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Restoration of sub-alpine shrub-encroached grasslands through pastoral practices: effects on vegetation structure and botanical composition

Marco Pittarello, Massimiliano Probo, Michele Lonati & Giampiero Lombardi

Pittarello, M. (Corresponding author, marco.pittarello@unito.it)[1]

Probo, M. (massimiliano.probo@unito.it)[1]

Lonati, M. (michele.lonati@unito.it)[1]

Lombardi, G. (giampiero.lombardi@unito.it)[1]

[1] Department of Agricultural, Forest and Food Sciences, University of Torino, Largo Braccini 2, Grugliasco, I 10095, Italy;

Abstract

Questions: The reduction of agro-pastoral activities in most of the European mountain areas has led to a widespread shrub-encroachment of former grasslands in recent decades, with a loss of many ecosystem services provided by open habitats. To reverse this process and restore grassland vegetation, two pastoral practices were implemented over shrub-encroached areas: the arrangement of temporary night camp areas (TNCA) and the strategic placement of mineral mix supplements (MMS) for cattle. The aim was to assess the effects produced on i) vegetation structure and ii) botanical composition by both pastoral practices, in order to identify their potential to reverse shrub-encroachment and restore semi-natural grassland vegetation.

Location: Shrub-encroached sites, predominantly composed of *Juniperus nana* Willd. and *Rhododendron ferrugineum* L., in Val Troncea Natural Park, south-western Italian Alps.

Methods: We measured the effects produced by both practices on vegetation cover and height, cover of the species belonging to different vegetation units, biodiversity indices (species richness and Shannon diversity index), forage pastoral value, and average indicator value for soil nutrient content, from 2011 to 2014, along permanent linear transects. Data were analyzed with Generalized Linear Mixed Models (GLMMs), Multiple Response Permutational Procedure (MRPP) and Principal Response Curve (PRC).

Results: Both practices were effective in reducing shrub cover and increasing the average height of the herbaceous layer, but within TNCA a marked increase in herbaceous cover was also evident. Moreover, the arrangement of TNCA increased the cover of meso-eutrophic grassland and fringe and tall herb plant species and decreased the cover of boreal-like shrubland and woodland species. The main effect produced by the placement of MMS on botanical composition was the increase in the cover of fringe and tall herb species. Plant biodiversity was enhanced by the arrangement of TNCA but not by the placement of MMS, and the implementation of both practices increased forage pastoral value and the average indicator value for soil nutrient content.

Conclusions: The arrangement of TNCA was the most effective pastoral practice to reverse shrub-encroachment, restore semi-natural meso-eutrophic grassland vegetation and increase plant diversity, herbage mass and forage quality.

Key words. Alpine pastures; Cattle; Endochory; Forage pastoral value; Meso-eutrophic grasslands; Plant Biodiversity; Principal Response Curve; Vegetation units, Woody plants

Nomenclature. Pignatti (1982) for plant species, Aeschimann et al. (2004) for plant communities

Abbreviations. TNCA: temporary night camp areas, MMS: mineral mix supplements

Running head: Pastoral practices for grassland restoration

Introduction

Shrub encroachment is a natural process common in different biomes globally, such as the arid and semiarid biomes of western United States, Australia, southern Africa, and the Mediterranean Basin (Eldridge et al. 2011) and the temperate environments of China (Li et al. 2013), South America (Cabral et al. 2003) and Europe (Prévosto et al. 2011). Shrub encroachment can be determined by a number of distinct factors and their interaction, such as global climate change (Gehrig-Fasel et al. 2007), reduced fire frequency (Scholes & Archer 1997), presence of exotic plant species (Archer 2010), and change in land use intensity (MacDonald et al. 2000). In the last decades, human depopulation and agro-pastoral abandonment reduced land use intensity in different European mountain chains, such as in the Carpathians (Galvánek & Lepš 2009), in the Pyrenees (Roura-Pascual et al. 2005), in the French Massif Central (Prévosto et al. 2006), and in the Alps (Bätzing 2005). The number of livestock farms, animals reared, and the extent of grasslands and meadows regularly exploited by pastoral activities has strongly declined (MacDonald et al. 2000; Probo et al 2013), resulting in widespread tree and shrub-encroachment of former open habitats. Consequently, these vegetation changes decreased the yield and nutritive value of forage, plant and animal diversity and nitrogen fixation, and increased the probability of wild-fires (Laiolo et al. 2004; Freléchoux et al. 2007; Lonati et al. 2015).

Livestock management could be a sustainable and effective tool to affect the cover and structure of vegetation by counteracting shrub-encroachment in rugged sub-alpine locations, where the steep slopes and the low number of roads often limit the possibility of implementing mechanical shrub clearing. Indeed, the reintroduction of grazing within abandoned semi-natural grasslands, as well as the implementation of a rotational grazing system with recommended stocking levels, were successful in reducing the possibility of shrub encroachment (Hansson & Fogelfors 2000; Krahulec et al. 2001; Probo et al. 2014). However, as livestock generally tend to concentrate within the flattest sites and to avoid the steepest sites (Bailey et al. 1996), they need to be forced or attracted towards these locations to exert their positive effect on vegetation structure and composition. Therefore, the implementation of specific pastoral practices using targeted grazing over steep and shrub-encroached areas is needed when the stage of shrub-encroachment is too advanced.

Practices such as the arrangement of temporary night camp areas (TNCA) and the strategic placement of mineral mix supplements (MMS) for cattle, can be used to reduce locally the shrub cover through the action of cattle trampling and restore grassland vegetation within the areas where shrubs were removed. These techniques, which have been described and implemented by Probo et al. (2013) and Tocco et al. (2013), have recently been supported by European agri-environmental payments in the Piedmont region (north-western Italy) in order to restore semi-natural grassland vegetation. The authors performed short-term monitoring mainly to evaluate the impact of TNCA and MMS on the reduction of shrub cover. However, to better understand the effects produced by restoration practices on vegetation structure and botanical composition, a longer period of monitoring is often needed (Bakker et al., 1996), above all at high-elevation locations, where environmental constraints (e.g. low temperatures and short growing season) slow down the ecological response of plant communities (Körner 2003). Therefore, in our research the effects of TNCA and MMS on semi-natural grassland restoration were monitored over a longer period (i.e. three years after treatment). The aim was to assess the effects produced on i) vegetation structure and ii) botanical composition, by both pastoral practices, in order to identify their potential to reverse shrub-encroachment and restore sub-alpine semi-natural grassland vegetation.

