







Quantifying establishment limitations during the ecological restoration of species-rich *Nardus* grassland

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Keywords

Community assembly; Establishment limitation; Germination; Intraspecific trait variation; Plant–soil interactions; Restoration ecology; Semi-natural *Nardus* grasslands

Abbreviations

AM fungi = arbuscular mycorrhizal fungi; Cb = bacterial concentration; Cf = fungal concentration; PFN, BFN, FFN, respectively = plant-, bacterial- and fungal-feeding nematodes; SLA = specific leaf area.

Nomenclature

Schaminée et al. (2010; 2015)

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Abstract

Aims: Successful establishment of species-rich *Nardus* grasslands on ex-agricultural land requires identification and removal of barriers to effective seed germination and seedling survival. Therefore, we investigate how germination and early development are affected by soil conditions from different restoration phases and how this relates to their specific plant strategies.

Location: Grasslands and experiments in northern Belgium.

Methods: We selected three grassland restoration phases (*Lolium perenne* grasslands, grass–herb mix grasslands and species-rich *Nardus* grasslands), which were characterized by a distinct plant community and soils with contrasting abiotic and biotic properties (respectively, eutrophic, mesotrophic and oligotrophic soils). In a first germination experiment we investigated the species-specific responses (germination, lag time and emergence rate) of 70 grassland species (that typically occur along the restoration gradient) in each of the selected soils. Second, a mesocosm experiment was set-up in which a mixture of 19 species (representative of the distinct grassland restoration phases) was grown together in the respective soils. Here, we analysed the intraspecific variation of plant growth, SLA and identified changes in community assembly.

Results: Irrespective of soil influences, *Nardus* grassland species had significantly lower germination potentials and longer germination lag times than *L. perenne* grassland species. Germination (and its lag time) of grass–herb mix grassland species were negatively affected by the oligotrophic soils. Soil factors determined early growth patterns during the emergence and establishment phase. *L. perenne* grassland species exhibited a more plastic growth response and were highly dependent on soil type. *Nardus* grassland species exhibited large intraspecific variation in SLA, which was found to be significantly lower in the oligotrophic soils. Even though the difference in bio-available P between mesotrophic and oligotrophic soils was minor, *Nardus* grassland species were only able to compete in the oligotrophic soils (no significant difference in biomass between communities). Mesotrophic mesocosms exhibited the highest species richness after 200 d of growth.

Conclusion: Plant species from the three grassland restoration phases display distinct germination strategies, irrespective of soil type. Interactions between growth strategies and soil factors determine competitive asymmetry and therefore shape community assembly in the distinct grassland phases.

Introduction

European semi-natural grasslands have a high conservation priority due to their high species diversity, not only in plants but also in invertebrate, fungal and microbial diversity (Rydin et al. 1997; Tilman et al. 2001; Vickery et al. 2001; Critchley et al. 2004). However, due to agricultural intensification and land-use change, species-rich grasslands on nutrient-poor soils have undergone a dramatic decline in the last century and have become rare and endangered in many parts of Europe (van Dijk 1991; Pärtel et al. 2005). For this reason, conservation and restoration of species-rich *Nardus* grassland is now recognized as a priority in European conservation policies, as reflected in the Habitats Directive 92/43/EEC (Silva et al. 2008; Convention on Biological Diversity 2014). Restoration management generally focuses on reducing grassland productivity (Oomes 1992). The cessation of fertilization combined with well-timed mowing of nutrient-rich grasslands (with dominating *Lolium perenne*), generally leads to herb-rich grassland within 5–10 yr (Schippers et al. 2012). However, the final attenuation and transition to nutrient-poor, species-rich *Nardus* grassland has been shown to take several decades, and success is generally limited (Walker et al. 2004; Schelfhout et al. 2017).

There is a consensus that successful restoration is often hampered by both a lack of propagules (dispersal limitation) and unsuitable conditions at the restoration sites (establishment/recruitment limitation; Öster et al. 2009). Habitat loss and fragmentation have impoverished the species pools and seed sources in remnant sites (Poschlod et al. 1996; Bullock et al. 2002). Furthermore, traditional farming practices that used to exchange grassland species between sites have disappeared (e.g. use of uncleaned seeds or hay, traditional manure spreading containing diaspores and artificial flooding techniques; Poschlod & Bonn 1998). However, dispersal limitation can be mitigated during grassland restoration through direct seeding, over-sowing, hay strewing (harvested from species-rich donor site) or turf translocation (Walker et al. 2004; Edwards et al. 2007; Kiehl et al. 2010). The recent debate concerning the pros and cons of assisted migration has now produced a useful framework to guide decisions as to when introducing species is desirable (Baasch et al. 2016). Establishment limitation, on the other hand, has been shown to be more difficult to overcome and is mainly caused by several abiotic and biotic filters through strong environmental legacies of the past agricultural land use (Öster et al. 2009).

Grassland restoration success is strongly related to resource availability. High species richness in semi-natural grasslands is generally associated with low levels of nutrient availability (Janssens et al. 1998). High residual soil

fertility is one of the most important abiotic factors constraining establishment opportunities of target grassland species, and thus grassland restoration (Bobbink et al. 1998; Walker et al. 2004; Gilbert et al. 2009). Accumulation of nutrients caused by repeated fertilization have been shown to promote the growth of a few competitive grasses, with certain life-history traits, which restricts opportunities for the establishment of more typical semi-natural grassland species (Grime 1973; Walker et al. 2004; Bobbink et al. 2010; Ceulemans et al. 2013). Species with an acquisitive resource use strategy dominate in fertile soils and usually exhibit a high SLA, high leaf N content and a short leaf life span. In contrast, species occurring on infertile soils require conservative resource strategies and usually exhibit a low SLA, low leaf N content and a long leaf life span (Wright et al. 2004; Laliberté et al. 2012). Soil fertility can thus act as a filter for species with economically competitive leaf investment strategies (Wright et al. 2004). This feedback loop can potentially trigger grasslands to shift to alternative states once they cross a specific threshold (Gilbert et al. 2009; Suding & Hobbs 2009; Ceulemans et al. 2017).

