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3   **Positive responses of coastal dune plants to soil conditioning by the invasive**

4   ***Lupinus nootkatensis***

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16   **Highlights**

17   Soil conditioned by an invasive alien lupin facilitated seedling growth of native dune species.

18   Soil-P mobilization by lupins was an important driver for plant responses.

19   Large growth differences among species were not explained by specific plant strategies.

20   No negative soil legacies were found.

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22

23   **Abstract**

24   Invasive nitrogen-fixing plants drive vegetation dynamics and may cause irreversible changes

25   in nutrient-limited ecosystems through increased soil resources. We studied how soil

26   conditioning by the invasive alien *Lupinus nootkatensis* affected the seedling growth of co-

27 occurring native plant species in coastal dunes, and whether responses to lupin-conditioned

28 soil could be explained by fertilisation effects interacting with specific ecological strategies of

29 the native dune species. Seedling performance of dune species was compared in a greenhouse

30 experiment using field-collected soil from within or outside coastal lupin stands. In associated

31 experiments, we quantified the response to nutrient supply of each species and tested how

32 addition of specific nutrients affected growth of the native grass *Festuca arundinacea* in

33 control and lupin-conditioned soil. We found that lupin-conditioned soil increased seedling

34 biomass in 30 out of 32 native species; the conditioned soil also had a positive effect on

35 seedling biomass of the invasive lupin itself. Increased phosphorus mobilisation by lupins was

36 the major factor driving these positive seedling responses, based both on growth responses to

37 addition of specific elements and analyses of plant available soil nutrients. There were large

38 differences in growth responses to lupin-conditioned soil among species, but they were

39 unrelated to selected autecological indicators or plant strategies. We conclude that *Lupinus*

40 *nootkatensis* removes the phosphorus limitation for growth of native plants in coastal dunes,

41 and that it increases cycling of other nutrients, promoting the growth of its own seedlings and

42 a wide range of dune species. Finally, our study indicates that there are no negative soil

43 legacies that prevent re-establishment of native plant species after removal of lupins.

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45 **Keywords**: dune restoration, invasive legume, lupin, nitrogen fixation, phosphorus

46 mobilization, soil legacy

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50 **1. Introduction**

51 Invasive alien plant species have the potential to alter nutrient dynamics in soils, affecting

52 both pool size and fluxes (Ehrenfeld 2003, 2010; Castro-Díez et al. 2014). These changes

have direct effects on plant-available nutrients (e.g. Rice et al. 2004), and on the size and

composition of soil microbial communities (Wang et al. 2012). Such changes can in turn

affect vegetation dynamics through plant–soil interactions (van der Heijden et al. 2008; Bever

et al. 2010; van der Putten et al. 2013), and feedbacks between vegetation and nutrient cycling

are frequent and well-documented (Ehrenfeld et al. 2005). Symbiotic nitrogen-fixing species,

in particular, lead to marked changes in the composition and function of soil biota and have

considerable impacts on nitrogen pools and fluxes (Ehrenfeld 2003; Liao et al. 2008; Corbin

& D'Antonio 2012; Hiltbrunner et al. 2014). As nitrogen availability has a strong impact on

plant population dynamics and vegetation succession, symbiotic nitrogen fixers may

transform nutrient-poor ecosystems by elevating plant-available nitrogen and increasing

nitrogen cycling in litter and soils (Rice et al. 2004; Harrison & Bardgett 2010).

Nutrient acquisition traits of *Lupinus* species involves both symbiotic nitrogen fixation

and a high ability to mobilise soil phosphorus and other bound elements using carboxylate

exudation from roots (Lambers et al. 2013). These traits increase the cycling of nutrients

through root exudates, leaf and root turnover, and explain how lupins contribute as ecosystem

engineers and facilitators of soil development in nutrient-poor systems (e.g. Lambers et al.

2012). Establishment of nitrogen-fixing species on nutrient-poor soils also modifies other soil

resources and environmental conditions such as soil structure and organic matter content

(Corbin & D'Antonio 2012). The altered soil conditions continue to be effective after removal

of the nitrogen-fixing species, leaving a set of marked soil legacies (Corbin & D'Antonio

2012). These legacies may persist for decades after removal of the invasive nitrogen fixers

(Maron & Jeffries 2001). However, in some cases these legacies are of short duration (e.g.

Malcolm et al. 2008; Grove et al. 2015), and show spatio-temporal patterns affected by

climate and other abiotic or biotic factors (Yelenik & Levine 2011; Konlechner et al. 2015).

Restoration of ecosystems invaded by nitrogen-fixing species may be difficult when

soil legacies of the alien species exist (Corbin & D'Antonio 2004). The changes in soil

79 resources and conditions may promote establishment of more competitive species, shift

80 dominance of co-occurring native species, favour other invasive species, and promote re-

81 establishment of the invasive species itself (e.g. Maron & Connors 1996; Maron & Jefferies

82 2001; Goergen & Chambers 2012). In addition, the invasive species may drive changes in soil

83 biota with accumulation of harmful or beneficial organisms causing interspecific plant–soil

84 feedbacks that affect vegetation dynamics (van der Putten et al. 2013). Management of soil

85 carbon using addition of activated carbon or labile sugars offers opportunities for control of

86 invasive species and restoration of ecosystems through effects on plant–microbe

87 communication and availability of N, P and organic compounds (e.g. Kulmatiski 2011;

88 Mitchell & Baker 2011; Nolan et al. 2015). Although the long-term effects may be difficult to

89 predict (Török et al. 2014), the approach has a potential to reduce the impact of soil legacies

90 after removal of invasive species.

91 Species-specific responses to soil conditions are central for restoration outcomes in

92 coastal dunes after removal of invasive lupins (Konlechner et al. 2015), as found in

93 restoration of other degraded ecosystems (e.g. Eschen et al. 2006, Abella et al. 2015). To

94 better understand the impact of removing invasive nitrogen-fixing species on restoration

95 dynamics, two questions are central: 1) Does the impact involve simple fertilisation effects, or

96 also more complicated species-specific plant–soil feedbacks, and 2) to what extent can

97 differences in responses among native species be explained by autecological indicators or

98 plant strategies. In the present study, we addressed these questions by testing how soil

99 conditioned by the invasive alien *Lupinus nootkatensis* affects the seedling growth of a set of

100 co-occurring coastal dune species in SW Norway. We predicted that lupin-conditioned soil

101 has a positive effect on seedling biomass of native species, and that the response to lupin-

102 conditioned soil is positively correlated with the response to nutrient availability.

