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What is the effect of 19 years of restoration managements on soil and vegetation on formerly improved upland grassland?

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1 **To what extent did 19 years of restoration managements on formerly improved upland**
2 **grassland alter soil and vegetation?**

3

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16

17

18 **ABSTRACT**

19

20 Finding the best management strategies to restore grassland diversity and achieve a
21 compromise between agricultural use and biodiversity protection is a global challenge. This
22 paper reports novel data relating to the impacts of 19 years of restoration managements
23 predicted to increase botanical diversity within reseeded upland temperate grassland common
24 in less favoured areas in Europe. The treatments imposed were: continuous sheep grazing,
25 with and without lime application; hay cutting only, with and without lime application; hay
26 cutting followed by aftermath grazing, with and without lime application; and a control

27 treatment continuing the previous site management (liming, NPK application and continuous
28 sheep grazing).

29 Defoliation type, irrespective of liming, was the key driver influencing plant species
30 diversity (hay cutting followed by aftermath grazing > hay cutting > grazing). Grazing only
31 managements supported grasses at the expense of forbs, and thus related plant species
32 diversity significantly declined. Limed treatments had higher concentrations of Ca and Mg in
33 the soil compared to those receiving no lime. However, no effects on species richness or plant
34 species composition were found. Potassium was the only element whose plant-available
35 concentration in the soil tended to decrease in response to cutting treatments with herbage
36 removal.

37 Postponing the first defoliation to the middle of the growing season enables forbs to
38 reach seed production, and this was the most effective restoration management option for
39 upland grassland (as hay cutting only, and as hay cut followed by aftermath grazing).

40 Although continuous low-density sheep grazing is often adopted as a means of improving
41 floristic biodiversity, deleterious effects of this on plant diversity mean that it cannot be
42 recommended as a means of long-term maintenance or restoration management of European
43 temperate grasslands.

44

45 **Keywords:** Cutting, Grazing, Liming, Nutrients, Restoration, Species richness, Sward

46

47

48 **1. Introduction**

49 Grasslands are among the most important biotopes in Central Europe, providing
50 habitats where almost two-thirds of endangered plant species occur (Jongepierová et al.,
51 2018), and some type of defoliation is required for their management (Bakker, 1989). Both
52 intensive farming practices (e.g. high application rates of N, P and K plus frequent offtake of

53 biomass) and cessation of management can cause a significant decline in related biodiversity.
54 Finding optimal ways to restore grassland floristic diversity is the subject of much debate
55 amongst land managers, ecologists, and conservationists. The overall aim of fertilizer and/or
56 lime application in intensive grassland management is to increase biomass yield, but
57 increasing nutrient availability simultaneously reduces plant species richness (Bakker et al.,
58 2002; Hejcman et al., 2010; Storkey et al., 2015; Goulding et al., 2016; Humbert et al., 2016;
59 Titěra et al., 2020). Cessation of fertilization will not lead to immediate restoration of species-
60 rich grassland due to a combination of a lack of diaspore sources together with high residual
61 levels of available nutrients and changes in soil microbial activity (Pegtel et al., 1996; Smith
62 et al., 2008; Pavlů et al., 2011).

63 There are several strategies that can be applied to reverse nutrient accumulation caused
64 by intensive grassland management. Long-term biomass removal by hay-making or grazing
65 without fertilization are both seen as potential ways to remove soil nutrients (Hansson and
66 Fogelfors, 2000; Van Diggelen and Marrs, 2003) and increase plant species diversity.
67 However, reducing concentrations of excess nutrients within the soil and increasing species
68 richness through taking two or more annual cuts can be a lengthy and difficult process (Pavlů
69 et al., 2011), clearly depending on the period over which any particular management regime
70 has been adopted, on soil nutrient status, and vegetation structure. Consequently, conservation
71 management after nutrient removal should include the traditional practices that have
72 historically contributed to the formation of the biological diversity of semi-natural grasslands
73 (Bonari et al., 2017).

74 According to some authors it is possible to reduce K concentrations in the soil
75 relatively quickly (Parr and Way, 1988; Schaffers et al., 1998; Pavlů et al., 2013). However, P
76 reduction is reported as being much slower (Perring et al., 2009). High concentrations of
77 plant-available P in the soil are particularly associated with low species richness and

78 dominance of highly productive species (Janssens et al., 1998; Hejerman et al., 2010; Klaus et
79 al., 2011) while, in contrast, high soil K concentrations are compatible with high values of
80 plant diversity (Janssens et al., 1998; Crawley et al., 2005). Although Janssens et al. (1998)
81 conclude N is the main element limiting plant diversity, its availability is considered to be
82 controlled by P as this is an important nutrient for the symbiotic fixation of atmospheric N in
83 legumes and for the mineralization of organic matter in soils. The interdependency of P and N
84 was highlighted by the studies of Crawley et al. (2005) and Klaus et al. (2013). Lime
85 application to increase soil pH is another wide-spread agricultural practice undertaken to
86 improve herbage production through enhancing nutrient availability in the soil (Holland et al.
87 2018) that has a long-lasting impact on grassland (Spiegelberger et al., 2006). Liming can
88 have both negative and positive effects on plant diversity depending on the number of species
89 with different pH optima in a species pool. However, in areas characterised by long-term
90 acidification, liming is used as an important tool in the restoration of species-rich grassland
91 habitats (de Graaf et al., 1998).

92 The restoration of species-rich grassland on previously agriculturally-improved
93 pastures has several further specific abiotic and biotic constraints, including a degradation
94 period. In general, re-establishment of species from the seed bank is considered to be poor if
95 this degradation phase takes more than a few decades. However, if the lost species are still
96 present in the locally surrounding vegetation there is a chance to restore degraded
97 communities (van Diggelen and Marrs, 2003 and citations therein). In situations where
98 inappropriate abiotic conditions and lack of propagules are not barriers to re-establishment of
99 desirable vegetation, management regime can be a key driver influencing floristic diversity
100 (Pavlů et al., 2011). As indicated previously, two basic defoliation options are hay-making
101 and grazing, which can also be used in combination (van Diggelen and Marrs, 2003).
102 However, the effects of grazing and hay-making on species richness and plant species

103 composition differ in several ways (Hansson and Fogelfors, 2000; Krahulec et. al., 2001;
104 Mogg et al., 2002; Mládková et al. 2015). During hay-making the above-ground biomass is
105 non-selectively cut and removed at the same time, while factors affecting vegetation under
106 grazing management include stocking rate, selective grazing, trampling and nutrient
107 enrichment (Wallis DeVries, 1998; Stewart and Pullin, 2008; Ludvíková et al., 2014). Sheep
108 are more selective of forbs and legumes than cattle, with preferential grazing encouraged by
109 low grazing pressure (Dummont et al., 2011). A combination of grazing with cutting is
110 generally recommended to minimize conservation risks (Krahulec et al., 2001).

