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13 **Restoration treatments to control *Molinia arundinacea* and woody and alien species**
14 **encroachment in *Calluna vulgaris* heathlands at the southern edge of their distribution**

15
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25

26 **Abstract**

27 Throughout the last decades, *Calluna vulgaris* (L.) Hull heathlands have declined across Europe
28 and nowadays their conservation is particularly challenging at the southern edge of their
29 distribution. In the Nature Reserve of Vauda (north-western Italy), six restoration treatments were
30 applied (extensive annual goat browsing, one-off mowing, annual mowing, one-off fire without and
31 with annual browsing, and annual fire) and their effects on plant diversity and the cover of *C.*
32 *vulgaris*, its competitor grass *Molinia arundinacea* Schrank, woody, and alien species were
33 monitored between 2005 and 2011. In the short-term, most of the treatments changed the vegetation
34 community, reducing *C. vulgaris* cover according to a gradient of increasing biomass removal. In
35 the mid-term, *C. vulgaris*, *M. arundinacea*, woody and alien species cover followed different
36 trajectories according to the treatment and functional group. Annual fire shifted the vegetation
37 towards a *M. arundinacea*-dominated community, while extensive annual browsing did not affect
38 the heathland community and resulted in the lowest increase in *M. arundinacea*, which showed a
39 remarkable fitness in these environments. Moreover, annual burning and mowing were effective in
40 reducing woody species encroachment ($p < 0.05$), and fire treatments triggered a peak in alien
41 species cover (mainly *Panicum acuminatum* Swartz) in the short-term. Six years after treatment,
42 species richness and Shannon index did not differ between treated and control sites ($p > 0.05$). In
43 conclusion, these results highlight the need and potential benefit of integrating multiple techniques
44 to preserve *C. vulgaris* heathlands at their southern edge.

45 **Keywords.** Goat browsing, mowing, *Panicum acuminatum*, plant diversity, prescribed burning.

46 1. Introduction

47 The conservation of threatened habitats is particularly challenging near their range edges, where
48 populations are smaller, fragmented, and more vulnerable to environmental changes (Sexton et al.,
49 2009). Techniques for habitat restoration, which are effective at the core of target species
50 distribution, may have unexpected outcomes at the distribution edges. This is the case of *Calluna*
51 *vulgaris* (L.) Hull heathlands, a key European cultural landscape and habitat (EU Council Directive,
52 1992), developed after human mediated disturbance regimes, such as grazing, burning, and mowing
53 (Davies et al., 2016; Fagundez, 2013). Nowadays, heathlands are declining in most European
54 countries due to different drivers of land use and environmental change. The abandonment of
55 traditional management has often led to their conversion to woodlands (Pywell et al., 2011).
56 Moreover, increasing atmospheric nitrogen deposition has favored the replacement of *C. vulgaris*
57 by grasses such as *Molinia* spp. (Bobbink et al., 2010; Terry et al., 2004).

58 Despite the extensive knowledge on heathland conservation measures in their main oceanic
59 distribution area (Davies et al., 2016; Littlewood et al., 2014; Pywell et al., 2011), very little work is
60 available for their southern edges (Fagundez, 2013). Here, these ecosystems often occur under
61 Continental rather than Atlantic climates and mineral rather than thick organic soils (Lonati et al.,
62 2009). At these southern margins, heathlands are facing major threats because of increased heath
63 fragmentation, minor adaptive capacity, and higher pressure by local and exotic grass, shrub and
64 tree encroachment (Bartolome et al., 2005; Borghesio, 2014). Indeed, woody encroachment happens
65 at faster rates (Ascoli and Bovio 2010), and *C. vulgaris* competes with vicariant and more
66 productive grasses, such as *Molinia arundinacea* Schrank rather than *Molinia caerulea* (L.) Moench
67 (Borghesio et al., 2014; Daněák et al., 2012). Consequently, techniques that are effective in the re-
68 establishment of the dominance of *C. vulgaris* (e.g. browsing, prescribed burning, mowing) may not
69 successfully achieve the target of restoring the composition of the whole plant community
70 (Littlewood et al., 2014) and may promote competitor and alien species (Davies et al., 2016).