103

2. Materials and methods

2.1. Study system

To test the response of sand dune plants to lupin-conditioned soil, we cultivated seedlings of 33 species in soil samples collected either within or outside patchy stands of *Lupinus nootkatensis* in extensive coastal sand dunes near Sele in SW Norway (58°49'31"N, 5°32'51"E), part of the Jæren protected landscape area. *Lupinus perennis*, another nitrogen-fixing species also introduced to bind sand along railways in the region about 100 years ago, was not present in the study area. The coastal climate of the area has average temperatures ranging from 0.6 °C in February to 14.4 °C in August (1961–1990). Monthly precipitation ranges from 50 mm in April to 156 mm in September, giving a total of 1180 mm per year. The natural vegetation is a relatively stable dune grassland with a diverse community of the grasses *Ammophila arenaria* and *Festuca rubra*, the legumes *Anthyllis vulneraria* and *Lotus corniculatus*, and other species such as *Campanula rotundifolia*, *Carex arenaria*, *Galium verum, Hieracium pilosella, Pimpinella saxifraga, Plantago lanceolata* and *Polygala vulgaris*. Locations dominated by lupins had a higher frequency of *Cirsium arvense, Draba incana*, *Hieracium umbellatum*, *Rumex acetosa* and grasses compared to locations without these invasive alien plants (H.M. Hanslin, unpubl. data). Nomenclature follows Lid & Lid (1994).

The lupins had developed a dense vegetation cover outcompeting the original dune vegetation. We do not know the precise age of the lupin stands studied, but they had most likely developed over decades. Management by repeated cutting is efficient in controlling lupins, but old stands are still dominated by nitrophilous species such as *Anthriscus sylvestris* and *Poa pratensis*. Thus, after local control of the lupins some soil legacies may persist, thus, preventing or delaying restoration of the original dune vegetation.

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2.2. Soil sampling

Soil was collected along three transects within a 30 m x 70 m area of patchy lupin stands.
Along each transect, 40 soil samples were collected within dense monospecific lupin stands
and 40 samples at least 5 m away from the nearest lupin plant in random locations with other
herbaceous vegetation. The three sampling transects were randomly selected from a set of
eight pre-marked transects. Soil samples of 10 cm x 10 cm were collected from 1–15 cm
depth and individually bagged. These samples were sieved through a 1-cm mesh to remove
large roots and large invertebrates, and used to fill 11 x 10 x 10 cm^3 pots.

A sampling distance of at least 5 m from the nearest lupin plant was chosen to
minimize the potential lupin impact on the control soil. Since the location had not previously
been managed and the turnover rate of lupin stands is low, any lupin effect on control soil was
unlikely. The sampled control soil was conditioned by several herbaceous species. To reduce
the variation among control soil samples, some standardisation in collection of control soil
was done by targeting mixed vegetation dominated by the grasses *Ammophila arenaria* and
Festuca rubra, and avoiding large stands of native legumes, the dwarf shrub *Empetrum*
nigrum or the fern *Polypodium vulgare*.

2.3. Test species

Seeds of a range of monocot and dicot species were collected in the year 2013 in the study
area (8–10 seed families per species), including all major species of coastal dunes and
different plant functional types (Table 1). The dunes were covered by a mosaic of vegetation
types. In the study region, only the test species *Ammophila arenaria*, *Carex arenaria*,
Corynephorus canescens, *Lathyrus japonicus* and *Leymus arenaria* can be considered as dune
specialists. The other species occur in a wider range of mesic to dry habitats, from heathlands
to grasslands. All species were perennials, except the annual *Trifolium arvense* and the

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biennial *Jasione montana*. Information on the ecological niche of the species and potential mycorrhizal associations was taken from Fitter and Peat (1994) and Hempel et al. (2013). In total, 25 seeds per species were dried at 60 °C for 48 h and weighed to get an estimate of seed size.

The seeds were surface-sterilised in 3% NaOCl solution for 5 min., washed, stratified for 6 weeks in darkness at 2 °C on moist filter paper, germinated in darkness or under low light (PAR 150 µmol m^{-2} s^{-1}, 20/15 °C day-night temperatures), and transplanted as young seedlings to pots with the field-collected soil. A few species without known stratification requirements were sown directly into the pots. Three pots were used for each soil type per species, each pot representing a unique soil sample, giving a total of 198 pots (2 soil types x 33 species x 3 replicates).

After 2 weeks, seedlings were thinned to the same number per pot within species, i.e. 2–6 seedlings per pot, depending on seedling size and expected growth rate. Germination was variable and slow in some species, so care was taken to compare even-aged seedlings within species. Recruitments from the seed bank, mainly *Draba incana, Epilobium* spp. and *Sedum acre*, were removed on a regular basis.

2.4. Experimental design

Seedlings were cultivated under controlled conditions in a greenhouse. The experiment started in early May 2014 and harvested from early July to early August 2014 depending on germination, growth rate and development of individual species. Harvest time was differentiated among species to get a better balance of biomass produced per volume of soil available. Pots were placed in individual trays on a table in a greenhouse compartment and given overhead watering with tap water (pH 7.2, electrical conductivity 0.2 mS cm^{-1}) using a hose sprinkler nozzle, mostly daily or twice daily in warm and sunny periods. Air temperature was kept below 25 °C by venting.

180   At harvest, roots were carefully washed free of substrate. The largest individual plant

181   per pot was dried at 60 °C for 48 h and biomass dry weight was determined for roots and

182   shoots separately. Total biomass and the root fraction of total biomass were estimated.

183   Flowering occurred only in a few individuals of *Galium boreale*, *Linaria vulgaris* and *Viola*

184   *tricolor* growing in lupin-conditioned soil, and thus this was not further analysed.

185

186   *2.5. Nutrient series*

187   To establish an index of nutrient response for each species, a separate experiment with a

188   fertiliser series was established in pure sand. Three levels of a diluted balanced liquid fertiliser

189   (7-1-6% N-P-K with micronutrients), split into three doses applied at 1, 3 and 6 weeks, gave a

190   total of 4, 8 and 12 mg nitrogen (N) per pot. Otherwise, the pots were established, maintained

191   and harvested as described for plants in field-collected soil for a total of 396 pots (33 species

192   x 3 levels x 4 replicates). Plants in this experiment were harvested at the same age as plants of

193   the same species growing in field-collected soils.