111 In this paper we describe grassland community status after 19 years of continual
112 exposure to various alternative restoration regimes. We compare the species composition and
113 richness, and soil chemical characteristics when managed according to one of seven regimes
114 that represent the common and best practices in less favoured areas dominated by temperate
115 European upland grassland. Within this context, we aimed to answer the following questions:
116 (i) what are the effects of long-term restoration managements on species richness, plant
117 species composition and soil chemical properties?, (ii) what is the effect of previous liming on
118 species richness, plant species composition and soil chemical properties?, and (iii) can any
119 regime can be recommended for restoring plant diversity of temperate upland grasslands?

120

121

122 **2. Materials and Methods**

123

124 *2.1. Experimental design*

125 The experimental plots used (the Brignant plots) were set up in 1994 to test the
126 effectiveness of various restoration management regimes in achieving reversion of upland
127 improved permanent pasture to semi-natural vegetation (Hayes and Tallwin, 2007). They

128 were established at the Pwllpeiran Upland Research Centre on permanent pasture that had
129 been ploughed and reseeded in 1973, and which had received regular inputs of fertilizer and
130 lime. Sown grass species still dominated the sward at the time the plots were established,
131 particularly *Lolium perenne*, at 58% cover. The plots are located at 310 m a.s.l. (O.S. Ref:
132 SN752757) on free-draining typical brown podzolic soils. The area receives a mean annual
133 rainfall of approximately 1850 mm and has average minimum and maximum air temperatures
134 of 5.2 °C and 11.9 °C respectively. The plots are arranged in a randomized block design with
135 three blocks and a total of seven grassland management regimes imposed (see Supplementary
136 materials Figs S1 and S2). The treatments, which have been running continuously since 1994,
137 are: sheep grazing, with (GL+) and without (GL-) lime application; hay cutting only, with
138 (HL+) and without (HL-) lime application; and hay cutting followed by aftermath sheep
139 grazing, with (HGL+) and without (HGL-) lime application. Control (CO) plots continuing
140 the previous site management (i.e. limed, fertilised and continually grazed by sheep) are also
141 included within each block. These receive an annual application of 60 kg ha⁻¹ N and 30 kg P
142 ha⁻¹, with K also applied as required to maintain an index of 2+ (ADAS, 1983). Soil samples
143 are taken in spring and tested for K concentration. Additional K is then applied by hand if the
144 concentration has fallen below that equivalent to an index of 2 (ADAS, 1983). All of the lime
145 treatment plots received a single application of lime in 1998 with the intention of maintaining
146 a soil pH of 6.0. Treatments are imposed on three replicate plots of 0.08 ha (hay cut only) or
147 0.15 ha (grazed) in size. The schematic block design of the experiments and an aerial photo
148 are provided in Appendix A, Figs S1 and S2.

149 From 1994 to 2012 the plots were stocked with ewes (usually Welsh Hill Speckled
150 Faced yearlings) with numbers adjusted to maintain a sward surface height of approximately 4
151 to 6 cm. Turnout occurred late April/early May when there was sufficient biomass to sustain
152 stock. There was no spring grazing of the HGL+ and HGL- treatments, to allow colonising

153 forb species an opportunity to establish. The HL+, HL-, HGL+, HGL- plots had a single hay
154 harvest taken annually after the 15th of July, as and when weather conditions allowed. Plots
155 were subsequently restocked on the HGL+ and HGL- treatments after a short period of re-
156 growth. All stock were removed at the end of September/early October, depending on
157 seasonal climatic conditions and related biomass growth.

158

159 *2.2. Measurements*

160

161 Data and samples were collected in July 2012, 18 years after the treatments were
162 imposed. Visual percentage cover of vascular plant species was estimated in ten randomly
163 located (0.4 m × 0.4 m) quadrats per plot in July 2012. The mean of ten quadrats of botanical
164 composition was used for statistical evaluation. The nomenclature of the plant species follows
165 Kubát et al. (2002). The plant species diversity was evaluated by plant species richness,
166 Shannon (H) species diversity index and Shannon (J) species evenness index (Begon et al.,
167 2005).

168 Fifteen individual soil cores were taken to a depth of 7.5 cm from randomly located
169 areas from within each plot. The soil cores per plot were bulked then air-dried, biomass
170 residues and roots were removed and the samples were then ground in a mortar to pass a 2
171 mm sieve. All chemical analyses were performed in an accredited laboratory of the Crop
172 Research Institute in Chomutov. Plant-available Ca, K, Mg and P were extracted by the
173 Mehlich III method (Mehlich 1984) and concentrations were determined by inductively
174 coupled plasma optical emission spectrometry (GBC Scientific Equipment Pty Ltd,
175 Melbourne, Australia). Determination of pH (CaCl₂) was done using a pH meter (Sentron
176 Welling, Leek, The Netherlands). Total N soil concentrations were determined using the
177 Kjeldahl method and organic C concentrations by the conventional oxidation procedure

178 incorporating chromo-sulphuric acid and colorimetry (AOAC, 1984).

179

180 2.3. Data analysis

181

182 A linear mixed-effects model (LMM) with fixed effects of treatment and random effect
183 of the block was used to analyse the effect of treatment on selected plant species, cover of
184 graminoids and forbs, species richness, Shannon (H) species diversity index, Shannon (J)
185 species evenness index, and soil chemical properties. If necessary, data was log-transformed
186 to meet LLM assumption requirements. Benjamini-Hochberg's procedure was applied to
187 control for false-discovery rate (FDR) (Verhoeven, Simonsen, & McIntyre 2005). To identify
188 significant differences between individual treatments a post-hoc comparison using Tukey's
189 range test was applied. All LMM analyses were performed in Statistica 13.1 (Dell Inc., Texas,
190 2016).

