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## A five-year rotational grazing changes the botanical composition of sub-alpine and alpine grasslands

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# UNIVERSITÀ DEGLI STUDI DI TORINO

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#### 8 A five-year rotational grazing changes the botanical composition of sub-alpine and alpine

- 9 grasslands
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#### 20 Abstract

- 21 Aim: The implementation of Grazing Management Plans (GMP), a specific policy and
- 22 management tool, aimed at enhancing farm productivity, while preserving plant diversity, soil,
- and landscape. The GMP are based on rotational grazing systems (RGS) with animal stocking
- rate adjusted to keep it balanced with grassland carrying capacity. The aim was to test the five-
- 25 year effects produced by GMP implementation on botanical composition, plant diversity, and
- 26 soil nutrient content on sub-alpine and alpine pastures.
- 27 Location: Val Troncea Natural Park, western Italian Alps.
- 28 Methods: A total of 199 vegetation transects was carried out in summer 2011 and repeated in
- summer 2016. The botanical composition was recorded and plant diversity indexes, i.e. species
- 30 richness and Shannon diversity (H' index) were computed. Moreover, the mean soil nutrient
- 31 content was indirectly estimated through the computation of Landolt N indicator value (N
- 32 index) for each transect.
- 33 Pair-sample statistical tests and PERMANOVA were perfored at different levels: on the whole
- 34 vegetation dataset, on vegetation communities (i.e. vegetation types and ecological groups), and
- 35 considering functional pools of species.
- 36 **Results:** Considering the whole vegetation dataset, species richness, H' index, and N index
- 37 significantly increased from 2011 to 2016. Moreover, species richness significantly increased in
- almost all the vegetation ecological groups, with the highest increase within mesotrophic one.
- 39 The H' index significantly increased in eutrophic, pre-forest, and thermic groups, while N index
- 40 increased in all the vegetation groups, except in the eutrophic and snow-bed ones. A significant
- 41 difference in botanic composition was detected within oligotrophic, mesotrophic, and thermic
- 42 groups. The number and cover of nitrogen-poor high-elevation species increased in all groups
- 43 and this result could be probably related to the effects produced by livestock, which promoted
- 44 seed transportation and increased connectivity amongst different communities. The meso-
- 45 eutrophic species number and cover significantly increased within thermic, mesotrophic, and
- 46 pre-forest groups, suggesting a greater use of such areas by livestock under RGS than
- 47 continuous grazing system.
- 48 **Conclusions:** The implementation of a RGS with stocking rate adjustments proved to be an
- 49 effective and a sustainable management tool to enhance botanical composition and plant
- 50 diversity of sub-alpine and alpine grasslands over a five-year span.

#### 51 Keywords

- 52 Alps; Cattle grazing; High-elevation; Livestock management; Meso-eutrophic species; Pasture;
- 53 Shannon diversity index; Species richness

#### 54 Nomenclature

55 Pignatti (1982) for plant species; Aeschimann et al. (2004) for plant communities

### 56 Abbreviations: CGS = Continuous Grazing System; RGS = Rotational Grazing System; GMP

57 = Grazing Management Plan

#### 58 Introduction

59 Permanent grasslands, both meadows and pastures, are spread worldwide and cover about 25%

of the earth's surface (FAOSTAT 2015, <u>http://www.fao.org/faostat/en/#data/RL;</u> accessed on 15

61 Dec 2017). They host a wide range of animal and plant species (Wilson et al. 2012; Dengler et

al. 2014), amongst which many exclusively dependent on these open habitats (Reitalu et al.

63 2008; Schmid et al. 2017). Moreover, they provide essential functions and services to human

64 and ecosystem health, such as animal production, carbon storage, nutrient cycling, pollination,

soil protection and water conservation, wild-fire risk mitigation, and tourism opportunities

66 (Silva et al. 2008; Harrison et al. 2010; Conant et al. 2017). However, permanent grasslands are

among the main threatened ecosystems, particularly the most extensively managed ones. Indeed,

68 in the last century, a massive decline of grasslands due to land-use changes has occurred (Dong

et al. 2011; Gillet et al. 2016), resulting in a loss of biodiversity (Wesche et al. 2012).

70 The role of grazing in preserving and improving grassland ecosystems has been broadly

studied (Collins et al. 1998; Adler et al. 2001; Sebastià et al. 2008; Li et al. 2017). Livestock

72 management influences plant species composition (Olff & Ritchie 1998), nutrient redistribution

73 (Malo & Suarez 1995; Dai 2000; Gaujour et al. 2012; Lonati et al. 2015), biomass removal

74 (Borer et al. 2014), and soil and plant species cover by grazing, trampling, excreta deposition,

and seed transportation (Pittarello et al. 2016 a, b; Probo et al. 2016). For all these reasons, it

can modify intraspecific and interspecific competition dynamics among plant species, often

77 favouring meso-eutrophic species dominance within regularly grazed grasslands (Niu et al.

78 2016; Nervo et al. 2017).

79 A dramatic decline in the overall area covered by grasslands has been also recorded for the 80 Italian Alps (Bätzing 2005; Orlandi et al. 2016). Alps are a biodiversity hotspot, with many rare 81 and endemic species, often relics of ice ages (Stehlik 2003). In the last decades, alpine semi-82 natural grasslands, shaped by millennia of extensive human activities and land management, 83 have undergone a process of agro-pastoral abandonment due to socio-economic changes (Probo 84 et al. 2013). As a consequence, undergrazing has occurred over large areas, i.e. often the 85 steepest and most marginal ones, resulting in widespread vegetation cover and composition 86 changes. Oligotrophic herbaceous species, shrubs and trees have encroached large areas of 87 semi-natural grasslands, decreasing plant and animal diversity, meso-eutrophic species cover,

forage mass and quality, and grassland carrying capacity (Probo et al. 2014). The carrying

89 capacity has been defined by Allen et al. (2011) as the maximum livestock stocking rate

90 achieving a target level of animal performance, in a specified grazing system, that can be

91 applied over a defined time without deterioration of the grazing land.