In grassland restoration management, succession is generally directed to regulate the transition towards the desired equilibrium and thus to prevent unwanted alternative states. Often, it is necessary to direct more than one factor, process or interaction that was altered or led to the degraded state (Suding et al. 2004). Soils are dynamic systems, and complex interactions between soil biogeochemistry, soil biota and vegetation determines community development (De Deyn et al. 2003, 2004; Wardle 2004). When plant communities react to altered soil processes, litter and root quality changes, which in turn alters the soil food web (Wardle 2004). The altered abundances and composition of the soil biotic community was shown to be a key component of environmental limitation, through reducing establishment opportunities of target grassland species (De Deyn et al. 2003; Van Der Heijden 2004; Kardol et al. 2006; Vergeer et al. 2006). Furthermore, changes in soil biota may lag behind during the restoration process (and the related change in above-ground vegetation) and can potentially cause hysteresis (thresholds of the restoration pathway are more difficult to overcome than in the degradation pathway; Holtkamp et al. 2008; Suding & Hobbs 2009). Plant–soil feedbacks induced by soil biota heavily depend on the competitive strategy of the individual species and are thus an important driver of the succession rate (Kardol et al. 2006). While much attention has been paid to restore soil conditions by lowering nutrient availabilities, the interaction between soil biota and the plant community within the context of restoration has received much less attention. Yet, soil biota are an integral part of plant–soil feedbacks, improve soil structure and

hence determine restoration success (Harris 2009; Kardol et al. 2015; Perring et al. 2015; Wubs et al. 2016).

In natural, as well as semi-natural systems, soil abiotic and biotic factors are interlinked and interactions have complex effects on plant performance, competition and, ultimately, community assembly (Wardle 2004). Walker et al. (2004) have stressed the need for experimental research on the performance of species, as well as the establishment of target species during grassland restoration in order to successfully integrate system feedback loops and thresholds in the directed succession towards semi-natural grasslands. Successful establishment depends on successful germination, emergence and establishment of desirable seedlings. However, no study has currently investigated how soils from different restoration phases influence the distinct life phases. Here, the focus lies on one particular grassland type: species-rich *Nardus* grasslands on siliceous substrates, which form a high priority European habitat type (Natura 2000 code: 6230).

In this study, we aim to investigate how soils from different grassland restoration phases on former agricultural land affect (1) species-specific germination, (2) initial establishment and (3) early community development when dispersal limitation is lifted. The distinctive restoration phases can be determined as eutrophic *Lolium perenne* grassland, mesotrophic grass-herb mix grassland (mosaic of various grasses with abundant herbs that are homogeneously dispersed) and oligotrophic species-rich *Nardus* grassland. To answer our two-first research questions, an extensive germination experiment was executed in which the emergence responses of 70 grassland species were tested in soils from each of three distinctive restoration phases (eutrophic, mesotrophic and oligotrophic soils). The second research question was addressed by conducting a mesocosm experiment in which mixtures of different plant species, characteristic of the different restoration phases, were grown together. Using a restoration gradient associated with different soil biotic and abiotic variables provides a solid case to test how different combinations of these factors (interaction) could affect the germination and establishment of grassland species.

We hypothesized that germination and establishment success of *Lolium perenne* grassland species and grass-herb mix grassland species would be lower in oligotrophic soils due to (a)biotic soil filtering effects. However, we did not expect mesotrophic and eutrophic soils to limit the establishment of *Nardus* grassland species until light competition becomes a limiting factor. Furthermore, we predicted that *L. perenne* grassland species and grass-herb mix grassland species would outperform the *Nardus* community on eutrophic and mesotrophic soils, since they are expected to exhibit a more plastic growth response depending on the

nutrient availability. On the other hand, *Nardus* grassland species were anticipated to be more resilient in oligotrophic soils due to a higher nutrient use efficiency, which should reflect in a lower SLA.

Methods

Site selection and soil analysis

In order to select grassland soils representative for three distinct restoration phases, we used a data set of 44 grasslands along a restoration gradient with contrasting soil nutrient availability and soil biotic community (S. Wasof et al. unpubl.). The studied grassland sites were selected across three regions (nature reserves Gulkse putten - GP, Turnhouts vennengebied - TV, Liereman - L) in northern Belgium. This data set contained site information on the percentage cover of plants, nematode counts at genus level, soil microbial community biomass using phospholipid fatty acid (PLFA) profiling and biogeochemical properties (P fractions, pH-KCl, CeC-Ca, Al_{CEC}). Analyses of these data showed a clear distinction in vegetation and soil (a)biotic variables between sites (Appendix S1; S. Wasof et al. unpubl.). This enabled us to distinguish three grassland restoration phases: (1) *L. perenne* grasslands, which resembled the basal community *Holcus lanatus*-*L. perenne* (syntaxon code: 16RG23 *sensu* Schaminée et al. 2015); (2) grass-herb mix grasslands, which resembled either the basal community of *Anthoxanthum odoratum* (16RG24) or *H. lanatus*-*Silene flos-cuculi* (syntaxon code: 16RG7); and (3) *Nardus* grasslands (syntaxon code: 19 *sensu* Schaminée et al. 2010), which were all well developed and species-rich. As a result, 19 grassland sites (out of the 44 sites) were the best candidates for setting up the germination and the mesocosm experiments (Appendix S1).

In each of the 19 selected sites, 30 L of soil were collected from the sandy topsoil (top 5–15 cm), including intermediate soil from the rhizosphere (and thus including its microbiome). Soil subsamples from the same region and the same restoration phase were mixed, resulting in a total of nine soils (3 regions × 3 restoration stages). The soil material was homogenized by sieving through a mesh size of 6.3 mm to remove coarse organic material.

Soil biogeochemical properties, nematode feeding types (Bongers & Bongers 1998) and the microbiotic composition (chemotaxonomic PLFA markers) were determined in order to quantify available soil filters (see Appendix S2 for details on methodology). Differences in soil factors between the different soil restoration phases (i.e. eutrophic, mesotrophic and oligotrophic) were determined with LMM and region was used as a random effect (*lme4*, R package v 1.1-12, R Foundation for Statistical Computing, Vienna, AT).