191 Redundancy analysis (RDA) in the CANOCO 5 program (ter Braak and Šmilauer,
192 2012) was used to evaluate multivariate vegetation and soil data. All cover, soil and herbage
193 chemical properties data in RDA were logarithmically transformed [$y = \log(y + 1)$]. For
194 multivariate data analyses, two approaches were taken: i) impact of liming (comparing
195 Limed+ versus Limed- treatments, regardless of cutting management); ii) impact of
196 defoliation management (comparing Grazing, Hay, and Hay + Grazing treatments, regardless
197 of liming). For all analyses 999 permutations were performed, with blocks used as covariables
198 to restrict permutations into blocks. To visualize the results of the RDA analyses standard
199 biplot ordination diagrams were generated.

200

201

202

203 **3. Results**

204

205 *3.1. Species richness*

206

207 Significant differences in the mean numbers of all vascular plant species, the mean
208 numbers of vascular plant species with cover $\geq 1\%$, and Shannon (H) species diversity indices
209 were recorded between treatments (Table 1). Shannon (J) species evenness indices did not
210 show significant differences between treatments. The mean numbers of all vascular plant
211 species, the mean numbers of vascular plant species with cover $\geq 1\%$ and the Shannon species
212 diversity indices were almost always significantly higher under treatments that included a
213 cutting regime (HL+, HL-, HGL+, and HGL-) than those which included grazing
214 management only (GL+, GL-, CO) (Fig. 1). Fertilizer applications and liming had no
215 significant influence on species richness or diversity (Table 1).

216

217 *3.2. Plant species composition*

218

219 Based on RDA the effect of liming on plant species composition was negligible, and
220 most of the variability in plant species composition was explained by defoliation regime
221 (Table 2, Analyses 1 – 3). The first axis of the RDA of the vegetation data (Table 2, Analysis
222 1) displayed a gradient of defoliation management (Fig. 2). Three groups of treatments with
223 similar plant species composition were identified on the ordination diagram: CO, GL+ and
224 GL- treatments as the first group; HL+ and HL- treatments as the second group; and HGL+
225 and HGL- as the third group. The first group was species poor (number of species <12) and
226 favoured grasses (especially *Poa pratensis*, *Agrostis capillaris*, *Festuca rubra*) and *Urtica*
227 *dioica*. The second group was medium species rich (number of species about 17), and

228 included *Alopecurus pratensis*, *Cerastium holosteoides*, *Cirsium palustre*, *Holcus mollis*. The
229 third group was the most species rich (number of species >19) and included the forb species
230 *Betonica officinalis*, *Hypochoeris radicata*, *Potentilla erecta*, *Ranunculus acris*, *Taraxacum*
231 spp.

232 Based on LMM results the cover of graminoids was significantly supported by grazing
233 only management regimes (CO, GL-, GL+) regardless of fertiliser and liming inputs (Table
234 1). The total cover of graminoids in these treatments ranged from 112.8 ± 3.9 % (GL-) to
235 117.3 ± 1.6 % (GL+), whereas the cover in the treatments that incorporated cutting ranged
236 from 46.0 ± 3.9 % (HGL) to 58.6 ± 4.0 % (HGL+). Grazing managements (CO, GL+, GL-)
237 supported the dominant grasses *A. capillaris* and *P. pratensis*. While there was a tendency for
238 *Anthoxanthum odoratum* to be suppressed by fertilizer applications (CO), *Festuca rubra*
239 tended to be supported by a grazing only management.

240 Unlike graminoids, forbs were significantly suppressed by grazing only management
241 (CO, GL-, GL+), regardless of fertilization and liming (Table 1). The total cover of forbs for
242 the grazed treatments ranged from 2.5 ± 1.5 % (GL+) to 3.3 ± 1.1 % (GL-), whereas for the
243 cut treatments the total cover ranged from 59.5 ± 4.8 % (HGL+) to 65.7 ± 3.3 % (HL+). The
244 main species (*Plantago lanceolata*, *Ranunculus repens*, *Rumex acetosa*), as well as other less
245 abundant species (such as *Ranunculus acris*, *C. holosteoides*, *Crepis capillaris*, *Leontodon*
246 *autumnalis*, *Rhinanthus minor* and *Taraxacum* spp.), were supported by cutting management
247 regimes, whereas *Trifolium repens* cover was not influenced by defoliation method.

248

249 3.3. Soil chemical properties

250

251 Redundancy analysis indicated that management regime explained the highest
252 proportion of the soil nutrient content variability in the first and all axes (Table 2, Analyses 4

253 – 6). The first axis of RDA displayed a gradient of soil pH (Fig. 3). Soil from the plots that
254 had received lime had a higher pH and plant available concentrations of Ca and Mg. Plant
255 available concentrations of P, K and organic C were positively correlated with the CO
256 treatment fertilized by N, P and K. Liming plot treatment was the second best explanatory
257 predictor for soil chemical properties (Table 2, Analyses 4 – 6).

258 Results from LMM show that management regime did not influence the
259 concentrations of N_{tot} , C_{org} , or the C:N ratio in the soil (Table 3). Concentrations of K in the
260 soil had a tendency ($P=0.040$) to be higher in the grazing only managements. Liming
261 influenced concentrations of Ca and Mg in the soil. The concentrations of Ca were highest in
262 all formerly limed treatments, and a similar response was found for Mg. The highest
263 concentration of P was found in CO plots. Plant species richness was negatively correlated
264 with soil K concentration ($P = 0.015$, $r = -0.52$), however, there was only a trend for P
265 concentration in the soil to impact species richness. Concentrations of other soil nutrients did
266 not influence species richness.

267

268 4. Discussion

269

270 4.1. Species richness and plant species composition

271

272 Our results showed that type of defoliation (cutting, grazing, and their combination)
273 influenced plant species composition, species richness and Shannon (H) species diversity
274 indices more than fertilizer applications (CO treatment) or liming. This is in accordance with
275 previous studies that found that changes in species composition (Köhler et al., 2001) and
276 species richness (Parr and Way, 1988) were more affected by type of disturbance than by
277 minor changes in soil nutrients.