92 For all these reasons, European Union latest policies aim to safeguard the remaining

grasslands and to restore the degraded ones (Mikkonen & Moilanen 2013; Mihók et al. 2017).

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94 Specifically, in the western Italian Alps (Piedmont Region) the implementation of Grazing 95 Management Plans (GMP) has been promoted. The GMP are a management tool aiming at enhancing farm productivity, while preserving biodiversity, soil, and landscape through the 96 97 application of farm-specific and sustainable grazing management actions (Lombardi et al. 2011; 98 Argenti & Lombardi 2012). To achieve these objectives, GMP implemented rotational grazing 99 systems (RGS) with animal stocking rate adjusted to keep it balanced with corresponding 100 grassland carrying capacity (Probo et al. 2014). The RGS improved the spatial distribution of 101 grazing cattle on rough sub-alpine (i.e. the ones located below the treeline) and alpine pastures 102 by homogenizing the selection of different vegetation communities by livestock when compared 103 to continuous grazing systems (CGS) (Probo et al. 2014). Indeed, free-roaming cattle under 104 CGS, generally with a stocking rate lower than grassland carry capacity, resulted in a more 105 uneven livestock distribution, with extensive undergrazing and overgrazing situations 106 determined by the exacerbated selection for preferred vegetation communities. However, the 107 effects produced by the implementation of GMP on plant diversity and botanical composition 108 were not taken into consideration in previous research. Actually, some studies assessed the 109 effects produced by RGS on plant and animal biodiversity, soil properties, vegetation cover, 110 animal productivity, and forage selection and quality (Morris et al. 2005; Jacobo et al. 2006; 111 Wrage et al. 2011; Teague et al. 2013; Ravetto Enri et al. 2017 a), but they were not conducted 112 on high-elevation grasslands. No research, to our knowledge, has been carried-out on the 113 medium-term effects (e.g. five years) of RGS on botanical composition, biodiversity indexes, 114 and soil nutrient content on sub-alpine and alpine grasslands. 115 With this goal, a research was conducted on sub-alpine and alpine pastures of Val Troncea 116 Natural Park (north-western Italy), which were exploited by a beef cattle herd, managed under 117 the implementation of a GMP for five consecutive years (2011-2015). To test the effectiveness 118 of GMP, the effects produced on plant diversity (species richness, Shannon diversity index, and 119 beta-diversity indexes), soil nutrient content, estimated indirectly by Landolt N indicator value 120 for soil nutrient, plant composition and species cover were assessed. It was hypothesized that 121 after five-year of GMP implementation: (1) species richness and Shannon diversity index would 122 enhance, (2) soil nutrient content would increase and homogenize across different vegetation 123 communities, and (3) meso-eutrophic species number and cover would increase.

6

#### 124 Methods

- 125 Study area and grazing management126 The study was conducted within Val Troncea Natural Park (Piedmont, north-western Italy,
- 127 latitude 44°57'N and longitude 6°57'E), which is included within the Site of Community
- 128 Interest and the Special Protection Area (code id: IT1110080) of Nature 2000 network
- 129 (92/43/EEC and 2009/147/EC directives) (Figure 1).
- 130 Dominant soils, originated from calcareous parent rock, were gravelly and nutrient-poor.
- According to the meteo station located at 2150 m a.s.l. (latitude 44°98'N and longitude 6°94'E),
- average annual temperature is about 4°C (February: -3.8°C; July: 12.6°C) and annual average
- 133 precipitation is 703 mm (mean from 2003 to 2015).
- 134 Within the Park boundaries, one study area of 448 ha was selected (Figure 1). The study area
- 135 was dominated by sub-alpine and alpine grasslands and shrublands, ranging from about 1900 to
- about 2820 m a.s.l. Grasslands were mainly dominated by Festuca curvula Gaudin, Carex
- 137 sempervirens Vill., Festuca nigrescens Lam. non Gaudin, Agrostis tenuis Sibth., and Poa alpina
- 138 L., while the shrub layer was predominantly composed by *Rhododendron ferrugineum* L.,
- 139 Juniperus nana Willd., Vaccinium myrtillus L., and Vaccinium gaultherioides Bigelow.
- 140 Until 2010, the study area was traditionally grazed by one free-ranging cattle herd of about 80
- 141 animal units (sensu Allen et al. 2011), composed by cows predominantly of Piedmontese breed.
- 142 From 2011 to 2015, with the implementation of a specific GMP (Probo et al. 2014), the area
- experienced a change in grazing management, since it was subdivided into four paddocks,
- 144 which were grazed under a RGS (starting in paddock 1 and ending in paddock 4, Figure 1),
- 145 from the beginning of July to the end of September, by 105 animal units, according to grassland
- 146 carrying capacity. The average annual carrying capacity of the four paddocks was calculated
- 147 using the method defined in Daget & Poissonet (1971), based on the multiplication of the
- 148 grazable area with pastoral value, which is a synthetic index of sward forage quality (Pittarello
- 149 et al. 2018), and altitudinal and slope coefficients.
- 150

