nutrient-rich soils (Carrillo-Garcia et al. 2000). Moreover, the inoculation with mycorrhiza increased performance of other rare plant species, especially in arid and low nutrient environments (Sylvia 1989; Fisher & Jayachandran 2002) and in multifunctional microbial combinations (Requena et al. 1997). Best results yielded whole inoculum of native microbes and even long-term benefits for facilitating the establishment of below- and aboveground interactions for up to several years were found in restored ecosystems (Maltz & Treseder 2015). However, also negative effects of home-soil and home-microbes were found for endangered violets and several other rare plant species, which was attributed to an accumulation of pathogens (Klironomos 2002; Eckstein & Otte 2005).

Thus, it has been shown that plant species propagation and reintroduction can in general benefit from the longtime of experience in agri- and horticultural research, but they also add another suite of challenges to researchers and practitioners, because plants are transplanted to natural habitats where abiotic and biotic factors are less controlled and species need to survive and compete with other species. Therefore, there is an urgent need to further investigate the potential use of ecological knowledge on plant-soil interactions and growth promoting microbial techniques from horticulture to enhance reintroduction success. The present study aims to shed more light on these issues, concentrating on the use of PGPR in combination with natural and artificial soil types. In addition to
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previous studies, we do not only measure plant growth to see effects, but also investigate the microbial community in the rhizosphere. In detail, we want to answer the following questions:

Do different soil types and the PGPR application have a short time effect on seedling growth and a long term effect on the growth of reintroduced plants in the field?

Do the soil communities differ between varying sample times, soil types and PGPR inoculation?

Are the PGPR among the dominant groups in the greenhouse or the field bacterial communities?

Do the soil types and PGPR influence nodule formation in a legume?

Methods

Plant species and study region

The study was carried out in the regions of Bremen and Lower Saxony in the northern lowlands of Germany. The area is nowadays dominated by agriculture and under high anthropogenic influence, with the remaining semi-natural habitats being heavily fragmented. As model organisms we chose three persistent herbaceous plant species from three different habitat types: Genista anglica (heathland), Geum rivale (forest) and Senecio paludosus (river corridors). The three species are generally rare in the study region, but seed donor populations were found that were sufficiently big to not be at risk by seed sampling. Seeds were sampled in summer 2013 and stored at 4 °C in the dark. Prior to sowing, seeds of S. paludosus were stratified in Petri dishes on moist paper for six weeks. G. anglica seeds were carefully sandpapered and watered for one week to facilitate germination.

Experimental setup

Stratified seeds were sown to big, shallow containers in March 2014 under six different treatment combinations, consisting of three soil types (see below) and the application of plant growth promoting rhizobacteria (PGPR) to half of the containers for each soil type, resulting in the treatments control (pure soil) and + PGPR (amended with PGPR). Seedlings of proper size were pricked and transferred to individual pots (0.5 l volume) containing the same treatment conditions as the sowing containers. Plants were kept in the greenhouse until June, then they were transferred to a cold frame to acclimatize to outside conditions. In September, plants were transferred to the field and planted to their new habitat, in three field locations each hosting two plots. Samples were collected from seedlings in the greenhouse and from adult plants having been in the field for one year. Adults’ fitness measurements were done in all locations and plots. At best, three bacteria samples were collected for
every treatment-plant combination at both sample times. In the field, all samples were taken from the same plot if possible, to reduce variation in the soil communities due to spatial distance (Appendix 7.1). Field locations were found suitable for transplantation when wild species were already present, species had successfully been reintroduced there before or habitat requirements of the species were met (estimated based on expert knowledge).

Plants were cultivated on three different soil types: natural, mixed and potting soil. Natural soil was collected shortly before sowing from the field location of the seed donor populations for each plant species. It was kept moist and sieved to exclude rock fragments, large roots and large soil animals from the experiment. Plants grown on this soil were supposed to find natural conditions regarding abiotic conditions and soil microbial communities. For the mixed soil treatment, the soil was artificially created to reflect several natural soil features, i.e. natural soil pH, organic matter content and soil texture. These soil features can easily be measured and are indicative of important soil properties, e.g. water holding capacity and nutrient availability. As a template, soil samples from natural soil were taken during seed sampling and analyzed. The mixed soil was created from various commercially available substrates: quartz sand, potting soil (KNO Bremen), peat, silt (Florisol® rubra) and clay (Florisol® TM profi, 10030.006, Stephan Schmidt Gruppe, Germany). These were mixed based on their dry weight and moistened only shortly before sowing (Appendix 6.4). Commonly available potting soil (KNO Bremen) was used for the potting soil treatment. It was mixed with quartz sand (approx. 50:50) for germination, to enhance water percolation, but was used purely after pricking. To prevent reduced growth due to nutrient shortage, all plants were provided with a small amount of slow-release fertilizer after pricking.

A commercially available mixture of plant growth promoting rhizobacteria (PGPR, Vitabac®, Bactivia GmbH, Germany) was used as the microorganism treatment, containing five species of rhizobacteria that can also be found naturally in regional soils. These bacteria show various plant growth promoting traits, e.g. stimulation of germination and root growth and an increase in nutrient availability (Table B). Being delivered as white powder, the mixture was diluted in water and applied by watering. The dilution was applied repeatedly to the pots after sowing and after pricking. Based on the producer’s instructions (personal communication), 0.4 g Vitabac® was applied for each liter of soil, corresponding to $2 \times 10^9$ colony-forming units. The soil of *S. paludosus* was very compact, caused by the high clay and silt content, and part of the inoculation passed the pots along the seam without percolating the soil. To ensure sufficient inoculation, the application was repeated, reaching a final rate of 1 g of Vitabac® per liter of soil.
Analysis of plant fitness and bacteria communities

To measure the seedlings’ biomass, plantlets were carefully extracted from the soil and cleaned, after which shoot and root material was separated. Samples were dried at 40°C and weighted. Because adult plants differed in their growth form, different fitness traits were assessed. Fitness in the bushy *G. anglica* was estimated by 2-4 independent persons on a 4-step ordinal scale (small, moderately large, large, very large) and averaged. In *S. paludosus* mean plant height was measured, whereas in *G. rivale* mean leaf length. Bacteria samples in the greenhouse were taken by carefully removing the seedling roots from the soil without destroying or touching them. In the field, samples were taken with a steel ring from the basis of the plant. For *G. rivale*, three additional wild growing individuals were sampled. Samples were transferred to the lab and the rhizosphere soil was carefully extracted. Bulk soil was used for the analysis of basic soil parameters. In addition, *Rhizobium* nodulation in *G. anglica* was estimated by counting the number of nodules.

DNA was extracted from rhizosphere samples using the NucleoSpin® Soil Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer’s instructions with minor modifications. The 16S rRNA genes were amplified by PCR using the primers GM5F and 907R for bacteria (Muyzer et al. 1995). The quantity of the extracted genomic DNA and the PCR products was measured by agarose gel electrophoresis. DGGE analyses were carried out on six sample subsets, because the number of lanes on each gel was limited. Subsets were created based on plant species and sampling time, to be able to quantitatively explore treatment effects. PCR-products were separated on a 6% polyacrylamide gel with a denaturing gradient of 50 to 70%, stained with 1× SYBR-gold, visualized by blue light excitation, and photographed. Selected bands were excised from DGGE gels, re-amplified by PCR without gc-clamp, and sequenced (Sanger sequencing, LGC Genomics, Berlin, Germany). Sequences were subjected to nucleotide BLAST (www.ncbi.nlm.nih.gov/BLAST/) for an identification of the nearest phylogenetic taxa. These sequences were aligned with the MUSCLE algorithm in MEGA 7 (Kumar et al. 2016). A more detailed description can be found in Appendix 7.3.

Statistical analysis

Plant fitness parameters were analysed with R (R Developmental Core Team 2016). Seedling biomass and nodule counts were analysed with generalized linear models, for Gamma distributed data and with negative binomial errors, respectively. Fitness of adult *G. anglica* individuals was compared with ordinal regression, whereas linear models were used for the other two species, because no indications of spatial autocorrelation could be found. Post-hoc tests were done with Tukey-Tests and automatically corrected for multi-testing. Soil parameters between treatments were compared with Wilcoxon signed-rank test. For cluster analysis, the presence or absence of DGGE bands was detected
manually, recorded in a zero/one matrix and used to calculate matrix clusters with Past 3.13 (Hammer et al. 2001) applying the integrated UPGMA algorithm with the Dice similarity index (classical clustering). The number of DGGE-bands were compared between sample times using Mann-Whitney U-test. The phylogenetic tree was calculated using the Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei 1993). The tree with the highest log likelihood is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 190 nucleotide sequences. With all positions containing missing data eliminated, the final dataset contained 370 positions.

Results

Soil

The soil pH was higher in all mixed soil treatments than expected, probably due to the high buffer capacity of the inorganic material (Appendix 7.2). Although the components of soil texture fell into the same size categories, the soil structure differed between mixed and natural soils for G. rivale and S. paludosus. Due to sieving, the natural soil consisted of loose fragments resulting in a crumbly structure, whereas the mixed soil formed a compact blocky structure within the germination containers, caused by the high clay and silt content. In G. anglica and G. rivale, nutrients differed significantly between soil treatments in the germination containers (all p < 0.03), with mostly higher nutrients in the potting and mixed soil, compared to natural soil. In S. paludosus, potting soil contained more nutrients than the other soil types (both p < 0.01). After having grown one year in the field, the nutrient content around the roots of plants grown on potting soil was still higher than in the other treatments (all p < 0.03). In G. anglica and S. paludosus, the nutrient content decreased after the plants had been in the field for one year. Surprisingly, the opposite was true for G. rivale, magnesium and calcium increased compared to the cultivation conditions.

Plant fitness

Shoot and root biomass of G. anglica were significantly influenced by the soil (shoot p = 0.002, root p = 0.049) and the PGPR treatment (shoot p = 0.003, root p < 0.001), including a significant interaction in roots (p < 0.001). Growth was similarly high in natural and potting soil, but reduced in mixed soil by up to 30%. The PGPR had a negative effect on root growth in natural and potting soil (reduction by approx. 43%), but no effect in mixed soil (Figure 7.1 a, upper panel). In adult G. anglica from the field,
the biggest plants were observed when plant were cultivated on natural soil without PGPR and the smallest plants grew on potting soil with and without the addition of PGPR (soil: $p < 0.001$; bacteria: $p = 0.007$). All other treatment combinations led to intermediate fitness levels (Figure 7.1 b, upper panel).

Figure 7.1: Fitness of three plant species (one from each ecosystem type) and all soil and bacterial treatments. A) Dry weight (mg) of root and shoot biomass at seedling stage, sampled during pricking. B) Fitness of adult plants having grown in the field for one year. Given are fitness levels of *Geum anglica* (upper panel, leaf length for *G. rivale* (in cm, middle panel) and plant height for *S. paludosus* (in cm, lower panel). The numbers indicate the number of replicates being measured.

In *Geum rivale* seedlings the PGPR inoculation did not influence shoot or root biomass overall, but a significant interaction with soil was found for shoot biomass ($p = 0.006$). The lowest mean biomass for shoots and roots was observed in natural soils ($p < 0.001$). Shoot biomass was superior in mixed soils, whereas in root biomass mixed and potting soil gave similar results. Overall, the influence of the soil treatment on seedling biomass was significant for both shoots and roots (both $p < 0.001$, Figure 7.1 a,
middle panel). No significant effect of the cultivation treatments on adult’s leaf size in the field were found (Figure 7.1 b, middle panel). The growth of *S. paludosus* seedlings was significantly influenced by the soil (shoot and root, both p < 0.001), but not by PGPR inoculation. Shoot growth was high on natural and potting soil and decreased on mixed soil by 62%. Mean root biomass was similar on natural and mixed soils, but much higher on potting soil (Figure 7.1 a, lower panel). In adult plants of *S. paludosus*, none of the treatments influenced fitness significantly (Figure 7.1 b, lower panel).

**Rhizosphere bacteria**

For all plant species higher overall cumulative numbers of different bands on the DGGE-gel were found in the greenhouse compared to the field (Table 7.1, #bands). Regarding the individual treatments, the mean number of bands was significantly lower in the greenhouse than in the field for *G. anglica* (p < 0.001), indicating that the soil treatments were dominated by different bacterial groups, whereas the field sample communities were more similar. The opposite trend was true for *G. rivale* (p = 0.036) and *S. paludosus* (p = 0.027). Neither the soil nor the PGPR treatment had an overall effect on the band counts.

Table 7.1: Mean number of DGGE-bands for each plant species, treatment and sampling time. Given are the maximum number of different bands for each plant species and sampling time (# bands), the mean number of bands for each treatment and the corresponding standard deviation (n = 3).

<table>
<thead>
<tr>
<th></th>
<th><em>Genista anglica</em></th>
<th><em>Geum rivale</em></th>
<th><em>Senecio paludosus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Greenhouse</td>
<td>Field</td>
<td>Greenhouse</td>
</tr>
<tr>
<td><strong>Samples</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural</td>
<td>17.0 ± 5.2</td>
<td>25.0 ± 1.0</td>
<td>22.3 ± 2.1</td>
</tr>
<tr>
<td>Natural + PGPR</td>
<td>18.7 ± 1.5</td>
<td>25.0 ± 2.0</td>
<td>20.0 ± 1.0</td>
</tr>
<tr>
<td>Mixed</td>
<td>10.0 ± 0.0</td>
<td>23.7 ± 0.6</td>
<td>25.3 ± 1.5</td>
</tr>
<tr>
<td>Mixed + PGPR</td>
<td>16.0 ± 1.7</td>
<td>26.0 ± 1.0</td>
<td>25.7 ± 1.5</td>
</tr>
<tr>
<td>Potting</td>
<td>19.7 ± 1.2</td>
<td>24.3 ± 0.6</td>
<td>22.7 ± 2.9</td>
</tr>
<tr>
<td>Potting + PGPR</td>
<td>17.7 ± 1.5</td>
<td>28.0 ± 2.0</td>
<td>25.0 ± 4.6</td>
</tr>
<tr>
<td>Wild</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>


Figure 7.2: Cluster of soil samples based on their bacteria community are given for *G. anglica* (a + b), *G. rivale* (c + d) and *S. paludosus* (e + f). For each species, samples were collected in the greenhouse and in the field one year after transplantation.
Based on the cluster analysis, the dominant groups of the bacteria communities differed strongly between the different soil treatments, but the PGPR inoculation showed only minor effects (Figure 7.2). In all plant species, the dominant bacteria in natural soils analyzed in the greenhouse form a clear cluster which shows very low similarities (0.1 – 0.4) with the clusters for mixed and potting soils, regardless of the PGPR treatment (Figure 7.2 a, c, e). The dominant groups in mixed and potting soil were more similar, which is not surprising as they both contain the same source of potting soil, but can still be clearly distinguished. Here, communities also seem to be influenced by the microbial treatment. Surprisingly, rhizosphere communities still displayed distinct clusters when plants had been grown in the field for one year already, although the similarity in general is higher compared to cultivation conditions. In the rhizosphere of G. anglica, natural soil was still separated from potting and mixed soils, whereas these two could not be differentiated clearly (Figure 7.2 b). In G. rivale, communities from the natural soil treatment were similar to communities found in wild plants, and dominant bacteria in potting soil samples were more similar to the ones in the natural soil treatment than to the ones in the mixed soil (Figure 7.2 d). S. paludosus rhizosphere communities could not be differentiated between potting and natural soil, but the mixed soil was clearly different (Figure 7.2 f).

