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Designing seed mixtures for restoration on alpine soils: who should your neighbours be?

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Abstract

Questions: (1) When alpine vegetation is actively restored by seeding, how is vegetation cover influenced by seeding treatments and soil conditions? (2) How does the cover of species differ when they are seeded in a mixture and how is their response influenced by soil conditions? (3) Do individual species perform better or worse in a mixture than when sown separately?

Location: Hjerkinn, Dovrefjell, Norway.

Methods: In a factorial, randomized field experiment, we recorded the percentage cover of *Festuca ovina*, *Luzula multiflora* ssp. *frigida,* and *Poa alpina* seeded in four different soil types for three years after seeding. We seeded the three species separately and in a mixture in organic topsoil, peat soil, mineral fine soil and mineral coarse soil. We also recorded seedling emergence in a greenhouse experiment, using the same seeding treatments.

Results: In the field experiment, vegetation cover established fastest when *F. ovina* was sown in monoculture followed by the seed mixture. After three years, mean cover of *F. ovina* was 1.4 higher than mean *P. alpina* cover and more than three times higher than mean *L. multiflora* cover for single-species treatments, and four (*P. alpina*) and 15 (*L. multiflora*) times higher when the species were seeded together. *L. multiflora* germinated slowly in the greenhouse experiment, which could partly explain its poor field performance. In the field experiment, establishment was faster in organic soils than mineral soils for all seeding treatments. The largest difference between *F. ovina* and *L. multiflora* performance in the mixture treatment was found in the organic soil types, where overall cover was larger than in the mineral soils. In the organic soils, *F. ovina* was slightly facilitated in the mixture treatment, while the opposite was found for *L. multiflora.*

Conclusion: When the restoration goal is to quickly establish a vegetation cover, seeding monocultures of rapidly establishing species may be more effective than seeding mixtures even in alpine sites where interspecific facilitation may prevail.

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Keywords: Competition, Facilitation; *Festuca ovina;* Field experiment; *Luzula multiflora* ssp. *frigida; Poa alpina;* Restoration; Seed mixture; Soil conditions;

Nomenclature: Lid &Lid (2005)

Running head: Designing seed mixtures for restoration

Introduction

Ecological restoration has become increasingly important following human ecosystem conversion and degradation (Roberts et al. 2009; Suding 2011) and can play an important role in ecosystem recovery (SER 2004; Jones & Schmitz 2009). Passive restoration is often preferable (Prach et al. 2001; Rydgren et al. 2013; Hagen et al. 2014). But active restoration may be needed if ecosystems are severely degraded and/or recovery will not be achieved within a reasonable period of time (Gretarsdottir et al. 2004; James et al. 2012) using methods like seeding, fertilization, turf transplants and mulching (Krautzer et al. 2011; Vega et al. 2015). This may be especially important in environments where abiotic conditions are stressful, such as arctic-alpine ecosystems (Prach & Hobbs 2008; Jorgenson et al. 2010)where biological processes are slow due to low temperatures and a short growing season (Billings & Mooney 1968). Under these conditions active restoration can speed up soil stabilization and vegetation recovery (Forbes & Jefferies 1999; Kearns et al. 2015).

A common approach on sparsely vegetated sites is seeding with commercially available seed (Younkin & Martens 1987; Tinsley et al. 2006; Kiehl et al. 2010; Kearns et al. 2015). This is a cheap and simple approach (Krautzer et al. 2011), but in recent years there has been increased emphasis on using seed mixtures of local origin (vander Mijnsbrugge et al. 2010; Krautzer et al. 2011; Hagen et

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al. 2014). Using native seed mixtures reduces the risk of introducing new genetic material that may threaten native populations (Hufford & Mazer 2003; Byrne et al. 2011), and site-specific populations may perform better than introduced ones over time (Sackville Hamilton 2001). Changes in conservation policy and practice are also increasing the need for native seed in ecological restoration projects (vander Mijnsbrugge et al. 2010; Jørgensen et al. 2016). Norway's Nature Diversity Act, for example, requires the exercise of due care if alien organisms are introduced, and this includes viable seeds (Anonymous 2009). Designing effective native species mixtures for restoration purposes requires an understanding of the establishment and interaction of native species under varying abiotic conditions, for example different soil types.

When bare soil is seeded for ecological restoration, the quality of microsites is of the utmost importance for seedling emergence, survival and growth (Jumpponen et al. 1999; Cooper et al. 2004; Donath & Eckstein 2010). In the first phase of vegetation recovery, microsite quality is largely determined by physical conditions, but living organisms subsequently have an increasing impact through direct plant–to-plant interactions and through indirect interactions via litter and soil properties (Facelli & Facelli 1993). In restoration projects the available substrate may vary both within and between sites depending on soils present at the site and the level of degradation. To acquire knowledge of how the seeded species, alone or in mixtures, perform under different soil conditions is therefore highly relevant.