Experimental design

Germination experiment

A pool of 70 plant species, which are known to occur along the restoration gradient, were selected (see Appendix S3 for a detailed description of the selection procedure and Appendix S4 for the species list) in order to test whether or not germination is affected by (a)biotic soil conditions from different restoration phases. A total of 50 seeds (2% resolution per unit) from each of the 70 grassland species (seeds originated from Germany and were provided by Rieger-Hoffman) were germinated in each of the nine soils (3 regions \times 3 restoration phases). Furthermore, all species were additionally germinated in a control (glass beads of 0.75–1.00 mm covered with distilled water) to analyse average germination chance, given there are no nutrients and soil biota present. This control also provided insight into the seedling biomass that can be acquired through the endosperm nutrient availability. In total, 700 [(9 soils + 1 control) \times 70 species] \times 50 seeds were hand-counted in order to select viable seeds (empty seed shells and discoloured seeds omitted). Each unit (700 Petri dishes of 100 mm \times 15 mm) was filled with a 1 cm substrate (soil or glass beads) and the moisture content of the soils were adapted to make sure all soils were equally saturated. All seeds were stratified in the allocated moist soils for 6 wk at 5 °C to break dormancy. The germination was completed under a controlled day–night rhythm of 16 hr day at 25 °C and 8 hr night at 15 °C.

Cold germination was calculated as the proportion of seeds germinated at the end of the stratification period, in order to assess which species germinate during winter or early spring. The germination lag time was based on the time it takes for dicots to produce their first cotyledons (in epigeal germination). In species with hypogeal germination (below-ground germination), the end of the lag time was determined when the first two leaves emerged. In monocots it was defined when the first leaf emerged from the coleoptile. Germination (potential) was defined as the highest count of emerged seedlings at the end of the experiment. The emergence growth was determined as the relative growth (length) of seedlings in the first 2 wk of emergence. The germination lag time was monitored daily, while germination and seedling growth were determined on a weekly basis.

Community mesocosm experiment

For the mesocosm experiment, the nine soils (samples) were split into three subsamples and as a result 27 mesocosms (3 restoration phases \times 3 regions \times 3 subsamples) were established in a controlled environment. Each mesocosm (35-L pots; 45 \times 40 \times 37 cm) was planted with a

mixed community composed of grasses and forbs characteristic of each of the grassland restoration phases: (1) *Dactylis glomerata*, *H. lanatus*, *L. perenne*, *Phleum pratense* and *Taraxacum officinale* (*L. perenne* grassland species), (2) *Achillea millefolium*, *Agrostis capillaris*, *Anthoxanthum odoratum*, *Centaurea jacea*, *Festuca rubra*, *Hypericum perforatum*, *Leontodon autumnalis*, *Plantago lanceolata* and *Poa pratensis* (grass–herb mix grassland species) and (3) *Calluna vulgaris*, *Hieracium pilosella*, *Luzula campestris*, *Nardus stricta* and *Veronica officinalis* (*Nardus* grassland species). These 19 species were selected out of the 70 species tested in the germination experiment based on viability and suitability of the species.

Pre-germination treatments were based on Kew's Seed Information Database (2015), Baskin (2014) and the TRY database (Green 2009; Kattge et al. 2011) in order of priority and adapted to feasibility (see Appendix S5). The germination took place in 0.75–1.00 mm glass beads filled with distilled water. Because not all species started to germinate immediately, seedlings were placed in a climate chamber (when they reached 1–5 cm) at 4 °C with 24 hr light (120 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD) until the start of the experiment to ensure that all species were of comparable size. The seedlings were planted at random in a fixed stratified grid (see Appendix S6). This configuration ensures minimized positioning effects. For each species, three seedlings were planted in the central zone and three in the edge zone. The decision to plant three individuals per species in both the central zone and the edge zone was made to prevent accidental mortality of a specific species. All further measurements at the species level were determined on individuals in the central area of the mesocosm to avoid edge effects. After 25 d, dead seedlings were replaced to avoid influences of transplant shock-related mortality on the experiment. Throughout the experiment, the controlled light regime amounted to 16 hr light with a temperature of 25 °C and 8 hr dark at 15 °C. During the first month of the experiment, mesocosms were watered daily until saturation. During the rest of the experiment (experiment lasted 200 d), mesocosms were saturated every 3 d. Unwanted seedlings recruiting from the seed bank were removed manually.

The species-specific growth rate (in $\text{mm}\cdot\text{d}^{-1}$) was determined 25 d after seedling establishment (planted) and was based on species height. This measurement was used as an indicator of above-ground competition because a rapid growth strategy allows species to quickly shade-out other species. Eutrophic soils were mown after 55 d to minimize light competition and to reflect realistic management ($\pm 4\text{--}5$ mowing periods $\cdot\text{yr}^{-1}$ in highly productive grasslands). All mesocosms were mown after 130 d (mesotrophic grasslands are normally mown about twice a year and oligotrophic grasslands once). The mown biomass was dried at 70 °C, weighed per mesocosm and the

concentrations of P (acid digestion in Teflon bombs and colorimetric determination of P using the malachite green method), C and N (elemental analysis by catalytic combustion) were determined.

Specific leaf area (SLA) is often considered a central trait in ecological selection, as it is one of the main determinants in positioning species along the leaf economic spectrum (Westoby 1998; Poorter & de Jong 1999; Wilson et al. 1999; Wright et al. 2004). SLA was determined after 200 d, in order to obtain information on the intra- and interspecific variation related to the different restoration phases. Leaf area was measured on one individual per species per mesocosm with imageJ (U.S. National Institutes of Health, Bethesda, MD, US, <http://imagej.nih.gov/ij/>, 1997–2016), and leaves were weighed with a precision of 0.1 mg. All biomass was separated per species, dried and weighed to obtain the dry mass. The community-specific biomass proportion was calculated per mesocosm to monitor differences in community assembly. Species richness was calculated as the sum of species that survived (out of the initial 19 planted) after 200 d.

Data analysis

The significance threshold was set at 0.05 (*P*-value) for all the statistical analyses. All data are represented with mean and its variability with the SE.

Germination experiment

All germination variables were analysed with GLMM using region and species identity as random effects (lme4, R v 1.1-12). The predictors were the plant community groups (i.e. *L. perenne* grassland species, grass-herb mix grassland species and *Nardus* grassland species), the soil restoration phases (i.e. eutrophic, mesotrophic and oligotrophic) and their interaction. A binomial error family was used for the germination percentage, while Poisson errors (count data) were used for the germination lag time and the cold germination (germination during the stratification period). Species that did not germinate were excluded from the data set (eight species; see Appendices S4, S11).