Among the strongest and most widespread bands, seven to 13 from each gel were excised, sequenced and used for the construction of a phylogenetic tree (Appendix 7.4). None of the five bacteria strains that were supposed to be in the PGPR treatment was found among the dominant bacteria species. There was no overlap in dominant bacteria species among plant species and sampling times (with one exception for the latter). In general, in greenhouse samples a variety of phyla were found among the dominant species, whereas field samples were mainly dominated by α- and β-Proteobacteria. Among the dominant bacteria species in the greenhouse rhizosphere of G. anglica there were members of the Bacteroidetes, the Firmicutes and the γ-Proteobacteria. Moreover, some members of β-Proteobacteria and α-Proteobacteria were present, of which Rhizobium sp. are especially important, because they can form a symbiosis with G. anglica. Interestingly, in field samples, the dominant nitrogen-fixing α-Proteobacteria shifted towards a dominance of Azospirillum sp. and Bradyrhizobium sp. Also in G. rivale the greenhouse rhizosphere is dominated by several phyla: Bacteroidetes, Firmicutes, Actinobacteria, γ-Proteobacteria and several β-Proteobacteria. The rhizosphere community from the field, again mainly contained α- and β-Proteobacteria as being dominant. For S. paludosus, several uncultured bacteria clones were identified, mainly from the β-Proteobacteria for greenhouse and field samples, with one exceptional Bacteroidetes being present in potting soils in the greenhouse.

Rhizobium nodulation of G. anglica juvenile roots differed strongly between soil treatments (p < 0.001), but not between PGPR treatments (p = 0.059). Most nodules were found in natural soils, with a mean
of 16 nodes per plant, followed by mixed soils with a mean of 4 nodes per plant. In potting soil, almost no nodulation occurred (mean nodes per plant were 0.6).

Discussion

The success rates in species reintroductions have been very low, which is mainly caused by the failure to establish to natural conditions at new sites. Thus, further research is urgently needed to understand plant-soil interactions, and to determine new and effective methods to increase this success (Drayton & Primack 2012). Expertise from the horti- and agricultural sector, for example the use of PGPR or specially adapted growing substrates, provides a basis to develop solutions also applicable in natural systems and reintroduction efforts.

The application of PGPR had no effect on the fitness of *G. rivale* and *S. paludosus* and a negative effect on the growth of seedlings and adults in *G. anglica*. Previous studies reported better plant growth, increased yield, enhanced root growth and an increased number of root hairs due to PGPR inoculation (Ahemad & Kibret 2014 and literature cited therein). Thus, we expected an overall positive effect of the PGPR on plant growth, at least during propagation in the greenhouse. As the introduced bacteria were not among the dominant ones in any of the soil treatments, we can think of four reasons why no (positive) effects of the inoculum were observed in the present study. First, applied PGPR might not have been able to establish, because they are free-living and thus sensitive to unfavorable soil conditions and competition (Garbaye 1994). Second, the PGPR may not have been able to establish a population big enough to promote plant growth. The final population size of the applied bacteria has been found to be critical for growth promotion (Holl & Chanway 1992). Third, plants respond differently to PGPR depending of substrate quality, with increasing responses caused by declining soil quality (Carrillo-Garcia et al. 2000). Thus, soil quality was probably too good for a distinct plant response. Fourth, the bacteria strains being present in the inoculum were not compatible with the plant species. Certain PGPR strains can have negative effects on some plants, although they have been beneficial to others before (Enebak et al. 1998).

The different soil types had a strong effect on all plant species, partly even long-term. It is widely known that soil properties like soil texture, pH, organic matter, nutrient content and compaction are important physicochemical soil properties influencing plant growth (Sinnett et al. 2008; Neumann et al. 2014; Quan & Liang 2017). In *G. anglica*, natural and potting soil led to good growth of seedlings, whereas it was lower on mixed soil. Natural and potting soil differed most strongly in nutrient content, texture and soil pH, thus physicochemical soil properties might not be responsible for growth differences in this case. In addition, no distinct pattern was found regarding dominant bacteria identified by sequencing, but in the mixed soil treatment there was a slightly lower number of DGGE
bands. Still, the mixed soil contained a high amount of potting soil as well, thus it appears to be unlikely that the microbial community differed strongly between these treatments. A possible reason for the reduced individual growth in the mixed treatment could be competition between seedlings, because here germination rates were highest (data not shown). On the long run, cultivation on natural soil led to enhanced growth even in the field, especially compared to potting soil. This was probably caused by a significantly higher number of root nodules already found on seedlings, which led to easier acclimatization to outside conditions, due to a better nitrogen supply. The seedlings of *G. rivale* grew best on mixed and potting soil, but growth was reduced on natural soil. This might have been caused by low nutrient contents in the germination containers, especially low values for P, but also by differing microbial communities. The taxonomic tree shows that some of the dominant bacteria differed between natural soil and the other treatments. Field soil was found to reduce growth of plant species due to undesirable pathogens that accumulate among natural plant populations (Packer & Clay 2000; Haskins & Pence 2012). In the present case, natural soil was collected from a dense stand of *G. rivale*, thus an accumulation of pathogens seems possible, although no well-known plant pathogen was identified from the sequences. Shoot and root biomass of *S. paludosus* was strongly decreased on mixed soil compared to the other soil treatments. Most likely, this was caused by the strong compaction of the mixed soil, which led to a suppression in root development, very low water percolation and thus to low soil aeration.

Despite its effect on plant growth, the soil treatment also strongly influenced the soil bacterial community, which is in accordance with previous studies (Kreitz & Anderson 1997; Buyer et al. 2002). In the greenhouse, the similarities between the community clusters were very low, especially between natural soil and the artificial soils. In part, even the PGPR treatment formed separate communities in mixed and potting soils. However, the fact that a similar clustering pattern could still be found in the field samples after one year is remarkable. Obviously, the soil type used during cultivations has a long-term effect on the rhizosphere microbial community of the reintroduced plant in the field. Overall similarity increased, which indicates that the introduced soil communities are slowly invaded by the natural communities occurring in the habitat. Based on the comparison with samples from wild individuals of *G. rivale*, the natural soil used for propagation is taken over most rapidly. It has been stated that the reestablishment of the native soil community could be a limiting factor in restoration success (Bever et al. 2010), thus using natural soil or natural soil inoculum might be advantageous. When bacteria are introduced to new habitats, e.g. in the rhizosphere of reintroduced plants, several effects might occur: they could either become invasive, or they could coexist with the native microbes, or they are outcompeted by the preexisting microbial community. Our results suggest that the latter mechanism became effective, but with a certain time lag. The introduced PGPR were not present among the dominant bacteria identified via sequencing. Moreover, most identified bacteria were
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found in soil with and without inoculum, thus the PGPR did not strongly influence the microbial community present in the different soil types, neither in the greenhouse, nor in the field. This finding is also supported by the fact that PGPR did not affect the number of nodules in the legume. Based on the taxonomic tree, dominant bacteria groups did not overlap between the three plant species, although the potting soil treatment was exactly the same. Plant species-specific rhizosphere communities have also been found in previous studies (Westover et al. 1997; Miethling et al. 2000) and have been attributed to variation in rhizodeposits (Grayston et al. 1998).

In summary, the application of unspecific PGPR had no clear effect on plant growth or rhizosphere communities in the present study. They do not seem to provide huge benefits to plant species reintroductions, probably because they are not able to compete with established microbial communities, or to cope with the huge variability in soil conditions found in natural systems. In contrast, different soil types influenced both, plant growth as well as bacterial communities, strongly and lasting even in the field. Inoculating plants with natural soil or natural soil microbial communities yields the risk of introducing pathogens, but it also promises to be highly beneficial in supporting plants to acclimatize to natural environmental conditions and thereby increasing reintroduction success.
References


Rhizosphere communities in reintroduction


Requena, N., Jimenez, I., Toro, M. & Barea, J.M. 1997. Interactions between plant-growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi and *Rhizobium* spp. in


Conserving biodiversity and saving species from extinction is a race against time. Conducting studies and experiments, however, is time consuming and translating scientific findings into political decisions is even more of a challenge. As scientists, we can only accelerate the first phase of this process. To advance more rapidly, it is important to share data and experiences from all around the world in easily accessible databases and intensify cooperation within and between scientific communities.
The loss of biodiversity and the extinction of species can only be slowed down if we understand the interactions between organisms and their environment, learn about the underlying causes for changes within these interactions, and develop effective methods to address these and ameliorate their negative effects. Local habitat specialists are especially vulnerable to ongoing habitat destruction and changing environmental conditions (McKinney & Lockwood 1999; Hewitt et al. 2010; Clavel et al. 2011). On smaller geographical scales, soil parameters are important factors influencing species distribution and rarity, however, our understanding about above-belowground interactions in natural systems is still rudimentary.

In the present thesis, the responses of forest and grassland species to edaphic gradients were analyzed, to clarify the role of the species soil niche as determinant of rarity and regional range size. Moreover, plant-soil interactions in the context of species reintroductions were studied to understand their impact on failure or success in this widely used method to fight species extinction. In this chapter, I will summarize my findings and discuss them in regard to ecological niche theory and their potential value for conservation and reintroduction efforts.

The nature of environmental gradients

In the present thesis, species responses were measured along soil pH (herbaceous forest species, chapter 3 – 5) and phosphorous gradients (grassland species, chapter 4). Austin (1980) developed a framework to categorize different types of gradients to which species might respond differently. He distinguished between direct, resource and indirect gradients. Along indirect gradients, the variable used to order the observations has no physiological effect on the plant. Species distributions vary along indirect gradients because of underlying correlations with environmental factors. Examples for indirect gradients are altitude, latitude or longitude. Direct gradients are those that do have a direct physiological influence on growth and survival but are no resource for plants. Temperature is an example for a direct gradient, as it is not changed by plants, but strongly influences them. Variables that are consumed by plants and for which competition might take place (also called “milieu factors” by Jansen and Oksanen (2013)), such as light or water, form a resource gradient. Another way to categorize gradients is by assessing the position of its variable in the chain of processes that affect the plants, varying between proximal and distal (Austin 2002). A proximal gradient represents the direct causal response of the plant, whereas a distal gradient acts more indirectly. For example, the available soluble nutrient concentration in the rhizosphere is a more proximal resource than total soil nutrient content. In this context, an indirect gradient is clearly distal, whereas direct and resource gradients are more proximal.
Another challenge in defining and comparing gradients is their length. In theory, to assess the full species response, it is necessary to evaluate the full gradient relevant to the species and beyond. However, often this is not possible in nature, because gradients are limited either naturally, e.g. when a mountain reaches the highest point, or arbitrary, when sampling effort is limited (Oksanen & Minchin 2002). Nevertheless, evaluating species responses in sections of different length or at different positions along the gradient might lead to the detection of different results (Austin 2013a). Based on these theoretical frameworks, the gradients used in the present study can be classified as followed:

The phosphorus (P) gradient used in chapter 4 is a resource gradient. P is measured as plant available phosphorus from two subsamples taken within a 1 m$^2$ plot (Diekmann et al. 2014). Thus, it is on an intermediate position concerning the direct causal response of the plant. The P gradient is naturally limited by the environmental conditions that are found in the study area for the sampled alliance (Bromion erecti).

The soil pH gradient from chapters 3-5 has been categorized as a direct gradient by Austin (1980), however in the study region it is more complex. The pH per se, i.e. the concentration of hydrogen ions, has a direct physiological effect on plants, but is no resource and thus a direct gradient (Andersson 1992). In addition, soil pH is also closely related to the soil nutrient status, because it controls the nutrients’ chemical form and solubility. Nutrient availability is changing along soil pH gradients, categorizing them as being indirect resource gradients. The fact that nutrient solubility does not change continuously with pH, but abruptly when reaching certain thresholds, further complicates the analysis of pH gradients. This is especially important considering aluminum toxicity in acidic soils. In addition, for the lowlands of Germany, a close correlation with soil moisture has been found for pH, which adds another indirect resource gradient (Peppler-Lisbach 2008a). However, in the uplands, the relationship between pH, moisture and nutrient content in soils is less strong. In conclusion, the pH gradient unites properties from indirect, direct and resource gradients. The classification regarding the causal position is also difficult, due to the plurality of the pH effects. The pH gradient used here has natural limits set by the available soil conditions found in deciduous forests in the study area.

The shape of species responses

Although the responses of species along gradients have been investigated for several decades, no general agreement on their shape has been found (Austin 2002). However, some hypotheses have evolved regarding the response of species in general and towards different gradient types (Austin & Smith 1990; Austin 2002). Thereby, it is important to differentiate between the physiological (fundamental) and the
ecological (realized) response, because species response modelling based on vegetation data can only describe the realized niche, but says little about the fundamental niche. In general, vegetation scientists agree that species respond in ecologically different ways to different gradients and that unimodal response curves are rare and skewed responses are more common (Austin 2002).

In the present case, the phosphorus gradient is a naturally limited resource gradient of intermediate causality. Based on niche theory, its fundamental response is of a limiting response type, which eventually declines reaching toxic levels beyond what is experienced naturally (Austin & Smith 1990). The ecological response, however, might be influenced by competitive interactions and is assumed to be skewed. It was hypothesized that curves might fade slowly towards mesic central gradient positions due to competition, and have steep declines towards the extreme ends due to physiological tolerances (Austin 2002). In the present analysis (Table 8.1, Box 3), 28% of the species did not respond towards the gradient, 32% had increasing or decreasing responses and 40% had plateau or unimodal response curves. Among the unimodal responses, most curves were symmetric, thus the expected dominance of skewed responses cannot be seen. Moreover, there are no obvious tendencies towards steeper declines to the extremes compared to the central position, based on our observational examination.

The pH gradient is a combination of an indirect, a direct and a resource gradient with natural limits. The response to an indirect gradient can take any shape and is controlled by the local correlation of several environmental factors (Austin 2002). Thus, it cannot be extrapolated beyond the range in which it was measured and has only local value for prediction (Austin & Smith 1990). However, an indirect gradient usually represents a combination of several direct gradients in a simple way and might thus be a very good predictor of species distributions (Guisan & Zimmermann 2000). For direct gradients, the response shape is mainly determined by the species’ tolerance towards the variables, and the responses of different species are spread along the gradient (Austin & Smith 1990). Response curves might have steep declines towards gradient extremes due to the limit of their physiological tolerance. Table 8.1 clearly shows that the communities of herbaceous forest species had very different responses in lowland and upland forests. Whereas the lowlands were clearly dominated by plateau and unimodal response types, all response types included in the HOF model approach could be found in almost equal parts in the uplands, except for type one. This result was expected when treating the pH gradient as indirect, because responses differ according to the differences in the underlying environmental gradients between lowlands and uplands. The fact that almost all species respond to the gradient further agrees with the previous findings from Guisan and Zimmermann (2000). Thus, in the present case the indirect pH gradient seems to be more similar to an ordination axis than to a direct or resource gradient, which should be taken into account.
considering further development of niche theory from response modelling. Nevertheless, the responses also met some expectations from direct gradients. For example, steep declines towards the acidic gradient extreme and smooth declines towards neutral conditions are especially pronounced in the lowlands.