71 To address these issues, a heathland restoration experiment was established in a highly
72 fragmented, continental dry heathland located on Po Plain lowlands, northern Italy (Ascoli et al.,
73 2009). The study aimed to assess the long-term effects (six years after treatments) of browsing,
74 prescribed burning, and mowing for the restoration of heathland vegetation, by answering the
75 following questions: i) what is the effect of single restoration techniques and their combination on
76 plant diversity and species community assemblage? ii) How does restoration affect the cover of *C.*
77 *vulgaris*, *M. arundinacea* and encroaching woody species? iii) Is there any restoration treatment
78 that triggers the invasion of alien species?

79

80 2. Materials and Methods

81 2.1 Study area, experimental design, and vegetation surveys

82 The study area was located within the Nature Reserve of Vauda, northwest Italy (7°41'17"E,
83 45°13'13"N), at an altitude ranging from 240 to 480 m a.s.l. The climate is continental, with 81%
84 of mean annual precipitation (1 000-1 100 mm) falling between April and November and mean
85 annual temperature about 12°C. The Reserve lies on a fluvio-glacial terrace, characterized by
86 ancient and leached soils with low pH (4.8), high clay content, and a thin organic layer (Borghesio,
87 2014). The Reserve was instituted in 1993 to maintain a relict heathland ecosystem. Despite
88 protection policies, in the last decades the heathland has declined because of *M. arundinacea* and
89 woody species encroachment (mainly European aspen *Populus tremula* L. and silver birch *Betula*
90 *pendula* Roth) due to the abandonment of traditional management (i.e. grazing and mowing).
91 Moreover, large and frequent pastoral uncontrolled fires during the winter dry season, when grasses
92 dry out, threaten the heathland (Ascoli and Bovio, 2010).

93 The experimental area was composed of *C. vulgaris* stands in the building phase (*sensu*
94 Watt, 1955) with an advanced encroachment of woody species, i.e. average (\pm SE) tree density and
95 basal area were $22,722 \pm 1518$ stems ha⁻¹ and 3.1 ± 0.4 m² ha⁻¹, respectively (Ascoli et al., 2013).
96 Six restoration treatments were applied from 2005 to 2011: 1) annual fire, 2) one-off fire, 3) annual

97 mowing, 4) one-off mowing, 5) extensive annual browsing, and 6) one-off fire + extensive annual
98 browsing. Annual fire was implemented every winter from 2005 to mimic current pastoral
99 practices, one-off fire once under prescribed burning conditions in winter 2005 (for details see
100 Lonati et al., 2009), annual mowing every spring, and one-off mowing once in spring 2005.
101 Mowing was performed mechanically at 8 cm height and included biomass harvesting. Annual and
102 one-off fire were carried out over eight 600 m² plots each, while annual and one-off mowing over
103 eight 100 m² plots each. A herd of about 100 goats exploited annual browsing and one-off fire +
104 annual browsing plots (which received a single winter prescribed burn in 2005) for 3.5 h and 3 h
105 day⁻¹, respectively, over a period of four weeks between April and May. Annual browsing and one-
106 off fire + annual browsing were carried out over 16 plots each (plots were 1250 m² and 1000 m²,
107 respectively), with a stocking density of about 135 Animal Units ha⁻¹ and a stocking rate of 0.05
108 AU ha⁻¹ year⁻¹ (*sensu* Allen et al., 2011). Moreover, eight untreated 300 m² plots were used as
109 control areas. Since we expected a higher variability of vegetation cover and composition after
110 treatment, due to the more heterogeneous effects produced by the selective feeding behavior of
111 goats (Iussig et al., 2015), the number of plots for extensive annual browsing and one-off fire +
112 extensive annual browsing was double compared to other treatments. All 72 experimental plots were
113 fenced and randomly selected within comparable *C. vulgaris* heathland patches, which were chosen
114 on the basis of similar vegetation cover and composition.

115 In each plot, botanical composition was determined using the vertical point-quadrat method
116 (Daget and Poissonet, 1971) along one fixed 10 m transect. In each transect, at 20 cm intervals, the
117 species touching a steel needle were identified and recorded (i.e. 50 points of vegetation
118 measurement). Since rare species are often missed by this method, a complete list of all other plant
119 species included within a 1 m buffer around the transect line was also recorded (Orlandi et al.,
120 2016). Vegetation surveys were conducted during summer 2004 (pre-treatment year), 2007, 2009,
121 and 2011 (i.e. two, four, and six years after treatments, respectively).
122

123 2.2 Statistical analyses

124 For each species recorded, the frequency of occurrence (number of occurrences/50 points) was
125 calculated for each transect and converted to percentage cover (%) (Pittarello et al., 2016). In
126 particular, the percentage cover of *C. vulgaris*, *M. arundinacea*, woody encroaching species, i.e.
127 species classified as chamaephyte, phanerophyte, or nanophanerophyte according to Raunkiaer
128 (1937), and alien species (Celesti-Grapow et al., 2009) was computed. Species richness and
129 Shannon diversity index were also calculated for each survey.