For emergence growth, the growth rate in the control was subtracted from the growth rates in the soils to distinguish the increment related to seed mass from the additive effects of abiotic and biotic soil factors. Species with e.g. low and variable germination were here excluded from the analysis to avoid inclusion of unreliable data (15 species excluded; see Appendix S11). The emergence growth was related to species' ecology in terms of the Ellenberg N indicator value and the soil restoration phases using LMM. The Ellenberg N expresses a species' affinity to productive vs unproductive environments and has been shown to

relate primarily to P availability in the soil (Diekmann 2003; Klaus et al. 2012). Ellenberg N values were retrieved for each species with the TR8 package (R package v 0.9; Fitter & Peat 1994; Hill et al. 1999). This metric was only used for the emergence growth (but not for growth in the mesocosms) due to the large number of species in this experiment and their more continuous relation to productivity. This approach also allows extrapolation of the mesocosm growth rate results to a larger more continuous species pool. For germination and its lag time, we were more interested in the overall patterns within communities, and group differences were thus more appropriate than regression.

Mesocosm experiment

Differences in biomass (DM in g), assimilated P and N (i.e. concentration in dry biomass expressed in $\text{mg}\cdot\text{kg}^{-1}$) and species richness between the soil restoration phases were analysed at the mesocosm level using a GLMM with soil code (9 levels: soil phase \times region) as random factor. Growth rates and SLA were analysed at the species level, with soil restoration phase, plant community groups and their interaction effect as fixed factors. Species identity and a nested term were used as random factors. Specifically, the mesocosm code (27 mesocosms) was nested in the soil code (nine soils) to account for the dependency between communities on the same soil within the mesocosms (lowest level of analysis). The species identity accounted for the variation among species when differences between plant community groups were analysed. Community biomass percentages were analysed using a negative binomial error distribution (glmmADMB, R package v. 0.8.3.3) and species identity was excluded to determine assembly patterns on a community level. Tukey's HSD test (multcomp, R package v 1.4-6) was used to test *post-hoc* differences among groups.

Results

Soil biogeochemistry and soil biotic composition

The soils from the three restoration phases did not differ in ammonium, nitrate and bacteria concentrations (Cb; see Table 1). Eutrophic soils contained significantly more bioavailable P and AM fungal concentrations (C_{AMF}), but lower fungal concentrations (C_{f}) than the oligotrophic soils (concentrations of mesotrophic soils were intermediate). Furthermore, oligotrophic soils were significantly more acid and contained significantly more exchangeable aluminium (Al_{CEC}) than the eutrophic and mesotrophic grasslands. In addition, oligotrophic soils had lower plant-feeding nematode concentrations (PFN), but higher fungal-feeding (FFN) and bacteria-feeding nematode

Table 1. Biogeochemical composition and soil biotic communities (mean \pm SE). Total P (P_{TOT}), bio-available P (BAP), Al cation exchange capacity (Al_{CEC}), ammonium (NH_4^+) and nitrate (NO_3^-) concentrations are depicted in $mg \cdot kg^{-1}$. C/N is C to N ratio, N/S the N to S ratio, Cf the fungal concentration (AM fungi not included) relative to total microbiota, C_{AMF} the arbuscular mycorrhizal fungal concentration relative to total fungi, PFN plant feeding nematodes relative to total nematode count, FFN fungal feeding nematodes relative to total nematode count and BFN bacterial feeding nematodes relative to total nematodes. Values on the same row with a distinct letter are statistically different as determined by Tukey HSD *post-hoc* tests.

	Eutrophic	Mesotrophic	Oligotrophic
BAP	88.61 \pm 2.26 ^a	11.77 \pm 0.44 ^b	4.8 \pm 1.74 ^c
P_{TOT}	784.7 \pm 180.40 ^a	243.48 \pm 15.33 ^b	76.01 \pm 31.14 ^b
Al_{CEC}	46.67 \pm 12.6 ^a	83.00 \pm 10.15 ^a	189.33 \pm 30.12 ^b
pH_{KCL}	4.6 \pm 0.14 ^a	4.37 \pm 0.08 ^a	3.77 \pm 0.02 ^b
NH_4^+	4.12 \pm 1.42 ^a	1.78 \pm 0.51 ^a	12.05 \pm 6.77 ^a
NO_3^-	0.22 \pm 0.13 ^a	0.07 \pm 0.00 ^a	3.16 \pm 2.68 ^a
C/N	10.49 \pm 0.74 ^a	11.96 \pm 0.95 ^a	9.94 \pm 0.36 ^a
Cb	88.18 \pm 0.40 ^a	85.91 \pm 1.10 ^a	84.85 \pm 1.20 ^a
Cf	6.35 \pm 0.15 ^a	8.71 \pm 0.84 ^b	11.49 \pm 0.75 ^c
C_{AMF}	46.00 \pm 1.92 ^a	38.44 \pm 2.12 ^b	23.48 \pm 2.15 ^c
PFN	22.74 \pm 1.49 ^a	20.52 \pm 1.24 ^a	12.58 \pm 1.36 ^b
FFN	5.09 \pm 1.12 ^a	6.11 \pm 1.08 ^a	11.23 \pm 1.9 ^b
BFN	26.23 \pm 3.13 ^{ab}	25.83 \pm 2.92 ^a	37.17 \pm 3.97 ^b

concentrations (BFN) compared to the eutrophic and mesotrophic soils.

Germination experiment

Differences in soil factors between the grassland restoration phases had no significant effect on germination and its lag time of *Nardus* grassland species and *L. perenne* grassland species (Fig. 1a). Interestingly, grass-herb mix grassland species had a significantly lower germination percentage and germinated slower in the oligotrophic soil than in the other two restoration phases (i.e. mesotrophic and eutrophic soils). Model results of the GLM displayed a significant lower germination percentage in *Nardus* grassland species compared to *L. perenne* grassland species in the eutrophic soils ($z = -2.16$, $P = 0.031$), oligotrophic soils ($z = -2.21$, $P = 0.027$) and a trend was observed in the mesotrophic soils ($z = -1.76$, $P = 0.078$). However, the multiple comparisons test (*post-hoc* Tukey HSD) reported no significant differences in this regard (Fig. 1a, Appendix S7) due to the family-wise error rate correction used in the Tukey HSD test. In addition, and irrespective of the soil restoration phase, *L. perenne* grassland species germinated significantly faster than grass-herb mix grassland species and *Nardus* grassland species (Fig. 1b). However, no significant differences were observed in the germination percentage or lag time between grass-herb mix grassland species and *Nardus* grassland species (Fig. 1a,b,

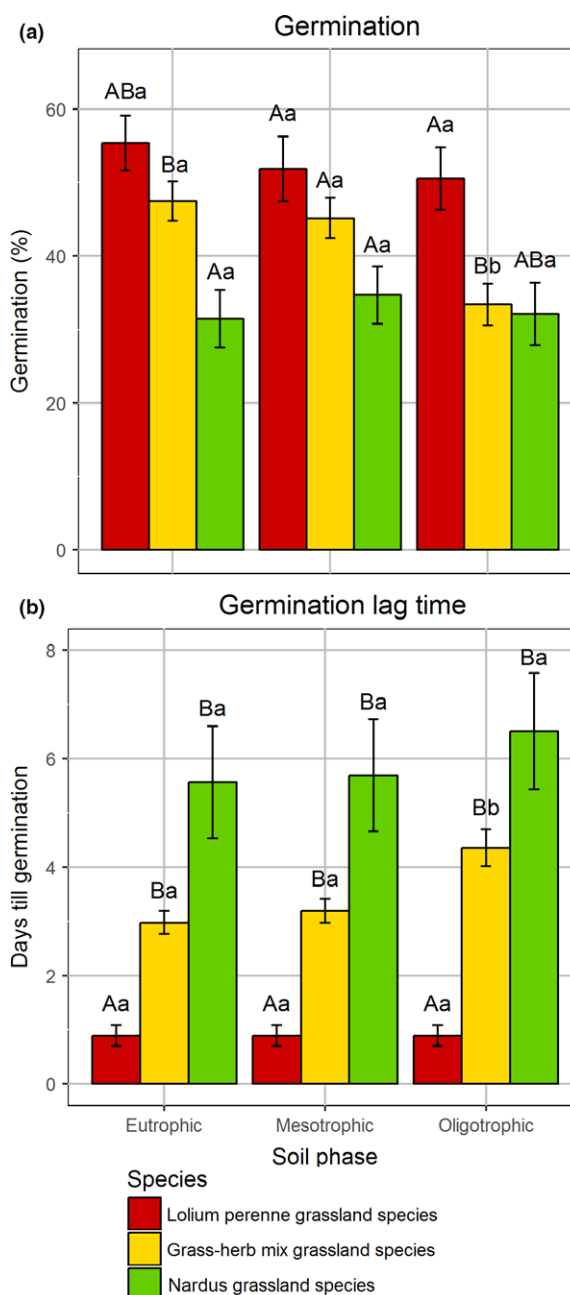


Fig. 1. Germination percentage (resolution = 2%) (a), and germination lag time (days to germinate after the stratification period) (b). Groups with distinct letters are significantly different at $P = 0.05$. Capital letters depict statistical comparison of means within each soil phase (i.e. eutrophic, mesotrophic and oligotrophic) and across plant community groups (species). Lowercase letters depict statistical comparison of means within each species group across the soil phases. Error bars are SE. [Colour figure can be viewed at wileyonlinelibrary.com]

Appendix S7). *L. perenne* grassland species germinated on average more during the stratification period ($73 \pm 5\%$) compared to grass-herb mix grassland species ($20 \pm 2.2\%$;

$t = -2.45$, $P = 0.037$) and *Nardus* grassland species ($13 \pm 0.03\%$; $t = -2.85$, $P = 0.01$).

The species' position along a productivity gradient was reflected in the Ellenberg N and was significantly different for each vegetation phase. *L. perenne* grassland species had an average Ellenberg N of 5.86 ± 0.09 , while grass-herb mix grassland species averaged 4.39 ± 0.07 and *Nardus* grassland species 2.33 ± 0.05 (group differences were all highly significant at $P < 0.001$). The effect of a species' position along a productivity gradient (as indicated by Ellenberg N) on the emergence growth rate (relative to control) was soil-dependent (Fig. 2). In oligotrophic soils, species characteristic for high productivity environments (high Ellenberg N) did not grow faster ($t = 0.53$, $P = 0.6$) compared to species preferring non-productive soils (low Ellenberg N). The growth rate in oligotrophic soils was on average lower than in the control ($-0.18 \pm 0.04 \text{ mm} \cdot \text{d}^{-1}$). In mesotrophic soils, the growth rate of species increased significantly with its Ellenberg N value ($t = 2.49$, $P = 0.013$). In eutrophic soils, the effect of the species' productivity requirements on emergence growth rate was strongest ($t = 3.88$, $P < 0.001$). There was a strong interaction effect (on emergence growth) between soil types and species' position along a productivity gradient ($X^2 = 50.67$, $P < 0.001$). This implies that the regression slopes differed significantly between the soil types.

Mesocosm experiment

At the mesocosm level, total biomass production was congruent with the P concentrations in plant biomass, but not

with N concentrations (no statistical difference in plant N between different soils; Table 2). This was also reflected in the N/P ratios, which were significantly higher in the oligotrophic soils compared to the mesotrophic and eutrophic soils. Species richness after 200 d was highest in mesotrophic soils with, on average, 18.67 ± 0.05 species (of a total of 19 species), and was significantly lower in oligotrophic soils (15.56 ± 0.13 species; $z = 3.226$, $P = 0.004$) and eutrophic soils (15.22 ± 0.13 species; $z = -3.572$, $P = 0.001$).

At a community level, *L. perenne* and grass-herb mix grassland species made up most of the biomass, with the latter being the most dominant in eutrophic and mesotrophic soils (Fig. 3a, Appendix S8). Relative biomass of *Nardus* grassland species made only a minor contribution to total biomass in these two soil types, but was significantly higher in the mesotrophic soils compared to the eutrophic soils. Most importantly, the biomass proportion of *Nardus* grassland species was 12.46 times higher in oligotrophic soils compared to mesotrophic soils. Furthermore, biomass proportions of *L. perenne* grassland species and grass-herb mix grassland species were significantly lower in oligotrophic soils compared to eutrophic, but not compared to mesotrophic soils.

There was a clear difference in species-specific growth rate of all plant community groups between soil restoration phases, with plant growth being lowest in oligotrophic soils compared to the other two restoration phases (see Fig. 3b, Appendix S8). In addition, while there was a significant difference in plant growth between the three plant community groups in the eutrophic soils, this difference was not significant between *L. perenne* grassland and grass-

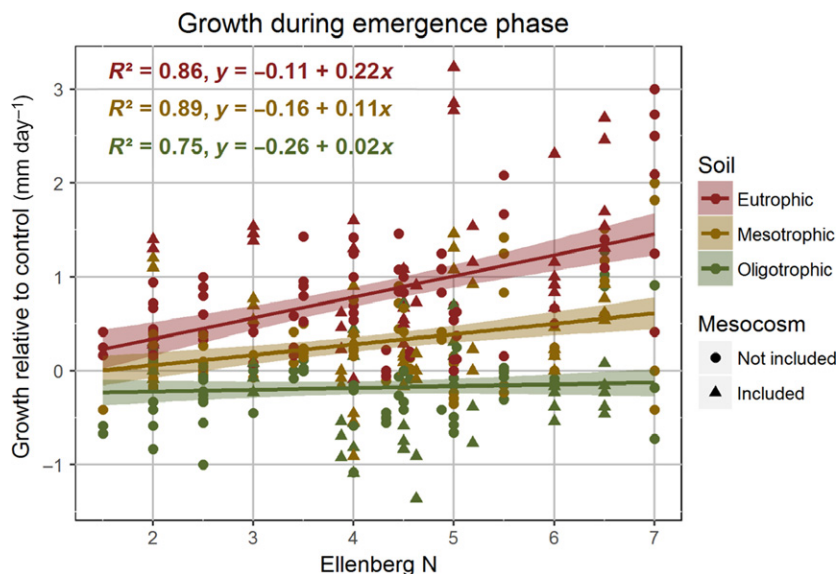


Fig. 2. Growth rate relative to control (in $\text{mm} \cdot \text{d}^{-1}$). The colour of the regression line depicts the soil medium. Symbols denote which species were used in the mesocosm experiment. The transparent colours surrounding the regression lines indicate SE. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 2. Total dry mass production over 200 d (DM in g), biomass total P concentrations (mg·kg⁻¹), total N concentrations (mg·kg⁻¹) and the N/P ratio. Values on the same row with a distinct letter are statistically different as determined by the Tukey HSD *post-hoc* test.

	Eutrophic	Mesotrophic	Oligotrophic
DM	66.00 ± 4.96 ^a	37.57 ± 2.62 ^b	3.73 ± 0.60 ^c
P	4271.35 ± 183.3 ^a	1900.35 ± 83.85 ^b	745.1965 ± 117.85 ^c
N	27 074.44 ± 935.15 ^a	17 324.44 ± 1139.27 ^b	26 006.67 ± 2127.58 ^{ab}
N/P	6.44 ± 0.36 ^a	9.26 ± 0.76 ^a	38.96 ± 4.12 ^b

herb mix grassland in the mesotrophic soils. Furthermore, species did not differ in their growth rate in oligotrophic soils.

The SLA values of all plant community groups were lower in oligotrophic soils compared to the other two soils restoration phases (Fig. 3c, Appendix S8). In addition, grass–herb mix grassland species had significantly lower SLA values in mesotrophic compared to eutrophic soils. However, while there was no difference in SLA values between plant community groups among restoration phases, *Nardus* grassland species had a lower SLA in the oligotrophic soils compared to the other plant community groups (i.e. *L. perenne* grassland and grass–herb mix grassland).

Discussion and conclusions

Germination strategies

Germination strategies were highly dependent on the species' affinities to a particular grassland restoration phase and this was irrespective of the soil phases. The observed rapid germination and high germination potential of *L. perenne* grassland species during the stratification pre-treatment (73 ± 5%) indicates that germination strategies are seasonally dependent. *L. perenne* grassland species occurring on eutrophic soils need to germinate before the sward canopy closes and thus escape light competition by germinating in the winter or early spring. *Nardus* grassland species occurring in oligotrophic soils, on the other hand, experience reduced exposure to light competition and are thus not restricted by an increased germination lag time. This is in line with the results of Olff et al. (1994), who found that changes in germination attributes occurred during the succession toward nutrient-poor grassland.

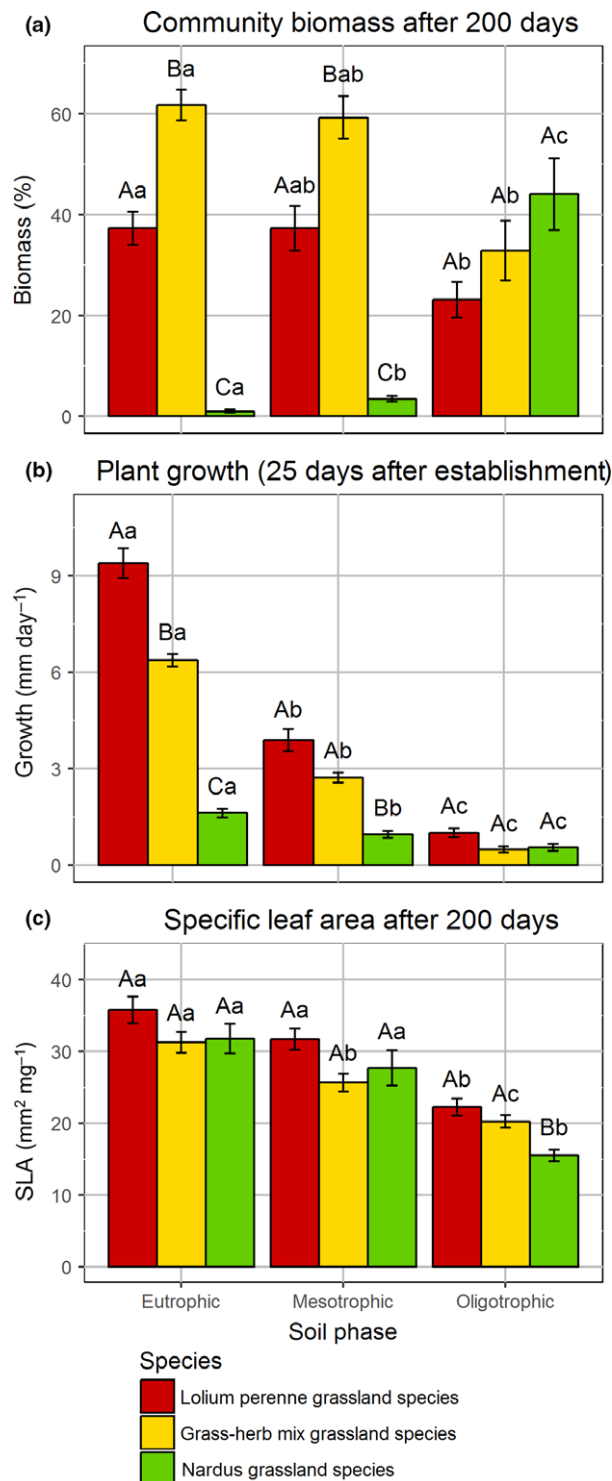
The postponed germination of *Nardus* grassland species indicates morphological and/or physiological dormancy. This mechanism is important to ensure favourable environmental conditions for germination. When *Nardus* grassland species come out of dormancy they enter a transitional state called conditional dormancy, where they only germinate under a narrow range of conditions (Baskin & Baskin 2004; Baskin 2014; Hoyle et al. 2015). The lower germination potential of *Nardus* grassland species thus indicates a trade-off in dormancy, where

conditional dormancy restricts germination potential under optimal conditions but, on the other hand, decreases mortality during unfavourable environmental conditions.

Abiotic and biotic filters during establishment

As expected, soil biogeochemistry and the soil biotic community were clearly distinct in the three grassland restoration phases (Table 1, Appendix S9; Cornelissen et al. 2001; Eisenhauer et al. 2011; Holtkamp et al. 2008). The distinct plant growth strategies of species from specific grassland restoration phases determine how they react to specific soil filters. Germination (and its lag time) of *Nardus* grassland species and *L. perenne* grassland species were not affected by the soils. Grass–herb mix grassland species, on the other hand, were negatively affected by oligotrophic soil properties. The oligotrophic soils had a pH-KCL of 3.77 (which relates to a pH-H₂O of 4.47; Van Lierop 1981) and were thus below the Al buffer range where exchangeable Al (Al³⁺) becomes soluble (also reflected in the high Al_{CEC} of 189.33 mg·kg⁻¹). Abedi et al. (2013) found detrimental effects of Al³⁺ on germination, although this was dependent on the Al tolerance of the plant species. Generally, an Ellenberg indicator value for environmental acidity (EIV R) of 4 or lower indicates Al tolerance (Abedi et al. 2013). Indeed, only *Nardus* grassland species approximated this indicator for Al tolerance (mean EIV R of 4.07 ± 0.19 compared to 5.89 ± 0.07 in grass–herb mix grassland species and 6.78 ± 0.07 in *L. perenne* grassland species). Surprisingly, germination of *L. perenne* grassland species was not affected by oligotrophic soils. Generally, root growth inhibition due to Al toxicity takes place 2–4 d after the initiation of seed germination (Rout et al. 2001) and rapid germination (±1 d in *L. perenne* grassland species) most likely prevented its limiting effects in this phase.

In oligotrophic soils, no significant difference in seedling growth rate was observed, even though species differed markedly in their growth efficiency (RGR generally increases with EIV N; Bartelheimer & Poschlod 2016). The competitive advantage of productive species during early establishment was most likely suppressed due to the low pH, which restricts the availability of various plant nutrients (Lucas & Davis 1961) and can cause high availability



of Al^{3+} (Bache 1985). Plants establishing under high Al^{3+} concentrations usually develop shallower root systems with reduced length of the root hair zone, which thus further reduced nutrient uptake (Delhaize & Ryan 1995; Ma et al. 2001). High observed seedling mortality of *L. perenne*

Fig. 3. (a) Community biomass shifts after 200 d. Species that died (no biomass) were removed from the analysis. (b) Plant growth as an indicator for light competition (25 d after establishment). (c) SLA per species after 200 d. *Achillea millefolium* and *Calluna vulgaris* were removed from the analysis due to the composite leaves. Values of *Taraxacum officinale* are missing in oligotrophic soils due to early mortality. Groups with distinct letters are significantly different at $P = 0.05$. Capital letters depict the statistical comparison of means within soil phases and across plant community groups (species). Lowercase letters show the statistical comparison of means within each species group across the soil phases. Error bars display SE. [Colour figure can be viewed at wileyonlinelibrary.com]

grassland species ($22.22 \pm 8.15\%$) and grass-herb mix grassland species ($43.24 \pm 4.7\%$) in the oligotrophic soils confirms the Al filter hypothesis, which states that only species with high physiological tolerance to Al can maintain root growth and persist through the seedling stage (Poozesh et al. 2007; Abedi et al. 2013). This pattern was similar in the establishment phase, where Al toxicity most likely damaged root tips and root cortex cells in non-resistant species (Rout et al. 2001). Furthermore, P is known to precipitate in Al-rich soils and thus further reduced P availability in the already oligotrophic soils (Wright 1943).

In addition, high AM fungal concentrations in the eutrophic soils, and to a lesser extent in mesotrophic soils, most likely increased P assimilation and lateral spread through the integration of plant species in the hyphal network (Hartnett et al. 1999; Van Der Heijden 2004; Gross et al. 2010). As expected, BAP concentration differences in the soil (Table 1) were not congruent with the average P concentrations in the biomass (Table 2; contrast BAP between mesotrophic and oligotrophic soils is smaller than divergence of P in the biomass). However, even though AM fungal concentrations most likely increased interspecific competition in our mesocosms, effects of AM fungi on growth patterns and thus on the competitive asymmetry between communities depend on the relative mycotrophy of dominant vs subordinate plants (Collins & Foster 2009).

Drivers of intraspecific trait variation during community development

The plastic growth response of *L. perenne* grassland species during community development (which was in line with the emergence growth response) was highly dependent on nutrient availability and thus increased competitive asymmetry in the eutrophic soils. The strategy of productive species (*L. perenne* grassland species) thus involves reaching maximum height as fast as possible (high observed cold germination) with an acquisitive resource-use strategy (high allocation of C to growth; see e.g. Wardle 2004). Growth rates of grass-herb mix grassland species were similar to *L. perenne* grassland species in the mesotrophic soils,

but no cold germination was observed. Grass–herb mix grassland species thus appear to exhibit a late, fast-growing strategy. Other characteristics such as stem density and phenology could possibly have an influence on their competitive advantage in mesotrophic soils (Sun & Frelich 2011). These traits tend to maintain an advantage over rapid-growing species during the middle of the growing season. The intraspecific variation of SLA was highest in *Nardus* grassland species and they maintained the lowest SLA in oligotrophic soils (values were comparable to those found in grasslands at the lowest end of productivity; Poorter & de Jong 1999). Low SLA is generally related to high nutrient use efficiency, with corresponding high leaf longevity and nutrient resorption from senescing leaves (Wright & Westoby 2003; Wright et al. 2004). *Nardus* grassland species thus exhibit a late (longer germination lag time) and slow growth strategy. The distinct growth strategies here observed in the grassland restoration phases are analogous to the growth strategies hypothesized for herbaceous grassland species (respectively, early and fast, late and fast and late and slow) by Sun & Frelich (2011). The conservative resource use strategy and its corresponding growth strategy of *Nardus* grassland species is most economically competitive in oligotrophic soils and enables them to compete against faster-growing acquisitive species.