We found that species response curves might take different shapes when modelled along different gradients or along the same gradient but in different areas. Moreover, the responses are rarely unimodal, not to mention symmetric unimodal. These results are in accordance with previous studies using GAM (Bio et al. 1998; Ejrnæs 2000). It might be argued that non-unimodality was found as a result of gradients being too short, however in all cases gradient length was limited due to the natural occurrence of environmental factors.

Table 8.1: Overview of model shapes in the studies in chapters 3 – 5. Given is the proportion (%) of species in the communities for every model type. Model type numbers resemble the following response curve shapes: 1 = no response, 2 = increasing/decreasing, 3 = plateau, 4 = symmetric unimodal, 5 = skewed unimodal, 6 = symmetric bimodal and 7 = skewed bimodal. Results for lowland and upland forest are also described in the results section of chapter 3.

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<td>Lowland forests</td>
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<td>3</td>
<td>Upland forests</td>
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<td>4</td>
<td>Calcareous grassland</td>
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Patterns in niche parameters

Several niche parameters are usually extracted from species response curves as numerical summaries of niche properties, which allows for comparisons between species. Commonly used parameters are the species optimum, describing the optimal conditions along the gradient, and the niche width, indicating the tolerance range of the species. The eHOF package for HOF models (Jansen & Oksanen 2013) gives a set of additional niche parameters – the outer and central borders, based on the work of Heegaard (2002). They measure relative tolerance limits based on the curve maxima. In chapters 3 and 4, we further introduce another measure of tolerance limits, the (fixed) upper and lower limits. They are defined by a fixed probability of occurrence, which marks the minimum requirements for feasible growth and translates into a minimum number of species being present in the habitat. Most niche parameters for forest species revealed different patterns in the lowlands and uplands, thus they are discussed separately below.
In grasslands, the majority of species, for which an optimum could be determined, had their preferred habitats in extremely low phosphorus concentrations (Box 3). Moreover, excluding those without a distinct response towards the P gradient, many species had narrow niches. This is in accordance with previous studies which found highest species richness and abundance in grasslands to be related to low P availability (Gilbert et al. 2009; Pannek et al. 2015), and identified P enrichment as being a major threat to grassland biodiversity in Europe (Ceulemans et al. 2014). Upper P limits are expected to be mainly determined by competition and are more or less evenly distributed along the gradient, without showing any special threshold value above which a community shift might take place. Ceulemans et al. (2014) tried to define a critical threshold for P pollution, but found no critical level of pollution below which no harm to biodiversity could be expected.

In lowland forest communities, most species find optimal conditions between pH 5 and 6 and have a niche width of 2 – 4 pH units. At slightly lower pH values compared to our study, highest species richness for herbaceous forest species in German lowlands was found by Peppler-Lisbach and Kleyer (2009), whereas a steady increase of species richness along a pH gradient was found in France by van Couwenberghe et al. (2011). As many species from chapters 3 and 4 showed plateau responses towards the base-rich side of the pH gradient, niche width of those species is underestimated, because it is restricted by the gradient limit on the right side and not by the species ecology. Accordingly, many more species had a lower limit compared to an upper limit. Similar to the grasslands, the upper limits were evenly distributed along the gradient and were probably determined by competition for the underlying nutrient gradients. In contrast, the lower limits and the lower central borders were clumped at pH values between 3 and 4.5, which is exactly the pH range where aluminum toxicity was found to cause problems to many plant species and where the highest species turnover occurs (Peppler-Lisbach & Kleyer 2009). Thus, we believe that the clumping of limits indicates a physiological threshold to the majority of the community.

In the uplands, most herbaceous forest species did not have a single optimum, but showed bimodal responses. However, the optima found were evenly distributed along the whole gradient, which is also applicable for the niche width and the upper limits for pH. Most of the lower limits were aggregated between pH 2.5 and 4.5, but this clumping was not significant. The upland patterns are more irregular than the lowland patterns, which is probably caused by the less stringent correlations between the underlying gradients. For a more detailed interpretation and comparison of the data, it is necessary to use multivariate modelling approaches, such as GAM, to account individually for the different environmental variables (nutrients, moisture, pH).
Box 3: Response curves and frequency distributions of species optima and niche width (based on the central borders) for the herbaceous forest and grasslands species from chapters 3 and 4. Rare and endangered species are marked in red (categories based on Garve (2004)).
Determination of range size and rarity

Key characteristics determining species resilience to global environmental change are abundance and range size. Species with small range sizes and low abundances were found to be especially at risk, because they are likewise vulnerable to stochastic effects and localized catastrophes (Rooney et al. 2004; Clavel et al. 2011). For conservation it is thus important to explore the causes of differing range size and abundance, and to explain why some species are rare and some are common. Several explanations for species rareness were proposed: Hanski et al. (1993) predicted that species using common resources are common as well (niche position hypothesis), whereas Brown (1984) postulated that species that are able to exploit a wider range of resources also have a wider distribution (niche breadth hypothesis). In addition, a low competitive ability and a low dispersal ability might also explain rareness (Kolb et al. 2006; Markham 2014). Species niche breadth and niche position were found to be good predictors of range size, but a combined approach, the available niche breadth, was superior compared to the single determinants (Gregory & Gaston 2000; Pannek et al. 2013; Markham 2014).

Another possibility to describe the species niche, when actual measurements are not available to calculate niche position and breadth, are environmental indicator values, such as the system developed and updated by Ellenberg et al. (2001) for Central Europe. They allow an estimation of environmental conditions in a vegetation plot based on the vegetation alone. Thus they are invaluable in vegetation ecology and have been widely and successfully used in numerous studies (Diekmann 2003). Because they are derived from expert experience, contain an element of circularity when used and do not account for niche shifts, their application has been criticized (Wamelink et al. 2002; Zelený & Schaffers 2012). Nevertheless, they are often well correlated to measured species optima, and might thus also be good predictor of their range size and rarity (Diekmann 1995; Schaffers & Sýkora 2000; Diekmann 2003).

On-going environmental changes and habitat losses may force species to live in environments that are closer to their physiological or ecological limits than to their optimal conditions. Thus, we hypothesized in chapters 3 and 4 that niche limits might be better predictors of species range size and abundance than optima, already at present and increasingly so in the future, making them more relevant to conservation (Falkengren-Grerup & Tyler 1993a). We tested the lower and upper limits of species for their ability to determine range size and several measures of rarity, and compared them to optima and Ellenberg indicator values.
In the lowland forests, lower limits, optima and Ellenberg R (soil reaction) values were all significantly correlated to range size, threat level and an expert-based estimation of population trend. In all cases, lower limits had higher correlation coefficients than the other niche measures, whereas optima and Ellenberg R were on a similar level. Herbaceous species intolerant to acidic soil conditions had a lower abundance, a smaller range size and showed a decreasing population trend in the lowland forests. In the uplands, only the lower limits were significantly correlated to regional range size, but not to the other rarity measures. This result is associated with a strong decline in moist, base-rich and fertile forest habitats in the German lowlands, mainly caused by land conversion and drainage (Döring-Mederake 1991). The soils of upland forests are stronger buffered due to the carbonate bedrock and offer overall better conditions for basiphilous species. Similar results in regard to soil type rarity was found for Swedish forest taxa (Gustafsson 1994).

In dry grasslands, species range size was positively correlated with the Ellenberg N (nitrogen or nutrients) value, species optima and their upper limits. Here, species with a higher nutrient demand were more common than species tolerant to low nutrient availability. Again, the correlation coefficient of the limits was much higher than for the other niche measures. In addition, we separately analyzed the limits of all species with an Ellenberg N value of two. Their tolerances towards P availability varied strongly, although they were supposed to be similar in their preference to low nutrient availability. The species that are most tolerant to low P availability and least competitive had the smallest range size, which is in accordance with the finding by Kleijn et al. (2008) and Markham (2014). Regarding species Red List status, threatened species had low Ellenberg N values or smaller upper limits than non-threatened species.

The results support our hypothesis that niche limits may be better predictors of plant distribution and rarity than species optima or Ellenberg values, because they performed consistently better in both habitat types. Moreover, they were also related to range size in species with similar optima. Niche limits, being critical levels at the end of niche breadth, were also suggested to be a main driver to species establishment in a community by Tilman (1988) and Paal et al. (2013). In addition, our results demonstrate the importance of soil variables in species distribution on regional scales.

Niche shifts

We found different species responses along the pH gradient in lowland and upland forests in chapter 3. Lower limits were correlated between the two regions, but more clumped in the lowlands than in the uplands. Upper limits were not related between regions and response curve shapes differed. Shifts in niche
parameters were also analyzed in various other studies on pH (Diekmann & Lawesson 1999; Gégout & Krizova 2003; Coudun et al. 2006; Hájková et al. 2008; Plesková et al. 2016; Reinecke et al. 2016; Wagner et al. 2017), moisture (Booth & Zygmunt 2005), altitude (Gégout et al. 2003), latitude (Wasof et al. 2013), Ellenberg indicator values (Diekmann & Lawesson 1999; Prinzing et al. 2002) and conductivity gradients (Hájková et al. 2008). Contradictory results were found for niche positions and niche width, being stable between regions in some studies, but shifting in others. Variation in species responses were discussed as being caused by local adaptation towards environmental factors (Wasof et al. 2013), competitive release towards areas with smaller species pools (Diekmann & Lawesson 1999), ecological marginality (Prinzing et al. 2002) and compensation for unfavorable climatic conditions (Hájková et al. 2008). However, shifts of species niches were found to be rather species specific instead of revealing a general directional trend, if they occurred at all. Interestingly, most studies used complex, indirect gradients (latitude, altitude, pH or ordination axes) to analyze species niche shifts. Considering the ecological theory concerning these gradient types, strong, non-directional variations in niches between studied species, habitats and geographical ranges are not surprising. The gradients’ correlation with species distribution depends on local correlations between environmental factors, thus responses to indirect gradients can take any shape and are unlikely to show a consistent pattern across studies (Austin 2002). Although biotic interactions and species traits surely play an important role in shaping species realized niches, ecological theory as well as the results of Wagner et al. (2017) strongly suggest underlying environmental factors explaining shifts in species responses modelled along indirect gradients. Our hypothesis that the lower pH limits from chapter 3 are relatively constant across regions is in accordance with ecological theory, because they are caused by a physiological threshold towards aluminum acidity and are thus conceived to have a direct impact. However, it still needs to be tested on larger geographical scales.

Reliability of response models

HOF models are effective tools to study species responses along gradients and performed better than all other types of response curve functions (Lawesson & Oksanen 2002; Oksanen & Minchin 2002; Jansen & Oksanen 2013). However, their results can be biased, such as in other ecological modelling systems, because of two reasons: (1) incorrect ecological assumptions and (2) various characteristics of the data basis used to fit the models. Regarding the first issue, by using HOF models we assume that species distributions are mainly distinguished by environmental factors, that species are in an equilibrium with their environment and that species response shapes take one of the seven pre-determined shapes. All these assumptions might be questioned, but this simplification is a reasonable compromise in exchange
Synthesis

for feasibility and interpretability when using HOF models. Concerning data characteristics, several error sources pose potential biases to species distribution models.

In chapter 5, we explicitly discuss the bias imposed by differing numbers of presences, frequencies (ratio between presences and absences) and sample sizes to HOF models and the niche parameters derived from them. The species optimum was the only parameter that was not affected by changing data characteristics. Re-prediction success of curve shapes among repetitive runs was low with small sample sizes and high frequencies. Moreover, the full set of model types was only exploited when frequencies were low and the sample size high. Also, niche width and niche boundaries were strongly influenced, shifting towards more extreme values with an increasing frequency and an increasing number of presences. Previous studies found unreliable results for logistic regression (Stockwell & Peterson 2002; Coudun & Gégout 2006) and low prediction accuracy for several species response models with small sample sizes (Pearce & Ferrier 2000; Wisz et al. 2008), as well as effects of frequency on the performance of distribution models (Fielding & Bell 1997). In addition, we found a directed shift in niche parameters, which might lead to wrong ecological interpretations when comparing rare and common species or results between studies. Thus, we generally agree with the suggestions made by previous authors that sample size should be large, that frequency (sampling prevalence) should be fixed in comparative studies and must be reported (McPherson et al. 2004), and that a minimum number of presences is needed irrespective of the general sample size (Coudun & Gégout 2006) to get reliable results from species response models.

However, with these guidelines the study presented in chapter 3 would not have been possible, because most of the rare species would have been rejected from the data set due to a too low number of presences. Thus, another way was chosen to see whether the patterns found were caused by statistical bias or had an ecological meaning. The prevalence-based thresholds were calculated, which identified those points along the gradient where the probability of occurrence becomes lower than by chance given the total frequency. The strong correlation between these thresholds and the evaluated limits demonstrated the ecological significance of the difference between rare and common species in the study.

When combining vegetation plot data from multiple sources, they probably have different sampling patterns, e.g. plots are sampled randomly and non-randomly. Non-random sampling usually results in a smaller number of common and a larger number of rare species to be found compared to random sampling (Diekmann et al. 2007). This leads to an overestimation in the probability of occurrence for rare species compared to common species and has been shown to decrease prediction accuracy in classification tree models (Edwards et al. 2006). However in response modelling, reliable conclusions about the distribution of species can only be drawn when an adequate number of samples covers the whole range of the
environmental variable (Austin & Heyligers 1989). Random sampling includes a considerable redundancy towards dominant habitat types or environmental conditions and is thus only suitable for describing the full variety of conditions in a region when sampling numbers are extremely high (Økland 1990; Diekmann et al. 2007). The forests in the study area are mainly growing on acidic soils, thus the data sets comprised from the literature were imbalanced towards the acidic side of the gradient. An additional vegetation survey focused on more base-rich sites to compensate this imbalance and led to a non-random sampling design in favor of a more evenly distributed sampling pattern along the gradient and the coverage of all pH values present in the area.

Another issue, which is especially apparent in soil factors, is small-scale heterogeneity in plots. Strong variations in soil pH and other soil parameters can occur within a few meters. In the data comprised from literature, plot size varied between 100 and 900 m$^2$, and although authors claimed their plots to be homogeneous, small-scale heterogeneity surely occurred. To what extent this might have influenced the models depends on the species capacity to integrate over the environmental heterogeneity found in plots (Palmer & Dixon 1990) and might thus be rather species and gradient specific. Several studies discovered limited tolerances of forest herbs, which are also included in the analyses in chapters 3 and 4, towards a soil pH gradient (Andersson 1992; Falkengren-Grerup & Tyler 1993a, b). Palmer and Dixon (1990) suggested a correction method for response curves based on abundance, but not for presence/absence data. Potential effects of within-plot heterogeneity of environmental parameters might be an overestimation of niche breadth, an underestimation of the maximum probability of occurrences and a shift in the species optima.

Surprisingly, species rarity was found to increase the accuracy of species distribution models, irrespective of the effect of sample prevalence (Pearce & Ferrier 2000; Franklin et al. 2009). It was suggested that rare species are often habitat specialists and that their niche is thus easier to define than the niche of common, generalist species. We have no means to estimate the effect of this bias on the results of the studies presented here. However, the fact that only forest specialist species were used probably limits potential problems in comparing species among each other on the one hand, and on the other hand increases the reliability of the results found.