Together with local environmental conditions, interspecific interactions among plants are important in determining community composition (Bertness & Callaway 1994; Eckstein 2005). Use of seed mixtures can therefore improve the efficiency of ecological restoration. Generally, positive interactions (facilitation) are assumed to increase with increasing physical stress (Bertness & Callaway 1994), as found in harsher environments (Callaway et al. 2002), and positive and negative (competition) interactions occur simultaneously, with varying relative importance along environmental gradients (Holmgren et al. 1997). We generally know little about how species

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commonly used in seed mixtures influence each other, even though this information is vital for ecological restoration. The competition hierarchy between species may vary depending on regional and local conditions, and we should ideally know how species in a given seed mixture interact under given field conditions, so that we achieve the defined restoration goals and avoid wasting money on unsuitable seeds.

In this study, we examined how cover of seeded native species developed during the initial restoration phase (the first three years). In particular, we studied their performance when seeded separately and in a mixture under different soil conditions, looking especially at facilitation or inhibition of individual species in the mixture. We compared seeding treatments using single species (*Festuca ovina*, *Luzula multiflora* ssp. *frigida* and *Poa alpina*) and a mixture of all three species on four different soil types in a randomized field experiment at an alpine site. All three species are common in the study area, and *F. ovina* and *P. alpina* have previously been seeded in restoration projects (Aamlid & Sæland 2010; Hagen et al. 2014). We also tested the emergence rates of the same species in a greenhouse experiment, both as single species and in a mixture.

We asked the following questions: (1) Does percentage cover of seeded vegetation differ between seeding treatments (three single species and one mixture) and soil conditions (from coarse and infertile mineral soils to more fertile organic topsoil and peat)? (2) How does the cover of species differ when they are seeded in a mixture and is their response influenced by soil conditions? (3) Do the individual species perform differently when seeded alone or in a mixture?

Materials and methods

Study area

In 2011, we established an experimental site within the former Hjerkinn military area (62°16’N, 9°27’E), altitude 1100 m, at Dovrefjell, Norway (Fig. 1). The area has been used for military training since before 1950’s up to 1999, and a number of technical constructions, like roads, buildings and

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other training facilities was located in the area. In 1999, the Norwegian parliament decided to restore the ecosystem to its original state (Hagen et al. 2014), by removing roads and other technical constructions. The surrounding vegetation is dominated by lichen and dwarf shrub heath with scattered herbs, grasses and mosses. Domestic sheep together with introduced musk oxen and occasionally wild reindeer graze the area in summer. The climate is dry and cold. Mean annual precipitation is 435 mm, mean annual temperature 0.0°C and mean July temperature 10.0°C at the closest weather station, Fokstugu, 17 km SW of Hjerkinn, elevation 973 m, for the normal period 1961–1990 (Aune 1993; Førland 1993). The experimental site is in the slightly continental section of the low alpine vegetation zone, and the growing season is about 115 days (Moen 1999). The bedrock in the Hjerkinn area is Precambrian, dominated by metamorphosed rock covered by calcium-poor glacial sediments (Sigmond et al. 1984).

Field experimental design and sampling

At the experimental site, we established a 12 × 30 m rectangle, divided into 160 squares (each 1.5 × 1.5 m) (Fig. 1). The site was an old road verge with hardly any natural reestablishment of vegetation cover over 40 years. Some scattered patches of vegetation (mainly *Festuca ovina* and some lichens) were removed by hand before establishing the experiment. The experiment included four seeding treatments and four soil types in a balanced and completely randomized factorial design. There were also unseeded reference plots for all soil types to gauge background colonization (not included in the statistical analysis). Two mineral soil types—coarse gravel and fine sand—and two organic soil types, —peat and topsoil—were tested. These soil types represent most situations of degraded, alpine land. All soils were obtained within 2 km of the experimental site. The peat soil was dug up from a depth of approximately 20 cm, while the topsoil was collected from the upper soil in moist heath and contained seeds and plant fragments. Both mineral soil types derived from disturbed sites that had been exposed during construction work in recent years, and therefore contained only limited

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numbers of seeds and plant fragments. The added soil layer was 10 cm deep, which we expected to be sufficient to minimize potential subsoil influence during the three-year experiment. The two mineral soil types had low total N (≤ 0.02%) and organic content (≤ 1.7%), and pH values of 5.4 (coarse) and 6.3 (fine). The organic soil types had pH values of 4.2 (peat) and 5.5 (topsoil), and higher total N (2.3% and 0.2%) and organic content (77.1% and 7.3%), the higher values being for peat soil. The soils were applied to the 160 squares on 10–12 August 2011, and commercial granulate fertilizer, 10 $g \cdot m^{-2}$ of NPK (22:3:10), was added to the experimental plots just before seeding on 24 August 2011.

