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5 Facilitating ecosystem assembly: plant-soil interactions as a restoration tool

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25

26 Abstract

27

28 Although plant-soil interactions are increasingly recognized as an important factor in ecosystem
29 restoration, their effects on community assembly during de novo ecosystem establishment are
30 largely unknown. In a heathland restoration trial after topsoil removal we introduced either only
31 aboveground heathland species with fresh herbage or both above- and belowground heathland
32 species with sods to facilitate community assembly. Sod inoculation increased resemblance of the
33 microbial community to the reference system, with a higher fungal and lower bacterial proportion to
34 the community structure. Also densities of bacteriophagous and phytophagous nematodes, Acari and
35 Collembola increased after sod inoculation. The cover of heathland plant species increased by 49%
36 after sod inoculation. The introduction of solely aboveground heathland species increased the cover
37 of these species by only 13%, and did not affect soil community assembly. Additionally, the increase
38 in cover of heathland species over time was inversely correlated to the cover of mesotrophic
39 grassland species. Inverse correlations were also observed between changes in fungal and bacterial
40 abundances. Simultaneous introduction of key species of both above- and below-ground
41 communities had a critical effect on the establishment of both communities, providing a potential
42 shortcut for successful restoration of target ecosystems on disturbed soils.

43

44 **Key words;** restoration; ecological filters; fungi; heathlands; mesofauna; bacteria

45

46 1. Introduction

47

48 Ecosystem assembly is a fundamental concept in ecology. Traditionally the focus has been on the
49 assembly of aboveground communities (Götzenberger et al. 2011), but in recent years the
50 importance of belowground community composition has become increasingly recognised (Reynolds
51 et al. 2003, Wardle et al. 2004). Two major pathways are identified in plant-soil interactions: a first,

52 direct, pathway is associated with the interaction between roots and soil organisms such as
53 symbionts and pathogens. A second, indirect, pathway includes interactions between decomposers
54 and plants and concerns nutrient cycling rates and soil formation (Wardle et al. 2004). The extent to
55 which aboveground community composition affects belowground development and vice versa is still
56 largely unclear. It is suggested that the soil community may either follow or facilitate vegetation
57 development, dependant on the ecosystem (Harris 2009).

58

59 Little is known about the sequence in which characteristic above- and below-ground species have to
60 establish for a smooth ecosystem development. While especially late-successional plants may need
61 particular soil organisms to function properly (De Deyn et al. 2003, Frouz et al. 2008), the
62 establishment of these soil organisms themselves may depend on the presence of characteristic
63 plant species which promote the development of a typical organic soil layer (Frouz et al. 2009).
64 Studies that included analysis of both above- and below-ground development during succession of
65 semi-natural grassland or dwarf shrub vegetation reported varying results: in some studies both
66 above- and below-ground communities develop along similar lines (Lozano et al. 2014), while others
67 report that belowground development either lags behind aboveground changes (e.g. Frouz et al.
68 2009, Holtkamp et al. 2008, Jangid et al. 2011) or precedes them (Van der Bij et al. 2016).

69

70 Filters are assumed to play an important role in vegetation assembly, especially abiotic conditions,
71 dispersal and establishment are considered critical factors (Van Diggelen & Marrs 2003, Cramer et al.
72 2008). A better understanding of how plant-soil interactions affect the establishment of
73 characteristic plant species would add significantly to this knowledge and has not only theoretical
74 value, but would also provide valuable insights for practical restoration, e.g. after topsoil removal.
75 There a bare substrate is created with suitable abiotic conditions and an opportunity for new species
76 to establish. Previous studies have shown that vegetation assembly can be facilitated by introducing
77 seeds of target species (Holtkamp et al. 2008, Kiehl et al. 2010, Klimkowska et al. 2010) and it sounds
78 reasonable that similar filters also apply for belowground community assembly. For example,
79 dispersal limitation is assumed to be strong for soil fauna as Acari (Lehmitz et al. 2012), one of the
80 most abundant soil fauna groups in oligotrophic systems (Wardle et al. 2004, Frouz et al. 2009).
81 Facilitation of soil community assembly would be a logical next step to further enhance ecosystem
82 restoration (Kardol & Wardle 2010). However, studies that explored this option by inoculation
83 experiments showed varying results (Pywell et al. 2007, Kardol et al. 2009, Wubs et al. 2016).

84

85 Although the extent to which plant-soil interactions affect ecosystem assembly remains largely
86 unknown, several papers emphasized their importance for restoration ecology (Harris 2009, Kardol &
87 Wardle 2010, Van der Putten et al. 2013). In the present study we assessed the potential of plant-soil
88 interactions in de novo heathland ecosystem establishment. In a field trial immediately after topsoil
89 removal we introduced either only aboveground species by means of fresh herbage, or
90 simultaneously both above- and below-ground species by means of sods. We monitored the parallel
91 development of vegetation and soil community to assess the following research question: does the
92 simultaneous introduction of above- and below-ground species in early succession have a synergistic
93 effect on heathland community assembly? We hypothesized that introduction of the soil community
94 in early succession would enhance vegetation assembly.