It was noted that the position of the species optimum along the ecological gradient has an influence on the derivation of response curves (Mohler 1983; Rydgren et al. 2003). It was shown that response curves tend to be asymmetric when the species optimum is near the gradient extremes (Austin et al. 1990; Austin et al. 1994). Unreliable results for curve parameters caused by extreme optima were also found in logistic regression by Coudun and Gégout (2006). Also, the data sets used in chapters 3 to 5 contained many
species with optima at the gradient extremes. However, intensive sampling at the gradient extremes, at least towards the acidic extreme in the forest data set, increased model accuracy at this point of the gradient, as suggested by Mohler (1983).

Implications for ordination

The fact that response shapes regularly deviate from a symmetric unimodal curve reveals some incompatibilities between ecological theory and statistical methods used in vegetation studies. Ordination is a widely used technique to order and summarize vegetation data. Several methodologies were developed, among which correspondence analysis (CA)-based approaches (CA, DCA, CCA) are most widely adopted (Austin 2013a). Other ordination methods are principal coordinates analysis (PCoA) and non-metric multi-dimensional scaling (NMDS). They all have in common that they assume the species responses to be unimodal along an ecological gradient, given it is of sufficient length (Austin 2013a). However, this situation can rarely be found in natural systems. In addition, other clear ecological assumptions were made for the canonical correspondence analysis (CCA) including equal niche breadth, homogeneously distributed optima and long gradients compared to the species niche breadth (ter Braak 1985, 1986). None of the communities analyzed in the present thesis meets these expectations and I feel certain that this is also the case in many other studies, species and systems. Moreover, rare and abundant species show different patterns in their niche parameters along gradients, which might add additional uncertainty to ordination results, but standardization techniques have been hypothesized to be useful counteractions (Austin 2013a). The apparent incompatibilities between ecological theory and ordination methods was called a Kuhnian research paradigm (Austin 2013a), where each participant in the paradigm tends to ignore inconvenient facts until the accumulation of exceptions produces a sudden switch to a new paradigm. Thus, the present thesis presents another exception to the assumptions made by common, CA-based ordination methods. Luckily, several authors have tested and compared ordination models for their robustness towards discrepancies between assumed and natural conditions, and have nominated NMDS with extended dissimilarity to be the best candidate for replacing the old paradigm (Minchin 1987; De’ath 1999; Mahecha et al. 2007; Austin 2013a).

The importance of soil in plant species distributions

Soil formation is driven by climate, parental material, topography and living organisms, and it is vertically and horizontally very heterogeneous on small scales (Hall et al. 2004; Thuiller 2013). However, there is
evidence from all around the world that soil factors, such as pH, nutrients or moisture, explain plant species distributions and diversity (chapters 3 and 4) (Hall et al. 2004; Coudun et al. 2006; Bertrand et al. 2011; Bertrand et al. 2012; Chambers et al. 2013) and even animal distributions (Titeux et al. 2009). Unfortunately, these ecophysiological meaningful variables have rarely been used in species distribution models so far, which was criticized by various authors (Bertrand et al. 2012; Thuiller 2013; Mod et al. 2016). Due to the complexity of soils, caution is needed when using distal gradients like topography or soil texture as simplified proxies for belowground resources, because this underestimates the importance of the edaphic dimension (Hall et al. 2004). But data on direct and resource gradients are hard to come by, especially at resolutions which accurately reflect the natural small scale heterogeneity of soils. The lack of appropriate soil data has been identified as being the main reason why these are missing from many distribution models (Dengler et al. 2011; Mod et al. 2016). In chapters 3 and 4, we join the call for considering edaphic factors in predicting species distributions more rigorously. To put this into practice, it is of utmost importance to continuously collect environmental data parallel to vegetation surveys and to share them in global databases. Moreover, we need to increase the collaboration with geo-environmental sciences, to combine knowledge and data from both fields (Mod et al. 2016), and establish standardized methodologies for data collection (Schuster & Diekmann 2003).

Soil pH is special among the edaphic factors. Due to its’ complexity, it was an excellent predictor of species distribution in many cases, especially in Central Europe. Here, species richness and diversity, overall but especially so for rare species, is generally much higher on calcareous site than on acidic sites (Chytrý et al. 2003; Pärtel et al. 2004; van Couwenberghe et al. 2011). For Central Europe, it was hypothesized that during the Pleistocene range contradictions more acidophilous than calciphilous species became extinct, because most refugial areas were on calcareous sites, e.g. being situated in southern European mountain ranges with abundant limestone and dolomite (Chytrý et al. 2003; Ewald 2003). A similar pattern was found all over the world by Pärtel (2002), who concluded that negative correlations between species richness and pH were caused by historical and evolutionary processes. However, other findings contradict the worldwide generality of the historical hypothesis and suggest more favorable nutrient conditions for plants as the underlying cause (Peet et al. 2003). Nevertheless, in Central Europe soil pH is a strong predictor of species richness, range size and rarity. This makes it a valuable environmental factor for conservation planning.
Modelling implications for conservation and reintroductions

Today, species distribution models (SDMs) are commonly used by scientists to examine the distribution of rare species and find potentially suitable habitats (e.g. Bourg et al. 2005; Olsson & Rogers 2009; Abeli et al. 2012). Many authors claim that their results are important for the conservation of the species and there is no doubt that the development of SDMs contributed significantly to our understanding of the relationship between species and their environment. A variety of potential benefits and applications of SDMs to conservation authorities have been proposed, such as guidance during the decision making processes (Guisan et al. 2013), finding suitable habitats for species reintroductions (Gogol-Prokurat 2011), indication of yet unknown populations (Wiser et al. 1998) and prediction of the impact of environmental changes or of the course of invasion (Guisan et al. 2013). Moreover, it is usually suggested that modelling is cost-effective and an alternative to expert consulting (Abeli et al. 2012; Gastón et al. 2014). However, at least in the published literature evidence of SDMs supporting actual conservation management is scarce. Guisan et al. (2013) provide one of the very few studies giving examples of management decisions that were explicitly guided by SDMs. Thus, to what extent SDMs can actually contribute to conservation remains elusive and will only become clear when there is a closer cooperation between decision makers and scientists.

In the present thesis, species responses were modeled along individual environmental gradients. In comparison to multivariate approaches, such as climatic envelope models or GAMs, the HOF models (and other univariate methods) do only provide limited direct potential guidance or use to conservation managers, because they do not consider the interactions between various environmental factors. This drawback can partly be bypassed when using indirect gradients or ordination axes, which summarize the most important environmental factors in one variable (such as soil pH in the lowlands), but this constrains our understanding of the importance of individual environmental factors in determining distributions. However, species response models can contribute indirectly to conservation by increasing our understanding of relationships between species and their environment. In more detail, univariate response models can be used (1) to develop sound ecological niche theory, which is the basis for more complex SDMs and other statistical methods, (2) to identify the environmental variables that are most important to species or communities, (3) to increase our understanding towards the direct effects of individual gradients and (4) to develop a new and more accurate set of species indicator values (Wamelink et al. 2005), which may supplement missing data.

As mentioned before, soil pH was found to be extraordinarily useful for the determination of species abundance and range size for forests in the study area (Peppler-Lisbach & Kleyer 2009; Pannek et al. 2013).
Thus, in the present case it is possible to construe some implications for regional conservation and reintroduction from the single gradient models. The results suggest that in the German lowlands, the few remaining deciduous forests on high pH soils are especially valuable for conserving the biodiversity of herbaceous forest plants. They usually provide the whole set of favorable environmental conditions needed by the majority of the plant community and the rare plant species. Moreover, a further reduction of soil pH, for example by an increased input of nitrogen (Grennfelt & Hultberg 1987), should be avoided, especially when soil pH is already approaching the threshold level of pH 4.5. Below this threshold, the herbaceous community will change completely towards an acid-tolerant, low-diversity community and probably all rare plant species will be lost. In the German upland range, species distribution cannot be explained reliably by soil pH alone. Here, more information on other environmental or management factors need to be taken into account before considering actions regarding the conservation of plant diversity. In Northern Europe, a significantly higher proportion of high pH soil is found in protected areas than it can be expected from the overall proportion of these soils (Pärtel et al. 2004), which is good news. However, this has not been analyzed for Central Europe, yet, thus we do not know in how far the regional or local forest biodiversity is protected. For forest species in the lowlands, habitat fragmentation is another major constrain, which needs to be considered in addition to soil factors (Kolb & Diekmann 2004, 2005).

Also soil P content (or content of macronutrients in general) has been shown to determine species diversity and community assembly in grasslands (Stevens et al. 2004; Gilbert et al. 2009; Stevens et al. 2011; Pannek et al. 2015). Our results that nutrient levels should be kept as low as possible if rare species shall be conserved are in accordance with the findings of other authors. In addition, we identified those species which are most vulnerable to increasing P levels: species with low P optima and additionally also low P upper limits. These species, such as *Euphrasia officinalis*, *Helictotrichon pratense* and *Hippocrepis comosa*, are already the least widespread and will most likely go extinct. These species might need additional assistance in the future, for example via translocation or reintroduction to nutrient-poor habitats.

**Studying reintroductions**

If there is no way to reduce the stress that threatens a species’ survival in its original habitat, we have no alternative to reintroducing this species to a new habitat to save it from extinction. For good reasons species reintroduction and associated methods are nowadays accepted techniques for mitigating plant species declines worldwide (US Fish and Wildlife Service 1999; Planta Europa Network 2008; IUCN/SSC 2013). However, overall success rates of reintroductions are varying between 33% and 78%, depending on the methodology used for data collection (Godefroid et al. 2011; Guerrant 2012). As reintroductions may
bear multiple risks and usually follow strict strategic and ethical guidelines, they are only considered when there are no alternatives to protect a threatened species from extinction (IUCN/SSC 2013). Thus, much higher success rates are highly desirable. In this context, it is surprising that published literature on plant reintroduction is scarce. Godefroid and Vanderborght (2011) found that only 82 papers on plant reintroductions were published between 1989 and 2009, although they knew about 653 reintroduction programs from 39 countries from an earlier practitioner survey. This lack of scientific or otherwise published literature is in accordance with my own experience from searching the literature during this thesis. Apart from the book “Plant reintroduction in a changing climate – Promises and perils” published by Maschinski and Haskins (2012), information on plant reintroductions was hard to find, especially in regard to certain topics, such as the effect of plant-soil interactions and bacteria. In my opinion, this lack of studies might be due to several reasons: (1) experiments on plant reintroductions are labor-intensive, costly and time-consuming; (2) either intensive preliminary studies or a lot of experience is needed to choose suitable techniques, species and habitats for the reintroduction; (3) to conduct large-scale studies, intensive cooperation with governmental agencies is needed and (4) the chances are high that negative or very specific results are achieved, which are difficult to publish. In addition to the reduced academic interest, it is believed that worldwide thousands of reintroduction projects are being conducted without publishing their results to the public (Godefroid & Vanderborght 2011), which all leads to a general scarcity of reintroduction literature. I support the call from Godefroid and Vanderborght (2011) for a change in attitude towards the publication or broadcasting of specific and especially negative results in this context. The chapters 6 and 7 of this thesis contribute to increasing the knowledge we have about species reintroductions and aim at increasing success rates of future reintroductions.

Table 8.2: Effects of the three soil treatments (natural, mixed, potting) on various growth traits during the reintroduction experiment described in chapters 6 and 7. Different letters (a, b, c) indicate significant differences between the treatments for each species and trait measured. “a” marks the most successful treatment, other letters follow in decreasing order.
<table>
<thead>
<tr>
<th>Species</th>
<th>Soil treatment</th>
<th>No. of seedlings</th>
<th>Height</th>
<th>Height</th>
<th>No. of branches</th>
<th>Height</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Euphorbia palustris</em></td>
<td>Natural</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td></td>
<td>Potting</td>
<td>ab</td>
<td>b</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Soil treatment</th>
<th>No. of seedlings</th>
<th>Shoot biomass</th>
<th>Root biomass</th>
<th>Height</th>
<th>No. of branches</th>
<th>Height</th>
<th>No. of branches</th>
<th>Growth</th>
<th>Flower</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Genista anglica</em></td>
<td>Natural</td>
<td>b</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>a</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>ab</td>
<td>b</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>Potting</td>
<td>c</td>
<td>a</td>
<td>a</td>
<td>c</td>
<td>c</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Soil treatment</th>
<th>No. of seedlings</th>
<th>Shoot biomass</th>
<th>Root biomass</th>
<th>Leaf length</th>
<th>Leaf length</th>
<th>Leaf size</th>
<th>Flower</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Geum rivale</em></td>
<td>Natural</td>
<td>a</td>
<td>c</td>
<td>b</td>
<td>a</td>
<td>c</td>
<td>b</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>b</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>Potting</td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>b</td>
<td>b</td>
<td>a</td>
<td>a</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Soil treatment</th>
<th>No. of seedlings</th>
<th>No. of leaves</th>
<th>Leaf length</th>
<th>No. of leaves</th>
<th>Leaf length</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phyteuma nigrum</em></td>
<td>Natural</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td></td>
<td>Potting</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>Soil treatment</th>
<th>No. of seedlings</th>
<th>Shoot biomass</th>
<th>Root biomass</th>
<th>Leaf length</th>
<th>Height</th>
<th>Leaf length</th>
<th>Height</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Senecio paludosus</em></td>
<td>Natural</td>
<td>a</td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>c</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>Potting</td>
<td>b</td>
<td>a</td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
</tbody>
</table>
The impact of soil and certain soil biota on plants during reintroduction

The failure of reintroductions is often attributed to insufficient knowledge about the species’ needs. Many individuals already die during the acclimatization phase shortly after being transplanted to their new habitats (Dalrymple et al. 2012; Haskins & Pence 2012). The adaptation to new environmental conditions is stressful for the species and, hence, a proper acclimatization procedure prior to transplanting might potentially prevent some of these early losses. Numerous modelling and experimental studies show that soil factors play an essential role in plant life (see literature cited above in this chapter or in chapter 1), however the acclimatization to soil factors has rarely been described in reintroduction literature so far. But several studies on ecosystem restoration show promising results regarding the inclusion of knowledge on plant-soil interactions (Eviner & Hawkes 2008; Heneghan et al. 2008; Hufford et al. 2014; Wubs et al. 2016; Koziol & Bever 2017). In the reintroduction experiment in chapter 6 and 7, we tested whether plants raised on different soil types (natural, mixed and potting soil) performed differently during cultivation and after transplanting. We also examined if the application of plant growth promoting rhizobacteria, which are known to promote plant growth from horti- and agricultural studies, would be of advantage for the plants and their acclimatization success after transplanting.

The influence of the soil treatment on plant growth at different stages during the reintroduction experiment described in detail in chapters 6 and 7 is summarized in Table 8.2. Although there are slight variations among growth traits measured at the same phase, for each individual species the responses during the greenhouse cultivation phase were similar, while the importance of the treatments are decreasing after transplanting to the field. All plant species grew best on natural soil, except for *Geum rivale* that grew least on natural soil. The use of potting soil in general led to intermediate growth rates, except for *Genista anglica*, whose natural soil conditions differed most strongly from potting soil. The artificially mixed soil was the best growing substrate for *G. rivale*, however it led to reduced growth of the riverside species, due to the high clay content of the mixture. The structure of the clayey mixed soil differed clearly from the natural soil, and the substrate in the pots was very compact (compare cover picture of chapter 6).