#### 151 Vegetation transects

- 152 The study area was subdivided into 150 x 150-m grid cells and a linear vegetation transect was
- 153 established in the centre of each grid cell using the vertical point-quadrat method (Daget &
- 154 Poissonet 1971; Pittarello et al. 2017). A total of 199 vegetation transects was carried out in
- summer 2011 and was repeated in summer 2016 to measure vegetation changes after five years
- 156 of GMP implementation. Each transect, 10-m long, was pinpointed thanks to a GPS device
- 157 (Stonex<sup>®</sup> S3). Vegetation transects were carried out from June to August, before livestock
- 158 grazing and at the flowering phenological stage of the dominant graminoids. At 50-cm interval
- 159 along each transect, plant species touching a steel needle were identified and recorded. Since

160 rare species are often missed by this method, a complete list of all other plant species included

161 within a 1-m buffer area around the transect line, was also recorded (Probo et al. 2017).

162

#### 163 Data analysis

- 164 The frequency of occurrence of each plant species recorded ( $f_i$  = number of occurrences/20
- 165 points of vegetation measurement), which is an estimate of species canopy cover, was
- 166 calculated for each transect and converted to percentage cover (Pittarello et al. 2016 a). Species
- 167 relative abundance  $(SRA_i)$  was determined in each transect and used to detect the proportion of
- 168 different species according to the equation of Daget & Poissonet (1971):

$$SRA_i = \frac{f_i}{\sum_{i=1}^n f_i} \times 100$$

169 where SRA<sub>i</sub> and  $f_i$  are species relative abundance and frequency of occurrence of species *i*.

- 170 Vegetation diversity was expressed according to two indexes: species richness and Shannon
- 171 diversity index. Shannon diversity index (H') was calculated for each transect according to the
- 172 equation (Magurran 1988):

$$\mathbf{H}' = -\sum_{i=1}^{l=n} \left\{ \frac{SRA_i}{100} \times \log_2\left(\frac{SRA_i}{100}\right) \right\}$$

173 where  $SRA_i$  is the species relative abundance of species *i*.

174 Moreover, beta-diversity indexes (Sørensen, Horn and Morosita-Horn) were computed for each

paddock, considering the multiple-community dissimilarity between transects (Chao et al. 2008;

176 Chao et al. 2012). The indexes differ in the weights attributed to SRA<sub>i</sub> (Magurran & McGill

177 2011), ranging from 0 (set of identical communities) to 1 (set of communities that share no

178 species).

179 Soil nutrient content was estimated indirectly by Landolt N indicator value for soil nutrient

180 (hereafter N index). Indeed, the N index can properly characterize an area (Tölgyesi et al. 2014)

and it is well correlated to the supply of several nutrients, such as nitrogen, phosphorous, and

182 potassium (Diekmann 2003). Each plant species was associated to the corresponding N value

183 (Landolt et al. 2010) and the mean N index was calculated for each transect, by averaging

184 species values weighted on their SRA (Ravetto Enri et al. 2017 b).

185 In order to identify different functional pools of species, which are characterized by similar

186 ecological needs, each plant species was classified according to its phytosociological optimum

- 187 at the class level, as identified by Aeschimann et al. (2004). Moreover, species belonging to
- 188 phytosociological classes having physiognomic, ecological, and floristic similarity were pooled
- according to Theurillat et al. (1995; Appendix S1). Six functional species pools out of the ten
- 190 detected were retained for further analyses, in order to focus on the ones more directly affected
- 191 by grazing management, i.e. excluding typical forest and rocky species.

192 Statistical analyses

193 Changes between 2011 and 2016 in plant diversity, soil nutrient content, and botanical

194 composition were assessed at different levels: on whole vegetation dataset, on vegetation

communities (i.e. vegetation types and ecological groups), and considering the functional pools

196 of species identified in Appendix S1.

197 Vegetation transects were classified into vegetation types and ecological groups, according to

198 Cavallero et al. (2007) and Probo et al. (2014) by hierarchical cluster analysis (Appendix S2).

199 The classification variable was SRA, the cluster method was Pearson correlation coefficient,

and the between group linkage was the resemblance coefficient.

To detect differences in plant diversity indexes and N index between 2011 and 2016, on the whole dataset and for each vegetation ecological group, pair-sample statistical tests were

203 performed. Vegetation variables were tested for normality and homogeneity of variance using

204 Shapiro-Wilk and Levene's test, respectively. When assumptions were not verified, data were

205 log-transformed and normality and homogeneity of variance were tested on log-transformed

206 data. Paired-sample *t*-tests and non-parametric Wilcoxon signed-rank tests (Sokal & Rohlf

207 1995) were used, depending if normality and homogeneity of variance were verified or not,

208 respectively. The same analysis was carried out to test the differences in beta-diversity between

209 2011 and 2016, considering paddock as the experimental unit.

210 A permutational analysis of variance (PERMANOVA), considering species percentage cover

as main variable, was performed using 9999 permutations to assess differences in botanical

212 composition for the whole dataset and for each vegetation ecological group identified, using

213 Bray-Curtis dissimilarity as measure of the vegetation changes between 2011 and 2016.

214 Moreover, changes in the total number and cover of species belonging to different functional

215 pools between 2011 and 2016 were tested by paired-sample *t*-tests and non-parametric

216 Wilcoxon signed-rank tests for each ecological group, except for the rocky one, since it proved

to be not significantly different between surveyed years.