278 The grazing only management regimes encouraged an increase in grasses at the
279 expense of forbs, regardless of fertilizer applications and liming, leading to decreasing species
280 richness and Shannon (H) species diversity indices. In contrast, hay cutting and hay cutting
281 followed by aftermath grazing showed the highest species richness, linked to increased forb
282 cover, which was likely the result of there being no sward disturbance until the middle of
283 July. Dominance by grasses is a typical response of temperate grassland to frequent sward
284 defoliation (Louault et al., 2005; Pavlů et al., 2007; Ludvíková et al., 2015). Likewise, grazing
285 management in comparison to cutting has been shown to promote dominance of grasses at the
286 expense of forbs (Krahulec et al., 2001; Dumont et al., 2011; Mládková et al. 2015), and
287 therefore mowing is generally advocated in situations where species richness maintenance is
288 the main goal of sward management (Hansson and Fogelfors, 2000). However, our data show
289 that similar outcomes can be obtained by hay cutting followed by aftermath grazing. Although
290 liming has been shown to frequently have positive effects on species richness in grasslands
291 (de Graaf et al., 1998; Holland et al. 2018), this was not evident during the current study. It is
292 likely that this is a result of the vegetation found in the surroundings of the experiment being
293 adapted to growth in the acidic brown podzolic soils at the site.

294

295 *4.2. Soil chemical properties*

296

297 Although it is generally considered that P and N_{tot} concentrations in the soil have
298 negative effects on grassland diversity (Janssens et al., 1998; Crawley et al., 2005; Hejcman et
299 al., 2010; Pruchniewicz and Żołnierz, 2014), the results of our experiment show that N did not
300 have any effect on plant species diversity. Low soil P concentrations in the locality were
301 likely responsible for only a negative tendency of P concentration in the soil to influence
302 species richness. Furthermore, Janssen et al. (1998) hypothesised that while N is the main

303 nutrient limiting plant diversity, its availability is controlled by P.

304 It is assumed that K concentrations in the soil do not strongly influence plant species
305 richness (Crawley, 2005) and that a higher K concentration is compatible with high levels of
306 sward diversity (Janssens et al., 1998). In our experiment, K concentration in the soil had a
307 tendency to be higher not only in the control treatment with fertilizer applications, but also in
308 both other grazing only treatments, where a proportion of the nutrients removed were returned
309 in sheep excreta. Cutting with biomass removal resulted in a decrease in soil K
310 concentrations, as has been reported previously (Parr & Way, 1988; Schaffers et al., 1998;
311 Alfaro et al., 2003, 2004; Hejzman et al., 2010; Pavlů et al., 2013; Pavlů et al., 2016). The
312 negative relationship between plant-available K and species richness was likely due to the
313 relatively high concentrations of K, even after the long-term removal of herbage biomass
314 from cut plots. So, although it appears that reductions in species richness are connected to
315 higher soil K concentrations, these decreases may be predominantly linked, once again, to
316 defoliation management.

317

318 *4.3. Management implications*

319

320 Restoration of improved permanent pasture is a long-term process that can be
321 successful if several conditions (as summarized by van Diggelen and Marrs, 2003) can be
322 fulfilled: (i) abiotic resource levels are within thresholds of target communities and species,
323 (ii) sufficient viable propagules of target species are available at a rate that supports rapid
324 establishment of desired species, and (iii) appropriate management regimes well adapted to
325 target species requirements are in place.

326 Although it is generally believed that extensive grazing is a suitable management
327 technique to maintain and restore plant diversity of temperate grassland (e.g. van Diggelen

328 and Marrs, 2003; Marriot et al., 2009; Jacquemyn et al., 2011; Ludvíková et al., 2015;
329 Moinardeau et al., 2016) it is necessary to consider factors such as type of grazing animal and
330 the grazing system. As indicated previously, sheep can be highly selective grazers and
331 consistently sort the best quality components from within multi-species swards (Garcia et al.,
332 2003). Thus, a low species richness is typical of swards managed by continuous sheep
333 grazing, regardless of grazing intensity (Marriot et al., 2009). While grazing by less selective
334 stock is preferable, economic and socioeconomic pressures have led to a reduction in cattle
335 numbers within marginal areas of the UK and similar regions within the EU, despite targeted
336 support within agri-environment schemes.

337 While the current study has established the comparative benefits to plant species
338 richness of hay cutting, the practicality of this management option in upland fringe areas is
339 commonly compromised by topography, terrain and climatic conditions (especially rainfall).
340 Alternatives based on delayed or rotational grazing systems should be explored if reliance on
341 support payments to offset reduced productivity is to be avoided.

342

343 **5. Conclusion**

344

345 Measurements carried out on this long-term restoration experiment provide several
346 clear messages. The first is that a higher species richness with a high proportion of forbs was
347 observed on treatments in which cutting with biomass removal is included, whereas
348 treatments with only grazing were linked to low species richness with a higher proportion of
349 grasses in the sward. Secondly, K was the only element whose plant available concentration
350 in the soil tended to decrease in response to cutting treatments with herbage removal. Higher
351 concentrations of Ca and Mg in the soil in treatments with former liming had no effect on
352 species richness and plant species composition. Finally, continuous sheep grazing is not

353 recommended as a means of maintaining or restoring plant diversity within temperate
354 grasslands. Postponing the timing of the first defoliation (hay cutting) to mid growing season,
355 thus allowing forbs to reach the reproductive stage, would be the most effective restoration
356 management option for upland grassland.

357

358 **CrediT authorship contribution statement**

359 **Lenka Pavlů:** Investigation, Conceptualization, Methodology, Writing-Original draft. **Vilém**

360 **V. Pavlů:** Investigation, Formal analysis, Visualization, Writing-Original Draft. **Mariecia D.**

361 **Fraser:** Investigation, Resources, Supervision, Writing- Reviewing and Editing.

362

363 **Declaration of competing interest**

364 The authors declare that they have no known competing financial interests or personal
365 relationships that could have appeared to influence the work reported in this paper.

366

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375 **Declaration of competing interest**

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379

380 **Appendix A. Supplementary data**

381

382 Supplementary data associated with this article can be found, in the online version, at

383 XXX

384

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535

536 **Table 1**

537 Cover (%) of the most abundant vascular plant species in alphabetical order; cover (%) of total graminoids and total forbs;
 538 number of all plants species and plant species $\geq 1\%$; Shannon index (H) and Shannon species evenness index (J) under the
 539 different treatments in 2012. Treatment abbreviations: CO = control, continuing the previous site management with liming,
 540 fertilising and grazing by sheep, GL+ = sheep grazing with liming application, GL- = sheep grazing without liming application,
 541 HL+ = hay cutting only with liming application, HL- = hay cutting only without liming application, HGL+ = hay cutting
 542 followed by aftermath sheep grazing with liming application, HGL- = hay cutting followed by aftermath sheep grazing
 543 without liming application. Numbers represent average of three replicates \pm standard error of the mean (SE); *F*-ratio = *F*-
 544 statistics for the test of a particular analysis; *P*-value = corresponding probability value. In cases of significant differences
 545 obtained by linear mixed-effects modelling after table-wise Benjamini-Hochberg's FDR correction (highlighted in bold), a
 546 post hoc comparison using the Tukey's HSD test was applied to identify significant differences between treatments.
 547 Differences are indicated by different small letters within rows.