Plants can be influenced by physicochemical and biological soil properties. By creating the mixed soil treatment, we planned to examine whether plant growth and acclimatization could be facilitated by replicating some important physicochemical soil parameters with easily accessible soil substrates. However, we failed at establishing natural pH levels and natural soil structure, probably because the substrate was not given enough time to undergo soil forming processes (Pronk et al. 2012). Thus, our results regarding the effect of artificially mixed substrate can only be seen as preliminary, especially
because specifically optimized growing substrates are widely and successfully used in horticultural and agricultural businesses. In addition to the three physicochemical soil parameters examined here (pH, organic matter content and soil texture), many more play essential roles in plant-soil-microbe interactions, especially soil nutrient availability, which needs to be analyzed in future studies on plant reintroductions.

Overall, results for natural soils were best and several other studies support the so-called “home soil advantage” (Carrillo-Garcia et al. 2000; Montalvo & Ellstrand 2000; Grøndahl & Ehlers 2008). In the present case, we cannot distinguish whether plant growth on natural soil was positively influenced by physicochemical or biological factors, because we did not compare untreated to sterilized natural soil. Based on published literature, growth was probably promoted by a combination of both factors, physicochemical and biological (Philippot et al. 2013; Neumann et al. 2014; Liao et al. 2015; Quan & Liang 2017). Important biological players in plant-soil interactions are mycorrhiza fungi (Sylvia 1989; Requena et al. 1997; Maltz & Treseder 2015; Koziol & Bever 2017), which might have been present in the natural soils, but maybe not or only to a lesser extent in the mixed and potting soil treatments. However, they were not studied in the experiment presented in this thesis, so interpretations in this direction are speculative. Contrary to all other species, the growth of *G. rivale* was strongly reduced on natural soil compared to the other treatments. Several other authors also found growth of herbaceous and tree species to be repressed on home soil, due to an accumulation of pathogens in the root vicinity of congeners (Packer & Clay 2000; Klironomos 2002; Eckstein & Otte 2005; Nijjer et al. 2007). We hypothesized that this could also have been the case in *G. rivale*, because soil was taken from a dense population, whereas all other species had only sparse occurrences at their sites. But at the analysis of the soil rhizobacterial community no known plant pathogen was found among the dominant bacteria groups. Nevertheless, we cannot reject this hypothesis based on the findings, because plant-bacteria interaction can be highly species-specific (Enebak et al. 1998) and to our knowledge no one has studied this relationship for *G. rivale*, yet. Moreover, soil fungi can also act as plant pathogens, but they were not analyzed in the present experiment.

Table 8.3: Effects of the inoculation with plant growth promoting rhizobacteria (PGPR) on multiple growth traits measured during the reintroduction experiment from chapters 6 and 7. Given is the information whether PGPR increased (green), decreased (red) or had no effect (violet) on plant growth for the three different soils.
<table>
<thead>
<tr>
<th>Species</th>
<th>Soil treatment</th>
<th>No. of seedlings</th>
<th>Height</th>
<th>Seedlings</th>
<th>No. of branches</th>
<th>Height</th>
<th>Transplants</th>
<th>Adult 1st year</th>
<th>Adult 2nd year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euphorbia palustris</td>
<td>Natural</td>
<td>no</td>
<td>decrease</td>
<td>increase</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>no</td>
<td>increase</td>
<td>increase</td>
<td>no</td>
<td>no</td>
<td>increase</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>Potting</td>
<td>no</td>
<td>decrease</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>decrease</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Genista anglica</td>
<td>Natural</td>
<td>no</td>
<td>decrease</td>
<td>decrease</td>
<td>no</td>
<td>decrease</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>no</td>
<td>decrease</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>Potting</td>
<td>no</td>
<td>decrease</td>
<td>decrease</td>
<td>no</td>
<td>increase</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Geum rivale</td>
<td>Natural</td>
<td>no</td>
<td>no</td>
<td>decrease</td>
<td>decrease</td>
<td>no</td>
<td>decrease</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>no</td>
<td>no</td>
<td>decrease</td>
<td>decrease</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>Potting</td>
<td>no</td>
<td>no</td>
<td>decrease</td>
<td>decrease</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Phyteuma nigrum</td>
<td>Natural</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
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<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Senecio paludosus</td>
<td>Natural</td>
<td>no</td>
<td>no</td>
<td>decrease</td>
<td>decrease</td>
<td>decrease</td>
<td>no</td>
<td>no</td>
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<td></td>
<td>Mixed</td>
<td>no</td>
<td>no</td>
<td>increase</td>
<td>decrease</td>
<td>decrease</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>Potting</td>
<td>no</td>
<td>no</td>
<td>decrease</td>
<td>no</td>
<td>decrease</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
</tbody>
</table>
In addition to the soil treatment, we also examined the effect of an inoculation with PGPR on the cultivation and acclimatization success in the reintroduction experiment (Table 8.3). In most cases, the PGPR had no effect on plant growth at all, and if there was an effect, it was mainly negative. Furthermore, the analysis of the rhizosphere community did not show any of the applied bacteria to be among the dominant ones, neither in the greenhouse, nor in the field. Thus, it is no surprise that no growth promoting effects have been found, as the final population size of the PGPR has been determined to be crucial for growth promotion (Holl & Chanway 1992). Other reasons for the ineffectiveness could be that the PGPR were not able to establish at all, due to unfavorable application or too much competition with natural bacteria. In a study on ecosystem restoration, inoculation effects were reduced by the soil legacy compared to bare soil (Wubs et al. 2016), thus we expected the PGPR to be more effective on the mixed and potting soil media compared to natural soil, but this was not the case. In the end, commercially available, unspecific PGPR do not seem to provide any advantages to the plants in the context of reintroduction, thus additional costs for the application of such PGPR inoculum should be saved.

Rhizosphere communities in reintroductions

Natural rhizosphere microbial communities contribute significantly to ecosystem processes (Högberg et al. 2001; Rillig & Mummey 2006) and may facilitate plant growth or act as pathogens (van der Heijden et al. 2008). They are affected by soil properties and plant exudates, but they are likewise able to make changes to these systems (Bever et al. 2010). Based on the increasing awareness of their influence on the outcome of ecosystem restorations (Suding et al. 2004; Kardol & Wardle 2010), we took a closer look at the processes in these communities during reintroduction in chapter 7. Bacterial communities in the rhizosphere differed significantly between soil types and host plants, which is in coherence with previous studies (Marschner et al. 2001; Buyer et al. 2002; Marschner & Timonen 2005; Berg 2009). It is not clear which of the two factors – soil or host – is more dominant in the present case, because communities differed between plant species on the same soil as well as between soils with the same host plant species. Soil pH is one of the most influential soil variables for bacteria, shaping diversity, size, activity, community structure and global distribution of bacteria (Lauber et al. 2009; King et al. 2010; Rousk et al. 2010). In the present experiment, however, we did not manage to establish or preserve natural pH conditions in the mixed and natural soil treatments, respectively. Thus the variations in pH between the soil types were very small and cannot explain differences in the rhizobacterial communities. Similar to the results in plant growth, the PGPR had no or only little effects on the rhizobacterial community.
The most important finding of the experiment was that different soil treatments, used during cultivation, still had a major effect on the soil microbial communities in the rhizosphere one year after transplanting. While long-term differences in growth traits based on soil treatments could only be found in *E. palustris* and *G. anglica*, the long-term impact on the microbial community was found for all studied plant species (*G. anglica*, *G. rivale*, *S. paludosus*). In *G. rivale* the rhizobacterial communities of wild individuals were analyzed in addition to the transplanted ones. Wild bacterial communities were very similar to the ones found in the natural soil treatment in regard to dominant bacteria groups. This shows that the natural soil treatment is more rapidly taken over by wild bacteria communities, than the artificial soils. Hence, it might be advantageous to use natural soil or natural soil inoculum in reintroduction to accelerate the establishment of natural microbial communities and thereby acclimatization to new habitats. However, caution is warranted, as natural soils might also contain plant pathogens and pests. Negative effects of natural soils could be seen in *G. rivale* plants, which grew least on natural soil. Thus, uncritical upscaling of the home soil advantage in reintroduction cultivations might cause heavy losses. Species specific preliminary studies need to be conducted to assess potential benefits and threats when using natural soils in reintroduction cultivations.

**Conclusions and perspectives**

The main result of this thesis is that plant – soil interactions, regarding physicochemical and biological factors, are important for plant life and play major roles in their distributions and rarity as well as in ecosystem restoration and species reintroductions. We identified two important variables – niche limits and soil microbes - which deserve more attention in future studies of rare plant species.

Models on species responses and distributions are powerful tools to predict species reactions to upcoming environmental changes and guide conservation decisions. However, these models and other statistical tools rely on assumptions that are not necessarily true, for example the unimodality of species responses along gradients. Surprisingly, most studies which contribute to the development of ecological niche theory use complex gradients, such as soil pH, which are difficult to interpret. One possible way to proceed in response modelling is to focus on direct or resource gradients, which are of sufficient length and have a very proximal effect. Another possibility to increase our understanding of the ecological niche is to conduct experiments with artificial environmental gradients. With regard to the limits of ecological niches, we need a discussion on ecologically viable population sizes to identify meaningful levels for setting the limits. Moreover, their comparability and usefulness across larger spatial scales needs further testing.

As the reintroduction or translocation of an endangered species is often the only chance to save it from extinction, more research is urgently needed in this field. Unfortunately, results are often very habitat
or species specific and it is difficult to generalize findings. Thus, this conservation technique is expensive in time and money and at the moment its use is completely constrained to rich, industrial countries. With regard to implementing plant-soil interactions to reintroductions, some promising successes are achieved by using LiDAR (light detection and ranging) technology for finding suitable habitats (Questad et al. 2014). Also the fast development in molecular techniques, such as the development of next generation sequencing, will contribute to our understanding of plant-soil interactions. In summary, there are many starting points for further research on the development of affordable techniques in species conservation.

Conserving biodiversity and saving species from extinction is a race against time. Conducting studies and experiments, however, is time consuming and translating scientific findings into political decisions is even more of a challenge. As scientists, we can only accelerate the first phase of this process. To advance more rapidly, it is important to share data and experiences from all around the world in easily accessible data bases and intensify cooperation within and between scientific communities.
References


Synthesis


Chapter 9

Appendices
Appendix 3.1: Details about the data collected from published sources and used in the article. The study sites are described by geographical region and forest type. Given are the number of plots used in this article and their plot sizes. Soil pH measurements are characterized by their sample design, as numbers of samples taken within every plot and mixed, the sampled soil horizon and the buffer solution used in the laboratory analysis. All information follow the author’s description within the particular study. Below the table the citation for every study is listed.

<table>
<thead>
<tr>
<th>Data sources</th>
<th>Geographical region</th>
<th>Forest type</th>
<th>No. of plots</th>
<th>Plot size (m$^2$)</th>
<th>pH extraction</th>
<th>Sample design</th>
<th>Soil horizon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gönnert (1989)</td>
<td>Aller Urstromtal + Bevenser Becken</td>
<td>deciduous, semi-natural</td>
<td>36</td>
<td>100-500, homogeneous</td>
<td>H$_2$O</td>
<td>mean of up to 16 samples</td>
<td>upper mineral soil layer</td>
</tr>
<tr>
<td>Heinken (1995)</td>
<td>lowlands of Lower Saxony</td>
<td>deciduous, semi-natural</td>
<td>419</td>
<td>250-900, homogeneous</td>
<td>H$_2$O, KCl</td>
<td>5 samples mixed</td>
<td>upper mineral soil layer</td>
</tr>
<tr>
<td>Huntke (2002)</td>
<td>Ammerland</td>
<td>deciduous</td>
<td>119</td>
<td>not mentioned</td>
<td>H$_2$O, KCl</td>
<td>3 samples mixed</td>
<td>topsoil</td>
</tr>
<tr>
<td>Michaelis et al. (2016)</td>
<td>Stader Geest</td>
<td>deciduous, semi-natural</td>
<td>573</td>
<td>within 4 m$^2$ around the plant</td>
<td>H$_2$O, KCl, CaCl$_2$</td>
<td>3 samples mixed</td>
<td>topsoil</td>
</tr>
<tr>
<td>Pannek et al. (2013)</td>
<td>Stader Geest</td>
<td>deciduous, semi-natural</td>
<td>88</td>
<td>500, homogeneous</td>
<td>CaCl$_2$</td>
<td>3 samples mixed</td>
<td>topsoil</td>
</tr>
<tr>
<td>Rüther and Peppler-Lisbach (2007)</td>
<td>Friesland</td>
<td>deciduous, semi-natural</td>
<td>108</td>
<td>100-400, homogeneous</td>
<td>H$_2$O, KCl</td>
<td>3-5 samples mixed</td>
<td>upper mineral soil layer</td>
</tr>
<tr>
<td>Wulf (1992)</td>
<td>Stader Geest</td>
<td>deciduous, semi-natural</td>
<td>127</td>
<td>100-150, homogeneous</td>
<td>CaCl$_2$</td>
<td>10 samples mixed</td>
<td>upper 0-10 cm</td>
</tr>
<tr>
<td>Mast (1999)</td>
<td>mainly highlands of Lower Saxony, Harz</td>
<td>not mentioned</td>
<td>505</td>
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<td>6-10 samples mixed</td>
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Appendices

Appendix 3.2: The frequency distribution of plots in relation to pH values, given separately for lowlands and uplands.

Appendix 3.3: Linear regressions of pH values of the same set of soil samples, equally spaced along the pH gradient, simultaneously measured in $\text{H}_2\text{O}$, $\text{CaCl}_2$ and $\text{KCl}$. The regression equations were used to convert all pH data to standardized values of $\text{pH}_{\text{CaCl}_2}$. 

\[ R^2 = 0.92 \ P < 0.001, \ N = 72 \]

\[ R = 0.99 \ P < 0.001, \ N = 72 \]
Appendix 3.4: Example of HOF models of types II-VII showing the responses of species along the pH gradient in the uplands (cf. Jansen & Oksanen 2013). None of the species complied with model I. a) Type II: monotonic sigmoid curve with an optimum at the extreme left or right of the gradient (*Chrysosplenium alternifolium*), b) Type III: monotonic sigmoid with a plateau, c) Type IV: unimodal symmetric response, d) Type V: unimodal skewed response, e) Type VI: bimodal with more or less symmetric optima, f) Type VII: bimodal with asymmetric optima. Black vertical solid lines describe the position of the optima as calculated for each model (not available for bimodal responses), grey vertical solid lines denote the upper and lower central borders (max * e-0.5), respectively. The dotted grey line corresponds to a probability of occurrence of y = 0.05, and its intersection(s) with the response curve marks the lower and/or upper limits.
Appendices

**Appendix 3.5**: Results from the modelling approach with eHOF for the lowlands and uplands of northern Germany. For each species, the table shows the number of occurrences in the dataset (#), the calculated HOF model type, HOF model optimum (Opt) as well as lower and upper central borders (LowCB and UppCB). LowLim and UppLim indicate the lower and upper calculated 0.05 limits, respectively. LowPrevT and UppPrevT are defined as those points where the response curve intersects the line representing the overall percent frequency in the data set. Range size gives the number of occupied grid cells in an Ordnance Survey map for the period 1982-2003 (Garve 2007), whereas threat level was obtained from the red list of vascular plants in Lower Saxony and Bremen (Garve 2004; NG = least concern, V = near-threatened, 3 = vulnerable, 2 = endangered). The change in range size was calculated by standardizing the number of abandoned and newly colonized grid cells in the time period before and after 1981 by the overall number of colonized grid cells in the latter time period, converting it into percentage increase and decrease of occupancy and subtracting these from each other. This was done for both regions separately. The experts’ estimation of the population trend (Expert trend) was taken from Cordes et al. (2006) and was only available for the lowlands (1 = increase, 0 = constant, -1 = decrease). Ellenberg’s values for soil reaction (R) were obtained from Ellenberg et al. (2001).