95

96 **2. Materials and methods**

97

98 *2.1 Site description*

99

100 The Dwingelderveld National Park (N 52°48'14.3, E 6°24'38.6) is a large lowland heathland (altitude
101 7m) in the Netherlands. It has a maritime temperate climate (Cfb) with an average annual
102 temperature of 8.8°C and an annual average rainfall of 783 mm ([http://en.climate-](http://en.climate-data.org/location/105881/)
103 [data.org/location/105881/](http://en.climate-data.org/location/105881/)). In the 1930's 200 ha in the centre of the area was converted from

104 heathland into agricultural grasslands and restored again in 2011-2012 with topsoil removal (30-40
105 cm), only road sides with mesotrophic grassland were left untouched. Compared to reference values
106 from the meta-analysis of De Graaf et al. (2009) and measurements in reference sites nearby (Table
107 1) pH and soil buffering were higher than in typical Dutch heathlands but after topsoil removal
108 nutrient levels lay well within the range of typical heathlands

109

110 2.2 Experimental setup

111

112 The experiment was installed in November 2011 immediately after topsoil removal. We manipulated
113 both the abiotic and the biotic environment in a full-factorial set up. The soil-pH was manipulated by
114 (1) addition of acid (150 g elemental S per m²), (2) addition of lime (200 g Dolokal per m²) or (3) left
115 untouched. We manipulated the biotic conditions by establishing three inoculation treatments: (1)
116 introduction of aboveground parts of heathland plant species, (2) addition of both plant species and
117 soil community or (3) control. We did not measure the effects of adding only the soil community,
118 because we were not capable to remove seeds from the added soil without severely disturbing the
119 soil community. Each combination of treatments consisted of 3 replicates. The experiment was set
120 up in 27 random plots of 15m x 15m with 2m buffers. In November 2011 we added elemental Sulfur
121 or Dolokal and in December 2011 we spread crumbled sods from nearby well-developed dry
122 heathlands. These sods contained the existing vegetation, the soil seed bank and the soil community.
123 Sods were collected by cutting the upper 5 cm of a nearby dry heathland and were added
124 immediately to the experimental plots in a ratio of 1:15 (i.e. donor material of 1 m² on 15 m²
125 experimental plot). Aboveground plant material was added via the introduction of fresh herbage
126 collected after seed setting of the dominant plant species *Calluna vulgaris* (L.) Hull in September
127 2012, the first opportunity after installing the experiment. This material was collected from a nearby
128 well-developed dry heathland and added at the plots immediately after the mowing in a ratio of 1:2.
129 Control plots remained unaltered after topsoil removal.

130

131 The number of germinable *C. vulgaris* seeds added per m² was expected to differ between both
132 inoculation treatments due to the different ratios in which the donor materials were added. We
133 assessed these figures by using data from Legg et al. (1992) on the number of viable seeds in the
134 seed bank and the annual seed production per m² for mature dry heathlands in combination with a
135 germination percentage of 75% of fresh heather seeds (Spindelbock et al. 2013). We calculated that
136 we added an average of 34125 germinable seeds per m² with fresh herbage and 15800 per m² with
137 sods. Since we introduced a high number of seeds in both treatments, we expected that seed
138 availability was not a limiting factor for the establishment of *C. vulgaris*.

139

140 2.3 Microbial community

141

142 In 2009, before topsoil removal, we took three soil samples in the agricultural grassland from a layer
143 just below the planned removal depth as starting point for microbial community development. Soil
144 samples from the experimental plots (5 cm depth) were taken immediately after topsoil removal
145 before the treatments were imposed and after 2 years in November 2013. Nearby dry heathlands
146 which were used as source for the sod-treatment were sampled as a reference soil (n=3) at the same
147 time. In each sampling point a composite sample of 3 x 100 cm³ soil was obtained with Kopecky rings.
148 Aliquots of the soil were refrigerated for the analysis of microbial biomass or freeze-dried for
149 phospholipid fatty acid (PLFA) analysis.

150

151 Microbial biomass-C was determined with the fumigation-extraction procedure (Jenkinson &
152 Powlson 1976) using K_{ec} of 0,45 (Vance & Jenkinson 1987). Microbial community phenotypic
153 structure was measured with PLFA analysis using a modified method from Frostegård et al. (1993)
154 according to the methods described by Courtney et al. (2014).

155

156 2.4 Soil fauna

157

158 Soil fauna was sampled together with the microbial samples. Samples were stored at 10°C for
159 nematode community analysis. Nematodes were extracted from 10 g soil with a modified Bergmann
160 funnel (Háněl 1995) for 48 hours, after which they were fixed with formaldehyde and transferred to
161 microscopic slides. Nematodes were divided into feeding groups according to Yeates et al. (1993).
162 Soil mesofauna groups (Acari and Collembola) were extracted with a Tullgren apparatus and sorted
163 under a dissection microscope as described by Frouz (1997).

164

165 2.5 Vegetation

166

167 Two permanent quadrats (2m x 2m) were established at the centre of each plot. In July-August of
168 each year we made vegetation relevés according to the Londo scale (Londo 1976). In the donor sites
169 for herbage and sods 4 vegetation relevés (2m x 2m) were made in August 2012. Plant species were
170 classified into 3 categories: characteristic heathland species, typical mesotrophic grassland species
171 species and other species. Species with a faithfulness of at least 10% to the dry heathland association
172 (SynBioSys, Hennekens et al. 2010) were labelled characteristic heathland species.