All stastistical analyses were performed using IBM SPSS Statistics 24.0 (IBM Corp. Released

219 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.), except the

220 PERMANOVA analysis, which was performed with Past software (PAST 3.16, Hammer et al.

221 2001) and beta-diversity computed using R 3.4.1 (R Core Team, 2017. R: A language and

222 environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

223 URL https://www.R-project.org/) with "SpadeR" package (Chao et al. 2016).

9

#### 224 **Results**

#### 225 Effects on plant diversity and N index

A total of 275 and 281 plant species was recorded, in 2011 and in 2016, respectively.

227 The complete species list and corresponding Landolt N indicator values, phytosociological

- 228 optima, and functional species pool attribution are available in Appendix S3. Vegetation
- transects recorded in 2011 were classified into 18 vegetation types and seven ecological groups
- 230 (Appendix S2). The thermic ecological group was the most widespread in the study area,
- 231 followed by oligotrophic and pre-forest and shrub-encroached ones (hereafter called pre-forest
- group), with eutrophic and mesotrophic groups together accounting for 22.7% of the total area.
- 233 Considering the whole vegetation dataset, species richness, Shannon diversity index, and N
- 234 index significantly increased from 2011 to 2016 (Table 1). Conversely, Sørensen, Horn and
- 235 Morosita-Horn indexes of beta-diversity did not show significant differences between the two
- 236 years (Table 1).

237 Species richness significantly increased in almost all the vegetation ecological groups except

within the rocky one, with the highest increase detected within mesotrophic group (+ 11.5

239 species, on average). H' index significantly increased in eutrophic, thermic, and pre-forest

- 240 groups, while N index increased in all the vegetation groups except in eutrophic and snow-bed
- 241 ones (Table 2).

#### 242 Effects on botanical composition

The PERMANOVA performed on the whole dataset did not show a significant difference between 2011 and 2016 (P-value = 0.262), while differences in species composition were

- 245 detected within mesotrophic, oligotrophic, and thermic groups (Table 3).
- 246 Considering the whole vegetation dataset, a significant increase in the number of species was
- 247 detected for all functional pools of species (Table 4). Conversely, species cover significantly
- 248 changed only for nitrogen-poor high-elevation and meso-eutrophic species pools.

249 The number and cover of species belonging to different pools highlighted predominant

- 250 changes within oligotrophic, thermic, and pre-forest ecological groups, while less pronounced
- changes were detected within other groups (Table 5). More in detail, in all the ecological groups
- the number of nitrogen-poor high-elevation species (e.g. *Myosotis alpestris* f.w. Schmidt,
- 253 Festuca violacea Gaudin, Agrostis alpina Scop., etc.) significantly increased. Moreover, some
- species belonging to the same pool significantly increased their cover (e.g. F. violacea, A.
- 255 alpina, Carex rosae (Gilomen) Hess et Landolt, etc.) in almost all the vegetation ecological
- groups. Conversely, the number of meso-eutrophic species (e.g. Poa alpina L., Achillea gr.
- 257 millefolium, Carum carvi L., etc.) increased only within thermic groups, while they increased
- their cover within mesotrophic, pre-forest, and snow-bed ones. The number of ruderal species

- 259 (e.g. Cerastium arvense L., Gagea fistulosa (Ramond) Ker.-Gawl., Cirsium eriophorum (L.)
- 260 Scop., etc.) increased in oligotrophic, thermic, and pre-forest groups, while their cover increased
- 261 in the mesotrophic one. Snow-bed species number (e.g. Salix herbacea L., Sibbaldia
- 262 procumbens L., Luzula alpino-pilosa (Chaix) Breistr.) mainly increased within the snow-bed
- 263 group. The total number of species belonging to the fringe and tall herb pool (e.g. Poa chaixii
- 264 Vill., Pulmonaria australis (Murr) Sauer, Cruciata glabra (L.) Ehrend., Geranium sylvaticum
- L., etc.) significantly increased only within pre-forest group, while the cover of these species
- 266 increased within the eutrophic one.

#### 267 Discussion

268 The five-year implementation of GMP provided very promising results for biodiversity 269 conservation in sub-alpine and alpine pastures. The implementation of a RGS with stocking rate 270 adjustments proved to be an effective management tool to enhance botanical composition and 271 plant diversity of sub-alpine and alpine grasslands. Indeed, beyond the improvement of grazing 272 spatial distribution on rough areas and the homogenization of the selection of vegetation 273 communities by cattle herds since the very beginning of their application (Probo et al. 2014), it 274 also significantly changed these communities during a five-year span. A more homogeneous 275 exploitation of sub-alpine and alpine grasslands by livestock led to an overall increase of species 276 richness, H' index, and soil nutrient content, as well as to remarkable changes in plant species 277 composition and cover, despite the well documented evidence of slow vegetation dynamics at 278 high elevation (Tasser & Tappeiner 2002; Körner 2003). Mesotrophic, oligotrophic, and thermic 279 groups showed significant changes in botanical composition. This result is partly consistent with 280 Pavlů et al. (2003), who studied the effects of different grazing practices on the vegetation of a 281 poorly improved upland pasture. These authors observed, in five years, a significant distinction 282 in plant species composition and structure between rotational grazing and continuous stocking, 283 but they did not detect a significant effect on species richness. Also Jacobo et al. (2006) 284 evaluated the effects of RGS compared to the traditional CGS on floristic composition dynamic 285 in a study conducted in the Flooding Pampa region, over a period of 4 years. The authors did not 286 detect plant species diversity changes due to grazing management, while other variables did, 287 e.g. functional group cover, and they ascribed this result to a new balance in the vegetation 288 community typical of temperate grasslands, where the increase of some species led to a decrease 289 of others. Conversely, in the present study, the change in grazing regime led also to an increase 290 in plant species richness within all the considered ecological groups, except in the rocky one, 291 which was little interested by grazing, due to its topographical and vegetation constraints (Probo 292 et al. 2014). Moreover, beta-diversity remained stable, highlighting that the increase in species 293 richness was homogeneously distributed across vegetation transects within paddocks.