Lowlands:

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### Appendices

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Appendices

Chapter 5

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**Appendix 5.1:** Model formulas for the seven different model types (modified from Jansen and Oksanen (2013)). Data were analyzed as presence/absence, hence M=1.

![Frequency distribution of soil pH measurements in 1219 plots of semi-natural deciduous forest in the Central Upland Range in Germany.](image)

**Appendix 5.2:** Frequency distribution of soil pH measurements in 1219 plots of semi-natural deciduous forest in the Central Upland Range in Germany.
Appendix 5.3: Literature survey on articles that used HOF models between 2011 and 2016. Data set attributes are given as published in the original articles and their appendices. NA means that no information was available. Given are number of observations (No obs), number of species or parameters considered (No spec/para), the minimum presences (Min pre) and minimum frequencies (Min fre) of the species in the used data sets.

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<td>plants</td>
<td>41*4</td>
<td>cover</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Suchrow et al.</td>
<td>5</td>
<td>shape, top, borders</td>
<td>Germany</td>
<td>salt marsh</td>
<td>plants</td>
<td>2691</td>
<td>richness</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Toledo et al.</td>
<td>5</td>
<td>shape</td>
<td>Bolivia</td>
<td>tropical forest</td>
<td>wooden plants</td>
<td>220</td>
<td>100</td>
<td>3</td>
<td>0.013</td>
<td>1.3</td>
</tr>
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<td>Uğurlu and Oldeland</td>
<td>5</td>
<td>shape, optimum</td>
<td>Turkey</td>
<td>forest</td>
<td>oak trees</td>
<td>1104</td>
<td>8</td>
<td>30</td>
<td>0.03</td>
<td>2.7</td>
</tr>
<tr>
<td>Visser et al.</td>
<td>5</td>
<td>shape</td>
<td>Malaysia</td>
<td>forest</td>
<td>Shorea leprosula</td>
<td>NA</td>
<td>1</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<td>5</td>
<td>shape, optimum</td>
<td>Namibia</td>
<td>rangeland</td>
<td>vascular plants</td>
<td>1269</td>
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<td>0.04</td>
<td>4.0</td>
</tr>
<tr>
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<td>7</td>
<td>shape, threshold</td>
<td>Greenland</td>
<td>arctic tundra</td>
<td>plants</td>
<td>NA</td>
<td>4</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<td>5</td>
<td>shape</td>
<td>Norway</td>
<td>lake sediment</td>
<td>pollen</td>
<td>321</td>
<td>183</td>
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<td>3.1</td>
</tr>
<tr>
<td>Cao et al.</td>
<td>5</td>
<td>shape, optimum, tolerance</td>
<td>China, Mongolia</td>
<td>NA</td>
<td>pollen</td>
<td>2626</td>
<td>156</td>
<td>10</td>
<td>0.004</td>
<td>0.4</td>
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<td>Parameters</td>
<td>Location</td>
<td>Ecosystems</td>
<td>Organisms</td>
<td>No obs</td>
<td>No spec/para</td>
<td>Min pre</td>
<td>Min fre</td>
<td>Min fre (%)</td>
</tr>
<tr>
<td>------------------------</td>
<td>--------------</td>
<td>-------------------------------------------</td>
<td>-------------------</td>
<td>-----------------</td>
<td>-----------</td>
<td>--------</td>
<td>-------------</td>
<td>---------</td>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>Engagement et al. (2014)</td>
<td>5</td>
<td>shape, optimum, tolerance</td>
<td>Finland</td>
<td>lake sediment</td>
<td>chironomid</td>
<td>298</td>
<td>40</td>
<td>10</td>
<td>0.03</td>
<td>3.4</td>
</tr>
<tr>
<td>Ferry et al. (2015)</td>
<td>7</td>
<td>shape</td>
<td>Southern Ocean</td>
<td>sea-floor sediment</td>
<td>diatoms</td>
<td>163</td>
<td>24</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Fortin et al. (2015)</td>
<td>5</td>
<td>shape</td>
<td>North America</td>
<td>lake sediment</td>
<td>chironomids</td>
<td>435</td>
<td>6</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Heathcote et al. (2015)</td>
<td>7</td>
<td>shape</td>
<td>USA</td>
<td>lake sediment</td>
<td>diatoms</td>
<td>56</td>
<td>88</td>
<td>10</td>
<td>0.18</td>
<td>17.9</td>
</tr>
<tr>
<td>Holmes et al. (2011)</td>
<td>5</td>
<td>shape</td>
<td>Norway, Iceland</td>
<td>lake sediment</td>
<td>chironomids</td>
<td>207</td>
<td>133</td>
<td>10</td>
<td>0.13</td>
<td>13.0</td>
</tr>
<tr>
<td>Mills and Ryves (2012)</td>
<td>5</td>
<td>shape</td>
<td>Uganda</td>
<td>crater lake</td>
<td>diatoms</td>
<td>64</td>
<td>57</td>
<td>10</td>
<td>0.16</td>
<td>15.6</td>
</tr>
<tr>
<td>Tian et al. (2014)</td>
<td>5</td>
<td>shape</td>
<td>Mongolia</td>
<td>lake sediment</td>
<td>pollen</td>
<td>90</td>
<td>20</td>
<td>17</td>
<td>0.19</td>
<td>18.9</td>
</tr>
<tr>
<td>Turner et al. (2016)</td>
<td>7</td>
<td>shape</td>
<td>Tibet</td>
<td>lake+ pond sediment</td>
<td>green algae</td>
<td>91</td>
<td>2</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Bojková et al. (2012)</td>
<td>5</td>
<td>shape, PropOcc, tolerance</td>
<td>Czech Republic</td>
<td>stream</td>
<td>stonefly larvae</td>
<td>170</td>
<td>18</td>
<td>11</td>
<td>0.06</td>
<td>6.5</td>
</tr>
<tr>
<td>Laporta and Sallum (2014)</td>
<td>7</td>
<td>shape</td>
<td>Brazil</td>
<td>tropical rain forest</td>
<td>mosquitos</td>
<td>30</td>
<td>73</td>
<td>3</td>
<td>0.10</td>
<td>10.0</td>
</tr>
</tbody>
</table>
Appendices

Appendix 5.4: Description of results for 51-parameters and visualized results of (generalized) linear mixed models and correlation matrices for all parameters that are not shown in the article.

Optimum$_{51}$

The mean optimum$_{51}$ showed similar patterns as described above, but were significantly influenced by presence, frequency and their interaction. On average, the optimum$_{51}$ decreased by 0.02 pH units with increasing number of presences (est. = -0.026, p = 0.039) and increased by 0.02 units with increasing frequency (est. = 0.028, p < 0.001), whereas this effect again leveled off with increasing numbers of presences (est. = -0.018, p = 0.004). Correlations between the different scenarios were even stronger than before (coefficients 0.87 to 0.99).

Fig 5.4.1. (left) Results of linear mixed models identifying the trends for the optimum$_{51}$ along the frequency gradient for all four presence scenarios. Colored lines show the regressions for the single species, whereas the black line shows the overall trend across species (population trend). (right) Spearman - correlation matrix for the mean optimum$_{51}$ showing all possible combinations of scenarios. Axes are sorted by frequency and number of presences. Given are the correlation coefficients.

LowLims$_{51}$

Although the number of observations was strongly reduced in the LowLims$_{51}$, the same significant relationships between Pre (est. = -0.004, p > 0.001), Fre (est. = -1.313, p < 0.001) and the limits could be
found as described above. The shift of the $\text{limits}_{51}$ towards lower pH values was even more pronounced, with a maximum difference of pH 1.1 units between the highest and the lowest estimated $\text{limits}_{51}$. Because the number of species having a $\text{LowLim}_{51}$ in the high frequency scenarios was reduced, only few significant correlations could be found for these scenarios, even if the correlation coefficient was high in some cases. Still, the correlations between scenarios with similar frequencies, compared to strongly differing frequencies, were much higher.

Fig 5.4.2. (left) Results of linear mixed models identifying the trends for the lower limits$_{51}$ along the frequency gradient for all four presence scenarios. Colored lines show the regressions for the single species, whereas the black line shows the overall trend across species (population trend). (right) Spearman correlation matrix for the mean lower limits$_{51}$ showing all possible combinations of scenarios. Axes are sorted by frequency and number of presences. Given are the correlation coefficients.

$\text{UppLims}_{51}$

Using $\text{UppLims}_{51}$ reduced the data set by 90%. Still, species $\text{UppLims}_{51}$ shifted to more base-rich soils under high presence numbers (est. = 0.169, p < 0.001) and a high frequency (est. = 1.223, p < 0.001). Both parameters significantly influenced the $\text{UppLims}_{51}$, as described for the $\text{UppLims}_{any}$, but the impact of frequency changes was even stronger. An increase in frequency in the Pre25 resulted in a shift of the $\text{UppLims}_{51}$ by as much as 2.5 pH units. The correlations between scenarios showed the same trend as mentioned for the $\text{UppLims}_{any}$, but with Pre25:Fre0.5 having unexpectedly low correlation coefficients.
Fig 5.4.3. (left) Results of linear mixed models identifying the trends for the upper limits_{55} along the frequency gradient for all four presence scenarios. Colored lines show the regressions for the single species, whereas the black line shows the overall trend across species (population trend). (right) Spearman correlation matrix for the mean upper limits_{55} showing all possible combinations of scenarios. Axes are sorted by frequency and number of presences. Given are the correlation coefficients.

Lower central borders

Fig 5.4.4. (left) Results of linear mixed models identifying the trends for the mean lower central border (LowCB) along the frequency gradient for all four presence scenarios. Colored lines show the regressions for the single species, whereas the black line shows the overall trend across species (population trend).
(right) Spearman - correlation matrix for the mean lower central border (LowCB) showing all possible combinations of scenarios. Axes are sorted by frequency and number of presences. Given are the correlation coefficients.

Upper central borders

Fig 5.4.5. (left) Results of linear mixed models identifying the trends for the mean upper central border (UppCB) along the frequency gradient for all four presence scenarios. Colored lines show the regressions for the single species, whereas the black line shows the overall trend across species (population trend). (right) Spearman - correlation matrix for the mean upper central border (UppCB) showing all possible combinations of scenarios. Axes are sorted by frequency and number of presences. Given are the correlation coefficients.
**Appendix 5.5:** Results of (Generalized) linear mixed models for all curve parameters. Given are estimates, standard errors and p-values for all fixed effects and variance and standard deviation for the random effects. The table also includes the number of observations, the assumed distribution and information about scaling (yes or no).

<table>
<thead>
<tr>
<th>Fixed effects</th>
<th>Intercept</th>
<th>Pre</th>
<th>Fre</th>
<th>Pre * Fre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>estimate</td>
<td>std. error</td>
<td>estimate</td>
<td>std. error</td>
</tr>
<tr>
<td>Mean niche width</td>
<td>3.195</td>
<td>0.071</td>
<td>0.2375</td>
<td>0.0114</td>
</tr>
<tr>
<td>Mean optimum&lt;sub&gt;any&lt;/sub&gt;</td>
<td>5.417</td>
<td>0.140</td>
<td>0.0001</td>
<td>0.0219</td>
</tr>
<tr>
<td>Mean optimum&lt;sub&gt;51&lt;/sub&gt;</td>
<td>5.529</td>
<td>0.160</td>
<td>-0.0256</td>
<td>0.0150</td>
</tr>
<tr>
<td>Nr of optima</td>
<td>3.997</td>
<td>0.056</td>
<td>0.0107</td>
<td>0.0194</td>
</tr>
<tr>
<td>Mean LowLim&lt;sub&gt;any&lt;/sub&gt;</td>
<td>3.361</td>
<td>0.029</td>
<td>-0.1523</td>
<td>0.0064</td>
</tr>
<tr>
<td>Mean LowLim&lt;sub&gt;51&lt;/sub&gt;</td>
<td>4.060</td>
<td>0.045</td>
<td>-0.0037</td>
<td>0.0003</td>
</tr>
<tr>
<td>Nr of LowLims</td>
<td>3.345</td>
<td>0.084</td>
<td>0.0773</td>
<td>0.0234</td>
</tr>
<tr>
<td>Mean UppLim&lt;sub&gt;any&lt;/sub&gt;</td>
<td>6.820</td>
<td>0.050</td>
<td>0.2918</td>
<td>0.0098</td>
</tr>
<tr>
<td>MeanUppLim&lt;sub&gt;51&lt;/sub&gt;</td>
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<td>0.139</td>
<td>0.1691</td>
<td>0.0195</td>
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<tr>
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<td>0.092</td>
<td>-0.3539</td>
<td>0.0364</td>
</tr>
<tr>
<td>Mean LowCB</td>
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<td>0.073</td>
<td>-0.0482</td>
<td>0.0130</td>
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<tr>
<td>Mean UppCB</td>
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<td>0.079</td>
<td>0.1576</td>
<td>0.0089</td>
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<tr>
<td>IQV</td>
<td>0.922</td>
<td>0.010</td>
<td>-0.0057</td>
<td>0.0006</td>
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### Random effects