Community assembly patterns

Although the mowing regime minimized light competition in the eutrophic and mesotrophic mesocosms, *Nardus* grassland species could not establish (low biomass; Fig. 3a). This was also reflected in the high mortality in eutrophic soils of the *Nardus* grassland species *Calluna vulgaris* (100%), *Hieracium pilosella* ($55 \pm 18\%$), *Veronica officinalis* ($44 \pm 18\%$), *Nardus stricta* ($33 \pm 17\%$) and of the grass–herb mix grassland species *Poa pratensis* ($56 \pm 0.18\%$), *Agrostis capillaris* ($22 \pm 15\%$) and *Festuca rubra* ($22 \pm 15\%$). In the eutrophic and mesotrophic soils, biomass production was N-limited (N:P ratio <14; Koerselman & Meuleman 1996). Although plant concentrations did not exceed the critical value for N limitation (<14 000 mg·kg⁻¹ N), addition of N would probably have increased the competitive exclusion by *L. perenne* grassland species (Wassen et al. 1995). In the eutrophic soils N limitation was also reflected in the yellow discoloration of leaves of e.g. *Taraxacum officinale*. Additional factors reducing the competitive exclusion of *L. perenne* grassland species were the higher initial relative dominance of grass–herb mix species (48 individuals compared to 30 individuals in each of the other communities) and the more intensive mowing regime in the eutrophic mesocosms (three times compared to

two). This is in line with what is observed in field studies, the mowing regime alone is sufficient to permit grass–herb mix grassland species to establish even in eutrophic situations (Bonanomi et al. 2006; Billeter et al. 2007). Furthermore, plants in mesotrophic soils experienced a lower intensity of ecological filters and thus maintained highest species richness after 200 d.

The oligotrophic soils experienced high P limitation (N: P > 16) and plant P concentrations approximated the critical value of 700 mg·kg⁻¹ P (Wassen et al. 1995). Poorter & de Jong (1999) related low biomass production in oligotrophic mesocosms (comparable to 42.6 g DM·m⁻²·yr⁻¹) to SLA values of ~10–15 across species within a habitat, which is in line with the low SLA observed among the *Nardus* grassland species. Highest mortality after 200 d was observed in the *L. perenne* grassland species *Taraxacum officinale* (100%), across the grass–herb mix grassland species *Plantago lanceolata* ($44 \pm 18\%$), *Hypericum perforatum*, *Poa pratensis*, *Leontodon autumnalis* ($33 \pm 17\%$), *Centaurea jacea* ($22 \pm 15\%$) and surprisingly also in the *Nardus* grassland species *Veronica officinalis* ($56 \pm 18\%$ vs 0% in mesotrophic soils). *Holcus lanatus*, *Festuca rubra* and *Dactylis glomerata* are known to be tolerant to Al, while *L. perenne* and *Phleum pratense* are moderately sensitive (Wheeler et al. 1993; Wheeler 1995). The unexpected low mortality of *L. perenne* grassland species in the oligotrophic mesocosms was thus most likely related to their Al tolerance (indicated sensitivity to Al toxicity of *L. perenne* grassland species in the germination experiment was calculated on a larger species pool). Phosphorus limitation thus enabled *Nardus* grassland species to compete as they have the most economical leaf investment strategy for this situation and because they most likely exhibited higher Al resistances than some of the species with a high leaf area (such as *Taraxacum officinale*, *Leontodon autumnalis* and *Plantago lanceolata*). Even though the difference in bio-available P between mesotrophic and oligotrophic soils was minor, *Nardus* grassland species were thus only able to compete in oligotrophic soils.

Management implications

Our results confirm the need to reduce bio-available P below the 10 mg·kg⁻¹ threshold or select grasslands below this threshold if the management objective is to restore species-rich *Nardus* grassland (Gilbert et al. 2009). Furthermore, the distinct soil biota observed in the oligotrophic soils is known to promote late successional species through positive plant–soil feedbacks (Kardol et al. 2006). Soil inoculations can assist restoration management in this regard (Middleton & Bever 2012; Wubs et al. 2016). When screening potential sites for *Nardus* grassland restoration management, a pH target of ~4.5 could be

useful as an additional soil filter to reduce competition between herb–grass mix grassland species and *Nardus* grassland species (only applicable when BAP approximates $10 \text{ mg}\cdot\text{kg}^{-1}$). *Nardus* grassland species appear to germinate less, irrespective of soil influences, and lifting seed limitation is thus an important factor in the restoration process. Finally, mowing in May during the transition period from grass–herb mix grassland to species-rich *Nardus* grassland could reduce the competitive disadvantage of the slower-germinating *Nardus* grassland species (± 1 mo compared to 2 wk).

Conclusion

The competitive ability of *Nardus* grassland species is constrained during the emergence phase due to inherently lower germination potentials, longer germination lag times and slower seedling growth rates. Competitive asymmetry is higher in fertile conditions, which is caused by the plastic growth response of productive species (high C use efficiency). *Nardus* grassland species only exhibit a lower SLA (high nutrient use efficiency) in oligotrophic situations and are only able to compete at the lowest levels of productivity. Oligotrophic soil filtering heavily constrains productive species and has the strongest effect on germination, emergence and establishment of grass–herb mix grassland species. Our study clearly shows that ecological restoration management should include knowledge of establishment limitation to increase efficiency in directing the non-random aspects of community assembly.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

- Appendix S1.** Grassland soil selection procedure.
- Appendix S2.** Methodology for determining soil biotic and abiotic factors.

- Appendix S3.** Species selection procedure.
- Appendix S4.** Species list for the experiments.
- Appendix S5.** Seed germination treatments of the mesocosm seedlings.
- Appendix S6.** Stratified random sampling design of the mesocosms.
- Appendix S7.** Mixed model results of germination and germination lag time.
- Appendix S8.** Mixed model results of mesocosm biomass, relative growth and SLA.
- Appendix S9.** Soil patterns.
- Appendix S10.** Primary data of soil biogeochemical analysis.
- Appendix S11.** Primary data of germination experiment.
- Appendix S12.** Primary data of species-specific analysis in the mesocosm experiment.
- Appendix S13.** Primary data of biomass analysis in the mesocosm experiment.