Our sampling unit was a 0.5 × 0.5 m plot in the centre of each square that was permanently marked and seeded. Consequently, the distance between seeded plots was 1 m. The plots were seeded with the perennial tussock forming graminoids *Festuca ovina*, *Poa alpina* and *Luzula multiflora* ssp. *frigida*, either separately or in a mixture of all three species produced by professional seed growers from seeds collected at Dovrefjell and nearby mountain areas. We added 2700 seeds to each plot corresponding to a seeding density of 10800 m^{-2} (based on seed weight), i.e. 5.08 $g \cdot m^{-2}$ for *F.ovina*, 5.88 $g \cdot m^{-2}$ for *P. alpina* and 3.68 $g \cdot m^{-2}$ for *L. multiflora* ssp. *frigida*. The seed mixture plots were seeded with 900 seeds of each species. All combinations of soil and seeding were replicated eight times, giving a total of 128 seeded plots and 32 untreated control plots (see Fig. 1). The seed density is based on previous field experiments in the same area (Hagen & Evju 2013), although slightly reduced. All three species have a broad distribution in Norway, but *L. multiflora* ssp. *frigida* has the most scattered distribution (Lid & Lid 2005). Locally, *F. ovina* prefer drier soils than the two other species, whereas *P. alpina* prefer slightly more basic soil conditions (Lid & Lid 2005).

We recorded species abundance of both sown and unseeded species as percentage cover in each plot in early August 2012–2014 but the cover of unseeded species were not included in our analyses.

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Greenhouse experiment

The germination rate and viability of seeds might affect the results in the field experiment. We therefore conducted a greenhouse experiment to provide optimal potential germination conditions to support the interpretation of field emergence of the sown species. Most species have a physiological optimum germination temperature of about 20°C (Hartmann et al. 2011), including arctic and alpine species (Gartner 1983). We used the same seeding treatments, the same batch of seeds and the same seeding density (168 seeds in each treatment) as in the field experiment, i.e. seeding with *Festuca ovina*, *Poa alpina* and *Luzula multiflora* ssp. *frigida*, either separately or in a mixture of all three species. We used a greenhouse at the Norwegian University of Life Sciences during winter from early November 2011 until mid-March 2012. The seeds were sown in pots (13.5 × 12 cm, height 6.5 cm), each combination replicated eight times, in standard soil (85 % *Sphagnum*, 10 % sand and 5 % clay, pH 5.5–6.5), watered regularly, and subjected to 16-hour day ($16^{o}C$) and 8-hour night ($13^{o}C$) with six lamps each of 400 watt on a 8 m^2 table. The experiment was repeated twice. We counted the emerged seedlings without removing them from day 5 and at 2–4 day intervals until day 29. For experiment one we also counted the number of seedlings on day 37 and 59. Experiment one only included single species. In the second experiment, we also included the mixed sowing treatment.

Statistical analyses

To compare the percentage cover of the three seeded species and the seed mixture in different soil types (questions 1 and 2), we used linear models. In analysis of the first question, we used a model with seeding treatment (four levels) and soil type (four levels) as main factors. Since percentage cover data is strictly bounded but non-binomial, we logit transformed the cover data (Warton & Hui 2011) before fitting the linear model. In the analysis of the second question, we used linear models

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to analyse the difference in log-transformed cover values between species in each seed mixture plot over the range of soil conditions (four levels); see Appendix S3, supplementary material, for interpretation of these analyses. We focused on the cover difference between *F. ovina* and *P. alpina*, and *P. alpina* and *L. multiflora* ssp. *frigida*, and used the last comparison between *F. ovina* and *L. multiflora* ssp. *frigida*, that is not independent from the two other analysis, as a check whether the result is consistent and a logical consequence of the two other analyses.

We used a simulation test to examine whether a species performed better or worse when grown in mixture compared to monoculture (question 3). We approached this question using proportional data (see below) because the number of seeds per species was three times higher in the single-species treatments than in the mixture treatment, and the sum of cover of the three species in the mixture was often higher than 100%. Since cover values are bounded, there cannot be a linear relationship between cover and "performance" and therefore not possible to correct for differences in seed number by dividing or multiplying cover values. We assumed that comparisons of proportions are sufficient to infer differences in performance between the two treatments. In each simulation (repeated 2000 times) we drew three plots from the single species data set, one for each species, and one plot from the mixture data set (all plots were drawn at random and with replacement). We recorded for each species whether its proportion of the total cover in the mixture (proportional cover in mixture) exceeded its proportion of the summed cover of the three single species plots (below referred to as the proportional cover when seeded alone). The number of times this happened among the 2000 simulations was used as test statistic. This analysis tests, for a given species, the null hypothesis that the proportional cover when seeded alone is the same as the proportional cover in mixture. We rejected the null hypothesis when the test statistic was ≥ 1950 or ≤ 50, corresponding to a significance level of 0.05. A large test statistic indicates that the species in question is facilitated by growing in a mixture rather than alone. Each soil type and year combination was analysed separately, giving 12 analyses.