194

195   *2.6. Response to specific nutrients*

196   A separate experiment was run to identify the nutrients causing the responses to lupin-

197   conditioned soil and especially to distinguish between potential effects lupins have on N and

198   P availability. Plant biomass in pots receiving N, PK, micronutrients or all combined

199   (N+PK+micronutrients) was compared to biomass in unfertilized pots for both lupin-

200   conditioned and control soil using *Festuca arundinacea* as a test plant. Activated carbon and

201   labile carbon were also added to additional pots to test their net effects on plant growth. Pots

202   were randomly allocated to treatments and placed in individual trays on a greenhouse table,

203   and care was taken (as in the other experiment) to prevent soil contamination among pots.

204   Activated carbon (10 g kg^{-1} dry soil) was mixed with soil before it was placed in pots,

205   while labile carbon was added as 10 ml sucrose solution (100 mg kg^{-1} dry soil). These

206   amounts are comparable to Kulmatiski (2011) for activated carbon and a lower than amounts

207   used for labile carbon (e.g. Török et al. 2014). For fertiliser treatments, solutions were given

208   as three biweekly 10 ml doses with a total of 5 mg N, 3 mg P or 3.8 mg K per pot.

209   Micronutrients were applied as 10 ml per pot of a 0.0005% dilution of a solution with DTPA

210   and EDTA-chelated iron (Fe), manganese (Mn), zinc (Zn), boron (B), copper (Cu) and

211   molybdenum (Mo) (Pioner Mikro with Fe; Brøste, Lyngby, Denmark). Surface-sterilised

212   seeds of *Festuca arundinacea* were sown and seedlings thinned to one individual per pot

213   giving a total of 112 pots (2 soils x 7 treatments x 8 replicates). Shoots were harvested after 8

214   weeks and biomass determined after drying at 60 °C for 48 h. In this experiment roots were

215   not harvested as they were difficult to separate from the activated carbon.

216   Nutrient levels in these dune soils were below detection levels for many elements in

217   standard soil analyses. Therefore, plant root simulator probes (PRS Probes; Western Ag

218   Innovations, Saskatoon, Canada) were inserted vertically in two pots each of the original

219   control and lupin-conditioned pots for 7 weeks and analysed for accumulation of nutrients on

220   a resin-based ion exchange membrane. Such probes were also installed in one pot of each of

221   the sucrose-treated lupine-conditioned and control soil.

222

223   *2.7. Data analyses*

224   The responses to lupin-conditioned soil were tested using the mixed effect ANOVA model:

225    $y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + T_k + \varepsilon_{ijk},$

226   with species (α, $i =33$), soil type (β, $j = 2$) and transect (T, $k = 3$). Species and transect were

227   considered random factors and soil type as fixed factor. Diagnostics of the ANOVA models

228   were made using QQ plots of residuals and plots of residuals against predicted values. Total

229   biomass was ln-transformed. Root fractions were mainly in the range 20–70% and inspection

230   of residual plots indicated that there was no need for transformation. Effect sizes of the soil

231   responses were estimated as Cohen's d with 95% CI for each species (Cohen 1988). The

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232   response to fertilisation was estimated for each species as the slope of the linear regression of

233   ln total biomass on nutrient level.

234   To test whether the response to lupin-conditioned soil could be explained by soil

235   nutrient availability, a regression was performed between Cohen's d and the slope of the

236   nutrient response; similarly, we tested for a relationship with seed biomass. CSR strategies

237   (Grime 1977) were extracted from literature data for all species and coded as X, Y, Z

238   coordinates according to Hunt et al. (2004) and summarized in Appendix A. The relationship

239   between CSR strategy and Cohen's effect size was analysed in a mixture design model with

240   main, two- and three-way interactions of C, S, and R using the DOE function in Minitab 17

241   (Minitab Ltd., Coventry, UK). Effect sizes were ln transformed before analyses to increase

242   normality and lower heteroscedasticity of the residuals. Effect sizes were also plotted against

243   biomass in control soil as a potential indicator of disproportionate relationships between

244   biomass and soil resources across species, but revealed no relationship between production

245   per pot and effect size.

246   Ln-transformed biomass data from the experiment on specific nutrients were analysed

247   as a completely randomised design using the ANOVA model:

248   $y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij},$

249   with soil type (α, $i = 2$, lupin-conditioned vs control) and fertiliser treatment ($\beta, j = 7$) as fixed

250   factors.

251

252   **3. Results**

253   *3.1. Responses to lupin-conditioned soil*

254   We found positive responses to lupin-conditioned soil in a large majority of the species (Fig.

255   1; mean Cohen's d = 2.68 ± 2.08 SD, 77.7 CV), but an interaction between soil type and

256   species (Table 2) indicated diverging responses among the 33 species tested. Two species,

257   *Linaria vulgaris* and *Trifolium arvense*, showed no (or a slightly negative) response to lupin-

258   conditioned soil, while moderate to strong positive responses were found in the remaining 31

259   species. *Achillea millefolium*, *Galium boreale*, *Hieracium pilosella* and *Jasione montana* were

260   the most responsive species. Lupin-conditioned soil also had a moderate positive effect on

261   *Lupinus nootkatensis* itself (Fig. 1).

262

263   *3.2. Effects of plant strategies and nutrient responses*

264   Comparing the major groups of vegetation in the dune system (graminoids, legumes and other

265   dicotyledon species), we found no difference in response to lupin-conditioned soil between

266   these groups (Fig. 2). Moreover, a comparison of responses across groups based on their

267   habitat preferences along gradients of soil humidity and nitrogen indicated no differences

268   (Fig. 2). These responses were unrelated to mycorrhizal status of the species based on

269   database information. Furthermore, no relationship was found between estimated effect size

270   of the response to lupin-conditioned soil and the slope of the nutrient response or seed size

271   (Fig. 3). In line with these findings, we found no clear relationships between CSR strategies

272   and the response to lupin-conditioned soil estimated as the Cohen's effect size, as there were

273   no significant linear or quadratic terms in the model and the model explained only 26% of the

274   variation in the dataset (Table 3).