Materials and methods

Study area

The study was conducted in Val Troncea Natural Park, south-western Italian Alps (latitude 44°57'N, longitude 6°57'E). Throughout recent decades, the Val Troncea Natural Park has experienced changes in shrubland extent and is therefore representative of habitat subject to shrub-encroachment and loss of grasslands due to pastoral abandonment. Annual average air temperature is 0.8° C (January: -8 °C, July: 9.5 °C) and annual average precipitation is 956 mm (Biancotti et al. 1998). The study area consisted of a large paddock (about 75 hectares), which was delimited with electric fences and was the most shrub-encroached of 18 paddocks managed under a rotational grazing system during summer. Within the paddock, elevations ranged from 1960 to 2360 m a.s.l. Grasslands were mainly dominated by *Festuca curvula* Gaudin, *Nardus stricta* L. and *Festuca* gr. *rubra*. The shrub layer was predominantly composed of *Juniperus nana* Willd. and *Rhododendron ferrugineum* L. The study area was grazed for 21 days (from 28 Jun to 18 Jul 2011) by 160 beef cows, corresponding to 135 animal units (AU - Allen et al. 2011), predominantly of the Piedmontese breed. The paddock was stocked at the same stocking rate (*sensu* Allen et al. 2011) in the same period in 2012, 2013 and 2014.

Arrangement of temporary night camp areas and placement of mineral mix supplements for cattle

Four TNCA were arranged and four MMS were placed within the study area. The TNCA were positioned within large patches of shrub-encroached grasslands with roughly the same slope (28 % on average). The TNCA were arranged from 30 Jun to 15 Jul 2011 and cattle were confined for two consecutive nights within each area, which was delimited with electric fences. The extent of TNCA was on average 1107 m^2, so a mean area of about 7 m^2 per night was available to each cow, resulting in a stocking density of 1200 AU ha^{-1}.

Mineral mix supplement sites were positioned within large patches of shrub-encroached grasslands with roughly the same slope (30 % on average). Within each site, cows were offered phosphate mineral mix supplements *ad libitum* for the whole grazing period. Mineral mix supplements were supplied in 5-kg blocks which were placed 5 m apart in pairs and had the same composition of the MMS used by Probo et al. (2013). In recent research conducted by Probo et al. (2013) within a similar study area and with the same experimental design of MMS placement and a similar herd, the stocking density measured within 45 m^2 of MMS through cattle GPS tracking systems was 58 AU ha^{-1}.

Each TNCA and each pair of MMS blocks was considered as a treatment site and was paired with a control site placed at a maximum distance of 130 m. Control sites of MMS and TNCA had approximately the same slope, area, soil cover, vegetation structure, botanical composition, and distance from regularly grazed pastures (i.e. 60 m) with respect to paired treatment sites.

Vegetation surveys

Botanical composition was determined using the vertical point-quadrat method (Daget & Poissonet 1971; Kohler et al. 2004) along permanent linear transects. Four transects were placed at each treatment and control site, simulating a cross with the center matched with the midpoint between the two MMS blocks and with the barycenter of the TNCA. Transects were 12.5 m long and surveys were carried out in late June in 2011, 2012, 2013 and 2014. In each transect, at every 50-cm interval, plant species touching a steel needle were identified and recorded. Since rare species are often missed by this method, a complete list of all other plant species included within a 1-m buffer area around the transect line was also recorded (Kohler et al. 2004). Within the same buffer, the percentages of shrub, herbaceous, and bare ground covers were visually estimated to give an estimate of basal cover. Furthermore, 20 measurements of the height of the herbaceous and shrub layers were randomly carried out with the sward stick method (Stewart et al. 2001).

In 2011, the extent of shrub-damaged areas modified by cattle trampling (i.e. the areas having broken shrub branches, signs of hoof-prints and faecal deposition) around MMS locations were visually estimated immediately after treatment, following the sharp fine-scale fragmentation of the original dense shrub cover. Fine-scale fragmentation is characterized by the lack of an intact core area and by the lack of difference between the edge and the core of the modified shrub area (Bar Massada et al. 2008).

Data analysis

Vegetation variables

To detect the effects on vegetation structure within both TNCA and MMS sites, the average annual heights of the shrub and the herbaceous layers were calculated for each vegetation transect as well as the average shrub, herbaceous and bare ground basal cover.

For each plant species recorded the frequency of occurrence (f_i = number of occurrences/25 points of vegetation measurement), which is an estimate of species canopy cover, was calculated for each transect and converted to percent cover (%SC). Species Relative Abundance (SRA) was determined in each transect and used to detect the proportion of different species according to the equation of Daget and Poissonet (1971):

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

where SRA_i and f_i are Species Relative Abundance and frequency of occurrence of the species *i*.

The phytosociological optimum was related to each plant species at the class level (including all subordinated syntaxa), according to Aeschimann et al. (2004). Groups of classes with physiognomic, ecological, and floristic similarity (called 'vegetation units') were defined according to Theurillat et al. (1995) and Aeschimann et al. (2004) (Table 1). The sum of the %SC of the species belonging to each vegetation unit was calculated for each transect to assess changes in the botanical composition.

Table 1. Vegetation units (*sensu* Theurillat et al., 1995) identified within the study sites, with the corresponding phytosociological classes (according to Aeschimann et al., 2004).

Vegetation unit	Phytosociological classes
Meso-eutrophic grasslands	*Molinio-Arrhenatheretea*
Nitrogen poor calcareous dry grasslands	*Festuco-Brometea*
Nitrogen poor calcareous high-elevation grasslands	*Elyno-Seslerietea variae*
Nitrogen poor acidic grasslands	*Juncetea-Trifidi, Nardetea-Strictae*
Fringe and tall herb grasslands	*Epilobietea-Angustifolii, Mulgedio-Aconitetea, Trifolio-Geranietea*
Boreal shrublands and woodlands	*Loiseleurio-Vaccinietea, Vaccinio-Piceetea excelsae*

Vegetation diversity was expressed according to two indices: species richness and Shannon diversity index. Shannon diversity (H’) was calculated for each transect according to the following equation:

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

where SRA_i is the Species Relative Abundance of species *i*.

Each species detected was classified according to its main dispersal strategy (Landolt et. al 2010): autochory, boleochory, dysochory, endochory, epichory, meteorochory, and myrmecochory.

Moreover, the Index of Specific Quality (ISQ) (Daget & Poissonet, 1971; Cavallero et al., 2007) was also attributed to each species. The ISQ is based on palatability, morphology, structure, and productivity of the plant species found in the Western Italian Alps, and it ranges from 0 (low) to 5 (high). In each transect, forage pastoral value, a synthetic value which summarizes forage yield and nutritive value ranging from 0 to 100, was calculated on the basis of the SRA and the ISQ according to Probo et al. (2013).

The demand of each plant species for nutrients was estimated according to the Landolt nutrient value (Landolt et al., 2010). The average indicator value for soil nutrient content, weighted for SRA, was calculated indirectly from the vegetation community for each transect to evaluate the overall effect of fertilization produced by pastoral practices on the soil.

Statistical analyses

Generalized Linear Mixed Models (GLMMs, Zuur et al., 2009) were used to test for annual differences between treatment and control sites for vegetation structure variables (i.e. shrub, herbaceous and bare ground basal cover and average herbaceous and shrub height) and botanical composition variables (i.e. sum of the %SC of species belonging to each vegetation unit, biodiversity indices, forage pastoral value, and average Landolt indicator value for soil nutrient content). In all GLMMs, treatment was considered as a fixed factor. Vegetation transect was considered as a random factor nested within area (where each area contained both a treatment and a control site, thus maintaining the paired structure). A Poisson distribution was specified for count variables which were not overdispersed (overdispersion in the data was tested by the *qcc* R package; Scrucca, 2004). If count data were overdispersed, a negative binomial distribution was used. For continuous data a normal distribution was specified when the normality of the distribution was met (the normality was tested using the Kolmogorow-Smirnov test), otherwise a gamma distribution was used. Generalized Linear Mixed Models were performed using the glmmADMB package (Fournier et al., 2012) of R statistical software, version 3.0.1. (R Development Core Team, 2012).

Multiple Response Permutation Procedures (MRPPs) were used to assess annual differences in the botanical composition of plant communities between TNCA or MMS sites and related control sites using the Bray-Curtis distance to calculate the distance matrix (McCune et al. 2002). The significance level was set to $P < 0.05$ (999 permutations). The MRPPs were performed using the ‘Vegan’ package (Oksanen et al., 2013) of R software.

A Principal Response Curve (PRC) analysis was performed to visualize the overall effect produced by pastoral practices on the botanical composition of treatment sites compared to that of control sites. As each TNCA and MMS site was paired with a specific control site, a covariate accounting for the site-pair was used in the PRC. Principal Response Curve analyses was performed using Canoco 5 (ter Braak & Smilauer 2012).

Results

Treatment and control sites in 2011 (i.e. pre-treatment) did not differ in vegetation structure (Table 2) and botanical composition (Table 3 and Fig.1). It was assumed, therefore, that treatment and control sites had roughly the same vegetation and ecological features before treatments.

Effects produced on vegetation structure

Areas modified by cattle around MMS sites had an elliptic shape, with the main axis placed along the contour lines, and with an average extent of 69 m^2. Three years after the implementation of both practices, the percentage of shrub cover was strongly reduced and the cover of bare ground increased, but a marked increase in the herbaceous cover was detected only within TNCA (Table 2). The average herbaceous height increased three years after treatment within both TNCA and MMS sites, whereas the average height of the shrub layer did not change over time.

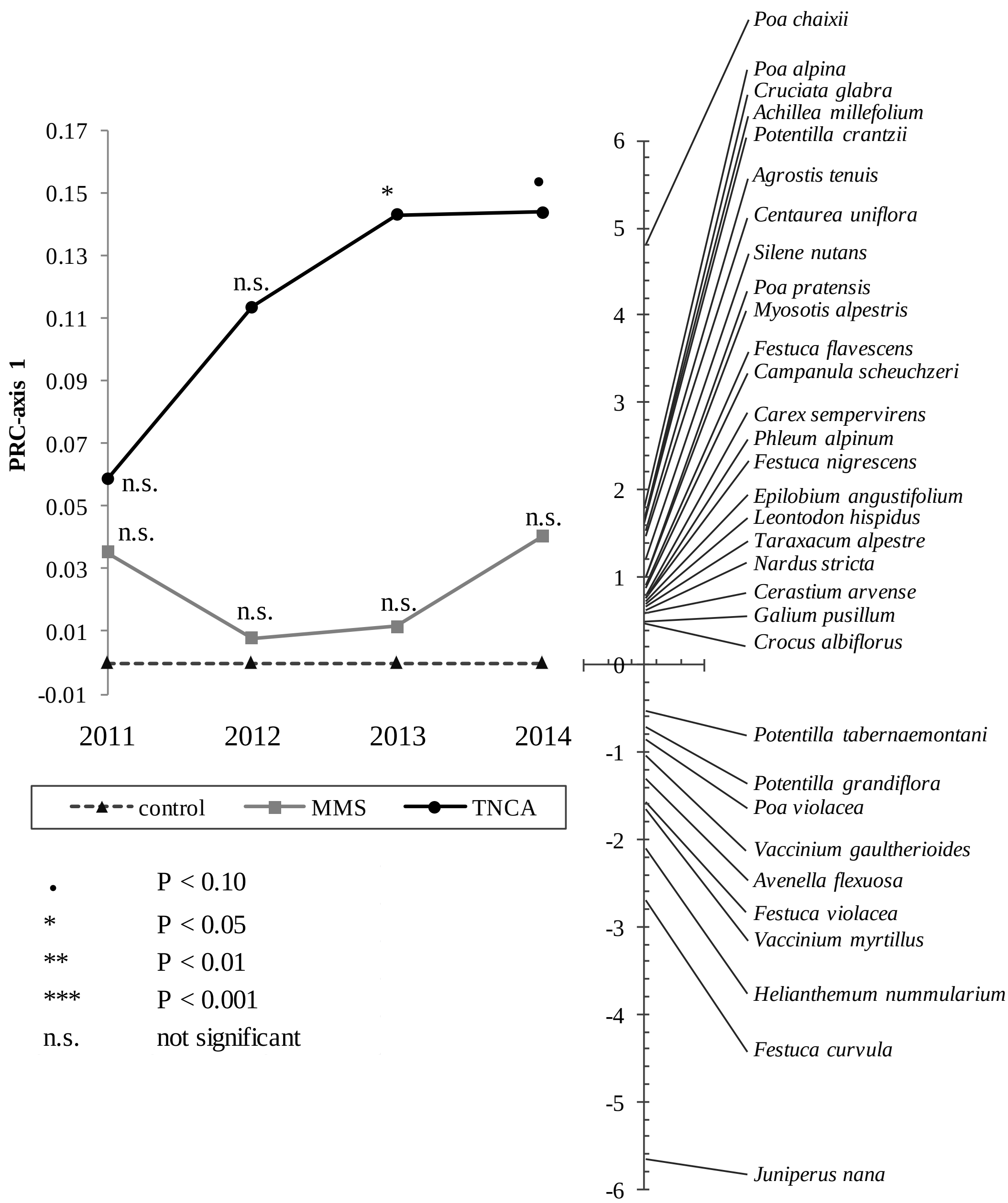
Table 2. Effects produced by the arrangement of four temporary night camp areas (TNCA) and the strategic placement of four mineral mix supplement (MMS) on vegetation structure variables with respect to paired control sites. Values shown are the mean and the standard error (SE) of the mean, and in 2011 they refer to pre-treatment. Asterisks represent the statistical significance level of differences between treatment and control sites: *** = P < 0.001; ** = P < 0.01; * = P < 0.05; n.s. = not significant (P > 0.05).

	TNCA							MMS						
	Treatment			Control				Treatment			Control			
	mean	±	SE	mean	±	SE	P	mean	±	SE	mean	±	SE	P
Shrub cover (%)														
2011	56	±	4	57	±	4	n.s.	74	±	3	73	±	2	n.s.
2012	29	±	5	57	±	5	***	49	±	4	72	±	2	***
2013	29	±	5	58	±	5	***	56	±	4	69	±	3	*
2014	21	±	5	59	±	5	***	52	±	4	67	±	4	*
Herbaceous cover (%)														
2011	33	±	3	32	±	5	n.s.	18	±	2	16	±	2	n.s.
2012	40	±	5	33	±	5	n.s.	17	±	3	16	±	1	n.s.
2013	52	±	6	33	±	4	*	23	±	3	21	±	2	n.s.
2014	64	±	5	33	±	4	***	28	±	3	23	±	4	n.s.
Bare ground cover (%)														
2011	10	±	1	11	±	2	n.s.	7	±	1	12	±	1	***
2012	31	±	4	11	±	2	***	33	±	3	12	±	1	***
2013	19	±	3	9	±	1	***	20	±	3	10	±	1	***
2014	16	±	2	8	±	1	***	20	±	3	10	±	1	***
Average herbaceous height (cm)														
2011	10	±	1	10	±	1	n.s.	9	±	1	9	±	1	n.s.
2012	13	±	1	11	±	1	*	10	±	1	9	±	1	n.s.
2013	16	±	2	10	±	1	**	15	±	1	10	±	2	***
2014	19	±	1	13	±	1	***	19	±	1	12	±	1	***
Average shrub height (cm)														
2011	29	±	2	29	±	2	n.s.	25	±	1	25	±	2	n.s.
2012	33	±	3	30	±	2	n.s.	26	±	1	26	±	2	n.s.
2013	28	±	2	27	±	3	n.s.	24	±	2	20	±	2	n.s.
2014	30	±	3	29	±	2	n.s.	29	±	2	27	±	2	n.s.

Effects produced on botanical composition

A total of 166 plant species was detected in botanical surveys. A complete list of plant species found with the corresponding phytosociological optimum and class is provided in the Supplementary material (S1). The MRPP analysis revealed that the botanical composition of plant communities within TNCA did not significantly differ between treatment and control sites in 2011 and 2012, but it differed both in 2013 and in 2014 (Fig. 1), whereas no significant changes between treatment and control sites over the three year of monitoring were detected for MMS sites. The PRC analysis showed an increase in the cover of herbaceous nutrient-demanding species within TNCA (e.g. *Poa chaixii* Vill., *Poa alpina* L., *Achillea millefolium* L., and *Agrostis tenuis* Sibth.), and a reduction of shrub and herbaceous nutrient-poor species (e.g. *J. nana*, *F. curvula*, *Festuca violacea* Gaudin, *Helianthemum nummularium* L., *Vaccinium. myrtillus* L., *Avenella flexuosa* L., and *Vaccinium gaultherioides* Bigelow) (Fig. 1).

Fig. 1. Principle Response Curve (PRC) (axis 1) showing the effects on the botanical composition of plant communities produced by the arrangement of temporary night camp areas (TNCA) and the placement of mineral mix supplements (MMS) with respect to untreated paired control sites. Species scores of the most affected species (species score > 0.5) are shown on the right side of the graph: positive values represent species whose canopy cover increased after treatment, whereas negative values represent species whose canopy cover decreased over time. Statistical significance is related to Multiple Response Permutation Procedures (MRPPs) results.



Changes in botanical composition were more pronounced within TNCA than within MMS sites (Table 3). Specifically, within TNCA both herbaceous species belonging to meso-eutrophic grassland and fringe and tall herb grassland vegetation units increased over time, while the species associated with boreal shrubland and woodland vegetation unit decreased. In contrast, within MMS sites only an increase in species belonging to fringe and tall herb grasslands was detected. Species belonging to the vegetation units of nitrogen poor (calcareous and acidic) grasslands did not increase over time both within TNCA and MMS.

Both biodiversity indices (number of species and Shannon diversity index) increased within TNCA three years after the treatment, whereas within MMS sites no significant variation was detected. In 2014, we detected on average 11.7 new species (i.e. species present in 2014 and missing in 2011) for each transect within TNCA, accounting for about one third of total species richness (Table 3). These species mainly belonged to nitrogen poor acidic (27%), meso-eutrophic (25%) and nitrogen poor calcareous dry (21%) grassland vegetation units and 35% of them were linked to the action of animals as dispersal vectors (30% by endochory and 5% by epichory).

Forage pastoral value significantly increased by 53 % and 24 % within TNCA and MMS sites three years after treatment, respectively. The average Landolt indicator value for soil nutrient content increased over time by 8 and 9 % within TNCA and MMS sites, respectively.

Table 3. Effects produced by the arrangement of four temporary night camp areas (TNCA) and strategic placement of four mineral mix supplement (MMS) on botanical composition, biodiversity indices, forage pastoral value, and average Landolt indicator value for soil nutrient content with respect to paired control sites three years after their implementation. Values shown are the mean and the standard error (SE) of the mean. Asterisks represent the statistical significance level of differences between treatment and control sites: *** = P < 0.001; ** = P < 0.01; * = P < 0.05; n.s. = not significant (P > 0.05).

	TNCA							MMS						
	Treatment			Control				Treatment			Control			
	mean	±	SE	mean	±	SE	P	mean	±	SE	mean	±	SE	P
Vegetation units														
Meso-eutrophic grassland species cover (%)														
2011	8.0	±	2.42	4.5	±	0.99	n.s.	0.8	±	0.39	1.5	±	0.86	n.s.
2012	14.8	±	4.33	8.5	±	2.42	n.s.	1.3	±	0.58	1.8	±	0.86	n.s.
2013	17.3	±	4.10	6.5	±	1.87	*	1.5	±	0.60	2.0	±	0.61	n.s.
2014	25.0	±	4.60	8.3	±	2.61	**	3.0	±	0.97	2.3	±	1.00	n.s.
Nitrogen poor calcareous dry grassland species cover (%)														
2011	12.8	±	2.77	15.3	±	2.48	n.s.	10.5	±	3.14	13.8	±	3.66	n.s.
2012	14.5	±	3.41	14.3	±	3.04	n.s.	10.8	±	2.95	18.3	±	4.32	*
2013	13.3	±	3.77	14.3	±	3.20	n.s.	11.5	±	3.69	20.8	±	5.83	n.s.
2014	20.3	±	4.29	22.0	±	5.04	n.s.	13.0	±	3.44	24.0	±	6.41	***
Nitrogen poor calcareous high-elevation grassland species cover (%)														
2011	20.8	±	4.79	24.8	±	3.03	n.s.	9.8	±	2.87	15.8	±	4.29	n.s.
2012	11.8	±	2.30	18.8	±	3.53	*	7.5	±	2.37	9.0	±	2.90	n.s.
2013	19.8	±	4.93	17.8	±	3.59	n.s.	9.0	±	2.49	10.8	±	3.35	n.s.
2014	20.5	±	4.41	15.8	±	2.90	n.s.	7.5	±	2.18	10.3	±	2.76	n.s.
Nitrogen poor acidic grassland species cover (%)														
2011	17.5	±	4.41	16.3	±	4.04	n.s.	8.8	±	1.94	7.8	±	2.54	n.s.
2012	23.8	±	5.94	24.3	±	4.16	n.s.	6.8	±	1.10	12.8	±	2.55	*
2013	19.5	±	5.17	19.3	±	4.17	n.s.	5.8	±	1.46	9.3	±	1.61	n.s.
2014	28.8	±	6.31	25.0	±	4.72	n.s.	16.3	±	2.73	19.8	±	4.28	n.s.
Fringe and tall herb grassland species cover (%)														
2011	14.5	±	3.43	12.3	±	3.40	n.s.	13.0	±	2.25	7.3	±	1.91	n.s.
2012	23.8	±	5.13	14.8	±	3.70	n.s.	11.8	±	2.28	8.3	±	1.89	n.s.
2013	32.5	±	6.22	13.8	±	3.20	***	16.8	±	3.03	9.0	±	2.05	*
2014	36.8	±	7.43	18.3	±	4.64	**	28.0	±	3.52	11.5	±	3.12	***
Boreal shrubland and woodland species cover (%)														
2011	71.8	±	8.13	73.3	±	5.50	n.s.	85.0	±	8.34	88.5	±	9.38	n.s.
2012	48.0	±	8.89	71.5	±	5.85	n.s.	76.0	±	8.95	81.8	±	8.90	n.s.
2013	44.0	±	7.85	75.3	±	5.80	***	72.0	±	5.41	82.3	±	7.76	n.s.
2014	44.8	±	8.24	76.0	±	6.81	***	74.8	±	8.04	89.3	±	8.53	n.s.
Biodiversity indices														
Shannon diversity index (*H'*)														
2011	2.8	±	0.23	2.8	±	0.15	n.s.	2.3	±	0.10	2.4	±	0.10	n.s.
2012	3.1	±	0.22	2.9	±	0.17	n.s.	2.3	±	0.10	2.5	±	0.12	n.s.
2013	3.0	±	0.23	2.8	±	0.18	n.s.	2.4	±	0.10	2.5	±	0.08	n.s.
2014	3.4	±	0.20	2.9	±	0.13	*	2.7	±	0.08	2.7	±	0.10	n.s.
Number of species														
2011	26.6	±	2.97	27.6	±	1.90	n.s.	18.2	±	1.22	18.5	±	0.98	n.s.
2012	31.9	±	3.22	31.6	±	2.17	n.s.	18.2	±	1.40	18.6	±	1.03	n.s.
2013	31.6	±	3.08	28.2	±	1.86	n.s.	19.9	±	1.16	18.8	±	1.28	n.s.
2014	34.8	±	3.42	26.9	±	1.66	**	21.3	±	1.32	19.0	±	1.12	n.s.

Forage Pastoral Value											
	2011	9.1	± 0.93	9.3	± 0.79	n.s.	6.6	± 1.02	6.7	± 1.07	n.s.
	2012	12.0	± 1.49	9.8	± 1.00	n.s.	5.6	± 0.72	6.2	± 0.78	n.s.
	2013	12.6	± 1.25	9.3	± 1.07	**	7.5	± 0.69	6.6	± 0.68	n.s.
	2014	13.9	± 1.40	9.9	± 0.90	*	8.2	± 0.58	6.4	± 0.61	*
Average Landolt indicator value for soil nutrient content											
	2011	2.3	± 0.05	2.2	± 0.05	n.s.	2.1	± 0.02	2.1	± 0.02	n.s.
	2012	2.3	± 0.05	2.3	± 0.04	n.s.	2.2	± 0.03	2.1	± 0.02	n.s.
	2013	2.5	± 0.05	2.2	± 0.04	***	2.2	± 0.02	2.1	± 0.01	*
	2014	2.5	± 0.05	2.2	± 0.04	***	2.3	± 0.02	2.1	± 0.01	**

Discussion

Temporary night camp area was the most effective pastoral practice to enhance vegetation structure and botanical composition, as it reversed shrub-encroachment, restored semi-natural meso-eutrophic grassland vegetation and increased plant diversity, herbage mass and forage quality three years after its implementation. Also the extent of the area modified by cattle, was much wider within TNCA than at MMS placement locations (i.e. 1107 m^2 vs 69 m^2 on average, respectively). However, even though strategic placement of MMS was less effective for grassland restoration, this practice produced some positive results and it can be considered as an alternative tool to reverse shrub-encroachment, above all in the more rugged and steeper locations, as it requires less labor to herd cattle and it is less costly than the arrangement of TNCA. These pastoral practices appeared to be less invasive than other techniques, such as burning, which produced negative effects on plant and animal diversity immediately after its implementation (Hansson & Fogelfors 2000; Lyet et al. 2009), and more feasible and less costly than shrub-clearing in rugged mountain areas. Moreover, as pointed out by Barbaro et al. (2001), a successful restoration of semi-natural grasslands is more achievable with a combination of shrub-clearing and grazing rather than by shrub-clearing without grazing. Indeed, livestock play an important role in the maintenance and enhancement of grassland biodiversity through defoliation, generation of gaps, nutrient cycling, and propagule dispersion (Gaujour et al. 2012).

The intense trampling action by cattle caused serious and widespread mechanical damage to the branches of the shrubs (e.g. *J. nana*), determining a strong decrease in their cover. Moreover, shrub cover decreased more intensively within TNCA, where trampling by livestock had been more intense due to the higher stocking density and shrub species did not demonstrate any sign of re-sprout. The bare ground cover noticeably increased one year after treatment within both TNCA and MMS sites, then it gradually reduced in the following years. The bare ground reduction was related to the process of gap recolonization by herbaceous species. This process was much more pronounced and faster within TNCA than at MMS sites, as within TNCA herbaceous cover progressively increased, almost doubling after three years and it was probably related to the more intense effect of fertilization occurred within the TNCA. However, these promising results confirmed that recolonization is a slow process at these elevations as vegetation communities are almost entirely composed of perennial species, with a negligible presence of annual fast-growing species (Körner, 2003).

The increase in the average herbaceous height within both TNCA and MMS, which determined an increase in forage yield, may be attributed to the increase in the average height of many species due to the intense fertilization effect and to the increase in the cover of tall herb species, such as *P. chaixii*. This tall graminoid species (up to 120 cm high) seems to colonize both abandoned sites after the reintroduction of grazing in the Carpathians (Krahulec et al., 2001) and underused sites where stocking density and related dung deposition is intensively increased in the Alps (Probo et al., 2013).

The greater effectiveness in the restoration of semi-natural grassland vegetation composition produced by the arrangement of TNCA with respect to MMS placement was confirmed by the increase in the cover of meso-eutrophic grassland species (belonging to the *Molinio-Arrhenatheretea* phytosociological class), which was three times higher compared to the pre-treatment state (i.e. 25% vs 8% in 2014 and 2011, respectively). Considering that agro-pastoral abandonment leads to a decrease of meso-eutrophic species in favor of nutrient-poor herbaceous and shrub species (Prévosto et al. 2006), these data represent a promising result to reverse the abandonment process. The greater availability of nitrogen in the soil deriving from intense dung and urine deposition by cattle (Aarons et al., 2004) favored the recolonization of the bare ground gaps created by livestock trampling by plant species usually associated with intense grazing or mowing and fertilization processes (e. g. *P. alpina*, *A. tenuis*, and *Poa pratensis* L.). Consequently, an increase in the average Landolt indicator value for soil nutrient content was detected. These species also have a high index of specific quality, so the increase in their cover can help to explain the improvement in forage quality within TNCA. A similar trend was assessed for the cover of fringe and tall herb grassland species (e.g. *P. chaixii*, *Epilobium angustifolium* L.), which are nutrient-demanding species, usually abundant in the early secondary stage of semi-natural grassland encroachment. Species belonging to nitrogen poor vegetation units did not increase either within TNCA or within MMS sites, probably because they were less efficient in responding to increased nutrient availability compared to nutrient-demanding species (Chapin III et al., 1986).

Two of the major problems in shrub-encroached grassland restoration are the transience of the seed bank under shrub canopy (Barbaro et al., 2001) and the low distance of seed dispersal for most of grassland species (Tackenberg & Stöcklin, 2008), so an external seed supply is often required to achieve an effective grassland restoration. In our study, the external supply was effectively met by the arrangement of TNCA, as plant biodiversity enhanced, above all thanks to the increased number of new species detected in the gaps created by livestock trampling. It is worth mentioning that many of these species were linked to the action of livestock as a vector for their dispersal from grazed pastures, mainly through the process of endochory, i.e. the ingestion by cattle of plant seeds and their excretion through faecal deposition (Traba et al., 2003). For these reasons, the long-term effectiveness of the restoration of treated areas should require the direct inclusion of restored areas within regularly grazed pastured (Winsa et al., 2015).

In conclusion, with the pastoral practices carried out (particularly with TNCA) it was possible to begin a process of semi-natural grassland restoration. By reducing the shrub cover, increasing the availability of nutrients into the soil and using livestock as a vector for herbaceous seed dispersal from adjacent grazed pastures, the meso-eutrophic species significantly started recolonizing former shrub-encroached areas and bare ground gaps. A regular grazing in the following years would help both to counteract a new woody species growth and to increase the cover of these meso-eutrophic species. Considering the rate of herbaceous recolonization and the bare ground cover still present (16-20%), it is possible to expect a further increase in the cover of the meso-eutrophic herbaceous species within treated areas in the years to come.

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Appendix S1.

Supporting information to the paper

Pittarello M., et al. Restoration of sub-alpine shrub-encroached grasslands through pastoral practices: effects on vegetation structure and botanical composition. *Applied Vegetation Science*

Appendix S1. List of the plant species recorded in the botanical surveys , with the corresponding phytosociological optimum and class. Species nomenclature follows Pignatti et al. (1982) and syntaxonomic nomenclature follows Aeschimann et al. (2004)

Plant species	Phytosociological optimum	Phytosociological class
Achillea millefolium	*Molinio-Arrhenatheretea*	*Molinio-Arrhenatheretea*
Acinos alpinus	*Elyno-Seslerietea variae*	*Elyno-Seslerietea variae*
Agrostis alpina	*Elyno-Seslerietea variae*	*Elyno-Seslerietea variae*
Agrostis tenuis	*Molinio-Arrhenatheretea*	*Molinio-Arrhenatheretea*
Ajuga pyramidalis	*Caricetalia-curvulae*	*Juncetea trifidi*
Alchemilla xanthochlora	*Molinio-Arrhenatheretea*	*Molinio-Arrhenatheretea*
Alopecurus gerardi	*Nardion strictae*	*Juncetea trifidi*
Alyssum alpestre	*Seslerion-variae*	*Elyno-Seslerietea variae*
Androsace brigantiaca	*Molinio-Arrhenatheretea*	*Molinio-Arrhenatheretea*
Androsace carnea	*Juncetea trifidi*	*Juncetea trifidi*
Antennaria dioica	*Nardetea strictae*	*Nardetea strictae*
Anthoxanthum alpinum	*Juncetea trifidi*	*Juncetea trifidi*
Arabis ciliata	*Elyno-seslerietea variae*	*Elyno-Seslerietea variae*
Arabis hirsuta	*Festuco-Brometea*	*Festuco-Brometea*
Armeria alpina	*Caricetalia-curvulae*	*Juncetea trifidi*
Arnica montana	*Nardion strictae*	*Juncetea trifidi*
Astragalus danicus	*Festuco-Brometea*	*Festuco-Brometea*
Astragalus sempervirens	*Avenion semperivirentis*	*Festuco-Brometea*
Avenella flexuosa	*Quercetea robori-sessiflorae*	*Quercetea robori-sessiflorae*
Avenula pubescens	*Triseto-Polygonion*	*Molinio-Arrhenatheretea*
Biscutella laevigata	*Seslerion-variae*	*Elyno-Seslerietea variae*
Botrychium lunaria	*Juncetea trifidi*	*Juncetea trifidi*
Bunium bulbocastanum	*Caucalidion lappulae*	*Stellarienea mediae*
Campanula scheuchzeri	*Juncetea trifidi*	*Juncetea trifidi*
Cardamine resedifolia	*Androsacetalia alpinae*	*Thlaspietea rotundifolii*
Carduus carlinaefolius	*Elyno-Seslerietea caeruleae*	*Elyno-Seslerietea variae*
Carex caryophyllea	*Brometalia erecti*	*Festuco-Brometea*
Carex ornithopoda	*Erico-Pinetalia, Elyno-Seslerietea variae*	*Elyno-Seslerietea variae*
Carex rosae	*Oxytropido-Elynion*	*Carici rupestris, Kobresietea bellardii*
Carex sempervirens	*Elyno-Seslerietea variae, Juncetea trifidi*	*Elyno-Seslerietea variae*
Carlina acaulis	*Mesobromion*	*Festuco-Brometea*
Centaurea uniflora	*Festucion-variae*	*Juncetea trifidi*
Cerastium arvense	*Agropyretea intermedii-repentis*	*Agropyretea intermedii-repentis*
Cerinthe glabra	*Rumicion alpini*	*Artemisietea vulgaris*
Cirsium acaule	*Festuco-Brometea*	*Festuco-Brometea*
Cotoneaster integerrimus	*Berberidenion*	*Cratego-Prunetea*
Crepis conyzifolia	*Festucion-variae*	*Juncetea trifidi*
Crocus albiflorus	*Triseto-Polygonion*	*Molinio-Arrhenatheretea*
Cruciata glabra	*Trifolio-Geranietea*	*Trifolio-Geranietea*
Cystopteris fragilis	*Asplenietea trichomanis*	*Asplenietea trichomanis*
Daphne mezereum	*Fagion sylvaticae*	*Carpino-Fagetea sylvaticae*
Dianthus neglectus	*Caricetalia-curvulae*	*Juncetea trifidi*
Draba aizoides	*Drabo-Seslerion variae*	*Elyno-Seslerietea variae*
Dryas octopetala	*Carici-Kobresietea*	*Carici rupestris, Kobresietea bellardii*
Epilobium angustifolium	*Epilobion angustifolii*	*Epilobietea angustifolii*
Erysimum jugicola	*Elyno-Seslerietea variae*	*Elyno-Seslerietea variae*
Euphorbia cyparissias	*Festuco-Brometea*	*Festuco-Brometea*

Plant species	Phytosociological optimum	Phytosociological class
Euphrasia alpina	*Juncetea trifidi*	*Juncetea trifidi*
Festuca curvula	*Seslerion-variae*	*Elyno-Seslerietea variae*
Festuca flavescens	*Piceion excelsae*	*Vaccinio-Piceetea excelsae*
Festuca nigrescens	*Nardion strictae*	*Juncetea trifidi*
Festuca quadriflora	*Seslerion-variae*	*Elyno-Seslerietea variae*
Festuca violacea	*Caricion ferrugineae*	*Elyno-Seslerietea variae*
Gagea fistulosa	*Rumicion alpini*	*Artemisietea vulgaris*
Galium album	*Arrhenatherion elatioris*	*Molinio-Arrhenatheretea*
Galium gr. *rubrum*	*Quercion robori-sessiliflorae*	*Quercetea robori-sessiflorae*
Galium pusillum	*Potentillion caulescentis*	*Asplenietea trichomanis*
Gentiana kochiana	*Nardion strictae*	*Juncetea trifidi*
Gentiana verna	*Elyno-Seslerietea varie*	*Elyno-Seslerietea variae*
Gentianella campestris	*Nardetea strictae*	*Nardetea strictae*
Geranium sylvaticum	*Mulgedio-Aconitetea*	*Mulgedio-Aconitetea*
Geum montanum	*Juncetea trifidi*	*Juncetea trifidi*
Globularia cordifolia	*Elyno-Seslerietea*	*Elyno-Seslerietea variae*
Helianthemum nummularium	*Festuco-Brometea*	*Festuco-Brometea*
Helianthemum oelandicum	*Ononidetalia*	*Festuco-Brometea*
Helictotrichon parlatorei	*Seslerietalia variae*	*Elyno-Seslerietea variae*
Helictotrichon sedenense	*Seslerietalia variae*	*Elyno-Seslerietea variae*
Hepatica nobilis	*Carpino-Fagetea sylvaticae*	*Carpino-Fagetea sylvaticae*
Hieracium glanduliferum	*Juncetea trifidi*	*Juncetea trifidi*
Hieracium pilosella	*Festuco-Brometea*	*Festuco-Brometea*
Hieracium prenanthoides	*Calamagrostietalia villosae*	*Mulgedio-Aconitetea*
Hieracium sylvaticum	*Carpino-Fagetea sylvaticae*	*Carpino-Fagetea sylvaticae*
Hieracium x *auriculiforme*	*Sedo-Scleranthetalia*	*Koelerio-Corynephoretea*
Hippocrepis comosa	*Festuco-Brometea*	*Festuco-Brometea*
Homogyne alpina	*Piceetalia excelsae*	*Vaccinio-Piceetea excelsae*
Hutchinsia alpina	*Thlaspion rotundifolii*	*Thlaspietea rotundifolii*
Hypochoeris maculata	*Festuco-Brometea*	*Festuco-Brometea*
Hypochoeris radicata	*Cynosurion*	*Molinio-Arrhenatheretea*
Juncus trifidus	*Juncetea trifidi*	*Juncetea trifidi*
Juniperus nana	*Loiseleurio-Vaccinietea*	*Loiseleurio-Vaccinietea*
Koeleria cenisia	*Seslerion-variae*	*Elyno-Seslerietea variae*
Lactuca perennis	*Festuco-Brometea*	*Festuco-Brometea*
Larix decidua	*Piceion excelsae*	*Vaccinio-Piceetea excelsae*
Leontodon autumnalis	*Cynosurion*	*Molinio-Arrhenatheretea*
Leontodon helveticus	*Juncetea trifidi*	*Juncetea trifidi*
Leontodon hispidus	*Arrhenatherion elatioris*	*Molinio-Arrhenatheretea*
Lonicera coerulea	*Piceetalia excelsae*	*Vaccinio-Piceetea excelsae*
Lotus alpinus	*Elyno-Seslerietea variae*	*Elyno-Seslerietea variae*
Lotus corniculatus	*Molinio-Arrhenatheretea*	*Molinio-Arrhenatheretea*
Luzula alpino pilosa	*Salicion herbaceae*	*Betulo-Alnetea viridis*
Luzula lutea	*Caricetalia-curvulae*	*Juncetea trifidi*
Luzula luzulina	*Piceion excelsae*	*Vaccinio-Piceetea excelsae*
Luzula multiflora	*Nardion strictae*	*Juncetea trifidi*
Luzula sieberi	*Piceion excelsae*	*Vaccinio-Piceetea excelsae*
Luzula spicata	*Juncetea trifidi*	*Juncetea trifidi*
Luzula sylvatica	*Luzulo-Fagion*	*Quercetea robori-sessiflorae*
Melampyrum sylvaticum	*Vaccinio-Piceetea excelsae*	*Vaccinio-Piceetea excelsae*
Myosotis alpestris	*Elyno-Seslerietea variae*	*Elyno-Seslerietea variae*
Nardus stricta	*Nardetea strictae*	*Nardetea strictae*
Nigritella nigra	*Seslerietalia variae*	*Elyno-Seslerietea variae*
Onobrychis montana	*Elyno-Seslerietea variae*	*Elyno-Seslerietea variae*
Orthilia secunda	*Piceetalia excelsae*	*Vaccinio-Piceetea excelsae*
Oxytropis halleri ssp. *velutina*	*Festucetalia valesiacae*	*Festuco-Brometea*

Plant species	Phytosociological optimum	Phytosociological class
Phleum alpinum	*Poion alpinae*	*Molinio-Arrhenatheretea*
Phyteuma betonicifolium	*Juncetea trifidi*	*Juncetea trifidi*
Phyteuma michelii	*Caricetalia-curvulae*	*Juncetea trifidi*
Phyteuma orbiculare	*Elyno-Seslerietea variae*	*Elyno-Seslerietea variae*
Pinus cembra	*Piceion excelsae*	*Vaccinio-Piceetea excelsae*
Pinus uncinata	*Picetalia excelsae*	*Vaccinio-Piceetea excelsae*
*Plantago alpina*s.l.	*Nardion strictae*	*Juncetea trifidi*
Plantago media	*Festuco-Brometea*	*Festuco-Brometea*
Poa alpina	*Poion alpinae*	*Molinio-Arrhenatheretea*
Poa carniolica	*Stipo-Poenion perconcinnae*	*Festuco-Brometea*
Poa cenisia	*Thlaspietalia- rotundifolii*	*Molinio-Arrhenatheretea*
Poa chaixii	*Calamagrostietalia villosae*	*Mulgedio-Aconitetea*
Poa pratensis	*Molinio-Arrhenatheretea*	*Molinio-Arrhenatheretea*
Poa violacea	*Juncetea trifidi*	*Juncetea trifidi*
Polygala alpestris	*Elyno-Seslerietea variae*	*Elyno-Seslerietea variae*
Polygonum viviparum	*Elyno-Seslerietea variae, Juncetea trifidi*	*Elyno-Seslerietea variae*
Potentilla aurea	*Juncetea trifidi*	*Juncetea trifidi*
Potentilla crantzii	*Seslerion-variae*	*Elyno-Seslerietea variae*
Potentilla grandiflora	*Festucion variae*	*Juncetea trifidi*
Potentilla tabernaemontani	*Brometalia erecti*	*Festuco-Brometea*
Pulmonaria australis	*Trifolio-Geranietea*	*Trifolio-Geranietea*
Pulsatilla vernalis	*Juncetea trifidi*	*Juncetea trifidi*
Ranunculus montanus	*Elyno-Seslerietea variae*	*Elyno-Seslerietea variae*
Ranunculus pyrenaeus	*Nardion strictae*	*Juncetea trifidi*
Rhododendron ferrugineum	*Piceion excelsae*	*Vaccinio-Piceetea excelsae*
Rumex acetosella	*Koelerio-Corynephoretea*	*Koelerio-Corynephoretea*
Salix helvetica	*Salicion helveticae*	*Betulo-Alnetea viridis*
Salix serpyllifolia	*Carici rupestris, Kobresietea bellardii*	*Carici rupestris, Kobresietea bellardii*
Salvia pratensis	*Festuco-Brometea*	*Festuco-Brometea*
Saxifraga paniculata	*Asplenietea trichomanis*	*Asplenietea trichomanis*
Sedum anacampseros	*Androsacetalia alpinae*	*Thlaspietea rotundifolii*
Sedum montanum	*Sedo-Scleranthetalia*	*Koelerio-Corynephoretea*
Sempervivum arachnoideum	*Sedo-Scleranthion*	*Koelerio-Corynephoretea*
Senecio incanus ssp. *incanus*	*Caricion curvulae*	*Juncetea trifidi*
Seseli libanotis	*Geranion sanguinei*	*Trifolio-Geranietea*
Sesleria varia	*Elyno-Seslerietea variae*	*Elyno-Seslerietea variae*
Sibbaldia procumbens	*Salicion herbaceae*	*Betulo-Alnetea viridis*
Silene nutans	*Mesobromion*	*Festuco-Brometea*
Soldanella alpina	*Caricetalia-curvulae*	*Juncetea trifidi*
Taraxacum alpestre	*Montio-Cardaminetalia*	*Montio-Cardaminetalia*
Taraxacum alpinum	*Thlaspetalia rotundifolii, Poion alpinae*	*Molinio-Arrhenatheretea*
Taraxacum laevigatum	*Festuco-Brometea*	*Festuco-Brometea*
Taraxacum officinale	*Arrhenatheretalia elatioris*	*Molinio-Arrhenatheretea*
Thesium alpinum	*Seslerion variae*	*Elyno-Seslerietea variae*
Thymus gr. *serpyllum*	*Festuco-Brometea*	*Festuco-Brometea*
Trifolium alpinum	*Juncetea trifidi*	*Juncetea trifidi*
Trifolium montanum	*Brometalia erecti*	*Festuco-Brometea*
Trifolium pratense nivale	*Poion alpinae*	*Molinio-Arrhenatheretea*
Trifolium repens	*Cynosurion*	*Molinio-Arrhenatheretea*
Trifolium thalii	*Caricion ferrugineae*	*Elyno-Seslerietea variae*
Vaccinium gaultherioides	*Loiseleurio-Vaccinietea*	*Loiseleurio-Vaccinietea*
Vaccinium myrtillus	*Vaccinio-Piceetea excelsae*	*Vaccinio-Piceetea excelsae*
Vaccinium vitis-idaea	*Vaccinio-Piceetea excelsae*	*Vaccinio-Piceetea excelsae*
Valeriana tripteris	*Asplenietea trichomanis*	*Asplenietea trichomanis*
Verbascum thapsus	*Atropion*	*Epilobietea angustifolii*
Veronica allionii	*Caricetalia-curvulae*	*Juncetea trifidi*

Plant species	Phytosociological optimum	Phytosociological class
Veronica alpina	*Androsacetalia alpinae*	*Thlaspietea rotundifolii*
Veronica chamaedrys	*Origanetalia vulgaris*	*Trifolio-Geranietea*
Viola biflora	*Betulo-Alnetea viridis*	*Betulo-Alnetea viridis*
Viola calcarata	*Elyno-Seslerietea variae*	*Elyno-Seslerietea variae*
Viola hirta	*Origanetalia vulgaris*	*Trifolio-Geranietea*
Viola riviniana	*Quercion robori-sessiliflorae*	*Quercetea robori-sessiflorae*
Vitaliana primulaeflora	*Androsacion alpinae*	*Thlaspietea rotundifolii*

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