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Differences in emergence rates for the four seeding treatments at the end of the greenhouse experiment were analysed using a general linear model (GLM). Since the emergence data are proportions, we used GLM with quasibinomial error (to account for overdispersion) and logit link function (Crawley 2013).

All simulations were performed by macro-programming in LOTUS 1-2-3 version 5.01 (Lotus Development Corporation 1991), while R version 3.2.2 (R Development Core Team 2015) was used for the other statistical analyses. We used the glm function in R in the statistical modelling where we for linear models assumed normal errors and used the identity link function. We started with models containing all factors and their interactions, and simplified them as much as possible to find the minimal adequate model by using a backward selection procedure with likelihood ratio tests (Crawley 2013).

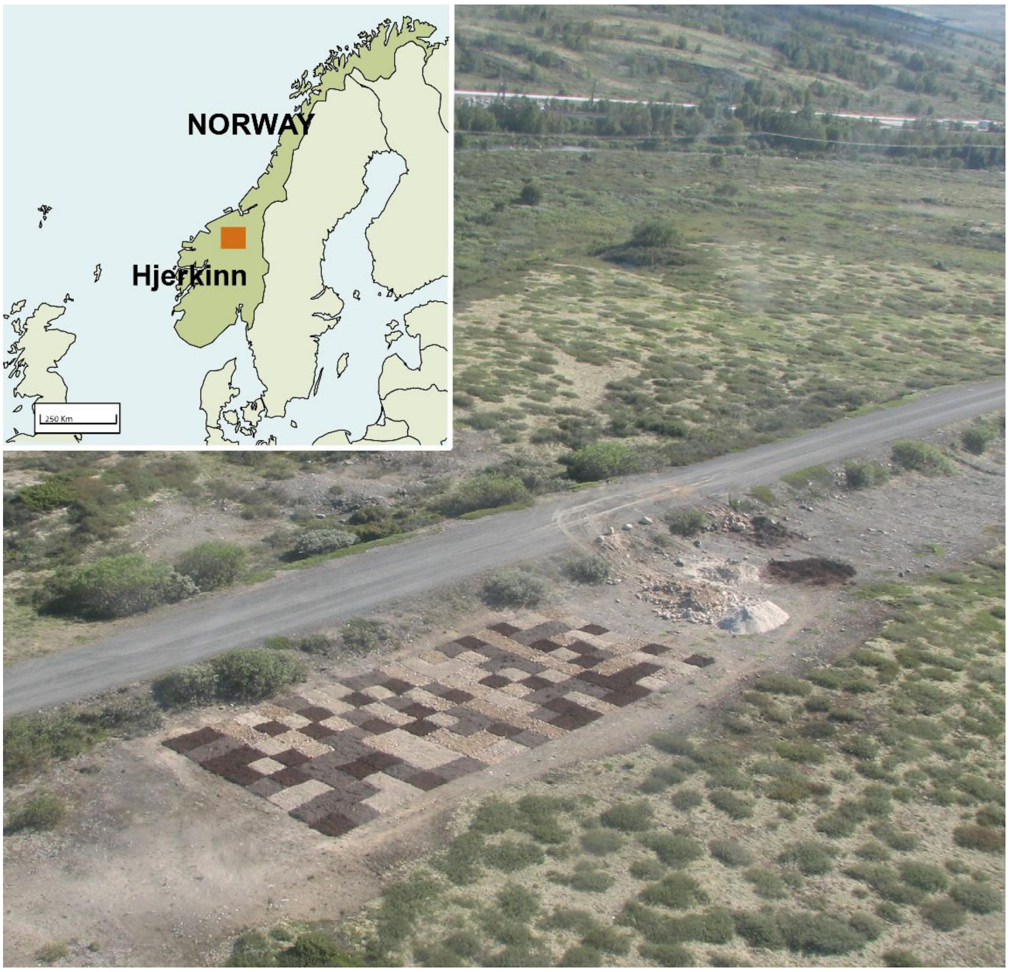

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Figure 1. Aerial view of the experimental study site at Hjerkinn, Dovrefjell, showing the 160 randomized squares (8 × 20), each measuring 1.5 × 1.5 m, with four different soil types and four different seeding treatments. Photo: Dagmar Hagen.

Results

In the field experiment, vegetation cover established fastest when *F. ovina* was sown in monoculture followed by the seed mixture treatment. According to the minimal adequate model, where the soil type consisted of two levels, organic soil and mineral soil, and where all interaction terms have been removed, the establishment was faster in organic soils than mineral soils for all seeding treatments (Fig. 2; first question, see Appendix S1-2). The species differed significantly in cover but followed the same pattern (Fig. 2.), i.e. the interaction soil type and seeding treatment was non-significant (Appendix S1). Single-species *F. ovina* had the highest mean cover, 57%, followed by the mixture and the single-species *Poa alpina*, with 41% and 33% cover respectively. The mean cover of single-species *Luzula multiflora* was only 17%.