275

276   *3.3. Effects on root allocation patterns*

277   Effects of soil conditioning on the allocation of biomass to roots varied among species (Table

278   2), with species-specific increased or reduced allocation to roots in lupin-conditioned soils.

279   Species such as *Carex arenaria*, *Festuca arundinacea*, *Galium verum*, *Linaria vulgaris,*

280   *Trifolium arvense* and *Viola tricolor* allocated less to roots in lupin-conditioned soil, while

281   *Jasione montana*, *Leymus arenaria* and *Plantago lanceolata* showed the opposite pattern. The

282   root fraction was independent of total plant biomass in field-collected soils. There was no

283   consistent relationship between the changes in root fraction in response to more nutrients in

284　the fertilisation series and the changes in root fraction in response to lupin-conditioned soil

285　(not shown).

286

287　*3.4. Response to addition of specific nutrients*

288　In the experiment with addition of different nutrients, the lupin-conditioned soil gave much

289　stronger growth of *Festuca arundinacea* than the control soil (Fig. 4). The effects of

290　fertilisation treatments differed between soil types (soil by treatment interaction $F_{6,98} = 18.5$, p

291　< 0.001, $R^2_{adj} = 0.89$), with no response to fertilisation in lupin-conditioned soil ($F_{6,49} = 2.1$, p

292　$= 0.07$, $R^2_{adj} = 0.11$). In contrast, PK fertilisation resulted in a strong growth response in

293　control soil ($F_{6,49} = 28.2$, $p < 0.001$, $R^2_{adj} = 0.75$), indicating that plants were not N-limited,

294　but limited by P or K. The effects of additional nitrogen and micronutrients on growth were

295　marginal. Addition of activated carbon increased plant biomass in control soil only, while

296　labile carbon did not influence growth in any of the soils. Analyses of plant-available

297　nutrients showed considerably higher availability of nitrate, P, Fe and Mn in lupin-

298　conditioned soil, and a tendency of higher Ca and S in control soil (Table 4). Labile carbon

299　also reduced soil nitrate by 25–30% in both lupin-conditioned and control soil, not affecting

300　the other measured nutrients.

301

302　**4. Discussion**

303　*4.1. Positive effects of lupin-conditioned soil*

304　The prediction that lupin-conditioned soil improves seedling growth was supported by our

305　findings of a positive effect on seedling performance in 30 out of 32 native species.

306　Combined, the results of our experiments support the conclusion that increased nutrient

307　availability and especially P mobilisation are strong drivers of seedling responses to lupin-

308　conditioning of sandy dune soil. Nutrient availability did, however, not explain all responses,

309　as the lack of relationship between Cohen's d estimates and the slope of the response to

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fertilization. Physio-chemical soil characteristics such as nutrient availability, soil organic

matter, soil aggregation etc. are improved by lupins and other nitrogen-fixing species on

infertile soils (Corbin & D'Antonio 2012). The changes in abiotic conditions caused by

nitrogen-fixers are followed by major changes in soil biota (Marchante et al. 2008). These

biotic changes can have an impact through effects on e.g. nutrient cycling, but also through

interspecific plant–soil feedbacks (van der Putten et al. 2013).

4.2. Plant responses to facilitation of abiotic conditions

The fertilization experiment with *Festuca arundinacea* showed that PK, and not N, limited

plant growth on control dune soil. Lupin-conditioned soil had more available P, nitrate, Fe

and Mn which promoted plant growth. Lupins have a high ability to mobilise soil phosphorus

using carboxylate exudation from specialist cluster roots and unspecialised roots (Lambers et

al. 2013). *Lupinus nootkatensis* does not develop cluster (-like) roots under low phosphorus

availability (H.M. Hanslin, unpubl. observ.), but our analysis showed a five-fold elevated

plant-available P in lupin-conditioned bulk soil. This indicates a strong ability to mobilise

phosphorus in this species. These results are in line with other studies reporting elevated

phosphorus in soil under lupins (Myrold & Huss-Danell 2003; Óskarsson & Sigurgeirsson

2004; Halvorson & Smith 2009). An on-going study has shown that lupin plants at the study

site have on average a $\delta^{15}N$ value of –0.83‰, i.e. considerably higher than in soil (–2.7‰) and

companion plants (–3.6‰; H.M. Hanslin, unpubl. data). Hence, nitrogen fixation makes a

significant contribution to the lupin nitrogen budget and cycling under the study conditions. N

was, however, not limiting for *Festuca arundinacea* growth in control or lupin-conditioned

soil. This is most likely due to deposition of N from long-range transport and local agriculture

at the study site. If the response of *Festuca arundinacea* is representative of the other tested

species, we infer that the interaction between P mobilization and N availability is an important

driver of interspecific effects of lupin-conditioned soil.

4.3. Potential soil feedbacks

We found that addition of activated carbon had a slight positive effect on growth of *Festuca arundinacea* in control soil, but no effect on growth in lupin-conditioned soil. Activated carbon can sequester organic compounds, interfere with plant–microbe communication, and reduce nitrogen and phosphorus availability and allelochemicals in soils (e.g. Kulmatiski 2011). As observed effects were of intermediate size, and opposite to what we expected, we do not conclude about potential impact. A more detailed study, isolating the different components and mechanisms would be needed to evaluate the use of activated carbon in vegetation restoration after lupin removal, potentially also including species covering the range of responses to lupin-conditioned soil.

4.4. No correlation between response to lupine-conditioned soil and fertilization response

We found no support for the prediction that the response to lupin-conditioned soil is positively correlated with the response to fertiliser addition. This result was unexpected as 1) the results from the experiment with *Festuca arundinacea* indicated that the improvement in nutrient conditions drives the response to lupin-conditioned soil, and 2) both our own field data and that of others (e.g. Grove et al. 2015) indicate facilitated establishment of nitrophilous species on soil conditioned by invasive nitrogen fixers. This suggests that the response to lupin-conditioned soil is a combination of species-specific responses to changes in both abiotic and biotic conditions in the soil, with species limited by different resources and plant-biota interactions. However, methodological issues cannot be discounted. Either the more nitrophilous species were not efficiently exploring the lupin-conditioned soil, or there is a possibility that the fertilisation series did not distinguish between nutrient demands among species. The higher soil organic matter content in field-collected soil also provides more beneficial soil water conditions and more steady nutrient release over time. These conditions

362 may favour less nutrient-demanding species relative to more nitrophilous species. Although

363 we found large effect sizes comparing growth in control and lupin-conditioned soil, estimates

364 are based on only three replicates per treatment per species, so some caution in interpretation

365 of responses of individual species is advised.