294 Both H' and N indexes showed the same increasing trend, considering the whole dataset, thus 295 confirming that grazing enhances species diversity and soil nutrient, homogenizing it across 296 different vegetation communities and supporting the first two experimental hypotheses. The 297 increase in the mean N value from 2.3 to 2.4 and the consequent biodiversity enhancement 298 measured are consistent with Pittarello et al. (2018), who found that species richness and H' 299 index peaked at an average N index value of 2.5 along an "humped-shape" curve in alpine 300 pastures. The highest increases of N value were recorded within two vegetation ecological 301 groups normally avoided and undergrazed by free-ranging animals, i.e. thermic and pre-forest 302 ones (Probo et al. 2014), highlighting an increased exploitation of these communities. 303 The third hypothesis was confirmed as well, i.e. meso-eutrophic species significantly 304 increased both in their number and cover. Particularly, they increased within mesotrophic, 305 thermic, and pre-forest groups, suggesting a greater use of such areas by livestock under RGS 306 than CGS. This is also supported by the increase of N index value in these groups, which was 307 probably related to the increase of soil nutrient content produced by the increase of excreta 308 deposition by livestock (Aarons et al. 2004; Güsewell et al. 2005). The increased nutrient 309 availability and cattle trampling favoured the establishment of meso-eutrophic more competitive 310 species (i.e. A. gr. millefolium, C. carvi, etc.), whose main dispersal mechanisms are endochory 311 and epichory (Traba et al. 2003; Cosyns et al. 2005).

312 The effect of the change in grazing management on botanical composition can be further 313 demonstrated by the increase both in number and in cover of plant species belonging to the 314 nitrogen-poor high-elevation pool within all vegetation ecological groups. The increase of these 315 species at lower elevation could be probably related to the seed transportation operated by 316 livestock. Indeed, the RGS implementation allowed livestock to homogenise the selection for 317 different areas within the paddock, even the most inaccessible, steep and high-altitude ones 318 (Bailey et al. 1998; Probo et al. 2014). Therefore, animals may have promoted seed 319 transportation and increased connectivity amongst different communities. The increase in 320 ruderal species number within communities generally avoided by free-ranging animals can also 321 be connected to the "disturbance" effect of the grazing livestock (Bullock et al. 2001; Teuber et 322 al. 2013). Although many of these species, characterized by relative short life, light-demanding 323 and which produce a large amount of seeds, are important for pollinators, some of them (i.e. 324 *Cirsium eriophorum* (L.) Scop., *Cirsium spinosissimum* (L.) Scop.) should be monitored in the 325 coming years to avoid their negative effect on forage quality and palatability (Pittarello et al. 326 2018). However, the ecological impact of these species is limited, since they are all 327 autochthonous species and any alien species was recorded in the study area due to high altitude 328 constraints (Marini et al. 2009).

- 329 To conclude, RGS with stocking rate adjustments was an effective and a sustainable
- 330 management tool to protect semi-natural grassland diversity threatened by the abandonment,
- 331 even in high-altitude grasslands. However, to assess the long-term effects produced by this
- 332 policy and management tool implementation, a longer monitoring of the vegetation and a deeper
- 333 assessment of other ecological variables and animal productivity would be advisable for future
- research. Specifically, the possibility that RGS promote a homogenization of botanical
- 335 composition across different vegetation communities within paddocks due to the loss of peculiar
- 336 ecological niches, should be further investigated on the long term.

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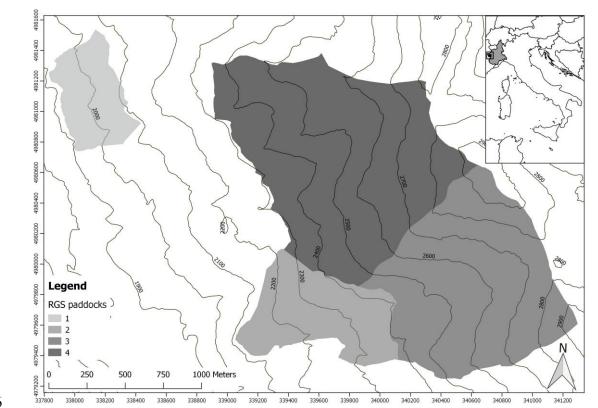
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- 536 Supplementary material
- 537 Appendix S1. Functional pools of species recorded in the study area with the corresponding
- phytosociological optimum at the class level. \* = groups taken into account for statistical
  analyses.
- 540 Appendix S2. Vegetation ecological groups and types (labelled according to dominant species
- names), corresponding phytosociological community, and area covered by each of them.
- 542 Appendix S3. List of the plant species recorded in the botanical transects, with the
- 543 corresponding phytosociological optimum and class, functional pool of species, and N index
- 544 (Landolt et al. 2010).