173

174 2.6 Data handling and statistics

175

176 Before analysis normality of the residuals and equality of variances were checked, nematode and
177 mesofauna data needed a $\ln(x+1)$ transformation to meet the criteria. We checked the effects of the
178 treatments on soil chemical characteristics with a linear mixed model. Addition of Sulfur or Dolokal
179 had a significant effect on soil pH and soil base status but did not affect plant nutritional parameters
180 (Table 1 in Appendix). Since the biotic treatments had no significant effects on any of the measured
181 soil chemical parameters we pooled the abiotic treatments and analysed the effects of the biotic
182 treatments only. Treatment effects and values of the reference heathlands were tested with an
183 Analysis of Variance (ANOVA), a post-hoc Tukey test was used to determine individual differences.
184 Only for microbial community composition pre-treatment measurements of the original agricultural
185 grasslands deep horizon were included as starting point. Since both vegetation and soil fauna of the
186 agricultural grassland were removed with topsoil removal, they did not represent the actual starting
187 points and were therefore not included in further analysis.

188

189 PLFA data were subjected to a Principal Component Analysis (PCA). We tested treatment effects with
190 a Multivariate Analysis of Variance (MANOVA) with PCA1 and PCA2 as dependent variables and
191 treatments (including starting points and reference heathlands) as fixed factor. On both PCA1 and
192 PCA2 treatment effects were determined separately with an ANOVA and a post-hoc Tukey test. We
193 determined correlations in the rate of change per year (Δ) between different species categories
194 within plant, microbial- and mesofauna communities and between those communities with a two-
195 sided Pearson correlation test. For statistics we used R (R Core Team 2016) and the nlme-package for
196 LME (Pinheiro et al. 2015).

197

198 3. Results

199

200 3.1 Microbial community

201

202 After topsoil removal microbial biomass was $70 \pm 13 \mu\text{g micC g}^{-1}$ soil, compared to $770 \pm 220 \mu\text{g micC g}^{-1}$
203 soil in the reference heathland (ANOVA, F: 6.26, $p < 0.0001$, Table 2). In the first two years of the
204 experiment microbial biomass increased only after sod inoculation, while there were no differences
205 over time in the other treatments (ANOVA, F: 3.10, $p: 0.017$, Tukey test, $p < 0.05$). Relative bacterial
206 contribution to the microbial community structure in the control and after herbage addition
207 remained similar to the deep horizon of the original grassland (Figure 1), while sod inoculation

208 reduced the bacterial contribution (ANOVA, F: 6.54, p: 0.019). In contrast, relative fungal
209 contribution increased significantly after sod inoculation compared to the other treatments (ANOVA,
210 F: 31.37, p<0.0001), although it was still lower than in the reference heathland (Tukey test, p<0.05).

211
212 A PCA based on all PLFA's showed a clear distinction between sod inoculation and the other
213 treatments (MANOVA, F: 8.37, p<0.0001). Microbial phenotypic composition changed in all plots
214 after topsoil removal compared to the original agricultural deep horizon (Figure 2). Sod inoculation
215 increased the resemblance of microbial phenotypic composition to the reference heathland within 2
216 years, after the addition of herbage it did not differ significantly from the control.

217 218 *3.2 Soil fauna*

219
220 After 2 years densities of all nematode feeding guilds except omnivores were significantly lower in
221 the experimental plots compared to the reference heathlands (Table 3, Tukey test, p<0.05). Total
222 nematode densities in the experimental plots reached maximal 7 % of the values of the reference
223 heathlands. Only bacteriophagous nematodes increased significantly after sod inoculation (ANOVA,
224 F: 32.37, p<0.0001). Although densities of other feeding guilds showed an increasing trend along the
225 inoculum gradient, there were no significant differences.

226
227 Densities of both Acari (ANOVA, F: 13.50, p<0.0001) and Collembola (ANOVA, F: 4.02, p: 0.017)
228 increased along the inoculum gradient (Figure 3), with higher densities after sod inoculation
229 compared to the control and intermediate values after the addition of herbage (Tukey test, p<0.05).
230 2 years after sod inoculation Collombola densities did not differ significantly from reference
231 heathlands, while densities of Acari were still much lower (Tukey test, p<0.05).

232 233 *3.3 Vegetation*

234
235 The cover of characteristic heathland species increased along the inoculum gradient (ANOVA, F:
236 120.33, p<0.0001), with a 13% increase after the addition of herbage and a further 36% increase
237 after sod inoculation (Figure 4). 3 years after sod inoculation characteristic heathland species
238 covered more than 50 percent of the surface. Typical mesotrophic grassland species showed the
239 opposite pattern (ANOVA, F: 13.78, p<0.0001), with significantly lower cover after both inoculation
240 treatments compared to the control. This contrast between heathland and mesotrophic grassland
241 species resulted in a different balance along the inoculum gradient: grassland species dominated the
242 control (ANOVA, F: 197.79, p<0.0001, Tukey test, p<0.05) while heathland species were dominant
243 after sod inoculation (ANOVA, F: 120.20, p<0.0001, Tukey test, p<0.05). After the addition of
244 herbage the cover of both categories was equal (ANOVA, F = 1.17, P = 0.287, Tukey test, P > 0.05).
245 Total herb cover reflected the increased cover of heathland species with significant higher values
246 after sod inoculation (ANOVA, F: 29.06, p<0.0001).