366   We expected the addition of labile carbon to lower the response to lupine-conditioned

367 soil, but found no effect on growth of the test species *Festuca arundinacea*. The addition of

368 labile carbon to soil has been found to immobilise inorganic nitrogen in microbial biomass

369 and to reduce plant growth (e.g. Eschen et al. 2006). Either the actual immobilisation was

370 marginal, or plant growth in the system was not limited by nitrogen. Unreplicated

371 measurements indicated that soil nitrate was reduced by 25–30% by sucrose addition in both

372 soils. Hence, we concluded again that growth was not limited by nitrogen availability, and the

373 lowering of available nitrogen by labile carbon was too small to have an effect.

374

375 *4.5. No relationship to plant strategies*

376 We found no simple relationships between seedling responses to lupin-conditioned soil and

377 their ecological strategies classified as Ellenberg indicator values or CSR strategies.

378 We expected that species with high score on the Ellenberg indicator value for soil nutrients

379 and species with C strategies would benefit more from the lupin-conditioned soil. One

380 explanation for this deviation may be that seedling responses differ from that of adult plants.

381 Another issue is the context-dependent valuation of plant strategies in the CSR scheme, as can

382 be seen in the differences in the allocated strategies between the original data from Hunt et al.

383 (2004) and e.g. the BIOLFLOR database (www.biolflor.de) calibrated for German conditions.

384 We may need a calibration to the study system and a higher resolution of coordinates to

385 achieve a better explanation of responses to lupin-conditioned soil using the CSR scheme.

386 The problem of potentially contrasting responses of seedlings and adult plants also applies

387   here. Even though we found no general trends with respect to ecological strategies, species

388   differed in the strength of their response to lupin-conditioned soil.

389

390   *4.6. Consequences for lupin invasiveness*

391   Soil conditioned by *Lupinus nootkatensis* had a positive effect on growth of conspecific

392   seedlings. Hence, the accumulation of soil-borne pathogens, parasites and herbivores was of

393   less importance for *Lupinus nootkatensis* than the sum of the abiotic soil modifications and

394   accumulation of beneficial organisms under the test conditions. Such positive intraspecific

395   plant soil feedbacks may promote dominance (Klironomos 2002). Further, N fixation has

396   been identified as an important trait for invasion of low-nutrient systems (Funk 2013). It has,

397   however, been suggested that the combined effect of N fixation and P mobilisation, and not

398   only the N fixation, is of high importance for lupin invasiveness on infertile soils (Lambers et

399   al. 2012; 2013). Although legumes with active N fixation excrete protons that acidify the root

400   zone and increase P availability in some soils (Hinsinger et al. 2003), the ability to mobilise

401   soil P efficiently is not widespread among the studied native coastal dune legumes (e.g. Davis

402   1991). Hence, lupins added both a new function and a new trait combination (N fixation and P

403   mobilisation) to the dune systems. This, in addition to the advantages of a taller canopy than

404   the competitors (asymmetric competition), contributes both discrete and continuous traits to

405   the system (Chapin et al. 1996) and explains much of the success and impact of *Lupinus*

406   *nootkatensis* on the invaded dune systems. Overall, the combination of N fixation, elevated P

407   mobilisation and net positive plant–soil feedbacks are three features that strongly modify soil

408   conditions and drive the invasiveness and dominance of *Lupinus nootkatensis* on coastal dune

409   soils in this region.

410

411   *4.7. Consequences for restoration and vegetation dynamics*

412 During succession on low-N soils, where nitrogen fixers such as lupins increase nutrient

413 availability, soil formation facilitates the establishment of other species (Walker et al. 2003;

414 Stinca et al. 2015). Lupin-conditioned soil had a positive net effect on seedling growth of

415 most species and there were no negative soil legacies that could prevent re-establishment of

416 native species after removal of lupins. Similar roles of lupins as ecosystem engineers and

417 facilitators of soil development can be found on low-P soils (Lambers et al. 2012). The effects

418 of lupins on nutrient availability through the combined N fixation and P mobilisation has

419 consequences for soil development (Ibekwe et al. 2007; Liao et al. 2008; Marchante et al.

420 2008) and thus vegetation dynamics through facilitation of more nutrient-demanding

421 vegetation (Myrold & Huss-Danell 2003; Hughes & Denslow 2005; Grove et al. 2015) due to

422 mid- to late-successional species outcompeting early-successional plants (Kardol et al. 2007).

423   Estimated Cohen's d had a large dispersion (CV = 77.7) across species. Hence,

424 although nearly all responses to lupin-conditioned soil were positive, there were considerable

425 differences among species in their ability to benefit from lupin-conditioned soil in the short

426 term. Shifts in plant–soil feedbacks by invasive plants may cause major changes and regime

427 shifts in ecosystems (Folke et al. 2004, Gaertner et al. 2014). *Lupinus nootkatensis* alters

428 feedbacks though nutrient cycling, but do these changes invoke regime shifts in the dune

429 systems or just speed up the return to the original vegetation? – Field observations indicate

430 that *Lupinus nootkatensis* causes large changes in vegetation composition and soil conditions

431 in coastal dunes in the study area; changes expected to promote resource-based facilitation of

432 succession and transitions to shrubland and forest (Kueffer 2010; Haubensak & D'Antonio

433 2011; Hiltbrunner et al. 2014). These changes also facilitate establishment of other invasive

434 plants (e.g. Maron & Connors 1996). While the destabilizing effect of increased nutrient

435 availability on vegetation dynamics (Cleland & Harpole 2010) could be counteracted by

436 negative plant–soil feedbacks (Bever et al. 2010), our results indicate a net positive

437 conspecific effect of lupins causing a more unstable community state. However, many abiotic

438 factors such as drought, salt spray and sand drift determine vegetation development on coastal

439 dunes and may prevent further encroachment. In addition, the leaching of nutrients in dunes is

440 high, so the elevated soil nutrient state may depend on the continued presence of lupins.