**Figure 1.** Location of the study area in Val Troncea, Piedmont, Italy (UTM zone 32 N, WGS84 datum).

**Table 1.** Mean species richness, Shannon diversity index (H' index), Landolt N indicator (N index) values, and beta-diversity (Sørensen, Horn and Morosita-Horn indexes) in 2011 and 2016 for the whole

547 548 549 550	<b>Table 1.</b> Mean species richness, Shannon diversity index (H' index), Landolt N indicator (N index) values, and beta-diversity (Sørensen, Horn and Morosita-Horn indexes) in 2011 and 2016 for the whole vegetation dataset. Asterisks represent the statistical significance level of differences between 2011 and 2016: *** $P < 0.001$ ; ** $P < 0.01$ ; * $P < 0.05$ ; ns, not significant ( $P > 0.1$ ).

2011		2016		
Mean	SE	Mean	SE	<i>P</i> -value
$28.72 \pm$	0.622	36.11 ±	0.814	***
3.35 ±	0.040	3.51 ±	0.042	***
2.32 ±	0.020	2.40 ±	0.025	***
0.22 ±	0.011	$0.22 \pm$	0.013	ns
0.45 ±	0.006	$0.46 \pm$	0.007	ns
0.75 ±	0.009	0.76 ±	0.008	ns
	Mean           28.72         ±           3.35         ±           2.32         ±           0.22         ±           0.45         ±	Mean         SE $28.72 \pm 0.622$ $3.35 \pm 0.040$ $2.32 \pm 0.020$ $0.22 \pm 0.011$ $0.45 \pm 0.006$	Mean         SE         Mean $28.72 \pm 0.622$ $36.11 \pm$ $3.35 \pm 0.040$ $3.51 \pm$ $2.32 \pm 0.020$ $2.40 \pm$ $0.22 \pm 0.011$ $0.22 \pm$ $0.45 \pm 0.006$ $0.46 \pm$	Mean         SE         Mean         SE $28.72 \pm 0.622$ $36.11 \pm 0.814$ $3.35 \pm 0.040$ $3.51 \pm 0.042$ $2.32 \pm 0.020$ $2.40 \pm 0.025$ $0.22 \pm 0.011$ $0.22 \pm 0.013$ $0.45 \pm 0.006$ $0.46 \pm 0.007$

551 552 553 554 Table 2. Mean species richness, Shannon diversity index (H' index) and Landolt N indicator value (N index) values in 2011 and 2016 vegetation transects. Asterisks represent the statistical significance level of differences between 2011 and 2016: \*\*\* P < 0.001; \*\* P < 0.01; \* P < 0.05; ns, not significant (P >

0.1).

		2011		2016	
Vegetation ecological group	Variables	Mean	SE	Mean SE	P-value
1. Eutrophic group	Sp. richness	28.14	± 1.133	$34.43 \pm 1.6$	06 ***
	H' index	3.51	$\pm 0.069$	$3.68 \pm 0.0$	94 *
	N index	2.70	$\pm 0.054$	$2.70 \pm 0.0$	89 ns
2. Mesotrophic group	Sp. richness	34.50	$\pm$ 3.864	$46.00 \pm 5.2$	*13 *
	H' index	3.73	$\pm 0.105$	$3.78 \pm 0.1$	26 ns
	N index	2.63	$\pm 0.057$	$2.83 \pm 0.0$	64 **
3. Oligotrophic group	Sp. richness	26.53	$\pm 0.908$	$33.50 \pm 1.5$	33 ***
	H' index	3.41	$\pm 0.062$	$3.51 \pm 0.0$	077 ns
	N index	2.28	$\pm 0.027$	$2.33 \pm 0.0$	31 *
4. Thermic group	Sp. richness	30.53	± 1.165	$38.27 \pm 1.3$	19 ***
	H' index	3.36	$\pm 0.062$	$3.59 \pm 0.0$	64 ***
	N index	2.14	$\pm 0.026$	$2.24 \pm 0.0$	33 **
5. Pre-forest group	Sp. richness	29.16	$\pm 1.505$	$36.56 \pm 1.9$	43 ***
	H' index	3.14	$\pm 0.134$	$3.38 \pm 0.1$	14 **
	N index	2.22	$\pm 0.024$	$2.31 \pm 0.0$	42 *
6. Snow-bed group	Sp. richness	26.75	$\pm 1.851$	$35.08 \pm 2.1$	55 **
	H' index	3.10	$\pm 0.177$	$3.11 \pm 0.2$	15 ns
	N index	2.39	$\pm 0.065$	$2.52 \pm 0.0$	89 ns
7. Rocky group	Sp. richness	20.50	$\pm 2.172$	$22.50 \pm 2.9$	52 ns
	H' index	2.83	± 0.244	$2.70 \pm 0.2$	02 ns
	N index	2.32	$\pm 0.089$	$2.09 \pm 0.0$	74 *

**Table 3.** Results of the PERMANOVA analysis for the ecological groups comparing botanical composition in 2011 and 2016 (Permutation number = 9999). Asterisks represent the statistical significance level of differences between 2011 and 2016: \*\*\* P < 0.001; \*\* P < 0.01; \* P < 0.05; ns, not significant (P > 0.1). SST = Total sum of squares; SSW = Within-group sum of squares. 557 558

Vegetation ecological group	SST	SSW	F	P-value
1. Eutrophic group	17.8	17.6	0.83	ns
2. Mesotrophic group	9.9	9.45	1.89	*
3. Oligotrophic group	18.1	17.6	2.35	**
4. Thermic group	41.9	41.2	1.83	*
5. Pre-forest and shrub-encroached group	24.0	23.7	0.93	ns
6. Snow-bed group	6.8	6.47	1.08	ns
7. Rocky group	2.6	2.4	0.97	ns