	Treatment								
	<i>F</i> -ratio	<i>P</i> -value	CO	GL+	GL-	HL+	HL-	HGL+	HGL-
Number of all plant species	15.43	<0.001	8.3 \pm 1.9 c	9.3 \pm 1.5 c	11.3 \pm 0.3 bc	17.3 \pm 1.2 ab	17.3 \pm 0.9 ab	19.3 \pm 1.2 a	20.3 \pm 0.7 a
Number of plant species $\geq 1\%$	14.39	<0.001	6.0 \pm 1.5 b	6.3 \pm 0.3 b	6.7 \pm 0.9 b	11.3 \pm 1.2 a	11.7 \pm 0.3 a	13.3 \pm 0.3 a	14.0 \pm 0.6 a
Shannon (H) diversity index	14.05	<0.001	1.4 \pm 0.2 c	1.6 \pm 0.1 c	1.7 \pm 0.1 bc	2.2 \pm 0.1 a	2.1 \pm 0.1 ab	2.3 \pm 0.1 a	2.4 \pm 0.1 a
Shannon (J) evenness index	1.70	0.20	0.7 \pm 0.04	0.7 \pm 0.04	0.7 \pm 0.05	0.8 \pm 0.03	0.7 \pm 0.01	0.8 \pm 0.01	0.8 \pm 0.01
Total graminoids	101.76	<0.001	116.6 \pm 1.9 a	117.3 \pm 1.6 a	112.8 \pm 3.9 a	52.1 \pm 3.8 b	55.6 \pm 4.1 b	58.6 \pm 4.0 b	46 \pm 3.9 b
Total forbs	159.35	<0.001	2.8 \pm 1.5 b	2.5 \pm 1.5 b	3.3 \pm 1.1 b	65.7 \pm 3.3 a	62.2 \pm 2.3 a	59.5 \pm 4.8 a	63.3 \pm 2.7 a
<i>Anthoxanthum odoratum</i>	4.08	0.018	0.43 \pm 0.4 b	13.3 \pm 5.2 ab	17.7 \pm 2.4 a	10.1 \pm 5.5 ab	12.2 \pm 4.4 ab	10.3 \pm 2.2 ab	8.9 \pm 4.8 ab
<i>Agrostis capillaris</i>	4.93	0.009	32.5 \pm 4.9 ab	36.0 \pm 5.1 ab	43.2 \pm 9.6 a	11.1 \pm 2.3 b	25.2 \pm 8.5 ab	18.9 \pm 4.4 ab	15.8 \pm 3.6 b
<i>Cynosurus cristatus</i>	2.26	0.10	0.0	0.7 \pm 0.7	0.3 \pm 0.3	1.1 \pm 0.95	0.4 \pm 0.4	3.3 \pm 1.3	0.4 \pm 0.2
<i>Festuca rubra</i>	3.26	0.038	29.2 \pm 4.5	28.7 \pm 0.7	21.8 \pm 3.8	18.6 \pm 2.1	12.0 \pm 6.1	16.9 \pm 6.5	14.4 \pm 5.0
<i>Holcus lanatus</i>	3.19	0.041	8.9 \pm 2.9	10.2 \pm 0.6	7.4 \pm 0.4	6.1 \pm 1.1	3.0 \pm 2.02	6.7 \pm 0.6	3.0 \pm 1.5
<i>Lolium perenne</i>	1.84	0.171	1.8 \pm 1.8	0.3 \pm 0.3	5.6 \pm 4.0	0.0	0.0	0.03 \pm 0.03	0.003 \pm 0.003
<i>Poa pratensis</i>	7.15	0.002	43.7 \pm 14.2 a	28.2 \pm 8.4 a	16.5 \pm 5.6 a	2.0 \pm 1.1 b	1.4 \pm 0.9 b	1.1 \pm 0.6 b	1.0 \pm 0.3 b
<i>Trifolium repens</i>	1.01	0.457	0.4 \pm 0.4	1.5 \pm 1.2	1.1 \pm 0.7	0.0	0.0	3.0 \pm 2.3	0.8 \pm 0.4

<i>Cerastium holosteoides</i>	3.33	0.036	0.0	0.0	0.0	0.5 ± 0.3	0.9 ± 0.3	0.13 ± 0.13	0.4 ± 0.4
<i>Crepis capillaris</i>	2.10	0.129	0.0	0.0	0.0	6.3 ± 4.1	1.7 ± 1.7	3.9 ± 1.9	5.3 ± 2.7
<i>Leontodon autumnalis</i>	1.97	0.148	0.0	0.0	0.0	3.9 ± 3.9	7.0 ± 3.6	1.1 ± 1.1	0.2 ± 0.2
<i>Plantago lanceolata</i>	10.53	<0.001	0.0 b	0.0 b	0.0 b	9.3 ± 3.9 ab	14.6 ± 2.1 ab	19.7 ± 6.0 a	22.0 ± 1.1 a
<i>Ranunculus acris</i>	8.98	<0.001	0.0 b	0.03 ± 0.03 b	0.0 b	2.7 ± 1.4 ab	1.4 ± 0.8 b	3.0 ± 0.4 ab	5.4 ± 1.3 a
<i>Ranunculus repens</i>	10.28	<0.001	0.1 ± 0.1 c	0.03 ± 0.03 c	0.4 ± 0.1 c	25.5 ± 7.0 a	18.6 ± 4.3 ab	13.1 ± 3.8 abc	8.3 ± 2.3 bc
<i>Rhinanthus minor</i>	1.24	0.35	0.0	0.0	0.0	0.6 ± 0.5	1.9 ± 1.9	0.3 ± 0.2	3.2 ± 2.6
<i>Rumex acetosa</i>	31.93	<0.001	1.0 ± 0.6 c	0.1 ± 0.1 c	1.4 ± 1.3 c	16.2 ± 2.4 a	14.7 ± 1.0 a	12.1 ± 2.6 ab	7.4 ± 1.6 b
<i>Taraxacum spp.</i>	2.33	0.099	0.0	0.0	0.0	0.4 ± 0.2	0.5 ± 0.2	1.3 ± 0.6	1.7 ± 1.0

548

549

550 **Table 2**

551 Results of redundancy analyses for six different H0 analyses (A1-A3 for vegetation, A4-A6 for
 552 soil); % expl. = explained variation by axis 1 (adjusted explained variation by all ordination
 553 axes), a measure of the explanatory power of the explanatory variables; *F*-ratio = *F*-statistics for
 554 the test of a particular analysis; *P*-value = corresponding probability value obtained by the Monte
 555 Carlo permutation test. Treatment abbreviations (CO, GL+, GL, HL+, HL-, HGL+, HGL-) are
 556 defined in Table 1.

Analysis	Expl. var.	Covariables	% expl.	<i>F</i> -ratio	<i>P</i> -value
Vegetation					
A1 Different grassland managed regimes have no effect on plant species composition	CO, HGL+, HGL-, HL+, HL-, GL+, GL-	blocks	58.8 (77.2)	18.6 (7.3)	0.001 (0.001)
A2 Different defoliation management regimes have no effect on plant species composition	Grazing, Hay, Grazing+Hay	blocks	58.6 (65.3)	24.0 (16.0)	0.001 (0.001)
A3 Liming has no effect on plant species composition	Limed+, Limed-	blocks	5.8	1.1	0.309
Soil					
A4 Different grassland managed regimes have no effect on soil properties	CO, HGL+, HGL-, HL+, HL-, GL+, GL-	blocks	44.8 (65.5)	10.6 (4.1)	0.002 (0.001)
A5 Different defoliation management regimes have no effect on soil properties	Grazing, Hay, Grazing+Hay	blocks	13.5 (15.4)	2.7 (1.5)	0.167 (0.179)
A6 Liming has no effect on soil properties	Limed+	blocks	42.3	13.2	0.001

557 **Table 3**

558 Soil characteristics per plot under the different treatments in 2012. Treatment abbreviations (CO, GL+, GL, HL+, HL-, HGL+,
 559 HGL-) are defined in Table 1. Numbers represent average of three replicates \pm standard error of the mean (SE); *F*-ratio = *F*-
 560 statistics for the test of a particular analysis; *P*-value = corresponding probability value. In cases of significant differences
 561 obtained by linear mixed-effects modelling after table-wise Benjamini-Hochberg's FDR correction (highlighted in bold), the
 562 post hoc comparison using the Tukey's HSD test was applied to identify significant differences between treatments.
 563 Differences are indicated by different small letters within rows.

	Characteristics	<i>F</i> -ratio	<i>P</i> -value	Treatment						
				CO	GL+	GL-	HL+	HL-	HGL+	HGL-
Soil up to 7.5 cm	pH	12.78	<0.001	4.73 \pm 0.05 ab	4.89 \pm 0.03 a	4.54 \pm 0.10 bc	4.91 \pm 0.01 a	4.41 \pm 0.09 c	4.74 \pm 0.05 ab	4.43 \pm 0.04 c
	C _{org} (%)	1.10	0.430	8.28 \pm 0.66	7.18 \pm 0.77	7.46 \pm 0.19	6.83 \pm 0.18	7.02 \pm 0.27	7.51 \pm 0.16	7.27 \pm 0.13
	N _{tot} (%)	0.41	0.854	0.51 \pm 0.11	0.53 \pm 0.05	0.56 \pm 0.02	0.66 \pm 0.11	0.55 \pm 0.03	0.58 \pm 0.01	0.57 \pm 0.02
	P mg kg ⁻¹	4.11	0.018	25.01 \pm 5.38 a	13.78 \pm 1.96 b	16.00 \pm 1.67 ab	16.65 \pm 1.02 ab	14.57 \pm 0.24 b	13.16 \pm 1.09 b	13.28 \pm 0.73 b
	K mg kg ⁻¹	3.22	0.040	172.53 \pm 7.11	153.07 \pm 17.27	175.91 \pm 24.86	121.63 \pm 9.26	121.72 \pm 4.95	141.47 \pm 3.99	122.62 \pm 6.41
	Ca mg kg ⁻¹	12.62	<0.001	1395 \pm 136 a	1390 \pm 115 a	813 \pm 112 bc	1415 \pm 123 a	773 \pm 213 c	1261 \pm 73 ab	714 \pm 144 c
	Mg mg kg ⁻¹	6.75	0.002	278.83 \pm 17.92 ab	290.38 \pm 25.82 a	207.31 \pm 17.36 abc	302.16 \pm 32.70 a	183.31 \pm 32.39 bc	269.93 \pm 20.57 abc	177.63 \pm 17.81 c
	C:N	2.07	0.103	18.0 \pm 3.6	13.7 \pm 0.7	13.3 \pm 0.8	11.6 \pm 1.8	12.9 \pm 0.5	12.9 \pm 0.2	12.8 \pm 0.4

564

565

566

567 **Fig. 1.** Species richness under different defoliation management regimes: sheep grazing, hay
568 cutting, hay cutting followed by aftermath sheep grazing. Error bars represent standard error
569 of the mean. Significant differences ($P < 0.05$) according to the Tukey post-hoc test are
570 indicated by different letters.

571

572 **Fig. 2.** Ordination diagram representing the results of redundancy analysis showing changes
573 in plant species composition, treatments were used as predictors. (see Table 2, Analysis 1 for
574 details).

575 Treatment abbreviations: CO = control, continuing the previous site management with liming,
576 fertilising and grazing by sheep, GL+ = sheep grazing with liming application, GL- = sheep
577 grazing without liming application, HL+ = hay cutting only with liming application, HL- =
578 hay cutting only without liming application, HGL+ = hay cutting followed by aftermath sheep
579 grazing with liming application, HGL- = hay cutting followed by aftermath sheep grazing
580 without liming application. Species abbreviations are based on the first four-letter of genera
581 and the four-letter of species name: Achilmile = *Achillea millefolium*, Agrocapi = *Agrostis*
582 *capillaris*, Aloppra = *Alopecurus pratensis*, Anthodor = *Anthoxanthum odoratum*, Betooffi =
583 *Betonica officinalis*, Brommoll = *Bromus mollis*, Cardprat = *Cardamine pratensis*, Ceraholo
584 = *Cerastium holosteoides*, Cirspalu = *Cirsium palustre*, Crepcapi = *Crepis capillaris*,
585 Cynocris = *Cynosurus cristatus*, Euphspp = *Euphorbia spp.*, Festrubr = *Festuca rubra*,
586 Holclana = *Holcus lanatus*, Holcmoll = *Holcus mollis*, Hyporadi = *Hypochoeris radicata*,
587 Leonautu = *Leotodon autumnalis*, Lolipere = *Lolium perenne*, Luzucamp = *Luzula*
588 *campestris*, Planlanc = *Plantago lanceolata*, Phleprat = *Phleum pratense*, Poaprat = *Poa*
589 *pratensis*, Poteerec = *Potentilla erecta*, Ranuacri = *Ranunculus acris*, Ranurepe =
590 *Ranunculus repens*, Rhinmino = *Rhinanthus minor*, Rumeacet = *Rumex acetosa*, Stelgram =