The three species differed significantly in cover at the end of the experiment when seeded together in the mixture (Fig. 3; second question Appendix S3). *F. ovina* reached a mean cover of 31%, whereas the other two species achieved a mean cover of only 8% (*P. alpina)* and 2% (*L. multiflora)*. The soil types influenced the better performance of *F. ovina* when compared to *P. alpina*; in the peat soil the difference in cover between these two species was significantly higher compared to the three other soil types (Fig. 3). The variation in soil types played a more important role for the cover differences between *P. alpina* and *L. multiflora. P. alpina* generally performed significantly better than *L. multiflora* except in the peat soil, and the largest difference between these species was in the organic topsoil (Fig. 3). *L. multiflora* performed significantly poorer in the organic topsoil in the mixture than when seeded alone (third question, Appendix S4). *F. ovina*

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showed a tendency, although not significant, to perform relatively better when seeded in the mixture in the organic soils (OP and OT). For *P. alpina* there were no significant differences between seeding alone and in the mixture (Appendix S4).

In the greenhouse experiment, *L. multiflora* had not germinated after nine days while the mean emergence rates of *F. ovina* and *P. alpina* were 39% and 72% respectively (Fig. 4). At the end of the experiment, after 59 days, the only significant difference ($P < 0.001$; appendix S5) in emergence rate was between *F. ovina* (mean 47%) and the other three treatments, which achieved mean rates of 79% (mixture), 83% (*L. multiflora*), and 89% (*P. alpina*).

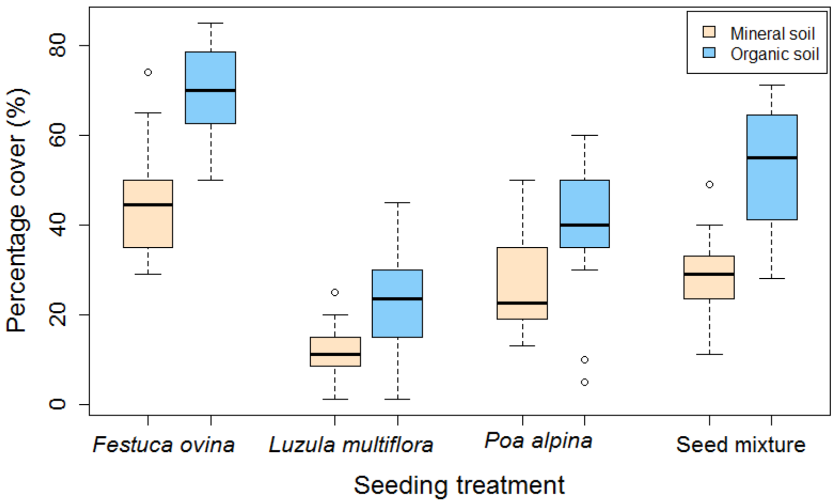

Figure 2. Differences in cover of seeded species among seeding treatments (*Festuca ovina*, *Luzula multiflora* ssp. *frigida*, *Poa alpina*, and a mixture of all three species) and soil types at the end of the experiment, in 2014, for the minimal adequate model. In this model, the two mineral soil types, mineral coarse and mineral fine, and the two organic soil types, peat soil and organic topsoil, were lumped together. The horizontal lines show the median whereas the boxes represent the inter-quartile range, whiskers extend to maximum and minimum values unless there are outliers (circles), i.e. data points that are 1.5 times the interquartile range.