247 248 *3.4 Within and between community linkage*

249
250 Although the magnitude smaller microbial biomass in the experiment compared to the reference
251 heathlands indicates that the microbial community was still far from the reference state, all
252 treatments showed a shift in the relative contribution of fungi and bacteria but the fastest changes
253 occurred in the soil addition treatment (Figure 5). The maximal herb cover in the experimental plots
254 after 3 years was between 60-70%, suggesting minimal competition for light and space. Remarkably,
255 also here an inverse correlation was found in the rate of change per year of the cover of heathland
256 and mesotrophic grassland species (Pearson correlation: -0.89, p<0.001). Both mesofauna groups
257 showed a positive correlation (Pearson correlation: 0.84, p<0.0001), suggesting minimal competition.
258 The rate of change per year between the cover of heathland species aboveground and the relative
259 contribution of fungi belowground showed a strong positive correlation (Pearson correlation: 0.69,

260 p<0.0001), as did the relative contribution of fungi and total mesofauna densities (Pearson
261 correlation: 0.63, p: 0.0006).

262

263 4. Discussion

264

265 4.1 Below-ground community assembly in relation to above-ground composition

266

267 After 2 years both the above- and below-ground community in the control treatment showed no
268 resemblance to the heathlands some 100 metres away but only to the mesotrophic grassland in the
269 road sides of the immediate surroundings, which suggests strong dispersal limitation for heathland
270 communities. In contrast, both inoculation treatments showed a clear development towards
271 heathland. Introduction of seeds by fresh herbage promoted aboveground community assembly, as
272 also reported in other studies (Holtkamp et al. 2008, Kiehl et al. 2010, Klimkowska et al. 2010), but
273 did not affect the belowground community. In contrast, sod inoculation did lead to increased
274 microbial biomass, fungal/bacteria ratio and soil fauna density and accelerated vegetation assembly
275 even further. These differences suggest that the belowground community does not automatically
276 follow the aboveground community and that only the simultaneous presence of both above- and
277 below-ground heathland species leads to a fast assembly towards the target ecosystem. Our results
278 show that plant-soil interactions can play a critical role in de novo ecosystem establishment. In the
279 short term, simultaneous introduction of target above- and belowground species has a synergistic
280 effect on both above- and below-ground community assembly.

281

282 The presence of above- and below-ground heathland species alone does not necessarily lead to
283 restored plant-soil interactions (Kardol & Wardle 2010). After 2 to 3 years vegetation cover and
284 microbial biomass were still far from the reference state, leading to conditions where competition
285 for light or space are likely still minimal. Nevertheless, an inverse correlation between the cover of
286 heathland and grassland species aboveground and fungi and bacteria belowground was present in
287 this stage, especially after sod inoculation. These results suggest that addition of above- and below-
288 ground heathland species not only reinforces their own establishment, but also reduces the
289 establishment of non-target species. In such situation, where competition between plants is probably
290 still very low, plant-soil interactions may be the main mechanism determining the balance between
291 grassland and heathland species (e.g. Kardol et al. 2006, Bonkowski & Roy 2012). This may lead to
292 priority effects that determine vegetation composition for decades (Cramer et al. 2008).

293

294 4.2 Assembly pathways

295

296 Three different assembly pathways developed in the different treatments, with the most distinct
297 differences between the control treatment and after sod inoculation. In the control treatment both
298 above- and below-ground communities showed high resemblance to mesotrophic grasslands. The
299 lack of sufficient seeds of heathland species combined with a high seed pressure of grassland species
300 from the immediate surroundings (Klimkowska et al. 2010) seems to direct vegetation development
301 towards a grassland. When the herb layer closes and recruitment gaps are no longer present,
302 heathland species are likely to have large difficulties to establish and might remain absent from the
303 community for a long time (Cramer et al. 2008).

304

305 A second pathway was followed after sod inoculation, where both above- and below-ground
306 communities showed a higher resemblance to reference heathlands. Further assembly may depend
307 on the infection rate of heather (*C. vulgaris*) by ericoid mycorrhiza. We did not measure mycorrhiza
308 separately but the lower overall fungal content as compared to reference heathlands does suggest a
309 low(er) infection rate in such former agricultural soils (Diaz et al. 2006, 2008). This interaction
310 between *C. vulgaris* and ericoid mycorrhiza may favour both sides by production of recalcitrant litter
311 by *C. vulgaris* and selective removal of labile nutrients by mycorrhiza (Read et al. 2004). When this

312 symbiotic relation establishes sufficiently, heathland species are likely to remain dominant in the
313 mid- to long term. This process might contribute to the inverse correlation between the cover of
314 grassland and heathland species, suggesting that sod inoculation not only facilitates community
315 assembly but also ecosystem functioning (Bever et al. 2010).

316
317 The third pathway, manifest after the addition of herbage, showed a mismatch between above- and
318 below-ground communities: aboveground heathland and grassland species had similar cover while
319 the community belowground was almost identical to that of an agricultural grassland. While after
320 both inoculation treatments the estimated number of *C. vulgaris* plants per area was similar, their
321 growth and thereby cover was lower after the addition of herbage, possibly reflecting a lower
322 mycorrhizal infection rate (Diaz et al. 2006). Mycorrhizal infection rate in the first decade could be
323 the tipping point for this pathway. A high rate might lead to heathland species gaining dominance
324 and ecosystem development converging with the pathway after sod inoculation. Alternatively, the
325 combination of an agriculturally-configured soil community and high cover of grassland species may
326 tip the balance in favour of a grassland system by a self-reinforcing feedback loop of higher
327 decomposition rates, higher productivity, higher litter quality and faster nutrient cycling (Bever et al.
328 2010, Kardol & Wardle 2010).