130 A Principal Response Curve (PRC) analysis was performed to visualize the overall effect
131 produced by treatments on the botanical composition of treated plots compared to that of control
132 plots over time. The PRC analysis was performed using Canoco 4.5 software (Ter Braak and
133 Šmilauer, 2009).

134 Generalized Linear Mixed Models (GLMMs) were used to test for differences of each
135 treatment against control for all the vegetation variables (i.e. species richness, Shannon diversity
136 index, *C. vulgaris* cover, *M. arundinacea* cover, woody species cover, and alien species cover) for
137 each of the four years during which vegetation surveys were carried-out. Each treatment was
138 considered as a fixed effect, with control used as a reference level for all the analyses. Poisson
139 distribution was specified for count variables and Gaussian or Gamma distributions were specified
140 for continuous data, depending if normality was met or not, respectively (normality was tested with
141 Kolmogorov-Smirnoff test). Significance tests were performed using the Wald statistic. The
142 GLMMs were carried out using R 3.0.3 (R Development Core Team, 2012), with the glmmADMB
143 package (Fournier et al., 2012).
144

145 3. Results and Discussion

146 A total of 66 plant species was detected in botanical surveys (Appendix 1). Six years after
147 treatments, species richness did not differ between treated and control sites, underlying the high

148 stability and resistance to treatments of the floristic composition of *C. vulgaris* heathlands at their
149 southern edge, though inter-annual fluctuation in species richness can occur among years (Figure
150 1a). In the short-term, most of the treatments changed the heathland community, reducing *C.*
151 *vulgaris* cover in 2007 (Figures 1c) according to a gradient of increasing biomass removal:
152 extensive annual browsing (which removed little biomass), one-off mowing, annual mowing, one-
153 off fire without and with annual browsing, and annual fire (which removed biomass repeatedly). In
154 the mid-term, we observed changes in *C. vulgaris*, *M. arundinacea*, woody and alien species cover,
155 which followed different trajectories according to treatment and functional group (Figure 1c-f).

156 *Molinia arundinacea* cover increased proportionally to biomass removal, as it produced
157 more biomass in southern edge heathlands as compared to *M. caerulea* in the Atlantic European
158 ones (Marrs et al., 2004) (Figure 1d). Annual fire, which simulated current uncontrolled pastoral
159 fires, repeatedly removed the heathland and shifted vegetation towards a *M. arundinacea*-
160 dominated community. Mowing treatments and one-off fire, combined or not with annual browsing,
161 initially reduced *C. vulgaris* cover (Figures 1c) and increased *M. arundinacea* cover in the short-
162 term (Figure 1d), but *C. vulgaris* started recovering at all sites after the first growing season mainly
163 by stump resprouting. However, six years later, one-off fire, combined or not with annual browsing,
164 displayed a lower *C. vulgaris* and a higher *M. arundinacea* cover in comparison to mowing
165 treatments. Indeed, graminoids benefited from both litter and crown biomass consumption in fire
166 treatments. Conversely, mowing did not completely remove the crown and left the litter, which
167 resulted in a higher *C. vulgaris* cover since 2007. In the following years, the recovery rate of *C.*
168 *vulgaris* in all these treatments was similar, but graminoids maintained a higher abundance in fire
169 treatments, in contrast to herbaceous forbs, as evidenced in Figure 2. Notably, one-off mowing did
170 not show significant differences in both *C. vulgaris* and *M. arundinacea* cover when compared to
171 control plots. Extensive annual browsing, the treatment with the lowest biomass removal, did not
172 affect the heathland structure as *C. vulgaris* is barely consumed by goats (Iussig et al., 2015), and it
173 resulted in the lowest increase of *M. arundinacea*, which was comparable to the one of the control.
174 Since heathland vegetation was always dominated by a low number of species (namely *C. vulgaris*,
175 *M. arundinacea*, and a few other graminoids, Figure 2 and Appendix 1), a situation comparable to
176 that of other heathlands (Hancock and Legg, 2012; Muñoz et al., 2012), Shannon diversity index
177 was not different between treatments and control at the end of the experiment (Figure 1b).