**Table 4.** Effects produced by Grazing Management Plan implementation on the total number and cover of species having the phytosociological optimum belonging to different functional species pools for the whole vegetation dataset. Asterisks represent the statistical significance level of differences between 2011 and 2016: \*\*\* P < 0.001; \*\* P < 0.01; \* P < 0.05; "+" P < 0.1; ns, not significant (P > 0.1). 561

			Spe	ecies nu	mb	er				Sp	pecies c	ove	er	
	2011			2016				2011			2016			_
Functional species pool	Mean		SE	Mean		SE	P-value	Mean		SE	Mean		SE	P-value
Ruderal plant species	0.8	±	0.05	1.1	±	0.06	***	2.9	±	0.44	3.2	±	0.39	ns
Snow-bed species	0.5	±	0.08	0.7	±	0.10	**	2.5	±	0.69	3.2	±	0.84	ns
Nitrogen-poor high-elevation species	15.7	±	0.42	20.4	±	0.50	***	99.1	±	3.30	120.4	±	3.91	***
Fringe and tall herb species	0.6	±	0.10	0.7	±	0.12	+	9.2	±	1.50	9.8	±	1.53	ns
Dry species	2.4	±	0.21	2.8	±	0.27	*	29.4	±	2.27	31.8	±	2.73	ns
Meso-eutrophic species	3.0	±	0.18	3.4	±	0.23	**	32.4	±	2.56	42.9	±	3.84	***

**Table 5.** Effects producted by Grazing Management Plan implementation on the total number and cover of species having the phytosociological optimum belonging to different functional species pools for each vegetation ecological group identified. Asterisks represent the statistical significance level of differences between 2011 and 2016: \*\*\* P < 0.001; \*\* P < 0.01; \* P < 0.05; "+" P < 0.1; ns, not significant (P > 0.1). 564

				Spe	ecies nu	mbe	r				Sp	oecies c	ove	r	
			2011			2010	6	_	2	201	l	2	201	6	_
Vegetation ecological group	Functional species pool	Mean		SE	Mean		SE	<i>P</i> -value	Mean		SE	Mean		SE	<i>P</i> -value
1. Eutrophic group	Ruderal species	1.1	±	0.16	1.4	±	0.17	+	8.9	±	2.23	6.5	±	1.26	ns
	Snow-bed species	1.4	±	0.37	2.1	±	0.49	ns	3.2	±	1.48	2.9	±	1.31	ns
	Nitrogen-poor high-elevation species	17.4	±	0.89	21.6	±	1.30	*	78.9	±	8.58	102.7	±	7.65	**
	Fringe and tall herb species	0.1	±	0.10	-	±	-	ns	2.3	±	0.97	7.5	±	2.55	*
	Dry species	1.0	±	0.30	0.8	±	0.28	ns	35.5	±	8.16	42.9	±	10.67	ns
	Meso-eutrophic species	2.6	±	0.17	2.8	±	0.30	ns	72.8	±	8.15	81.9	±	14.48	ns
2. Mesotrophic group	Ruderal species	1.4	±	0.46	2.1	±	0.23	ns	1.2	±	0.67	3.7	±	1.32	+
	Snow-bed species	0.3	±	0.25	-	±	-	ns	0.0	±	0.00	0.0	±	0.00	ns
	Nitrogen-poor high-elevation species	12.6	±	1.15	19.8	±	1.35	**	111.5	±	9.28	138.7	±	11.88	ns
	Fringe and tall herb species	2.9	±	0.90	3.3	±	1.10	ns	36.2	±	7.46	36.3	±	7.93	ns
	Dry species	4.6	±	1.12	5.6	±	1.51	ns	29.8	±	4.28	30.5	±	9.40	ns
	Meso-eutrophic species	8.8	±	1.15	10.8	±	2.08	ns	82.3	±	10.82	117.1	±	17.78	*
3. Oligotrophic group	Ruderal species	0.7	±	0.08	1.2	±	0.10	***	2.9	±	0.70	3.4	±	0.82	ns
	Snow-bed species	0.3	±	0.11	0.6	±	0.15	*	0.4	±	0.21	1.4	±	1.07	ns
	Nitrogen-poor high-elevation species	17.9	±	0.57	22.2	±	0.92	***	148.4	±	5.00	160.7	±	5.93	+
	Fringe and tall herb species	0.1	±	0.05	0.1	±	0.06	ns	0.0	±	0.00	0.5	±	0.53	ns
	Dry species	1.3	±	0.25	1.4	±	0.27	ns	12.9	±	2.15	14.5	±	3.09	ns
	Meso-eutrophic species	2.2	±	0.16	2.3	±	0.18	ns	18.7	±	3.11	21.8	±	3.19	ns
4. Thermic group	Ruderal species	0.8	±	0.07	1.1	±	0.09	*	2.5	±	0.69	3.4	±	0.75	ns
	Snow-bed species	0.3	±	0.09	0.3	±	0.11	ns	0.9	±	0.41	0.9	±	0.52	ns
	Nitrogen-poor high-elevation species	16.4	±	0.80	21.5	±	0.87	***	104.9	±	4.51	125.2	±	6.28	**
	Fringe and tall herb species	0.4	±	0.12	0.4	±	0.13	ns	4.7	±	1.64	2.4	±	0.97	+