329
330 The simultaneous introduction of key species from both above- and below-ground with sod
331 inoculation enhanced and accelerated ecosystem assembly towards the target system, and
332 demonstrates the potential of plant-soil interactions in early succession (Harris 2009). Without
333 introduction of key species, both above- and below-ground communities remained stuck in an
334 agricultural setting despite favourable abiotic conditions for heathland development. The trajectory
335 after the addition of herbage might either converge with the pathway after sod inoculation when
336 specific plant-soil interactions establish or switch towards a grassland in their absence.

337 338 *4.3 Implications for ecosystem restoration*

339
340 Our results show that the simultaneous introduction of key above- and below-ground species
341 enhances and accelerates the restoration of oligotrophic systems after soil disturbance. We found
342 that addition of the belowground community has a significant effect on vegetation composition
343 (Wubs et al., 2016). Such method provides a potential shortcut for quickly re-establishing target
344 oligotrophic ecosystems after topsoil removal, on post-mining sites or other newly created surfaces.
345 To maximize restoration success, sufficient material from both above- and below-ground
346 communities, ideally in the form of sods, is to be added immediately after soil disturbance.

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354 355 **6. References**

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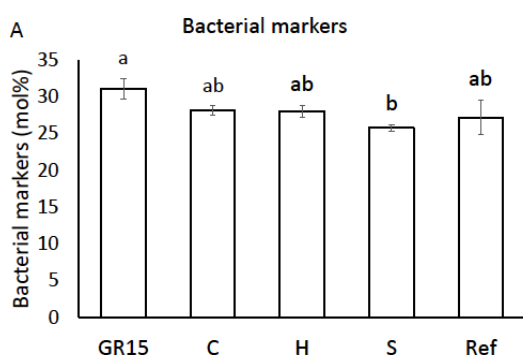
503 Table 1. Soil parameters in experimental site immediately after topsoil removal (Means \pm S.E. ; n=27)
 504 as compared to reference sites nearby (range; n=3).
 505

Site	Soil pH- H ₂ O	Exchangeable base cations $\mu\text{eq/kg soil}$	Plant available phosphorus $\mu\text{mol/kg soil}$	N mineral (NO ₃ + NH ₄) $\mu\text{mol/kg soil}$	Organic matter % dry soil
Experiment	5.61 (0.03)	10304 \pm 894	296.0 \pm 48.6	40.4 \pm 16.6	2.1 \pm 0.2
Dry heath reference	3.8-4.9	485-7690	100-700	1-220	1.6-11.9

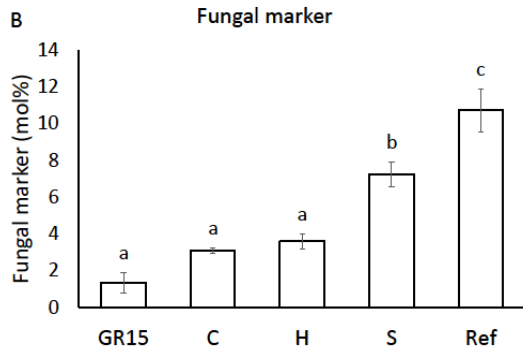
506
 507 Table 2. Results of ANOVA-models of the inoculum gradient including reference heathlands and only
 508 for microbial community the deep original grassland horizon. Statistics of solely the experimental
 509 treatments are included in Table 2 of the Appendix.

Component	ANOVA model		
	df	F	p
Microbial community			
Fungal marker	4	31.37	<0.0001
Bacterial markers	4	6.54	0.019
Nematodes			
Bacteriophagous	3	32.37	<0.0001
Phytophagous	3	7.96	0.0006
Mycophagous	3	10.63	<0.0001
Omnivores	3	2.15	0.119
Total	3	15.67	<0.0001
Mesofauna			
Acari	3	13.50	<0.0001
Collembola	3	4.02	0.017
Vegetation			
Cover heathland species	3	120.33	<0.0001
Cover grassland species	3	13.78	<0.0001
Total cover	3	29.06	<0.0001

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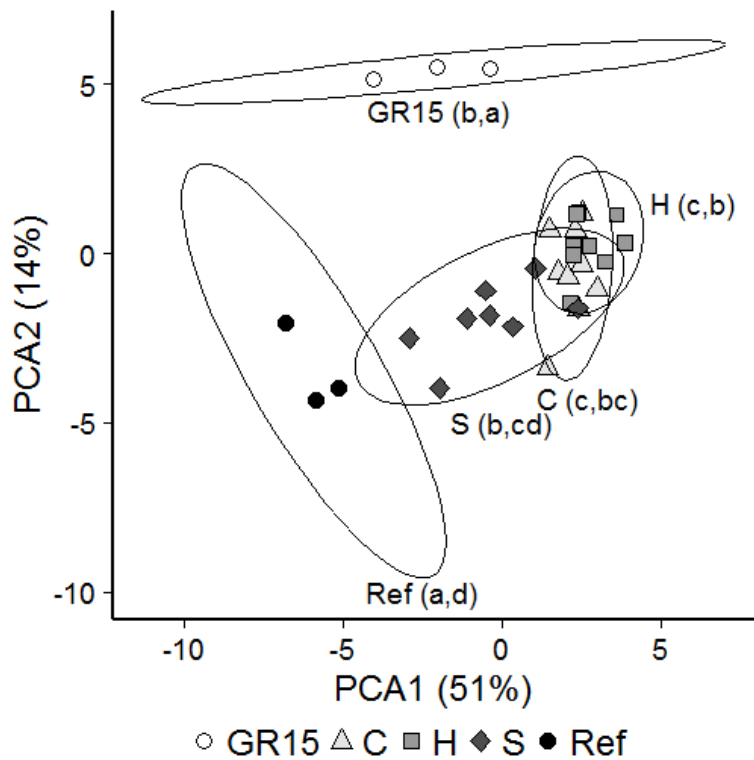