591 *Stellaria graminea*, *Taraspp* = *Taraxacum spp.*, *Triferep* = *Trifolium repens*, *Veroserp* =

592 *Veronica serpyllifolia*, *Veroarve* = *Veronica arvensis*, *Urtidioi* = *Urtica dioica*

593

594 **Fig. 3.** Ordination diagram representing the results of redundancy analysis showing changes

595 in nutrient concentrations in the soil at a depth of 0-7.5 cm, treatments were used as

596 predictors. (see Table 2, Analysis 4 for details). Treatment abbreviations (CO, GL+, GL,

597 HL+, HL-, HGL+, HGL-) are defined in Figure 1. Abbreviations: pH-soil acidity, Corg-

598 organic carbon; Ntot-total nitrogen in the soil; P, K, Mg, Ca-plant available nutrients; C:N-

599 ratio in the soil

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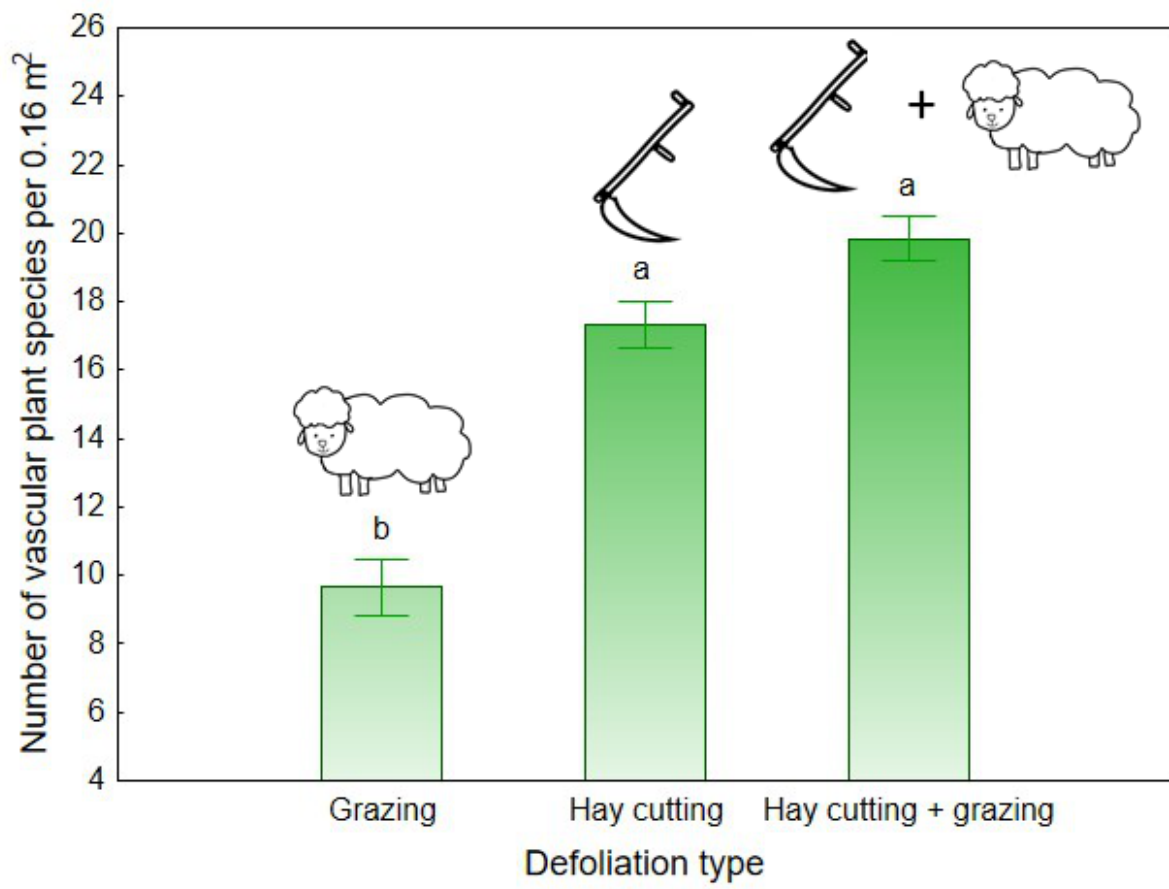
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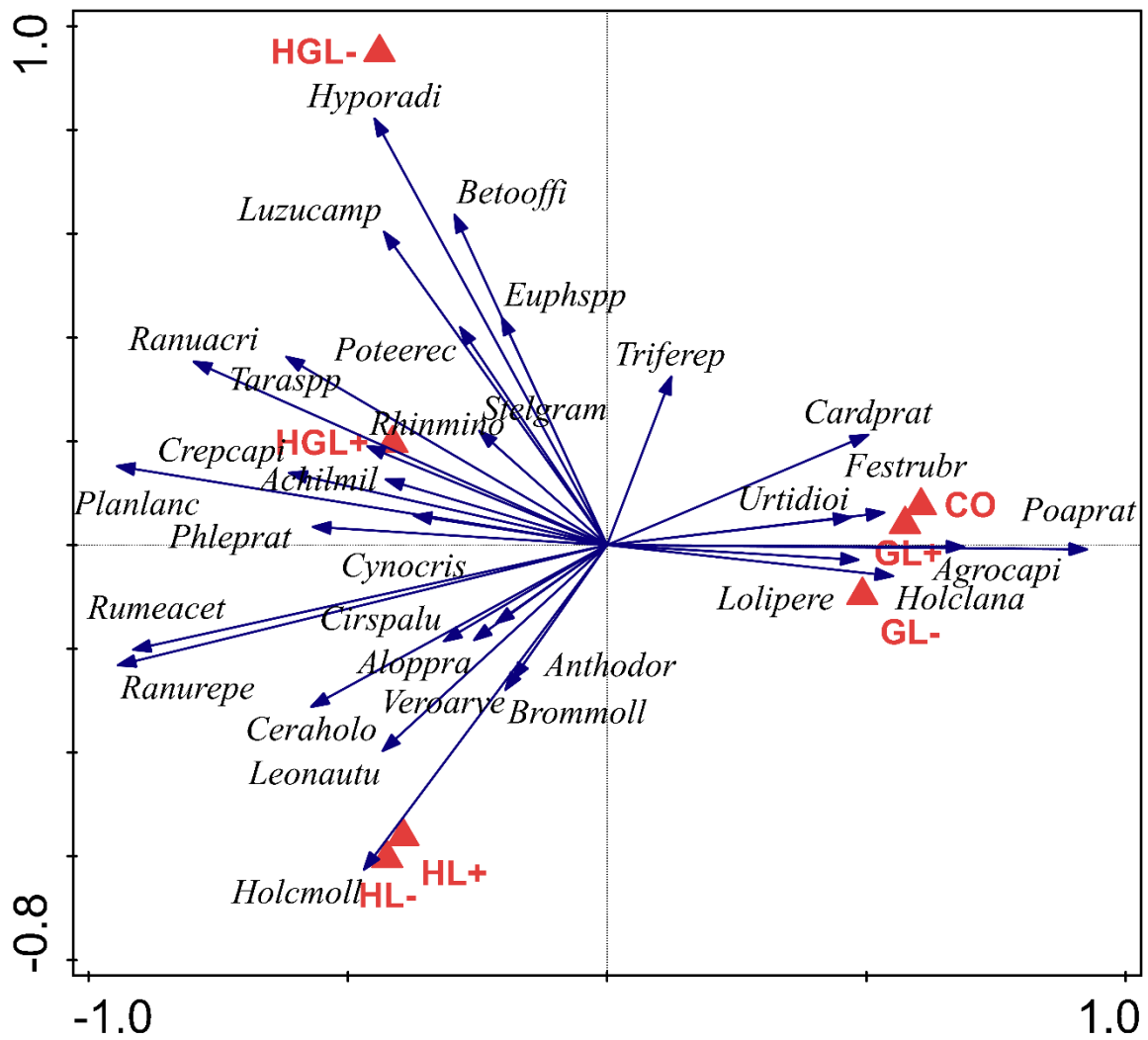
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612 Figure 1

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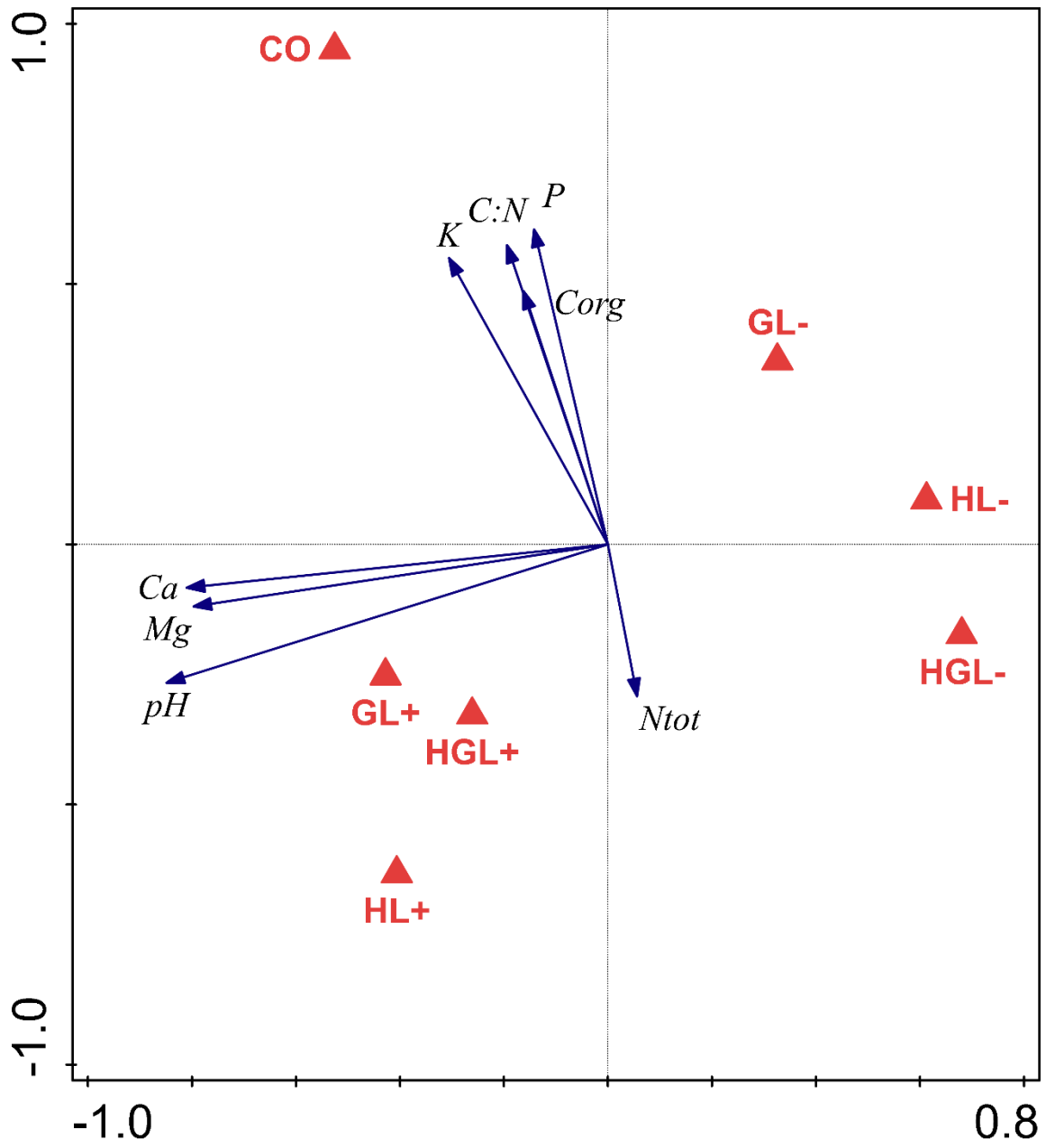
615 Figure 2

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620 Figure 3