	Dry species	3.5	±	0.36	4.2	±	0.45	*	46.9	±	4.11	48.5	±	4.59	ns
	Meso-eutrophic species	2.8	±	0.26	3.6	±	0.30	**	19.2	±	2.60	26.9	±	3.75	*
5. Pre-forest and shrub-encroached	Ruderal species	0.6	±	0.13	0.9	±	0.16	*	0.7	±	0.35	1.6	±	0.70	ns
group	Snow-bed species	0.0	±	0.04	0.2	±	0.10	ns	0.0	±	0.00	1.0	±	0.76	ns
	Nitrogen-poor high-elevation species	13.4	±	0.96	17.6	$\pm$	1.24	***	62.2	±	6.20	85.3	±	9.75	**
	Fringe and tall herb species	1.5	±	0.32	2.0	±	0.36	+	22.1	±	5.15	25.3	±	5.19	ns
	Dry species	2.6	±	0.59	3.5	±	0.87	*	25.1	±	5.68	30.3	±	6.18	ns
	Meso-eutrophic species	3.1	±	0.37	3.1	±	0.35	ns	17.4	±	3.01	26.9	±	5.61	+
6. Snow-bed group	Ruderal species	0.8	±	0.21	0.8	±	0.21	ns	2.3	±	1.08	0.4	±	0.38	+
	Snow-bed species	1.8	±	0.34	2.7	±	0.36	*	25.0	±	7.70	28.1	±	8.94	ns
	Nitrogen-poor high-elevation species	14.8	±	1.67	20.4	$\pm$	1.53	*	69.6	$\pm$	8.96	106.9	±	17.96	+
	Fringe and tall herb species	-	±	-	-	±	-	ns	0.9	±	0.92	1.5	±	1.54	ns
	Dry species	0.8	±	0.30	0.4	±	0.15	ns	6.0	±	3.55	1.5	±	0.67	ns
	Meso-eutrophic species	2.9	±	0.29	3.4	±	0.31	ns	31.1	±	6.94	55.4	±	14.75	*

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**Appendix S1.** Functional pools of species recorded in the study area with the corresponding phytosociological optimum at the class level. \* = groups taken into account for statistical analyses. Syntaxonomic nomenclature follows Aeschimann et al. 2004.

Functional species pool	Phytosociological class
Dry species*	Festuco-Brometea
Meso-eutrophic species*	Molinio-Arrhenatheretea
Snow-bed species*	Salicetea herbaceae
Nitrogen-poor high-elevation species	Juncetea trifidi,
(acidic and calcareous)*	Nardetea strictae,
	Carici rupestris-Kobresietea bellardii,
	Elyno-Seslerietea variae
Fringe and tall herb species*	Epilobietea angustifolii,
	Filipendulo-Convolvuletea,
	Mulgedio-Aconitetea,
	Trifolio-Geranietea sanguinei
Ruderal species*	Agropyretea intermedii-repentis,
	Artemisietea vulgaris,
	Polygono-Poetea annuae,
	Stellarietea mediae
Boreal forest species	Erico-Pinetea,
	Pyrolo-Pinetea,
	Vaccinio-Piceetea excelsae
Boreal shrubland species	Betulo carpaticae-Alnetea viridis,
	Crataego-Prunetea,
	Roso pendulinae-Pinetea mugo
Broad-leaved forest species	Carpino-Fagetea,
	Quercetea robori-sessiliflorae
Rocky species	Asplenietea trichomanis,
	Thlaspietea rotundifolii

**Appendix S2.** Vegetation ecological groups and types (labelled according to dominant species names), corresponding phytosociological community, and area covered by each of them. Vegetation ecological groups and types follows Cavallero et al. (2007), species nomenclature follows Pignatti et al. 1982, syntaxonomic nomenclature follows Aeschimann et al. 2004. 570

Vegetation ecological group	Vegetation type	Phytosociological plant community	Area (ha)	Are (%)
1 - Eutrophic group			58.5	13.
	Dactylis glomerata	Polygono-Trisetion	22.5	5.0
	Poa alpina	Crepido-Festucetum commutatae (=Poetum alpinae)	36.0	8.0
2 - Mesotrophic group			42.8	9.6
	Festuca gr. rubra and Agrostis tenuis	Nardo-Agrostion tenuis	42.8	9.6
3 - Oligotrophic group			85.6	19.
	Nardus stricta	Nardion strictae	58.5	13.
	Carex sempervirens	Caricion curvulae	6.8	1.5
	Trifolium alpinum and Carex sempervirens	Caricion curvulae	20.3	4.5
4 - Thermic group			139.6	31.
	Carex rosae	Seslerion variae	13.5	3.0
	Elyna myosuroides	Oxytropido-Elynion	13.5	3.0
	Festuca quadriflora	Caricion firmae	22.5	5.0
	Helianthemum nummularium	Transition between <i>Seslerion variae</i> and <i>Caricion ferrugineae</i>	78.8	17.
	Helianthemum oelandicum	Seslerion coeruleae (transition towards Caricion ferrugineae)	4.5	1.(
	Sesleria varia	Seslerion variae	6.8	1.5
5 – Pre-forest and shrub-encroached group			78.8	17.
	Calamagrostis villosa	Calamagrostion villosae	4.5	1.(
	Juniperus nana	Junipero-Arctostaphyletum	33.8	7.:
	Vaccinium gaultherioides	Loiseleurio-Vaccinion	40.5	9.0
6 - Snow-bed group			29.3	6.5
	Plantago alpina	Transition between Salicion herbaceae and Nardion strictae	18	4.0
	Salix herbacea	Salicion herbaceae	11.3	2.5
7 - Rocky group			13.5	3.0
	Saxifraga oppositifolia	Thlaspion rotundifolii	13.5	3.0
			448.1	10

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