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514 Figure 1. The sum of the relative contribution of the bacterial PLFA's (A) and the fungal PLFA (B).
 515 Means \pm S.E., letters indicate Tukey outcomes. GR15: deep horizon grassland; C: control; H: herbage;
 516 S: sods and Ref: reference heathland.
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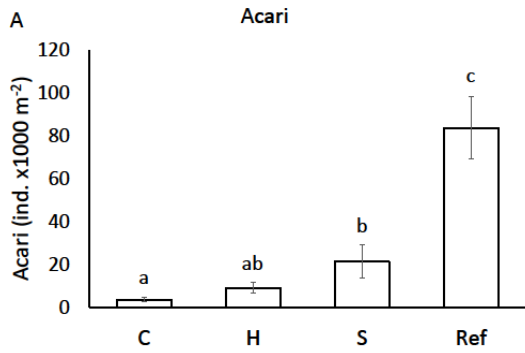


520
 521 Figure 2. A PCA based on all measured PLFA's. GR15: deep horizon original grassland; C: control; H:
 522 addition of herbage; S: sod inoculation and Ref: reference heathland. Ellipses represent 95%
 523 confidence intervals. Letters indicate Tukey outcomes from PCA 1 and PCA2.
 524

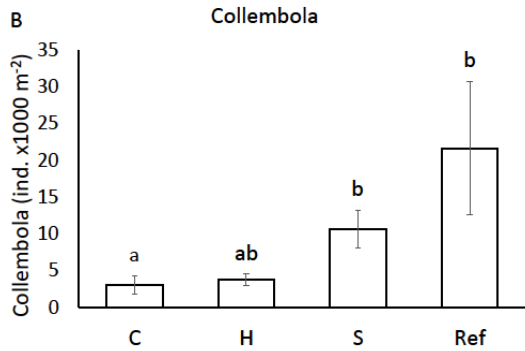
525 Table 3. Nematode densities for feeding guilds per 10 g dry soil. Means \pm S.E., letters indicate Tukey
 526 outcomes.

Feeding guild	Control	Herbage	Sods	Reference
Bacteriophagous	1.67 \pm 0.24 (a)	3.00 \pm 0.99 (ab)	5.67 \pm 1.21 (b)	168 \pm 104 (c)
Phytophagous	0.00 \pm 0.00 (a)	0.11 \pm 0.11 (a)	0.67 \pm 0.29 (a)	4.67 \pm 2.40 (b)
Mycophagous	0.11 \pm 0.11 (a)	1.33 \pm 0.90 (a)	3.22 \pm 1.58 (a)	25.67 \pm 12.91 (b)
Omnivores	2.44 \pm 0.80 (a)	3.67 \pm 0.96 (a)	4.67 \pm 1.91 (a)	10.00 \pm 3.46 (a)
Total	4.22 \pm 0.80 (a)	8.11 \pm 1.94 (a)	14.22 \pm 3.61 (a)	209 \pm 117 (b)

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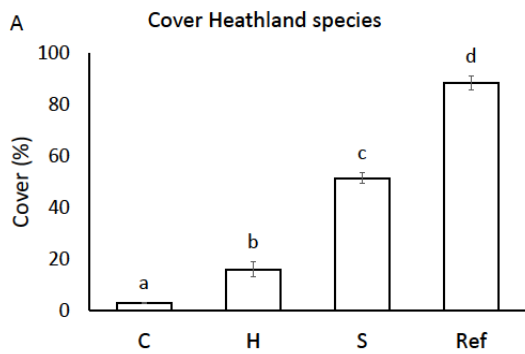


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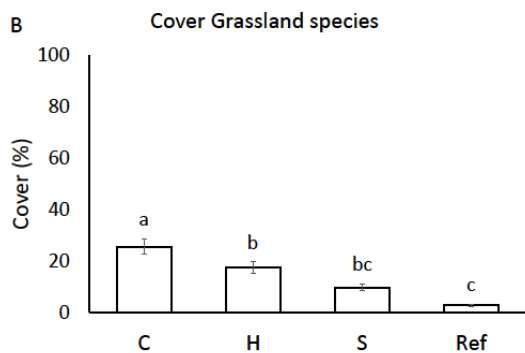
532 Figure 3. Acari (A) and Collembola (B) densities along the inoculum gradient after 2 years compared
 533 to reference heathlands. Means \pm S.E., letters indicate Tukey outcomes. C: control; H: herbage; S:
 534 sods and Ref: local reference heathland.

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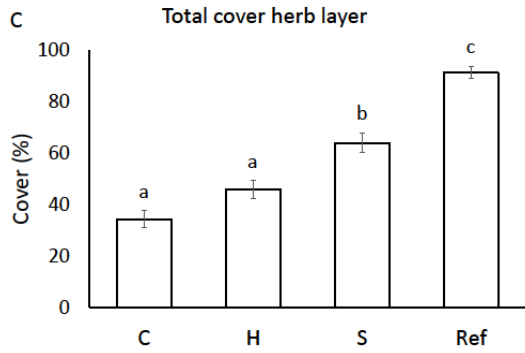
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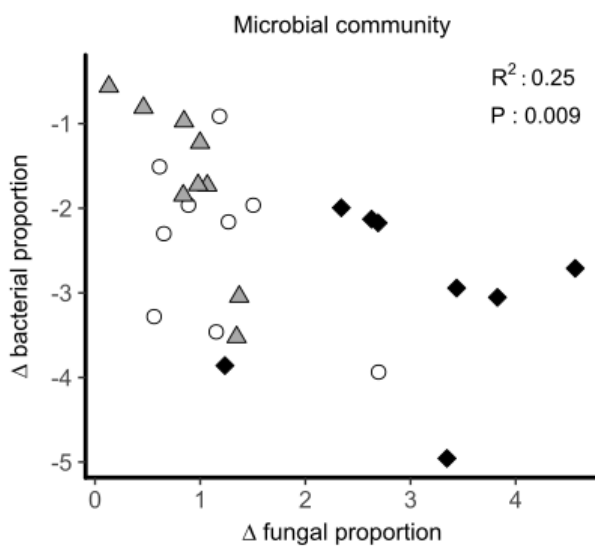
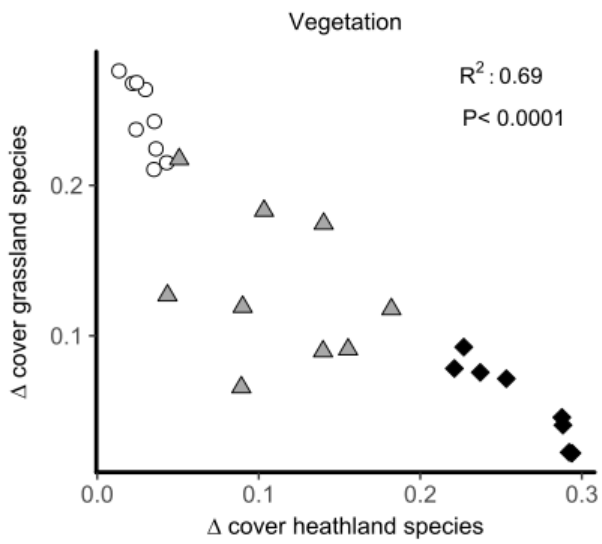


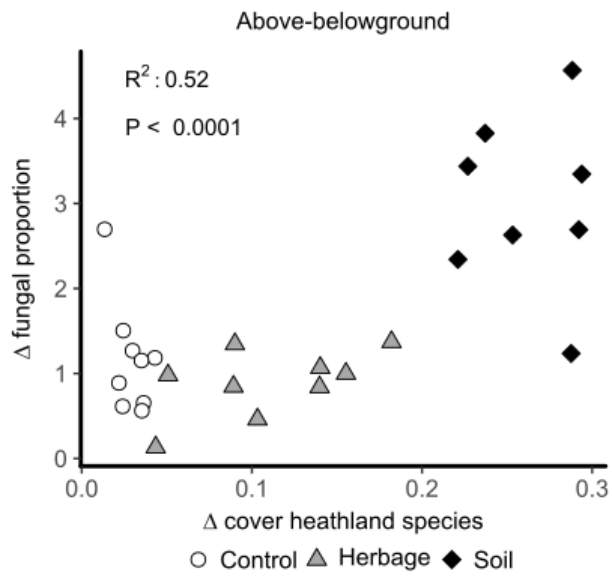
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Figure 4. Cover of heathland species (A), mesotrophic grassland species (B) and total herb cover (C) after 3 years. Means \pm SE, letters indicate Tukey outcomes. C: control; H: herbage; S: sods and Ref: reference heathland.





544

545 Figure 5. Correlations in the rate of change per year (Δ) within the above- (A) and below-ground (B)
 546 communities and between above- and belowground target species (C). Δ cover plant species in
 547 fraction of total cover per year, Δ microbes in mol% per year.

548

549

550 **Appendix. Statistical analysis**

551

552 *Table 1. Linear Mixed model results soil chemistry*

**IBM SPSS version 21
Mixed Model Analysis**

Treatments (Full factorial, random design, n=3)

Dry site only

Fixed:	treatment (9 treatments)	Acidified	elemental S	1500 kg/ha
Random:	Block	Limed	Dolokal	2000 kg/ha
	Time	Control-pH	No addition	-
Sample dates:	4-4-2012	Fresh Hay		1:2
	17-10-2012	Sods		1:15
	9-4-2013	Control-Biota	No addition	-
	29-10-2013			

Parameter Treatment		Treatments (full factorial)		Effect Biota-treatment only	
		F	p	F	P
Organc matter	%	1,039	,416		
Total-P	%	,830	,590		
Total-N	%	,651	,726		
Total-C	%	,444	,879		
NO3+NH4	µmol/kg soil	1,943	,062	2,212	,115
Olsen-P	µmol/kg soil	,699	,692		
pH_H2O		8,521	,000	,585	,559
Exchangeable Base Cations*	µeq/kg soil	8,527	,000	,869	,422