441   To conclude, our study showed that *Lupins nootkatensis* removes the phosphorus

442 limitation of plant growth in coastal dunes and increases cycling of other nutrients, promoting

443 the growth both of its own seedlings and a wide range of dune species. There are no negative

444 soil legacies that could prevent re-establishment of native species after removal of lupins.

445

446 **Acknowledgements**

449 **Appendix A. Supplementary material.**

450 Supplementary data for calculation of the CSR mixture model can be found in Appendix A

451

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588   **Table 1.** List of the plant species used in a greenhouse experiment to test the response to

589   lupin-conditioned coastal dune soil. The type (AM, arbuscular mycorrhiza; ECM,

590   ectomycorrhiza; NM, non-mycorrhiza) and frequency of mycorrhizal symbiosis (F_{myc}), and

591   Ellenberg indicator values for soil nitrogen, moisture, reaction are shown (E_{nutri}, E_{moist}, E_{react}); -

592   , missing data (Fitter and Peat 1994; Hempel et al. 2013).

Species	Family	Mycorrhiza type	E_{nutri}	E_{moist}	E_{react}	F_{myc}
Graminoids						
Ammophila arenaria	Poaceae	AM	3	4	6	3
Anthoxanthum odoratum	Poaceae	AM	3	6	4	3
Carex arenaria	Cyperaceae	AM/NM	2	3	5	1
Corynephorus canescens	Poaceae	AM/NM	1	1	3	1
Festuca arundinacea	Poaceae	AM	6	6	7	3
Festuca rubra	Poaceae	AM/ECM/NM	5	5	6	3
Leymus arenaria	Poaceae	AM/NM	6	5	7	1
Legumes						
Anthyllis vulneraria	Fabaceae	AM	2	4	7	2
Cytisus scoparius	Fabaceae	AM	4	5	4	3
Lathyrus japonicus	Fabaceae	AM	6	5	7	3
Lotus corniculatus	Fabaceae	AM	2	4	6	3
Lupinus nootkatensis	Fabaceae	NM	-	-	-	0
Trifolium arvense	Fabaceae	AM	2	3	5	-
Other dicotyledons						
Achillea millefolium	Asteraceae	AM	4	5	6	3
Campanula rotundifolia	Campanulaceae	AM	2	4	5	3
Draba incana	Brassicaceae	NM	2	5	7	0
Galium boreale	Rubiaceae	AM	3	5	7	3
Galium verum	Rubiaceae	AM	2	4	6	3
Hieracium pilosella	Asteraceae	AM	2	4	-	2
Hypochaeris maculata	Asteraceae	AM	3	4	8	-
Hypochaeris radicata	Asteraceae	AM/NM	3	4	5	3
Jasione montana	Campanulaceae	AM/NM	2	4	4	2
Leontodon autumnalis	Asteraceae	AM	4	6	6	3
Linaria vulgaris	Scrophulariaceae	AM/NM	6	4	8	2
Pimpinella saxifraga	Apiaceae	AM	3	4	7	3
Plantago lanceolata	Plantaginaceae	AM	4	5	6	3
Plantago maritima	Plantaginaceae	AM	4	7	6	2

Sanguisorba officinalis	Roseceae	AM	5	7	6	3
Sedum acre	Crassulaceae	AM/NM	2	2	7	2
Silene uniflora	Caryophyllaceae	NM	4	6	6	0
Taraxacum sp. Sect. *Spectabilia*	Asteraceae	AM	7	5	7	3
Valeriana sambucifolia	Valerianaceae	AM	5	8	6	3
Viola tricolor	Violaceae	AM/NM	4	4	6	1

593

594   **Table 2.** Response of 33 coastal dune species to lupin-conditioned soil in a greenhouse

595   experiment. Plants were cultivated in soil from within and outside lupin stands collected along

596   three transects at the field site. Plant responses were evaluated as amount of total biomass (ln

597   transformed) and root fraction of total biomass (error df = 130, total df = 197).

Source	df	Total biomass		Root fraction	
		F	P	F	P
Species	32	26.80	0.000	17.26	0.000
Soil	1	435.2	0.000	10.35	0.002
Species x Soil	32	4.91	0.000	4.19	0.000
Transect	2	38.71	0.000	11.28	0.000
R^2 adj		88		77	

598

599 **Table 3.** ANOVA results of an mixture regression of how the CSR space affects seedling

600 responses to lupin-conditioned soil measured as ln transformed Cohens' d effect sizes (R_2 adj,

601 = 26%, total df = 31, error df = 25).

Source	df	F	P
Regression	6	2.80	0.032
Linear	2	0.60	0.554
Quadratic	3	4.16	0.016
C*S	1	0.04	0.844
C*R	1	3.32	0.081
S*R	1	0.92	0.347
C*S*R	1	1.34	0.258
Lack of fit	5	1.48	0.239

602

Table 3. ANOVA results of an mixture regression of how the CSR space affects seedling

603 **Table 4.** Nutrient supply rates (mean ± SD) in pots with control and lupin-conditioned soil

604 during a greenhouse experiment. Supply rates were estimated as μg 10 cm^{-2} for a period of 7

605 weeks using PRS probes with ion exchange membrane. P values for the tests of differences

606 between soil types are shown.