<table>
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<tr>
<th></th>
<th>Species</th>
<th>Pre</th>
<th>Fre</th>
<th>Pre */+ Fre</th>
<th>No obs</th>
<th>Scaling</th>
<th>Assumed distribution</th>
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<tr>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>variance std.dev.</td>
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<td>0.734</td>
<td>0.048</td>
<td>0.220</td>
<td>0.061</td>
<td>0.248</td>
<td>0.025</td>
</tr>
<tr>
<td>Mean optimum_{any}</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>normal</td>
</tr>
<tr>
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<td>1.437</td>
<td>0.043</td>
<td>0.208</td>
<td>0.018</td>
<td>0.133</td>
<td>0.006</td>
</tr>
<tr>
<td>Mean optimum_{51}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>normal</td>
</tr>
<tr>
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<td>1.506</td>
<td>0.017</td>
<td>0.130</td>
<td>0.009</td>
<td>0.097</td>
<td>0.002</td>
</tr>
<tr>
<td>Nr of optima</td>
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<td></td>
<td></td>
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<td>normal</td>
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<tr>
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<td>0.037</td>
<td>0.192</td>
<td>NA</td>
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<td>NA</td>
</tr>
<tr>
<td>Mean LowLim_{any}</td>
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<td>normal</td>
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<td>0.339</td>
<td>0.001</td>
<td>0.025</td>
<td>0.014</td>
<td>0.118</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean LowLim_{51}</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>normal</td>
</tr>
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<td>0.362</td>
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<td>NA</td>
<td>0.414</td>
<td>0.644</td>
<td>NA</td>
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<tr>
<td>Nr of LowLims</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>poisson</td>
</tr>
<tr>
<td>variance std.dev.</td>
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<td>0.857</td>
<td>0.049</td>
<td>0.221</td>
<td>0.089</td>
<td>0.298</td>
<td>NA</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td>normal</td>
</tr>
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<td>0.003</td>
<td>0.052</td>
<td>0.044</td>
<td>0.210</td>
<td>0.005</td>
</tr>
<tr>
<td>Mean UppLim_{51}</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>normal</td>
</tr>
<tr>
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<td>0.083</td>
<td>0.897</td>
<td>0.947</td>
<td>0.081</td>
</tr>
<tr>
<td>Nr of UppLims</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>poisson</td>
</tr>
<tr>
<td>variance std.dev.</td>
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<td>0.936</td>
<td>0.117</td>
<td>0.342</td>
<td>0.060</td>
<td>0.245</td>
<td>NA</td>
</tr>
<tr>
<td>Mean LowCB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>normal</td>
</tr>
<tr>
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<td>0.747</td>
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<td>0.127</td>
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<td>0.233</td>
<td>0.009</td>
</tr>
<tr>
<td>Mean UppCB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>normal</td>
</tr>
<tr>
<td>variance std.dev.</td>
<td>0.689</td>
<td>0.830</td>
<td>0.033</td>
<td>0.182</td>
<td>0.041</td>
<td>0.201</td>
<td>0.017</td>
</tr>
<tr>
<td>IQV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>normal</td>
</tr>
<tr>
<td>variance std.dev.</td>
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<td>0.087</td>
<td>0.000</td>
<td>0.006</td>
<td>0.035</td>
<td>0.187</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Example model build in R with the package "lme4"

```r
model1 <- lmer(MeanOptimum ~ scaledPre * scaledFre + (1 + scaledPre * scaledFre | Species), data = dataframe1)
```

response: MeanOptimum

explanatory: scaledPre * scaledFre

random intercept: Species
Appendix 5.6: Frequency distribution of model types chosen in four exemplary model scenarios with three different information criteria (AIC, AICc and BIC). Thirty species were modelled following the procedure described in the article, but with differing information criteria for model selection.

In the published article of chapter 5, five additional appendices are presented, which cannot be shown here due to their format (e.g. GIF-files). However, they can be found online via open access at:

**Chapter 6**

**Appendix 6.1:** Summary of some important species characteristics, including family, the habitat type in which the species is found in the study region, red list status in the study region (Garve 2004), Raunkiær life form and form of reproduction.

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Habitat type</th>
<th>Red list status</th>
<th>Life form</th>
<th>Reproduction</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Euphorbia palustris</em></td>
<td>Euphorbiaceae</td>
<td>River corridors</td>
<td>Endangered</td>
<td>Geophyte</td>
<td>Seeds and vegetative</td>
</tr>
<tr>
<td><em>Genista anglica</em></td>
<td>Fabaceae</td>
<td>Heathlands</td>
<td>Vulnerable</td>
<td>Hemiphanerophyte</td>
<td>Seeds</td>
</tr>
<tr>
<td><em>Geum rivale</em></td>
<td>Rosaceae</td>
<td>Wet deciduous forest</td>
<td>Vulnerable</td>
<td>Hemicryptophyte</td>
<td>Seeds and vegetative</td>
</tr>
<tr>
<td><em>Phyteuma nigrum</em></td>
<td>Campanulaceae</td>
<td>Moist deciduous forest</td>
<td>Near threatened</td>
<td>Hemicryptophyte</td>
<td>Seeds</td>
</tr>
<tr>
<td><em>Senecio paludosus</em></td>
<td>Asteraceae</td>
<td>River corridors</td>
<td>Endangered</td>
<td>Hemicryptophyte</td>
<td>Seeds and vegetative</td>
</tr>
</tbody>
</table>
Appendices

Appendix 6.2: Pictures of the studied plant species

<table>
<thead>
<tr>
<th>Species</th>
<th>Seedlings</th>
<th>Transplants</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euphorbia palustris</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>Genista anglica</td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>Geum rivale</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
</tr>
<tr>
<td>Phyteuma nigrum</td>
<td><img src="image10.png" alt="Image" /></td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
<tr>
<td>Senecio paludosus</td>
<td><img src="image13.png" alt="Image" /></td>
<td><img src="image14.png" alt="Image" /></td>
<td><img src="image15.png" alt="Image" /></td>
</tr>
</tbody>
</table>
**Appendix 6.3:** Approximate number of seeds sown (first number), number of germinated seedlings within the first eight weeks (second number) and number of plants surviving to the adult stage and transplanted to the field (third number), given separately for each species and treatment combination.

<table>
<thead>
<tr>
<th></th>
<th>E. palustris</th>
<th>G. anglica</th>
<th>G. rivale</th>
<th>P. nigrum</th>
<th>S. paludosus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>with PGPR</strong></td>
<td>Natural</td>
<td>300/20/45</td>
<td>500/308/45</td>
<td>500/209/45</td>
<td>2000/135/41</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>300/7/28</td>
<td>500/360/45</td>
<td>500/158/45</td>
<td>2000/44/32</td>
</tr>
<tr>
<td><strong>without PGPR</strong></td>
<td>Natural</td>
<td>300/19/45</td>
<td>500/304/45</td>
<td>500/266/45</td>
<td>2000/108/42</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>300/6/28</td>
<td>500/347/45</td>
<td>500/150/45</td>
<td>2000/36/16</td>
</tr>
<tr>
<td></td>
<td>Potting</td>
<td>300/17/45</td>
<td>500/271/44</td>
<td>500/199/45</td>
<td>2000/45/8</td>
</tr>
</tbody>
</table>
Appendix 6.4: Overview of soil parameters for natural soils, mixed soils and the substrates used to create the mixed soils.

Soil texture, pH, organic matter content and C/N ratio from natural soil (home soil) collected near the seed donor populations.

<table>
<thead>
<tr>
<th>Soil texture</th>
<th>Species</th>
<th>Sand (%)</th>
<th>Silt (%)</th>
<th>Clay (%)</th>
<th>FAO Soil</th>
<th>Ct [%]</th>
<th>Nt [%]</th>
<th>C/N ratio</th>
<th>OM (%)</th>
<th>pH (CaCl2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Euphorbia palustris</td>
<td>19</td>
<td>38.1</td>
<td>42.9</td>
<td>Clay</td>
<td>6.229</td>
<td>0.58</td>
<td>10.7</td>
<td>10.7</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Genista anglica</td>
<td>96.8</td>
<td>1.4</td>
<td>1.8</td>
<td>Sand</td>
<td>2.876</td>
<td>0.171</td>
<td>11.7</td>
<td>19.3</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>Geum rivale</td>
<td>69.3</td>
<td>15.5</td>
<td>15.2</td>
<td>Sandy loam</td>
<td>11.228</td>
<td>0.96</td>
<td>16.8</td>
<td>4.9</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Phyteuma nigrum</td>
<td>60.7</td>
<td>26.9</td>
<td>12.4</td>
<td>Sandy loam</td>
<td>7.784</td>
<td>0.524</td>
<td>14.9</td>
<td>13.4</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>Senecio paludosus</td>
<td>69.9</td>
<td>17.5</td>
<td>12.6</td>
<td>Sandy loam</td>
<td>3.156</td>
<td>0.271</td>
<td>11.6</td>
<td>5.4</td>
<td>5.7</td>
</tr>
</tbody>
</table>

Composition of the substrate of the mixed soil treatment.

<table>
<thead>
<tr>
<th>Soil texture</th>
<th>Anorganic soil components [ % ]</th>
<th>Organic soil components [ % ]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overall 01S 38M 36M Sand Peat Potting soil</td>
<td>Overall Peat Potting soil</td>
</tr>
<tr>
<td>Euphorbia palustris</td>
<td>90 27 18 45 0 10 2 8</td>
<td></td>
</tr>
<tr>
<td>Geum rivale</td>
<td>81 8 16 8 48 19 0 19</td>
<td></td>
</tr>
<tr>
<td>Genista anglica</td>
<td>95 0 2 2 91 5 4 1</td>
<td></td>
</tr>
<tr>
<td>Phyteuma nigrum</td>
<td>87 31 22 0 35 13 0 13</td>
<td></td>
</tr>
<tr>
<td>Senecio paludosus</td>
<td>95 24 5 9 57 5 0 5</td>
<td></td>
</tr>
</tbody>
</table>
### Appendix 6.5: Data on soil conditions found in the germination bowls. Samples were taken parallel to pricking the seedlings. Data on P, Mg, Ca and K content are given in mg/100g soil.

<table>
<thead>
<tr>
<th>Species</th>
<th>Soil</th>
<th>PGPR</th>
<th>P</th>
<th>Mg</th>
<th>Ca</th>
<th>K</th>
<th>pH</th>
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<tbody>
<tr>
<td>Phyteuma nigrum</td>
<td>natural</td>
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<td>1.47</td>
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<td>206.26</td>
<td>7.28</td>
<td>4.94</td>
</tr>
<tr>
<td></td>
<td>natural</td>
<td>+</td>
<td>1.55</td>
<td>19.96</td>
<td>209.87</td>
<td>8.09</td>
<td>4.84</td>
</tr>
<tr>
<td></td>
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<td>-</td>
<td>66.21</td>
<td>41.45</td>
<td>860.14</td>
<td>738.76</td>
<td>6.00</td>
</tr>
<tr>
<td></td>
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<td>75.30</td>
<td>44.75</td>
<td>677.51</td>
<td>1469.48</td>
<td>6.00</td>
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<tr>
<td></td>
<td>mixed</td>
<td>-</td>
<td>10.84</td>
<td>24.30</td>
<td>200.98</td>
<td>10.93</td>
<td>6.69</td>
</tr>
<tr>
<td></td>
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<td>+</td>
<td>11.03</td>
<td>28.09</td>
<td>180.97</td>
<td>10.38</td>
<td>6.56</td>
</tr>
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<td>-</td>
<td>1.21</td>
<td>31.52</td>
<td>151.02</td>
<td>5.27</td>
<td>4.58</td>
</tr>
<tr>
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<td>+</td>
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<td>34.73</td>
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<td>6.07</td>
<td>4.57</td>
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<td>55.06</td>
<td>43.35</td>
<td>618.94</td>
<td>855.85</td>
<td>5.98</td>
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<td>5.81</td>
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<td>11.57</td>
<td>203.48</td>
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<td>6.16</td>
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<td>533.09</td>
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<td>41.70</td>
<td>663.99</td>
<td>567.30</td>
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<td>7.29</td>
<td>16.43</td>
<td>147.83</td>
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<td>6.93</td>
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<tr>
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<td>-</td>
<td>0.90</td>
<td>6.17</td>
<td>134.38</td>
<td>1.98</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>natural</td>
<td>+</td>
<td>0.88</td>
<td>6.04</td>
<td>133.00</td>
<td>2.26</td>
<td>5.66</td>
</tr>
<tr>
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<td>-</td>
<td>60.09</td>
<td>39.98</td>
<td>688.32</td>
<td>595.03</td>
<td>5.95</td>
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<tr>
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<td>+</td>
<td>63.75</td>
<td>38.18</td>
<td>667.48</td>
<td>1021.89</td>
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</tr>
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<td>47.08</td>
<td>6.51</td>
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<td>10.52</td>
<td>27.16</td>
<td>163.44</td>
<td>24.24</td>
<td>6.5</td>
</tr>
<tr>
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<td>-</td>
<td>2.27</td>
<td>1.91</td>
<td>15.31</td>
<td>0.78</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
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<td>+</td>
<td>1.82</td>
<td>1.75</td>
<td>14.85</td>
<td>0.82</td>
<td>4.93</td>
</tr>
<tr>
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<td>11.16</td>
<td>92.37</td>
<td>469.09</td>
<td>652.41</td>
<td>5.95</td>
</tr>
<tr>
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<td>potting</td>
<td>+</td>
<td>69.78</td>
<td>44.67</td>
<td>613.35</td>
<td>15.89</td>
<td>5.91</td>
</tr>
<tr>
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<td>-</td>
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<td>6.17</td>
<td>77.87</td>
<td>1.58</td>
<td>5.82</td>
</tr>
<tr>
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<td>+</td>
<td>1.82</td>
<td>6.93</td>
<td>96.18</td>
<td>1.56</td>
<td>6.33</td>
</tr>
</tbody>
</table>
Appendices

Appendix 6.6: Species of bacteria included in the PGPR treatment and their plant growth promoting traits.

<table>
<thead>
<tr>
<th>Species</th>
<th>Plant growth promoting traits</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Azospirillum brasilense</strong></td>
<td>IAA, P solubilization, N-fixation, antibiotic resistance</td>
<td>(Thakuria et al. 2004)</td>
</tr>
<tr>
<td></td>
<td>IAA, gibberellin, N-fixation</td>
<td>(Steenhoudt &amp; Vanderleyden 2000)</td>
</tr>
<tr>
<td><strong>Azotobacter chroococcum</strong></td>
<td>Gibberellin, kinetin, IAA</td>
<td>(Verma et al. 2001)</td>
</tr>
<tr>
<td></td>
<td>P solubilization</td>
<td>(Kumar et al. 2001)</td>
</tr>
<tr>
<td><strong>Bacillus sp. (B. subtilis &amp; B. megaterium)</strong></td>
<td>Antifungal activity</td>
<td>(Cazorla et al. 2007)</td>
</tr>
<tr>
<td></td>
<td>IAA, P solubilization</td>
<td>(Zaidi et al. 2006)</td>
</tr>
<tr>
<td></td>
<td>Siderophores, antifungal activity</td>
<td>(Ahmad et al. 2008)</td>
</tr>
<tr>
<td><strong>Pseudomonas fluorescence</strong></td>
<td>IAA, siderophores, P solubilization</td>
<td>(Gupta et al. 2005)</td>
</tr>
<tr>
<td></td>
<td>Induced systemic resistance, antifungal activity</td>
<td>(Saravanakumar et al. 2007)</td>
</tr>
<tr>
<td></td>
<td>IAA, siderophores, antifungal activity</td>
<td>(Dey et al. 2004)</td>
</tr>
<tr>
<td></td>
<td>ACC deaminase, P solubilization</td>
<td>(Shaharoona et al. 2008)</td>
</tr>
</tbody>
</table>

Bacterial Indole-3-acetic-acid (IAA, auxin) might influence the following physiological processes in the plant: alters endogenous pool of IAA in the plant; affects cell division, extension and differentiation; stimulates seed germination; increases root and xylem development; controls vegetative growth; initiates lateral and adventitious root formation; mediates responses to light, gravity and florescence; affects photosynthesis, pigment formation, biosynthesis of several metabolites and stress resistance. By cracking inorganic, insoluble forms of phosphorus bacteria can increase P availability for plants (P-solubilization). Nitrogenase activity and nitrogen fixation increase the nitrogen availability for plants, whereas siderophores build iron complexes which can be taken up by plants. ACC deaminase reduces the ethylene concentration and facilitates stress tolerance.
Appendix 6.7: Sampling times for germination rates, fitness parameters and bacteria samples. Dark grey parameters were analyzed statistically, whereas light grey parameters are descriptive only.