553

		Mean						
pH-treatment		Acidified			Control-pH			Li
Biota-treatment		Control_Biota	Fresh Hay	Sod	Control_Biota	Fresh Hay	Sod	Control_Biota
Organc matter	%	2,02	2,09	2,58	1,84	2,26	1,75	3,47
Total-P	%	0,86	1,02	0,9	0,98	1,32	0,81	1,23
Total-N	%	0,06	0,07	0,06	0,06	0,05	0,04	0,05
Total-C	%	1,25	1,58	1,24	1,31	1,06	0,98	0,97
NO3+NH4	µmol/kg soil	39,5	42,8	38,6	35,9	46,8	30,7	46,6
Olsen-P	µmol/kg soil	193	236	245	265	296	264	226
pH_H2O		5,5	5,67	5,52	5,91	5,8	5,82	6,22
Exchangeable Base Cations	µeq/kg soil	8205	10251	8555	8602	11437	9278	14790

554

555 Table 2. Results ANOVA model on inocula gradient with pooled pH data. In separate analyses either differences along the
556 inoculation gradient within the experiment were tested after which they were compared to the reference heathlands. For
557 the soil community $n = 9$ per treatment, for the vegetation $n = 18$, for the reference heathlands $n = 3$. *: $\ln(x+1)$ transformed.
558 C: control; H: hay addition, S: sod inoculation, GR: deep horizon original agricultural grassland (only microbes) and Ref:
559 reference heathlands.

Component	Parameter	ANOVA model experiment						ANOVA model experiment + reference								
		Inocula			Tukey test			Inocula			Tukey test					
		d.f	F	P	C	H	S	d.f	F	P	GR	C	H	S	Ref	
Microbes	Fungal marker	2	25.15	<0.0001	a	a	b	4	31.37	<0.0001	a	a	a	b	c	
	Bacterial markers	2	3.90	0.0348	a	a	a	4	3.54	0.0190	a	a	a	b	ab	
Nematodes	Bacteriophagous*	2	4.79	0.0178	a	a	b	3	32.37	<0.0001		a	a	b	c	
	Phytophagous*	2	4.06	0.0304	a	a	b	3	7.96	0.0006		a	a	a	b	
	Mycophagous*	2	2.75	0.0841	a	a	a	3	10.63	<0.0001		a	a	a	b	
	Omnivorous*	2	0.69	0.5111	a	a	a	3	2.15	.1185		a	a	a	a	
	Total*	2	2.84	0.0779	a	a	a	3	15.67	<0.0001		a	a	a	b	
Mesofauna	Acari*	2	6.63	0.0051	a	a	b	3	13.50	<0.0001		a	a	b	c	
	Collembola*	2	3.80	0.0369	a	a	b	3	4.02	0.0173		a	a	b	b	
	Total*	2	7.47	0.0030	a	a	b	3	13.38	<0.0001		a	a	b	c	
Vegetation	Cover heatland species	2	81.01	<0.0001	a	b	c	3	120.33	<0.0001		a	b	c	d	
	Cover grassland species	2	12.44	<0.0001	a	b	c	3	13.78	<0.0001		a	b	b	c	
	Total cover herb layer	2	17.82	<0.0001	a	a	b	3	29.06	<0.0001		a	a	b	c	

560

561

562 Table 3. Correlations between different parameters within and between communities. Paired values of all experimental plots
 563 (n = 27). Probabilities marked with an asterisk are significant after application of a Bonferroni correction to control the Type
 564 I error rate

Interaction	Level	Parameter 1	Parameter 2	Correlation	P
Within communities	Microbes	Fungal marker	Bacterial markers	-0.635	0.0005*
	Nematodes	Bacteriophagous	Mycophagous	0.627	0.0005*
		Bacteriophagous	Phytophagous	0.349	0.0742
		Mycophagous	Phytophagous	0.493	0.0090
		Total nematodes	Bacteriophagous	0.897	<0.0001*
		Total nematodes	Phytophagous	0.463	0.0151
		Total nematodes	Mycophagous	0.704	<0.0001*
	Mesofauna	Acari	Collembola	0.841	<0.0001*
		Total mesofauna	Acari	0.988	<0.0001*
		Total mesofauna	Collembola	0.916	<0.0001*
	Vegetation	Cover heathland	Cover grassland	-0.595	0.0011*
		Total cover	Cover heathland	0.858	<0.0001*
		Total cover	Cover grassland	-0.199	0.3206
Between communities	Microbes - vegetation	Fungal marker	Cover heathland	0.753	<0.0001*
		Fungal marker	Cover grassland	-0.342	0.0876
		Bacterial markers	Cover heathland	-0.462	0.0176
		Bacterial markers	Cover grassland	-0.008	0.9711
	Microbes - nematodes	Fungal marker	Mycophagous	0.321	0.1101
		Bacterial markers	Bacteriophagous	-0.154	0.4538
	Microbes - mesofauna	Fungal marker	Acari	0.589	0.0015*
		Fungal marker	Collembola	0.753	<0.0001*
		Fungal marker	Total mesofauna	0.656	0.0003
		Bacterial markers	Acari	-0.320	0.1111
		Bacterial markers	Collembola	-0.489	0.0112
		Bacterial markers	Total mesofauna	-0.380	0.0557
	Nematodes - vegetation	Phytophagous	Total cover	0.321	0.1027
		Phytophagous	Cover heathland	0.336	0.0866
		Phytophagous	Cover grassland	0.161	0.4218
		Total nematodes	Total cover	0.329	0.0939
	Mesofauna - vegetation	Acari	Cover heathland	0.342	0.0810
		Collembola	Cover heathland	0.361	0.0642
		Total mesofauna	Total cover	0.239	0.2299

565

566