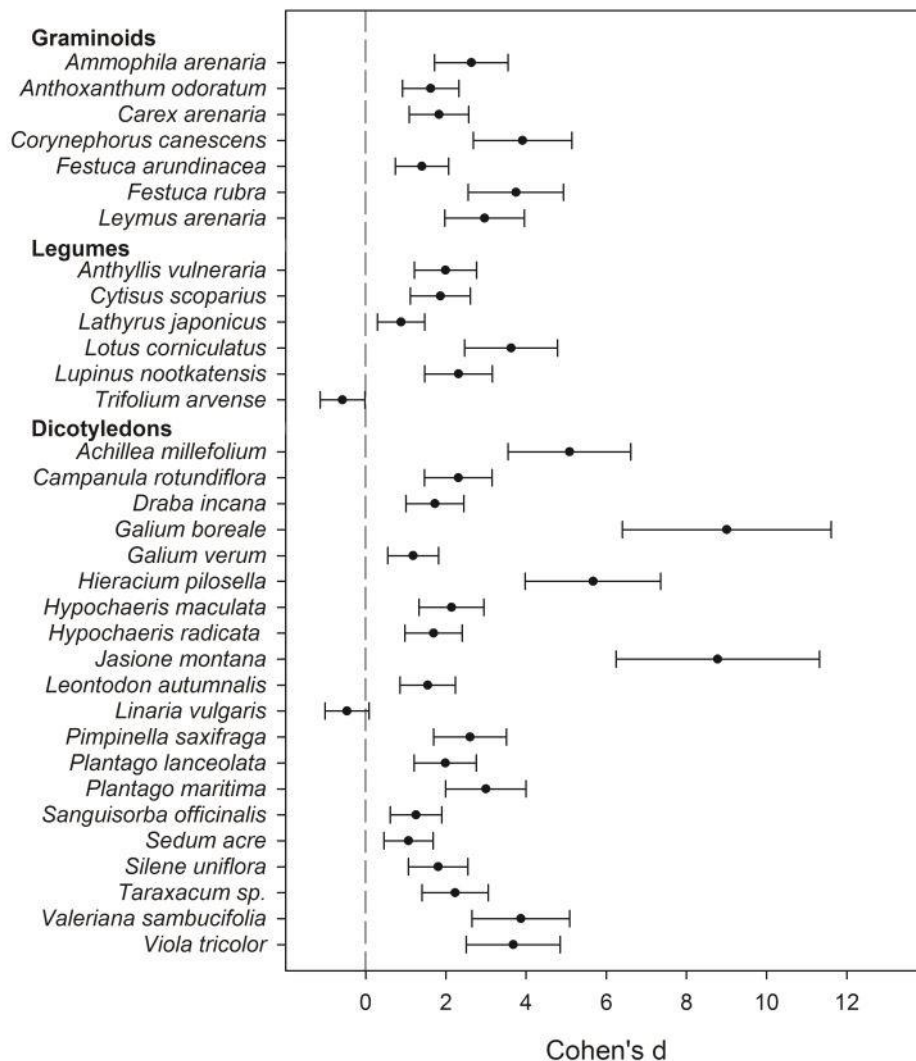|	Control soil	Lupine soil	P value
NO$_3$-N	212 ± 44	675 ± 161	0.059
NH$_4$-N	3.69 ± 0.78	3.04 ± 0.06	0.360
P	1.47 ± 0.81	8.28 ± 1.24	0.023
K	111 ± 13	102 ± 12	0.548
Ca	1793 ± 714	839 ± 169	0.208
Mg	153 ± 87	447 ± 142	0.131
S	46.2 ± 6.1	35.6 ± 1.7	0.139
Al	14.6 ± 5.3	19.7 ± 1.9	0.334
Fe	5.36 ± 3.70	70.4 ± 17.9	0.037
Mn	0.00 ± 0.00	6.89 ± 0.11	0.000
Zn	3.18 ± 1.45	6.74 ± 2.69	0.240
B	1.12 ± 0.12	0.93 ± 0.51	0.667
Cu	0.37 ± 0.23	0.31 ± 0.02	0.752

607

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609

610   **Figures**

611

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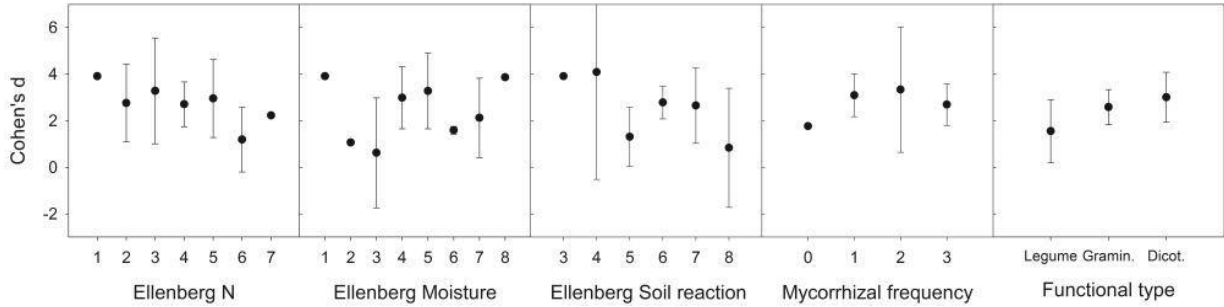614   **Fig. 1** Estimated effect size (Cohen's d; mean and 95% CI) of the responses in a set of coastal

615   dune species to lupin-conditioned soil. Plants grouped as graminoids, legumes and other

616   dicotyledon species.

617

618

619

620

Fig. 2. Estimated effect size of the growth responses to lupin-conditioned soil (Cohen's d with 95% CI) across functional types, habitat preferences grouped as Ellenberg values for soil nitrogen, moisture content and soil reaction, frequency of mycorrhizal infections and functional type of vegetation in a set of 32 coastal dune species from SW Norway. The response of the invasive alien *Lupinus nootkatensis* is not included in the estimates. Ellenberg values and data on mycorrhizal association are from Fitter and Peat (1994). Spearman rank correlation coefficients showed only weak relationships ($r_s = -0.005$ to -0.16) between Cohen's d and Ellenberg values for nitrogen, moisture and soil reaction.

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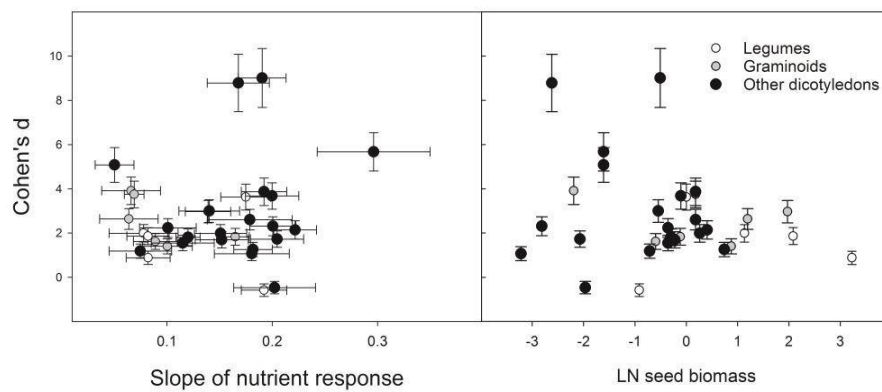
Fig. 3. Relationships between estimated effect size (Cohen's d ± SE) of the growth responses

of 33 dune plants to lupin-conditioned soil and the responses to soil nutrients and relationship

to seed biomass (1000 seeds). Responses to soil nutrients were estimated as the slope of ln-

transformed total biomass to increasing nutrient availability in a controlled experiment.