<table>
<thead>
<tr>
<th>Fitness parameters</th>
<th>Germination</th>
<th>Pricking</th>
<th>Transplants</th>
<th>1 year in field</th>
<th>2 years in field</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Euphorbia palustris</strong></td>
<td>Number of seedlings &amp; germination rates</td>
<td>Dark grey</td>
<td>Light grey</td>
<td>1 year in field</td>
<td>2 years in field</td>
</tr>
<tr>
<td>Plant height</td>
<td>1 year in field</td>
<td>2 years in field</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of shoots</td>
<td>1 year in field</td>
<td>2 years in field</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of flowering individuals</td>
<td>1 year in field</td>
<td>2 years in field</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival in the field</td>
<td>1 year in field</td>
<td>2 years in field</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Genista anglica</strong></td>
<td>Number of seedlings &amp; germination rates</td>
<td>Dark grey</td>
<td>Light grey</td>
<td>1 year in field</td>
<td>2 years in field</td>
</tr>
<tr>
<td>Plant height</td>
<td>1 year in field</td>
<td>2 years in field</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of shoots</td>
<td>1 year in field</td>
<td>2 years in field</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fitness scale</td>
<td>1 year in field</td>
<td>2 years in field</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of flowering individuals</td>
<td>1 year in field</td>
<td>2 years in field</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flowering intensity</td>
<td>1 year in field</td>
<td>2 years in field</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival in the field</td>
<td>1 year in field</td>
<td>2 years in field</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Geum rivale</strong></td>
<td>Number of seedlings &amp; germination rates</td>
<td>Dark grey</td>
<td>Light grey</td>
<td>1 year in field</td>
<td>2 years in field</td>
</tr>
<tr>
<td>Mean leaf length</td>
<td>1 year in field</td>
<td>2 years in field</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of inflorescences shoots per individual</td>
<td>1 year in field</td>
<td>2 years in field</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival in the field</td>
<td>1 year in field</td>
<td>2 years in field</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Phyteuma nigrum</strong></td>
<td>Number of seedlings &amp; germination rates</td>
<td>Dark grey</td>
<td>Light grey</td>
<td>1 year in field</td>
<td>2 years in field</td>
</tr>
<tr>
<td>Number of leaves</td>
<td>1 year in field</td>
<td>2 years in field</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean leaf length</td>
<td>1 year in field</td>
<td>2 years in field</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of flowering individuals</td>
<td>1 year in field</td>
<td>2 years in field</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of inflorescences shoots per individual</td>
<td>1 year in field</td>
<td>2 years in field</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of inflorescences per individual</td>
<td>1 year in field</td>
<td>2 years in field</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Inflorescence length</td>
<td>1 year in field</td>
<td>2 years in field</td>
<td></td>
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<tr>
<td>Survival in the field</td>
<td>1 year in field</td>
<td>2 years in field</td>
<td></td>
<td></td>
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<tr>
<td><strong>Senecio paludosus</strong></td>
<td>Number of seedlings &amp; germination rates</td>
<td>Dark grey</td>
<td>Light grey</td>
<td>1 year in field</td>
<td>2 years in field</td>
</tr>
<tr>
<td>Mean leaf length</td>
<td>1 year in field</td>
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</tr>
<tr>
<td>Plant height</td>
<td>1 year in field</td>
<td>2 years in field</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of flowering individuals</td>
<td>1 year in field</td>
<td>2 years in field</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of inflorescences per individual</td>
<td>1 year in field</td>
<td>2 years in field</td>
<td></td>
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<tr>
<td>Survival in the field</td>
<td>1 year in field</td>
<td>2 years in field</td>
<td></td>
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**Appendix 6.8:** (Statistical) methods applied to analyze the different fitness parameters and plant species. # indicates that data was available for multiple sampling times, but each sampling times was analyzed separately.

<table>
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<th>Fitness parameter</th>
<th>Euphorbia</th>
<th>Genista</th>
<th>Geum</th>
<th>Phyteuma</th>
<th>Senecio</th>
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<tr>
<td><strong>Seeding stage</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Number of seedlings</td>
<td>GLM (Poisson) + Tukey post-hoc test</td>
<td>GLM (Poisson) + Tukey post-hoc test</td>
<td>GLM (Poisson) + Tukey post-hoc test</td>
<td>GLM (Poisson) + Tukey post-hoc test</td>
<td>GLM (Poisson) + Tukey post-hoc test</td>
</tr>
<tr>
<td></td>
<td>LMM; random factors were grazing effect, plot and sampling time</td>
<td>LM #</td>
<td>LM #</td>
<td>LM #</td>
<td></td>
</tr>
<tr>
<td><strong>Individual fitness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Plant height</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of shoots</td>
<td>GLM (negative binomial) + Tukey post-hoc test</td>
<td>GLM (Poisson) #</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fitness scale</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of leaves</td>
<td>OrdReg + Tukey post-hoc test #</td>
<td>GLM (negative binomial)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mean leaf length</td>
<td></td>
<td></td>
<td>LM</td>
<td>GLM (negative binomial)</td>
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<td>Flowering intensity</td>
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<td>Number of flowering individuals</td>
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<td>GLM (negative binomial)</td>
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<tr>
<td>Number of flower stands per individual</td>
<td>GLM (negative binomial)</td>
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<tr>
<td>Number of inflorescences per individual</td>
<td>GLM (negative binomial)</td>
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Appendix 7.1: Numbers of replicates used in the analyses for all plant - treatment combination. In the greenhouse, the roots of three seedlings were combined to one rhizosphere sample, thus overall 9 seedlings were sampled for each combination. In the field, again three replicates (1, 2, 3) were sampled, here based on one plant each. If possible, all plants were taken from the same plot (1, 2, 3 / x), but in some cases this was not possible. Different plots are indicated by a "/", e.g. in "2, 3 / 1" the replicates 2 and 3 are taken from the same plot, whereas replicate 1 is taken from another.

<table>
<thead>
<tr>
<th></th>
<th>Genista</th>
<th>Geum</th>
<th>Senecio</th>
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</thead>
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<tr>
<td></td>
<td>Natural</td>
<td>Mixed</td>
<td>Potting</td>
</tr>
<tr>
<td>number of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>replicates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>greenhouse</td>
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<td></td>
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</tr>
<tr>
<td>plant fitness measurements</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>bacterial community</td>
<td>3x3</td>
<td>3x3</td>
<td>3x3</td>
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<tr>
<td>field</td>
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<tr>
<td>plant fitness measurements</td>
<td>29</td>
<td>31</td>
<td>24</td>
</tr>
<tr>
<td>bacterial community</td>
<td>1, 2, 3 / x</td>
<td>1, 2, 3 / x</td>
<td>1, 2, 3 / x</td>
</tr>
</tbody>
</table>

The numbers of replicates for each plant species are as follows:

- **Genista**
  - Greenhouse:
    - Plant fitness measurements: 20 replicates
    - Bacterial community: 3x3 replicates
    - Nodule counts: 25 replicates
  - Field:
    - Plant fitness measurements: 29 replicates
    - Bacterial community: 1, 2, 3 / x

- **Geum**
  - Greenhouse:
    - Plant fitness measurements: 20 replicates
    - Bacterial community: 3x3 replicates
  - Field:
    - Plant fitness measurements: 19 replicates
    - Bacterial community: 1, 2, 3 / x

- **Senecio**
  - Greenhouse:
    - Plant fitness measurements: 20 replicates
    - Bacterial community: 3x3 replicates
  - Field:
    - Plant fitness measurements: 11 replicates
    - Bacterial community: 1, 2, 3 / x
Appendix 7.2: Soil nutrients in the greenhouse and in the field for all plant-treatment combinations.

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<th>Species</th>
<th>Soil</th>
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<th>Calcium</th>
<th>Potassium</th>
<th>C/N</th>
<th>pH</th>
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</thead>
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<td></td>
<td></td>
<td></td>
<td>Greenhouse</td>
<td>Field</td>
<td>Greenhouse</td>
<td>Field</td>
<td>Greenhouse</td>
<td>Field</td>
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<td>0.81</td>
<td>1.91</td>
<td>1.72</td>
<td>15.31</td>
<td>10.93</td>
<td>0.78</td>
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<td>1.82</td>
<td>0.28</td>
<td>1.75</td>
<td>1.19</td>
<td>14.85</td>
<td>5.77</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>mixed -</td>
<td>2.41</td>
<td>0.32</td>
<td>6.17</td>
<td>1.64</td>
<td>77.87</td>
<td>8.26</td>
<td>1.58</td>
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<tr>
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<td>1.82</td>
<td>0.23</td>
<td>6.93</td>
<td>1.43</td>
<td>96.18</td>
<td>11.12</td>
<td>1.56</td>
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<td>potting -</td>
<td>11.16</td>
<td>1.53</td>
<td>92.37</td>
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<td>469.09</td>
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<td>3.01</td>
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<td>613.35</td>
<td>213.36</td>
<td>15.89</td>
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<td>Geum rivale</td>
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<td>2.56</td>
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<td>4.52</td>
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<td>703.43</td>
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<td>3.52</td>
<td>39.98</td>
<td>24.22</td>
<td>688.32</td>
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<td>14.42</td>
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<td>667.48</td>
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<td>4.67</td>
<td>NA</td>
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<td>170.52</td>
<td>NA</td>
<td>4.22</td>
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<tr>
<td></td>
<td>natural +</td>
<td>7.38</td>
<td>2.79</td>
<td>11.57</td>
<td>3.77</td>
<td>203.48</td>
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<td>70.28</td>
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<td>1.65</td>
<td>38.40</td>
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<td>661.51</td>
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<td>663.99</td>
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**Appendix 7.3:** Detailed description of the methods used to analyze the bacterial rhizosphere communities.

**Rhizosphere extraction**

To remove the rhizosphere soil, remaining bulk material was carefully removed from the roots. Roots were transferred to sterile 50 ml tubes filled with phosphate buffered saline solution (PBS; 137 mM NaCl, 2.7 mM KCl, 8.5 mM Na2HPO4, and 1.5 mM KH2PO4, pH 7.3) and washed on a rotary shaker for 15 minutes. Afterwards, roots were removed and tubes were centrifuged for 10 minutes at 8000 x g. The resulting pellet was defined as the rhizosphere sample and stored at -18 °C for further processing.

**DNA extraction and determination of quality and purity**

DNA was extracted from soil samples using the NucleoSpin® Soil Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer’s instructions with minor modifications. The soil (fresh weight, 500 mg) was transferred to the bead tube type A and lysis buffer SL1 and Enhancer SX were added. The physical disruption was done for 35 s at 25 Hz using a mixer mill type MM200 (Retsch, Haan, Germany). The DNA was eluted in 100 µL SE buffer and all DNA extracts were stored at -20 °C. The quantity of the extracted genomic DNA and the PCR products was measured by agarose gel electrophoresis using a Horizon® 58 horizontal gel electrophoresis apparatus (Biometra GmbH, Göttingen, Germany) and gels with 1% agarose in 1× TAE buffer. The DNA was stained with ethidium bromide (0.5 µg mL\(^{-1}\)), visualised by UV light, and photographed. The intensities of the bands were compared to the O’GeneRuler™ 1kb Plus DNA Ladder (Fermentas, St. Leon-Rot, Germany) to use equal amounts of DNA for subsequent methods.

**PCR**

The 16S rRNA genes were amplified by PCR using a Mastercycler Gradient (Eppendorf, Hamburg, Germany) and the primers GM5F and 907R for bacteria (Muyzer et al. 1995). The gc-clamp (Muyzer et al. 1993) was located at the 5’ end of the forward primer (GM5F). All oligonucleotides were synthesised by Biomers (Ulm, Germany). PCR was performed in 50 µL reactions which contained 5 µL of DreamTaq PCR-buffer, 1.25 U DreamTaq DNA-Polymerase and 20 µg of BSA (Fermentas, St. Leon-Rot, Germany). The final concentrations were 0.5 µmol L\(^{-1}\) of each primer and 50 µmol L\(^{-1}\) of each nucleotide. A touchdown program was conducted with an initial denaturation at 95 °C for 60 s, followed by 13 cycles of 30 s denaturation at 95 °C, annealing for 25 s at 57 °C with a decrement of 0.5 °C per cycle and an extension at 72 °C for 13 s. Additional 20 cycles were conducted with 20 s of denaturation, 25 s of
annealing, and 13 s of extension. A final extension of 30 min was applied for all PCR reactions to eliminate artefactual double DGGE bands resulting from possible heteroduplexes (Janse et al. 2004).

**DGGE and sequencing**

Equal amounts of PCR-products (approximately 400 ng) were separated for 18 h at 70 V on a 6% polyacrylamide gel with a denaturing gradient of 50 to 70% (100% denaturant contained 7 M urea and 40% (v/v) deionised formamide) using a DGGE 2001 apparatus (C.B.S. Scientific, Del Mar, CA, USA). The bands were stained with 1× SYBR-gold, visualized by blue light excitation, and photographed.
References


Appendices


Erklärung

Hiermit erkläre ich, Jana Michaelis, dass ich die hier vorgelegte Dissertation ohne unerlaubte fremde Hilfe angefertigt habe. Es wurden ausschließlich die angegebenen Quellen und Hilfsmittel verwendet und die den benutzten Werken wörtlich und inhaltlich entnommenen Stellen sind als solche kenntlich gemacht.

Die Kapitel 3 – 7 sind als eigenständige Artikel jeweils von mehreren Autoren verfasst worden. Die Anteile der Autoren sind im Folgenden dargelegt.


Kapitel 4: Fr. Pannek und ich haben die meisten Analysen durchgeführt, die Daten für die Waldarten beigetragen, Abbildungen angefertigt und Verbesserungsvorschläge zum Manuskript gemacht. Prof. Diekmann war maßgeblich für das Konzept, das Schreiben und das Veröffentlichen zuständig.


Declaration on the contribution of the candidate to a multi-author article/manuscript which is included as a chapter in the submitted doctoral thesis

Contribution of the candidate is given in % of the total work load (up to 100% for each category)

**Chapter 1**

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Data analysis and interpretation 100 %
Preparation of figures and tables 100 %
Drafting of the manuscript 100 %

Chapter 7

Experimental concept and design 90 %
Experimental work and/or acquisition of (experimental) data 70 %
Data analysis and interpretation 50 %
Preparation of figures and tables 50 %
Drafting of the manuscript 100 %

Chapter 8

Experimental concept and design 100 %
Experimental work and/or acquisition of (experimental) data 100 %
Data analysis and interpretation 100 %
Preparation of figures and tables 100 %
Drafting of the manuscript 100 %

Chapter 9

Experimental concept and design
Experimental work and/or acquisition of (experimental) data
Data analysis and interpretation
Preparation of figures and tables
Drafting of the manuscript

See individual chapters

Date:
Signature: