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CONSERVATION AND RESTORATION OF DEGRADED SEMI-NATURAL GRASSLANDS WITH TWO LIVESTOCK SPECIES.

Study of vegetation dynamics and animal spatial distribution

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Preface

Semi-natural European grasslands are undoubtedly important for human beings. For millennia they were managed with low-intensity agricultural practices (such as extensive grazing and mowing), resulting in grassland ecosystems characterized by high natural value. Starting from the mid XX century, the intensification of agriculture, which resulted from the development of the food industry, induced a dramatic decline of extensive agricultural systems, such as those based on semi-natural grasslands, and the loss of traditional and sustainable food production supply chains. The society had to face a crucial challenge, consequently: the conservation of semi-natural grasslands, which are more and more threatened by both the abandonment on the one hand, and intensification on the other. This challenge cannot be met without working for more resilient, more profitable, and more environmentally sustainable grazing systems.

This thesis addresses the issue of conservation and restoration of semi-natural grasslands through livestock grazing, in two different environments and with two different livestock species. The author hopes that this contribution could increase the body of knowledge on this topic, highlighting the importance of extensive grazing systems in our society, and providing practical implications for the grassland management.

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1. Introduction

1.1. Threats and challenges of European grasslands

Permanent grasslands occur over 62 million ha in Europe (EU-28; Eurostat data 2021), corresponding to 34.5% of the total utilized agricultural area (UAA). Among EU-28 countries, the highest proportion is found in Ireland (90.2% of UAA) and the lowest in Finland (1% of UAA). Permanent grasslands play a crucial role in the European economy and food production, yet their surface declined in the last 60 years (-30% from 1967 to 2007 in EU-6, i.e. Belgium, France, Italy, Luxemburg, The Netherlands, and West Germany; Peeters 2015).

Semi-natural European grasslands are a hotspot of biodiversity (Dengler et al. 2014; Habel et al. 2013). They host the highest plant species richness in the world at plot size <100 m^2 (116 and 98 vascular plants per 25 and 10 m^2, respectively; Wilson et al. 2012; Dengler et al. 2012). This huge diversity is associated with an intermediate disturbance level, according to the Intermediate Disturbance Hypothesis (Grime 1973). Indeed, traditional low intensity grazing and mowing can be considered intermediate disturbances, which ensure species richness to peak as a result of the co-existence of several species due to ecological niche overlaps (Pittarello et al. 2018; Škornik et al. 2010). For instance, European calcareous dry grasslands were managed by shepherding for centuries, resulting in an outstanding hotspot of biodiversity (Poschlod & WallisDeVries 2002). The link between agro-pastoral management and high natural value of semi-natural habitats has been clearly emphasized by Halada et al. (2011), who reported 28 grassland habitats out of 32 listed in Annex I (92/43/CEE ‘Habitats Directive’) to be fully (16 habitats) or partly (12 habitats) dependent on low intensity agricultural management for their existence. Semi-natural European grasslands are not only a biodiversity hotspot, but play also a key role for the provision of several other ecosystem services, such as food production, pollination, nutrient cycle regulation, CO_2 stock, landscape quality, and cultural heritage (Bengtsson et al. 2019; Villoslada Peciña et al. 2019).

According to the EU Red List of Habitats, almost half of the grassland habitats are threatened (Janssen et al. 2016). Such threats are mostly driven by opposite trends: intensification and abandonment of agricultural practices (Hülber et al. 2017; Isselstein et al. 2005; Valkó et al. 2018). On one hand, intensification through fertilization, increase of stocking rates, mechanization, irrigation, and water drainage caused widespread loss of species-rich grassland habitats. On the other hand, the abandonment of marginal areas (e.g., mountain regions) caused fragmentation and shift to shrublands and forests. Other threats include nitrogen and phosphorus deposition and climate change (Ceulemans et al. 2011; Schirpke et al. 2017; Stevens et al. 2011).

In the Alps, the abandonment of grazing and mowing traditional practices is a widespread socio-economic process that caused loss of grassland habitats and diversity (Maurer et al. 2006; Orlandi et al. 2016; Tasser & Tappeiner 2002). Indeed, the lack of management leads to litter accumulation, dominance of a few competitive species, and shrub and tree encroachment, which in turn adversely affect plant diversity and composition (Bohner et al. 2020; Pittarello et al. 2016b; Tasser & Tappeiner 2002). For instance, from 1985 to 2013, shrublands and forests increased by 10.6% across Switzerland, at the expense of open habitats (Abegg et al. 2020). Moreover, also the reduction in stocking rates and inappropriate management practices contributed to grassland degradation in the Alps. When the stock is managed with a low stocking rate over large pastures (such as in continuous grazing systems), the surface is unevenly exploited by the animals, resulting in over and under-grazing situations at the same time (Perotti et al. 2018; Probo et al. 2014).

To face the challenging decline of grassland diversity in marginal areas, such as in mountain regions, the development of sustainable and resilient extensive grazing systems is a major priority. Grazing has proven to be effective for both conservation, improvement, and restoration of grasslands: it can slow down or reverse secondary succession, while favoring diversity by reducing light competition and increasing spatial heterogeneity (Dostálek & Frantík T. 2008; Rupprecht et al. 2016; Tälle et al. 2016; Borer et al. 2014). Moreover, the balanced management of grasslands can be enhanced by adopting specific management practices, which can promote a more efficient exploitation of these systems. For instance, the implementation of rotational grazing systems has proven to be effective for the improvement of botanical composition, diversity, and forage quality (Perotti et al. 2018). Also strategically placed attractive points can be used to influence animal spatial distribution and reduce over and under-grazing situations (Pittarello et al. 2016a).

1.2. Relationships between vegetation and livestock spatial distribution

The effects of grazing on vegetation result from the combination of defoliation, trampling, and nutrient addition through feces and urine depositions (Lezama & Paruelo 2016). The impacts of such factors change according to grazing intensity, resulting in different plant species compositions. For instance, high nitrogen inputs and treading under high grazing intensity could favor nutrient-demanding species and rosette and prostrate growth forms. On the other hand, woody growth forms can dominate under-exploited areas, where defoliation is very limited (Škornik et al. 2010; Pizzio & Herrero-Ja 2016). Also grazing selectivity changes at different grazing intensities, with lower selection expressed at higher grazing intensity (Pittarello et al. 2017). The importance of grazing intensity in shaping plant composition in alpine grasslands has been recently highlighted by Pittarello et al. (2020), who found that it has a significant influence on plant diversity and forage quality.

The distribution of livestock is generally uneven over a pasture, resulting in areas characterized by different livestock site use intensities (Bailey 2005). Livestock spatial distribution is affected by several factors at pasture-scale: environmental characteristics (i.e., slope, aspect, terrain roughness, accessibility), attractive points (e.g., water and supplement sources, night sheds, milking barns), and vegetation (e.g., palatability, forage quality and quantity) (Dorji et al. 2013; Probo et al. 2014; Putfarken et al. 2008). This especially applies in mountain regions, which are characterized by high spatial heterogeneity.

The relationship between vegetation and livestock spatial distribution is thus bidirectional: on one hand, livestock spatial distribution influences vegetation, with more intensively used areas characterized by different plant assemblages than under-used areas; on the other hand, plant communities affect animal spatial distribution, with high quality forage being more attractive than low quality one (Bailey et al. 2005). These complex relationships, i.e. which are the drivers of livestock spatial distribution and which are the effects on vegetation, should be taken into account for a sustainable management of semi-natural grasslands.

The spatial distribution of livestock can be measured both directly and indirectly: direct methods include visual observations and GPS collars; indirect proxies include distance from congregation areas, counting of dung pats, and vegetation-derived indices (Bailey et al. 2018; Dorji et al. 2013; Pittarello et al. 2021). Among these, the use of GPS collars in research studies has greatly increased the knowledge on animal movements and behavior, providing useful information for land managers and conservationists (Swain et al. 2011). The first studies with GPS collared cows were performed more than 20 years ago in North American rangelands (Bailey et al. 2018; Swain et al. 2011; Turner et al. 2000). This technology allows to gather a huge quantity of data, for long periods, and with a minimal effort from the researcher in the field. By combining GPS data positioning and environmental and vegetation information, it is possible to investigate the complex relations among multiple drivers of livestock spatial distribution and inspect site use intensity over the pasture and during the grazing season (Bailey et al. 2015; Probo et al. 2014; Swain et al. 2011).

1.3. Overview of the two case-studies

In this thesis, two case-studies focusing on the conservation and restoration of semi-natural grasslands through livestock grazing are presented. The first one is about the reintroduction of sheep grazing for the conservation and restoration of species-rich dry grasslands (6210* and 6240* EU Habitats) in the Susa Valley, Italian Alps (Figure 1). The second one concerns the use of Highland cattle for the restoration of *Alnus viridis*-encroached subalpine pastures in the Italian and Swiss Alps (Figure 2). The outcomes of these studies are included in four scientific papers (two per each case-study) and are herein reproduced in Chapter 2 and Chapter 3, as follows:

2. Conservation of semi-natural dry grasslands with sheep grazing
    - 2.1 Environmental and management factors affecting site use intensity by sheep. This sub-chapter is a reproduction of the paper '*Distance from night penning areas as an effective proxy to estimate site use intensity by grazing sheep in the Alps*' (2019), published on *Agronomy*.
    - 2.2 Effects of sheep grazing and wildfire on dry grassland diversity and composition. This sub-chapter is a reproduction of the paper '*Sheep grazing and wildfire: disturbance effects on dry grassland vegetation in the Western Italian Alps*' (2021), published on *Agronomy*.
3. Restoration of *Alnus viridis*-encroached pastures with Highland cattle grazing
    - 3.1 Environmental and vegetation factors affecting Highland cattle spatial distribution. This sub-chapter is a reproduction of the paper '*Spatial distribution of Highland cattle in Alnus viridis encroached subalpine pastures*' (2021), published on *Frontiers in Ecology and Evolution*.
    - 3.2 Effects of Highland cattle on *Alnus viridis*-encroached pastures with the use of attractive supplements. This sub-chapter is a reproduction of the paper '*Use of molasses-based blocks to modify grazing patterns and increase Highland cattle impacts on Alnus viridis-encroached pastures*', submitted to *Frontiers in Ecology and Evolution*.

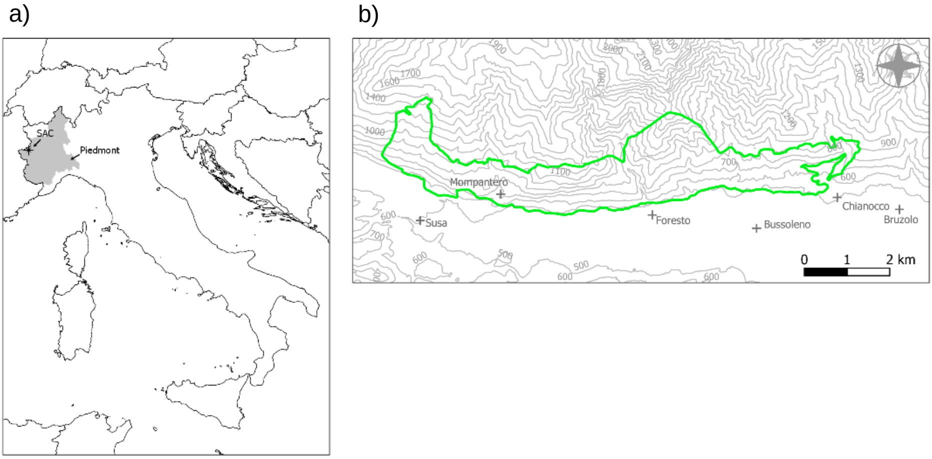

Figure 1 – (a) Location and (b) map of the Special Area of Conservation (SAC) ‘Oasi xerotermiche della valle di Susa – Orrido di Chianocco’ where the first case-study was conducted in the Susa Valley (Piedmont Region), Italian Alps.

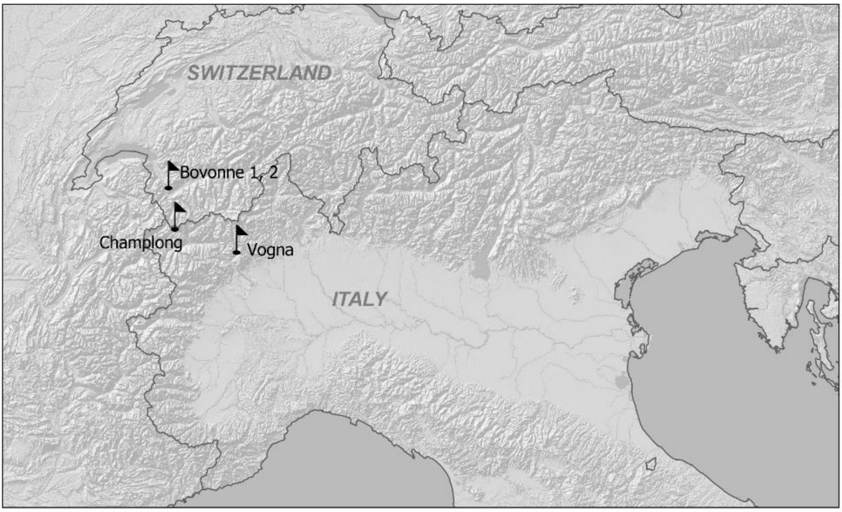

Figure 2 – Location of the sites where the second case-study was conducted in the Italian (Vogna) and Swiss Alps (Bovonne 1, Bovonne 2, Champlong)

The case-studies are representative of general situations that can be observed throughout the Alps: since the mid XX century, semi-natural dry grasslands were largely abandoned due to their low biomass production and difficult mechanization and intensification (steep slopes, shallow soil, paucity of water sources etc.); subalpine pastures faced either a reduction of stocking rates and the abandonment of the most isolated areas due to difficult accessibility and poor road network (Tasser & Tappeiner 2002).

In each case-study, different grazing systems and livestock species are described, according to the characteristics of the sites. In the first case-study, sheep were used for the exploitation of dry grasslands characterized by low quality vegetation, steep slopes and rough terrain. They were managed by the shepherd throughout the day, and confined in temporary penning areas at night. In the second case-study, Highland cattle were managed with rotational grazing in large paddocks and with the use of strategically-placed feed supplements. The Highland breed is characterized by high adaptability to cold weather, low feeding selectivity, and ease movement in rough and rocky terrain, which are typical conditions of the alpine environment (Pauler et al. 2020a, b).

These researches aimed at increasing the knowledge about the role of extensive grazing systems in the safeguarding of mountain semi-natural grasslands threatened by land abandonment. More specifically, in both case-studies, two main topics were explored: (1) the environmental, management, and vegetation drivers of livestock spatial distribution and (2) the effects produced by livestock grazing on vegetation, also in relation to different grazing intensities. The first topic was addressed through the use of GPS collars. In the first case-study, the obtained data were used to produce a simple proxy to estimate site-use intensity by grazing sheep, while in the second case-study they were used to investigate the ability of Highland cattle to exploit *A. viridis*-encroached pastures and the effectiveness of feed supplements to modify their spatial distribution. The second topic was addressed by studying the effects of sheep grazing on plant diversity and composition along a gradient of increasing disturbance (first case-study) and by exploring the grazing impacts induced by the use of attractive supplements on *A. viridis* plants and vegetation cover (second case-study).

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2. Conservation of semi-natural dry grasslands with sheep grazing

2.1. Environmental and management factors affecting site use intensity by sheep

This sub-chapter is a reproduction of the scientific paper published on *Agronomy*, 2019, 9, 333, with permission from the authors:

Distance from Night Penning Areas as an Effective Proxy to Estimate Site Use Intensity by Grazing Sheep in the Alps.

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Abstract

Livestock site use intensity can vary widely across a grazing area due to several factors such as topography and distance from sheds and water sources. However, an accurate approximation of animal site use should be assessed for each part of the grazing area to apply effective management strategies. In the Alps, shepherds manage sheep through lenient supervision during the day and confining the animals in temporary night penning areas (TNPA) at night. In our case study, we assessed sheep site use over the grazing area with global positioning system (GPS) collars and calculated the sums of inverse distances from all TNPA (unweighted and weighted on the number of penning nights) and from all water sources, as well as the slope, on 118 sample points. We assessed the relative importance of these variables in affecting site use intensity by animals using different sets of models. Both the unweighted and weighted distances from TNPA were found to be the most important factors. The best fitting model accounted for the weighted distance from TNPA and the distance from water, but the latter showed a lower relative importance. Our study suggests that using the distance from TNPA, preferably weighted on the number of penning nights, is an effective proxy to estimate the spatial variability of sheep stocking rate during grazing in the Alps.

Keywords: drinking sources; GIS; grazing behavior; pastures; spatial distribution; stocking rate

Introduction

Livestock grazing is useful for the implementation of management strategies that address the restoration, improvement, or maintenance of grassland vegetation (Metera et al. 2010). As a basic criterion, the effectiveness of such strategies depends on livestock stocking rate, which can strongly affect nutrient availability, plant species diversity, and vegetation dynamics (Perotti et al. 2018; Pittarello et al. 2016b). Stocking rate can be quantified for the entire grazing area as the overall average number of animals per hectare and time unit. However, the overall stocking rate dismisses animal site use intensity, which can have wide variability over the grazing area due to differences in forage quality and quantity, topography (e.g., slope), animal behavior, and the presence of attractive points such as drinking troughs, sheds, and milking areas (Bailey et al. 1996; Manthey & Peper 2010; Pittarello et al. 2016a; Probo et al. 2014; Russell et al. 2012; Svoray et al. 2009). Animal site use can be measured directly at each site of the grazing area through global positioning system (GPS) collars, visual observations, etc. Otherwise, it can be estimated through indirect measures (proxies) such as the distance from congregation areas like sheds or water sources. These proxies usually assume that animal site use decreases with increasing distances from congregation areas (Tarhouni et al. 2010). More specifically, several authors (Fernandez-Gimenez & Allen-Diaz 2001; Manthey & Peper 2011; Wesuls et al. 2013) proved that inverse distances from congregation areas were linearly related to animal site use. However, the reliability of such proxies was rarely validated with direct measurements (Putfarken et al. 2008; Svoray et al. 2009) and, to date, no comparative studies have been conducted to assess the different proxies.

In the Alps, a reliable proxy to estimate site use intensity by animals would be particularly useful since pastures are characterized by a high spatial heterogeneity due to changes in topography and vegetation. In these environments, sheep flocks are commonly managed by shepherds in a daily routine, which entails lenient supervision during the day and confinement in temporary areas during the night (temporary night penning areas—TNPA) (Lombardi 2005). TNPA confine flocks to areas of about 1–3 m^2/sheep by means of electrified fences, and they are moved over the grazing area every one-four days, generally located in sites with homogeneous topographic conditions and limited presence of rocks.

TNPA help to prevent wolf attacks and, occasionally, contrast shrub encroachment and improve grassland vegetation due to livestock trampling and dung deposition (Espuno et al. 2004; Pittarello et al. 2017). TNPA, as well as water sources, gentle terrains, and milking areas, can therefore be considered the main congregation areas that affect grazing sheep site use in the Alps.

Our study aims to implement a method that uses a GPS/GIS assessment to determine the relative importance of distance from TNPA, distance from water sources, and slope in affecting sheep site use intensity during grazing.

Materials and Methods

The study was conducted in the northwestern Italian Alps (45°08′ N, 7°06′ E) in the Site of Community Interest ‘Oasi xerotermiche della Valle di Susa—Orrido di Chianocco e Foresto’ (SCI IT1110030), an area characterized by a xerothermic and sub-Mediterranean climate with an average annual temperature of 11 °C and an average annual precipitation of 670 mm (Biancotti et al. 1998). Slopes ranged from 4° to 65° (average 28°) and the elevation ranged from 510 to 1260 m a.s.l. The grazing area was characterized by homogeneous seminatural dry grasslands dominated by *Stipa pennata* L., *Bromus erectus* Hudson, and *Festuca ovina* s.l.

From 15 April to 16 May 2015, a flock of 250 Bergamasca (meat breed) sheep grazed over a 45 ha area. Fourteen TNPA (average area: 737 ± 74.0 m^2) were progressively set out over the area and each was used to fence the sheep in from two to three consecutive nights (2.3 ± 0.47; mean ± standard deviation). During the period, four water sources homogeneously distributed over the grazing area were also made available to the sheep.

Ten randomly selected sheep were equipped with GPS collars (Model Corzo, Microsensory SLL, Fernàn Nùñez, Spain; 5 m accuracy) and tracked at 15 min intervals for the entire duration of the experiment. The tracked sheep were dry ewes, two to four years old, weighing approximately 70 kg, and regularly fed on Alpine pastures during the summer. During the study, the flock experienced this specific grazing area for the first time. We assumed the 10 selected sheep as representative of the entire flock, since sheep are livestock characterized by a highly cohesive grazing behavior.

We randomly generated 160 sample points over the grazing area and assessed the number of GPS fixes within a 30 m buffer zone around each of them as a direct measurement of the site use intensity by grazing sheep (Pittarello et al. 2017; Putfarken et al. 2008). The 30 m distance was considered to encompass a zone with homogeneous vegetation and topographic conditions. The sample points were spaced 60 m apart to avoid overlaps between buffers. When a buffer zone exceeded the grazing area, the number of GPS fixes included was weighted by the within-the-grazing-area portion and rounded to the nearest integer value. Forty-two sample points were excluded from further analysis, as they exceeded the boundaries of the grazing area for more than 25% of their buffer zone, so 118 sample points were retained.

According to the following formulas, for each sample point we calculated:

(i) the sum of inverse distances from all TNPA (hereafter ‘unweighted distance from TNPA’)

$$(1) \qquad Unweighted\ d_{TNPA} = \sum_{i=1}^{i=14} \left(\frac{1}{d_i}\right)$$

where d_i is the distance from each TNPA;

(ii) the sum of inverse distances from all TNPA weighted on the number of consecutive penning nights for each of them (hereafter ‘weighted distance from TNPA’)

$$(2) \qquad Weighted\ d_{TNPA} = \sum_{i=1}^{i=14} \left(\frac{n_i}{d_i}\right)$$

where d_i is the distance from each TNPA and n_i is the corresponding number of nights;

(iii) the sum of inverse distances from all water sources (hereafter ‘distance from water’)

$$(3) \qquad d_{water} = \sum_{j=1}^{j=4} \left(\frac{1}{d_j}\right)$$

where d_j is the distance from each water source;

(iv) the slope, which is assessed as the average value of the buffer zone using a 10 m resolution digital terrain model (Regione Piemonte 2019).

Geographical analyses were conducted using Quantum GIS version 2.18.26 (QGIS Development Team 2016).

To assess the relative importance of (i) distance from TNPA (weighted and unweighted), (ii) distance from water, and (iii) slope in predicting the actual site use intensity by the sheep during grazing, we ran 11 generalized linear models (GLMs). We set the sheep site use intensity (i.e., the count of GPS fixes within each buffer zone) as response variable and set the distances from TNPA and from water as well as the slope in all possible combinations as explanatory variables. We specified a negative binomial error distribution for the GPS count and a logarithmic link function (McCullagh & Nelder 1983). All explanatory variables were standardized (Z-scores) before performing GLMs to allow for the analysis of effect size by scrutinizing model parameters (β coefficients). Autocorrelation was tested using Pearson's correlation before running the GLMs. Residual deviance, percent of explained deviance (D^2), Akaike information criterion with small-sample correction (AICc), and Bayesian information criterion (BIC) were used to compare the goodness of the model fit. D^2 was calculated according to the following formula:

$$\mathrm{D}^2 = \frac{\text{null deviance} - \text{residual deviance}}{\text{null deviance}}$$

where null deviance is the deviance of an intercept-only GLM and residual deviance is the deviance that remains unexplained after the model fit. Statistical analyses were performed using SPSS 25 (SPSS Inc., Chicago, IL, USA).

Results

The daily acquisition rate of the GPS devices refers to the total potential of daily fix acquisitions, and was 44.2 ± 2.54% (mean ± standard error). Explanatory variables showed a not significant ($p \geq 0.05$) or weak ($R \leq 0.25$) autocorrelation and all of them were retained in the models. Average values for the response and explanatory variables in buffer zones are provided in Table 1. According to the performed GLMs, the sheep site use was significantly related to the selected predictors (Table 2). More particularly, it was always positively affected by both unweighted and weighted distances from TNPA and the distance from water sources, but negatively by the slope. However, the slope effect was not significant when the distances from TNPA were weighted on the number of penning nights (M7 and M8). Among the explanatory variables, the distance from TNPA had the most influence (highest β coefficients) in all the models, followed by the distance from water and then by the slope. β coefficients increased for the distance from water and the slope in M9, M10, and M11, but their effect size was lower than those of the distances from TNPA.

Lower AICc and BIC scores were obtained in models including distances from TNPA and specifically, in models based on weighted distances (M5, M6, M7, and M8). The lowest values in terms of residual deviance and D2 were performed by M8, which considered weighted distance from TNPA, distance from water, and slope, although this latter variable was not significant. The same model deprived of slope (M6) showed the best fit according to AICc and BIC values.

Table 1. Summary statistics for the dependent and explanatory variables used in the models. Values apply to the 30 m buffer zone around the 118 random points. Numbers in brackets refer to formulas detailed in the Methods section. TNPA refers to temporary night penning areas.

Variable	Minimum	Mean	Maximum
Site use intensity (global positioning system (GPS) count)	0.00	35.14	434.00
Distance from TNPA—unweighted (m^{-1}) (1)	0.01	0.04	0.14
Distance from TNPA—weighted (m^{-1}) (2)	0.03	0.08	0.29
Distance from water (m^{-1}) (3)	0.02	0.10	1.67
Slope (°)	10.92	28.41	43.87

Table 2. Summary of generalized linear models (GLMs) of sheep site use intensity by different sets of explanatory variables. Numbers in brackets refer to formulas in the Methods section. β coefficients and significance levels are provided for each variable as results of the related GLM. Goodness of model fit (best values are highlighted in bold): D^2, explained deviance; AICc, Akaike's information criterion with small-sample correction; BIC, Bayesian information criterion. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; *ns*, $p \geq 0.05$.

Generalized Linear Model	**Distance from TNPA**	**Distance from Water**	**Slope**	**Residual Deviance**	**D^2 %**	**AICc**	**BIC**
M1: Distance from TNPA (unweighted) (1)	1.43 ***	-	-	111.5	63.1	892.7	898.1
M2: Distance from TNPA (unweighted) (1) + distance from water (3)	1.41 ***	0.18 **	-	102.6	66	885.9	894
M3: Distance from TNPA (unweighted) (1) + slope	1.40 ***	-	−0.20 *	109.2	63.9	892.5	900.6
M4: Distance from TNPA (unweighted) (1) + distance from water (3) + slope	1.36 ***	0.21 **	−0.21 *	100.4	66.8	885.8	896.5
M5: Distance from TNPA (weighted) (2)	1.47 ***	-	-	107.4	64.5	888.6	894
M6: Distance from TNPA (weighted) (2) + distance from water (3)	1.43 ***	0.21 **	-	99.2	67.2	**882.5**	**890.6**
M7: Distance from TNPA (weighted) (2) + slope	1.44 ***	-	−0.12 *ns*	106.5	64.8	889.7	897.8
M8: Distance from TNPA (weighted) (2) + distance from water (3) + slope	1.39 ***	0.23 **	−0.15 *ns*	**98.2**	**67.5**	883.6	894.4
M9: Distance from water (3)	-	0.76 ***	-	282.6	6.5	1063.8	1069.2
M10: Slope	-	-	−0.62 ***	267.8	11.4	1048.9	1054.4
M11: Distance from water (3) + slope	-	0.96 ***	−0.63 ***	243.3	19.5	1026.6	1034.7

Discussion

All GPS devices worked as expected with a satisfactory acquisition rate. However, the harsh morphology of the study area (i.e., very rocky, rough, and a steep mountainous environment) had a negative effect on the accuracy of GPS fix acquisition. This determined that the signal bounced off a considerable proportion of GPS fixes out of the study area borders, so these were excluded from the analyses.

The present research highlighted the remarkable relationships that exist among site use intensity by grazing sheep and specific environmental/management predictors, namely, distance from night penning areas, distance from water, and slope. As expected, site use intensity was inversely related to the slope and directly related to the distance from TNPA and water sources in all GLMs we performed (Amiri 2009; Svoray et al. 2009). Specifically, the models that included the distance from TNPA (both unweighted and weighted, i.e., from M1 to M8) explained remarkable percentages of deviance (>63%, higher than shown in Putfarken et al. 2008 and in Dorji et al. 2013), which proved the pivotal role of TNPA in affecting sheep distribution during grazing. Instead, the distance from water sources and the slope showed a weaker influence, as demonstrated by their lower β coefficients. This was also observed in models based only on these variables (M9, M10, and M11), which had the lowest explained deviances, confirming the findings of other authors (Dorji et al. 2013; Putfarken et al. 2008). Nevertheless, unlike our study, previous studies did not compare different regressive models that consider environmental and management variables.

The models that included distances from TNPA (M1 to M8) also achieved the best fitting results in terms of AICc and BIC, which varied within a range of 10 points. Therefore, according to Burnham & Anderson (2002), all of them can be considered as having comparable reliability. The limited differences among the models including the unweighted and weighted distances from TNPA may be due to the low variability in the number of penning nights among TNPA used in Equation (2), which resulted in distributions with a high similarity. Nevertheless, M6 (weighted distance from TNPA + distance from water) can be considered as the best fitting model as it presented the lowest AICc and BIC scores. Moreover, in this model, the relative importance of distance from water was lower (β coefficient was sevenfold smaller) than that of the weighted distance from TNPA, suggesting that the implementation of predictive models that include the distance from water sources could be of limited effectiveness. This finding was in contrast to the results of the study by Putfarken et al. (2008), which highlighted a higher relative importance of the distance from the drinking trough as compared to the distance from the sheep shed. Nevertheless, in their trial study, they tested the effects of only one drinking trough and one sheep shed in different management conditions (i.e., higher stocking rate, longer grazing season, lowland mesotrophic grasslands, and with a cow and sheep mixed grazing system). Moreover, the distance of a given site from all available water sources could be difficult to assess in some situations, e.g., when linear water sources like mountain streams are available for livestock over the grazing area. Therefore, according to our results, the distance from TNPA, preferably weighted on the number of penning nights (Equation (2)), can be reasonably considered as the main driver and a suitable and easily measured proxy to estimate the spatial variability of sheep stocking rate during grazing.

Future research should avoid some shortcomings still evident in our study, such as (i) the short duration of the experiment, (ii) the low grassland forage variability related to the occurrence of one vegetation community, (iii) the limited number of tracked animals (avertible by selecting rotating collared sheep), and (iv) the lack of information about animal behavior activity (i.e., resting, grazing, and traveling categories). Nonetheless, the approach we propose, which is based on a comparison among different models including environmental and management predictors, could also be applied for other livestock species and categories, shepherding managements, vegetation types, and environments.

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2.2. Effects of sheep grazing and wildfire on dry grassland diversity and composition

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Sheep Grazing and Wildfire: Disturbance Effects on Dry Grassland Vegetation in the Western Italian Alps

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Abstract

Land abandonment represents a major threat to the conservation of European semi-natural dry grasslands. To ensure biodiversity and habitat conservation, in this study, sheep grazing was reintroduced in abandoned *Festuco-Brometea* dry grasslands of a western Italian alpine valley. The experimental design included three treatments, along a gradient of increasing disturbance: (i) not-grazed, (ii) grazed, and (iii) penning areas. Unexpectedly, two years after grazing reintroduction, a wildfire hit the study area. To investigate the short-term effects of grazing and wildfire disturbances on vegetation, 62 permanent transects were monitored from 2014 to 2018. Vegetation cover, plant diversity, and richness and abundance of three functional groups of species were analyzed through generalized linear mixed models. Grazing caused little changes in vegetation, with the greatest effects observed in penning areas: the pioneer xerothermic species group, including many rare plant species, benefited from the reduction in the litter layer and the opening of gaps in the sward induced by trampling. The wildfire led to an increase in plant diversity and to changes in plant composition: dry grassland species decreased, while pioneer xerothermic and ruderal species increased their abundance. Short-term results suggest that both disturbances may foster the conservation of alpine dry grasslands.

Keywords: biodiversity conservation; Bromus erectus; burning; night pens; priority habitats; Stipa pennata; vegetation dynamics

Introduction

Since the 1950's, socio-economic transformations have resulted in a pronounced migration of people from rural to industrialized urban areas, resulting in widespread abandonment of managed semi-natural habitats (Poschlod et al. 2002; Prévosto et al. 2011). The negative effects of this agro-pastoral abandonment are widely recognized as crucial issues affecting the conservation of European semi-natural grasslands (Valkó et al. 2018), and are two-fold. Firstly, the lack of biomass removal in semi-natural grasslands leads to litter accumulation and the modification of plant interspecific competition (Bohner et al. 2020; Enyedi et al. 2008). As a consequence, a few highly competitive grasses often increase and become dominant, while less competitive species are suppressed (Giarrizzo et al. 2017; Hegedüšová & Senko D. 2011). Secondly, the processes of natural succession result in a gradual but widespread shrub and tree encroachment (Giarrizzo et al. 2017; Orlandi et al. 2016; Pittarello et al. 2016). Ultimately, both changes in interspecific competition and woody species encroachment lead to a dramatic decrease in plant diversity (Bohner et al. 2020; Görzen et al. 2019).

The interruption of management practices is a major threat for semi-natural dry grasslands, traditionally managed through extensive mowing and lenient grazing. Among them, *Festuco-Brometea* grasslands are valuable for their high plant diversity and richness in rare plant species (Dengler et al. 2012; Wilson et al. 2012) and provide habitat for several animal species, such as endangered birds and butterflies (Calaciura & Spinelli 2008; WallisDeVries & Van Swaay 2009). During the last few decades, several programs concerning the reintroduction of grazing in abandoned dry grasslands have been successfully implemented in Europe to support biodiversity and rare species conservation and to control invasive shrubs and highly competitive grass species (Dostálek & Frantík 2008; Elias et al. 2018; Elias & Tischew 2016; Schwabe et al. 2013). Selective grazing, trampling, defecation, and heterogeneous distribution of the stocking rates enhance the sward's structural heterogeneity, thus supporting the ecological needs of a rich flora (Godó et al. 2017; Pittarello et al. 2017; Rupprecht et al. 2016). Moreover, biomass removal and trampling by grazing animals, which open the sward and reduce litter accumulation, play a crucial role in the germination and establishment of short-lived and light-demanding species (Elias et al. 2018; Ruprecht et al. 2010). Indeed, these species tend to disappear in abandoned grasslands because of the reduction in their competitive abilities (Bohner et al. 2020; Rupprecht et al. 2016).

Most of the studies concerning the management and conservation of European dry grasslands refer to Central and Eastern Europe, e.g., Dostálek & Frantík 2008, Elias et al. 2018, and Ruprecht et al. 2016. In contrast, little research has focused on the management of the dry grasslands in inner-alpine valleys, despite their high conservation interest. Such species-rich communities, broadly threatened by agro-pastoral abandonment (Schwabe & Kratochwil 2004), harbor many rare elements of the steppic and Mediterranean flora (Braun-Blanquet 1961; Dengler et al. 2019; Royer 1991). Given the conservation interest in these plant communities, sheep grazing was reintroduced in abandoned *Festuco-Brometea* dry grasslands of a western Italian alpine valley in 2015, with the support of the EU LIFE program (project LIFE12 NAT/IT/000818 'Xero-grazing'). Until the mid of the 20th century, these grasslands had been traditionally maintained through extensive sheep grazing and (in the flattest areas) mowing. However, after the Second World War, this area was rapidly abandoned, and shrubs and trees progressively encroached on the grasslands, causing reduction and fragmentation of these habitats. In 2017, two years after grazing was reintroduced, an unexpected wildfire hit the entire area. Wildfires are a common disturbance in dry grasslands and contribute to keep habitats open by counteracting shrub and tree encroachment (Deák et al. 2019; Stavi 2019). However, wildfires may turn into a severe threat for habitats and biodiversity in areas encroached by shrubs and with litter accumulation as a result of land abandonment (Valkó et al. 2014). By monitoring vegetation on permanent transects from 2014, i.e., before grazing implementation, to 2018, i.e., after the wildfire, this study provides the unique opportunity to investigate the short-term effects of two different types of disturbance on vegetation.

The objectives of this paper were to analyze the effects of sheep grazing reintroduction, along a gradient of increasing grazing disturbance, and of the wildfire, on vegetation cover, plant diversity and richness and abundance of three functional groups of species (i.e., dry grassland, pioneer xerothermic and ruderal species) in abandoned *Festuco-Brometea* dry grasslands of an inner-alpine valley.

Materials and Methods

Study Area

The study was conducted within the Special Area of Conservation (SAC) "Xerothermic Oases of Susa Valley-Orrido of Chianocco" (SAC IT1110030), North-Western Italian Alps (45°08' N, 7°06' E). The area is characterized by a xerothermic and sub-Mediterranean climate, with an average annual air temperature of 11 °C and average annual precipitation of 670 mm (Biancotti et al. 1998). The geological substrate consists mainly of Mesozoic limestones and dolomites.

Semi-natural dry grasslands belonging to *Festuco-Brometea* Br.-Bl. and Tüxen ex Br.-Bl. 1949 class and downy oak (*Quercus pubescens* Willd.) woodlands are the most represented plant communities in the SAC. *Festuco-Brometea* communities include *Stipo-Poion carniolicae* Br.-Bl. 1949 and *Xerobromion erecti* Br.-Bl. and Moor 1938 phytosociological alliances, which belong, respectively, to 6240* and 6210* priority habitats under the Habitat Directive 92/43/EEC (Biondi et al. 2009). *Stipa pennata* L. s.l., together with *Festuca ovina* L. s.l, and *Bromus erectus* Hudson, together with *F. ovina* s.l., are the dominant species of the *Stipo-Poion carniolicae* grassland type (hereafter, '*Stipo-Poion*') and of the *Xerobromion erecti* grassland type (hereafter, '*Xerobromion*'), respectively. Both grassland types host several plant species listed in National and Regional Red Lists, such as *Euphorbia sulcata* Loisel. (Conti et al. 1997), or protected by the Piedmont Regional Law (LR 32/1982), such as *Brassica repanda* (Willd.) DC., *Echinops ritro* L., and all Orchidaceae species. Moreover, some species are considered of conservation interest due to their rarity in the Alps, such as the Mediterranean species *Asterolinon linum-stellatum* (L.) Duby, *Helianthemum salicifolium* (L.) Mill., *Linaria simplex* (Willd.) DC., *Linum strictum* L., and *Ononis reclinata* L. (Pignatti et al. 2017).

Experimental Design

The study was conducted in a 63 ha area within the SAC, consisting of 41 ha of *Stipo-Poion* and 22 ha of *Xerobromion* not managed since the 1950s (Figure 1). Altitude ranged from 550 to 1300 m a.s.l. and the mean slope and aspect were 30.6° and 211° N, respectively. A 43 ha area was grazed by sheep in 2015, 2016 and 2017, while a 20 ha area was left ungrazed. The presence of some wild ungulates was observed in the area, but the effects of their exploitation were negligible. Grazing was applied at low intensity (stocking rate ranging from 0.064 to 0.073 LU ha^{-1} year^{-1}, with 1

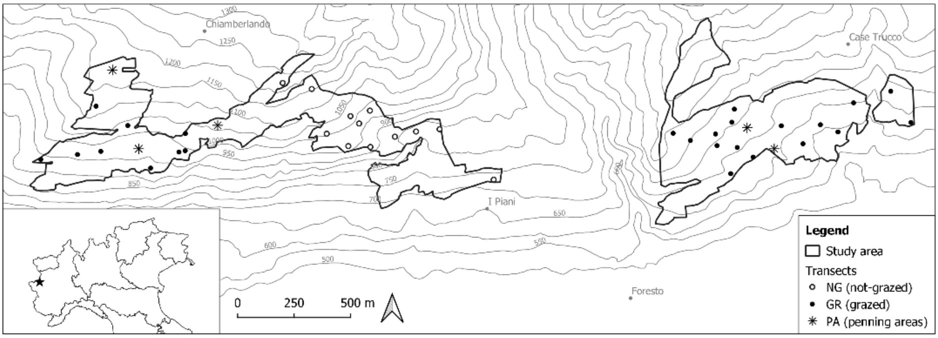

Figure 1. Map of the study area in the Western Italian Alps and location of the permanent transects. Each penning area (PA) includes five permanent transects.

sheep = 0.15 LU) and the grazing period ranged from April to June (i.e., at the peak of plant biomass production for these grasslands). The flock consisted of 250 Bergamasca sheep managed by the shepherd in a daily routine, entailing lenient supervision during the day and confinement in temporary penning areas at night (Ravetto Enri et al. 2019). Penning areas were fenced with an electrified net that delimited an area of about 1–3 m^2 $sheep^{-1}$. During each grazing year, they were moved over the pasture every 1–4 days, avoiding previously used locations. Temporary penning areas are commonly used in the Alps to prevent wolf attacks and, occasionally, to contrast shrub encroachment and improve grassland vegetation through dung deposition and trampling (Pittarello et al. 2016). In this study, two penning areas in *Stipo-Poion* and three in *Xerobromion* were selected for monitoring. Each of them was used by the flock in the first grazing year (i.e., 2015) at a high stocking rate (0.83 LU ha^{-1} $year^{-1}$) and then regularly grazed in the following years. Therefore, the experimental design included three treatments for each grassland type, along a gradient of increasing disturbance: (i) not-grazed (NG), (ii) grazed (GR), and (iii) penning areas (PA).

From 22 to 30 October 2017, an unexpected wildfire occurred in the SAC, affecting the entire study area. To prevent soil erosion due to animal trampling and to encourage the spontaneous recovery of vegetation, grazing was not applied in 2018. The effects of the wildfire, which was assumed to have evenly hit the three grazing treatments, were assessed in the first after-fire season.

Vegetation transects

A total of 62 linear permanent transects of 12.5 m length was used to assess botanical composition (Table 1 and Figure 1). For both GR and NG treatments, transects were set approximately every 1.5 ha into homogeneous and representative *Stipo-Poion* and *Xerobromion* patches. In each of the five selected PA, five permanent transects were placed at a minimum distance of 5.5 m from each other. The ends of each transect were marked with rebars to ensure its precise re-location.

Vegetation transects were surveyed between April and May (i.e., at the flowering phenological stage of the dominant species) throughout four years: one year before the reintroduction of grazing (2014, T0), one (2016, T1) and two (2017, T2) years after the grazing implementation, and in the first vegetative season following the wildfire (2018, T3). In T1 and T2, transects were surveyed just before the annual grazing.

Botanical composition was assessed along each transect using the vertical point-quadrat method (Daget & Poissonet 1971), with 50 points spaced at 25 cm intervals. At each point, plant species touching a steel needle were identified and recorded. Moreover, total plant cover (%) was visually estimated within a 2 m × 12.5 m area centered on the transect line (i.e., 25 m^2 vegetation plot). All plant species included within the vegetation plot were also recorded to account for occasional species. Species nomenclature followed Landolt et al. (2010).

Table 1. Number of permanent transects per grassland type and treatment (NG = not-grazed, GR = grazed, PA = penning areas).

	Stipo-Poion carniolicae	***Xerobromion erecti***	**Total**
NG	7	5	12
GR	17	8	25
PA	10	15	25
Total	34	28	62

Data analysis

At each transect, species relative abundance (SRA) of each recorded i species was calculated according to the equation of Daget and Poissonet (1971):

$$(1) \qquad \mathrm{SRA}_i = \frac{f_i}{\sum_{i=1}^{n} f_i} \times 100\ (\%)\,,$$

where f_i is the frequency of occurrence (number of occurrences / 50 points) of the species i.

A SRA_j = 0.3% was attributed to all occasional *j* species found in the vegetation plot but not recorded along the transect, according to Tasser and Tappeiner (2005). As the overall SRA_{i+j} (hereafter SRA) was greater than 100%, the SRA_i were rescaled, while maintaining SRA_j = 0.3%, to obtain a SRA equal to 100% at each plot.

At each vegetation plot, diversity was expressed in terms of total species richness, i.e., the total number of species found within the vegetation plots, and Shannon diversity index (Magurran 1988). Additionally, each plant species was associated with its phytosociological optimum (at the class level) according to Aeschimann et al. (2004) to identify groups of species characterized by similar ecological needs. Then, plant species were pooled into three functional groups: (i) 'dry grassland species' for plant species with ecological optimum in *Festuco-Brometea* and *Lygeo-Stipetea* classes, which correspond to the typical species of the habitats; (ii) 'pioneer xerothermic species' for plant species with ecological optimum in *Koelerio-Corynephoretea* and *Thero-Brachypodietea* classes, which include the rare Mediterranean annual species; and (iii) 'ruderal species' for plant species with ecological optimum in *Agropyretea intermedii-repentis*, *Artemisietea vulgaris* and *Stellarietea mediae* classes, which correspond to typical species of disturbed habitats that negatively impact on habitat conservation. Species richness and SRA of the three functional groups were computed for each vegetation plot. The list of all plant species and their corresponding phytosociological optimum and SRA for each treatment and year is provided in Table S1 (Appendix).

Statistical analysis

Generalized linear mixed models (GLMMs) were used to assess the effects of grazing treatments and wildfire on grassland vegetation. All analyses were conducted separately for *Stipo-Poion* and *Xerobromion* grassland types. Total plant cover, total species richness, Shannon diversity index, and species richness and SRA of dry grassland, pioneer xerothermic, and ruderal species were considered as response variables. Treatment, year, and their interaction were set as fixed factors, while the plot was specified as a random factor to account for the repeated measure structure over the years. Tukey's post hoc tests on treatment x year interactions were performed to analyze significant differences amongst treatments within each year and significant differences amongst years within each treatment. Shannon diversity index, being a continuous variable, was modeled with both normal and gamma distributions, while total species richness and richness of the three functional groups, being count variables, were modeled with both Poisson and negative binomial distributions. The model resulting in the lowest Akaike's Information Criterion value for each analysis was considered as the best fitting one and retained (Zuur et al. 2009). Total plant cover and the SRA of the functional groups, being percentage data, were rescaled between 0 and 1 to be modeled with a Beta distribution. Before the analysis, the SRA of pioneer xerothermic and ruderal species were transformed according to the formula proposed by Cribari-Neto and Zeileis (2010) to rescale them and avoid 0 and 1 values, which are not allowed by Beta distribution.

The R software (R Core Team 2018) was used for statistical analyses. GLMMs were run with the 'glmmTMB' package (Brooks et al. 2017) and Tukey's post hoc tests were computed with the 'emmeans' package (Length 2018).

Results

The results of the GLMMs were scrutinized focusing on two periods of the temporal series: (i) period T0-T2, to assess the effects of grazing treatments, and (ii) period T2-T3, to assess the effects of the wildfire.

Before the implementation of grazing (T0), the average total plant cover was 74% in *Stipo-Poion* and 88% in *Xerobromion*, respectively (Figure 2a,b). During the grazing period (T0-T2), no difference in total plant cover was observed, either among treatments or among years, in both grassland types (except for a decrease in the night pens of *Xerobromion* at T1). After the wildfire, the total plant cover did not change in *Stipo-Poion*. However, in *Bromus*-dominated grasslands, values decreased in NG and PA, but treatments did not differ among each other after the wildfire.

A total of 237 plant species belonging to 47 botanical families were recorded in the study area (Table S1, Appendix). In both grassland types, total species richness did not differ yearly among treatments and did not change within each treatment either after two years of grazing (except for an increase in the penning areas of *Xerobromion*) or after the wildfire (Figure 3a,b). The yearly species richness of all functional groups did not differ among treatments. The richness of dry grassland species did not vary throughout time as well, while some variations within treatments were found for pioneer xerothermic and ruderal species (Figure 3c–h). After two years of grazing, the richness of pioneer xerothermic species increased in PA in both grassland types. In *Stipa*-dominated grasslands, it also increased in GR. No variations in the richness of pioneer xerothermic species were detected after the wildfire. The richness of ruderal species did not change during grazing (except for an increase in the penning areas of *Stipo-Poion*), but it increased after the wildfire in every treatment in *Xerobromion* (average increase +2.6 species).

Shannon diversity index did not differ yearly among treatments during grazing, despite a significant increase in GR in *Stipo-Poion* (Figure 4a,b). After the wildfire, it increased in NG in *Stipo-Poion* and in all treatments in *Xerobromion*.

The SRA of the functional groups showed opposite trends throughout time: in both grassland types, dry grassland species decreased, while pioneer xerothermic and ruderal species increased (Figure 4c–h). More specifically, the SRA of dry grassland species decreased two years after grazing in PA in both grassland types and in GR in *Stipa*-dominated grasslands. However, treatments did not differ at T2. A further reduction was observed in all treatments in both grassland types following the wildfire (on average, −17.0% and −11.9% in *Stipo-Poion* and *Xerobromion*, respectively). In contrast, the SRA of pioneer xerothermic species increased in PA and (only in *Stipo-Poion*) in GR during the grazing period. In the penning areas of *Stipo-Poion* such increase (+12.0%) resulted in a significantly higher value compared to the other treatments at T2. After the wildfire, a further increase in the SRA of pioneer xerothermic species was recorded in all the treatments in both grassland types (+12.2% and +5.0% in *Stipo-Poion* and *Xerobromion*, respectively). Ruderal species SRA increased in the penning areas of both grassland types at T2, even though treatments did not differ among each other. After the wildfire, ruderal species SRA significantly increased in most treatments, i.e., NG and GR in *Stipa*-dominated grasslands (+6.7%) and NG and PA in *Bromus*-dominated grasslands (+3.5%).

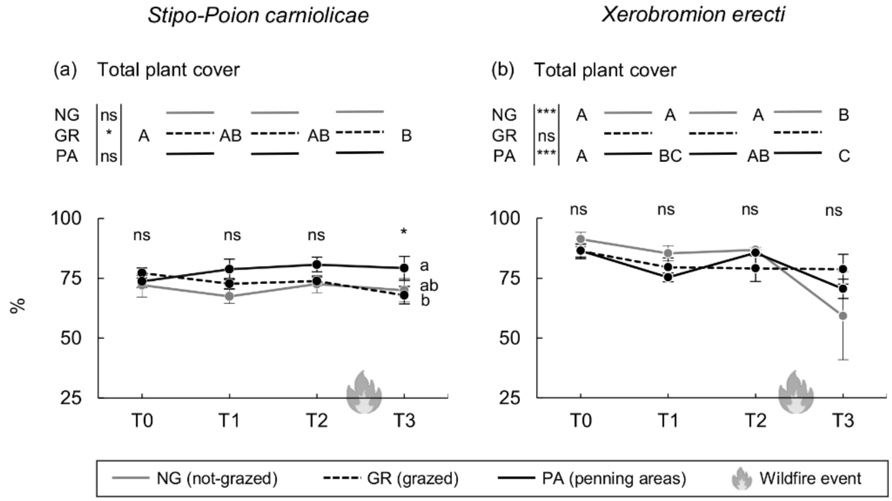

Figure 2. Results of the generalized linear mixed models performed on total plant cover in (a) *Stipo-Poion carniolicae* and (b) *Xerobromion erecti* grassland types. Uppercase letters indicate significant differences among years within each treatment while lowercase letters indicate significant differences among treatments within each year, according to Tukey’s post hoc tests. Years: T0, before grazing implementation; T1 and T2, after one and two years of grazing; T3, after the wildfire. Significance levels: ns, $p \geq 0.05$; *, $p < 0.05$; ***, $p < 0.001$.

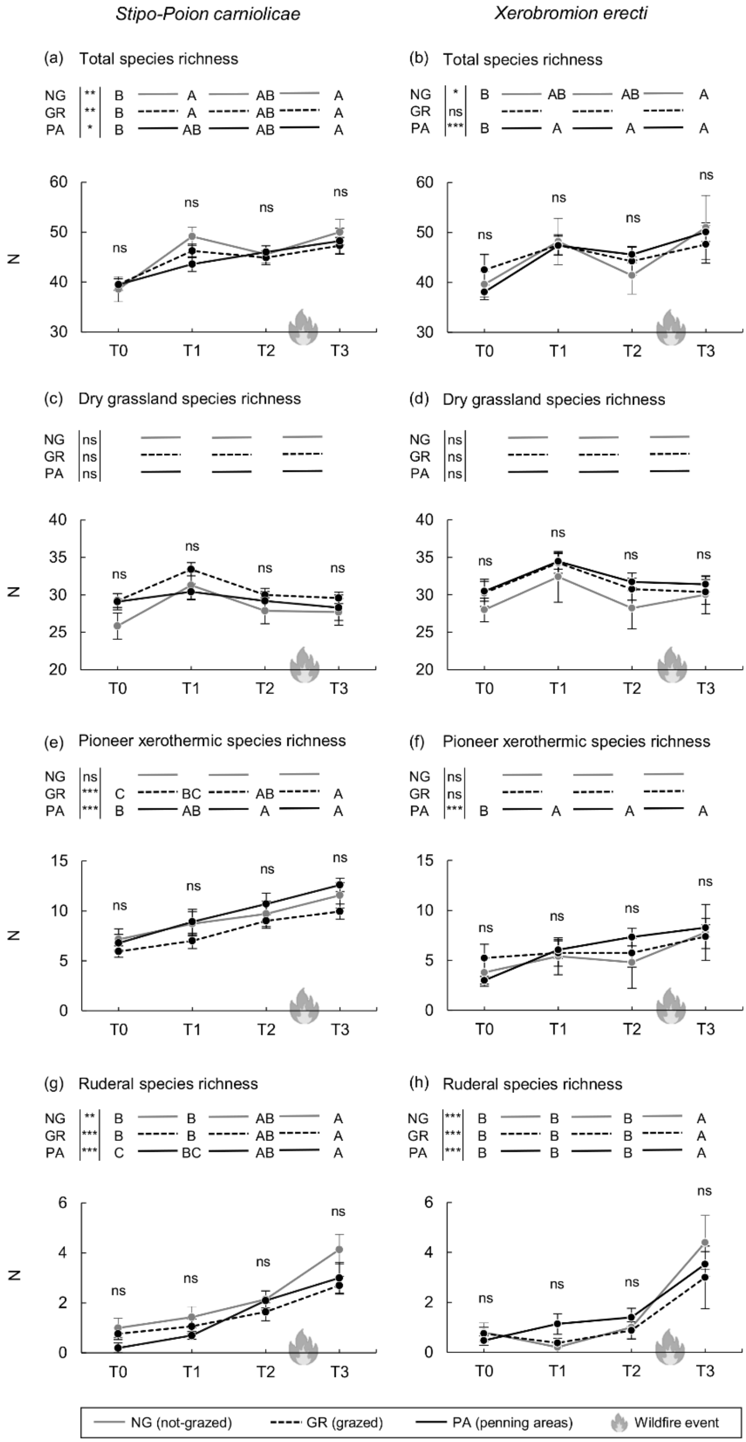

Figure 3. (The caption of Figure 3 is reported on page 37).

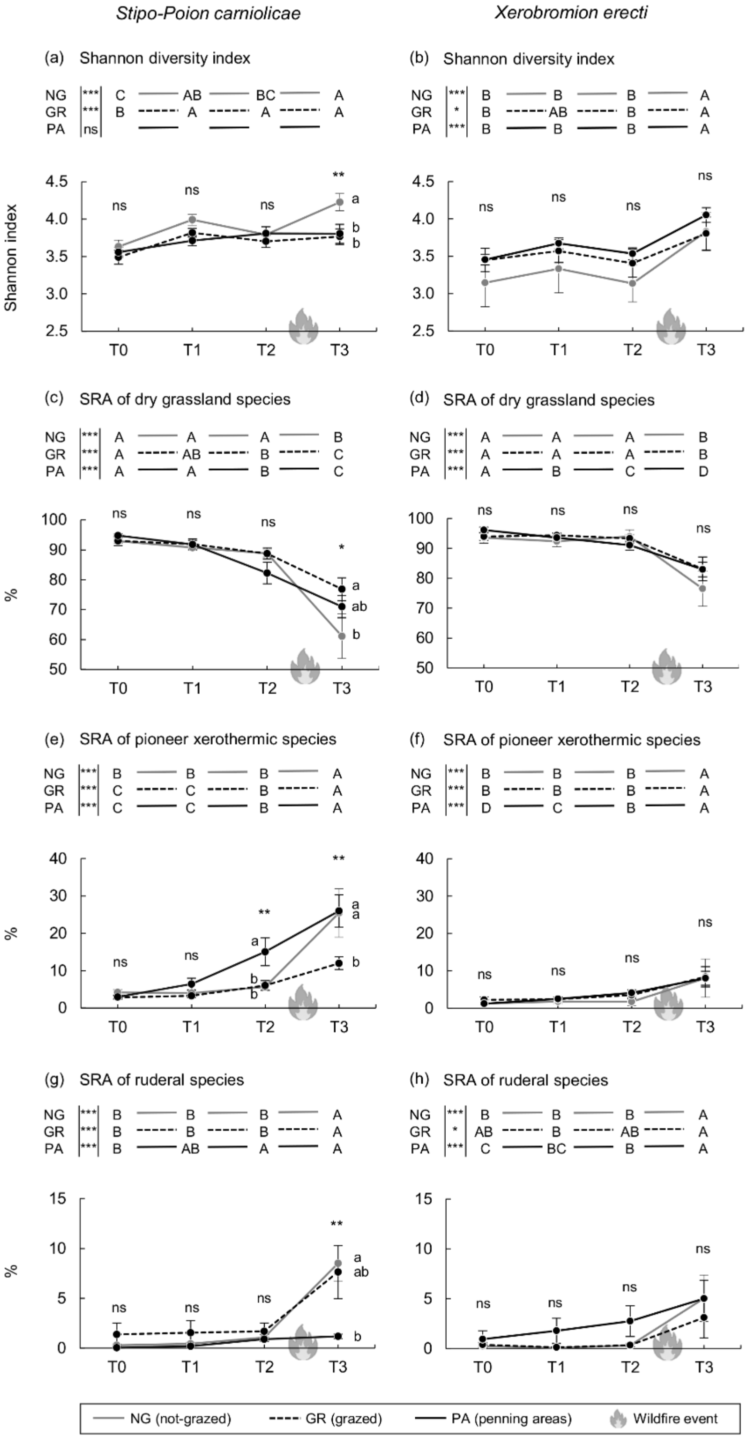

Figure 4. (The caption of Figure 4 is reported on page 37).

Figure 3. Results of the generalized linear mixed models performed on total species richness (a,b) and richness of dry grassland species (c,d), pioneer xerothermic species (e,f) and ruderal species (g,h) in *Stipo-Poion carniolicae* and (b) *Xerobromion erecti* grassland types. Uppercase letters indicate significant differences among years within each treatment according to Tukey's post hoc tests. Years: T0, before grazing implementation; T1 and T2, after one and two years of grazing; T3, after the wildfire. Significance levels: ns, $p \geq 0.05$; *, $p < 0.05$; ***, $p < 0.001$.

Figure 4. Results of the generalized linear mixed models performed on Shannon diversity index (a,b), SRA of dry grassland species (c,d), pioneer xerothermic species (e,f) and ruderal species (g,h) in *Stipo-Poion carniolicae* and (b) *Xerobromion erecti* grassland types. Uppercase letters indicate significant differences among years within each treatment, while lowercase letters indicate significant differences among treatments within each year, according to Tukey's post hoc tests. Years: T0, before grazing implementation; T1 and T2, after one and two years of grazing; T3, after the wildfire. Significance levels: ns, $p \geq 0.05$; *, $p < 0.05$; ***, $p < 0.001$.

Discussion

The reintroduction of sheep grazing in *Festuco-Brometea* dry grasslands of an inner-alpine valley caused few changes in their vegetation in the short term. Indeed, after two years of grazing, total plant cover, species richness, and Shannon diversity index did not differ among treatments. The SRA of functional groups, instead, changed in the night pens, where dry grassland species decreased and pioneer xerothermic and, slightly, ruderal species were favored. Most of the pioneer xerothermic species are annual and light-demanding and require bare ground microsites to germinate and grow. In penning areas, their competition was probably enhanced by the opening of gaps in the sward and the reduction in the litter layer induced by trampling, which provided suitable germination microsites (Godó et al. 2017; Rupprecht et al. 2016; Schwabe et al. 2013). In particular, *Alyssum alyssoides* (L.) L., *Arenaria serpyllifolia* L., *H. salicifolium*, *O. reclinata*, and *Petrorhagia saxifraga* (L.) Link were the most abundant pioneer xerothermic species in the penning areas at the end of the grazing period (Table S1, Appendix). Among these, the Mediterranean species *H. salicifolium* and *O. reclinata* are considered rare in the Alpine region.

The absence of a remarkable grazing effect on vegetation, despite defoliation, trampling and feces deposition, could be related to the harsh ecological conditions characterizing dry ecosystems (e.g., shallow soils, summer droughts, etc.). Indeed, these ecological constraints can slow down or prevent an effective vegetation response to grazing management

(Sternberg et al. 2000; Török et al. 2014). Moreover, interannual fluctuations in temperature and precipitation could affect the plant species composition in dry grassland communities (Dostálek & Frantík 2011), and interact with and confound the effects of treatments in the short term (Ruprecht et al. 2016; Sternberg et al. 2000).

Although the interannual variability could not be disentangled due to the absence of unburned control areas, the predominance of the wildfire effects on the vegetation changes between T2 and T3 was assumed. After the wildfire, total plant cover did not change in most treatments, with some reductions observed only in *Xerobromion*. Total species richness and the richness of functional groups were poorly affected by fire disturbance as well. The only variation concerned the recruitment of some new ruderal annuals from the seed bank, such as *Ajuga chamaepitys* (L.) Schreb., *Chaenorrhinum minus* (L.) Lange, *Chenopodium album* L., and *Turritis glabra* L. (Table S1, Appendix). In agreement with the results of Valkó et al. (2016) and Vidaller et al. (2019), instead, the Shannon diversity index was enhanced by the wildfire (especially in *Xerobromion*), resulting in a much more balanced proportion of species. Such an effect reflected the trend of the SRA of functional groups: in both grassland types, dominant dry grassland species decreased, while those groups that were poorly represented before the wildfire (i.e., pioneer xerothermic and ruderal species) increased their relative abundance. Specifically, in both grassland types, the pioneer xerothermic species accounting for most of the SRA after the wildfire were *A. alyssoides*, *A. serpyllifolia*, *Echium vulgare* L., *P. saxifraga*, and the rare Mediterranean species *L. simplex* and *O. reclinata*. The species accounting for most of the SRA of ruderal species were *Carduus nutans* L., *Elymus repens* (L.) Gould, *Erodium cicutarium* (L.) L'Hér., *Reseda lutea* L., and *R. phyteuma* L. (Table S1, Appendix). These pioneer xerothermic and ruderal species probably benefited from both the litter removal and the damage caused by burning to perennial species (e.g., dominant tussock-forming grasses belonging to the dry grassland functional group), that created bare ground areas suitable for their germination and propagation (Deák et al. 2014; Valkó et al. 2016). The increase in *E. repens* after the wildfire can be related to its stoloniferous life form, as highlighted also by Ruprecht et al. (2016). The occurrence of both pioneer xerothermic and ruderal species in the first stages of a post-fire chronosequence was also reported by Lonati et al. (2013) in Scots pine forests of an inner-alpine dry valley in the Western Italian Alps.

The positive effects on biodiversity and pioneer xerothermic species, and the substantial recovery of plant cover in the first after-fire season, might be taken into account in case of application of prescribed burning for the conservation of these grasslands. This practice has been proposed as a cost-effective management tool for the maintenance of different open habitats, such as dry grasslands and heathlands (Ascoli et al. 2013; Valkó et al. 2014; Vidaller et al. 2019), even if some authors highlighted possible negative outcomes on habitat conservation status (Silva et al. 2020). Still, in most of the European countries, the implementation of prescribed burning may be laborious because of strict regulations.

Conclusions

This study showed that the plant biodiversity of *Festuco-Brometea* dry grasslands of an inner-alpine valley was preserved after both grazing and burning disturbances. The main effects on vegetation concerned the abundance of functional groups rather than their species richness, and the magnitude of such effects was greater after the wildfire than in the previous grazing period. Changes in plant composition intensified along the gradient of increasing grazing disturbance and suggested a similar pattern under both burning and grazing disturbances, i.e., a decrease in dry grassland species and an increase in pioneer xerothermic and ruderal species. Future studies should be performed in a longer time span, to assess long-term vegetation responses and disentangle treatment effects from climatic influences.

Appendix: Table S1, plant species recorded in each treatment and year with corresponding phytosociological optimum and average species relative abundance; File S1, R codes used to run the Generalized Linear Mixed Models. File S1 can be found online at https://www.mdpi.com/2073-4395/11/1/6/s1

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Conflicts of Interest: The authors declare no conflict of interest.

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Appendix

Table S1. Average Species Relative Abundance (SRA) of all plant species per year (T0, before grazing; T1 and T2, after one and two years of grazing; T3, after the wildfire) and treatment (NG, not-grazed; GR, grazed; PA, penning areas) in *Stipo-Poion carniolicae* and *Xerobromion erecti* habitats. Plant species are grouped by functional groups. Phytosociological optimum (opt.) refers to Aeschimann et al. (2004). FB = *Festuco-Brometea*, KC = *Koelerio-Corynephoretea*, TB = *Thero-Brachypodietea*, Ag = *Agropyretea intermedia-repentis*, Ar = *Artemisietea vulgaris*, St = *Stellarietea mediae*, As = *Asplenietea trichomanis*, CF = *Carpino-Fagetea sylvaticae*, CP = *Crataego-Prunetea*, ES = *Elyno-Seslerietea variae*, Ep = *Epilobietea angustifolii*, EP = *Erico-Pinetea*, LV = *Loiseleurio-Vaccinetea*, MA = *Molinio-Arrhenatheretea*, PR = *Pistacio lentisci-Rhamnetea alaterni*, PP = *Pyrolo-Pinetea*, Qp = *Quercetea pubescentis*, Qr = *Quercetea robori-sessiflorae*, Ro = *Rosmarinetea*, Th = *Thlaspietea rotundifolii*, and TG = *Trifolio-Geranietea*.

		Stipo-Poion carniolicae												*Xerobromion erecti*											
		T0			T1			T2			T3			T0			T1			T2			T3		
Plant species	opt	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA
Dry grassland species																									
Stipa pennata s.l.	FB	13.3	24.8	13.6	11.2	19.5	16.9	13.6	23.9	17.7	9.9	22.3	16.7	2.8	10.6	8.9	1.3	7.5	16.1	2.5	10.6	20.5	3.2	12.9	20.5
Festuca ovina s.l.	FB	17.3	10.4	16.7	16.7	10.6	12.3	18.0	8.5	9.2	8.8	5.1	6.9	15.6	10.0	30.1	15.8	11.7	21.7	19.2	9.1	20.1	8.0	5.7	11.0
Bromus erectus	FB	6.2	4.4	11.5	6.3	5.3	15.2	5.2	4.7	14.0	1.2	1.7	10.1	33.0	27.5	14.0	30.0	32.0	14.8	29.3	30.3	13.0	19.7	22.3	8.2
Carex humilis	FB	9.8	7.2	0.5	8.1	6.8	1.3	9.6	7.4	1.2	3.0	7.0	0.8	12.6	11.4	2.9	10.8	7.8	3.9	12.5	8.5	4.5	10.8	9.2	3.5
Carex liparocarpos	FB	3.7	2.7	10.7	2.6	3.6	8.1	3.6	4.2	9.4	5.9	5.2	9.2	1.1	1.3	5.2	2.3	1.3	5.8	1.3	2.6	8.4	2.2	3.0	5.3
Chrysopogon gryllus	FB	3.3	9.6	3.6	2.1	7.4	4.7	2.6	8.2	3.7	2.2	10.1	4.9	1.2	4.8	-	1.8	3.5	-	0.2	4.8	-	1.9	5.3	0.2
Teucrium chamaedrys	FB	6.9	4.7	4.7	9.8	5.5	2.7	4.9	4.9	2.3	0.6	1.2	0.7	5.0	4.4	7.4	3.8	4.4	4.4	3.2	4.4	3.3	0.6	1.3	1.3
Anthericum liliago	FB	7.4	3.1	0.2	4.4	2.4	0.2	8.1	1.7	0.0	8.5	3.7	0.2	3.1	1.6	0.1	1.5	1.2	0.1	2.8	0.6	0.0	2.7	2.7	0.0
Fumana spp.*	FB	1.8	3.9	3.9	1.6	5.2	0.5	1.0	4.9	0.7	0.6	1.1	0.2	0.3	1.5	2.6	0.2	2.2	1.0	0.3	2.0	0.7	2.0	0.6	1.1
Brachypodium rupestre	FB	0.5	-	0.1	1.5	0.0	0.1	2.3	-	0.1	2.0	0.0	0.0	7.1	5.2	1.3	10.5	3.9	2.3	9.7	4.3	1.7	7.4	3.2	1.1
Koeleria vallesiana	FB	2.7	4.4	3.9	2.2	4.6	3.7	1.4	3.0	0.2	0.4	0.9	1.0	0.3	1.1	1.1	0.3	0.5	1.1	0.1	0.9	0.6	0.1	0.9	0.1
Thymus serpyllum s.l.	FB	1.3	2.0	2.7	2.7	2.5	3.2	1.0	2.1	2.1	0.4	1.3	1.6	0.8	1.6	0.6	1.7	1.6	0.7	2.0	1.1	0.9	1.3	1.1	0.7
Artemisia campestris	FB	1.8	0.6	0.6	1.8	0.6	1.0	1.8	0.7	3.7	1.6	0.8	0.7	0.2	0.6	1.9	0.5	0.7	2.0	0.3	0.5	1.6	1.2	0.7	3.0
Helianthemum apenninum	FB	1.0	1.3	4.5	2.4	1.3	1.5	1.2	0.7	1.1	2.0	2.3	0.9	0.1	0.8	1.4	0.4	0.4	0.8	0.1	0.6	0.4	0.2	0.4	1.0
Potentilla pusilla	FB	1.7	1.2	0.8	1.1	1.5	1.7	1.4	1.2	0.7	0.5	0.6	0.4	0.6	0.5	2.4	0.5	0.7	2.0	0.3	0.8	1.4	2.1	0.5	1.5
Thymus oenipontanus	FB	0.6	1.1	0.9	0.9	1.0	1.6	0.3	0.7	0.5	0.4	0.5	0.7	1.2	0.7	1.8	1.4	0.6	2.0	0.5	1.1	1.6	0.7	0.7	2.6
Phleum phleoides	FB	1.2	0.8	2.9	1.6	1.2	2.2	1.9	0.8	1.8	1.7	0.6	1.9	0.7	0.6	1.0	0.7	0.7	0.9	1.5	0.4	0.6	0.9	0.7	0.6
Bothriochloa ischaemum	FB	0.8	1.4	1.0	1.0	1.5	3.3	1.5	1.8	1.7	0.5	0.9	1.0	0.8	0.2	0.7	0.2	0.7	0.7	-	0.6	0.6	0.1	0.2	0.4
Koeleria pyramidata	FB	-	0.4	3.2	0.0	0.3	3.9	-	0.8	3.6	0.0	0.1	1.7	0.1	1.0	0.2	-	2.5	0.1	-	2.3	0.2	-	1.0	0.1
Trinia glauca	FB	0.3	0.7	0.6	0.3	0.8	0.7	0.5	1.0	0.6	0.6	1.1	0.7	0.6	0.5	0.9	0.4	0.9	0.6	0.9	0.3	0.7	0.2	0.5	1.3
Cleistogenes serotina	FB	2.5	0.6	0.4	3.1	1.1	0.3	3.0	0.4	0.4	0.8	-	0.0	-	0.0	0.1	-	0.1	0.3	-	0.1	0.5	-	0.0	2.1
Astragalus onobrychis	FB	0.4	0.3	0.1	0.4	0.2	0.2	0.4	0.0	0.1	0.2	0.2	0.1	0.2	0.1	1.8	0.4	0.3	2.3	0.5	0.2	1.0	0.2	0.2	2.0
Eryngium campestre	FB	0.5	0.4	0.2	0.4	0.4	0.4	0.3	0.4	0.5	0.2	0.7	1.1	0.2	0.5	0.3	0.2	0.4	0.5	0.2	0.3	0.9	0.2	0.6	2.0
Onobrychis arenaria	FB	0.3	0.3	0.2	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.4	0.3	0.3	1.0	1.0	0.3	0.6	0.8	0.5	0.9	0.9	1.5	0.7	1.4
Helianthemum nummularium	FB	0.4	0.1	1.0	0.2	0.2	0.8	0.2	0.1	0.3	0.1	0.4	0.2	0.5	0.4	1.0	0.6	1.3	0.8	0.2	0.4	0.5	0.9	0.8	1.0
Lactuca perennis	FB	1.0	0.2	0.1	1.0	0.3	-	0.5	0.5	0.1	1.0	1.1	0.1	0.2	0.0	0.4	0.4	0.1	0.3	0.4	0.0	0.4	0.4	0.1	1.0

* including *F. ericoides* and *F. procumbens*

		Stipo-Poion carniolicae												*Xerobromion erecti*											
		T0			T1			T2			T3			T0			T1			T2			T3		
Plant species	opt	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA
Silene otites	FB	0.2	0.4	0.2	0.5	0.7	0.3	0.3	0.5	0.2	1.2	0.8	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.1	0.8	0.3	1.2	0.4	0.4
Poa perconcinna	FB	0.0	0.0	0.9	0.0	0.0	0.2	-	0.0	1.4	0.0	0.1	4.6	-	0.0	0.0	-	0.0	0.0	-	0.1	0.1	0.1	0.5	0.1
Ononis pusilla	FB	0.2	0.3	0.7	0.3	0.4	0.7	0.2	0.3	0.7	0.4	0.3	0.5	0.5	0.2	0.3	0.3	0.4	0.2	0.1	0.2	0.2	0.4	0.1	0.2
Asperula aristata	FB	0.1	0.2	0.5	0.6	0.8	0.4	0.2	0.5	0.3	0.2	0.3	0.1	-	0.0	0.3	0.1	0.4	0.4	0.1	0.2	0.3	0.1	0.4	0.4
Galium lucidum s.l.	FB	0.8	0.1	-	0.6	0.2	0.1	0.4	0.1	0.0	0.9	0.1	0.1	0.6	0.7	0.3	0.3	0.5	0.1	0.3	0.9	0.1	0.4	1.0	0.4
Allium sphaerocephalon	FB	0.1	0.1	0.3	0.3	0.2	0.3	0.3	0.2	0.2	0.7	0.5	0.4	0.2	-	0.0	0.3	0.1	0.3	0.1	0.1	0.4	0.3	0.4	0.7
Helianthemum italicum	FB	0.3	0.5	-	0.2	0.4	-	0.1	0.3	-	0.4	0.1	-	0.1	0.9	0.7	0.2	0.7	0.2	0.1	0.3	0.2	0.1	0.2	0.1
Centaurea scabiosa	FB	0.4	0.2	-	0.2	0.2	-	0.2	0.3	-	0.2	0.3	-	0.5	0.2	0.2	0.3	0.2	0.2	0.5	0.1	0.2	2.2	0.0	0.7
Sanguisorba minor	FB	0.2	0.3	0.2	0.8	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.1	0.2	0.2	0.1	0.2	0.3	0.1	0.2	0.3	0.2	0.5	0.3
Scorzonera austriaca	FB	-	0.3	0.4	0.0	0.3	0.3	0.0	0.2	0.6	-	0.4	0.5	0.2	0.3	0.1	0.2	0.2	0.1	0.4	0.2	0.2	0.1	0.6	0.1
Inula montana	FB	0.1	0.5	0.4	0.2	0.5	0.4	0.1	0.2	0.4	0.1	0.3	0.4	0.3	0.2	0.0	0.3	0.3	0.0	0.5	0.2	0.0	0.1	0.2	-
Galium verum	FB	-	-	-	0.0	-	-	-	-	-	0.0	0.1	-	-	0.0	1.4	-	0.0	1.3	-	0.2	0.4	0.2	0.1	0.2
Hippocrepis comosa	FB	0.2	0.5	0.1	0.3	0.4	0.1	0.0	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.2	0.1	0.4	0.4	0.1	0.1	-	0.0	0.4
Stachys recta	FB	0.7	0.1	0.0	0.2	0.1	-	0.3	0.1	0.0	1.1	0.7	0.0	0.1	0.1	0.0	0.1	0.2	0.0	0.3	0.2	0.0	0.8	0.6	0.1
Anthyllis vulneraria	FB	0.2	0.2	0.4	0.2	0.5	0.0	0.1	0.3	0.0	0.3	0.3	0.0	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.3	0.1	0.2	0.2	0.3
Ononis procurrens	FB	0.3	0.1	-	0.2	0.2	0.1	0.0	0.1	0.1	0.4	0.5	0.1	-	0.0	0.1	-	0.2	0.2	-	0.1	0.1	-	0.3	0.6
Hieracium pilosella	FB	0.2	0.3	0.0	0.2	0.4	0.0	0.2	0.4	0.1	-	0.2	0.0	0.5	0.1	0.2	0.7	0.1	0.2	0.2	0.0	0.1	0.1	0.0	0.0
Ononis natrix	FB	0.1	0.1	-	0.0	0.0	-	-	0.0	-	0.2	0.3	-	-	0.1	0.3	-	0.0	0.2	-	0.0	0.1	0.2	0.2	1.1
Globularia bisnagarica	FB	0.1	0.2	0.2	0.0	0.3	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.1	-	-	0.1
Achillea tomentosa	FB	0.1	0.1	0.1	0.1	0.1	0.3	0.2	0.1	0.3	0.1	0.1	0.2	-	0.2	0.3	-	0.3	0.3	-	0.1	0.2	-	0.0	0.1
Ranunculus bulbosus	FB	-	-	0.0	0.1	-	0.0	0.1	-	0.0	0.0	-	0.1	0.2	0.1	0.2	0.2	0.1	0.3	0.2	0.1	0.3	0.1	0.1	0.7
Teucrium montanum	FB	0.8	0.2	0.0	0.2	0.3	0.1	0.1	0.2	0.0	0.1	0.1	-	-	0.3	0.1	0.1	0.2	0.1	-	0.2	0.0	0.1	-	0.0
Orchis tridentata	FB	-	0.0	0.2	0.0	0.1	0.3	0.1	0.1	0.2	-	0.0	0.1	0.1	0.1	0.3	0.3	0.1	0.3	0.1	0.1	0.3	-	-	-
Pseudolysimachion spicatum	FB	-	0.0	-	-	0.0	-	-	0.0	0.1	-	-	0.1	0.1	0.0	0.2	0.1	0.2	0.1	0.2	0.1	0.4	0.1	0.1	0.5
Dianthus carthusianorum	FB	-	0.1	0.2	-	0.0	0.2	-	0.0	0.3	-	0.0	0.3	-	0.3	0.0	-	0.2	0.0	-	0.1	0.0	-	0.1	0.0
Knautia mollis	FB	0.0	0.1	-	0.0	0.1	-	0.0	0.1	0.0	0.0	0.1	-	0.2	0.1	0.0	0.4	0.1	0.1	0.4	0.1	0.1	0.1	0.3	0.1
Campanula sibirica	FB	0.0	0.1	0.1	0.2	0.3	0.2	0.2	0.2	0.1	-	0.0	0.1	0.1	0.0	-	0.2	0.1	-	-	-	-	-	0.0	-
Centaurea valesiaca	FB	-	0.1	-	-	0.0	-	-	0.1	-	0.0	0.3	-	-	-	-	-	-	0.0	-	-	-	0.3	0.2	0.4
Taraxacum laevigatum s.l.	FB	-	-	-	-	0.0	-	0.0	0.1	-	0.0	0.1	0.0	-	-	0.1	0.1	0.0	0.2	0.1	0.0	0.1	0.2	0.1	0.3

		Stipo-Poion carniolicae												*Xerobromion erecti*											
		T0			T1			T2			T3			T0			T1			T2			T3		
Plant species	opt	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA
Scabiosa vestita	FB	0.1	0.1	-	0.2	0.1	-	0.1	0.1	-	-	0.1	-	-	0.1	0.1	0.1	0.1	0.1	-	0.1	0.1	-	0.1	-
Linum appressum	FB	0.1	0.1	0.1	0.0	0.1	-	0.1	0.1	-	-	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.0	-	-	0.1
Coronilla minima	FB	-	0.1	-	-	0.1	-	-	0.0	-	0.0	0.0	-	0.4	0.1	0.1	0.1	0.2	0.1	0.1	0.2	0.1	-	0.1	0.0
Koeleria macrantha	FB	-	0.2	-	0.0	0.1	-	-	0.4	-	-	0.0	0.3	-	-	-	-	-	-	-	-	0.0	-	-	-
Erysimum rhaeticum	FB	-	0.1	-	0.0	0.1	-	0.0	0.1	-	0.1	0.1	-	-	-	0.1	-	-	0.1	-	-	0.1	0.1	0.2	0.2
Pimpinella saxifraga	FB	0.1	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.1	0.1	-	0.1	0.1	-
Leontodon crispus	FB	0.1	0.1	-	0.0	0.0	-	-	0.0	-	0.0	-	-	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.0	-
Orobanche sp.	FB	-	0.2	-	0.1	0.1	-	-	0.1	-	-	-	-	-	0.2	-	0.1	0.0	0.1	-	0.0	0.0	-	0.0	0.0
Arabis hirsuta	FB	-	-	0.1	-	0.0	0.1	-	-	0.0	-	-	0.1	0.1	0.0	0.1	0.3	-	0.1	-	0.0	0.1	-	0.0	0.0
Anacamptis pyramidalis	FB	0.0	0.1	0.1	-	0.1	0.1	-	0.1	0.2	-	-	-	0.1	0.0	0.1	-	-	0.1	-	0.0	0.0	-	-	-
Thesium divaricatum	FB	0.3	0.0	-	0.0	0.0	-	0.0	0.1	-	-	0.0	-	0.1	0.3	-	0.1	0.1	-	0.1	0.0	-	0.1	-	-
Heteropogon contortus	FB	-	0.1	0.0	-	0.1	0.0	-	0.1	0.0	-	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-
Cuscuta epithymum	FB	0.0	0.2	-	0.2	0.1	-	-	0.0	-	-	0.0	-	-	0.0	-	-	0.1	0.0	-	-	-	-	-	0.0
Centaurium erythraea	FB	-	0.1	0.2	0.0	0.1	0.1	-	-	-	-	0.1	-	-	0.0	-	-	0.1	-	-	-	-	-	-	-
Prunella grandiflora	FB	-	-	0.1	-	-	0.1	-	-	0.0	0.0	-	-	0.1	0.1	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.1	0.1	0.0
Herniaria incana	FB	-	-	-	-	-	-	-	0.0	-	-	0.0	-	-	0.0	-	-	-	0.1	-	0.1	0.1	-	0.0	0.2
Thesium linophyllon	FB	-	0.2	-	-	0.1	-	-	0.0	-	-	-	-	0.1	-	-	0.1	-	-	0.1	-	-	-	-	-
Odontites luteus	FB	-	0.1	0.1	-	0.0	-	-	0.0	0.2	-	0.0	-	-	0.0	-	-	-	-	-	-	-	-	-	-
Biscutella cichoriifolia	FB	0.1	0.0	-	0.1	-	-	0.1	-	-	0.2	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-
Salvia pratensis	FB	-	-	-	0.0	-	-	0.0	-	-	-	-	-	0.1	0.0	0.0	0.1	0.0	-	0.1	0.0	0.0	0.1	0.0	0.0
Melica ciliata	FB	-	-	-	0.1	-	-	0.2	0.1	-	0.0	0.1	-	-	-	-	-	-	-	-	-	-	-	-	0.0
Silene vulgaris	FB	-	0.0	-	0.0	0.0	-	0.0	0.0	-	0.0	0.1	-	0.1	-	-	-	-	-	0.1	-	-	0.1	0.0	0.0
Orchis morio	FB	-	-	0.0	-	-	0.1	-	-	0.1	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-
Lavandula angustifolia	FB	-	-	-	-	-	-	-	-	-	-	0.0	-	-	0.0	0.0	-	0.0	0.0	-	0.0	0.0	-	-	-
Trifolium montanum	FB	-	-	-	-	-	0.0	-	0.0	0.0	-	-	-	-	0.0	-	-	0.0	-	-	0.0	0.0	-	0.0	-
Plantago serpentina	FB	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	0.0	-	0.0	0.1	-	-	-	-	-	-
Briza media	FB	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	0.2	0.1	0.0	0.1	-	-	-	-	-
Linum tenuifolium	FB	-	0.0	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	-
Euphorbia cyparissias	FB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	0.1	-	0.1	-	-	-	-	-	-

		Stipo-Poion carniolicae												*Xerobromion erecti*											
		T0			T1			T2			T3			T0			T1			T2			T3		
Plant species	opt	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA
Muscari racemosum	FB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	-	-	0.0	-	-	0.1	-	-	-
Campanula rotundifolia	FB	0.0	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-
Polygala comosa	FB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	-	-	0.0	-	-	0.0	-	-	-
Veronica prostrata	FB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	-	-	0.0	-	-	-	-	-	-
Medicago lupulina	FB	-	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-	-	-	-	-	0.0	0.0
Ophrys holosericea	FB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	-	-	0.0	-	-	-	-	-	-
Aster linosyris	FB	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-
Brachypodium sylvaticum	FB	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-
Crepis albida	FB	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Orchis ustulata	FB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-
Plantago media	FB	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Echinops ritro	LS	0.0	0.1	0.2	0.0	0.1	-	0.1	0.1	-	0.2	0.0	-	0.1	0.1	0.3	-	0.1	0.3	-	0.1	0.2	-	0.2	0.8
Muscari comosum	LS	-	-	0.5	-	-	0.1	-	-	0.2	0.0	-	0.2	-	-	-	-	-	-	-	-	0.0	-	-	0.0
Pioneer xerothermic species																									
Arenaria serpyllifolia	KC	0.2	0.2	0.3	0.3	0.3	1.4	1.1	1.4	6.4	7.7	5.4	16.3	0.1	0.1	0.1	0.1	0.3	0.4	0.3	1.3	1.6	2.8	4.6	2.3
Petrorhagia saxifraga	KC	0.9	0.3	0.7	0.7	0.5	0.7	0.9	0.5	1.2	2.1	1.4	1.2	0.1	0.3	0.1	0.1	0.4	0.3	0.1	0.6	0.3	0.2	0.5	0.2
Alyssum alyssoides	KC	0.5	0.2	0.2	0.5	0.2	0.2	0.3	0.3	1.1	4.8	0.5	0.4	0.1	0.2	0.1	0.1	0.1	0.4	0.1	0.1	0.7	0.4	0.4	2.6
Echium vulgare	KC	0.3	0.2	0.0	0.4	0.1	0.1	0.6	0.2	0.1	2.6	0.3	0.1	-	-	-	-	0.1	0.1	-	0.0	0.1	1.1	0.2	0.2
Sempervivum tectorum	KC	0.1	0.5	0.2	0.4	0.4	-	0.3	0.2	-	0.3	0.1	-	0.4	0.2	0.4	0.4	0.3	0.2	0.2	0.1	0.1	0.1	0.2	0.1
Acinos arvensis	KC	0.2	0.1	0.1	0.2	0.1	0.2	0.3	0.2	0.5	0.3	0.2	0.3	-	0.1	0.1	0.1	0.1	0.2	-	0.2	0.2	0.1	0.1	0.6
Hornungia petraea	KC	-	0.0	0.0	-	-	0.1	-	0.1	0.2	0.0	0.2	1.7	0.1	-	-	0.1	0.0	-	0.2	0.2	0.0	0.7	0.6	0.1
Dianthus sylvestris	KC	0.5	0.3	-	0.2	0.3	-	0.1	0.3	0.1	-	-	0.1	0.1	0.2	0.0	0.2	0.1	0.1	0.1	0.1	0.0	-	-	0.1
Arabis auriculata	KC	-	0.0	-	0.0	0.0	-	0.1	0.1	0.1	0.3	0.2	0.1	-	0.1	-	-	0.0	0.0	-	0.0	0.2	0.2	0.5	0.2
Minuartia rubra	KC	-	0.0	-	-	0.0	0.0	-	0.1	0.1	-	0.3	0.3	-	0.1	-	-	0.1	0.0	-	0.1	0.1	0.1	0.2	0.1
Sedum montanum	KC	0.1	-	0.2	0.2	0.1	0.1	0.1	0.0	0.1	0.1	0.0	-	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.0	0.1	0.1	-	0.1
Sedum album	KC	0.0	0.0	-	0.0	0.1	0.0	0.1	0.0	-	0.2	0.0	-	0.1	0.0	-	0.1	0.0	0.0	0.1	-	-	0.3	0.0	-
Cerastium brachypetalum	KC	-	-	0.1	-	-	0.1	-	-	0.1	-	-	0.2	-	0.0	-	-	0.0	-	0.1	0.0	0.0	-	-	-
Poa bulbosa	KC	-	-	0.2	-	-	0.0	-	-	0.1	-	0.0	0.1	-	0.0	-	-	-	-	-	-	-	-	-	0.0
Trifolium arvense	KC	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-	-	-	-	0.0	-	0.2	0.1

		Stipo-Poion carniolicae												*Xerobromion erecti*											
		T0			T1			T2			T3			T0			T1			T2			T3		
Plant species	opt	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA
Cerastium arvense	KC	0.1	0.0	-	0.0	0.0	0.1	0.1	0.0	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arabidopsis thaliana	KC	-	-	-	-	-	-	-	-	-	-	0.0	0.2	-	-	-	-	-	-	-	-	-	-	-	-
Petrorhagia prolifera	KC	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	0.0	-
Silene armeria	KC	-	0.0	-	-	0.0	-	-	0.0	-	-	-	-	-	-	-	0.1	-	-	0.1	-	-	-	-	-
Potentilla argentea	KC	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	0.0	-	0.0	-	-	-	-	-	-	-
Filago vulgaris	KC	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Saxifraga tridactylites	KC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-
Ononis reclinata	TB	0.3	0.1	0.4	0.2	0.4	2.1	0.9	1.0	2.4	2.1	0.7	1.5	0.1	0.1	0.0	0.2	0.1	0.1	0.1	0.3	0.0	0.6	0.2	0.1
Euphorbia sulcata	TB	0.2	0.2	0.1	0.2	0.3	0.2	0.2	0.4	0.5	1.9	0.9	0.4	-	0.0	-	-	0.1	0.1	-	0.1	0.1	0.1	0.2	0.3
Linaria simplex	TB	0.0	0.1	0.1	0.1	0.1	0.0	0.2	0.1	0.1	1.3	1.0	0.7	0.1	0.1	-	0.1	0.0	-	0.1	0.1	0.1	0.7	0.3	0.2
Bupleurum baldense	TB	0.2	0.1	0.2	0.1	0.1	0.4	0.1	0.2	0.4	0.2	0.1	0.3	0.1	0.5	0.1	0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.1	0.2
Linum strictum	TB	-	0.2	0.1	-	0.1	0.1	-	0.6	0.2	-	0.1	0.3	-	0.0	-	-	0.2	0.0	-	-	0.1	-	-	-
Helianthemum salicifolium	TB	-	-	-	0.0	0.0	0.3	0.0	0.0	1.0	0.0	0.0	0.5	-	-	-	-	0.0	-	-	0.0	0.0	-	0.1	0.1
Crupina vulgaris	TB	0.2	0.1	-	0.3	0.1	-	0.2	0.1	-	0.6	0.1	0.0	0.1	0.0	0.1	0.1	-	0.1	0.1	-	0.1	0.1	-	0.1
Medicago minima	TB	-	-	-	0.0	-	0.1	0.0	0.0	0.2	0.1	0.1	0.7	-	-	-	0.1	-	0.1	0.1	-	0.1	0.1	0.0	0.2
Asterolinon linum-stellatum	TB	-	0.0	0.1	-	0.0	0.2	-	0.1	0.2	-	0.2	0.5	-	0.0	-	-	-	0.0	-	-	0.0	0.2	0.0	0.0
Micropus erectus	TB	-	-	-	-	0.0	0.1	-	0.1	0.1	0.3	0.1	0.2	-	-	-	-	0.0	0.0	-	-	0.0	-	-	0.0
Crucianella angustifolia	TB	0.2	-	-	-	-	-	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ruderal species																									
Elymus repens	Ag	-	1.1	-	-	1.3	-	-	1.0	-	0.0	1.8	-	0.1	0.2	0.8	-	-	1.5	0.1	0.0	2.4	0.1	0.3	2.5
Poa compressa	Ag	-	-	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Reseda lutea	Ar	-	0.1	-	0.1	0.1	0.1	-	0.3	0.3	0.9	3.9	0.3	-	0.0	0.0	-	0.0	0.0	-	0.1	0.0	0.7	0.2	0.1
Carduus nutans	Ar	0.1	0.0	-	0.1	0.1	-	0.5	0.1	-	5.8	0.2	0.1	0.1	-	0.0	-	0.0	0.1	0.1	-	0.1	2.2	0.5	1.2
Tragopogon dubius	Ar	-	0.1	-	-	0.1	-	-	0.0	-	-	-	-	0.1	-	0.0	-	-	0.1	-	-	0.0	-	-	0.0
Turritis glabra	Ar	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	0.2	0.1	0.1
Erigeron annuus	Ar	0.0	-	-	0.0	-	-	-	-	0.1	-	-	-	-	-	-	0.1	-	-	-	-	0.0	-	-	-
Galium aparine	Ar	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.4	-	-
Convolvulus arvensis	Ar	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-	-	-	0.0	-
Melilotus officinalis	Ar	-	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-

		Stipo-Poion carniolicae												*Xerobromion erecti*											
		T0			T1			T2			T3			T0			T1			T2			T3		
Plant species	opt	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA
Crepis foetida	St	0.1	0.1	-	0.1	-	0.0	0.3	0.1	0.1	1.1	0.2	0.1	-	0.0	-	-	-	0.0	0.1	0.0	-	0.1	-	0.0
Reseda phyteuma	St	-	-	-	-	0.0	-	-	0.0	-	0.1	0.7	-	-	-	-	-	-	0.0	-	-	0.0	0.4	0.4	0.1
Erodium cicutarium	St	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-	0.0	-	-	-	-	-	0.0	-	0.6	0.5
Viola arvensis	St	-	-	-	-	-	-	-	0.0	-	-	0.2	0.1	-	-	-	-	-	-	0.1	-	0.0	0.7	-	0.1
Orlaya grandiflora	St	0.0	-	-	-	0.0	-	0.1	0.1	0.1	0.1	0.0	0.1	-	-	0.0	-	0.0	0.0	-	-	0.0	-	0.1	0.0
Ajuga chamaepitys	St	-	-	0.0	0.0	-	0.0	0.0	0.0	-	0.1	0.0	0.1	-	-	-	-	-	0.0	-	-	-	0.1	0.0	0.1
Chaenorrhinum minus	St	-	-	-	-	-	-	-	-	-	0.2	0.2	0.0	-	-	-	-	-	-	-	-	-	0.1	-	-
Sherardia arvensis	St	-	0.0	-	-	0.0	0.1	-	0.0	0.2	-	-	0.1	-	-	-	-	-	-	-	0.0	-	-	-	-
Vicia sativa s.l.	St	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-	-	-	0.6	-
Sonchus oleraceus s.l.	St	-	-	-	-	-	-	0.1	-	0.1	0.0	0.1	0.0	-	-	-	-	-	-	-	0.0	-	-	-	-
Chenopodium album	St	-	-	-	-	-	-	-	-	-	0.0	0.0	0.0	-	-	-	-	-	-	-	-	-	0.1	0.0	0.1
Fumaria officinalis	St	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	-
Anagallis arvensis	St	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	0.1	-	-
Bunium bulbocastanum	St	-	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-	-	0.1	-	-	-	-
Bromus hordeaceus	St	-	-	0.0	-	-	-	-	0.0	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-
Capsella bursa-pastoris	St	-	-	-	-	-	-	-	-	-	0.0	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-
Scleranthus annuus	St	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-
Adonis flammea	St	-	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-
Malva neglecta	St	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0
Polygonum aviculare	St	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-
Senecio vulgaris	St	-	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-
Others																									
Galium pusillum	As	-	-	0.0	-	-	0.0	0.0	0.0	0.0	-	-	-	-	0.0	-	0.1	-	-	-	-	-	-	-	-
Sedum dasyphyllum	As	-	0.0	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alyssoides utriculata	As	-	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Asplenium ruta-muraria	As	-	-	-	-	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cephalanthera longifolia	CF	-	-	-	-	-	-	-	-	0.0	-	-	0.0	0.1	0.0	-	0.1	0.1	-	0.1	0.1	-	-	-	-
Platanthera chlorantha	CF	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	0.0	-	-	0.0	-	-	-	-
Viola alba	CF	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	0.1	0.0	0.0

		Stipo-Poion carniolicae												*Xerobromion erecti*											
		T0			T1			T2			T3			T0			T1			T2			T3		
Plant species	opt	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA
Prunus avium	CF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-	-
Tamus communis	CF	-	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rosa canina s.l.	CP	-	0.1	0.0	-	0.0	-	-	0.0	-	-	0.0	-	-	0.1	0.1	-	0.1	0.1	-	0.0	0.0	0.1	-	0.0
Prunus spinosa	CP	-	0.0	-	-	-	-	-	-	-	-	-	-	-	0.1	0.1	-	0.1	0.1	-	0.1	0.1	-	0.2	0.1
Crataegus monogyna	CP	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	0.1	-	-	0.1	-	-	-	-	-
Ligustrum vulgare	CP	0.0	-	-	0.0	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rubus fruticosus s.l.	CP	-	0.0	-	-	-	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Amelanchier ovalis	CP	-	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bupleurum ranunculoides	ES	-	-	-	0.0	-	-	-	-	0.2	-	-	0.0	0.2	0.1	-	0.1	-	0.0	-	0.0	0.1	-	-	-
Globularia cordifolia	ES	-	0.0	0.0	-	0.1	0.0	-	0.1	0.0	-	-	-	0.1	-	-	0.1	-	-	-	0.1	-	-	-	-
Arabis ciliata	ES	-	-	-	-	-	-	-	-	-	-	0.0	0.0	0.1	-	0.1	0.1	-	0.0	0.1	0.0	0.0	0.1	0.1	-
Ranunculus montanus s.l.	ES	-	-	-	-	0.0	-	-	0.0	-	-	0.0	-	-	-	0.0	-	-	0.0	-	-	0.0	-	-	-
Verbascum thapsus	Ep	0.0	-	-	-	-	-	0.0	-	-	0.2	0.0	-	-	-	-	-	-	-	-	-	-	0.2	0.0	0.3
Epipactis atrorubens	EP	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-	-
Polygala chamaebuxus	EP	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-
Arctostaphylos uva-ursi	LV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	-	-	0.1	-	-	-	-	-
Dactylis glomerata	MA	1.0	1.3	0.3	2.3	1.6	0.4	2.6	1.7	0.4	2.0	1.6	0.2	0.7	0.3	0.4	1.0	0.3	0.4	0.5	0.6	0.4	0.6	0.9	0.9
Plantago lanceolata	MA	0.1	0.1	-	0.2	0.1	-	0.1	0.1	-	0.7	0.2	-	0.4	0.1	0.2	0.2	0.1	0.3	0.2	0.1	0.4	0.2	0.1	0.6
Lotus corniculatus	MA	-	0.0	-	0.0	0.0	-	-	0.0	-	-	0.1	-	0.3	0.3	0.1	0.5	0.3	0.1	0.3	0.2	0.1	0.2	0.8	0.1
Centaurea jacea s.l.	MA	-	0.0	-	-	0.0	-	-	-	-	-	0.1	-	0.3	0.2	-	0.2	0.1	0.0	0.3	0.1	-	0.1	0.0	0.0
Festuca rubra	MA	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-	-	-	-	-	0.1	0.6	0.1
Achillea millefolium s.l.	MA	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.1	0.0	0.1	0.1	0.0	0.1	0.1	0.0	-	0.1	0.0
Taraxacum officinale s.l.	MA	-	-	-	0.0	-	-	0.0	0.0	-	0.0	-	0.0	-	-	-	0.1	-	-	0.1	-	-	0.1	0.0	0.1
Poa pratensis s.l.	MA	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.0	-	0.0	-	-	0.0	0.0	-	0.0	0.0
Arrhenatherum elatius	MA	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	-	-	0.1	-	-	0.0	-	-	0.0	-
Hypochaeris radicata	MA	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	0.1	-
Stachys officinalis	MA	-	-	-	0.0	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-
Viola tricolor	MA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-
Geranium molle	MA	-	-	-	-	-	-	-	-	0.0	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-

		Stipo-Poion carniolicae												*Xerobromion erecti*											
		T0			T1			T2			T3			T0			T1			T2			T3		
Plant species	opt	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA
Trifolium campestre	MA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0
Gymnadenia conopsea	MA	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-	-	-	-	-
Pimpinella major	MA	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-	-	-	-	-
Juniperus oxycedrus	PR	-	0.0	-	-	0.1	-	-	0.0	-	-	-	-	-	-	-	0.1	0.1	-	-	0.0	-	-	-	-
Saponaria ocymoides	PP	0.0	0.0	-	0.1	-	-	0.0	-	-	0.3	-	-	0.1	0.1	-	0.1	-	0.1	0.1	-	0.0	7.0	0.4	0.6
Astragalus monspessulanus	PP	0.3	0.0	-	0.1	0.0	-	0.1	0.0	0.0	0.0	0.0	-	-	-	0.2	-	-	0.3	-	-	0.2	-	0.0	0.0
Quercus pubescens	Qp	0.1	-	0.1	0.2	-	0.0	0.1	0.0	0.1	0.1	0.0	0.0	-	-	0.0	-	-	-	-	-	-	0.1	-	-
Arabis turrita	Qp	-	-	-	-	-	-	-	-	-	0.2	-	0.1	-	0.0	-	0.1	-	-	-	-	-	0.1	0.0	0.1
Acer opalus	Qp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-	-	-	0.0
Hippocrepis emerus	Qp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0
Galium rubrum s.l.	Qr	-	-	-	-	-	-	-	-	-	-	-	0.1	0.4	0.0	-	0.1	-	-	0.1	0.0	-	0.2	-	-
Betula pendula	Qr	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-
Ononis minutissima	Ro	-	-	-	-	0.1	0.1	-	0.0	-	-	-	-	-	-	-	0.1	-	0.0	0.1	-	-	-	-	-
Leuzea conifera	Ro	-	-	-	-	0.0	-	-	0.0	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-	-
Fumana thymifolia	Ro	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aethionema saxatile	Th	0.3	0.1	0.1	0.3	0.1	0.0	0.2	0.1	0.0	0.3	0.3	-	0.1	0.1	0.0	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.1
Brassica repanda	Th	-	0.1	-	-	0.1	-	-	0.2	-	0.0	0.1	-	-	0.1	-	-	0.1	-	-	0.0	0.0	-	0.1	-
Vincetoxicum hirundinaria	Th	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.2	0.0	-	0.2	0.0	0.0	0.2	0.0	-	0.2	0.0	-
Gypsophila repens	Th	-	0.2	-	-	0.2	-	-	0.1	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	0.0	0.0
Erucastrum nasturtiifolium	Th	-	0.0	-	0.0	0.0	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Calamintha nepeta s.l.	Th	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-
Peucedanum oreoselinum	TG	0.1	0.3	0.2	0.1	0.3	0.2	0.1	0.5	0.1	0.1	0.4	0.2	1.8	0.6	0.1	1.6	0.6	0.2	1.4	0.4	0.2	0.8	1.1	0.4
Hypericum perforatum	TG	0.1	0.3	0.5	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.2	0.2	0.3	0.2	0.2	0.2	0.1	0.1	0.2	0.2	0.2
Origanum vulgare	TG	-	0.0	0.6	0.2	0.0	0.2	0.2	0.0	0.3	0.0	0.0	0.3	-	0.1	0.1	0.1	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Verbascum chaixii	TG	0.0	-	-	0.2	-	-	0.1	-	-	0.2	0.0	0.0	-	0.0	0.0	-	0.1	0.0	-	0.1	0.0	-	0.0	0.0
Laserpitium siler	TG	0.2	-	-	0.1	0.0	-	0.2	0.0	-	0.0	-	-	-	-	0.0	-	-	-	0.1	-	-	-	-	0.0
Clinopodium vulgare	TG	0.1	-	-	-	-	-	-	-	-	-	-	-	0.1	0.0	-	-	-	-	-	0.0	-	-	-	-
Peucedanum cervaria	TG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	-	-	0.0	-	-	0.0	-	-	0.0
Thalictrum minus	TG	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	-	-	0.0	-	-	0.1	-	-	0.0	-

		Stipo-Poion carniolicae												*Xerobromion erecti*											
		T0			T1			T2			T3			T0			T1			T2			T3		
Plant species	opt	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA	NG	GR	PA
Inula spiraeifolia	TG	-	-	-	-	-	0.0	-	-	0.1	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-
Aster amellus	TG	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Agrimonia eupatoria	TG	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	-	-	0.0	-	-	0.0	-	-	-	-
Centaurea triumfettii	TG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	0.0	-	0.0	-	-	-	-
Vicia onobrychioides	TG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	-	-	0.0	-	-	0.0	-
Viola hirta	TG	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	0.1	-	-	0.1	-	-	-	-	-
Geranium sanguineum	TG	-	-	-	-	-	-	0.0	-	-	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Silene nutans	TG	-	-	-	-	-	-	-	0.0	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-
Erysimum diffusum	TG	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	-	-	-	-	-	-	-	-	-	-
Polygonatum odoratum	TG	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-
Securigera varia	TG	-	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-

3. Restoration of *Alnus viridis*-encroached pastures with Highland cattle grazing

3.1. Environmental and vegetation factors affecting Highland cattle spatial distribution

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Spatial Distribution of Highland Cattle in *Alnus viridis* Encroached Subalpine Pastures

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Abstract

Green alder (*Alnus viridis*) is a shrub species that has expanded over former pastures in Central Europe due to land abandonment, leading to negative agri-environmental impacts, such as a reduction in forage yield and quality and an increase in nitrate leaching. Robust livestock breeds such as Highland cattle could be used to control *A. viridis* encroachment. The objectives of this study were to investigate the impact of *A. viridis* encroachment on plant community composition and diversity and to map the spatial distribution of Highland cattle in *A. viridis*-encroached pastures. During the summer of 2019, three different Highland cattle herds were placed along an *A. viridis* encroachment gradient. A total of 58 botanical surveys were carried out before grazing to assess plant community composition, pastoral value, and ecological indicator values. The spatial distribution of cattle was studied during the whole grazing period by monitoring six to eight cows equipped with GPS collars in each herd. Plant species associated with higher pastoral values of the vegetation were found in areas with lower *A. viridis* cover, while highly encroached areas were dominated by a few nitrophilus and shade-tolerant broad-leaved species and by ferns. Cattle spent more time in areas with higher pastoral value but did not avoid areas with high cover of *A. viridis*, on steep slopes or far from water. These results show that Highland cattle are able to tolerate harsh environmental conditions and that they can exploit *A. viridis*-encroached pastures. This suggests that they have a high potential to reduce *A. viridis* encroachment in the long-term.

Keywords: biodiversity, GPS tracking, grassland, robust livestock, INLA, Alps, green alder, grazing

Introduction

Throughout the last decades, socioeconomic changes have led to a reduction in agro-pastoral activities in European mountain areas, triggering profound landscape modifications, and widespread natural reforestation and shrub encroachment in former pastures (Estel et al. 2015). For instance, from 1985 to 2013, shrublands and forests have increased by 10.6% across Switzerland, with the largest increases in the Alps (Abegg et al. 2020). Shrublands now cover an area of 679 km^2 in Switzerland, with about 70% of them dominated by pure stands of green alder [*Alnus viridis* (Chaix.) D. C.], which is the most rapidly expanding shrub species in Central Europe (Anthelme et al. 2007). *A. viridis* is a pioneer species, which lives in symbiosis with the N2- fixing actinomycete *Frankia alni* (Huss-Daniel 1997), and its presence is determined by land use intensity (Caviezel et al. 2017). Encroachment by *A. viridis* can have several negative agri-environmental impacts: in particular, *A. viridis*-dominated shrublands have lower plant and animal diversity and forage quality than open grazed areas (Anthelme et al. 2001). A recent study showed that *A. viridis* encroachment resulted in a rapid decline in plant species richness, with dense *A. viridis* patches hosting 62% less species than adjacent open pastures (Zehnder et al. 2020). Moreover, there is high nitrate and dissolved organic carbon leaching from *A. viridis* shrublands, which results in water pollution, soil acidification, hydrological drawbacks, and gaseous nitrogen losses (Bühlmann et al. 2016; van der Bergh et al. 2018). It is therefore important to find methods that control *A. viridis* encroachment in order to reverse biodiversity losses and to protect key ecosystem services. Controlling shrub encroachment is challenging because mechanical removal is extremely difficult and expensive in harsh and marginal mountain conditions due to steep slopes and a lower density of road network. Moreover, many shrub species are not palatable to grazing animals, since their foliage has low digestibility, mostly due to high tannin concentrations (Waghorn 2008). However, some robust livestock species and breeds can forage on shrubs and digest their leaves, thanks to ruminal bacterial populations, which can degrade lignified material and which have high resistance to tannins (Manousidis et al. 2016). Highland cattle are a robust breed that are increasingly being reared in different European alpine countries due to their ability to forage on poor quality and shrub-encroached pastures (Pauler et al. 2020a, b). As a consequence, the particular grazing behavior of Highland cattle can also result in distinct pasture botanical composition, with reduced woody plant species cover in the long term (Pauler et al. 2019). However, the impact of robust livestock on shrub encroachment may depend on environmental and management conditions, such as slope, pastoral value of the vegetation, and distance to water sources and attractive supplements for livestock (Probo et al. 2014; Homburger et al. 2015; Pittarello et al. 2016). It is therefore important to evaluate the conditions under which robust livestock breeds are able to effectively reduce shrub encroachment in alpine pasture. However, to the best of our knowledge, no study has assessed in detail the drivers related to plant community composition and diversity in *A. viridis*-encroached pastures nor the potential of Highland cattle to reduce *A. viridis* encroachment. For this reason, in the present study, we aimed (i) to disentangle the topographic and ecological drivers of plant community composition and diversity in *A. viridis*-encroached pastures, and (ii) to measure the spatial distribution of Highland cattle in *A. viridis*-encroached pastures to determine whether they are attracted to or avoid *A. viridis* patches. Here, we hypothesized that spatial distribution of Highland cows would vary depending on *A. viridis* cover and other factors such as distance to water, slope, and the pastoral value of the vegetation. More specifically, we expected cows to spend more time in areas around water sources and in more valuable pasture and to avoid steep slopes.

Materials and Methods

To investigate vegetation features and cattle spatial distribution, three Highland cattle herds were placed in four different *A. viridis*-encroached paddocks in the Swiss and Italian Alps in the summer of 2019. Three paddocks were located in Switzerland: Bovonne 1 and Bovonne 2 (in the canton of Vaud) and Champlong (in the canton of Valais). Bovonne 1 and Bovonne 2 were adjacent paddocks, grazed one after the other by the same herd. The fourth paddock was located in Val Vogna (in the province of Vercelli) in the Italian Alps (Table 1). Highland cattle grazed each paddock for approximately 1 month: in July (Bovonne 1), from mid-July to late-August (Champlong), and in August (Bovonne 2 and Val Vogna). All the herds included suckler cows, heifers, and calves, varying in age from 6 months to 17 years. The paddocks had similar environmental conditions in terms of elevation and slope, were grazed at comparable livestock stocking rates, and were representative of a gradient of *A. viridis* encroachment, i.e., with *A. viridis* cover values ranging from 20 to 70% (Table 1). A water trough was installed in each of the two paddocks in Bovonne, while many natural streams were present both in Val Vogna and Champlong paddocks.

In order to characterize the drivers of plant community composition and diversity within each paddock, the botanical composition was determined along 12.5-m transects. Transects were placed in patches (1.08 ± 0.22 ha, Table 1) with homogeneous botanical composition and vegetation structure, and the vertical point-quadrat method was used to record plant species (Daget & Poissonet 1971). At 50-cm intervals along each transect, the plant species touching a steel needle were identified and recorded (i.e., 25 points per transect). Since rare species are often missed by this method, all other species within a 1-m buffer area around the transect were also recorded (Kohler et al. 2004). A total of 58 vegetation transects were surveyed in the four paddocks (Table 1). Plant species nomenclature followed Aeschimann et al. (2004). The relative abundance of each plant species was computed by dividing species frequency of occurrence by the sum of frequency of occurrence values for all species in the transect and multiplying it by 100. A species relative abundance value of 0.3% was assigned to all the species found only in the buffer zone and not in the main transect, following Pittarello et al. (2016). We then calculated the pastoral value (PV) of the vegetation, based on the species composition. The PV is a synthetic index summarizing forage yield, quality, and palatability for livestock (Pittarello et al. 2018). To estimate PV, we attributed each species an index of specific quality (Cavallero et al. 2007). The index of specific quality depends on the preference, morphology, structure, and productivity of the plant species, and it ranges from 0 (low) to 5 (high). The PV, which ranges from 0 to 100, was calculated as follows (Daget & Poissonet 1971):

$$PV = \sum_{i=1}^{n} (SRA_i \times ISQ_i) \times 0.2$$

where SRA_i is the species relative abundance, and ISQ_i is the index of specific quality value of the species *i*.

Table 1. Topographical, management, and vegetation characteristics of the four *A. viridis*-encroached paddocks. Cover of *Alnus viridis*, distance to water, elevation, slope, and pastoral value of the vegetation are mean values (+SD) measured at a 10-m x 10-m cell scale over the whole paddock area. One livestock unit = 1 animal of 500 kg.

	Bovonne 1	**Bovonne 2**	**Champlong**	**Val Vogna**
Coordinates	N46°16'9.857" E7°6'44.252"	N46°16'12.118 E7°6'58.814"	N45°56’ 2.306" E7°12’ 14.333"	N45°46’ 18.815" E7°54’ 9.198"
Average elevation (m a.s.l.)	1745 ± 46	1789 ± 32	2012 ± 36	1897 ± 67
Average slope (°)	23 ± 8	21 ± 10	22 ± 8	21 ± 6
Average distance to water (m)	116 ± 44	156 ± 3	18 ± 19	107 ± 75
Grazable area (ha)	8.26	7.67	5.99	17.88
Grazing days	28	24	40	28
Number of livestock units	23.5	23.5	11.3	71.2
Stocking rate (livestock units ha^{-1} year^{-1})	0.22	0.2	0.21	0.3
Number of GPS-equipped cows	8	8	7	6
Cover of *Alnus viridis* (%)	61 ± 32	71 ± 28	44 ± 30	20 ± 34
Number of vegetation transects	11	11	12	24
Average vegetation patch size (ha)	0.69 ± 0.12	0.59 ± 0.08	0.50 ± 0.09	1.87 ± 0.53
Pastoral value (PV)	6.5 ± 6.1	5.6 ± 6.5	10.4 ± 6.3	18.3 ± 11.9
Species richness of paddocks	71	96	123	157

Moreover, we used the indicator values of Landolt et al. (2010) for each plant species. These indicate the environmental conditions in the habitats in which the given species occurs and are a measure of its environmental preferences. We used the light (L), soil moisture (F), and nutrient value (N) (nitrogen) indicators. We then determined the mean ecological conditions of each vegetation patch, as the mean Landolt value for L, F, and N by multiplying each species indicator value by its relative abundance. For each vegetation transect, two plant diversity indexes were computed: species richness and Shannon diversity index H' (Magurran 1998). *A. viridis* canopy cover (%) was visually estimated within each vegetation patch. Each paddock was subdivided into 10- × 10-m grid cells, and the distance to water sources (i.e., streams and water troughs), the aspect, elevation, and slope were calculated for the centroid of each cell using QGIS and SAGA software. Moreover, *A. viridis* cover and PV were calculated for each grid cell using the values of the corresponding vegetation patch. If a cell covered more than one vegetation patch, the patch accounting for most of the cell area was considered. To avoid issues with circular variables (where high and low values are close together), aspect was transformed into southness (180 − | aspect − 180|) (Chang et al. 2004). In each herd, six to eight cows were equipped with GPS collars (Followit Tellus GPS collars) that recorded their position every 10 min during the whole grazing period, with an average accuracy of 2–5 m (Table 1). The number of GPS fixes was calculated for each grid cell.

All statistical analyses were performed using R version 3.4.4. A partial canonical correspondence analysis (CCA, vegan package), taking into account the spatial distance between botanical surveys, was performed to describe how PV, biodiversity indices, ecological indicators (i.e., Landolt indexes), and topography (i.e., slope and aspect) were related to plant community composition (after Hellinger transformation). Moreover, relationships between *A. viridis* cover and diversity indexes and PV were analyzed with generalized linear mixed models with Poisson (species richness and PV) and quasi-Poisson (Shannon diversity index H') distributions, with paddock considered as a random factor (lme4 package). The spatial distribution of cattle calculated from GPS data (i.e., the number of GPS fixes counted in each grid cell) was investigated separately for three distinct periods within each paddock, i.e., the beginning (P1), middle (P2), and end (P3) of the grazing period. These periods had equal lengths within each paddock and varied between 1 and 2 weeks depending on the length of the grazing period (Table 1). The impacts of slope, *A. viridis* cover, distance to water, and PV on the number of GPS fixes were assessed using linear regression models with zero-inflated negative binomial likelihood, as this error distribution accounted for overdispersion in the response variable (tested using the AER package). Homburger et al. (2015) found this likelihood structure to be appropriate for GPS counts in zones incompletely exploited by cattle. In order to account for the spatial autocorrelation of data, a spatial structure error term (using a two-dimensional random walk of second order) was included, and the model was fitted by Integrated Nested Laplace Approximation (INLA).

Results

We found a total of 252 plant species in all the paddocks, with the highest species richness detected in Val Vogna (157 species, Table 1). The results of the CCA analysis highlighted different ecological, topographical, and vegetation impacts on plant community composition (Figure 1), which significantly explained the variation in community composition across the paddocks (CCA 1 = 20.9%, CCA2 = 19.0%, CCA3 = 12.9%, CCA 4 = 10.9%, and total = 84.6%). Vegetation patches within the two Bovonne paddocks were characterized by higher *A. viridis* cover and nitrogen enrichment (i.e., higher values for N indicator). In contrast, the Champlong paddock had many open pasture patches (with higher light availability), while Val Vogna patches were more heterogeneous. Four patches in Val Vogna were well distinguished and corresponded to vegetation dominated by dwarf shrubs, such as *Vaccinium vitis-idaea*, *Juniperus nana*, and *Rhododendron ferrugineum*, with low PV (Figure 1B). *A. viridis* cover had a significant negative effect on plant species richness, Shannon diversity index H’, and PV ($p < 0.001$). Indeed, plant species associated with higher PV (e.g., *Festuca pratensis* and *Trifolium pratense*) were often found in the most open areas, characterized by a lower *A. viridis* cover, as well as typical small sized pasture species (e.g., *Medicago lupulina* and *Polygala alpestris*), which were associated with the highest light availability and biodiversity (both in terms of species richness and Shannon diversity index H’). Conversely, areas with high levels of *A. viridis* encroachment and with high indicator values for N were dominated by a few tall broad-leaved species, such as *Adenostyles alliariae* and *Cicerbita alpina*, together with ferns (mainly *Athyrium filix-femina* and *Dryopteris dilatata*).

Estimates of linear regression models showed that livestock spatial distribution was often positively influenced by PV. Cattle spent more time in high PV areas in Bovonne 1 (during periods 1 and 2), Champlong (period 3), and Val Vogna (periods 2 and 3) (Figure 2). In Bovonne 2, the PV did not influence cattle spatial distribution. The cover of *A. viridis*, slope, and distance to water sources did not generally affect livestock spatial distribution. *A. viridis* cover and slope only had significant effects in the Champlong paddock during the third and the first grazing period, respectively; in both cases, cattle spent more time in areas with high *A. viridis* cover and on steeper slopes. Distance to water had a negative effect only in periods 1 and 2 in Bovonne 1 paddock, where water sources were less abundant, meaning cattle spent more time close to water.

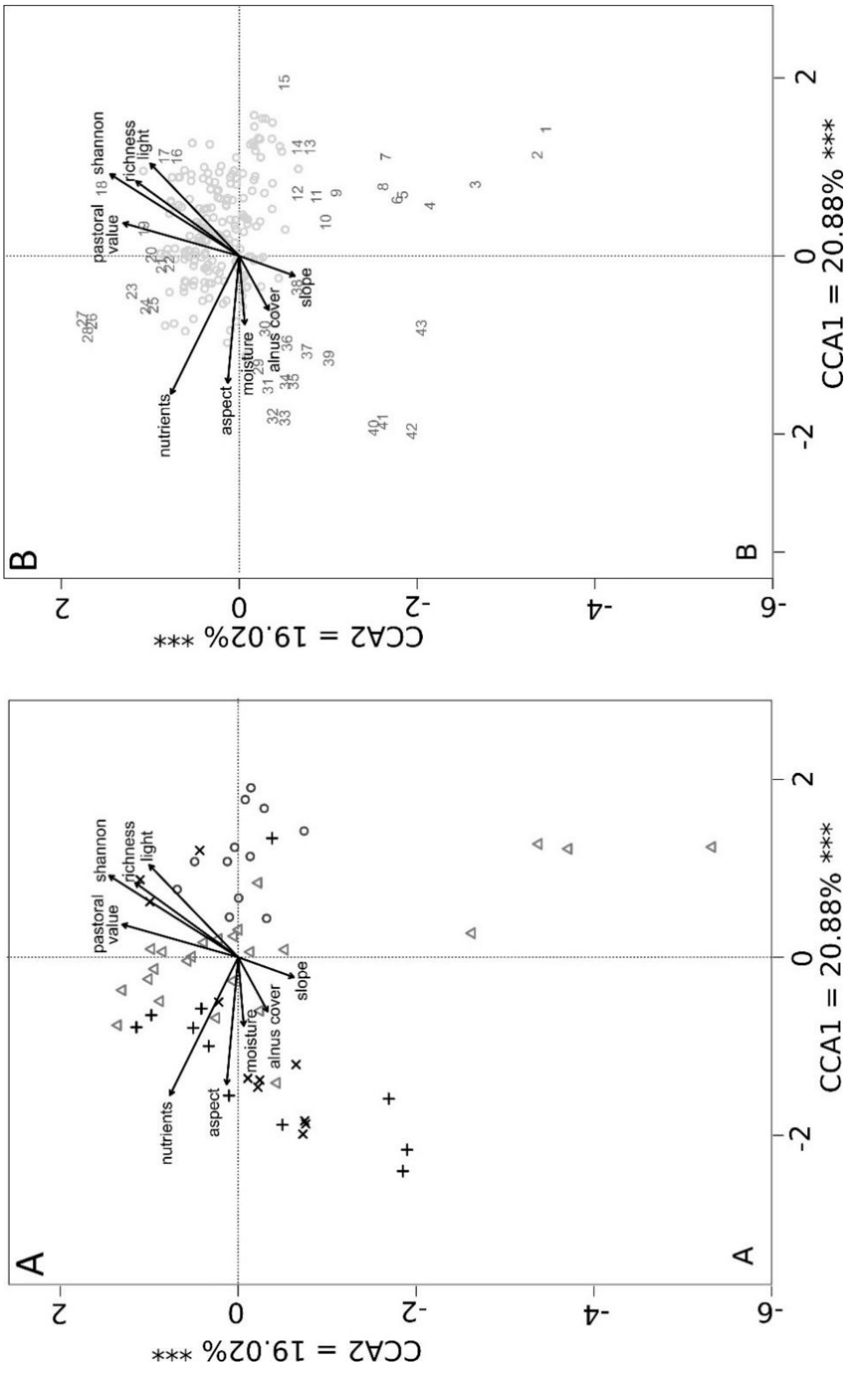

Figure 1. (The caption of Figure 1 is reported on page 63).

Figure 1. Partial canonical correspondence analysis (CCA) showing plant community composition explained by Alnus cover, slope, aspect, pastoral value, species richness, Shannon index and ecological indicators for nutrients, light and moisture (Landolt indexes). Both dimensions presented are significant ($p<0.001$). (A) Botanical surveys are represented for Bovonne 1 (+), Bovonne 2 (x), Champlong (o) and Val Vogna (Δ). (B) Plant species are represented by numbers (detailed below) for the most important species and by dots for the remaining species. 1 *Vaccinium vitis-idaea,* 2 *Juniperus nana,* 3 *Cryptogramma crispa,* 4 *Gymnocarpium dryopteris,* 5 *Astrantia minor,* 6 *Rhododendron ferrugineum,* 7 *Arnica montana,* 8 *Avenella flexuosa,* 9 *Festuca scabriculmis,* 10 *Rosa penduline,* 11 *Vaccinium myrtillus,* 12 *Calamagrostis villosa,* 13 *Melampyrum sylvaticum,* 14 *Plantago lanceolata,* 15 *Epilobium montanum,* 16 *Medicago lupulina,* 17 *Thlaspi montanum,* 18 *Polygala alpestris,* 19 *Molinia arundinacea,* 20 *Hypericum richeri,* 21 *Festuca pratensis,* 22 *Trifolium pratense,* 23 *Arabis ciliate,* 24 *Rumex alpestris,* 25 *Campanula latifolia,* 26 *Capsella bursa-pastoris,* 27 *Chenopodium bonus-henricus,* 28 *Trifolium badium,* 29 *Cardamine resedifolia,* 30 *Adenostyles alliariae,* 31 *Acer pseudoplatanus,* 32 *Rubus saxatilis,* 33 *Thalictrum aquilegiifolium,* 34 *Saxifraga rotundifolia,* 35 *Cicerbita alpina,* 36 *Urtica dioica,* 37 *Dryopteris dilatate,* 38 *Rubus idaeus,* 39 *Athyrium filix-femina,* 40 *Prenanthes purpurea,* 41 *Sorbus aucuparia,* 42 *Picea abies,* 43 *Oxalis acetosella*.

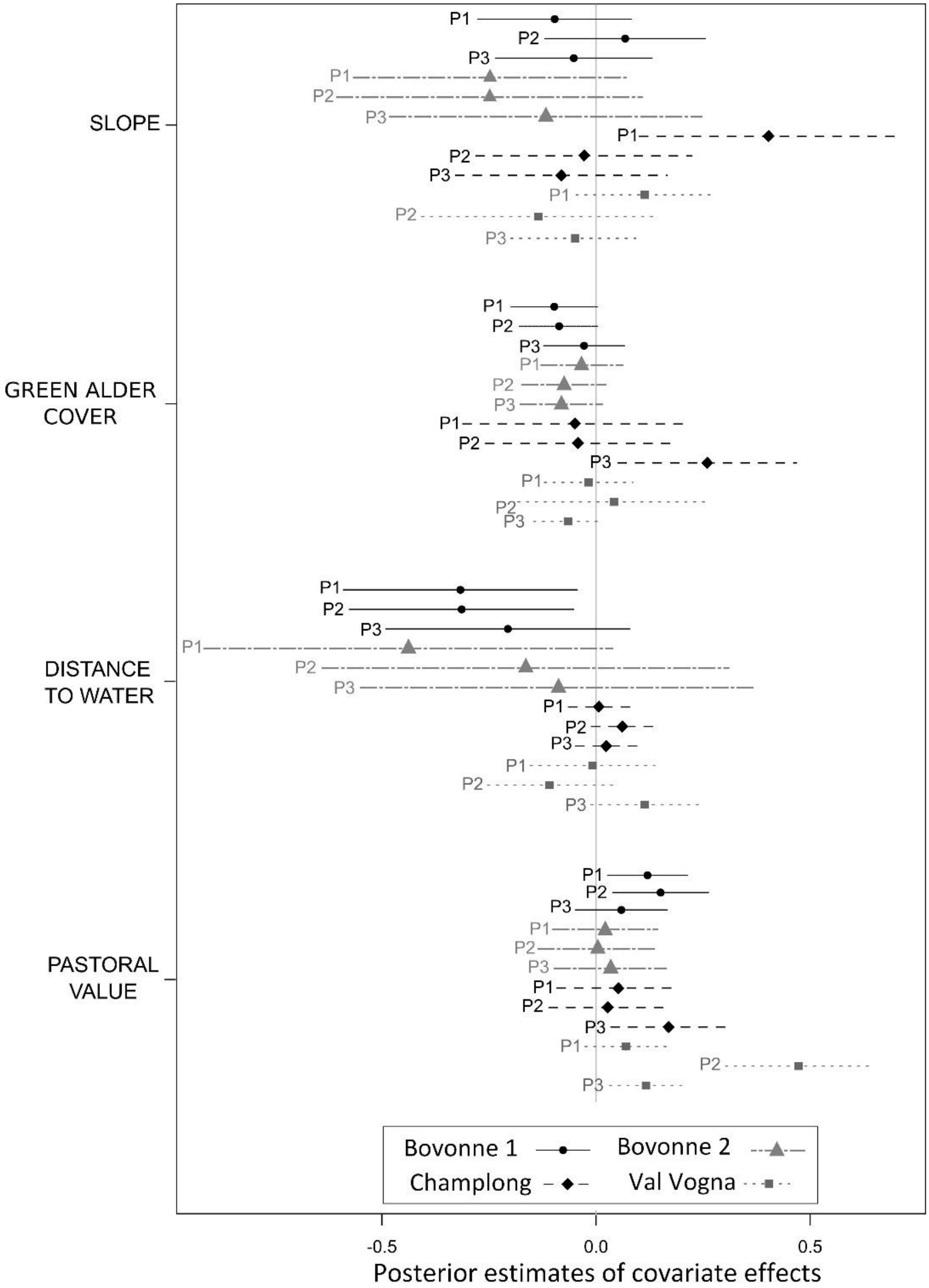

Figure 2. Effects of four covariates on livestock spatial distribution. The dots show the mean estimated effects, and the line the 95 % confidence interval, in the paddocks of Bovonne 1 (black line), Bovonne 2 (grey dashed line), Champlong (black dashed line) and Val Vogna (grey dotted line) for the three grazing periods (P1, P2, P3).

Discussion

Overall, plant communities with higher *A. viridis* cover had lower plant diversity and were dominated by a few broad-leaved species, like *A. alliariae* and *C. alpina*, which prefer N-enriched conditions. These results support the findings of Anthelme et al. (2001) and Zehnder et al. (2020), who also showed a decline in alpine plant diversity with *A. viridis* encroachment. The few species that survive in *A. viridis*-dominated areas are able to take advantage of the N-enrichment and increased shading, and they replace many typical pasture species that are highly light demanding. Interestingly, other than broad-leaved species, the understory vegetation of many *A. viridis* patches was dominated by ferns (in particular, *A. filix-femina* and *D. dilatata*). These ferns are also highly shade tolerant, but they typically prefer areas with lower N levels, compared to the broad-leaved species (Landolt et al. 2010). They may therefore be more abundant in areas recently invaded by *A. viridis* where N levels have not increased so much. These results show that encroachment by *A. viridis* threatens the diversity of Alpine pastures and leads to a loss of typical pasture species. The Highland cattle did not avoid patches with a high density of *A. viridis*. In fact, they were able to graze in most *A. viridis* covered areas, therefore indicating that they could be valuable in grazing and reducing *A. viridis* patches. In contrast to many other breeds (e.g., Homburger et al. 2015), Highland cattle were also capable of grazing on the steepest slopes and were able to spend time in areas further from water sources, as they were not significantly influenced by these harsh conditions. This is in line with the observations of Pauler et al. (2020a) who found that Highland cattle used space more evenly and avoid steep slopes less than other breeds. Moreover, cattle even preferred *A. viridis* patches and steep slopes in Champlong, during certain grazing periods, which shows that cattle are able to move to areas with the most unfavorable conditions and can stay there for relatively long periods (approximately 2 weeks). Cattle also responded differently to the various factors during the time they were on the paddocks. This indicates that the herds may explore the paddock intensively at the beginning of the grazing period before selecting particular areas later on. However, despite the fact that Highland cattle graze less selectively on plants compared to other breeds (Pauler et al. 2020b), they were still mostly attracted to valuable pastures. Thus, providing a mosaic of open pasture areas with higher quality forage in *A. viridis*-encroached paddocks may be a good solution to respect grazing preferences while increasing grazing pressure on *A. viridis*-encroached patches. Moreover, this type of setting would enhance the potential for seed translocation through endo- and epi-zoochory by Highland cattle (Mouissie 2004; Cosyns et al. 2005). Indeed, in the study of Pauler et al. (2019), plant species relying on epizoochory were significantly more frequent in pastures grazed by Highland cattle than in paddocks grazed by other breeds, probably because the long fur of this breed is ideal for transporting seeds. Animal movements might thus provide additional seed translocation fluxes from pastures to encroached areas to facilitate the restoration of typical pasture vegetation in the long-term (Pittarello et al. 2016). However, future research is needed to assess the simultaneous effects of cattle grazing, trampling, and seed transportation on the restoration of pasture vegetation in *A. viridis*-encroached areas. We observed considerable impact of the cattle on vegetation within the encroached areas (data not shown), including intense defoliation of *A. viridis* shrubs and a reduction of their canopy cover, due to browsing, as well as damage to branches due to cattle movement and scratching. Moreover, an increase in bare soil due to livestock trampling and a considerable number of dung pats was observed within *A. viridis*-encroached areas. If repeated for several years, these effects of the cattle could provide favorable conditions for the recolonization of typical pasture vegetation in formerly encroached areas. The repetition of the botanical surveys along the fixed vegetation transects installed during this study could allow us to assess the medium- and long-term impacts produced by Highland cattle on vegetation. In addition, testing improved techniques to attract livestock to the most encroached areas, such as the placement of specific attractive points (Pittarello et al. 2016; Bailey et al. 2019), might be relevant to further increase the grazing pressure and thus livestock impacts on *A. viridis*-encroached subalpine pastures. In conclusion, our results show that Highland cattle can graze in harsh environmental conditions and exploit *A. viridis*-encroached pastures. This suggests that they have a high potential to reduce *A. viridis* encroachment in the long-term.

Data availability statement: The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: http://doi.org/10.5281/zenodo.4420882.

Ethic statement: The animal study was reviewed and approved by Direction générale de l’agriculture, de la viticulture et des affaires vétérinaires du canton de Vaud. Written informed consent was obtained from the owners for the participation of their animals in this study.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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3.2. Effects of Highland cattle on *Alnus viridis*-encroached pastures with the use of attractive supplements

This sub-chapter is a reproduction of the scientific paper (author's version) submitted to *Frontiers in Ecology and Evolution*, with permission from the authors:

Use of molasses-based blocks to modify grazing patterns and increase Highland cattle impacts on Alnus *viridis*-encroached pastures

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Abstract

Alnus viridis is a pioneer species that has expanded in Central Europe in the last decades, resulting in a series of negative agro-environmental impacts. Robust livestock grazing could be used as a targeted tool to reduce its encroachment but there is a lack of information on the best approach to achieve this. In this study, we assessed the potential of attractive points (i.e. molasses-based blocks) to lure Highland cattle in *A. viridis*-encroached areas of the paddocks and monitored associated after-grazing impacts on vegetation. In 2019 and 2020, two Highland cattle herds equipped with GPS collars were placed in three paddocks along an *A. viridis*-encroachment gradient in the Swiss and Italian Alps. In 2020, attractive points were added to highly encroached areas within each paddock to attract the herds to graze on *A. viridis*. Botanical surveys were carried out before and after grazing around attractive points and control areas. Highland cattle grazed significantly more around attractive points (up to 50 m from the attractive points) compared to the previous year (i.e., same area without attractive points) and to control areas. The increased grazing around attractive points led to a significant decrease in herbaceous cover and an increase in bare soil compared to control areas. Livestock grazing and trampling significantly reduced the cover of ferns and megaphorbs, forbs and woody species around attractive points compared to control areas. *A. viridis* leaves and branches were significantly more consumed and damaged up to 10 meters from the attractive points, due to the more intense livestock grazing. Overall, the presence of attractive points was efficient in attracting cattle toward highly encroached areas, where they successfully consumed and damaged *A. viridis* trees. Such results highlighted the potential of this management regime to reduce *A. viridis* encroachment in alpine grasslands in the long-term.

Keywords: alpine grassland, green alder, robust livestock, GPS-tracking, targeted grazing

Introduction

During the last century, socio-economic transformations have led to a large-scale decrease in agro-pastoral activities across mountain areas of Europe, causing many challenges for grassland management and biodiversity conservation (MacDonald et al. 2000; Valkó et al. 2018). For instance, in Switzerland, the last 30 years have witnessed a significant decrease in grassland area in alpine regions (Strebel and Bühler 2015; Zehnder et al. 2016). The reduction of livestock farming has caused profound landscape modifications and widespread shrub and tree encroachment in former meadows and pastures (Estel et al. 2015). For example, from 1985 to 2013, shrublands and forests increased by 10.6% across Switzerland, with the largest increases in the Alps (Abegg et al. 2020). Woody species encroachment of alpine grasslands has been even more pronounced in the Italian Alps (Orlandi et al. 2016). Shrublands now cover an area of 679 km^2 in Switzerland, with about 70% of them dominated by pure stands of green alder (*Alnus viridis* (Chaix.) D. C.), which is among the most rapidly expanding shrub species in Central Europe (Anthelme et al. 2007). *Alnus viridis* is a pioneer shrub species that lives in symbiosis with the N_2-fixing actinomycete *Frankia alni* (Huss-Daniel 1997). It is found mostly in steep, north and west-facing slopes, but it can easily expand into more habitats in alpine environments, thanks to its efficient colonization ability and substantial seed production (Caviezel et al. 2017; Farmer et al. 1985). Its presence is strongly affected by land-use intensity, as land-abandonment is an essential driver of its spread (Caviezel et al. 2017).

Encroachment by *A. viridis* can have several negative agro-environmental impacts on mountain grasslands, which in turn reduce the provision of key ecosystem services for society. For instance, *A. viridis* encroachment causes nitrogen enrichment in soils, leading to increased nitrate and dissolved organic carbon leaching (Bühlmann et al. 2016). This can result in soil acidification, water pollution and gaseous nitrogen losses (Bühlmann et al. 2016; Caviezel et al. 2014; van den Bergh et al. 2018). Moreover, *A. viridis*-dominated shrublands are characterized by a lower forage quality and host lower animal and plant diversity than adjacent grasslands (Anthelme et al. 2001; Bühlmann et al. 2014; Svensk et al. 2021). A recent study by Zehnder et al. (2020) showed that *A. viridis* encroachment resulted in a rapid reduction in plant species richness, with dense *A. viridis* patches hosting 62% less species than nearby open pastures. This decrease in biodiversity is linked to the reduction in solar radiation under highly encroached areas, as well as to soil nitrogen enrichment. Indeed, only a few tall and shade-tolerant plants with broad leaves are able to adapt to these ecological conditions and dominate the understory vegetation of *A. viridis* shrublands, such as *Adenostyles alliariae* (Gouan) A. Kern and *Cicerbita alpina* L. (Wallr.), together with a few fern species (Svensk et al. 2021). Furthermore, in contrast to coniferous forests, *A. viridis* stands do not provide protection against erosion and avalanches on steep slopes, mostly due to the elasticity of their branches that easily bend under snow pressure (Caviezel et al. 2014). The resistance to this environmental pressure enables a better adaptation of *A. viridis* in these areas, compared to other trees that can easily break under such stress. Finally, the encroachment by *A. viridis* can also adversely impact landscape quality in subalpine areas, resulting in reduced attractiveness for tourists.

Because *A. viridis* predominantly establishes on steep slopes and marginal locations where road network is limited or absent, the mechanical removal of this shrub species can be technically difficult, expensive and time consuming. One alternative and sustainable nature-based solution to counteract shrub encroachment could be the use of targeted grazing (Elias et al. 2018; Elias and Tischew 2016). However, many shrub species are not palatable for production-oriented livestock due to low foliage digestibility. Indeed, their leaves can contain high lignin and tannin concentrations that reduce digestibility and palatability, due to their interaction with digestive molecules, and can even cause toxicity in some cases (Papanastasis et al. 2008). Nevertheless, some robust livestock species and breeds have higher resistance to tannins, with ruminal bacterial populations that can better degrade lignified material, allowing them to feed on shrubs and digest their leaves (Berry et al. 2002; Marques et al. 2017). For instance, previous studies have shown that goats can efficiently forage on woody plants and significantly reduce their cover (Álvarez-Martínez et al. 2016; Iussig et al. 2015). Some cattle breeds can also forage on shrub species (Pauler et al. 2019; Zehnder et al. 2016). For example, Highland cattle, a robust breed originating from Scotland, are able to graze on shrubs (Pauler et al. 2020a) due to their low maintenance energy requirements, which allow them to graze on poor quality forage while maintaining good nutrient supply (Berry et al. 2002). This has recently led, together with the low costs of their maintenance and care, to an increased rearing of this livestock breed in alpine regions (Pauler et al. 2020b). Previous studies have demonstrated their ability to reduce woody plant species cover over time, with a turnover in plant diversity (Pauler et al. 2020a, 2019). Moreover, in addition to the direct grazing of the leaves, Highland cattle are also able to damage shrub branches and trunks thanks to their long horns (Svensk et al. 2021). Recently, Svensk et al. (2021) pointed out the ability of Highland cattle to graze over steep subalpine pastures characterized by high *A. viridis* cover and vegetation of low forage quality. Therefore, the use of Highland cattle grazing could be an efficient and sustainable tool to reduce *A. viridis* encroachment and restore encroached pastures in the long-term.

Together with cattle feeding behavior, livestock management techniques are also a key to increase livestock impacts on targeted shrub-encroached vegetation. For instance, livestock supplements could be used to change animal spatial distribution and attract them to underused and shrub-encroached locations (Probo et al. 2016, 2013). Different types of dietary supplementation exist, mainly composed of salt (i.e. mineral mix supplements) or sugar (i.e. molasses-based blocks). In the Alps, the use of mineral supplements is far more popular than other supplement types, and previous studies already demonstrated their efficacy in attracting beef cattle herds within steep alpine shrub-encroached areas (Pittarello et al. 2016; Probo et al. 2013). For example, in a 5-year study, Probo et al. (2016) highlighted a significant reduction in shrub cover, together with the establishment of typical pasture species with higher forage quality, thanks to the effects produced by grazing, trampling, seed and dung translocation at mineral supplement locations. Dehydrated molasses-based blocks were also proven to be very efficient in influencing cattle grazing patterns in unfavorable field conditions (i.e. steep slopes, far from water sources and usually undergrazed zones) and across large areas in North American Desert rangelands (Bailey et al. 2001; Bailey & Welling 1999), but so far, they have never been tested in alpine shrub-encroached areas, despite their lower cost and labor than fencing and herding. Using molasses-based blocks for cattle grazing management provides different advantages, such as an enrichment of cattle diets through mineral supplementation, that can also reduce the risks of foot pathology and mycotoxicosis, and an enhancement of the intake of forage with low digestibility and palatability, thanks to their appetizing role (Mordenti et al. 2021). The evaluation of the effectiveness of grazing management practices to modify vegetation cover and composition in alpine environments is generally carried out over a medium- to long-term period, since the growing season and vegetation cycles are slow at high altitudes (Körner 2003). However, due to the knowledge gap regarding the use of molasses-based supplements in alpine environments, even the assessment of the short-term effects produced on vegetation by this management practice could allow the identification of key impacts on the cover of target plant species or functional groups, providing perspectives on their long-term restoration potential. Indeed, previous studies, e.g. Tocco et al. (2013), showed that the analysis of functional group cover in the short-term can be essential to assess the effectiveness of targeted grazing techniques in a longer term.

In the present study, we strategically placed attractive molasses-based blocks on *A. viridis*-encroached pastures to attract Highland cattle herds and increase their impact on shrub vegetation. Specifically, we aimed to investigate: (i) the effect of strategically placed molasses-based blocks on Highland cattle spatial distribution and (ii) the after-grazing impacts produced by livestock around attractive points on *A. viridis* trees, understory plant functional group and soil cover. We hypothesized that (i) Highland cattle would significantly increase the use of areas around molasses-based blocks, and (ii) their grazing and trampling pressure would induce a higher impact on *A. viridis* trees around molasses-based blocks, with an increased consumption of leaves and damages on trunks and branches compared to control areas. Simultaneously, we expected (iii) the understory vegetation to be more impacted around attractive points, with an increase in bare soil due to livestock grazing and trampling, which could lead to a potential re-colonization of those areas by typical pasture species in the long-term.

Materials and Methods

Study areas and grazing management

During the summer seasons of 2019 and 2020, two Highland cattle herds were placed in three *Alnus viridis*-encroached paddocks in the Swiss and Italian Alps. The first paddock (Paddock 1, 17.88 ha) was located in Val Vogna, in the province of Vercelli (Italy), while the other two paddocks (Paddock 2, 8.26 ha and Paddock 3, 7.67 ha) were located in Bovonne, in the canton of Vaud (Switzerland) and were grazed one after the other by the same herd. All paddocks were grazed at a comparable stocking rate between years (Table 1) and had similar topographical conditions, with an average elevation of 1861 ± 45m a.s.l (mean ± s.e.) and an average slope of 23 ± 8° (mean ± s.e.). The three paddocks were representative of an *A. viridis* cover gradient, as Paddock 1, 2 and 3 had an average *A. viridis* cover of 20%, 61%, and 71% respectively. The herds grazed in the summer pastures from the middle of June to the beginning of September (Table 1). All the herds included suckler cows, heifers, and calves, varying in age from 6 months to 17 years (with an average of 5 years for Paddock 1 and 4 years for Paddock 2 and 3) and about 70% of the animals were present in both years in the same site. A water trough was installed in Paddock 2 and 3, while natural streams were present in Paddock 1 . In each herd and during both years, six to ten cows (Table 1) were equipped with GPS collars (Followit AB ©, Tellus GPS System collars) that recorded their position every 10 minutes during the whole grazing period, with an accuracy of two to five meters.

Table 1. Grazing periods, grazing days, livestock units (LU), grazable area, livestock stocking rates, and number of GPS collars in the study areas in 2019 and 2020.

Year	Paddock	Grazing period	Grazing days	LU[1]	Grazable area (ha)	Stocking rate[2]	N° GPS collars
2019	1	July 19th to September 3rd	44	45.4	17.88	0.31	6
	2	July 2nd to 19th	17	29.8	8.26	0.17	8
	3	July 30th to August 17th	19	29.8	7.67	0.19	8
2020	1	July 20th to August 18th	29	70.4	17.88	0.31	8
	2	June 15th to July 2nd	17	29.6	8.26	0.17	10
	3	July 2nd to 20th	18	29.6	7.67	0.19	10

[1] LU = Livestock Unit. One livestock unit = 1 animal of 500 kg.
[2] LU/ha * year

In 2020, five molasses-based blocks of 22.5 kg each were added to each paddock as attractive points. They were mostly composed of sugar (40%) and contained mineral supplements which are often lacking in natural alpine environments (Schlegel & Kessler 2001), thus complementing cattle feeding (detailed composition available in Appendix, Table S1). The number of blocks was defined based on the average consumption by cattle declared by the producer (i.e., 35-50 g/calf * day, 100 g/heifer * day, and 150-200 g/cow * day). The consumption of molasses was monitored every week in each paddock to check that enough molasses was available during the grazing period. At the end of the grazing period, the blocks were weighed and the average consumption per animal was estimated by dividing the total amount consumed by the number of animals and grazing days. The attractive points were placed at five points along a 40-meter line, lying along a contour line, and separated by 10 meters from each other (Appendix, Figure S1), in highly *A. viridis* encroached areas (i.e., patches with more than 66% of *A. viridis* canopy cover). A control line was also established in each paddock in areas with comparable *A. viridis* cover, slope, botanical composition and distance to water sources.

Moreover, to avoid any pre-existing site effect on cattle distribution, we also checked that the attractive points and control locations were similarly exploited by livestock in 2019, before the attractive points were established. Around control and attractive point lines, buffers with a radius of 10 and 50 meters were created in a GIS environment, and the number of GPS positions was calculated for each cow within each buffer (using QGIS 3.6 software). The 10-meter buffer was used to assess the attractive effect in a small area, in which livestock were likely present to actively consume molasses, while the 50-meter buffer was used to estimate the attractive effect on a larger scale, i.e., including areas where livestock grazed and walked around supplement locations.

Vegetation surveys

Around both attractive and control points, botanical surveys were carried out in 2020 both before grazing (June-July) and after grazing (August), on cross-shaped vegetation transects of 10 meters (see Appendix, Fig. S1), using the vertical point-quadrat method (Daget & Poissonet 1971). At 50 cm intervals along each transect, the vascular plant species touching a steel needle up to a grazable height (i.e., 1.8 m) were identified and recorded. To account for rare species around the transect, all other species within one meter buffer around the transect were also recorded (Kohler et al. 2004). Each line of attractive and control points in all three paddocks was made up of 16 transects (i.e., 48 vegetation transects in control areas and 48 in attractive points areas in total). The percentages of herbaceous and bare soil cover were visually assessed before and after grazing within a one-meter buffer around each transect, to assess the changes in vegetation cover produced by livestock grazing and trampling. Plant species nomenclature followed Aeschimann et al. (2004).

Along the vegetation transect, the percentage species cover (%SC) was calculated by converting the recorded frequency of occurrence of each plant species to 100 measurements. A %SC of 0.3% was assigned to the species found only in the one-meter buffer zone and not along the transect, following Pittarello et al. (2016). Species were grouped in three main functional groups that were common among all paddocks and typical of the understory of *A. viridis*-encroached pastures (Svensk et al. 2021): 1) ferns and megaphorbs, i.e., all ferns and tall broad-leaved forbs having a leaf diameter higher than 10 cm and a plant height higher than 40 cm, following Pignatti (1982); 2) forbs, i.e., other non-graminoid species that were not classed as megaphorbs; and 3) woody, i.e., all woody species (including *A. viridis*). Graminoids were not taken into account as they were a minor component of the understory vegetation, representing only on average 5.92 ± 3% (mean ± SE) of the vegetation cover in each paddock (with a maximum of 11.5% in Paddock 1).

Along each transect, three *A. viridis* trees were randomly selected every three to four meters and marked. One branch for each tree, having the leaves within a grazable height (i.e., 1.8 m), was selected to assess the number of living leaves before and after grazing, and thus estimate the consumption of *A. viridis* leaves by Highland cattle. Whenever the branch was broken and untraceable after grazing, the number of remaining leaves was considered equal to zero. The damages made on *A. viridis* branches through grazing, scratching or trampling were also visually assessed with the following scale: 0 (no damages), 1 (moderate damage), 2 (intense damage) and 3 (branch totally damaged or broken). The maximum height at which damages were observed on each branch was also recorded.

For the analyses of soil and functional group cover as well as leaf consumption, a response ratio (RR) was computed for all the assessed variables according to the following formula:

$$RR = \frac{Value_{Post} - Value_{Pre}}{Value_{Pre}}$$

where “ValuePost” is the value measured after grazing and “ValuePre” the value measured before grazing. This formula provides an indication of the effect size which is not biased by the initial (before grazing) differences among replicates. A negative RR indicates that the value decreased after grazing, while a positive RR indicates that the value increased after grazing.

Statistical analysis

All statistical analyses were performed using R version 3.4.4 (R Development Core Team 2017). The effects of attractive points (control versus attractive points), paddocks (1, 2, 3), year (2019, 2020) and their interactions on the spatial distribution of cows (i.e., total raw GPS location counts of each collared cow within the buffer) were tested separately for the 10- and 50-m buffers by using a generalized linear model (GLM) with a negative binomial distribution to take into account the significant over-dispersion of the data. As this analysis treats each cow as independent, we repeated it randomly selecting 50% of the cows, to account for herd possible interaction and pseudoreplication issues. As the results did not change the significance of the effects (see Appendix, Table S2), we are confident that our results are not affected by issues of pseudoreplication. The effects of attractive points, paddocks and their interactions on 2020 soil cover (RRs of herbaceous and bare soil cover) and on functional group cover (RRs of ferns and megaphorbs, forbs, and woody species cover) were tested using generalized least square model (GLS), with treatment (control versus attractive points) and paddock (1, 2, 3) as fixed factors and coordinates of the transects nested in paddock as random effects to take into account spatial correlation amongst transects. The effect of attractive points, paddock, distance of trees along the transect and their interactions on the RR of *A. viridis* leaves were tested using the same type of GLS model with coordinates of the transects nested into paddock. Finally, we carried out a conservative analysis in which we analyzed only the average RR in each area of the paddocks (i.e. 3 control points vs. 3 attractive points, one control and one attractive point from each paddock: 6 data points in total), using ANOVA, to check that our results were robust to any spatial pseudoreplication. Post-hoc tests were performed for the models when significant differences were detected (Tukey's test, $P < 0.05$), and additional student's test (t-test) were performed to assess the difference of RRs from zero.

Results

Effects of attractive points placement on livestock spatial distribution

Based on the remaining molasses after grazing, we calculated that each animal consumed 134 g of molasses per day on average. All molasses-based blocks (except two placed in Paddock 3) were completely consumed at the end of each grazing period. There were no significant differences in the number of GPS locations between control and attractive point areas in 2019, in both 10-m ($P = 0.47$) and 50-m buffers ($P = 0.21$, Fig. 1), indicating that the locations were equally attractive to livestock, before the molasses blocks were placed.

When we analyzed 10-m buffers around the attractive points, there was a significant increase of livestock use in attractive points areas in 2020 compared to 2019 in all paddocks, (AP x Y, $P < 0.001$, +959% on average; Fig. 1A). In 2020, the number of GPS fixes was significantly higher around attractive points than in control areas (+443% on average, $P < 0.05$). There was also a significant increase in livestock use in 2020 compared to 2019 in all paddocks, when we analyzed 50-m buffers (Y, $P < 0.05$, +226% on average). The effect of the attractive points was different among paddocks (AP x P, $P < 0.01$) in both 10- and 50-m buffers: the livestock use in 2020 was significantly higher around attractive points compared to control areas in Paddock 1 and 2 ($P < 0.05$), while there was no difference between attractive points and control areas in Paddock 3 ($P = 0.99$, Fig. 1B). The results from the analysis performed on 50% of the herd did not lead to different results (see Appendix, Table S2).

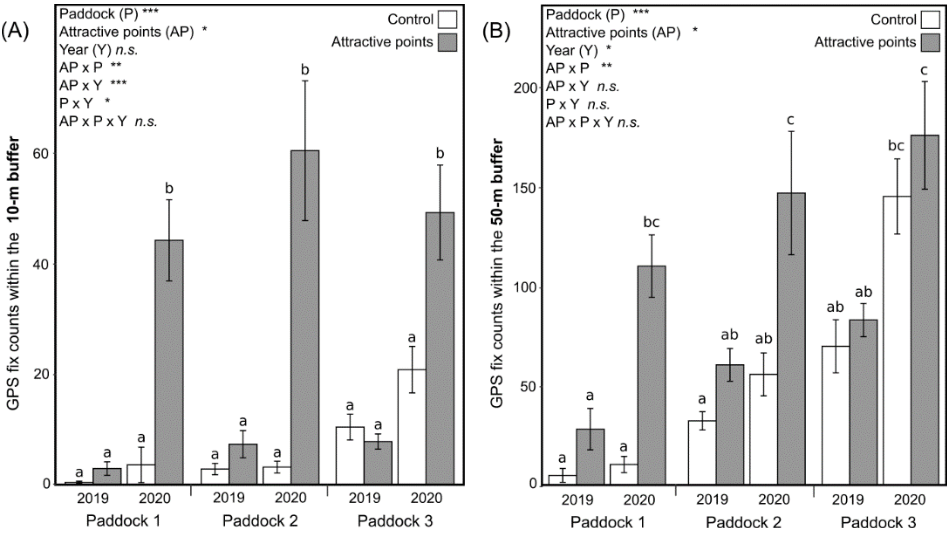

Figure 1. Average count (± SE) of GPS locations per cow (GPS fixes taken every 10 minutes during the grazing period), in 10-meters buffers (A) and 50-meters buffers (B) in 2019 and 2020 in all paddocks for both attractive points (grey) and control (white) areas. Different letters indicate significant differences (Tukey's test, P < 0.05) between areas and years for each paddock.

Effects produced by livestock on soil cover

Soil cover was highly impacted by livestock grazing and trampling. There was a significant decrease in herbaceous cover and a significant increase in bare soil cover in all paddocks after grazing, in both control and attractive points areas, with response ratios always significantly different from zero ($P < 0.001$, Fig. 2). The impacts of livestock around the attractive points always had a significant effect on both herbaceous and bare soil cover in all the paddocks ($P < 0.001$, Fig. 2), with a higher impact on the soil cover around attractive points compared to control areas. Indeed, the herbaceous cover had a RR of -0.77 on average around attractive points and a RR of -0.39 on average in control areas, while the bare soil cover had a RR of 5.4 on average around attractive points and a RR of 1.68 on average in control areas. Concerning the herbaceous cover, the significant interaction between attractive points and paddocks ($P < 0.05$) highlighted differences in the amplitude of this effect among paddocks, with a higher difference between attractive points and control areas within Paddock 2 (Fig. 2A). The Anova analysis performed on the average RR of soil cover provided the same results, except for bare soil cover for which the effect of AP was marginally significant (see Appendix, Table S3).

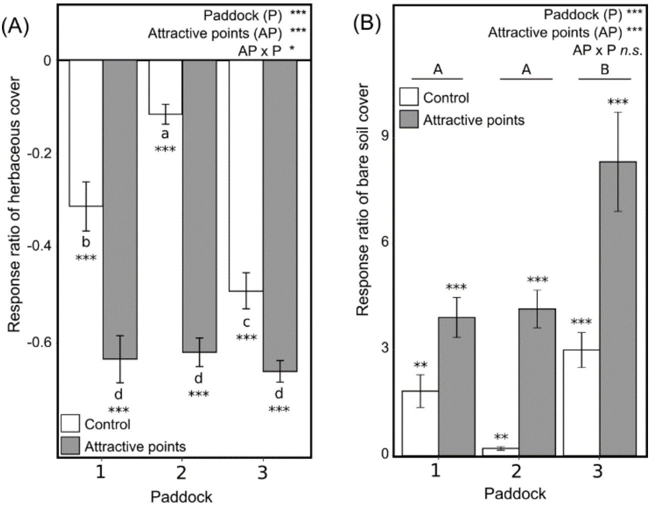

Figure 2. Average response ratio (± SE) of herbaceous (A) and bare soil (B) cover on all paddocks in both attractive points (grey) and control (white) areas. *P < 0.05, **P < 0.01, ***P < 0.001, and n.s. (non-significant) indicate any significant differences from zero (t-tests). Different letters indicate significant differences (Tukey's test, P < 0.05) between areas for each paddock, with lower case letters representing differences between buffers, and upper case letters representing differences between paddocks.

Effects of livestock on Alnus viridis trees

Alnus viridis trees were highly damaged by Highland cattle grazing. In Paddocks 1 and 3, there was a significant decrease in the number of *A. viridis* leaves after grazing in both control and attractive points areas, with negative response ratios significantly different from zero (P < 0.001, Fig. 3). In Paddock 2, the decrease was only observed around attractive points, as there was no significant change in the number of leaves after grazing in control areas (P = 0.43). The effect of attractive points on leaves differed among paddocks (AP x P, P < 0.001), as leaves around attractive points in Paddock 1 and 2 were significantly more impacted by grazing than in control areas, while both areas were equally impacted in Paddock 3 (Fig. 3). In all paddocks, the distance of the trees from the attractive points did not impact the grazing effect on *A. viridis* leaves, up to 10 meters (P = 0.81). However, the interaction between attractive points and distance was significant (P < 0.01). In addition to leaf consumption, frequent damages on *A. viridis* branches were assessed at attractive point locations at an average height of 1.48 ± 0.53 m (mean ± SE), with 68% of branches having damage scores between 1 and 2 (moderate to intense damages) and 10% with damage scores of 3 (very severe damage or broken branches). Reducing our dataset to 6 data points and analyzing the average RR of number of *A. viridis* leaves showed that the effect of AP was also significant (see Appendix, Table S3).

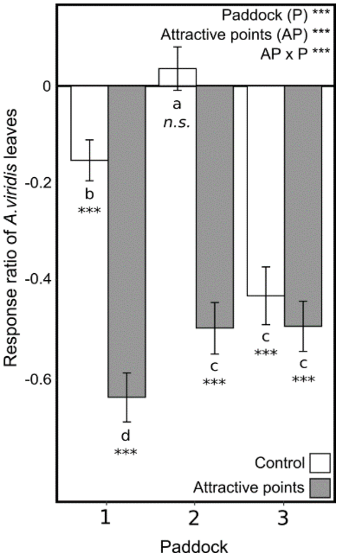

Figure 3. Average response ratio (± SE) of *A. viridis* remaining living leaves in all paddocks in both attractive points (grey) and control (white) areas. *P < 0.05, **P < 0.01, ***P < 0.001, and n.s. (non-significant) indicate any significant differences from zero (t-tests). Different letters indicate significant differences (Tukey's test, P < 0.05) among the areas of each paddock.

Effects of livestock on the understory plant functional group cover

A total of 149 plant species were recorded during the botanical surveys in all paddocks (the species list and their corresponding functional group are available in Appendix, Table S4). Ferns and megaphorbs included 37 species and had the highest percentage cover (44.4% per paddock, on average), with *Adenostyles alliariae* (14.1% per paddock on average), *Athyrium filix-femina* (L.) Roth (10.4% per paddock on average) and *Dryopteris dilatata* (Hoffm.) A. Gray (4.3% per paddock on average) being the most dominant species. The highest number of species (78) belonged to forbs (23.5% of the total percentage cover), with *Ranunculus aconitifolius* L. (8.7% per paddock in average), *Stellaria nemorum* L. (2.7% per paddock in average) and *Viola biflora* L. (2.2% per paddock in average) being the most dominant ones. Woody species (26.1% of percentage cover) comprised 14 species, including *Alnus viridis* (18.5% per paddock in average), *Vaccinium myrtillus* L. (2.0% per paddock in average) and *Rhododendron ferrugineum* L. (1.5% per paddock in average). Graminoids were not abundant in the botanical surveys (20 species and 5.9% of the average cover).

The increased grazing and trampling around attractive points had a significant impact on understory plant functional groups. There was a significant decrease in ferns and megaphorb species cover in all paddocks after grazing, in both control and attractive points areas, with negative response ratios always significantly different from zero ($P < 0.001$, Fig. 4A). The effect of attractive points on ferns and megaphorbs differed among paddocks (AP x P, $P < 0.001$), as attractive points areas of Paddock 2 and 3 were significantly more impacted by grazing than control areas, while both areas were equally impacted within Paddock 1 (Fig. 4A).

Similarly, there was a significant decrease in forb species cover after grazing in both control and attractive points areas in Paddock 2 and 3, with negative response ratios significantly different from zero (P < 0.001, Fig. 4B). In Paddock 1, forbs were less impacted compared to other paddocks, and they only significantly decreased in attractive points areas (Fig. 4B). The presence of attractive points always had a significant effect on forbs in all paddocks, leading to a higher decrease compared to control areas (P < 0.001, Fig. 4B).

In all paddocks, woody species decreased significantly more around attractive points than in control areas (P < 0.001; Fig. 4C), in which they were not significantly damaged, with response ratios not different from zero (-0.11 ± 0.07, P = 0.10).

Reducing our dataset to only 6 data points and analyzing the average RR of functional groups showed the same results, except for ferns and megaphorbs for which the effect of AP was not significant (see Appendix, Table S3). This shows that our results were weakly affected by any spatial pseudoreplication.

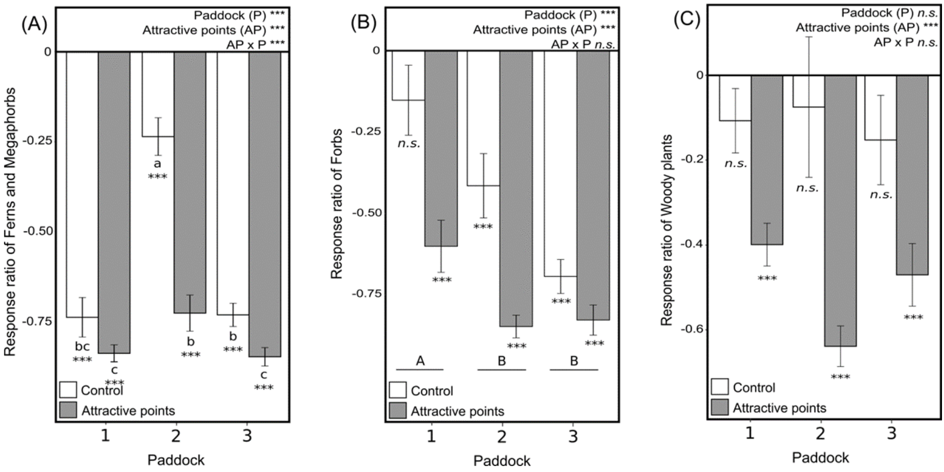

Figure 4. Average response ratio (± SE) of ferns and megaphorbs (A), forbs (B) and woody species (C) cover on all paddocks for both attractive points (grey) and control (white) areas. *P < 0.05, **P < 0.01, ***P < 0.001, and n.s. (non-significant) indicate any significant differences from zero (t-tests). Different letters indicate significant differences (Tukey's test, P < 0.05) among areas for each paddock, with lower case letters representing differences between buffers, and upper case letters representing differences between paddocks.

Discussion

Overall, the strategic placement of molasses-based blocks significantly modified Highland cattle spatial distribution, with an increased livestock exploitation of areas around attractive point locations, compared to the previous year and to control areas. This result confirmed our first hypothesis that Highland cattle were attracted to molasses-based blocks and that they were able to exploit steep and highly encroached *A. viridis* areas, as recently pointed out by Svensk et al. (2021). The results on the effectiveness of molasses-based blocks are thus consistent with those found by Bailey & Welling (1999), who showed that they could attract herds into underused rangelands with poor forage quality vegetation. Those authors assessed the efficiency of dehydrated blocks in un-encroached zones with gentle to moderate slopes, while our study showed that such strategic placements can attract herds even in areas with steeper slopes and high levels of woody species encroachment, where grazing conditions could be more challenging. When analyzing the attractive effect at different spatial scales, a significant increase in the use of areas up to 50-m from attractive points locations compared to control areas was detected in most of the paddocks. This large-scale effect of attractive points underlines the potential of such management techniques to increase livestock use over large *A. viridis* encroached patches. In the study of Bailey et al. (2001) in extensive pastures without shrub-encroached vegetation, the authors demonstrated that molasses based-blocks could have a significant influence on cattle grazing up to 600-meters, even on the steepest terrain. The effect of attractive points within 10-m buffers was even more significant, with a highly increased livestock use at a close range near the molasses-based blocks. Such measurements at different spatial scales highlight the influence of attractive points on Highland cattle spatial distribution, with this type of management enabling cattle to increase their visits to the surrounding encroached zones once they are attracted by the molasses. Other studies have also shown high potential for different strategic placements to reduce shrub-encroachment at different scales, and to attract herds into under-grazed areas (Bailey & Jensen 2008; Bailey & Welling 2007; Pittarello et al. 2016). In particular, Pittarello et al. (2016) found a significant attractive effect at 10 and 50 meters around attractive points in dwarf-shrub encroached subalpine pastures, using a different supplement type (mineral mix supplements) for another cattle breed (Piedmontese breed). In our study, under comparable mountain topographic conditions, livestock use and supplement consumption were much higher, with 134 g of molasses consumed per animal per day, compared to 13.7 g of mineral mix supplement in Pittarello et al. (2016), suggesting a higher attractiveness and efficiency of molasses-based than mineral mix blocks in modifying livestock spatial distribution. Indeed, when considering all paddocks, the average time spent around attractive points up to 50 meters was 74 min^{1}·cow^{-1}·day^{-1}, corresponding to about 5.2% of the grazing period on average, compared to 39 min^{1}·cow^{-1}·day^{-}1 around control points (i.e., 2.7% of the grazing period on average). In comparison, Pittarello et al. (2016) found an average time spent of 18 min^{1}·cow^{-1}·day^{-}1 around mineral mix supplements in a 50-m buffer. Furthermore, the familiarity of the cattle with the paddock characteristics might be an important factor to consider when implementing such management in the long term, as the exploration behavior could increase over time and have an enhanced effect on vegetation. Further studies are needed to investigate cattle behavior and different management types, in which the choice of the cows composing the herd could also be a key element (Sueur et al. 2018).

Overall, the increased use by Highland cattle of areas with attractive points led to significant consumption of leaves and damages to the branches of *A. viridis* trees. Moreover, it resulted in a decrease in herbaceous cover and an increase in bare soil cover after grazing, compared to control areas. Moreover, the cover of the three investigated functional groups was significantly reduced by the increased livestock use of attractive point areas. These impacts were related both to grazing and to the mechanical damages caused by Highland cattle once attracted to the attractive point areas. Such damages may result from trampling, scratching and breaking branches while walking, as recently observed by Svensk et al. (2021). The significant effect of the interaction between attractive points and the distance of trees suggests a different effect of the distance between control and attractive point areas. Indeed, large paths created by the animals were observed between attractive point blocks and the surrounding area, due to grazing activity and movement from attractive points to water sources. While we could not differentiate between effects of grazing and trampling on the vegetation, Highland cattle were observed to graze on species with particularly low forage quality, such as ferns (*A. filix-femina*, *D. dilatata*) and megaphorbs (*A. alliariae*). This demonstrates the ability of this breed to forage not only on *A. viridis* trees but also on other unpalatable understory species, which is consistent with our second and third hypotheses. Indeed, previous studies have shown that Highland cattle tend to be less selective with regard to forage quality compared to other breeds, and are able to forage on woody plants (Pauler et al. 2020a, b). Moreover, they tend to be more adapted to low quality vegetation and lower nutrient intake, as they continue to gain weight under such constraining conditions (Berry et al. 2002). *A. viridis* leaves and the understory vegetation have also proven to be a valuable forage resource for certain robust cattle breeds such as Dexter cattle (Zehnder et al. 2016). Even if only measured in the short-term, the intense impacts exerted on *A. viridis* trees and woody species-group indicate the potential for Highland cattle to reduce woody species encroachment in the medium- and long-term time.

Maintaining this management regime over several years could enable typical pasture species to colonize areas where plant functional group cover was highly impacted and new bare soil gaps created. Although we saw a decrease in the cover of all plant functional groups in the short-term after cattle grazed the plots in our study, it is expected that continuation of grazing by cattle could promote an increase in plant diversity in the long term. Indeed, several studies have shown that long-term targeted grazing by goats led to increased light conditions in formerly highly shrub-encroached pastures, with the establishment of light-demanding, low-growing and less competitive grassland species (Elias et al. 2018; Köhler et al. 2020; Silva et al. 2019). Similarly, we can expect cows to alleviate light competition for the understory vegetation cover and this is likely to result in a shift in plant functional groups in the long term, with an increase in plant diversity (Borer et al. 2014). Moreover, to avoid possible risks of localized erosion linked to excessive livestock trampling, it would be advisable to regularly move attractive points to different shrub-encroached areas along the grazing season. This would also allow to expand the restoration effect on a wider area (Probo et al. 2013). The placement of permanent botanical transects to repeat surveys every year would be necessary to assess these possible changes in vegetation cover and botanical composition. Moreover, as Highland cattle periodically travelled from open pastures to highly covered zones (Svensk et al. 2021), the restoration of former pastures could be enhanced by seed translocation through endo- and epi-zoochory. Indeed, a recent study (Pauler et al. 2019) demonstrated that a higher number of epizoochoric plant species were found in pastures grazed by Highland cattle compared to pastures grazed by production-oriented cattle breeds, since the long fur of the Highland cattle resulted in more efficient seed dispersal. The same study also found that the less selective Highland cattle grazing behavior led to higher plant species richness in pastures, as Highland cattle consumed a larger range of species compared to other breeds. They were thus able to graze on less palatable dominant species and reduce their abundance for the benefit of other herbaceous species. Moreover, an important number of dung-pats was already observed around attractive points after grazing, which could further promote seed dispersal from adjacent pastures through endozoochory (Cosyns et al. 2005). Indeed, while other management strategies such as prescribed burning or clear-cutting have proven effective at reducing shrub encroachment in subalpine environments, targeted grazing seems to be best suited to restore subalpine pastures, especially considering the enhanced plant dispersion by livestock (Alados et al. 2019). In addition, the mechanical damage applied to *A. viridis* branches by livestock might increase pathogens attacks on this shrub species and therefore contribute to reduce its spread. For example, fungi species of the genus *Phytophthora* can spread through water and have the potential to infect bark lesions of a plant (Perry 2006), and have been reported to have severe impacts on alder trees including *A. viridis*, sometimes leading to mortality (Bregant et al. 2020; Pisetta et al. 2012). Nevertheless, further studies are needed to assess the potential risk of pathogens to infect *A. viridis* after Highland cattle grazing impacts. In conclusion, our findings demonstrate that the strategic placement of molasses-based blocks was efficient in attracting Highland cattle toward areas highly invaded by *A. viridis*, despite the harsh terrain conditions. Highland cattle successfully foraged on, and damaged, *A. viridis* trees around the attractive points, thus significantly altering the understory vegetation through foraging and trampling, in just one year. Highland cattle could thus have the potential to effectively reduce *A. viridis*-encroachment in the long term, and, by increasing light conditions for understory species and translocating seeds, they could promote subalpine pasture restoration.

Appendix: Table S1, Composition of molasses-based blocks; Table S2, Additional analysis made on cows spatial distribution (GLM with 50% of cows); Table S3, Additional analysis made on soil cover, *A. viridis* trees, and plant functional groups cover (ANOVAs); Table S4, List of plant species and corresponding functional group; Figure S1, Scheme of the cross-shaped vegetation transects.

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Appendix

Table S1. Composition of molasses-based blocks

Components (%)		**Additives per kg (mg)**	
Raw protein	3.0%	Inorganic zinc	900
Raw fat	2.0%	Inorganic manganese	900
Raw ash	24.0%	Inorganic iodine	60
Sugar	40.0%	Inorganic cobalt	12
Calcium	4.0%	Inorganic selenium	9
Phosphorus	2.0%		
Magnesium	0.2%		
Sodium	2.5%		

Table S2. Results of the additional analyses (Generalized Linear Model) made on spatial distribution with 50% of the cows randomly selected in each paddock

		Spatial distribution	
		10 m	**50m**
	Df	*p*	*p*
Attractive points (AP)	1	2.65E-07	0.001786
Paddock (P)	2	0.04978	2.34E-05
Year (Y)	1	9.53E-13	0.002477
AP x P	2	0.00037	0.014911
AP x Y	1	0.07794	0.260557
P x Y	2	0.07751	0.379016
AP x P x Y	2	0.95897	0.506013

Table S3. Results of the additional analyses (Attractive Points vs. Control areas, ANOVAs) made on soil cover, *A. viridis* trees, and plant functional groups cover.

	Df	*p*
Vegetation cover		
Herbaceous cover	1	0.0313
Bare soil cover	1	0.0566
A. viridis leaves	1	0.0479
Plant functional groups		
Megaphorbs	1	0.1260
Forbs	1	0.0240
Woody plants	1	0.0063

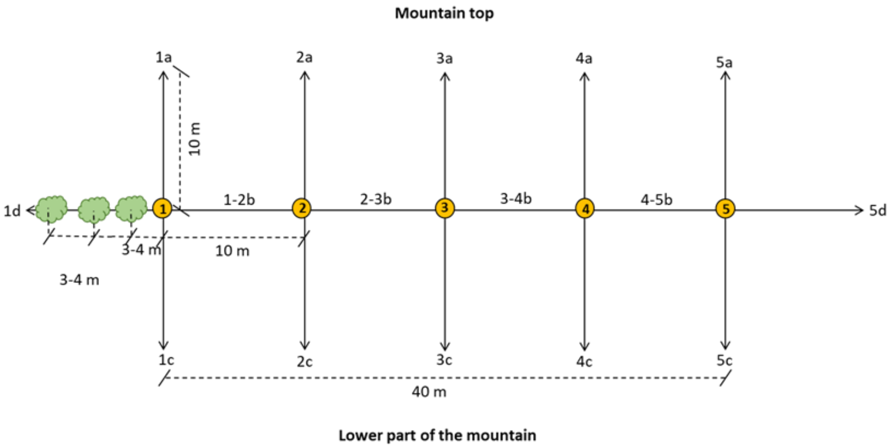
Table S4 - List of all species found in the botanical surveys made in both 2019 and 2020 in all paddocks combined, sorted by their corresponding functional groups. Plant species nomenclature follows Aeschimann et al. (2004).

Plant species	**Group**	**Plant species**	**Group**
Achillea macrophylla	Ferns and Megaphorbs	*Veronica urticifolia*	Ferns and Megaphorbs
Aconitum lamarckii	Ferns and Megaphorbs	*Achillea millefolium*	Forbs
Aconitum napellus	Ferns and Megaphorbs	*Ajuga reptans*	Forbs
Adenostyles alliariae	Ferns and Megaphorbs	*Alchemilla alpina* aggr.	Forbs
Alchemilla xanthochlora	Ferns and Megaphorbs	*Alchemilla pentaphyllea*	Forbs
Asplenium viride	Ferns and Megaphorbs	*Anthyllis vulneraria*	Forbs
Astrantia major	Ferns and Megaphorbs	*Aposeris foetida*	Forbs
Athyrium filix-femina	Ferns and Megaphorbs	*Aster bellidiastrum*	Forbs
Campanula latifolia	Ferns and Megaphorbs	*Astrantia minor*	Forbs
Carduus defloratus	Ferns and Megaphorbs	*Caltha palustris*	Forbs
Chaerophyllum hirsutum	Ferns and Megaphorbs	*Campanula scheuchzeri*	Forbs
Cicerbita alpina	Ferns and Megaphorbs	*Campanula trachelium*	Forbs
Cirsium oleraceum	Ferns and Megaphorbs	*Cardamine amara*	Forbs
Cryptogramma crispa	Ferns and Megaphorbs	*Cardamine resedifolia*	Forbs
Dryopteris dilatata aggr.	Ferns and Megaphorbs	*Cerastium vulgare*	Forbs
Dryopteris filix-mas	Ferns and Megaphorbs	*Crocus albiflorus*	Forbs
Epilobium angustifolium	Ferns and Megaphorbs	*Cuscuta europaea*	Forbs
Equisetum sylvaticum	Ferns and Megaphorbs	*Dactylorhiza fuchsii*	Forbs
Gentiana lutea	Ferns and Megaphorbs	*Dactylorhiza maculata*	Forbs
Gymnocarpium dryopteris	Ferns and Megaphorbs	*Daucus carota*	Forbs
Heracleum sphondylium	Ferns and Megaphorbs	*Epilobium alpestre*	Forbs
Hieracium prenanthoides	Ferns and Megaphorbs	*Epilobium obscurum*	Forbs
Huperzia selago	Ferns and Megaphorbs	*Euphorbia cyparissias*	Forbs
Lamium galeobdolon aggr.	Ferns and Megaphorbs	*Galeopsis tetrahit*	Forbs
Oreopteris limbosperma	Ferns and Megaphorbs	*Galium pusillum* aggr.	Forbs
Petasites albus	Ferns and Megaphorbs	*Gentiana purpurea*	Forbs
Peucedanum austriacum	Ferns and Megaphorbs	*Geranium sylvaticum*	Forbs
Peucedanum ostruthium	Ferns and Megaphorbs	*Geum montanum*	Forbs
Phegopteris connectilis	Ferns and Megaphorbs	*Geum rivale*	Forbs
Polystichum lonchitis	Ferns and Megaphorbs	*Hieracium intybaceum*	Forbs
Prenanthes purpurea	Ferns and Megaphorbs	*Hieracium murorum* aggr.	Forbs
Rumex alpestris	Ferns and Megaphorbs	*Homogyne alpina*	Forbs
Rumex alpinus	Ferns and Megaphorbs	*Hypericum maculatum*	Forbs
Seseli libanotis	Ferns and Megaphorbs	*Hypericum richeri*	Forbs
Urtica dioica	Ferns and Megaphorbs	*Knautia dipsacifolia*	Forbs
Veratrum album	Ferns and Megaphorbs	*Leontodon helveticus*	Forbs

Table S4 – continued.

Plant species	**Group**	**Plant species**	**Group**
Leucanthemum vulgare agg	Forbs	*Valeriana tripteris*	Forbs
Lotus alpinus	Forbs	*Veronica chamaedrys*	Forbs
Orchis sp.	Forbs	*Veronica serpyllifolia*	Forbs
Oxalis acetosella	Forbs	*Viola biflora*	Forbs
Paris quadrifolia	Forbs	*Acer pseudoplatanus*	Woody
Pedicularis tuberosa	Forbs	*Alnus viridis*	Woody
Phyteuma betonicifolium	Forbs	*Larix decidua*	Woody
Phyteuma hemisphaericum	Forbs	*Lonicera caerulea*	Woody
Phyteuma spicatum	Forbs	*Lonicera nigra*	Woody
Pimpinella saxifraga	Forbs	*Picea abies*	Woody
Plantago atrata	Forbs	*Rhododendron ferrugineum*	Woody
Polygonum viviparum	Forbs	*Rosa pendulina*	Woody
Potentilla crantzii	Forbs	*Rubus idaeus*	Woody
Potentilla aurea	Forbs	*Rubus saxatilis*	Woody
Potentilla erecta	Forbs	*Salix hastata*	Woody
Primula veris	Forbs	*Sorbus aucuparia*	Woody
Pulsatilla montana	Forbs	*Sorbus chamaemespilus*	Woody
Ranunculus aconitifolius	Forbs	*Vaccinium myrtillus*	Woody
Ranunculus montanus aggr.	Forbs	*Agrostis capillaris*	Graminoids
Rhodiola rosea	Forbs	*Agrostis schraderiana*	Graminoids
Rumex scutatus	Forbs	*Anthoxanthum alpinum*	Graminoids
Saxifraga rotundifolia	Forbs	*Avenella flexuosa*	Graminoids
Saxifraga stellaris	Forbs	*Calamagrostis villosa*	Graminoids
Sedum acre	Forbs	*Carex flacca*	Graminoids
Sempervivum montanum	Forbs	*Carex leporina*	Graminoids
Silene dioica	Forbs	*Carex sp.*	Graminoids
Silene nutans	Forbs	*Dactylis glomerata*	Graminoids
Silene rupestris	Forbs	*Deschampsia cespitosa*	Graminoids
Silene vulgaris	Forbs	*Festuca rubra aggr.*	Graminoids
Soldanella alpina	Forbs	*Festuca scabriculmis*	Graminoids
Solidago virgaurea	Forbs	*Luzula alpinopilosa*	Graminoids
Streptopus amplexifolius	Forbs	*Luzula campestris aggr.*	Graminoids
Stellaria nemorum	Forbs	*Luzula sylvatica*	Graminoids
Taraxacum officinale aggr.	Forbs	*Phleum rhaeticum*	Graminoids
Thalictrum aquilegiifolium	Forbs	*Poa chaixii*	Graminoids
Trifolium badium	Forbs	*Poa nemoralis*	Graminoids
Trifolium pratense	Forbs	*Poa pratensis*	Graminoids
Trifolium repens	Forbs	*Trisetum flavescens*	Graminoids
Trollius europaeus	Forbs		

Figure S1 – Scheme of the 16 cross-shaped vegetation transects performed in attractive points and control areas. Yellow numbers represent the attractive points (molasses-based blocks) or the control points, depending on the area. The green figures represent the *Alnus viridis* trees selected along each transect. Alphanumeric codes are transects ID numbers.



4. Conclusions

4.1. Environmental, vegetation and management factors drive livestock spatial distribution

This thesis addressed the relative importance of several drivers of animal spatial distribution in two different environments and with two different livestock species. In the first case-study (Chapter 2.1), sheep site-use intensity was mainly affected by the distance from night penning areas, whereas other management (distance from water troughs) and topographic variables (slope) had a minor influence. The solely distance from penning areas, weighed on the number of nights, explained 65% of the model's deviance. Similarly, also Putfarken et al. (2008) highlighted that the distance from sheep sheds had a great influence on animal distribution. In the second case-study (Chapter 3.1), the vegetation forage quality significantly increased site frequentation by Highland cattle, even though not in all the periods and paddocks. Contrarily, other factors such as topographic variables and shrub cover had generally no or low influence. The addition of molasses-based supplements in target shrub-encroached patches induced significant changes in Highland cattle spatial distribution, since the buffer areas around attractive points were more used compared to the control ones (fourfold more, on average). These results increased our understanding of livestock spatial distribution in extensive grazing systems, which results in practical implications for grassland conservation and restoration.

In this thesis, the distance from night penning areas proved to be an effective proxy to estimate site-use intensity by sheep, highlighting the remarkable influence of congregation areas (such as night pens, pastoral buildings, and water and mineral supplements) on livestock spatial distribution. Recently, Pittarello et al. (2021) proposed the use of the distance from pastoral buildings (together with slope) as an effective proxy of site-use intensity by cattle in alpine summer pastures. Such proxies, which are rather easy to quantify, can provide useful information for the optimal management of grasslands. For instance, in the sheep grazing system, the careful arrangement of penning areas could be used to strategically modulate grazing pressure over the pasture. Also the use of molasses-based blocks proved to be an efficient technique to increase the frequentation of the less exploited pasture areas by Highland cattle. Management practices could thus be efficiently used to modulate livestock spatial distribution for specific conservation and restoration purposes, such as to increase the exploitation of target vegetation (e.g., oligotrophic vegetation on steep slopes, shrub-encroached patches etc.) or to reduce the pressure on vulnerable sites (e.g., specific rare species populations, alpine bogs etc.) (Bailey 2005). Moreover, the results of this thesis suggest that molasses-based supplements, although not conventionally used by alpine farmers, can be more efficient than typical salt-based ones to attract cows in target areas (cf. Pittarello et al. 2016a). Molasses (a sugar byproduct) is an energetic feed supplement, rich in minerals and vitamins. Due to its sweet taste, it has an appetizing role for animals. By acting at ruminal and intestinal level, the addition of molasses in the diet can provide several advantages: (i) increase dry matter intake and ingestion of low-quality forage, (ii) increase digestibility of dry matter, fiber, nitrogen, and fats, (iii) improve protein and vitamin synthesis, and (iv) improve animal health status (reduction of metabolic disorders) (Mordenti et al. 2021). By enhancing the value of an industrial byproduct, the use of molasses could also meet the principles of circular economy (Mordenti et al. 2021).

The effectiveness of restoration actions in specific sites and conditions is also dependent on livestock species and breed (Lamoot et al. 2005; Pauler et al. 2019). Indeed, certain species and breeds are more able to graze in shrub-encroached vegetation, on steep slopes and face other environmental constraints. In Chapter 3.1, the results showed a great adaptability of Highland cattle breed to various environmental and vegetation conditions, since cows were mostly not influenced by slope, *Alnus viridis* cover, and distance from water, whereas forage pastoral value was often an important driver of their movements. Surprisingly, in a specific paddock and grazing period, site frequentation even increased with increasing slope or increasing *A. viridis* cover. This behavior highlighted a higher robustness of Highland cattle compared to other cattle breeds. For instance, Probo et al. (2014) observed that the spatial distribution of Piedmontese cows exploiting an alpine pasture was negatively influenced by slope, while Pauler et al. (2020a) observed that Highland cattle visited the most distant and steepest areas compared to Braunvieh and Angus *x* Holstein breeds.

Grazing animals are important for enhancing habitat connectivity in grasslands. By moving over the pasture, they can carry plant seeds in the feces, fur and hoofs, and move genetic pools from one place to another and *vice versa*. For instance, Rico et al. (2014) and Wagner et al. (2013) demonstrated that flock movements were responsible for the dispersal of many plant species in fragmented calcareous grasslands, resulting in increased species richness in the re-connected patches. Other than seeds, grazers are also carriers of fertility. Indeed, they typically concentrate dung in the most frequented areas, such as night pens, water sources and other attractive points. Therefore, the modulation of livestock spatial distribution through management practices will also affect seeds and fertility flows, with positive consequences for genetic and species diversity and restoration aims. In shrub-encroached patches, the increased frequentation by livestock can favor the establishment of the typical pasture vegetation due to the improved fertility and the transfer of pasture species from surrounding areas, as observed by Pittarello et al. (2016b) and Probo et al. (2016).

4.2. Impacts of livestock grazing on dry and *A. viridis*-encroached grasslands

In this thesis, the effects of sheep grazing were investigated in understudied alpine dry grasslands of extraordinary natural value (up to 71 vascular plant species per 25 m^2), increasing the knowledge about the grazing-related vegetation dynamics in these environments (Chapter 2.2). Despite these grasslands were abandoned for about 50 years, the results showed only a few changes in vegetation after sheep grazing was reintroduced. The scarce level of response of these plant communities to grazing disturbance could be related to both the short term monitoring and the environmental constraints (water deficiency, shallow soils, summer droughts etc.). These environmental conditions, and especially water deficiency, are indeed important determinants of plant assemblages in dry ecosystems, and can slow down or prevent a prompt and distinct vegetation response to grazing management (Török et al. 2014). Also the addition of nutrients could produce only few changes when water is the limiting factor (Ren et al. 2015). However, as expected, grazing intensity proved to be an important element affecting vegetation composition. In night penning areas, where stocking density was high, vegetation composition modified towards an increase of pioneer xerothermic, and, slightly, ruderal species. The abundance of some rare species also increased in the penning areas. These outcomes were likely related to the trampling effect exerted by sheep, which opened the vegetation structure, reduced the litter layer and created bare ground germination microsites (Godó et al. 2017; Schwabe et al. 2013). For instance, Elias et al. (2018) demonstrated that goat grazing, at high grazing pressure, had a positive effect on the performances of the rare species *Gagea bohemica*, due to the increased light availability. Vegetation changes related to increased soil fertility were not observed in the night pens, maybe because of water limitation, or because of the short term monitoring period.

In the second case-study, the increased grazing intensity induced by the use of molasses-based attractive points produced remarkable effects in *A. viridis*-encroached areas (Chapter 3.2). Indeed, around attractive points, bare soil markedly increased and *A. viridis* plants were strongly damaged (many leaves eaten and trunks and branches destroyed). These impacts are expected to induce significant changes in vegetation, particularly towards an increase of typical pasture species, forage quality and plant diversity. As demonstrated in Chapter 3.1, *Alnus*-dominated areas are characterized by lower diversity and forage value compared to surrounding pasture areas. The exploitation of *Alnus*-dominated patches by Highland cows is expected to increase fertility, reduce light competition, and transfer seeds from more valuable grassland areas, as already observed by Pittarello et al. (2016b) in dwarf shrub vegetation. In Chapter 3.2, post-grazing changes were analyzed as an early indicator of future vegetation dynamics, and showed an increase in bare soil and a decrease of herbaceous and woody plant cover. Interestingly, preliminary results from the next vegetative season (i.e., one year after the experiment was set) already showed a significant colonization of attractive point areas by pasture species (Annex 1). In Vogna study site, the abundance of some high-quality grasses (*Poa pratensis*), legumes (*Lotus alpinus* and *Trifolium pratense*), and nitrophilous species (*Rumex alpestris*) increased around attractive points and that of *A. viridis* reduced compared to the pre-experiment conditions. On the contrary, no changes in botanical composition were observed in the control areas. It is likely that pasture species were transferred to attractive point areas through the dung, but also through the long fur of Highland cows. Pauler et al. (2019) demonstrated the higher ability of Highland cows to transfer epizoochoric plant species compared to other cattle breeds. Moreover, also the reduced competition for light induced by the changes in vegetation structure (increase in bare soil, reduction of woody species cover) and the addition of nutrients can favor the colonization of these areas by the typical pasture species available in the seed bank or transferred by the cows.

The suitability of Highland cattle for the restoration of shrub-encroached and degraded alpine pastures goes far beyond their physical characteristics (i.e., long horns and fur, lightness, small size, agility in rough terrain). Pauler et al. (2020b) demonstrated their lower selection for plant traits (such as leaf dry matter content, nitrogen leaf content, spines) compared to other cattle breeds, resulting in higher ability to forage on low-quality vegetation and woody species. For instance, the results of Chapter 3.2 showed that Highland cows foraged on *A. viridis* foliage. In Annex 2, the preliminary results of on-going research from four woody-encroached sites (namely, ‘Almese’, ‘Casteldelfino 1’, ‘Casteldelfino 2’, and ‘Bovonne’) show that woody species accounted for 15-46% of the Highland cow diet. In Bovonne, *A. viridis* made up 12% of their diet, while *Rubus idaeus* achieved 40% in Casteldelfino 2. Despite the low selectivity towards herbaceous species (Pauler et al. 2020b), they preferentially ate certain woody species (e.g., *Frangula alnus*, *Rhamnus alpinus*, and *Fraxinus excelsior*), and avoided others (e.g., *Corylus avellana* and *Crataegus monogyna*) (Annex 2). The ability of Highland cows to forage on shrubs and trees highlights their plasticity to exploit otherwise poorly used resources. Typically, shrub and tree foliage account for a minor portion of cattle diet, mainly due to low palatability and toxicity issues (e.g., high fiber content, lignin, tannins, noxious chemicals etc.) (Vandermeulen et al. 2018). However, some woody species can also represent a very good quality feedstuff, rich in minerals and proteins, and may be the only resource available during critical periods of grass shortages (Hejcmanová et al. 2014; Ravetto Enri et al. 2020; Vandermeulen et al. 2018). The remarkable consumption of woody plants by Highland cattle suggests they might have a ruminal and intestinal microbial population which allow them to digest this feedstuff. For instance, other livestock species, such as goats, use to forage on woody and toxic plants due to their ability to detoxify plant secondary metabolites (Giger-Reverdin et al. 2020; Iussig et al. 2015). The exploitation of these resources by livestock can contribute to farming system sustainability, as highlighted e.g. by European Union policies encouraging agroforestry systems (Mosquera-Losada et al. 2018; Rigueiro-Rodríguez et al. 2009; Santiago-Freijanes et al. 2018).

4.3. Final considerations

The two case-studies included in this thesis highlighted the essential role of livestock grazing for the conservation and restoration of semi-natural grasslands. Particular attention has to be paid to the choice of livestock species and breed when planning the management of semi-natural grasslands, since some can be more suitable to certain environments, or differ in the effects produced on vegetation. This thesis also highlighted the importance of livestock grazing intensity, not just averaged at the pasture/farm-scale, but at a finer plot-scale, for the achievement of specific goals. The modulation of livestock grazing intensity through management practices is thus a key element for the effectiveness of conservation and restoration actions.

The outcomes of this thesis suggest interesting directions for future research studies. Firstly, the study of Highland cattle feeding preferences could provide novel insights on the ability of this breed to exploit marginal and woody-encroached pastures. This topic has been investigated by the author in an on-going research conducted in four woody-encroached sites in the Western Alps (see Annex 2 for the preliminary results), and could be extended in further study areas and vegetation communities where grazing with Highland cattle might be adopted as a conservation and restoration tool (e.g. heathlands). Moreover, similar research could also be extended to other understudied robust cattle breeds. For instance, other than Highland cattle, the iGRAL project aimed at studying the use of Sarda cattle, a robust breed native to Sardinia Region, for the restoration of degraded wood-pastures and grasslands. The forage quality of shrub and tree species consumed by Highland cows (e.g., protein and fiber content, digestibility, minerals, toxic compounds etc.), their feed intake, their growing performances, and the quality of the final meat products should also be investigated to have a more comprehensive knowledge on the potential of this farming system. Currently, the author and colleagues are planning to perform the analysis of the forage quality of *A. viridis* leaves collected in Bovonne and Val Vogna (second case-study) as well as the analysis of the meat quality (i.e., macronutrients, acidic profile, vitamins, cholesterol and color) of the Val Vogna cows. Another topic of great importance would be the monitoring of vegetation dynamics through time. This is particularly important after the application of an experimental treatment, such as the attractive points used in this thesis. For this reason, the author and colleagues have planned to survey the vegetation around molasses attractive points in the next years, in order to follow the changes in botanical composition and forage quality. Also the monitoring of the soil parameters would increase the understanding of grazing impacts around feeding supplement areas. Moreover, the study of the ability of livestock to transport the seeds of pasture species in the dungs is also an interesting and understudied research topic, especially for restoration goals, which requires further attention. Finally, the use of livestock GPS collars can help explore some open research questions concerning animal spatial behavior, such as the differences in grazing patterns among different species, breeds, and individuals, in different seasons and weather conditions. For instance, behavioral differences among individuals may be useful to select more robust genotypes. Recently, some innovative applications of GPS tracking systems, such as ‘real-time tracking’ and ‘virtual fencing’, have been developed to support farmers' work, and their effectiveness and applicability should be tested in rough alpine environments.

4.4. Bibliography

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Annex 1

Comparison of species richness and botanical composition in 2020 vs. 2021 in Attractive Points and Control areas in Vogna (*Preliminary results of on-going research*)

To scrutinize the changes in vegetation composition and diversity one year after the point attractive (molasses blocks) experiment, species richness and botanical composition were compared in 2020 vs. 2021 for both Attractive Points and Control areas in Vogna study site. Species richness was compared by paired t-tests, with transects as repetitions (n=16 per each year). Changes in botanical composition were investigated by *indicator species analysis* using species relative abundance data, and transects as repetitions (n=16 per each year). Paired t-tests were performed with Past 4.01 statistical software. The indicator species analysis was performed using the '*indicspecies*' package in R.

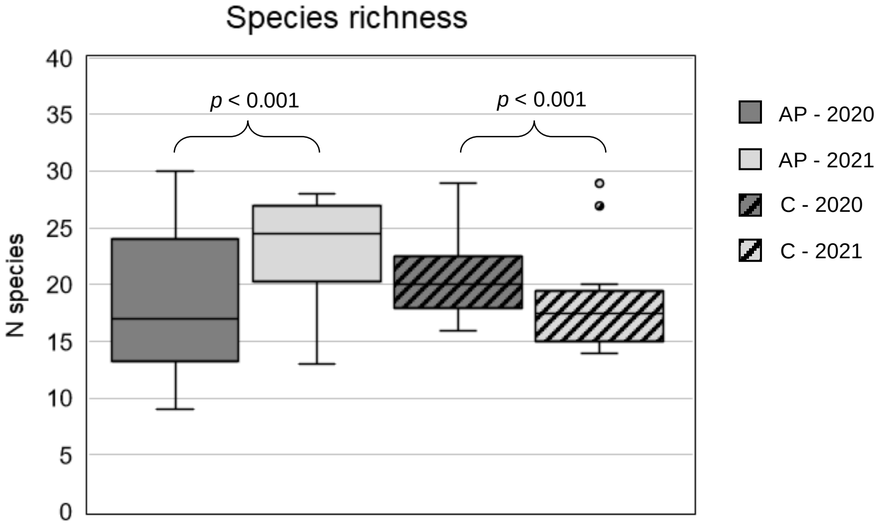

Figure 1. Species richness in Attractive Points (AP) and Control (C) areas in 2020 and 2021 in Vogna. P values of paired t-tests 2020 vs. 2021 are shown in the graph.

Table 1. Indicator value (IndVal) and p-values of the plant species associated to 2020 vs. 2021 in Attractive Points in Vogna, according to the '*indicator species analysis*'. Any species was associated to 2020 vs. 2021 in Control areas. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$.

	2020 IndVal	2021 IndVal
Alnus viridis	0.807*	
Poa pratensis		0.953***
Larix decidua		0.678*
Lotus alpinus		0.678*
Rumex alpestris		0.679*
Silene vulgaris		0.911**
Trifolium pratense		0.661**
Veronica serpyllifolia		0.707**

Annex 2

Diet composition and feeding preferences of Highland cows in four woody-encroached sites (*Preliminary results of on-going research*).

Herein are shown the preliminary results of a study about Highland cattle diet and feeding preferences, which was conducted as part of the iGRAL project. The data were collected in 2021 in four woody-encroached sites extensively grazed by Highland cows: Almese (Susa Valley, Italy, 450 m a.s.l.), Casteldelfino 1 (Varaita Valley, Italy, 1350 m a.s.l.), Casteldelfino 2 (Varaita Valley, Italy, 1250 m a.s.l.) and Bovonne (Canton of Vaud, Switzerland, 1750 m a.s.l.). Bovonne study site was the same of the studies in Chapter 3.1 and 3.2.

To investigate cattle diet and feeding preferences, we used direct observations. We chose a focal animal and recorded its behavior during 15 sec. observation units, followed by a 20 sec. interval between each observation. For each observation, we recorded (i) the plant species consumed and (ii) all the plant species available in a 1-m buffer area around the head of the cow. Moreover, we attributed a ‘consumption value’, if any, (= relative consumption on a 0-10 scale) and an ‘abundance value’ (= relative abundance on a 0-10 scale) to each plant species. Total monitoring days and hours were 12 and 150, respectively.

Based on these data, we calculated the relative contribution of herbaceous and woody species in the diet, as the proportion of the sum of consumption values of each species and the sum of all consumption values (Figure 1). Secondly, we calculated the Jacob’s Selectivity Index[1] for the most frequent plant species (Figure 2). The Index was calculated for each cow in each day, according to the following formula:

$$Jacob's\ Selectivity\ Index_{xyz} = \frac{(C_{xyz} - A_{xyz})}{(C_{xyz} + A_{xyz} - 2 \cdot C_{xyz} \cdot A_{xyz})}$$

where C_{xyz} and A_{xyz} are the relative consumption and the relative abundance of the species $_x$ for the cow $_y$ in the day $_z$, respectively.

Jacob’s Selectivity Index is bounded between -1 and 1, with 0 as the threshold between preference and avoidance. When the value is > 0, the species is preferred, when it is < 0, the species is avoided. When the value is 0, the species is consumed proportionally to its abundance.

[1] Jacobs, J. (1974). Quantitative measurement of food selection. *Oecologia* 14(4): 413-417. doi:10.1007/BF00384581

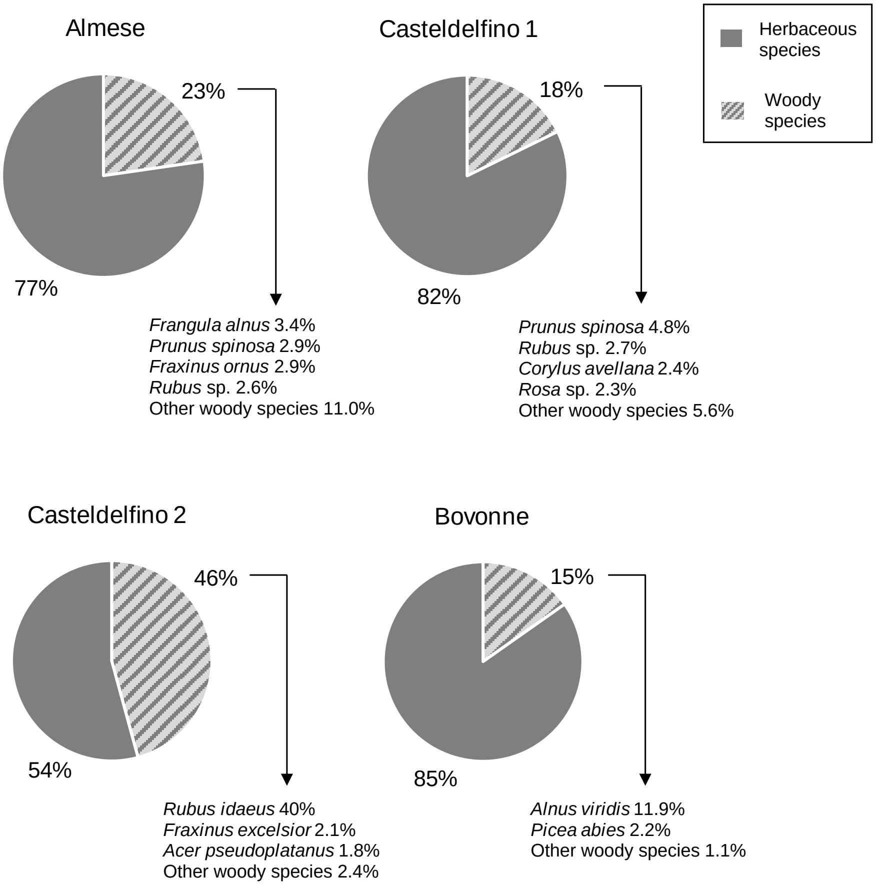

Figure 1. Relative proportion of herbaceous and woody species in Highland cows diet in four woody-encroached sites.

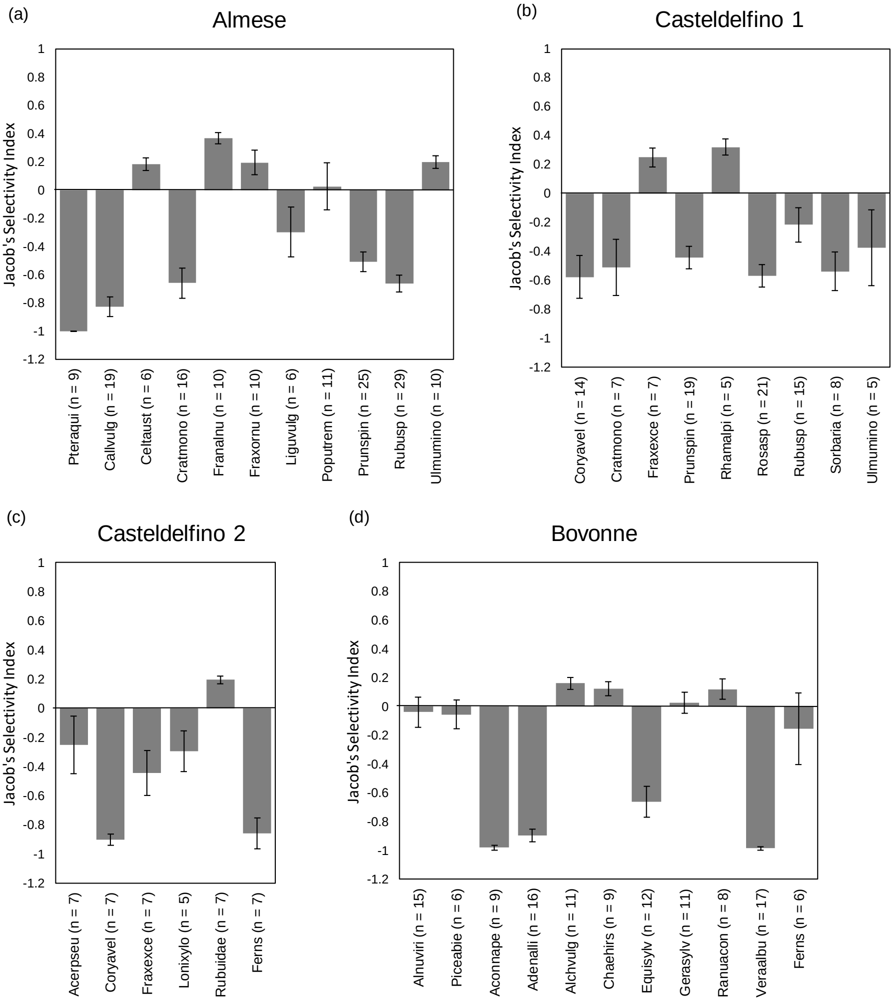

Figure 2. Jacob's Selectivity Index of some woody and herbaceous species expressed by Highland cows in four woody-encroached sites. Bars are mean values and error bars are standard errors. The number of repetitions (i.e., cows) is reported in brackets. Acerpseu = *Acer pseudoplatanus*, Aconnape = *Aconitum napellus*, Adenalli = *Adenostyles alliariae*, Alchvulg = *Alchemilla vulgaris*, Alnuviri = *Alnus viridis*, Callvulg = *Calluna vulgaris*, Celtaust = *Celtis australis*, Chaehirs = *Chaerophyllum hirsutum*, Coryavel = *Corylus avellana*, Cratmono = *Crataegus monogyna*, Equisylv = *Equisetum sylvaticum*, Franalnu = *Frangula alnus*, Fraxexce = *Fraxinus excelsior*, Fraxornu = *Fraxinus ornus*, Gerasylv = *Geranium sylvaticum*, Liguvulg = *Ligustrum vulgare*, Lonixylo = *Lonicera xylosteum*, Piceabie = *Picea abies*, Poputrem = *Populus tremula*, Prunuspin = *Prunus spinosa*, Pteraqui = *Pteridium aquilinum*, Ranuacon = *Ranunculus aconitifolius*, Rhamalpi = *Rhamnus alpinus*, Rosasp = *Rosa* sp., Rubuidae = *Rubus idaeus*, Rubusp = *Rubus* sp., Sorbaria = *Sorbus aria*, Ulmumino = *Ulmus minor*, Veraalbu = *Veratrum album*.