636
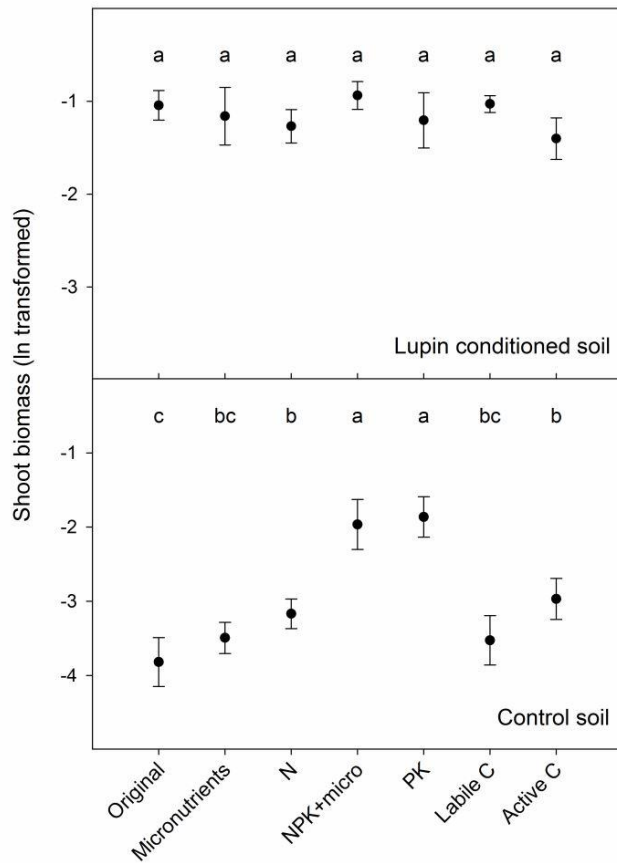
Fig. 4. Effects of fertilisation on growth (mean and 95% confidence intervals) of the grass *Festuca arundinacea* in control soil or lupin-conditioned soils with addition of micronutrients, nitrogen (N), phosphorus (P) and potassium (K) and combinations of these, labile carbon and activated carbon. Letters (a, b, c) indicate results of multiple comparisons within soil type.

APPENDIX A

644   Analyses of the relationship between CSR strategies according to Grime (1977) and the

645   response to lupin-conditioned soil measured as the Cohen's effect size of the difference in

646   growth responses between lupine-conditioned soil and control soil. CSR strategies were

647   extracted from literature data for all species and coded as X, Y, Z coordinates according to

648   Hunt et al (2004). The relationship between CSR strategy and Cohen's effect size was

649   analysed in a mixture design model with main and two-way interactions of C, S, and R using

650   the DOE function in Minitab 17. Effect sizes were LN (+1) transformed before analyses to

651   increase normality and lower heteroscedasticity of the residuals.

652

653   **Table A1.** CSR coordinates used in the analyses, based on Hunt et al. (2004) and

654   supplemented with data from the BIOLFLOR database (www.biolflor.de) * *Trifolium arvense*

655   was excluded from the analyses, due to strongly deviating residuals.

Species	Cohen's d	Strategy	C	S	R
Achillea millefolium	5.08123	C/CSR	0.6667	0.1667	0.1667
Ammophila arenaria	2.63390	C/CSR	0.6667	0.1667	0.1667
Anthoxanthum odoratum	1.61976	SR/CSR	0.1667	0.4167	0.4167
Anthyllis vulneraria	1.98807	CSR	0.3333	0.3333	0.3333
Campanula rotundifolia	2.30873	S/CSR	0.1667	0.6667	0.1667
Carex arenaria	1.82817	SC/CSR	0.4167	0.4167	0.1667
Corynephorus canescens	3.91028	SC	0.5000	0.5000	0.0000
Cytisus scoparius	1.86229	SC	0.5000	0.5000	0.0000
Draba incana	1.72662	S/SR	0.0000	0.7500	0.2500
Festuca arundinacea	1.40283	SC/CSR	0.4167	0.4167	0.1667
Festuca rubra	3.74530	SC/CSR	0.4167	0.4167	0.1667
Galium boreale	9.00564	SC/CSR	0.4167	0.4167	0.1667
Galium verum	1.18347	SC/CSR	0.4167	0.4167	0.1667
Hieracium pilosella	5.67092	S/CSR	0.1667	0.6667	0.1667
Hypochaeris maculata	2.14114	CSR	0.3333	0.3333	0.3333
Hypochaeris radicata	1.69245	CSR	0.3333	0.3333	0.3333
Jasione montana	8.77721	S/SR	0.0000	0.7500	0.2500
Lathyrus japonicus	0.88095	SC	0.5000	0.5000	0.0000
Leontodon autumnalis	1.54679	R/CSR	0.1667	0.1667	0.6667
Leymus arenaria	2.96494	SC	0.5000	0.5000	0.0000
Linaria vulgaris	-0.46443	CR	0.5000	0.0000	0.5000
Lotus corniculatus	3.62492	S/CSR	0.1667	0.6667	0.1667
Lupinus nootkatensis	2.31454	C	1.0000	0.0000	0.0000
Pimpinella saxifraga	2.60183	SR/CSR	0.1667	0.4167	0.4167
Plantago lanceolata	1.98458	CSR	0.3333	0.3333	0.3333
Plantago maritima	2.99566	S	0.0000	1.0000	0.0000
Sanguisorba officinalis	1.25495	CSR	0.3333	0.3333	0.3333
Sedum acre	1.06806	S	0.0000	1.0000	0.0000
Silene uniflora	1.80644	CSR	0.3333	0.3333	0.3333
Taraxacum sp.	2.23050	R/CSR	0.1667	0.1667	0.6667
Trifolium arvense	-0.57831	SR[*]	0.0000	0.2500	0.7500
Valeriana sambucifolia	3.86706	C	1.000	0.0000	0.0000

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657