178 Woody species displayed opposite responses to treatments in comparison to graminoids
179 (Figure 1e). Annual burning and mowing were effective in reducing woody species encroachment.
180 One-off fire and mowing top-killed trees, but aspen and birch sprouted vigorously and only
181 subsequent annual browsing effectively controlled shoot growth. Annual browsing had a delayed
182 effect and woody species cover reached the same level as one-off fire + annual browsing by the end
183 of the study. PRC analysis (Figure 2) highlighted significant differences in the botanical
184 composition between treated and untreated plots ($p < 0.01$), with a marked increase in woody
185 species cover in unmanaged control plots, as showed by the trend of phanerophytes (*P. tremula*,
186 *Frangula alnus* Miller, and *B. pendula*).

187 Five alien species were inventoried, but only *Panicum acuminatum* Swartz reached a
188 noticeable percentage cover (Appendix 1). Interestingly, *P. acuminatum* was triggered only by fire
189 treatments, displayed a peak in the short-term and decreased to the level of control in 2011 (Figure
190 1f), remaining higher only in the annual fire treatment (Figure 2). In North America *P. acuminatum*
191 showed a great fitness after annual fire (Walsh, 1995), a trait maintained also outside its natural
192 distribution area (Lonati et al., 2009). However, our results confirm that this invasive species
193 quickly declines when fire frequency is low and it does not become important in terms of density
194 and biomass (Walsh, 1995). Under a long-term perspective, prescribed burning might have the
195 effect in rejuvenating the *P. acuminatum* seed bank rather than considerably increasing its
196 vegetation cover, which is however unfavorable to the control of this alien species.

197

198 4. Conclusions

199 Both frequent fires simulating current uncontrolled pastoral burns and lack of management
200 promote heathland losses at the southern edge of their distribution, stressing the need for
201 conservation measures. Moreover, the encroaching grass *Molinia arundinacea*, woody and alien
202 species, such as *Populus tremula* and *Panicum acuminatum*, appear to have a high resilience to
203 different restoration treatments. Results evidence the need and potential benefit of integrating
204 multiple techniques to preserve southern edge fragmented heathlands. The restoration of these
205 habitats may not be effective with just one of the tested treatments, since all the techniques involve
206 trade-offs between undesired effects, efficacy and operational difficulties. However, six years after
207 treatments, extensive goat browsing and annual mowing provided the best results for the
208 maintenance of *Calluna vulgaris* and kept woody and alien species under a critical level. Likewise
209 in Atlantic heathlands, prescribed burning may be also valuable for *Calluna* heathlands restoration
210 at their southern range, but only when applied with long return intervals (i.e. longer than six years,
211 but further research is needed to establish a suitable return interval). A higher caution in the use of
212 fire is mandatory because of the presence of encroaching species with marked fire-traits adapted to
213 a more fire-prone environment when compared to Atlantic regions, which can benefit greatly from
214 repeated burns at the expense of *Calluna* heaths.