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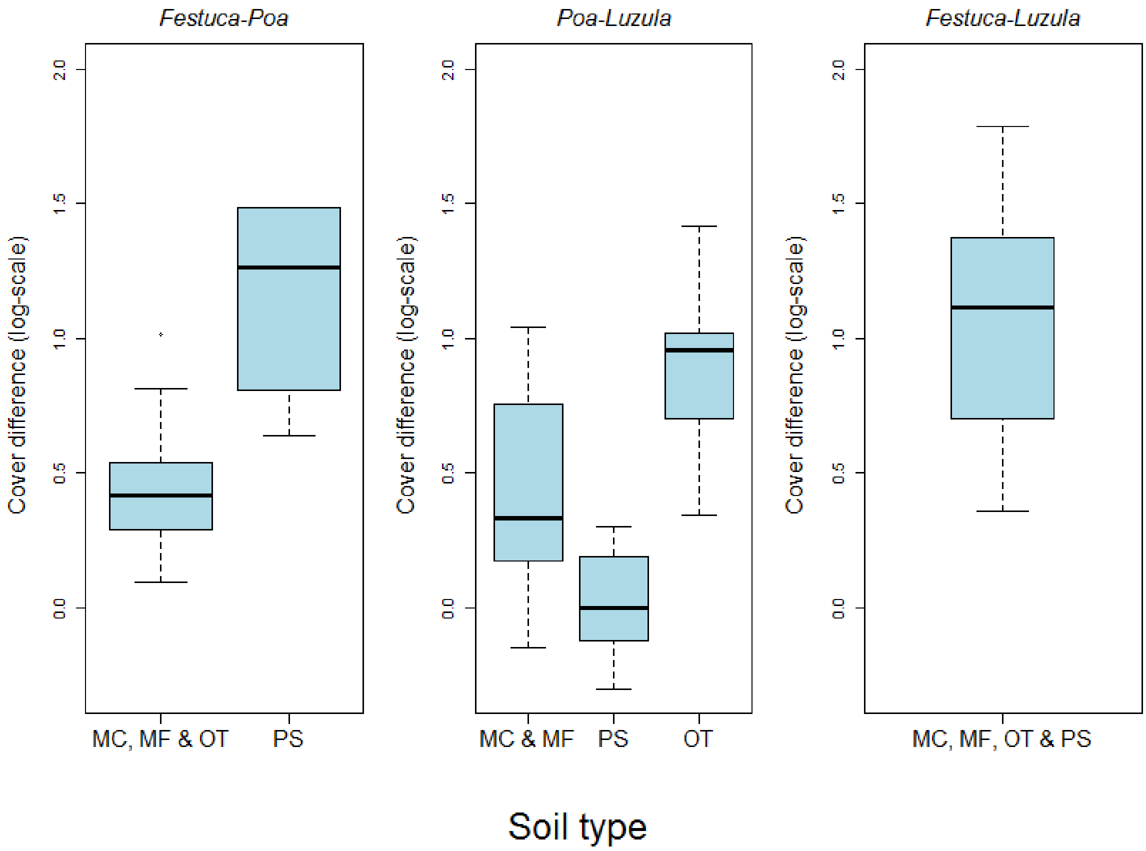

Figure 3. Differences in log-transformed cover values between pair of species (*Festuca ovina*, *Luzula multiflora* ssp. *frigida*, *Poa alpina*) in the mixture seeding treatment in the four different soil types at the end of the experiment, in 2014, for the three minimal adequate models (lumping together non-significant soil types). MC = mineral coarse, MF = mineral fine, PS = Peat soil, and OT = Organic topsoil. The horizontal lines show the median whereas the boxes represent the inter-quartile range, whiskers extend to maximum and minimum values unless there are outliers (circles), i.e. data points that are 1.5 times the interquartile range.

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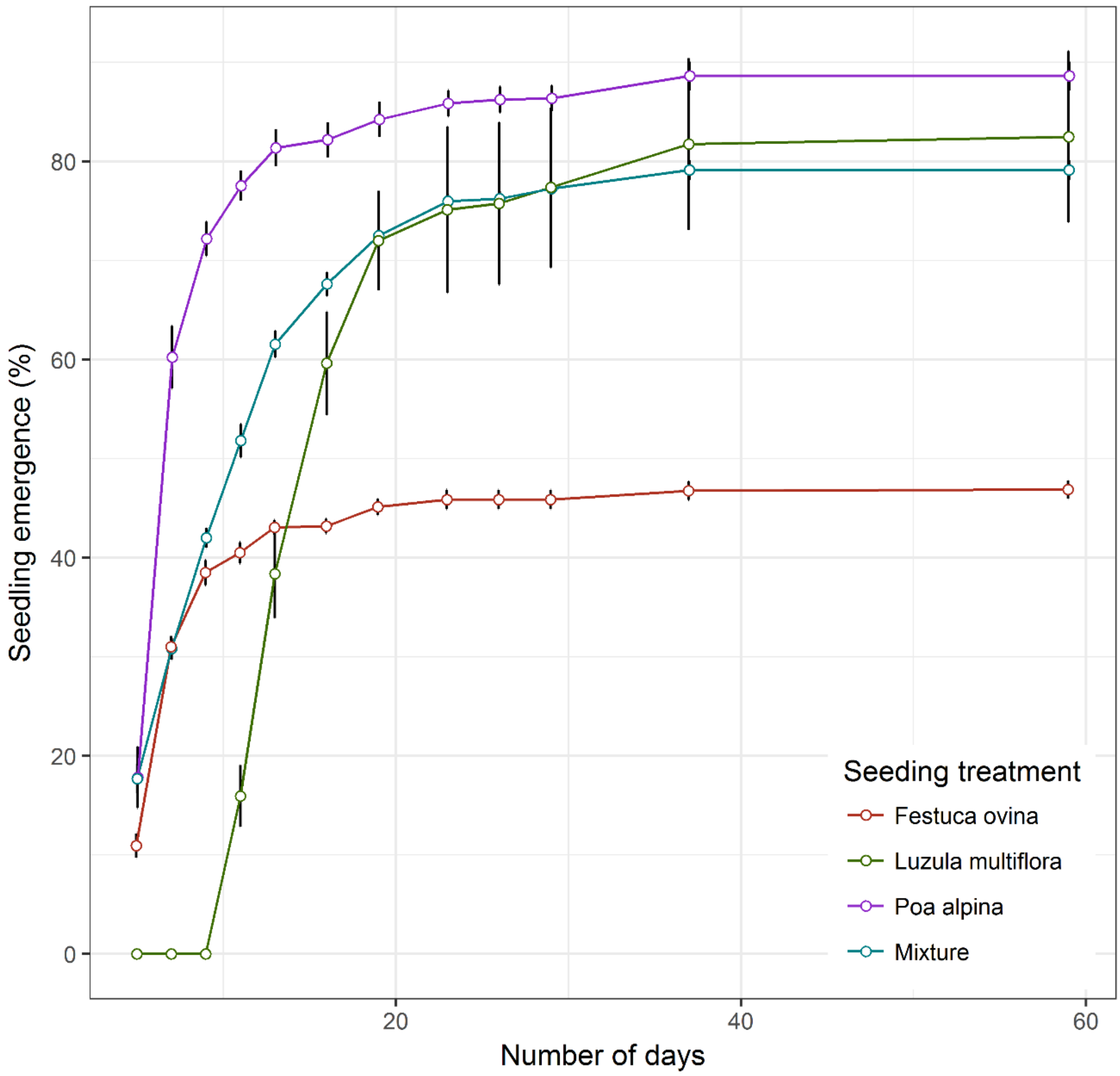

Figure 4. Emergence rate (%) of *Festuca ovina*, *Luzula multiflora* ssp. *frigida*, *Poa alpina* and the species mixture over the course of the greenhouse experiment. Error bars show the standard errors.

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Discussion

The field experiment showed that one of the monoculture seeding treatments established vegetation cover more quickly than the seed mixture. The best performing species, *F. ovina,* performed even slightly better in the mixture than in the monoculture for the organic soil but not so much that it outweighed the poorer performance of the two other species, in particular the poor performance of *L. multiflora*. Soil conditions at the field site also affected the cover; all species had higher cover in organic soils than in mineral soil, and the differences between performance in the mixture and single seeding between *F. ovina* and *L. multiflora* were largest in the organic soil types.

In field sowing experiments, seed germination and seedling establishment can be of considerable importance for the outcome (Ross & Harper 1972; Miller 1987), with long-lasting effects on community structure (Vaughn & Young 2015). In our experiment, the poor establishment of *L. multiflora* when seeded alone was evident after only one year, indicating either low germination rate or poor seedling establishment (high mortality). In the greenhouse experiment, *L. multiflora* seedlings emerged later than the two other species. The slow emergence of *L. multiflora* may have contributed to its poor performance in the field experiment, by reducing its establishment success during too short growing season at our alpine field site. Other studies have shown that even small differences in germination date can translate into large differences in performance between species (Miller 1987; Bergelson 1996; von Gillhaussen et al. 2014). However, caution is needed in using greenhouse results to interpret outcomes under field conditions (see Verdú & Traveset 2005) due to different soil and climatic conditions. Furthermore, *Luzula multiflora* prefers a slightly moister soil (Lid & Lid 2005) than the conditions at the experimental site, which might also hamper its emergence and establishment. Another explanation for the low establishment of *L. multiflora* is its low seed mass, as seedling establishment is often positively associated with seed mass (Weiher et al. 1999; Moles & Westoby 2006). The overall emergence rate may contribute to differences in establishment success between species seeded singly. This was, however, not supported by our

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greenhouse experiment, where *F. ovina* had the lowest overall emergence rate of the three species. We observed more tillering by *F. ovina* than the other two species, which therefore expanded quite quickly despite the low emergence rate. In the field experiment, however, we recorded the cover of the species and not the number of established seedlings. Of the three species, *F. ovina* prefers the driest soil (Lid & Lid), and has xeropylly and an early shoot phenology which gives success in dry habitats (Grime et al. 2007).

Soil conditions clearly affect performance, and the lower cover on mineral soils than on organic soils was as expected. Vegetation development on soils with low organic matter content is usually slow (Jorgenson & Joyce 1994; Kidd et al. 2006; Rydgren et al. 2013; Rydgren et al. 2016), because poor water- and nutrient-holding capacity results in low availability of macro nutrients like N and P (Reid & Naeth 2005). In the harsh environment at the field site, density-independent processes may be relatively more important in the first year in single-species treatments, and throughout the experiment in plots with low percentage cover. There was probably marked density-independent seedling mortality because this life stage is particularly vulnerable and often experiences high mortality in alpine environments (Evju et al. 2010; Erschbamer & Mayer 2011; Marcante et al. 2012). The stronger species × soil interaction in the mixture treatment than in the single-species treatment could be explained by a relatively greater negative effect of interspecific competition on *L. multiflora* under more favourable conditions in organic soils than in the nutrient-poor mineral soils. This fits with the results of a greenhouse experiment by Hagen et al. (2014), who found that competition between seeded alpine species is stronger in “favourable” organic soils. In the first year, *L. multiflora* seedlings in the mixture treatment may already have experienced strong competition from the faster germinating, larger seeded *F. ovina* and *P. alpina* (Black 1958; Leishman 1999). We expect that direct competition—for soil nutrients, water and space—increased in intensity over time as *F. ovina* and *P. alpina* cover increased (their cover totalled 51% in the organic soils and 27% in the mineral soils in 2014). In the last two years, emergence of the remaining *L. multiflora* seeds may also have been hampered by indirect competition (cf. Donath & Eckstein 2010;

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Loydi et al. 2013) mediated through the litter layer accumulating in the organic soil plots (see Appendix S2). It is well known that litter can act as a mechanical barrier to seedling emergence, change physical conditions, or release allelochemicals (Xiong & Nilsson 1999; Loydi et al. 2013 and references therein).

In contrast to *L. multiflora, F. ovina* tended to perform relatively better in the mixture than seeded alone in the organic soils. Although the underlying mechanism of this pattern is hard to deduce from our experiments, a likely explanation is asymmetrical and reduced competition in mixture. Consequently, the competition experienced by *F. ovina* is reduced in mixture compared to monoculture because intraspecific competition is more intense than interspecific competition.

The three species performed very differently in the cold, dry environment at Dovrefjell. Some authors have suggested that interspecific interactions may be consistent across scales (Peltzer & Wilson 2001), whereas others have suggested that competitive ability varies with the environment (Tilman 1988), and even from year to year (Knappová et al. 2013). Clearly, the outcome of field experiments may depend on regional-scale variables (climate), fine-scale variables (like physical and chemical properties of the soil), as well as the seeding rate, the species selected and their competitive abilities under the given conditions (Schippers et al. 1999). Knowledge of such factors should be taken into consideration when seeding is used in restoration projects.

In ecological restoration, seeding is often used to enhance establishment of vegetation cover and stabilize soils (Aradottir & Hagen 2013). When the goal is to establish a vegetation cover as fast as possible, the species or combination of species that perform best should be used. In extreme habitats, like alpine sites, it is reasonable to assume that a well designed mixture will be a better choice (due to interspecific facilitation) than single seeded species but as we show it is not always so. The choices of what seeds to use should therefore be based on knowledge of how species perform singly or together under the given field conditions. Our study contributes to this knowledge base. However, in many instances the seeded species are intended to facilitate the establishment of local

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species, often concomitant with reduction in their own cover as shown by Gretarsdottir et al. (2004). Nevertheless, if the seeded species are strong competitors, they may persist and even dominate the vegetation (Kearns et al. 2015; Rydgren et al. 2016).Thus, the purpose for using seed mixtures in ecological restoration must be clear; sometimes weak competitors are wanted whereas strong competitors are preferred in other cases. There is considerable inappropriate use of seed mixtures in restoration projects (Fagan et al. 2008; Bochet & García-Fayos 2015). This includes: using seed mixtures at sites where seeding is unnecessary and natural recovery (passive restoration) is more appropriate; using unsuitable seed mixtures so that species interactions alter the outcome or increase the expense; using unsuitable seed mixtures containing species that are not well adapted to environmental conditions at the site in question; and using introduced seeds of species that are native to the area, causing genetic risk (Falk et al. 2006; Byrne et al. 2011). Knowledge can be gained by closer collaboration between practitioners and researchers and by using restoration projects as field experiments (see also McKay et al. 2005). This will give us better insight into how to design appropriate seed mixtures for specific given field conditions to achieve restoration goals when seeding is necessary.

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Supporting Information

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

Appendix S1. Statistical analysis of the field experiment: model parameter estimates of the analysis of seeding treatment consisting of four levels (single species and mixture seeding).

Appendix S2. Selected photos of the plots at the field experiment.

Appendix S3. Statistical analysis of the field experiment: model parameter estimates of the analysis of the mixture seeding.

Appendix S4. Statistical analysis of the field experiment: summary of the simulation analysis of differences in percentage cover of each species when seeded alone and in the mixture with the other two species for the four soil types.

Appendix S5. Statistical analysis of the greenhouse experiment: model parameter estimates of the analysis of seeding treatment consisting of four levels (single species and mixture seeding).

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