215

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221

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226

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228 **6. References**

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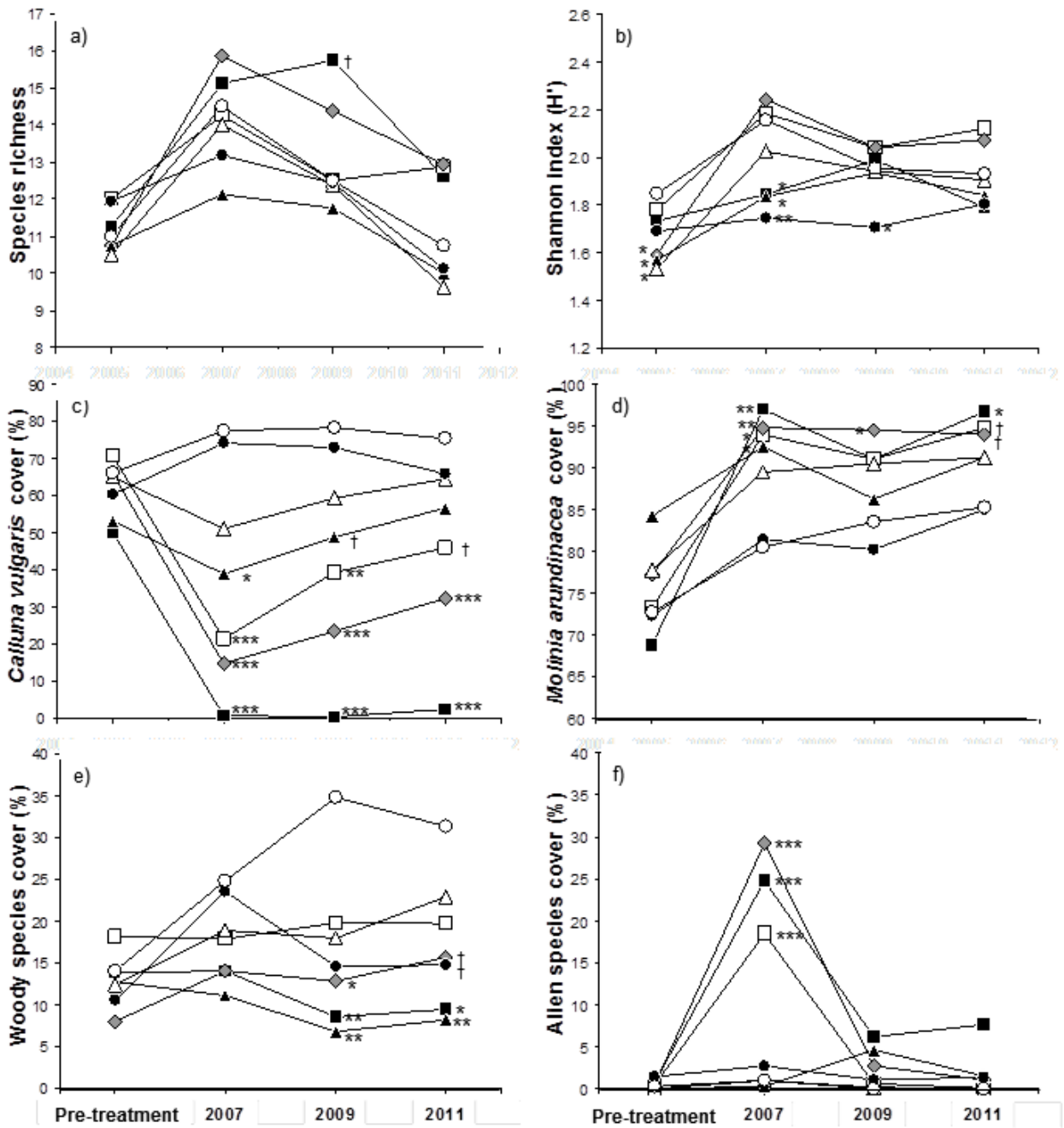
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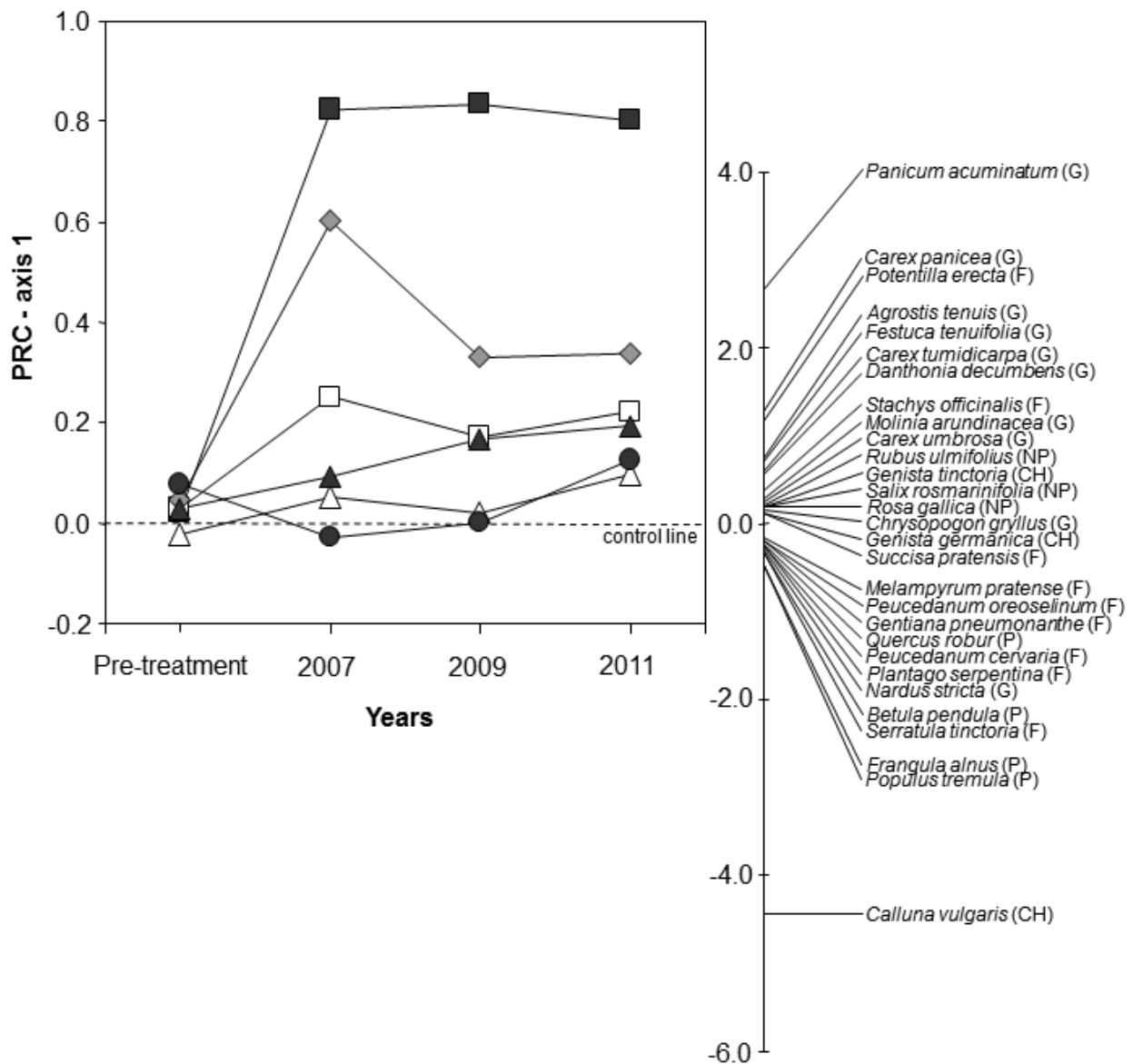
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309
 310 **Fig. 1.** a) Species richness, b) Shannon diversity index, c) *Calluna vulgaris* cover (%), d) *Molinia*
 311 *arundinacea* cover (%), e) woody species cover (%), and f) alien species cover (%) of *C.*
 312 *vulgaris* heathlands, untreated (i.e. control plots) or subjected to six restoration treatments. †
 313 = $p < 0.1$; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$. Statistical significance values are
 314 related to differences of each treatment against control. ■ = annual fire; □ = one-off fire;
 315 ◆ = one-off fire + annual goat browsing; ▲ = annual mowing; △ = one-off mowing; ● =
 316 annual goat browsing; ○ = untreated.



317
 318 **Fig. 2.** PRC (axis 1) showing effects on botanical composition of *C. vulgaris* heathlands produced
 319 by the implementation of six restoration treatments with respect to untreated control (control
 320 line) from pre-treatment stage (2004) to 2011. The scores of the most frequent species
 321 (namely, species present in at least the 14 plots, i.e. the 5% of total plots) are shown on the
 322 right side of the graph: positive values represent species whose canopy cover increased after
 323 treatments, whereas negative values represent species whose canopy cover decreased over
 324 time. Letters within brackets indicate: G = graminoid, F = herbaceous forb, NP =
 325 nanophanerophyte, CH = chamaephyte, P = phanerophyte. ■ = annual fire; □ = one-off fire;
 326 ◆ = one-off fire + annual goat browsing; ▲ = annual mowing; △ = one-off mowing; ● =
 327 annual goat browsing; ○ = untreated.

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9. Highlights

1. Six restoration treatments were applied in southern *Calluna vulgaris* heathlands
2. Annual fire shifted the vegetation towards a *Molinia arundinacea*-dominated community
3. In the short-term, fire treatments triggered a peak in alien species cover
4. Six years after treatments, plant diversity did not differ between treated and control sites
5. Six years after treatments, goat browsing and annual mowing provided the best results